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Feasibility of Generating Renewable Energy from Wastewater Treatment Process Using Microbial Fuel Cells: The West Bank as Case Study

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Dedication

To my mother, father, brothers and sister

Acknowledgements

First of all, praise to Allah for helping me in making this research possible. I would like to extend thanks to many people who helped me during my research work. Special mention goes to my enthusiastic supervisor Prof. Dr. Marwan Haddad, not only for his tremendous academic support, but also for giving me many wonderful opportunities during my work in Water and Environmental Studies Institute (WESI).

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الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان:

Feasibility of Generating Renewable Energy from Wastewater Treatment Process Using Microbial Fuel Cells: The West Bank as Case Study

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تم الإشارة اليه حيثما ورد ، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification

Student's Name: **اسم الطالب:**

Signature: **التوقيع :**

Date: **التاريخ :**

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List of Abbreviations and Nomenclature

Abbreviation	*Meaning*
BOD	Bio-Chemical Oxygen Demand
COD	Chemical Oxygen Demand
MAC-MFC	Multiple Anode Chamber MFC
MDCs	Microbial Desalination Cells
MFCs	Microbial Fuel Cells
MPL	Micro Porous Layer
Mt	Million tonnes
Mtoe	million tonnes of oil equivalent
OsMFCs	Osmotic Microbial Fuel Cells
PCR	power to cost ratio
PEM	Proton Exchange Membrane
pH	Potential Hydrogen (Acidity or Alkalinity Scale)
POME	palm oil mill effluent
PVDF- AC	Activate Carbon with Polyvinylidene Fluoride as diffusion layer
TSS	Total Suspended Solids
WW	Wastewater
WW-MFC	MFC operated by wastewater as substrate
WWTP	Wastewater Treatment Plant

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Feasibility of Generating Renewable Energy from Wastewater Treatment Process Using Microbial Fuel Cells: The West Bank as Case Study

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Abstract

Microbial fuel cells (MFCs) technology, is an innovative and relatively new technology by which organic matter can be simultaneously biodegraded anaerobically and generate electrical energy directly. MFCs can be used in various applications such as: water desalination, biosensors as well as water and wastewater treatment. Through this study, MFCs as a wastewater treatment system was investigated for the first time in Palestine.

Palestine is a developing country that suffers from improper wastewater collection and treatment systems in addition to water supply shortage that can cause increasing organics concentrations in the discharged wastewater.

MFC model used in this research was double chambered-MFC (DS-MFC), it was operated by primary effluent wastewater as substrate, salt bridge was used as proton exchange media and water saturated of dissolved oxygen was used as cathodic solution.

This research consists of two main parts: First, investigation of many parameters that may affect MFCs efficiency, such as: electrode material type, electrode size, salt bridge diameter, type of salt solution that used in salt bridge and concentration of salt solution used in the salt bridge. This part was conducted by constructing and operating different MFCs to investigate

each parameter (variable) individually. All conditions, except the concerned parameter, were fixed for each parameter experiment. Three duplicates-MFCs for each variable value were used to obtain reliable results. Output open circuit voltage (OCV) was measured one time per day and for one week for each variable and then the obtained output voltage data were analyzed as a trial to find the most suitable conditions.

The second part is aimed to understand and model the relationship between COD of substrate in MFC at any time and output voltage from the MFC at the same time in addition to define the kinetic reaction order of COD removal process. Four different COD-MFCs were constructed, three duplicates for each. Initial COD value was approximately fixed for each 3 duplicates, and other parameters were set and fixed as found from the first part of this research. After 15 days-startup period the MFCs were operated for 30 days. COD was measured for the twelve MFCs each two days and output voltage was measured each 24 hours.

Analysis of the obtained data from performed experiments, showed that MFCs with copper electrode produce output voltage significantly higher than MFCs with carbon brushes electrodes which, in turn, achieved output voltage significantly higher than both that achieved by MFCs with zinc electrodes and MFCs with manufactured carbon electrodes. It was found that diameter of salt bridge affects the output voltage of MFCs; MFCs with 10 mm salt bridge shown significantly higher output voltage than MFCs with both 16 and 24 mm salt bridges. This behavior could be interpreted by increasing the electrical resistance when the diameter is increased.

It was found that KCl salt bridge is significantly more efficient than NaCl salt bridges when they were used in MFCs for wastewater treatment. It was found also that MFCs with 1M KCl salt bridges can produce output voltage significantly higher than that produced by MFCs with 3M KCl salt bridges. Results revealed that the COD of the substrate used in MFC at any time is related proportionally to output voltage from that MFC at the same time, this relationship can be fitted as natural logarithmic model as following:

COD (mg/L) = 229.85 Ln (V)-1039.6; where V is output voltage (mV), and this model can be used with large number of limitations to indicate COD for a wastewater sample by measuring output voltage of MFC operated by that sample.

Maximum COD removal percentage achieved in this study was 87.1 % which is comparable to published achievements. It was found that COD removal behavior in this study was ranged between 1^{st} and 2^{nd} order kinetic reactions. A maximum output power achieved was 0.585 W/m^3 with an average output power of 0.251 W W/m^3.

Introduction

1.1. Wastewater Treatment and Energy Issues

Wastewater treatment has its positive impacts on sustainable water management and environment protection; however, it is considered an energy-intensive process **(Foley, 2010; Gikas and Tsoutsos, 2015)**. Conventional wastewater treatment requires circa 1.1-2.4 MJ/m^3 of electrical energy **(Tchobanoglous et al., 2003).** Energy needs could be higher significantly for small wastewater treatment plants i.e. for wastewater treatment plant with flow less than 5000 m^3/day **(Gikas and Tsoutsos, 2015)**.

As more investments are needed to increase the wastewater treatment capacity and efficiency, it becomes a growing challenge to meet the associated energy demands. In future, energy demand for wastewater treatment is expected to grow due to increasing restricts on discharge requirements and resulted in increasing required treatments and energy needs. Inevitable population growth and consequently increasing of wastewater generation could increase energy needs for wastewater treatment in future.

Studies reveals that new pollutants such as endocrine disrupting materials, pharmaceuticals and personal care products are contributing to increase energy need for wastewater treatment; because removal of such pollutants can’t be performed efficiently using conventional wastewater treatment processes; so other energy-intensive and special treatment processes are

required **(Adam et al., 2002; Petrovicè et al., 2001; Ternes et al., 2002; Westerhoff et al., 2005; Zwiener and Frimmel, 2000)**.

Current global energy consumption is about 9301 million tonnes of oil equivalent (1 ton=11,630 kWh) "Mtoe", 84.5 % of the global consumption is of non-renewable resources **(International Energy Agency, 2015)**. **Figure 1.1** shows world total energy consumption from 1971 to 2013.

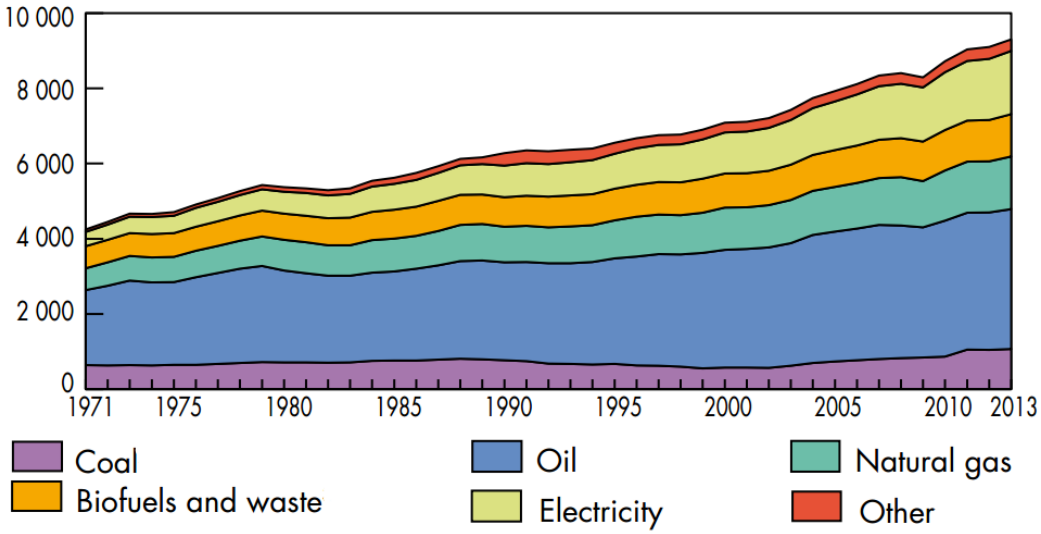

Figure 1.1 World total final energy consumption (Mtoe) through 1971 to 2013

Source: **(International Energy Agency, 2015)**

Non-renewable sources of energy are dramatically decreasing worldwide; due to the heavily increasing consumption of energy worldwide. Moreover, increasing carbon emissions from fossils fuels resources (**Figure 1.2**), followed by change in carbon percentage in the atmosphere have a great input into global climate change; so extensive research was started globally to find renewable, cheap and sustainable sources of energy.

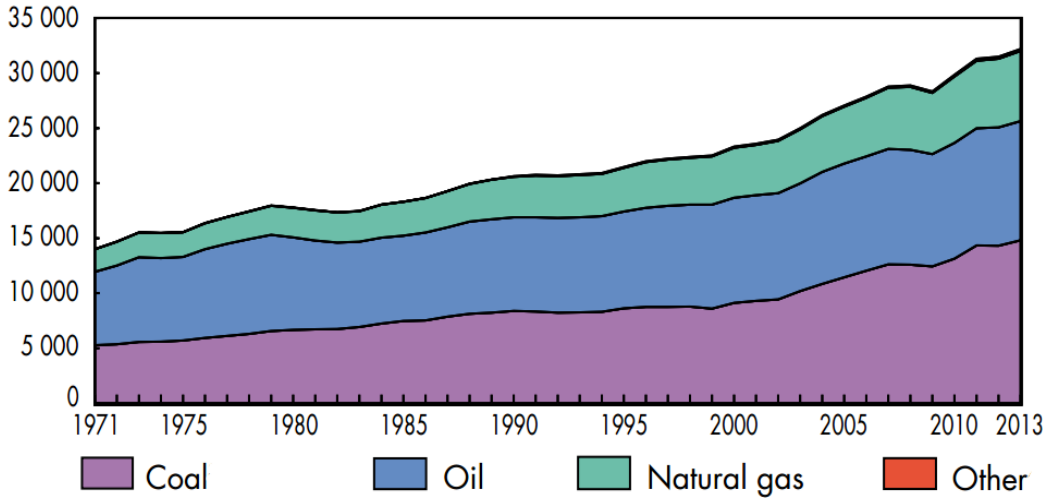

Figure 1.2 CO_2 emissions from energy consumption (Mt of CO2) through 1971 to 2013

Source: **(International Energy Agency, 2015)**

Renewable energy is the energy that created by naturally replenished sources such as: winds, sunlight, tides, etc. Other energy sources such as: biomass, anaerobic digestion and biofuel are widely considered renewable energy **(You, 2016)**.

Wastewater went to be considered as a resource for water, energy and plant nutrients more than as waste. Actually, wastewater contains energy folds of that is required for its treatment **(Gude, 2015; McCarty et al., 2011; Shoener et al., 2014)**.

Net-zero energy wastewater treatment or net-positive energy wastewater treatment is an evolving trend that could be achieved through: energy conservation in water and wastewater treatment, hydraulic energy recovery, heat recovery, anaerobic digestion, algae growth for biofuels, microbial fuel cells (MFCs), and microbial desalination cells (MDCs), etc. **(Gude, 2015)**

Microbial Fuel Cells (MFCs) technology provide an innovative option in generating sustainable energy from waste water simultaneously with its

treatment; so wastewater treatment energy needs might be partially self-covered. Microbial Fuel Cells (MFCs) are devices used to generate electrical energy in a biochemical process; in which living microorganisms digest and oxidize organic matters and producing electrons and protons which could be exploited (Borah et al., 2013; Du et al., 2007; Fenget al., 2009; Hisham, et al., 2013; Huggins et al., 2013; Karmakar et al., 2010; Kim et al., 2007; Lee et al., 2012; Lovley, 2006; Logan et al., 2006).

1.2. Importance of the Research in Palestine

To our knowledge, this is the first research on MFCs conducted in Palestine. Palestine is a developing country that suffers from Israeli occupation. Energy situation in Palestine is very critical; due to absence of conventional fossil fuels resources and the lack to import 100 % petroleum needs and 92% of electrical needs from Israel.

Wastewater treatment sector in Palestine is a budding sector and needs enormous efforts and budgets to be developed to satisfy environmental standards and policies. In the developed countries energy needs for wastewater treatment is significant portion of the total energy needs.

MFCs could be an innovative solution to treat wastewater and produce energy that could cover large portion of wastewater treatment energy needs, and so could be of high economic and technical value for Palestine.

1.3. Objectives and Research Questions

The main objectives for this research are:

1) Design, construct and investigation the behavior of microbial fuel cells for primary effluent wastewater treatment.
2) Data acquisition from pilot experiments: COD removal and output voltage data will be collected.
3) Manage data obtained from pilot experiment: including statistical analysis and modeling to characterize MFC behavior.

This research will try to answer following key questions:

1) Which are the most suitable operating conditions and settings for MFC (salt bridge characteristics and electrodes material) that would reflect optimal energy generation and treatment efficiency in wastewater treatment?
2) What is the relationship between organic content of wastewater and output energy from MFC?

2. Literarture Review

2.1. Background

MFCs are devices that directly convert the chemical energy stored in the organic matters into electrical energy **(Mahendra and Mahavarkar, 2013; Min et al., 2005)**.

Typical MFC consists of two compartments: Anodic chamber and cathodic chamber, separated by proton exchange membrane (PEM) **(Karmakar et al., 2010; Mahendra and Mahavarkar, 2013; Du et al., 2007)**.

Substrate (organic-rich matter) is added to the anodic chamber, where oxidation of organic matter occurs by anaerobic microorganisms **(Delaney et al., 1984; Mathuriya and Sharma, 2009)**.

Electrons and protons are produced from oxidation half-reaction of the substrate, electrons transfer from the anode to the cathode through external electrical load (resistance); where protons transfer to the cathode through PEM or salt bridge **(Lithgowet al., 1986; Mohan et al., 2007)**. **Equation 1** shows the typical anode reaction using acetate as example reaction.

$$CH_3COO^- + 2H_2O \text{ (anaerobic microorganisms)} \rightarrow 2CO_2 + 7H^+ + 8e^- \quad (1)$$

Through digestion of organic matters, electrons are transferred to the anode by microorganisms in three mechanisms: First, by exogenous mediators, which are added externally, such as Thionine. Second, Mediators naturally produced by microorganisms. Third, direct transfer from the cytochromes to

the anode **(Min and Logan, 2004)**. One of the disadvantages of synthetic mediators is that they are unstable and toxic to most microorganisms.

In the cathodic chamber; where reduction half-reaction occurs; catholite solution is added and it is working as electron acceptor. The most common electron acceptor (catholites) are ferricyanide ($K_3[Fe(CN)_6]$) or oxygen, which is the most suitable electron acceptor due to its high oxidation potential. At the cathodic chamber electrons and protons are combined with oxygen to form water as illustrated in **Figure 2.1** (Lithgow et al., 1986; Rabaey et al., 2004). **Equation 2** shows the typical cathodic reaction using acetate as example reaction.

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \qquad (2)$$

MFCs that contains two chambers, are called double-chambered MFCs (DS-MFC), see **Figure 2.2**. Cathodic chamber can be removed, and the cathode is placed at the side of the anodic chamber and exposed to air at the other side, then it will be called single-chambered MFC (SC-MFC) or air-cathode MFC **(Karmakar et al., 2010)**.

Figure 2.1 shows the main parts and processes in a typical MFC, where **Figure 2.2** show schematic drawing for single chambered microbial fuel cell.

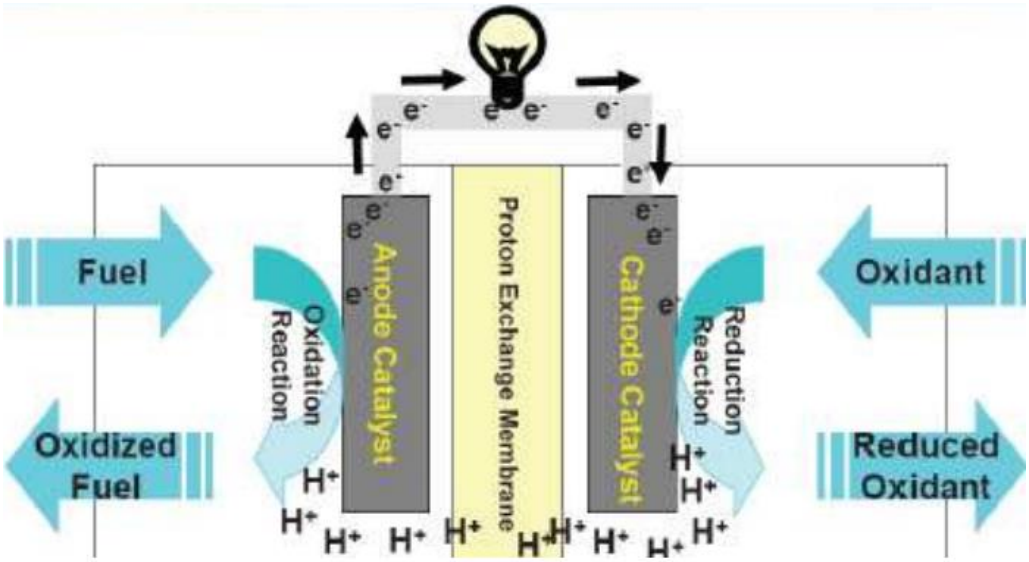

Figure 2.1 Typical microbial fuell cell

Source: **(Oji et al., 2012)**

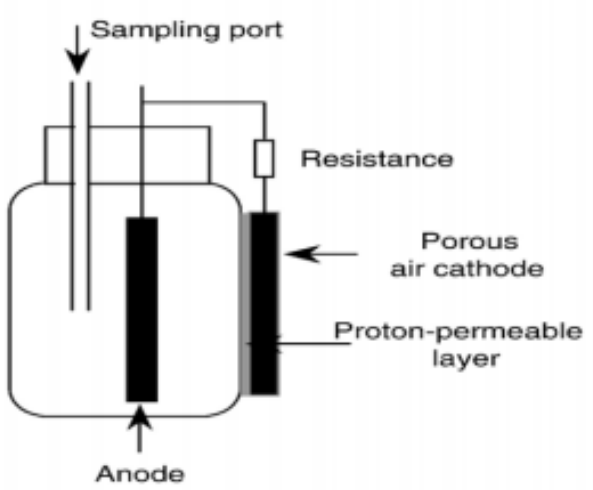

Figure 2.2 Schematic design of single chambered MFC

MFCs have many advantages comparing to conventional anaerobic digestion processes: MFCs are applicable for low concentration substrates and they can be operated at ambient temperatures 20ºC and below **(Pham et al., 2006)**.

MFCs have many potential applications including: wastewater treatment, biofuel production, water desalination, remote power sources and biosensors. In wastewater treatment MFCs can remove organics and inorganics such as nitrate and sulfide. Non-conventional applications and functions of MFCs were navigated; Zhen He studied the merger of MFC and reverse osmotic pressure to create osmotic microbial fuel cells (OsMFCs) to produce high quality water simultaneously with generating electrical energy **(He, 2012)**.

2.2. History and Development of MFCs

The linkage between chemistry, biology and electricity isn't an emerging discovery; scientists have long known about this relationship **(Potter, 1911)**. In the late 1700s, Luigi Galvani, noted that a detached frog's leg twitched due to the electrical charges in the atmosphere; this discovery leaded to find electro-chemistry Science **(Rogers, 2010)**.

In the beginnings of the 20th century, researches published on electricity production using microorganisms. The first attempt proved that biological processes could produce electrical energy was in 1911 by Potter; he published the earliest report on the ability of producing electrical energy from oxidizing organic matters by microorganisms and he had demonstrated it **(Bullen et al., 2006; Shuklaet al., 2004)**. However, this attempt was neglected for about two decades until Cohen approved that a batch biological fuel cell could produce more than 35 volts **(You, 2016; Cohen, 1931)**.

The USA NASA space program in the sixth decade of the previous century, was interested in biological fuel cell technology to produce energy in space ships from organic wastes **(Putnam, 1971)**.

A clear principle for biological fuel cells was identified in 1976 by Rao **(Rao et al., 1976; You, 2016)**. In the 1980s, Bennetto succeeded to extract electrical current from MFCs that were operated using wastewater, pure cultures and artificial electron mediators; to facilitate electrons transfer to the anode **(Bennetto et al., 1985; Bennetto et al., 1983)**.

In the 1980's, it was discovered that the generated electricity could be significantly increased if electron mediators were added **(Du et al., 2007)**. The outer layers of the majority of microorganisms consists of non-conductive lipid membrane, peptididoglycans and lipopolysaccharides which hinder the direct electron transfer to the anode. Electron mediators such as: thionine, methyl blue, methyl viologen, humic acid, neutral red and others can accelerate the transfer **(Davis and Higson, 2007)**.

Only anodophiles (exoelectrogens) such as *Shewanella putrefaciens, Geobacteraceae sulfurreducens, Geobacter metallireducens and Rhodoferax ferrireducens* are bioelectrochemically active organisms and they can form a biofilm on the anode surface and also transfer electrons to the anode directly by conductance through their cell membrane **(Du et al., 2007)**.

Habermann and Pommer demonstrated that some microorganisms can produce electron shuttles naturally, so no exogenous mediators are necessary **(Habermann and Pommer, 1991)**.

However, in the last two decades, too many scientists and researchers have worked on development of MFCs by attempting to increase efficiency of treatment and output power.

2.3. Review of the Latest Research on MFCs

MFCs technology is an innovative solution for bioelectricity production from wastewater treatment simultaneously with its treatment; so, energy needs of wastewater treatment could be partially or totally recovered from the wastewater itself. Today many researchers are working on developing MFC's and other electrochemical technologies.

As a comparison between MFCs and conventional aeration treatment of wastewater, it was found that both of them able to remove more than 90% of COD; but with shorter time in conventional aeration (8 days) than MFCs (10 days) **(Huggins et al., 2013)**. In the case of high COD concentration, MFCs showed lower removal efficiency compared to aeration, but much higher efficiency for low COD concentration. Suspended solids measurements showed that MFCs reduced sludge production by 52-82 % compared to aeration and MFCs also can save energy consumption in aeration since that MFC process is anaerobic **(Huggins et al., 2013)**.

MFCs can be operated by a wide range of substrates as long as they contain organic matters **(You, 2016)**.

Lee with his partners used sulfide as substrate, which is considered as one of the common inorganic pollutants in the wastewater from livestock farming. In Lee's study, sulfide was added to a synthetic wastewater with a

concentration of sulfide ranged from 50 to150 ppm and autotrophic denitrifier "*Pseudomonas"* was used as pure culture. They used two types of electrodes; carbon cloth electrode and carbon felt electrode and found that carbon cloth electrode can generate more voltage than the carbon felt **(Lee et al., 2012)**.

The achieved voltage density, power density, current density, percentage of sulfide converted to thiosulfate and percentage of sulfide converted to elementary sulfur in Lee's study were 65 mV/m^2 of anode area, 29.3 mW/m^2 of anode area, 108 mA/m^2 of anode area, 40.7% and 57.6 % respectively **(Lee et al., 2012)**.

Hisham with his co-authors used three different substrates to operate MFCs using carbon paper as electrodes for 96 hours; they were activated sludge, palm oil mill effluent (POME) and leachate from food waste. The highest voltage among three substrates was obtained by leachate-MFC (0.455 V), followed by POME-MFC (0.444 V) and then activated sludge-MFC (0.396 V). Where the highest treatment efficiency in terms of COD was achieved by activated sludge-MFC (37.5%), then leachate-MFC (6.11%) and POME-MFC achieved zero-COD removal. In terms of nitrogen removal, Sludge-MFC achieved the highest efficiency (65.28%) followed by POME-MFC (48.12%) and leachate-MFC (25.15%) **(Hisham et al., 2013)**.

Guo with his partners, used anaerobic sludge as substrate with adding glucose as extra carbon source in the startup period, nitroaromatic antibiotic chloramphenicol solutions with different concentrations were added to the sludge in order to study its removal efficiency **(Guo et al., 2016)**.

Four types of MFCs were used in Guo's experiments: normal MFC, open-circuit MFC, no extra carbon source MFC and abiotic (anaerobic sludge was autoclaved before inoculation) MFC. Results showed that the maximum removal efficiency of nitroaromatic antibiotic chloramphenicol was achieved in normal MFC, followed by open circuit MFC, then abiotic MFC and the minimum removal efficiency was achieved in MFC with no extra carbon source; given that equal initial concentration was applied. Results also showed an inverse relationship between removal efficiency and initial concentration of nitroaromatic antibiotic chloramphenicol **(Guo et al., 2016)**.

Table 2.1 shows various types of substrates used in previous MFC studies and power generation for each type **(Maria and Dharmendra, 2016)**.

Table 2.1 Achieved power output in various substrates used in MFCs

Substrate	MFC Type	Power generation	Reference
Swine	Single chamber	261 mW/m^2	**(Köroğlu et al., 2014; Min et al., 2005)**
Swine	Double chamber	45 mW/m^2	**(Köroğlu et al., 2014; Min et al., 2005)**
Saline domestic sewage sludge	Double chamber	41 W/m^3	**(Karthikeyan et al., 2016)**
Nitrogen containing organic compounds (pyridine and methyl orange)	Single chamber	502.5 mW/m^2	**(Wang et al., 2015)**
Nitrogen containing organic compounds (pyridine and methyl orange)	Two single chambers connected in series	401.6 mW/m^2	**(Wang et al., 2015)**
Sulfide containing synthetic WW	Double chamber	29.3 mW/m^2	**(Lee et al., 2012)**
Paper recycling WW+ 100 mM PBS	Single chamber	672 mW/m^2	**(Huang and Logan, 2008)**
Urine	Double chamber	8 mA/m^2	**(Ieropoulos et al., 2012)**
Lemon peel waste	Double chamber	371 mW/m^2	**(Miran et al., 2016)**
Domestic WW	Air-cathode	422 mW/m^2	**(Köroğlu et al., 2014; Ahn and Logan, 2010)**
Leachate	Air-cathode	344 mW/m^2	**(Köroğlu et al., 2014; Puig et al., 2011)**
Starch	Air-cathode	239.4 mW/m^2	**(Köroğlu et al., 2014; Lu et al., 2009)**
Beer brewery	Air-cathode	205 mW/m^2	**(Köroğlu et al., 2014; Feng et al., 2008)**
Beer brewery	Single chamber	170 mW/m^2	**(Köroğlu et al., 2014; Feng et al., 2008)**
Textile WW	Single chamber	812 mW/m^2	**(Mise and Saware, 2016)**

A Multiple anode chamber MFC (MAC-MFC) design (see **Figure 2.3**) was developed by Mathuriya and its performance was compared to a single cathode chamber MFC (SC-MFC). During 60 days of operation with different wastewaters, it was found that the MAC-MFC generated stable and higher power outputs in comparison to SC-MFC **(Mathuriya, 2016)**.

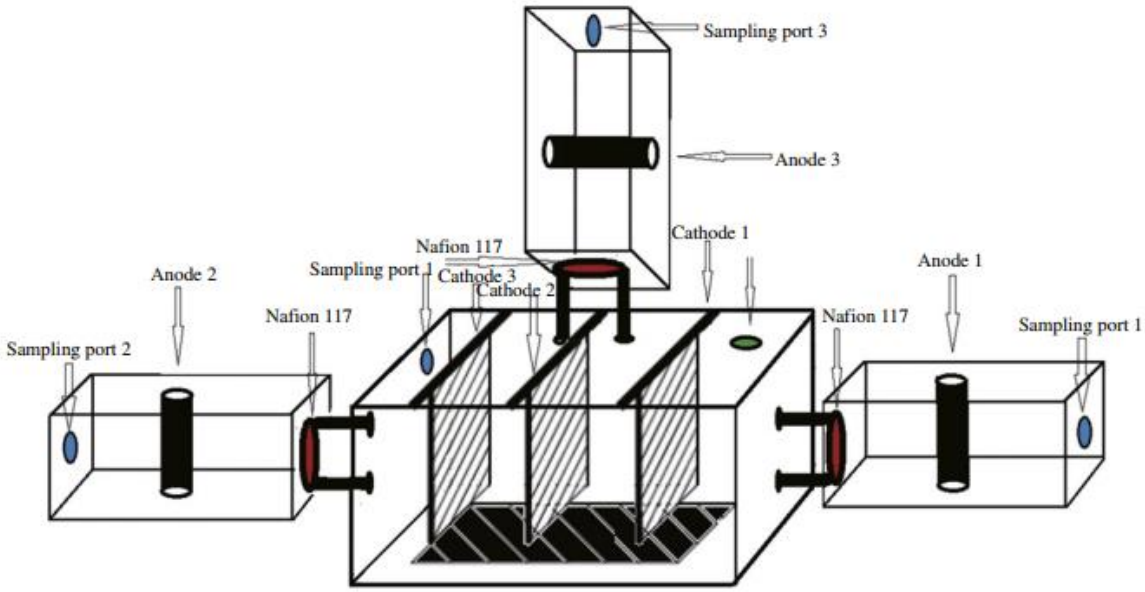

Figure 2.3 Schematic design of multiple anode chamber MFC developed by Mathuriya

MFCs can be operated by pure culture, but it is more suitable to be operated by mixed cultures especially when the used substrate is complex such as wastewater **(Kim et al., 2007; Prasad et al., 2006)**.

Researchers have done intensive research on the cultures that could operate MFCs. Pure cultures such as *Clostridium acetobutylicum, Clostridium thermohydrosulfuricum, Shewanella putrefaciens, Geobacter sulfurreducens, Desulfobulbus propionicus, Rhodoferax ferrireducens and Saccharomyces cerevisae* (yeast) are used in bioelectricity production **(Mathuriya and Sharma, 2009; Mokhtarian et al., 2012; Mathuriya and Sharma, 2010)**.

Borah with his co-workers, isolated *Bacillus megaterium* and other 24 types of microorganisms from soil, and they used each type to operate individual MFC. They found that *Bacillus megaterium* is the most efficient type among the 25 types **(Borah et al., 2013)**.

Lee used autotrophic denitrifier, isolated from an expanded granular sludge, sp. C27, to operate an MFC. *Pseudomonas sp. C27 can* convert sulfide to elementary sulfur, the generated power density was 29.3 mW/m^2 (Lee et al., 2012). Lee's findings are considered as an introduction for treatment of sulfides containing wastewater simultaneously with harvesting electricity.

Sheikh et al. used isolated facultative anaerobic bacteria as biocatalysts in MFCs. These MFCs were operated by various substrates. They achieved a maximum voltage of 0.30 V using urea as substrate **(Sheikh et al., 2015)**.

Enterobacter sp. ALL-3 culture was used by Tkach and his coworkers to operate SC-MFC at different temperatures (ranges from 5 ^oC to 25 ^oC), using acetate as substrate. The maximum achieved power densities at 5 ^oC, 10 ^oC and 25 ^oC were 293, 213 and 84 mw/m^2 respectively **(Tkach et al., 2016)**.

Startup temperature in Tkach's experiment was 5 ^oC for 30 days, a maximum voltage of 500 mV was achieved. After 8-10 days, the voltage was reduced to less than 50 mV and the MFC was resembled again. Then Temperature was increased to 10 ^oC and a maximum voltage achieved was 530 mV. Finally, temperature was increased to 25 ^oC and the maximum voltage achieved was 480 mV. It can be noticed that the time in which acetate was consumed at 10 ^oC range was less than that for 25 ^oC; this is because digestion of acetate was quicker. But still these results can be accredited for *Enterobacter sp. ALL-3* culture and can't be generalized for all cultures **(Tkach et al., 2016)**.

Genetically engineered bacterial strain was used to operate an MFC with urine substrate and was observed by Shreeram and his partners. An increase

of 2.7-fold in peak power density was achieved using genetically engineered strain comparing to the wild-type strain **(Shreeram et al., 2016)**.

Type of microorganisms doesn't alone affect MFC efficiency; cells count and biofilm density also have effects on output power generated by MFC. Power density depends directly on biofilm growth; so, it is increased significantly during initial growth period of biofilm **(Ramasamy et al., 2008)**.

Previous research found that using ferricyanide solution as cathodic solution (electron acceptor) is more efficient, in terms of both treatment and power generation, than using oxygen. Mohan, with his partners, tested both cathodic solutions under the same conditions, they found that generated power is slightly lower in case of using dissolved oxygen. Where COD removal percentage is significantly higher in the case of using ferricyanide solution **(Mohan et al., 2007)**.

Studies demonstrated that nitrite can be used as cathodic solution (electron acceptor). Ammonium-containing effluent from the carbon-utilizing anode was fed to external biofilm-based aerobic reactor for nitrification, the nitrified liquor was then fed to the cathodic chamber in an MFC in order to reduce nitrate **(Virdis et al., 2008)**.

A maximum power of 34.6 ±1.1 W/m3, maximum current of 133.3 ±1.0 A/m3 and nitrite removal rate of 0.41 kg NO3—N/m3.day of net volume of the cathodic chamber were achieved **(Virdis et al., 2008)**. COD removal rate was 2 kg COD/m3.day of the net volume of the cathodic chamber. Achieved

COD/N ratio in MFCs was 4.5 compared to COD/N ratio in conventional aeration processes of more than seven.

It is important to mention that the electrodes, especially anode, are the key components to set the efficiency of MFC's **(Logan et al., 2006; Offei et al., 2016)**. Many types of electrodes are available and can be used; e.g. graphite, zinc, copper, aluminum, carbon, stainless steel, mild steel, etc. The electricity generation from MFCs is directly proportional to the surface area and type of the anode **(Ashoka et al., 2012)**.

Achieved power densities of previous studies on MFCs, using the most common electrodes, are summarized in **Table 2.2**.

Table 1.2 Recorded power densities for various electrode materials

Electrode Material	Power Density	Reference
Carbon paper	148 mW/m^2	**(Thygesen et al., 2011)**
Graphite fiber brush	422 mW/m^2	**(Ahn and Logan, 2010)**
Granular activated carbon	2981 mW/m^2	**(Nam et al., 2010)**
MPL-Carbon viel	60.7 mW/m^2	**(You et al., 2014)**
MPL-Carbon cloth	50.6 mW/m^2	**(You et al., 2014)**
PVDF-AC	1400 mW/m^2	**(Yang et al., 2015)**
Carbon felt	356 mW/m^3	**(Aelterman et al., 2008)**
Graphite felt	386 mW/m^3	**(Aelterman et al., 2008)**
Graphite wool	321 mW/m^3	**(Aelterman et al., 2008)**
Graphite granules	257 mW/m^3	**(Aelterman et al., 2008)**
Activated carbon	1740 mW/m^3	**(Offei et al., 2016)**

Mediator-less DS-MFC, using carbon nanotube CNT-doped PEM, carbon zinc electrodes and *E. coli* culture was constructed by Vijay and others. Maximum output voltage and maximum current were 1.23 volts and 8.08 mA respectively **(Vijay et al., 2014)**.

Electrodes used in MFCS should have satisfy various conditions to be efficient, such as: reasonable cost, good electrical conductivity, high surface area, low electrical resistance, non-corrosive, biocompatible, distance between electrodes should be as close as possible and they should be chemically and mechanically stable **(Jang et al., 2004; Ashoka et al., 2012; Singh et al., 2010)**. The maximum current was achieved in the literature when the platinum-coated graphite was used as electrode **(Ashoka et al., 2012)**.

Another study focused on electrodes used in MFCs, MFCs were constructed and monitored using cowdung as substrate, nylon as PEM and methylene blue mediator. Five different metals were used as electrodes: copper (Cu), aluminum (Al), stainless steel (SS), zinc (Zn), carbon (C) and mild steel (MS). Twenty-one electrodes combinations were applied, with the same surface area of each electrode. The highest four combinations were Cu/Zn, SS/SS, C/C and Al/SS respectively **(Ashoka et al., 2012)**. However, other questions should be raised; does copper stay stable without reactions with substrate? If there were any reactions, how do these reactions affect the microorganisms?

Activated carbon (AC) are now used frequently as electrodes for MFCs, due to its low price and good catalytic activity **(Wang et al., 2013; Offei et al., 2016)**.

Output power from MFC follows saturation kinetics as a function of provided substrate (anodic solution). Generated voltage from MFC is decreased linearly with time **(Barua and Deka, 2010)**.

MFC is anaerobic process that has many advantages such as: it produces less carbon emissions, requires less energy, produces less sludge through treatment, requires less nutrients and higher potential energy recovery because most the organics transferred to energy **(Hwang et al., 2004; Du et al., 2007)**.

Conditions that affect MFC efficiency are: pH, temperature, proton exchange material, electrode material, ratio of membrane surface to anodic chamber volume, ratio of anode surface area to the volume of anodic chamber and dissolved oxygen concentration in the cathodic chamber **(Karmakar et al., 2010; Oji et al., 2012)**.

Feng et al. tried to operate MFC under different temperatures, they used three temperatures: 30, 20 and 15 ^{o}C. They found that generated energy is significantly reduced when temperature reduced by 5 ^{o}C (from 20 to 15 ^{o}C), and is reduced less significantly when temperature reduced from 30 to 20 ^{o}C. For COD removal efficiency, they found that slight differences between the 3 temperatures **(Feng et al., 2009)**.

Protons exchange from the anode to cathode can be achieved through PEM mainly, the main disadvantage of PEM is the high cost **(Du et al., 2007)**. To overcome cost problem of PEM, salt bridges can be used for protons exchange but with less efficiency **(Sevda and Sreekrishnan, 2012)**.

Nafion membrane is the most popular PEM due to its highly selective permeability of protons **(Du et al., 2007)**. Park and Zeikus used porcelain septum made from kaolin as proton exchange media, a maximum power and

voltage achieved, using sewage sludge as substrate, were 788 mW/m^2, 045 V **(Park and Zeikus, 2003)**.

Grzebyk and Poźniak have prepared interpolymer cation exchange membranes with polyethylene/poly (styrene-co-divinylbenzene) by their sulfonation with a solution of chlorosulfonic acid in 1,2-dichloroethane, and they used them in MFC as protons exchange media; but they achieved low power output **(Grzebyk and Poźniak, 2005)**.

Min et al. compared between salt bridges and PEM as proton exchange systems in MFCs, using both pure and mixed cultures, and they found that generated power from PEM system, 38 mW/m^2, was order of magnitude higher than power generated from salt bridges, 2.2 mW/m^2. According to their conclusion, salt bridge has internal electrical resistance that is extremely higher than that for membrane **(Min et al., 2005)**.

However, other researchers have worked on development of salt bridges and improvement of its efficiency. Sevda and sreekri have investigated the effect of salt concentration of salt bridges on electricity generation from synthetic WW-MFC. Maximum output power, was achieved at 5% concentration of NaCl, was 84.99 mW/m^2 with 88.41 % COD removal **(Sevda and Sreekrishnan, 2012)**.

To our knowledge, there is no previous research on the effect of salt concentration in salt bridges on output power when natural wastewater is used as substrate. Also, no previous studies performed on the effect of salt concentration used in salt bridges for other salts such as potassium chloride.

COD removal characteristics in SC-MFC were studied by Zhang with his partners **(Zhang et al., 2015)**. However, to our knowledge, COD removal characteristics for DS-MFC were not studied before and this gap will be covered by this study.

However, to our knowledge, there are no previous research on other conditions that affect MFC efficiency.

2.4. Summary

It can be notice from the performed literature review that much subjects regarding MFCs and its application in wastewater treatment were covered in previous research. Latest research is mainly covering: substrates used to operate MFCs, microorganisms' cultures, electrode material, system temperature, cathodic solutions, proton exchange mechanism and COD removal characteristics for single chambered MFCs.

In this research, many new topics is to be covered; i.e. wastewater mixed with anaerobic sludge will be used as substrate, salt bridge characteristics effects on MFC behavior and COD removal characteristics for double chambered MFCs.

This research is good to be conducted in Palestine for the first time, and could be a starting point for further research for Palestine in future. MFCs could be an innovative solution for wastewater treatment and energy problems occur in Palestine.

3. Study Area and Methodology

3.1. Study Area

Occupied Palestinian Territories consists of two disconnected parts; Gaza Strip and the West Bank, including East Jerusalem, with a total land area of 6,020 Km^2 (See **Figure 3.1**). It has a total population of 4.55 million: 2.79 million in the West Bank 2.79 and 1.76 million in the Gaza Strip **(Palestinian Central Bureau of Statistics, 2015)**.

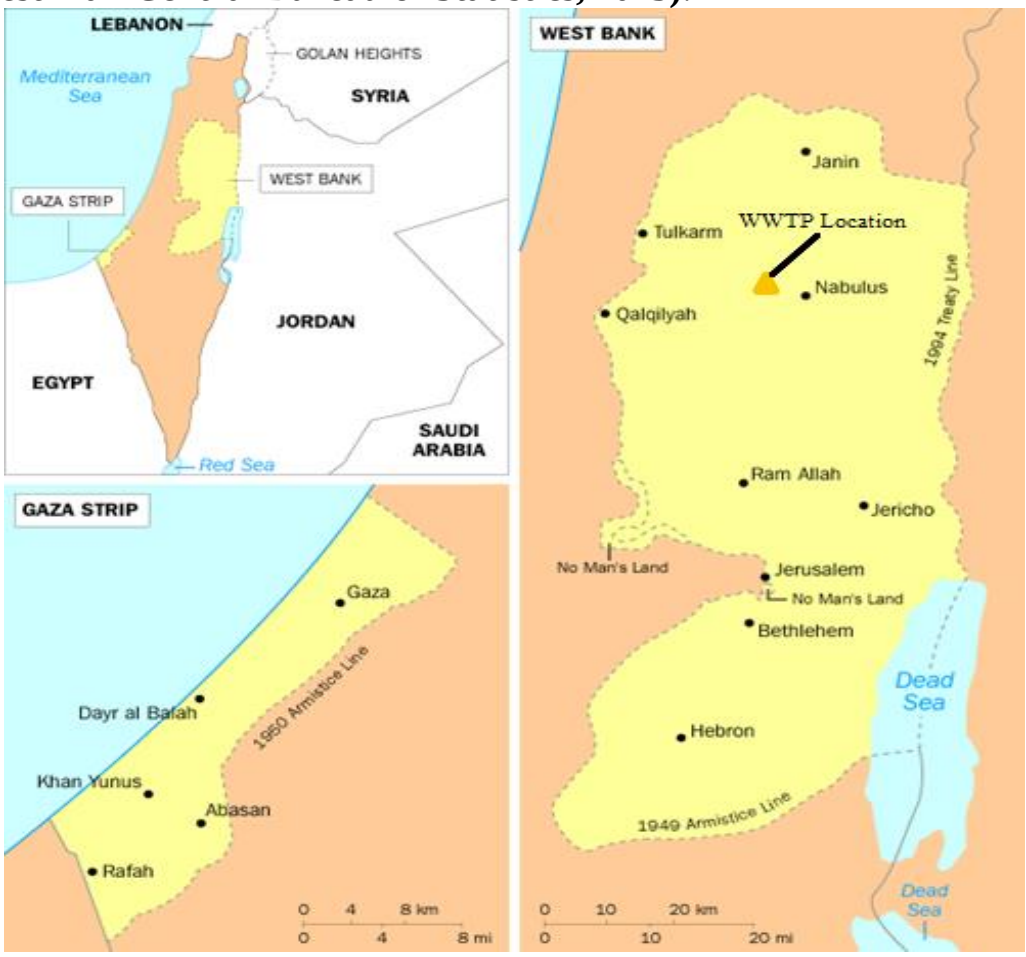

Figure 3.1 Maps and main districts of Gaza Strip and West Bank

(Source: www.globalsecurity.org/military/world/palestine/images/palestinemap.gif)

The wastewater samples were collected from Nablus West wastewater treatment plant. This WWTP is located to the west of Nablus as shown in **Figure 3.1**.

In the West Bank, the annual discharge of wastewater was estimated at about 62 million m^3, about 31% of the communities are connected to the sewer system **(Palestinian Water Authority , 2011)**. About 15% of this wastewater is treated in six major wastewater treatment plants (capacities range from 1,500 to 15,000 m^3/d) and 11 small wastewater treatment plants (10-120 m^3/d capacity) **(Judeh, 2015)**. One of the major WWTP use solar energy as energy source which is Jericho WWTP, which has 440 PV solar panels generating approximately 100 kWh per day in summer days **(Khalaf, 2015)**.

Nablus West wastewater treatment plant serves the western parts of Nablus city and the nearby villages. It was constructed by the German Government fund through the German Development Bank (KfW) **(Palestinian Water Authority , 2011)**. West Nablus WWTP serves a total population of 110,000 capita. Treatment system is conventional Activated Sludge System, with an actual average flow of about 11,000 m^3/day where the design flow is 15,000 m^3/day **(Homaeidan, 2014)**.

Nablus West plant contains two main lines; first one is wastewater treatment line including: grit chamber, primary sedimentation tank, aeration tanks, final sedimentation tanks, filtration and disinfection. Where the second one is sludge treatment line including thickener, anaerobic digester, sludge

drying basin, sludge storing, liquor storage tank, gas holder and gas flare (Abu-Ghosh et al., 2014).

Influent wastewater to West Nablus WWTP is mostly domestic wastewater with few slaughter houses wastewater and three halva factories.

Average influent characteristics are: COD=990 mg/L, BOD=400 mg/L, TSS=410 mg/L, pH=7.8 and conductivity=1500 μs/cm **(Nablus Municapility, 2016)**.

Samples were collected from the effluent of primary sedimentation tank; in order to get rid of unnecessary solids (See **Figure 3.2**).

Figure 3.2 Wastewater sampling from primary effluent from Nablus West WWTP

3.2. Methodology

This study was performed mainly by laboratory work, all laboratory work was done in Environmental Engineering Laboratory of the Civil Engineering Department at An-Najah National University, Nablus.

Laboratory work was performed in two stages: the first stage consists of all experiments that have be done to investigate parameters that affect MFC performance, i.e. salt bridge diameter and electrode materials. While in the second stage, four different COD primaries effluent wastewater-MFCs, for each concentration three MFCs duplicates were constructed, all MFCs were operated and monitored for 30 days in addition to 15 days-startup period. The research methodology is presented in the following sections:

3.2.1. Experimental Setup and Design of Experiments

The main goal of this study is to investigate MFCs behavior under different parameters and trying to characterize the relation of the output power of MFCs with substrate COD. Double chambered-MFCs were used, with carbon brushes electrodes (purchased from pumps spare parts market), salt bridges for protons exchange, primary effluent wastewater as substrate and anaerobic sludge as source of anaerobic microorganisms; it worth to mention that sludge will cause substantial increase of the substrate COD.

To obtain various range of data, four MFCs were fed with different COD-substrate's. To obtain more precise results, three duplicates of each concentration were used; so, twelve MFCs were constructed and operated at

total. **Table 3.1** shows the details of the used substrates for each concentration.

COD for the anaerobic sludge and the used wastewater were measured using titrimetric method according to "Standard Methods for the Examination of Water and Wastewater" **(American Public Health Association, American Water Works Association, & Water Environment Federation, 2012)**; they were found to be 48,000 mg/L and 547 mg/L respectively as presented in **Appendix A**.

Volume of substrate used for each MFC is 800 mL, COD for the mixed sludge-wastewater was selected to be within 300-1700 mg/L; volume of the sludge was calculated using equation 3.

$$\text{COD total} = \frac{(\text{WW VolumeX WW COD}) + (\text{Sludge Volume X Sludge COD})}{Total\ volume} \quad (3)$$

Table 3.1 Details of substrates used in the MFCs

Number	WW Volume (mL)	Distilled Water Volume (mL)	Sludge Volume (mL)	Expected Mixture COD (mg/L)
1	500	300	0	342
2	795	0	5	844
3	785	0	15	1437
4	780	0	20	1733

3.2.1.1. Preparation of Chambers

MFCs were constructed using glass jars for both anode and cathode chambers as shown in Figure 3.3. Volume of the used jars was 1 liter,

whereas the used volume was 800 mL; this is to prevent substrate and/ aerated water from dropping outside the jars during mixing/shaking.

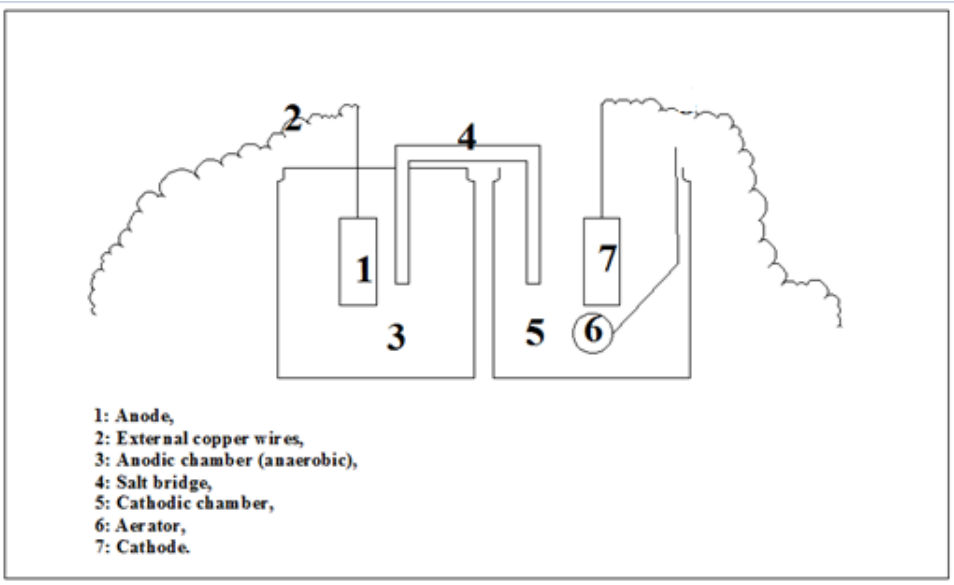

Figure 3.3 Schematic of DS-MFC used

3.2.1.2. Electrodes Preparation

The first experiment of the part was aimed to investigate different electrode materials effect on MFCs performance. Four types of electrodes (Zinc plate, Copper plate, Carbon brushes and pre-treated Carbon electrodes) were prepared and inserted into twelve different WW-MFCs, three duplicates for each one of the four electrodes, dimensions of all electrodes were 7 cm X 24 cm. DS-MFC were used in this step, salt bridges of 16 mm diameter and filled with KCl salt solution with 1 M concentration used and dissolved oxygen in distilled water by fish aerators was used as cathodic solution. Area of anodes where equal in all MFCs and equal to 28 cm^2.

Carbon brushes were selected to be used in MFCs in the second stage of this study. Carbon brushes electrodes were treated by soaking them in 1 M-HCl

solution for 24 hours in order to increase their electro-efficiency. Electrodes were then soaked into mixed solution of anaerobic sludge and primary effluent wastewater (20% sludge in terms of volume) at 35 ^{o}C for 3 days; in order to allow culture to form on the anodes surface.

3.2.1.3. Preparation of Salt Bridges

Salt bridges were used as proton exchange media due to its low cost and availability comparing to PEMs.

Pyrex Glass tubing were used as structure of the bridges; because it is an inert material chemically and electrically. U-shaped salt bridges were constructed from straight glass tubes and using Bunsen flame for bending to form U-shaped tubes (See **Figure 3.4**).

Procedure followed in preparing of 1 M KCl-salt bridges can be summarized as:

- Preparation of glass tubes (30 cm length for each).
- Use Bunsen flame to bend glass tubes at two points (first and second thirds along tubes), bending angle was 90^{o} to form U-shaped tubes.
- Allow bent tubes to cool in room temperature for excessive time of 24 hours (See **Figure 3.4**).
- Preparation of salt solution by weighing 74.5513 gm of 99% pure KCl using accurate balance (ABT 100-5M) and then adding salt to 1 L distilled water in a 2 Liter beaker.
- Salt solution was heated and mixed using hot plate mixer, then agar is added gradually to the solution (3 % weight to volume ratio).

- The beaker was kept covered during heating and mixing.
- Keep the solution heated and stirred until all the agar become dissolved, the solution become clear and bubbles are just beginning to form.
- Once the solution prepared, filling U-shaped tubes with solution was performed immediately using funnel and 200 mL-beaker.
- Mineral wool was used to close the ends of the glass tubes, and then salt bridges were immediately place in 1M-KCl solution to prevent agar in the tubes from shrinking during cooling (See **Figure 3.5**).
- After cooling (approximately 2 hours), salt bridges were stored in the refrigerator for few days before using them.

Figure 3.4 Prepared glass U-shaped tubes for use in salt bridges

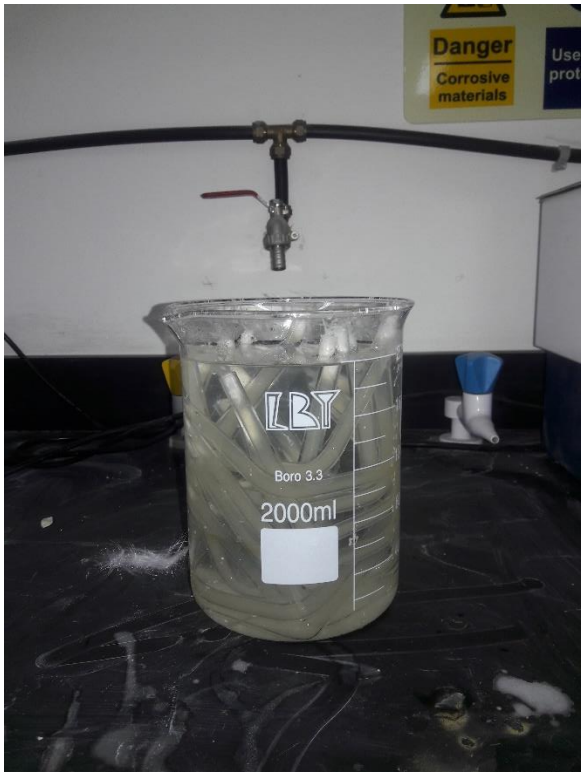

Figure 3.5 Prepared salt bridges soaked in KCl solution

3.2.1.4. Temperature Control System

Temperature control system was constructed and applied to the hood were all MFCs were put; in order to keep temperature within 34-36 ^{o}C during operation of the MFCs. Temperature control system consists of: Arduino, two water proof temperature sensors, two air sensors, heater, Bluetooth device for monitoring and microcontroller.

3.2.1.5. Mixing System

One of the obstacles that was faced in this study is mixing of WW and sludge. Hot plate stirrer was non-practical solution to solve this dilemma due to two reasons: required number of stirrers was 12 which is impossible to get, the second reason that the mechanism by which hot plate stirrer works is

questionable for our application; i.e. hot plate stirrer works by applying magnetic field which can affect electrical behavior of the MFCs.

Mechanical system was the main suggestion to solve mixing problem. The first idea was to construct vertical or horizontal mixers that connected to the 12 MFCs with one strong motor; but this idea was rejected because of the existence of salt bridges and electrodes in the anodic chambers.

Finally, shaking plate was proposed and shown to be the best solution. A mechanical shaker was designed and constructed to perform mixing task for the samples, it consists of: geared motor, transition mechanism, bearing, caster wheel and movable box. See **Figure 3.6**.

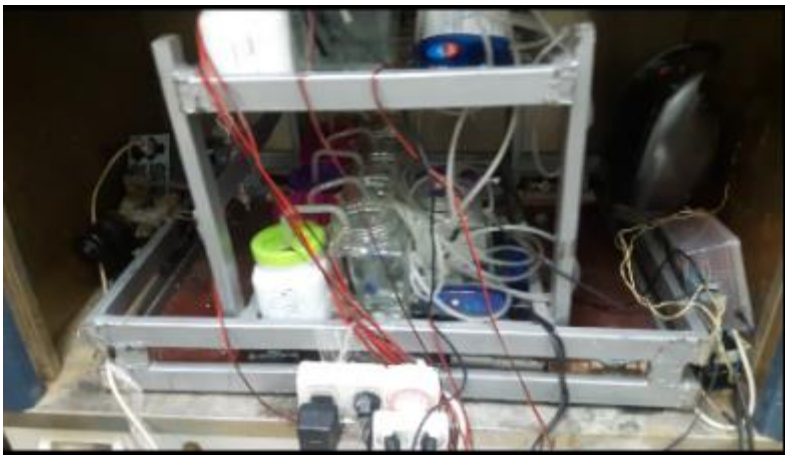

Figure 3.6 Used mixing system

3.2.1.6. Wastewater and sludge Sampling

Primary effluent wastewater was used as substrate for all the performed experiments in this thesis, the reason for that was to avoid solids in the primary sludge despite that some organics are lost with sludge; i.e. COD is expected to be decreased by about 20-30 % through primary sedimentation.

Since that many areas in Nablus have combined sewer system for WW and storm water, and storm water can enter the WWTP and then cause dilution for the WW, sampling of wastewater from Nablus West WWTP was done in non-rainy days, and after at least 72 hours of any raining fall, to assure that no storm water is mixed with the collected wastewater.

Sampling of WW was done from the weirs of the primary sedimentation tank in the WWTP and from various locations along the weir.

WW is collected in a cleaned plastic container with a volume of 10 liters, and then stored at 35 ^{o}C till use after one-two days, in order to assure keeping microorganisms activity.

Sludge was collected in glass container from the anaerobic digester in the WWTP and stored at 35 ^{o}C until use after one-two days.

Sampling from MFCs was performed using pipette; in all sampling times, approximately half of the sample is collected from the first top third of the anodic chamber and the rest from the second top third of the chamber.

3.2.1.7. Cathodic chamber preparation

Oxygen is the best efficient electron acceptor as mentioned before. In this experiment, the cathodic solution used was aerated water. Distilled water was used, 800 mL distilled water was used in each cathodic chamber. Evaporation of distilled water was noticed due to heater and aeration effect; so cathodic chambers were refilled on daily basis.

Fishing aerators were applied to aerate the cathodic chambers; operating was done for 15 minutes intervals; each operating interval was followed by

15 minutes break in order to prevent exhausting of the fishing aerators. It was assured that the amount of aeration is approximately equal in all cathodes.

3.2.1.8. Electrical Panel Preparation

To ease the voltage and power measurements, an electrical panel was prepared.

For each MFC, a 1000-ohm resistance was fixed at the electrical panel and connected with the electrodes with copper wires, for all MFCs the length of the copper wires was equal (1 meter).

3.2.2. Experimental Program

The experimental program is discussed in the following sections.

3.2.2.1. Investigation of Effect of Electrode Material

MFCs with different electrodes (Zinc, copper, manufactured carbon and carbon brushes) were operated for 7 days, and behavior of each MFC was inspected in terms of output voltage. Voltcraft M-3860M multimeter (manufacturer: METEX) was used to measure output open circuit voltage on daily basis.

3.2.2.2. Investigation of Effect of Salt bridges characteristics

Three different diameters salt bridges (10, 16 and 24 mm) were studied in DS-MFCs using carbon brushes electrodes and 1M-KCl salt. They were compared to each other in terms of output power.

Two types of salt solutions, 1 M KCl and 1 M NaCl, were used in WW-MFC and compared each to other in terms of output voltage.

Then, three different concentrations of KCl solutions (1M, 2M, and 3M) were used in WW-MFCs, three duplicates for each concentration, and compared each to other in terms of output voltage.

Efficiency of salt bridges depends on its diameter, length, and type of salt used and concentration of salt solution. Salt bridges length was specified practically, in a way that the ends of the bridges were approximately reach the middle of the chambers. For the selection of the other characteristics of the salt bridges, three experiments were performed. All output voltage measurements were performed on daily basis for all salt bridge experiments.

3.2.2.3. Operation of the Second Stage Experiment

Twelve MFCs were constructed and fed mainly with wastewater and small quantity of anaerobic sludge. Metabolic behavior is highly affected by the surrounding conditions such as temperature and pH. Anaerobic sludge was used in this study as source of anaerobic microorganisms, it was collected from anaerobic digester in which the temperature is more than 40 ^{o}C and it was there for 12–60 days depending on the temperature.

Since that temperature in the anaerobic digester is near thermophilic conditions (40 ^{o}C – 60 ^{o}C), then it is expected that mesophilic

microorganisms (20 ^{o}C – 40 ^{o}C) are very weak in the anaerobic sludge, therefore a startup period is required in order to assure that mesophilic microorganisms were become strong enough **(Bitton, 2005)**.

After mixing WW with anaerobic sludge, 15 days startup period was performed at 35 ^{o}C temperature and without connecting salt bridges and electrical circuit in order to allow microorganisms to adapt with the experiment conditions.

After startup of the experiment, COD for each MFC was measured each two-day using titrimetric method. Output voltage was measured on daily basis using Voltcraft M-3860M multimeter.

3.2.3. Analytical Procedures and Measurements:

Since that the main idea of this research is to find the relationship between organic contents in the different COD-WW samples and the output power, then two types of measurements were concerned; quality (environmental) measurements and energy measurements. It worths to mention that environmental measurements were performed according to standard methods for examination of water and wastewater **(American Public Health Association, American Water Works Association, & Water Environment Federation, 2012)**. Details of all measurements performed are summarized in the following sections.

3.2.3.1. Environmental Measurements

The main environmental/quality measurement in this study is organic matter contents or COD. COD measurements were taken one time each 48 hour as following:

- COD measurement:

COD was measured on 48 hours basis during the operating of the MFCs started at the end of startup period in the second stage.

COD measurements were performed according to standard methods for examination of water and wastewater (see **Appendix A**) **(American Public Health Association, American Water Works Association, & Water Environment Federation, 2012).**

COD was calculated according to the following equation:

$$\text{COD (mg } O_2\text{/l)} = \frac{(A-B)\ X\ M\ X\ 8000}{mL\ sample}$$

Where:

A: mL of F.A.S used for blank,

B: mL of F.A.S used for the sample,

M: molarity of F.A.S (0.05 M) and

8000: milliequivalent weight of oxygen.

3.2.3.2. Voltage and Power Measurement

Voltcraft M-3860M Multimeter was used to measure output voltage (See **Figure 3.7**).

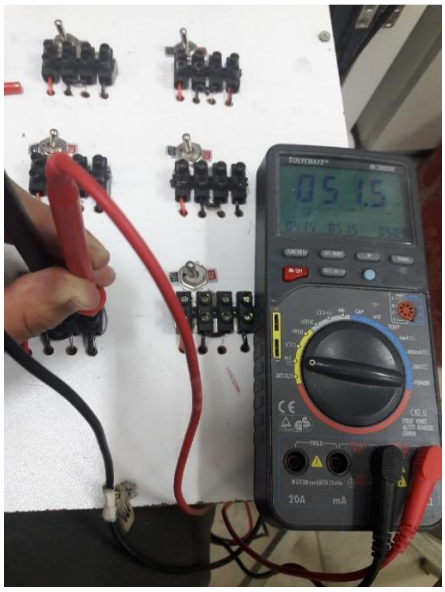

Figure 3.7 Output voltage measurement

Writing down the measurements was performed after the reading fixed on the multimeter screen.

3.2.4. Kinetic models for COD decay in the MFCs

The commonly used kinetic models for environmental applications are 0, 1st, 2nd and 3rd order kinetic equations:

Zero order kinetic reaction: $C_A = C_o - Kot$

First order kinetic reaction: $C_A = C_o e^{-K1t}$

Second order kinetic reaction: $(1/C_A) = (1/C_o) + K_2t$

Third order kinetic reaction: $(1/C_A)^2 = (1/C_o)^2 + 2K_3t$

Since we have COD vs time data, we can use the data to obtain the most suitable kinetic model for each MFC. The four kinetic equations can be linearized by finding Kt value for each point (COD, time) and plotting t vs

Kt for the four kinetic models and determine which the most representative model is.

Linearize kinetic models, obtained:

- 0 order: $(C_o - C_A) = K_o t$,
- 1st order: $Ln(C_o / C_A) = K_1 t$
- 2nd order: $((C_o/C_A)-1)/ C_o = K_2 t$,
- 3rd order: $0.50(C_A^{-2} - C_o^{-2}) = K_3 t$.

3.2.5. Data management and analysis:

Obtained measurements data were analyzed statistically using testing hypothesis procedures in order to obtain reliable results. One-way ANOVA was used as a tool for statistical analysis to find the significant differences between different conditions investigated.

Linearization using EXCEL was utilized to characterize COD kinetic model. Excel was used also to find the relationship between COD of substrate in MFC and output voltage of the same MFC.

4. Results and Discussion

This study consists of two stages; first one is to investigate the effect of different parameters/conditions on the performance of MFC where the second one is to characterize the relationship between organic content in the substrate used in MFC with output power generated by MFC. Results of both stages are summarized in the following sections.

4.1. Effect of different conditions on MFC performance

In this stage four individual parameter were investigated, one experiment is performed to investigate each parameter.

4.1.1. Effect of electrode material on output voltage

In this experiment four different electrodes, were used in 4 different MFCs, three MFC-duplicates for each electrode. Used electrode materials were: Zinc sheets, Copper sheets, manufactured pretreated carbon electrodes and Carbon brushes. All used electrodes have the same dimensions (7 cm X 4 cm).

Figure 4.1 represents the measurements of the output voltage for each electrode material during 7 days of operation. Output voltage measurements are provided in **Table A1** provided in **Appendix A**.

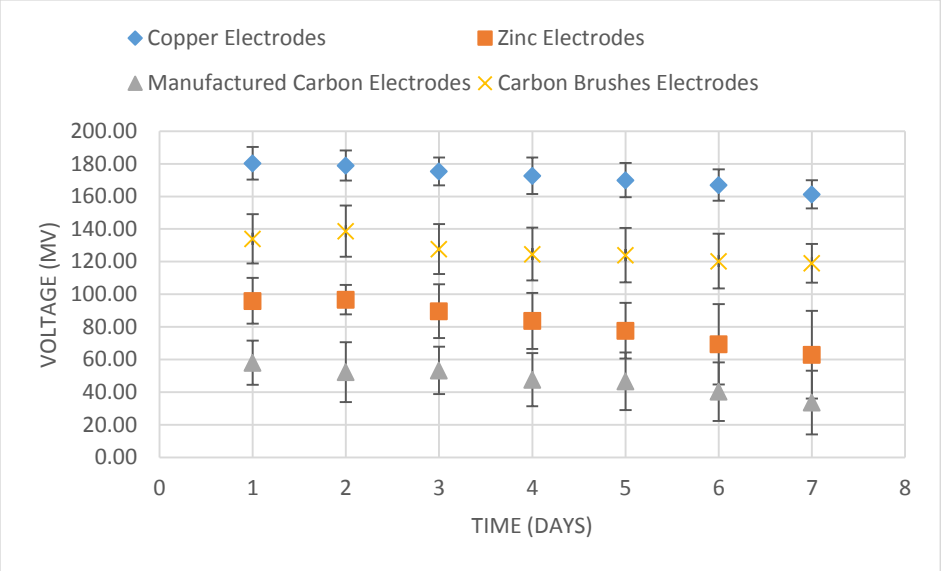

Figure 4.1 Output voltage in MFCs of different electrode materials

As shown in **Figure 4.1**, Copper sheets electrode-MFC looks to be the most efficient one, then Carbon brushes-MFC, then Zinc sheets-MFC and finally manufactured Carbon electrodes-MFC.

Statistical analysis is used to approve or to disclaim the initial conclusion from the previous figure. One-way ANOVA and post hoc tests are used here applying IBM SPSS statistics 2.0 software.

The purpose here is to check if there is a significant difference between the different electrodes materials used and to allocate where the difference is. In these experiments, all conditions were fixed except the electrode material; output voltage vs time was plotted for each electrode type. Since we have one independent variable here, i.e. electrode material, we can use One Way ANOVA test.

Checking the ANOVA assumptions:

- Continuous dependent variable: Output voltage data can be considered as continuous data-interval continuous variable.

- Independent variable should consist of two or more groups: here we have 4 groups (4 electrode materials).
- Independence of observations, which means that there is no relationship between the observations in each group or between the groups themselves: this condition is satisfied here.
- There should be no significant outliers: SPSS was used to check outliers and found that there are no outliers in the data as shown in steam and leaf plots for the data provided in **Appendix B**, **Figure B1**.
- Dependent variable should be approximately normally distributed: Shapiro-Wilk test was used to check normality and it was found that all the distributions are approximately normal distributed (All sig. values were more than 0.05 as shown in **Appendix B**, **Table B1**).
- Homogeneity of variances: Levene's test for homogeneity of variances was used in SPSS to check for homogeneity and it was found that this condition was satisfied as shown in **Appendix B**, **Table B2**.

As a result, One Way ANOVA can be used here to compare between the 4 groups we have; Copper, Zinc, Manufactured Carbon and Carbon Brushes.

Using ANOVA in SPSS it was found that there is a significance difference between electrode materials used in terms of output voltage (significance less than 0.05) as shown in **Appendix B**, **Table B9**.

To allocate which electrode materials have significance difference between them, Post Hoc tests was performed on SPSS. Results are summarized in **Appendix B**, **Table B10**. It can be noticed that Copper electrodes-MFCs are significantly more efficient than Carbon brushes-MFCs and Zinc electrodes-

MFCs. Carbon brushes-MFCs are significantly more efficient than zinc electrodes-MFCs; whereas Zinc-MFCs are significantly more efficient than manufactured carbon-MFCs in the first two days only.

It can be noticed that, in general, output voltage is decreasing with time; this can be justified by the decreasing of nutrients with time in the substrate. Variations in output voltage between duplicates for the same electrode material can be attributed to many causes: variation in substrate constituents, variation in microorganism's cultures, and variation in salt bridges efficiency and variation in electrode position in the anode.

4.1.2. Effect of salt bridge diameter on output voltage

In this experiment three salt bridges diameters, 10 mm, 16 mm and 24 mm, were used in 3 different MFCs, three MFC-duplicates for each diameter.

Nine DS-MFCs were constructed, 1M-KCl salt was used, Carbon brushes electrodes were used, area of each electrode was 28 cm^2 and temperature maintained to 35 ^oC. Primary effluent wastewater was used as substrate (790 mL) in addition to anaerobic sludge (10 mL). Dissolved oxygen in water was used as cathodic chamber and this experiment was running for one week. **Figure 4.2** represents the results of this experiment. Detailed results of this experiment are shown in **Appendix A**, **Table A2**.

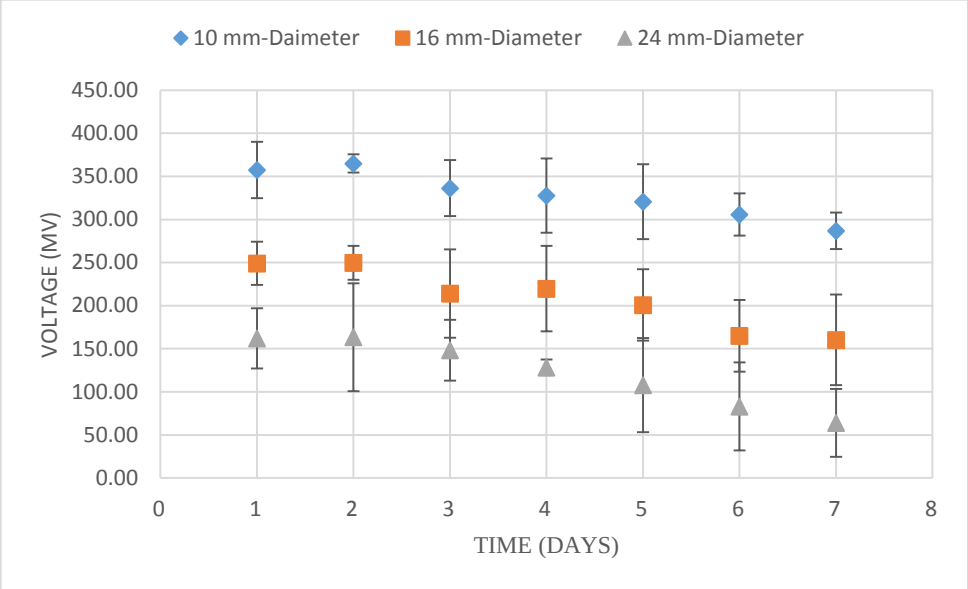

Figure 4.2 Output voltage of MFCs with different salt bridge diameters

As shown in **Figure 4.2**, and based on statistical analysis performed, the best output voltage is achieved by the 10-mm salt bridge-MFC, then by 16 mm and the 24-mm salt bridge-MFC.

The purpose here is to check if there is a significant difference between the different diameters of salt bridges used and to allocate where the difference is. In these experiments, all conditions were fixed except the salt bridge diameter; output voltage vs time was plotted for each electrode type. Since we have one independent variable here, i.e. salt bridge diameter, we can use One Way ANOVA test.

Checking the ANOVA assumptions:

- Continuous dependent variable: Output voltage data can be considered as continuous data-interval continuous variable.
- Independent variable should consist of two or more groups: here we have 3 groups (3 salt bridge diameters).

- Independence of observations, which means that there is no relationship between the observations in each group or between the groups themselves: this condition is satisfied here.
- There should be no significant outliers: SPSS was used to check outliers and found that there are no outliers in the data as shown in steam and leaf plots for the data captioned from SPSS output provided in **Appendix B**, **Figure B2**.
- Dependent variable should be approximately normally distributed: Shapiro-Wilk test was used to check normality and it was found that all the distributions are approximately normal distributed (All sig. values were more than 0.05 as shown in **Appendix B**, **Table B3** obtained from SPSS output).
- Homogeneity of variances: Levene's test for homogeneity of variances was used in SPSS to check for homogeneity and it was found that this condition was satisfied as shown in **Table B4** in **Appendix B**.

As a result, One Way ANOVA can be used here to compare between the three groups we have; 10 mm, 16 mm and 24 mm salt bridges. Using ANOVA in SPSS it was found that there is a significance difference between salt bridges diameters used in terms of output voltage (significance less than 0.05) as shown in **Table B11**, **Appendix B**.

To allocate which diameters have significant difference between them, Post Hoc tests was performed on SPSS. Results are summarized in **Table B12** in **Appendix B**. It can be noticed that 10 mm salt bridges-MFCs are significantly more efficient than 16 mm salt bridges-MFCs; whereas 16 mm

salt bridges-MFCs are significantly more efficient than 24 mm salt bridges-MFCs in the first one days only.

It can be noticed that, in general, output voltage is decreasing with time; this can be justified by the decreasing of nutrients with time in the substrate. Variations in output voltage between duplicates for the same electrode material can be attributed to many causes: variation in substrate constituents, variation in microorganism's cultures, and variation in salt bridges efficiency and variation in electrode position in the anode.

4.1.3. Effect of salt bridge solution on output voltage

In this experiment two salt solutions were used in salt bridge preparation, KCl and NaCl. Six DS-MFCs were constructed, 1M-KCl and 1M-NaCl salts was used in 10 mm-salt bridges, Carbon brushes electrodes were used, area of each electrode was 28 cm^2 and temperature maintained to 35 ^oC. Primary effluent wastewater was used as substrate (790 mL) in addition to anaerobic sludge (10 mL). Dissolved oxygen in water was used as cathodic chamber and this experiment was running for one week. **Figure 4.3** represents the results of this experiment. Detailed results of this experiment are shown in **Appendix A**, **Table A3**.

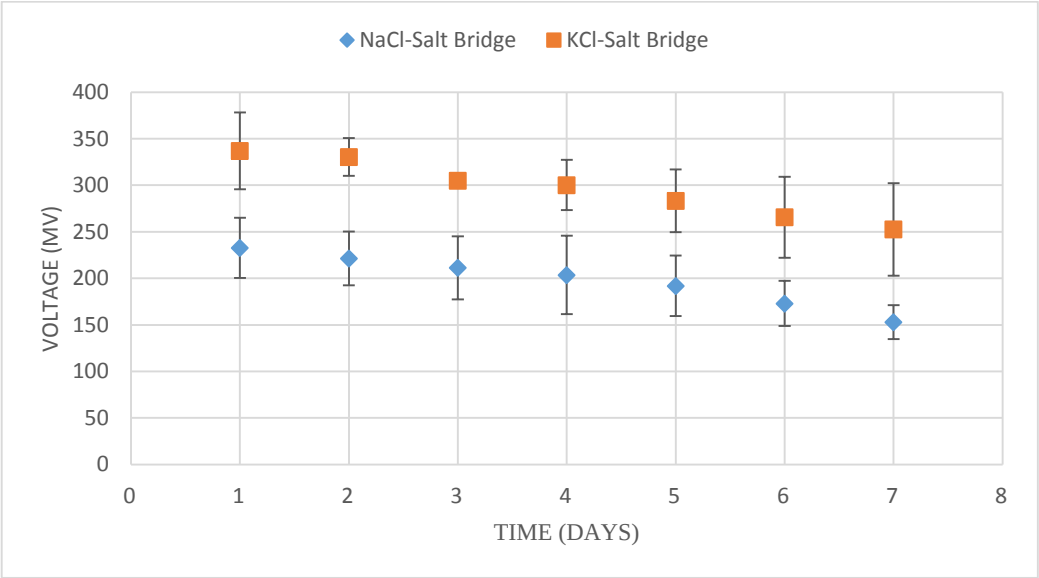

Figure 4.3 Output voltage for KCl and NaCl Salt bridges

As shown in **Figure 4.3** the best output voltage looks to be achieved by the KCl salt bridge-MFC.

The purpose here is to check if there is a significant difference between the two different salts used. In these experiments, all conditions were fixed except the salt type; output voltage vs time was plotted for each salt type. Since we have one independent variable here, i.e. salt type, we can use One Way ANOVA test.

Checking the ANOVA assumptions:

- Continuous dependent variable: Output voltage data can be considered as continuous data-interval continuous variable.
- Independent variable should consist of two or more groups: here we have 2 groups (2 salt types).
- Independence of observations, which means that there is no relationship between the observations in each group or between the groups themselves: this condition is satisfied here.

- There should be no significant outliers: SPSS was used to check outliers and found that there are no outliers in the data as shown in steam and leaf plots for the data captioned from SPSS output provided in **Appendix B**, **Figure B3**.
- Dependent variable should be approximately normally distributed: Shapiro-Wilk test was used to check normality and it was found that all the distributions are approximately normal distributed (All sig. values were more than 0.05 as shown in **Appendix B**, **B6** obtained from SPSS output).
- Homogeneity of variances: Levene's test for homogeneity of variances was used in SPSS to check for homogeneity and it was found that this condition was satisfied as shown in **Table 2.15**, **Appendix B**.

As a result, One Way ANOVA can be used here to compare between the 2 groups we have; NaCl and KCl salt bridges-MFCs. Using ANOVA in SPS it was found that there is a significance difference between both salts as shown in **Table B13**, **Appendix B**.

4.1.4. Effect of salt concentration on output voltage

In this experiment three KCl salt concentrations, 1M, 2M and 3M, were used in salt bridge preparation. Nine DS-MFCs were constructed, 10 mm-salt bridges were used, Carbon brushes electrodes were used, area of each electrode was 28 cm^2 and temperature maintained to 35 ^oC. Primary effluent wastewater was used as substrate (790 mL) in addition to anaerobic sludge (10 mL). Dissolved oxygen in water was used as cathodic chamber and this

experiment was running for one week. **Figure 4.4** represents the results of this experiment. Detailed results of this experiment are shown in **Appendix A**, **Table A4**.

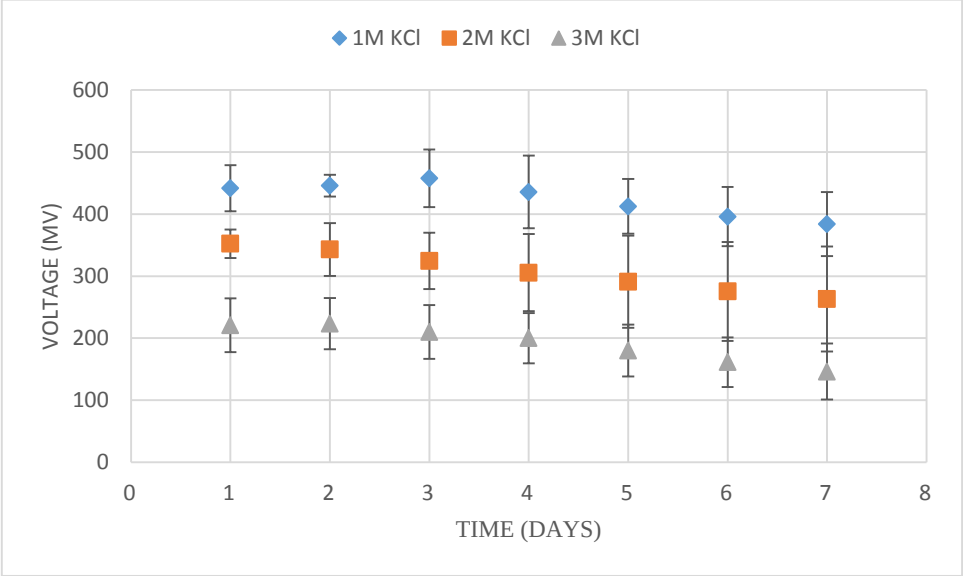

Figure 4.4 Output voltage for KCl and NaCl Salt bridges

As shown in **Figure 4.4** and based on statistical analysis the best output voltage is achieved by the KCl salt bridge-MFC.

The purpose here is to check if there is a significant difference between the different salt concentrations used and to allocate where the difference is. In these experiments, all conditions were fixed except the salt concentration in the salt bridges; output voltage vs time was plotted for each concentration. Since we have one independent variable here, i.e. salt concentration, we can use One Way ANOVA test.

Checking the ANOVA assumptions:

- Continuous dependent variable: Output voltage data can be considered as continuous data-interval continuous variable.

- Independent variable should consist of two or more groups: here we have 3 groups (3 concentrations).
- Independence of observations, which means that there is no relationship between the observations in each group or between the groups themselves: this condition is satisfied here.
- There should be no significant outliers: SPSS was used to check outliers and found that there are no outliers in the data as shown in steam and leaf plots for the data captioned from SPSS output provided in **Appendix B**, **Figure B4**.
- Dependent variable should be approximately normally distributed: Shapiro-Wilk test was used to check normality and it was found that all the distributions are approximately normal distributed (All sig. values were more than 0.05 as shown in **Table B7**, **Appendix B** obtained from SPSS output).
- Homogeneity of variances: Levene's test for homogeneity of variances was used in SPSS to check for homogeneity and it was found that this condition was satisfied as shown in **Table B8**, **Appendix B**.
- As a result, One Way ANOVA can be used here to compare between the 3 groups we have; 1M, 2M and 3M KCl salt bridges-MFCs. Using ANOVA in SPSS it was found that there is a significance difference between salt concentrations used for the salt bridges in terms of output voltage (significance less than 0.05) as shown in **Table B14**, **Appendix B**.

- To allocate which groups have significance difference between them, Post Hoc tests was performed on SPSS. Results are summarized in **Table B15**, Appendix B. It can be noticed that 1M KCl salt bridges-MFCs are significantly more efficient than 3M KCl salt bridges-MFCS. 1M KCl salt bridges-MFCs are significantly more efficient than 2M KCl salt bridges-MFCs in the first three days only; the same thing as in a comparison between 2M KCl salt bridges-MFCs and 3M KCl salt bridges-MFCs.

4.2. COD Determination and Determining Kinetic Models for COD Removal

COD was measured using titrimetric method according to "Standard Methods for the Examination of Water and Wastewater" **(American Public Health Association, American Water Works Association, & Water Environment Federation, 2012)**. Dilution of samples using distilled water was applied when the expected COD higher than the allowed range (40-400 mg/L).

Samples were mixed with: standard potassium dichromate digestion solution, sulfuric acid reagent and sulfamic acid in quantities as given in the standard methods. After heating of mixture for 2 hours at 150 ^{o}C and cooling, the mixture was titrated against standard ferrous ammonium sulfate (F.A.S) and using ferroin as indicator. COD was measured for all MFCs substrates day after day, at each time a blank sample was prepared from distilled water and the same reagents used for the samples.

- COD measurement -COD of anaerobic sludge used

Since that the expected COD of the sludge is too large; it is required to dilute sludge samples using distilled water. 10 mL of anaerobic sludge were taken from the well mixed sample using 10 mL-graduated cylinders, and diluted with 990 mL distilled water. The resulted dilution factor is:

P_1= initial volume of sample/ final volume

= 10/ (990+10) = 0.01

The resulted 1 L sample was well mixed using magnetic stirrer and three COD samples, each 1 mL, were taken. The samples were taken from top, middle and bottom of the beaker containing sample.

According to **Table 4.2220**: I in "Standard Methods for the Examination of Water and Wastewater", when ampules are used the required volumes of reagent are: 2.5 mL sample, 1.5 mL digestion solution and 3.5 mL sulfuric acid reagent. Lower sample volume can be taken and diluted to 2.5 mL to detect higher COD values. Sample volume taken from diluted sludge were 1 mL for each, so the total dilution factor is:

$P=P_1 \text{ X } P_2= .01 \text{ X } (1/2.5) = 0.004$

Results are represented in the following table:

Table 2.1 Summary of sludge COD measurement

Sample	A (mL)	B (mL)	A-B (mL)	Dilution factor	COD (mg/L)
1	2.9	1.6	1.3	0.004	52,000
2	2.9	1.8	1.1	0.004	44,000
3	2.9	1.7	1.2	0.004	48,000
Blank	**2.9**				
Average COD for the three samples (mg/L)					48,000

- COD measurement -COD of used wastewater

COD of the primary effluent was expected to be around 500-700 mg/L. Dilution was performed to assure that the measured COD is within allowed range (40-400 mg/L).

Volume of sample, taken from the well mixed 10 L origin sample, was 1 mL and diluted into 2.5 mL using distilled water; so, dilution factor is 0.40. Results are presented in the following table.

Table 4.2 Summary of wastewater COD measurement

Sample	A (mL)	B (mL)	A-B (mL)	Dilution factor	COD (mg/L)
1	2.7	1.1	1.6	0.4	640
2	2.7	1.4	1.3	0.4	520
3	2.7	1.5	1.2	0.4	480
Blank	2.7				
Average COD for the three samples (mg/L)					547

- Sampling calculations

Since that titrimetric method for COD determination is applicable within the range (40 mg/L < COD < 400 mg/L) and dilution factor was taken 0.40 (1 mL sample diluted into 2.5 mL) then the expected COD of samples should be lie in this range and considering used dilution factor.

At determined COD= 40 mg/L and dilution factor= 0.40, the origin COD is 40/0.4 = 100 mg/ L. The same as at COD=400 mg/L and dilution factor= 0.2, the origin COD is 2000 mg/L. Resulting that COD of samples should be within the range 200 to 2000 mg/L. 300, 900, 1400 and 1700 mg/L were selected considering the previous points.

Samples contain WW and sludge, considering that sludge will increase total COD of the samples and using the following equation:

$$\text{COD} = \frac{(\text{WW Volume X WW COD}) + (\text{Sludge Volume X Sludge COD}) + (\text{D.W. } VolumeXD.W.\ COD)}{Total\ volume}$$

Where:

WW volume: volume of WW used in the mixture (mL),

WW COD: COD of the influent WW, measured to be 547 mg/L,

Sludge volume: volume of sludge used in the mixture (mL),

Sludge COD: COD of the used sludge and equals 48,000 mg/L,

D.W. Volume: volume of distilled water used to dilute samples in low COD samples (mL),

D.W. COD: COD of distilled water and equals to 0 mg/L,

Total volume: volume of the total mixture and equals to 800 mL.

To determine the kinetic models for COD decay in each MFC, using the approach discussed in section 3.2.4.

After linearization of each model for each MFC, find R^2 value for each model using Excel as shown in **Tables D1-D4** (**Appendix D**) and **Figures 4.5-4.20**:

MFCs 1, 2 and 3

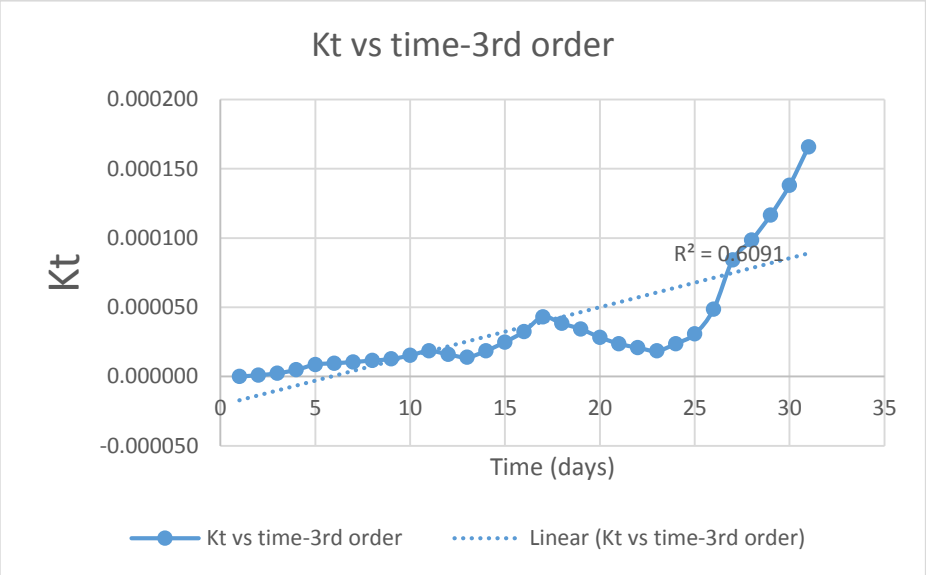

Figure 4.5 Comparison between Kt values of third order reaction and linearized Kt values for COD behaviour in MFCs 1,2 and 3

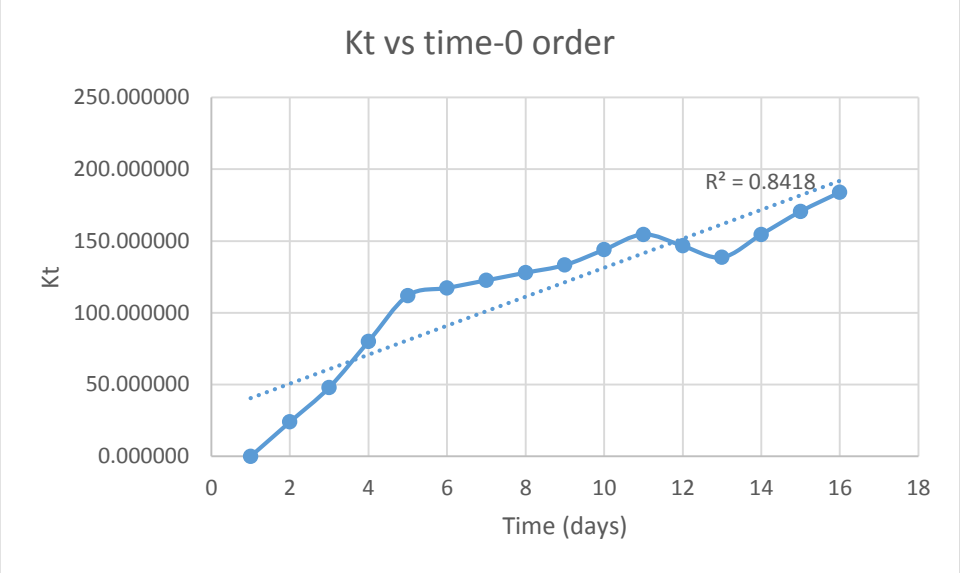

Figure 4.6 Comparison between Kt values of zero order reaction and linearized Kt values for COD behavior in MFCs 1, 2 and 3

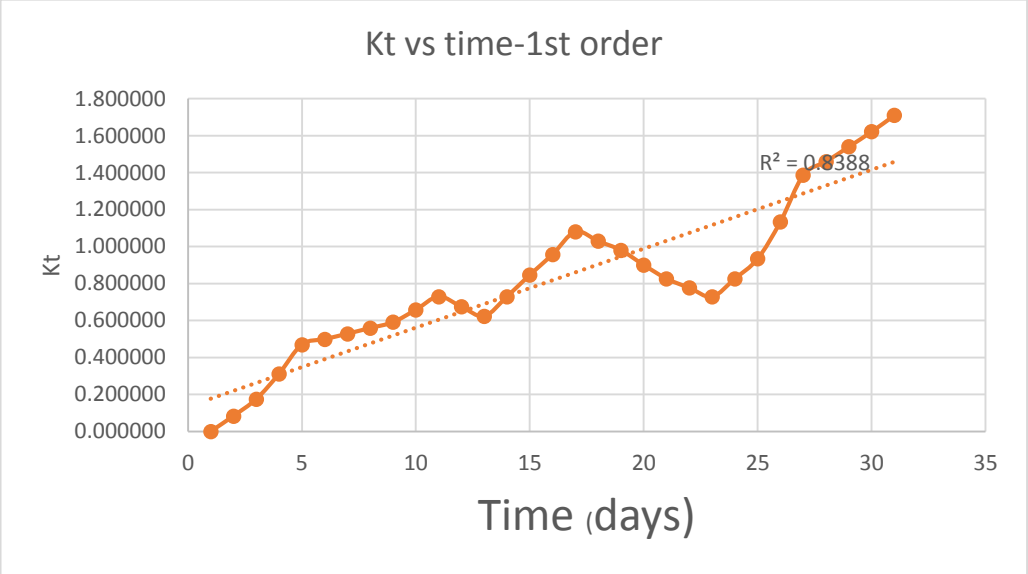

Figure 4.7 Comparison between Kt values of first order reaction and linearized Kt values for COD behavior in MFCs 1, 2 and 3

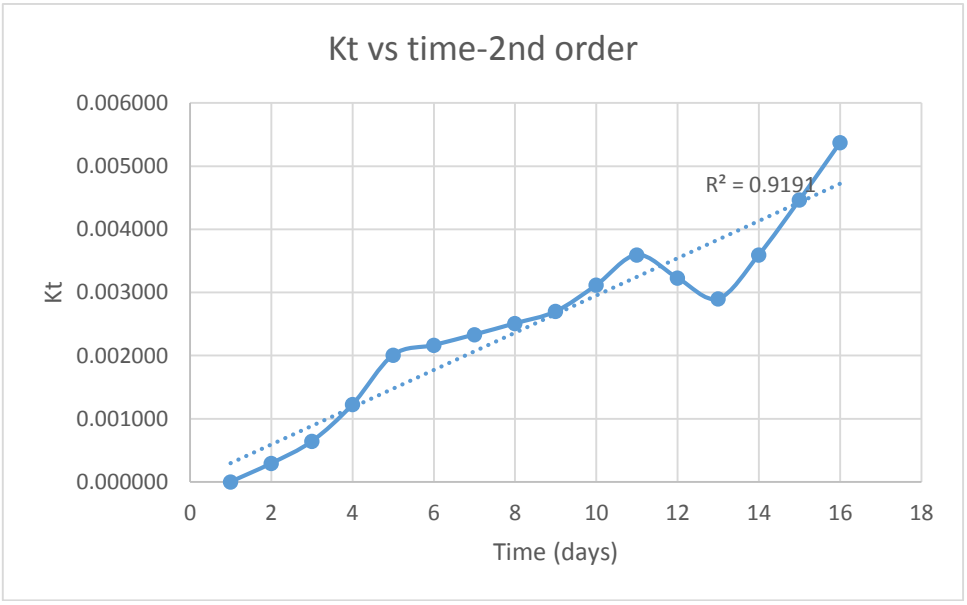

Figure 4.8 Comparison between Kt values of second order reaction and linearized Kt values for COD behavior in MFCs 1, 2 and 3

2nd order is the best suitable model for MFC1.

MFCs 4, 5 and 6:

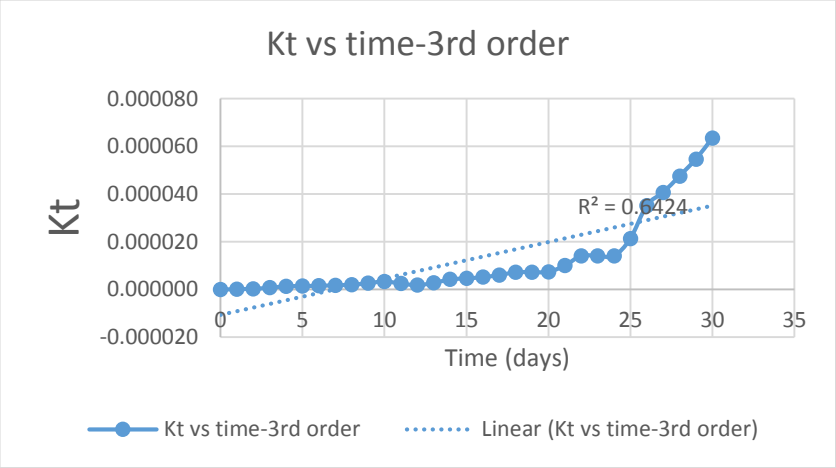

Figure 4.9 Comparison between Kt values of third order reaction and linearized Kt values for COD behavior in MFCs 4, 5 and 6

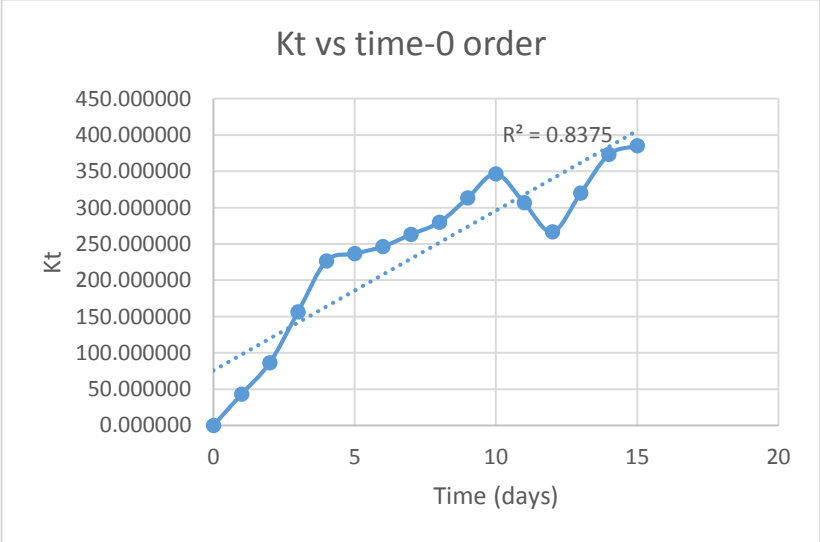

Figure 4.10 Comparison between Kt values of zero order reaction and linearized Kt values for COD behavior in MFCs 4, 5 and 6

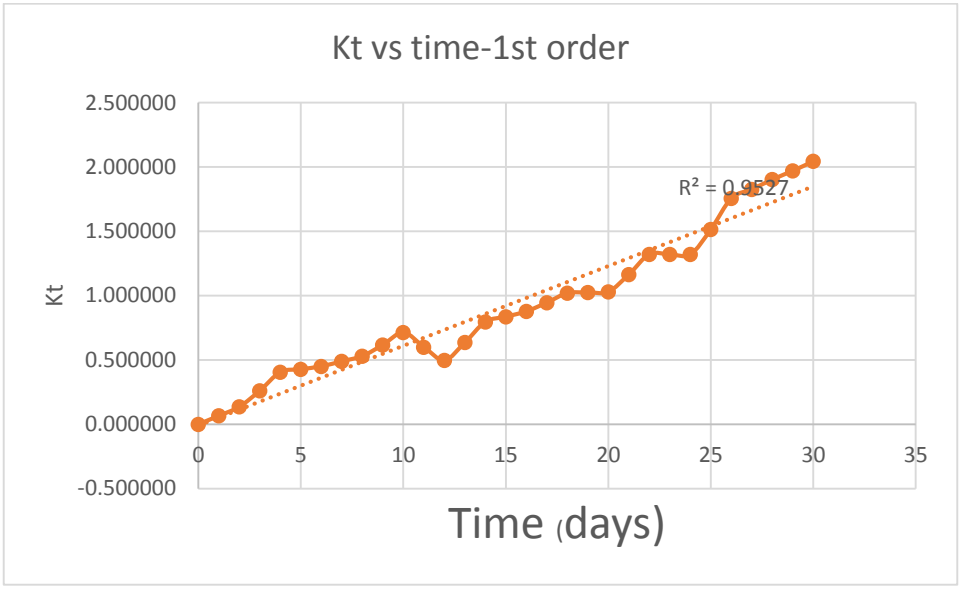

Figure 4.11 Comparison between Kt values of first order reaction and linearized Kt values for COD behavior in MFCs 4, 5 and 6

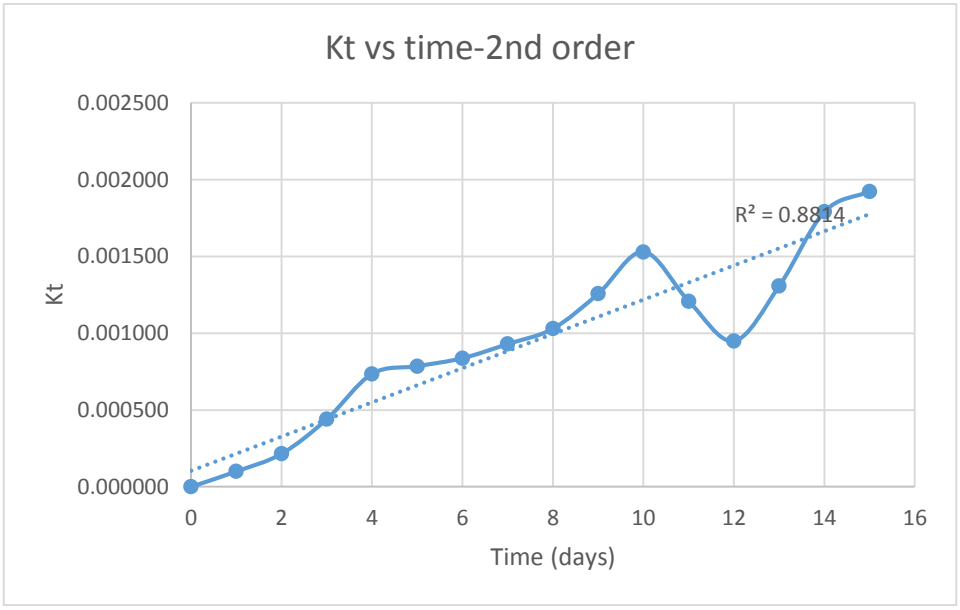

Figure 4.12 Comparison between Kt values of second order reaction and linearized Kt values for COD behavior in MFCs 4, 5 and 6

1st order kinetic model is the best suitable model for MFC2.

MFCs 7, 8 and 9

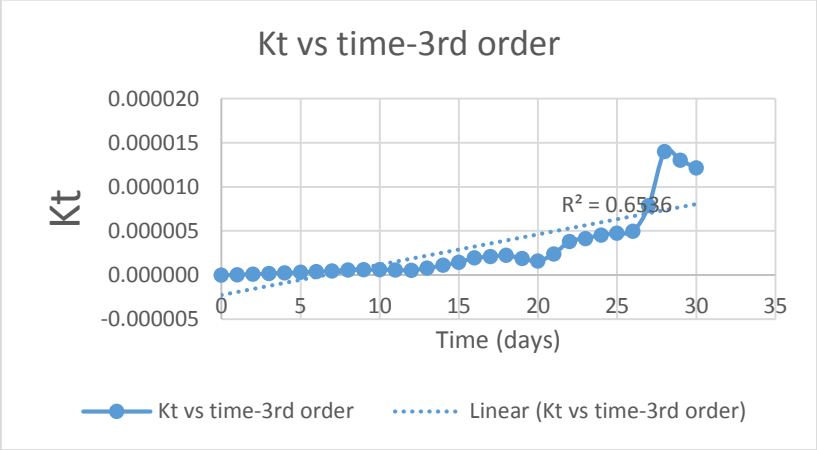

Figure 4.13 Comparison between Kt values of third order reaction and linearized Kt values for COD behavior in MFCs 7, 8 and 9

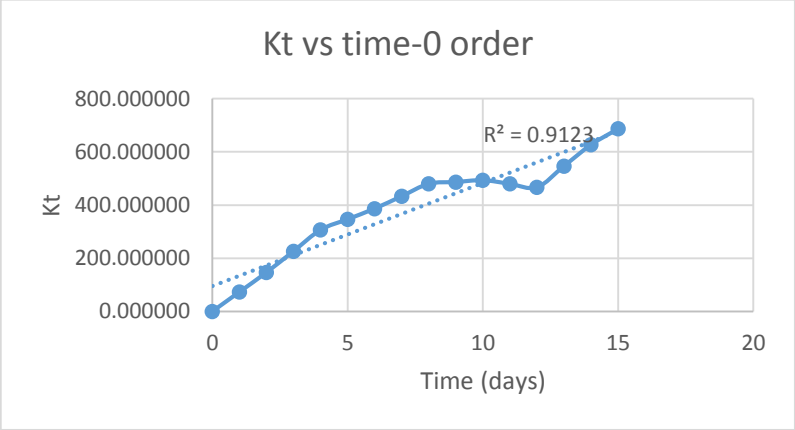

Figure 4.14 Comparison between Kt values of zero order reaction and linearized Kt values for COD behavior in MFCs 7, 8 and 9

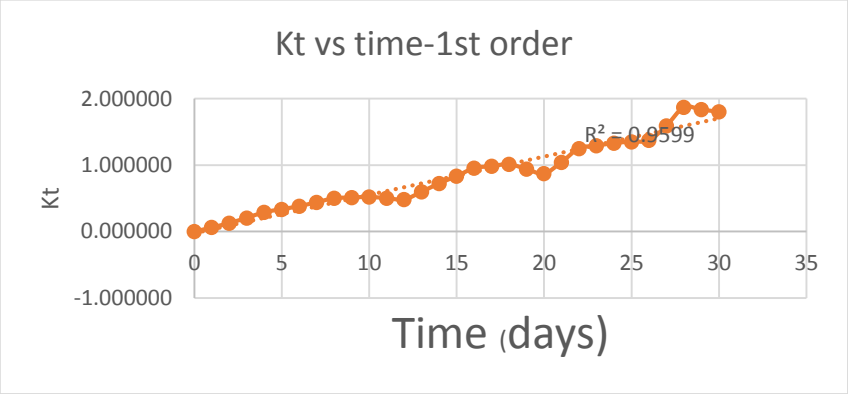

Figure 15 Comparison between Kt values of first order reaction and linearized Kt values for COD behavior in MFCs 7, 8 and 9

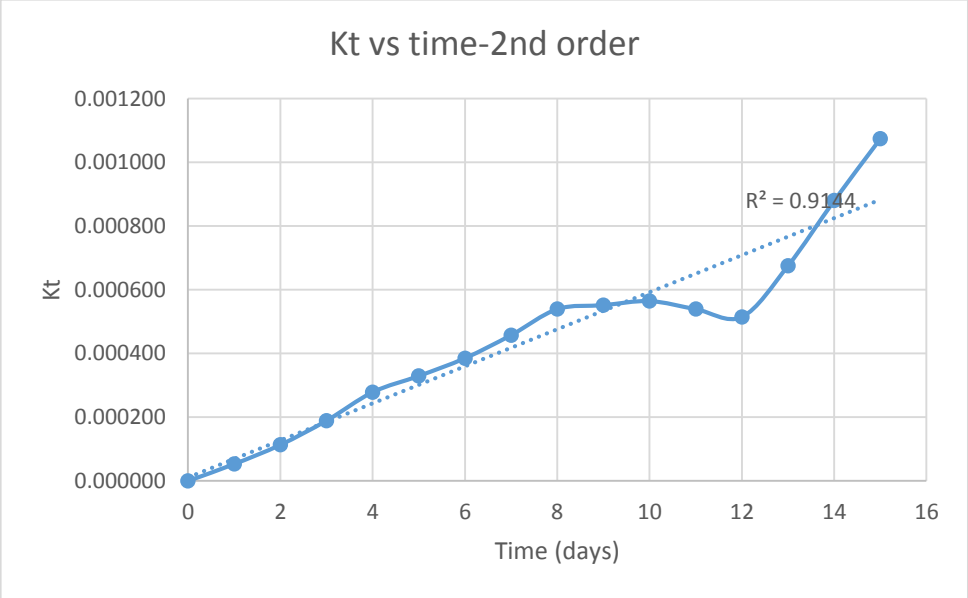

Figure 16 Comparison between Kt values of second order reaction and linearized Kt values for COD behavior in MFCs 7, 8 and 9

1st order is the most suitable model for MFC3.

MFCs 10, 11 and 12:

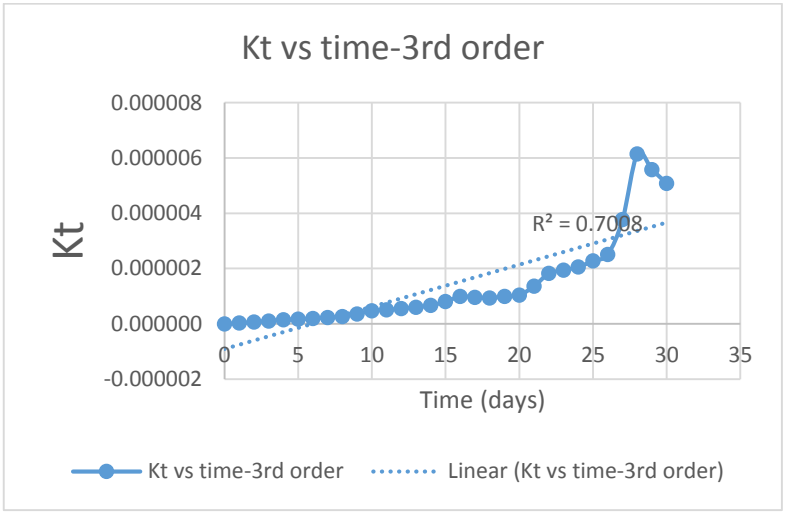

Figure 4.17 Comparison between Kt values of third order reaction and linearized Kt values for COD behavior in MFCs 10, 11 and 12

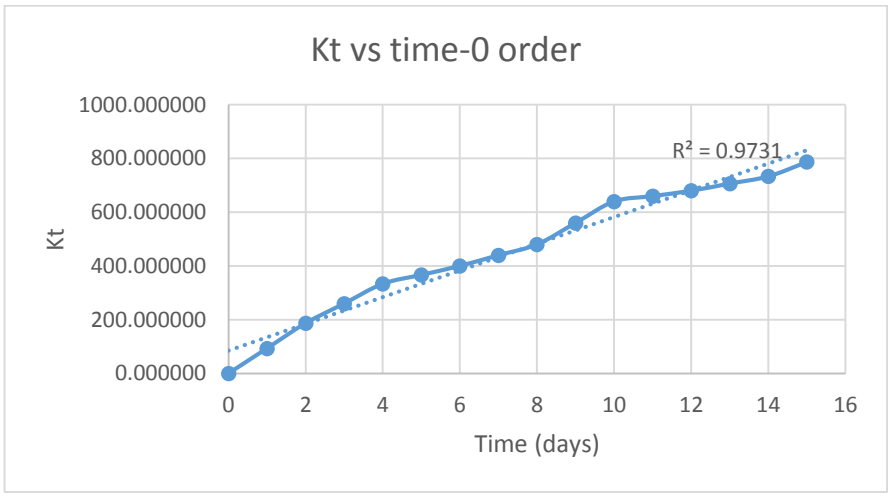

Figure 4.18 Comparison between Kt values of zero order reaction and linearized Kt values for COD behavior in MFCs 10, 11 and 12

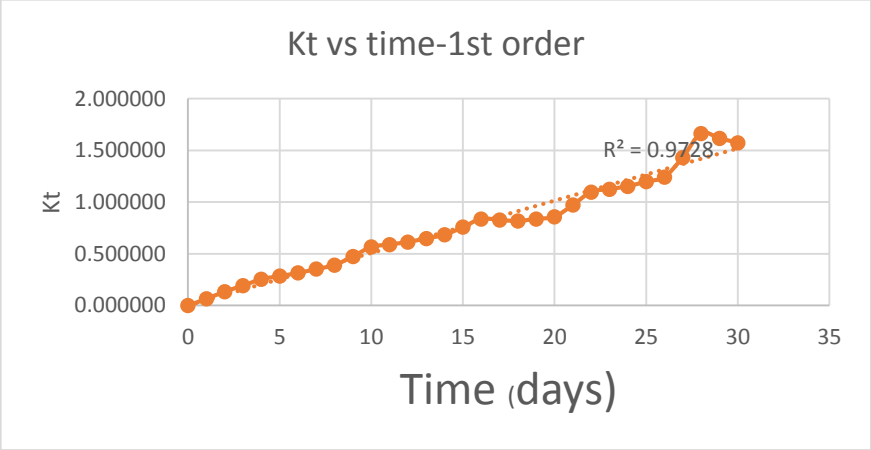

Figure 4.19 Comparison between Kt values of first order reaction and linearized Kt values for COD behavior in MFCs 10, 11 and 12

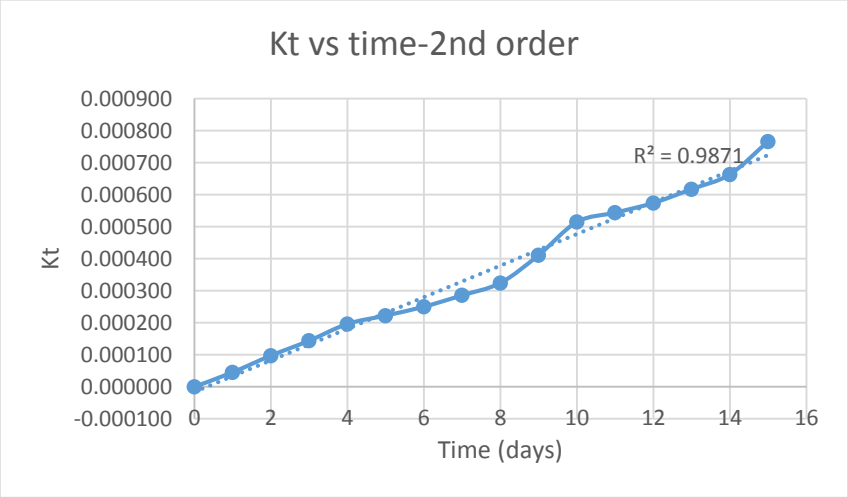

Figure 4.20 Comparison between Kt values of second order reaction and linearized Kt values for COD behavior in MFCs 10, 11 and 12

2nd order kinetic reaction is the most suitable one for MFC4.

4.3. Relationship between output voltage and substrate COD

Data obtained from the second part of this study are provided in Appendix D. It can be noticed that there is a relationship between COD and output voltage. **Figure 4.21** shows the plot of all data points obtained.

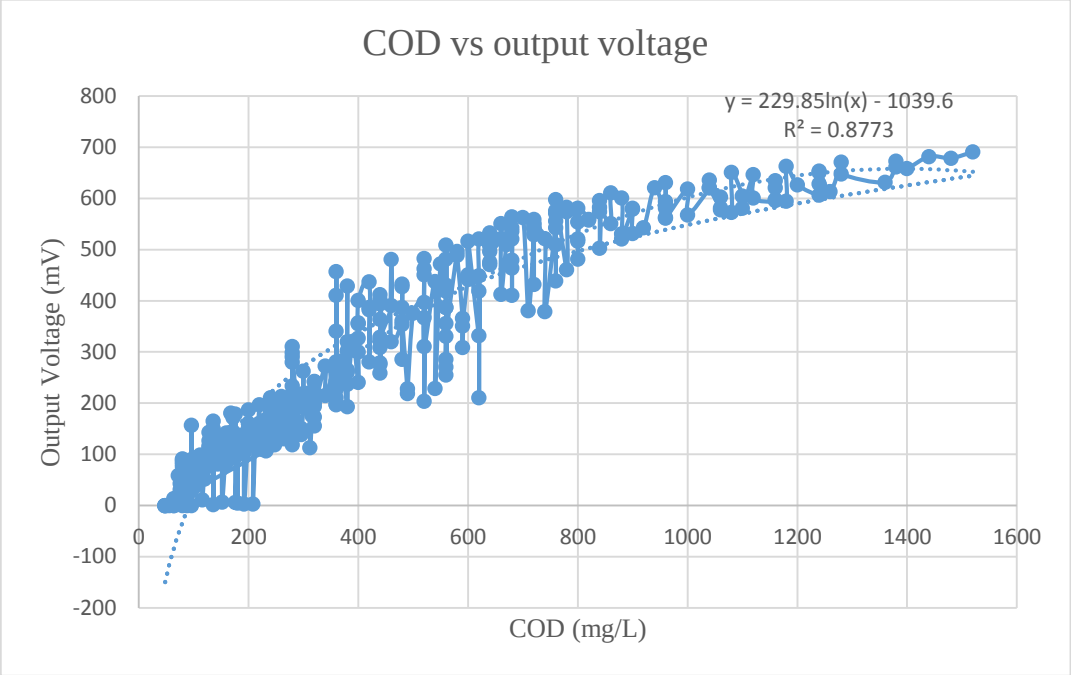

Figure 4.21 Relationship between COD and output voltage

Since that this is a nature process, the best mathematical model represents this data is natural logarithmic model.

COD (mg/L) = 229.85 Ln (V)-1039.6; where V is output voltage (mV).

This model can be used to indicate COD of a certain wastewater sample, by measuring the output voltage of a MFC operated by that sample, and considering all conditions of the experiments performed to obtain this model. It worth to mention that COD removal behavior for the four different MFCs was ranged between 1st and 2nd order kinetic reaction. Actually, COD behavior is 1st order kinetic. In the analysis of COD data with time, it was found that MFC 1 and 4 are following 2nd order with a minor difference from 1st order. Where in MFC 2 and 3 are following1st order reaction. These results indicate that our data, to somehow, are logical.

4.4. Achieved output power and feasibility estimation

Output power can be calculated as:

P = I X V

Where:

P: output power, watt,

I: Electrical current through the load (resistance), Amber,

V: output voltage, Volt

Electrical current can be calculated as:

I=V/R

Where R is the applied resistance, Ohm

Used Resistances were 1000 ohm. Maximum output voltage achieved in this study was 0.684 volt. Calculating power for the maximum voltage:

I=0.684/1000=0.684 mA=0.000684 A.

P=0.000684 X 0.684=0.00046786 Watt.

Normalize the output power to the anode surface area, anode surface area was 28 cm^2= 0.0028 m^2, resulting:

Normalized power = P/ Anode surface area

= 0.00046786/ 0.0028

= 0.71 W/m^2

Normalized power to the used volume of WW= P/volume

= 0.00046786/ 0.0008

= 0.585 W/m^3.

During 30 days of operation, average output voltage is 448 mV; so average P= 0.000201 W and volume normalized power is 0.2509 W/m^3, calculate output power for 30 days:

Output power= 0.2509 W/m^3 X 30 days X 24 hr/day = 140.4 Wh =180.65 WH/m^3.

Comparing these results to the data presented in Table 1 and Table 2, found that maximum output power in this research is higher than several previous results (Thygesen et al., 2011; Ahn and Logan, 2010; You, et al., 2014; Aeltermanet al., 2008). However, maximum output power in this research attached with higher substrate COD and different electrode material.

To estimate the feasibility of output power of applying MFC technology in Nablus West WWTP, assuming that MFC is practically applicable without any obstacle:

Average volume of WW influent is 11,000 m3/day and 330,000 m3/month. Potential output power monthly can be estimated by multiplying monthly influent by output power normalized to the volume of wastewater and assuming that efficiency is 70% as:

Monthly output power= 70% X 330,000 m3 /month X 0.18065 kWh/m3

=41,730 kWh/month

= 41.73 MWh/month.

Monthly consumed power in the Nablus West WWTP is 130-240 MWH, 185 MWH/month as an average. So, purchased electricity could be decreased to 77%.

Each KWH costs 0.6134 NIS, so the power generated form MFCs will save 41,730 X 0.6134=36,566 NIS/ month which is equivalent to $ 87,761/year; assuming that operation and maintenance costs of MFC processes is equal to that for secondary treatment used in the plant.

As a rough estimation of the capital cost of inserting MFCs in Nablus-West WWTP using the similar approach that used in (Patra, 2008) by estimation power to cost ratio (PCR).

PCR and capital cost estimation:

Average output power= 0.000201 W= 0.201 mW.

Assume that carbon brushes were used as electrodes and applying MFCs on the final sedimentation tank (volume=3859 m3) with same settings used before, for one MFC:

Cost of (7 cm X 4 cm)-carbon bush= 20 NIS= $ 5.71.

Cost of salt bridge= 15 NIS= $ 4.30.

Cost of containers= 30 NIS= $ 8.6.

Cost of aerator= 35 NIS= $ 10.

Cost of shaking system= 37 NIS= $ 10.6.

Cost of temperature control system=33 NIS= $ 9.4.

Cost of electrical connections= 4.5 NIS= $ 1.3.

Total cost= $ 49.91.

PCR=0.201/49.91= 0.004 mW/$.

Number of required MFCs (volume= 0.80 Litre) = 3859/0.80=4824 MFCs.

Produced power= 0.201 mW/MFC X 4824 MFC = 970 mW.

Estimated operation cost= Produced power/PCR= 970/.004= $ 242, 500.

Assuming upscaling the system will require a reduplication of the total cost by three times. However inserting MFC system into a small scale WWTP will be much easier than large-scale WWTP; with higher PCR ration in large scale WWTP. So, the capital cost of inserting MFC system into the plant = $ 727,500 **(Li et al., 2014)**.

The total cost (investment) necessary to achieve the above savings is about $ 727,500. The economic feasibility of the project is estimated using payback period analysis:

Payback period (years)= Investment/ Savings (per year)

=727500/ 87,761

=8.3 years.

Since that the payback period of the project is more than 5 years, then the project is not feasible economically. However, from an environmental point of view and energy perspective this project can be implemented to overcome energy and environmental challenges.

5. Conclusions and Recommendations

- Out of the four-electrode material applied in MFCs, copper electrode was the best followed by carbon brushes.
- The smaller the salt bridge diameter used in the MFC, the best output voltage achieved.
- Salt bridge made of potassium chloride salt used in MFC, proved to be more efficient than that made of sodium chloride salt in output voltage.
- The smaller the salt concentration filled in the salt bridges that used in MFC the better output voltage achieved.
- Fixing of optimally obtained operating parameters and varying COD concentration of the substrate revealed that a relationship exists between output voltage and COD value in MFC.
- A first order kinetic reaction of the COD removal was predominating for all MFCs behavior.
- It is recommended to develop COD vs voltage models for MFCs under different conditions and parameters in a trial to generalize such model.
- the project is not feasible economically. However, from an environmental point of view and energy perspective this project can be implemented to solve energy and environmental challenges.

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Appendices

Appendix A: Achieved output voltage for first stage experiments

Table A1 Achieved output voltage for different electrodes used in MFCs

	Electrode Material	MFC number	Time (Day)						
			1	2	3	4	5	6	7
Voltage (mV)	Copper	1	73.00	71.00	68.00	65.00	61.00	60.00	54.00
		2	93.00	89.00	85.00	87.00	82.00	79.00	71.00
		3	81.00	83.00	79.00	72.00	73.00	68.00	65.00
		average	82.33	81.00	77.33	74.67	72.00	69.00	63.33
	Zinc	4	82.00	77.00	78.00	72.00	65.00	62.00	57.00
		5	56.00	60.00	55.00	51.00	47.00	43.00	38.00
		6	60.00	63.00	46.00	38.00	31.00	13.00	4.00
		average	66.00	66.67	59.67	53.67	47.67	39.33	33.00
	Manufactured Carbon	7	71.00	73.00	68.00	66.00	67.00	61.00	56.00
		8	44.00	38.00	39.00	35.00	36.00	29.00	20.00
		9	59.00	46.00	53.00	42.00	37.00	31.00	25.00
		average	58.00	52.33	53.33	47.67	46.67	40.33	33.67
	Carbon Brushes	10	83.00	81.00	73.00	70.00	68.00	66.00	59.00
		11	76.00	88.00	74.00	72.00	73.00	68.00	66.00
		12	54.00	58.00	47.00	43.00	42.00	38.00	43.00
		Average	71.00	75.67	64.67	61.67	61.00	57.33	56.00

Table A2 Achieved output voltage for different salt bridge diameters used in MFCs

	Salt Bridge Diameter	MFC number	Time (Day)						
			1	2	3	4	5	6	7
Voltage (mV)	10 mm	1	353.00	364.00	338.00	313.00	314.00	293.00	279.00
		2	392.00	355.00	368.00	376.00	367.00	334.00	311.00
		3	327.00	376.00	303.00	294.00	281.00	290.00	271.00
		average	357.33	365.00	336.33	327.67	320.67	305.67	287.00
	16 mm	4	273.00	252.00	229.00	241.00	215.00	185.00	179.00
		5	251.00	268.00	256.00	255.00	233.00	193.00	201.00
		6	223.00	229.00	157.00	163.00	154.00	117.00	101.00
		average	249.00	249.67	214.00	219.67	200.67	165.00	160.33
	24 mm	7	123.00	94.00	108.00	129.00	47.00	25.00	19.00
		8	190.00	180.00	164.00	119.00	123.00	103.00	81.00
		9	173.00	216.00	173.00	137.00	153.00	121.00	92.00
		average	162.00	163.33	148.33	128.33	107.67	83.00	64.00

Table A3 Achieved output voltage for different salts used in salt bridges for MFCs

Voltage (mV)	Salt Bridge Solution	MFC number	Time (Day)						
			1	2	3	4	5	6	7
	NaCl	1	269	254	237	246	228	200	173
		2	222	211	224	203	183	166	149
		3	207	199	173	162	165	153	137
		average	233	221	211	204	192	173	153
	KCl	4	381	353	311	327	314	297	289
		5	299	314	297	273	247	216	196
		6	331	324	307	301	289	284	273
		average	337	330	305	300	283	266	253

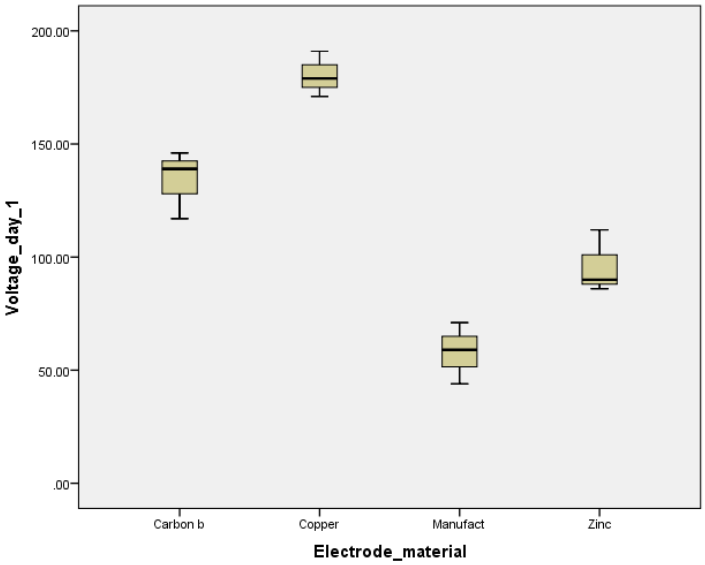
Table A4 Achieved output voltage for different salt concentrations used in salt bridges for MFCs

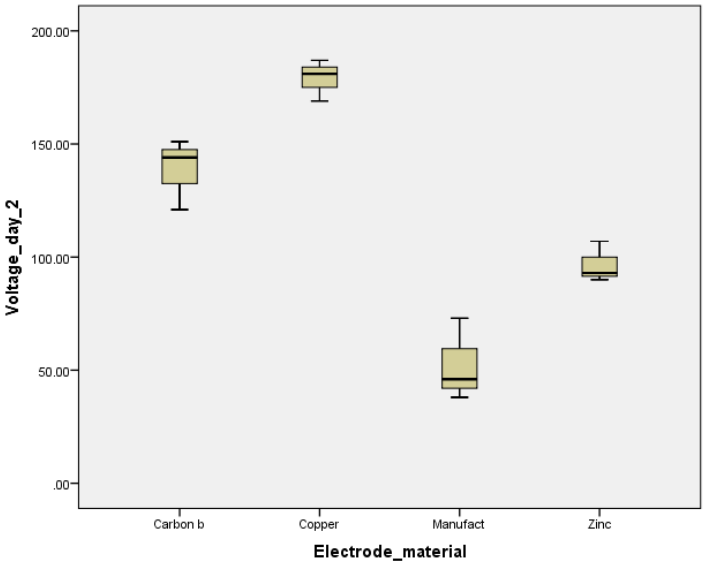
Voltage (mV)	KCl concentration	MFC number	Time (Day)						
			1	2	3	4	5	6	7
	1 M	1	431	459	429	397	382	363	348
		2	411	426	433	407	392	374	361
		3	483	453	511	503	463	451	443
		average	441.7	446	457.7	435.7	412.33	396	384
	2 M	4	353	335	323	311	318	305	298
		5	375	389	371	365	348	336	325
		6	329	305	280	241	207	185	167
		average	352.3	343	324.7	305.7	291	275.3	263
	3 M	7	261	271	257	244	226	199	189
		8	175	195	172	165	144	119	99
		9	227	205	201	191	171	166	151
		average	221	223.7	210	200	180.33	161.3	146

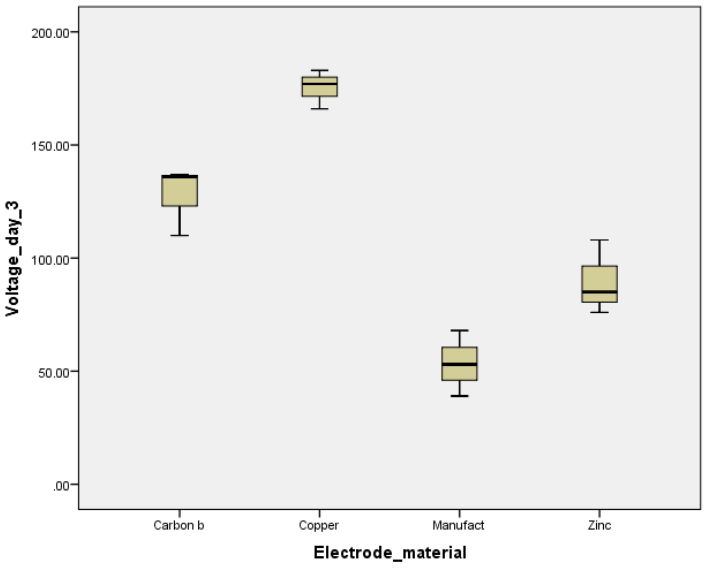
Appendix B: Statistical Analysis

- **Check for outliers of obtained data using SPSS through steam and leaf plots**

  - Effect of electrode material on MFC output voltage






200.00
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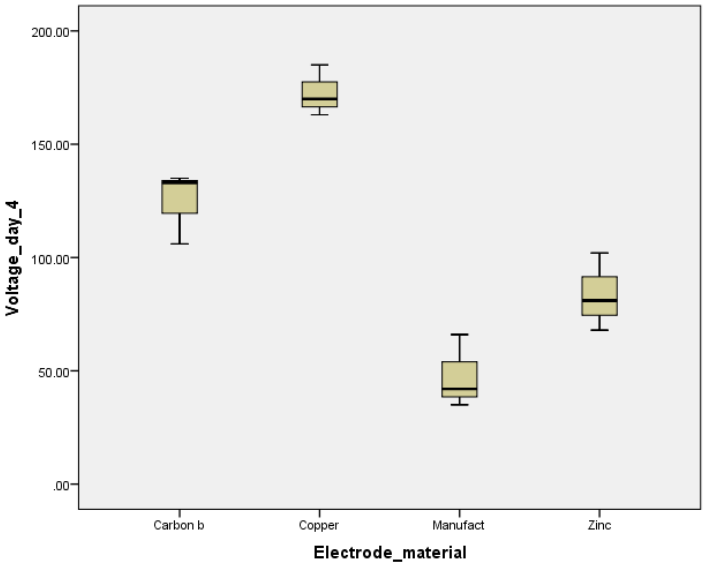
Carbon b
Copper
Manufact
Zinc
Electrode_material


200.00
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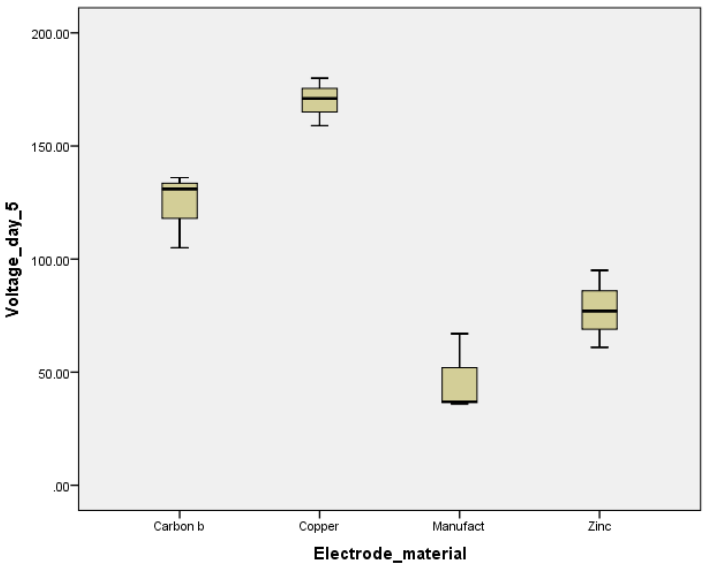
Voltage_day_4
Carbon b
Copper
Manufact
Zinc
Electrode_material


200.00
150.00
100.00
50.00
.00
Voltage_day_5
Carbon b
Copper
Manufact
Zinc
Electrode_material

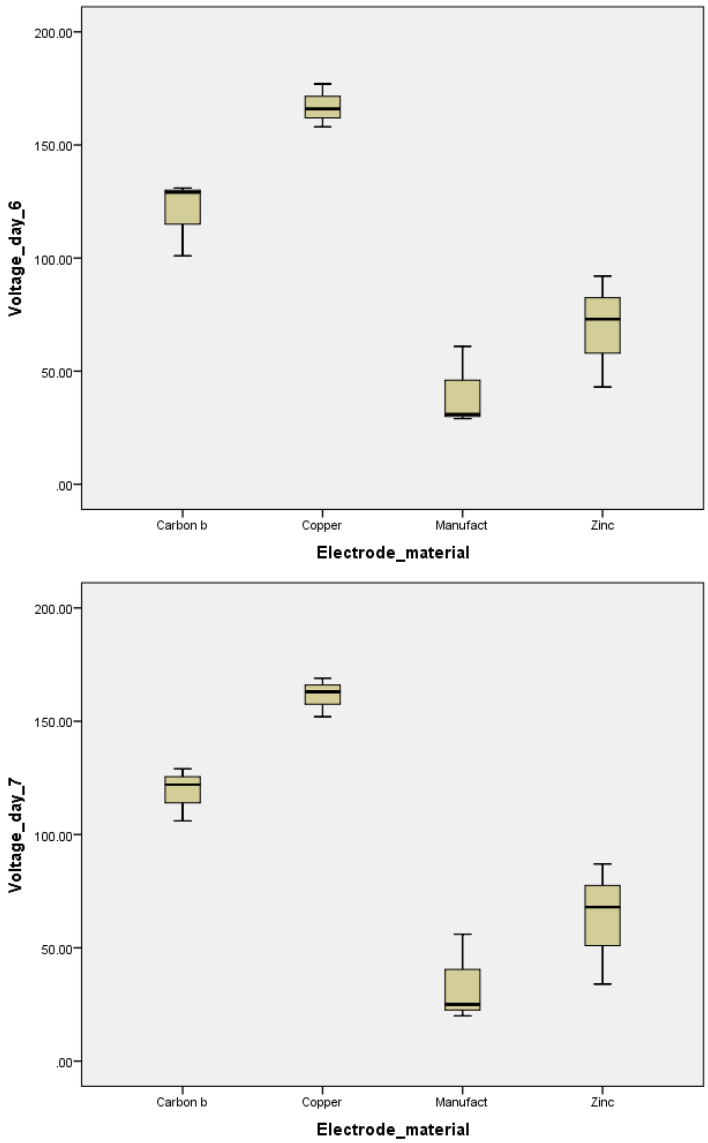
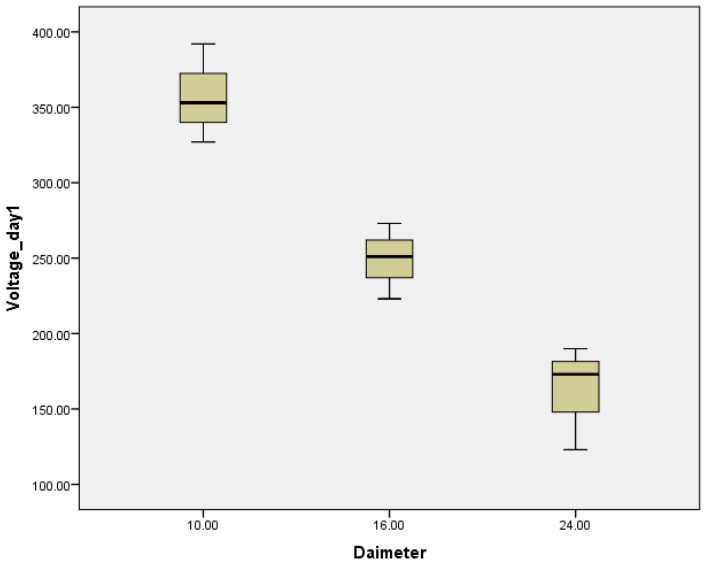
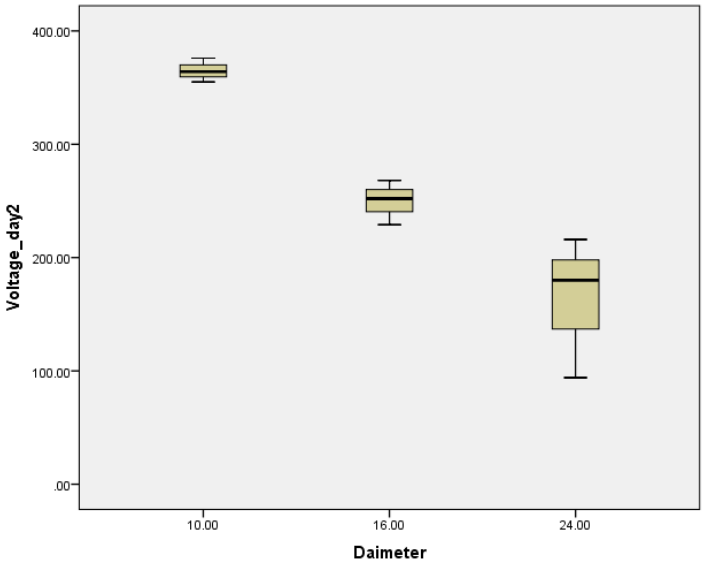
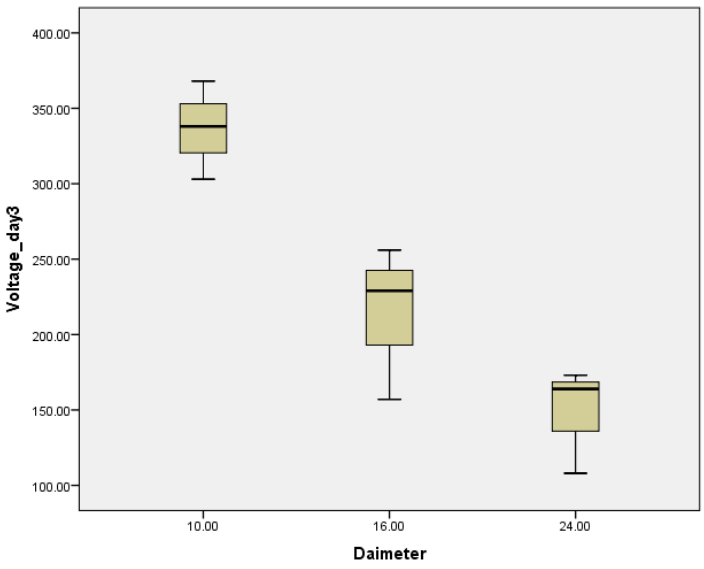


Figure B1 Steam and leaf plots for electrode materials experiment data to check for outliers using SPSS during the seven days of operation

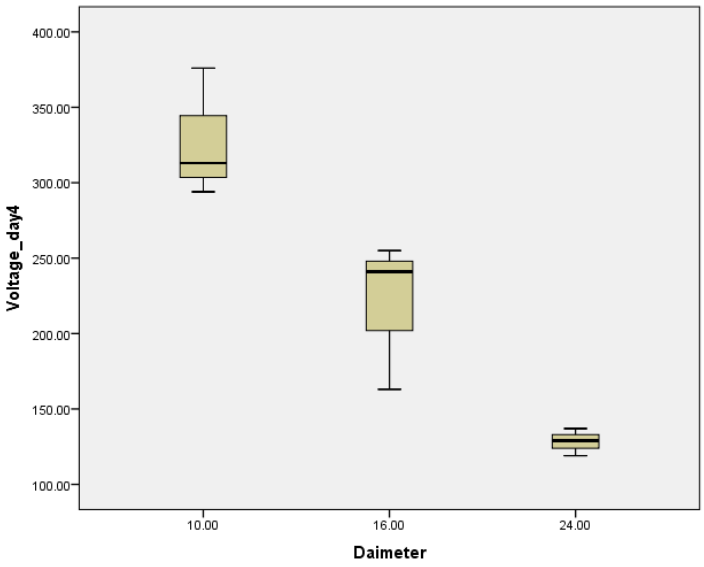
- Effect of salt bridge diameter on MFC output voltage:

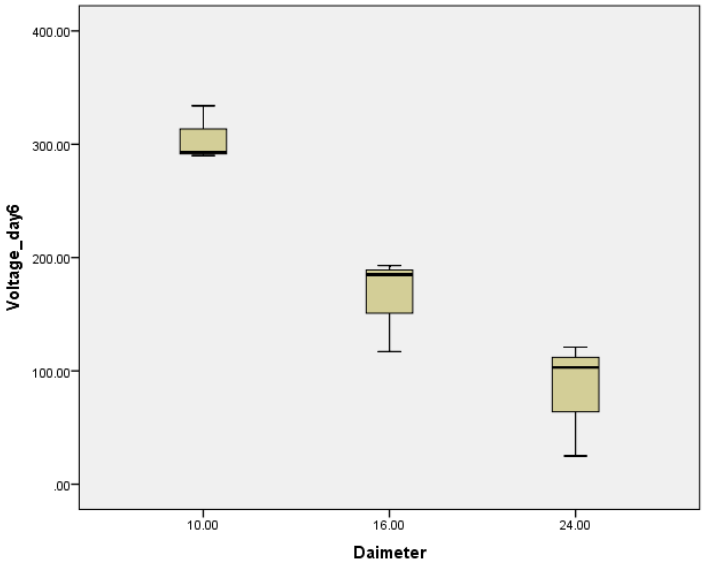

400.00
350.00
300.00
250.00
200.00
150.00
100.00
Voltage_day1
10.00
16.00
24.00
Daimeter


400.00
300.00
200.00
100.00
.00
Voltage_day2
10.00
16.00
24.00
Daimeter


400.00
350.00
300.00
250.00
200.00
150.00
100.00
Voltage_day3
10.00
16.00
24.00
Daimeter

-


400.00
350.00
300.00
250.00
200.00
150.00
100.00
Voltage_day4
10.00
16.00
24.00
Daimeter


400.00
300.00
200.00
100.00
.00
Voltage_day6
10.00
16.00
24.00
Daimeter

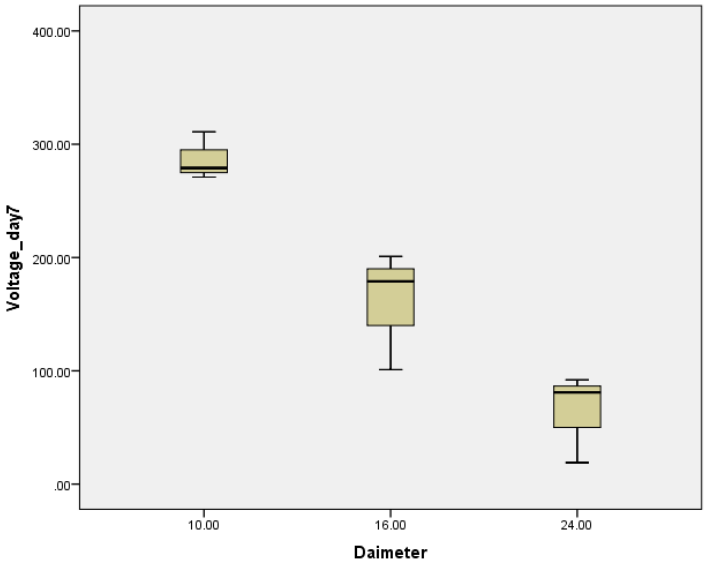
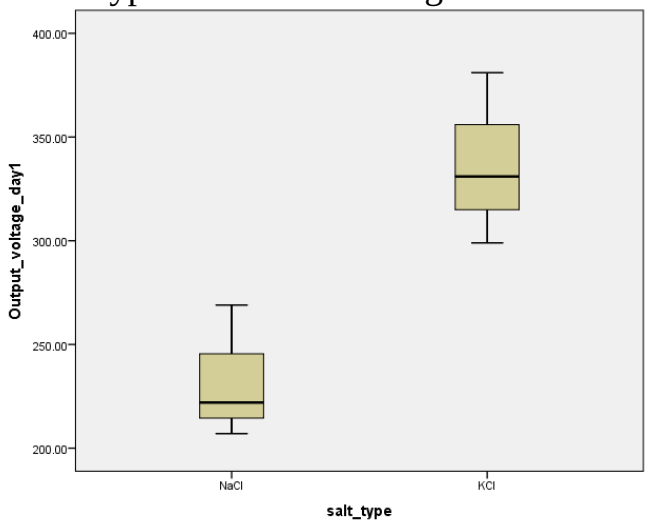
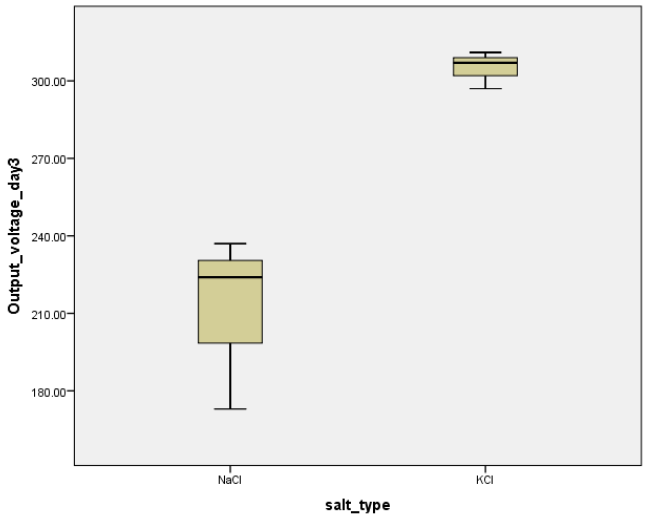
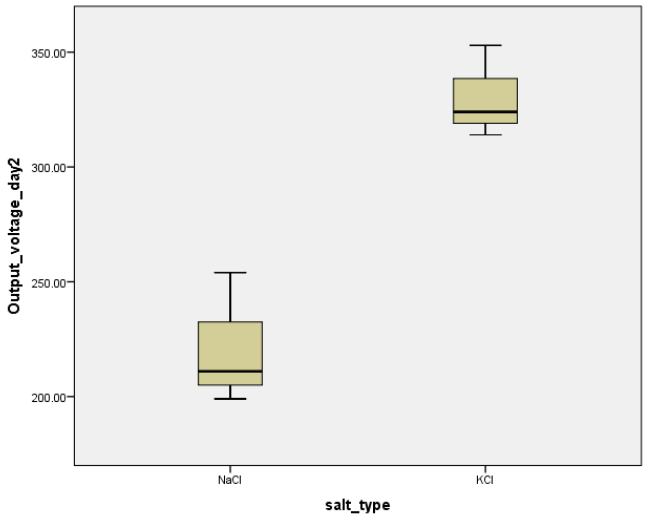

400.00
300.00
200.00
100.00
.00
Voltage_day7
10.00
16.00
24.00
Daimeter

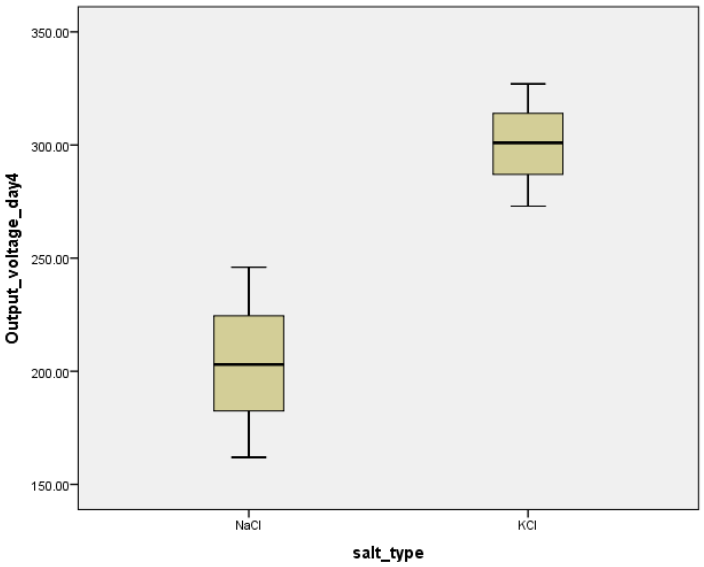
Figure B2 Steam and leaf plots for salt bridge diameter experiment data to check for outliers using SPSS during the seven days of operation

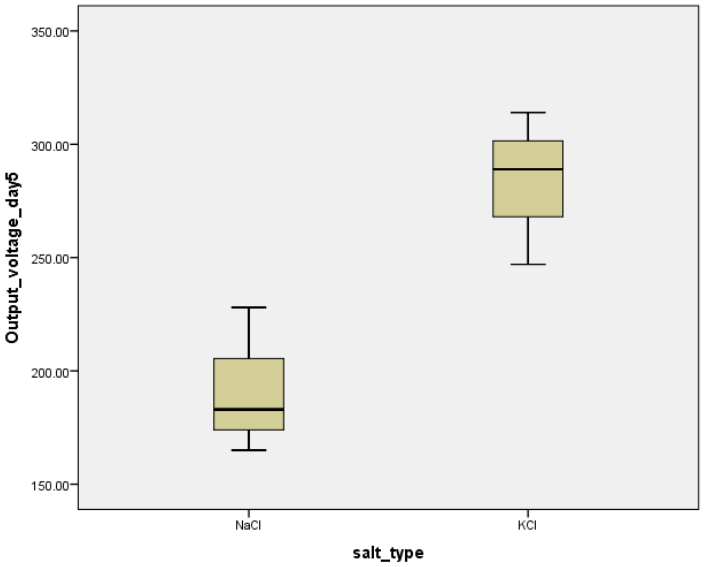
- Effect of salt types used in salt bridges on MFC output voltage:

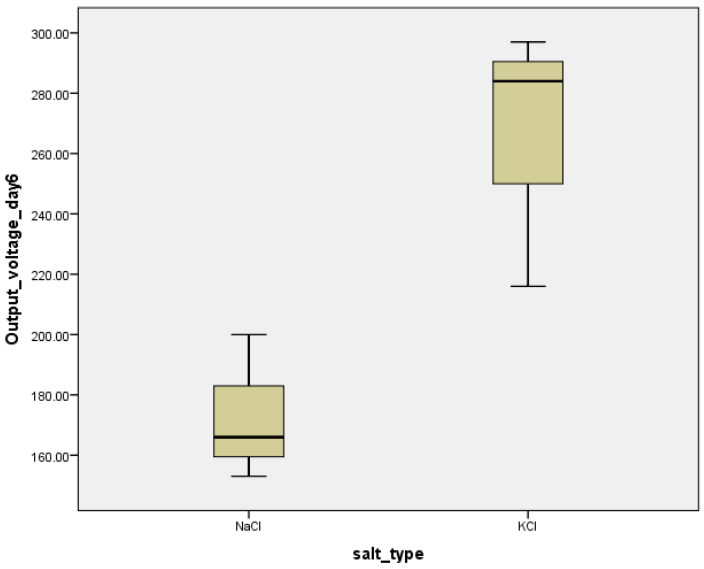







350.00
300.00
250.00
200.00
150.00
Output_voltage_day4
NaCl
KCl
salt_type


350.00
300.00
250.00
200.00
150.00
Output_voltage_day5
NaCl
KCl
salt_type


300.00
280.00
260.00
240.00
220.00
200.00
180.00
160.00
Output_voltage_day6
NaCl
KCl
salt_type

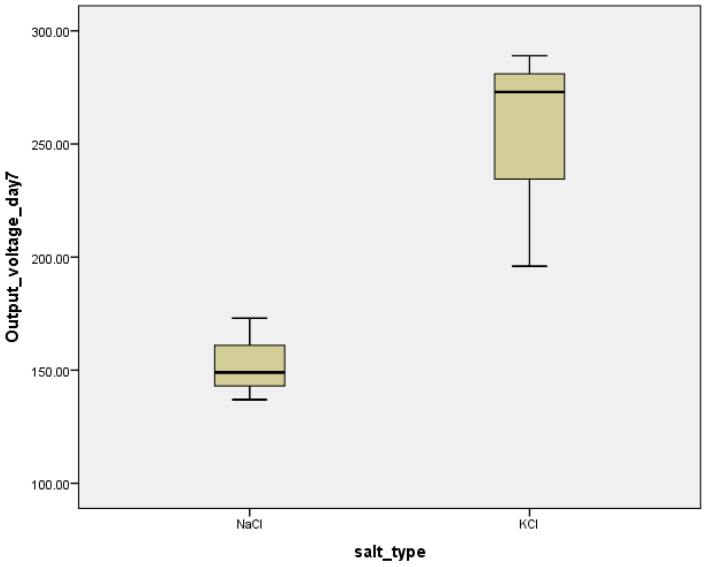
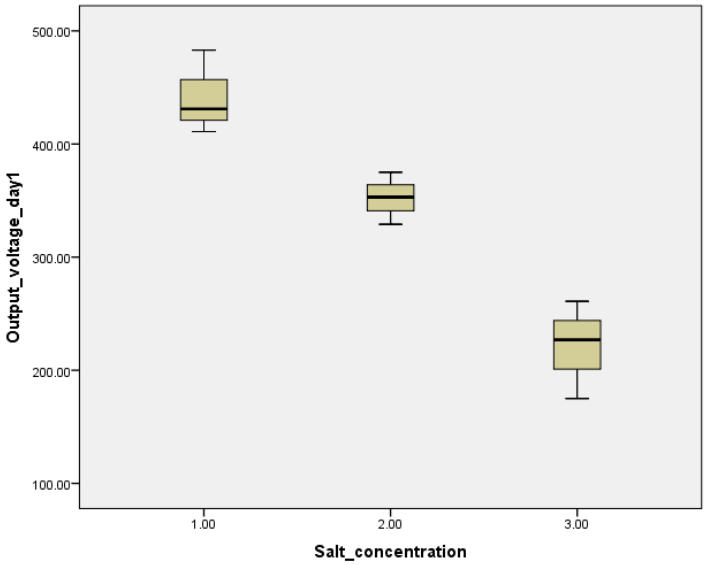
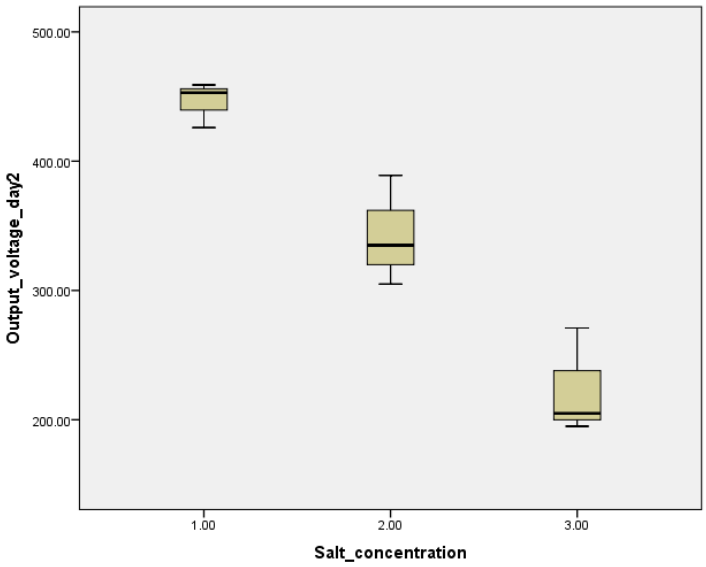
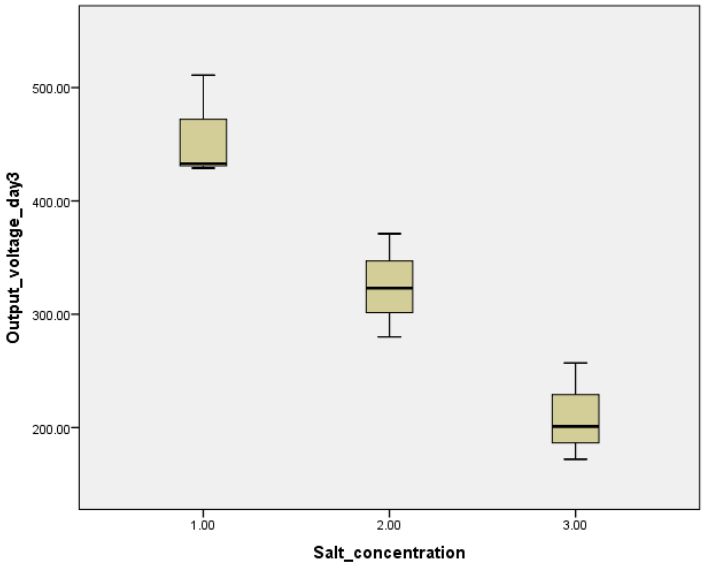


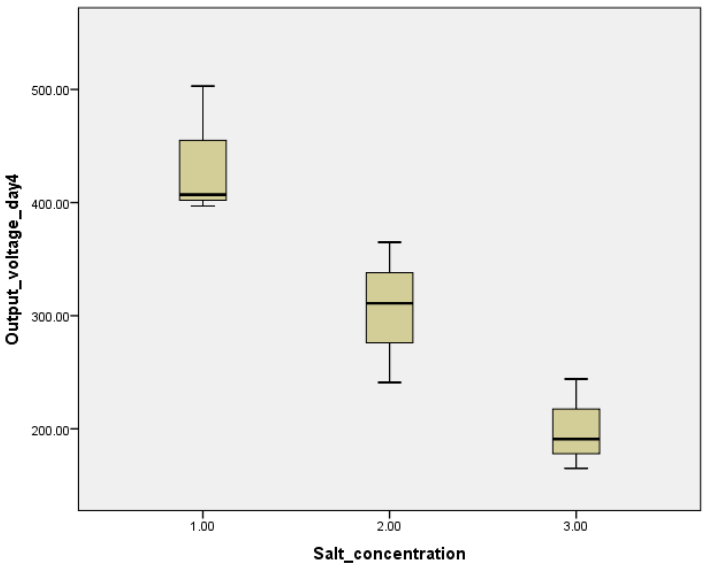
Figure B3 Steam and leaf plots for salt bridge type experiment data to check for outliers using SPSS during the seven days of operation

- Effect of salt solution concentration used in salt bridges on MFC output voltage:




500.00
400.00
300.00
200.00
Output_voltage_day2
1.00
2.00
3.00
Salt_concentration


500.00
400.00
300.00
200.00
Output_voltage_day3
1.00
2.00
3.00
Salt_concentration


500.00
400.00
300.00
200.00
Output_voltage_day4
1.00
2.00
3.00
Salt_concentration

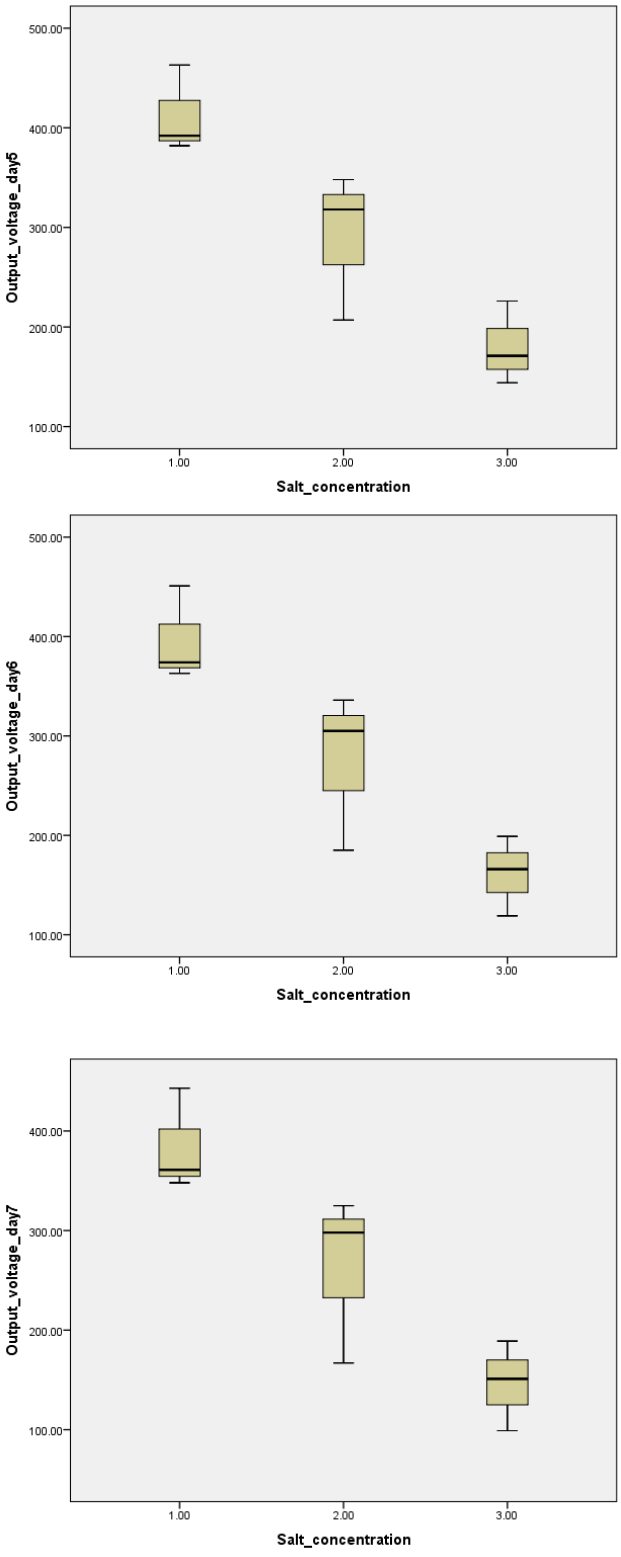


Figure B4 Steam and leaf plots for salt concentration used in salt bridges experiment data to check for outliers using SPSS during the seven days of operation

- **Check for normality and homogeneity of variances using Shapiro-Wilk test and Levene's test respectively on SPSS**

  - Electrode material effect on MFC output voltage

Table 3 Shapiro-Wilk test results on SPSS to check normality of the data obtained from electrode materials experiment

	Electrode_material	Shapiro-Wilk		
		Statistic	df	Sig.
Voltage_day_1	Carbon b	.918	3	.446
	Copper	.987	3	.780
	Manufact	.996	3	.878
	Zinc	.862	3	.274
Voltage_day_2	Carbon b	.913	3	.430
	Copper	.964	3	.637
	Manufact	.911	3	.420
	Zinc	.878	3	.317
Voltage_day_3	Carbon b	.778	3	.062
	Copper	.972	3	.679
	Manufact	1.000	3	.962
	Zinc	.940	3	.527
Voltage_day_4	Carbon b	.801	3	.118
	Copper	.958	3	.605
	Manufact	.909	3	.414
	Zinc	.982	3	.742
Voltage_day_5	Carbon b	.867	3	.288
	Copper	.993	3	.843
	Manufact	.774	3	.054
	Zinc	.999	3	.935
Voltage_day_6	Carbon b	.800	3	.114
	Copper	.992	3	.826
	Manufact	.797	3	.107
	Zinc	.983	3	.754
Voltage_day_7	Carbon b	.951	3	.576
	Copper	.972	3	.679
	Manufact	.852	3	.246
	Zinc	.974	3	.691

Table B2 Levene's test results from SPSS to check homogeneity of variances of electrode material experiment

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Voltage_day_1	.298	3	8	.826
Voltage_day_2	1.285	3	8	.344
Voltage_day_3	.569	3	8	.651
Voltage_day_4	.302	3	8	.824
Voltage_day_5	.478	3	8	.706
Voltage_day_6	1.015	3	8	.435
Voltage_day_7	1.696	3	8	.244

- Salt bridge diameter on MFC output voltage

Table B3 Shapiro-Wilk test results on SPSS to check normality of the data obtained from salt bridge diameter experiment

	Daimeter	Shapiro-Wilk		
		Statistic	df	Sig.
Voltage_day1	10.00	.987	3	.780
	16.00	.995	3	.868
	24.00	.925	3	.471
Voltage_day2	10.00	.993	3	.843
	16.00	.989	3	.803
	24.00	.947	3	.556
Voltage_day3	10.00	.998	3	.915
	16.00	.936	3	.510
	24.00	.852	3	.245
Voltage_day4	10.00	.912	3	.426
	16.00	.861	3	.271
	24.00	.996	3	.878
Voltage_day5	10.00	.982	3	.745

	16.00	.910	3	.418
	24.00	.941	3	.531
Voltage_day6	10.00	.801	3	.117
	16.00	.828	3	.183
	24.00	.885	3	.339
Voltage_day7	10.00	.893	3	.363
	16.00	.905	3	.403
	24.00	.860	3	.268

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Voltage_day1	.272	2	6	.771
Voltage_day2	4.575	2	6	.062
Voltage_day3	.638	2	6	.561
Voltage_day4	4.088	2	6	.076
Voltage_day5	.215	2	6	.813
Voltage_day6	1.427	2	6	.311
Voltage_day7	1.865	2	6	.234

- Salt solution type used in salt bridges effect on MFC output voltage

Table B4 Shapiro-Wilk test results on SPSS to check normality of the data obtained from salt solution type used in salt bridges experiment

Tests of Normality				
	salt_type	Shapiro-Wilk		
		Statistic	df	Sig.
Output_voltage_day1	NaCl	.918	3	.447
	KCl	.984	3	.759
Output_voltage_day2	NaCl	.904	3	.399
	KCl	.927	3	.476
Output_voltage_day3	NaCl	.895	3	.369
	KCl	.942	3	.537
Output_voltage_day4	NaCl	1.000	3	.974
	KCl	1.000	3	.959
Output_voltage_day5	NaCl	.942	3	.537
	KCl	.979	3	.722
Output_voltage_day6	NaCl	.938	3	.518
	KCl	.867	3	.286
Output_voltage_day7	NaCl	.964	3	.637
	KCl	.875	3	.309

Table B5 Levene's test results from SPSS to check homogeneity of variances of salt solution type used in salt bridges experiment

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Voltage_day1	.134	1	4	.733
Voltage_day2	.692	1	4	.452
Voltage_day3	7.049	1	4	.057
Voltage_day4	.375	1	4	.574
Voltage_day5	.000	1	4	.986
Voltage_day6	1.953	1	4	.235
Voltage_day7	4.471	1	4	.102

- Salt solution concentration used in salt bridges effect on MFC output voltage

Table B6 Shapiro-Wilk test results on SPSS to check normality of the data obtained from salt solution concentration used in salt bridges experiment

	Salt_concentration	Shapiro-Wilk		
		Statistic	df	Sig.
Output_voltage_day1	1.00	.938	3	.520
	2.00	.999	3	.952
	3.00	.986	3	.770
Output_voltage_day2	1.00	.881	3	.328
	2.00	.974	3	.688
	3.00	.847	3	.232
Output_voltage_day3	1.00	.786	3	.083
	2.00	.999	3	.939
	3.00	.967	3	.654
Output_voltage_day4	1.00	.820	3	.163
	2.00	.994	3	.858
	3.00	.963	3	.628
Output_voltage_day5	1.00	.841	3	.217
	2.00	.901	3	.388
	3.00	.963	3	.628
Output_voltage_day6	1.00	.842	3	.220
	2.00	.896	3	.374
	3.00	.990	3	.808
Output_voltage_day7	1.00	.850	3	.242
	2.00	.874	3	.306
	3.00	.992	3	.829

Table B7 Levene's test results from SPSS to check homogeneity of variances of electrode material experiment

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Voltage_day1	.656	2	6	.553
Voltage_day2	1.449	2	6	.307
Voltage_day3	.047	2	6	.955
Voltage_day4	.360	2	6	.712
Voltage_day5	1.234	2	6	.356
Voltage_day6	1.524	2	6	.292
Voltage_day7	1.428	2	6	.311

- **One way ANOVA test to check for significance differences and Tukey HSD test to locate where the significance difference is:**

  - Effect of electrode material on MFCs output:

Table B8 One-way ANOVA test results for data obtained from electrode materials experiment using SPSS

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Voltage_day1	Between Groups	24666.250	3	8222.083	46.365	.000
	Within Groups	1418.667	8	177.333		
	Total	26084.917	11			
Voltage_day2	Between Groups	26724.667	3	8908.222	47.574	.000
	Within Groups	1498.000	8	187.250		
	Total	28222.667	11			
Voltage_day3	Between Groups	24588.333	3	8196.111	41.429	.000
	Within Groups	1582.667	8	197.833		
	Total	26171.000	11			
Voltage_day4	Between Groups	26067.000	3	8689.000	36.688	.000
	Within Groups	1894.667	8	236.833		
	Total	27961.667	11			
Voltage_day5	Between Groups	26205.583	3	8735.194	35.377	.000
	Within Groups	1975.333	8	246.917		
	Total	28180.917	11			

Voltage_day6	Between Groups	28202.250	3	9400.750	28.837	.000
	Within Groups	2608.000	8	326.000		
	Total	30810.250	11			
Voltage_day7	Between Groups	29278.917	3	9759.639	29.695	.000
	Within Groups	2629.333	8	328.667		
	Total	31908.250	11			

Table B9 Tukey HSD test for the data obtained from electrode materials experiment using SPSS

Multiple Comparisons				
Tukey HSD				
Dependent Variable	(I) electrode_ material	(J) electrode_material	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day1	Copper	Zinc	.000*	49.5141
		Manufactured Carbon	.000*	87.5141
		Carbon brushes	.012*	11.5141
	Zinc	Copper	.000*	-119.1525
		Manufactured Carbon	.033*	3.1808
		Carbon brushes	.033*	-72.8192
	Manufactured Carbon	Copper	.000*	-157.1525
		Zinc	.033*	-72.8192
		Carbon brushes	.001*	-110.8192
	Carbon brushes	Copper	.012*	-81.1525
		Zinc	.033*	3.1808
		Manufactured Carbon	.001*	41.1808
Voltage_day2	Copper	Zinc	.000*	46.5538
		Manufactured Carbon	.000*	90.8872
		Carbon brushes	.028*	4.5538
	Zinc	Copper	.000*	-118.1128
		Manufactured Carbon	.017*	8.5538
		Carbon brushes	.023*	-77.7795
	Manufactured Carbon	Copper	.000*	-162.4462
		Zinc	.017*	-80.1128
		Carbon brushes	.000*	-122.1128
	Carbon brushes	Copper	.028*	-76.1128
		Zinc	.023*	6.2205
		Manufactured Carbon	.000*	50.5538
Voltage_day3	Copper	Zinc	.000*	48.8899
		Manufactured Carbon	.000*	85.2233

		Carbon brushes	.014*	10.8899
	Zinc	Copper	.000*	-122.4434
		Manufactured Carbon	.053	-.4434
		Carbon brushes	.043*	-74.7767
Multiple Comparisons				
Tukey HSD				
Dependent Variable	(I) electrode_ material	(J) electrode_material	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day3	Manufactured Carbon	Copper	.000*	-158.7767
		Zinc	.053*	-73.1101
		Carbon brushes	.001*	-111.1101
	Carbon brushes	Copper	.014*	-84.4434
		Zinc	.043*	1.2233
		Manufactured Carbon	.001*	37.5566
Voltage_day4	Copper	Zinc	.000*	48.7612
		Manufactured Carbon	.000*	84.7612
		Carbon brushes	.021*	7.7612
	Zinc	Copper	.000*	-129.2388
		Manufactured Carbon	.080*	-4.2388
		Carbon brushes	.046*	-81.2388
	Manufactured Carbon	Copper	.000*	-165.2388
		Zinc	.080*	-76.2388
		Carbon brushes	.001*	-117.2388
	Carbon brushes	Copper	.021*	-88.2388
		Zinc	.046*	.7612
		Manufactured Carbon	.001*	36.7612
Voltage_day5	Copper	Zinc	.000*	51.2469
		Manufactured Carbon	.000*	82.2469
		Carbon brushes	.029*	4.9135
	Zinc	Copper	.000*	-133.4198
		Manufactured Carbon	.151*	-10.0865
		Carbon brushes	.028*	-87.4198
	Manufactured Carbon	Copper	.000*	-164.4198
		Zinc	.151*	-72.0865
		Carbon brushes	.001*	-118.4198
	Carbon brushes	Copper	.029*	-87.0865
		Zinc	.028	5.2469
		Manufactured Carbon	.001*	36.2469
Multiple Comparisons				
Tukey HSD				

Dependent Variable	(I) electrode_ material	(J) electrode_material	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day6	Copper	Zinc	.001*	50.4569
		Manufactured Carbon	.000*	79.4569
		Carbon brushes	.053*	-.5431
	Zinc	Copper	.001*	-144.8765
		Manufactured Carbon	.275*	-18.2098
		Carbon brushes	.035*	-98.2098
	Manufactured Carbon	Copper	.000*	-173.8765
		Zinc	.275*	-76.2098
		Carbon brushes	.003*	-127.2098
	Carbon brushes	Copper	.053*	-93.8765
		Zinc	.035*	3.7902
		Manufactured Carbon	.003*	32.7902
Voltage_day7	Copper	Zinc	.001*	50.9308
		Manufactured Carbon	.000*	80.2642
		Carbon brushes	.081*	-5.0692
	Zinc	Copper	.001*	-145.7358
		Manufactured Carbon	.270*	-18.0692
		Carbon brushes	.022*	-103.4025
	Manufactured Carbon	Copper	.000*	-175.0692
		Zinc	.270*	-76.7358
		Carbon brushes	.002*	-132.7358
	Carbon brushes	Copper	.081*	-89.7358
		Zinc	.022*	8.5975
		Manufactured Carbon	.002*	37.9308

- Effect of salt bridge diameter on MFCs output:

Table B10 One-way ANOVA test results for data obtained from salt bridge diameter experiment using SPSS

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Voltage_day1	Between Groups	57460.222	2	28730.111	29.605	.001
	Within Groups	5822.667	6	970.444		
	Total	63282.889	8			
Voltage_day2	Between Groups	61424.667	2	30712.333	20.823	.002
	Within Groups	8849.333	6	1474.889		
	Total	70274.000	8			
Voltage_day3	Between Groups	54621.556	2	27310.778	16.661	.004
	Within Groups	9835.333	6	1639.222		
	Total	64456.889	8			
Voltage_day4	Between Groups	59739.556	2	29869.778	20.454	.002
	Within Groups	8762.000	6	1460.333		
	Total	68501.556	8			
Voltage_day5	Between Groups	68418.000	2	34209.000	15.592	.004
	Within Groups	13164.000	6	2194.000		
	Total	81582.000	8			
Voltage_day6	Between Groups	76091.556	2	38045.778	23.047	.002
	Within Groups	9904.667	6	1650.778		
	Total	85996.222	8			
Voltage_day7	Between Groups	75053.556	2	37526.778	23.660	.001
	Within Groups	9516.667	6	1586.111		
	Total	84570.222	8			

Table 4 Tukey HSD test for the data obtained from salt bridge diameter experiment using SPSS

Multiple Comparisons				
Tukey HSD				
Dependent Variable	(I) Salt_bridge_diameter	(J) Salt_bridge_diameter	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day1	10 mm	16 mm	.013*	30.2903
		24 mm	.001*	117.2903
	16 mm	10 mm	.013*	-186.3763
		24 mm	.033*	8.9570
	24 mm	10 mm	.001*	-273.3763
		16 mm	.033*	-165.0430
Voltage_day2	10 mm	16 mm	.024*	19.1216
		24 mm	.002*	105.4549
	16 mm	10 mm	.024*	-211.5451
		24 mm	.074	-9.8784
	24 mm	10 mm	.002*	-297.8784
		16 mm	.074	-182.5451
Voltage_day3	10 mm	16 mm	.024*	20.9031
		24 mm	.003*	86.5697
	16 mm	10 mm	.024*	-223.7636
		24 mm	.196	-35.7636
	24 mm	10 mm	.003*	-289.4303
		16 mm	.196	-167.0969
Voltage_day4	10 mm	16 mm	.031*	12.2642
		24 mm	.002*	103.5975
	16 mm	10 mm	.031*	-203.7358
		24 mm	.060	-4.4025
	24 mm	10 mm	.002*	-295.0692
		16 mm	.060	-187.0692
Voltage_day5	10 mm	16 mm	.046*	2.6544
		24 mm	.003*	95.6544
	16 mm	10 mm	.046*	-237.3456
		24 mm	.111	-24.3456
	24 mm	10 mm	.003*	-330.3456
		16 mm	.111	-210.3456
Multiple Comparisons				
Tukey HSD				

Dependent Variable	(I) Salt_bridge_diameter	(J) Salt_bridge_diameter	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day6	10 mm	16 mm	.013*	38.8795
		24 mm	.001*	120.8795
	16 mm	10 mm	.013*	-242.4538
		24 mm	.106*	-19.7871
	24 mm	10 mm	.001*	-324.4538
		16 mm	.106*	-183.7871
Voltage_day7	10 mm	16 mm	.019*	26.8931
		24 mm	.001*	123.2265
	16 mm	10 mm	.019*	-226.4402
		24 mm	.057	-3.4402
	24 mm	10 mm	.001*	-322.7735
		16 mm	.057	-196.1069

- Effect of salt solution type used in salt bridges on MFCs output:

Table 5 One-way ANOVA test results for data obtained from salt solution type used in salt bridge experiment using SPSS

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Voltage_day1	Between Groups	16328.167	1	16328.167	11.856	.026
	Within Groups	5508.667	4	1377.167		
	Total	21836.833	5			
Voltage_day2	Between Groups	17821.500	1	17821.500	28.591	.006
	Within Groups	2493.333	4	623.333		
	Total	20314.833	5			
Voltage_day3	Between Groups	13160.167	1	13160.167	22.001	.009
	Within Groups	2392.667	4	598.167		
	Total	15552.833	5			

Voltage_day4	Between Groups	14016.667	1	14016.667	11.242	.028
	Within Groups	4987.333	4	1246.833		
	Total	19004.000	5			
Voltage_day5	Between Groups	12512.667	1	12512.667	11.379	.028
	Within Groups	4398.667	4	1099.667		
	Total	16911.333	5			
Voltage_day6	Between Groups	12880.667	1	12880.667	10.382	.032
	Within Groups	4962.667	4	1240.667		
	Total	17843.333	5			
Voltage_day7	Between Groups	14900.167	1	14900.167	10.611	.031
	Within Groups	5616.667	4	1404.167		
	Total	20516.833	5			

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- Effect of salt solution concentration used in salt bridge on MFCs output:

Table 6 One-way ANOVA test results for data obtained from salt solution concentration used in salt bridges experiment using SPSS

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Voltage_day1	Between Groups	73922.667	2	36961.333	29.283	.001
	Within Groups	7573.333	6	1262.222		
	Total	81496.000	8			
Voltage_day2	Between Groups	74281.556	2	37140.778	29.120	.001
	Within Groups	7652.667	6	1275.444		
	Total	81934.222	8			
Voltage_day3	Between Groups	92176.222	2	46088.111	22.753	.002
	Within Groups	12153.333	6	2025.556		
	Total	104329.556	8			
Voltage_day4	Between Groups	83604.222	2	41802.111	14.072	.005

	Within Groups	17823.333	6	2970.556		
	Total	101427.556	8			
Voltage_day5	Between Groups	80792.889	2	40396.444	13.153	.006
	Within Groups	18427.333	6	3071.222		
	Total	99220.222	8			
Voltage_day6	Between Groups	82624.889	2	41312.444	12.061	.008
	Within Groups	20551.333	6	3425.222		
	Total	103176.222	8			
Voltage_day7	Between Groups	84734.889	2	42367.444	10.738	.010
	Within Groups	23673.333	6	3945.556		
	Total	108408.222	8			

Table 7 Tukey HSD test for the data obtained from salt solution concentration used in salt bridges experiment using SPSS

Dependent Variable	(I) Salt_bridge_diameter	(J) Salt_bridge_diameter	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day1	1M KCl-salt bridge	2M KCl-salt bridge	.049*	.3279
		3M KCl-salt bridge	.001*	131.6612
	2M KCl-salt bridge	1M KCl-salt bridge	.049*	-178.3388
		3M KCl-salt bridge	.009*	42.3279
	3M KCl-salt bridge	1M KCl-salt bridge	.001*	-309.6721
		2M KCl-salt bridge	.009*	-220.3388
Voltage_day2	1M KCl-salt bridge	2M KCl-salt bridge	.029*	13.5296
		3M KCl-salt bridge	.001*	132.8629
	2M KCl-salt bridge	1M KCl-salt bridge	.029*	-192.4704
		3M KCl-salt bridge	.015*	29.8629
	3M KCl-salt bridge	1M KCl-salt bridge	.001*	-311.8037
		2M KCl-salt bridge	.015*	-208.8037
Voltage_day3	1M KCl-salt bridge	2M KCl-salt bridge	.026*	20.2489
		3M KCl-salt bridge	.001*	134.9156
	2M KCl-salt bridge	1M KCl-salt bridge	.026*	-245.7511
		3M KCl-salt bridge	.047*	1.9156
	3M KCl-salt bridge	1M KCl-salt bridge	.001*	-360.4177
		2M KCl-salt bridge	.047*	-227.4177
Voltage_day4	1M KCl-salt bridge	2M KCl-salt bridge	.060	-6.5424
		3M KCl-salt bridge	.004*	99.1243
	2M KCl-salt bridge	1M KCl-salt bridge	.060	-266.5424
		3M KCl-salt bridge	.120	-30.8757

	3M KCl-salt bridge	1M KCl-salt bridge	.004*	-372.2090
		2M KCl-salt bridge	.120	-242.2090
Voltage_day5	1M KCl-salt bridge	2M KCl-salt bridge	.081	-17.5033
		3M KCl-salt bridge	.005*	93.1633
	2M KCl-salt bridge	1M KCl-salt bridge	.081	-260.1700
		3M KCl-salt bridge	.109	-28.1700
	3M KCl-salt bridge	1M KCl-salt bridge	.005*	-370.8367
		2M KCl-salt bridge	.109	-249.5033
Multiple Comparisons				
Tukey HSD				
Dependent Variable	(I) Salt_bridge_diameter	(J) Salt_bridge_diameter	Sig.	95% Confidence Interval
				Lower Bound
Voltage_day6	1M KCl-salt bridge	2M KCl-salt bridge	.099*	-25.9533
		3M KCl-salt bridge	.006*	88.0467
	2M KCl-salt bridge	1M KCl-salt bridge	.099*	-267.2866
		3M KCl-salt bridge	.118*	-32.6199
	3M KCl-salt bridge	1M KCl-salt bridge	.006*	-381.2866
		2M KCl-salt bridge	.118*	-260.6199
Voltage_day7	1M KCl-salt bridge	2M KCl-salt bridge	.123*	-36.6964
		3M KCl-salt bridge	.008*	80.3036
	2M KCl-salt bridge	1M KCl-salt bridge	.123*	-278.0297
		3M KCl-salt bridge	.135*	-40.3630
	3M KCl-salt bridge	1M KCl-salt bridge	.008*	-395.0297
		2M KCl-salt bridge	.135*	-274.3630

Appendix C: COD calculations

Table C1 Details of COD calculations for all measurements

Day	MFC number	Required F.A.S initial read.	Required F.A.S final read.	Volume of F.A.S	A-B	sample volume	dilution factor	COD (mg/L)
	Blank	0	2.8	2.8	-	-	-	-
	MFC 1	2.8	3.8	1	1.8	2.5	1	288
	MFC 2	3.8	4.75	0.95	1.85	2.5	1	296
	MFC 3	4.75	5.6	0.85	1.95	2.5	1	312
	MFC 4	5.6	6.75	1.15	1.65	1	0.4	660
	MFC 5	6.75	7.8	1.05	1.75	1	0.4	700
	MFC 6	7.8	8.9	1.1	1.7	1	0.4	680
	MFC 7	8.9	10.1	1.2	1.6	0.5	0.2	1280
	MFC 8	10.1	11.35	1.25	1.55	0.5	0.2	1240
	MFC 9	11.35	12.75	1.4	1.4	0.5	0.2	1120
	MFC 10	12.75	13.65	0.9	1.9	0.5	0.2	1520
	MFC 11	13.65	14.65	1	1.8	0.5	0.2	1440
0	MFC 12	14.65	15.6	0.95	1.85	0.5	0.2	1480
	Blank	0	2.65	2.65	-	-	-	-
	MFC 1	2.65	3.8	1.15	1.5	2.5	1	240
	MFC 2	3.8	4.8	1	1.65	2.5	1	264
	MFC 3	4.8	5.9	1.1	1.55	2.5	1	248
	MFC 4	5.9	7.1	1.2	1.45	1	0.4	580
	MFC 5	7.1	8.15	1.05	1.6	1	0.4	640
	MFC 6	8.15	9.4	1.25	1.4	1	0.4	560
	MFC 7	9.4	10.7	1.3	1.35	0.5	0.2	1080
	MFC 8	10.7	11.9	1.2	1.45	0.5	0.2	1160
	MFC 9	11.9	13.35	1.45	1.2	0.5	0.2	960
	MFC 10	13.35	14.45	1.1	1.55	0.5	0.2	1240
	MFC 11	14.45	15.4	0.95	1.7	0.5	0.2	1360
2	MFC 12	15.4	16.45	1.05	1.6	0.5	0.2	1280
	Blank	0	2.9	2.9	-		-	-
	MFC 1	2.9	4.8	1.9	1	2.5	1	160
	MFC 2	4.8	6.3	1.5	1.4	2.5	1	224
	MFC 3	6.3	8.1	1.8	1.1	2.5	1	176
	MFC 4	8.1	9.7	1.6	1.3	1	0.4	520
	MFC 5	9.7	11.5	1.8	1.1	1	0.4	440
	MFC 6	11.5	13.4	1.9	1	1	0.4	400
	MFC 7	13.4	15.05	1.65	1.25	0.5	0.2	1000
	MFC 8	15.05	16.75	1.7	1.2	0.5	0.2	960
4	MFC 9	16.75	18.7	1.95	0.95	0.5	0.2	760

	Day	MFC number	Required F.A.S	Volume of F.A.S	A-B	sample volume	dilution factor	COD (mg/L)
	MFC 10	18.7	20.05	1.35	1.55	0.5	0.2	1240
	MFC 11	20.05	21.5	1.45	1.45	0.5	0.2	1160
	MFC 12	21.5	23.1	1.6	1.3	0.5	0.2	1040
	Blank	0	2.6	2.6	-		-	-
	MFC 1	2.6	4.1	1.5	1.1	2.5	1	176
	MFC 2	4.1	5.5	1.4	1.2	2.5	1	192
	MFC 3	5.5	7.1	1.6	1	2.5	1	160
	MFC 4	7.1	8.6	1.5	1.1	1	0.4	440
	MFC 5	8.6	10	1.4	1.2	1	0.4	480
	MFC 6	10	11.65	1.65	0.95	1	0.4	380
	MFC 7	11.65	13.15	1.5	1.1	0.5	0.2	880
	MFC 8	13.15	14.7	1.55	1.05	0.5	0.2	840
	MFC 9	14.7	16.35	1.65	0.95	0.5	0.2	760
	MFC 10	16.35	17.55	1.2	1.4	0.5	0.2	1120
	MFC 11	17.55	18.95	1.4	1.2	0.5	0.2	960
6	MFC 12	18.95	20.1	1.15	1.45	0.5	0.2	1160
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	4.4	1.7	1	2.5	1	160
	MFC 2	4.4	6	1.6	1.1	2.5	1	176
	MFC 3	6	7.7	1.7	1	2.5	1	160
	MFC 4	7.7	9.4	1.7	1	1	0.4	400
	MFC 5	9.4	11	1.6	1.1	1	0.4	440
	MFC 6	11	12.8	1.8	0.9	1	0.4	360
	MFC 7	12.8	14.5	1.7	1	0.5	0.2	800
	MFC 8	14.5	16.3	1.8	0.9	0.5	0.2	720
	MFC 9	16.3	18.15	1.85	0.85	0.5	0.2	680
	MFC 10	18.15	19.5	1.35	1.35	0.5	0.2	1080
	MFC 11	19.5	21	1.5	1.2	0.5	0.2	960
8	MFC 12	21	22.5	1.5	1.2	0.5	0.2	960
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	4.5	1.8	0.9	2.5	1	144
	MFC 2	4.5	6.2	1.7	1	2.5	1	160
	MFC 3	6.2	8.1	1.9	0.8	2.5	1	128
	MFC 4	8.1	9.9	1.8	0.9	1	0.4	360
	MFC 5	9.9	11.9	2	0.7	1	0.4	280
	MFC 6	11.9	13.7	1.8	0.9	1	0.4	360
	MFC 7	13.7	15.45	1.75	0.95	0.5	0.2	760
	MFC 8	15.45	17.25	1.8	0.9	0.5	0.2	720
	MFC 9	17.25	19.1	1.85	0.85	0.5	0.2	680
10	MFC 10	19.1	20.65	1.55	1.15	0.5	0.2	920

	Day	MFC number	Required F.A.S	Volume of F.A.S	A-B	sample volume	dilution factor	COD (mg/L)
	MFC 11	20.65	22.4	1.75	0.95	0.5	0.2	760
	MFC 12	22.4	24.05	1.65	1.05	0.5	0.2	840
	Blank	0	2.8	2.8	-		-	-
	MFC 1	2.8	4.7	1.9	0.9	2.5	1	144
	MFC 2	4.7	6.3	1.6	1.2	2.5	1	192
	MFC 3	6.3	8.2	1.9	0.9	2.5	1	144
	MFC 4	8.2	10.1	1.9	0.9	1	0.4	360
	MFC 5	10.1	12.25	2.15	0.65	1	0.4	260
	MFC 6	12.25	13.5	1.25	1.55	1	0.4	620
	MFC 7	13.5	15.2	1.7	1.1	0.5	0.2	880
	MFC 8	15.2	17.1	1.9	0.9	0.5	0.2	720
	MFC 9	17.1	19.1	2	0.8	0.5	0.2	640
	MFC 10	19.1	20.8	1.7	1.1	0.5	0.2	880
	MFC 11	20.8	22.7	1.9	0.9	0.5	0.2	720
12	MFC 12	22.7	24.5	1.8	1	0.5	0.2	800
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	4.6	1.9	0.8	2.5	1	128
	MFC 2	4.6	6.3	1.7	1	2.5	1	160
	MFC 3	6.3	8.4	2.1	0.6	2.5	1	96
	MFC 4	8.4	10.3	1.9	0.8	1	0.4	320
	MFC 5	10.3	12.4	2.1	0.6	1	0.4	240
	MFC 6	12.4	14.2	1.8	0.9	1	0.4	360
	MFC 7	14.2	16.1	1.9	0.8	0.5	0.2	640
	MFC 8	16.1	18.1	2	0.7	0.5	0.2	560
	MFC 9	18.1	20.1	2	0.7	0.5	0.2	560
	MFC 10	20.1	21.8	1.7	1	0.5	0.2	800
	MFC 11	21.8	23.7	1.9	0.8	0.5	0.2	640
14	MFC 12	23.7	25.4	1.7	1	0.5	0.2	800
	Blank	0	2.9	2.9	-		-	-
	MFC 1	2.9	5	2.1	0.8	2.5	1	128
	MFC 2	5	7.25	2.25	0.65	2.5	1	104
	MFC 3	7.25	9.7	2.45	0.45	2.5	1	72
	MFC 4	9.7	10.7	1	1.9	2.5	1	304
	MFC 5	10.7	11.9	1.2	1.7	2.5	1	272
	MFC 6	11.9	13.1	1.2	1.7	2.5	1	272
	MFC 7	13.1	14.6	1.5	1.4	1	0.4	560
	MFC 8	14.6	16.3	1.7	1.2	1	0.4	480
	MFC 9	16.3	18.3	2	0.9	1	0.4	360
	MFC 10	18.3	20.25	1.95	0.95	0.5	0.2	760
16	MFC 11	20.25	22.5	2.25	0.65	0.5	0.2	520

	Day	MFC number	Required F.A.S	Volume of F.A.S	A-B	sample volume	dilution factor	COD (mg/L)
	MFC 12	22.5	24.6	2.1	0.8	0.5	0.2	640
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	4.6	1.9	0.8	2.5	1	128
	MFC 2	4.6	6.5	1.9	0.8	2.5	1	128
	MFC 3	6.5	8.7	2.2	0.5	2.5	1	80
	MFC 4	8.7	9.7	1	1.7	2.5	1	272
	MFC 5	9.7	11.2	1.5	1.2	2.5	1	192
	MFC 6	11.2	12.2	1	1.7	2.5	1	272
	MFC 7	12.2	13.5	1.3	1.4	1	0.4	560
	MFC 8	13.5	15.3	1.8	0.9	1	0.4	360
	MFC 9	15.3	17	1.7	1	1	0.4	400
	MFC 10	17	18.3	1.3	1.4	1	0.4	560
	MFC 11	18.3	19.3	1	1.7	1	0.4	680
18	MFC 12	19.3	20.2	0.9	1.8	1	0.4	720
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	4.6	1.9	0.8	2.5	1	128
	MFC 2	4.6	6.6	2	0.7	2.5	1	112
	MFC 3	6.6	8.35	1.75	0.95	2.5	1	152
	MFC 4	8.35	10	1.65	1.05	2.5	1	168
	MFC 5	10	11.2	1.2	1.5	2.5	1	240
	MFC 6	11.2	11.9	0.7	2	2.5	1	320
	MFC 7	11.9	13.2	1.3	1.4	1	0.4	560
	MFC 8	13.2	14.6	1.4	1.3	1	0.4	520
	MFC 9	14.6	16.2	1.6	1.1	1	0.4	440
	MFC 10	16.2	17.35	1.15	1.55	1	0.4	620
	MFC 11	17.35	18.2	0.85	1.85	1	0.4	740
20	MFC 12	18.2	19.6	1.4	1.3	1	0.4	520
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	4.75	2.05	0.65	2.5	1	104
	MFC 2	4.75	6.7	1.95	0.75	2.5	1	120
	MFC 3	6.7	8.1	1.4	1.3	2.5	1	208
	MFC 4	8.1	9.7	1.6	1.1	2.5	1	176
	MFC 5	9.7	11.6	1.9	0.8	2.5	1	128
	MFC 6	11.6	12.8	1.2	1.5	2.5	1	240
	MFC 7	12.8	14.3	1.5	1.2	1	0.4	480
	MFC 8	14.3	16.3	2	0.7	1	0.4	280
	MFC 9	16.3	18.3	2	0.7	1	0.4	280
	MFC 10	18.3	19.6	1.3	1.4	1	0.4	560
	MFC 11	19.6	21.2	1.6	1.1	1	0.4	440
22	MFC 12	21.2	22.7	1.5	1.2	1	0.4	480

	Day	MFC number	Required F.A.S	Volume of F.A.S	A-B	sample volume	dilution factor	COD (mg/L)
	Blank	0	2.6	2.6	-		-	-
	MFC 1	2.6	4.65	2.05	0.55	2.5	1	88
	MFC 2	4.65	6.7	2.05	0.55	2.5	1	88
	MFC 3	6.7	8.2	1.5	1.1	2.5	1	176
	MFC 4	8.2	10.2	2	0.6	2.5	1	96
	MFC 5	10.2	11.4	1.2	1.4	2.5	1	224
	MFC 6	11.4	12.6	1.2	1.4	2.5	1	224
	MFC 7	12.6	14.5	1.9	0.7	1	0.4	280
	MFC 8	14.5	16.1	1.6	1	1	0.4	400
	MFC 9	16.1	18	1.9	0.7	1	0.4	280
	MFC 10	18	19.2	1.2	1.4	1	0.4	560
	MFC 11	19.2	20.7	1.5	1.1	1	0.4	440
24	MFC 12	20.7	22.3	1.6	1	1	0.4	400
	Blank	0	2.8	2.8	-		-	-
	MFC 1	2.8	5.2	2.4	0.4	2.5	1	64
	MFC 2	5.2	7.6	2.4	0.4	2.5	1	64
	MFC 3	7.6	9.8	2.2	0.6	2.5	1	96
	MFC 4	9.8	11.6	1.8	1	2.5	1	160
	MFC 5	11.6	13.7	2.1	0.7	2.5	1	112
	MFC 6	13.7	16	2.3	0.5	2.5	1	80
	MFC 7	16	17.9	1.9	0.9	1	0.4	360
	MFC 8	17.9	20.1	2.2	0.6	1	0.4	240
	MFC 9	20.1	22.1	2	0.8	1	0.4	320
	MFC 10	22.1	23.6	1.5	1.3	1	0.4	520
	MFC 11	23.6	25.6	2	0.8	1	0.4	320
26	MFC 12	25.6	27.3	1.7	1.1	1	0.4	440
	Blank	0	2.7	2.7	-		-	-
	MFC 1	2.7	5.1	2.4	0.3	2.5	1	48
	MFC 2	5.1	7.4	2.3	0.4	2.5	1	64
	MFC 3	7.4	9.6	2.2	0.5	2.5	1	80
	MFC 4	9.6	11.5	1.9	0.8	2.5	1	128
	MFC 5	11.5	13.6	2.1	0.6	2.5	1	96
	MFC 6	13.6	15.8	2.2	0.5	2.5	1	80
	MFC 7	15.8	18	2.2	0.5	1	0.4	200
	MFC 8	18	20.3	2.3	0.4	1	0.4	160
	MFC 9	20.3	22.5	2.2	0.5	1	0.4	200
	MFC 10	22.5	24.6	2.1	0.6	1	0.4	240
	MFC 11	24.6	26.6	2	0.7	1	0.4	280
28	MFC 12	26.6	28.5	1.9	0.8	1	0.4	320
30	Blank	0	2.6	2.6	-		-	-

	Day	MFC number	Required F.A.S	Volume of F.A.S	A-B	sample volume	dilution factor	COD (mg/L)
	MFC 1	2.6	4.9	2.3	0.3	2.5	1	48
	MFC 2	4.9	7.1	2.2	0.4	2.5	1	64
	MFC 3	7.1	9.35	2.25	0.35	2.5	1	56
	MFC 4	9.35	11.35	2	0.6	2.5	1	96
	MFC 5	11.35	13.4	2.05	0.55	2.5	1	88
	MFC 6	13.4	15.5	2.1	0.5	2.5	1	80
	MFC 7	15.5	17.7	2.2	0.4	1	0.4	160
	MFC 8	17.7	19.8	2.1	0.5	1	0.4	200
	MFC 9	19.8	21.8	2	0.6	1	0.4	240
	MFC 10	21.8	23.6	1.8	0.8	1	0.4	320
	MFC 11	23.6	25.4	1.8	0.8	1	0.4	320
	MFC 12	25.4	27.3	1.9	0.7	1	0.4	280

Appendix D: COD and Output Voltage data for all MFCs

(Underlined COD values was found by averaging upper and lower values)

Table D1 COD and output voltage measurements for MFC 1, 2 and 3

MFCs (1,2,3), CODo=342 mg/L								
	COD (mg/L)				voltage (mV)			
Day	1.0	2.0	3.0	average	1.0	2.0	3.0	average
0.0	288.0	296.0	312.0	298.7	140.0	151.0	113.0	134.7
1.0	**264.0**	**280.0**	**280.0**	**274.7**	153.0	139.0	119.0	137.0
2.0	240.0	264.0	248.0	250.7	147.0	131.0	119.0	132.3
3.0	**200.0**	**244.0**	**212.0**	**218.7**	143.0	143.0	117.0	134.3
4.0	160.0	224.0	176.0	186.7	131.0	132.0	124.0	129.0
5.0	**168.0**	**208.0**	**168.0**	**181.3**	129.0	119.0	108.0	118.7
6.0	176.0	192.0	160.0	176.0	138.0	121.0	103.0	120.7
7.0	**168.0**	**184.0**	**160.0**	**170.7**	143.0	117.0	96.0	118.7
8.0	160.0	176.0	160.0	165.3	132.0	103.0	101.0	112.0
9.0	**152.0**	**168.0**	**144.0**	**154.7**	136.0	93.0	91.0	106.7
10.0	144.0	160.0	128.0	144.0	118.0	107.0	87.0	104.0
11.0	**144.0**	**176.0**	**136.0**	**152.0**	128.0	115.0	96.0	113.0
12.0	144.0	192.0	144.0	160.0	131.0	102.0	81.0	104.7
13.0	**136.0**	**176.0**	**120.0**	**144.0**	151.0	95.0	73.0	106.3
14.0	128.0	160.0	96.0	128.0	127.0	84.0	60.0	90.3
15.0	**128.0**	**132.0**	**84.0**	**114.7**	113.0	97.0	72.0	94.0
16.0	128.0	104.0	72.0	101.3	96.0	76.0	59.0	77.0
17.0	**128.0**	**116.0**	**76.0**	**106.7**	104.0	63.0	43.0	70.0
18.0	128.0	128.0	80.0	112.0	99.0	77.0	21.0	65.7
19.0	**128.0**	**120.0**	**116.0**	**121.3**	87.0	79.0	11.0	59.0
20.0	128.0	112.0	152.0	130.7	82.0	61.0	7.0	50.0
21.0	**116.0**	**116.0**	**180.0**	**137.3**	73.0	69.0	5.0	49.0
22.0	104.0	120.0	208.0	144.0	54.0	52.0	3.0	36.3
23.0	**96.0**	**104.0**	**192.0**	**130.7**	29.0	38.0	3.0	23.3
24.0	88.0	88.0	176.0	117.3	13.0	26.0	6.0	15.0
25.0	**76.0**	**76.0**	**136.0**	**96.0**	5.0	31.0	2.0	12.7
26.0	64.0	64.0	96.0	74.7	1.0	14.0	0.0	5.0
27.0	**56.0**	**64.0**	**88.0**	**69.3**	0.0	4.0	0.0	1.3
28.0	48.0	64.0	80.0	64.0	0.0	2.0	0.0	0.7
29.0	**48.0**	**64.0**	**65.0**	**59.0**	0.0	0.0	1.0	0.3
30.0	48.0	64.0	50.0	54.0	0.0	0.0	0.0	0.0

Table D2 COD and output voltage measurements for MFC 4, 5 and 6

MFCs (4,5,6), CODo=844 mg/L								
	COD (mg/L)				voltage (mV)			
Day	1	2	3	average	1	2	3	average
0	660	700	680	680	551	563	480	531.3333
1	**620**	**670**	**620**	**636.667**	521	510	449	493.3333
2	580	640	560	593.3333	489	471	419	459.6667
3	**550**	**540**	**480**	**523.333**	472	438	387	432.3333
4	520	440	400	453.3333	451	403	355	403
5	**480**	**460**	**390**	**443.333**	433	391	321	381.6667
6	440	480	380	433.3333	412	361	303	358.6667
7	**420**	**460**	**370**	**416.667**	383	320	283	328.6667
8	400	440	360	400	357	279	261	299
9	**380**	**360**	**360**	**366.667**	320	247	243	270
10	360	280	360	333.3333	281	218	227	242
11	**360**	**270**	**490**	**373.333**	255	185	219	219.6667
12	360	260	620	413.3333	211	181	211	201
13	**340**	**250**	**490**	**360**	215	193	228	212
14	320	240	360	306.6667	203	181	198	194
15	**312**	**256**	**316**	**294.667**	221	165	176	187.3333
16	304	272	272	282.6667	213	153	163	176.3333
17	**288**	**232**	**272**	**264**	198	168	147	171
18	272	192	272	245.3333	191	139	131	153.6667
19	**220**	**216**	**296**	**244**	197	142	139	159.3333
20	168	240	320	242.6667	181	134	156	157
21	**172**	**184**	**280**	**212**	172	127	132	143.6667
22	176	128	240	181.3333	179	117	118	138
23	**136**	**176**	**232**	**181.333**	165	108	107	126.6667
24	96	224	224	181.3333	157	129	110	132
25	**128**	**168**	**152**	**149.333**	143	109	99	117
26	160	112	80	117.3333	126	99	91	105.3333
27	**144**	**104**	**80**	**109.333**	109	93	85	95.66667
28	128	96	80	101.3333	88	81	89	86
29	**112**	**92**	**80**	**94.6667**	93	85	81	86.33333
30	96	88	80	88	90	79	76	81.66667

Table D3 COD and output voltage measurements for MFC 7, 8 and 9

MFCs (7,8,9), CODo=1437 mg/L								
	COD (mg/L)				voltage (mV)			
Day	1	2	3	average	1	2	3	average
0	1280	1240	1120	1213.333	671	651	647	656.3333
1	**1180**	**1200**	**1040**	**1140**	663	627	621	637
2	1080	1160	960	1066.667	651	621	631	634.3333
3	**1040**	**1060**	**860**	**986.667**	636	603	611	616.6667
4	1000	960	760	906.6667	619	587	598	601.3333
5	**940**	**900**	**760**	**866.667**	621	579	576	592
6	880	840	760	826.6667	601	583	556	580
7	**840**	**780**	**720**	**780**	596	574	541	570.3333
8	800	720	680	733.3333	581	559	564	568
9	**780**	**720**	**680**	**726.667**	583	551	543	559
10	760	720	680	720	571	543	536	550
11	**820**	**720**	**660**	**733.333**	558	550	522	543.3333
12	880	720	640	746.6667	531	533	508	524
13	**760**	**640**	**600**	**666.667**	510	511	517	512.6667
14	640	560	560	586.6667	476	482	509	489
15	**600**	**520**	**460**	**526.667**	451	463	481	465
16	560	480	360	466.6667	431	428	457	438.6667
17	**560**	**420**	**380**	**453.333**	422	437	429	429.3333
18	560	360	400	440	387	411	401	399.6667
19	**560**	**440**	**420**	**473.333**	356	394	387	379
20	560	520	440	506.6667	331	367	364	354
21	**520**	**400**	**360**	**426.667**	311	327	341	326.3333
22	480	280	280	346.6667	286	299	311	298.6667
23	**380**	**340**	**280**	**333.333**	265	273	291	276.3333
24	280	400	280	320	234	241	280	251.6667
25	**320**	**320**	**300**	**313.333**	213	220	263	232
26	360	240	320	306.6667	197	192	242	210.3333
27	**280**	**200**	**260**	**246.667**	172	161	213	182
28	200	160	200	186.6667	151	142	187	160
29	**180**	**180**	**220**	**193.333**	131	138	161	143.3333
30	160	200	240	200	111	119	149	126.3333

Table D4 COD and output voltage measurements for MFC 10, 11 and 12

MFCs (10,11,12), CODo=1733 mg/L								
	COD (mg/L)				voltage (mV)			
Day	1	2	3	average	1	2	3	average
0	1520	1440	1480	1480	691	682	679	684
1	**1380**	**1400**	**1380**	**1386.67**	673	659	662	664.6667
2	1240	1360	1280	1293.333	654	632	648	644.6667
3	**1240**	**1260**	**1160**	**1220**	629	614	635	626
4	1240	1160	1040	1146.667	607	598	621	608.6667
5	**1180**	**1060**	**1100**	**1113.33**	595	578	605	592.6667
6	1120	960	1160	1080	601	562	596	586.3333
7	**1100**	**960**	**1060**	**1040**	581	581	582	581.3333
8	1080	960	960	1000	573	563	594	576.6667
9	**1000**	**860**	**900**	**920**	568	551	581	566.6667
10	920	760	840	840	543	543	574	553.3333
11	**900**	**740**	**820**	**820**	532	522	559	537.6667
12	880	720	800	800	521	542	554	539
13	**840**	**680**	**800**	**773.333**	503	521	517	513.6667
14	800	640	800	746.6667	482	533	521	512
15	**780**	**580**	**720**	**693.333**	461	497	529	495.6667
16	760	520	640	640	439	483	497	473
17	**660**	**600**	**680**	**646.667**	413	442	464	439.6667
18	560	680	720	653.3333	387	411	432	410
19	**590**	**710**	**620**	**640**	366	381	419	388.6667
20	620	740	520	626.6667	332	379	397	369.3333
21	**590**	**590**	**500**	**560**	309	351	376	345.3333
22	560	440	480	493.3333	286	329	354	323
23	**560**	**440**	**440**	**480**	271	309	322	300.6667
24	560	440	400	466.6667	255	276	300	277
25	**540**	**380**	**420**	**446.667**	229	261	281	257
26	520	320	440	426.6667	204	243	259	235.3333
27	**380**	**300**	**380**	**353.333**	193	219	237	216.3333
28	240	280	320	280	211	203	201	205
29	**280**	**300**	**300**	**293.333**	182	208	192	194
30	320	320	280	306.6667	173	194	184	183.6667

Appendix E: Determining Kinetics Models for COD Removal

Table E1 Kt values for COD reduction in MFC1 to determine the kinetic model

		Kt value			
t(day)	COD(mg/L)	zero order	1st order	2nd order	3rd order
1	298.6666667	0.000000	0.000000	0.000000	0.000000
2	**274.6666667**	24.000000	0.083770	0.000293	0.000001
3	250.6666667	48.000000	0.175204	0.000641	0.000002
4	**218.6666667**	80.000000	0.311780	0.001225	0.000005
5	186.6666667	112.000000	0.470004	0.002009	0.000009
6	**181.3333333**	117.333333	0.498991	0.002166	0.000010
7	176	122.666667	0.528844	0.002334	0.000011
8	**170.6666667**	128.000000	0.559616	0.002511	0.000012
9	165.3333333	133.333333	0.591364	0.002700	0.000013
10	**154.6666667**	144.000000	0.658056	0.003117	0.000015
11	144	154.666667	0.729515	0.003596	0.000019
12	**152**	146.666667	0.675448	0.003231	0.000016
13	160	138.666667	0.624154	0.002902	0.000014
14	**144**	154.666667	0.729515	0.003596	0.000019
15	128	170.666667	0.847298	0.004464	0.000025
16	**114.6666667**	184.000000	0.957299	0.005373	0.000032
17	101.3333333	197.333333	1.080913	0.006520	0.000043
18	**106.6666667**	192.000000	1.029619	0.006027	0.000038
19	112	186.666667	0.980829	0.005580	0.000034
20	**121.3333333**	177.333333	0.900787	0.004894	0.000028
21	130.6666667	168.000000	0.826679	0.004305	0.000024
22	**137.3333333**	161.333333	0.776917	0.003933	0.000021
23	144	154.666667	0.729515	0.003596	0.000019
24	**130.6666667**	168.000000	0.826679	0.004305	0.000024
25	117.3333333	181.333333	0.934309	0.005175	0.000031

26	**96**	202.666667	1.134980	0.007068	0.000049
27	74.66666667	224.000000	1.386294	0.010045	0.000084
28	**69.33333333**	229.333333	1.460402	0.011075	0.000098
29	64	234.666667	1.540445	0.012277	0.000116
30	**59**	239.666667	1.621791	0.013601	0.000138
31	54	244.666667	1.710344	0.015170	0.000166

Table E2 Kt values for COD reduction in MFC2 to determine the kinetic model

		Kt value			
t(day)	COD(mg/L)	zero order	1st order	2nd order	3rd order
0	680	0.000000	0.000000	0.000000	0.000000
1	**636.6666667**	43.333333	0.065847	0.000100	0.000000
2	593.3333333	86.666667	0.136336	0.000215	0.000000
3	**523.3333333**	156.666667	0.261874	0.000440	0.000001
4	453.3333333	226.666667	0.405465	0.000735	0.000001
5	**443.3333333**	236.666667	0.427771	0.000785	0.000001
6	433.3333333	246.666667	0.450586	0.000837	0.000002
7	**416.6666667**	263.333333	0.489806	0.000929	0.000002
8	400	280.000000	0.530628	0.001029	0.000002
9	**366.6666667**	313.333333	0.617640	0.001257	0.000003
10	333.3333333	346.666667	0.712950	0.001529	0.000003
11	**373.3333333**	306.666667	0.599621	0.001208	0.000003
12	413.3333333	266.666667	0.497838	0.000949	0.000002
13	**360**	320.000000	0.635989	0.001307	0.000003
14	306.6666667	373.333333	0.796331	0.001790	0.000004
15	**294.6666667**	385.333333	0.836248	0.001923	0.000005
16	282.6666667	397.333333	0.877824	0.002067	0.000005
17	**264**	416.000000	0.946144	0.002317	0.000006
18	245.3333333	434.666667	1.019475	0.002605	0.000007

19	**244**	436.000000	1.024925	0.002628	0.000007
20	242.6666667	437.333333	1.030404	0.002650	0.000007
21	**212**	468.000000	1.165507	0.003246	0.000010
22	181.3333333	498.666667	1.321756	0.004044	0.000014
23	**181.3333333**	498.666667	1.321756	0.004044	0.000014
24	181.3333333	498.666667	1.321756	0.004044	0.000014
25	**149.3333333**	530.666667	1.515912	0.005226	0.000021
26	117.3333333	562.666667	1.757074	0.007052	0.000035
27	**109.3333333**	570.666667	1.827691	0.007676	0.000041
28	101.3333333	578.666667	1.903677	0.008398	0.000048
29	**94.66666667**	585.333333	1.971731	0.009093	0.000055
30	88	592.000000	2.044756	0.009893	0.000063

Table E3 Kt values for COD reduction in MFC3 to determine the kinetic model

		Kt value			
t(day)	COD(mg/L)	zero order	1st order	2nd order	3rd order
0	1213.333333	0.000000	0.000000	0.000000	0.000000
1	**1140**	73.333333	0.062343	0.000053	0.000000
2	1066.666667	146.666667	0.128833	0.000113	0.000000
3	**986.6666667**	226.666667	0.206794	0.000189	0.000000
4	906.6666667	306.666667	0.291352	0.000279	0.000000
5	**866.6666667**	346.666667	0.336472	0.000330	0.000000
6	826.6666667	386.666667	0.383725	0.000386	0.000000
7	**780**	433.333333	0.441833	0.000458	0.000000
8	733.3333333	480.000000	0.503526	0.000539	0.000001
9	**726.6666667**	486.666667	0.512659	0.000552	0.000001
10	720	493.333333	0.521875	0.000565	0.000001
11	**733.3333333**	480.000000	0.503526	0.000539	0.000001
12	746.6666667	466.666667	0.485508	0.000515	0.000001

13	**666.6666667**	546.666667	0.598837	0.000676	0.000001
14	586.6666667	626.666667	0.726670	0.000880	0.000001
15	**526.6666667**	686.666667	0.834559	0.001075	0.000001
16	466.6666667	746.666667	0.955511	0.001319	0.000002
17	**453.3333333**	760.000000	0.984499	0.001382	0.000002
18	440	773.333333	1.014352	0.001449	0.000002
19	**473.3333333**	740.000000	0.941327	0.001289	0.000002
20	506.6666667	706.666667	0.873273	0.001150	0.000002
21	**426.6666667**	786.666667	1.045124	0.001520	0.000002
22	346.6666667	866.666667	1.252763	0.002060	0.000004
23	**333.3333333**	880.000000	1.291984	0.002176	0.000004
24	320	893.333333	1.332806	0.002301	0.000005
25	**313.3333333**	900.000000	1.353859	0.002367	0.000005
26	306.6666667	906.666667	1.375365	0.002437	0.000005
27	**246.6666667**	966.666667	1.593089	0.003230	0.000008
28	186.6666667	1026.666667	1.871802	0.004533	0.000014
29	**193.3333333**	1020.000000	1.836711	0.004348	0.000013
30	200	1013.333333	1.802809	0.004176	0.000012

Table E4 Kt values for COD reduction in MFC4 to determine the kinetic model

		Kt value			
t(day)	COD(mg/L)	zero order	1st order	2nd order	3rd order
0	1480	0.000000	0.000000	0.000000	0.000000
1	1386.666667	93.333333	0.065139	0.000045	0.000000
2	1293.333333	186.666667	0.134819	0.000098	0.000000
3	1220	260.000000	0.193191	0.000144	0.000000
4	1146.666667	333.333333	0.255183	0.000196	0.000000
5	1113.333333	366.666667	0.284684	0.000223	0.000000
6	1080	400.000000	0.315081	0.000250	0.000000
7	1040	440.000000	0.352821	0.000286	0.000000

8	1000	480.000000	0.392042	0.000324	0.000000
9	920	560.000000	0.475424	0.000411	0.000000
10	840	640.000000	0.566395	0.000515	0.000000
11	820	660.000000	0.590493	0.000544	0.000001
12	800	680.000000	0.615186	0.000574	0.000001
13	773.3333333	706.666667	0.649087	0.000617	0.000001
14	746.6666667	733.333333	0.684179	0.000664	0.000001
15	693.3333333	786.666667	0.758286	0.000767	0.000001
16	640	840.000000	0.838329	0.000887	0.000001
17	646.6666667	833.333333	0.827966	0.000871	0.000001
18	653.3333333	826.666667	0.817710	0.000855	0.000001
19	640	840.000000	0.838329	0.000887	0.000001
20	626.6666667	853.333333	0.859383	0.000920	0.000001
21	560	920.000000	0.971861	0.001110	0.000001
22	493.3333333	986.666667	1.098612	0.001351	0.000002
23	480	1000.000000	1.126011	0.001408	0.000002
24	466.6666667	1013.333333	1.154182	0.001467	0.000002
25	446.6666667	1033.333333	1.197985	0.001563	0.000002
26	426.6666667	1053.333333	1.243794	0.001668	0.000003
27	353.3333333	1126.666667	1.432385	0.002155	0.000004
28	280	1200.000000	1.665008	0.002896	0.000006
29	293.3333333	1186.666667	1.618488	0.002733	0.000006
30	306.6666667	1173.333333	1.574036	0.002585	0.000005

جامعة النجاح الوطنية
كلية الدراسات العليا

جدوى توليد الطاقة المتجددة من معالجة المياه العادمة باستخدام خلايا الوقود الحيوية: الضفة الغربية كحالة دراسة

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في هندسة المياه والبيئة بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس – فلسطين.
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الملخص

خلايا الوقود الحيوية هي تكنولوجيا ناشئة، من خلال هذه التكنولوجيا يمكن هضم المواد العضوية لاهوائيا بالتزامن مع انتاج الطاقة الكهربائية مباشرة. يوجد عدة تطبيقات لخلايا الوقود الحيوية، منها: تحلية المياه، المجسات الحيوية بالاضافة الى معالجة المياه العادمة. خلال هذه الدراسة سيتم بحث استخدام خلايا الوقود الحيوية في معالجة المياه العادمة ولأول مرة في فلسطين.

فلسطين هي دولة نامية تعاني من عدم توفر أنظمة ملائمة لتجميع ومعالجة المياه العادمة بالاضافة الى نقص المياه والذي بدوره يؤدي الى زيادة تركيز المواد العضوية في المياه العادمة المنتجة.

في هذه الدراسة سيتم استخدام خلايا الوقود الحيوية ثنائية الحجرات وباستخدام المياه العادمة الخارجة من حوض الترسيب الأولي كمصدر للمواد العضوية في هذه الخلايا، واستخدام القنطرة الملحية كوسط ناقل للبروتونات بالاضافة الى استخدام الماء المشبع بالأكسجين المذاب كمحلول الحجرة السالبة.

يتكون هذه البحث من جزئين رئيسين: الأول بحث تأثير عدة عوامل على كفاءة خلايا الوقود الحيوية في انتاج الفولتية الكهربائية، وهذه العوامل هي: نوع مادة القطب المستخدم، قطر القنطرة الملحية المستخدمة، نوع الملح المستخدم في القنطرة الملحية و تركيز الملح المستخدم في القنطرة الملحية. وقد تم تنفيذ هذا الجزء من خلال بناء و تشغيل خلايا وقود حيوية ثنائية الحجرات ليتم بحث تأثير كل عامل على حدى مع تثبيت

العوامل الأخرى. و لقد تم إستخدام ثلاث خلايا متكررة لكل متغير للحصول على نتائج موثوقة. وقد تم قياس فرق الجهد للدارة الكهربائية المفتوحة. بحيث تم أخذ قراءات فرق الجهد لكل خلية كل يوم بمعدل قراءة واحدة يومية و لمدة أسبوع. ومن ثم تم تحليل البيانات الناتجة لإيجاد الظروف المثلى لتشغيل خلايا الوقود الحيوي.

أما الجزء الثاني فهو عبارة عن محاولة لفهم ونمذجة العلاقة بين استهلاك الأكسجين الكيميائي كمؤشر على المواد العضوية الموجودة و الفولتية المخرجة من خلايا الوقود الحيوية. بالإضافة لمحاولة فهم النموذج الحركي لتحلل المواد العضوية الموجودة في المياه العادمة. في هذا الجزء من الدراسة، تم تجهيز 4 خلايا وقود حيوية بحيث تختلف فيما بينها بتركيز المواد العضوية (استهلاك الأكسجين الكيميائي). وقد تم تحضير 3 خلايا متكررة لكل تركيز. وقد تم حصر و تثبيت معظم العوامل التي قد تؤثر على كفاءة التحلل. قبل البدء بهذه التجربة لقد تم تجهيز الخلايا وحقنها بالمياه العادمة واخضاعها لنفس ظروف التجربة و لمدة 15 يوما قبل البدء بالتجربة و ذلك من أجل تجهيز وتنشيط الكائنات الدقيقة الموجودة فيها. لقد تم إجراء فحص التحلل الكيميائي للاكسجين لكل خلية من الاثني عشر خلية بمعدل مرة واحدة كل يومين، أما قياس فرق الجهد لكل خلية فقد تم اجرائه بشكل يومي وبمعدل مرة واحدة يوميا.

التحليل الاحصائي للبيانات الناتجة عن التجارب، أثبتت أن أقطاب النحاس أكثر فعالية من أقطاب فرش الكربون التي بدورها أكثر فعالية من أقطاب الزنك. قطر القنطرة الملحية يؤثر ايضا على الفولتية الناتجة، فقد تبين أن القنطرة الملحية ذات قطر 10 مم هي أعلى كفاءة من القنطرة ذات قطر 16 و 24 مم. وقد وجد أيضا أن استخدام ملح كلوريد البوتاسيوم في القنطرة الملحية يولد فولتية كهربائية أعلى من حالة استخدام ملح كلوريد الصوديوم. الفولتية الكهربائية الناتجة تتأثر أيضا بتركيز الملح في القنطرة الملحية، حيث ان استخدام ملح كلوريد البوتاسيوم بتركيز 1 مول\التر أكثر كفاءة من استخدام تركيز 2 و 3 مول\التر.

من مخرجات هذه الدراسة أيضا إيجاد أن قيمة الاستهلاك الكيميائي للأكسجين لعينة المياه العادمة عند أي زمن تتناسب مع الفولتية الناتجة عن خلية الوقود الحيوية في نفس الزمن. وقد تم ايجاد أن هذه العلاقة هي علاقة لوغاريتم طبيعي كما يلي:

كمية الاستهلالك الكيميائي للأكسجين (ملجالتر)= 229.85 لو$_{هـ}$(ج) – 1039.6

حيث أن ج هي الفولتية الكهربائية الناتجة من خلية الوقود الحيوية، ويمكن استخدام هذا النموذج تبعا لمحددات كثير لتقدير قيمة الاستهلاك الكيميائي للأكسجين لعينة المياه العادمة من خلال قياس الفولتية الكهربائية الناتجة عن خلية وقود حيوية يتم تشغيلها باستخدام هذه العينة.

القيمة القصوى لنسبة ازالة تلوث الاستهلاك الكيميائي للأكسجين التي تم الوصول اليها في هذه الدراسة هي 87.1 % وهي قيمة قابلة للمقارنة مع النتائج المنشورة السابقة. جدير بالذكر أن سلوك قيمة الاستهلاك الكيميائي للأكسجين في التجارب المشغولة يتبع النموذج الحركي من الدرجة الأولى والثانية. القيمة العظمى للقدرة الكهربائية التي تم الحصول عليها هي 0.585 واطام^3 اما معدل انتاج القدرة الكهربائية في جميع الخلايا هو 0.251 واطام^3.