WASTEWATER SLUDGE IN BIOELECTRICITY GENERATION USING
MICROBIAL FUEL CELLS

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ABSTRACT

WASTEWATER SLUDGE IN BIOELECTRICITY GENERATION USING MICROBIAL FUEL CELLS

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Today, the majority of world's energy is provided by fossil fuels which soon will be exploited. This pressure forces countries to investigate renewable energy technologies like microbial fuel cells (MFCs). MFCs can convert chemical energy in organic matter into electricity via microbial activity. The objective of this study was to investigate the operational parameters and interactions within the MFCs to overcome the challenges that limit their full scale applications.

This study started with optimization experiments by testing the type of materials. However, the power density (PD) values (~5.5 mW/m^2) showed that performance depends on microbial degradation and electron transfer. Then, the system was redesigned considering electron losses, membrane fouling and internal resistance (IR). The maximum PD was calculated as 464 mW/m^2.

Following optimization experiments, the system was fed with different sludges (municipal, poultry, beverage, petrochemical and textile) to test the suitability of MFCs. The PD decreased to 205.93 mW/m^2 with municipal sludge, since the sludge contains numerous ions, increasing IR. Applying 10 and 20 min sonication increased PD to 225.23 mW/m^2 and 281.54 mW/m^2, respectively. PD dropped to 45.88 mW/m^2

when poultry sludge was fed. Beverage sludge, on the other, increased the PD to 360.91 mW/m^2, while PD was calculated as 21.17 mW/m^2 and 31.44 mW/m^2 for petrochemical and textile sludge, respectively.

One final objective was to operate a laboratory scale anaerobic digester to compare the performance of two competing bioenergy technologies. MFCs did not require additional heating even if the output was comparably small with more rapid organic conversion.

Keywords: Anaerobic digestion, bioenergy, microbial fuel cell, power density, sewage sludge

ÖZ

MİKROBİYAL YAKIT HÜCRELERİNİ KULLANARAK ATIKSU ÇAMURU İLE BİYOELEKTRİK ÜRETİMİ

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Günümüzde, dünyanın büyük bir çoğunluğu yakın gelecekte tükenmesi beklenen fosil yakıtları enerji kaynağı olarak kullanmaktadır. Bu baskı, ülkeleri mikrobiyal yakıt hücreleri (MYH) gibi yenilenebilir enerji teknolojilerini araştırmaya itmektedir. MYH'ler organik maddenin içerisindeki kimyasal enerjiyi mikrobiyal aktivite ile elektriğe dönüştürebilmektedirler. Bu çalışmanın amacı, MYH'lerin gerçek ölçekli kullanımını engelleyen zorlukların üstesinden gelmek için işletim parametrelerini ve sistem içerisindeki etkileşimleri araştırmaktır.

Bu çalışma, kullanılan malzemelerin test edildiği optimizasyon deneyleri ile başlamıştır. Ancak, elde edilen güç yoğunluğu (GY) değerleri (~5.5 mW/m^2), performansın daha çok mikrobiyal parçalanma ve elektron transferine bağlı olduğunu göstermiştir. Bu nedenle, sistem, elektron kayıplarını, membran tıkanmasını ve iç direnci (İD) göz önüne alarak yeniden tasarlanmıştır. En yüksek GY değeri 464 mW/m^2 olarak hesaplanmıştır.

Optimizasyon deneylerini takiben, sistem farklı çamurlar (kentsel, kümes hayvanları, içecek, petrokimya ve tekstil tesisleri) ile beslenerek, MYH'lerin değişik arıtma sistemlerine uygunluğu test edilmiştir. Kentsel çamurda GY değeri 205.93 mW/m^2

değerine düşmüş ve bunun sebebi de çamurun içeriğindeki sayısız iyonlara bağlı artan İD'tir. 10 ve 20 dk sonikasyon uygulandığında ise GY 225.23 mW/m^2 ve 281 mW/m^2 değerlerine yükselmiştir. Kümes hayvanları tesisinden alınan çamurla beslenen sistemde ise GY 45.88 mW/m^2'ye düşmüştür. Petrokimya ve tekstil çamurlarında elde edilen GY 21.17 mW/m^2 ve 31.44 mW/m^2 iken, içecek çamurunda GY değeri 360.91 mW/m^2'ye kadar yükselmiştir.

Bu çalışmanın en son hedefi ise laboratuar ölçekli bir anaerobik çürütücü işleterek, bu iki rakip biyoenerji teknolojisini karşılaştırmaktır. MYH'ler, enerji çıktıları karşılaştırılabilir ölçüde düşük olmasına rağmen ek ısıtmaya ihtiyaç duymamaktadırlar, ayrıca, organik dönüşümü MYH'lerde daha hızlı gerçekleşmektedir.

Anahtar Kelimeler: Anaerobik çürütme, arıtma çamuru, biyoenerji, güç yoğunluğu, mikrobiyal yakıt hücresi

To my family…

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LIST OF ABBREVIATIONS

ADS   : Anaerobically Digested Sludge

AEM   : Anion Exchange Membrane

AFB   : Anaerobic Fluidized Bed

BOD   : Biological Oxygen Demand

CD   : Current Density

CE   : Coulombic Efficiency

CEM   : Cation Exchange Membrane

COD   : Chemical Oxygen Demand

DET   : Direct Electron Transfer

DWW   : Domestic Wastewater

F/M   : Food to Microorganism Ratio

GHG   : Greenhouse Gas

HFC   : Hydrogen Fuel Cell

HRT   : Hydraulic Retention Time

IWW   : Industrial Wastewater

IR   : Internal Resistance

LCC   : Layered Corrugated Carbon

MET   : Mediated Electron Transfer

MFC   : Microbial Fuel Cell

MWW   : Municipal Wastewater

OCV   : Open Circuit Voltage

PBS   : Phosphate Buffer Solution

PD   : Power Density

PEM   : Proton Exchange Membrane

sCOD   : Soluble Chemical Oxygen Demand

SRT   : Solids Retention Time

SWW   : Synthetic Wastewater

TCD   : Thermal Conductivity Detector

TS   : Total Solids

TSS   : Total Suspended Solids

VFA   : Volatile Fatty Acid

VS   : Volatile Solids

VSS   : Volatile Suspended Solids

WAS   : Waste Activated Sludge

WWTP   : Wastewater Treatment Plant

CHAPTER I

INTRODUCTION

Today, the majority of the energy demand of the community is being met by petroleum, coal and natural gas (Du et.al., 2007). Currently, to meet the energy demand of the growing population, power plants are in the edge of exploiting these non-renewable resources. This unfortunate practice will soon create a pressure on the energy market and not only affect the electricity in our houses but also bring the global economy to a point of no return since the production will be directly affected. Besides, power plants utilizing coal especially, are creating atmospheric pollution, threatening both environment and human health (Zhou et.al., 2014). All these drawbacks are now forcing governments to investigate renewable and sustainable energy resources like wind, solar or biomass.

Although wind and solar power are more popular on the outside, while some people call it waste, biomass resources contain a great treasure that would solve two problems: waste disposal and energy bottleneck. Bioenergy techniques convert the energy inside the waste (agricultural, municipal, etc.), wastewater and sludge into heat, electricity and fuel, and can reduce greenhouse gas (GHG) emissions significantly. Anaerobic digestion and microbial fuel cells (MFCs) are the two most popular bioenergy technologies that are being the center of many research today. MFCs can generate electricity from the chemical energy present in the bonds of organic substrate via microbial utilization (Logan et.al., 2006; Ömeroğlu and Sanin, 2016). Producing energy using biomass actually been known for almost a century, in fact, the first MFC was operated by M.C. Potter in 1910 which was then improved by Barnet Cohen in 1931 (Cohen, 1931; Potter, 1911). But neither of these studies drew attention until the last energy crisis in 1980s. The basic design that is still being used

today was developed back then by M.J. Allen and H.P. Bennetto (Rajalakshmi and Dhathathreyan, 2008). Nowadays, there is an increasing attention towards the topic to use MFCs as a renewable energy alternative.

MFCs can utilize different materials as fuel and luckily this list includes wastewater and sludge. Conventional wastewater treatment plants (WWTPs) and sludge stabilization processes require high amounts of energy which makes the whole process very expensive. On the other hand, wastewater contains a hidden energy that is almost nine times higher than the energy required to treat itself (i.e. aeration, thermal processes, dewatering, etc.) (Abourached et.al., 2014). Even better, at the end of biological treatment, when wastewater is settled, this energy is concentrated in sludge. Considering these, MFC seems to be a sustainable and useful form of bioenergy technology to compensate the overall energy demand of a WWTP (Ting and Lee, 2007). But even if, MFC is a promising alternative, the full scale application is still limited and anaerobic digesters seem to dominate the market (Du et.al., 2007; Ömeroğlu and Sanin 2016).

To overcome the problems associated with the operation of an MFC, first the working principle of the system must be well understood. Regardless of the configuration, the working principle is theoretically common. The fuel (wastewater/sludge) is fed to anode chamber where microorganisms degrade it into electrons (e^-) and protons (H^+). The electrons travel within the anolyte solution to anode electrode and then to cathode electrode to generate current while protons pass through the membrane separating the chamber, to meet the final electron acceptor which is usually oxygen (Du et.al., 2007; Ömeroğlu and Sanin, 2016). For this transfer to happen, anode chamber must be completely anaerobic to prevent the interaction of electrons with any sort of electron acceptors such as SO_4 and NO_3.

The performance of an MFC depends on many parameters: (i) reactor configuration, (ii) membrane type (fouling and internal resistance-IR), (iii) anode and cathode electrodes (electron transfer and current), (iv) substrate (electron source), (v) pH, (vi) temperature, (vii) type of microorganism (degrade and carry electrons) and (viii)

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mediator. Each of these parameters has an important role during the operation of an MFC since the bioelectrochemical interactions within the system are directly affected. Especially, in terms of electron transfer, electrodes-microorganisms-mediator work in group to liberate and transfer the electrons. If the electrons are not properly transferred to anode electrode, then current flow and therefore, energy production decreases. For this reason, sometimes external agents become necessary such as mediators. These are synthetic or naturally produced molecules that help the transfer of electrons to anode electrode if the microorganisms are not anodophilic (cannot conduct electrons due to their non-conductive lipid membranes) (Du et.al., 2007; Rabaey and Verstraete, 2005). In this case, mediators transfer the electrons (capture the electron-transfer to anode surface-reduce itself-capture another electron to transfer) and improve the energy conversion efficiency.

MFCs embody many advantages compared to the rest of the bioenergy technologies. For starters, they have very low carbon emissions unlike fossil fuel based power plants and their energy conversion efficiencies are not limited to Carnot cycle since there is no combustion process involved (Rabaey and Verstraete, 2005). The chemical energy is directly converted to electricity to minimize the losses. They can utilize any substrate type including the ones with inhibitory content like pulp and paper (Chaudhuri and Lovley, 2003; Mathuriya and Sharma, 2009). But they are still limited in application, even in pilot scale, due to their high initial and operational costs due to the materials used like membrane (Nafion 117), metal electrodes and synthetic mediators. That's why, it is necessary to test different materials and select the cheapest one while achieving maximum energy output. However, an optimization experiment solely based on materials will not be successful considering the fact that MFC operations rely on both biological and electrochemical reactions which could be easily affected by any change in environmental circumstances. Therefore, microbial inoculum, electron transfer and bioelectrochemical interactions must be also very well described for the specific system being operated.

Considering these, this study aims to optimize the operational conditions and start-up materials of an MFC to maximize energy production while minimizing the overall

operational cost of the system. To achieve this, a dual chamber MFC was constructed since it is easier to control compared to single chamber air-cathode systems. The optimization experiments were divided into two for better monitoring of system performance with changing parameters. During the first set of experiments (preliminary studies) only the materials making up the physical MFC were tested and this included electrodes, Pt catalyst concentration and wire connecting the electrodes. Considering the corrosive constituents in wastewater and sludge, especially with industrial origin, electrodes must be resistant both to minimize the cost and sustain high power output. In this study, different carbon based materials (low cost) were investigated and optimum one was selected. However, producing the maximum power in an MFC does not only depend on using the best material, if it was so, then the problem with MFC operation would be easily solved so far. For this reason, during the second stage of optimization experiments, operational parameters such as reactor insulation to minimize electron loss, concentration and organic content of carbon source, membrane surface properties and microbial inoculum were studied in detail. The main idea behind this set of optimization experiments was to optimize the conditions to liberate electrons (microbial degradation) and carry them with minimum loss (insulation and membrane fouling) to maximize current flow.

Once the optimization studies were completed, the second and the main objective of this study was investigated, the impact of different sources of wastewater sludges on energy production potential of an MFC using mixed microbial cultures. Even though the use of different substrates to feed MFCs is one of the most important advantages, depending on the constituents sometimes the energy production may be enhanced, sometimes inhibited. That's why; type of substrate is one of the operational parameters affecting the amount of electricity generated that must be studied in detail. In this study, MFCs were fed with wastewater and sludge samples taken from (i) municipal WWTP, (ii) beverage industry WWTP, (iii) petrochemical industry WWTP, (iv) poultry industry WWTP and (v) textile industry WWTP. Samples taken from the municipal WWTP served as baseline since it neither contained high concentration of organics nor inhibitory substances. Different from the rest, the

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municipal sludge was also subjected to ultrasonication to see the impact of pretreatment on power generation. Textile and petrochemical sludge contains numerous heavy metals and long chain hydrocarbons that resist degradation. Feeding an MFC with these samples helped us to see if inhibition took place or MFCs were able to generate electricity and resist toxicity. Beverage and poultry sludges were rich in organic content and were expected to enhance the electricity generation. The electricity generation was monitored by current/voltage production and the results showed the impact of substrate constituents on power output.

One final and minor objective of this study was the comparison of MFC with anaerobic digestion. Since, today, MFCs cannot reach the same potential as the chemical fuel cells, they are expected to coexist with anaerobic digestion technology in WWTPs. Methane produced via anaerobic digestion of sewage sludge, especially sludges with high organic content, has considerable energy content. To see the difference, the energy potential of the samples from the municipal WWTP (baseline) was anaerobically digested and the energy yields of both systems were compared.

This study will certainly fill the gap in the literature on MFC optimization because so far majority of the studies have focused on the materials making up an MFC rather than the circumstances enhancing electron liberation (microbial degradation) and transfer. The findings reported in the literature relied on similar electrodes and membranes with little information on microbial activity, biodegradation and electron transfer unlike this study. Besides, few studies have demonstrated substrate comparison and this might be the only one that focused on MFCs fed with various sludges to compare both the constituents and pretreatment. This provides a diverse comparison of substrate effect on MFC performance. In addition to this, there is lack of evidence in the previous work done on MFCs regarding their battle with dominant bioenergy technologies such as anaerobic digestion. The final part of this study provided valuable information on the energy balance, stabilization extent and many other advantages/disadvantages of both systems for a healthy comparison.

CHAPTER II

LITERATURE REVIEW

2.1.Wastewater-Sludge-Energy Nexus

The demand for energy has grown tremendously within the last decade due to the massive increase in population and industrialization (Birol, 2007; Mercuri et.al., 2016). Currently, the energy resources being utilized in power plants are petroleum, coal and natural gas, which are scarce and non-renewable (Du et.al., 2007; Logan, 2009). The practices exploiting these resources has accelerated within the last couple of decades, triggering a global energy crisis (Du et.al., 2007). In addition to limited energy resources, current energy practices rely on the combustion of fossil fuels which then release carbon, sulfur and nitric oxide compounds into the atmosphere resulting in GHG emission and atmospheric pollution (Zhou et.al., 2014). Considering the exploitation of resources together with the environmental adverse impacts of the power plants operated today, it is mandatory that the existing energy system should become renewable and sustainable to overcome the energy insecurity and climate change challenges (Bocci et.al., 2014).

Today, biomass resources are becoming popular as "green" energy resources and many different types of wastes and wastewater/sludge (i.e. industrial, urban, domestic, etc.) are being accepted as suitable substrates for bioenergy production (Octave and Thomas, 2009). Although waste and wastewater have been regarded as unwanted materials so far, now they are considered as misplaced resource containing valuable products and energy (Gao et.al., 2014). Wastewater treatment is a must to protect the environment and water resources that will be used as potable water

(Gude, 2016). Unfortunately, WWTPs are chemical and energy intensive, resulting in high operational and maintenance costs (Sustarsic, 2009). The aeration in a WWTP can count up to 75% and 60% of the total energy requirements in a WWTP, respectively (Gude, 2016).

The wastewater and sludge have high organic content, which can yield an energy almost 9 times higher than the amount required for their treatment and 60% of this energy is concentrated in sludge (Abourached et.al., 2014; Ting and Lee, 2007). Therefore, this hidden energy should be extracted and used to create energy neutral WWTPs. The energy present in the wastewater and sludge can be present in three different forms: (i) organic matter (ii) nutrients (N and P) and (iii) thermal energy (McCarty et.al., 2011). The first two (organic matter and nutrients) can be categorized as chemical energy and counts up to 26% of the total energy present in wastewater and sludge. The rest 74% is thermal energy but it cannot be as efficiently harvested as chemical energy (Gude, 2016). Traditionally, the aim of a WWTP is usually to meet the discharge standards to protect environment. But today, considering the scarce water and energy resources and environmental pollution problems, the efforts are devoted to develop a sustainable wastewater treatment technique to recover resources and minimize energy needs (Gude, 2015).

Harvesting the chemical energy in wastewater and sludge is only possible via bioenergy technologies which will play a key role to reduce global dependency on fossil fuels and minimize atmospheric emissions. The most well-known bioenergy technology is anaerobic digestion where the organic carbon content of sludge is converted into methane (CH_4) through a series of reactions (hydrolysis, acidogenesis, acetogenesis and methanogenesis). The biogas production via anaerobic digestion has been known to be the most energy-efficient and environmentally beneficial technology but there exist other bioenergy technologies that can contribute to renewable energy production through higher chemical-to-electrical energy conversion efficiencies (Weiland, 2010). One of these bioenergy technologies is MFC, a bioreactor, which can convert the chemical energy in organic wastes into electrical energy via the help of microorganisms and catalysts (Du et.al., 2007).

MFCs can utilize various types of organic matter including wastewater and sludge, that's why, they are promising alternatives in terms of both energy production and wastewater treatment. Unfortunately, today, MFCs are still not integrated into WWTPs due to economic and technical limitations, and still anaerobic digesters seem to dominate although their energy conversion efficiency is much lower (Abourached et.al., 2014; Du et.al., 2007). To overcome these challenges, the operational parameters, microbial kinetics and bioelectrochemical reactions taking place between electron donor, acceptor and microorganisms should be handled in greater details.

2.2. Energy Recovery from Sludge

2.2.1. Anaerobic Digestion

Energy recovery using anaerobic digesters is a well-known technology. Anaerobic digestion of energy crops, solid wastes, leachate and sludge is becoming popular to minimize GHG emissions and preserve water/energy resources (Weiland, 2010). In 2007, the biogas production using anaerobic digestion reached 6 million tons of oil equivalents (EurObserver, 2008). Germany is the leading country in biogas production among Europe, with a total 4,000 agricultural biogas plants integrated to German farms (Weiland, 2010). In addition to agricultural biogas production, it is possible to generate 350 kWh of electricity for each million gallons of wastewater (Burton, 1996). This value can go up to 491 kWh-525 kWh if the system is coupled with a micro-turbine or internal combustion engine (Stillwell et.al., 2010).

The anaerobic degradation pathway of organic matter is a multi-step process (Figure 2.1). During the hydrolysis stage, polymers (protein, lipid, carbohydrate) are degraded via the help of exo-enzymes to produce smaller molecules that can pass

through the cell membrane. During this process, proteins, polysaccharide (carbohydrate) and lipids are converted into amino acids, simple sugars and long chain fatty acids, respectively. When the solids content of wastewater/sludge is high, hydrolysis usually becomes the rate-limiting step. At the end of hydrolysis stage, the substrate becomes readily degradable for acidogenesis (Van Lier et.al., 2008).
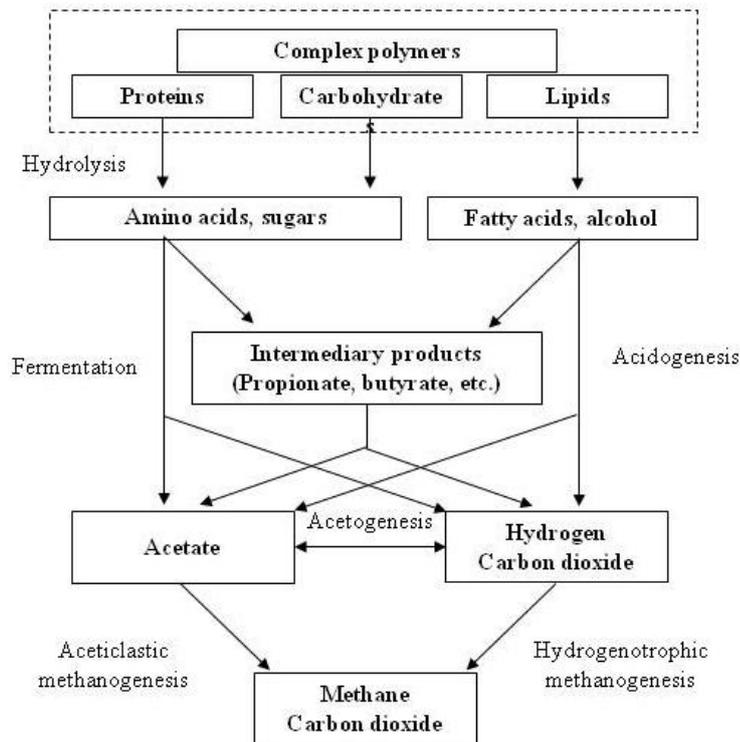
Figure 2.1. Schematic representation of the reactions taking place during anaerobic digestion (figure drawn after Gujer and Zehnder, 1983; Van Lier et.al., 2008)

Following hydrolysis, acidogenesis/fermentation takes place. At this stage, the hydrolyzed products diffuse into bacterial cells and fermented or anaerobically oxidized to form volatile fatty acids (VFAs) (acetate and higher organic acids such as propionate and butyrate) (Figure 2.1). The fatty acids, other than acetate (i.e. propionate and butyrate), are then converted into acetate, hydrogen and carbon dioxide through acetogenesis reactions (Van Lier et.al., 2008; Vesilind, 2003).

Finally, methanogenic bacteria start to dominate the system and accomplish the final conversion of organic matter into methane and carbon dioxide (Vesilind, 2003). At this stage, two important reactions take place (Van Lier et.al., 2008):

Reaction (2.1): $CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$

Reaction (2.2): $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

The biogas produced at the end of anaerobic digestion mostly composed of methane (CH_4) and carbon dioxide (CO_2) but also contain smaller concentrations of ammonia, hydrogen sulfide and water (gaseous/vapor) and it must be desulfurized biologically prior to gas utilization units to prevent any kind of damage, by injecting air into raw biogas to convert H_2S into elementary sulfur (Weiland, 2010).

The amount of CH_4 produced and electrical energy generation can be calculated theoretically using the stoichiometric conversion between chemical oxygen demand (COD) and mL methane production at 35°C (T) and 1 atm (P) (Tchobanoglous et.al., 2004):

Equation (2.1): $PV = nRT$, where, $V = 0.4$ L CH_4/g COD, $R = 0.082$ L.atm/K.mol

Using Equation 2.1, the moles of CH_4 produced per g of COD degraded can be found as 1.6×10^{-2} moles. Using the molecular weight of CH_4 (16 g/mole), g CH_4 produced/g COD reduced can be calculated as 0.25 g CH_4/g COD. Assuming the energy content of methane as 50.4 MJ/kg, and 35% conversion efficiency in an alternator, the electricity production by an anaerobic digester can be found as 1.2 kWh/kg COD. Since anaerobic digestion is a stabilization technique monitored based on volatile suspended solids destruction, the energy production potential should be also calculated based on volatile suspended solids (VSS) reduction. Using the ratio between COD/VSS as 1.5, the electricity production by an anaerobic digester per VSS destroyed is 1.86 kWh/kg VSS destroyed (Gude, 2016; Parker et.al., 2008).

The performance of an anaerobic digester is affected by several parameters such as: pH, alkalinity, temperature, solids and hydraulic retention time (SRT and HRT) (Appels et.al., 2008). Although each microorganism has a certain pH range to function properly, methanogens are extra sensitive to pH with a range 6.5-7.2 (Boe 2006; Turovskiy and Mathai, 2006). Temperature is a key parameter affecting the microbial kinetics (growth rate, metabolism and digestion of substrate) (Appels et.al., 2008). Just like pH, the methanogen population is again the most sensitive group to temperature changes in an anaerobic digester and favor higher temperatures. At higher temperatures, reaction rates increase, pathogens are destroyed much more effectively and substrate is solubilized for bacterial uptake (Appels et.al., 2008). But sometimes, higher temperatures may also inhibit the digestion process through the formation of free ammonia at high concentrations (Boe, 2006).

SRT is an important parameter in the operation of an anaerobic digester. Low SRT values affect the extent of stabilization since each sludge removal removes a certain portion of microorganisms and growth rate cannot compensate removal rate resulting in process failure (washout) (Appels et.al., 2008; Turovskiy and Mathai, 2006).

2.2.2.Microbial Fuel Cells

2.2.2.1.History of Microbial Fuel Cells

Although the ability to generate electricity using biomass as fuel has drawn attention after the last energy crisis in 1980s, the very first MFC was constructed almost a century ago by M.C. Potter (Durham University) in 1910 (Potter, 1911). Potter used *Escherichia coli* and *Saccharomyces* cultures to generate electricity and became successful at his idea of harvesting bioenergy for human use (Bullen et.al., 2006; Potter, 1911). The design of Potter was primitive and still needed improvement and

only after 20 years later, in 1931, Barnet Cohen achieved 35 volts and 2 milliamps of electrical energy production by connecting MFCs in series (Cohen, 1931). The interest in fuel cells accelerated after the USA space program in 1950s to use MFCs during space flights to dispose waste and generate electricity (Bullen et.al., 2006). Unfortunately, valuable studies focusing on MFCs did not generate much interest until the energy crisis in 1980s, when M.J. Allen and H.P. Bennetto (Kings College London) started to study MFCs in more detail to produce the basic MFC design that is still in use today (Rajalakshmi and Dhathathreyan, 2008). Years after, in 1990s, a new type of MFC was born "mediator-less MFC" with the discovery of B.H. Kim (Korean Institute of Technology). Kim discovered certain species of bacteria can transport electrons (electrochemically active microorganisms) and didn't require the presence of a mediator for electron transport (Kim et.al., 1999).

Although many scientists started to search MFC technology, many more questions are still present on their operation and large scale applications. Today, scientists are trying to optimize the MFC materials, membranes, types of microorganisms and electron transport mechanisms. Although the idea of energy production by bacteria is known fact for almost 100 years, scientists have recently started to understand the MFC technology and how to show to its true potential.

2.2.2.2. Working Principle of Microbial Fuel Cells

MFCs are electrochemical devices that can convert the intrinsic chemical energy in organic matter into electrical energy with the help of microorganisms and Pt catalyst (Logan et.al., 2006). Regardless of the configuration, the working principle and reactions taking place are common. A typical MFC has two separate chambers, anode and cathode, and a membrane separating them. The connection between anode (negative electrode) and cathode electrodes (positive electrode) are responsible for current flow (Logan et.al., 2006). In a dual chamber MFC, the fuel or substrate (i.e.

glucose, acetate, wastewater, sludge, etc.) is fed to the anode chamber where it is degraded under anaerobic conditions by microorganisms to produce electrons (e$^-$) and protons (H$^+$) as shown in Figure 2.2 (Ömeroğlu and Sanin, 2016). The example reaction showing the oxidation of glucose in anode chamber is (Pham et.al., 2006):

Reaction (2.3): $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$
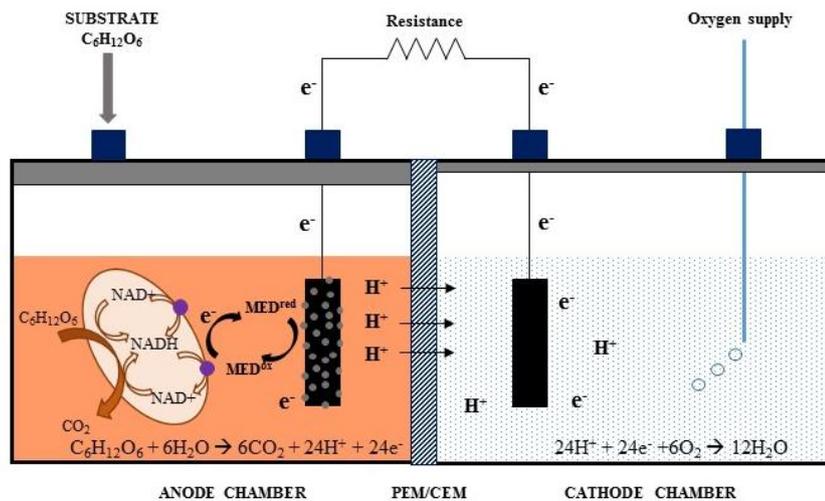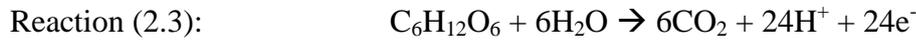
Figure 2.2. Schematic representation of the working principle of a typical dual chamber MFC (figure drawn with modifications after Rabaey and Verstraete, 2005)

Reaction 2.3 must take place under anaerobic circumstances, because if there is another electron acceptor, the electrons will be absorbed before they can reach to anode electrode (Zhao et.al., 2009). The presence of another electron acceptor, especially oxygen, will affect the anode potential. Anode potential is an important parameter controlling the synergistic relationship between the final bacterial electron shuttle and electron liberating capacities of the biocatalyst (Aelterman et.al., 2008; Ömeroğlu and Sanin, 2016). There are three different metabolic routes to describe the importance of anode potential on current generation: (i) high redox-oxidative metabolism, (ii) medium to low redox oxidative metabolism and (iii) fermentation (Rabaey and Verstraete, 2005). If the chamber is completely anaerobic, then the microorganisms can use respiratory chain in an oxidative metabolism and transport

electrons using NADH, dehydrogenase, ubiquinone, coenzyme Q and cytochrome (high anode potential) (Beliaev et.al., 2001; Champine et.al., 2000; Nevin and Lovley, 2002). But when anode potential is low, meaning there are electron acceptors other than the electrode itself, such as nitrate, sulfate, etc., then electrons will interact with these and methane formation occurs (Kim et.al., 2004; Kim et.al., 2005). If neither of these conditions prevails, but anode potential is still low, then fermentation dominates as follows (Logan, 2004):

Reaction (2.4): $C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_4O_2$

Reaction (2.5): $C_6H_{12}O_6 \rightarrow 2H_2 + 2CO_2 + C_4H_8O_2$

If fermentation dominates, instead of high-redox oxidation, then current flow will be limited since two-thirds of the electrons will remain in fermentation products, only one-third of electrons will be available for electricity production (Logan, 2004).

Under ideal conditions, the electrons will be transferred to the anode electrode and finally reach to cathode electrode via an external circuit to generate electricity (Zhou et.al., 2014). The way electrons are transferred to the anode electrode is an important factor in MFC performance. There exist two basic mechanisms: (i) direct electron transfer (DET) and (ii) mediated electron transfer (MET). DET occurs when the cell wall or pilus of the microorganism gets in direct contact with the electrode surface (Zhou et.al., 2014). Only if the microorganism transferring the electron is anodophilic then DET can occur since the outer layer of most microbial species are composed of non-lipid conductive lipid membrane limiting electron transfer (Davis and Higson, 2007). When the surface of anodophilic microorganisms contact the anode electrode surface, their cytochromes or pilus/pilu (conductive nanowire) can transfer the electrons (Figure 2.3) (Rinaldi et.al., 2008). But if the microbial species present are not anodophilic (i.e. *Escherichia coli, Pseudomonas* sp., *Proteus* and *Bacillus*), then DET cannot place and the transfer of electrons can only occur in the presence of a mediator (Lovley, 2006). Mediators are synthetic or naturally produced compounds that accelerate electron transfer (Rabaey and Verstraete, 2005). They are

present in oxidized form and reach the outer surface of the microorganisms, penetrate and capture the electrons to reduce themselves (Du et.al., 2007). Then these mediators travel to the anode electrode surface and re-oxidize themselves by liberating the electrons (Zhou et.al., 2014). This helps the safe electron transfer while the re-oxidized mediator interacts with the microorganisms, continuing the redox cycle (Figure 2.3) (Neto et.al., 2010; Rabaey et.al., 2005b). A good mediator should be: (i) a good electron carrier, (ii) soluble, (iii) non-toxic, (iv) non-degradable and (v) inexpensive (Ieropoulos et.al., 2005a). Typical synthetic mediators are: thionine, neutral red, phenanzines and methylene blue (Zhou et.al., 2014). Unfortunately, these mediators are not suitable for practical applications because of their cost and toxicity (Du et.al., 2007). Luckily, some microorganisms can produce endogenous mediators like humic acids, anthraquionine and thiosulphate (Lovley, 1993).
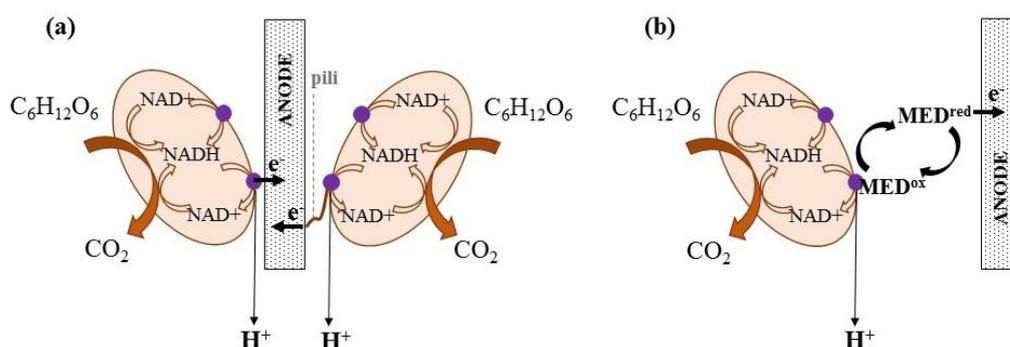
Figure 2.3. Mechanisms of electron transfer (a) DET and (b) MET (figure drawn with modifications after Zhou et.al., 2014)

The protons, on the other hand, pass through the membrane to react with the electron acceptor, usually oxygen, to produce water. The reaction taking place in the cathode chamber is given below (Pham et.al., 2006):

Reaction (2.6): $24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$

Both reactions, Reaction 2.3 and Reaction 2.6 require catalysts, anodic reaction requires biofilm catalyst on electrode surface for oxidation and electron transfer while cathodic reaction requires Pt to accelerate oxygen reduction rate and reduce activation energy (Zhou et.al., 2014). Sometimes alternative catalysts are also used since Pt is expensive for practical applications, (i.e. MnO_x (where x is 2 or 4), PbO_2, Fe (II), phtalocyanine and CoTMPP (cobalt tetramethylphenylporphyrin)) (Roche and Scott, 2009; Zhou et.al., 2011).

The O_2 molecule in Reaction 2.6 acts as an electron acceptor and it is the most commonly used one due to its high redox potential (0.82 mV). Oxygen itself and the reaction product H_2O are both non-toxic, which makes O_2 the ideal electron acceptor (Franks and Nevin, 2010). But it has one important drawback: reduction rate of O_2 is very slow, causing high overpotential, limiting the performance of MFCs (Gil et.al., 2003). There exist other electron acceptors such as ferric cyanide and potassium permanganate, but they are expensive (require regeneration) and may diffuse back affecting anode potential negatively (Logan et.al., 2006; Zhou et.al., 2014).

The membrane separating the chambers has a crucial role in MFC performance. Usually proton exchange membranes (PEM) or cation exchange membranes (CEM) are used. The membrane should be selectively permeable so that only the protons can pass through while the oxygen flux is limited to preserve anaerobic conditions in anode chamber (Logan, 2008). In conventional hydrogen fuel cells (HFCs), PEMs are known to perform better than CEMs, since the system separates H_2 and O_2 in gaseous form and PEM is required to conduct protons in between (Logan, 2008). In MFC, on the other hand, the water conducts the protons and PEM surface gets fouled with cation species other than H^+ when in contact with wastewater/sludge (contains higher amounts of Na^+, K^+, NH_4^+, Ca^{2+} and Mg^{2+} than H^+ in anolyte), reducing the performance (Rozendal et.al., 2006). The most commonly preferred type of PEM is Nafion 117 (DuPont, Wilmington, Delaware) because of its high selective permeability (Du et.al., 2007). But it has one major drawback: its cost. While Nafion 117 can cost $1400/m^2$, a well-known CEM, CMI-7000 (Membranes International, Inc.) cost much less ($80/m^2$) (Logan, 2008). That's why, in most of the

wastewater/sludge applications, CMI-7000 was preferred. These membranes were shown to have very close performances (in terms of IR and power density-PD) in previous studies (Kim et.al., 2007b).

Overall, Reaction 2.3 and Reaction 2.6 are thermodynamically favorable redox reaction (half reactions), which gives the aerobic oxidation of glucose. The potentials (E_{anode} and $E_{cathode}$), calculated from the Gibbs free energy of each reaction taking place, helps to calculate the overall theoretical cell potential ($E_{emf} = E_{anode} - E_{cathode}$) (Logan, 2008). The theoretical maximum voltage calculated using these thermodynamic relationships is usually higher than the measured maximum working voltage (0.3-0.7 V) or open circuit voltage (OCV) (0.8 V) (Kim et.al., 2007a; Logan, 2008). The voltage generation by an MFC is a very complicated concept since it depends on various parameters compared to HFCs such as bacterial metabolism, electron transfer mechanisms, enzymatic reactions, thermodynamic balances and components of an MFC. That's why; better understanding of the working principle and bacterial kinetics in an MFC will be helpful in improving the performance.

2.2.2.3.Design and Configuration of Microbial Fuel Cells

There exist various types of MFC bioreactors, including, H-type (salt bridge), dual chamber (cubic or bottle), single chamber air-cathode, miniature, upflow and stacked (Zhou et.al., 2014). Dual chamber systems are the most commonly used type of MFC since they are much easier to control compared to single chamber air-cathode systems (Pham et.al., 2005). Dual chamber systems can take various shapes: cylindrical or rectangular (Figure 2.4). Cylindrical MFCs are composed of two bottles connected to each other with a membrane (PEM/CEM) or salt bridge (concentrated NaCl solution) to enhance proton diffusion (Du et.al., 2007; Kim et.al., 2007b). This system was basically called as H-type/salt bridge MFCs. An advanced salt bridge MFC was operated by Min et.al. (2005), using synthetic wastewater

(SWW) and domestic wastewater (DWW). The low power densities obtained in this study was directly attributed to the high IR of the NaCl solution compared to membrane (19920Ω and 1286Ω, respectively) (Min et.al., 2005). That's why, today, salt bridge systems are not so common. Another form of cylindrical MFCs is miniature MFC. Although this system has a diameter of 2 cm, significant power densities were reported in the literature (Figure 2.4) (Ringeisen et.al., 2006). The small size and high PD make this type of MFCs perfect candidate as sensors in areas where access is limited (Du et.al., 2007). The rectangular, membrane MFCs are currently used in laboratories and run in batch mode with SWW as substrate (Figure 2.4) (Du et.al., 2007).

Rectangular MFCs are usually separated with a membrane (PEM or CEM) instead of salt bridge and they are very easy to control but usually expensive because of the additional aeration costs (in cathode chamber). For this reason single chamber air-cathode MFCs were developed to eliminate the extra aeration cost since cathode is directly exposed to air (only anode chamber exists) (Figure 2.4) (Abreyava et.al., 2015). While this design offers significant cost savings, the formation of thick carbonate salt layer on cathode electrode adversely affects the cathode reaction, therefore, current flow (Pham et.al., 2005). The working principle of this system is similar to the one described in Section 2.2. Here, the substrate is again fed to the anaerobic anode chamber and oxidized to form protons and electrons. The air cathode surface is porous to allow proton transfer (Park and Zeikus, 2003). This system basically consists of five parts: (i) PEM/CEM, (ii) catalyst layer, (iii) cathode, (iv) diffusion layer and (v) anode. The membrane and cathode electrode are bonded to each other and called as electrode/PEM assembly (He et.al., 2017).

Upflow MFCs could be more suitable for wastewater treatment because they are easier to scale up compared to rest of the configurations (He et.al., 2005). In some of the upflow MFC systems, effluent is recirculated to anode chamber (to treat wastewater, not to generate power), and this may bring extra pumping and electricity costs when integrated to WWTPs (Du et.al., 2007). Sometimes, anode and cathode chambers are separated from each other using glass wool/beads (physical separation),

and electrodes are placed accordingly. The influent enters from bottom and after passing through cathode, exists at the top continuously (Du et.al., 2007). Therefore, the anolyte and catholyte are not separate as in membrane-less MFCs, but still the physical separation can provide oxygen gradient for proper operation.

Single chamber upflow systems are also being used (Figure 2.4) and called as tubular single chamber air-cathode MFCs. Here a tubular MFC has an outer cathode exposed directly to air (cylindrical) and an inner anode with graphite granules is placed in anode chamber (fully anaerobic) (Rabaey et.al., 2005a). To keep the cathode from drying up (direct contact with air), the cathode electrode is usually dripped with electrolyte during operation (Du et.al., 2007).

Since MFCs are very small devices, the voltage output is as small as their size. That's why, to generate higher amounts of current density (CD) and PD, usually several MFCs are connected in series or parallel (Figure 2.4) (Aelterman et.al., 2006). The study of Aelterman et.al. (2006) showed that stacked MFCs could have greater ability in producing higher power but the overpotential (increase in series connection) may sometimes limit current flow (He et.al., 2017). Stacked MFCs can be applied to various configurations including air-cathode tubular systems (Zhuang and Zhou, 2009).
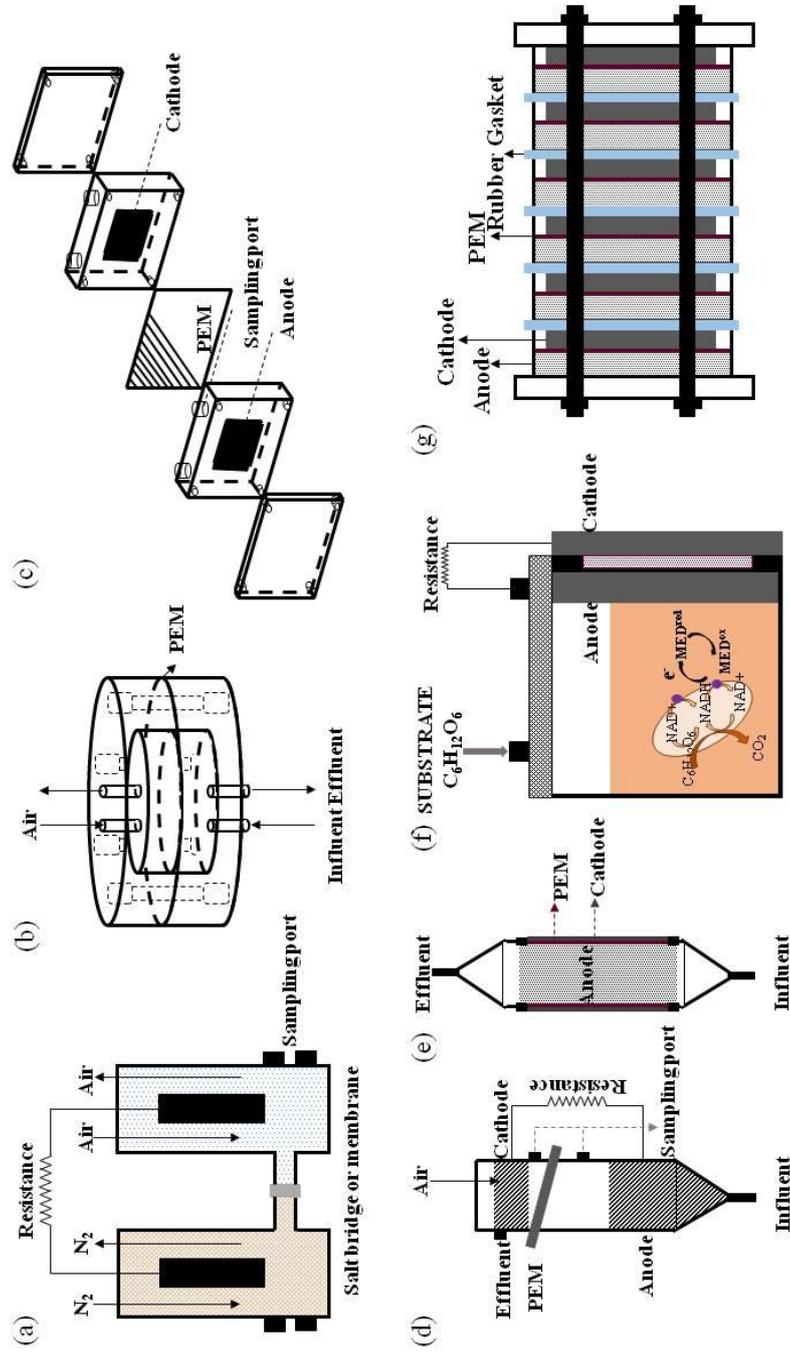
Figure 2.4. Schematic representation of (a) H-type/salt bridge MFC, (b) miniature MFC (dual chamber), (c) rectangular/cubic MFC, (d) upflow single chamber air-cathode (tubular) MFC, (e) upflow single chamber air-cathode MFC, (f) single chamber air-cathode MFC and (g) stacked MFC (a,b, d, e, g-drawn with modifications after Du et.al., 2007, c-drawn with modifications after Allen and Benneto, 1993, f-drawn with modifications after Du et.al., 2007 and Rabaey and Verstraete, 2005)

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2.3. Factors Affecting the Performance of Microbial Fuel Cells

The ideal performance of an MFC actually depends on the bioelectrochemical interactions between the substrate/fuel (low potential) and oxygen (final electron acceptor, high potential) (Rabaey and Verstraete, 2005). Although, anode and cathode potentials presented in literature, enables us to calculate the theoretical cell potential (E_{emf}), still there are uncertainties on ideal cell voltage due to complex nature of substrate-electron-microorganism interactions and respiratory chain that varies from microbe to microbe (Du et.al., 2007). Some microorganisms may be able to transfer the electrons via cytochrome or pili (anodophilic), while the others may not. That's why, to boost the performance of an MFC, first the working principle and microbial kinetics must be understood and improved. Then, the rest of the parameters, mostly arising from the operational conditions, should be discussed.

So far, the working principle and basics of the reactions taking place in anode and cathode electrodes have been discussed and evaluated (Section 2.2). Now, the rest of the factors affecting the power generation in an MFC will be discussed.

When the ideal performance (E_{emf}) was compared to the findings in the literature, it was shown that the laboratory MFCs had much lower performances (Du et.al., 2007). This mostly arises from the operational conditions and materials used but there are also other factors listed as follows (Du et.al., 2007; Gude, 2016; Liu et.al., 2005a):

- Reactor configuration (discussed in Section 2.3)
- Type and concentration of substrate
- Type of microorganisms
- Anode/cathode electrode materials
- Membrane
- pH
- Temperature
- Mediator

2.3.1.Type of Substrate Fed to the System

The type of substrate used in an MFC is a crucial factor determining the performance since it is responsible for the amount of electrons liberated. Various substrates can be utilized by MFC: (i) simple carbohydrates (i.e. acetate, butyrate, glucose), (ii) DWW and (iii) complex mixtures of industrial wastewater (IWW) (i.e. sugar industry, pulp and paper industry, etc.) (Chaudhuri and Lovley, 2003; Mathuriya and Sharma, 2009; Liu et.al., 2005b; Lui et.al., 2004; Pandey et.al., 2016).

The most well-known metabolic fuels are carbohydrates, fatty acids and amino acids, which are present in complex solutions of wastewater and sludge, but the most abundant group among these is carbohydrates (Pandey et.al., 2016). The electricity generation using different types of carbohydrates as carbon source in SWWs has been tested for years (Table 2.1). For instance, Lee et.al. (2008) compared the energy conversion efficiency in a dual chamber MFC of two different substrates: (i) non-fermentable acetate and (ii) fermentable glucose. It was shown that the performance of an MFC utilizing acetate was better than glucose fed MFC (360 mW/m^2 and 9.8 mW/m^2, respectively) due to the differences in anode potential (methane formation was observed in glucose fed MFC). Acetate is a simple substrate to be used as carbon source. It is readily biodegradable since it is an end product of metabolic pathways of complex carbohydrates like in anaerobic digestion (Biffinger et.al., 2008). In the study of Çatal et.al. (2008), six hexoses (D-glucose, D-galactose, D(-)-levulose, L-fucose, L-rhamnose and D-mannose), three pentoses (D-xylose, D(-)-arabinose and D(-)-ribose), two uronic acids (D-galacturonic acid and D-glucuronic acid) and one aldonic acid (D-gluconic acid) were tested using mixed bacterial culture enriched with acetate. The maximum PDs were in the range of 1240-2770 mW/m^2. D-mannose showed the poorest performance whereas D-glucuronic acid generated highest power. This study indicated that most of monosaccharides (including lignocellulosic materials) can be used in an MFC. Glucose is the second most commonly used substrate in MFCs. In the study of Rabaey et.al. (2003), glucose was

utilized in dual chamber MFC using mixed culture and achieved 3500 mW/m^2 maximum PD. As one may see, even if the same substrate was used or identical MFCs were operated, still there may be at least one order of magnitude difference in the PD (Kim et.al., 2007a). Especially, when compared to HFCs utilizing carbon (methanol), the PD of MFCs are several orders of magnitude lower (1000-100,000) due to various constraints (Liu et.al., 2005b).

Today, vast amounts of wastewater and sludge are being produced and they cannot be discharged without taking the necessary precautions. Treating wastewater or stabilizing sludge, both are energy intensive and expensive processes (Sustarsic, 2009). Luckily, both wastewater and sludge can be used as fuel in an MFC, so that energy requirement can be met. Today, MFCs are still not integrated into WWTPs but there exist numerous laboratory studies showing the utilization of different types of wastewater/sludge by MFCs (Table 2.2). For example, in the study of Mansoorian et.al. (2013), food processing IWW was used as carbon source and electron donor in a dual chamber MFC to generate electricity. In this study, the effluent from anode chamber was also sampled to measure the extent of treatment in terms of COD, biological oxygen demand (BOD), total suspended solids (TSS) and VSS. 86% COD and 79% BOD removal was achieved in this study, which was significant (without recirculation). Since food processing IWW contains high concentrations of sulfate, methane inhibitor was also added. The maximum PD measured was 230 mW/m^2. In a different study, conducted by Huang et.al. (2011), a dual chamber anaerobic fluidized bed (AFB) type of MFC was operated with distillery wastewater and achieved a maximum PD of 124.03 mW/m^2. This study demonstrated that AFB-MFC system can be used for simultaneous wastewater treatment and power generation and when compared to a conventional MFC system, AFB-MFC showed higher COD removal efficiencies making it suitable for high strength wastewaters.

Brewery wastewater has been studied many times due to its high organic content and absence of inhibitory compounds. In the study of Feng et.al. (2008), a single chamber air-cathode MFC was fed with brewery wastewater and both treatment and electricity production efficiencies were studied. Maximum PD was 205 mW/m^2, which

decreased to 170 mW/m^2 when temperature was reduced from 30°C to 20°C, respectively. When phosphate buffer solution (PBS) (200 mM) was added to adjust the pH, PD reached to 528 mW/m^2. This study did not only show how effective MFC utilized brewery wastewater but also the impact of pH and temperature on MFC performance. Another study on brewery wastewater was conducted by Wen et.al. (2010). Just like Feng et.al. (2008), in this study also the impact of PBS addition and substrate concentration were evaluated. Findings showed that both PBS and substrate concentration increase had a positive impact on power output.

So far, the wastewaters with high organic and low inhibitory content have been discussed. Will MFCs be able to operate with, for instance, pharmaceutical wastewaters or refinery wastewaters? The study carried out by Sun et.al. (2009), answered this question. The focus of the experiments was decolorization of active brilliant red X-3B (ABRX3) dye in a single chamber MFC. When glucose was used (no ABRX3 addition), the maximum PD was 274 mW/m^2. But when ABRX3 was added (minimum concentration 300 mg/L), the PD dropped to 234 mW/m^2. As dye concentration increased further (i.e. 1500 mg/L), PD decreased to 110 mW/m^2. Decolorization was not affected by the initial dye concentration; however, electricity generation was affected by increasing dye concentrations. It was concluded that this was mostly due to the competitive inhibition of azo-dye for electrons.

Refinery effluents contain long chain hydrocarbons and many metals that may inhibit microbial growth and metabolism. Chandrasekhar and Mohan (2012), studied petroleum sludge, not wastewater, and achieved a maximum PD of 53.11 mW/m^2. Considering the fact that, sludge contains higher concentrations of every unwanted material, this study seems to be successful in terms of remediation and energy production.

MFCs are not only used to treat wastewaters but also sludges. To illustrate, Xiao et.al. (2013) carried out experiments on the electricity output of an MFC fed with alkaline pretreated sludge. In this study, waste activated sludge (WAS) obtained from a WWTP was subjected to alkaline pretreatment and a maximum PD of 65.49

mW/m^2 was recorded. Methane production was observed but the authors stated that they didn't find out a direct correlation between electricity and methane production. However, they showed that the production of humic-like substances during biodegradation enhanced the electricity production (natural mediator).

In another study on sludge-fed MFCs, Jiang et.al. (2009) demonstrated that pretreatment has a positive impact on performance. Ultrasonic pretreatment increased the soluble COD (sCOD) concentration and the impact was directly observed in the final power output (80.5 W/m^3, not mW/m^2 since the surface area of graphite fiber brush anode electrode was not presented by the authors). The authors also claimed that MFC performance is affected by substrate concentration, catholyte concentration and anodic pH. Years after this research, the same study group, Jiang et.al. (2011), examined the impact of pretreatment further and compared raw sludge with ultrasonically pretreated sludge (>0.6 W/mL) and showed that not only carbohydrates, but also, aromatic proteins, microbial by-products, carboxylic and aliphatic components were also solubilized, enhancing power output.

Zhang et.al. (2012) showed that using biocathodes, oxidation reactions within an MFC can be facilitated and higher power densities can be obtained. In this study, a three chamber system was constructed (one additional cathode chamber) and WAS was used as anodic inoculum and substrate. The findings showed that biocathode improved the electricity production around 55% compared to conventional MFCs.

Today, MFCs not only serve as a method for wastewater treatment or sludge stabilization but also a technique to reuse DWWs as in the study of Ge et.al. (2013). Ge et.al. (2013) showed that with the help of osmotic MFCs both electricity generation and wastewater reuse was possible. Water flux of 1.06-1.49 L/m^2/h were achieved with 24.3-72.2% reductions in wastewater effluent. Reactor configuration, membrane fouling and concentration polarization were found to be main parameters affecting the performance of the system. The energy analysis carried out showed that osmotic MFC can produce considerable energy, just like the usual MFCs, but this time with the advantage of wastewater reuse.

Table 2.1. Details of MFCs fed with SWWs containing simple carbohydrates as carbon source/electron donor

Inoculum	MFC Type	Carbon Source	Anode Electrode	Cathode Electrode	Wire	External Resistance	Membrane	PD (mW/m²)	Reference
Mixed (ADS)	Dual chamber	Petroleum hydrocarbons	Carbon felt	Carbon felt	Cu	1000 Ω	CMI-7000	0.91	Adelaja et.al., 2015
Mixed (sediment)	Dual chamber	Glucose	Pt	Ti-TiO₂	Cu	10 Ω	CMI-7000	642	Akman et.al., 2013
Mixed	Single chamber	Glucose	Non-wet proof carbon cloth	30% wet proofed carbon cloth	*	1000 Ω	Nafion (binder)	766	Cheng et.al., 2006a
Mixed (DWW)	Single chamber	Glucose	Non-wet proof carbon cloth	Carbon cloth coated with PFTE and Pt (wet-proofed)	*	1000 Ω	Nafion (binder)	2160	Çatal et.al., 2008
		Galactose						2090	
		Fructose						1810	
		Fucose						1760	
		Ramnose						1320	
		Mannose						1240	
		Xylose						2330	
		Arabinose						2030	
		Ribose						1520	
		Galacturonic acid						1480	
		Glucuronic acid						2770	
		Guluconic acid						2050	

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Table 2.1. Details of MFCs fed with SWWs containing simple carbohydrates as carbon source/electron donor (cont'd)

Inoculum	MFC Type	Carbon Source	Anode Electrode	Cathode Electrode	Wire	External Resistance	Membrane	PD (mW/m²)	Reference
Mixed (WAS)	Dual chamber	Glucose + gulatamate	Graphite felt	Graphite felt	Pt	10 Ω	None	1.3	Jang et.al., 2004
Mixed	Dual chamber	Sucrose	Carbon paper	Carbon paper	*	1000 Ω	PEM (no specification)	74.65	Jia et.al., 2014
Mixed (ADS)	Dual chamber	Acetate	Graphite rod	Graphite rod	*	100 Ω	CMI-7000	360	Lee et.al., 2008
		Glucose						9.8	
Mixed (ADS)	Dual chamber	Sodium acetate	Carbon cloth	Carbon cloth	Ti	1000 Ω	CMI-7000	580	Liu et.al., 2013
Mixed	Dual chamber	Glucose + gultamate	Graphite felt	Graphite felt	*	3-110000 Ω	*	560	Moon et.al., 2006
Mixed (ADS)	Dual chamber	Glucose	Graphite rod	Graphite rod	Cu	1000 Ω	Nafion 117	10.28	Oh et.al., 2014
		Acetate						9.76	
Mixed	Dual chamber	Glucose	Plain graphite	Plain graphite	Plain graphite rod	*	CMI-7000	3500	Rabaey et.al., 2003

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Table 2.2. Details of MFCs fed with wastewater and sludge as carbon source/electron donor

Inoculum	MFC Type	Carbon Source	Anode Electrode	Cathode Electrode	Wire	External Resistance	Membrane	PD (mW/m^2)	Reference
Mixed	Single chamber	Brewery wastewater	Non-wet proof carbon cloth	Wet-proofed carbon cloth (PTFE)	Ti	1000 Ω	*	205	Feng et.al., 2008
Mixed	AFB dual chamber	Distillery wastewater	Carbon fiber paper	Carbon fiber paper	Cu	120 Ω	Nafion 117	124.03	Huang et.al., 2011
Mixed (ADS)	Dual chamber	Raw wastewater	Ti-TiO$_2$	Ti-TiO$_2$	Cu	10 Ω	CMI-7000	26.62	Köroğlu et.al., 2014
		Brewery wastewater						0.158	
Mixed	Single chamber	Starch processing wastewater	Carbon paper	Carbon paper	*	1000 Ω	Nafion 117	239.4	Lu et.al., 2009
Mixed	Dual chamber	Food processing wastewater	Graphic sheet	Graphic sheet	Cu	20,000 Ω	Nafion 117	230	Mansoorian et.al., 2013
Mixed (ADS)	Dual chamber	WAS	Carbon felt	Carbon paper	Cu	1000 Ω	Nafion 117	65.49	Xiao et.al., 2013

*not indicated in the original study

2.3.2.Type of Microorganism

Microorganisms are the backbone of MFC operation since they are responsible for biodegradation of substrate and electron transfer. Many microorganisms have the ability to transfer the electrons liberated from substrate degradation to anode electrode surface (Du et.al., 2007). However, nowadays, studies are focusing on identification of microorganisms that are able to produce and transfer electrons are being discussed (Holmes et.al., 2004; Logan et.al., 2005). As mentioned before, the interactions between the substrate and microorganisms in the anode chamber are the fundamentals of MFC operation, and the most important mechanism responsible for electricity generation at this point is the electrons shuttles between microorganisms/mediator and anode electrode (Du et.al., 2007). There exist specific microbial strains in the literature that are known to carry electrons in their cell structure. Mostly metal reducing microorganisms such as *Geobacter*, *Shewanella*, *Rhodoferax* species have this ability since they produce energy (ATP) through the dissimilatory reduction of metal oxides under anaerobic conditions and transfer the electrons to anode electrode as the final electron acceptor in anode chamber (Du et.al., 2007; Holmes et.al., 2004; Lovley et.al., 2004; Vargas et.al., 1998).

Mixed cultures are also known to have good performances in MFCs since they allow wider substrate utilization. Because in mixed cultures, both anodophiles and electrophiles exist together allowing better degradation and electron transfer (Du et.al., 2007). These organisms can also work well with naturally existing mediators such as humic acid or sulphate/sulphide improving power output (Ieoropoulos et.al., 2005b). A list of specific microorganisms screened and identified in MFCs operations in the literature are given in Table 2.3.

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Table 2.3. Type of microorganisms and substrates used in MFC operation

Microorganism	Substrate	Reference
Geobacter sulfurreducens	Acetate	Bond and Lovley, 2003
Rhodoferax ferrireducens	Glucose	Chaudhuri and Lovley, 2003
Shewanella putrefaciens	Lactate	Kim et.al., 2002
Geobacter metallireducens	Acetate	Min et.al., 2005
Clostridium butyricum	Starch, glucose, lactate, molasses	Niessen et.al., 2004
Aeromonas hydrophila	Acetate	Pham et.al., 2003
Pseudomonas aeruginosa	Glucose	Rabaey et.al., 2004
Shewanella oneidensis	Lactate	Ringeisen et.al., 2006

2.3.3.Anode/Cathode Electrode Materials

The main challenge in an MFC operation is the identification of anode/cathode materials and reactor configuration to maximize power output while minimizing the cost so that the system can be integrated into a WWTP. The use of better performing anode and cathode electrode materials can significantly enhance the electricity generation in an MFC since they affect the activation polarization losses (Du et.al., 2007). Activation losses refer to the amount of energy lost during the transfer of electrons from the bacterial cell protein to the anode electrode surface (Logan, 2008). These losses can be easily minimized via accelerating the electron transfer to anode surface or increasing the Pt catalyst concentration on the cathode electrode surface. To maximize PD, any kind of electron loss must be minimized in an MFC. That's why, selection of electrode materials is crucial in terms of MFC performance.

An ideal anode electrode should be: (i) highly conductive, (ii) physically and chemically stable, (iii) non-corrosive, (iv) inexpensive and (v) non-fouling (Dumitru and Scott, 2016; Logan, 2008). The IR caused by anode electrode should be as low as possible while the surface area is high (Zhou et.al., 2011). Although, electrical conductivity is the most important property of anode electrode, the non-corrosivity rules out many good metals. To illustrate, stainless steel is a well-known conductive material but not suitable for MFC operation since its surface does not allow microbial attachment (biofilm formation for electron transfer) and easily corroded when in contact with wastewater and sludge (Logan, 2008).

Several different materials are being used as anode electrode. Today, carbonaceous materials show good biocompatibility and conductivity. In addition to this, they are relatively cheap, therefore, widely used (Hernandez-Fernandez et.al., 2015). The surface of carbon anode electrodes (i.e. carbon, graphite, reticulated vitreous carbon) is suitable for biofilm formation, enhancing electron transfer (Logan, 2008). Carbon materials can exist in different forms such as plain (paper, mesh, felt, sheet), brush, wet-proofed or foam (Dumitru and Scott, 2016; Logan, 2008). Anode electrodes in the form of carbon paper are usually very brittle, although they have high surface area and this definitely affects microbial attachment. Carbon cloth is much more durable but they are slightly expensive (Dumitru and Scott, 2016). Graphite electrodes have been also used in a variety of studies (Moon et.al., 2006; Oh et.al., 2014). Graphite materials are highly conductive and have high surface area but their low porosity results in less power compared to carbon-felt materials (Logan, 2008).

In the last few years, non-conventional metal electrodes are also being discussed (i.e. stainless steel, titanium). Metals have much higher conductivity, but as stated before their surface properties does not allow biofilm formation and some metals are easily corroded and toxic to microorganisms (i.e. copper) (Dumitru and Scott, 2016).

Natural anode materials are also being used in MFC operation created by the synthesis of anode electrode using natural and recyclable materials (Sonawane et.al., 2017). An example can be the layered corrugated carbon (LCC) anode electrode

produced from packaging waste by carbonization. Chen et.al. (2012) showed that LCC shows better performance compared to conventional graphite felt electrode.

Cathode electrode, on the other hand, has a different characteristic since a tri-phase chemical reaction between electrons, protons and oxygen takes place on its surface in the presence of a catalyst (Logan, 2008). The cathode electrode should have high redox potential and easily capture protons passing through the membrane (Zhou et.al., 2011). Carbonaceous materials are the most commonly used ones (i.e. carbon felt, carbon cloth, graphite rod, granular graphite, etc.) due to their chemical stability, biocompatibility and high conductivity since expensive precious metals are not suitable for wastewater treatment (Bajracharya et.al., 2016). Carbon cloth electrodes usually have high porous surfaces and higher flexibility (Zhang et.al., 2010). High porosity cathode materials usually perform better compared to flat ones, due to available high surface areas (Bajracharya et.al., 2016). Speaking of high surface areas, graphite fiber brush cathodes also have high surface areas and porosity, which are produced from shredded carbon fibers attached to a Ti wire core (Wei et.al., 2011). In addition to these, packed and tubular cathode electrodes are also available. The main idea behind the discovery of these two electrodes was to produce high PD by increasing the available surface area (Bajracharya et.al., 2016).

Cathode electrode directly affects the performance of an MFC and that's why, its surface is modified with Pt, usually, in order to reduce the cathodic activation energy and accelerate the reaction. When pure graphite cathode electrode was compared to a Pt coated one, it was shown that Pt graphite felt cathode showed three times higher performance compared to pure graphite cathode electrode (Moon et.al., 2006). But Pt is expensive, which is one of the economic limitations in MFC applications. Today, several researchers are studying the use of alternative catalysts (non-Pt). CoTMPP, FePc (iron phthalocynaine), rutile and mangangeses oxides are now demonstrated to be inexpensive and effective alternative catalysts (Cheng et.al., 2006b; Yu et.al., 2007; Zhou et.al., 2011).

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2.3.4.Type of Membrane Used

Ideally, the membrane separating the anode and cathode chambers (or anode chamber and cathode electrode in single chamber air-cathode MFCs) should be permitting the transport of protons from anode to cathode chamber while blocking the oxygen reflux to anode chamber. The membrane should be selectively permeable, since any ion can affect the electrochemical balance. The membrane should be ionically conductive, durable, chemically stable, biocompatible, unsusceptible to fouling and inexpensive. In HFCs, PEM is used as a layer to conduct the protons in between H_2 and O_2, but in MFCs, since water/wastewater/sludge conducts the protons, use of PEM is not mandatory (Logan, 2008). CEMs are also preferred, since the most widely used PEM, Nafion 117, costs $1400/m^2$, while CEM (i.e. CMI-7000) costs far less, $80/m^2$ (Logan, 2008). The high cost and susceptibility to fouling are the economic limitations in the way of WWTP and MFC integration.

As stated before, the most popular type of PEM is Nafion 117, where 117 describes the membrane thickness (Logan, 2008). It is usually preferred due to its high selective permeability but today, less expensive and more durable solutions are present (Du et.al., 2007). The major drawback of Nafion 117 is the instantaneous fouling of the membrane in contact with wastewater/sludge. Although it is designed to conduct protons, in wastewater/sludge, other cations (i.e. Na^+, Mg^{2+}, K^+, NH_4^+) exist at concentrations 10^5 higher and tend to occupy the active sites of Nafion 117, resulting in increased IR and reduced PD (Du et.al., 2007; Kim et.al., 2007a; Rozendal et.al., 2006). If protons cannot migrate to cathode chamber, pH will decrease affecting bacterial metabolism and therefore current flow. At the same time, catholyte pH will change limiting the mass transfer (Logan, 2008). To overcome this problem, pH buffer solutions are usually used.

CMI-7000 (manufactured by Membrane International, Inc.) is currently being used in most of the MFC studies since it is much cheaper and has IR close that of the Nafion 117 (Kim et.al., 2007b). CMI-7000 is a strong acid polymer membrane (formed from

large amounts of sulfonic acid groups) and has a thick and stiff structure (mechanically durable) when compared to sensitive Nafion 117 (Logan, 2008; Scott, 2014). CMI-7000 allows all sorts of cation transport and blocks the unwanted reflux to anode chamber. This time, since all cations can pass through, instantaneous fouling becomes significant compared to Nafion 117 (Logan, 2008).

In addition to CEM and PEM, anion exchange membranes (AEM) and bipolar membranes also exist. AEMs are solid polymer electrolyte membranes with quaternary ammonium functional groups (Scott, 2014). Under alkaline conditions, AEMs are known to catalyze fuel cell reactions (lower activation loss). The most well known AEM is AMI-7000 by Membranes International, Inc. (Logan, 2008). Bipolar membranes, on the other hand, consist of AEM and CEM in series. In these systems, anions such as OH^- are transported to anode, while protons reach cathode to balance the charge in between the chambers (Logan, 2008).

2.3.5.pH and Temperature

pH difference in between anode and cathode chambers affects the rate of proton transport. Theoretically, after a certain drop during the fermentation of the substrate, the pH should start to increase as more acetate is removed and electrons and protons are transferred. However, during the operation of an MFC, pH gradually drops as H^+ is produced and accumulate within the anolyte since the proton transfer through the membrane (fouling) is slower than its production (Du et.al., 2007; Squadrito and Cristiani, 2016). Although, pH difference may seem like the driving force in proton transfer, fouling of the membrane limits the transfer, increasing the IR (Du et.al., 2007). In addition to this, abrupt changes in pH affects the microbial activity and buffer solutions are used during most MFC operations. Gil et.al. (2003) compared MFCs with and without buffer. Without buffering, they observed a 4.1 pH difference. In the buffered MFC, the pH difference was around 0.5, but the voltage

output increased 2 times. This was due to two possible reasons: (i) a more stable environment was provided to the microorganisms and (ii) slow proton transfer problem was solved and more H^+ became available for cathodic reaction. To conclude, proton availability is one of major limitations in the cathode reaction and pH adjustment is an important factor affecting current flow in an MFC.

Temperature is an operational parameter in every system involving microorganisms. As stated before, microorganisms are the backbone of MFC operation in terms of biodegradation (electron liberation) and electron transfer to anode electrode. Higher temperatures are usually not favored in MFC operation since methanogens may grow and inhibit electron transfer (Jadhav and Ghangrekar, 2009). Electrochemically active bacteria dominate at ambient temperature (25-30°C) and this is the major advantage of MFC. Unlike anaerobic digestion, MFCs do not require additional heating/energy, so, the energy produced can be used in WWTPs when needed, instead of compensating its own energy needs (Rabaey and Verstraete, 2005).

2.3.6. Mediator

Some microorganisms (anodophiles) are able to transfer electrons due to the differences in their cell structure. DET occur when the cell wall can form a direct physical contact with the anode electrode or can form a pilus to transfer electrons in between (Zhou et.al., 2014). But in some cases this type of electron transfer is not possible and an agent becomes necessary. MET takes place via: (i) exogeneous mediators, (ii) primary metabolites and (iii) secondary metabolites. As described previously (Section 2.2), exogeneous mediators penetrate through microbial cell wall to capture the electron and become reduced. As they reach to the anode electrode's surface they release the electron and become oxidized again to transfer more electrons, in the form of a continuous redox cycle (Neto et.al., 2010; Rabaey et.al., 2005b). Exogeneous mediators include thionine, neutral red, phenanzines, quinines

and methylene blue (Zhou et.al., 2014). Use of synthetic mediators is expensive, that's why natural mediators or MET via primary/secondary metabolites are the focus of today's research. Some microorganisms are able to produce fermentation products (H_2, H_2S, alcohols and ammonia), which act like a mediator (Erable et.al., 2010). For example, *Proteus vulgaris*, *Escherischia coli*, *Desulfovibrio desulfuricans* can produce sulfide as mediator (Bullen et.al., 2006; Schröder, 2007). MET via secondary metabolites occur when the microorganisms produce their own mediator such as phenanzine derivatives (Osman et.al., 2010). To illustrate, *Shewanella oneidensis* can produce exogeneous mediator, flavins. Since synthetic mediators are very expensive, MET via secondary metabolites is crucial in electron transfer. Mediators increase PD significantly, especially when specific bacterial strains are used that are not anodophilic. The only drawback is the cost, which is now being eliminated via the use of secondary metabolites such as phenanzines or humic acid.

2.4. Applications of Microbial Fuel Cells

2.4.1. Electricity Generation

The chemical energy stored in the organic matter is directly converted to electricity in an MFC, without combustion. Therefore, the energy conversion efficiency in an MFC is not limited to Carnot cycle (Du et.al., 2007). The electron yield in an MFC can go up to 80-89% (Chaudhuri and Lovley, 2003; Rabaey et.al., 2003). Unfortunately, the power output of MFC is still low since the rate of current flow is small compared to HFCs (Du et.al., 2007). This problem can be solved by using capacitors to store the electricity generated. Since MFCs are very small devices, the electricity generated can be used to power a certain process in a WWTP, small telemetry systems and wireless sensors in remote locations (Du et.al., 2007). Today, robots that are fueled with sugar, waste, fruit, and energy crops can be powered using

MFCs. The plantation of mini MFC in human body is also possible. Using the nutrients, MFCs can be used as implantable medical device (Du et.al., 2007).

2.4.2.Wastewater and Sludge Treatment

MFCs can be used as biological reactors during wastewater treatment and sludge stabilization (Du et.al., 2007). The electricity generated in a WWTP using MFC can reduce the energy-related costs significantly. Therefore, the advantages of MFC-WWTP can be listed as follows:

- Electricity production: Today, in US, almost $25 billion is being spent annually for water/wastewater treatment. Generating the electricity necessary for WWTP is a great opportunity to reduce cost (Logan, 2008; WIN, 2001).
- Reduction in the aeration requirements: In a typical WWTP with aerobic treatment, 1 kWh energy is required per kg of substrate oxidized. The energy used for aeration can reach 30kWh/capita annually (Rabaey and Verstraete, 2005). If single chamber air-cathode MFCs are integrated into WWTPs, aeration-related costs can be eliminated (Logan, 2008).
- Reduction of the solids: Sludge handling in a WWTP is a difficult and expensive process. MFCs can reduce the solid production up to 50-90%, which automatically decreases sludge handling costs (Holzman, 2005).

2.4.3.Bioremediation

Water scarcity is an emerging global problem for the last decade, and access to clean water has become a severe challenge and worldwide concern. Therefore, remediation of any surface that's in contact with water resources is a crucial concern. The

methods of cleaning include: dredging, ozonation, electrochemical degradation which are expensive and aggressive physico-chemical methods of cleaning (Hashim et.al., 2011; Yeung, 2011). Luckily, bioremediation methods that rely on microbial activity (i.e. decomposition, detoxification) are becoming more and more popular each day due to low cost and environmentally safe nature (Li and Yu, 2015). MFCs can also be used for bioremediation. But at this point, they no longer serve as energy technologies, power is used to drive degradation reactions (Gregory and Lovley, 2005; Logan and Regan, 2006). In the study of Gregory and Lovley (2005), uranium was directly precipitated on cathode plate charged by electrode potentials in an MFC. In the study of Gregory et.al. (2004), microbial electrodes were used as electron donors for microbial respiration to reduce nitrate to nitrite via electric current. The findings of this study involved clues on producing current from anaerobic sediments and bioremediation of oxidized pollutants (Gregory et.al., 2004).

2.4.4.Biosensors

Monitoring and gathering data on environment are essential tools in understanding the ecological responses to human activity, but to do this, sensors are necessary. Sensors require power for operation and MFCs can be used for this purpose also (Logan and Regan, 2006). There are two aspects of using MFCs as biosensors: (i) direct correlation between coulombic yield and oxygen demand and (ii) directly powering the sensors (Du et.al., 2007; Logan and Regan, 2006). Sediment MFCs are developed to monitor the quality of rivers, sea and oceans and are powered up by the organic content of sediments (Reimers et.al., 2001; Tender et.al., 2002).

2.4.5.Biohydrogen Production

MFCs can be used to generate hydrogen instead of electricity by removing the oxygen at cathode and applying external potential (Logan and Regan, 2006). Under normal operational circumstances, protons combine with oxygen to form water in the cathode chamber and hydrogen production is thermodynamically unfavorable (Du et.al., 2007). To overcome the energy barrier, cathode potential can be increased via an external potential supply and hydrogen can be produced (Liu et.al., 2005c). Theoretically, the external potential required for this reaction to proceed is 0.11 V but practically more than 0.25 V is necessary (Logan and Regan, 2006; Liu et.al., 2005c). This number is still lower than the 1.2 V potential needed for the direct electrolysis of water to produce hydrogen (Du et.al., 2007). Biohydrogen production using MFCs is longer necessary, eliminating the aeration costs. In addition to this, the produced hydrogen can be stored for future use.

CHAPTER III

MATERIALS AND METHODS

3.1.Sludge Sample

WAS and anaerobically digested sludge (ADS) samples used as inoculum and substrate during MFC optimization studies plus substrate impact experiments (municipal WWTP sludge) and laboratory scale anaerobic digester operation were obtained from Ankara Central WWTP, which has a current flowrate of 765,000 m^3/day (ASKI, 2017). WAS samples were collected from the return activated sludge line of the biological treatment process while ADS samples were collected from the anaerobic digester directly.

The sludge samples used to test the impact of substrate type on MFC performance were taken from different industrial WWTPs around Turkey. The samples used to determine the enhancing effect of high organic concentration were obtained from: (i) a beverage factory and (ii) a poultry industry. The samples were taken from the WAS line of both WWTPs. The sludge sample used to test the impact of inhibitory contents in substrate on MFC performance were taken from (i) a textile factory WWTP and (ii) a petrochemical industry WWTP.

All collected samples were left to settle to achieve the required solids concentration by removing the top supernatant except for the poultry WWTP sludge, since its initial solids concentration was already too high. During the settlement of solids, the samples were kept at 4°C refrigerator and dark, so that microbial activity could be

minimized and the contents of the sludge were kept as close as it could be to its original source.

3.2.Chemicals

All chemicals used during the preparation of SWW, PBS and saline solution were supplied from Merck KGaA, Germany. The membranes, Nafion 117 and CMI-7000, used in the MFC set-up were purchased from DuPont, USA and Membranes International, respectively. The anode and cathode electrodes (carbon cloth, carbon paper, Pt coated carbon cloth) were all purchased from Fuel Cell Store (Texas, USA).

The 0.45µm, white gridded 47 mm diameter filters used during the solids determination were purchased from Merck Millipore KGaA, Germany. The ready-to-use COD kits (LCK 514, 100-2000 mg/l O_2) were supplied from Hach Lange.

3.3.Experimental Set-Up of Microbial Fuel Cells

3.3.1.Microbial Fuel Cell Configuration

In this study, a dual chamber MFC made from plexiglass was used. The dimensions of anode and cathode chambers were 8 cm x 10 cm x 7.5 cm (widthxlengthxdepth) and the total volume of each chamber was 600 mL (Figure 3.1). Providing 150 mL of headspace, the working volume was kept as 450 mL. The chambers were separated from each with a membrane which was either a PEM (Nafion 117) or CEM (CMI-7000) and the membrane was held in between two rubber gaskets bolted to the outer

surface of the chambers to prevent liquid and air leaks and fractures. There were two ports at the top of each chamber. In the anode chamber, one of the ports was used for substrate feed while the other one was used to submerge the anode electrode. In the cathode chamber, on the other hand, one of the ports was used to aerate the system (connected to an air pump) while the other one was used to submerse the cathode electrode, similar to anode chamber. The electrodes were connected to each other using a copper (Cu) wire with an external resistance device in between to support current flow. The generated voltage was measured and recorded continuously using a calibrated digital multimeter (8846A Fluke Digital Multimeter) purchased from Netes Electronic, Ankara.
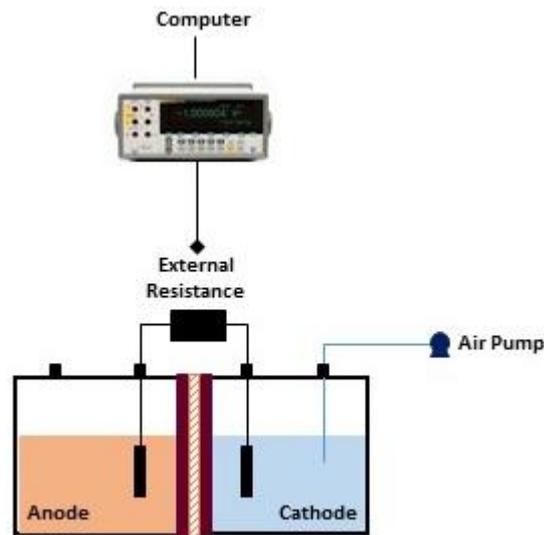
Figure 3.1. Experimental set-up of dual chamber MFC used in the study

3.3.2. Characteristics of Anode and Cathode Electrodes Used

As stated before, electrodes play a crucial role in the transfer of electrons (current flow) from anode to cathode chamber in an MFC. The anode potential must be high to attract the electrons and minimize their loss. For this reason, it must be conductive,

non-corrosive (when in contact with wastewater and sludge) and provide surface for microbial attachment and biofilm formation (high surface area and porosity). In this study, well-known carbon based electrodes were used. Non-wet proofed and wet-proofed carbon cloth and carbon paper were tested in terms of their performance. Carbon paper has a stiff and brittle structure but it is easier to connect it to a wire using epoxy resin cover. Carbon cloth, on the other hand, is much more flexible and has higher porosity that promotes microbial attachment (Logan, 2008). Carbon cloth is thinner than carbon paper and both electrode types have their own differences in transport, porosity and conductivity. Carbon cloth is mechanically more durable compared to carbon paper, which is a significant advantage for sludge fed systems.

The characteristic of cathode electrode was also important to maximize the PD during the experiments. Its surface was coated with Pt to act as a catalyst. Again, carbon based materials were used as cathode electrode and carbon cloth and carbon paper were compared with Pt concentrations of 0.5 mg/cm^2 and 0.4 mg/cm^2, respectively. 0.5 mg/cm^2 was the most commonly used concentration for Pt coating but then since Pt is an expensive metal, it was decided to test a lower concentration like 0.4 mg/cm^2. However, concentrations lower than 0.4 mg/cm^2 were not tested since decreasing Pt levels resulted in lower PD values during the experiments. All electrodes were purchased from Fuel Cell Store via Referans Kimya Co..

At the end of optimization experiments, it was demonstrated that the non-wet proofed carbon cloth and 0.5 mg/cm^2 Pt coated carbon cloth serve as the best anode and cathode electrodes, respectively. The size of both electrodes was 3 cm x 5 cm (widthxlength) with a surface area of 15 cm^2.

3.3.3.Membrane

The membrane separating a dual chamber MFC serves for two purposes: (i) to ensure the passage of protons (H^+) from anode to cathode and (ii) to limit the transfer of

oxygen from cathode to anode so as to create anaerobic environment with no alternative electron acceptors inside the anode chamber other than the anode electrode itself (high anode potential). If protons cannot pass through the membrane in between, like in the case of fouling, then the electrochemical reactions will be inhibited which will in turn increase IR and reduce the energy production. That's why selecting the type of membrane is a critical issue and must be handled with care. In this study, Nafion 117 and CMI-7000 membranes were subjected to test. Nafion 117 is a specifically designed PEM which only allows proton passage while CMI-7000 is a CEM (all cations can pass through it), so their surface properties and diffusion characteristics are completely different from each other. The code 117 refers to the thickness of the membrane which is 0.019 cm (Logan, 2008). This membrane was actually prepared to be used in HFCs, so it was expected to work in a high proton concentration environment with controlled humidity. But when used in MFCs, with direct contact with wastewater, a neutral pH is produced resulting in a negative change in membrane functioning. But still, Nafion 117 is preferred due to its high selective permeability, especially in HFCs but in MFCs since less expensive and more durable solutions are being investigated, CMI-7000 also proven to work. CMI-7000 is much thicker and has a more rigid structure compared to Nafion 117. Since it is structurally stronger with similar IR at low cost, nowadays it is highly preferred in MFC studies.

The size of each membrane was 10 cm x 7.5 cm, based on the size of the opening in between the chambers. Prior to use, both membranes were stored at dark, inside closed containers without any direct contact with the surrounding environment since the moisture may affect the size of the membrane. The size of the Nafion 117 membrane is based on dry product conditioned at 23°C and 50% relative humidity before cutting. But since after, in contact with wastewater or water, the dimensions of the membrane will be altered and not symmetrically, certain conditioning procedures must be applied. So once the membrane is opened and exposed to the environment, its dimensions change and needs to be preconditioned for at least 24 hours at hot water (80-100°C) with H_2O_2 in. Unfortunately, this method, especially

the temperature, seriously damaged the active sites of the membrane and resulted in instantaneous fouling during operation. Nafion 117 is a very sensitive membrane, easily affected by the moisture and many other environmental circumstances. So considering the sensitive nature of the membrane, the pretreatment temperature was reduced to 40°C to ensure the expansion of the membrane while protecting the active sites of it.

CMI-7000, on the other hand, has a different pretreatment technique compared to Nafion 117. CMI-7000 has a more solid and durable structure but it still requires pretreatment both for expansion and activate the cation exchange sites by adding NaCl (Akman et.al., 2013). Initially, the membrane was subjected to 40°C distilled water, 5% NaCl solution but then, within time, the NaCl (Na^+ and Cl^- ions) was thought to occupy the active sites, shortening the fouling time (Akman et.al., 2013). The final method of pretreatment was decided to be 3% NaCl (w/v) at room temperature for 48 hours.

3.3.4. Contents of the Anode and Cathode Chambers

All throughout the optimization experiments (both stages), the dual chamber MFC was fed with SWW (anode chamber) to eliminate the impact of wastewater constituents on current and voltage results since the main variables analyzed were operational conditions and materials used. The carbon and nitrogen sources were selected as glucose ($C_6H_{12}O_6$) and ammonium chloride (NH_4Cl), except for the MFC-11 operation where carbon source was sodium acetate (CH_3COONa). To ensure proper enzymatic functioning of the microorganisms Mg, Fe, Mn and Zn complexes were also added. SWW was prepared by dissolving the constituents listed in Table 3.1 in PBS solution at a pH of 7.5. In addition to this, microbial inoculum was also added. The inoculation volume was adjusted to keep the volatile solids (VS) concentration as 510 mg VS per 450 mL active MFC volume (Lobato et.al., 2012).

That's why, prior to inoculation the solids content of the sludge samples were measured and brought to similar levels via concentrating by physical means (settlement or centrifuge-3000 rpm, 5 min). The supernatants were stored if dilution is necessary to achieve the target VS concentration. Removing the top supernatant and concentrating the sludge sample helped to reduce the matrix effect of the complex sludge constituents and provide high concentration of microorganisms necessary for substrate degradation and electron transfer.

Table 3.1. SWW constituents used in optimization studies

Constituents	Concentration (mg/L)		
	SWW-1	SWW-2	SWW-3
$C_6H_{12}O_6$	4500	4500***	4500
NH_4Cl	500	500	500
$MgSO_4.7H_2O*/MgCl_2.6H_2O**$	100*	100**	100**
$FeSO_4.7H_2O*/FeCl_2.4H_2O$	5*	50	50**
$MnSO_4.7H_2O*/MnCl_2.4H_2O**$	5*	5**	-
$ZnSO_4.7H_2O*/ZnCl_2**$	5*	5**	-
$CaCl_2.2H_2O$	20	20	20
$NaCl$	30	30	30
$CoCl_2.2H_2O$	0.5	0.5	0.5
$CuCl_2.5H_2O$	1	1	1
H_3BO_3	1	1	1

*** Glucose concentration was decreased to 2500 mg/L (SWW-2) at MFC-16

Initially, SO_4^{2-} forms of Mg, Fe, Mn and Zn were preferred however, it was understood that following biodegradation SO_4^{2-} acts as an electron acceptor reducing the electron transfer efficiency to cathode. That's why, after preliminary trial experiments, Cl forms of Mg, Fe, Mn and Zn were started to be used.

Once the optimization experiments were completed, SWW use was ended and the sludge samples collected from Ankara Central WWTP and different industrial WWTPs were fed to anode chamber. The solids concentration of each substrate (sludge samples) was brought to same level via concentration or dilution so as to eliminate the impact of fouling and focus on the constituents that may inhibit or enhance power output of the system. Five different types of sludge samples collected from WWTPs were used at this point: (i) municipal wastewater (MWW) sludge, (ii) beverage IWW sludge, (iii) textile IWW sludge, (iv) petrochemical IWW sludge and (v) poultry sludge. All systems were inoculated with WAS from Ankara Central WWTP.

Throughout the MFC operations, the cathode chamber was filled with a mixture of 0.1 M PBS (25°C) at pH 8.0 and 80 mM NaCl solution.

3.3.5. Ultrasound Pretreatment

During the operation of municipal sludge-fed MFCs, it was decided to investigate the impact of ultrasound pretreatment on electron liberation and eventually on PD. The concentrated 250 mL sludge samples were subjected to ultrasonication inside 500 mL volume beakers placed in ice baths to eliminate the effect of heating due to vigorous sonication through the metal probe. Sartorius Labsonic P (Sartorius AG, Germany) was used to sonicate the sludge samples, with a 22 mm probe of 255 W sonication power. The details of the device are given in Table 3.2. The sonication times were 10 min and 20 min. The sludge used to inoculate the system was not sonicated in order to keep the microbial population alive.

Table 3.2. Features of Sartorius Labsonic P sonication device used in the experiments

Sonication Frequency	24 kHz
Probe Size	22 mm
Sonication Power	255 W
Sludge Volume	250 mL
Sonication Density	0.73 W/mL
Duration	10 and 20 min

3.3.6.Set-Up of Microbial Fuel Cells

Dual chamber MFCs are easier to control and operate compared to single chamber air-cathode systems and because of this important concern, in this study, dual chamber MFC was operated. The anode chamber of an MFC is the part that most affects the electron transfer efficiency within the system and it must be free of all sorts of potential electron acceptors. For this reason it was sealed and checked against any kind of gas and liquid leakage to make sure anaerobic conditions prevail inside. Cathode chamber, on the other hand, was exposed to atmospheric pressure with an air pump connected for aeration to provide a terminal electron acceptor, oxygen, to complete the reaction.

The system was operated at 25°C and insulated with teflon tape and non-conductive epoxy resin to prevent electron escape. Anode chamber was mixed using a magnetic stirrer to preserve homogeneity. The electrodes were submerged through the ports on top of each chamber using a copper wire insulated with plastic coating to prevent electron escape. The external resistance to the electrodes was supplied using a resistance decade box (Lutron RBOX 408) purchased from Lutron Electronic Enterprise Co., Ltd.. The current produced was measured via a digital multimeter

(8846 A Fluke Digital Multimeter, Netes Electronic, Ankara). The data was recorded continuously (15 min intervals) using a computer connected to the multimeter (Figure 3.2). The anode chamber was filled with SWW initially, during the optimization studies, and inoculated with WAS while the cathode chamber was filled with PBS and NaCl (80 mM) solution. The details of the reactor set-ups (electrode/membrane types, carbon source and PD) are given in Table 3.3 and Table 3.4.

Once the optimization studies were completed, the rest of the reactors (sludge-fed) were operated using carbon cloth as anode electrode and Pt loaded (0.5 mg/cm^2) carbon cloth as cathode electrode with CMI-7000 membrane in between. During this stage of experiments, the anode chamber was inoculated with WAS regardless of the type of sludge fed. Everything was kept same in the cathode chamber with PBS and NaCl solution as it was during the optimization experiments. The details of these reactors are given in Table 3.5.
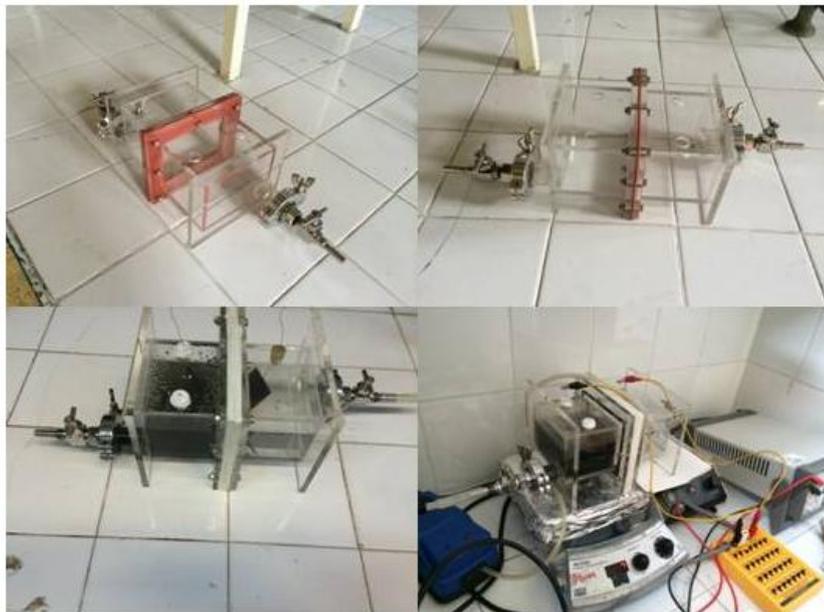
Figure 3.2. Dual chamber MFC reactor configuration and set-up details

Table 3.3. Details of MFC set-ups during preliminary experiments

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW
MFC-1	Glucose	C cloth	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	10,000	SWW-1
MFC-2	Glucose	C cloth	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Pt	10,000	SWW-2
MFC-3	Glucose	C cloth	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	10,000	SWW-2
MFC-4	Glucose	Wet proofed C cloth	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	10,000	SWW-2
MFC-5	Glucose	C paper	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	10,000	SWW-2
MFC-6	Glucose	C cloth (BIOFILM)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	10,000	SWW-2*
MFC-7	Glucose	C cloth (BIOFILM)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	10,000	SWW-2*

*Mg, Mn and Zn concentrations were halved.

Table 3.4. Details of MFC set-ups during optimization experiments

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW
MFC-8	Glucose	C paper	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	3000	SWW-2*
MFC-9	Glucose	C cloth (BIOFILM)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-2*
MFC-10	Glucose	C cloth (BIOFILM)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-2*
MFC-11	Sodium Acetate	C cloth (BIOFILM)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-2*
MFC-12	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3
MFC-13	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3
MFC-14	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3

*Mg, Mn and Zn concentrations were halved.

Table 3.4. Details of MFC set-ups during optimization experiments (cont'd)

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW
MFC-15	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3
MFC-16	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**
MFC-17	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**
MFC-18	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**
MFC-19	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**
MFC-20	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	300	SWW-2**
MFC-21	Glucose	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	300	SWW-2**
MFC-22	Glucose	C paper	C paper (0.4 mg/cm^2 Pt)	CMI-7000	Cu	300	SWW-2**

**Glucose concentration was halved.

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Table 3.5. Details of MFCs fed with different types of sludges

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	Inoculum
MFC-23	WAS	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	500	WAS
MFC-24	Sonicated WAS (10 min)	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	500	WAS
MFC-25	Sonicated WAS (20 min)	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	WAS
MFC-26	Poultry Sludge	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	2000	WAS
MFC-27	Beverage IWW Sludge	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	300	WAS
MFC-28	Petrochemical IWW Sludge	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	2000	WAS
MFC-29	Textile IWW Sludge	C cloth (BIOFILM)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	1500	WAS

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3.4. Experimental Set-Up of Laboratory Scale Anaerobic Digesters

The final stage of this study was to operate laboratory scale anaerobic digesters using the same MWW sludge in MFCs so as to compare these two technologies. These reactors were set-up using glass reactors with a working volume of 3.2 L and a total volume of 5 L (1.8 L headspace). Two replicate reactors were set-up and fed with concentrated WAS and ADS to achieve a food to microorganism ratio (F/M) of 1 g VS WAS/g VSS ADS (VS refers to volatile solids). The sludge concentrations were adjusted by settling and centrifuge (4000 rpm for 5 min). The details of the initial reactor set-up are given in Table 3.6.

Table 3.6. Initial pH, COD and solid measurements of the laboratory scale anaerobic digesters

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)
Reactor-1 (R-1)	7.32	27325	28135	16275
Reactor-2 (R-2)	7.50	26150	28190	16670

Prior to set-up, the reactors were subjected to sealing test, both gas and liquid, to make sure that the system was leak-proof and anaerobic. This test was carried out after sealing the connection points of the reactors with teflon tape and silicone, using soap and nitrogen gas. If there was any leak within the system points then bubbles should have formed when nitrogen flows through the reactor. Luckily, in this system, there were no leaks detected initially and in order to prevent any potential ones in the future, the reactors were sealed in layers every day of sampling since sometimes at high temperatures silicone might expand.

The top caps of the glass reactors were connected to glass graduated cylindrical gas collection units to measure the volume of methane produced each day. These gas collection units were placed in 10% NaCl (w/v) and 2% H_2SO_4 (v/v) solution to

prevent the dissolution of the gas produced and measure the volume of gas production accurately. The reactors were placed on magnetic stirrers to ensure homogeneity, otherwise, gas production would be inconsistent. Both reactors were operated in a constant temperature room of 35°C. Prior to operation, they were purged with nitrogen gas for 10 min to remove the oxygen within the system. The set-up is shown in Figure 3.3.

Figure 3.3. Reactor set-up including the gas collection unit

The sampling was done using the port at the bottom of the reactor using a plastic syringe which had an opening size same as the port so that air would not leak when placed. The biogas production was monitored using the graduated gas collection unit since as produced it pushed the brine solution down from point zero. After necessary measurements were done and samples were collected, a vacuum pump was used to revert the brine solution to its original level so that the next day new gas amount could be measured. The reactors were operated until the gas produced became negligible based on the cumulative gas production data. The operation lasted for 65

days and the parameters measured were solids concentration, COD, pH, gas volume and gas composition. During the first 12 days, daily sampling was done since the biogas production was high but then frequency of sampling decreased as daily changes became insignificant.

3.5.Analytical Methods

The parameters measured all throughout this study were total solids (TS), VS, TSS, VSS, COD, pH, gas volume and composition and voltage/current. Solids concentration (TS, VS, TSS, VSS), pH and COD were measured in both MFC and laboratory scale anaerobic digesters while voltage/current were measured for all MFCs (preliminary experiments, optimization experiments, substrate fed systems) and gas composition and volume measurements were done for only digester reactors. In this section, the above listed parameters and the associated measurement techniques will be described.

3.5.1.Solids Determination

In all reactors (MFCs and digesters) solids concentrations of the sludge fed and microbial inoculum were measured to make a sound comparison between each set. If the solids concentration was not adjusted then this would make another variable affecting the findings and comparing each set with each other would be impossible. TS and VS concentrations were analyzed using the Standard Method 2450B and 2540E, respectively. TSS and VSS concentrations were measured using Standard Method 2450D and 2540E, respectively (APHA, AWWA, WEF, 2005).

3.5.2.Chemical Oxygen Demand

COD was an important parameter in this study since it was used to determine the theoretical amount of electrons liberated in MFCs and the ratio of methane produced with respect to the amount of COD degraded during the operation of anaerobic digesters. Total COD of ADS, WAS and SWW samples were done using Hach LCK-514 COD kits (100-2000 mg/L O_2) and Hach DR3900 spectrophotometer (Hach Company, USA). All measurements were done after calibrating the device and in duplicates to monitor the accuracy of analyses.

3.5.3.pH Measurements

pH measurements were done for two different purposes: (i) to determine the time of membrane fouling in MFCs and (ii) to monitor the anaerobic transformation in digesters. The analysis was done according to Standard Method 4500+ using a pH meter of Cyberscan PC 510 and probe EC-PH 510/21S supplies from Eutech Instruments, Spain. Prior to each sampling, the pH meter was calibrated using standard solutions of pH 4, 7 and 10 (APHA, AWWA, WEF, 2005).

3.5.4.Gas Volume and Composition

For the anaerobic digesters, a graduated cylindrical gas collection unit was used to record the volume of biogas produced by measuring the displacement of brine solution. The composition, on the other hand, was determined using Agilent Technologies 6890N Gas Chromatograph with thermal conductivity detector (TCD). The column used was helium (29 cm/s). The oven program was as follows: 45°C for

1 min, from 45°C to 65°C at a rate of 10°C/min. The device was calibrated using two different calibration gas mixtures: (i) 65% methane, 25% carbon dioxide, 10% nitrogen and (ii) 25% methane, 55% carbondioxide, 20% nitrogen. All measurements were done in triplicates.

3.5.5. Voltage/Current Measurements

The electricity generated in each MFC was measured through voltage using 8856A/SU Fluke Digital Multimeter. The device was connected to the resistance box to measure the current in between the electrodes and the data was recorded continuously. The obtained data was then used to calculate the PD and coulombic efficiency (CE):

Equation (3.1) : Voltage (V) = Current (A) x Resistance (Ω)

Equation (3.2) : PD (mW/m^2) = A x V / Cross Section Area of Anode (m^2)

Equation (3.3) : $CE = \dfrac{MW \times \int_0^t I\,dt}{Fbv\Delta COD} \times 100$ (%)

$\quad\quad\quad\quad\quad$ Where, the integral function represents current integrated over time

$\quad\quad\quad\quad\quad\quad$ MW is the molecular weight of substrate (g/mole)

$\quad\quad\quad\quad\quad\quad$ F is Faraday's constant (96500 C/mole electron)

$\quad\quad\quad\quad\quad\quad$ b is moles of electrons released from the substrate

$\quad\quad\quad\quad\quad\quad$ v is the volume of anode chamber (0.45 L)

$\quad\quad\quad\quad\quad\quad$ ΔCOD is the amount of COD utilized (COD_i – COD_f) mg/L

CE is an important parameter in evaluating MFC performance and is the percentage of electrons recovered from substrate to the theoretical amount of electrons liberated (Çatal et.al., 2008). For optimization sets the MW was 180 g/mole for glucose and the moles of electrons liberated were 24 based on stoichiometry (see Reaction 2.3, Chapter 2).

3.5.6. Polarization Curve and Internal Resistance Calculations

Internal resistance (IR, R_{int}) was an important parameter in this study since it is an indicator of system performance in MFCs. If IR value is small and close to the external resistance applied then it shows that the system works close to its optimum level. Membrane fouling, microbial activity, material performance and many more parameters that are effective on MFC operation can be effectively compared using internal resistance. In order to maximize the PD in an MFC, first internal resistance must be determined and this can be done by plotting a polarization curve. Polarization curve is an essential tool that helps to calculate IR by measuring the voltage and CD (A/m^2) with respect to different external resistance values (R_{ext}). Different regions on the polarization curve describe different types of losses within the system that impede performance. A typical polarization curve is given in Figure 3.4. Here, the rapid drop following OCV (E^0_{cell}, black dot on Figure 3.4) indicates the activation losses which are due to the energy lost during electron transfer to the anode electrode surface (Region 1-rapid voltage losses, low current). These losses can be lowered by increasing Pt catalyst concentration on cathode electrode or adding mediator.

The second region on the polarization curve (Figure 3.4), region of constant voltage drop), represents ohmic losses, which arise from proton accumulation in anolyte, membrane fouling and problems in electron transfer between the electrodes (current flow). These losses can be minimized by changing the type of membrane, increasing its lifetime using different preconditioning methods or choosing electrodes with low electrical resistivity. The slope of this region actually gives us the internal resistance.

The third region of the curve (Figure 3.4, rapid voltage drop, high current) shows concentration or mass transfer losses which occur when the flux of reactants to the electrode or the flux of product from the electrode are insufficient and limit the rate of reaction. The flow of protons from anode to cathode chamber is mainly responsible for this since the accumulating protons affect bacterial metabolism

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negatively and electron transfer. To overcome these losses, buffer solutions are usually used but this is only effective to a certain extent since after a certain H^+ concentration, electron transfer is inhibited.
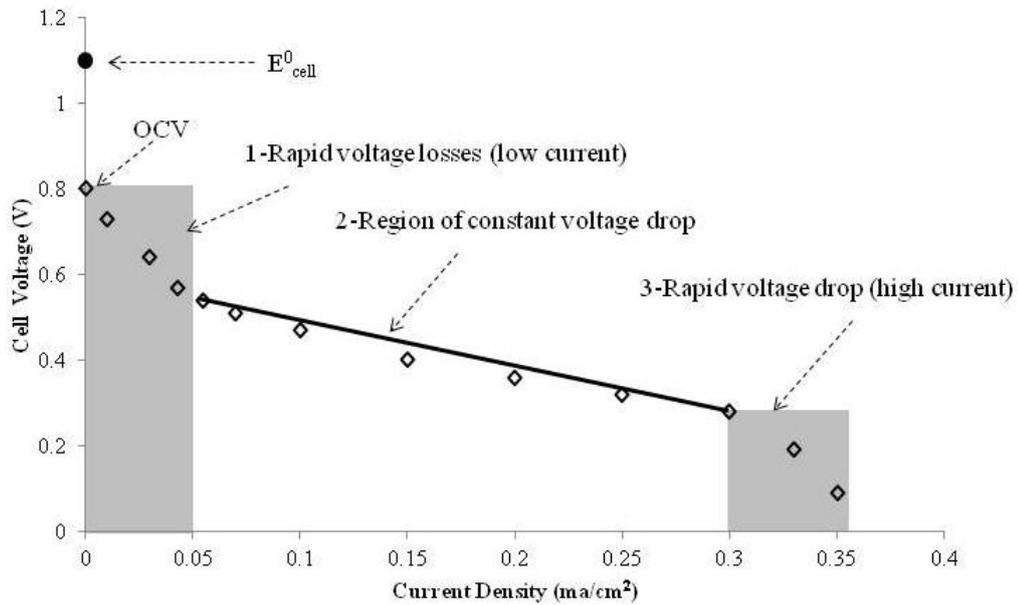
Figure 3.4. Characteristics of a typical polarization curve (drawn with modifications after Logan, 2008)

As stated before, the slope of the second region gives us the internal resistance and to enhance the performance and maximize PD, this value must be reduced. But in addition to this, there also exists an empirical formula to calculate the internal resistance which is sometimes found to be more reliable in scientific circles (Logan, 2008):

Equation (4.1): $\dfrac{V}{OCV} = \dfrac{Rext}{(Rext+Rint)}$ where V is the voltage measured at maximum PD

CHAPTER IV

RESULTS AND DISCUSSION

4.1.Microbial Fuel Cell Operation

The aim of this study was to investigate the impact of different sources of wastewater sludges on energy production potential of an MFC using mixed microbial cultures. Although the use of different sludges as substrate (energy sources) in an MFC is an important advantage, depending on the constituents, sometimes the energy production may be enhanced, sometimes inhibited. For this reason, type of substrate is one of the crucial operational parameters affecting the PD. In this study, a dual chamber MFC was fed with sludges from: (i) beverage industry WWTP, (ii) a poultry industry WWTP, (iii) a textile factory WWTP, (iv) a petrochemical industry WWTP and (v) a municipal WWTP (MWWTP) (serves as baseline).

Although the main objective of this study was to investigate the impact of substrate type on MFC performance, as in any other biological reactor operation, MFC set-up and operational conditions had to be optimized first. The parameters tested during the optimization studies were: (i) membrane (PEM/CEM), (ii) anode/cathode electrode material, (iii) mediator, (iv) wire material (Pt or Cu), (v) Pt concentration and (vi) external resistance (Ω). None of these parameters are independent of each other since MFCs work in a systematic way through the interaction of each parameter. For instance, if the mediator is not effective at transferring the electrons, then the quality of anode electrode does not enhance the current flow. Therefore, each parameter must be in harmony with the rest to obtain the optimum results. That's why, set of detailed optimization experiments are necessary.

4.1.1.Preliminary Microbial Fuel Cell Experiments

The optimization studies were divided into two as: (i) preliminary MFC operation and (ii) MFC optimization studies. The reason behind this was the difference in the contents of optimization experiments. Initially, the experience on MFC operation was limited and it was thought that the system can only be optimized by testing different materials. That's why the variable of concerns were anode and cathode electrode materials, wire connecting the electrodes and SWW constituents during preliminary experiments and the findings were compared based on PD only to progress faster. During the optimization experiments, on the other hand, based on the data of preliminary experiments, it was decided to focus on operational parameters and system design rather than materials. This was necessary in order to enhance microbial activity and minimize electron loss.

The first reactor (MFC-1) was set-up using SWW-1 and glucose as the carbon source. The anode and cathode electrodes were plain carbon cloth and Pt coated carbon cloth (0.5 mg/cm^2), respectively, and Cu wire (0.5 mm in diameter) was used to connect them. Nafion 117 was used as membrane and an external resistance of 10,000 ohms was supplied to the system. Initial measurements prior to the set-up of MFC-1 are given in Table 4.1. For this system only, the initial COD of ADS used as microbial inoculum was very high since it was set-up using a very concentrated sludge.

Table 4.1. Initial pH and COD measurements (t=0 day) for MFC-1

	pH			COD (mg/L)		
	SWW	ADS	Mix	SWW	ADS	Mix
Analysis-I	7.30	8.40	7.50	8470	35450	14670
Analysis-II	7.40	8.40	7.40	8015	35810	14260
Average	7.35	8.40	7.45	8242.5	35630	14465

The pH of the SWW was set to approximately 7.4 using PBS and because of this the pH was not affected when mixed with ADS. The pH of the mixture was within the range of optimum pH range 6.5-8.2 for microbial activity (Speece, 1996). Microbial degradation and electron transfer in anode was very important and that's why keeping pH and temperature suitable for microorganisms was crucial in this study.

Although SWW was fed to MFC system and solids concentration became negligible when inoculum was added, still TS, VS, TSS and VSS were measured to understand the content of inoculums better and decide the inoculation volume (Table 4.2).

Table 4.2. Initial solids concentration measurements (t=0 day) for MFC-1 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	41700	21450	28050	15990
Analysis-II	40880	21520	28170	15920
Average	41290	21485	28110	15955

Throughout the reactor operation, COD and pH (of the anolyte) were measured based on the voltage production trend. The first measurement was done when the acclimation period was over and after voltage production reached its maximum level (t=4 days). Then on days 8, 12 and 18, same measurements were repeated as the voltage declined and became stable (Table 4.3).

Table 4.3. pH and COD measurements for MFC-1 during operation

	t = 4 days		t = 8 days		t =12 days		t = 18 days	
	pH	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**
Analysis-I	7.20	8240	6.90	6310	6.60	5920	6.80	5870
Analysis-II	-	8310	-	6270	-	5970	-	5750
Average	7.20	8275	6.90	6290	6.60	5945	6.80	5810

The most important parameter in this was voltage and it was measured continuously using a multimeter. The voltage is not presented directly; it was converted to PD to make comparison easier between the reactors (normalizing the data based on the external resistance and surface area of electrode). The PD vs. time (min) is presented in Figure 4.1.
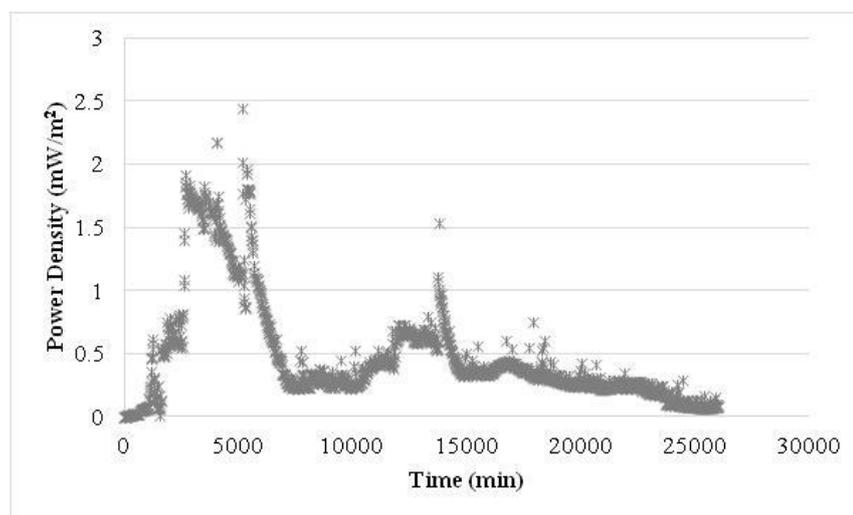
Figure 4.1. Power density (mW/m^2) with respect to time (min) graph for MFC-1

MFC-1 was operated for 18 days. The acclimation period lasted for 1 day, and PD started to increase immediately afterwards. This period was short since the organic matter was readily available and soluble in the form of glucose to be easily taken up by the microorganisms. The maximum PD was measured on 3.5 days as 2.44 mW/m^2 with a voltage of 191 mV. At this point the COD reduction was 42.8%. After the 4th day of operation, the voltage output remained stable around 150-170 mV and at day 5.5 voltage started to drop to 60 mV and remained stable. The reason for this decrease could be the fast uptake of readily available organic matter within the first couple of days, resulting in membrane fouling due to high concentration of H$^+$ ions released. Besides this amount of COD reduction is not expected in anaerobic systems within such short time interval, therefore, the system may have faced anoxic or aerobic conditions due to SWW constituents (SO$_4^{-2}$) or air leak during set-up or

sampling. CE(%) also seemed to support this since it was calculated as 0.02%, which indicates that the electrons released by the oxidation of glucose (Reaction 2.3) was not transferred to cathode electrode and was either taken up another electron acceptor within the anode chamber or Cu wire was unsuccessful in the transfer (conductivity problem). This finding actually once proved the reason why anode chamber must be kept strictly anaerobic. Since the voltage production did not increase afterwards, the reactor was terminated and the COD reduction at this point was 59.8%. Unfortunately, this MFC operation did not give much clue about the system kinetics or optimization of the operational parameters.

Considering the drawbacks during the MFC-1 operation, MFC-2 was set-up, after testing the gas and liquid leakage prior to operation. In MFC-2, membrane and anode/cathode electrodes were kept the same, but this time, the SWW content changed (SWW-2 was used) to eliminate any potential electron acceptors (increase anode potential), chlorinated forms of each chemical were used instead of SO_4^{-2}. In addition to this, Pt wire was used instead of Cu. The initial pH, COD and solids concentration measurements are given in Table 4.4 and Table 4.5.

Table 4.4. Initial pH and COD measurements (t=0 day) for MFC-2

	pH			COD (mg/L)		
	SWW	ADS	Mix	SWW	ADS	Mix
Analysis-I	7.43	7.57	7.56	7320	23750	8010
Analysis-II	7.40	7.57	7.55	7940	23550	7920
Average	7.42	7.57	7.56	7630	23650	7965

Table 4.5. Initial solids concentration measurements (t=0 day) for MFC-2
(inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	19760	10280	17780	9700
Analysis-II	20250	11540	16980	8990
Analysis-III	20720	11600	17020	9530
Analysis-IV	20170	10480	17100	9240
Average	20225	10975	17220	9365

During the operation of MFC-2, the part of the Pt wire that was in direct contact with the SWW and ADS mixture ruptured. That's why the voltage was not monitored for a long time, even the acclimation period was not completed. The final data obtained did not make much sense due to this problem during the operation.
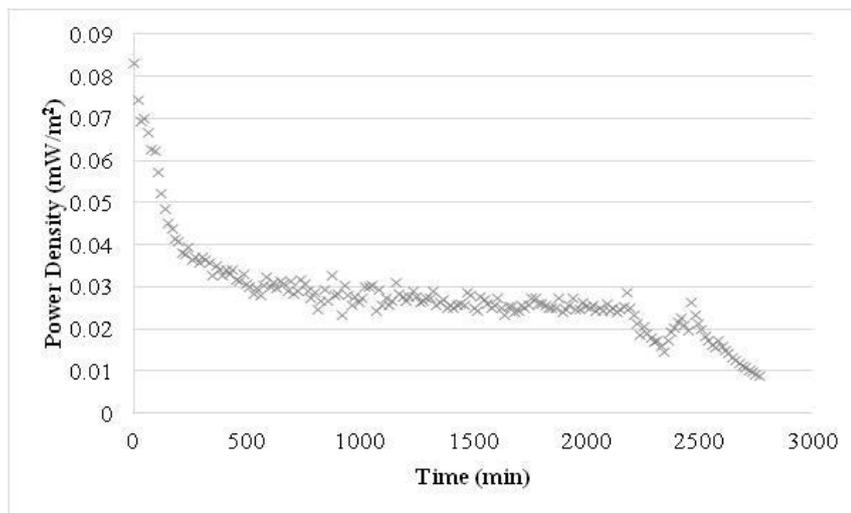
Figure 4.2. Power density (mW/m^2) with respect to time (min) graph for MFC-2

Following the operation of MFC-2, using the same ADS sample and SWW (SWW-2) prepared for MFC-2, MFC-3 was set-up (Table 4.4-4.5). In this system, everything were the same with the MFC-1 (anode/cathode electrode, membrane, Cu wire, same sludge was used so COD and VS values were also same). In this system, the

acclimation period lasted for 3.5 days, which was an indicator of persistence of anaerobic conditions, unlike MFC-1. The voltage production started to rise afterwards with a constant external resistance of 10,000 ohms. The graphical representation of PD is given in Figure 4.3. As can be seen from the figure, the maximum PD measured was 5.54 mW/m^2 at 4.5 days (288.2 mV) which was almost about the twice of the value obtained during the first MFC operation. The COD reduction at this point was 16% (Table 4.6).
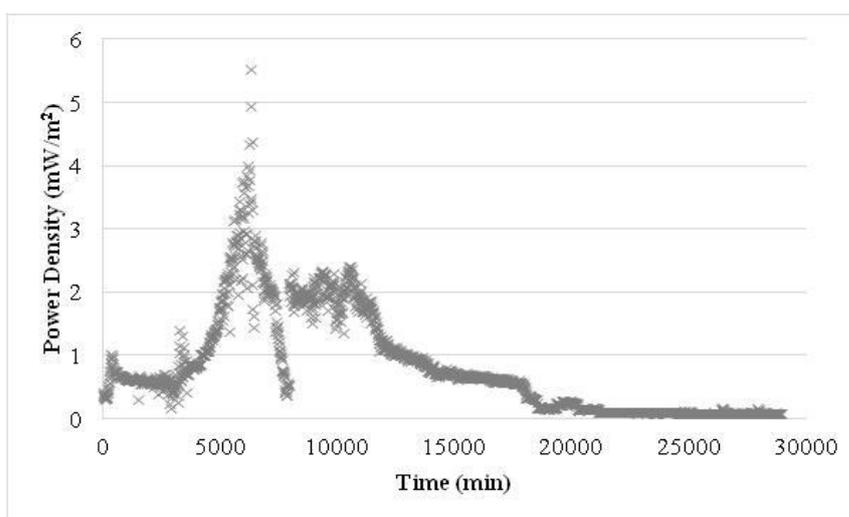
Figure 4.3. Power density (mW/m^2) with respect to time (min) graph for MFC-3

Table 4.6. pH and COD measurements for MFC-3 during operation

	t = 4 days		t = 8 days		t =12 days		t = 18 days	
	pH	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**
Analysis-I	7.43	6780	6.98	5550	6.81	3900	6.70	3110
Analysis-II	-	6600	-	5460	-	3710	-	3170
Average	7.43	6690	6.98	5505	6.81	3805	6.70	3140

This high voltage couldn't be pursued for long periods of time and declined immediately. At this point the pH of the anolyte solution also decreased (Table 4.6). This was an indicator of membrane fouling since the protons present in the anolyte

solution couldn't pass through Nafion 117 due to the accumulation of rest of cations resulting from microbial degradation. This increased internal resistance and prevented the system from reaching its potential. Unfortunately, CE (%) of this system was also very low, 0.08% at peak PD, but then stabilized to 0.06% with a total COD reduction of 60%, similar to MFC-1. This also showed that sampling results in air leak, which in return, decreases CE since the system starts to act aerobically.

MFC-4 was operated using different materials, since the power density and CE of the previous systems were lower than the values observed in previous research (see Table 2.1). In this reactor, wet-proofed (30%) carbon cloth and Pt-coated carbon cloth (0.5 mg Pt/m^2) were used as anode and cathode electrode, respectively. The initial pH, COD and solids concentration measurements are given in Table 4.7 and Table 4.8.

Table 4.7. Initial pH and COD measurements (t=0 day) for MFC-4

	pH			COD (mg/L)		
	SWW	ADS	Mix	SWW	ADS	Mix
Analysis-I	7.50	7.73	7.54	7740	17850	7830
Analysis-II	7.49	7.70	7.50	7200	18350	7500
Average	7.50	7.72	7.52	7470	18100	7665

Table 4.8. Initial solids concentration measurements (t=0 day) for MFC-4 (inoculum-ADS)

	Concentration (mg/L)			
	TS	VS	TSS	VSS
Analysis-I	18770	10700	15460	9080
Analysis-II	19380	12010	16480	9620
Analysis-III	18220	10590	16520	9900
Analysis-IV	20020	12210	15620	9300
Average	19110	11377.5	16020	9475

This reactor set was operated for 17 days, and the maximum voltage and PD measured were 196.2 mV and 2.57 mW/m^2 which were still not satisfactory compared to the previous sets (Figure 4.4). The maximum PD was reached on 6th day, which was around 4th day for the previous sets. It was thought that, since the constituents of wastewater and sludge are corrosive, non wet-proof plates would have a longer lifetime and could produce higher power densities. But this was not the situation.
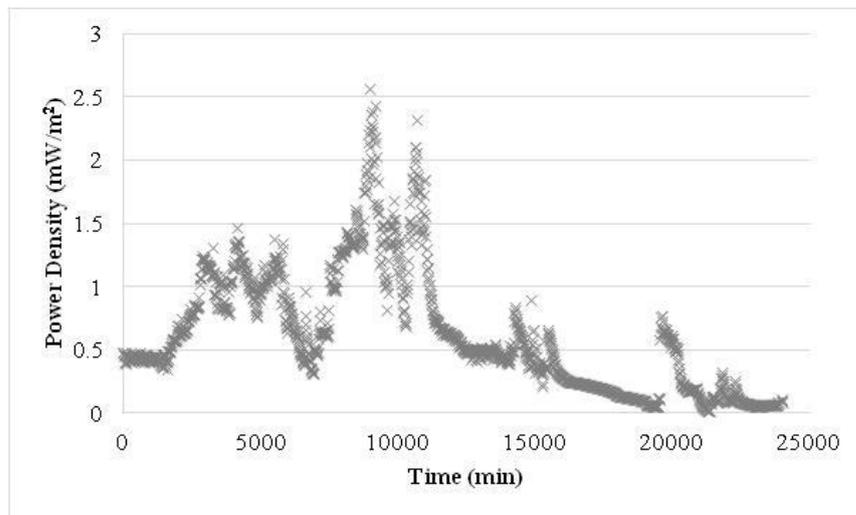
Figure 4.4. Power density (mW/m^2) with respect to time (min) graph for MFC-4

In this set there occured a number of peaks which were very close to each other. This was due to the problem in the magnetic stirrer, which was replaced with a new one during reactor operation but resulted in a declined voltage output for 1.5 days due to the sedimentation of sludge inoculum and heterogeneity of the anolyte solution. During the operation of MFC-4, there occurred fluctuations in the voltage production, and it was due to the change in temperature. Unfortunately, due to the reactor geometry and need for magnetic stirrer, it was impossible to use hot water bath to keep the temperature constant and also due to the fragile characteristic, it couldn't be placed in the constant temperature room. That's why, the temperature was tried to be kept constant using air conditioner and heater but the reactor

operation was still affected from the low temperatures during night time. Once, the impact of temperature on power output was realized, the impact of temperature on voltage output was tried to be minimized and the room temperature was kept between 23-25°C. Once the stirrer was replaced, the voltage and PD increased (second peak, Figure 4.4), but again declined (although organic matter degradation continued) due to the fouling of the membrane and accumulation of H^+ in the anode chamber increasing pH.

Table 4.9. pH and COD measurements for MFC-4 during operation

	t = 4 days		t = 8 days		t =12 days		t = 17 days	
	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.61	6780	6.91	4890	6.74	4000	6.78	3460
Analysis-II	-	6690	-	5130	-	3840	-	3210
Average	7.61	6735	6.91	5010	6.74	3920	6.78	3335

The time at which maximum PD was measured the COD reduction was approximated via interpolation (since measurement was not done on that day) as 23.4% which was much higher than that of the previous three reactors. The reason for this could be the change in the anode electrode since its surface characteristic was different; it may have decelerated the electron transfer within the anolyte solution. For this reason, the time to reach maximum PD was longer. The overall COD reduction of the system (when the reactor terminated) was 56.5% similar to the rest of the reactor operations. The CE (%) was calculated as (overall) 0.05% which was lower than MFC-3.

MFC-5 was operated using carbon paper as anode and Pt coated carbon paper as cathode electrode (0.4 mg Pt/cm^2). The external resistance was 10,000 ohms and Cu wire was used to connect the electrodes. Carbon source was glucose and SWW-2 mixture was added as substrate. The membrane separating the chambers was Nafion 117 like the rest. The initial measurements are given in Table 4.10 and Table 4.11.

Table 4.10. Initial pH and COD measurements (t=0 day) for MFC-5

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.60	7.31	7.45	7530	18900	8280
Analysis-II	7.51	7.28	7.46	7740	19440	7620
Average	7.55	7.30	7.46	7635	19170	7950

Table 4.11. Initial solids concentration measurements (t=0 day) for MFC-5 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	20288	13390	15336	8664
Analysis- II	20616	13730	16861	9464
Analysis-III	21256	14130	17088	8904
Analysis-IV	20904	13920	16668	8712
Average	20766	13792.5	16488.3	8936

In MFC-5 both electrodes and Pt loading were different and in this set the acclimation period was longer than the rest when PD was analyzed (Figure 4.5). This does not solely mean that glucose was not biodegraded by microorganisms, since PD graph gives us an idea on electron transfer, the long acclimation period can be interpreted as the electron transfer rate due to different electrode materials. The time required for biofilm formation on carbon paper was longer compared to carbon cloth. In direct contact with wastewater, carbon paper is brittle and the surface is less porous which inhibits microbial attachment and biofilm formation.
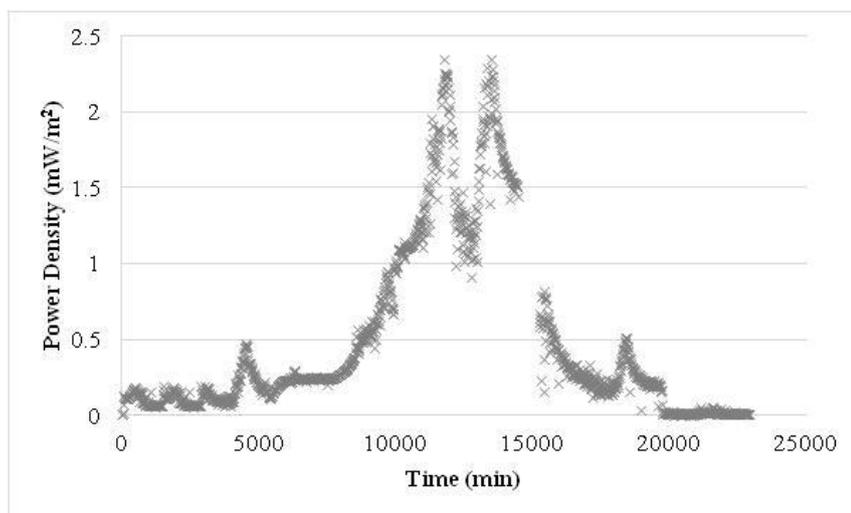
Figure 4.5. Power density (mW/m^2) with respect to time (min) graph for MFC-5

The maximum voltage and PD measured for this set were 188 mV and 2.36 mW/m^2 on day 8 which was twice the time when maximum PDs were observed for MFC-1 and MFC-3. Just like MFC-4, the reason for this was the different surface characteristics of the electrodes which slowed down the electron transfer. The CE (%) was calculated 0.04% similar to MFC-4 but lower than that of MFC-3. The overall COD reduction was 57% (Table 4.12).

Table 4.12. pH and COD measurements for MFC-5 during operation

	t = 4 days		t = 8 days		t =12 days		t = 16 days	
	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.31	7080	6.79	5080	6.65	3900	6.57	3390
Analysis-II	-	6930	-	4720	-	4080	-	3450
Average	7.31	7005	6.79	4900	6.65	3990	6.57	3420

On day 10, the voltage produced couldn't be measured due to an upset in the multimeter function (Figure 4.5). The programme in the multimeter required update which resulted in the loss of connection between the device and the computer. After the set-up of the multimeter from scratch, the problem was solved. The similar

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temperature fluctuations, as in MFC-4, were also observed in the voltage production graphs of MFC-5 (close peaks).

In all five systems, for the first couple of days, the voltage production was low which was due to the bacterial acclimation period. This period lasted between 1 to 5 days depending on the speed of biofilm formation (anode electrode surface structure) and how fast the microorganisms transfer the electrons to anode surface. Once, maximum value was reached, voltage production started to decline which was thought to be due to the fouling of the membrane. Once the membrane surface is filled with protons and some of the cations present in the synthetic wastewater, H^+ remained in the anolyte solution, decreasing the pH and voltage produced eventually since the electrons no longer transported to the anode surface but react with H^+.

Considering the importance of microbial attachment on anode electrode for fast and efficient transfer of electrons, in MFC-6, carbon cloth (plain, non wet-proofed, anode) was submerged in a brown bottle (to minimize the intrusion of light) containing ADS and SWW-2 solution for 3 days at 35°C to enhance biofilm formation on the surface. This time, the $MgCl_2.6H_2O$, $MnCl_2.4H_2O$ and $ZnCl_2$ were halved to 50 mg/L, 2.5 mg/L and 2.5 mg/L, respectively, to decrease the interference of cations with Nafion 117 surface. The resistance was still kept as 10,000 ohms and Cu wire was used to connect anode electrode to carbon paper (0.4 mg Pt/cm^2) cathode electrode. The initial measurements are given in Table 4.13 and Table 4.14.

Table 4.13. Initial pH and COD measurements (t=0 day) for MFC-6

	pH			COD (mg/L)		
	SWW	ADS	Mix	SWW	ADS	Mix
Analysis-I	7.54	7.20	7.51	6710	20800	7180
Analysis-II	7.51	7.20	7.50	6560	22150	6900
Average	7.53	7.20	7.51	6635	21475	7040

Table 4.14. Initial solids concentration measurements (t=0 day) for MFC-6 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	16900	11780	13980	6980
Analysis-II	17200	11100	12080	6800
Analysis-III	16800	10450	11000	6090
Analysis-IV	17340	11870	13240	7150
Average	17060	11300	12575	6755

The maximum voltage and PD measured in this set were 183.2 mV and 2.24 mW/m^2, respectively (Figure 4.6). This reactor was operated for a much shorter time (3 days) and the acclimation period did not even lasted a day long, it was hours and the maximum PD was measured at 1st day of operation. This was due to the presence of biofilm on anode electrode surface, and this is a certain proof of the necessity of microbial attachment for electron transfer. But the maximum PD was still low showing that solely biofilm formation was still not satisfactory enough to boost the performance of the system.
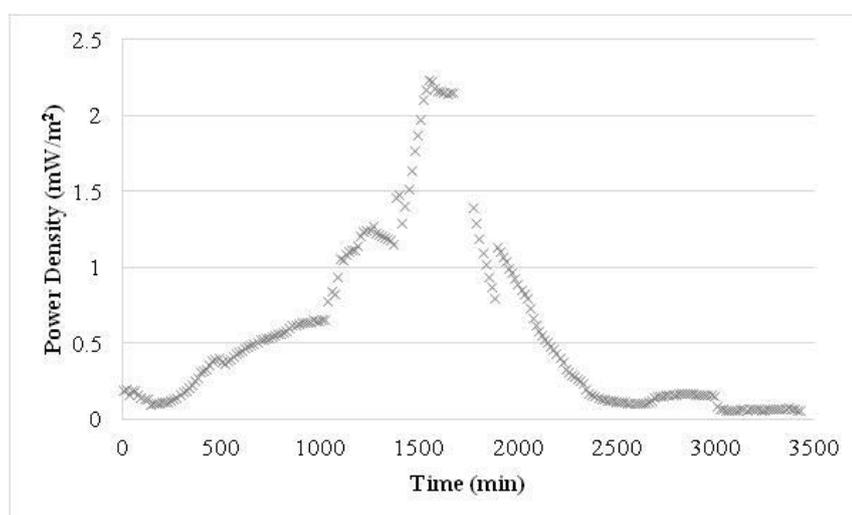
Figure 4.6. Power density (mW/m^2) with respect to time (min) graph for MFC-6

The overall COD reduction was 24% since the reactor was terminated in a very short time interval (Table 4.15). In the previous sets, even if the PD stayed stable, the system was operated to see if there would be any changes. That's why, previously, pH used to drop together with COD reduction, H^+ liberation and fouling of the membrane. But in order to move fast and test the rest of the parameters in time, it was decided to check the PD and voltage and terminate the system to move on. The CE (%) calculated for MFC-6 was 0.017%, probably because the reactor was terminated too early (1/6 of the time), since while calculating CE, current is integrated over time and if the duration of operation is longer, much more current passes, increasing the CE. If the reactor was assumed to be operated for 18 days like the previous six sets and the current remained the same for the rest of the operation with a COD removal of about 55%, then the CE could reach 0.05%. Therefore, the operational cycle (min) is also important in calculating the CE.

Table 4.15. pH and COD measurements for MFC-6 during operation

	t = 1.5 days		t = 3 days	
	pH	**COD (mg/L)**	**pH**	**COD (mg/L)**
Analysis-I	7.46	5930	7.41	5510
Analysis-II	-	6010	-	5210
Average	7.46	5970	7.41	5360

MFC-7 was similar to MFC-6 except for the NaCl addition to cathode chamber together with PBS. The initial measurements are given in Table 4.16 and Table 4.17.

Table 4.16. Initial pH and COD measurements (t=0 day) for MFC-7

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.49	7.12	7.47	6410	19250	7220
Analysis-II	7.50	7.06	7.47	7030	18550	6960
Average	7.50	7.09	7.47	6720	18900	7090

Table 4.17. Initial solids concentration measurements (t=0 day) for MFC-7 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	18350	13050	13640	6300
Analysis-II	18010	13560	13240	6460
Analysis-III	16990	13000	13900	7300
Analysis-IV	18340	13330	12980	6180
Average	17922.5	13235	13440	6560

The maximum voltage and PD measured were 281.2 mV and 5.27 mW/m^2, respectively, which were close to the values obtained in MFC-3 (Figure 4.7). But in this system, the distribution over time was different. The reactor kept transferring current at significant levels compared to peak for a day. Therefore, it could be stated that the addition of NaCl solution was definitely effective on the electron transfer.
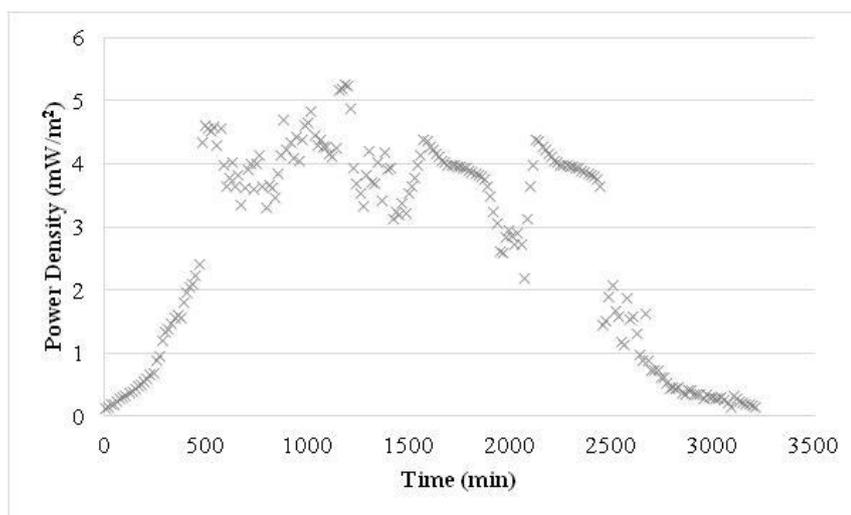
Figure 4.7. Power density (mW/m^2) with respect to time (min) graph for MFC-7

Just like MFC-6, this system was also operated over a very short period of time. That's why, a significant pH drop was not observed since before membrane fouling started to rise H^+ concentration. The overall COD reduction was 29.5%, slightly

higher than MFC-6, probably because the temperature of the room where the reactor operated was adjusted at 25°C and stabilized using external heaters.

Table 4.18. pH and COD measurements for MFC-7 during operation

	t = 1 days		t = 2 days	
	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.46	5410	7.42	5420
Analysis-II	-	6150	-	5000
Average	7.46	5780	7.42	5210

Since, the power densities of the reactors were in the range of 2.2-5.5 mW/m^2, which was far from the values obtained in the literature (Table 2.1, Chapter 2), it was decided to focus on the microbial activity (circumstances to enhance microbial degradation and electron transfer) and insulation of the MFC to keep the free electron transfer limited between anode and cathode. In addition to these, it was thought that H$^+$ ions couldn't pass through the membrane not only because of the accumulation of cations on the surface but also due to the glassification of the active sites of Nafion 117 at 80°C pretreatment. The H$^+$ remaining in the anolyte solution tend to interact with the free electrons and decrease power output. Considering all these, some changes have been made during the set-up of each MFC and this made up the second part.

Table 4.19. Results of MFC set-ups during preliminary experiments

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW	PD (mW/m²)	Changes
MFC-1	Glucose	C cloth	C cloth (0.5 mg/cm² Pt)	Nafion 117	Cu	10,000	SWW-1	2.4	-
MFC-2	Glucose	C cloth	C cloth (0.5 mg/cm² Pt)	Nafion 117	Pt	10,000	SWW-2	-	SWW-2 was used (chlorinated forms).
MFC-3	Glucose	C cloth	C cloth (0.5 mg/cm² Pt)	Nafion 117	Cu	10,000	SWW-2	5.5	Cu wire was used instead of Pt.
MFC-4	Glucose	Wet-proofed C cloth	C cloth (0.5 mg/cm² Pt)	Nafion 117	Cu	10,000	SWW-2	2.6	Wet-proofed anode electrode was used.
MFC-5	Glucose	C paper	C paper (0.4 mg/cm² Pt)	Nafion 117	Cu	10,000	SWW-2	2.4	Both electrodes were changed to C paper.
MFC-6	Glucose	C cloth (Biofilm)	C paper (0.4 mg/cm² Pt)	Nafion 117	Cu	10,000	SWW-2*	2.24	Biofilm formed on anode electrode. Mn, Mg and Zn concentrations were halved.
MFC-7	Glucose	C cloth (Biofilm)	C paper (0.4 mg/cm² Pt)	Nafion 117	Cu	10,000	SWW-2*	5.47	20 mM NaCl solution was added to anolyte.

*Mg, Mn and Zn concentrations were halved.

4.1.2. MFC Optimization Experiments

Preliminary studies were the part of this research where the system was thought to be enhanced only by testing different electrodes or wire materials. However, the results showed that there could be other factors behind the performance of an MFC. That's why, the first 7 reactors are called preliminary sets since the experience and understanding on the system was limited during their operation. Following the preliminary reactor operations, operational changes were made to prevent electron loss and enhance microbial degradation and electron transfer. These changes can be listed as:

- Insulation of the stainless steel sampling ports using teflon tape (outside) and non-conductive epoxy resin (inside).

- Insulation of the wire connecting the anode and cathode electrodes using plastic coating.

- Insulation of the connection points on the top of chambers of the reactor using plastic stoppers, non-conductive epoxy resin and teflon tape.

- Change in the pretreatment temperature of membranes (both Nafion 117 and CMI-7000) to make sure that surface properties were preserved and membrane fouling was delayed.

- Increase in the concentration of NaCl solution in cathode chamber since during preliminary studies it was shown that it had a positive impact on the electrical conductivity and power output.

- Decrease in the concentration of $MgCl_2.6H_2O$, $MnCl_2.4H_2O$ and $ZnCl_2$ so that they won't occupy the active sites of the membranes, especially CMI-7000, or act as an alternative electron acceptor.

- Decrease in the concentration of glucose (carbon source) so as to decelerate H^+ release and prevent instantaneous fouling of the membrane (increase membrane lifetime, therefore, cell lifetime).

- Addition of vitamin and mineral solution to the anolyte to improve microbial activity both in terms of biodegradation and electron transfer.

- Use of WAS instead of ADS as microbial inoculum since it was believed to be more active and diverse microbial community (WAS) would be more effective compared to a stabilized group of microorganisms (ADS) during electron transfer.

- Decrease in the external resistance since resistance and current flow are inversely proportional to each other and if external resistance was reduced it was expected to reach the optimum potential.

Based on these changes, several MFCs were operated. First change was the lowered external resistance during the operation of MFC-8 from 10,000 ohms to 3000 ohms. MFC-8 was set-up using SWW-2 but $MgCl_2.6H_2O$, $MnCl_2.4H_2O$ and $ZnCl_2$ concentrations halved. Anode and cathode were both carbon paper. NaCl solution concentration was increased to 50 mM since it was shown to have a positive impact on the system. The initial measurements are given in Table 4.20 and Table 4.21.

Table 4.20. Initial pH and COD measurements (t=0 day) for MFC-8

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.57	7.25	7.56	6960	21000	7550
Analysis-II	7.60	7.18	7.56	7240	20540	7680
Average	7.59	7.22	7.56	7100	20770	7615

Table 4.21. Initial solids concentration measurements (t=0 day) for MFC-8 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	14940	8706.7	13420	7420
Analysis-II	14993.3	8466.7	13780	7480
Analysis-III	15020	8420	13760	7680
Analysis-IV	14640	8140	-	-
Average	14898.3	8433.4	13653.3	7526.7

The maximum voltage and PD measured were 161.7 mV and 5.81 mW/m^2, respectively, which was unacceptable considering the outputs of the previous studies (Figure 4.8). It was understood that, without a mediator, it was a must to form a biofilm on anode electrode. From this point on, it was decided to continue using carbon cloth as anode electrode and form biofilm on the surface prior to reactor operation. Similar to MFC-7, the reactor kept transferring current at significant levels compared to peak for a day.
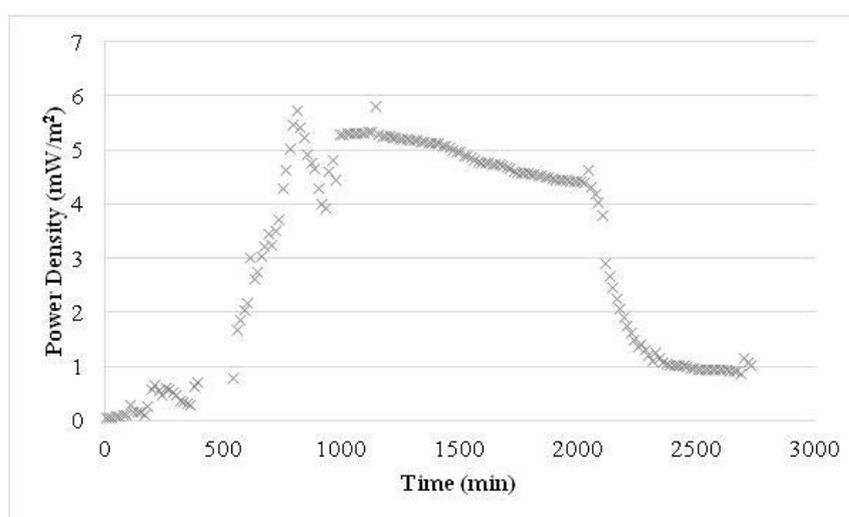
Figure 4.8. Power density (mW/m^2) with respect to time (min) graph for MFC-8

This system was again operated for a very short time to see the impact of the change immediately and move on to the next parameter. The COD reduction was 28.8% similar to MFC-7, after fixing the temperature problem. Due to the length of operation, a significant pH drop was not observed since before membrane fouling started to rise H^+ concentration (Table 4.22).

Table 4.22. pH and COD measurements for MFC-8 during operation

	t = 1 days		t = 2 days	
	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.54	5960	7.53	5430
Analysis-II	-	6460	-	5410
Average	7.54	6210	7.53	5420

Following MFC-8, since the PD was still not satisfactory, it was decided to go back to carbon cloth as anode electrode since during preliminary studies it was shown that biofilm formation certainly had a positive impact on the power output of the system. In addition to this, the external resistance was further reduced to 1000 ohms from 3000 ohms, since resistance and current inversely affect each other. NaCl concentration was kept the same at 50 mM. The cathode electrode was again carbon paper (0.4 mg Pt/cm^2). This way MFC-9 was started. The initial measurements are given in Table 4.23 and Table 4.24.

Table 4.23. Initial pH and COD measurements (t=0 day) for MFC-9

	pH			COD (mg/L)		
	SWW	ADS	Mix	SWW	ADS	Mix
Analysis-I	7.55	7.34	7.55	7010	25920	7340
Analysis-II	7.55	7.27	7.52	7140	25200	7400
Average	7.55	7.31	7.54	7075	25560	7370

Table 4.24. Initial solids concentration measurements (t=0 day) for MFC-9
(inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	20120	13560	15600	9940
Analysis-II	18960	14220	13880	8680
Analysis-III	20950	15080	13800	8020
Analysis-IV	19530	14250	14780	9200
Average	19890	14277.5	14515	8960

The maximum voltage and PD measured during the operation of MFC-9 were 107.1 mV and 7.71 mW/m^2, respectively (Figure 4.9). There was almost no acclimation period since during biofilm formation this time the electrode was kept in the SWW+ADS containing bottle for 1 week, and this probably enhanced both biofilm formation and the familiarity of microorganisms to the existing medium. This MFC run showed that both biofilm formation and decreased external resistance had positive impact on PD since both enhance current flow. The PD dropped gradually, with continuous current flow, probably due to increased conductivity and microbial transfer. After the 4th day of reactor operation, PD decreased sharply due to membrane fouling which can be also supported with the decline in pH (Table 4.25). The overall COD removal at the end of reactor operation (day 7) was 42.9% and CE (%) was 0.2% which was almost 10 times higher than the value calculated for preliminary sets. This certainly showed that the efficiency of electron transfer (in other words current flow) improved with the changes made.
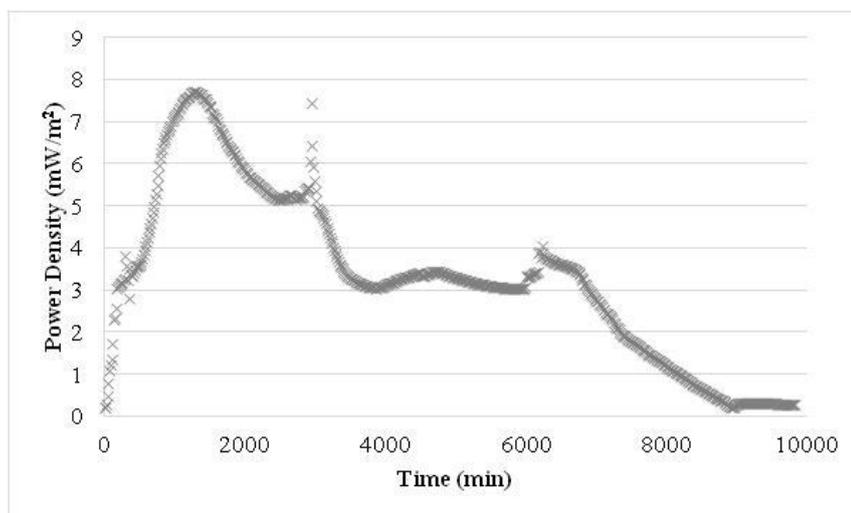
Figure 4.9. Power density (mW/m^2) with respect to time (min) graph for MFC-9

Table 4.25. pH and COD measurements for MFC-9 during operation

	t = 1.5 days		t = 4 days		t = 7 days	
	pH	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**
Analysis-I	7.52	6170	7.08	5680	6.90	4160
Analysis-II	-	6240	-	5710	-	4260
Average	7.52	6205	7.08	5695	6.90	4210

MFC-9 showed better performance when compared to preliminary experiments but still, PD was not satisfactory and need to be improved. The reason for low voltage outputs were thought to be the escape of free electrons released from organic matter degradation. To overcome this, MFC-10 was set-up after proper insulation. The sampling ports were sealed using teflon tape and glass wool. In addition to this, at the end of MFC-9 operation it was understood the resistance box stopped functioning properly and replaced with a new one. The rest of the parameters (anode/cathode electrode, membrane and external resistance) were same with MFC-9. The initial measurements of pH, COD and solid concentration are given in Table 4.26 and Table 4.27.

Table 4.26. Initial pH and COD measurements (t=0 day) for MFC-10

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.54	7.39	7.53	7330	23560	7410
Analysis-II	7.51	7.36	7.53	6990	24380	7470
Average	7.53	7.37	7.53	7160	23970	7440

Table 4.27. Initial solids concentration measurements (t=0 day) for MFC-10
(inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	14500	8740	12200	8340
Analysis-II	15370	9020	10780	6960
Analysis-III	15020	9010	11840	7200
Analysis-IV	14950	8830	11980	7340
Average	14960	8900	11700	7460

This system was operated for 4 days and the overall COD reduction was 29.4% (Table 4.28). The maximum voltage was 166.8 mV and PD was 18.55 mW/m^2 (Figure 4.10). The reactor showed better performance compared to the rest with a slightly higher CE (%) as 0.3 % (compared to MFC-9). In this system, in addition to PD a new parameter started to be measured which was OCV. OCV is the potential measured when the system is not connected to any load (resistance box) in a circuit. It gives us the maximum voltage available. OCV is a measure of full potential within a system, for instance, if one has a 1.5 V battery, its OCV should also be 1.5 V. In this system, OCV was measured as 391 mV. The highest OCV obtained for an MFC so far was 800 mV as reported in the literature, but the OCV of MFC-10 was 391 mV which was almost half of the highest value reported (Kim et.al., 2007a). This clearly showed that there still was a problem with electron transfer resulting from either insulation or membrane fouling.
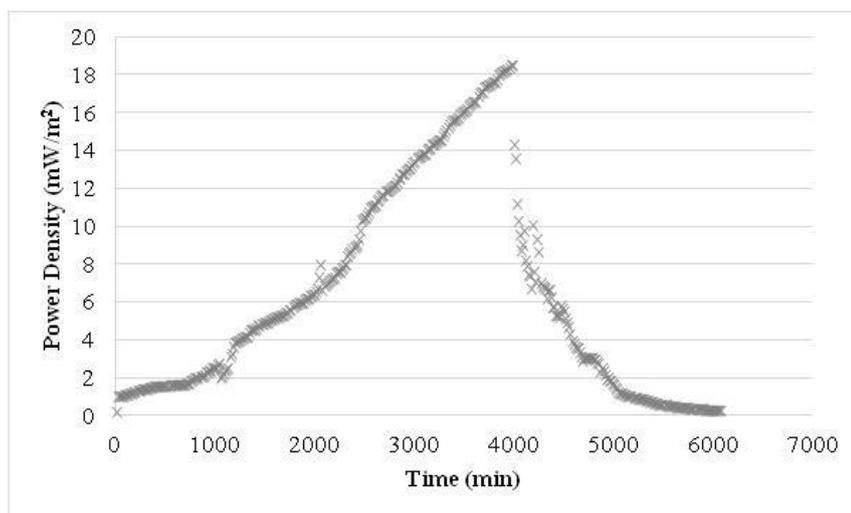
Figure 4.10. Power density (mW/m^2) with respect to time (min) graph for MFC-10

Table 4.28. pH and COD measurements for MFC-10 during operation

	t = 1 days		t = 3 days		t = 4 days	
	pH	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**
Analysis-I	7.52	6550	7.24	5580	7.18	5300
Analysi-II	-	6510	-	5640	-	5200
Average	7.52	6530	7.24	5610	7.18	5250

Due to the length of operation, a significant pH drop was not observed since before membrane fouling started to rise H$^+$ concentration, the reactor was terminated.

As stated before, PEM has a significant role in MFC operation and its sulfonated sites are actually responsible from proton transfer (Logan, 2008). Once these sites are fouled, H$^+$ ions start to accumulate, blocking electron transfer. Nafion 117 is a very sensitive membrane, and should be handled gently prior to set-up. Because of this, it was decided to decrease the temperature of the distilled water and H$_2$O$_2$ solution's temperature from 80°C to 40°C during MFC-11 set-up. The pH of PBS was also adjusted to 8.0, to create H$^+$ gradient between the chambers and enhance H$^+$ transfer. In addition to these, sodium acetate was used as carbon source, instead of glucose,

since in some studies it was reported that anaerobic microorganisms can take up acetate easier. The initial measurements of this MFC are given in Table 4.29 and Table 4.30.

Table 4.29. Initial pH and COD measurements (t=0 day) for MFC-11

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.61	7.08	7.57	6880	10500	8320
Analysis-II	7.57	-	7.60	7030	10400	7800
Average	7.59	7.08	7.59	6955	10450	8060

Table 4.30. Initial solids concentration measurements (t=0 day) for MFC-11 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	13640	7160	12500	6600
Analysis-II	13650	7170	13260	7100
Analysis-III	13410	7060	12920	6560
Analysis-IV	13670	7160	12780	7080
Average	13592.5	7137.5	12685	6835

MFC-11 was operated for 6 days, the maximum voltage and PD were 258.4 mV and 44.52 mW/m^2, respectively (Figure 4.11). Compared to MFC-8 and MFC-9, PD increased almost 2.5 times but still the OCV measured (476 mV) was low, indicating insulation problems within the system. COD reduction was 35.2% at the end of 6 days (Table 4.31). pH reduction recorded on day 6, showing that membrane fouling started blocking H$^+$ transfer. Therefore, two main problems had to be solved following this set: (i) membrane lifetime and (ii) insulation to transfer electrons at a higher rate.
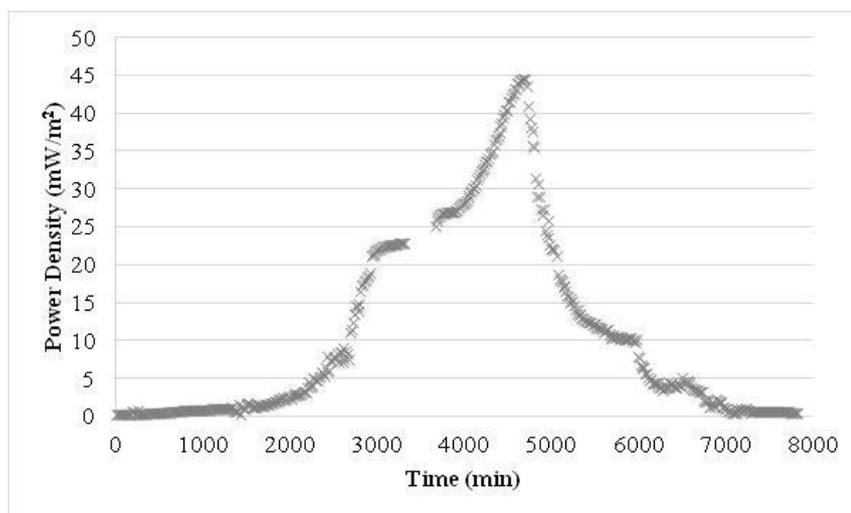
Figure 4.11. Power density (mW/m^2) with respect to time (min) graph for MFC-11

Table 4.31. pH and COD measurements for MFC-11 during operation

	t = 1.5 days		t = 3 days		t = 6 days	
	pH	**COD (mg/L)**	**pH**	**COD (mg/L)**	**pH**	**COD (mg/L)**
Analysis-I	7.50	6710	7.17	5980	6.97	5630
Analysis-II	-	6530	-	6090	-	4810
Average	7.50	6620	7.17	6035	6.97	5220

MFC-12 was operated using glucose as the carbon source since the main problem of the system was thought to be electron escape not the carbon source. This time, in order to increase conductivity and therefore, electron movement, NaCl concentration (in catholyte) was increased to 80 mM. Plus, 10 mL vitamin and mineral solution, also known as Wolfe's solution, was added to anolyte. This was added in order to enhance microbial activity. Since this solution contained high concentrations of many items present in SWW, a different type of SWW was used in this set (SWW-3) eliminating MnCl$_2$.4H$_2$O and ZnCl$_2$.

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Insulation was a key item during the set-up of MFC-12 and for this purpose, the inner and outer surfaces of the sampling ports were covered with plastic and teflon, respectively. The Cu wire was insulated using plastic cover.

The initial measurements done for MFC-12 are given in Table 4.32. Solids concentrations were same with MFC-11, since WWTP had a maintenance and didn't allow sampling so stored sludge was used.

Table 4.32. Initial pH and COD measurements (t=0 day) for MFC-12

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.56	7.08	7.50	7240	10500	8410
Analysis-II	7.56	-	7.51	7550	10400	8600
Average	7.56	7.08	7.51	7395	10450	8505

This set was operated for 5 days and the COD reduction was 39.4%, similar to MFC-11 (Table 4.33). Unlike MFC-11, pH drop was not observed, so despite the addition of mineral solution, the membrane surface was not fouled in a short time interval. This once again shows the dynamic nature of MFCs in all aspects. This was actually a big advantage for the cell since pH drop also affects microbial activity adversely. Enhanced microbial activity means, enhanced biodegradation and electron transfer and this was clearly shown during MFC-12 operation. The highest voltage recorded was 459.9 mV and maximum PD was 141.01 mW/m^2 (Figure 4.12). CE(%) was 0.8%. OCV was 688 mV which was significantly higher than the previous sets showing the success of insulation since it is a good indicator of electron escape. MFCs are very small systems and any sort of conductive surface may capture the electrons moving between anode and cathode electrodes. Therefore, insulation is an effective precaution in improving system performance. The findings showed that the loss of electrons was the main cause behind the low power densities and performance of microorganisms must be enhanced for better electron transfer and organic matter degradation. But to make sure that whether this performance increase was due to microbial activity or NaCl concentration or insulation, during the 5th day of reactor

operation, NaCl concentration was reduced to 50 mM so as to narrow down the causes without a new reactor operation. This change resulted in a voltage decrease of 29%, showing that NaCl has a positive impact on power output but not enough to increase the PD from 44.52 to 141.01 mW/m^2, therefore a new reactor distinguishing between insulation and microbial activity had to be operated.
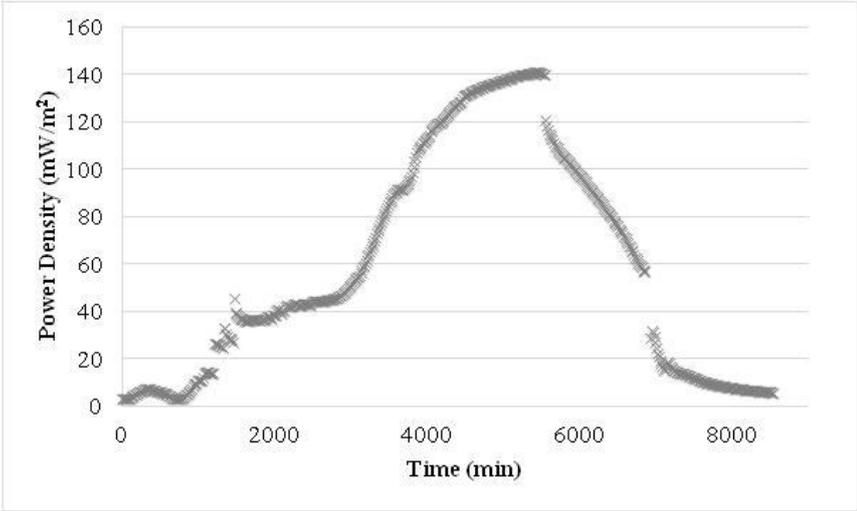
Figure 4.12. Power density (mW/m^2) with respect to time (min) graph for MFC-12

Table 4.33. pH and COD measurements for MFC-12 during operation

	t = 1.5 days		t = 2.5 days		t = 5 days	
	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.53	6720	7.49	6230	7.20	5110
Analysis-II	-	6750	-	6100	-	5190
Average	7.53	6735	7.49	6165	7.20	5150

MFC-13 was identical to MFC-12 in terms of electrodes, membrane type, SWW and insulation techniques but this time mineral solution was not added to see whether the improvement was due to insulation or microorganisms had a share on the success. The initial pH, COD and solids concentration measurements are given in Table 4.34 and Table 4.35.

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Table 4.34. Initial pH and COD measurements (t=0 day) for MFC-13

	pH			COD (mg/L)		
	SWW	**ADS**	**Mix**	**SWW**	**ADS**	**Mix**
Analysis-I	7.55	7.03	7.49	7200	10220	8220
Analysis-II	7.58	-	7.52	7260	10160	8190
Average	7.57	7.03	7.51	7230	10190	8205

Table 4.35. Initial solids concentration measurements (t=0 day) for MFC-13 (inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	13250	7080	11760	6640
Analysis-II	13150	6910	12020	7040
Analysis-III	13090	7100	12300	7440
Analysis-IV	13280	7040	12120	7045
Average	13200	7032.5	12050	7045

This set was operated for 9 days and the maximum voltage and PD measured were 374.3 mV and 93.41 mW/m^2, respectively (Figure 4.13). The OCV was recorded as 659 mV, close to MFC-12, showing the effectiveness of insulation once again. The decrease in the voltage and PD, on the other hand, clearly showed that microorganisms are the backbone of an MFC and their viability enhance electricity generation. The CE(%) was calculated as 0.5%, lower compared to MFC-12, and this showed that microbial activity was limited in terms of electron transfer. The COD reduction was 39.7%, lower than the sets operated for a shorter period of time (MFC-11 and MFC-12).
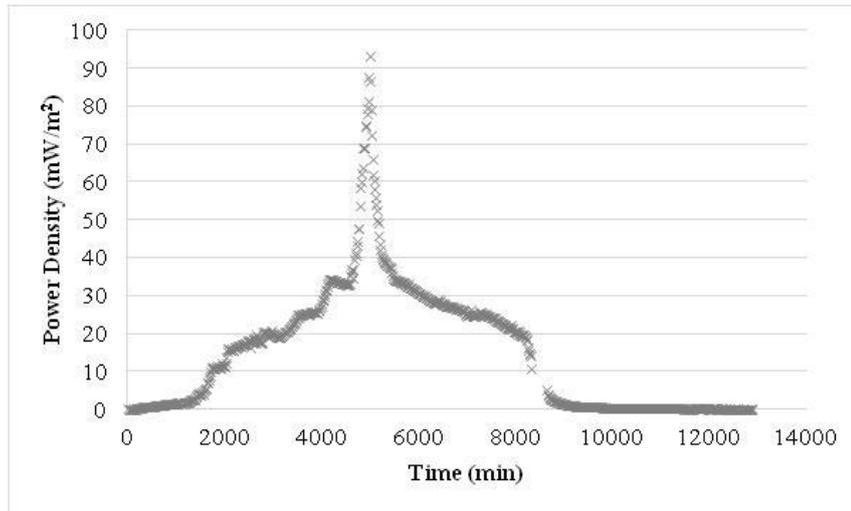
Figure 4.13. Power density (mW/m^2) with respect to time (min) graph for MFC-13

Table 4.36. pH and COD measurements for MFC-13 during operation

	t = 1 days		t = 2 days		t = 4 days		t = 6 days		t = 9 days	
	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.49	6720	7.48	6580	7.36	6430	7.20	5620	7.05	5010
Analysis-II	-	6840	-	6680	-	6450	-	5550	-	4890
Average	7.49	6780	7.48	6630	7.36	6440	7.20	5585	7.05	4950

MFC-14 was operated using the same materials and operational conditions as MFC-12. The purpose of this reactor was to demonstrate the repeatability of MFC-12 findings. The initial measurements are given in Table 4.37 and Table 4.38.

Table 4.37. Initial pH and COD measurements (t=0 day) for MFC-14

	pH			COD (mg/L)		
	SWW	ADS	Mix	SWW	ADS	Mix
Analysis-I	7.52	7.12	7.51	7610	10220	8500
Analysis-II	7.52	7.14	7.50	7560	10040	8510
Average	7.52	7.13	7.51	7585	10130	8505

Table 4.38. Initial solids concentration measurements (t=0 day) for MFC-14
(inoculum-ADS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	10880	7400	10000	5100
Analysis-II	10930	7760	10320	5140
Analysis-III	10900	6230	9460	5200
Analysis-IV	11000	7570	9940	5120
Average	10927.5	7240	9930	5140

This reactor was operated for 6 days with a COD removal of 39.4%. A slight pH drop was observed from 7.50 to 7.19, not so sharp but enough to observe membrane fouling together with the decline in PD (Figure 4.14). The maximum PD was 150.84 mW/m^2, close to MFC-12 (141.01 mW/m^2) showing the repeatability of reactor operation.
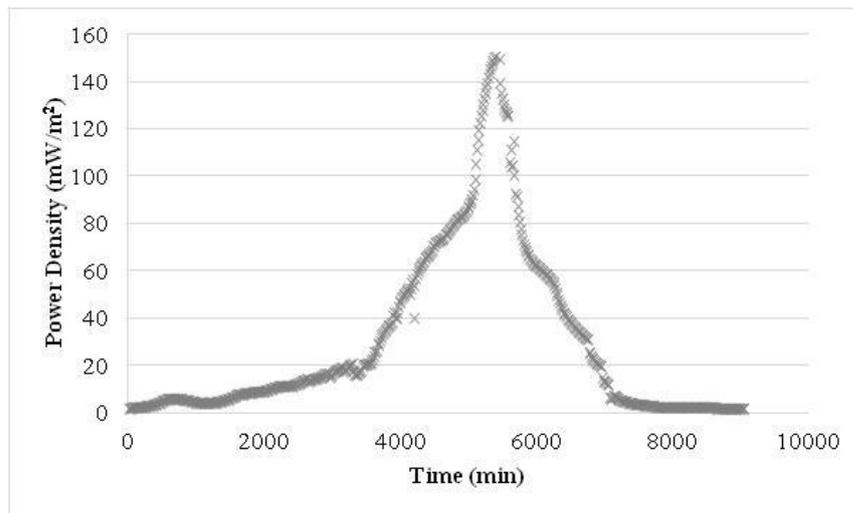
Figure 4.14. Power density (mW/m^2) with respect to time (min) graph for MFC-14

Table 4.39. pH and COD measurements for MFC-14 during operation

	t = 1 days		t = 2.5 days		t = 3.5 days		t = 6 days	
	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.51	6770	7.47	6240	7.40	6120	7.18	4980
Analysis-II	-	6840	-	6180	-	6060	-	4780
Average	7.51	6805	7.47	6210	7.40	6090	7.18	4880

MFC-15 was operated to test whether mineral supplement can be eliminated if a more viable and versatile inoculums were used instead of ADS, like WAS. ADS is a more stabilized and less viable form of sludge when compared to WAS and in addition to this, WAS contains some nutrients that match with some of the constituents in vitamin and mineral solution. Therefore, the microbial inoculum at this point was the mixture of WAS and ADS and WAS was purged with nitrogen to remove the oxygen and protect the anode chamber. This reactor also had one more difference from the rest: humic acid (1 g/L solution) addition as natural mediator which is much cheaper than a synthetic one. Mostly, mixed cultures are not dominated with anodophilic microorganisms that can transfer electrons easily. At that point, mediators come to help to carry the electrons but they are mostly synthetic with a quinine functional group. But these synthetic mediators are very expensive and therefore structurally similar natural ones must be investigated. Degradation products of lignocellulostic compounds have polyphenolic structure which makes them very similar to phenolic mediator "thionine". Humic acids are in this form and due to their complex chemistry they cannot be degraded easily, therefore, do not interfere with the electron formation but rather take place in electron transfer stage of the MFC's metabolic pathway. Taking this into consideration, MFC-15 was started to be operated with 1 g/L humic acid solution addition. The initial measurements are given in Table 4.40 and Table 4.41 (ADS was same with MFC-14). WAS was concentrated to reach a certain solids concentration using centrifuge as described in Chapter 3. So at this point, it was also aimed to reduce the cost of the system by eliminating the vitamin+mineral solution and using WAS and humic acid instead.

Table 4.40. Initial pH and COD measurements (t=0 day) for MFC-15

	pH			COD (mg/L)		
	SWW	**WAS**	**Mix**	**SWW**	**WAS**	**Mix**
Analysis-I	7.56	7.00	7.49	10040	20000	11310
Analysis-II	7.53	7.02	7.49	10140	19640	11350
Average	7.55	7.01	7.49	10090	19820	11380

Table 4.41. Initial solids concentration measurements (t=0 day) for MFC-15 (inoculum-WAS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	19540	13910	11940	7880
Analysis-II	19680	14000	12080	7920
Analysis-III	19760	14060	12120	7900
Analysis-IV	19700	13950	11860	7820
Average	19670	13980	12000	7880

The OCV measured was 726 mV and the maximum PD was 203.72 mW/m^2. The overall COD reduction was 45.5% at the end of 8 days and the CE (%) was calculated as 0.7%. Findings of MFC-15 demonstrated that viability of microbial culture and transfer of electrons within the anode chamber at the beginning are crucial for a higher power output. But the inclusion of ADS in the inoculum without the addition of mineral solution definitely dropped the CE (%) since half of the microorganisms were not as effective as the rest in transferring the liberated electrons. That's why, in the next set, it was decided to completely remove ADS from the inoculum and move on with WAS.
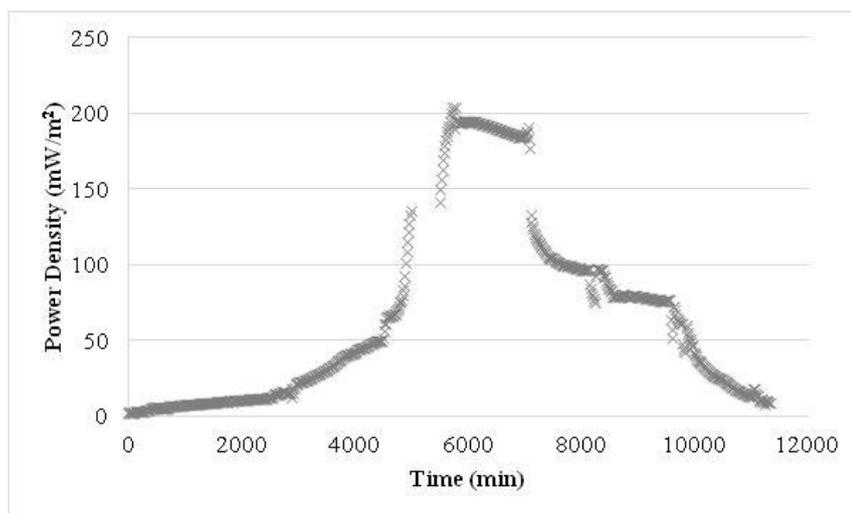
Figure 4.15. Power density (mW/m^2) with respect to time (min) graph for MFC-15

Table 4.42. pH and COD measurements for MFC-15 during operation

	t = 1 days		t = 2.5 days		t = 5 days		t = 8 days	
	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)	pH	COD (mg/L)
Analysis-I	7.49	9680	7.40	8650	7.22	7410	6.97	6230
Analysis-II	-	9710	-	8610	-	7440	-	6170
Average	7.49	9695	7.40	8630	7.22	7425	6.97	6200

MFC-16 was established using WAS as the only inoculum (in MFC-15 it was WAS and ADS mixed together). The major difference of this reactor was the use of CMI-7000, instead of Nafion 117, as membrane. According to Logan (2008), the internal resistances and power densities attained by MFCs with CMI-7000 or Nafion 117 are actually very close to each other in dual chamber systems when compared to conventional HFCs. The reason for this is the direct contact of the Nafion 117 membrane with liquid, and the fouling of surface with wastewater constituents. Besides, CMI-7000 usually performs close to Nafion 117 in MFCs. The glucose concentration in this set was 2500 mg/L to prevent the instantaneous fouling of the membrane since CMI-7000 is not as selective as Nafion 117, and all cations will

attack the surface. Humic acid was added like MFC-15. The initial measurements are given in Table 4.43 and Table 4.44.

Table 4.43. Initial pH and COD measurements (t=0 day) for MFC-16

	pH			COD (mg/L)		
	SWW	**WAS**	**Mix**	**SWW**	**WAS**	**Mix**
Analysis-I	7.53	7.06	7.50	4900	20420	5820
Analysis-II	7.53	7.08	7.49	5130	20600	5980
Average	7.53	7.07	7.50	5015	20510	5900

Table 4.44. Initial solids concentration measurements (t=0 day) for MFC-16 (inoculum-WAS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	20940	14400	13360	7900
Analysis-II	20800	14290	13440	8160
Analysis-III	20880	14330	13480	8080
Analysis-IV	20820	14260	13520	8260
Average	20860	14320	13450	8100

The OCV of this system was measured as 609 mV, lower than MFC-15. The maximum PD measured was close to MFC-15, as it was measured as 258.71 mW/m^2. In terms of OCV, this system did not show much of a difference when compared to MFC-12 or MFC-14 (slightly lower than these two) but the PD obtained was higher. The reason for lower OCV could be the lower organic content. But the PD was higher since as the external resistance was lowered, it became easier for the microorganisms and mediator to carry the electrons to anode electrode surface. Previously (MFC-12, MFC-14), although the organic content was higher (so as the amount of free electrons), the electrons couldn't be carried effectively. In this case (MFC-16), although the amount of free electrons was lower, they were carried much more effectively and therefore yielded higher PD.
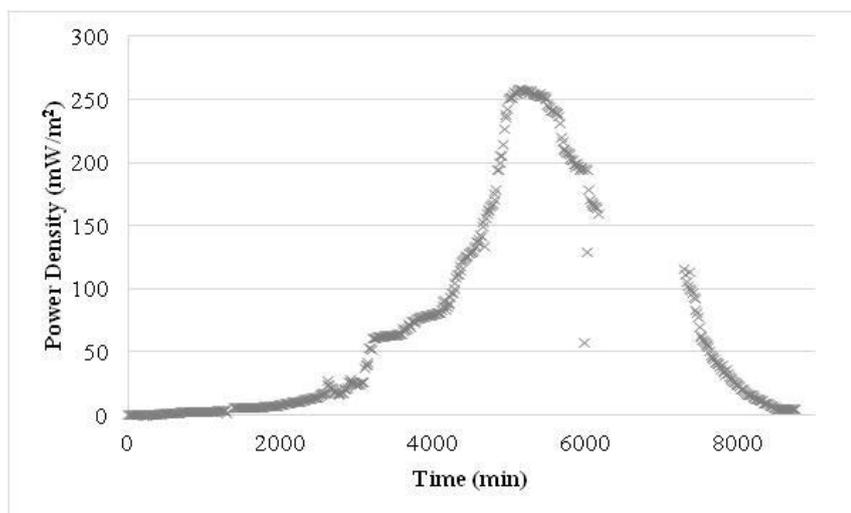
Figure 4.16. Power density (mW/m^2) with respect to time (min) graph for MFC-16

The initial COD was lower compared to MFC-15, since glucose was halved. The overall COD removal was 35.9% at the end of 6th day (COD measured was 3780 mg/L). The significant pH drop (pH was measured as 6.83) was an indicator of membrane fouling. Since in this reactor CMI-7000 was used, all types of cations present attacked the surface and passed through it, and resulted in a faster surface accumulation inhibiting the transfer of the rest of the protons. This decreased the pH of the system. The measurements were done at the end of reactor operation to prevent air leak and the CE (%) certainly proved that it worked. CE was calculated as 2.2%. Previously, it was 0.7%, almost 1/3 of the value calculated for MFC-16. This showed that sampling during reactor operation results in air leak, inhibiting electron transfer. For this reason, in MFC-16, both PD and CE were improved.

Some of the MFCs were operated in identical set-up during this study and MFC-17 was one of them. It was identical to MFC-16 in all aspects to test the accuracy and repeatability. The initial measurements are given in Table 4.45 and Table 4.46.

Table 4.45. Initial pH and COD measurements (t=0 day) for MFC-17

	pH			COD (mg/L)		
	SWW	WAS	Mix	SWW	WAS	Mix
Analysis-I	7.55	6.93	7.53	4770	11820	4930
Analysis-II	7.55	-	7.54	4850	11680	4980
Average	7.55	6.93	7.54	4810	11750	4955

Table 4.46. Initial solids concentration measurements (t=0 day) for MFC-17
(inoculum-WAS)

	Concentration (mg/L)			
	TS	VS	TSS	VSS
Analysis-I	19770	10490	14220	5880
Analysis-II	19850	10520	14300	6040
Analysis-III	19890	10560	14360	6080
Analysis-IV	19810	10590	14280	6000
Average	19830	10540	14290	6000

MFC-17 was identical to MFC-16, but the PDs were completely different from each other. Maximum PD was 150.52 mW/m^2, 1.7 times lower than the maximum PD calculated for MFC-16 (258.71 mW/m^2). The main reason behind this was the differences in sludge sampling used for inoculation. As you can see in Table 4.46, there was a distinctive reduction in the VS content of sludge compared to MFC-16. VS content is an important parameter and is an indicator of the organic content of the sludge and microorganisms also a part of this organic content. Therefore, for one more time the importance of microbial activity for MFC operation was shown. Lower VS content resulted in slow microbial degradation and electron transfer, decreasing PD. The acclimation period was also very long compared to the rest of the reactors as can be seen from Figure 4.17. These findings were also consistent with COD reduction which was 17.5% at the end of 7th day of reactor operation. Generally, the average COD reduction in this study was approximately 35-45%.
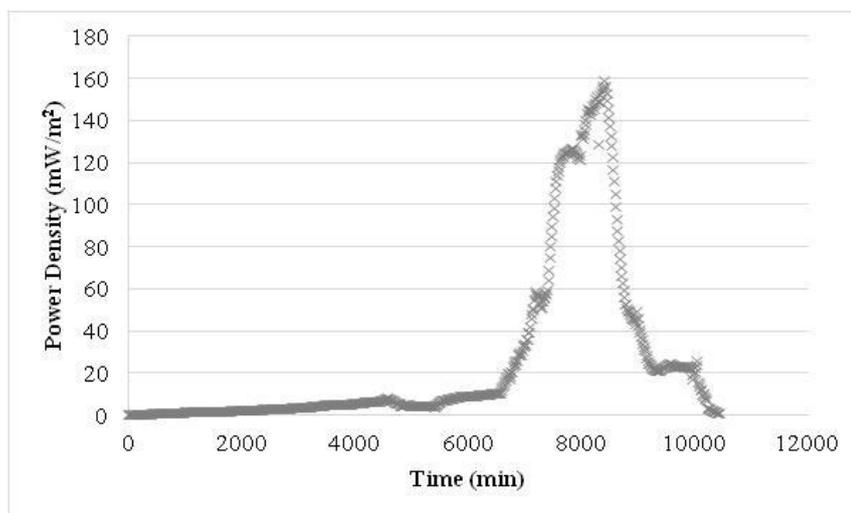
Figure 4.17. Power density (mW/m^2) with respect to time (min) graph for MFC-17

Starting with MFC-18, to make a better judgment on the system parameters that needs optimization, internal resistance was decided to be calculated and for this reason, MFC-18 was not operated at a single external resistance but rather at a range of external resistance values between 400 ohms and 25,000 ohms. The initial measurements are given in Table 4.47 and Table 4.48. WAS was again used as microbial inoculum, and the volume of inoculation was determined based on the initial VS measurements. The anode and cathode electrodes were both carbon cloth (only cathode electrode contained 0.5 mg Pt/cm^2). CMI-7000 was again used to separate the chambers. The system was tested against gas and liquid leakage and insulated properly to prevent electron escape.

Table 4.47. Initial pH and COD measurements (t=0 day) for MFC-18

	pH			COD (mg/L)		
	SWW	**WAS**	**Mix**	**SWW**	**WAS**	**Mix**
Analysis-I	7.50	7.03	7.51	4920	18400	5150
Analysis-II	7.51	7.06	7.51	4880	18520	5210
Average	7.51	7.05	7.51	4900	18460	5180

Table 4.48. Initial solids concentration measurements (t=0 day) for MFC-18 (inoculum-WAS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	23130	15810	16660	9880
Analysis-II	23060	15750	16600	9800
Analysis-III	23100	15830	16540	9700
Analysis-IV	22990	15690	16640	9820
Average	23070	15770	16610	9800

The maximum PD measured for this system was 346.56 mW/m^2 at 400 ohms. The polarization curve is given in Figure 4.18, since 400 ohms was the lowest resistance tested, regions 1 and 2 are well-observed, but the region of concentration losses (region 3) were not clearly observed.
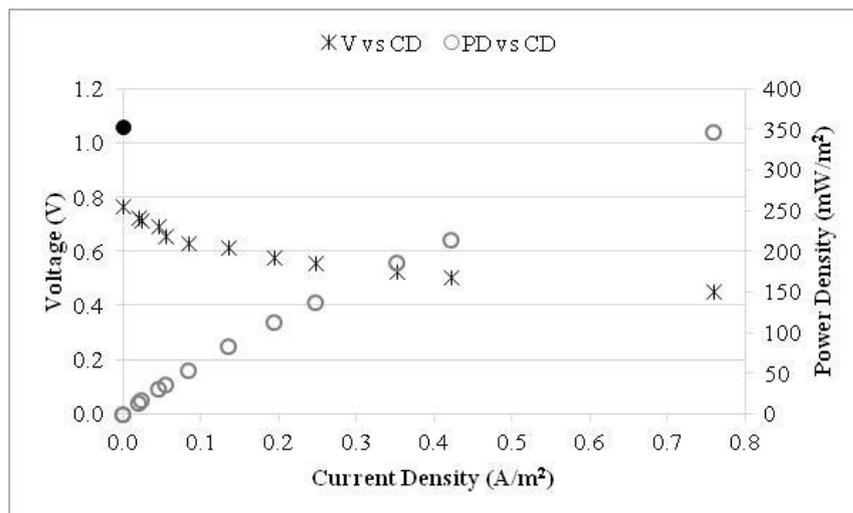
Figure 4.18. Polarization curve plotted for MFC-18

The circular marker on Figure 4.18 indicates the theoretical potential of MFC (E_{emf}), and it was calculated as 1.055 V using the following formula (Logan, 2008):

Equation (4.2): $E_{emf} = E_{cathode} - E_{anode}$

The potentials of anode (E_{anode}) and cathode ($E_{cathode}$) were retrieved from the studies of Rittmann and McCarty (2001) and Logan et.al. (2006) as -0.428 V and 0.627 V, respectively. Since this is the theoretical potential of the cell itself (the maximum attainable voltage that could be measured), these numbers were taken from literature based on the fact that glucose is the carbon source (E_{anode} depends on it) and oxygen was used as the terminal electron acceptor in the cathode chamber ($E_{cathode}$ depends on this).

The internal resistance of the system was calculated as 250 ohms and 270 ohms using the slope (remember Figure 3.4, Section 3.5.5) and the empirical formula (Equation.4.2), respectively. However, there is also one additional method to estimate the internal resistance of a fuel cell; which is the resistance at which the maximum PD was measured. According to this, MFC-18 had an internal resistance of 400 ohms. This clearly showed that the range of external resistances applied using the Lutron RBOX 408 device must be enlarged to capture every detail and to obtain possibly a better result at a lower resistance value.

The measured OCV of MFC-18 was 769 mV which was lower than the theoretical value (cell potential), 1055 mV (1.055 V as given before). The OCV measured in MFC is always lower than the theoretical potential due to the maximum potentials of bacterial enzymes or oxygen reduction within the cathode chamber.

This reactor was terminated on day 11 and the pH was 6.82 on this day of measurement indicating a possible membrane fouling and decline in system performance. The COD was 3020 mg/L with a total reduction of 41.7%. The CE (%) was calculated as 2.5% with a slight increase compared to MFC-16, mostly resulting from finding the optimum (and significantly lower) external resistance value that allow current flow.

MFC-19 was operated again to test repeatability, just like MFC-17. But this time sampling was done more carefully. It was identical to MFC-18, except for one difference. The reactor was operated at a single external resistance value, 400 ohms. The initial measurements are given in Table 4.49 and Table 4.50.

Table 4.49. Initial pH and COD measurements (t=0 day) for MFC-19

	pH			COD (mg/L)		
	SWW	**WAS**	**Mix**	**SWW**	**WAS**	**Mix**
Analysis-I	7.50	7.06	7.49	5010	19060	5390
Analysis-II	7.50	7.09	7.50	5050	18940	5270
Average	7.50	7.08	7.50	5030	19000	5330

Table 4.50. Initial solids concentration measurements (t=0 day) for MFC-19 (inoculum-WAS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	19440	13790	13540	8020
Analysis-II	19550	13860	13500	8080
Analysis-III	19470	13830	13560	8120
Analysis-IV	19620	13920	13400	7900
Average	19520	13850	13500	8030

The maximum voltage measured was 429.1 mV and the maximum PD was calculated as 306.94 mW/m^2. These values were close to MFC-18 showing the system was under control. The acclimation period, however, showed a strange pattern like a ladder, unlike the rest of the reactors and its length was similar, around 1-2 days.

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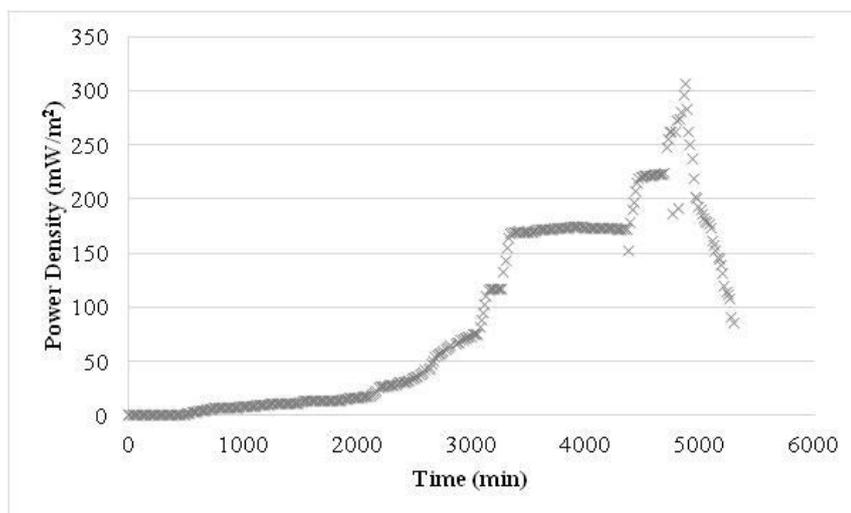
Figure 4.19. Power density (mW/m^2) with respect to time (min) graph for MFC-19

This set was operated for 4 days, it was terminated immediately after the voltage started to drop. The pH and COD were measured as 7.27 and 3900 mg/L, respectively. The duration of reactor operation did not allow us to see the pH drop due to proton accumulation. The overall COD reduction was 26.8%, lower than the rest because of the duration just like pH. Because even if the voltage drops due to limited electron transfer (resulting from H$^+$ accumulation) the microorganisms still continue to degrade the organic matter and therefore, COD.

The polarization curve plotted for MFC-18 had some shortcomings as discussed previously. The regions of losses were not clear as the range of external resistances tested was narrow. Due to this, during the operation of MFC-20, the range was enlarged and kept between 150 ohms and 30,000 ohms. In addition to this, some operational changes were made during the set-up of MFC-20. Also to obtain a higher power output WAS used for microbial inoculation was concentrated so as to obtain higher concentration of microorganisms at a smaller inoculation volume and decrease the interference of the sludge matrix with the membrane compared to higher volumes of inoculation. In addition to this, since the major parameter affecting the internal resistance is membrane it was decided to apply the changes in pretreatment of Nafion 117 that lead to higher power densities to CMI-7000 and decrease the

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pretreatment temperature to room temperature (25°C). Also, to prevent the occupation of active sites of CMI-70 with Na^+ and Cl^- ions prior to use (which reduces efficiency to cation transfer), the concentration of NaCl used during pretreatment was decreased from 5% to 3%. The initial measurements done are given in Table 4.51 and Table 4.52.

Table 4.51. Initial pH and COD measurements (t=0 day) for MFC-20

	pH			COD (mg/L)		
	SWW	**WAS**	**Mix**	**SWW**	**WAS**	**Mix**
Analysis-I	7.50	7.02	7.51	5090	26500	5620
Analysis-II	7.51	7.05	7.50	5130	26100	5740
Average	7.51	7.04	7.51	5110	26300	5680

Table 4.52. Initial solids concentration measurements (t=0 day) for MFC-20 (inoculum-WAS)

	Concentration (mg/L)			
	TS	**VS**	**TSS**	**VSS**
Analysis-I	28800	22190	19480	12040
Analysis-II	28730	22130	19360	11880
Analysis-III	28880	22240	19460	12000
Analysis-IV	28830	22200	19500	12120
Average	28810	22190	19450	12010

The OCV was measured as 773 mV, again lower than the theoretical potential of the cell, as expected. The maximum PD in the system was calculated as 464.11 mW/m^2 at 300 ohms external resistance. This, once more, proved that the decrease in external resistance definitely enhances current flow and in order to plot a more accurate polarization curve, MFC must be operated at a wide range of resistance values from very low to extremely high. The slope of the polarization curve given in Figure 4.20 gave an internal resistance value of 207 ohms while the empirical formula yielded 210 ohms. Therefore, the optimum resistance value must be somewhere around 200-250 ohms. The PD calculated for 150 ohms was 410.74 mW/m^2 which was very

close to 464.11 mW/m^2 showing that somewhere in between the optimum value would be identified. Unfortunately, the resistance box device had a mechanical problem that resulted in short-circuiting during the adjustment of 200 ohms and that was why in the first place it was not included in the range. Based on these results, it was decided to operate the system at 300 ohms external resistance.
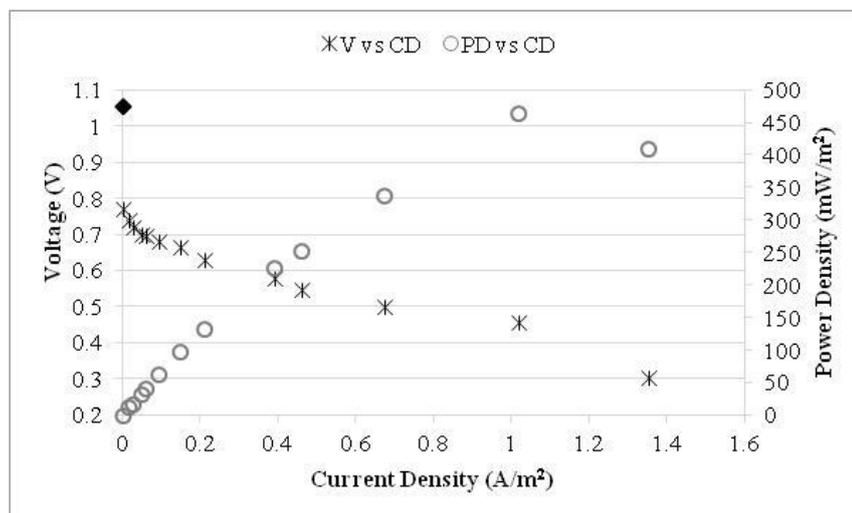
Figure 4.20. Polarization curve plotted for MFC-20

MFC-20 was operated for 8 days. pH and COD were measured as (when terminated) 6.99 and 3410 mg/L, respectively. pH dropped because of membrane fouling and accumulation of H^+ in anolyte. COD, on the other hand, was degraded by microorganisms to generate electrons and create current and also to preserve their metabolic activities. The overall COD reduction was 40%. In the light of this information and current flow, CE (%) was calculated as 3%, which was significantly higher than the rest of the reactors, especially the preliminary sets, proving the improvement within the system. But still, the majority of COD was used for microbial metabolism or electron transfer was significantly inhibited by membrane fouling due to high concentration carbon source and its instantaneous degradation.

MFC-21 was identical to MFC-20 and operated to test repeatability. Initial measurements are given in Table 4.53 and Table 4.54.

Table 4.53. Initial pH and COD measurements (t=0 day) for MFC-21

|	pH			COD (mg/L)		
	SWW	WAS	Mix	SWW	WAS	Mix
Analysis-I	7.51	7.00	7.51	5050	28180	5720
Analysis-II	7.52	6.98	7.51	5110	28320	5780
Average	7.51	6.99	7.51	5080	28250	5750

Table 4.54. Initial solids concentration measurements (t=0 day) for MFC-21 (inoculum-WAS)

	Concentration (mg/L)			
	TS	VS	TSS	VSS
Analysis-I	31300	21900	20800	12440
Analysis-II	31250	21890	20840	12340
Analysis-III	31210	21770	20700	12400
Analysis-IV	31280	21840	20860	12380
Average	31260	21850	20800	12390

The OCV was measured as 766 mV and the maximum PD in the system was calculated as 432.18 mW/m^2 at 300 ohms external resistance, which was similar to MFC-20. The slope of the polarization curve given in Figure 4.21 gave an internal resistance value of 210 ohms while the empirical formula yielded 220 ohms. At the end of reactor operation pH and COD were measured as 7.09 and 3625 mg/L, respectively. The overall COD reduction in this set was 37% which was close to MFC-20. Considering the fact that there could be several order of magnitude differences between identical MFC operations, as reported in the literature by Kim et.al. (2007a), these two reactors showed great repeatability with an error of ±22.6.

It is obvious that the changes made on the pretreatment method and inoculation were affecting in reducing the internal resistance via preserving the membrane's cation exchange performance and bacterial electron formation (biodegradation) and transfer. The maximum PD obtained was 464.11 mW/m^2 and 432.18 mW/m^2 for MFC-20 and MFC-21, respectively, at an external resistance of 300 ohms. It can be said that both systems were operated close to internal resistance values but maybe

higher power densities could have been obtained if they were operated at 200 ohms since the values obtained at 150 ohms and 300 ohms were very close to each other. The values obtained at the end of these sets were very close to the ones obtained in the previous studies (Table 2.1, Chapter 2). So it was decided that optimization studies were successful, especially in terms of improving the conditions for microorganisms and reducing internal resistance and it was time to test the rest of the materials that were planned at the very beginning of the study.
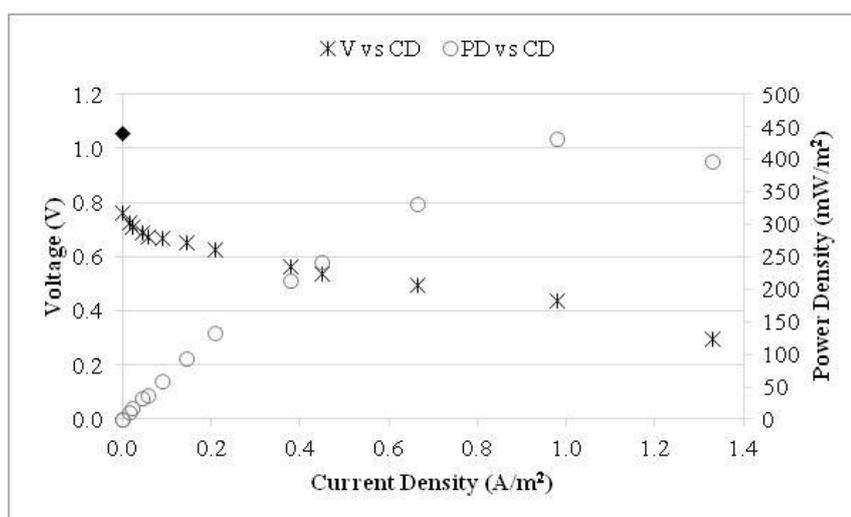
Figure 4.21. Polarization curve plotted for MFC-21

MFC-22 was set-up using different types of anode and cathode electrodes to test if there would be any changes in system performance. The difference of MFC-22 from preliminary experiments was, initially the operational conditions were not considered at all, only materials were tested. But at this point of the study, operational conditions regarding insulation, SWW concentrations, IR, membrane and external resistance were improved and it was thought that would there be any changes if the materials change. Anode electrode was made of carbon paper, which was just the opposite of carbon cloth, disabling biofilm formation, to see its impact under optimized conditions. Cathode electrode was again made of carbon paper with a lower concentration of Pt on its surface (0.4 mg/cm^2). CMI-700 was used again as the

membrane and Cu wire was used to connect the electrodes. Glucose was selected as the carbon source (2500 mg/L). External resistances were between 150-30,000 ohms. The initial measurements done prior to reactor set-up are given in Table 4.55 and Table 4.56.

Table 4.55. Initial pH and COD measurements (t=0 day) for MFC-22

	pH			COD (mg/L)		
	SWW	WAS	Mix	SWW	WAS	Mix
Analysis-I	7.53	7.12	7.53	4910	33880	5880
Analysis-II	7.53	7.10	7.53	4850	34340	5920
Average	7.53	7.11	7.53	4880	34110	5900

Table 4.56. Initial solids concentration measurements (t=0 day) for MFC-22 (inoculum-WAS)

	Concentration (mg/L)			
	TS	VS	TSS	VSS
Analysis-I	29130	20660	19660	13180
Analysis-II	29270	20750	19560	13040
Analysis-III	29220	20800	19580	13000
Analysis-IV	29180	20710	19680	13220
Average	29200	20730	19620	13110

The maximum PD measured for this system at 300 ohms external resistance was 310.77 mW/m^2. The internal resistance was calculated as: (i) 310 ohms (slope of the polarization curve) and (ii) 293 ohms (empirical formula). As can be seen from the findings obtained, the system performance was not as good as it was when carbon cloth was used as electrode, allowing biofilm formation and enhanced electron transfer (MFC-20 and MFC-21). This certainly increased internal resistance since electrodes and electron transfer are two major items of internal resistance (Logan, 2008). As a result, PD decreased (PD and internal resistance are inversely proportional to each other).
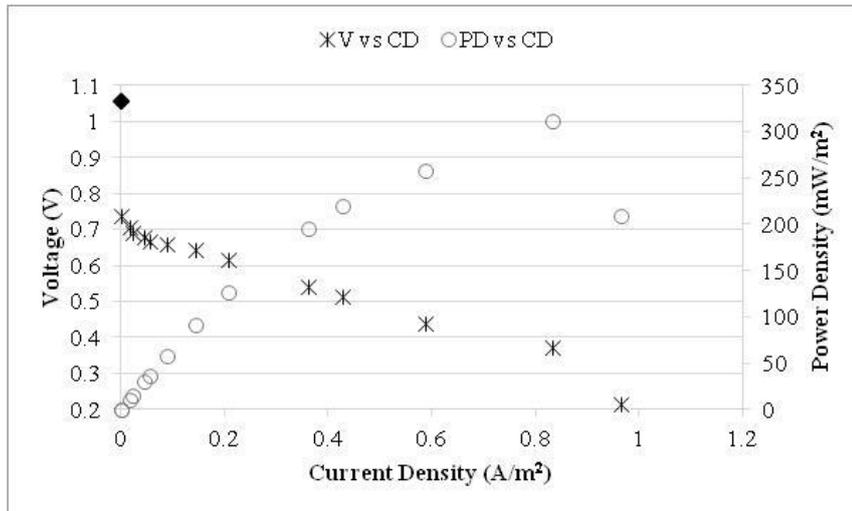
Figure 4.22. Polarization curve plotted for MFC-22

MFC-22 was operated for 8 days and at the end of reactor operation the pH dropped to 7.05, due to H^+ accumulation after membrane surface was fouled. Final COD and COD reduction (%) were 3550 mg/L and 39.8%, respectively. COD reduction was similar to MFC-20 and MFC-21 but due to material difference, the electrons liberated were not transferred effectively. This was also reflected to CE (%) which was calculated as 2.1%.

Considering these findings and the timeline of the study, it was concluded that the optimization studies should be completed and it was time to move onto the next phase: operating the dual chamber MFC using different sludge samples as substrate. It was finally decided to operate the upcoming reactors using carbon cloth as anode, Pt loaded carbon cloth as cathode and CMI-7000 as the membrane in between and Cu wire to connect the electrodes.

Table 4.57. Results of MFC set-ups during optimization experiments

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW	PD (mW/m^2)	Changes
MFC-8	Glucose	C paper	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	3000	SWW-2*	5.8	50 mM NaCl solution was added to anolyte.
MFC-9	Glucose	C cloth (Biofilm)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-2*	7.71	Identical to MFC-8, only anode electrode was changed.
MFC-10	Glucose	C cloth (Biofilm)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-2*	18.55	Resistance box was replaced, 50 mM NaCl was added, sampling ports were insulated.
MFC-11	Sodium acetate	C cloth (Biofilm)	C paper (0.4 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-2*	44.52	pH of PBS was increased to 8.0, Nafion 117 was pretreated at 40°C.
MFC-12	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3	141.01	80 mM NaCl solution was added. Vitamin + mineral solution and SWW-3 was added to anode.

*Mg, Mn and Zn concentrations were halved.

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Table 4.57. Results of MFC set-ups during optimization experiments (cont'd)

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW	PD (mW/m^2)	Changes
MFC-13	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3	93.41	Vitamin + mineral solution was excluded.
MFC-14	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3	150.84	Identical to MFC-12.
MFC-15	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	Nafion 117	Cu	1000	SWW-3	203.72	WAS and ADS mixture as used as inoculum. Humic acid was added as mediator.
MFC-16	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**	258.71	Resistance and membrane were changed, SWW-2 was added but glucose was halved. WAS was used as inoculum.

**Glucose concentration was halved.

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Table 4.57. Results of MFC set-ups during optimization experiments (cont'd)

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)	SWW	PD (mW/m^2)	Changes
MFC-17	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**	160	Identical to MFC-16.
MFC-18	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**	347.5	Humic acid concentration was increased, CMI-7000 was pretreated at room temperature.
MFC-19	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	SWW-2**	307	Identical to MFC-18.
MFC-20	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	300	SWW-2**	464	Inoculum was concentrated, NaCl used in CMI-7000 pretreatment decreased.
MFC-21	Glucose	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	300	SWW-2**	432	Identical to MFC-20.
MFC-22	Glucose	C paper	C paper (0.4 mg/cm^2 Pt)	CMI-7000	Cu	300	SWW-2**	304	Electrodes changed.

**Glucose concentration was halved.

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4.1.3.Operation of Microbial Fuel Cells with Different Types of Sludge Samples

One of the objectives of this study was to identify the impact of different types of sludges (substrates) on energy production, as stated at the very beginning. However, this could be only done after the first goal was accomplished which was MFC optimization. Taking into consideration the timeline of the study, it was time to start operating the system using different sludges the PD values obtained during optimization studies reached a level close to previous studies in the literature.

The starting point of this part of the study was MWWTP sludge obtained from Ankara Central WWTP, WAS line. The set up was called as MFC-23. As decided at the end of the optimization experiments, the anode and cathode electrodes using in MFC-23 were carbon cloth and the membrane in between was CMI-7000. The system was insulated again, as usual, and operated in a constant temperature room. It was inoculated using the WAS inside the biofilm bottle. The initial measurements done (for WAS) are given in Table 4.58.

Table 4.58. Initial pH, COD and solids concentration measurements for MFC-23

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
Analysis-I	7.09	29260	24960	18130	17140	14700
Analysis-II	-	29420	24750	17980	17060	14680
Analysis-III	-	-	24980	18140	17020	14660
Analysis-IV	-	-	24830	18110	17080	14640
Average	7.09	29340	24880	18090	17100	14670

This sludge sample was kept at 4°C (dark) in a refrigerator for 24 hours and the top supernatant was continuously removed with 3 hours intervals to concentrate the sludge. Then it was centrifuged to concentrate further up to 2.5% solids concentration. Then prior to reactor set up, it was purged with N_2 gas and fermented

at dark for another 48 hours. The anode chamber was filled with this sludge while the cathode was filled with PBS and 80 mM NaCl solution.

The external resistance was 300 ohms initially (based on the outputs of the optimization tests), but since the acclimation period was much longer compared to previous MFCs, the external resistance was increased to 500 ohms which indicates the increase in internal resistance and the maximum PD was obtained at 500 ohms. The internal resistance of the system was calculated based on three methods (as done previously): (i) slope of the polarization curve, (ii) empirical formula and (iii) the resistance at which the highest PD was measured. In the light of this information, the internal resistance was calculated as: (i) 450 ohms, (ii) 540 ohms and (iii) 500 ohms. On the average, it can be said that the system had an internal resistance of about 500 ohms. As the internal resistance increased in this case, the PD decreased. Actually, increase in internal resistance was expected since there were so many interferences in the case of sewage sludge as a substrate (unlike glucose which was readily available and pure) and it was not easy to degrade it, electrons were not easily liberated. In addition to this, numerous ions present in sludge also affected proton transfer through CMI-7000 (increase in internal resistance).
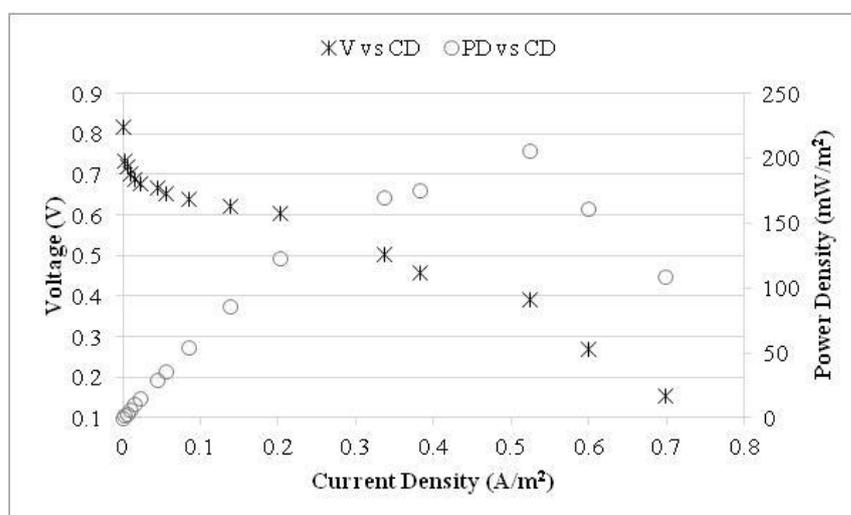
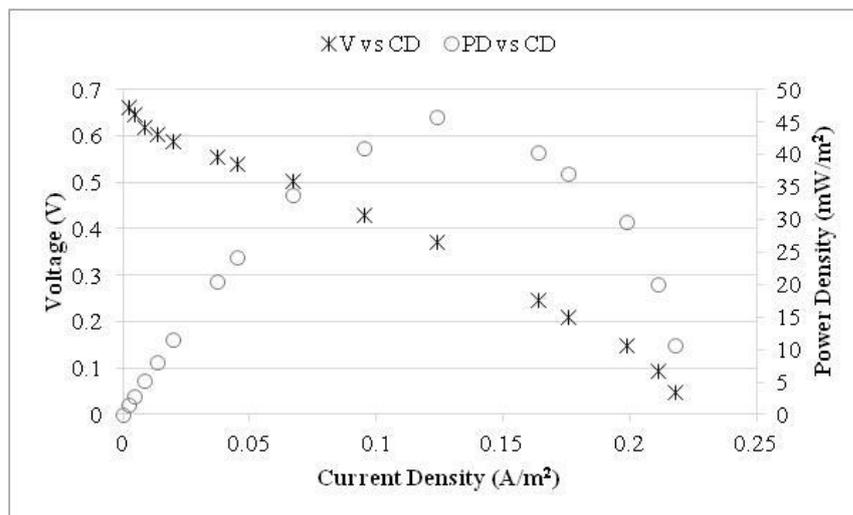**Figure 4.23.** Polarization curve plotted for MFC-23

The maximum PD calculated was 205.93 mW/m^2 at 500 ohms, and at the end of reactor operation the COD was measured as 16080 mg/L with a total reduction of 45.2%. When pure carbon source was used (as in optimization studies) PD was 464 mW/m^2 which was almost 2 times of the value calculated here. The drop in PD was due to the increasing IR with all the cations and interferences present in sludge. The COD reduction, on the other hand, was close to optimization studies and this showed that although sludge was degraded by microorganisms, electron was not transferred as effectively as it was during optimization experiments (due to membrane fouling, sludge matrix effect, etc.). The CE (%) was not calculated since the exact structure and molecular weight of the sludge were not precisely known. The pH drop was more significant in this case since the anolyte was not buffered, and final pH was measured as 6.38 at the end of 12 day reactor operation.

Following the operation of MFC-23, another sampling was done at the Ankara Central WWTP, but this time the aim was to investigate the impact of ultrasound pretreatment on power output of MFC. The sludge sample brought to the laboratory was again concentrated to 2.5% TS concentration and had similar properties compared to the sludge used in MFC-23. Since the characteristic of sludge in both MFC operations were similar, it was thought that the only variable affecting energy production would be ultrasound pretreatment. The initial measurements are given in Table 4.59.

Table 4.59. Initial pH, COD and solids concentration measurements for MFC-24

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
Analysis-I	7.03	31260	25210	17620	17200	13880
Analysis-II	-	31420	25280	17680	17340	14000
Analysis-III	-	-	25160	17570	17280	13940
Analysis-IV	-	-	25230	17650	17260	14100
Average	7.03	31340	25220	17630	17270	13980

The sludge sample was subjected to 10 min ultrasound pretreatment using Sartorius Labsonic P (Sartorius AG, Germany), with a 22 mm probe at a power of 255 W. The microbial inoculum was added separately without ultrasonication. The reactor was again purged with nitrogen gas. The acclimation period was longer compared to SWW fed MFCs but 1.5 days shorter when compared to MFC-23. The reason for this was the breakdown of complex organics within the sludge via ultrasound pretreatment, enhancing biodegradation. However, the ease of biodegradation was not reflected to current flow and the maximum PD obtained was 225.23 mW/m^2 with an IR of 400 ohms (Figure 4.24). Even if the internal resistance was reduced to 100 ohms compared to MFC-23, there was no significant improvement in reactor performance. That's why, it was decided to operate another set of reactor increasing the duration of ultrasound pretreatment to 20 min (MFC-25).

Figure 4.24. Polarization curve plotted for MFC-24

MFC-25 was identical to MFC-23 in all aspects except for the duration of pretreatment. This time, the sludge sample was subjected to 20 min ultrasound pretreatment using Sartorius Labsonic P (Sartorius AG, Germany). The microbial inoculum was added separately without ultrasonication. The initial measurements are presented in Table 4.60.

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Table 4.60. Initial pH, COD and solids concentration measurements for MFC-25

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
Analysis-I	7.07	30920	24820	17490	16180	13140
Analysis-II	-	30840	24730	17400	16060	13040
Analysis-III	-	-	24850	17470	16000	13060
Analysis-IV	-	-	24800	17480	16160	13120
Average	7.07	30880	24800	17460	16100	13090

The internal resistance measured was reduced to 340 ohms with a PD of 281.54 mW/m^2. The internal resistance was slightly reduced compared to 10 min sonicated set but the change in the performance was not satisfactory. The expected outcome was a significant decrease in internal resistance and increase in PD since ultrasonic pretreatment improves biodegradability, therefore, the amount of electron liberated for energy production. The reason why internal resistance couldn't be lowered with the help of ultrasonication was probably due to the instant release of electrons with rapid biodegradation and the release of ions that fouled the membrane, preventing the system reach its full capacity. The polarization curve of MFC-25 is given in Figure 4.25.
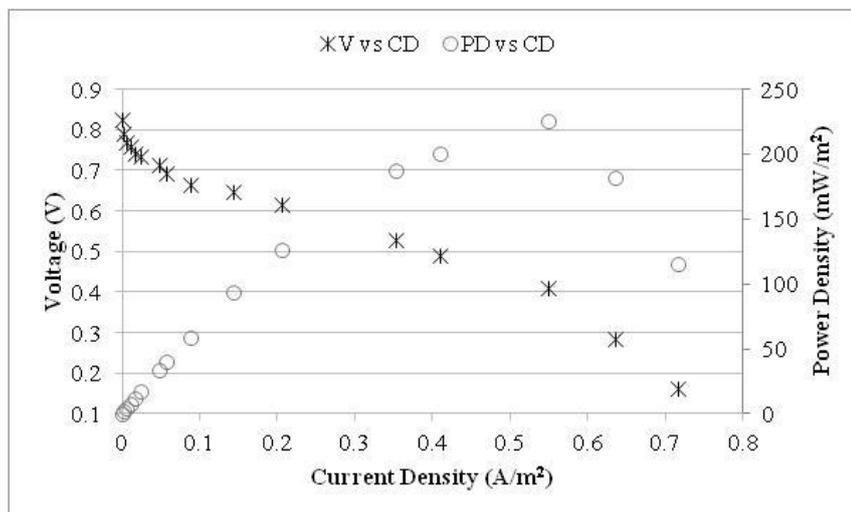
Figure 4.25. Polarization curve plotted for MFC-25

The final COD of the system was 14590 mg/L with a total reduction of 52.8%, which was slightly higher than the rest since the biodegradability of sludge was increased with sonication and it was readily taken up by microorganisms and degraded. The final pH was 6.69 which showed the accumulation of H^+ ions due to membrane fouling, like in the rest of the MFCs operated.

Following MFC-25, a sludge sample was obtained from a poultry industry. This sludge sample was very dense in terms of solids concentration (there was no supernatant on top to dilute the wastewater and with distilled water the particles started to float) and for this reason the TSS and VSS concentrations were not measured (it was impossible to filter the sample through the 0.45µm membrane). The electrodes, membrane and connecting wire were kept the same. The reactor was inoculated using the sludge (WAS) inside the biofilm bottle. The initial measurements are given in Table 4.61.

Table 4.61. Initial pH, COD and solids concentration measurements for MFC-26

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)
Analysis-I	7.63	54450	47000	32920
Analysis-II	-	53950	47130	33000
Analysis-III	-	-	47090	33110
Analysis-IV	-	-	47060	33090
Average	7.63	54200	47070	33030

As can be seen from Table 4.61, poultry WW sludge was a very thick (~5% solids concentration) and had very high organic content which was expected to increase the performance of MFC. However, things did not go as expected. Although the organic content of poultry sludge was very high compared to MFC-23, due to the complex nature, it took longer time for the microorganisms to degrade it and for this reason the acclimation period was long. In addition to this, the storage of this sludge and set up of the system was very difficult since methanogens were very active and favored the substrate (due to high organic content).
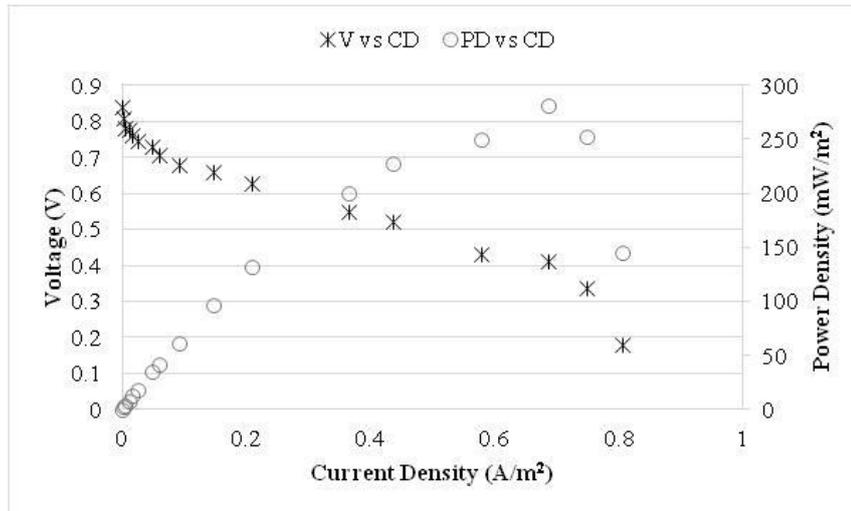
Figure 4.26. Polarization curve plotted for MFC-26

The acclimation period was long but the time of membrane fouling was very short due to the nature of the substrate (release of high concentrations of protons and clogging of membrane surface due to high solids concentration). The maximum PD was measured to be 45.88 mW/m^2 (at 2000 ohms) and the internal resistance was 1600 ohms (slope of the polarization curve, Figure 4.26). When calculated using the empirical formula, the internal resistance was 1700 ohms. The significant increase in internal resistance and decrease in PD was actually not surprising considering the high solids and organic content of the sludge. These two important parameters directly affect the membrane, which is the major internal resistance item.

MFC-26 was operated for 18 days due to long acclimation period. At the end of the reactor operation the pH was measured to be 6.49 and COD was 23840 mg/L with a total reduction of 56%. This is not a surprising result considering the length of operation and the active nature of methanogens inside the poultry sludge. Due to the solids and organic content and its initial characteristics, the sludge was already producing methane and at the end of reactor operation, the membrane bended towards the cathode chamber due to gas (methane) accumulation. This certainly inhibited electron transfer and current flow. Eventually, together with membrane

fouling due to high organic loading, the system performance decreased with increasing internal resistance.

MFC-27 was operated using sludge from a beverage WWTP, WAS line, as substrate. The sludge sample sent was initially very dilute and in order to bring all the MFCs operated to the same level, the sludge was concentrated using centrifuge but due to the available sample volume a TS concentration of 2% was achieved (Table 4.62). The cathode chamber was filled with PBS and 80 mM NaCl solution. To plot an accurate polarization curve the reactor was operated at different external resistance values between 150 ohms and 200,000 ohms.

Table 4.62. Initial pH, COD and solids concentration measurements for MFC-27

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
Analysis-I	7.19	52050	20230	14500	13820	11460
Analysis-II	-	50450	20270	14550	13980	11600
Analysis-III	-	-	20110	14400	13900	11560
Analysis-IV	-	-	20070	14470	13940	11540
Average	7.19	51250	20170	14480	13920	11540

This reactor operation yielded a maximum PD of 360.91 mW/m^2 at an external resistance of 300 ohms. The internal resistance was calculated as 270 ohms using the slope of the polarization curve given in Figure 4.27.

The acclimation period for MFC-27 was 4 days, which was shorter compared to MFC-23 (due to higher organic content) and longer compared to optimization studies (due to complex nature of sludge). The PD was much higher compared to the previously operated MFCs using domestic and poultry sludge since the organic content was high and the sludge was dilute (considering poultry sludge). This reactor operation showed that at optimum solids concentration and high organic content, the performance of an MFC can be enhanced.
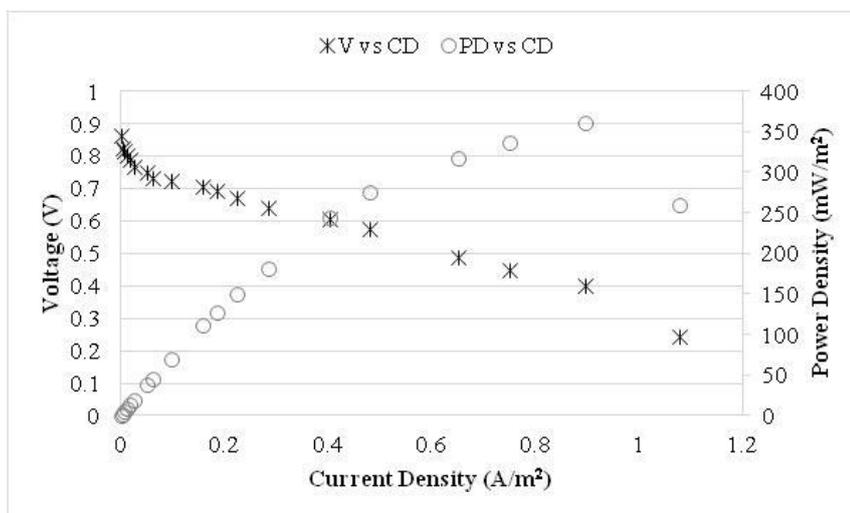
Figure 4.27. Polarization curve plotted for MFC-27

This reactor was operated for 15 days to see the general trend and membrane fouling time. At the end of reactor operation pH dropped to 6.71. The overall COD reduction in this set was 52.3%, with a concentration of 24440 mg/L at the end of reactor operation. pH drop in sludge fed systems were more significant compared to optimization studies since sludge contains electron acceptors that allow acidogenesis and methane formation within the anode chamber in addition to H^+ accumulation. Due to this, also the internal resistance increased in each set decreasing the PD.

Enhancement of MFC performance using organic rich sludge and sonication was shown in this study and it was time to investigate the inhibiting impact of sludge composition on PD. For this purpose two different sampling were done: one from a petrochemical plant and the other from a textile industry. MFC-28 was operated using the petrochemical sludge. The sludge sample was very thick and dry (impossible to filter, not fluid) so it was diluted using distilled water to a TS of 2.4%. The microbial inoculum was obtained from Ankara Central WWTP WAS line. The system was operated at different external resistance values between 150 ohms and 200,000 ohms. The initial pH and solids concentration measurements are given in Table 4.63. COD couldn't be measured since even after dilution it contained large particulates leading to heterogeneity.

Table 4.63. Initial pH and solids concentration measurements for MFC-28

	pH	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
Analysis-I	7.07	23440	13370	15080	9180
Analysis-II	-	23330	13260	15160	9280
Analysis-III	-	23410	13390	15200	9240
Analysis-IV	-	23380	13300	15040	9120
Average	7.07	23390	13330	15120	9200

MFC-28 had an acclimation longer than MFC-24 (poultry sludge) mainly due to the inhibitory constituents (complex hydrocarbons) present in petrochemical sludge. The maximum PD was measured as 21.17 mw/m^2 at 2000 ohms. This number is way below the power densities calculated for the rest of the MFC operations. The reason for such a sharp drop in PD was obviously the complex and inhibitory constituents of petrochemical sludge that couldn't be degraded by the microorganisms to produce electrons and generate current. In addition to this, the sludge was diluted with distilled water leading to large flocs and heterogeneity inside the reactor slowing down disintegration. The internal resistance was calculated as 1900 ohms and this high internal resistance was observed as instant membrane fouling. But this time, different from the rest of the MFC operations, membrane fouling was not due to high release of protons and electrons, but due to structure and constituents of sludge itself.
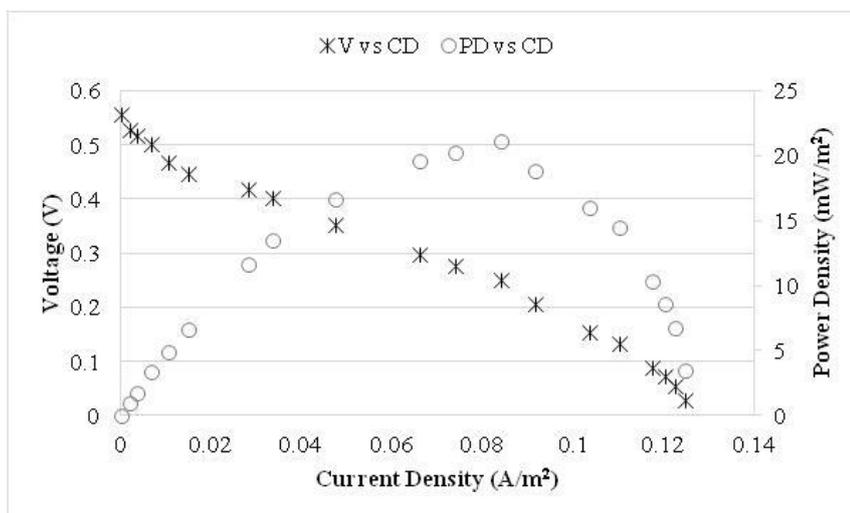
Figure 4.28. Polarization curve plotted for MFC-28

Petrochemical sludge is a very extreme sample since its organic content is lower and contains high concentrations of long chain hydrocarbons that cannot be degraded by microorganisms. Considering this, another sludge sample that still contains inhibitory materials but still can be tolerated by microorganisms was decided to be tested. For this purpose sludge from a textile industry's WWTP was used as substrate. Unfortunately, the sample was very dilute and couldn't be concentrated using centrifuge or any other means (bulking problem). So the experiments were done at a dilute concentration.

MFC-29 was operated identical to the rest of the reactor operations (in terms of electrodes and membrane). The system was operated at different external resistance values between 150 ohms and 200,000 ohms. The initial pH, COD and solids measurements are given in Table 4.64.

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Table 4.64. Initial pH, COD and solids concentration measurements for MFC-29

	pH	COD (mg/L)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
Analysis-I	6.99	7990	10380	6460	9140	5920
Analysis-II	-	8120	10470	6510	9200	5860
Analysis-III	-	-	10440	6400	9300	6000
Analysis-IV	-	-	10410	6550	9280	5960
Average	6.99	8055	10425	6480	9230	5935

The acclimation period was not as long as the petrochemical or poultry sludge but it was longer than MFC-23, MFC-25, MFC-26 and MFC-27. The polarization curve is given in Figure 4.29.
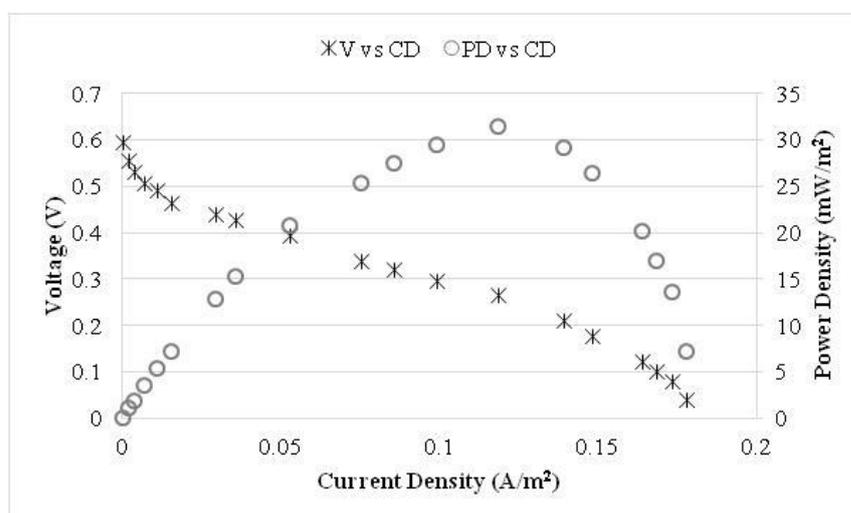
Figure 4.29. Polarization curve plotted for MFC-29

The maximum PD measured was 31.44 mW/m^2 at 1500 ohms. The internal resistance was calculated as 1390 ohms. The difference in power densities and internal resistances of MFC-28 and MFC-29 could be: (i) substrate constituents and (ii) solids content. Since the solids content of this set is not similar to the rest and fouling due to high solids loading has a significant impact on membrane fouling and therefore internal resistance.

At the end of reactor operation (19 days), the pH and COD were measured as 6.41 and 5020 mg/L with a total COD reduction of 37.7%. COD reduction was lower compared to sludge fed MFCs or optimization studies since textile sludge is not as easily biodegradable as municipal sludge or glucose itself. This low organic utilization was also reflected to internal resistance and low PD with reduced current flow.

Table 4.65. Results of MFCs fed with different types of sludges

MFC	C Source	Anode	Cathode	Membrane	Wire	Resistance (Ω)*	Inoculum	Maximum PD (mW/m^2)
MFC-23	WAS	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	500	WAS	205.93
MFC-24	Sonicated WAS (10 min)	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	500	WAS	225.23
MFC-25	Sonicated WAS (20 min)	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	400	WAS	281.54
MFC-26	Poultry sludge	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	2000	WAS	45.88
MFC-27	Beverage IWW sludge	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	300	WAS	360.91
MFC-28	Petrochemical IWW sludge	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	2000	WAS	21.17
MFC-29	Textile IWW sludge	C cloth (Biofilm)	C cloth (0.5 mg/cm^2 Pt)	CMI-7000	Cu	1500	WAS	31.44

*The resistance at which maximum PD was measured.

The experiments conducted in this study showed that MFC performance does not only depend on the type of material used. Any mechanism that affects electron liberation (microorganisms) and transfer directly impacts the power generation. To come up with a functioning MFC, first the system must be very well insulated and must be kept away from any conductive surfaces to prevent electron escape from the reactor. Secondly, the parameters effective on IR must be investigated in detail. For instance, membrane pretreatment technique definitely affected the surface properties and therefore IR and power output. Considering many more details as discussed in optimization experiments, then material differences can be discussed. And once the system is settled, it can be fed to stabilize and generate electricity. But, the type and concentration of substrate is also important as shown in this study. For instance, when beverage IWW sludge was fed with high organic content compared to textile or petrochemical IWW sludge, then considerable PD values and small IR values could be possible (due to better biodegradation). However, solely organic nature of sludge was not satisfactory, sometimes, like in the poultry sludge case, high concentration of organics and solids content could also impede system performance. Therefore, not only substrate constituents but also feed solids concentration is also important which will in turn affect the order of treatment in a WWTP. After a vigorous sludge dewatering process, using an MFC will not be appropriate.

4.2. Operation of Laboratory Scale Anaerobic Digesters

One of the objectives of this study was to operate a lab-scale anaerobic digester and compare the findings with MFCs. For this purpose, using the sludge sample collected from the anaerobic digester and WAS line of Ankara Central WWTP, duplicate reactors were set up. The anaerobic digesters were operated in a constant temperature room at 35°C using 5 L glass bottles (active volume was 3.2 L). The total duration was 65 days and the parameters of concern were: (i) solids concentration, (ii) COD, (iii) gas volume and (iv) gas composition. The F/M ratio (g VS/g VSS) was kept as 1

based on previous research to ensure maximum methane production could be achieved (Köksoy and Sanin, 2010).

Biogas produced was collected in gas collection units, placed in brine solution to prevent gas dissolution. For the first 12 days, daily sampling was done since the biogas production was high. Then the frequency of sampling decreased as the daily changes became insignificant (Figure 4.30). Within the first 12 days almost 95% of the biogas was produced and this showed that the biodegradable portion of sludge was taken up by the microorganisms and converted into biogas. Both reactors showed the same pattern indicating the repeatability of the operation. For the first couple of days, since the reactors were purged with nitrogen gas, the majority of the biogas produced was nitrogen and methane was around 5-7%. But when the nitrogen accumulated within the system was discarded, methane production started to dominate the system, making up almost 60% of the total biogas (Figure 4.31). The biogas and methane production values reached to 12 L and 7 L, respectively., when the reactors were terminated and these results were similar to the ones obtained in the study of Çelebi (2015), where anaerobic batch digesters utilizing sludge from Ankara Central WWTP were operated.
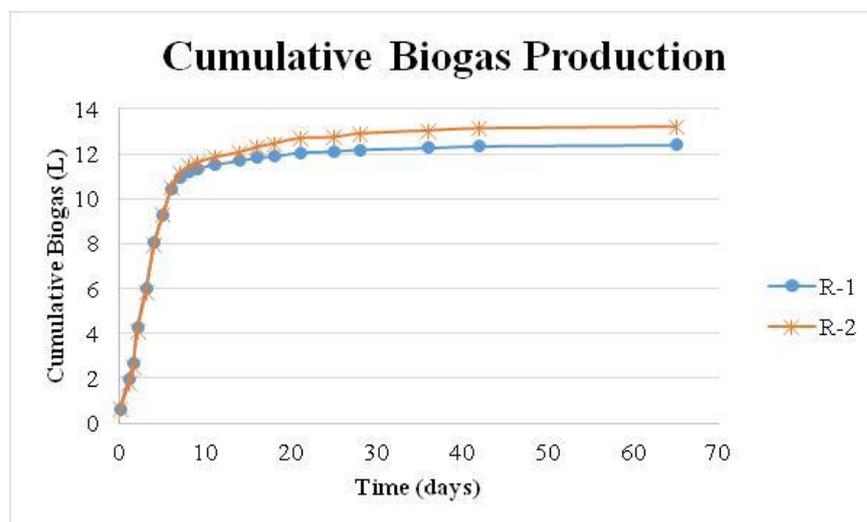
Figure 4.30. Cumulative biogas production over time for R-1 and R-2
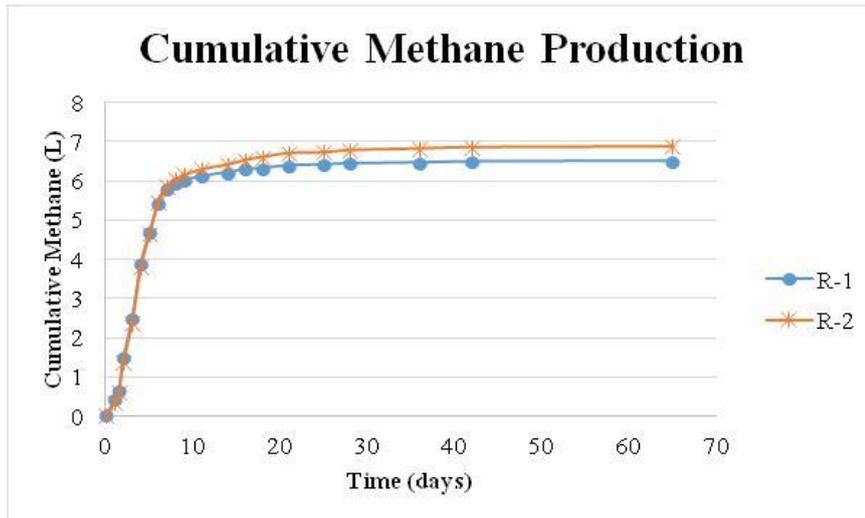
Figure 4.31. Cumulative methane production over time for R-1 and R-2

As can be seen from the graphs, biogas and methane production reached a stagnant level after day 16, showing that the biodegradable portion of the substrate was consumed and these findings were consistent with solids concentration and COD (Figure 4.32-4.34). The initial TS concentrations were 28135 mg/L and 28190 mg/L for R-1 and R-2, respectively. On day 16, the solids concentrations, both TS and VS, reached a stagnant level and at the end of reactor operation the total TS and VS reductions were 33% and 51%, respectively, while COD reduction was 43%.
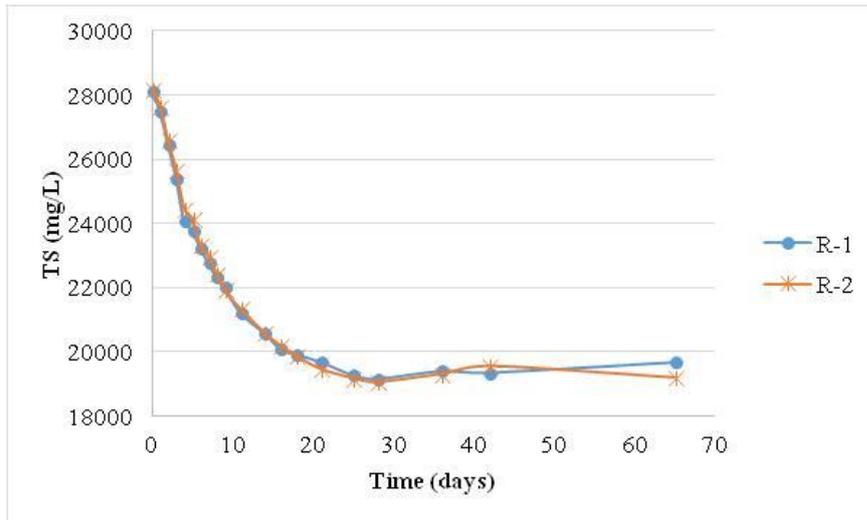
Figure 4.32. Total solids concentration with respect to time graph for lab-scale anaerobic digesters
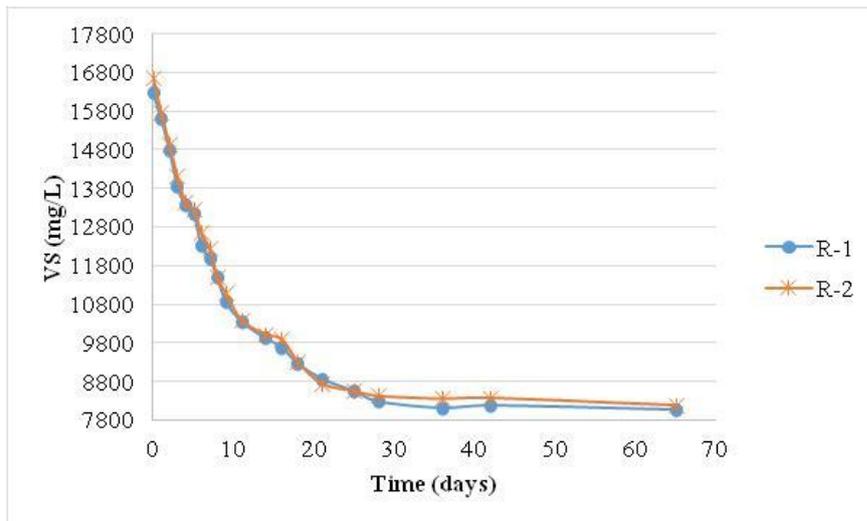
Figure 4.33. Volatile solids concentration with respect to time graph for lab-scale anaerobic digesters
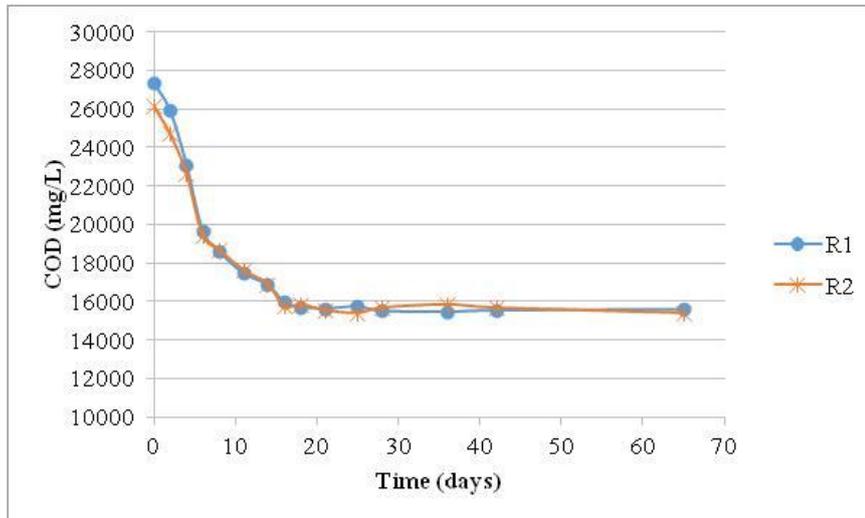
Figure 4.34. COD concentration with respect to time graph for lab-scale anaerobic digesters

4.3. Energy and Performance Comparison of Microbial Fuel Cells and Anaerobic Digesters

Anaerobic digesters are considered as sustainable solution for sludge management in terms of stabilization and biogas production, and are very mature and popular technologies nowadays. However, they also have some drawbacks such as long SRTs, large area requirements, complex process of hydrolysis, acetogenesis, acidogenesis and methanogenesis which require high level of maintenance and some in-plant safety issues (Khan et.al., 2017; McCarty et.al., 2011; Xin et.al., 2018; Zhang et.al., 2014). MFCs, on the other hand, directly convert the organic matter into electrical energy without any intermediate or complex processes (Xin et.al., 2018). However, the amount of energy produced is determined by the extent of biodegradation and electron transfer rate (Song et.al., 2015). This brings the problems related with the repeatability of the system, which can be solved via culturing specific organisms within the anolyte and on the biofilm. Compared to

anaerobic digestion, MFCs have small footprint and have shorter SRTs (Xin et.al., 2018). The first difference in between the two methods is the length and steps of the processes (Figure 4.35).
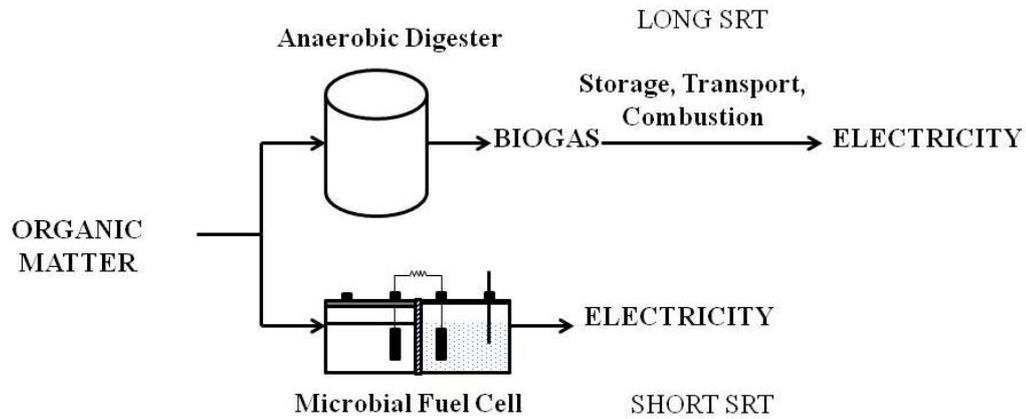
Figure 4.35. Schematic comparison of anaerobic digestion and MFC technology

First and foremost, the most important parameter in the comparison of MFC and anaerobic digestion is the amount of electricity generated per kg COD removed (kWh/kg COD). To make this comparison, an MFC and lab-scale anaerobic digester were operated with the same municipal sludge obtained from Ankara Central WWTP. The findings of these reactor sets were discussed in the previous sections. The energy yields of the systems were calculated using two different formulas as given below (Xin et.al., 2018):

Equation (4.3) : $E_{MFC} = \dfrac{VxV \cdot \frac{t}{R}}{(CODi-CODf)\,x\,v\,x\,3600000}$

where, E_{MFC} is the kWh/kg COD electricity produced by MFC

V is the voltage at maximum PD (V)

Volume (v) is 0.45 L

3600000 is the unit conversion coefficient (MJ to kWh)

t is the duration of MFC operation is seconds

R is the external resistance at maximum PD

Equation (4.4) : $E_{AD} = V_{methane} \times (-\Delta U) \times A \times B$

where, E_{AD} is the kWh/kg COD electricity produced by digester

$V_{methane}$ is the total volume of methane produced (m^3)

$-\Delta U$ is the energy of combustion (40 MJ/kg.m^3)

A is the methane to electricity conversion coefficient (0.35)

B is the unit conversion coefficient

Anaerobic digester not only generates electricity but uses it also to heat the reactor (unlike MFC; since MFCs are operated at ambient temperatures. Some portion of this energy is also lost through the surface of the reactor (45% of total heat energy). This is best represented by the following equation (Tchobanoglous et.al., 2004):

Equation (4.5) : $H = c \times M_{solids} \times \Delta T$

where, H is the amount of energy used for heating (Joules)

c is the specific heating coefficient (4200 J/kg.°C)

ΔT is the temperature difference to reach the designated temperature level (°C)

M_{solids} is the mass of solids within the digester (kg)

MFC-23 was operated using MWWTP sludge from WAS line. This reactor was operated for 12 days, therefore, t in Equation 4.3 is 1.04×10^6 seconds. The initial COD (COD_i) was 29340 mg/L and final COD was (COD_f) 16080 mg/L. Then the electricity produced by MFC-23 was:

$$E_{MFC} = \frac{0.393 \, V \times 0.393 \, V \times 1.04 \, x\frac{10^6}{500 \, ohms}}{\left(29340\frac{mg}{L}-16080\frac{mg}{L}\right)x\frac{1kg}{10^6 mg} \times 0.45 \, L \times 3600000\frac{J}{kwh}} = 0.015 \text{ kWh/kg COD removed}$$

Since MFC does not require any additional energy to run the system (unlike anaerobic digestion) this is the total amount of electricity for the system. This number may look small but if the CE (%) could be improved, this number would also

increase. Stacked configurations are usually used for this purpose, since usually the power output of a single MFC is small (Pham et.al., 2006).

The anaerobic digesters produced 7 L of methane at the end of reactor operation with an initial solids concentration of 2.8%. Initially, the sludge sample was kept at 4°C but left to reach room temperature at the laboratory prior to set-up (25°C). Therefore, the energy produced and heating requirements can be calculated as follows:

$$H = 4200 \text{ J/kg.°C} \times 28000 \text{ mg/L} \times 1 \text{ kg}/10^6 \text{ mg} \times 3.2 \text{ L} \times (35\text{-}25)\text{°C} = 3763.2 \text{ J}$$
$$= 0.001 \text{ kWh}$$

Some of the above energy is lost (45%) (Tchobanoglous et.al., 2004). Therefore, the total amount of energy required for heating is:

$$H = 0.001 \text{ kWh}/0.45 = 0.002 \text{ kWh}$$

The energy produced by anaerobic digestion is:

$$E_{AD} = 7 \text{ L} \times (1 \text{ m}^3/1000 \text{ L}) \times (40 \text{ MJ/m}^3) \times 0.35 \times 0.28 = 0.027 \text{ kWh/kg COD reduced}$$

The total energy balance of anaerobic digesters is as follows:

$$E_{AD, \text{ Total}} = 0.027 \text{ kWh/kg COD} - (0.002 \text{ kWh}/(0.012 \text{ kg COD/L} \times 3.2 \text{ L}))$$
$$= -0.025 \text{ kWh/ kg COD}$$

The results above show that, in addition to the energy produced, anaerobic digesters require energy to operate the system (heat the sludge). This number was calculated according the basic insulation and configuration (45% heat loss), therefore, the energy requirements can be reduced by better insulation and improved design. However, still the whole energy would be used to operate the system. In MFC, on the

other hand, the energy produced might be small (still could be improved by system insulation, changing the organic loading and system control), the energy can be used for other purposes since the system does not require any additional energy input for heating or other purposes like mixing within the WWTP. The energy generated by a single MFC was small since the voltage generated is between 0.3 V and 0.7 V (OCV) but stacked systems can multiply this number which allows us to generate beyond 100 V. If the system configuration is changed (single chamber air-cathode), other cost items like aeration could be also eliminated for MFCs. MFCs have the potential for improvement and promise a lot considering their advantages. Besides, anaerobic digestion produces methane, and gas must be combusted to generate electricity, while MFCs directly convert to electricity as described before (Figure 4.35).

The amount of electricity generated might be an important parameter in comparing the systems but it was not the sole one. Both reactors were operated using the same sludge sample to compare the COD reduction efficiencies. MFC required 12 days to achieve a COD reduction of 45.2% while anaerobic digesters degraded 43% of the COD within the first 16 days, showing similarity. But when MFCs were operated for longer periods, as described before, the COD reduction could reach up to 56%. This is an indicator of the rapid metabolism of the mixed culture compared to methanogens in anaerobic digesters. Even if the operation of MFCs were shorter compared to anaerobic digestion to achieve same level of organic removal, still the total operation cost of the system is much higher. At the end of each operation, the membrane and electrodes within the MFCs must be replaced but for anaerobic digestion continuous feed except for a couple of maintenance within the digester insulation. Due to this expensive nature of MFC operation actually the full scale application of the system is limited except for a couple of examples. Today, to overcome this material related economic limitations, mediator-less MFC systems are being studied but these systems are much more difficult to control in terms of oxygen reflux and preserving the anaerobic conditions in anode chamber.

In addition to the numerical parameters such as power and COD reduction, there exist other differences between anaerobic digestion and MFC technology. Microorganisms play an important role in both systems but their interactions differ in each system. For instance, during anaerobic digestion process a complex food chain type microbial consortium is present, starting with hydrolysis of substrate to methanogenesis (Angenent et.al., 2004; Pham et.al., 2006). The survival of methanogens is very important during digestion, that's why the system is very sensitive. In MFCs, mixed culture is present and there is no hierarchy like anaerobic digestion. There are numerous studies which operated MFCs with axenic cultures (Kim et.al., 1999; Bond and Lovley, 2003). Either way, the system is operated with an electrochemically active consortium to promote electron transfer and current flow.

In terms of organic loading, the operation of anaerobic digestion and MFC show differences. Anaerobic digestion can handle both low and high COD concentrations, but requires high temperatures to utilize them (Pham et.al., 2006). The organic loading rate in an MFC is more crucial compared to anaerobic digestion. When influent COD increases, membrane fouling occurs rapidly and % CE drops as happened in this study with poultry sludge.

To conclude, anaerobic digestion is more mature and technically and economically feasible compared to an MFC but MFCs are being improved continuously like single chamber air-cathode systems or mediator-less MFCs to minimize internal resistance and operational costs. To increase the PD, several MFCs are connected in series in the form of a stack. The economic limitations still remain, but solutions to this are also being investigated by scientists with attempts to replace the materials used. These challenges must be solved first to scale the reactors from laboratory to WWTPs treating thousands of cubic meters wastewater.

CHAPTER V

CONCLUSION

The aim of this study was to optimize a dual chamber MFC based on several factors such as: (i) anode/cathode electrode materials, (ii) membrane type (Nafion 117 or CMI-7000) and its pretreatment technique, (iii) reactor design and insulation, (iv) content of anolyte and catholyte solution and (v) type of microbial inoculum. The aim of these experiments was to maximize the power generation while minimizing the costs associated with the materials used. For this purpose, using low cost electrodes and membranes, the environmental conditions affecting the microbial activity was improved so as to enhance degradation and electron transfer. This was the most critical outcome of this study since even if the same materials were used and identical MFCs were operated, if the conditions affecting microbial activity are not suitable, then one cannot expect a functioning MFC, which was the case during preliminary experiments. This, the very first part of this study, was also crucial to proceed to the next steps.

The second objective of this study was to investigate the impact of different substrates (sludges) on PD. At this stage five different types of sludge were fed to the system. To make a healthy comparison, MWW sludge was selected as the baseline since it does not contain extreme concentrations of any specific material like in the case of poultry or petrochemical IWW sludge. But it was fed in three forms: (i) MWW sludge, (ii) 10 min sonicated MWW sludge and (iii) 20 min sonicated MWW sludge to see whether the dissolution of sludge could improve PD. 10 minutes sonication increased the power density to 225.23 mW/m^3, and the internal resistance decreased to 400 ohms (it was 500 ohms for municipal sludge with no sonication). When the sonication time increased to 20 minutes, the PD increased to 281.54

mW/m^2 and the internal resistance decreased further to 340 ohms. The dissolution of sludge and its organics certainly improved the performance but the changes were not significant and considering the fact that sonication is an energy intensive and expensive process to apply in full scale, it was decided to use substrates without any kind of pretreatment.

Substrate composition is an important parameter affecting MFC performance. Depending on the constituents it may either limit or boost the electricity production. In order to see both sides, poultry sludge, beverage IWW sludge, petrochemical IWW sludge and textile IWW sludge were used. Using poultry and beverage sludges as substrate it was expected to see an increase in PD and decrease in the IR. This was valid for the beverage IWW sludge where IR dropped to 300 ohms with a PD of 360.91 mW/m^2 (which was the highest value in this study for sludge fed MFC experiments). But, poultry sludge had different characteristics with a thicker nature which limited the energy production and resulted in extreme membrane fouling increasing the IR value up to 1600 ohms. Textile and petrochemical sludges, on the other hand, showed results that was identical what was expected from them. The internal resistances increased up to 1400 ohms showing that the electron transfer and probably microbial degradation leading to electron liberation were limited to the constituents of the sludge samples. This was another critical outcome of this study: substrate fed to the system must have an optimum solids concentration (which was identified as 2% TS in this study) regardless of the organic content to achieve a satisfactory energy production.

The starting point of this study was the limitations in the use of MFCs although they were promising technologies. Unfortunately, they need to compete with anaerobic digestion. To see the advantages and disadvantages of both systems, it was necessary to operate an anaerobic digester with an MFC operated at the same time, with the same sludge (MWW sludge). At the end, the findings were compared and an energy balance was done. The kWh electricity/kg COD value for the MFC was much lower compared to the electricity output of anaerobic digesters but since digesters require heating for operation, it was found that in addition to the electricity generated, extra

energy was required in anaerobic digesters while, even small amount, MFCs could directly use this energy for other purposes. Besides, the electricity of an MFC could be improved using different configurations and series connections (stacked MFCs), promising an effective bioenergy technique for future. But still, the economic limitations, especially the lifetime of membranes, limit the integration of MFCs into WWTPs.

CHAPTER VI

FUTURE STUDIES AND RECOMMENDATIONS

MFCs are great solutions to WWTPs in terms of both energy supply and treatment/stabilization. Unfortunately, their use is still limited since the materials used in their set-up are very expensive and require renewal during operation. For instance, membrane is the most expensive item of an MFC and due to its instantaneous fouling in contact with wastewater or sludge; it needs to be replaced during continuous operation. Since the contents of wastewater cannot be modified in a WWTP depending on the MFC materials used, then MFC must be improved regarding this problem.

To overcome this challenge, alternative materials must be constructed, meaning, instead of testing the existing materials, new ones must be developed. To illustrate, the surface characteristics of membrane surface could be improved to prevent the accumulation of ions and decrease IR. Pt catalyst is also another expensive item of MFC and maybe further research can be done to replace it with another catalyst that is cheaper. To conclude, the next step of the future of MFC technology relies on material technology and chemistry of materials to minimize the operational cost of the system set-up.

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WORK EXPERIENCE

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FOREIGN LANGUAGES

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PUBLICATIONS

1. **Ömeroğlu S.**, Sanin F.D. (2016) "Bioelectricity Generation from Wastewater Sludge Using Microbial Fuel Cells: A Critical Review", CLEAN-Soil, Air, Water, 44 (9), pp.1225-1233.

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2. **Ömeroğlu S.**, Sanin F.D., "A Comparison of Different Sewage Sludges Used in Microbial Fuel Cells for Energy Generation" European Biosolids and Organic Resources Conference, November 15-16 2016, Edinburgh, Scotland (Oral presentation).

3. **Ömeroğlu S.**, Sanin F.D., "Power Generation in a Microbial Fuel Cell Fed with Synthetic Wastewater", IWA World Water Congress and Exhibition 2016, October 9-14, 2016, Brisbane, Australia (Oral Presentation).

4. **Ömeroğlu S.**, Sanin F.D., "Microbial Fuel Cells: Sustainable Applications in Wastewater Treatment and Energy Production", 5th Turkish-German Water Partnership Days, October 6-8, 2015, Konya, Türkiye (Invited Speaker).

5. **Ömeroğlu, S.**, Kara Murdoch, F. and Sanin, F. D. "Development of Practical Extraction and Analysis Methods for Nonylphenol Compounds in Water and Wastewater" International Water Association World Water Congress and Exhibition, September 21-26 2014, Lisbon, Portugal (poster presentation).

6. Kara Murdoch, F., **Ömeroğlu, S.**, Bozkurt, H., Ahmad, M., and Sanin, F. D. "The Fate of Nonylphenolic Compounds in Water and Wastewater Systems". 17th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region. September 28- October 1, 2013, Istanbul, Turkey (poster presentation).

7. **Ömeroğlu, S.**, Bozkurt, H. and Sanin, F. D. "Fate of Nonylphenol Compounds during Aerobic and Anaerobic Digestion of Sludge" WEF Residuals and Biosolids 2013: Emerging Opportunities for Sustainable Resource Recovery, Nashville, TN, USA, May 5-8, 2013 (Oral Presentation).

8. **Ömeroğlu, S.** and Sanin, F. D. "Fate of Nonylphenol Compounds in Aerobic Batch Digesters" ECSM 3rd European Conference on Sludge Management, 6-7 Sept. 2012, Leon, Spain (Oral Presentation).

9. Bozkurt, H., **Ömeroğlu, S.**, Ahmed, M. Kara, F. and Sanin, F. D. "Nonylphenols in Sludge: Measurement and Removal Methods" V. Turkish German Solid Waste Days, TAKAG 2011, 27-30 September 2011, Stuttgart, Germany (Oral Presentation).

10. **Ömeroğlu S.**, Kara F., ve Sanin F. D., "Nonilfenol Bileşiklerinin Arıtma Çamurlarında Ölçümüne Yönelik Metotların Geliştirilmesi ve Uygulanması", EKK 2012 İTÜ XIII. Endüstriyel Kirlenme Kontrolü Sempozyumu, 17-19 Ekim 2012, İstanbul, Türkiye (Oral Presentation).

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AWARDS

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2. **Ömeroğlu S.** "Microbial Fuel Cells: A Sustainable Method for Wastewater Treatment and Energy Production", German Water Partnership Award Turkey 2015, April 2015.

PROJECTS

1. BAP-07-02-2014-007-090, "Mikrobiyal Yakıt Hücrelerini Kullanarak Atıksu Çamuru ile Biyoelektrik Üretimi", Graduate Thesis Project, 01.2014-12.2014.

2. BAP-03-11-2015-002, "Mikrobiyal Yakıt Hücreleri Aracılığıyla Atıksu Çamurundan Enerji Eldesi", Researcher, 01.2015-12.2016.