Copper recovery in a litre-scale Microbial Fuel Cell
Performance under different conditions
Master's thesis in the Master's Programme Infrastructure and Environmental Engineering and Water Resources Engineering and Management

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Department of Civil and Environmental Engineering
Division of Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 2016
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Examensarbete BOMX02-16-82/ Institutionen för bygg- och miljöteknik,
Chalmers tekniska högskola 2016

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Cover:
The cover shows a schematic diagram of the Microbial Fuel Cell used in this study.
Chalmers Reproservice, Göteborg, Sweden, 2016
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ABSTRACT

Modern-day use of metals and their improper disposal have created many environmental problems including toxicity due to uncontrolled metal leaching. Metal recovery from contaminated soil or incineration ash leachates by methods such as electro-winning is very energy intensive, although the contaminated sites contain amounts comparable to some workable ores.

Microbial fuel cells (MFCs) have been examined and deemed viable for energy-efficient recovery of metals from wastewater. This study aimed at upscaling previous viability studies of copper recovery with ml-scale cells from highly acidic leachate to a litre-scale reactor. Furthermore, the scope also included to investigate the operation and control measures necessary for this type of MFC.

Synthetic medium as well as municipal wastewater amended with NaHCO₃ and NaCH₃COO were used as the media for a carbon felt anode. A titanium cathode was fed with a simulated leachate medium containing NaCl, HCl, and CuSO₄. The separator between them was an anion exchange membrane (AEM). In addition to investigating operation and control of both reactor chambers, diffusion of ions through the AEM was tested in a small scale abiotic reactor.

The results showed steady start-up and stable performance of the MFC under controlled conditions. However, unfavorable conditions decreased the performance very rapidly. The most challenging aspect in the operation of the reactor was to control the pH in the cathode and anode chambers. High cathode pH resulted in precipitation of copper e.g. Cu(OH)₂, whereas very low pH led to acid leaking into the anode chamber inhibiting efficient bioelectrochemical activity. Moreover, the anode was susceptible to very high bicarbonate concentration. Furthermore, the reactor, especially the anode, had high voltage losses associated with mass transfer suggesting improvement in design and operation is required. Copper recovery was achieved by under-sizing the cathode substantially and consequently increasing the current density. On the other hand, cathode potential decreased with decreasing cathode size limiting the maximum current density achievable. Recirculation of the catholyte seemed to be an important aspect to reduce mass transfer losses. The membrane potential across the AEM had a positive effect on the total cell voltage.

Sustainable copper recovery is possible with completely developed and optimized bio-anodes and titanium cathode with adequate current density over 1 A/m².

Key words: Microbial fuel cell, copper recovery, operation and control, pH, current density
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Author’s Statement

I hereby certify that I have prepared this Master’s Thesis independently, and that only those sources, aids and advisors that are duly noted herein have been used and/or consulted.

NAFIS FUAD

Signature:

Date:
Preface

This Master’s Thesis was carried out as a part of the requirements of the Double Master’s Degree in Water Resources Engineering and Management (WAREM) from the University of Stuttgart, Germany and Infrastructure and Environmental Engineering (MPIEE) from Chalmers University of Technology, Sweden. The work is a part of a research project concerning remediation of contaminated sites. The project is carried out at the Department of Civil and Environmental Engineering, Division of Water Environment Technology, Chalmers University of Technology, Sweden. The project is carried out within a joint project with the Swedish Geological Survey (SGU) and the Regional Board in Västra Götaland (länsstyrelsen) financed by the Swedish Environmental Protection Agency.

This part of the project has been carried out with Nafis Fuad as a Master’s Degree candidate and Associate Professor Oskar Modin, Chalmers University of Technology and Mr. Carsten Meyer, University of Stuttgart as supervisors. The experiments described in the thesis were carried out from December 2015 to May 2016. All experiments were carried out in the Environmental Chemistry Laboratory of Department of the Civil and Environmental Engineering at Chalmers University of Technology.

Finally, it should be noted that the tests could never have been conducted without the sense of high quality and professionalism of the laboratory staff.

Göteborg June 2016

Nafis Fuad
**Notations**

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>AEM</td>
<td>Anion exchange membrane</td>
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<tr>
<td>Ag/AgCl</td>
<td>Silver-silver chloride reference electrode</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BES</td>
<td>Bioelectrochemical systems</td>
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<tr>
<td>CEM</td>
<td>Cation exchange membrane</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>E</td>
<td>Potential</td>
</tr>
<tr>
<td>$E^0_{AN}$</td>
<td>Standard anode potential</td>
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<tr>
<td>$E_{AN}$</td>
<td>Theoretical anode potential</td>
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<tr>
<td>EUR</td>
<td>Euro</td>
</tr>
<tr>
<td>F</td>
<td>Faraday’s constant</td>
</tr>
<tr>
<td>I</td>
<td>Current</td>
</tr>
<tr>
<td>$K_{sp}$</td>
<td>Solubility product constant</td>
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<tr>
<td>MFC</td>
<td>Microbial fuel cell</td>
</tr>
<tr>
<td>NHE</td>
<td>Normal hydrogen electrode</td>
</tr>
<tr>
<td>OCV</td>
<td>Open circuit voltage</td>
</tr>
<tr>
<td>OCP</td>
<td>Open circuit potential</td>
</tr>
<tr>
<td>R</td>
<td>Gas constant</td>
</tr>
<tr>
<td>SEK</td>
<td>Swedish Kronor</td>
</tr>
<tr>
<td>SHE</td>
<td>Standard hydrogen electrode</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
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$n$ Number of electron involved in a reaction
1 Introduction

Industrial activities in the last century have had severe impacts on the environment. They have made us very much dependent on fossil fuel, an unsustainable and non-renewable energy source with intense environmental impacts. They have also led to, deposition and accumulation of heavy metals and chemical compounds, both organic and inorganic in nature, that are severely toxic to the environment (Rahimnejad et al. 2015). Being an unsustainable and finite source fossil fuel has its inherent limitations even if the efficiency is increased substantially (Oliveira et al. 2013; Logan 2008). This has led to research and development of many renewable energy sources. Although some renewables such as wind and hydropower are used for a long time, they have their own limitations in terms of mobility. Furthermore, they also have impacts on the environmental to some extent. In contrast there are renewable sources comparatively new such as solar energy and nuclear energy which are limited by their dependency on precious material and problems related to waste disposal. In consequence the quest for finding other renewable sources for energy production is as strong as ever.

An ideal solution to this burning question of meeting the energy demand sustainably would be the use of a fuel that is renewable and reduce environmental impact upon its use. Since natural processes are very efficient within their range, the best way to find the solution would be to try to mimic and intensify the existing natural processes to meet up the demands created by the human activities. This principle is already in use in some of the technology existing today such as wastewater treatment plants and even for energy production with biogas plants.

Microbial fuel cell (MFC) is such a sustainable technology that uses waste and wastewater, renewable resources produced everyday by human activities that need to be taken care of. MFCs produce sustainable electrical energy, which is compatible with almost any device needing energy that we use today, with the expense of some external resources such as wires and electrical equipment, representing an insignificant share in the whole sustainability context (Oliveira et al. 2013). The fact that the a very wide range of organic substances can be used as fuel in MFCs makes it a strong candidate in regard to sustainable energy production (Logan 2009).

Although energy production has been the main objective of MFC research to date, the technology can be used to serve other purposes as well. These include, but are not limited to, recovery of precious or toxic metals from solutions though reduction at the cathode, hydrogen production at the cathode etc. (Modin et al. 2012; Rozendal et al. 2008). The goal of this study is to go forward with the research and development of MFCs for recovery of toxic as well as valuable metal copper from leachates. The focus of the study is on issues related to scale-up of reactors.

1.1 Background

About 1 million potentially polluted sites have been identified in Europe and the number is even bigger for North America (Dermont et al. 2008) The numbers for highly industrialized countries can be very high such as a quarter of a million for France and not far from a hundred thousand for Sweden (Fedje et al. 2013). Contaminated sites both in Sweden and the United States are predominantly polluted with heavy metals (Dermont et al. 2008; Khmilkovska 2014), due to earlier industrial activities. Heavy metals include
metals with high relative molecular weight, such as copper, zinc, lead etc. and their metalloids. Heavy metal contamination has toxic effect on the environment. Extensive use of chemicals containing heavy metals for a long time leads to the accumulation of the metals in a contaminated site (Bäckström & Sartz 2011; Paradelo et al. 2013).

This thesis focuses on a site “Köpmannebro”, predominantly contaminated with copper from CuSO₄·5H₂O, a hydrated compound with the trade name “Blue Vitriol” which was used for almost over a century in a wood impregnation factory. The contaminated area is about 8000m² with mostly contaminated bark and soil layers (Khmilkovska 2014). Previous work has successfully extracted copper from samples from this site (Fedje et al. 2013) which led to this project in order to attempt the recovery of the copper from the extracted leachate. Furthermore, copper is one of the heavy metals with a predicted stock of less than 50 years (Fedje et al. 2013), making the recovery attempt worthwhile.

1.2 General practice

The general practice for solution of contaminated sites is excavation and landfilling also known as “Dig and Dump” process (Dermont et al. 2008; Fedje et al. 2013). The reason for this choice is the convenience, swiftness, low requirements in terms of both technology and expertise, and well developed know-how for this method (Khmilkovska 2014). Although being by far the most used method for remediating contaminated sites, this method is not sustainable in nature, since it is not technically a treatment and is a mere transfer of the problem from a site with higher significance to a that with a lower one (Khmilkovska 2014). This can even be considered a spread of the contamination since the landfill can leach heavy metals to the groundwater of the new site, just as the old one, contaminating the nearby environment. This has led us to find other ways for proper treatment of the contaminated soil either by restricting or at the very least minimizing the leaching of heavy metals or by extracting the metal content from the soil or sediments (Fedje et al. 2013; Khmilkovska 2014). The first method, known as immobilization or isolation, although a method used to control contamination of various kinds such as those from nuclear waste, has some inherent drawbacks. These drawbacks include the fact that this method is temporary by every means since the metals are non-degradable, the storage space for the immobilized or stabilized contamination is not infinite and the long term effects of this methods are yet to be known (Dermont et al. 2008; Fedje et al. 2013). Furthermore, depending on the degree and the spread of the contamination the method has to adopt if the contamination can be transferred to another site known as ex situ version of the method or it has to be stabilized under in situ conditions on the site (Dermont et al. 2008). In the latter case although the problem is being taken care of, the site poses high risk for being used for sensitive purposes. In this regard extraction seems to be the only permanent solution which however uses different techniques including soil washing, physical separation, phytoextraction and thermal treatment etc. depending on conditions (Dermont et al. 2008; Khoshnoodi et al. 2013). Nevertheless, the difficulties and costs associated with metal extraction has made the use of “Dig and dump” very popular until now.

1.3 Scope of the study

The viability of recovery of copper by reduction on a cathode in an MFC has been studied before (An et al. 2014; Cheng et al. 2013; Fedje et al. 2015; Modin & Fedje 2012; Modin et al. 2012; Rodenas Motos et al. 2015; Ter Heijne et al. 2010). If this can be used in full
scale, this has the potential to become established as a sustainable solution for treating sites contaminated with copper.

The scope of the study was to assess the viability of a full scale MFC designed and operated for copper recovery from soil leachate heavily contaminated with copper. Furthermore, the scope of the study included to investigate the operation and control measures necessary for the MFC with a copper reducing cathode.

1.4 Aim of the study

The aim of this study was to investigate a litre-scale MFC for copper recovery from leachate. Specific objectives included:

- Optimize current generation and copper recovery in the MFC.
- Identify challenges associated with operation of a scaled-up MFC for copper recovery.
- Calculate the external resources required for the process.

1.5 Limitations

- Anode was maintained as the non-limiting electrode by feeding substrate (acetate) and alkalinity (bicarbonate).
- Coulombic efficiency was not optimized.
- Power derived from the MFC was not optimized. Only current generation was optimized since that increases the recovery of copper.
- Real leachates could not be used in this study due to an underperforming reactor and the low concentration of copper in the real leachate.
- MFC performance might slow down with decreasing copper concentration.
2 Theory

Fuel cell is a technology for electrochemical production of electricity from chemical fuels, such as hydrogen gas and methanol, through catalytic oxidation at the anode and chemical reduction at the cathode. Microbial fuel cells (MFCs) are unique and novel version of fuel cells which can biologically oxidize fuel at the anode without the need of any metal catalyst and now are being considered as a modern technology which can be a viable source of renewable electricity production since they can use waste as fuel and bacteria as generators (Dewan et al. 2008; Logan 2008; Oliveira et al. 2013). Even though the phenomenon that bacteria can produce electrons and essentially current was known for more than a century, it took some more than 4 decades for the field to develop practically and it is less than two decades since the process was revolutionized (Logan 2008). Bioelectrochemical systems (BESs), a general term to describe MFCs and related technology, are capable of producing electrons by biochemical degradation or biological oxidation of organic compounds by microorganisms at the anode of the system. From this anode, termed as bio-anode, the produced electrons are transferred to a positive electrode, the cathode, to facilitate a reduction reaction (Logan et al. 2006; Logan 2008; Rodenas Motos et al. 2015). The cathode where this reduction occurs can however be used in various ways for different purposes such as metal reduction to recover metals or hydrogen production etc. resulting in a sustainable electrochemical process (Logan et al. 2006; Logan 2008; Rodenas Motos et al. 2015). Different researchers have studied reduction of different metal such as \( \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+}, \text{Pb}^{2+}, \text{Fe}^{3+} \) as the cathode reaction (Modin et al. 2012; Ter Heijne et al. 2006). When the system is capable of producing energy from oxidation of the systematically replenished anode fuel and subsequent reduction at the anode in a sustainable way, it can be called as a microbial fuel cell (MFC) (Logan et al. 2006; Rodenas Motos et al. 2015). If electrical energy is put into the system to drive the electrochemical reactions, the system is called a microbial electrolysis cell.

In an MFC, microorganisms, more precisely bacteria, oxidize substrates in the form of organic and inorganic matter, releasing electrons produced from the oxidation which are transferred to the anode used as the negative electrode of the cell, after contributing to the energy production process in the microbial cell (Logan et al. 2006; Logan 2008), creating a syntrophic system in MFCs (Dolfing 2014). Alike other battery cells the electrons are then transferred to the cathode through an external circuit containing a load or a resistor, where they are reduced producing a current flow towards the anode conventionally (Logan et al. 2006; Oliveira et al. 2013; Rahimnejad et al. 2015). A schematic diagram of a typical MFC is shown in Figure 2.1. Applications of MFCs are very widespread starting from domestic and industrial wastewater treatment to landfill leachate treatment, electricity generation, hydrogen production, biosensor for pollutant analysis etc. (Du et al. 2007) and they can be used to achieve other goals such as metal reduction or desalination (An et al. 2014; Ter Heijne et al. 2011).
The performance of an MFC depends on the efficiency of the reactions in the anode and the cathode. The theoretical thermodynamic values applicable for each reaction is never achievable practically owing to the inherent losses occurring in MFCs that irreversible in nature (Oliveira et al. 2013). These reactions depend on materials and configurations (Logan et al. 2015) chosen for the separators and the electrodes and the reaction efficiencies change depending on many physical conditions such as pH, temperature, organic load, feed rate, shear stress etc. (Kim et al. 2007; Oliveira et al. 2013).

2.1 Microorganisms

Microorganisms are the driving force of an MFC since they act as biocatalysts in MFCs producing the electrons that run the cell (Sharma & Kundu 2010). Although it is known that some bacteria are capable of transferring electrons outside their cell, the exact pathway through which the electrons reach the anode connector is not certain yet (Logan et al. 2006; Logan 2008). The electrons that are used as the fuel in an MFC are generated by bacteria known as exoelectrogens (Logan 2008). Mixed culture of microorganisms has shown better performance than pure cultures (Du et al. 2007; Logan 2008). However, the development of microbial communities in MFCs is highly dependent on substrate and conditions used. For specific media used in this study having acetate as a substrate in a mediator-less MFC, the dominance of different strains of Geobacter can be expected (Du et al. 2007). Being metal reducing microorganisms Geobacter metallireducens produce ATP energy under anaerobic condition while facilitating metal reduction by the release of electrons to the metal oxides. This process of electron transfer is simulated in a MFC.
where the anode has the function of the final electron acceptor (Du et al. 2007; Venkata Mohan et al. 2008).

2.2 Separators

In basic MFCs, separators have the function to separate the electrolytes since the catholyte can inhibit biochemical activities leading to current production at the anode. In presence of oxygen from the cathode, the substrates at the anode could be oxidized chemically. Other electrochemical phenomena can also inhibit current production as described later in 2.5.1. Any kind of membrane such as cation exchange membrane, anion exchange membrane, bipolar membrane, microfiltration membrane, ultrafiltration membranes, or porous fabrics, glass fibers, J-Cloth, and salt bridge can be used provided that it separates the anode where oxidation of substrate by bacteria occurs and the cathode where reduction is facilitated as well as allows the selective transport of ions through it (Logan 2008). However, different characteristics such as proton transfer capacity and leakage increase the internal resistance of the MFC (Li et al. 2011; Rozendal et al. 2006). In specific applications such as this study other important functions are to separate copper from the bacteria since even a trace amount is toxic for them (Logan et al. 2006) as well as to separate the anode and cathode since the cathode is highly acidic which is not favorable for bacterial activity. Nevertheless membrane-less and single chamber MFC are also viable (Ghangrekar & Shinde 2007; Tartakovsky et al. 2008).

2.3 Basic electrochemical theory

The whole reaction, both the anode and cathode half reactions, needs to be thermodynamically favorable for a bioelectrochemical system to function as a MFC and produce electric current by electron flow from the negative electrode, anode, towards the positive electrode, cathode (Logan et al. 2006).

The maximum work that is possible to extract from the chemical potential can be calculated from the formation energy of the chemicals under different conditions. This is termed as Gibbs free energy, $\Delta G_r$, and can be calculated from the following equation:

$$\Delta G_r = \Delta G_r^0 + RT \ln(\pi) \tag{1}$$

where, $\Delta G_r (J)$ is the Gibbs free energy under the specific conditions, $\Delta G_r^0 (J)$ is the Gibbs free energy under standard conditions, usually defined as 298.15 K, 1 bar pressure, and 1 M concentration for all species, $R (8.31447 \text{ J mol}^{-1} \text{ K}^{-1})$ is the is the universal gas constant, $T (\text{K})$ is the absolute temperature, and $\pi$ (unitless) is the reaction quotient calculated as the activities of the products divided by those of the reactants.

The standard reaction Gibbs free energy can be calculated from information on energies of formation for organic compounds in water adopted from different reference texts.

However, these energies are not quantities easily measurable. Furthermore, they have thermodynamic relationships with other measurable quantities such as the electromotive force, $E_{emf} (V)$, which drives the current or electrons across the external electric circuit. The cell $E_{emf}$ is the difference between the cathode potential and the anode potential. The work derived from the cell is equal to the Gibbs free energy and since the work is
done for transferring electrons, it can be calculated from the cell potential and the charges transferred from the anode to the cathode.

Following the sign convention, the relation can be expressed as below:

\[ W = E_{emf} Q = -\Delta G_r \]  

(2)

where Q is the charge transferred in the reaction expressed in Coulomb (C), which is the product of the number of electrons exchanged in per reaction mole, n and the Faraday’s constant (96485.3 C/mole), F.

Rearranging the equation (2) the theoretical value of a cell under specific conditions can be calculated from the following equation:

\[ E_{emf} = \frac{-\Delta G_r}{nF} \]  

(3)

From equation (1) and equation (3) a generalized expression of the \( E_{emf} \) (V) can be derived as:

\[ E_{emf} = E^0_{emf} - \frac{RT}{nF} \ln(\Pi) \]  

(4)

This equation is known as Nernst equation where, \( E^0_{emf} \) is the standard electromotive force calculated from the \( \Delta G^0_r \) value.

The electromotive force can also be calculated for the half cell reactions at anode and cathode instead of the whole cell. In that case they are called electrode potential or anode and cathode potentials respectively.

By definition the cell electromotive force can be reformulated as:

\[ E_{emf} = E_{CAT} - E_{AN} \]  

(5)

where, \( E_{CAT} \) and \( E_{AN} \) are cathode and anode potentials respectively.

However, all the potentials and the electromotive force expressed so far are theoretical representing ideal and lossless conditions. In reality internal losses are associated with both the electrode potentials making the actual potentials measured less than those calculate theoretically. Hence, the actual voltage measured across the cell is also less than that calculated. The voltage measured in the cell in absence of current, termed as open circuit voltage (OPV), should theoretically approach the \( E_{emf} \), which is usually substantially lower due to the losses of potential occurring at the electrodes. This loss is termed as electrode overpotential. Electrode overpotential can be of very high values signifying a very high potential loss at an electrode, for example an oxygen reducing cathode with a pH around neutral typically has as high as 75% overpotential (Logan 2006). Consequently, values for potentials and electromotive forces calculated theoretically can only be used to quantify the losses to optimize the cell.

Thus the real voltage measured across the electrodes is defined as the cell voltage considering the overpotentials of both the anode and the cathode as well as other losses termed as ohmic losses. All the losses combined are termed as internal losses of the MFC.
Since the ohmic losses are related to current generated proportionally the proportionality constant is termed as the ohmic resistance of the system, \( R_Ω \). The cell voltage \( E_{\text{cell}} \) can therefore be expresses in the following way:

\[
E_{\text{cell}} = E_{\text{emf}} - (\sum \eta_a + |\sum \eta_c| + IR_Ω)
\]

(6)

where, \( \sum \eta_a \) and \( |\sum \eta_c| \) are anode and cathode overpotentials respectively. Although, generally they depend on the current, some overpotential exist even in absence of current or in open circuit condition. Separating these overpotential components the equation (6) can be reformulated as below:

\[
E_{\text{cell}} = OCV - IR_{\text{int}}
\]

(7)

where, \( R_{\text{int}} \) is defined as the internal resistance of the system, which includes the current dependent components of the overpotentials as well as the ohmic losses.

Both equations (6) and (7) represent MFC performances yet in different ways. For the first equation \( E_{\text{emf}} \) is a thermodynamic value which depends on conditions and the quantities are measurable. In the case of the latter equation both the quantities are measurable.

2.4 Losses

Among the losses occurring in MFCs the most significant losses that can be identified to be limiting the performance of the MFCs are ohmic losses, activation losses, metabolic loses of the microorganisms, and concentration losses. Except ohmic losses the other losses contribute to the overpotential of the electrodes. Although the minimum is the deviation from the ideal the better is the performance, these losses cause the potential of the cell to be lower than the ideal value calculated from the thermodynamic equations. They can be quantified by different techniques such as analyzing polarization curves, voltammograms, current interruption techniques etc. (Logan et al. 2006; Oliveira et al. 2013). Different measures can be taken depending on the extent of the loss occurring to minimize them generally by optimizing the design of the fuel cell, and choice of materials both for construction and operation as well as selection of suitable microorganisms (Oliveira et al. 2013).

2.4.1 Activation loss

The energy that any reactant needs to overcome to facilitate reaction which depends on adsorption and desorption of reagents, electron transfer, and the electrode used (Oliveira et al. 2013) is termed as activation loss or activation polarization (Logan et al. 2006). In the case of a bio-anode microorganisms needs to overcome an energy barrier to be able to release electrons to the anode. Ways to reduce anodic activation loss in MFCs with bio-anode is by using mediators to minimize the energy barrier (Oliveira et al. 2013), increasing the operating temperature, increase electrode surface area and growth enrichment of active biofilm (Logan et al. 2006). In case of abiotic electrodes these losses can be reduced by increasing electrode surface area and temperature, also improving electrode catalyst (Logan et al. 2006). Activation loss is governing in MFCs during low current densities (Logan et al. 2006; Oliveira et al. 2013).
2.4.2 Ohmic loss

Ohmic losses are defined by those losses occurring owing to conduction of electrons and other ions (Oliveira et al. 2013). These losses depend on the conductivity of the solutions that the species need to move in and the distance that needs to be covered by them (Oliveira et al. 2013). These losses dominate in the central operating range when the current density is neither very high nor very low (Oliveira et al. 2013). Ohmic losses can be reduced by increasing the conductivity for ions of the electrolytes, choosing low resistance membranes and optimizing the MFC design with reduced electrode spacing (Oliveira et al. 2013) and establishing effective contacts to the circuit (Logan et al. 2006).

2.4.3 Concentration loss

This kind of loss occurs if the initial concentration of substrates cannot be maintained or the transport of mass is limited (Oliveira et al. 2013). Concentration losses are usually substantial during high current densities (Oliveira et al. 2013). For the anode oxidized species needs to move away from the surface to create space for reduced species, substrate in this case, for the oxidation to function continuously. Also reduced species needs to get contact to the electrode in adequate rate. Otherwise the electrode potential increases for the anode and decreases for the cathode (Logan et al. 2006). If the electrolytes are not homogeneous because of inadequate mixing, diffusion gradients may limit the availability of different species in different location of the bulk (Logan et al. 2006). Recirculation or stirring or aeration with an optimum cell design favors the minimization of these losses (Oliveira et al. 2013).

2.4.4 Metabolic losses of the microorganisms

The electron transferred to the anode by the microorganisms is a byproduct of microbial metabolic activity. In the process of acquiring metabolic energy they liberate electrons by oxidizing substrate which travels through their metabolic pathways inside their cells to finally be discharged to an external acceptor having a higher potential outside the cells (Logan 2008). In the case of an MFC the electrons are released to the anode electrode. Hence, the anode electrode needs to stay at a potential lower than the substrate redox potential to facilitate the metabolic activity, which in terms run the whole process in an MFC. These difference of anode potential and the substrate redox potential is the losses occurring for microbial metabolism (Logan et al. 2006). Despite being losses this difference is essential, which will be explained further in 2.5.2.

2.5 Anode

The anode material needs to be conductive to be able to transfer the electrons that are generated in the anode to the cathode though the external circuit. Moreover, the material needs to be biocompatible to facilitate bioactivity i.e. oxidation of substrate by the microorganisms. Furthermore, the anode needs to remain chemically stable within the range of conditions between which the anode chamber is operated. Different kinds of anodes have been used for previous similar studies such as graphite felt, graphite paper, carbon fiber brush, graphite plate etc. (Karra et al. 2013; Rodenas Motos et al. 2015; Ter Heijne et al. 2008). The size of anode also varies depending on the scale of the reactors used in studies (Rodenas Motos et al. 2015). The substrate used in the bio-anode can be of both organic and inorganic origin (Aelterman et al. 2008). The most commonly used
substrate is acetate, an organic substrate (Aelterman et al. 2008; An et al. 2014; Logan et al. 2006; Logan 2008; Rodenas Motos et al. 2015; Ter Heijne et al. 2006).

The anode determines the electron production in an MFC depending on the microbial activity which is dependent on the anode potential (Aelterman et al. 2008). The anode operation is highly pH sensitive since microbial activity is very responsive to change in pH and suitable between a narrow range of 6-9 (Oliveira et al. 2013).

2.5.1 Substrate Oxidation in the Anode

Substrate oxidation by microorganisms, more specifically exoelectrogenic bacteria, is the process by which the electrons are released at the anode electrode. Although with different performances, any source of biodegradable organic matter can be used in an MFC as fuel from specific substrate such as salts of organic acids, carbohydrates and proteins to substrates of complex composition as wastewaters (Huang et al. 2008; Logan 2009) and landfill leachates (Gálvez et al. 2009; Greenman et al. 2009; Li et al. 2010; Puig et al. 2011). Studies on MFC evolved from the ones using additional mediators for carrying electrons from inside the cell to the electrode, to the discovery of power producing capability without mediators (Venkata Mohan et al. 2008). Moreover, the hypothesis of power production being limited to only certain microorganisms was proven not correct by diverse microbial communities found in MFCs which was later supported by the studies proving power producing abilities of many different bacteria as bio-anodes (Logan 2009).

The substrate oxidation reaction, the anode half reaction, for acetate as a substrate can be written as:

\[ \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + 9\text{H}^+ + 8e^- \] (8)

This reaction equation shows the degradation of acetate by the microorganisms by which the electrons are released. This reaction also releases 7 extra protons which is responsible for the acidification of the anode.

This system is most efficient in anaerobic conditions since oxygen reduces the number of coulombs available current generation by consuming electrons acting as a final acceptor at the anode. Besides, the high standard reduction potential of oxygen (Hoare 1968) can increase anode potential (Chen et al. 2014) having a negative effect as described in 2.5.2.

2.5.2 Anode Potential

The anode potential regulates the mode and amount of energy that can be harvested by the microorganisms present (Aelterman et al. 2008). This energy can be calculated using the following thermo-dynamic equation (Aelterman et al. 2008):

\[ \Delta G^{\circ'} = -n.F.(E^{'\circ}_{\text{donor}} - E^{'\circ}_{\text{acceptor}}) \] (9)

where, \( \Delta G^{\circ'} \) is the change in Gibbs free energy at a certain condition, \( n \) is the number of electrons associated, \( F \) is the Faraday constant and \( E^{'\circ} \) is the standard potential of the anolyte used.
The anode potential has a role in determining both the growth of the microorganisms as well as the output of electrical energy (Aelterman et al. 2008). Although the lower the anode potential the lower is the amount of energy obtainable by the microorganisms required for metabolism and growth, the ideal condition for a maximum energy output at a certain current from the MFC is to have as low anode potential as possible (Aelterman et al. 2008). On the contrary to generate higher current the anode needs to be at a higher potential, providing more energy to the microorganisms for metabolic activities and replication (Aelterman et al. 2008). Because of this difference in requirements for different objectives an optimum anode potential for the bio-anode is necessary to maintain. In case of MFC this can be controlled by selecting suitable resistances in the external circuit. Research has shown that an anode potential approximately 0 mV vs SHE can result in enhancement in the growth of electrochemically active microorganisms, increasing their density to speed up the start-up period and also higher current generation (Aelterman et al. 2008).

The anode potential for a specific set of conditions can be calculated as Table 2.1 by modifying the equation (4) for the half cell reaction, anode oxidation. In the case of acetate oxidation in presence of bicarbonate and proton the equation can be formulated as below:

\[
E_{AN} = E_{AN}^0 - \frac{RT}{nF} \ln \left( \frac{[CH_3COO^-]}{[HCO_3^-][H^+]^n} \right)
\]  

(10)

Table 2.1 Calculation of Anode Potential (Logan et al. 2006)

<table>
<thead>
<tr>
<th>Quantities</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{AN}^0)</td>
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</tr>
<tr>
<td>(R)</td>
<td></td>
<td>8.31447</td>
</tr>
<tr>
<td>(T)</td>
<td>K</td>
<td>298</td>
</tr>
<tr>
<td>(N)</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>(F)</td>
<td>C/mole</td>
<td>96485.3</td>
</tr>
<tr>
<td>([CH_3COO^-])</td>
<td>mM</td>
<td>20</td>
</tr>
<tr>
<td>([HCO_3^-])</td>
<td>mM</td>
<td>10</td>
</tr>
<tr>
<td>([H^+])</td>
<td>M</td>
<td>10^(-7)</td>
</tr>
<tr>
<td>(E_{AN})</td>
<td>V</td>
<td>-0.296</td>
</tr>
</tbody>
</table>

2.6 Cathode

An MFC can be designed with both bio-cathodes (Chen et al. 2008; Huang et al. 2011; Rozendal et al. 2008) and metal cathodes. In the cathode, the positive electrode of the cell, the electrons generated in anode oxidation are reduced by electron acceptors.
many electron acceptors with a potential higher than that of the anode can accept these electrons, the cathode in an MFC is very flexible as well. Generally, the MFCs are designed to have oxygen as an electron acceptor, owing to its abundance and high standard redox potential (Rismani-Yazdi et al. 2008) and the possibility to use an air-cathode (Yoo et al. 2011). The oxygen acceptor can take different reduction pathways. The ideal pathway being the complete reduction. Incomplete reduction leads to low energy conversion efficiency (Rismani-Yazdi et al. 2008). Cathodes can be both aerobic or anaerobic. In the case of a bio-cathode in aerobic conditions the electron acceptor is preferably oxygen; though other electron acceptors such as permanganate has also been used along with oxygen (Rismani-Yazdi et al. 2008). If the bio-cathode is at anaerobic conditions the final electron acceptors can be other species such as nitrate (Rismani-Yazdi et al. 2008). In the case of an abiotic cathode, metal cathode, oxygen or metal ions can both be used as electron acceptors. In the case of oxygen, it is reduced either completely to water molecules or partially to hydrogen-peroxide. In the case of metal ions, they can either be reduced to the metal itself, electroplating the cathode surface with the pure metal or be partially reduced to some other ion or compound depending the concentration of other species and the cathode potential. Cathode operation can also be very much pH dependent depending on the ionic species present in the catholyte and the potential under which the cathode is operated.

2.6.1 MFCs with copper treatment at the cathode

In the case of a copper reducing cathode the reduction reaction can be written in a following way:

\[ Cu^{2+} + 2e^- \rightarrow Cu \ (s) \]  

(11)

From the reformulated Nernst equation the \( E_{\text{CAT}} \) cathode reduction half reaction can be estimated as to be +0.286 V vs SHE for 1 g/L Cu\(^{2+}\) (Ter Heijne et al. 2010).

However, cathode reduction of copper can take other paths as well, for example:

\[ 2Cu^{2+} + H_2O + 2e^- \rightarrow C_2O + 2H^+ \]  

(12)

\[ C_2O + 2e^- + 2H^+ \rightarrow 2Cu(s) + H_2O \]  

(13)

The reactions described by equation (12) and (13) have the standard potentials of 0.207 V and 0.059 V vs SHE respectively.

Cathode potential is a very important parameter, which can change depending on the activity of the ions which eventually depends on the temperature and pH of the catholyte solution.

Pure copper or copper with very little impurity with flower-like formation, crystal formation and dendritic formation have been reported to be accumulated on the cathode surface (Rodenas Motos et al. 2015; Ter Heijne et al. 2010; Wu, Pan, Huang, Quan, et al. 2015).

In addition to copper reduction copper can also be removed from the solution by chemical adsorption or deposition at the cathode surface (Cheng et al. 2013; Wu, Pan, Huang, Quan, et al. 2015). Different materials have been used as a cathode electrode such as
copper plate, graphite paper, carbon rod, graphite plate, titanium wire, titanium sheet, stainless steel mesh etc. and have shown different results (An et al. 2014; Cheng et al. 2013; Rodenas Motos et al. 2015; Tao et al. 2011; Wu, Pan, Huang, Quan, et al. 2015; Wu, Pan, Huang, Zhou, et al. 2015).

### 2.6.2 Conditions effecting copper reduction at the cathode

An aspect that affects copper reduction at the cathode is the pH of the catholyte. High cathode pH will result in precipitation of copper oxides e.g. Cu$_2$O, Cu(OH)$_2$ whereas very low pH will lead to dissolution of accumulated copper back into the solution as Cu$^{2+}$ ions. The precipitation of copper occurs owing to different speciation of Cu$^{2+}$ ions depending on the pH and the potential of the cathode electrode (Ter Heijne et al. 2010; Wu, Pan, Huang, Quan, et al. 2015); however, in pH range 3-4.5 copper will be present either as Cu$^{2+}$ ions or as solid copper accumulated on the cathode (Ter Heijne et al. 2010).

Aerobic and anaerobic conditions can effect copper reduction since oxygen will be reduced at the anode if available in dissolved form in the solution.

### 2.7 Transient Current

Transient current is defined as the average of the current logged for 20 minutes from connecting the circuit (Aelterman et al. 2008) following a two-hour period of open circuit condition. This current could be a result of substrate storage inside the cell in the form of polymers (Aelterman et al. 2008) which does not represent the actual conditions and thus may not be considered.

### 2.8 Performance and Scaling up of MFCs

Scaling up MFCs is a challenging task (Logan 2010) since the performance of an MFC is dependent on many physical factors such as pH, temperature, external load, substrate concentration, electrode size and distance (Jadhav & Ghangrekar 2009; Liu et al. 2005; He et al. 2008), feed rate, shear stress, anodic electron transfer processes, cathodic limitations such as irreversible reactions causing inactive layer on the cathode surface, separator properties and configuration, cathode specific surface area etc. (Oliveira et al. 2013; Ter Heijne et al. 2011) all of which can vary between a wide range for different scales. Change in operating temperature can decrease current generation by regulating microbial activity as well as increase the same by facilitate change in microbial communities in an MFC (Jadhav & Ghangrekar 2009). Temperature also effects chemical reaction rates, mass transfer and fluid conductivity, ionic conductivity which decreases system losses. Studies have found that electrogenesis is optimum in the temperature range 30-45°C. Furthermore, start-up temperature was found to be dictating MFC performance (Oliveira et al. 2013). The pH of both the anode and cathode is critical for MFC performance. Anode pH is optimum for microbial activity of the electroactive bacteria between a very fine range of 6.0-7.0 (Jadhav & Ghangrekar 2009). High cathode pH has been found to be limiting cathodic reduction thermodynamically resulting in decreased current generation for cathode reactions involving protons. Furthermore, pH gradient between anode and cathode has a major impact on MFC performance (Oliveira et al. 2013). Substrate loading rate generally has a positive effect on current production by increasing the substrate availability and ionic strength of the anolyte (Oliveira et al. 2013). Higher flow rate has been linked positively with power output, and negatively with carbon...
removal at the anode. Furthermore, high shear stress from high flow rates train the microorganisms to establish a firm connection with the anode material and can lead to existence of selective microbial communities, the ones that are more adaptive (Oliveira et al. 2013).

Researchers have shown that scaling up electrode area does not increase power generation proportionally, owing to mass transport limitations for larger electrodes; the trend is rather logarithmic (Dewan et al. 2008; Oliveira et al. 2013); Moreover, increase of cathode area is more responsive than that of anode (Oliveira et al. 2013). It has been hypothesized that the mechanism of electron transfer differs from small size electrodes to the large ones (Dewan et al. 2008). Several studies found that cathode was the limiting factor in scaled up MFCs (Dewan et al. 2008; Ter Heijne et al. 2011). Separators have a significant limitation while scaling up MFCs. Besides high cost of high performing separators they control the ionic transfer which can be obstructed by membrane fouling or biomass growth on the surface leading to ohmic losses and overpotential (Li et al. 2011). Optimization of the control measures is necessary for upscaling (Oliveira et al. 2013). Due to these factor related to large MFC design, an effective alternate is considered to stack many small scale MFCs in different configurations to achieve power aimed at upscale MFCs (Oliveira et al. 2013). However, there are difficulties in operating all the cells at the same performance in a stacked MFC, leading to cell reversal. Nevertheless, it has been shown that power densities comparable to that of lab-scale MFCs are viable for scaled up MFCs as well; Furthermore, energy efficiency of MFCs can comparable with that of anaerobic digesters (Ter Heijne et al. 2011).
3 Materials and methodology

The design and setup of the MFC as well as the operation required some specific materials and chemicals. The design, materials and equipment and operation strategy used to conduct the experiments as well as the analysis of the results are described below.

3.1 Microbial Fuel Cell design

The Bioelectrochemical reactor used as MFC was designed with three compartments using the outer compartments as anode chambers, which were hydraulically connected, and the compartment in between as a cathode chamber to utilize both surface of the cathode to increase the active surface area 1.5 times compared to the total volume of the reactor. Each compartment had a size of 2 cm x 10 cm x 26 cm. The design was changed several times to optimize the operation and monitoring and finally the reactor was set-up with inflow feeds and recirculation and outflows for both anode and cathode.

3.2 Materials and equipment used

Materials and equipment used in this study are described briefly below:

3.2.1 Frame

The frame was constructed with 5 parts made of Plexiglas and sealant to accommodate the two anodes and the cathode inside the compartment parts and the AEM. The frame was equipped with sampling ports for all the chambers and recirculation tubing orifices for the anode. Later the cathode sampling ports were used as recirculation ports as well.

3.2.2 Membrane

Two anion exchange membrane (AMI-7001S) manufactured by Membranes International Inc. were used as the actual separator in the MFC to separate the anode and cathode. These membranes function as strong base anion exchange membranes which have a polymer structure of gel polystyrene cross linked with divinyl benzene with a functional group of quaternary ammonium. The size and thickness of the active membrane were 300x50 mm and 0.45±0.025 mm respectively. The membrane is chemically stable between pH of 1-10 (Membranes International n.d.). Other properties of the membrane were within the requirements for the MFC (Membranes International n.d.). The membrane was immersed in a 5% NaCl solution for 12 hours to allow for membrane hydration and expansion (Membranes International n.d.).

3.2.3 Carbon Felt anode

Carbon felt (43199, Alfa Aesar) was used as the bio-anode electrode which facilitates the attachment of the microorganisms to its fiber surface. The size and thickness of the active anode were 300 x 50 mm and 3.18 mm respectively. The choice of the material was based on the surface characteristics of the material with its higher surface area compared to other materials of the same size. Higher surface area creates more space to allocate more microorganisms on the anode surface; consequently, increases microbial activity of the bio-anode. Figure 3.1 shows the surface of carbon felt acquired by scanning electron microscopy.
3.2.4 Titanium cathode

The cathode used in this study was built by connecting a 30 cm x 5 cm titanium sheet of 0.25 mm thickness to a titanium wire. The cathode surface was washed with acid to remove any impure particles or passive chemical layers from the surface.

3.2.5 Titanium wiring

Titanium wire (267912-23G, Aldrich) with an area of 0.515 mm$^2$ was used to connect the anodes and the cathode to an external load.

3.2.6 Anode feed

Depending on the operating conditions and characteristics necessary at the anode the anode feed was changed several times. The compositions of these media are described below and summarized in Table 3.1. Anode feeds were kept under sealed containers in order to restrict oxygen to enter the anode and later was flushed with Argon (Ar) gas to maintain anaerobic conditions.

3.2.6.1 Synthetic Anode medium - 1

This media was designed by modifying the nutrient medium described by (Rozendal et al. 2008) containing 2100 mg/L KH$_2$PO$_4$, 6020 mg/L K$_2$HPO$_4$, 191 mg/L NH$_4$Cl which

![Figure 3.1 Carbon Felt.](image)
was supplemented by 1640 mg/L (20 mM) CH$_3$OONa and 200 mg/L yeast extract. The medium was prepared in tap water assuming it contains the necessary trace elements for the microorganisms.

3.2.6.2 Synthetic Anode medium - 2
This medium was designed by modifying the Synthetic Anode medium – 1. In this medium the buffer system was changed from phosphate to bicarbonate system owing to economic reasons, containing NaHCO$_3$ which varied in concentration following the change of current logged through the external circuit, also 200 mg/L CaCl$_2$·2H$_2$O, 200 mg/L MgSO$_4$·5H$_2$O and 50 mg/L KH$_2$PO$_4$ was used in tap water.

3.2.6.3 Synthetic Anode medium – 3
This medium was similar to the Synthetic Anode medium – 2 except from the concentration of NaHCO$_3$ which was 10 mM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Synthetic anode medium -1</th>
<th>Synthetic anode medium -2</th>
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</tr>
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<td>CH$_3$OONa</td>
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<td>20 mM</td>
<td>20 mM</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>3.6 mM</td>
<td>3.6 mM</td>
<td>3.6 mM</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>15.44 mM</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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</tr>
<tr>
<td>NaHCO$_3$</td>
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<td>10 mM</td>
</tr>
<tr>
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<td>0.9 mM</td>
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</tr>
<tr>
<td>MgSO$_4$·5H$_2$O</td>
<td>-</td>
<td>0.95 mM</td>
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</tr>
</tbody>
</table>

3.2.6.4 Wastewater
Wastewater was collected from the Rya wastewater treatment plant in Gothenburg and kept under refrigeration. The raw wastewater had a conductivity of 7.70 $\mu$S/s and a pH of 7.0.

3.2.7 Cathode feed
The cathode feed consisted of different concentration of HCl, NaCl, CuSO$_4$·5H$_2$O as described in 3.4. The concentration of acid was calculated from Table A.2 in order to achieve different target pH.
3.2.8 Pump

Maximum 5 pumps were used to operate the MFC at any given time. These included both continuous operation as well as programed feeding. Watson-Marlow (120S/DV) pumps were used to recirculate the anode and cathode feed as well as feed the anode chamber systematically.

3.2.9 pH controller

A pH controller (Hanna Instruments® Blackstone pH measuring and dosing system, BL 7916) was used to control the pH of the cathode to a desired level. The accuracy was ±0.01 pH and it included provisions to adjust the flow rate when the pH approaches the target value in order to prevent over dosage.

3.2.10 Electrical equipment

Among mentionable electrical equipment multi-meter, multi-channel signal logger (NI USB-6211, National Instruments), potentiostat (KP07, Bank Electronik – intelligent controls GmbH), Ag/AgCl reference electrodes with +0.200 (±0.008) V against Normal Hydrogen Electrode (NHE) or Standard Hydrogen Electrode (SHE) at 25°C and resistors of 5000 Ω, 3000 Ω, 2000 Ω, 1000 Ω, 470 Ω, 100 Ω, 56 Ω, 18 Ω, 1 Ω.

3.3 Reactor

The reactor was designed to have two anode chambers, one on each side of the single cathode chamber as shown in Figure 3.2. Two different anode chambers were used to intensify the electron generation and increase the voltage and thus the copper recovery. The anolyte was recirculated continuously to facilitate the organic carbon diffusion to the anode through both the anode chambers (Logan 2008).

![Figure 3.2 Schematic Diagram of the experimental setup.](image-url)
3.3.1 Volumes

Although the volumes of the three chambers were made identical, the difference in salt concentration might have facilitated the passage of water through the membranes, which resulted in the shift of the membrane. AEM are known to be more susceptible to deformation bent away from the cathode (Li et al. 2011). As a consequence of the described phenomenon the volumes of different solutes were different. The volume for the anolyte was calculated together with the pipe connecting both anode chambers, since the anolyte has the same residence time in both the anode chambers and the pipes.

From the Start-up 1 to the Run 2 the total volume of anolyte was 844 ml and catholyte was 430-500 ml. Later with introduction of pH controller in Run 3 the catholyte volume was increased to 1130 ml in total.

3.3.2 Flow

During the whole experiment period the flows were adjusted along with the experimental setup.

The anolyte was recirculated between the two anode chambers at a rate of 100 ml/min. The anode was fed with anode feed with a rate of 40 ml/min for 1 minute at every 3 hours resulting in 320 ml/day and cathode was fed with 20 ml/min for one minute at a same interval resulting in 160 ml/day inflow. The extra acetate flow was restricted to 5 ml once a day. The cathode out flow was set to keep the catholyte to 430 ml at any given time.

3.3.3 Hydraulic residence time (HRT)

During the batch mode runs the hydraulic residence time of the electrolytes were varied depending on the consumption of substrate in at the anode or copper removal at the cathode. The anolyte HRT was from one day to seven days.

During the feed mode the anolyte HRT was varied from one day up to three days.

3.4 Operation

As stated before the experimental setup and the operating conditions were changed several times during the duration of the experiments carried out. Different conditions along with their duration is shown in Figure 3.3.
3.4.1 Start-up 1

The run start-up 1 was performed under batch mode with a recirculation of the anolyte through a bottle open to air with a rate of 144 L/d. The external electric circuit was operated under 100 Ω. In the beginning of the start-up 1 the synthetic anode medium-1 and wastewater was used in a ratio of 10:1 as anolyte. The catholyte was chosen to be a solution of 1 M HCl containing 16.5 g/L NaCl, 1966 mg/L CuSO₄·5H₂O, equivalent to 500 mg/L Cu²⁺. This phase continued for 94 hours.

After monitoring the current generation lower than expected and observing the anode pH to be less than 1, the conditions were changed. Moreover, diffusion test was performed to investigate diffusion of Cl⁻ ions from 1M solution through the AEM. This test was performed on a lab-scale apparatus with 2 chambers having a volume 100 ml each. The AEM was placed between the chambers having an effective area of 7 cm². The test was run for 5 days and the solutions were sampled on day 0, 1, 3 and 5.

In the next phase the catholyte was changed to one without HCl containing the same concentration of NaCl and CuSO₄·5H₂O, also the anolyte was switched to a 1:1 mixture of synthetic anode medium-1 and wastewater. Under this condition the reactor was run...
for 505 hours. During this run the anolyte was analyzed regularly for the substrate concentration and was changed accordingly to keep the substrate availability to an abundance. The reactor was considered to be started up after the current generated reached 0.655 mA under 100 Ω.

3.4.2 Run 1 – with manual pH control

After a successful start-up the reactor was inspected and the cathode was found to have precipitates which were presumed to be copper hydroxides after the pH of the cathode was found to be 7.5-8. This led to the change of the experimental setup from being a batch mode reactor to a feed operated reactor. The external electric circuit load remained 100 Ω.

The anode recirculation was changed from the external bottle to internal recirculation from one anode chamber to the other with a same rate as the start-up 1, 144 L/d. Besides, an anode feed consisting of wastewater supplemented with 1640 mg/L CH3OONa was added to the anode chamber. The feed rate was 160 ml/day with 20ml feed every 3 hours.

The cathode was fed with a solution having a 1M concentration of HCl and the same concentration of NaCl and CuSO4·5H2O, with the same rate as the anode feed. However, after observing sharp decrease in current within few hours the feed was changed to a lower strength with 0.5 M HCl.

The duration of this run was 183 hours until the logged current decreased to zero.

3.4.3 Start-up 2

Start up 2 was performed under feed mode as Run 1. The external electric circuit load was changed from 100 Ω to 1000 Ω after 53 hours to aid the growth of the microorganisms which was kept for the rest of the duration of this run. The anode feed was modified from Run 1 by adding 23.81 mM NaHCO3 as pH buffer for the anolyte.

During the whole run the catholyte feed was varied by adjusting the HCl concentration from calculating the theoretical rate of basification of the catholyte estimated from the current logged in the external circuit. Moreover, 2 M HCl was injected to bring the cathode pH to desired value when the feed was not enough to control the pH. Furthermore, the whole catholyte was changed from time to time when the pH was very high and all copper was observed to be precipitated. The concentration of CuSO4·5H2O and NaCl was the same as Run 1. The total run was continued for 411 hours until the current logged reached a value of 0.638 mA under 1000 Ω.

3.4.4 Run 2 - manual pH control by intermittent feeding

Run 2 was very similar to the start-up 2. One of the differences was the resistances in the external circuit which changed stepwise from 470 Ω to 100 Ω and finally 56 Ω. Another difference was the concentration of HCO3- which was varied following the current generation during this run which was used to supplement the wastewater used along with 20 mM.
Near the end of this run the performance of the reactor was tested with copper free cathode by changing the catholyte as well as the cathode feed to solutions consisting of only HCl and NaCl of the same concentration as Start-up 2. This run continued for 402 hours.

3.4.5 Run 3 - with automatic pH control

During Run 3 an automatic pH controller was introduced to the setup to control the pH of the catholyte between 3.5-3.7, where the copper remains dissolved in the catholyte solution as well as the accumulated copper does not dissolve into the catholyte (An et al. 2014; Rodenas Motos et al. 2015; Ter Heijne et al. 2010). There were three different phases in this run.

During the first phase the cathode pH was controlled with a feed of 0.01 M HCl, 16.5 g/L NaCl, 1966 mg/L CuSO₄·5H₂O for 45 hours. Then the acidic strength was changed to 0.1 M keeping the NaCl concentration same. However, the concentration of CuSO₄·5H₂O was changed after 188hours to 3922 mg/L. These conditions remained unchanged until the end of this phase. The anode was fed with a feed comprised of Synthetic Anode Medium-2; however, the feeding rate was 0.360 L/day until 188 hours which was then changed to 1 L/day to decrease the hydraulic residence time and kept same for the rest of the phase. This phase marked its end at 574 hours into the run duration. During this phase the resistor in the external circuit was gradually decreased from 1000 Ω to 1 Ω with intermediate resistors of 470 Ω, 100 Ω, 56 Ω, 18 Ω keeping each of them for a duration of 3days, which is three times the HRT of the anolyte.

Diffusion test were performed to investigate diffusion of HCO₃⁻ ions against counter diffusion of Cl⁻ ions from 15mM solution through the AEM. This test was performed, similarly as the other diffusion tests, on a lab-scale apparatus with 2 chambers having a volume 100 ml each. The AEM was placed between the chambers having an effective area of 7 cm². The test was run for 2 days and the solutions were sampled on day 0,1 and 2.

The next phase was distinguished by its anode feed and cathode size. After the observation of decrease in current logged and relating it to the very high bicarbonate concentration of 119 mM, which was increased following the current generation, the anode feed was changed to having a fixed bicarbonate concentration of 10mM in the Synthetic Anode Medium – 3. Starting from the anode was rendered strictly anaerobic in nature by flushing both the anode and the anode feed with argon gas. Furthermore, owing to current density recorded being lower than that required the cathode surface area was reduced to 100cm² at the beginning of the phase. The resistors at the external circuit was increased this time from 1 Ω to 18 Ω and 56 Ω in ascending order. This phase was continued for 138 hours.

After the second phase copper removal was yet to be observed which was related to insufficient current density (Rodenas Motos et al. 2015). The last phase of the Run 3 was conducted to test the viability of copper removal on Titanium cathode surface. Hence, to increase the current density even under lower current than that was expected the cathode surface area was reduced to 7.63 m². With the observation that the conductivity of the catholyte was enough without the NaCl, it was excluded from the catholyte in this phase.
3.5 Copper recovery in a lab-scale fuel cell

Since the MFC performance did not reach the expected level within the time allocated for the study, a lab-scale test with an abiotic electrowinning cell was performed to calculate the rate of recovery of copper on a titanium cathode under target current densities in order to estimate the operation strategy and costs for a well performing litre-scale MFC.

3.6 Data acquisition and analysis

Many kinds of data and samples were gathered to successfully monitor and analyses the performance of the MFC and recovery of copper. These included electrical measurements, electrolyte sampling, volume measurements and treatment of raw data from electrical measurements and analyzing the samples taken.

3.6.1 Cell voltage

As described before cell voltage is a very valuable information that can be measured directly. Cell voltage can be measured by the difference of potentials of the anode and the cathode directly with a voltmeter or a multi-meter. In this study the cell voltage was logged with a multi-channel signal logger operated with a computer that logged the voltage every 10 seconds. The logs were saved later as raw data in excel files from where they were treated later according to different requirements. The open circuit voltage was measured by directly connecting the anode to the negative channel and the cathode to the positive channel of the logger. The cell voltage at different stages during current production was measured by connecting the channels across the resistor used in the external circuit.

3.6.2 Electrode potential

Electrode potentials are also very important parameters for assessing the performance and quantify losses of an MFC. However, they are not direct measurable quantities (Logan et al. 2006). Since they are components of the voltage they can be measured by measuring voltage, but by relative measurement to a known one (Logan et al. 2006). There are electrodes which have constant and known potentials known as reference electrodes. In this study a practically convenient reference electrode, Ag/AgCl, was used. A multi-meter was used to measure the potential of the electrodes against the reference electrode and later it was logged with the multi-channel signal logger. Although the potentials were logged against a reference electrode they were converted and reported against SHE, which conventionally has a zero (0) potential, throughout the report, unless mentioned otherwise. The Ag/AgCl reference electrode has a potential of +0.2 V vs SHE.

3.6.3 Membrane Potential

The membrane potential is defined as the loss of potential across the membrane (Rodenas Motos et al. 2015). This depends on the size and capacity of the membrane as well as the electrolytes. Membrane potential can be calculated from the cell voltage and electrode potential information or directly measured by using two reference electrodes across the membrane i.e. in the anolyte and the catholyte. A positive membrane potential indicates a contribution towards the current generation by the membrane whereas a negative membrane potential indicated resistance created by the membrane. In this study the membrane potential was measured as well as calculated and compared within the values
obtained in both the ways. Although membrane resistance contributes to the ohmic losses it is not a measure of the ohmic loss. Membrane potential can be expressed as:

\[ E_{\text{cell}} = E_{\text{CAT}} - E_{\text{AN}} - E_{\text{mem}} \]  

(12)

where, \( E_{\text{mem}} \) is the membrane potential.

3.6.4 Electrolyte sampling

Both the electrolytes were sampled regularly during both the batch mode and the feed mode of operations. During the batch mode samples were collected from the anode and cathode chambers in volumes of 5ml and 2ml respectively. During the feed mode the anode and cathode discharge were sampled, 14ml each. Occasionally the chambers were also sampled with 1.5ml. The anode samples were labeled and refrigerated whereas the cathode samples were stored after labeling for further tests.

3.6.5 Polarization curves and voltammograms

Polarization curves and voltammograms are tools to analyze the performance of MFCs or the electrodes and characterize the losses. Polarization curves for the MFC were recorded at different times by varying the external load both in ascending and descending order in the circuit and plotting the cell voltage against the current calculated using Ohms law. The values were accepted after a comparatively steady voltage was observed for some minutes under each resistance. Moreover, the data logged during Run 3 was used to plot a polarization curve (Ter Heijne et al. 2011). Since the polarization curves represent voltage as a function of current, internal resistance of the system can be estimated from the curves. Voltammograms were plotted by potential sweep technique with a potentiostat. The show the peaks for oxidation and reduction reactions at the electrodes for redox active compounds (Logan et al. 2006).

3.6.6 Analysis

The different equipment and methods used to analyze the samples collected and data acquired are described below:

Both anolyte and catholyte were examined for the pH value with a pH meter (VWR-pH 1000L, pHenomenal®), calibrated before the study.

Anolyte samples were analyzed for acetate concentration with High Performance Liquid Chromatography and UV detection (Shimadzu LC-20AD/T) after filtering the samples through 0.45 nm filters.

Catholyte samples were analyzed for decrease in copper concentration from correlating the concentration to an absorbance of 610 nm measured with a Shimadzu UV-1800 UV-VIS spectrophotometer. The samples were prepared by adding 2 M NaOH and 1M ammonia solution in a ratio of sample: NaOH: NH\(_3\) = 1:0.6:1 which gives a dark blue color after reaction.

Different ion concentrations were measured using ion chromatography (DIONEX ICS-900). The samples were diluted to, the detection capacity of the instrument, a concentration range between 0.2-3 mM.
The recorded voltage data were averaged for every hour using MATLAB®. Moreover, the voltage data were integrated numerically with MATLAB using trapezoidal integration technique in order to calculate charger transferred per day, which later were used to calculate moles of electron transferred per day and was correlated with moles of HCl used.

The current density was calculated as:

\[ \text{Current density, } J = \frac{E_{\text{cell}}}{R \cdot A} \left( \frac{A}{m^2} \right) \]  

(13)

where, R (Ω) is the resistor used in the external circuit and A (m²) is the surface area of the cathode (copper reducing electrode).

Coulombic efficiency per day was estimated as:

\[ \varepsilon_{cb} = \frac{\int V}{F \cdot R \cdot V_{\text{AN}} \cdot \Delta C_{\text{acetate}}} \]  

(14)

Where, \( \int V \) (mV·s) is the integral of voltage recorded over a day, \( V_{\text{AN}} \) (m³) is the total anolyte volume, \( \Delta C_{\text{acetate}} \) is the change of acetate concentration over a day, F and R, are as described before.

3.7 Monitoring and maintenance

Monitoring of the MFC included monitoring of both the operation and performance. The MFC was regularly checked for overflows, leakages, electrical connections, wear of tubing and clog etc. The current logged was also constantly monitored for any abrupt decrease. Maintenance of the reactor included replenishing anode and cathode feeds, disposal of discharges from anode and cathode, sludge removal when accumulated in excess etc.
4 Results and Discussion

The voltage signal recorded from the data logger was analyzed. The results were presented in accordance to operational phases described in 3.4.

4.1 Start-up 1

As described before, the start-up period was divided into two phases. The first phase was unsuccessful which led to changes in strategy and some additional investigations.

4.1.1 Phase 1

During the first phase, which lasted four days, the current generated in the reactor was negligible. The pH of the anode was found to be very low (<1). Figure 4.1 shows the current logged during this phase.

![Figure 4.1 Current during very low pH at the anode.](image)

From Figure 4.1 it is evident that there was very negligible current and presumably no microbial activity in the anode. This may be attributed to the very low pH value which was the cause of inhibition of microbial activity (Oliveira et al. 2013). The reason of this low pH in the anode was investigated further.

The flow of a low current despite inhibition of microbial activity can be explained by the membrane potential caused by the existence of a high salinity gradient owing to the high concentration of Cl⁻ in the catholyte (Nijmeijer & Metz 2010).

This phase has shed light on the performance of the membrane used in the study. Diffusion tests performed have shown very rapid diffusion of (less than an hour) of acid through the membrane when subjected to a pH lower than 1.

4.1.2 Diffusion tests

Diffusion tests performed showed the diffusion rate of anions through the membrane. Figure 4.2 shows the diffusion of Cl⁻ ions from a chamber with 1000 mM HCl solution to a chamber with 10 mM NaCl solution. While Figure 4.3 shows a very low diffusion of
Cl\(^-\) ions from a 15 mM solution to a 0 mM solution through the membrane. Figure 4.4 shows very low diffusion rate of sulfate ions through the AEM.

**Figure 4.2** Diffusion of Cl\(^-\) through AEM in the case of high concentration gradient. K is a diffusion constant.

**Figure 4.3** Diffusion of Cl\(^-\) through AEM in the case of low concentration gradient.
The results from the tests suggesting diffusion of acid from the catholyte to the anolyte which was attributable to the decrease of anode pH observed before. The small change in concentration of sulfate and chloride in Figure 4.3 and Figure 4.4 respectively can be neglected and attributed to the precision of the measurements since the initial concentration was 15 mM which was detected as 19 mM and 25 mM respectively.

Diffusion tests have shown diffusion of Cl\textsuperscript{-} ions with a high concentration gradient across the membrane. However, with a low concentration gradient the diffusion was found to be negligible in case of both Cl\textsuperscript{-} and SO\textsubscript{4}\textsuperscript{2-}, which could also be a result of different counter ions used for different tests. In the case of HCl protons can accompany Cl\textsuperscript{-} ions which is difficult in the case of NaCl.

4.1.3 Phase 2

The actual start-up phase having a catholyte with no acid, which lasted 21 days, the MFC showed to be performing consistently. The electrolytes were changed following the depletion of substrate in the anode and copper in the cathode which were traced by the analysis of the samples as well as the decrease of current. The change resulted in subsequent increase in current each time. The cell voltage reached as high as 70 mV from an initial value of less than 10 mV. Figure 4.5 shows the logged current during this phase and the change of electrolytes. While changing the electrolytes it was observed that the concentration of copper decreased to a very low value each time. However, there was no copper found on the cathode. Furthermore, blue precipitates were found in the cathode surface, on the membrane surface in the cathode, and the bottom of the cathode. The pH of the anode was found to be 7.1 – 7.75, normal range for facilitating microbial activity. However, the pH of the catholyte was found to be increased to a value around 7.5-8.

Another phenomenon noticed during this run was the sudden jolt in voltage difference across the circuit even with slight turbulence in the cathode chamber such as sampling with a syringe. This was investigated by aerating the cathode compartment for some time which resulted in a sudden increase of voltage difference across the circuit, which decreased again to a stable value after a short time. Figure 4.6 exhibits this event.
Figure 4.5 Hourly average of current logged during Start-up 1. The first arrows show the change of anolyte only whereas the others represent the change of both anolyte and catholyte.

Figure 4.6 Voltage across the circuit logged with aeration in the cathode compartment. The green arrows represent the start of aeration around 433 hours in the run.

Figure 4.5 shows the stable start-up of the MFC. The start-up time was typical (Aelterman et al. 2008; Saheb Alam et al. 2015). The use of substrate by the microorganisms can be correlated with the decreased current generated by the MFC. Since the cathode can reduce other oxidants such as O2, the anode oxidation can be assumed to be the limiting feature for the MFC. Batch feeding of substrate, marked with arrows in Figure 4.5, clearly shows the response of the microorganisms to substrate availability. Moreover, change of catholyte replacing the previous catholyte, from where most of the copper was precipitated, should have increased the cathode conductivity. Furthermore, dissolution of the copper precipitate should have removed any obstruction created for the ion transfer capacity of the membrane. However, since the MFC was still starting up at this stage this was assumed to be within the required ion transfer capacity and the anode was presumed to be the current limiting factor.

Nevertheless, the precipitation of copper for the catholyte solution was investigated further. This phenomenon can be attributed to the increase of pH in the cathode which
under a certain cathode potential range can precipitate different copper salts depending on the availability of other ions (Beverskog & Puigdomenech 1998). From the solubility product (Ksp is 2.2E-20) constant it was calculated that around a pH of 7.55 Cu(OH)₂ will form at 25°C. This increase of pH can be related to the reduction of oxygen to produce water molecule after combining with protons and also diffusion of Cl⁻ ions towards the anode which might cause the counter-diffusion of OH⁻ ions to the cathode (An et al. 2014).

The effect of aerating the cathode compartment can be attributed to some factors such as decrease of concentration loss by increase of mass transport of ions towards the cathode and mobilization of copper precipitates from the surface of the cathode to facilitate reduction on the cathode.

This phase has provided information about the current production and consumption of substrates as well as the start-up time for a MFC. Furthermore, the importance of catholyte recirculation was realized in this phase.

4.2 Run 1 - with manual pH control

During the Run 1, the attempt to keep the pH low to inhibit the precipitation of copper had different effects on the reactor performance. For the first 64 hours the dosage of 2 M HCl had positive effect on the current generated. However, the performance was found to be decreased over time within as short time as 24 hours. The pH was found to be increased in the cathode chamber to a value as high as 7.3 from a value of 3.18 in only 24 hours. Furthermore, the cathode was observed to have the precipitates of blue color each time. Figure 4.7 shows the current logged as well as the amount of HCl fed to the cathode either on a daily basis or by a feed at a regular interval for the whole run.

Following the attempt of feeding 1 M HCl at regular intervals instigated, a development of the reactor with a high cell voltage and subsequent current generation as high as 5.42 mA was observed. However, after the 95th hour a very steep drop in the current production was observed. The pH of the cathode as well as the anode at that point of time was measured to be very low. The cathode pH decreased to a value less than 1.0 and the pH of the anode was found to be less than 3.0. Flushing of the anode with new anolyte and changing the cathode feed to first 1 M HCl, 20 ml a day for 3 days first recovered the performance of the MFC, which was then followed by steady decrease of current generation. Following the further change of the cathode feed to 0.5 M HCl solution 20 ml 3 times in a day resulted in sharp increase in current generation, followed by a sharp fall in current production leading to complete cease of current flow through the circuit. The anode feed however was not observed to have any impact on the performance of the MFC. This point after 183 hours was decided to be the end of the run 1. One of the noticeable phenomena observed in this run was the increase of current to 3.75 times within 23 hours.
Figure 4.7 shows the effect of dosing acid on the current generation by the MFC. The sudden jump in the current at hours 18 and 41 can be related to the acid doses at those times. These phenomena can be explained by the dissolution of the copper precipitates upon the dosage of acid which presumably had brought back the conductivity of the catholyte as well as the ion transfer capacity of the membrane that were lost owing to the precipitation of copper. This can also be correlated to the moles of acid needed to bring down the pH to the desired value, which was more than the value calculated theoretically. The excess amount of acid was believed to be consumed to dissolve the precipitates. Although the reasons are not yet known, the sharp increase of current can be attributed to some hypotheses made by (Aelterman et al. 2008) (a) the organization of the microorganisms into an effective biofilm at the electrode surface, (b) achieving a sufficient high cell density, and (c) possibly the (quorum sensing regulated) onset of the production of endogenous mediating molecules or structures.

This run has provided information about the sensitivity of the microbial community to changes in pH and the difficulty in maintaining appropriate anolyte and catholyte pH manually.

4.3 Start-up 2

Following the deceased current generation caused by the ceased microbial activity, the reactor was set again for a second start up run. During the first 51 hours of this run the reactor did not show steady performance. Subsequent 355 hours, when the reactor was run under a load of 1000 Ω instead of the initial 100 Ω, it showed a small but steady increase of current generated. During the last 20 hours of the run the reactor showed a sharp increase in current production again. The maximum current generated in this stage was as high as 0.638 mA which corresponds to a cell voltage of 638 mV, which can be considered a close value to the open circuit voltage of a value around 800 mV measured for the MFC at different times. At this point the reactor was considered to be started up again. Figure 4.8 shows the current logged as well as the concentration of HCl increased in the cathode by a feed at a regular interval along with some excess amount to control the pH below a desired level for the whole run.
This start up time shows an improvement from the previous one and is comparable to some previous studies (Aelterman et al. 2008; Saheb Alam et al. 2015). Some of the microorganisms present throughout the first run could have survived the acidic environment and helped the second start up time to be faster. The sharp increase at the end of the run supported the hypothesis that was used to explain sharp current increase before, i.e. the formation of an efficient bioelectrochemically active biofilm on the anode surface. The addition of acid to the catholyte was calculated based on the expected current; however, this amount did not suffice to keep the pH within the desired level. This might have been caused by the diffusion of HCO$_3^-$ ions from the anolyte through the membrane, neutralizing the acid in the catholyte. HCO$_3^-$ was used as a pH buffer and proton carrier in the anolyte.

Diffusion tests performed for HCO$_3^-$ ions by counter-diffusion of Cl$^-$ ions showed a very high rate of diffusion through the membrane even under a moderate concentration gradient of 23.81 mM in Figure 4.9.
This run has provided information on the acidification of the anode and the basification of the cathode due to the oxidation and reduction reactions taking place and the diffusion of selective ions through the membrane.

4.4 Run 2 - manual pH control by intermittent feeding

Following the second start up period when the reactor was run under loads reduced gradually starting from 470 Ω down to 56 Ω resulted in increased current production. The peak current recorded in this run was as high as 5.74 mA (0.185 A/m²). The reactor showed stable performance under different loads during the whole run of 140 hours. Figure 4.10 shows the current logged as well as the amount of HCl fed and conc. of HCO₃⁻ maintained during this run. However, no copper removal was observed. The strategy of feeding acid to the cathode following the current generation did not have significant effect since the pH of the cathode showed significant increase resulting in precipitation of copper. Substantial amount of sludge was observed to accumulate on the anode side of the membranes and the piping for anode recirculation and feed.

![Figure 4.10](image)

Figure 4.10 Current logged, conc. of HCO₃⁻ and HCl changed vs time during the 2nd run with manual pH control. The green arrows mark the change of resistance across the circuit.

Although the peak current achieved in this run was higher than the previous run, it was attained under a load of lower resistance (56 Ω) than that of the previous (100 Ω). This can be related to the fact that the copper precipitates on the membrane might have reduced their ion exchange capacity. Furthermore, the sludge accumulation could have created a hinder against the membrane.

This run has shown the viability of using wastewater as a feed; however, the necessity of a pre-treatment of the wastewater for particle removal such as sedimentation, in order to avoid clogging the system, was also realized in this run.

4.5 Comparison of MFC with and without copper in the catholyte

The attempt to compare the performance of the MFC with and without the presence of copper in the catholyte was not completely successful. During this tenure a leakage of electrolyte feeds were observed. No data on feed was acquired for that reason. However, the current was logged during this time. Figure 4.11 shows the current logged for
catholytes both without and with respectively. The results show gradual decrease of performance of the MFC over time without copper. It should be noted that the leakage was spotted and taken care of in this time; however, the current generation had steady decrease with almost the same slope for around 40 hours both before and after the leakage. The introduction of copper decreased the current generation even further.

![Figure 4.11 Current logged, vs time during the trial run to compare the effect of copper in the catholyte. The green arrows mark the introduction of copper in the catholyte.](image)

The results from this run gives an idea that an oxygen reducing cathode will have higher current generation at the peak than a mixed copper and oxygen reducing cathode. Which can be justified by the electromotive force of an oxygen reducing cathode and a copper reducing cathode calculated theoretically.

### 4.6 Run 3 - with automatic pH control

The third run, when the pH was controlled between 3.5-3.7 with the same solution as the initial catholyte, resulted in much higher current generation. This run provided an idea about the amount of HCl required to control the pH of the cathode to a desired range. Although the actual HCl fed by the pH controller was higher than the amount estimated from the current logged, the trend showed a proportional relation with the current through the circuit as well as the bicarbonate buffer fed to the anodes. Some observations that can be emphasized are the increased HCl feed with increasing NaCl concentration in the catholyte, increased HCl feed with increasing NaHCO₃ concentration in the anolyte despite decreased current generation, and lastly an increase in feed after a leakage was spotted and repaired in the reactor. Figure 4.12 and Figure 4.13 shows the current logged with different resistors used in the circuit over the entire duration of the run. These plots show that the highest current achieved was as high as 9.22 mA with a 18 Ω resistor; however, this current was maintained for only a short time. A somewhat more stable current around 8 mA, over one hydraulic retention time of the anolyte, was observed with a 56 Ω resistor. The most stable current achieved over three times the hydraulic retention time of the anolyte was around 6 mA with a 100 Ω resistor. After the peak current with the 18 Ω resistor the current generation started to decrease for rest of the run. Finally with a 1 Ω resistor the current was as low as 2.78 mA. The noticeable phenomenon close to
the end of the run was the decrease of current generation along with increase of concentration of NaHCO$_3$ in the anode feed was observed in Figure 4.14. The change of anolyte to a lower bicarbonate concentration and reversal of resistance in the circuit to higher resistors did not bring the current generation back to previous order. Under any higher resistors the current generation was limited to approximately 1 mA compared to 9 mA generated previously.

Figure 4.12 Current logged under descending resistors across the circuit vs time during the 3rd run.

Figure 4.13 Current logged under ascending resistors across the circuit vs time during the 3rd run.
Much better performance of the reactor under controlled environment was observed in this run. However, it shows a sharp decrease in current production after a sudden increase of bicarbonate concentration. This can be attributed to the fact that such high concentration of bicarbonate can inhibit microbial activity (Corral et al. 1988; Fallanaj et al. 2016; Miyasaki et al. 1986; Silhacek & Taake 2005; Yaganza et al. 2009) or shift the microbial community towards a condition where methanogens outgrow exoelectrogens reducing electrogenesis (Oliveira et al. 2013). The latter case is more probable since the substrate consumption was still observed, indicating some microbial activity.

The open circuit potential of the MFC was measured as high as 850 mV with an open circuit anode and cathode potential of -275 mV and 540 mV respectively. The measure of potential of the anode and the cathode showed abrupt rise in anode potential during the high dosage of bicarbonate from -85 mV vs SHE to as high value as +470 mV vs SHE. Figure 4.15 and Figure 4.16 show the current against the potential of anode and cathode for both performing and non-performing MFC. Cyclic voltammetry conducted to check the microbial activity did not show current peaks in the voltammogram plotted in Figure 4.17 and Figure 4.18. The cathodic voltammogram is shown in Figure 4.19, which indicates a very high cathodic current capacity below 0 mV vs SHE. While running the cyclic voltammetry sweep, a very rapid deposition of copper on the cathode surface was observed within minutes.
Figure 4.15 Current vs anode and cathode potential of performing MFC.

Figure 4.16 Current vs anode and cathode potential after fall of MFC performance.

Figure 4.17 Voltammogram of anode Run 3 phase 2 (721 hours), OCP -0.31 V vs Ag/AgCl electrode.
The potential measurements for both anode and cathode showed that the peak current was generated with an anode potential of -100 mV vs SHE. This shows the peak microbial activity around this potential which is common (Aelterman et al. 2008). The plot of current vs potential for the period after the fall of the performance of the MFC can be related to very little microbial activity. This can be observed even more clearly from the voltammogram in Figure 4.17 and Figure 4.18 which do not show any peak which would be expected from a well-developed acetate oxidizing bio-anode (Aelterman et al. 2008). This can be explained by the probable inhibition of microbial activity owing to high bicarbonate concentration. Furthermore, the fact that decrease of bicarbonate concentration could not bring back the previous performance of the MFC can be hypothesized by the change of community of microbes during this inhibition period or the anode lost its adaptive characteristics to higher current densities and concede additional energy loss (Ter Heijne et al. 2011). Moreover, the feed being synthetic and very selective with only acetate as the carbon source the microorganisms surviving the inhibition period might have been of a kind that has very low activity. The cathode with a high current peak indicated that the cathode was not the limiting electrode and reduction of copper occurs at high currents, 150 mA (15 A/m² in this case). Moreover, the OCP of
the cathode (540mV) indicated reduction of other species, possibly oxygen, was going on as the cathodic reaction (Ter Heijne et al. 2010). Also the lower OCP of the cathode reduced in size can be attributed to the increase in mass transport losses.

This run shows the conditions such as electrode potentials and external circuit resistors at which the highest current was obtained.

4.7 Anaerobic Anode run

The attempt to render the anode system completely anaerobic during the phase 2 of the run 3 also showed some interesting results. Although the current produced was not at the peak due to degradation of the anode microorganisms, copper removal was observed in this phase. The open circuit potential was still recorded to be higher for the anode than that recorded earlier; consequently, the open circuit voltage was also lower. This might be the result of low microbial activity present at that time.

4.8 Effect of different conditions

The change of many conditions made during the whole run showed interesting effects on the performance of the MFC.

4.8.1 Aeration of cathode

As stated before recirculation of catholyte increased the current generation which was attributed to increase of mass transport and mobilization of possible copper precipitates formed under different conditions, facilitating contact of the catholyte to the cathode electrode. Furthermore, increasing the availability of oxygen at the cathode had presumably raised the electrode potential since oxygen has a higher standard reduction potential.

4.8.2 Salinity of the catholyte

The attempt to increase the conductivity of the catholyte with high concentration of NaCl, 46 hours in the Run 3, showed little effect on current generation. However, it showed some increase on the HCl fed to the cathode by the automatic pH controller. Figure 4.20 shows the effect of introduction of NaCl, which was very little. Furthermore, Figure 4.21 shows the effect of removing NaCl from the catholyte to compare the current generation under a comparable conditions under 100 Ω before and after removal, which shows a very little increase in current after removal of NaCl. A noticeable change for this two conditions is the open circuit potential which increased after the removal of NaCl.
Figure 4.20 Effect of cathode salinity.

Figure 4.21 Effect of removing salinity from the catholyte.

The effect of introduction of NaCl can be explained by the increase of conductivity which was realized by slight increase in current generation. However, later the increase of the current generation can be attributed to the rise of open circuit potential which may have been caused by the change in concentration of the ionic species present in the catholyte. Since, depending on the presence of anions different species can be formed under different cathode potential (Beverskog & Puigdomenech 1998). Moreover, since the potential depends on the activity of ions rather than mere concentration of them and the activity can reduce in presence of high salinity, the increase of potential with removal of salinity can be anticipated.

4.8.3 Change in bicarbonate concentration

The bicarbonate concentration had a considerable effect on the performance of the MFC. Increase in bicarbonate concentration from a value as low as 4.06 mM to a value of 12.74 mM showed a significant increase in current within 3 days which can be observed in Figure 4.22. However, increase of bicarbonate to a very high concentration (119 mM) had an adverse effect on the MFC performance which is shown in Figure 4.23.
The effect of bicarbonate can be attributed to the control of pH and the increase of proton conductivity of the anolyte. Furthermore, the negative effect of excess bicarbonate can be attributed to inhibition of bacterial activity as stated before.

4.9 Membrane Potential

As described in 3.6.3 membrane potential measured directly and calculated from other potentials at different times were found to be between a range of 18 mV to 43 mV (±8 mV). This can be attributed to the concentration gradient maintained for Cl⁻ ions (Nijmeijer & Metz 2010; Scialdone et al. 2014).

4.10 Removal of copper

Copper removal was not observed with a cathode having the same size as the anodes. The cathodic current density was found to be very low. The peak current density was found to be 0.15 A/m² with the large sized cathode. The attempt to increase current density by decreasing the cathode surface area resulted in current density up to 1.5 A/m². Copper removal was also observed during this phase shown in Figure 4.24.
The lab-scale abiotic test performed showed a copper recovery rate of 200 mg/L in four hours under 20 A/m² current density. However, from the theoretical current achievable and the practical current highest reported in scaled up MFCs capable of copper recovery until now a stable current density of 8.33 A/m² was estimated to be achievable with this experimental setup. This indicates a 10 hours run to recover 200 mg copper from 1L leachate.

Figure 4.24 Copper accumulated on the Titanium cathode surface (7.63 cm²).

The reason for having copper removal only after decreasing the cathode area could be that at low current density all the electrons are used to reduce oxygen. In a previous study, a current density of 1 A/m² was also shown to be necessary for copper reduction in a MFC (Rodenas Motos et al. 2015). Although a current density as high as 23 A/m² was reported for copper recovery in MFCs, it was for a brief time which started decreasing afterwards (Rodenas Motos et al. 2015). Hence the stable current achieved in this study was chosen to calculate the expected current density.

4.11 Operational control

The major parameter needed to be controlled was pH of both anode and cathode. As stated before the strategy to control anode pH within the limits which is favorable for microbial activity was using a bicarbonate buffer because of its buffer range as well as low cost. HCl was used to control the cathode pH since the real leachate contains a high concentration of HCl. Figure 4.25 shows the amount different chemicals used in comparison with electrons produced which was calculated from the current logged and demonstrates that the HCl fed to the cathode is more than the electron transferred to the cathode. However, it is also noticeable that it was distinctively high when there was an increased NaCl concentration in the catholyte or increased bicarbonate concentration in the anolyte and leakage in the system. A relation was observed between electrons generated and acid fed from Figure 4.26; however, not linear and with an offset of 13.25 mmoles/day for the acid. This indicates acid is necessary to control the cathode pH even when there is no or very little current generated in the reactor. Three points in Figure 4.26, the days when the feed of bicarbonate was very high at the anode side, show very high acid feed compared to the other days.
The excess amount of HCl than the necessary value calculated, fed to the system can be attributed to the diffusion of bicarbonate from the anode towards the cathode through the AEM. The increase of feed following increase of NaCl in the catholyte can be explained by the diffusion of Cl⁻ ions towards anode resulting in decrease in anode pH and consequently increase in cathode or pH split (Li et al. 2011). The acid fed during no current generation can also be explained by the ion diffusion due to a very large concentration gradient of ions between the chambers separated by the membrane. Furthermore, the three points with high acid feed also supports the explanation of bicarbonate diffusion through the membrane.

4.12 Substrate consumption at the anode and coulombic efficiency

Substrate consumption was monitored in order to keep the substrate availability high to restrict it from limiting the performance of the MFC. It was observed that although the current produced was low at different stages the substrate was consumed regularly. The coulombic efficiency calculated was found to be very low, less than 1% during Start-up 1 which was operated in batch mode. Although the exact coulombic efficiency could not
be calculated during the feed mode due to the sampling limitations, it was estimated over a day using the feed and anode chamber carbon concentrations, and was found to be, 12.5%.

The results can be explained by the very low electrogenesis during the start-up phase and the lack of wash-out of microorganism not taking part in this process, allowing them to use up the substrate available. On the other hand, during the feed mode the current was high suggesting the existence of electrogenesis, making the coulombic efficiency higher.

4.13 Internal Resistance of the MFC

Internal resistance of the reactor was estimated from polarization curves plotted at different stages either synthetically by changing the external resistor after a short period with pseudo steady state achieved or from data logged over the run under different external resistors (Run 3).

Figure 4.27, Figure 4.28 and Figure 4.29 shows the polarization curves for Run 1, Phase 1 and Phase 2 of the Run 3 respectively. The system resistance calculated from the slope of the polarization curve in the mid-range of current was found to be 200 Ω and 222.2 Ω for the Run 1 and Phase 2 of the Run 3, whereas Phase 1 of Run 3, when the current was at peak, showed a more practical internal resistance of about 20 Ω. The tail of the polarization curve in Figure 4.28 indicate high mass transfer loss (Rismani-Yazdi et al. 2008).

Figure 4.27 Polarization curve for Run 1.
The internal resistance found can be explained by the microbial activity present at the anode. During the Start-up 1 and phase 2 of the Run 3 the performance of the MFC was very low, presumably owing to immature microbial activity. However, during Phase 1 of the Run 3 the low internal resistance can be attributed to the high microbial activity observed by the current logged. The internal resistance estimated were comparable with previous studies (Cheng et al. 2013). The mass transport losses are related to cathodic limitation of facilitating the reduction reaction at the cathode surface (Rismani-Yazdi et al. 2008).
5 Outlook

The above results show many aspects of a MFC with a copper reducing cathode. The discussion of the results allows to assess the study in brief. Different aspects of the study with an outlook to improve the tests performed are presented below.

5.1 Performance of the MFC

The overall performance of the MFC used in this study can be characterized as underperforming. Several reasons can be attributed to this such as several start-ups with insufficient startup time, improper control of the environment, and frequent changes in environment etc. Nevertheless, the MFC performance was comparable to a previous successful study (Rodenas Motos et al. 2015) in high rate copper recovery in MFC until 384 hours into the Run 3 (Phase 1) under external resistor of 56 Ω. In fact, it showed better performance than the previous study during this time, before losing performance owing to over dosage of bicarbonate, presumably. This led to the assumption that high rate copper recovery is viable under the conditions such as titanium cathode, cathode pH range of 3.5-3.7, acetate as a substrate and bicarbonate as anode pH buffer applied in this study. The peak current generation expected in the MFC is about 250 mA with a current density of 8.33 A/m². The open circuit cathode potential indicates competitive reduction of oxygen at the cathode, a limiting factor for copper recovery; however, since current will drop with decreasing copper concentration in solution oxygen reduction will aid the current generation at low copper concentration. Nevertheless, in comparison to previous studies on litre-scale microbial fuel cell the current density and power density recorded in this study was very little since larger electrodes were used and similar or lower current was achieved. A comparison of the current and current density achieved in this research to some previous studies using copper reduction is presented in the Appendix I.

In order to improve the performance of the MFC it is necessary to ensure a very controlled start-up of the reactor. The temperature of the reactor needs to be controlled at a higher temperature during the start-up period, which can later be operated under room temperature to reduce energy usage. A better start-up of the reactor will contribute to a better performance while performing continuous experiment. A better design of the reactor with provisions of homogenous substrate diffusion towards the anodes and uniform catholyte recirculation to both sides of the cathode will increase the performance. Moreover, safeguards against accidents such as leakage and overflow need to be included. They include the measurement, sampling and feed ports of both the anode and cathode to be constructed in such a way that they cannot be mixed under any circumstance. The distance between the electrodes needs to be further decreased in order to decrease internal resistance. The experimental conditions should not be changed very frequently which will lead to a more stable performance throughout the run. In order to prevent over dosage of external chemicals both for good performance and sustainable resource usage bicarbonate pH buffer needs to be kept under concentration of 50 mM which has been proven to provide favorable conditions for Geobacter, the exoelectrogens expected with the substrate used (Du et al. 2007; Saheb Alam et al. 2015). If still necessary other strategies such as reducing the hydraulic residence time for the anolyte can be used to control the pH at the anode. Furthermore, since the mass transport losses increase significantly at high current densities an optimum external resistor needs to be selected to achieve the maximum current with a minimum mass transport loss. Since the cathode size did not
affect the current generation an optimum anode to cathode size ratio needs to be established (Cheng et al. 2013).

5.2 COD removal at the anode
Removal of substrate was found to be very fast during the batch mode runs even with very low current. This suggests presence of microorganisms in the bulk liquid consuming substrate as well as microbial activity before commencement of electrogensisis process. However, during feed mode owing to substrate availability in excess, the treatment efficiency was not assessed properly. To improve COD removal further, substrate diffusion needs to be homogenized by a better reactor design.

5.3 Sustainability of the process
The overall process is sustainable in regards to economic, social and environmental aspects. Since the general practice regarding contaminated site remediation does not account for proper treatment by either complete removal or recovery of the metal content, this technique of soil washing followed by recovery in MFC is sustainable is every way. The fuel being the COD present in wastewater or solid waste is cheap and available in abundance. Furthermore, this technique provides the COD removal as an additional aspect along with treatment of contaminated leachate. Apart from management of the high amount of sludge produced, wastewater can be used as a sustainable fuel for this type of MFCs.

There is no byproduct other than sludge in the anode outflow stream. The anolyte gets treated. The use of bicarbonate buffer decreases the risk of byproducts and can be useful to increase the alkalinity of receiving stream.

Since the most effective way of soil washing is with acid, leachates are already acidic which can be used as copper feed as well as to control pH. Since copper removal is slow cathode feed can also be recirculated.

Although, additional chemicals needed to control pH such as bicarbonate and acid, are not sustainable, the overall use of the MFC to recover copper overcomes the impacts of use of external chemicals. Furthermore, acidic leachates reduce the requirement of use of acid significantly.

5.4 Treatment limitations
There are several limitations in the treatment of both the electrolytes that was realized.

- Since no treatment for nitrogen and phosphorus was facilitated in the system, the wastewater discharge from the anode might need further treatment in the case of high concentrations in the influent and strict emission limits.
- The actual leachate treated at the catholyte may need further treatment for other metals washed, in the case that leachate was contaminated with other metals.
- Furthermore, as the treated leachate will have a low pH (below 4.0) it may not be suitable for direct disposal.
5.5 Cost assessment

Under the assumption of viability of high rate copper recovery adopted in 5.1 and using results for chemicals used and time required to recover copper from a synthetic solution with a lab-scale test, the costs for construction and operation of a batch operated litre-scale MFC with a total reactor volume of 5100 cm$^3$ containing 2000 ml electrolytes treating 1 L of a leachate (catholyte) containing 200 mg/L copper, estimated to take 10 hours, was calculated.

The construction cost of the reactor was calculated to be about 32000 SEK (3500 EUR). The breakdown of the estimated costs is provided in Table A.3.

However, the operation cost was calculated to be very low. Using wastewater amended with bicarbonate buffer as anode feed, the acid needed to control the pH during very high current was found to contribute the most to the operation costs. The operation costs estimated for operating such a litre-scale reactor was predicted to be about 1000 SEK (100 EUR) per run with copper contaminated leachates. However, in the case of a full functioning reactor the MFC needs to run continuously, which can be done with oxygen reducing cathode system, the operation costs will continue. Nevertheless, owing to the acidic nature of the leachates, the necessity to use acid ceases, operation costs will be negligible, about 10 SEK (1 EUR) per day. The breakdown of the operating costs is described in the Table A.4. The price of copper, which is 38 SEK (4 EUR) per kg of copper, can also be added to the cost benefit equation.

The foremost economic benefit of this process over the others is the electric energy that is obtained from COD removal, since COD from wastewater or solid waste is available without any monetary compensation, whereas in other techniques such as electrowinning this energy is considerably high and needs to be provided externally (Rodenas Motos et al. 2015).
6 Conclusions

From the above discussion of the results and assessment of different aspects of the study some conclusions were drawn upon which are pointed out below.

- Start-up takes a considerable amount of time for an MFC of this size. Start-up of a previously working reactor takes less time.
- From the comparison of performance of the MFC during different stages, a litre-scale MFC was found to be viable for treating copper contaminated leachates once the reactor is at peak performance and enough current is generated.
- Results obtained from small-scale and batch mode studies are not directly comparable with that of large-scale and continuous studies such as this study. The performance of different sizes of MFCs varies highly depending on the rates of ongoing reactions as well as internal resistance generated in the reactor.
- From the different tests performed in different scales and reported results of previous studies treatment of 1L of a leachate containing 200 mg/L copper was estimated to take 10 hours under peak current density predicted.
- From the results of effects of recirculation of the catholyte and change in size of the cathode mass transport losses were found to be a restricting factor, which is expected in higher currents.
- Significantly larger electrodes did not have significant increase in terms of voltage and associated current than that of previous studies proving the non-linear relationship of MFC performance with size of electrodes.
- From both the lab-scale test and the litre-scale test it was concluded that the cathode must be designed to allow a current density > 1A/m² in order to facilitate copper recovery.
- The cost was found to be feasible to operate the reactor even in a litre-scale MFC to treat the leachate obtained after soil washing.
- The overall process of leachate treatment for copper after soil washing is more sustainable than the general practice of remediation of contaminated sites such as “dig and dump” or transfer of contaminated soil. Moreover, the copper recovery in an MFC from leachate is sustainable than other techniques such as electrowinning.
- Some external resources such as pH buffer in the anode and acid in the cathode is necessary to run the reactor. These are the major contributors to the cost for operation.
- There is a relation between the current generated and the external resources used; however, the relation is not linear and they are needed to operate the reactor even with very little or virtually no current generated.
7 Recommendations

From the experience during the study some recommendations were suggested in case of future studies with similar scope. These include operation and control measures for better performance, tools and methods of analysis for better understanding of the results, improvements in materials and design.

- Impedance spectroscopy can be a powerful tool for better understanding and characterization of the bioelectrochemical system.
- Rendering the complete electrolyte system to an anaerobic system could aid the microbial electrogensis as well as eliminate oxygen as a competitive electron acceptor at the catholyte facilitating higher copper availability and less mass transport losses.
- Particle removal, e.g. by sedimentation, is recommended to keep the inflow system free from clogging as well as sludge removal in regular intervals to restrict clogging of the reactor and discharge system.
- Use of complex substrate system as anode feed can be investigated to achieve a true mixed culture microbial community, which has been proven to perform better.
- A more stable membrane under the target conditions (e.g. pH) can be investigated to prevent any accidents from effecting the microbial activity (e.g. leakage of acid into the anode chamber).
- Microbial analysis from samples of both the anode and the bulk can be performed to understand the microbial community evolution in order to predict the performance comparing with literature. Also, tracking the growth of biomass can provide information on yield coefficients to predict sludge production.
- Organic carbon measurements in the effluent can be automatized to calculate coulombic efficiency properly.
- Flow optimization ensuring sheer stress to remove microorganism from the bulk and restrict clogging of the reactor.
8 Reference


Li, W.-W. et al., 2011. Recent advances in the separators for microbial fuel cells. Special Issue: Biofuels - II: Algal Biofuels and Microbial Fuel Cells, 102(1), pp.244–252.

Li, Y. et al., 2010. Microbial fuel cells using natural pyrrhotite as the cathodic heterogeneous Fenton catalyst towards the degradation of biorefractory organics in landfill leachate. Electrochemistry Communications, 12(7), pp.944–947.


Modin, O. et al., 2012. Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute


Appendices

The appendix or attachments contains the significant photos, calculation sheets, program codes used for this study, but was deemed unnecessary to be used along with the text. These are attached to facilitate ease of detailed verification or future reproduction of the work performed.

**Appendix I**

**Comparison of studies**

*Table A.1 Cathode size used in different studies*

<table>
<thead>
<tr>
<th>Study</th>
<th>Cathode size (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(An et al. 2014)</td>
<td>3</td>
</tr>
<tr>
<td>(Rodenas Motos et al. 2015)</td>
<td>100</td>
</tr>
<tr>
<td>(Ter Heijne et al. 2010)</td>
<td>22</td>
</tr>
<tr>
<td><strong>This study</strong></td>
<td>300</td>
</tr>
</tbody>
</table>

*Figure A.1 Current density achieved in different studies.*

*Figure A.2 Current achieved in different studies.*
Appendix II Photos

There were several photos taken during the study to document different phenomena observed such as precipitation of copper, sludge accumulation etc. also the actual reactor was photographed.

Figure A.3 Reactor Assembly of the MFC.

Figure A.4 Precipitation of copper as Cu(OH)$_2$ at the cathode during high pH.
Appendix III  Calculation tables

Some detailed calculation about the calculation of the cathode feed and cost estimation are presented here.

Table A.2 Calculate pH Values of Hydrochloric acid (OxyChem 2015)

<table>
<thead>
<tr>
<th>Wt.% HCl</th>
<th>pH</th>
<th>Normality (eq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.647</td>
<td>0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>2.500</td>
<td>0.16</td>
<td>0.694</td>
</tr>
<tr>
<td>2.000</td>
<td>0.26</td>
<td>0.554</td>
</tr>
<tr>
<td>1.500</td>
<td>0.38</td>
<td>0.414</td>
</tr>
<tr>
<td>1.000</td>
<td>0.56</td>
<td>0.275</td>
</tr>
<tr>
<td>0.500</td>
<td>0.86</td>
<td>0.137</td>
</tr>
<tr>
<td>0.3647</td>
<td>1.00</td>
<td>0.100</td>
</tr>
<tr>
<td>0.2500</td>
<td>1.16</td>
<td>0.0685</td>
</tr>
</tbody>
</table>
The breakdown of the costs for constructing the reactor is presented below.

Table A.3 Construction costs

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Amount</th>
<th>Cost (SEK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plexiglas frame</td>
<td>total</td>
<td>1</td>
<td>3000</td>
</tr>
<tr>
<td>Titanium cathode</td>
<td>sheet</td>
<td>1</td>
<td>1250</td>
</tr>
<tr>
<td>Titanium wire</td>
<td>roll</td>
<td>1</td>
<td>1380</td>
</tr>
<tr>
<td>Carbon felt</td>
<td>pack</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>Pumps</td>
<td>number</td>
<td>3</td>
<td>10000</td>
</tr>
<tr>
<td>Tubing &amp; connections</td>
<td>total</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Feed container</td>
<td>number</td>
<td>2</td>
<td>1000</td>
</tr>
<tr>
<td>Data logger &amp; electrical equipment</td>
<td>total</td>
<td>-</td>
<td>4000</td>
</tr>
<tr>
<td>pH controller</td>
<td>number</td>
<td>1</td>
<td>9000</td>
</tr>
<tr>
<td>Other costs</td>
<td>total</td>
<td>-</td>
<td>1000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>31230</strong></td>
</tr>
</tbody>
</table>

Table A.4 Operation costs per day for an expected current of 250mA (8.33 A/m²)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Cost (SEK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>3.5 g</td>
<td>9</td>
</tr>
<tr>
<td>Electrical energy</td>
<td>1.2 kWh</td>
<td>1</td>
</tr>
<tr>
<td>Acid (if applicable)*</td>
<td>1 L, 2 N</td>
<td>1000*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><em><em>10 (1010</em>)</em>*</td>
</tr>
</tbody>
</table>

Appendix IV  MATLAB code used to treat the data logged by the data logger

```matlab
[FileName,PathName] = uigetfile('*.xlsx','Select the MATLAB code file');
A=xlsread(FileName);
figure
plot(A(:,1),A(:,2));
side=input('select side','s');
ds=length(A);
h=floor(ds/360);
d=floor(h/24)+1;
if side=='s'
    B=A(1:h*360,:);
elseif side=='e'
    B=A((ds-h*360+1):ds,:);
```
end
mvolt=B(:,2)*1000;
rsmvolt=reshape(mvolt,360,h);
C=[mvolt;zeros(d*8640-length(mvolt),1)];
rsC=reshape(C,8640,d);
idv=trapz(rsC)*10/96485.3; % integrated daily voltage
hmvolt=mean(rsmvolt,1);
Y=transpose(hmvolt);
figure
plot(0:(h-1),Y, 'k+-', 2, 2);
title(FileName);
xlim([0 h+1]);
ylim([min(Y) max(Y)+1]);
grid on;