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Investigation Of Heavy Metal Removal From Synthetic Desalter Effluent Using a Two-Step Approach by Micellar-Enhanced Ultrafiltration and Microbial Fuel Cell

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Abstract

Heavy metals in wastewater streams negatively affect the environment due to their high toxicity. Non-conventional heavy metal removal methods show higher efficiencies for the remediation of these pollutants. In this investigation, a two-step approach using micellar-enhanced ultrafiltration (MEUF) and microbial fuel cell (MFC) was investigated to remove copper, manganese, and zinc from a synthetic salt wastewater containing magnesium, sodium, and phenol. This synthetic solution was used to simulate refinery wastewater streams such as desalter effluent. The study was carried out in three phases. In the first phase, a flat plate polyether sulfone membrane was investigated for the MEUF process with different concentrations of rhamnolipid biosurfactant. The process was performed using a transmembrane pressure of 2.5 bar for an average time of 2 h. MEUF showed a maximum heavy metal removal efficiency of 99% for copper with a rhamnolipid concentration of 300 mg/L. In the second phase, a dual chamber MFC inoculated in the anode with *Shewanella oneidensis* MR-1 demonstrated a maximum removal efficiency of 93% and 98% for copper in the anode and cathode chambers, respectively. During the operation, the biofilm produced bacterial nanowires on the surface of the carbon felt electrodes. These bacterial nanowires enhanced electron transport, and maximum open-circuit voltages (OCV) of 516.6 mV in the anode and 127.7 mV in the cathode were obtained. Finally in the third phase, the complex rhamnolipid metals mixture was applied to the MFC system. A maximum metal removal of 84% for manganese was observed. Furthermore, it was found that MFC efficiency was lower and bacterial growth was inhibited at high rhamnolipid concentrations. In conclusion, a rhamnolipid concentration of 100 mg/L was optimal for bacterial growth in the MFC, resulting in a maximum OCV of 335.5 mV. Moreover, the synthetic wastewater solution in the anode chamber facilitated the formation of bacterial nanowires. The bacterial nanowires are formed due to the higher toxicity of heavy metals. The specific growth rate for the wastewater was 0.11 h^{-1}. The study led to a novel combined approach using MEUF and MFC for heavy metal remediation.

Keywords

Metal removal, wastewater, micellar-enhanced ultrafiltration, rhamnolipid, microbial fuel cell, *Shewanella oneidensis* MR-1, bacterial nanowires.

Summary for Lay Audience

Heavy metals in wastewater harm the environment due to their high toxicity. New approaches for heavy metal removal show higher efficiencies for the remediation of these pollutants. In this investigation, a two-step approach using filtration and bacterial-based technology was investigated to remove copper, magnesium, manganese, and zinc from synthetic salt wastewater containing hydrocarbons. The study was carried out in three phases. In the first phase, a filtration membrane was investigated with biosurfactant. The process showed maximum heavy metal removal efficiency for copper. In the second phase, a battery based on bacteria demonstrated a maximum removal efficiency for copper as well. In the third phase, the battery based on bacteria showed a higher removal efficiency for manganese, using the discharge wastewater from phase 1. Finally, the bacterial battery demonstrated voltage generation and metal removal for phases 2 and 3. The study demonstrated an efficient method for metal remediation and voltage production using two different processes.

Co-Authorship Statement

In the experimental work from this research 4 papers were written, the collaboration and authorship of them are described below:

Chapter 2

- **Title:** An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production.
- **Status:** Published in *Science of the Total Environment*, 2021, Vol. 754, pg. 142429
- **Carlos Munoz-Cupa:** Technical and theoretical advisor, literature review, writing and corrections of drafts and final paper.
- **Yulin Hu:** Technical and theoretical advisor, corrections of drafts and final paper.
- **Amarjeet Bassi:** Technical and theoretical advisor, corrections of drafts and final paper.
- **Charles Xu:** Technical and theoretical advisor, corrections of drafts and final paper.

Chapter 3

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- **Carlos Munoz-Cupa:** Experimental design, laboratory work, data analysis, and paper writing.
- **Amarjeet Bassi:** Technical and theoretical advisor, corrections of drafts and final paper.
- **Lei Liu:** Technical and theoretical advisor, corrections of drafts and final paper.

Chapter 4

- **Title:** Investigation of heavy metal removal from salty wastewater and voltage production using *Shewanella oneidensis* MR-1 nanowires in a dual-chamber microbial fuel cell.
- **Status:** Accepted in *Environmental Progress & Sustainable Energy*, 2023.
- **Carlos Munoz-Cupa:** Experimental design, laboratory work, data analysis, and paper writing.
- **Amarjeet Bassi:** Technical and theoretical advisor, corrections of drafts and final paper.

Chapter 5

- **Title:** Investigation of rhamnolipid addition on the microbial fuel cell permoance and heavy metal capture in metal laden wastewater.
- **Status:** Submitted under peer review in *Journal of Water Process Engineering*, 2023.

- **Carlos Munoz-Cupa:** Experimental design, laboratory work, data analysis, and paper writing.
- **Amarjeet Bassi:** Technical and theoretical advisor, corrections of drafts and final paper.

Dedication

To my mother Maria Cupa and my father Gratiniano Muñoz for their support and unconditional love.

(A mi madre Maria Cupa y mi padre Gratiniano Muñoz por su apoyo y amor incondicional)

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Nomenclature

List of abbreviations

MEUF	Micellar-enhanced ultrafiltration
MFC	Microbial fuel cell
PEM	Proton exchange membrane
PES	Polyether sulfone
CMC	Critical micellar concentration
OCV	Open-circuit voltage
ICP-OES	Inductively coupled plasma optical emission spectroscopy
TSB	Tryptic soy broth
OD	Optical density
PBS	Phosphate buffer solution
COD	Chemical oxygen demand
TOC	Total organic carbon
SDS	Sodium dodecyl sulfate
CTAB	Cetrimonium bromide
SDBS	Sodium dodecyl benzene sulfate
CPC	Cetylpyridinium chloride
TX-100	Triton X-100
RO90	Nonaoxyethylene oleylether carboxylic acid
JBR 425	Biosurfactant JBR 425
DPCl	Dodecylpyridinium chloride
BCl	Benzethonium chloride
ODA	Octadecylamine
PEUF	Polymer-enhanced ultrafiltration
PEI	Polyethyleneimine
PSA	Poly(sodium acrylate)
PSS	Poly(sodium 4-styrenesulfonate)
PAA	Polyacrylic acid
PAASS	Polyacrylic acid sodium salt
HMDS	Hexamethyldisilazane
SEM	Scanning electron microscopy
RHL	Rhamnolipid

List of symbols

P	Power density (mW/m^2)
I	Current density (mA/m^2)
V	Voltage (mV)
R	Resistance (Ω)
A	Surface area (m^2)

t	Time (h)
°C	Temperature in Celsius degrees
rpm	Revolutions per minute
J_P	Permeate flux (L/m2h)
V_P	Volume of the permeate (L)
A_M	Area of the membrane (m2)
R	Removal efficiency (%)
C_P	Concentration in the permeate (mg/L)
C_F	Concentration in the feed (mg/L)
C_R	Concentration in the retentate (mg/L)

List of Greek symbols

Ω	Resistance units
μ	Specific growth rate (h^{-1})
μP	Viscosity

List of subscripts

ext	External
int	Internal
ox	Oxidation
red	Reduction

Chapter 1

1 Introduction

Water pollution due to industrialization is increasing, and effluent contamination with toxic compounds and heavy metals is affecting the environment. In Ontario for example, a total of 1391.5 million m^3 of effluent were discharged from industrial processes (Minister of Industry, 2012). At the same time, wastewater treatments processes have been investigated to mitigate the impact of toxic compounds and remove them before discharge to the environment. Some industrial processes like desalinization of crude oil have high content of salts, hydrocarbons, phenols, and heavy metals.

Wastewater treatment of heavy metals has been carried out using approaches such as electrochemical processes, ion exchange, electrodialysis, and others. Previously, membrane separation using nanoparticles and polymers to bind heavy metal ions have been widely studied due to their higher selectivity and removal efficiency as well. To improve the selectivity of heavy metals, polymers have been used to form complex metal-polymers that efficiently reject toxic compounds by ultrafiltration, this process is called micellar-enhanced ultrafiltration (MEUF).

In this study, a process to remove heavy metals from wastewater containing salt and phenol was conducted. Then, a biosurfactant – metal complex was applied in ultrafiltration. The recovered retentate from the MEUF was then further investigated for metal removal and voltage generation using a two-chamber microbial fuel cell (MFC) inoculated with *Shewanella oneidensis* MR-1. Specifically, the goals were to investigate the MEUF process using rhamnolipid to complex heavy metals from wastewater and to investigate the bioremediation of the retentate using a MFC.

1.1 Research structure

The study was divided into i) the use of MEUF with rhamnolipid to form the complex metal-biosurfactant, here the filtrate was the treated wastewater and the retentate was the solution of metal and rhamnolipid micelles, ii) the cultivation of *S. oneidensis* in the MFC

with metal wastewater, and iii) the investigation of retentate from MEUF with rhamnolipid-metals complex in the anode chamber of the MFC.

In the first phase, synthetic desalter effluent was treated by ultrafiltration. The complexation between the metals and the rhamnolipid was evaluated under different conditions of pH, biosurfactant concentration, and volume ratio between the rhamnolipid and wastewater.

In the second phase, the synthetic desalter effluent before the ultrafiltration treatment was evaluated in the anode and cathode chamber of the MFC. In the third phase, the retentate from the MEUF process was investigated in the anode chamber of the MFC. In both phases, the anode chamber was inoculated with *Shewanella oneidensis* MR-1. Moreover, heavy metal removal, voltage generation, biofilm formation, and MFC performance were evaluated for phases two and three.

1.2 Objectives

1.2.1 Overall objective

Investigate heavy metal removal from synthetic desalter effluent via two-step approach, using micellar enhanced ultrafiltration (MEUF) and microbial fuel cell (MFC).

1.2.2 Specific objectives

- To critically review and summarize the existing literature on MEUF and MFC as alternatives for metal remediation.
- To investigate the heavy metal removal by complexation of biosurfactant and heavy metals using MEUF at different process conditions.
- To investigate the heavy metal removal and power generation of synthetic desalter effluent containing metals using the MFC approach at the anode and cathode chambers.
- To study the heavy metal removal and power generation of retentate from the ultrafiltration process using MFC at the anode.

1.3 Thesis overview

The following thesis is structured according to the results and information reported in Chapters 1 to 6. Chapter 1 provided an introduction and structure for the thesis. In Chapter 2, a literature review on heavy metal removal from desalter effluent, MEUF and MFC was performed. In Chapter 3, the development of MEUF with biosurfactant to remove metals from synthetic desalter effluent was investigated. In Chapter 4, metal removal from synthetic desalter effluent using MFC inoculated with *Shewanella oneidensis* was studied. Chapter 5 detailed the removal of metal ions from the retentate of the ultrafiltration process by MFC inoculated with *S. oneidensis*. In chapter 6, conclusions and recommendations were provided.

1.4 Contribution and novelty

- Heavy metal removal through ultrafiltration coupled with environmentally friendly biosurfactants such as rhamnolipid has a high removal efficiency at low metal concentrations.

- The performance of *Shewanella oneidensis* MR-1 in dual chamber MFC under different wastewater conditions was evaluated. Bacterial and MFC performance were evaluated under synthetic desalter effluent in the anode and cathode chamber.

- For the first time, the investigation of the retentate performance in the anode chamber of the MFC was analyzed, the best open-circuit voltage and *S. oneidensis* growth under the lowest and highest rhamnolipid concentrations from MEUF process were evaluated.

- A novel two-step approach using environmentally friendly processes such as MEUF coupled with rhamnolipid biosurfactant and MFC was studied. This process can increase the low sludge generation.

Chapter 2

2 Literature review

The information provided in this chapter provides a literature review regarding MEUF and MFC processes. The MFC information is based on the paper: **Munoz-Cupa, C., et al. (2021). An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production.** ***Science of the Total Environment,*** **754, 142429.** The review represents the culmination of objective 1 (see section 1.2).

2.1 Introduction

The increase in human population is directly proportional to the pollution of water streams due to industrial processing. Some of these pollutants are very toxic to humans such as heavy metals that have negative effects in the environment and nowadays is a major concern due to their toxicity. Some of the toxic heavy metals include lead, thallium, cadmium, chromium, copper which are commonly used in different processes (Mitra et al., 2022). Heavy metals into water streams are toxic at low concentrations between 1.0 - 10.0 mg/L (R. Chakraborty et al., 2022). Some industries like refineries significantly release heavy metals into the environment, which makes the study of this type of effluent an increasing research area.

Refinery processes are very important to convert petroleum crude oil into high value products like gasoline, diesel, and polymers. However, the refining process generates wastewater streams ranged from 1.5 to 13.2 mega millions of L/day (State, 2002) and contains different contaminants like heavy metals. These heavy metals have negative effects on the environment and nowadays which is a major concern due to their toxicity (White et al., 1995). The desalter unit is the first operation in the petroleum refinery applied to remove contaminants and water from crude oil. The main objective of this process is to avoid corrosion and further problems related to the quality of the final product. Some of these impurities include salts in approximately quantities of 28.5 to 713.2 mg/L (IPIECA, 2010).

Due to the salt characteristics present, desalter effluent is a highly polluting stream, and this effluent is the largest volume stream in the refinery processes with a discharge of 7.6 – 9.5 L of wastewater per barrel of crude oil fed to the unit (State, 2002). The current technologies for heavy metal removal and desalter effluent treatment include coagulation, flocculation, chemical precipitation, flotation, membrane filtration, ion exchange, electrodialysis, photocatalysis and, adsorption (Barakat, 2011). However, some technologies have issues related to the high cost, maintenance and further problems with chemicals disposal.

An effective approach for heavy metal removal is MEUF. The process uses an ultrafiltration membrane that rejects particles with higher molecular weight using complexation of metal ions with macromolecular species, for example, biosurfactants (Trivunac & Stevanovic, 2006). Biosurfactants applied in ultrafiltration are biodegradable and create micelles that increase their molecular weight, which is why they can be used in MEUF (El Zeftawy & Mulligan, 2011). Thus, the combination of this process with bioremediation increased the heavy metal removal and the possibility of high value products from salty metal wastewater treatment.

Bioremediation using bacteria and enzymatic processes is an environmentally friendly process where the reduction and biosorption of Cd, Cr, Cu, Ni, As, Pb, Zn, Mn and Fe is a natural process in some microorganisms. Some of these have use the metabolic pathways to transfer electrons beyond the inner membrane (Barakat, 2011; Baron et al., 2009). Additionally, microorganisms can use these metabolic pathways, because they can act as catalysts and are stabilizers of proteins in cell walls (Bruins et al., 2000). However, some microorganisms can also use heavy metal ions as electron acceptors in their respiration pathway under anaerobic conditions.

However, the high energy usage and the operational cost are the major challenges for the current wastewater treatment technologies. It has been estimated that the treatment cost is high, which is around 3% of the world’s electricity being used, and sludge disposal costs are 50% of wastewater treatment (Saba et al., 2017; Ye, Ngo, Guo, Chang, et al., 2019). Energy consumption is not only an issue, but the ineffective treatment of the produced

Green House Gases and dissolved substances like ammonia and phosphates are another technical bottleneck that conventional wastewater treatment faces (W. W. Li et al., 2014). Moreover, the recovery of high value products has been lately investigated in MFCs due to the formation of Hydrogen (H_2), Methane (CH_4) in the anode chamber through microbial degradation of organic carbon. Moreover, hydrogen peroxide (H_2O_2) production in the cathode chamber has also been studied. Other high value recovery products like heavy metals, especially gold and mercury (Jadhav et al., 2017). Consequently, MFCs have become a promising solution to produce bioelectricity with many benefits such as cleanliness, effectiveness, recyclability, and less toxic products (Mansoorian et al., 2013).

MFCs are capable of oxidizing simple organic compounds to carbon dioxide (CO_2) and allows biochemical reduction to transport electrons (Rahimnejad et al., 2012). This electron transport is similar to electrochemical batteries where the substrates located on the anode side are the fuel needed for the conversion from chemical energy to electrical energy (Oliveira et al., 2013). MFCs generate electrons and protons through the degradation of substrates in the anode side, and these electrons and protons transport via a resistor and a proton exchange membrane, respectively, to the cathode where they react with oxygen (O_2) and in response of these electron and proton transport electricity is generated (Das & Mangwani, 2010; Y. Sharma & Li, 2010). The main advantages of MFCs technology include: high conversion efficiency from substrates to energy, sludge volume reduction, and high-value products recovery such as gold and nanoparticles (Do et al., 2018; Rabaey & Verstraete, 2005; Y. Zhang et al., 2019).

However, there are still some bottlenecks faced by MFCs like low power output and the high electrode and catalyst costs (Xia et al., 2018). In this sense, it is a necessity to improve efficiency and develop new configurations with microorganisms that achieve higher electron transport such as *Shewanella* sp. Moreover, the low cost-effective and energy-efficient production process in MFC, take into consideration the study of different parameters that are important in the MFCs design. For example, the oxidation reduction reactions between the organic substances and the electron acceptors like O_2 are a key factor in the bioelectricity production (Oliveira et al., 2013). In addition, proton transport, circuit

resistance, electron transport, electrode materials, and external operation conditions directly affect the electricity generation in MFC technology (M. Zhou et al., 2011).

New approach to remove heavy metals from salty metal wastewater using two-step approach MEUF and MFC inoculated with *S. oneidensis*. Combining these two processes would increase the metal removal efficiency and produce added value from salty metal wastewater treatment. Additionally, *S. oneidensis* and its characteristic as metal reducing bacteria will increase the efficiency and resistance to the toxic metals in the wastewater.

2.2 Desalter effluent

Contaminants like sand, asphaltene, inorganic salts, clay, and water-soluble trace metals are present in petroleum crude oil (Aryafard et al., 2015; Dadari et al., 2016). These impurities impact refinery processes, decreasing the crude oil value, causing corrosion, and increasing the heat consumption (Aryafard et al., 2015). That is the reason why desalter units are the first step into the crude oil refinery, cleaning the crude oil upstream.

Desalter operation consists of a water washing process where the contaminants are removed by contact and the mixing of heated crude oil and water separating them in vessels (Pereira et al., 2016). To improve the separation, processes like demulsification or electrical desalination can achieve this. In demulsification, to break the emulsification created by the contact between water and crude oil, demulsifiers or surfactants are added to break the emulsion (Pereira et al., 2016; State, 2002). Electrical desalination uses high voltage to precipitate suspended solids (Pak & Mohammadi, 2008). However, to improve the efficiency in the desalination process, most of the refineries use the two processes achievement a cleaner crude oil and time processing (Pereira et al., 2016).

Desalter effluent removes contaminants from petroleum crude oil, where the chemical composition has dissolved metals and organic compounds. Thus, the reason why its correct disposal has been investigated through different processes. Desalter effluent treatment is expensive and can have logistical and environmental issues related with its process (Pak & Mohammadi, 2008).

2.2.1 Metal wastewater treatment

Wastewater from desalter unit must be treated prior discharge through conventional wastewater to avoid high concentrations of pollutants like heavy metals that can affect the environment. Some approaches have been developed, like reverse osmosis with a composite polyamide membrane to treat salty metal wastewater. With this process the effluent from PETRONAS located in Malaysia had a removal of COD, Na^{+}, Ca^{2+}, Mg^{2+}, Cl^{-}, F^{-}, Br^{-} and SO_4^{2-} between 97.5 - 100% (Borhan et al., 2019). Also, improvements of PES membranes increasing membrane surface hydrophilicity have been investigated, for example, the use of ferroxane nanoparticles improves COD removal by 94%, however, the agglomeration of nanoparticles is a big issue in this technology (Dadari et al., 2016).

Another membrane process to treat salty metal wastewater is to use nano-porous membrane-powered activated carbon as a hybrid system that avoids membrane fouling and enhance organic removal efficiency to 90.2% and 97.6% for COD and TOC respectively (Sarfaraz et al., 2012). Ultrafiltration has been used as a process for removal of emulsified oil and total dissolved solids over 95% (Norouzbahari et al., 2009).

2.3 Conventional processes for heavy metal removal

Heavy metals are pollutants of environmental concern, they cannot be degraded and are added permanently to the environment by bioaccumulation or bioaugmentation (Kahlon et al., 2018). Conventional processes for removing heavy metals are commonly used in industry to mitigate the impact of the metals in the environment. The treatment involves physical or chemical processes and are made to decrease the amount of metal toxicity in water (Fu et al., 2012).

Chemical precipitation is a process which is widely used for its relative simplicity, low cost, and ease of pH control (Fu et al., 2012), where the pH control (pH=11) is the parameter that increases or decreases heavy metal removal enhancing the efficiency of the process (Barakat, 2011). The removal rates depend on the conversion from soluble ions to insoluble that are removed by sedimentation (L. K. Wang et al., 2005). The process requires large quantities of chemicals to reduce metals for disposal which makes the precipitation process slow (Kurniawan et al., 2006). However, to overcome this issue different

configurations have been adapted like advanced Fenton-chemical precipitation process that uses zero-valent iron, hydrogen peroxide and alkali for precipitation and chelating agent dissolution (Fu et al., 2012).

Currently, the mixture of conventional treatments is the best way to improve their efficiency. The physicochemical process of coagulation-flocculation is one of them and the main function is to destabilize colloidal particles by adding coagulants, and after the particle size is increased by flocculation (Kurniawan et al., 2006). This combination of coagulation and flocculation has high removal efficiency (Semerjian & Ayoub, 2003), and it is an alternative to remove pollutants from wastewater.

The general overview of the process involves pH adjustment and the addition of ferric or alum salts as a coagulant to overcome the repulsive forces between particles (Kurniawan et al., 2006). The addition of the appropriate coagulant and flocculant is the greatest challenge of the process (Tatsi et al., 2003). The flocculation process at pH 9 optimize the coagulation and heavy metal removal around 99% and coagulation of organic compounds is achieved with polydadmac as flocculant (López-Maldonado et al., 2015). However, the use of this treatment requires chemicals and the transfer of toxic compounds to the solid phase. Also, other separation systems like filtration or sedimentation are needed thus those are the main disadvantages of the system (Gunatilake, 2015).

Flotation is as a process that has been used for recovery of high-value ions like gold or silver (RUBIO et al., 2002). The process consists in the separation of heavy metals from a liquid phase using bubble attachment, where the attached particle is separated by the bubble rise (Fu & Wang, 2011). Flotation can be classified: a) Dispersed-air flotation; b) Dissolved-air flotation; c) Vacuum air flotation; and e) Electro-floatation (Kurniawan et al., 2006).

Dissolved-air flotation and electro-floatation are commonly used to remove heavy metal ions. In the first one, the bubbles are accumulated in the surface for the lower density of ions. The second one, use of surfactants makes the ions hydrophobic for the separation with bubbles (Fu & Wang, 2011). Due to its characteristics, floatation can be applied in the

industry because it has low cost, better removal of small particles and short retention times (Kurniawan et al., 2006).

Membrane filtration is a common process in wastewater treatments because it can remove suspended solids, organic compounds, and inorganic pollutants like heavy metals (Kurniawan et al., 2006). This system depends on the size of the particle and is easy to manage. Some advantages are its high efficiency and space saving (Fu & Wang, 2011). Furthermore, there are different types of filtration systems that are classified according to the membrane size, like ultrafiltration, nanofiltration and reverse osmosis (Barakat, 2011).

In summary, ultrafiltration uses the transmembrane pressure in a membrane pore size smaller than the dissolved metal ion-polymer complex, where the complex is retailed while small ions would pass through the pores of the membrane (Fu & Wang, 2011). The pore size could be between 5-20 nm and the molecular weight of the complexing polymers could be between 1000 – 100,000 Da (Barakat, 2011). Nanofiltration is a mechanism that involves steric (sieving) and electrical effects, a small pore and membrane surface charge which allows charged metal ions to be rejected (Kurniawan et al., 2006).

Ion exchange is another process that has high recovery from metal ions from a solution and is based on the polarity of the ions (Fu & Wang, 2011). Therefore, in this process the removal of ionic pollutant and the complete deionization are an important mechanism of selection that depends mainly on the heavy metal ion concentration in wastewater (Dąbrowski et al., 2004). It is a process with an interchange of ions between solid and liquid phases, while an insoluble compound (resin) removes ions from an electronic solution (Barakat, 2011). It has been demonstrated the efficiency for heavy metal removal at low concentrations from positive charges of Ni, Cu, and Zn to negative charges of chromate, sulfate, nitrate, cyanides and dissolved organic carbon (Gunatilake, 2015). However, ion exchange resins are not available for all heavy metals making this process expensive in capital and operational costs (Fu & Wang, 2011), so the viability of the system depends on external factors like low-cost materials.

Electrodialysis consists in the separation between ionic components and an aqueous solution through membranes with an electrical driving force (Akhter et al., 2018). Most of

the time the membranes are made for plastic materials with anionic or cationic characteristics, these types of membranes avoid the unnecessary transport of ions (Akhter et al., 2018; Barakat, 2011). The applicability of the process can be enhanced increasing voltage and temperature to treat wastewater with high concentration of heavy metals (Gunatilake, 2015). **Table 2.1** shows the advantages and disadvantages of conventional heavy metal removal processes.

Table 2.1 Comparison of conventional processes for heavy metal removal.

Heavy metal removal method	Advantage	Disadvantage	Ref.
Chemical precipitation	Availability of equipment and chemical for industry scale. Low maintenance	Extra coagulation process. Large quantities of chemicals required. Large sludge generated. Not effective at low heavy metal concentrations	(Akpor & Muchie, 2010; Crini & Lichtfouse, 2019; Kurniawan et al., 2006)
Coagulation – Flocculation	Simple process. Wide range of chemicals. Good sludge settling.	Selection of coagulant according to metals. Filtration needed. Low arsenic removal.	(Crini & Lichtfouse, 2019; Gunatilake, 2015; Tatsi et al., 2003)
Flotation	Efficient removal of small particles. Short retention time. Metal selective.	High operational cost. Chemical requirement.	(Crini & Lichtfouse, 2019; Kurniawan et al., 2006)
Membrane Filtration	Efficient removal of particles and suspended solids. No chemical requirement. Low solid generation. Rapid and efficient.	High capital cost. High maintenance. Limited flow rates.	(Crini & Lichtfouse, 2019; M. Zhao et al., 2016)

Heavy metal removal process	Advantage	Disadvantage	Ref.
Ion exchange	Easy control and maintenance. Easy to use with other processes. Can be used as a batch and continuous operation. Metal selective. Efficient removal of low heavy metal concentration.	High capital and operational cost. Saturation of cationic exchange. Elimination of the resin.	(Crini & Lichtfouse, 2019; Dąbrowski et al., 2004; Fu & Wang, 2011; Gunatilake, 2015)
Electrodialysis	Increase degradability. Avoid unnecessary transport of ions. Recovery of value metals	Not feasible for high heavy metal concentrations. Corrosion and membrane replacement. Chemical requirement. High capital cost. Require pos-treatment.	(Akhter et al., 2018; Crini & Lichtfouse, 2019; Gunatilake, 2015)

2.4 Surfactants

Surfactants are compounds with a hydrophobic chain and a hydrophilic head group (amphiphilic compounds). Surfactants are widely used in the industry as adhesives, flocculants, foaming agents and penetrans (Mulligan, 2005). They can be divided according to their head group in anionic, cationic, nonionic and amphoteric (Schwarze, 2017). Where the selection of the surfactant is based on the cost and the applicability that can have in the process.

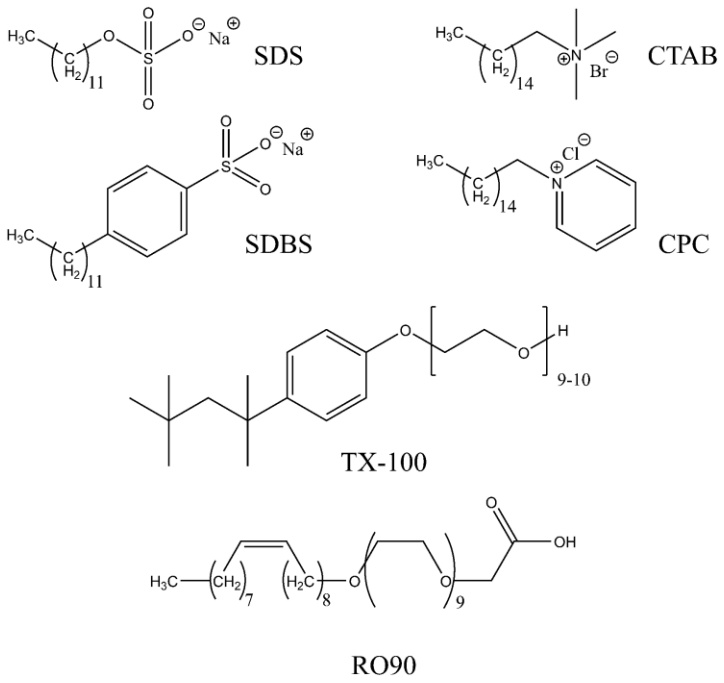

Figure 2.1 Main surfactants chemical structures (Schwarze, 2017)

2.4.1 Biosurfactants

Petroleum based surfactants are not the only ones available to bind metal ions, biosurfactants are also available for metal complexation above the critical micellar concentration (CMC). Biosurfactants such as rhamnolipid, surfactin and sophorolipid can be used as MEUF with the advantage of higher biodegradability in comparison with conventional surfactants (El Zeftawy & Mulligan, 2011; Mulligan et al., 1999).

Biosurfactants are produced by microorganisms like yeast or bacteria, however some of them have been produced from plants (Mulligan, 2005; Pacwa-Płociniczak et al., 2011). These surfactants are classified as glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids, polymeric and particulate compounds. The classification is according to their molecular weight and the functional groups of their hydrophobic part (Mulligan, 2005).

Reduction of surface and interfacial tension by biosurfactants in the interface between two fluids is given by the decreasing of intermolecular forces between solvent molecules (Jahan et al., 2020; Pacwa-Płociniczak et al., 2011). Biosurfactants have been widely used for bioremediation. Thus, hydrocarbon removal by mobilization, solubilization, and

emulsification depending on the molecular weight of the biosurfactant have been investigated (Pacwa-Płociniczak et al., 2011; Urum & Pekdemir, 2004). Metal removal by biosurfactants is another bioremediation application. Biosurfactants such as surfactin and rhamnolipid have been used for this application, due to their characteristic of complexation and adsorption of anionic biosurfactants (Mulligan et al., 2001).

2.4.1.1 Rhamnolipid

Rhamnolipids are biosurfactants classified as glycolipids and according to their head group are nonionic at low pH and anionic at higher pH (Mulligan, 2005; Pacwa-Płociniczak et al., 2011). The reason why this biosurfactant is very useful in terms of recovery, is because with acidification it can be recovered decreasing the pH to unbound metal ions (Schwarze, 2017). Generally, this biosurfactant is produced by microorganisms like *Pseudomonas aeruginosa* where R1 is a monorhamnolipid and R2 a dirhamnolipid (**Figure 2.2**).

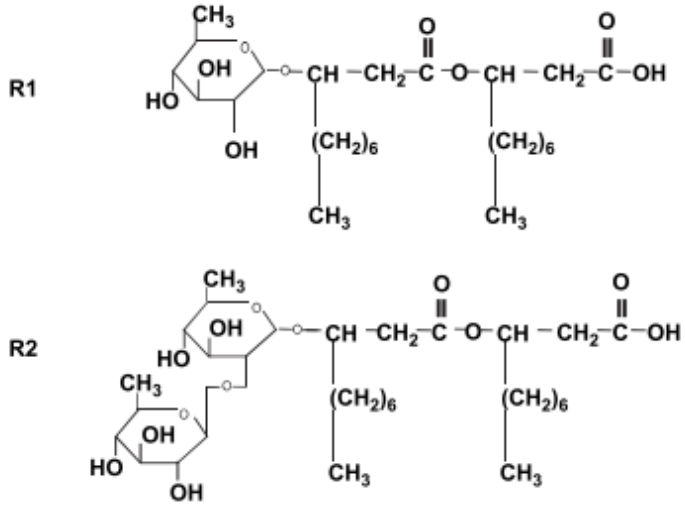

Figure 2.2 Structure of rhamnolipid produced by *Pseudomonas aeruginosa* (Abbasi-Garravand, 2012; Jahan et al., 2020; Mulligan, 2005).

Previous studies have shown the effectiveness for heavy metal removal using rhamnolipid and Micellar Enhanced Ultrafiltration for removal of Cd, Cu, Zn, Cr, Ni and Pb (Abbasi-Garravand & Mulligan, 2013; Mulligan et al., 2001; Shojaei & Khoshdast, 2018; Verma & Sarkar, 2018a, 2019). The cations from low to high affinity for rhamnolipid are $K^+ < Mg^{2+}$

< Mn^{2+} < Ni^{2+} < Co^{2+} < Ca^{2+} < Hg^{2+} < Fe^{3+} < Zn^{2+} < Cd^{2+} < Pb^{2+} < Cu^{2+} < Al^{3+} (Mulligan, 2005). This process decreases operating pressures, therefore reduce costs and energy requirements in heavy metal removal (A. H. Ali et al., 2017).

Rhamnolipids above Critical Micellar Concentration (CMC) form micelles, significantly enhancing the complexation between the biosurfactant and the metal. The CMC depends on the type of rhamnolipid, chemical composition and can range from 1 to 200 mg/L (Jahan et al., 2020; Verma & Sarkar, 2019). Different surfactants with their CMCs are shown in **Table 2.** Rhamnolipids have been used to remove heavy metals using different technologies like washing process to remove Zn^{2+} with a removal efficiency of 98.83% (Aşçi et al., 2008). The other process used by rhamnolipid bioremediation is using the biosurfactant as a precipitate collector from the floatation process to remove Cr with an efficiency of 96.75% (Shojaei & Khoshdast, 2018). However, recently MEUF has been investigated due to the effectiveness of the removal of heavy metals by complexation of polymers with metal ions and the separation through membrane filtration. Rhamnolipid has been reported as an efficient polymer for MEUF process to remove metals like Cd^{2+} (Verma & Sarkar, 2017a, 2018a, 2019, 2020).

Table 2.2 Critical micellar concentrations for different surfactants.

Surfactant	CMC	Ref.
JBR 425	30 mg/L	(Abbasi-Garravand & Mulligan, 2014)
CPC	0.9 mM	(Bahmani et al., 2019)
Rhamnolipid	22.5 mg/L	(W. Chen et al., 2017)
SDS	8.15 mM	(Ghazi & Qomi, 2015)
DPCl	2.45 mM	(Gokcek & Uzal, 2020)
BCl	1.47 mM	(Gokcek & Uzal, 2020)
ODA	296.9 mg/L	(Grzegorzek & Majewska-Nowak, 2018)
CPC	322.2 mg/L	(Grzegorzek & Majewska-Nowak, 2018)
RO90	0.02 mM	(Schwarze, 2017)
CTAB	0.9 mM	(Schwarze, 2017)
TX-100	0.25 mM	(Schwarze, 2017)
Rhamnolipid	50 mg/L	This study

2.5 Ultrafiltration

Ultrafiltration is a process where particles with higher molecular weight are rejected by a membrane (**Figure 2.3**). Ultrafiltration uses high cross flow minimizing the formation of filter cake decreasing accumulation in the membrane (Belter et al., 1988). Membranes generally have a pore size between 20 – 1000 Å using convection and diffusion as transport mechanism.

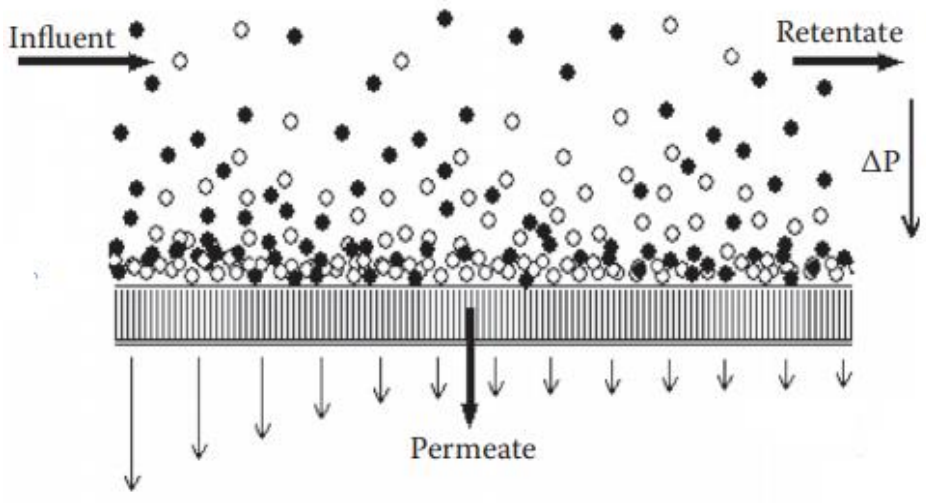

Figure 2.3 Membrane filtration in ultrafiltration **(De & Mondal, 2012)**

Nowadays the technique of complexation – membrane filtration allows the increase of the molecular weight of compounds like metal ions where the fixation on macromolecular species, for example, surfactants increasing their molecular weight (Trivunac & Stevanovic, 2006). The advantages of this process are the low energy requirements and the high removal efficiency due to the metal binding of the surfactant or polymer with high molecular weight (Juang et al., 2003).

Polymer enhanced ultrafiltration (PEUF) use polymers to bind metal ions allowing un-complexed components and water to go through the membrane (Baharuddin et al., 2014). However, the efficiency of the separation by PEUF depends on different parameters, such as: properties of the polymer or complexation agent, pH of the metal solution, complexation agent / metal ratio, and properties of the membrane (Borbély & Nagy, 2009).

The PEUF process can be done with different polymeric ligands like polyethyleneimine (PEI), poly(sodium acrylate) (PSA), poly(sodium 4-styrenesulfonate) (PSS) (Korus, 2018), polyacrylic acid (PAA), and polyacrylic acid sodium salt (PAASS) (Baharuddin et al., 2015).

2.5.1 Micellar-enhanced ultrafiltration

MEUF is a membrane separation where small compounds or ions are rejected by a membrane due to the bond with larger molecules like surfactants that can be biodegradables like rhamnolipids type I and type II (El Zeftawy & Mulligan, 2011; Schwarze, 2017). The process removes single or multiple heavy metal ions using surfactant concentration above CMC (Verma & Sarkar, 2019). Above CMC surfactants create structures called micelles that change the physicochemical properties of the solution as is shown in **Figure 2.4**.

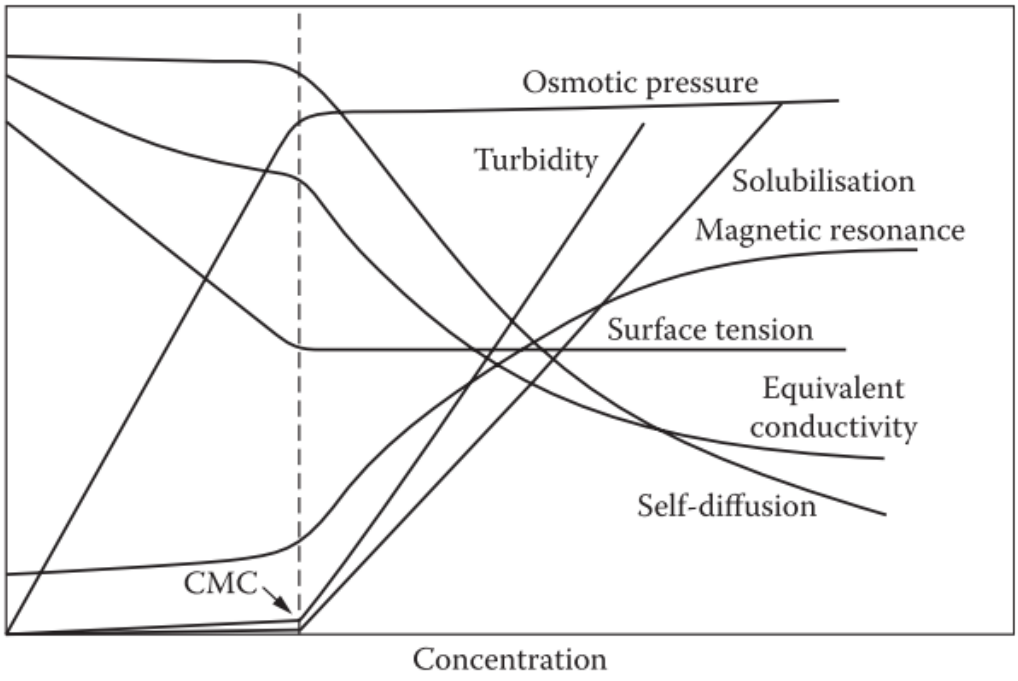

Figure 2.4 Changes in physicochemical properties above CMC **(De & Mondal, 2012)**

In MEUF the solution with micelles is added to the metal ion solution where the ions are solubilized in the micelles (Staszak et al., 2010). The general structure of MEUF is shown in **Figure 2.5**.

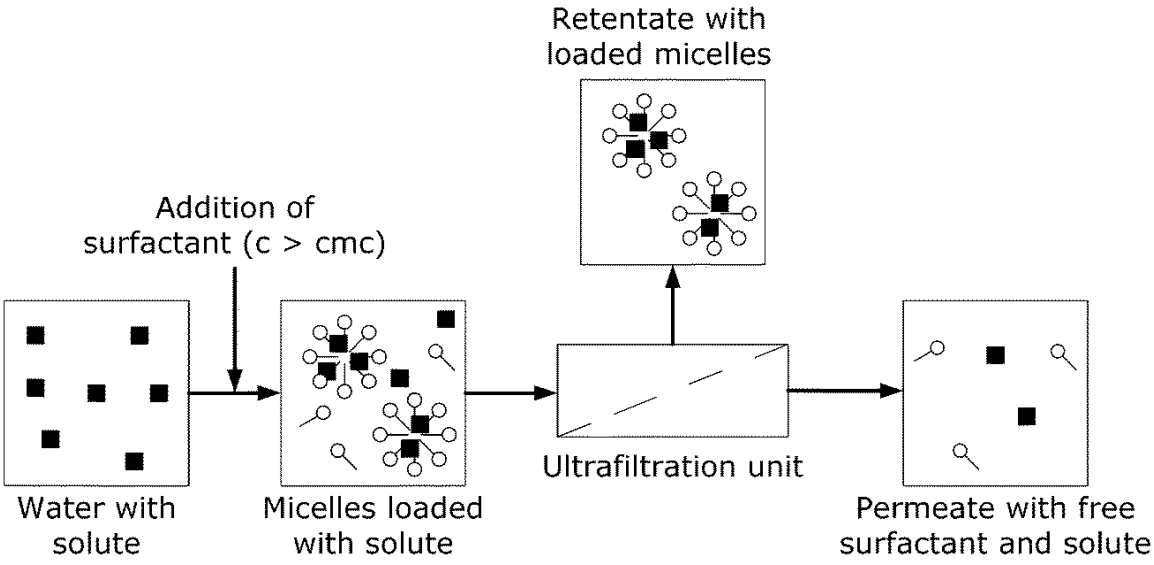

Figure 2.5 MEUF process (Schwarze, 2017).

Complexation between polymers and heavy metals has been investigated to treat wastewater increasing the selectivity and efficiency of ultrafiltration processes. **Table 2.3** shows the different heavy metal complexation with polymers in ultrafiltration.

Table 2.3 Previous studies on surfactant - heavy metal complexation for removal by ultrafiltration.

Polymer	Heavy metal / Pollutant	Removal	Ref.
Rhamnolipid	Cd (II) / Crystal Violet	98%	(Verma & Sarkar, 2020)
Polyacrylic acid sodium (PAAS)	Co (II)	97%	(J. Gao et al., 2019)
Cetylpyridinium chloride (CPC)	As (V) $NO3^-$	96.9% 90.5%	(Bahmani et al., 2019)
Polyethyleneimine (PEI)	Cr (III) / Cr (IV)	100%	(Aroua et al., 2007)
Polyacrylic acid (PAA)	Cd (II) Pb (II)	80% 20%	(Cañizares et al., 2008)
Carboxy methyl cellulose	Ni (II) Cu (II) Cr (III)	99.1% 97.6% 99.5%	(Barakat & Schmidt, 2010)

Polymer	Heavy metal / Pollutant	Removal	Ref.
Ethoxylated polyethyleneimine (PEPEI)	Cu (II)	97%	(Llanos et al., 2008)
Sodium dodecyl sulfate (SDS)	Zn (II)	99%	(Landaburu-Aguirre et al., 2009)
Chitosan	Cd (II)	98.5%	(Llorens et al., 2004)
Sodium dodecyl benzene sulfate (SDBS)	Cu (II)	90%	(Juang et al., 2010)
Rhamnolipid (JBR 425)	Cr (VI) Cr (III)	98.7% 99.4%	(Abbasi-Garravand & Mulligan, 2014)
Sodium dodecyl sulfate (SDS)	Zn (II)	86.67%	(S. H. Lee & Shrestha, 2014)
Nonaoxyethylene oleylether carboxylic acid (RO90)	Mg (II) Ni (II) Zn (II) Cd (II) Cu (II) Fe (II)	37.5% 61% 67.5% 72.5% 97.5% 99%	(Schwarze et al., 2015)
Polyethyleneimine (PEI) Polyvinylamine (PVAm) Polyacrylic acid (PAA)	Hg (II)	95% 99% 97%	(Y. Huang, Du, et al., 2016)
Polyethyleneimine (PEI)	Cr (VI)	80%	(S. Chakraborty et al., 2014)
Amino functionalized metal organic frameworks (MOFs)	Pb (II)	61.4%	(N. Yin et al., 2016)
Sodium dodecyl sulfate (SDS)	Cd (II)	90%	(J. Huang et al., 2019)
Polyacrylic acid sodium (PAAS)	Cd (II)	99.7%	(L. Chen & Qiu, 2019)
Polyacrylic acid sodium (PAAS)	Cu (II)	99.6%	(S. Y. Tang & Qiu, 2018)
Polyacrylic acid sodium (PAAS)	Ni (II) Cr (III)	99.5% 99.8%	(Q. Zhang et al., 2019)

Polymer	Heavy metal / Pollutant	Removal	Ref.
Polysodium 4-styrenesulfonate (PSS)	Pb (II)	91%	(M. Chen et al., 2018)
	Cu (II)	82%	
Sodium dodecyl sulfate (SDS)	Cu (II)	20%	(J. Huang, Yuan, et al., 2017)
	Cd (II)	20%	
	Zn (II)	25%	
	Pb (II)	45%	
Chitosan	Al	91%	(Lam et al., 2018)
	B	6%	
	Ca	22%	
	Co	45%	
	Fe	87%	
	K	11%	
	Mg	29%	
	Mn	39%	
	Na	12%	
	Ni	69%	
	S	59%	
	Si	34%	
	Sr	24%	
	Zn	64%	
Copolymer of maleic acid and acrylic acid	Cu (II), Zn (II), Ni (II), Mn (II)	98.8%	(Qiu & Mao, 2013)
Polyvinylamine (PVAm)	Pb (II), Cu (II), Fe (II)	99%	(Y. Huang, Wu, et al., 2016)
Sodium dodecyl sulfate (SDS)	Cd (II)	84.3%	(Landaburu-Aguirre et al., 2012)
	Cu (II)	75%	
Humic	Cr (III)	97.8%	(Alpatova et al., 2004)
	Pb (II)	92.5%	
	Cu (II)	76%	
	Ni (II)	73.6%	
	Co (II)	66.7%	
Unmodified starch	Zn (II)	96%	(Baharuddin et al., 2015)
	Pb (II)	66%	
Polyethyleneimine (PEI)	Cu (II), Ni (II)	98%	(Zamariotto et al., 2010)

However, the use of surfactants can increase the cost of the system, that is why an alternative economically viable is to recover surfactants to reuse them in the process of metal complexation. One method is changing the Kraft point if SDS is used as biosurfactant or change the pH to unbind the complex metal-surfactant. The un-complexed ions can pass through the membrane in the ultrafiltration process with efficiencies higher than 90% (Schwarze, 2017). Alkaline treatment is another alternative to precipitate Cd^{2+} ions from the complex metal-rhamnolipid. The process is followed by acidification of supernatant and centrifugation to precipitate rhamnolipid that finally is dried at 50°C. The recovery rhamnolipid is reused in MEUF to decrease the cost of the ultrafiltration process (Verma & Sarkar, 2019).

The mathematical equations to verify the MEUF experiments are summarized as following (Schwarze, 2017), J_P is the permeate flux in (L/m^{2}h) is defined in Equation (2.1):

$$J_P = \frac{V_p}{t \cdot A_M} \tag{2.1}$$

Where V_p is the permeate volume in (L), t is the filtration time in (h) and A_M is the specific membrane area in (m^2). To calculate the resistance of the membrane when a solution is filtered, it can be calculated from J_P, the difference of pressures (ΔP) and the viscosity of the solution (μ_P) as indicated in Equation (2.2):

$$J_P = \frac{\Delta P}{\mu_P \cdot R_\Sigma} \tag{2.2}$$

Where R_Σ is $R_S + R_M$ and R_M is given in Equation (2.3) applying only pure water through the membrane and R_S is calculated from Equation (2.4) assuming R_M as a constant:

$$J_W = \frac{\Delta P}{\mu_W \cdot R_M} \tag{2.3}$$

$$J_P = \frac{\Delta P}{\mu_P (R_S + R_M)} \tag{2.4}$$

The removal efficiency for the ultrafiltration separation is defined in Equations (2.5) and (2.6) for rejection and retention respectively:

$$R = \left(1 - \frac{C_P}{C_F}\right) \quad (2.5)$$

$$R = \left(1 - \frac{C_P}{C_R}\right) \quad (2.6)$$

Where C_P is the concentration in the permeate, C_F in the feed and C_R in the retentate (Schwarze, 2017).

2.6 *Shewanella oneidensis* MR-1

Shewanella oneidensis is a facultative aerobic gram-negative bacterium with a respiration pathway that involves the extracellular electron transport (EET) between the substrates and inorganic compounds such as metals (Heidelberg et al., 2002). *S. oneidensis* optimal growth conditions are at 30°C and, in addition can reduce nitrate to nitrite (Venkateswaran et al., 1999).

S. oneidensis is a bacterium that under aerobic conditions uses oxygen as an electron acceptor but under anaerobic conditions reduces alternative electron acceptors such as oxidized metals (Heidelberg et al., 2002). The respiration versatility of the microbe is reflected in the diversity electron transport system that uses cytochromes located in the cell wall to perform the respiration pathway (Myers & Myers, 2003). Additionally, *S. oneidensis* can produce sulfite that immobilizes toxic metals through the formation of metal sulfides (Heidelberg et al., 2002). Another advantage of this microorganism is the ability to tolerate toxic compounds that can be dangerous for most of the bacteria. For instance, the resistance to arsenic and its methylation into a less toxic compound has been demonstrated (J. Wang et al., 2016).

The EET of *S. oneidensis* is facilitated by cytochromes, reductases, iron-sulfur proteins and quinones (Heidelberg et al., 2002), where this pathway composed of cytochromes (*OmcA*, *MtrA* and *MtrC*) forms a conduit where electrons can move from menaquinone across the outer membrane (Brutinel & Gralnick, 2012). In addition, *MtrC* is a key factor in growth when the media with metal ions serves as an electron acceptor, meanwhile, *OmcA* is required for efficient electron transport (Mitchell et al., 2012). These quinones have an important role in the external electron transport because without them the reduction of

heavy metals is not possible, these reactions are involved in the cell membrane (Lall & Mitchell, 2007).

However, the efficiency not only depends on the quinones, but also the electron donor has a special function. The secretion of riboflavin as an electron shuttle is varied, that depends on the substrate used as an electron donor, fumarate and lactate do not affect the secretion of this compound, thus, the electron transport does not have negative reactions (C. Wu et al., 2013). On the other hand, enzymes such as hydrogenases or nucleases help the transport and assimilation of nutrients from the external cell to the internal membrane.

Hydrogenases are the common enzymes that are used for the bacteria to electron transport. *S. oneidensis* has (NiFe) hydrogenase and a (Fe) hydrogenase that are important to the metal reducing capacity (Heidelberg et al., 2002). These hydrogenases work on formation and oxidation of hydrogen from the substrate (fumarate, lactate or formate) using the substrates as electron donors. Then metal can be oxidized by (Fe) hydrogenase, therefore, the reduced metal products can be accumulated in the periplasm (C. K. Ng et al., 2013).

S. oneidensis has been studied widely for its reduction and accumulation applications in environmental remediation of toxic compounds. Also, nanoparticles production from reduction of heavy metals can be enhanced using the biosorption characteristics of the cell wall (De Corte et al., 2011). **Table 2.4** shows the different reduction of contaminants by *S. oneidensis*.

Table 2.4 Reduction or biodegradation of contaminants by *S. oneidensis.*

Contaminant	Characteristic	Ref.
Cr (VI)	Reduction in presence of goethite and humic acid. 65% of Cr (VI) reduction.	(Mohamed et al., 2020)
Nitroaromatic compounds (NACs)	Reduction mechanism according to molecular weight and polarizability.	(H. Wang et al., 2020)
Hg (II)	Increase rate of Hg reduction in presence of Elliott soil humic acid (ESHA). 88.8% of Hg (II) reduction.	(S. Lee et al., 2018)

Contaminant	Characteristic	Ref.
Sulfapyridine (SPY) – sulfamethoxazole (SMX)	Degradation of 23.91% for SPY and 59.88% for SMX.	(Mao et al., 2018)
Tellurite	Reduce to elemental Te in 25%.	(Valdivia-González et al., 2018)
Pd (II)	Biosorption of Pd (II) with capacity of 158.48 mg/g.	(H. Xu et al., 2018)
Sulfamethoxazole (SMX) and Fe (III)	SMX degradation of 38.5% at small concentrations.	(C. Zhou et al., 2018)
Cd-loaded polyferric flocs	The reduction of the flocs release Fe (II) and Cd (II) in goethite and magnetite forms to immobilize Cd.	(C. Li et al., 2016)
As (III) and As (V)	Reduction of As into less toxic organoarsenic compounds in presence of Fe (II).	(J. Wang et al., 2016)
U (VI)	Removal rate of U (VI) was 99% mediated by anthraquinone – 2 – sulfonate (AQS) under anaerobic conditions.	(J. X. Liu et al., 2015)
Cr (VI) and Te (IV)	Reduce Cr (III) complexed with Te (IV) creates nanoparticles that are accumulated on the cell surface.	(D. H. Kim et al., 2014)
Thiosulfate	Precipitation of Zn (II) as zinc sulfide nanocrystals.	(X. Xiao et al., 2015)
U (VI)	High concentration of NaHCO3 decrease U (VI) reduction	(Sheng & Fein, 2014)
Pd (II)	Reduction of Pd (II) to catalytic Pd nanoparticles on the cell wall.	(C. K. Ng et al., 2013)
Au (III)	Production of gold nanoparticles by biosorption on the cell wall.	(De Corte et al., 2011; J. W. Wu & Ng, 2017)

2.6.1 Impact of heavy metals in *Shewanella oneidensis*

Heavy metals are toxic compounds that can affect the morphology or characteristics of the bacteria; under large exposures of Cr (VI) genes with functions of metabolism, cell division and membrane response are regulated, while genes responsible of transport proteins and motility are repressed (Chourey et al., 2006). If Cr (VI) is added during the log phase under

anaerobic conditions, immediately cession of growth is overcome with the reduction of Cr (VI). On the other hand, under aerobic conditions the addition of Cr (VI) decreases the growth rate gradually (Viamajala et al., 2004). These impacts are common in microorganisms, however, conventional processes to remove heavy metals have similar issues related with the accumulation of metals in the membranes, or heavy metal disposal after processing. Thus, it is necessary maintenance and other treatments that make those options expensive.

2.6.2 Bacterial nanowires

Substrate utilization and communication between bacteria cells for better metabolic pathways is often related to mixing in the bioreactor. However, under anaerobic conditions *S. oneidensis* reduce metals in the extracellular phase due to the lack of O_2 for reduction. Under this condition, bacterial nanowires increase the surface area for substrate oxidation and increase the resistance to toxic environments.

2.6.2.1 Characteristics of bacterial nanowires

Bacterial nanowires are not only created by *S. oneidensis*, but they can also be observed in other facultative gram-negative bacteria such as *G. sulfurreducens, Bacillus,* as well as others. These nanowires are an extension of the periplasm and are made of cytochromes and proteins (Sure et al., 2016).

Nanowires of *S. oneidensis* are not solid, homogeneous filamentous, but instead are membrane vesicles and have diameters around 10 nm (Creasey et al., 2018). The main function of nanowires is to act as a conduit of electrons between cells and extracellular acceptors/donor at significant distances. Thus, nanowires act like a bridge between cells, for this characteristic can protect the cell wall avoiding periplasmic accumulation of toxic metals (Sure et al., 2016).

The transport of electrons in bacterial nanowires is an electron hopping between cytochromes (Malvankar & Lovley, 2014). Hence, the conductive characteristics of nanowires have been tested in various studies, for example, Leung 2011 used conducting-probe atomic force microscopy and constructing field-effect transistors to study the use of

nanowires as semiconducting materials for the interaction of electron transport chains (K. M. Leung et al., 2013).

The external electron transport between cytochromes in the periplasm and nanowires is the key factor through which heavy metal removal can be improved. This is because the interchange of electrons made the reduction of metal ions faster acting as an electrical network between the substrates (electron donor/acceptor) and the surface area of the bacteria. Here is where the capacity and efficiency of metal remediation has high value.

The applicability of bacterial nanowires in energy generation by microbial fuel cells (MFC) from wastewater and the possibility of using them as biosensors has high value byproducts for further perspectives (Malvankar & Lovley, 2014). Also, *S. oneidensis* nanowires have mechanical properties that can be used as building block for electronic devices and its physiological role avoiding accumulation of toxic compounds in the periplasm of the cells is very important for the survival of the microorganisms in waste toxic effluents (Sure et al., 2016).

2.6.3 *Shewanella oneidensis* nanowires formation

Nanowires from *S. oneidensis* transfer electrons where lactate is the electron donor and oxygen the electron acceptor. However, electron acceptor limitation increases the production of nanowires, which has been investigated in steady chemostat cultures (Pirbadian et al., 2014).

The production of nanowires emerges in the response of oxygen limitation, where the injection of oxygen is immediately consumed by cells that means 0% of dissolved oxygen (K. M. Leung, 2011). This suggests that nanowires formation takes place under limitation of oxygen but not necessarily under anaerobic conditions.

2.7 Microbial fuel cell design

MFCs are like batteries, where anode and cathode are connected through an external circuit and separated by a proton exchange membrane (PEM). However, in MFCs, microbes generate electrons in the anode chamber, and then these electrons are transported to the

cathode chamber where O_2 is reduced. The typical configuration also can be reformed into different designs like single chamber, up flow mode and stacked MFCs.

Hence, MFC design is a key factor in the synthesis and production of electricity to obtain high efficiencies and reduce costs for industrial applications. A typical configuration of MFC is shown in **Figure 2.6**, the system is composed of two chambers separated by a PEM (Das & Mangwani, 2010; Du et al., 2007; Rahimnejad et al., 2015; Slate et al., 2019; Xia et al., 2018).

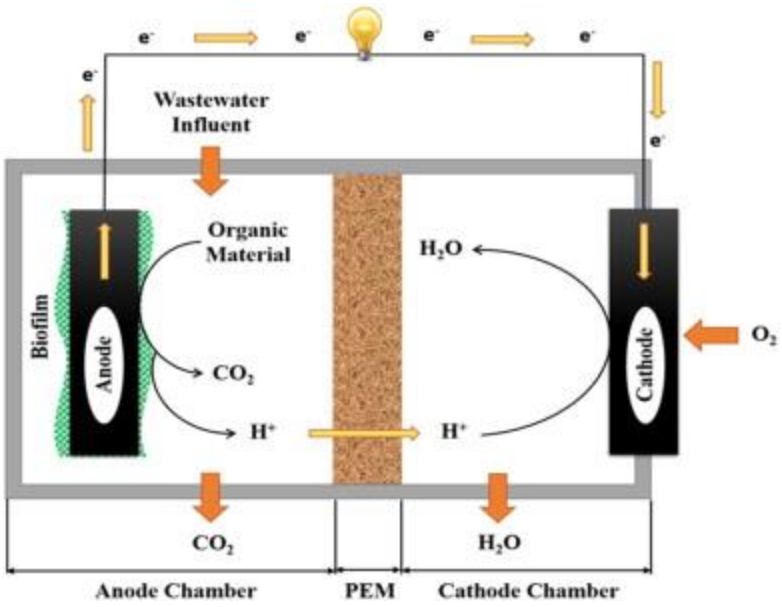

Figure 2.6 Typical configuration of MFC (ElMekawy et al., 2015).

Dual chamber MFCs are the most used design due to their applicability in the wastewater treatment and energy generation; however, this MFC design is limited to lab-scale (Das & Mangwani, 2010). Dual chamber is typically employed in batch mode. It consists of two electrodes separated by a membrane that transports electrons between the chambers where the proton exchange membrane can either be salt or a porous ceramic (Logan, 2005). The membrane allows the transportation of protons from the anode to the cathode, moreover, the movement of substrates and microorganisms between the electrodes is not allowed by the membrane (Du et al., 2007; Logan, 2005; Slate et al., 2019). The electrodes can be made of any conductive and non-corrosive materials. Thus, the anode should contain

substrates immersed in water with microorganisms and the cathode electrolyte solutions to facilitate the electron transport and the oxidation mechanism (Logan, 2005).

Some issues of this technology like high cost for aeration in the cathode chamber for reduction reactions, limit the large-scale application of dual chamber MFCs (Das & Mangwani, 2010; Oh et al., 2010). To overcome this, different models or configurations of dual-chamber MFCs have been developed, like cylindrical shape, rectangular shape, miniature shape which was investigated for biomedical applications, and flat plate MFCs (Du et al., 2007; Janicek et al., 2014; Slate et al., 2019).

Single-Chamber MFCs are simple configurations in comparison with dual-chamber because the cathode is exposed directly to the air, thus, there is no presence of cathode solution decreasing the overall costs (**Figure 2.7**) (Das & Mangwani, 2010; Slate et al., 2019). Electrons are transported to the high porous cathode where air is the electron acceptor, avoiding the use of aeration in the cathode chamber (Das & Mangwani, 2010; Du et al., 2007; Logan, 2005). Sevda et al., 2013 used a single MFC to treat three different compositions of wastewater showing that the electricity productions simultaneously obtained with wastewater treatment in terms of COD and Nitrogen (N) removal.

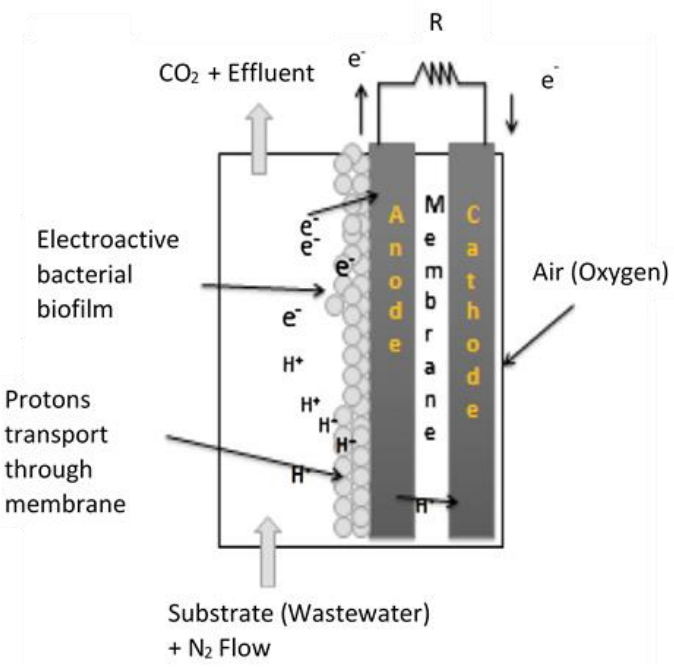

Figure 2.7 Single chamber MFC (Sevda et al., 2013)

However, single MFCs are not feasible for high power densities since it is important to have the anode and cathode closed, hence the working volume must be small (Fornero et al., 2010). Whereas, for large-scale applications, a large working volume is required to connect to achieve enough power density for the wastewater treatment.

The use of different electrode materials can enhance power production. Graphite fibers have been used as the anodes in a single chamber MFC to treat wastewater from olive mills, and the results showed that the power generation capacity was 124.6 mW/m^2 and COD removal was 65% (Pepé Sciarria et al., 2013). Carbon fibers have been investigated (activated carbon powder and polytetrafluoroethylene) to treat brewery wastewater, and a power generation of 24.1 W/m^3 was achieved (Wen, Wu, et al., 2010). Except for different electrode materials, new configuration designs have been developed to improve the performance of single MFC. For example, the use of microfiltration membranes as the PEM could increase the power production till 214 mW/m^2 (J. Sun et al., 2009).

Improvements to single chamber technology have been implemented to reduce the spacing between the anode and the cathode, and internal resistance. The separation of electrodes

can be enhanced by low-cost non-woven cloth separator. Non-woven separators have some advantages over membrane separator, like easy clean and reformation and the fouling is decreased with these separators (Abourached et al., 2016; Oliveira et al., 2013; X. Xiao et al., 2015). Some authors have reported studies using non-woven separators. Y. Park, Cho, et al., 2017; Y. Park et al., 2018; Y. Park, Park, et al., 2017 and C. Gao et al., 2017 used polypropylene non-woven material, and Wang et al. used a non-woven fabric material with a density of 400 g/m^3.

2.8 Wastewater treatment by microbial fuel cells

The major disadvantages of aerobic wastewater treatment are the high cost relating to the energy consumption and operational costs (Aelterman et al., 2006). On the contrary, MFC is a cost-effective approach to remove pollutants from wastewater by producing energy. However, COD for electron transport in the MFC varies from sources. For example, food processing, agricultural, animal, and municipal have easily degradable carbohydrates and high quantities of COD (Fornero et al., 2010).

2.8.1 Recovery and removal of pollutants

Recovery of other valuable compounds present in wastewater such as gold can increase the economic viability of MFCs. High strength wastewaters from food, animal, and agricultural industries have high concentrations in nutrients like phosphorus and nitrogen (Gude, 2016). These nutrients are essential in agricultural processes due to the use as fertilizers (Ye, Ngo, Guo, Chang, et al., 2019; Ye, Ngo, Guo, Liu, et al., 2019). Phosphorus removal and recovery by MFCs can be increased due to nitrification process, this P can be precipitated in the cathode surface in the form of struvite ($NH_4MgPO_4 \cdot 6H_2O$) (Gude, 2016; Jadhav et al., 2017). Nitrogen in the form of (NH_4^+-N) can be removed from wastewater by ammonification, nitrification and denitrification (Yakar et al., 2018). The removal of NH_4^+-N from wastewater is performed by the microorganisms such as microalgae species, then are transported to the cathode chamber (Ye, Ngo, Guo, Chang, et al., 2019). Ammonium transport from anode to cathode is carried out by diffusion and migration. Gradient concentration of ammonium manages the diffusion and electricity is the driven parameter in migration, thus, the amount of ammonium in the cathode chamber is

proportional to the MFC performance electricity generation (Ye, Ngo, Guo, Liu, et al., 2019). Compounds like nitrate decrease the power production when they are in the cathode, however, nitrate has been reported to be an electron acceptor in the anode reduction and 7.2 and 502.5 mW/m^2 of power can be generated (Chaturvedi & Verma, 2016). Moreover, supplementary aeration increases the ammonium removal to nitrate that is used as an electron acceptor in the respiration of anaerobic denitrifies (Teoh et al., 2020). Other recovery of products using MFCs increases the feasibility of this technology for by-products production. H_2O_2 is one of them and is used for chemical applications as a raw material, the recovery of this product happens for the cathodic reduction of substrates. Also, CH_4 recovered using methanogenic microbes can be used for further energy applications (Jadhav et al., 2017). Hiegemann et al. (2016) reported CH_4 production of 13.3 L/day, and Liu et al. (2017) showed a composition of 45% in CH_4 in the gas phase.

Recovery of Cr and Ag from wastewater using it as catholyte in MFCs is a process that can be achieved and be cost-effective for MFCs application (J. Ali et al., 2019; C. Kim et al., 2017). Cr is a heavy metal extensively present in industrial wastewater and poses a threat to human health and environment due to its toxicity. Therefore, Cr^{+6} treatment by reduction with MFC can be performed according to the microorganism characteristics in air-cathode MFCs, however, the power generation is lower in comparison with dual-chamber MFCs (Chaturvedi & Verma, 2016). Polyhydroxyalkanoates (PHAs) is a polymer with rhelogical properties such as polypropylene. Synthesis of PHAs by micro-aerophilic biofilm in the cathode, however, the carbon source is CO_2, and it has not been developed with wastewater (Jadhav et al., 2017). **Table 2.5** shows some products recovery from wastewater using MFCs.

Table 2.5 Recovery of products from wastewater using MFCs.

Recovery product	Quantity	Ref.
Phenol	85%	(Srikanth et al., 2016)
NH_4^+-N	68%	(Ge & He, 2016)
NH_4^+-N	57%	(Ye, Ngo, Guo, Chang, et al., 2019)
PO_4^{3-}	89%	(Ye, Ngo, Guo, Liu, et al., 2019)
Cr_2O_3	57.9%	(C. Kim et al., 2017)
Ag	67.8%	(J. Ali et al., 2019)

Modification in MFCs to produce hydrogen from the metabolic pathways as a complementary product. To produce H_2 in MFCs, the O_2 supply in the cathode is not necessary and the anodic potential must increase to 0.23 V or higher (Do et al., 2018). Biohydrogen is a clean fuel that can be storage and be used for further industrial applications (S. Li et al., 2019). The use of urban wastewater for biohydrogen production with a loading rate of 2 gCOD/L-d has a total of 57.5 kg CO_2-eq/kgH_2 of global warming potential (J. Chen et al., 2019).

As it was detailed heavy metals like Cr and Ag, and another by-product like CH_4 and H_2 can be recovered by MFC, however, specific conditions like microorganism growth, anode and cathode media, and concentration must be considered to ensure a high-power density production. MFCs have different characteristics that affect the wastewater treatment and energy production according to their operation and the viability of the anode and cathode chambers.

2.9 Microbial fuel cell operation and characteristics

MFC can operate in batch and continuous mode, which varies according to the type of substrate and microorganisms in the anode chamber.

2.9.1 Batch operation

Batch operation allows the accumulation of biomass and produces soluble redox mediators (Du et al., 2007; Rabaey & Verstraete, 2005). One of these applications is the use of iron reducing bacteria to improve MFC performance where each batch volume was 50 mL (J. R. Kim et al., 2005). However, the use of other substrates like olive mill wastewater in combination with domestic wastewater is required with an aim for achieving a higher COD removal efficiency (60%) and for swine wastewater (22%) (Pepé Sciarria et al., 2013). One advantage of batch mode is that the removal of nitrates and COD is higher compared to continuous operation because of the longer cycle and retention times (Ahn & Logan, 2010). However, this is only possible when the anodic chamber is working under batch mode, implying that the microorganisms are removed constantly in order to have a uniform growth and can manage a higher quantity of wastewater (Daniel et al., 2009).

2.9.2 Continuous operation

Continuous operation enhances the biofilm formation that is used for electrode transporting with the use of mobile shuttling molecules increasing the power production (Rabaey & Verstraete, 2005). However, the increase in the flow rate can lower COD removal and coulombic efficiency because the time for substrate degradation by microorganisms is short (Oliveira et al., 2013; Villaseñor et al., 2013). For example, Jayashree et al., 2015 reported a decreasing of COD removal from 79% to 32% when loading rate changed from 0.45 gCOD/L to 2.69 gCOD/L and coulombic efficiency decreased from 8% to 0.8% with the same change in the loading rate. Nevertheless, the highest power density of 254 mW/m^2 was performed at 2.69 gCOD/L. (Tamilarasan et al., 2017) also reported the same relation between the loading rate and the COD removal, the change of COD removal from 1.9 gCOD/Ld to 2.8 gCOD/Ld was 69% to 42% respectively.

Up flow MFCs technology also adopts continuous feed supply (Du et al., 2007), to enhance the performance of wastewater treatment (Ahn & Logan, 2010). Wastewater treatment performance and energy production can be improved with the combination of other processes like anaerobic hydrogen bioreactor (Y. Sharma & Li, 2010), and installment of multiple units (Rahimnejad et al., 2012).

2.10 The role of microorganisms

The selection of microorganisms depends on their capacity to transport electrons from substrates to the anode. The mixed cultures of microorganisms from different sources like marine sediment or soil have been widely used for biological stability. However, some microorganisms like *Shewanella putrefaciens* could not degrade complex organic compounds in the wastewater due to the strict requirement of electron donors in the substrate (H. J. Kim et al., 2002).

The metabolism of microorganisms is the key factor to understand how transfer of electrons occurred (aerobic or anaerobic). Aerobic bacteria prevent transferring of electrons from organic compounds to O_2 (electron acceptor), while anerobic bacteria, use alternative electron acceptors such as nitrate or solid electrodes because O_2 is not present (Fornero et al., 2010). The reduction of electrons happens in the extracellular wall where the

cytochromes reduce extracellular electrons from the substrate, where energy (ATP) can be produced and thus meet the need for the growth and reproduction of bacteria (**Figure 2.8**) (Du et al., 2007).

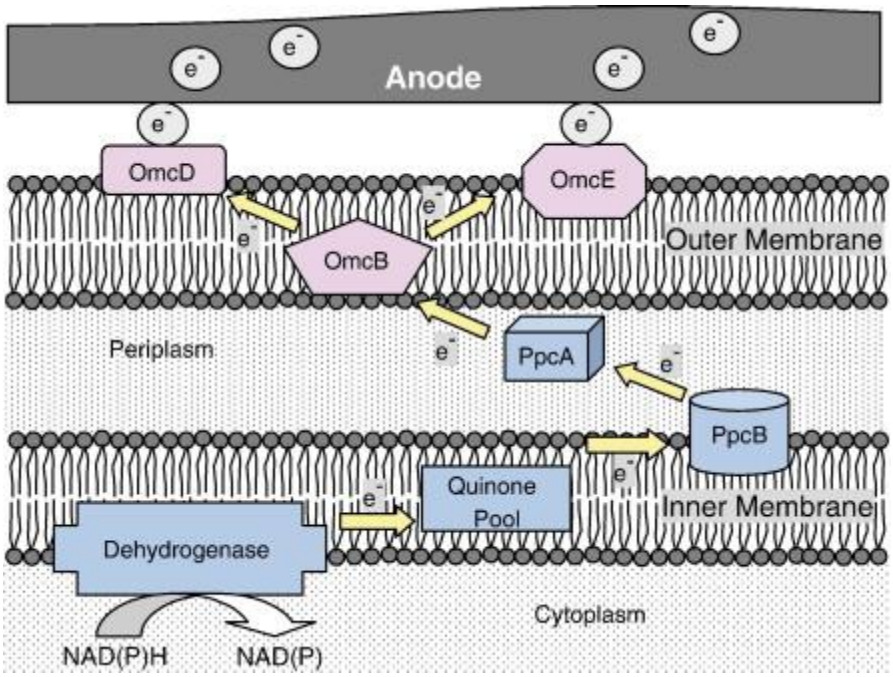

Figure 2.8 Respiration pathway for bacteria (Du et al., 2007).

The metabolism involved in the respiration pathway of microorganisms uses nicotinamide adenine dinucleotide (NADH) as the energy source for bacteria. NADH has a theorical voltage potential expressed in Equations (2.7), (2.8), and (2.9) where the biological standard potential ($E^{or}[V]$) has a relation with NADH and O_2 as electron acceptor (Fornero et al., 2010).

$$NAD^+ + H^+ + 2e^- \rightarrow NADH\ E^{or} = -0.320\ V \quad (2.7)$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-\ E^{or} = +0.820\ V \quad (2.8)$$

$$+0.820\ V - (-0.320\ V) = 1.14\ V \quad (2.9)$$

In the absence of electron acceptor, microbes transfer electrons directly to the anode surface; however, some surfaces are not allowed to transport electrons. Thus, the use of mediators such as Mn^{4+}, Fe^{3+}, or neutral red are necessary, and they are incorporated in the

anode chamber to facilitate the transport of electrons (Das & Mangwani, 2010; Fornero et al., 2010; B. H. Kim et al., 2007). Mediators use redox compounds from bacteria like quinones or flavin, which can be reduced to generate electrons toward anode surface (**Figure 2.9**) (Das & Mangwani, 2010; Du et al., 2007).

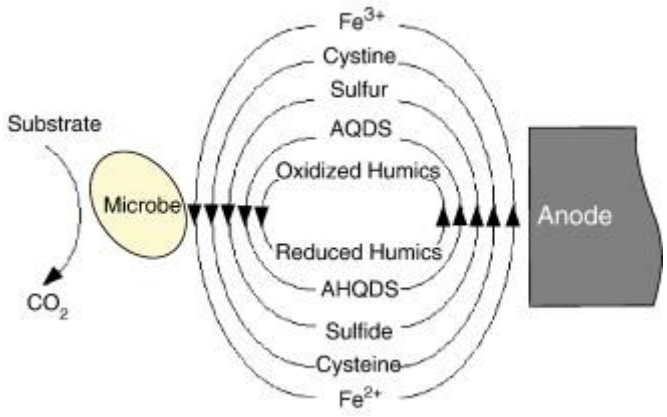

Figure 2.9 Electron transport to anode surface using mediators (Du et al., 2007).

2.11 Electrode materials

Electrode materials are very important for the performance of MFC, anode and cathode must be chosen according to the electrical conductivity, biocompatibility, chemical stability, corrosion, surface area and mechanical properties (Slate et al., 2019; M. Zhou et al., 2011). Electrode materials have been studied according to the characteristics of microorganisms and/or the electron transport in cathode.

2.11.1 Anode

The main characteristic for anode materials is the compatibility with microorganisms. Therefore, platinum, gold, stainless steel, and copper are good conductive materials, but cannot be used in the anode surface due to their high cost and inhibition properties which avoid cell division due to cisplatin that acts as antimicrobial (D. Leung & Xuan, 2015; Slate et al., 2019). Carbon based materials arise as an alternative with high electrical conductivity, biocompatibility, chemical stability, and low cost. Among them, graphite rod, graphite fiber brush, carbon cloth, carbon paper, carbon felt and reticulated vitreous carbon

(RVC) are the most studied (D. Leung & Xuan, 2015; Ping & Kang, 2014; Ramachandran et al., 2015; M. Zhou et al., 2011).

Carbon nanotubes are an alternative for anode materials as they have good conductivity, high surface area and compatibility with microorganisms. However, the high cost and the technical issues for producing carbon nanotubes make them less attractive for wastewater treatment and the energy generation (Mustakeem, 2015; Ramachandran et al., 2015; M. Zhou et al., 2011). The typical anode materials and their major characteristics are summarized in **Table 2.6**.

Table 2.6 Typical anode materials with advantages and disadvantages (Mustakeem, 2015).

Anode material	Advantage	Disadvantage
Stainless steel	High conductivity, not expensive	Low surface area, inhibition problems, corrosion
Graphite rod	High conductivity, chemical stable, not expensive	Difficult to increase surface area
Graphite brush	High surface area, easy construction	Clogging
Carbon cloth	Large porosity	Expensive
Carbon paper	Easy wire connection	Fragile
Carbon felt	Large surface area	High resistance
RVC	High electrical conductivity	Fragile, large resistance
Carbon nanotubes[a]	Excellent electrochemical activity, high conductivity	Expensive

[a]Ref. (Ramachandran et al., 2015; Slate et al., 2019)

To enhance conductivity or the performance of MFC, physical and chemical changes of the anode are required. Porous electrode materials are desirable since microorganisms can be attached to the anode surface and transport electrons by direct contact. Different treatments like the use of ammonia gas to enhance adhesion of the microorganisms can increase power density about 48% (S. Cheng & Logan, 2007; D. Leung & Xuan, 2015; Mustakeem, 2015; Slate et al., 2019). Other surface electrodes treatment uses inorganic acids to increase the positive charges (Feng et al., 2010; Mustakeem, 2015). Physical treatments like heat have been developed to improve power density and surface area due to the cleaning of impurities (M. Zhou et al., 2011).

2.11.2 Cathode

Cathode electrode is where O_2 is reduced in three-phase interfaces (gas, liquid and solid) to form water or hydrogen peroxide. O_2 corresponds to gas phase, electrolyte to liquid and electrode to solid phase (Mustakeem, 2015). The materials applied for the cathode are usually the same as those of anode. However, the catalytic properties of carbon materials must be enhanced when using catalysts like platinum (Lefebvre et al., 2011; Mustakeem, 2015; M. Zhou et al., 2011). For wastewater treatment, carbon-based materials coated with platinum are commonly used as electrodes in the cathode chamber.

Except for platinum, metal oxides like MnO_2 have also been used to treat wastewater, and the COD removal efficiencies and power density production are found to be 77.1% and 175.7 W/m^2, respectively. However, the high-cost catalysts including titanium, platinum or oxides make it a necessity to develop cheap and effective catalysts. Microorganisms can enhance the reduction process in the cathode. Biofilm can be formed over cathode by biocatalyst and then catalyze the reduction reaction. Previous studies observed that the use of yeast as the biocatalyst showed an improvement of 55% in power density, along with a decrease in the raw material costs (Mustakeem, 2015; G. Zhang et al., 2012; Zhuang et al., 2012). Pt is a material that has high conductivity and enhances electron transport; however, the high cost makes it economically infeasible and thus carbon-based materials are more common for electrodes in cathode chamber for MFC. **Table 2.7** shows different configurations of anode and cathode electrodes used in wastewater treatment by MFCs.

Table 2.7 Anode and cathode materials with catalysts used in wastewater treatment.

Anode	Cathode material	Type of wastewater	Power Output	Ref.
Graphite fiber brush	Graphite felt	Synthetic	3006 mW/m^3	(J. Ali et al., 2019)
Graphite rod	Graphite rod	Domestic	27 mW/m^3	(Cecconet et al., 2018)
Activated carbon	Activated carbon + Stainless steel	Azo dye	0.852 W/m^3	(Z. Fang et al., 2015)
Graphite	Graphite	Spent caustic	36.7 mV	(Fazli et al., 2018)

Anode	Cathode material	Type of wastewater	Power Output	Ref.
Titanium rod	Carbon cloth	Vegetable oil	2166 W/m^2	(Firdous et al., 2018)
Carbon fiber veil	Activated carbon	Self-powered	1.71 mW	(Gajda et al., 2017b)
Carbon brush	Carbon felt	Municipal	200 mW	(Ge & He, 2016)
Graphite fiber brush	Carbon cloth + 0.5 mg/cm^2 Pt	Municipal	0.36 kW	(Hiegemann et al., 2016)
Carbon brush + twined titanium	Carbon cloth + Pt crystal	Seafood	340 mW/m^2	(Jamal et al., 2020)
Plain graphite sheet	Plain graphite sheet	Retting	254 mW/m^2	(Jayashree et al., 2015)
Activated carbon fiber felt	Activated carbon fiber felt	Seafood	105 mW/m^2	(Jayashree et al., 2016)
Graphite fiber brush	PDVF + Activated carbon	Domestic	1.36 mW	(K. Y. Kim et al., 2015)
Pt foil	Gas diffusion electrode	Synthetic	0.7 mA/cm^2	(Krieg et al., 2017)
Granular graphite	Carbon fiber + PDVF[a] + C-Mn-Fe-O	Synthetic	1358 mW/m^3	(Y. Li, Liu, et al., 2015)
Granular carbon + stainless steel mesh	Carbon cloth + Vulcan carbon	Domestic	135 mW	(Linares et al., 2019)
Stainless steel	Activated carbon	Yogurt	1043 mW/m^2	(Luo et al., 2017)
Carbon brush	Carbon cloth + 0.35 mg/m^2 Pt	Swine	650 mW	(J. Ma et al., 2016)
Graphite felt	Carbon cloth + 0.35 mg/m^2 Pt + 5% Nafion	Domestic	1.4 mA	(Y. Park, Cho, et al., 2017)
Carbon felt	Carbon E4 + Mn	Synthetic	10 mW	(Recio-Garrido et al., 2017)
Carbon cloth pre-treated	Carbon cloth + Gas diffusion layer	Synthetic	88 mW/m^2	(Tanikkul & Pisutpaisal, 2018)

Anode	Cathode material	Type of wastewater	Power Output	Ref.
Carbon cloth	Cu-B Alloy	Municipal	6.1 mW	(Włodarczyk & Włodarczyk, 2019)
RVC[b]	RVC	Synthetic	175 mW/m^2	(He et al., 2005)
Graphite felt	PTFE[b]	Synthetic	150 mW/m^2	(Miyahara et al., 2013)
Carbon cloth	Platinum bar	Sewage	382.5 mW/m^2	(Sevda et al., 2013)
Graphite felt	Carbon cloth + 8 mg/cm^2 MnO_2	Swine	225 mW/m^2	(Zhuang et al., 2012)

[a]Polyvinylidenefluoride, [b]Reticulated vitreous carbon, Polytetrafluoroethylene

2.12 Electricity generation

MFCs generate electricity with the use of substrates as fuel for microorganisms located in the anode chamber, therefore microbes oxidize organic substrates using the EET and transport them though the electrode. Then, the electrons go to the cathode and reduce the electron acceptors (O_2). Protons from the anode chamber pass through the PEM to the cathode chamber facilitated the reduction process. In Equations (2.10) and (2.11), acetate is applied as the substrate for the electrode reactions, and sucrose is employed as the substrate for Equations (2.12) and (2.13) (Das & Mangwani, 2010).

$$Anode: CH_3COO^- + H_2O \xrightarrow{Microorganism} 2CO_2 + 2H^+ + 8e^- \quad (2.10)$$

$$Cathode: O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad (2.11)$$

$$Anode: C_{12}H_{22}O_{11} + 13H_2O \xrightarrow{Microorganism} 12CO_2 + 48H^+ + 48e^- \quad (2.12)$$

$$Cathode: O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad (2.13)$$

The output voltage in MFCs is calculated by the external resistance multiple by the current, which has a direct effect on the cathode and anode potential (Equation 2.14). This equation explains the relationship between activation overpotential and current density (Xia et al., 2018).

$$V_{cell} = V_c - V_a - \eta_{ohm} \quad (2.14)$$

Where, V_c is the cathode potential, V_a is the anode potential, and η_{ohm} is the ohm overpotential. However, most of the voltage measurements have used quantification instruments like voltmeters. Equation 2.15 can be used to calculate the power density.

$$P = \frac{IV}{A} \quad (2.15)$$

Where I (A) is the current, V (V) is the voltage that could be measured using a multimeter and A (m^2) is the surface area of the anode (Min et al., 2005).

2.12.1 Anode characteristics

Interaction between microorganisms, substrate, mediators (if needed) and electrode in the anode facilitates the oxidation and electron transport from the anode chamber. Glucose and carboxylic acids show higher coulombic efficiencies in comparison with real wastewater because the polymers are less complex. Hence, the conversion into acetate and hydrogen for electron transport is faster than complex organic compounds (Fornero et al., 2010).To have stable electron generation, it is critical to maintain a stable anode potential in accordance with the work of (J. Liu et al., 2014). It is also considered effective for biofilm formation and electrons generation to ensure constant COD removal and voltage output (Venkata Mohan, Mohanakrishna, et al., 2008). The electron transport between microbes and electrode can be achieved by either direct contact or the use of mediators and nanowires. The basic working principle of the direct contact and the use of nanowires is that biofilms can ensure the microorganism to be attached to the electrode surface. Nevertheless, biofilms could negatively affect the electron transport, thus, some improvements like nanowires decrease microbes' adhesion to the biofilm and thus facilitates the local potential in the anode, thereby increasing electron transport (Franks & Nevin, 2010; W. W. Li et al., 2014; Oh et al., 2010; Rabaey & Verstraete, 2005; Rahimnejad et al., 2015; Slate et al., 2019).

Rahimnejad et al., 2012 determined that microorganisms attached to the anode surface growth uniform in the anode surface performing uniform electrical behavior in the MFC. Parameters like production of methane in the anode can decrease the coulombic efficiency

which can be overcome with thin biofilms; thus, they reduce methanogens and consequently gas production (Fornero et al., 2010). Another important factor to be considered is that the contact time between biofilm and substrate must not be shortened, because otherwise there would be a reduction (Y. Sharma & Li, 2010). The metabolism and electron transfer behavior in molecular level, microbial community and biofilm levels, and three mechanisms of electron transport are illustrated in **Figure 2.10**.

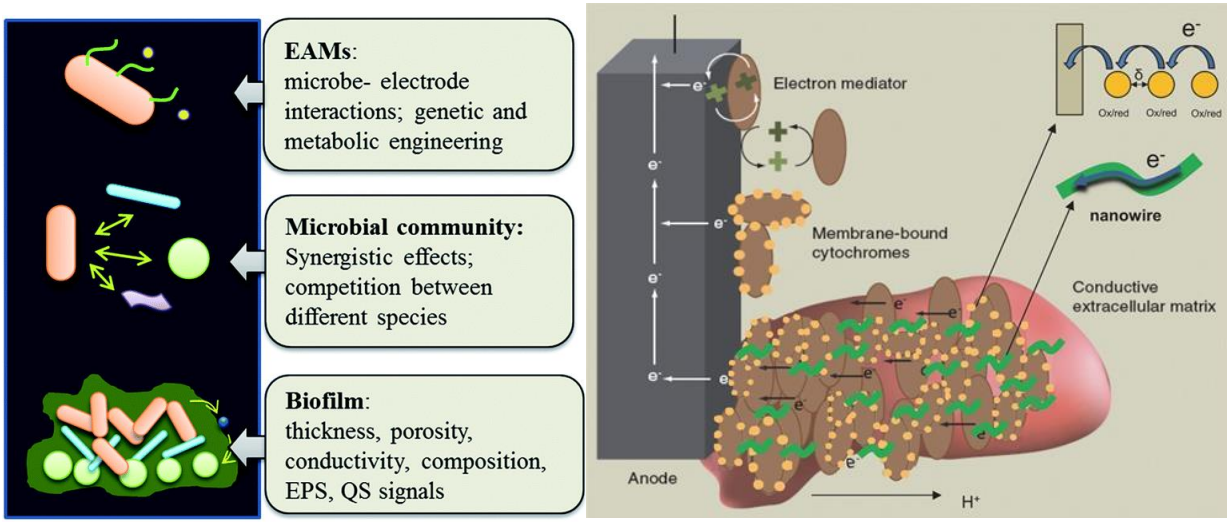

Figure 2.10 Electron transport. a) Molecular, microbial and biofilm levels; b) Electron mediator, direct contact through extracellular membrane, nanowires (Kumar et al., 2012; W. W. Li et al., 2014).

Bacterial nanowires can be observed in the facultative gram-negative bacteria like *Geobacter*, *Bacillus*, and *Shewanella*. These nanowires are an extension of the periplasm and are made of cytochromes and proteins. The main function of nanowires acts as a conduit for electrons between cell and extracellular acceptors/donor and hence performs like a bridge between cells (Sure et al., 2016). The transport of electrons in bacterial nanowires is an electron hopping between cytochromes (Malvankar & Lovley, 2014). Hence, the conductive characteristics of nanowires have been tested in various studies. In a study conducted by Leung (2011), conducting-probe atomic force microscopy and constructing field-effect transistors were used to study the use of nanowires as the semiconducting materials for the interaction of electron transport chains. The results

showed a linear curve between current and voltage confirming the conductivity and the resistivity of the nanowires with a result of 147 Ω·cm (K. M. Leung, 2011). Electron transport can be reduced by the biofilm because of the reduction of mass transfer rate. One possible solution is to modify the microorganisms that without the use of biofilms transfer electrons (Du et al., 2007). Power production not only depends on the anode interaction but also on the cathode interaction and the characteristics of it, thus the cathode chamber is studied.

2.12.2 Cathode characteristics

The cathode chamber is composed of: (i) the catholyte solution or O_2 and (ii) the electrode. The electrochemical relation with O_2 is a Monod-type kinetic due to its high oxidation potential as the electron acceptor, however, some catholyte solutions such as ferricyanide have higher redox potential which increases the reduction reactions (Du et al., 2007; Franks & Nevin, 2010; Rahimnejad et al., 2015). This relation happens at very slow rates, which can be explained as follows: catalysts like Pt and MnO_2 are often used to enhance the reduction of O_2 to H_2O or H_2O_2 (Franks & Nevin, 2010). Conventional carbon electrodes like graphite paper, carbon paper and carbon felt have been utilized to remove heavy metals in the cathode chamber, showing removal efficiencies for Cr ranging from 26.2% to 86.3%, and power production from 291.6 mW/m^2 to 348 mW/m^2 in the first 2 hours (Z. Li et al., 2008).

Cathode chambers with microorganisms can produce other valuable products and consequently decrease the costs related with production and operation in comparison while using Pt as the catalyst. The mechanism of electron transport is similar to anode interface where it can be through the cytochromes of the extracellular membrane or the use of mediators present in the catholyte solution (J. Huang et al., 2011; Rahimnejad et al., 2015; Watanabe, 2008). Also, biocathodes result in lower internal resistances than other conventional cathodes, thereby increasing the power density in MFC (G. Zhang et al., 2012).

Catholytes in solution can be salts like NaCl, the main function of this type of catholyte solution is to compensate the proton transport and control the pH in the cathode chamber

(Gil et al., 2003). The use of this solution in the cathode is not common because single chamber MFCs have been widely employed.

2.12.3 Proton transport

The oxidation of organic compounds produces electrons and protons (H^+), where protons have a direct effect on the internal resistance and power density of MCFs. Proton transports from the outside of the biofilm in the anode to the cathode at a lower rate than that of electron transport. Thus, to facilitate proton transport, the surface area of the membrane must be larger than that of the electrode area. Nafion and Ultrex membranes are commonly used in all MFCs systems due to their superior proton selectivity, i.e., separation of substrates to the cathode and O_2 to the anode. Nevertheless, other cations like Na^+, K^+, NH_4^+ Ca^{2+}, and Mg^{2+} can be transported by PEM membranes since cation is a significant criterion for selecting membrane rather than proton (Du et al., 2007; Franks & Nevin, 2010; Rabaey & Verstraete, 2005; Rahimnejad et al., 2015).

PEM can be divided according to the materials or the pore size; these membranes are cation exchange membranes (CEM), anion exchange membranes (AEM), bipolar membranes (BPM), microfiltration membranes (MFM), and ultrafiltration membranes (UFM) (D. Leung & Xuan, 2015). The selection of PEM is mainly based on the cost and effectiveness to transport H^+ through the membrane instead of other cations. Operational conditions have a direct effect on the transport of electrons and protons from anode to cathode (Gil et al., 2003). **Table 2.8** shows the proton exchange membranes used in wastewater treatment by MFCs.

Table 2.8 Membrane separator in MFCs for wastewater treatment.

Membrane	Wastewater	Ref.
CEM (CMI-7000)	Combined industrial	(Abbasi et al., 2016)
	Dairy	(Cecconet et al., 2018)
	Vegetable oil	(Firdous et al., 2018)
	Municipal	(Ge & He, 2016)
	Landfill leachate	(Nguyen et al., 2017)

Membrane	Wastewater	Ref.
PEM (Nafion 117)	Brewery	(Çetinkaya et al., 2015)
	Paper	(F. Chen et al., 2020)
	Retting	(Jayashree et al., 2015)
	Fecal	(H. Liu et al., 2017)
	Kitchen	(Naina Mohamed et al., 2020)
	Distillery	(Naina Mohamed et al., 2015)
	Petroleum refinery	(Srikanth et al., 2016)
	Surgical cotton	(Tamilarasan et al., 2017)
	Synthetic	(Tian et al., 2015)
AEM (AMI-7000)	Swine	(D. Ma et al., 2016)
Quartz sand chamber	Synthetic	(C. Gao et al., 2017)
Per fluorinated membrane - Nafion	Seafood	(Jayashree et al., 2016)
Polypropylene non-woven	Domestic	(Y. Park et al., 2018)
Plexiglass annulus	Domestic	(Yang et al., 2018)

2.13 Operation conditions

MFCs under different operation conditions have effects on the COD removal and power density. Thus, in this section, several important operating parameters including substrate, pH, temperature, time, external resistance, and aeration or O_2 are thoroughly discussed, and their effects on the power generation is also covered.

2.13.1 Effect of substrate

Wastewater has organic compounds that can be degraded by microorganisms, and thus COD concentration has effects on the time processing and consequently on the MFC efficiency. COD removal and power production have different responses to the initial COD concentration. To have higher electricity productions, the substrate needs to be oxidized completely, if not energy would be lost, hence high substrate and high active microbial community increase the conversion of substrate to energy (Krieg et al., 2017). To overcome this issue, high loading rate and enriched mixed microflora improve the substrate oxidation and thus the electricity production (Venkata Mohan, Veer Raghavulu, et al., 2008). However, MFC performance can be affected by undesirable bioprocesses done by other microorganisms like methanogenic strains; these microbes can use substrates as a source for production of CH_4 using electrochemical inactive reduction, thus, a reduction of

electricity is achieved (Koók et al., 2016). Different substrates have been investigated for electricity production from MFCs, moreover, wastewater as a substrate has different characteristics that can increase or decrease power generation. Non-fermentable substrates like glucose and acetate have better performances for power production in comparison with fermentable wastewaters such as brewery substrates, for example, Yu et al. (2015) showed a change from 1519 as (Naik & Jujjavarappu, 2020) evaluated different types of wastewaters using the same MFC. Powers of 0.86, 1.3 and 1.42 V were reported for pond water, cement plant water and sugar industry wastewater respectively, the results showed the better performance of sugar wastewater due to the highest levels of COD suitable for power performance.

Naina Mohamed et al. (2020) investigated the effect of COD concentration in wastewater on power production and COD removal using 2500 mgCOD/L and 5000 mgCOD/L. At 2500 mgCOD/L the power and COD removal were 37.2 mW/m^2 and 73.5% respectively and for 5000 mgCOD/L it was reported 27.2 mW/m^2 and 68.5% for power density and COD removal respectively. Changes in COD concentration reported by Naina Mohamed et al., 2015 showed an increase from 168 to 428 nW/m^2 in power density with a COD from 3.2 to 9.6 g/L. Pannell et al. (2016) reported an increase of current density from 3.2 to 10.9 A/m^2, the increasing in this value is due to the substrate accumulation from day 0 to day 90 as increasing the loading rate until 12 g/L-day. Samsudeen et al. (2015) increased the COD concentration from 800 to 2500 mg/L, the authors demonstrated an increase of power density from 49 to 83.2 mW/m^2 and COD removal from 55.7% to 71.8% for 800 mg/L and 2500 mg/L respectively. Nevertheless, high concentration of substrate may inhibit the reduction mechanism of the microorganism, because the microorganisms are unable to take compounds from the substrate due to a substrate saturation, which affects the electron transport (Koók et al., 2016).

2.13.2 Effect of pH

Low proton transport rate between anode and cathode can change the pH and affects the MFC performance for two reasons: (i) the acidification in the anode by accumulation of protons decreases the pH; low pH can inhibit the growth of microorganism, for example electrogenic microbes growth in an optimal pH of 8 and 11; and (ii) the occurrence of

alkalinization in the cathode is caused by the consumption of protons by O_2 reduction; high pH in cathode decreases the current generation because an increased pH value leads to a reduction in the O_2 potential (Du et al., 2007; Oliveira et al., 2013; Samsudeen et al., 2015). However, high salinity increases power production because the electrogenic microorganism growth under these optimal conditions of pH, above 7.5 (Aghababaie et al., 2015; H. M. Singh et al., 2019).

Samsudeen et al. (2015) reported a maximum power of 645 mV in comparison with 595 mV at pH of 8 and 6 respectively, this author also determined that a pH of 8 the COD removal was 63.5% in comparison with pH of 6 with a COD removal of 54.3%. Dual MFCs can use buffer and catholyte solutions in anode and cathode chamber, respectively, keeping pH values at optimal conditions to avoid acidification and alkalinization. However, in the case of single MFCs, the maximum conditions of pH value are between 8 and 11 for anode and cathode (Oliveira et al., 2013). These optimal conditions enhance the growth of electrogenic microorganisms that enhance the electron transfer, hence the substrate degradation and the power production would increase.

2.13.3 Effect of temperature

Temperature affects kinetics, conductivity of the solution, activation energy, electrode potentials and behavior of the microorganisms. Each microorganism has an optimal temperature for growth and metabolism pathways. Temperatures between 30 – 45 °C can facilitate biofilm formation and enhance bio–electrocatalytic activity. While a minimal decrease of temperature during MCF process can lower the cost for heating and leads to no significant influence on the power generation (Feng et al., 2008; Heidrich et al., 2014; Moon et al., 2006; Oliveira et al., 2013). However, some studies have reported the use of mesophilic temperatures at low temperatures with MFCs. Ahn & Logan, 2010 reported that the mesophilic temperatures have a direct effect on the substrate degradation and electricity production. Heidrich et al., 2014 studied the performance of MFC with temperatures between 1 and 5°C and it was observed that the MFC performance (48.7%) was low, moreover, start-up of MFC at low temperatures needs more time for stabilize the culture (Aghababaie et al., 2015). However, authors such as Jamal et al., 2020; Jayashree et al.,

2016; Tamilarasan et al., 2017 performed MFC for wastewater treatment at room temperature.

2.13.4 Effect of external Resistance

Low external resistance equal to internal resistance increases the power density generation at its maximum point. Also, the oxidation of organic substrates is faster because the metabolism of microorganisms stimulates the electron transport and high external resistance takes more (F. Chen et al., 2020; Ghangrekar & Shinde, 2007; Gil et al., 2003; Mansoorian et al., 2013). Mansoorian et al. (2013) showed that a 20-kohm the power production is high in comparison with 40-kohm, similar results are proved by Gil et al. (2003), where the current is higher with 10-ohm than 1000-ohm. Chen et al. (2020) reported a maximum of 56.2 mW/m^2 of power density with an external resistance between 200 and 300 ohms, a resistance of 1000 ohm decreased the power density to 23.5 mW/m^2 approximately. The variations reported by Kloch & Toczylowska-Maminska, 2020 showed the highest power density of 440 mA/m^2 at 100 ohms in comparison with 500, 1000 and 2000 ohms.

2.14 Challenges in microbial fuel cells and micellar-enhanced ultrafiltration

The main weakness of MFCs process is the high capital cost. Generally, electrodes required for anode and cathode are expensive, and the use of catalysts like Pt increase considerably the inversion cost, thereby making the process not feasible for industrial or wastewater treatments. However, operational cost can be reduced with MFC's power output, and the process does not generate great quantities of sludge that is required to be further treated before discharging into the environment.

Another technical issue for MCFs technology is scale-up, which is attributed to the need of the capacity and the areas to ensure an efficient electron transport of electrodes. The use of biofilms for electron transport in the anode and slow kinetic rates of microbes could negatively affect the electron transferring to cathode. This can be tackled by using bacteria with a higher transport rate like *Geobacter* and *Shewanella*, or genetic engineering of microorganisms. Nevertheless, the use of microbial consortium of microorganisms is better

because they are extracted from real wastewater. This combination of microbes enhances the electrochemical reactions; hence the oxidation of organic compounds is faster which then improves electron transport. Another way to improve electron transport is the biofilm thickness, which could allow the migration of substrate and electron transfer (Du et al., 2007; Fornero et al., 2010; Oh et al., 2010).

Electrodes distance affects the MFC efficiency because they can increase the internal resistance and power production. The reduction in the distance between anode and the cathode can solve these issues without having any extra cost for design. Use of metallic catalysts like Pt increase considerably the cost, so the use of biocathodes can increase the O_2 reduction in carbon-based materials and can produce additional valuable products through the recovery of microorganisms from the cathode (Fornero et al., 2010; Janicek et al., 2014; M. Zhou et al., 2011). Therefore, a maximized electrode area can enhance power production, thus it is significant to find cost-effective electrode materials. So, the use of carbon-based materials with high affinity and resistance to fouling, and good performance of electron transferring from anode to cathode could be one of future research direction (Du et al., 2007; M. Zhou et al., 2011).

Fouling in membrane separator in MFCs negatively affect the cation transfer and consequently the power production is reduced (Çetinkaya et al., 2015). For instance, Dannys et al. (2016) reported a reduction of 33% after 90 days of operation because of the membrane fouling. The use of membranes coated with different catalysts like multiwalled carbon nanotubes and the H_2O_2 production in the cathode chamber by electrodes with Pt catalysts may avoid fouling formation in the membrane (Yuan et al., 2016).

Power production from wastewater is low, which can be explained as follows: (i) wastewater has terminal electron acceptors like nitrate, soluble Fe^{3+}, and sulfates that affect the electron transport rate to the cathode; (ii) the high energy input of microorganisms; and (iii) the emission of compounds like hydrogens. These problems can be solved by using acidification that reduce the harboring methanogens and consequently gas production (Fornero et al., 2010).

2.15 Conclusions

Wastewater from refinery processes containing high levels of metals and salts is highly toxic for the environment. The treatment of this wastewater is imperative because of the large quantities produced in refinery processes. Moreover, its treatment and electricity production by MFC is a technology that offers many benefits and can be used to make processes environmental-friendly. During MFC process, the power produced can be recirculated back to the process and thus decreases costs associated with the energy consumption and avoids the use of fossil fuels for heating the process.

Conventional heavy metal removal treatments have been investigated and implemented in industry to decrease toxic compounds. These conventional treatments like chemical precipitation, coagulation, ion exchange, electrodialysis and floatation have advantages that can be applied in different places. However, some disadvantages like cost expensive, chemical generation and secondary treatments made necessary the development of new trends like MEUF and bioremediation.

New approaches like MEUF improve membrane ultrafiltration processes using surfactants to complex or bound toxic compounds. Biosurfactants have been studied because they are environmentally friendly and some of them like rhamnolipid can be recovered with the acidification process. Rhamnolipid forms complexed with heavy metal ions increasing the particle size allowing the water without complex or micelles go through the membrane. Thus, the heavy metal ions are bound to the rhamnolipid.

Bioremediation of heavy metals ions produces high value products, and the recovery of these metals can be achieved by different microorganisms like *S. oneidensis*. This bacterium is capable of surviving in toxic environments and can reduce heavy metal ions by its respiration pathway and the specific characteristics in its extracellular membrane.

Recovery of high value by-products like heavy metals increases the feasibility of MFC application for wastewater treatment. Moreover, other by-products like CH_4 and H_2 can be used as an energy source. Future research on recovery of high value products from wastewater using MFC should be directed into the high efficiency of the recovery

decreasing the alternative chemicals for precipitation of these products and improving reduction reactions from substrates and catholytes solution.

Low sludge generation in comparison with activated or anerobic treatment is an advantage for MFCs technology by lowering the cost used for the subsequent sludge treatment. The technical challenges caused by the presence of other pollutants in the wastewater can be addressed by using pre-treatments. In addition, other related issues like electrode spacing can be solved using MFC connected in series, thereby decreasing electrodes distance, and keeping the working volume.

The energy densities produced by MFC cannot meet the need to solely supply the wastewater treatment, because the power output is not high in comparison with the power input. Further studies to increase efficiency and store energy in capacitors or batteries should be carried out to increase the energy availability for wastewater treatment operation.

Based on the literature review some gaps were detected in heavy metal remediation. The purpose of this study is to the development of a new approach for metal remediation with environmentally friendly processes with high value by-products. The new approach proposed is the combination of separation methods such as MEUF and MFC for wastewater treatment.

2.16 References

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Chapter 3

3 Investigation of micellar-enhanced ultrafiltration using rhamnolipid for heavy metal removal from synthetic desalter effluent

The information provided in this chapter is based on the paper: **Munoz-Cupa, C., et al. (2022). Investigation of micellar-enhanced ultrafiltration (MEUF) using rhamnolipid for heavy metal removal from desalter effluent.** ***The Canadian Journal of Chemical Engineering*****, 100 (9), 2332-2330.** The results presented in Chapter 3 fulfill the competition of objective 2 (see section 1.3).

3.1 Abstract

The removal of heavy metals from synthetic desalter effluent is an important issue due to the toxicity of these pollutants. In this study, micellar-enhanced ultrafiltration (MEUF) is applied for the removal of heavy metals from salty metal wastewater. MEUF is a process in which surfactants above their critical micellar concentration (CMC) form micelles. Micelles bind compounds with low molecular weight; then, these are rejected by a semi-permeable membrane. Rhamnolipid is an anionic biosurfactant that has advantages such as biodegradability and low CMC. Rhamnolipid complexation with salty metal wastewater demonstrates high efficiency for heavy metal removal. The highest overall removal efficiency was reached at a rhamnolipid concentration of 300 mg/L with 94%, 82%, 99%, 76%, and 42% for Zn, Mg, Cu, Mn, and Na, respectively. Additionally, loading capacity on rhamnolipid micelles was evaluated to have better selectivity for Mn > Cu > Zn > Mg > Na. The results also showed that phenol has a significant effect on heavy metal removal, decreasing CMC, which increases micelles. Finally, the permeate flux decreases after increasing the rhamnolipid concentration due to the formation of the gel layer, creating an additional resistance to the permeate flux that goes through the membrane. This work shows that MEUF with rhamnolipid is a reliable process for heavy metal removal from salty metal wastewater.

3.2 Introduction

Crude oil has impurities that a desalter unit removes. These impurities impact refinery processes by decreasing oil quality, causing corrosion, and increasing heat consumption (Aryafard et al., 2015). The desalter unit is the first process to avoid contamination in further-downstream operations. Desalter operations consist of a water-washing process in which the contaminants are removed by contact and mixing of heated crude oil and water, which separates them into vessels (Abdel Aal et al., 2018). Some of these contaminants, like heavy metals, have negative impacts on the environment due to their accumulation (Mehta & Saini, 2017).

Current technologies for the removal of heavy metals from salty metal wastewater treatment include coagulation–flocculation, chemical precipitation, floatation, membrane filtration, ion exchange, electrodialysis, photocatalysis, adsorption, and electrocoagulation (Bijani & Khamehchi, 2019; Mehta & Saini, 2017; Pérez et al., 2016). The investigation of membrane technologies for salty metal wastewater treatment has also increased. Hybrid ultrafiltration (UF) with reverse osmosis has oil rejection of over 95% (Norouzbahari et al., 2009). Nanofiltration using a polyethersulphone (PES) membrane for chemical oxygen demand (COD) treatment has oil rejection of over 94% (Dadari et al., 2016). Additionally, micellar-enhanced UF (MEUF) was investigated for the removal efficiency of Ni, Pb, Cd, and Cr with 96%, 95%, 92%, and 86%, respectively, with sodium dodecyl sulphate (SDS) as a surfactant (Hashemi et al., 2018).

Alternative approaches are needed to overcome issues related to high cost, maintenance, and further problems like the disposal of chemical surfactants. The study of new technologies and innovations for current processes to obtain high-value products or improve efficiencies through environmentally friendly treatments has gained importance. Among them, UF is a process with high recovery efficiency. However, pore sizes between 0.01 µm and 100 µm are too large for heavy metal removal by themselves (Abdullah et al., 2019). Thus, the use of surfactants to enlarge metal ions by complexation of macromolecules can facilitate heavy metal removal. MEUF uses anionic and cationic surfactants that, above critical micellar concentration (CMC), aggregate into spherical micelles composed of a polar hydrophilic head and a hydrophobic tail in the core. These

micelles bind with the metal ions, forming larger molecules, that are rejected by the UF membrane (Abdullah et al., 2019; Carolin et al., 2017; Lin, 2020; Schwarze, 2017).

MEUF is affected by different parameters such as ion concentration, surfactant concentration, pH, transmembrane pressure, and feed flow rate (Lin et al., 2017). Some studies used SDS as a surfactant for metal removal (Yaqub & Lee, 2020). Cu, Ni, Co, Pb, Cr, and Zn also have been removed at percentages above 80% with this surfactant (Hashemi et al., 2018; J. Huang et al., 2019; Lin et al., 2017; Tortora et al., 2016). Biosurfactants such as rhamnolipid (Verma & Sarkar, 2020) and Cr^{+6} with 98.7% removal efficiency in single wastewater mixtures (Abbasi-Garravand & Mulligan, 2014). Other biosurfactants like saponin, which is inexpensive, has been used in MEUF for the removal of methyl violet, and a combination of rhamnolipid and saponin enhances the removal of Cu, Zn, Cr, Pb, Ni, and Mn from sludge due to their binding characteristics with these ions (Samal et al., 2017; J. Tang et al., 2019). Rhamnolipids are biosurfactants, which are classified as glycolipids with one or two rhamnoses (hydrophilic head) and β-hydroxy fatty acid (hydrophobic tail). These biosurfactants above CMC create micelles that complex with metals (W. Chen et al., 2017; Verma & Sarkar, 2020). Moreover, biosurfactants have advantages like biodegradability, low CMC, and high efficiency in complexation with metal ions (El Zeftawy & Mulligan, 2011; Haryanto & Chang, 2015; Verma & Sarkar, 2018b).

In this chapter, the removal of heavy metals from salty metal wastewater using rhamnolipid in the MEUF process is investigated, and various parameters that impact the efficiency of removal are reported.

3.3 Materials and methods

3.3.1 Chemicals and membrane

Rhamnolipid (average MW of 650 g/mol, 90% purity, solid/granular, and CMC of 50 mg/L (**Appendix 3**), zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$), manganese sulphate monohydrate ($MnSO_4H_2O$), magnesium sulphate ($MgSO_4$), copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium hypochlorite (NaClO, 5% solution) were purchased from Sigma Aldrich. Sodium chloride

(NaCl), phenol (C_6H_5OH), and ethanol (C_2H_5OH, 99% purity) were obtained from Fisher Chemical. The wastewater was obtained from Imperial Oil refinery, Sarnia, Ontario, Canada, and is identified as Avis and Coker brine. A vivaflow 200 Crossflow PES membrane (MWCO of 3000 Da) supplied by Sartorius with an effective membrane area of 0.02 m^2 was used for MEUF experiments without any pre-treatment.

3.3.2 Synthetic desalter effluent preparation

The concentration of salty metal wastewater metals was analyzed using inductively coupled plasma–optical emission spectrometry (ICP-OES) from Bureau Veritas. Synthetic desalter effluent was prepared according to the real salty metal wastewater concentrations shown in Table 3.1. $ZnSO_4 \cdot 7H_2O$ (13.5 mg/L), $MnSO_4H_2O$ (7.5 mg/L), $MgSO_4$ (415 mg/L), $CuSO_4 \cdot 5H_2O$ (10.5 mg/L), NaCl (2410 mg/L), and C_6H_5OH (354 mM) were used. The solutions with metals were mixed at 25°C until the dissolution of chemicals with deionized water.

Table 3.1 Metal concentration in salty metal wastewater

Total Metal	Avis brine (mg/L)	Coker brine (mg/L)
Cu	0.22	0.06
Mg	8.31	10.32
Mn	0.15	0.23
Na	241.40	76.36
Zn	0.27	0.63

3.3.3 Heavy metal removal by micellar-enhanced ultrafiltration

The MEUF experiments used a crossflow membrane with the feed pumped through the system. The volume of the feed flask or retentate was kept constant, with deionized water under pressure at 2.5 bar and 25°C. The recirculated line was accumulated in the feed or retentate container with the permeate going to another flask. The initial volume for the experiments was 250 ml of metal solution and biosurfactant. The contact time between rhamnolipid and metal solutions varied. However, mixtures were stirred at 200 rpm and at ambient temperature. **Figure 3.1** shows the setup of the experiments.

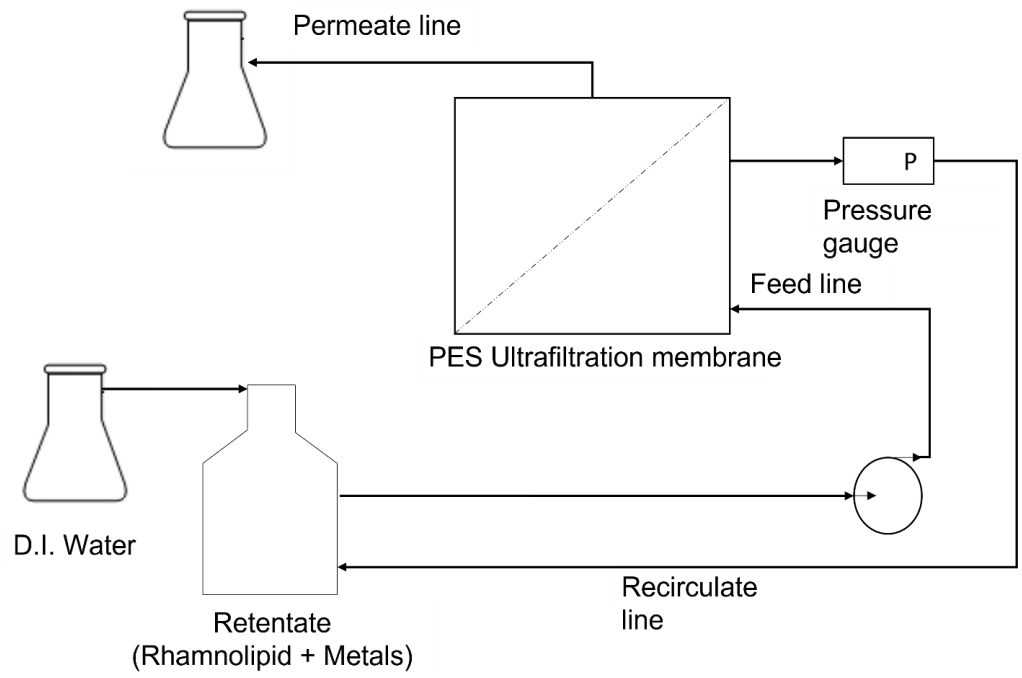


Figure 3.1 Micellar-enhanced ultrafiltration process set-up

The biosurfactant used in these experiments was rhamnolipid with pH between 6.5 and 7.5, which enhanced the process (Haryanto & Chang, 2015; Verma & Sarkar, 2017a). Rhamnolipid was added to the salty metal wastewater according to the required concentration and mixed at 25°C until dissolution occurred. The pH of the solution was controlled using 0.5 N NaOH and 0.1 N HCl. The processing time through the UF membrane was 2 h under a constant transmembrane pressure of 2.5 bar and room temperature. After every batch experiment, the membrane was cleaned with NaCl 0.5 mM, NaOH 0.5 M, and deionized water, according to the supplier's instructions.

After every batch UF process, the permeate was measured to find the permeate flux (J_P) using Equation (3.1):

$$J_P = \frac{V_P}{A \times t} \qquad (3.1)$$

where V_P is the volume of permeate, A is the area, and t is the UF processing time. The adsorption capacity of metals on rhamnolipid micelles (τ_i) was calculated using Equation (3.2):

$$\tau_i = \frac{C_{Oi} - C_{Pi}}{S_O - CMC} \quad (3.2)$$

where C_{Oi} and C_{Pi} are the concentrations of metals in feed and permeate, respectively, S_O is the concentration of rhamnolipid in feed, and CMC is 50 mg/L. The metal removal was calculated using Equation (3.3).

$$\%Removal = \frac{C_{Oi} - C_{Pi}}{C_{Oi}} \times 100 \quad (3.3)$$

All the experiments were done in duplicate, and the results reported are the average of the experiments.

3.4 Results and discussion

Metal removal, loading capacity of metals on rhamnolipid vesicles, and permeate flux were investigated under different conditions using rhamnolipid as a biosurfactant. First, one metal (Zn^{+2}); second, two metals (Zn^{+2} and Mg^{+2}); third, four metals (Zn^{+2}, Mg^{+2}, Mn^{+2}, and Cu^{+2}); then, five metals (Zn^{+2}, Mg^{+2}, Mn^{+2}, Cu^{+2}, and Na^{+}); and finally, the five metals (Zn^{+2}, Mg^{+2}, Mn^{+2}, Cu^{+2}, and Na^{+}) and C_6H_5OH were applied for synthetic desalter effluent containing metal simulation.

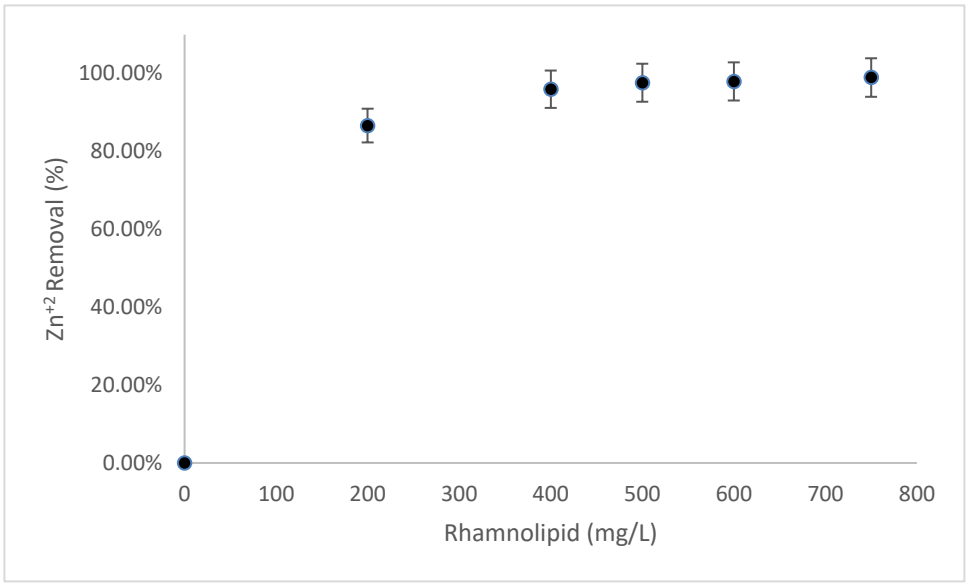
3.4.1 Metal removal by micellar-enhanced ultrafiltration

First, Zn^{+2} was used in a single solution to determine the removal rates using rhamnolipid at different concentrations above the CMC. The removal rates were 87% and 99% for 200 and 750 mg/L of rhamnolipid, respectively (**Figure 3.2a**). Similarly, 96.0% of Zn^{+2} removal was reported by Baharuddin et al., 2015 with unmodified starch at 0.4 mg/L.

Additionally, different zinc concentrations were evaluated with 300 mg/L of rhamnolipid to determine Zn^{+2} removal (**Figure 3.2b**). Removal rates were 99% and 99% for 0.05 and 0.01 M of Zn^{+2} concentration, respectively. However, at concentrations higher than 0.05 M, Zn^{+2} removal was under 54%, which explained that the rhamnolipid micelles do not bind metal ions at higher concentrations with the same efficiency as lower concentrations because of the reduced number of free micelles for complexation with metal ions.

Moreover, high concentrations of surfactant may increase fouling (Abdullah et al., 2019). This shows that MEUF is optimal for the removal of metal ions at low concentrations.

a)



b)

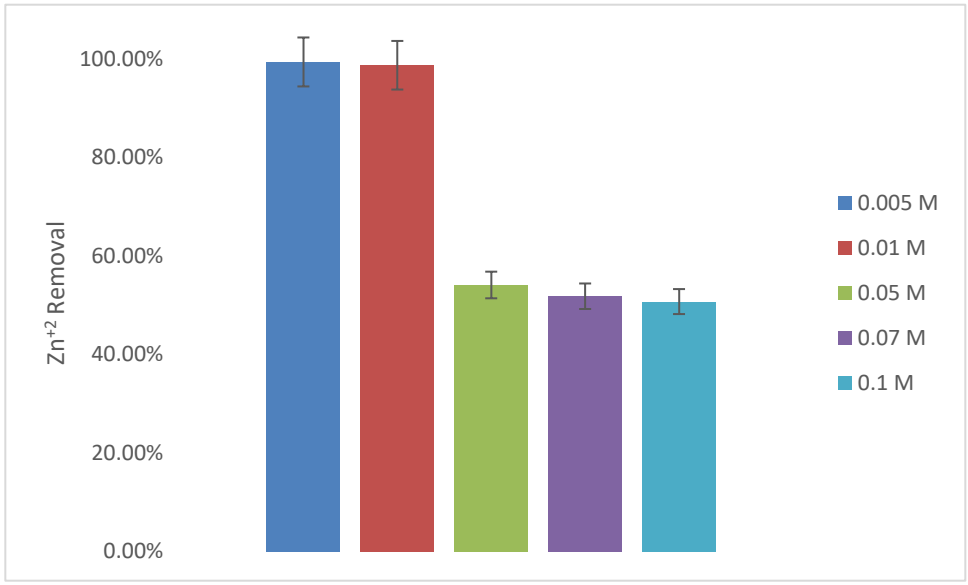
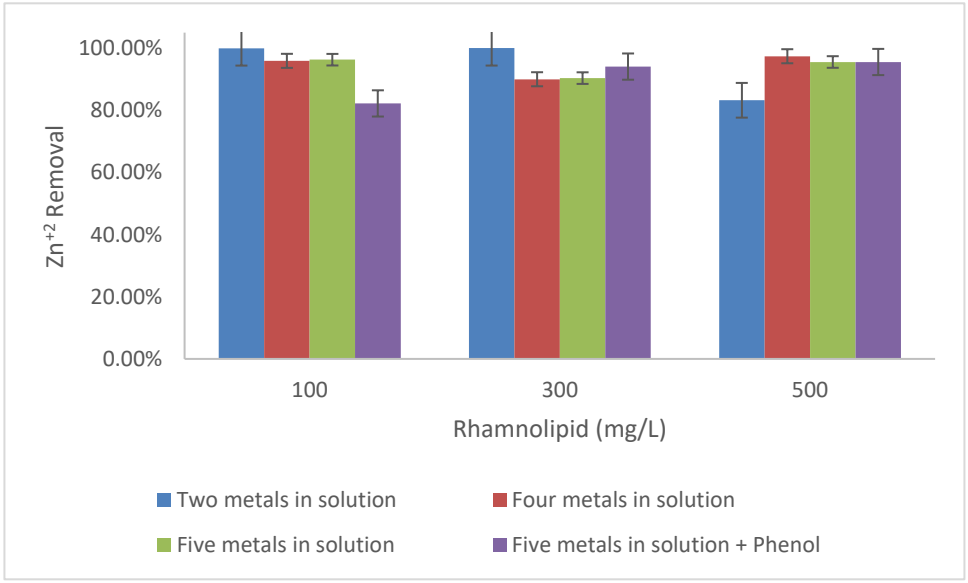


Figure 3.2 Removal of Zn^{+2}. a) At different rhamnolipid concentrations, and b) at rhamnolipid concentration of 300 mg/L

The removal of zinc and magnesium ions was analyzed using different mixtures of salty metal wastewater and different rhamnolipid concentrations (**Figure 3.3a and 3b**). Zn^{+2} and Mg^{+2} removal at 100 and 300 mg/L were close to 100%. However, at 500 mg/L, the

removal decreased to 83% and 78% for Zn^{+2} and Mg^{+2}, respectively, in a mixture of two metal cations in solution. salty metal wastewater of four and five metal cations showed removal of Zn^{+2} without significant change in the removal. However, Na concentration decreased Mg^{+2} removal by 7% and 13% in comparison with mixtures of four and two metals, and phenol reduced Mg^{+2} removal by 11% and 16% when compared with mixtures of four and two metals, respectively. Consequently, rhamnolipid micelles show competition between sodium and magnesium cations. Removal above 90% for both types of solutions shows that Na does not highly affect zinc removal. Moreover, the removal efficiency with phenol in the solution increases from 82.2% to 95.6% as the rhamnolipid concentration increases from 100 mg/L to 500 mg/L. Similarly, (El Zeftawy & Mulligan, 2011) reported Zn^{+2} removal close to 100% with rhamnolipid (0.13 mM). Higher zinc removal above 90% using SDS as a surfactant was investigated by (Ghadge et al., 2015; J. Huang, Qi, et al., 2017). Lower Zn^{+2} removal (74%) using a mixture of rhamnolipid and saponin was studied by (J. Tang et al., 2019). Schwarze et al., 2015 used nonaxoyethylene oleylether carboxylic acid (RO90) with zinc and magnesium removals of 70% and 40%, respectively. These are similar to the results reported in this work. However, low Zn^{+2} removal efficiency was reported by Elouzi et al., 2012 with a value of 7% with rhamnolipid.

a)



b)

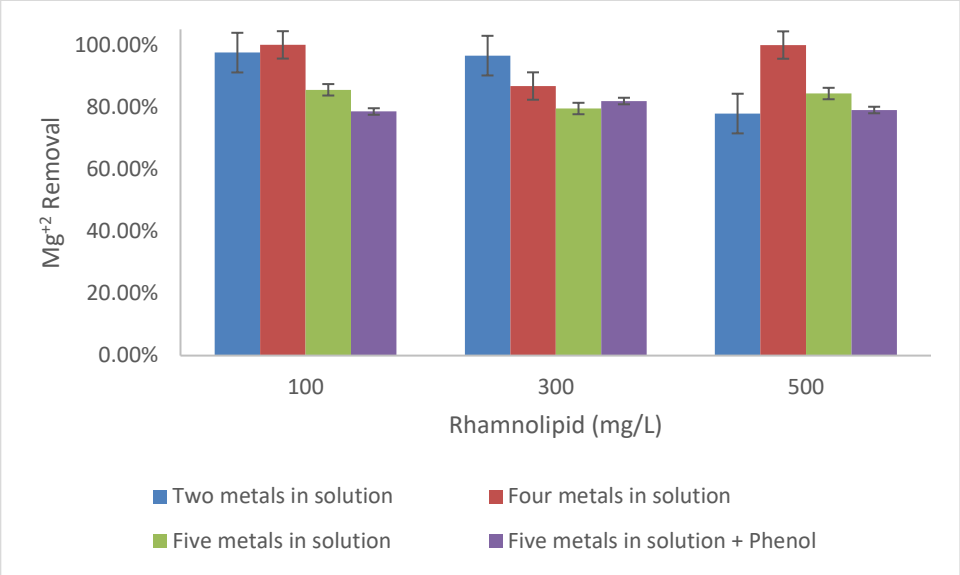


Figure 3.3 Metal removal at rhamnolipid concentrations of 100, 300 and 500 mg/L with different mixtures of metal in synthetic desalter effluent. a) Zn^{+2} removal, and b) Mg^{+2} removal

Sodium and phenol concentration do not impact the Cu^{+2} removal (**Figure 3.4**). Copper removals of 99% show that rhamnolipid vesicles bind it efficiently. Other studies reported results like this study. For example, Cu^{+2} removal 98% with rhamnolipid at 0.13 mM (El Zeftawy & Mulligan, 2011), 95% using RO90 (Schwarze et al., 2015), above 90% with SDS (Kowalska & Klimonda, 2017; Lin et al., 2017), and close to 100% with Tween-80 (Ghadge et al., 2015).

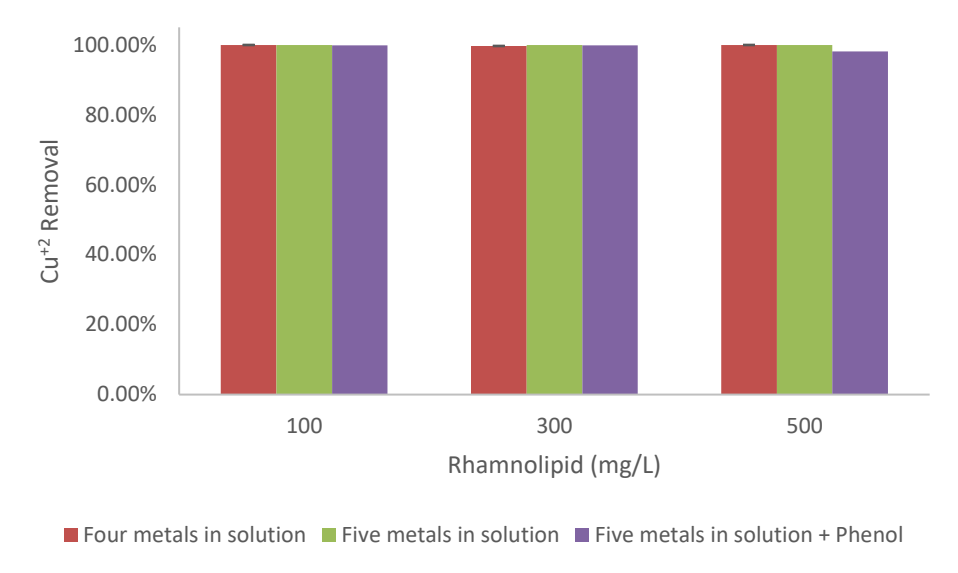
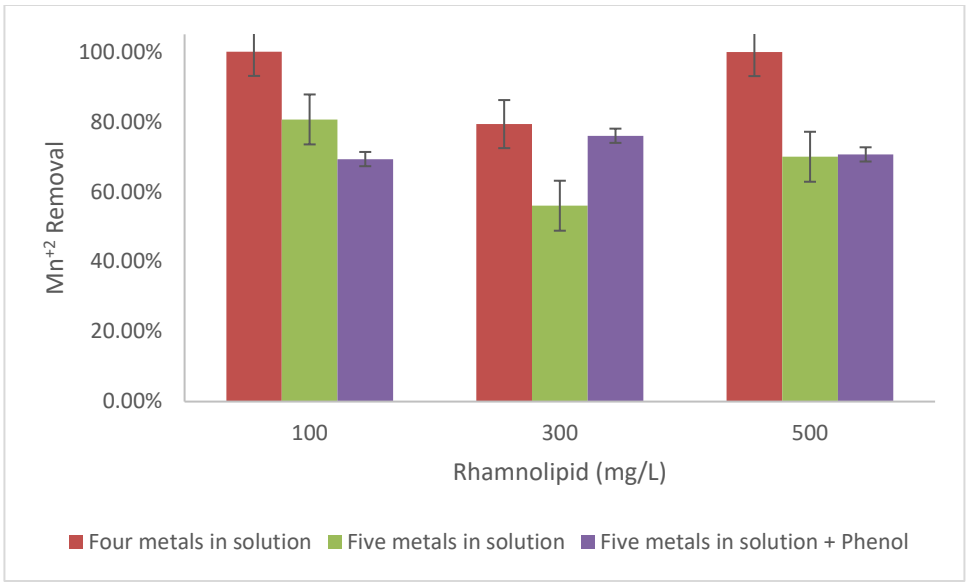


Figure 3.4 Cu^{+2} removal at rhamnolipid concentrations of 100, 300 and 500 mg/L in salty metal wastewater

Similar behavior in Mn^{+2} and Na^{+} removal was observed. It showed higher removal efficiency above 69% and 46% for Mn^{+2} and Na^{+}, respectively, at a rhamnolipid concentration of 100 mg/L. However, it lowered at 300 mg/L and increased at 500 mg/L. In **Figure 3.5a**, Mn^{+2} removal is close to 100%. **Figure 3.5b** shows Na^{+} removal close to 70%. These removal rates occurred at rhamnolipid concentrations of 100 and 300 mg/L. However, when the salty metal wastewater had salt cations, the removal efficiency for Mn^{+2} was reduced. Other studies reported higher Mn^{+2} removal efficiencies. However, some of these studies did not use other cations in the salty metal wastewater, which may explain their better results. For example, (Ghazi & Qomi, 2015) achieved a Mn^{+2} removal of 99.8% with SDS as a surfactant.

a)



b)

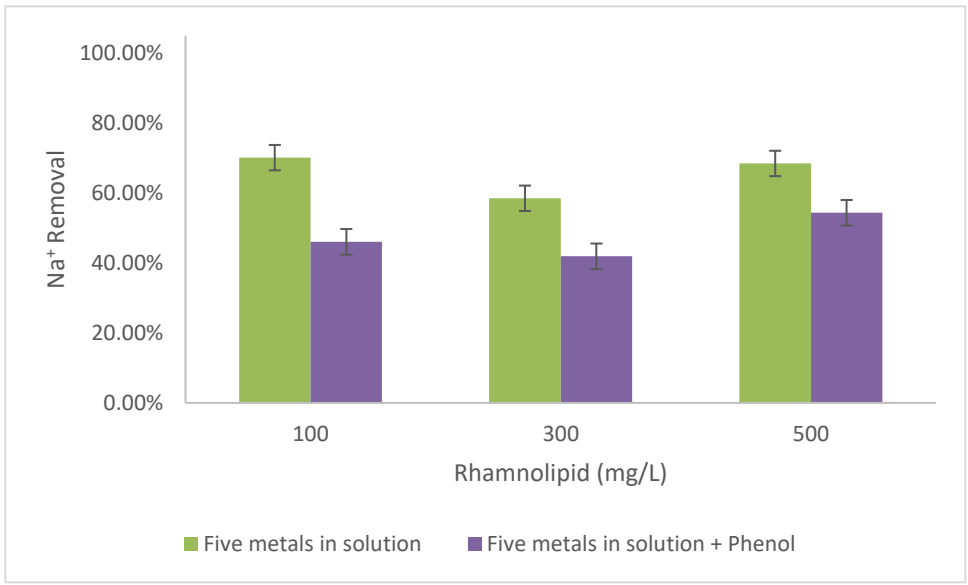


Figure 3.5 Metal removal at rhamnolipid concentrations of 100, 300 and 500 mg/L in synthetic desalter effluent. a) Mn^{+2} removal, and b) Na^{+} removal

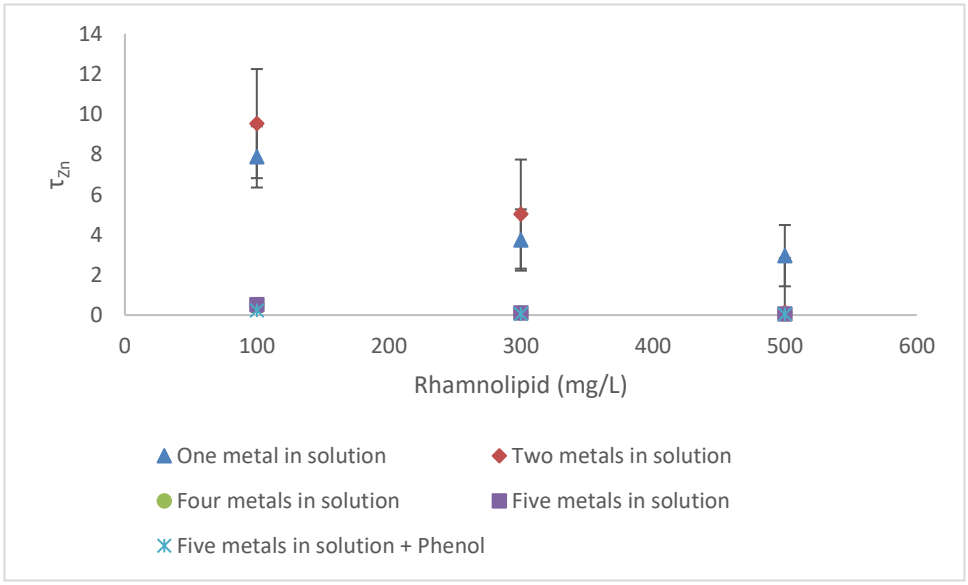
Furthermore, Na^{+} removal was not as high as the removal for other metals reported in this study, with a maximum removal of 70% (**Figure 3.5b**). This result is mainly due to the competition between Mn^{+2} and Na^{+} cations in binding in the micelles. Moreover, Cl^{-} and metals can react and form salts that can reduce the metal removal and reduce the efficiency of the overall process. Phenol does not have a significant effect on metal removal; however, the removal of Na^{+} is reduced by 28% on average for all rhamnolipid concentrations. These

results are expected due to the reactions of Na^{+} with phenol to sodium phenoxide, reducing the removal of Na^{+} by MEUF. On the other hand, phenol does not affect the characteristics of rhamnolipid micelles, which is observed in the removal of metals as was reported by (Z. Liu et al., 2017).

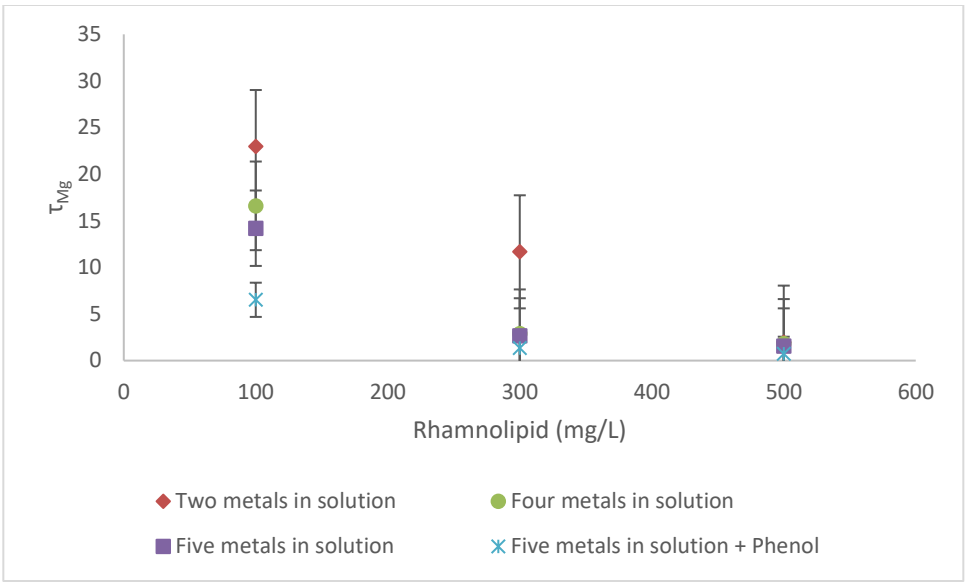
3.4.2 Rhamnolipid micelles loading capacity (τ)

The loading vesicle capacity measures the binding of the metals to the micelles. The results are reported individually for each metal cation according to their concentrations in the feed and the permeate. Zn^{+2} loading in rhamnolipid micelles is shown in **Figure 3.6a**. For mixtures of five metals, values of 0.52, 0,10, and 0.03 for rhamnolipid concentrations of 100, 300, and 500 mg/L respectively, were observed. Additionally, phenol does not significantly affect loading capacity. Thus, with one and two metals in the salty metal wastewater, loading capacities of 7.9 and 9.5 respectively, were determined, indicating that the micelles have lower binding for zinc in salty metal wastewater with more metal cations (Schwarze, 2017). Similarly, Mg^{+2} shows a behavior like Zn^{+2} (**Figure 3.6b**). However, Mg^{+2} has higher values for rhamnolipid loading capacity, which shows that rhamnolipid micelles bind with higher selectivity to zinc over magnesium.

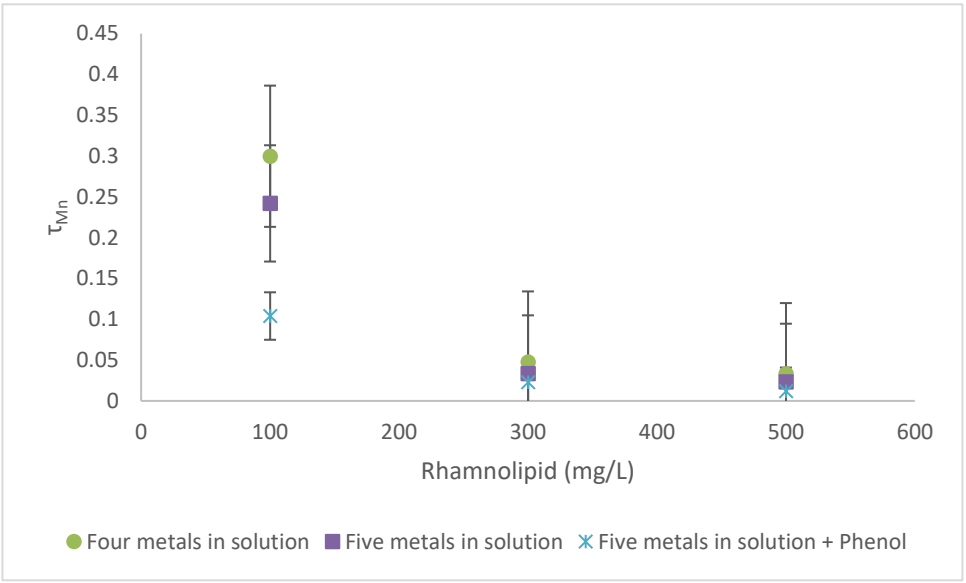
a)



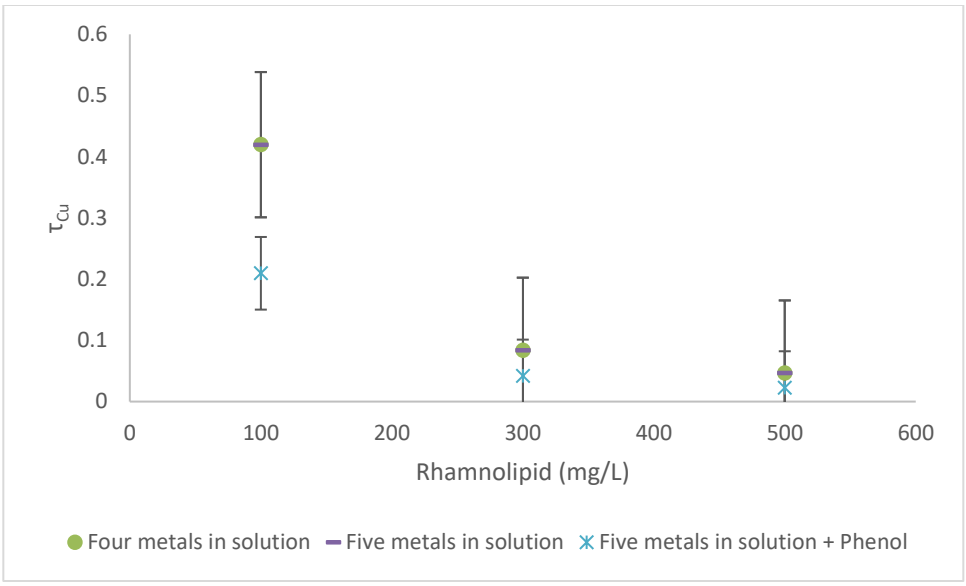
b)


35
30
25
20
15
10
5
0
τMg
0
100
200
300
400
500
600
Rhamnolipid (mg/L)
Two metals in solution
Four metals in solution
Five metals in solution
Five metals in solution + Phenol

c)


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
τMn
0
100
200
300
400
500
600
Rhamnolipid (mg/L)
Four metals in solution
Five metals in solution
Five metals in solution + Phenol

d)



e)

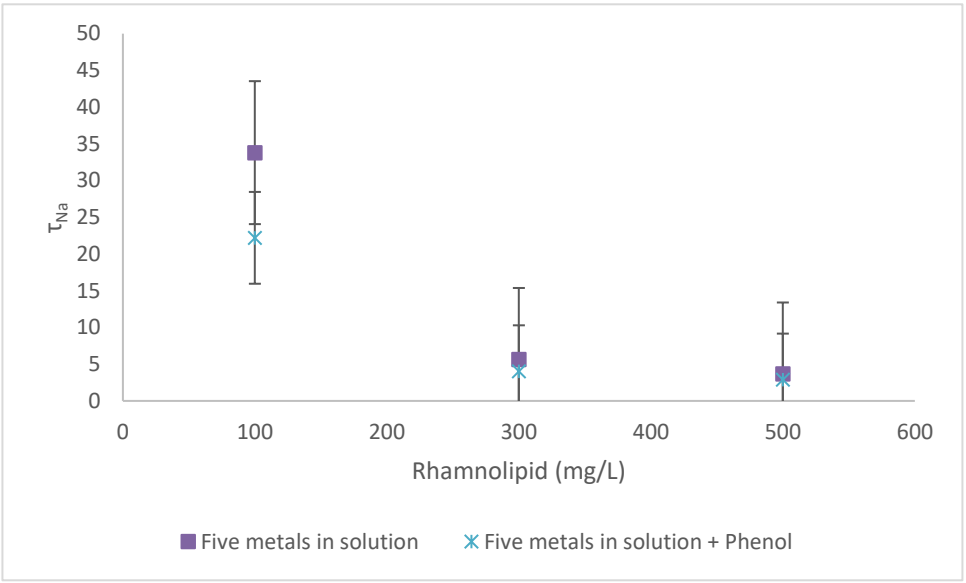


Figure 3.6 Rhamnolipid loading capacity for different metals. a) Zn^{+2}, b) Mg^{+2}, c) Cu^{+2}, d) Mn^{+2}, and e) Na^{+}.

Cu^{+2} cations with four and five metal cations in salty metal wastewater have similar values because the concentration in the permeate for both conditions is very close (**Figure 3.6c**). Also, Cu^{+2} has lower loading to micelles, indicating that micelles bind copper with higher efficiency, with values between 0.42 and 0.02. This characteristic is found with Mn^{+2} as well (**Figure 3.6d**). With Mn^{+2} loading, values are 0.29 for the highest and 0.01 for the lowest. On the contrary, Na^{+} cations have high values for rhamnolipid loading on micelles

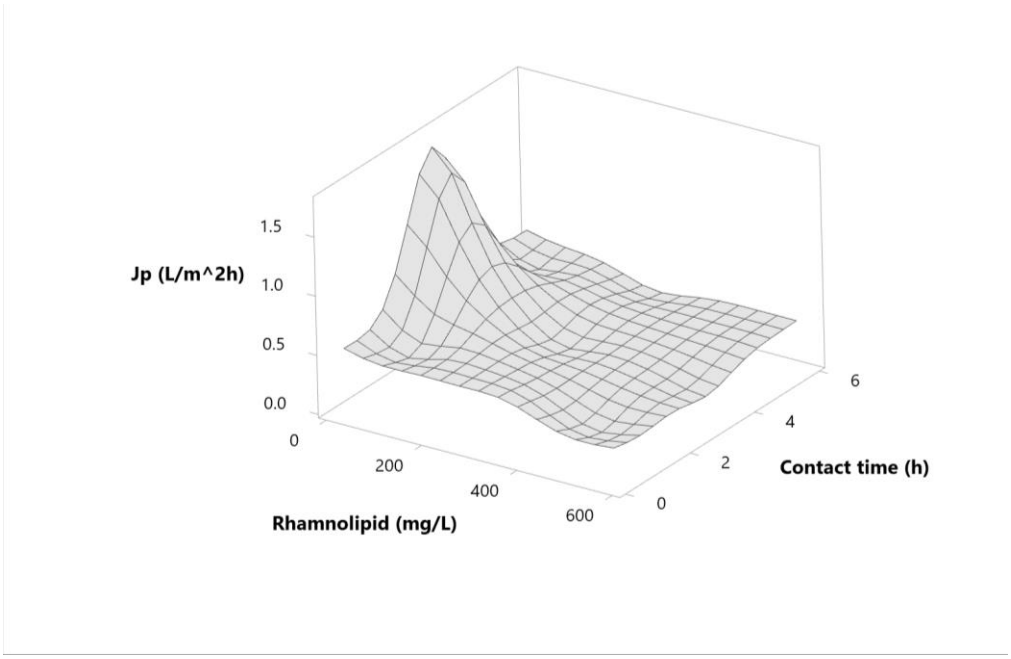
(**Figure 3.6e**). These values are between 33.8 and 2.9 for rhamnolipid concentrations of 100 and 500 mg/L, respectively. These results are consistent with the low sodium removal reported in this work. (Verma & Sarkar, 2017b) reported loading values decreasing from 1.2 to 0.1 for Cd^{+2} when rhamnolipid concentration increased from 50 to 650 mg/L.

3.4.3 Permeate flux

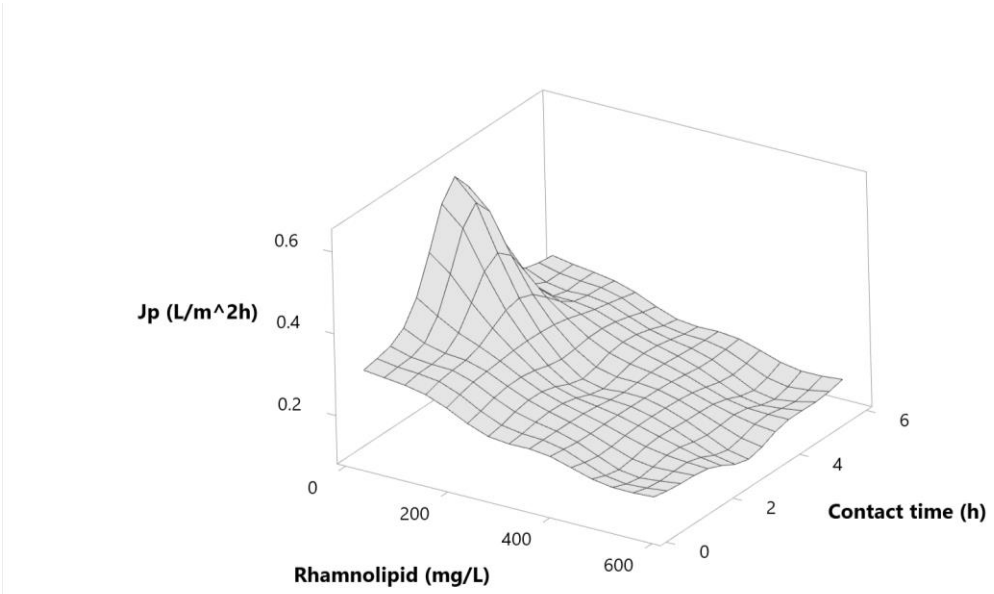
Permeate flux measures the quantity of permeate that goes through the membrane and is proportional to biosurfactant concentration. With one metal in the solution (Zn^{+2}), J_P decreased from 1.3 to 0.4 L/m^2h. This phenomenon is due to the increased viscosity of the solution in proportion with rhamnolipid concentration. El Zeftawy & Mulligan, 2011 reported a decrease in the permeate flux with zinc cations from 120 to 40 L/m^2h with a membrane of 10000 MWCO. The same effects were observed with copper and rhamnolipid complexes, reducing J_P from 23.1 to 17.3 L/m^2h (Ridha, 2011).

At a rhamnolipid concentration of 750 mg/L, the quantity of micelles increases. Consequently, the complexation between zinc and rhamnolipid micelles is higher, affecting the polarization effect on the membrane and enhancing the membrane's rejection of complexed micelles. However, some of these complexes generate a gel layer on the surface membrane that, with the addition of the hydrodynamic resistance of the membrane, reduces the permeate flux (El Zeftawy & Mulligan, 2011).

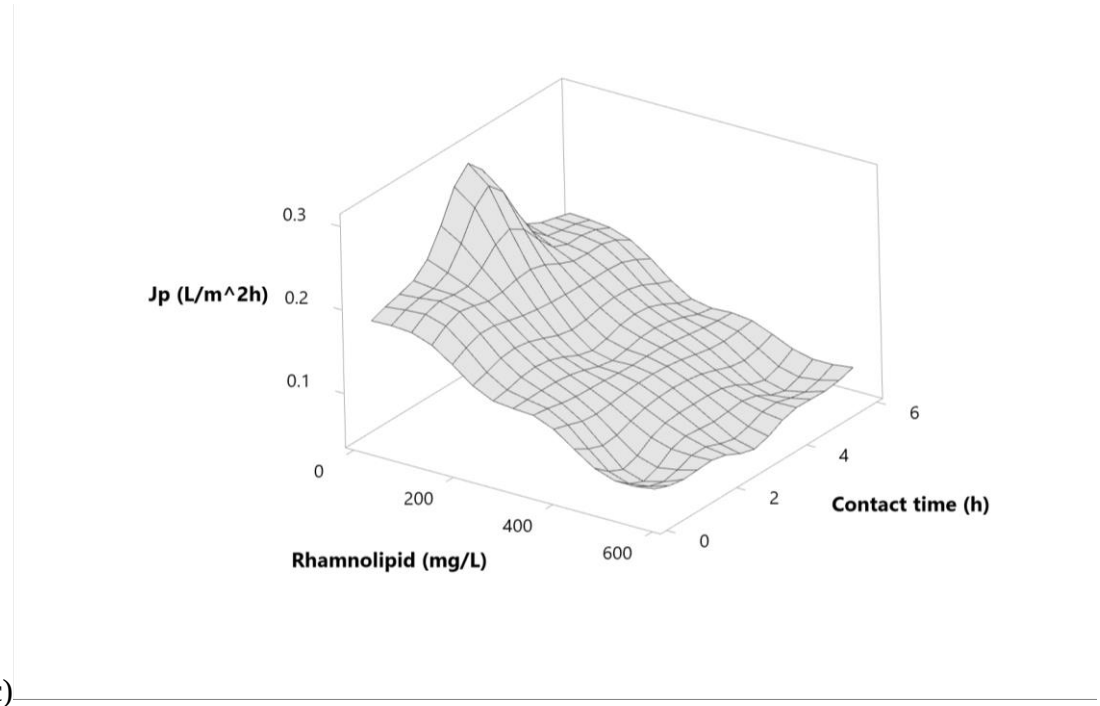
Figure 3.7 shows the change in the permeate flux according to the contact time and the rhamnolipid concentration. As the rhamnolipid concentration increases, the permeate flux decreases. Additionally, higher and lower contact times decrease the permeate flux considerably. Therefore, the highest permeate flux is when the concentration of rhamnolipid is below 200 mg/L and the contact time is between 2 and 4 h. This statement applies to either four or five metals in solutions.


Jp (L/m^2h)
1.5
1.0
0.5
0.0
0
200
400
600
Rhamnolipid (mg/L)
0
2
4
6
Contact time (h)

a)


Jp (L/m^2h)
0.6
0.4
0.2
0
200
400
600
Rhamnolipid (mg/L)
0
2
4
6
Contact time (h)

b)

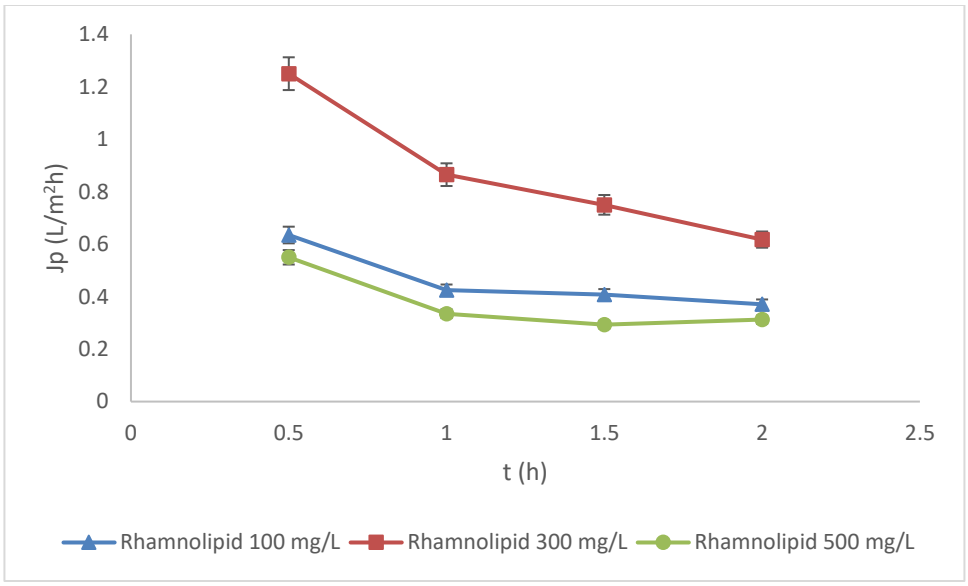


c)

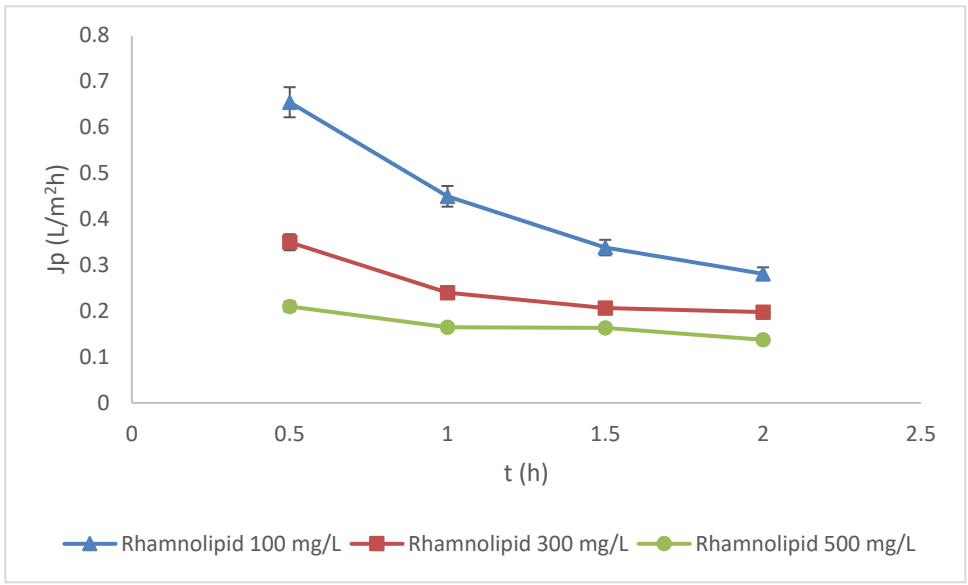
Figure 3.7 Permeate flux (J_P) vs rhamnolipid concentration and contact time. a) Zn^{+2}, Cu^{+2}, Mg^{+2}, and Mn^{+2} in solution; b) Zn^{+2}, Cu^{+2}, Mg^{+2}, Mn^{+2}, and Na^{+} in solution; c) Zn^{+2}, Cu^{+2}, Mg^{+2}, Mn^{+2}, and Na^{+} in solution with 477 mg/L of phenol.

Figure 3.7b and **Figure 3.7c** show a lower permeate flux in comparison with the results in **Figure 3.7a**. The highest permeate flux is close to 0.6 and 0.3 L/m^{2}h for five metals in the salty metal wastewater and with the presence of phenol concentration, respectively (**Figure 3.7a**). Furthermore, the salt concentration is responsible for the significant difference between **Figure 3.7a–c**. Sodium increases the hydrophobic behavior of surfactants and the electrostatic repulsion between micelles (Grzegorzek & Majewska-Nowak, 2018). Reduction in the permeate flux answers the relationship between phenol and CMC. High phenol concentration reduces CMC, and consequently, micelles concentration reduces the permeate flux.

SDS and CPC surfactants showed a reduction in the permeate flux when their concentration increased (Aryanti et al., 2017; Ghazi & Qomi, 2015; Grzegorzek & Majewska-Nowak, 2018; Jung et al., 2008). **Figure 3.8** shows the decline of permeate flux at different concentrations of rhamnolipid with the mixtures of metals in solution.


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Jp (L/m²h)
0
0.5
1
1.5
2
2.5
t (h)
Rhamnolipid 100 mg/L
Rhamnolipid 300 mg/L
Rhamnolipid 500 mg/L

a)


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Jp (L/m²h)
0
0.5
1
1.5
2
2.5
t (h)
Rhamnolipid 100 mg/L
Rhamnolipid 300 mg/L
Rhamnolipid 500 mg/L

b)

c)

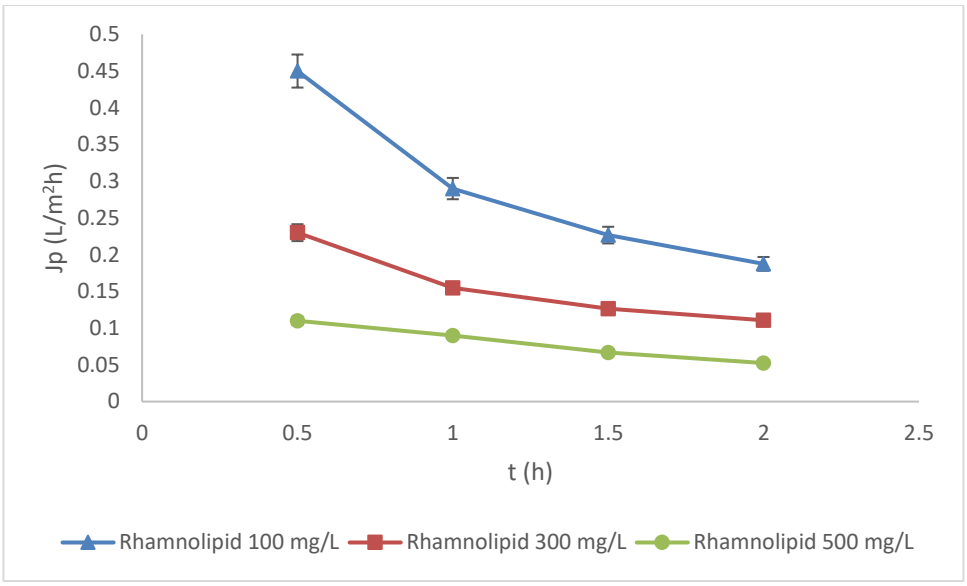


Figure 3.8 Permeate flux (*JP*) at different times with different rhamnolipid concentrations. a) Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}; b) Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, Na^{+}; c) Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, Na^{+}, Phenol

Figure 3.8a–c shows a decrease in the permeate flux over the 2 h of the experiment. This change is specially noted during the first 30 min, which shows a similar trend in comparison with the 3–5 min flux decrease observed by (Verma & Sarkar, 2018b). Additionally, flux decline in **Figure 3.8** indicates the formation of micelles that attach metal ions, increasing the membrane fouling when the concentration in the retentate increases over time. This statement was also proved by (J. Huang et al., 2019). Moreover, a rhamnolipid concentration of 500 mg/L decreases the permeate flux by 49%, 51%, and 72% from the maximum point after 2 h for the solutions with four metals (Zn^{+2}, Mg^{+2}, Cu^{+2}, and Mn^{+2}), five metals (Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, and Na^{+}), and five metals and phenol (Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, Na^{+}, and C_6H_5OH), respectively. This shows the relationship between the membrane fouling and the reduction of the permeate flux due to the absorption of metals and resistance to the flow due to the clogging of pores (M. Chen et al., 2020; X. Fang et al., 2017; Norouzbahari et al., 2009; Tortora et al., 2016). Independently of the surfactant, membranes lowered the permeate flux and fouled due to the gel layer composed of the complex micelle-metal on their surface.

3.5 Conclusions

The removal of heavy metals from salty metal wastewater was studied using rhamnolipid for the MEUF process. The removal of cations (Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, and Na^{+}) was evaluated in different mixtures of salty metal wastewater. The results indicated that at rhamnolipid concentrations above CMC (50 mg/L), the removal rates are highly efficient above 41.91%. For synthetic desalter effluent with one metal (Zn^{+2}), the highest removal rate (99%) was reached at a rhamnolipid concentration of 500 mg/L. High removal efficiencies of Zn^{+2} for synthetic desalter effluent with four and five metals were found at 100 mg/L for mixtures of Zn–Mg, Zn–Mg–Cu–Mn, and Zn–Mg–Cu–Mn–Na, and at 500 mg/L for Zn–Mg–Cu–Mn–Na with an initial phenol concentration of 477 mg/L. Mg^{+2} and Cu^{+2} removal were high at a rhamnolipid concentration of 100 mg/L. There were no significant changes in copper removal between the different rhamnolipid concentrations. This result indicates that rhamnolipid has more selectivity for Cu than the other cations studied. Mn^{+2} and Na^{+} had the lowest removal due to competition between cations in the metal–micelle complex. Additionally, salt concentration reduced the removal efficiency by 12.8% in Mg^{+2} due to Cl^{-} binding with cations to form metallic salts. However, MEUF with rhamnolipid is a reliable process for the removal of heavy metals with efficiencies over 90%.

The loading capacity on rhamnolipid micelles determined the selectivity of micelles to the cations studied in this work. At a rhamnolipid concentration of 500 mg/L, the loading is the lowest for all metal cations, explaining the higher concentration of micelles in the process leading to a sedimentation of rhamnolipid micelles. Thus, the complexation of micelle–metal is higher, resulting in better loading capacity values. According to the results, overall loading capacity has a selectivity of $Mn^{+2} > Cu^{+2} > Zn^{+2} > Mg^{+2} > Na^{+}$.

A higher concentration of rhamnolipid leads to more micelles, reducing the permeate flux because of the formation of a gel layer on the membrane surface. Additionally, phenol increases micelles, which contributes to the low permeate flux. However, rhamnolipid concentration and contact time between micelles and metals have similar characteristics to permeate flux behavior. It is observed that the highest permeate flux is up to 200 mg/L of rhamnolipid concentration and between 2 and 4 h of contact time.

MEUF using rhamnolipid is a reliable process for heavy metal removal; however, membrane fouling demonstrated by the average reduction of permeate flux by 52%, 60%, and 43% for rhamnolipid concentrations of 100, 300, and 500 mg/L, respectively, and the high cost of rhamnolipid are the main limitations for large-scale application.

3.6 References

Abbasi-Garravand, E., & Mulligan, C. N. (2014). Using micellar enhanced ultrafiltration and reduction techniques for removal of Cr(VI) and Cr(III) from water. *Separation and Purification Technology*, *132*, 505–512. https://doi.org/10.1016/j.seppur.2014.06.010

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Chapter 4

4 Investigation of heavy metal removal from synthetic desalter effluent using a microbial fuel cell with *Shewanella oneidensis* MR-1

The information of this chapter is based on the paper: **Munoz-Cupa, C., et al. (2023). Investigation of Heavy Metal Removal from Salty Wastewater and Voltage Production Using *Shewanella oneidensis* MR-1 Nanowires in a Dual-Chamber Microbial Fuel Cell. *Environmental Progress & Sustainability*. Submitted after peer review.** The sections in this chapter presented the results for the competition of objective 3 (see section 1.2).

4.1 Abstract

Heavy metal removal and simultaneous electricity generation were studied using a dual chambered microbial fuel cell inoculated with *Shewanella oneidensis* MR-1 in the anode. wastewater was prepared with Cu (II), Mg (II), Mn (II), Zn (II), Na, and phenol based on salty metal wastewater from refinery processes at different metal concentrations. In this study, a maximum open-circuit voltage of 517.6 mV was reached at Conc. 5 with wastewater in the anode chamber and 127.7 mV at Conc. 3 was generated with the wastewater in the cathode chamber. Moreover, μ at Conc. 5 was 0.11 h^{-1}, demonstrating bacterial growth under synthetic desalter effluent concentrations. The highest metal removal in the anode for Cu, Mg, Mn, Zn, and Na was 93%, 85%, 93%, 88%, and 42%, respectively. In the cathode chamber the removal of Cu, Mg, Mn, Zn, and Na was 98%, 49%, 47%, 59%, and 36%, respectively. During the operation in the anode with wastewater, SEM images showed that the bacterial nanowires are formed as response of toxic and anaerobic environments which contribute to the bacterial growth. These nanowires increased the metal removal and the voltage production as consequence of a higher rate of electrons from the anode to the cathode due to the higher extracellular membrane surface area. *Shewanella oneidensis* is a bacterium with metal-reducing characteristics in the extracellular membrane, and it is suitable for metal removal and

electron transport from carbon sources, demonstrated in electricity generation in microbial fuel cells.

4.2 Introduction

Synthetic desalter effluent polluted with metal salts is an increasing problem due to the toxicity of these effluents which are produced by industrial processes such as crude oil refining. Conventional processes for heavy metal removal such as precipitation, absorption, or membrane technologies are commonly used in industry. However, they have disadvantages such as high cost and low efficiency (Ezziat et al., 2019; D. T. Sun et al., 2018). Additionally, conventional processes have issues in their operation, such as chemical disposal and sludge production (Kaushik & Singh, 2020). Non-conventional processes arise as solutions for heavy metal pollution in wastewater (Wei et al., 2020). Among them, bioremediation processes using metal resistant bacteria have gained importance due to the varied respiratory pathways allowing reduction of inorganic compounds such as heavy metals. Metal resistant bacteria repair heavy metal pollution due to their ability to immobilize, bio-absorb and reduce them from wastewater. Moreover, high resistance to heavy metals from bacterial communities such as *Bacillus firmus, Shewanella loihica, Shewanella oneidensis, Viridibacillus arenosi,* among others, facilitate the treatment of wastewater with multiple heavy metals and organic pollutants (Rahman, 2020).

Shewanella oneidensis MR-1 uses intracellular reductases for reduction of these metals (Y. Yin et al., 2022). Intracellular reductases composed of proteins MtrA, MtrB, and MtrC facilitate the metal reduction; however, other genes such as *cymA* and *omcA* are used when fumarate concentration is high in the media solution (Saffarini et al., 2015). Additionally, *S. oneidensis* species can utilize substrates as electron acceptors easing the transfer from the cell to the anodes, generating electricity in microbial fuel cells (MFC). Cytochromes promote extracellular electron transport pathways that increase the electron conductivity from the cell to the outside medium or electrode in the MFC (Kouzuma et al., 2015). The electron acceptors can be heavy metals, flavins, tellurite, graphite, and carbon (Beblawy et al., 2018). *S. oneidensis* can produce nanowires under oxygen limitation conditions. *S. oneidensis* nanowires are periplasmic extensions with high cytochromes concentration

(Creasey et al., 2018). Furthermore, transport chain by electron hopping between cytochromes spaced at 1-2 nm increase the ability to transport electrons from the membrane to the anode (Sure et al., 2016). In addition, nanowires increase the surface area which enhances the bioelectricity production in MFC (L. Xu et al., 2018).

MFCs have gained importance due to their ability to generate electricity while removing pollutants. The process uses the oxidation and reduction of carbon sources and heavy metal ions producing voltage in response to the electron transport from anode to cathode (Logan, 2008; Munoz-Cupa et al., 2021). In general, MFCs have a liquid nutrient solution in the anode that is called anolyte which supports microbial growth and provides the electrons required for the extracellular electron transport. Efficiency in MFCs is determined for the energy content from substrates (Obileke et al., 2021). Microorganisms in the anode catalyze the substrate (electron donors) for the utilization by extracellular metabolism to the electrode surface (Abbas et al., 2018; al Shahrani, 2020; Mahmoud et al., 2022). MFCs are composed of anode and cathode with electrodes such as carbon felt, graphite, carbon cloth, etc., and a proton exchange membrane (PEM) that separates anode and cathode.

Single chamber MFC uses O_2 in the air as electron acceptor in the cathodic reaction with anolyte solution in the anode chamber (Munoz-Cupa et al., 2021; Obileke et al., 2021). Dual chamber MFCs use anolyte and catholyte solutions separated by a proton exchange membrane (PEM). The cathodic reaction uses the protons transported through the PEM that in combination with the electrons from the anode and O_2 forms water molecules. This configuration is more complex than single chamber MFCs, and the power production is lower (Bose et al., 2022; Mahmoud et al., 2022; Maqsood et al., 2022; Munoz-Cupa et al., 2021; Obileke et al., 2021). However, it can be used for the study and investigation of a variety of anolyte and catholyte solutions simultaneously or even with biotic cathodes. Besides single and dual chamber MFCs, sediment MFCs have been used for degradation of organic pollutants from the soil (Bose et al., 2021). Different parameters such as substrate, membrane, electrodes, pH, and temperature have an impact on the MFC performance. These parameters affect the microorganism’s growth, membrane fouling, and internal resistances, which significantly reduce power generation in MFCs (Obileke et al., 2021).

Catholyte reduction and precipitation of Fe (III), Cu (II), and Zn (II) were investigated using mixed cultures in the anode with a tubular dual chamber MFC (Gajda et al., 2017a). Additionally, Cr (VI) reduction in the biotic cathode with *S. oneidensis* (Hsu et al., 2012; Xafenias et al., 2013), and in an abiotic cathode (M. Li et al., 2018) with a dual MFC having removal efficiencies over 80% for both cases were studied. Other MFC configurations using hybrid electrolysis cell systems were used for Cr (VI), Pb (II), and Ni (II) removal (Y. Li, Wu, et al., 2015). Furthermore, sediment MFCs do not require PEM developing the removal of Cr (VI), Cu (II), and Pb (II) for soil remediation applications (Abbas et al., 2017, 2018; Habibul et al., 2016).

The investigation of heavy metal removal using MFC with different microorganisms and configurations expresses a wide application of heavy metal treatment with more toxicity resistance. (Y. Wu et al., 2018) investigated Cu removal using mixed cultures of *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Acidobacteria* in a single chamber MFC (Y. Wu et al., 2018). Additionally, biocathodes for Cr (VI), Cd (II), and Ni (II) removal using *Corynebacterium vitaeruminis*, *Ochrobactrum* and *Halomonas* were investigated with removal efficiencies over 90% for Cr and Ni, and above 80% for Cd (A. Singh & Kaushik, 2021; S. Zhao et al., 2021). Moreover, mixed cultures have a relatively higher efficiency for heavy metal removal; for instance, marine sediments treated with bacterial communities in anode and cathode for Cr (VI), Ni (II), and Cu (II) removal (Abbas et al., 2018). Additionally, mixed bacterial cultures enriched with sewage sludge for the operation of MFC and removal of Cd (II) and Zn (II) (Abourached et al., 2014).

MFC shows potential for power generation and oxidation/reduction of heavy metals simultaneously; this characteristic enhances the use of the technology for wastewater treatment and power devices such as sensors. However, in some cases, the high reduction potential of Pb, Cd, and Ni is the main disadvantage in catholyte reduction overcoming the power generation in the MFC (I. Chakraborty et al., 2020). On the other hand, Cr (VI), Fe (III), Ag (I), Cu (II), V (V), Zn (II), and Cu (II) have shown removal and power production in dual-chamber MFC with average removal efficiencies above 87.6% and an average power generation of 378.7 mW/m^2 (Ezziat et al., 2019; Gajda et al., 2017a; Lim et al., 2021; X. Wang et al., 2016).

New technologies for heavy metal removal such as MFC use microorganisms for metal removal and power generation simultaneously. Thus, in this chapter a salty metal wastewater composed of Cu (II), Zn (II), Mg (II), Mn (II), Na and phenol was treated in the anode and cathode chamber. The biggest difference in this study to previous studies is the removal of mixture of different heavy metals and the nanowires formation on the biofilm, which increases the power generation and the substrate oxidation in the anode chamber inoculated with *S. oneidensis* MR-1. In addition, OCV, metal removal, and polarization curves were analyzed in the biotic anode chamber and in the cathode chamber.

4.3 Materials and methods

4.3.1 Wastewater preparation

This work evaluated heavy metals concentration in salty metal wastewater (Munoz-Cupa et al., 2022). The wastewater was prepared according to the following concentrations: $ZnSO_4 \cdot 7H_2O$ (13.5 mg/L), $MnSO_4H_2O$ (7.5 mg/L), $MgSO_4$ (415 mg/L), $CuSO_4 \cdot 5H_2O$ (10.5 mg/L), NaCl (2410 mg/L), and C_6H_5OH (354 mM). For experiments with twice or three times the initial concentration for $ZnSO_4 \cdot 7H_2O$, $MnSO_4H_2O$, $MgSO_4$, and $CuSO_4 \cdot 5H_2O$. The previous compounds were mixed with deionized water at 25°C until dissolution.

4.3.2 Microbial culture conditions

A frozen *S. oneidensis* culture (ATCC® 700550™) was inoculated with tryptic soy broth (TSB) for 24 h, 150 rpm, and 29 °C in a Max Q 4000 incubator. After 24 h of inoculation, the liquid inoculum was stored in agar plates made from TSB and agar for immobilization of cells in colonies. The growth rate was analyzed using a UV spectrophotometer at optical density (OD_{600nm}). The specific growth rate was calculated from the optical density using the growth curve of the microorganisms during a period of 108 h of cultivation.

4.3.3 Microbial fuel cell device

A dual chamber MFC constructed from two borosilicate 500 mL bottles divided with a cation exchange membrane (CMI-7000A) at a diameter of 3.3 cm. The membrane has a polymer structure based on gel polystyrene cross linked with divinylbenzene, with

thickness of 0.45±0.025 mm. The membrane was washed at 80°C with H_2O_2 (3%), D.I. water, H_2SO_4 500 mM, and D.I. water for 1 h each for expansion and hydration. Carbon felt electrodes (6 cm x 6 cm x 0.8 cm) positioned in each chamber were connected through an external resistance by copper wires.

4.3.4 Microbial fuel cell operation

Liquid inoculum at OD_{600nm} between 0.2 - 0.3 was fed to the anode with a concentration of 30% v/v. Then, the anode electrode was cultivated for 24 h with inoculum connection in the anode with the salty metal wastewater for the anode experiments or with liquid media for the cathode experiments at 70% v/v. wastewater with different metal concentrations (**Table 4.1**) was used in the cathode and anode. Furthermore, anode solution was mixed with TSB, sodium-lactate (18 mM), and M9 medium (1000 mM) (Y. H. Park et al., 2016; S. Xu et al., 2016; P. Zhang et al., 2017). The catholyte solution was $K_3Fe(CN)_6$ with a concentration of 50 mM dissolved in 100 mM PBS (Biffinger et al., 2009; Dai et al., 2021; Ringeisen et al., 2006). The connection of the MFC was through an external resistance of 1 kΩ (Dai et al., 2021; M. Li et al., 2018; Xafenias et al., 2013; J. Zhang et al., 2020; N. Q. Zhou et al., 2016). The pH of the anode chamber was initially 7.0±0.5 and controlled using NaOH (500 mM) and HCl (500 mM). In addition, the MFC was operated under anaerobic conditions to fulfill better electron transport and bacterial nanowires formation.

Table 4.1 wastewater composition used for the MFC experiments.

Compound	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5
Zn	13.5 mg/L	27.0 mg/L	40.5 mg/L	500 mg/L	13.5 mg/L
Mn	7.5 mg/L	15.0 mg/L	22.5 mg/L	500 mg/L	7.5 mg/L
Mg	415.0 mg/L	830.0 mg/L	1245.0 mg/L	500 mg/L	415.0 mg/L
Cu	10.5 mg/L	21.0 mg/L	31.5 mg/L	500 mg/L	10.5 mg/L
Na	2410.0 mg/L	2410.0 mg/L	2410.0 mg/L	500 mg/L	2410.0 mg/L
C_6H_5OH	NA	NA	NA	NA	1

4.3.5 Data collection and SEM preparation

Polarization curves were measured by varying the external resistance from 0.1 to 100 kΩ. The activation, ohmic and concentration losses were evaluated according to the voltage dropping in the polarization curves. Data collection was performed using a data logger

multimeter OW18 series with accuracy of 0.5%±2 until stabilization of maximum voltage for each resistance value. Current density (*I*) and power density (*P*) were calculated using Equations 4.1 and 4.2, respectively (Logan, 2008). Where V is the voltage (mV), R_{ext} is the external resistance (Ω), and A is the electrode area (m^2). After stabilization and data collection for polarization curves, the voltage was recorded every 60 minutes.

$$I = \frac{V}{R_{ext} \times A} \quad (4.1)$$

$$P = V \times I \quad (4.2)$$

Internal resistance was measured using the power density peak method, which consists of the use of the maximum power density with the external resistance where this point is reached in the power density curve (Equation 4.3) (Logan, 2008), Where R_{int} is the internal resistance in Ω.

$$R_{int} = \frac{P}{I^2} \quad (4.3)$$

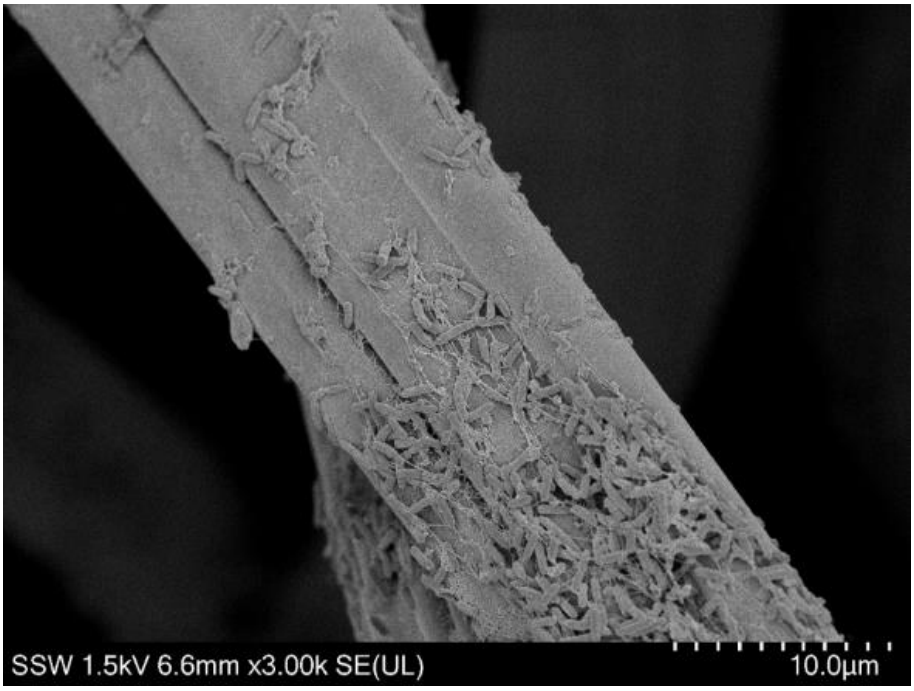
Biofilms in electrodes were fixed for 48 h at 4 °C in glutaraldehyde 2% in 100 mM phosphate buffer (pH 7.4). After fixation, the samples were pre-treated in Biotron at Western. Samples were washed 3 times with 100 mM PBS and 1 time with DI water. Then, samples were fixed in 2% Osmium for 1 hour, and they were rinsed 3 times for 10 min each with 0.1 phosphate buffer. Then the samples were gradually dehydrated in increasing ethanol concentrations (30%, 50%, 70%, 90%, and 100%) for 10 min each. Finally, samples were dried by ascending hexamethyldisilazane (HMDS). After dehydration, samples were coated with gold and SEM was done at Surface Science Western using the instrument Hitachi SU8230 Regulus Ultra High-Resolution Field Emission SEM.

4.4 Results and discussion

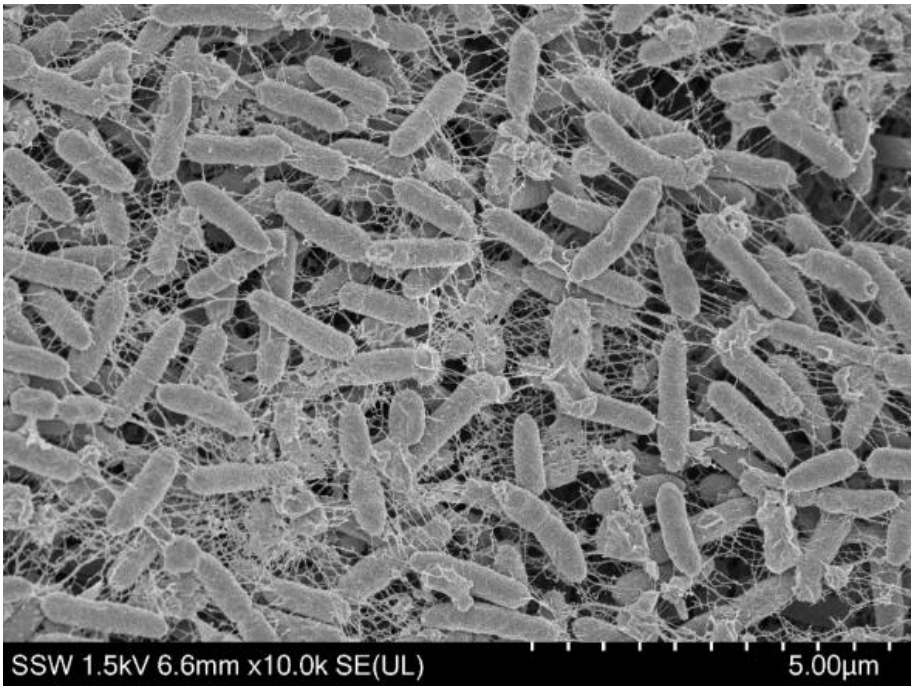
4.4.1 Biofilm and bacterial nanowires

Biofilm formation was observed as a single layer on the electrode surface after 7 days. The carbon felt fibers on the electrode facilitate the attachment of bacteria to the surface (**Figure 4.1a**). Biofilm formation in carbon felt in a sediment MFC showed a lighter bacteria

concentration with *S. oneidensis* (Shi et al., 2019). Moreover, same species with single chamber MFC showed higher biofilm formation with fumarate as electron donor in the anode (P. Zhang et al., 2017). However, with aerobic conditions nanowires formation is limited. Furthermore, graphite felt with *S. oneidensis* have shown some bacterial nanowires with lower connections and cells widespread around the fibers (Bretschger et al., 2007; Dai et al., 2021; Pinto et al., 2018). Nevertheless, the biofilm without coating on the electrode surface is thick (M. Sun et al., 2010), enhancing the electron transport from the media to the electrode. The external resistance (1 kΩ) contributes to the formation of thin biofilm. Moreover, biofilm showed a higher concentration (**Figure 4.1b**) at external resistance of 1 kΩ. However, at 2 kΩ biofilm in carbon cloth showed a low number of cells (T. Liu et al., 2016), but in our research, a high number of cells were observed with lower resistance due to presence of anaerobic conditions and the toxic environment due to the presence of heavy metals, sodium, and phenol. Additionally, biofilm acts as the mediator for easy electron transportation from the medium to the electrode, and the electrons are taken directly from the biofilm (Vanegas-Hernández et al., 2020).

a) 

b)



c)

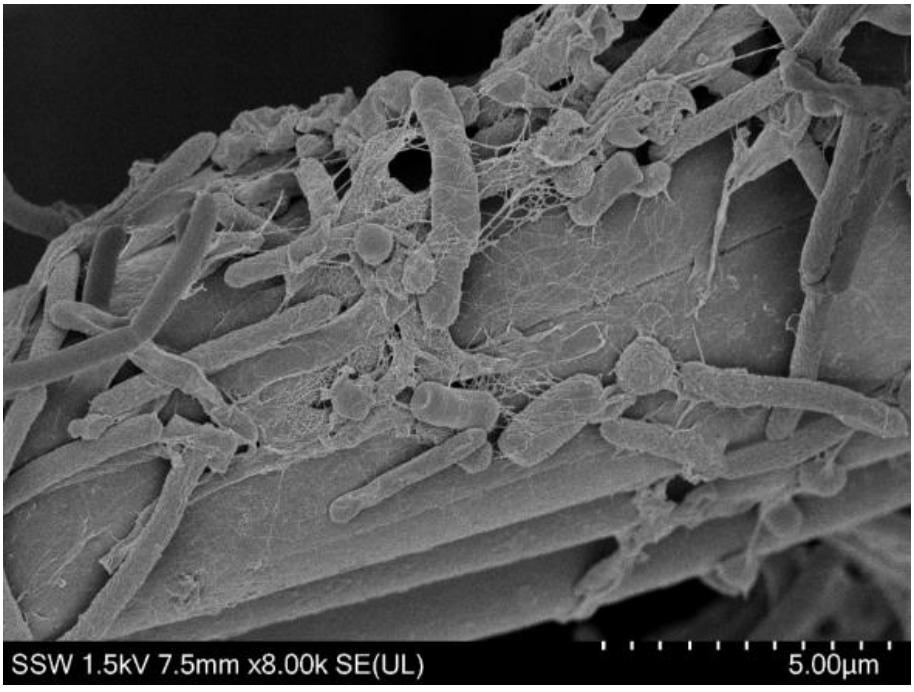


Figure 4.1 *Shewanella oneidensis* biofilm attached to carbon felt fiber in the anode chamber. a) Carbon felt fibers with biofilm, c) *S. oneidensis* biofilm with nanowires, and c) *S. oneidensis* biofilm in control solution.

Bacterial nanowires demonstrate the connectivity between cells allowing higher electron transport between the medium and the electrode (**Figure 4.1b**). *S. oneidensis* produce

nanowires with biofilm formation when added evenly to a mixed culture with an o-xylene medium (You et al., 2020), suggesting the bacteria adapts to adverse conditions. Additionally, microbial nanowires increase the resistance to metal toxicity and might help in metal reduction due to the increase in surface area (Sure et al., 2016). Moreover, it was demonstrated that the bacteria could grow under different metal concentrations (**Figure 4.2**). The specific growth rate for growth under standard conditions or media and concentration 5 were similar, with results of 0.1103 h^{-1} and 0.1133 h^{-1}, respectively.

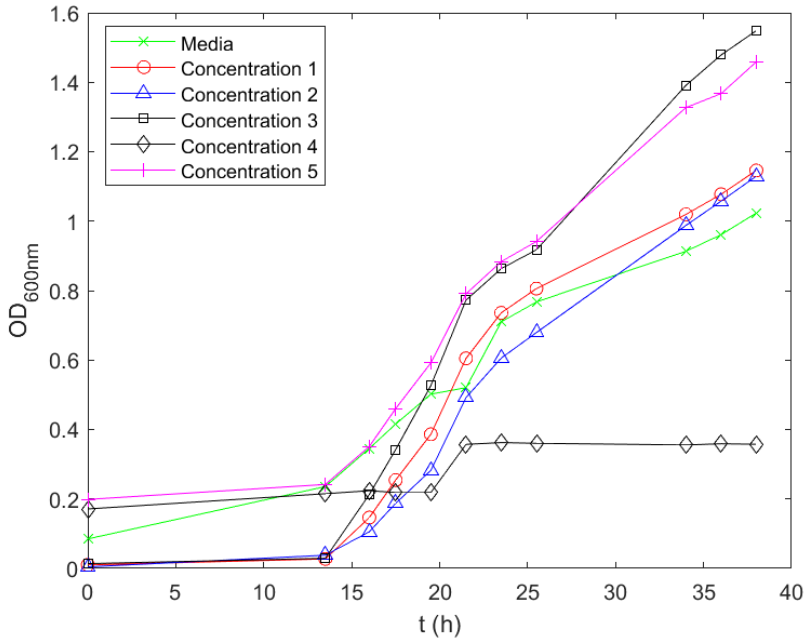


Figure 4.2 *Shewanella oneidensis* MR-1 growth under different metal concentrations.

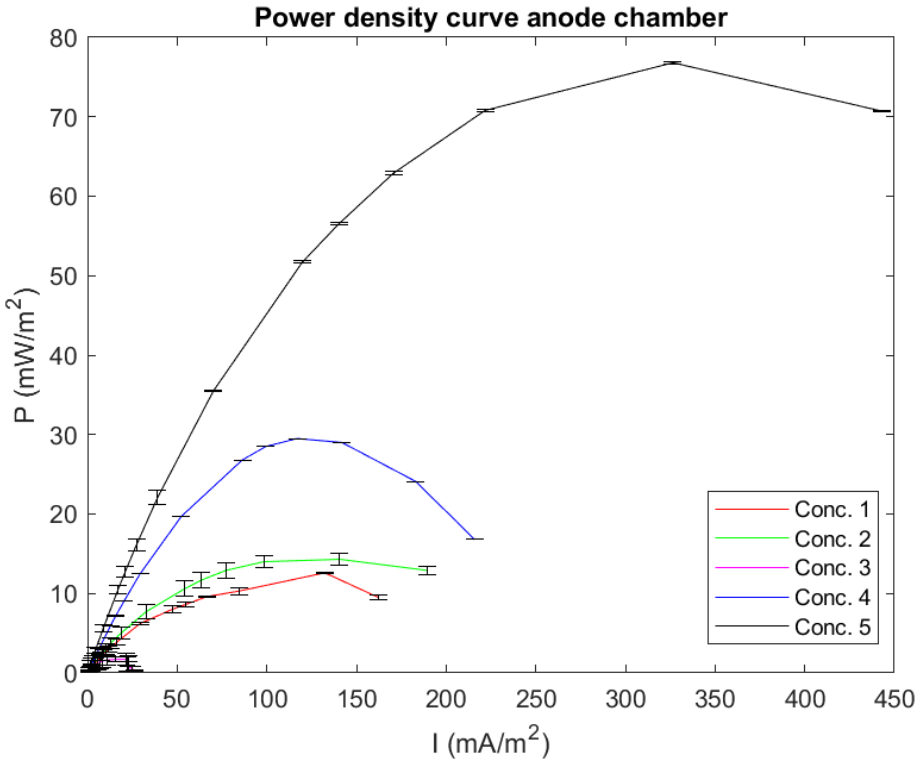
S. oneidensis nanowires are an extended part of the periplasmic membrane, with a similar composition to the cell membrane, leading to a higher electron transport with high conductivity (0.01 – 1 S/cm) (Creasey et al., 2018). This characteristic assists the electron transport mechanism which consists in direct electrons transfer to the electrode through the outer cell membrane (L. Xu et al., 2018). Heavy metal concentration and adverse conditions in the cultivation such as anaerobic conditions and phenol concentration increase the nanowires concentration. The presence of metals increases microbial nanowires concentration; however, less adverse conditions showed a lower microbial

nanowires concentration (**Figure 4.1c**). Bacterial nanowires act as a response to adverse conditions with high toxic compounds such as heavy metals.

4.4.2 Power density and polarization curves

Power density curves have trends showing the maximum power density that every concentration in the anode and cathode produced at different external resistances (**Figure 4.3**). The maximum power density of 76.8 mW/m^2 at a current density of 326.5 mA/m^2 was reached with Conc. 5 in the anode chamber (**Figure 4.3a**). Similarly, dual MFCs using *S. oneidensis* for the removal of Cu (II) and Cd (II) reported a maximum power density of 92.4 mW/m^2 (Y. S. Xu et al., 2016). Furthermore, mixed cultures used in MFCs for the removal of metal cations from sediments and effluents have reached maximum power densities of 31.9 mW/m^3 for Cu (Y. Wu et al., 2018), 1221.9 and 3781.3 mW/m^2 for Cr (Abbas et al., 2018; M. Li et al., 2018), 3.7 W/m^2 for Cd (II) and Zn (II) (Abourached et al., 2014), 722 mW/m^2 for Cu (II) (A. Singh & Kaushik, 2021), and 189.4 mW/m^2 for Cr (VI), Cd (II), and Ni (II) (Y. Li, Wu, et al., 2015). Additionally, MFC inoculated with *Corynebacterium vitaeruminis* reported a maximum power density of 252.36 mW/m^2 for the removal of Cr (S. Zhao et al., 2021). The data showed similar power densities to MFC treating heavy metals in the cathode chamber.

Higher metal concentration (Conc. 2, Conc. 3 and Conc. 4) significantly reduces the power density, which might contribute to a reduction in electron transport due to high deposition in the bacteria surface or biosorption, especially with high concentrations of Mg. Additionally, with concentration 4 (**Figure 4.3a**) the maximum power density was 29.5 mW/m^2 which is the second highest. These results indicate heavy metal inhibition in the extracellular electron transport, because of their toxicity leading to lower power densities and less metabolic pathways for the bacteria.



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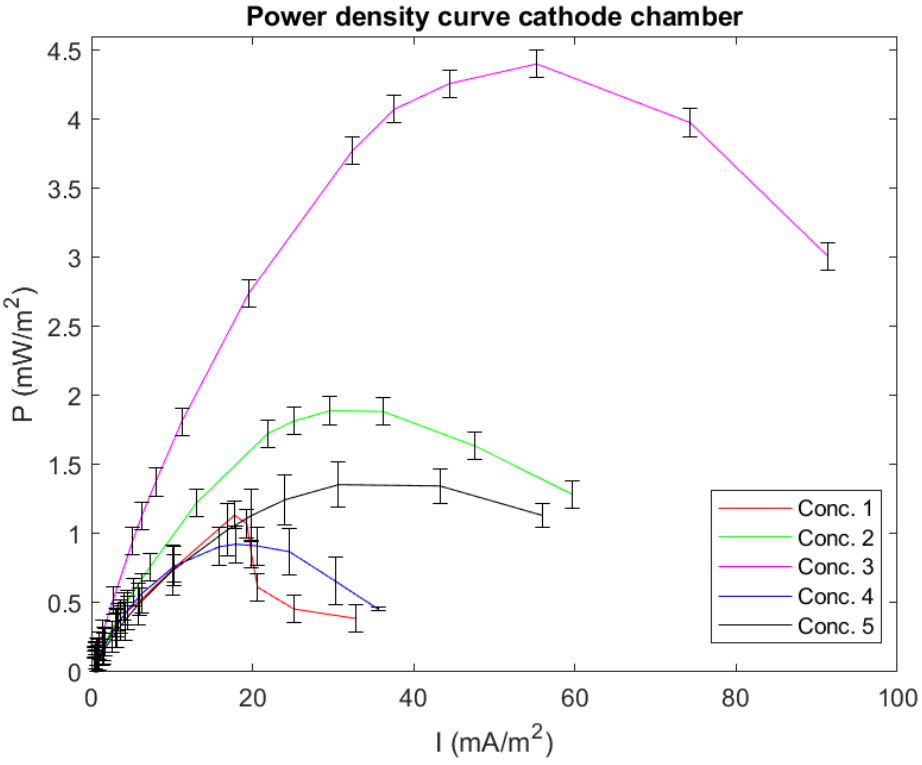
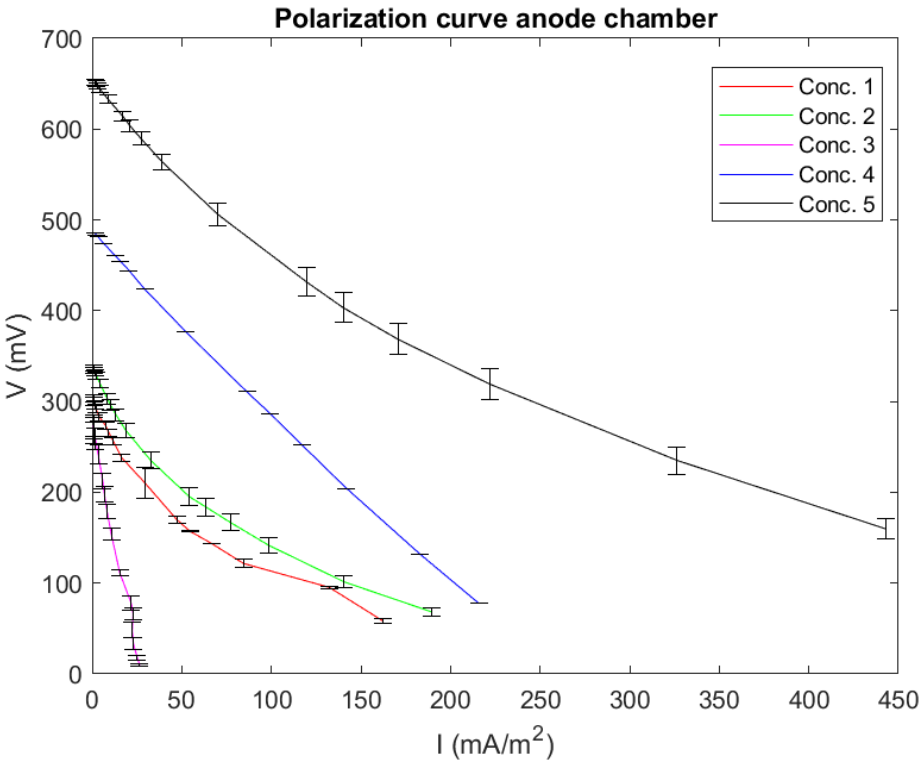


b)

Figure 4.3 Power density curves of metals concentrations in anode and cathode chambers varying external resistance from 100 Ω to 100000 Ω. a) Metal concentrations in anode chamber, and b) metal concentrations in cathode chamber.

Power density curves for metal solutions present in the cathode without the presence of catholyte solution presented a similar behavior in comparison with metals in the anode chamber. A maximum power density of 4.4 mW/m^2 at a current density of 55.3 mA/m^2 was reached with Conc. 3 in the cathode chamber (**Figure 4.3b**). A high concentration of metals increases the cations, allowing the reduction of them, resulting in a higher power output. However, metal concentration 4 (**Figure 4.3b**) affected the reduction process in the cathode due to the negative redox potential of Zn (II), Mg (II), Mn (II), and Na with a high concentration of these metals. Metal concentrations did not significantly affect the power densities between 0.9 and 1.4 mW/m^2 for the concentrations 1, 2, 4, and 5.



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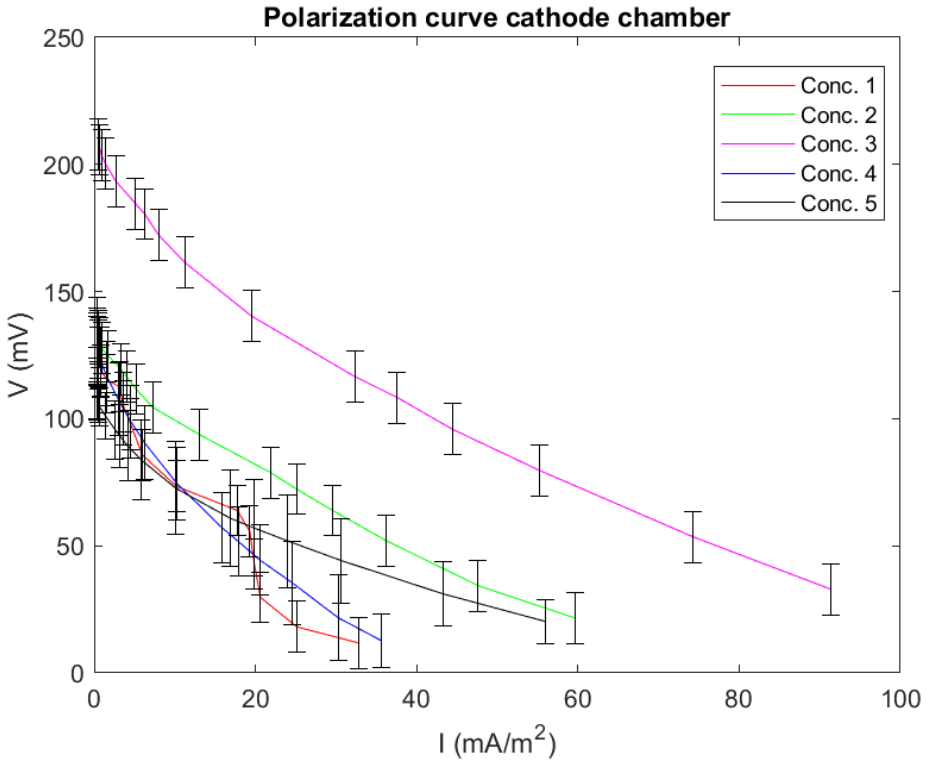


Figure 4.4 Polarization curves of metals concentrations in anode and cathode chambers varying external resistance from 100 Ω to 100000 Ω. a) Metal concentrations in anode chamber, and b) metal concentrations in cathode chamber.

Polarization curves showed a negative correlation with current that is similar to data reported in previous research with mixed cultures (Abbas et al., 2018; Baniasadi & Vahabzadeh, 2021; Kondaveeti et al., 2020; Y. Li, Wu, et al., 2015). The highest voltage at lower resistances is a response of better electron discharge with low stabilization of them through the external circuit. Different metal concentrations in the anode showed a higher voltage potential (**Figure 4.4a**) than the metals in the cathode (**Figure 4.4b**). Additionally, $K_3Fe(CN)_6$ stabilized the voltage at higher resistances due to the higher redox potential of this salt in comparison with the metals in the cathode (**Figure 4.4b**). Conc. 1 had a higher voltage potential of 651.8 mV in the anode because of the lower metal toxicity for the bacteria. This result is higher in comparison with MFC with Cd and Ni removal in the biocathode with a maximum voltage of 525.0 mV (A. Singh & Kaushik, 2021). Furthermore, these results are comparable with the voltage produced using Cu and Cd in the biotic anode with a maximum voltage of 676 mV (Y. S. Xu et al., 2016). Furthermore,

pure cultures with no heavy metals in the solution showed slightly higher voltages (Watson & Logan, 2010), because of the lowest toxicity in the environment. wastewater as catholyte solution showed a lower voltage production (**Figure 4.4b**) with a maximum of 207.9 mV. This result is much lower than data reported using Cr in the cathode with values between 1000 - 1200 mV (M. Li et al., 2018; Y. Li, Wu, et al., 2015), Cr redox potential is higher than the metals studied in this work, increasing the electron transport resulting in higher output voltage. In addition, metal reducing bacteria such as *S. oneidensis* increase the extracellular electron transport with anaerobic and toxic conditions which enhances the output voltage generated with wastewater in the anode chamber.

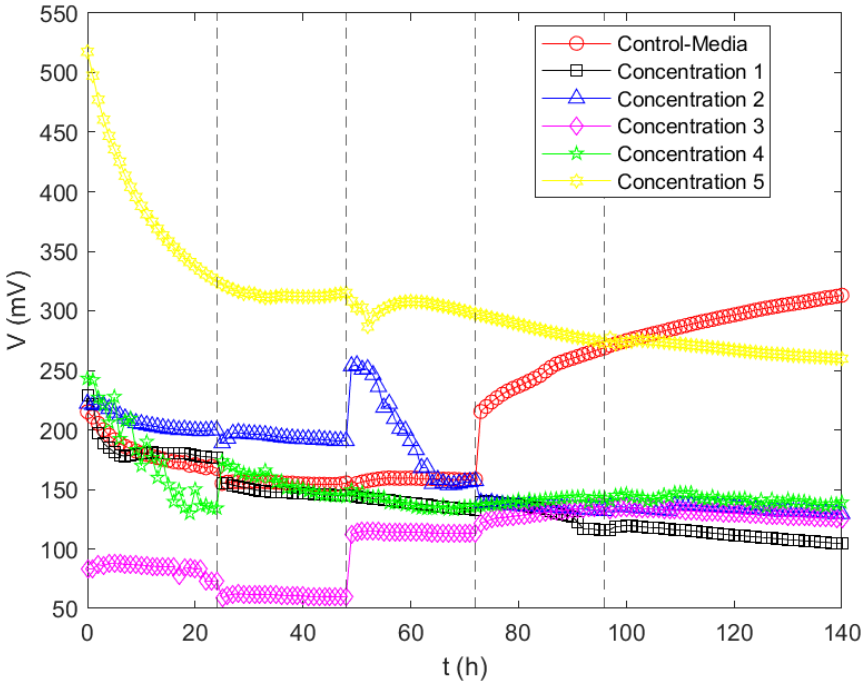
Activation losses at low R_{ext} in the initial stage of the polarization curves showed a decrease in the voltage, then at higher R_{ext} over 800 Ω, the voltage is further decreasing due to ohmic losses represented as the most linear part of the polarization curves, leading to a movement of electrons from substrates through the circuit. Therefore, concentration losses occurred due to the lack of organic carbon source (Bhunia & Dutta, 2018). The reduction of activation, ohmic and concentration losses can be managed by improving the configuration of the MFC. In this investigation, we used $K_3Fe(CN)_6$ in the cathode chamber because it has a better redox potential than oxygen. In addition, the use of *S. oneidensis* with high extracellular electron transport and a continued substrate feeding to avoid concentration losses due to lack of nutrients.

4.4.3 Open-circuit voltage

Open-circuit voltage (OCV) was evaluated using different metal concentrations in the anode and the cathode chambers. Control was performed without metals and ferricyanide on the cathode side. The maximum OCV was at the beginning of the experiment for Conc. 5, Conc. 4, and Conc. 1 with results of 517.6 mV, 243.2 mV, and 517.6 mV, respectively (**Figure 4.5a**). At t = 50 h and t = 96 h, maximum OCV for Conc. 2 and 3 were 254.4 mV and 135.8 mV, respectively. The maximum voltage reached in this research is similar to the maximum values between 453 – 507 mV using *S. oneidensis* in previous studies (Baniasadi & Vahabzadeh, 2021; Bian et al., 2018). However, in these studies the riboflavin concentration was controlled increasing the overall power production of the fuel cell, and a 3D porous carbon electrode was used to facilitate the biofilm formation on its

surface. Moreover, the evaluation of the specific growth rate (μ) for each concentration showed that Conc. 5 has a μ of 0.1133 h^{-1}, which is similar to the control media (0.1103 h^{-1}). Conc. 1, 2, 3, and 4 with μ of 0.1770 h^{-1}, 0.1953 h^{-1}, 0.2036 h^{-1}, and 0.2459 h^{-1}, respectively, demonstrates that the voltage production is directly proportional to the growth of the bacteria.

Metal concentration might be toxic for the bacteria growth, and the stabilization of the bacteria reached after 72 h in the absence of metals and phenol. In these optimal conditions and with a μ of 0.1103 h^{-1}, *S. oneidensis* accelerates the electron transfer reducing the resistance losses and increasing the output voltage (Dai et al., 2021; Y. H. Park et al., 2016; P. Zhang et al., 2017). Additionally, the voltage decreased for all the metal concentrations and stabilization was reached after 72 h of operation, which in this case was the MFC stabilization. Sodium lactate feeding every 24 h for 4 days did not help with the increase in power production because of the overload capacity in the *S. oneidensis* metabolism leading to a reduction of voltage generation.



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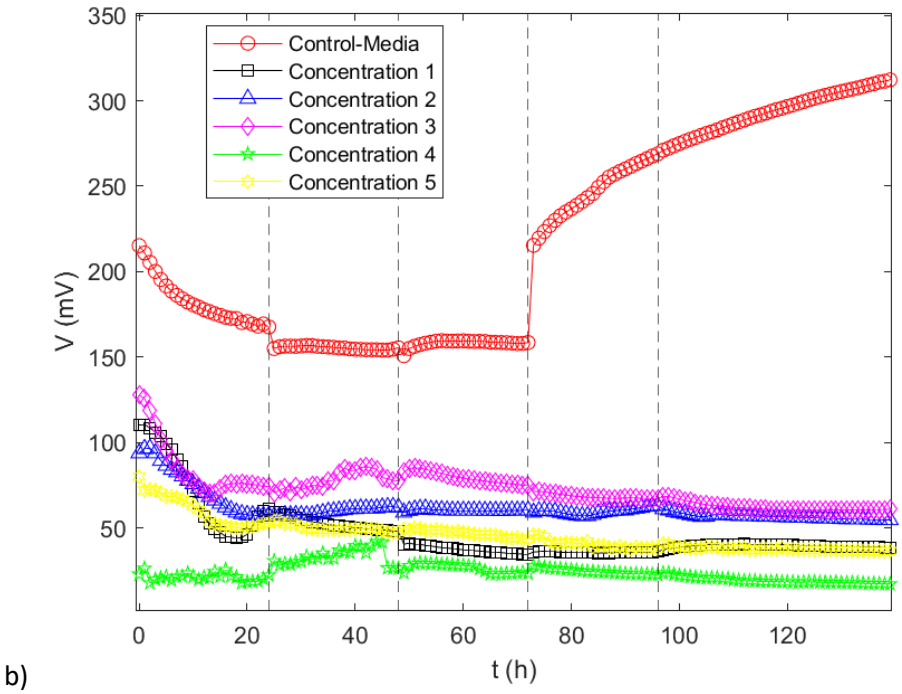

Figure 4.5 Open-circuit voltage (OCV) for MFC operation of 140 h. a) Metals in the anode chamber, and b) metals in the cathode chamber

Metals in the cathode showed a lower voltage production in comparison with the control experiments (**Figure 4.5b**). The maximum OCV was 127.7 mV for Conc. 3, and the minimum was 17.1 for Conc. 4. However, these results revealed a higher voltage production with metals in the cathode versus 0.9 mW in a MFC with electrocoagulant for metal removal in the cathode (Gajda et al., 2017a). In addition, high zinc concentration in the cathode chamber showed a correlation between voltage and time with the same trend as this research (Lim et al., 2021). After 72 h a constant trend in the voltage produced was observed, similar to the results reported with metals in the anode chamber. The constant trend revealed stabilization of the electron transport from the bacteria through the anode and cathode chambers which is shown as OCV.

Voltage dropping was observed to get a constant trend after 72 h (**Figures 4.5a and 4.5b**). The anode revealed a higher OCV than the cathode, indicating a better electron transport with heavy metals in the anode. Moreover, the decrease in power production is affected by nutrient consumption, high concentration of dead cells, and metal inhibition in the *S. oneidensis* respiration (H. J. Kim et al., 2002; Y. Wu et al., 2018). This voltage dropping

also is a response of ohmic, activation and concentration losses that affect the microbial growth and the electron transport. In addition, fouling in cation exchange membrane affects the proton transport from anode to cathode chamber which also affects the OCV.

4.4.4 Heavy metal removal

Heavy metal removal from wastewater was evaluated in the anode and cathode chambers. *S. oneidensis* facilitated metal reduction with the highest concentration removal of Cu which was 93% at Conc. 4, Mg of 97% at Conc. 3, Mn of 98% at Conc. 3, Zn of 96% at Conc. 4, and Na of 61% at Conc. 4 (**Table 4.2**). Phenol concentration in the wastewater achieved metal removals greater than 85%. However, the initial concentration of sodium was high, which resulted in a low removal of 42%. The metal removal was facilitated by oxygen limitation conditions that produced bacterial nanowires that share metabolic pathways with the extracellular membrane (Abbas et al., 2018). The *S. oneidensis* respiration pathway enhances electron transport because of the production of riboflavin that reduces metals simultaneously, as consequence metals can also be deposited on the electrode surface (Abbas et al., 2017). Higher concentrations of metals and phenol do not affect metal removal significantly, indicating the ability of the bacteria to uptake metals for their reduction with higher efficiencies and the capacity of phenol as a carbon source for the bacteria. This result showed that the ability to reduce metals by *S. oneidensis* is improved with higher concentrations of metal acceptors without affecting the toxicity resistance of the bacterium.

Table 4.2 Metal removal after 140 h of MFC operation.

		Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5
Anode Chamber	**Cu**	25%	50%	71%	93%	93%
	Mg	65%	91%	97%	96%	85%
	Mn	79%	88%	98%	96%	93%
	Zn	60%	73%	83%	96%	88%
	Na	44%	45%	45%	61%	42%
Cathode Chamber	**Cu**	60%	82%	92%	99%	98%
	Mg	40%	54%	64%	24%	49%
	Mn	48%	51%	59%	33%	57%
	Zn	58%	61%	47%	39%	59%
	Na	69%	52%	26%	56%	36%

Diverse studies have investigated the removal of single metals in the bio-anode. For example, Ni (II) removal achieved a maximum of 75.0% in the bio-anode (Y. Liu et al., 2019), Cu (II) removal in a soil MFC (J. Zhang et al., 2020) accomplished a maximum of 41.6%, Cu (II) removal in single chamber MFC with an efficiency of 98.3% (Y. Wu et al., 2018). Mixed metals showed a removal above 72.7% in a sedimental MFC for Cr (VI), Cu (II), and Ni (II) (Abbas et al., 2018), 90.0% in a single-chamber MFC for Cd (II) and Zn (II) (Abourached et al., 2014), and 31.0% in the bio-anode of dual MFC for Cd (II) and Pb (II) (Habibul et al., 2016). Our results revealed higher removal rates between 60% and 99% for the mixture of metals Cu, Mg, Mn, and Zn in the wastewater (**Table 4.2**). Additionally, utilization of mixed cultures might enhance the toxicity tolerance of microorganisms improving the efficiency and the metabolism resistance to metals. However, single cultures like *S. oneidensis* enhances metal removal, due to their metal reducing characteristics in the extracellular membrane with the cytochromes *MtrC* and *OmcA* that act as metal terminals for the extracellular reduction (Gude, 2016; Han et al., 2017). The mechanism used by *S. oneidensis* to reduce heavy metals is detailed in **Figure 4.6**.

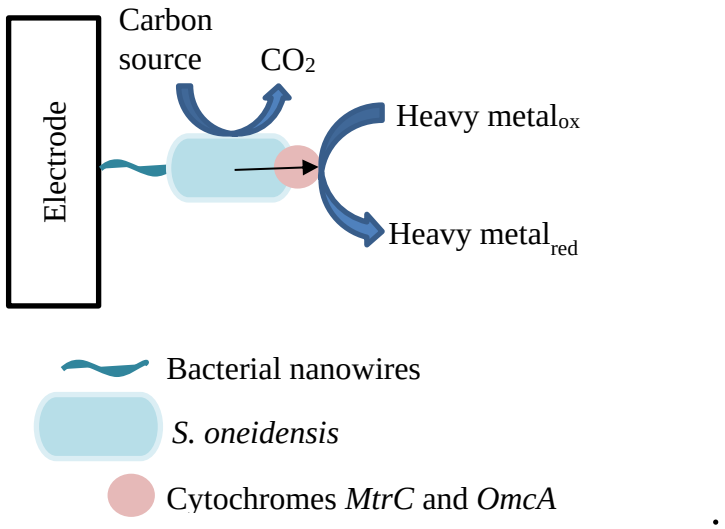

Figure 4.6 Heavy metal removal by extracellular reduction of *Shewanella oneidensis*

Metal removal in the cathode chamber reached a maximum removal for Cu at 99% with Con. 4, Mg at 64% with Con. 3, Mn at 59% with Conc. 2, Zn at 61% with Conc. 2 and Na at 69% with Conc. 5 concentration does not affect metal removal as the values reported are

like those without phenol (**Table 4.2**). The higher redox potential of Cu (II) (+0.34 V) indicates that the reduction of this compound took more electrons for reduction over the other metals, observing higher removals of Cu over Mg, Mn, and Zn. However, Na removal was higher in Conc. 1 due to the high concentration of salt in this solution and competitivity inhibition to metal removal. Additionally, higher metal removal from the anode versus the cathode was related to the higher internal resistance that benefited the metal reduction in the anode (Kaushik & Singh, 2020). The results reported in this study were able to achieve without the use of an external power supply for the reduction of metals with low redox potential such as Zn, Mg, Mn, and Na. Reduction of Zn (II) with 96% of removal efficiency (Lim et al., 2021) is comparable to the Zn removal efficiencies in **Table 4.2**. Moreover, the reduction of a mixture of metals in the cathode with Zn, Cu, and Cd with the removal of 92%, 87%, and 84%, respectively in 2 dual MFCs in parallel (X. Wang et al., 2016). Biocathode with high metal removal efficiency could lead to an MFC with bioanode and biocathode for metal removal in both chambers, improving the overall removal and the efficiency of this treatment.

4.5 Conclusions

MFCs inoculated with *S. oneidensis* in the anode chamber showed a high metal removal efficiency of over 80%. Moreover, bacterial nanowires allowed a higher electron transport which was traduced in better power and OCV, this performance was better at Conc. 5 that has phenol concentration of 354 mM in the anode chamber. It was demonstrated that metal wastewater in the cathode chamber generated lower power and OCV. However, heavy metal removal had comparable efficiencies to the wastewater fed in the anode chamber. Voltage generated was directly proportional to the bacterial growth, showing that control media and Conc. 5 had specific growth rate close to 0.1100 h^{-1}. Dual chamber MFC is convenient for heavy metal removal in anode and cathode chamber; however, our results reported better performances using the synthetic desalter effluent in the anode chamber and $K_3Fe(CN)_6$ in the cathode chamber.

4.6 References

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Chapter 5

5 Investigation of rhamnolipid – metal complex from retentate for heavy metal removal in microbial fuel cell with *Shewanella oneidensis* MR-1

The information of this chapter is based on the paper: **Munoz-Cupa, C., et. al. (2023). Investigation of Rhamnolipid Addition on the Microbial Fuel Cell Performance and Heavy Metal Capture in Metal Laden Wastewater. *Water Process Engineering*. Submitted.** The sections in this chapter present the results for the competition of objective 4 (see section 1.2).

5.1 Abstract

Wastewater loaded with metals was investigated in this study for their remediation, it was used a dual chamber microbial fuel cell aided with rhamnolipid biosurfactant at 100 mg/L and 500 mg/L in the anode and inoculated with *Shewanella oneidensis* MR-1, and $K_3Fe(CN)_6$ 50 mM in 100 mM of PBS was used in the cathode chamber. The bacteria demonstrated a higher specific growth rate of 0.11 h^{-1} and better biofilm formation analyzed by the SEM. The highest power density was 13.9 mW/m^2 determined at 100 mg/L of rhamnolipid. Furthermore, the internal resistance for the same concentration was 2.8 Ω, which indicates the low resistance of electrons through the external circuit. Metal removal had the highest removal of 60.2%, 89.5%, 83.9%, 90.6%, and 47.9% for Cu, Mg, Mn, Zn, and Na, respectively, corresponding to the wastewater coupled with rhamnolipid at concentration of 500 mg/L. Furthermore, COD removal was 28.7% and 17.0% for biosurfactant concentrations of 100 mg/L and 500 mg/L, respectively. These results demonstrated the high efficiency of microbial fuel cells with wastewater loaded with rhamnolipid.

5.2 Introduction

Water pollution from heavy metals and phenolic compounds have a toxic impact on water streams. These pollutants have bioaccumulation due to their non-biodegradability in the environment (Kahlon et al., 2018). Moreover, heavy metals like lead and mercury are

highly toxic at low concentrations and impact human health, affecting cells and producing adverse effects like cancer (Zamora-Ledezma et al., 2021). Industrial processes have increased the metal concentrations in water streams, increasing negative impacts. Conventional heavy metal removal methods such as adsorption, ultrafiltration, reverse osmosis, electrodialysis, precipitation, coagulation, flotation, and ion exchange are selected according to operational costs, the concentration of metal ions, environmental impact, chemicals added, and removal efficiency (Qasem et al., 2021). These methods have disadvantages, such as toxic chemical sludge and low efficiencies at low heavy metal concentrations (K. Yin et al., 2019).

Non-conventional heavy metal removal methods have gained importance due to their characteristics related to biodegradability, high removal efficiencies, and low cost. These non-conventional methods include microbial remediation, surfactant aid removal, and fuel cells. Recently, surfactant remediation was investigated to remove heavy metals from wastewater streams, mine water, and industrial sludge (Elouzi et al., 2012). Among them, biosurfactants such as glycolipids, lipopeptides, fatty acids, and phospholipids bind heavy metals (Mulligan, 2005). Rhamnolipid (RHL) is an anionic biosurfactant produced by *Pseudomonas* species. It comprises one or two rhamnose sugar heads with one or two b-hydroxy decanoic acid tails (Jahan et al., 2020). The metabolic pathway involves 3 reactions for the synthesis of the fatty acid tail by Rh1A genes. The genes Rh1B uses deoxythymidine diphosphate (dTDP)-L-rhamnose sugars and 3-(3-hydroxyalkanoyloxy)alkanoic acid (HAA) for mono-rhamnolipid production. Finally, mono-rhamnolipid and dTDP-L-rhamnose with Rh1C gene is used for di-rhamnolipid generation, which is commercially available for laboratory experiments (Soberón-Chávez et al., 2005). Moreover, RHL concentration above critical micellar concentration (CMC) forms micelles, enhancing the binding of metals to their structure (Verma & Sarkar, 2019). The metal-micelle complex can be separated by physical processes like ultrafiltration, where the filtrate is the clean wastewater, and the retentate has the metal-micelle complex accumulated (Lin et al., 2017).

Bioremediation of heavy metals using bacteria has the advantage of fast growth rates and wide range of applications due to their metabolic pathways that can degrade organic

pollutants. Moreover, mechanisms for metal remediation, such as absorption and extracellular reduction, are commonly found in some species, such as *Bacillus*, *Shewanella*, and *Pseudomonas* (K. Yin et al., 2019). Bioremediation by electrochemical systems such as microbial fuel cells (MFCs) has gained importance due to their simultaneous power generation and pollutant remediation.

MFCs are divided into two main types: single chamber and dual chamber. Single chamber MFCs where the anode is separated from the cathode by a membrane; however, the cathode is exposed directly to air indicating no requirement for a cathode section (Munoz-Cupa et al., 2021; Ramya & Senthil Kumar, 2022). Dual chamber MFCs are the most used and consist of two sections separated by a semipermeable membrane; one section is the anode with the nutrients and the microorganism, and the second is the cathode with a catholyte solution or oxygen as reduction agents. Additionally, MFCs are composed of electrodes in the anode and cathode chambers connected through an external circuit (Greenman et al., 2021). The microorganisms oxidize the anolyte solution, and the electrons generated from this process move through the external circuit to the catholyte solution for reduction. The electrons' movement through the circuit generates electricity; however, electron transportation requires biofilm formation on the electrode (Ramya & Senthil Kumar, 2022).

MFCs have recently gained attention for heavy metal remediation in the anode and cathode chambers. For example, copper, chromium, cadmium, zinc, and iron were investigated for treatment using single-chamber MFCs (Ezziat et al., 2019). Nevertheless, chromium has been widely investigated due to its high redox potential and high toxicity (Mathuriya & Yakhmi, 2014). However, most studies have used cathodic reduction reactions for heavy metal remediation (C. Fang & Achal, 2019). MFCs for heavy metal remediation in soil have shown viability in their use independently of the mixed cultures involved in soil (Gustave et al., 2021; Y. Sun et al., 2022). Soil remediation has shown removals for Cu and Cr of 87.62% and 52.35%, respectively (J. Zhang et al., 2020, 2022). Moreover, the removal of heavy metals in the cathode chamber has been proven by other authors. Cr (VI) removal of 98.63% with a dual MFC inoculated with *Corynebacterium vitaeruminis* showed a higher performance in the bio-cathode (S. Zhao et al., 2021). Moreover, Ag removal of 99.8% with mixed culture (Almatouq et al., 2022), Zn removal of 96% from

industrial wastewater (Lim et al., 2021), and Au removal efficiency of 95% with yeast in the anode chamber (Al-Asheh et al., 2022), showed high efficiencies for single metal-polluted wastewaters at the cathode chamber. In the anode chamber, wastewater polluted with Cr and Cu showed a removal efficiency of 80.70% and 72.72%, respectively (Abbas et al., 2018).

Surfactants such as sodium dodecyl sulfate (SDS) were investigated for removal from water in MFCs. The study showed the removal of 70% of SDS, demonstrating the ability of mixed cultures to take complex organic molecules for their metabolism (I. Chakraborty et al., 2021). Moreover, palladium α-lipoic acid nano-complex compound (PLAC) added to the anode inoculated with *Shewanella oneidensis* demonstrated higher power production due to higher cell permeability (Gomaa et al., 2021). Also, the addition of SDS showed a power 98 times higher than without surfactant in single chamber MFC. In addition, the process performed oil wastewater treatment contributed to higher efficiency in using MFC (Hwang et al., 2019). Furthermore, adding biosurfactants such as trehalose lipids in MFC with *Rhodococcus pyridinivorans* showed high electricity production (P. Cheng et al., 2018). Rhamnolipid enhanced electroactive microorganisms such as *Geobacter* and *Desulfovibrio*. Consequently, hydrocarbons' power and removal are enhanced (Ambaye et al., 2022). Biosurfactant-producing bacteria (*Brevibacillus*) enhance power production while producing biosurfactants in the MFC anode (Naik & Jujjavarapu, 2021). Rhamnolipid was also added to *Shewanella* species proving a higher efficiency for oil remediation from soil (Joe et al., 2019).

This chapter proposes a MFC process for heavy metal remediation using MFC inoculated with *Shewanella oneidensis*. In this investigation, the heavy metal wastewater comprised copper, zinc, magnesium, manganese, sodium, and phenol. wastewater simulates industrial wastewater with high salt concentrations. The heavy metal wastewater is complexed with rhamnolipid to increase metal removal and voltage generation. The process was performed under batch conditions in the anode chamber. The operation of the MFC was evaluated with polarization curves, open-circuit voltage, biofilm formation, and heavy metal removal.

5.3 Materials and methods

5.3.1 Synthetic desalter effluent

Heavy metals concentration in the wastewater loaded with rhamnolipid was utilized from previous MEUF ultrafiltration study (Munoz-Cupa et al., 2022). The initial concentration of the metals is reported in **Table 5.1**. The solutions were mixed with rhamnolipid concentrations of 100 and 500 mg/L. The rhamnolipid solutions were prepared using the volume from the retentate with rhamnolipid at these two concentrations, the procedure is explained in a previous study with the wastewater.

Table 5.1 Total metal ions fed into the anode chamber.

Compound present in wastewater	Rhamnolipid Concentration 100 mg/L	Rhamnolipid Concentration 500 mg/L
Cu	10.48	10.30
Mg	331.00	324.00
Mn	5.40	5.60
Zn	11.00	12.80
Na	1210.00	1210
Phenol	354 mM	354 mM

5.3.2 Microbial culture

Shewanella oneidensis MR-1 from culture ATCC® 700550™ was cultivated with tryptic soy broth (TSB) for 24 h, at 150 rpm and 29°C. The bacteria growth was evaluated by UV spectrophotometer at 600 nm, and the optimal growth for MFC inoculation was between 0.2 and 0.4. To ensure aseptic conditions, the cultivation of the single culture was performed in a laminar flow to avoid cross contamination with other microorganisms. Moreover, the MFC operation was performed in an incubator to avoid external contamination and to keep a constant temperature of 28 °C.

5.3.3 Microbial fuel cell device

A dual chamber MFC fabricated by borosilicate glass with a volume capacity of 500 mL separated by a cation exchange membrane (CMI-7000A) with an area of 8.55 cm^2. The anode and cathode chambers used carbon felt electrodes (36 cm^2), These were connected using copper wires and an external resistance box (**Figure 5.1**). The Anode chamber was

inoculated with *S. oneidensis* and M9, TSB, and sodium lactate solution. Catholyte solution of $K_3Fe(CN)_6$ 50 mM in 100 mM PSB was fed into the cathode chamber.

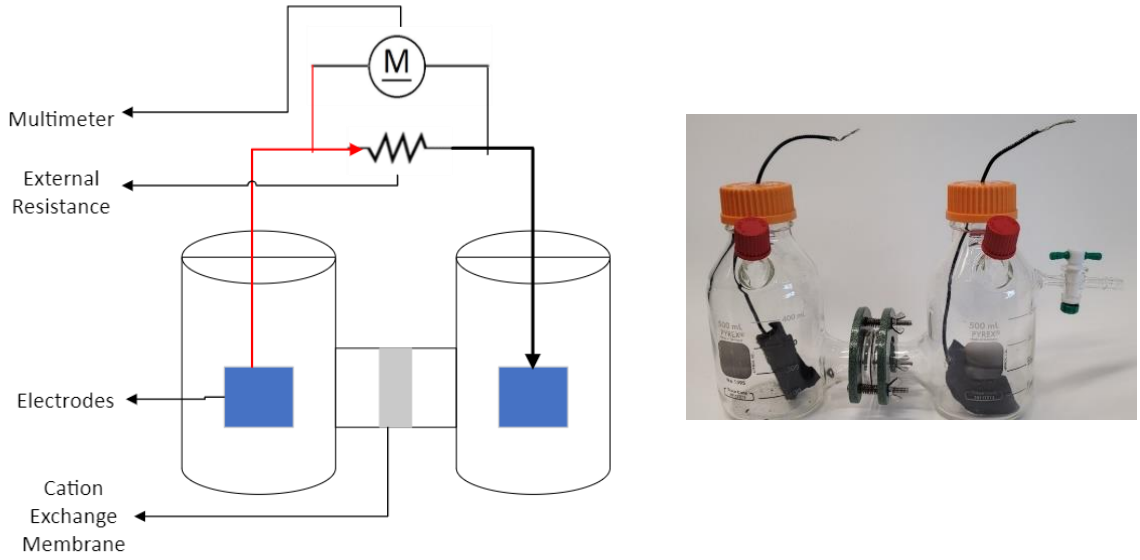


Figure 5.1 Dual-chamber microbial fuel cell set-up.

5.3.4 Microbial fuel cell operation and data acquisition

The Anode chamber was inoculated with retentate wastewater containing metals and rhamnolipid with TSB and M9 for 12 h before data uptake from voltage generation. The inoculum at optical density (OD_{600nm}) between 0.3 – 0.5 was used in the anode chamber. The catholyte solution was composed of $K_3Fe(CN)_6$ at 50 mM dissolved in 100 mM PBS. A cation exchange membrane CXM-200S (CMI-7000S) with a thickness of 0.45±0.025 separates the anode and cathode chambers. The membrane was pre-treated with washes of H_2O_2 (3%), deionized water (DI) water, and H_2SO_4 (500 mM) for one h each at 80 °C. Additionally, the anode chamber with wastewater was used in this study due to the higher redox potential of $K_3Fe(CN)_6$ for the cathode chamber.

The anode and cathode chamber were connected through an external resistance box with a constant resistance of 1000 Ω. Voltage and power generation were recorded using a data logger multimeter OW18 with an accuracy of 0.5%±2. Polarization and power density curves were determined by varying the external resistance from 0.1 kΩ to 100 kΩ. Current

density (I) and power density (P) were calculated using Watt's and Ohms. The internal resistance (Ri) was calculated from the polarization curves using the slope at current densities of 0 (Equation 5.1). After stabilization for 12 h, the voltage was measured every hour for 140 h in each experiment.

$$Ri = -\frac{\Delta V}{\Delta I} \qquad (5.1)$$

Biofilms in electrodes were fixed for 48 h at 4 °C in glutaraldehyde 2% in 0.1 M phosphate buffer (pH 7.4). After fixation, the samples were pre-treated in Biotron at Western. Samples were washed three times with 0.1 M phosphate buffer and one time with DI water. Then, samples were fixed in 2% Osmium tetroxide (aqueous) for 1 hour. After fixation, samples were rinsed three times for 5 min with phosphate buffer. Then the samples were gradually dehydrated in ethanol solutions with increasing concentrations (30%, 50%, 70%, 90%, and 100%) for 5 min each. Finally, samples were dried by ascending concentrations of hexamethyldisilazane (HMDS) diluted in 100% ethanol (25%, 50%, 75%, 90%, and 100%). The samples were coated with 4 nm of iridium for the scanning electron microscopy (SEM) analysis taken in Surface Science Western using the instrument Hitachi SU8230 Regulus Ultra High-Resolution Field Emission SEM.

Rhamnolipid concentration was evaluated using the methylene blue complexation method (Pinzon & Ju, 2009; F. Zhao et al., 2016). The samples were adjusted to a pH of 2.3±0.2 with 1 N HCl. 100 µL of methylene blue (1 g/L) previously adjusted to a pH of 8.6±0.2 were added to a 5 mL of acidified sample. Then, 200 µL of borax solution 50 mM and 4 mL of chloroform ($CHCl_3$) were added to the previous mixture. The samples were vigorously mixed for 1 min and left to stand for 5 min. The chloroform phase was then read using a UV spectrophotometer to measure the absorbance at 638 nm. A calibration curve (**Appendix 2**) was used for the conversion of absorbance into rhamnolipid concentrations; the curve was measured using standard rhamnolipid solutions at different rhamnolipid concentrations. After every batch cycle, the samples were centrifuged at 7000 g for 20 min. The supernatant was evaluated using ICP-OES for the determination of total metals in the liquid solution. The COD was evaluated using a HACH spectrophotometer DR 3900, with COD kit TNT 822. The vial kit was turned upside down before putting 2

mL of sample, the vial containing sample was mixed and leave in a digestor for 2 h at 150 °C. The digested vial was left at room temperature for 20 minutes and placed into the HACH spectrophotometer for reading.

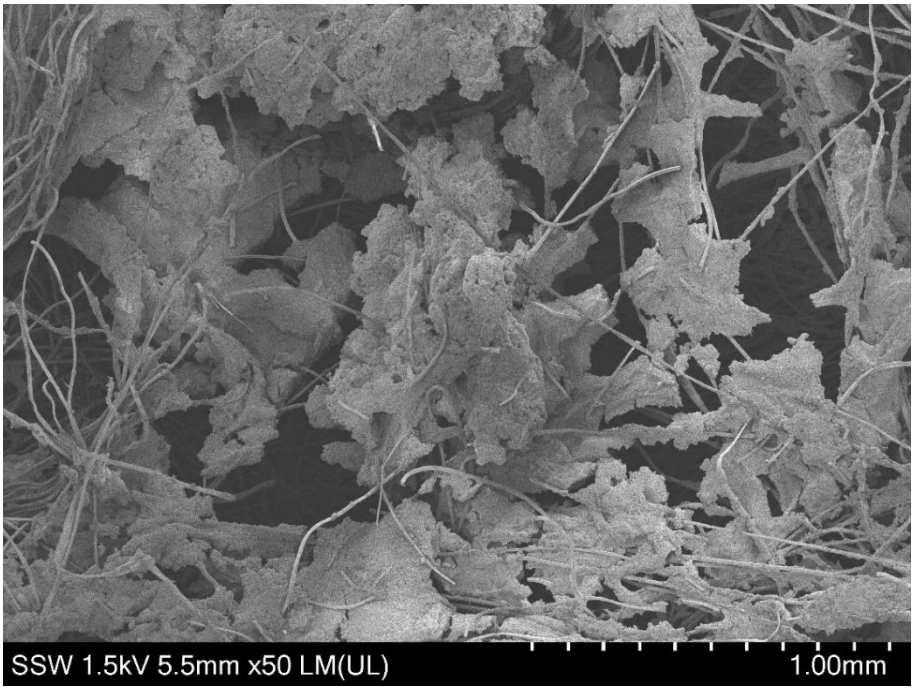
5.4 Results and discussion

5.4.1 Rhamnolipid effect on biofilm and bacterial grow

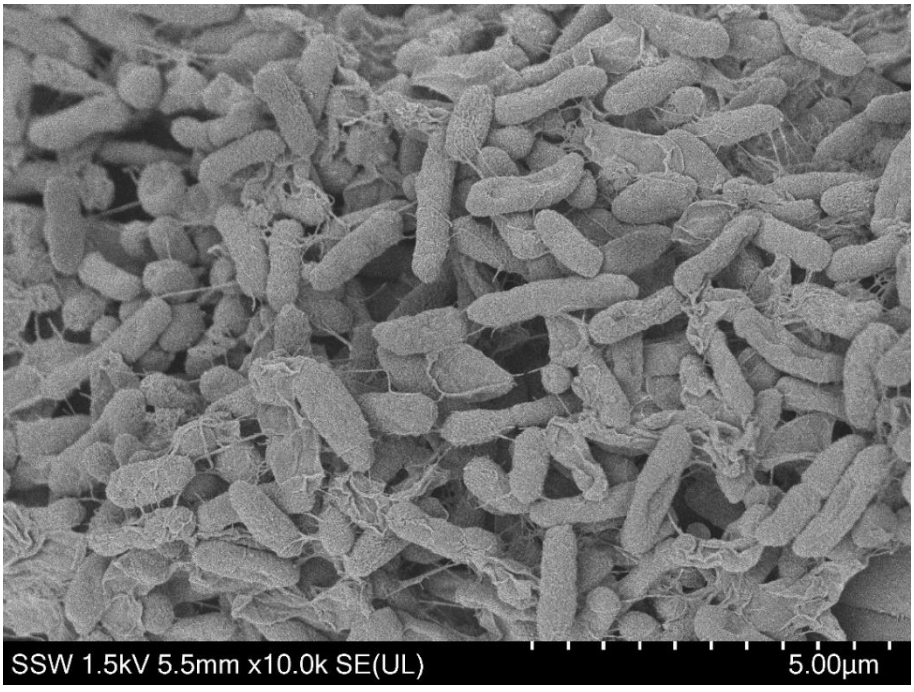
Figure 5.2 indicates the anode chamber's SEM images of the biofilm on the electrode surface. The biosurfactant forms clusters in the carbon felt fibers (**Figure 5.2a**), which increase the attachment to the electrode surface. *S. oneidensis* nanowires were observed in the biofilm at a rhamnolipid concentration of 100 mg/L (**Figure 5.2b**), representing a higher extracellular electron transport. Bacterial nanowires are periplasmic extensions of the extracellular membrane (Pirbadian et al., 2014). Additionally, some species, such as *Shewanella xiamenensis*, have metal resistance, and some of them increase the presence of bacterial nanowires such as Zn and Cu (I.-S. Ng et al., 2015).

At 500 mg/L, the biosurfactant harms the extracellular membrane. It was observed that the permeability of the extracellular membrane is high. Consequently, the membrane is broken, which affects the bacteria's growth (**Figure 5.2c**). In another study, rhamnolipid biofilm with *Geobacter sulfurreduncens* was found to be low at 40 mg/L of biosurfactant (Y. Zhang et al., 2018). Moreover, a previous study observed that at a rhamnolipid concentration of 120 mg/L, the biofilm clusters have a high concentration comparable to SEM reported in this research (Y. Zhang, Jiang, et al., 2017). Also, other authors reported that low concentrations of rhamnolipid with *Bacillus subtilis* demonstrated an outer coat in the cells (Sotirova et al., 2012). Other surfactants reported in previous studies revealed higher biofilm formation on the electrode surface at low concentrations. For example, the use of polysorbate detected better biofilm formation than no use of this surfactant (Tominaga et al., 2022). However, when the concentration of surfactants such as SDS is high, the population found in the biofilm is less; this was observed in a previous study (Song et al., 2015). The appearance of the cells also is affected, as shown in **Figure 5.2b**, which shows a biosurfactant coating compared with the bacteria with no presence of rhamnolipid in the anode chamber (**Figure 5.2d**).

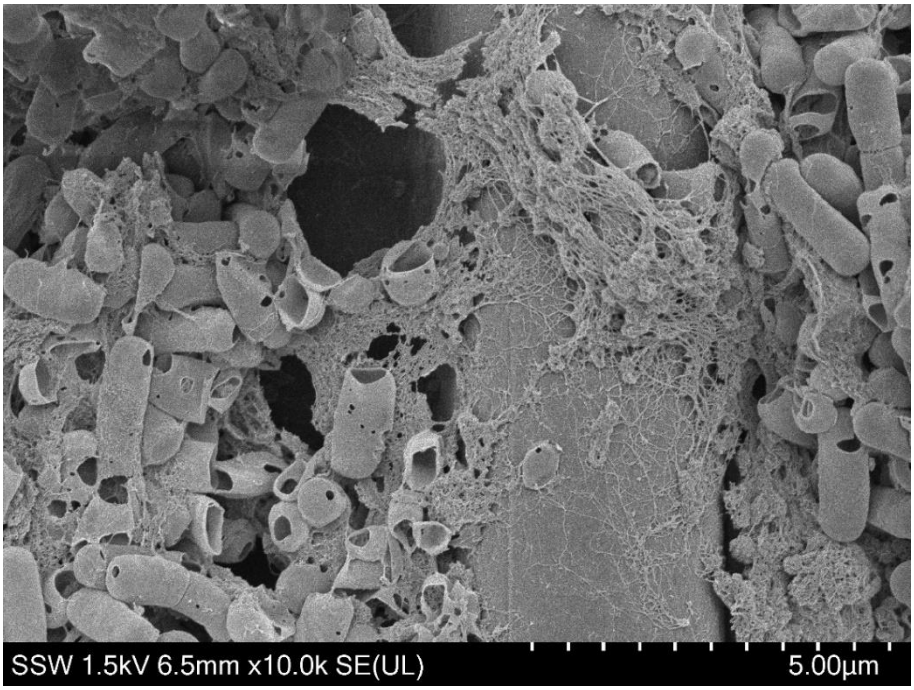
a)



b)



c)



d)

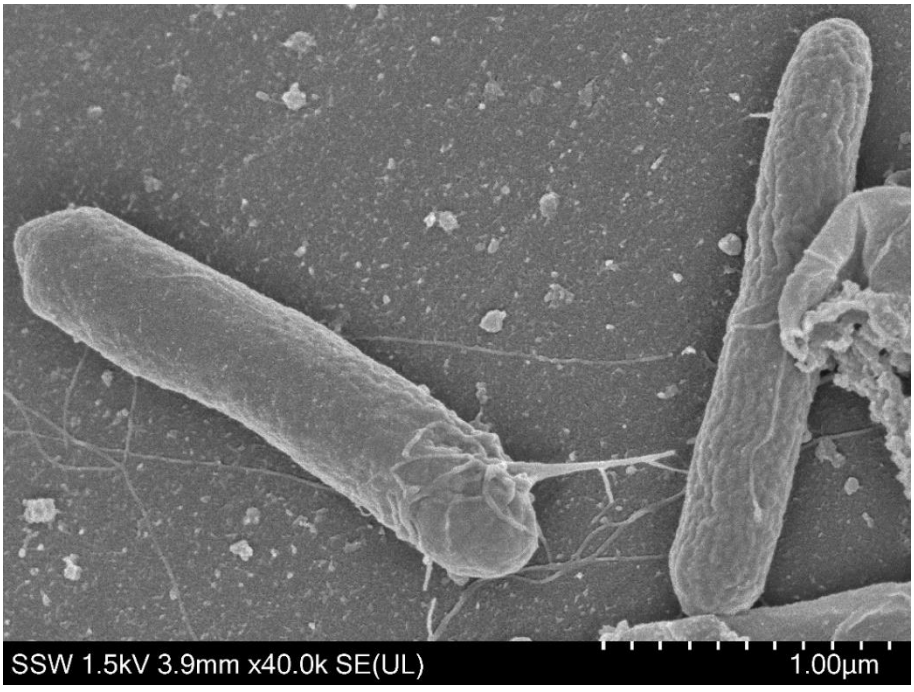


Figure 5.2 SEM images from carbon felt electrodes from anode: a) surfactant – bacteria biofilm on carbon felt fibers, b) bacteria – rhamnolipid clusters on carbon felt demonstrating presence of bacterial nanowires, c) extracellular membrane damaged by high rhamnolipid concentration (500 mg/L), and d) bacteria without presence of rhamnolipid in the anode chamber**.**

The results in a biofilm also are comparable to the bacterial growth analyzed by the specific growth rate (μ) and the double time (t_d), calculated from the exponential phase in the growth curve at an optical density of 600 nm which is presented in **Figure 5.3** and **Table 5.2**. The specific growth rate has similar results in the media and the rhamnolipid concentrations of 100 mg/L, indicating the viability of the bacteria to grow under this biosurfactant concentration. Moreover, the double time is approximately 6 h, the same concentrations of 100 mg/L with wastewater and the media. However, at a rhamnolipid concentration of 500 mg/L, the bacteria require more time to grow with double times above 17.3 h, which confirms the break in the extracellular membrane observed in **Figure 5.2c** and the low growth under this biosurfactant condition.

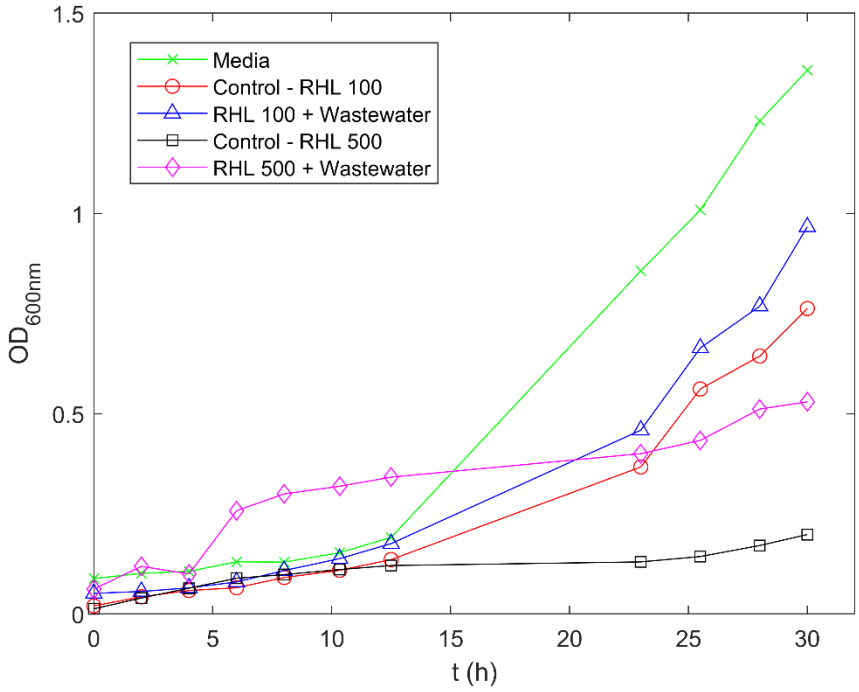


Figure 5.3 *Shewanella oneidensis* MR-1 growth under different rhamnolipid – metal complex concentrations.

Table 5.2 Specific growth rate (μ) and double time (t_d) under different rhamnolipid conditions and tryptic soy broth media.

	Media	RHL 100 mg/L - Control	RHL 100 mg/L + Wastewater	RHL 500 mg/L - Control	RHL 500 mg/L + Wastewater
μ (h^{-1})	0.112	0.105	0.110	0.030	0.040
t_d (h)	6.2	6.6	6.5	23.5	17.3

5.4.2 Polarization curves and voltage generation

Figure 5.4 shows the power density and polarization curves, which were evaluated by varying the external resistances from 0.1 kΩ to 100 kΩ. The maximum power densities observed in the control experiments using rhamnolipid without wastewater concentration were 67.8 mW/m^2 and 13.9 mW/m^2 for rhamnolipid concentrations of 100 mg/L and 500 mg/L, respectively (**Figure 5.4a**). Moreover, with the addition of metal wastewater, the maximum power densities were 37.6 mW/m^2 and 13.6 mW/m^2 for rhamnolipid concentrations of 100 mg/L and 500 mg/L, respectively (**Figure 5.4b**). Wastewater showed a reduction with rhamnolipid concentration of 500 mg/L of 24.0 mW/m^2. However, at a rhamnolipid concentration of 100 mg/L, the power density decreased by 56%. In addition, voltage dropping in polarization curves demonstrated a similar trend for control and wastewater experiments. The maximum voltage was for rhamnolipid at 100 mg/L with 408.0 and 360.2 mV values for control and wastewater, respectively.

Previous studies demonstrated different power densities using surfactants and biosurfactants to treat hydrocarbons and organic pollutants in soil and wastewater (**Table 5.3**). From these studies, trehalose lipids showed an excellent performance increasing the power density from 54.7 mW/m^2 to 324.0 mW/m^2. The higher power production at 100 mg/L of rhamnolipid proves a better energy conversion at lower rhamnolipid concentrations due to a less toxic environment and less damage to the *S. oneidensis* extracellular membrane, in comparison to the 500 mg/L rhamnolipid concentration. Removal of organic compounds using rhamnolipid addition have been investigated for a period of 20 days using soil in a dual MFC (Ambaye et al., 2022), 15 h with single-chamber MFC with glucose (Wen, Kong, et al., 2010), and 30 days with a dual MFC with oily wastewater coupled with rhamnolipid (Y. Zhang, Zhao, et al., 2017). However, long-term

and continuous operation requires further investigation to evaluate the possible effects of rhamnolipid in bacteria growth and microbial fuel cell performance.

Table 5.3 Comparison of maximum power density generated with different surfactants.

Surfactant	Maximum Power Density (mW/m^2)	Type of MFC	Reference
SDS	12.7	Cylindrical MFC	(I. Chakraborty et al., 2021)
SDS	221.5	Single-chamber MFC	(Hwang et al., 2019)
Triton X-100	2.5±5	Single-chamber MFC	(Hwang et al., 2019)
Trehalose lipid	324	Single-chamber MFC	(P. Cheng et al., 2018)
Sophorolipid	15.29	Single-chamber MFC	(Shen et al., 2014)
Biosurfactant	10.6	Dual-chamber MFC	(J. Liu & Vipulanandan, 2017)
Rhamnolipid	67.8	Dual-chamber MFC	This study

The results indicated that low biosurfactant concentration in the anode allows better electron transport due to lower resistance values than 500 mg/L of biosurfactant added. The internal resistance calculated by the slope method from the polarization curve (voltage dropping) showed a low resistance at rhamnolipid at 100 mg/L with results of 2.13 Ω and 2.83 Ω for control and wastewater, respectively. At 500 mg/L, 9.27 Ω and 14.83 Ω were observed for internal resistances. Previous studies demonstrated that rhamnolipid and Tween 80 in air-cathode MFC decreased the internal resistance from 29 Ω to 5 Ω and from 27 Ω to 5.7 Ω, respectively (Wen et al., 2011; Wen, Kong, et al., 2010). Furthermore, other biosurfactants studied, such as sophorolipid, showed low resistance to electron flow (Shen et al., 2014). Other authors indicated low internal resistances of 254.1 Ω (I. Chakraborty et al., 2021), 366 Ω (P. Cheng et al., 2018), and 15 Ω with wastewater and high content of SDS (Radeef & Ismail, 2021), SDS at 20 mg/L showed internal resistance of 33.1 Ω (Song et al., 2015), suggesting that the addition of surfactants decrease the internal resistances which is related to the increase in electron transport due to high permeability in the extracellular membrane.

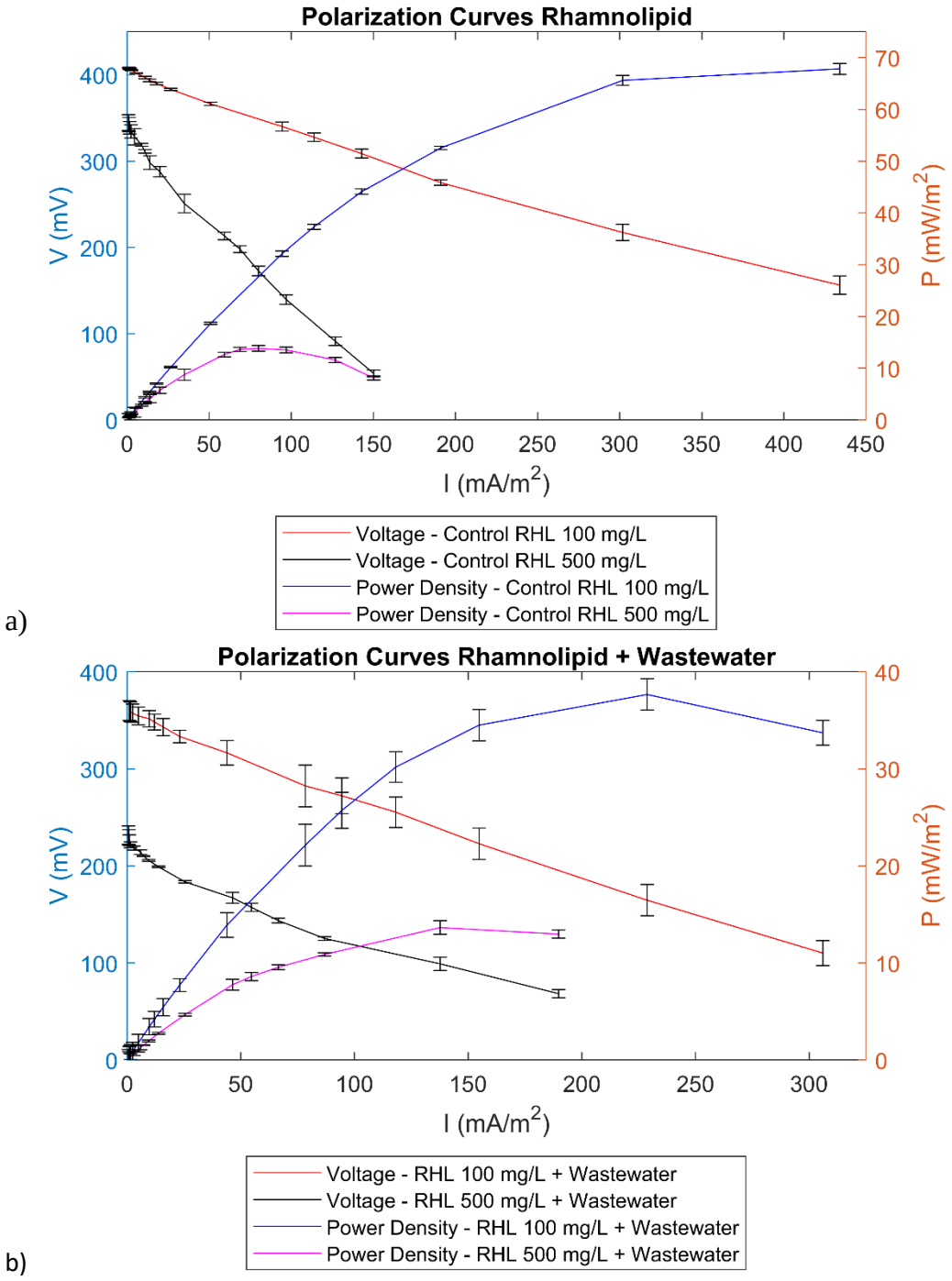


Figure 5.4 Polarization and power density curves for rhamnolipid concentrations of 100 mg/L and 500 mg/L for: a) rhamnolipid fed into the anode chamber (control), and b) rhamnolipid and wastewater fed into the anode chamber.

Figure 5.5 shows the open-circuit voltage (OCV) trend during 140 h of MFC operation with data acquisition every hour for rhamnolipid concentrations in the anode chamber of

100 mg/L and 500 mg/L. The maximum OCV was 344.0 mV, and 335.5 mV for control and wastewater with 100 mg/L of biosurfactant added. At 500 mg/L, the maximum OCV was 213.7 mV and 189.5 mV for control and wastewater, respectively. TSB and sodium lactate 18 mM were added every 24 h for 4 days; this procedure revealed different voltage peaks due to more availability of electrons from the substrate to be oxidized by the bacteria. However, the OCV did not increase significantly with the addition of substrate to the anode chamber because the OCV is a response of the redox potential between anode and cathode and not the concentration of the substrate. Additionally, the increase in the substrate or electron donor was not significant to change the redox potential enough for increasing the OCV (Logan, 2008; Mostafa et al., 2010). Moreover, in previous studies, this performance was observed with SDS and sophorolipid evaluated in 4 phases with 4 peaks at the beginning of each cycle (I. Chakraborty et al., 2021; Shen et al., 2014). The biosurfactant increased the extracellular electrochemical activity in the bacteria at 100 mg/L concentration. However, higher biosurfactant concentrations negatively impact the OCV with a reduction in the maximum output of 37.9% and 43.5% for control and wastewater in the anode, respectively.

High voltage generation was observed in contaminated soil with hydrocarbons and biosurfactant addition for high voltage generation due to the increase in the extracellular permeability and reduction in internal resistance (Ambaye et al., 2022; X. Li et al., 2018; Wen, Kong, et al., 2010; X. Xu et al., 2015; Y. Zhang, Jiang, et al., 2017). In addition, the dropping in the voltage shows the slow mass transfer from the substrates to the bacteria due to concentration losses and ohmic losses (Logan, 2008; Y. Xiao et al., 2017). The results consistently indicate that rhamnolipid at 100 mg/L performs better in the OCV due to less extracellular damage in the bacteria. However, not all surfactants in MFC enhance efficiency; SDS and Triton X-100 added into a single chamber MFC for wastewater treatment showed much lower OCV with a maximum of 55 mV (Hwang et al., 2019).

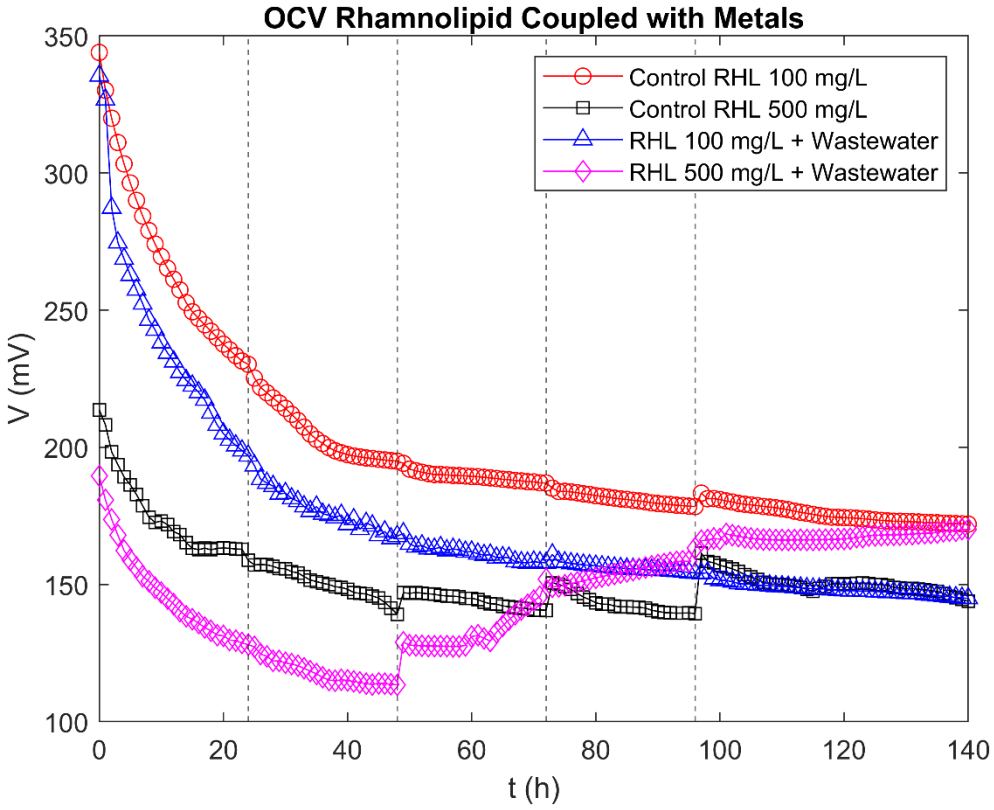


Figure 5.5 OCV in dual MFC for rhamnolipid concentrations of 100 mg/L and 500 mg/L in control and wastewater fed into the anode chamber.

5.4.3 Effect of rhamnolipid concentration on heavy metal removal and COD

ICP-OES evaluated metals on the wastewater after 140 h of MFC operation in Bureau Veritas. The metals evaluated were Cu, Mg, Mn, Zn, and Na. Metal removal from metal wastewater solutions was found to be the highest for Mg and Zn, with values of 79%±0.2 and 89%±1 for rhamnolipid concentrations of 100 mg/L and 500 mg/L, respectively. The lowest removal efficiencies were for Na, with values of 26% and 48% at 100 mg/L and 500 mg/L, respectively (**Figure 5.6**). High biosurfactant concentrations have better removal efficiencies due to the higher micelles' formation. Other authors reported metal removal efficiencies for different metals in wastewater and soil (**Table 5.3**).

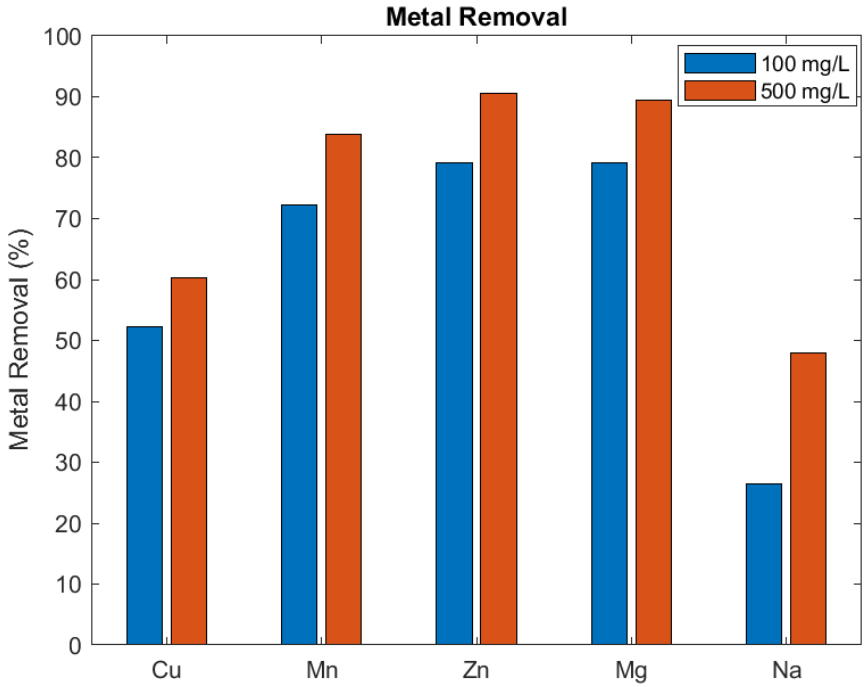


Figure 5.6 Metal removal efficiency in the anode chamber after 140 h of operation, at rhamnolipid concentrations of 100 mg/L and 500 mg/L.

Table 5.4 Metal removal efficiencies reported by other authors.

Metal	Removal Efficiency	Microorganism	MFC	Reference
Cr Mn	82.2% 61.2%	*Shewanella oneidensis*	Dual MFC	(Juliastuti et al., 2019)
Cu	99.9%	*Synergistacege, Desulfobacterium, Desulfovibrio*	Dual MFC	(S. Wu et al., 2022)
Cd Zn	90.0% 97.0%	Mixed bacteria culture	Single MFC	(Abourached et al., 2014)
Au	95.00, In cathode	Yeast	Dual MFC	(Al-Asheh et al., 2022)
Ag	99.8%, in cathode	*Butyricoccus, Petrimonas, Desulfomicrobium, Desulfovibrio*	Dual MFC	(Almatouq et al., 2022)
Cr	98.6%, in cathode	*Corynebacterium vitaeruminis*	Dual MFC	(S. Zhao et al., 2021)

COD removal for rhamnolipid 100 mg/L with wastewater was 29%, and for rhamnolipid 500 mg/L with wastewater was 17%. The removal is not high, indicating that the wastewater's rhamnolipid, substrate, and phenol require more oxygen to be treated. Moreover, the low concentrations and low activity of microorganisms reduce the ability of biodegradation of organic matter which is an indicator for low COD removal. Our results are low in comparison to COD removal efficiencies in MFC without surfactants, such as 70.0% (S. Wu et al., 2022), and with surfactants with results of 75.0% (K. Sharma et al., 2022), 87.0% with SDS from carwash wastewater (Radeef & Ismail, 2021), 71.2% with SDS in bilge water (Hwang et al., 2019), 79.1% with SDS and wastewater (I. Chakraborty et al., 2021). Other authors have reported the degradation of hydrocarbons from soil using anionic surfactants such as SDS (X. Li et al., 2018). These findings and the results reported in this research demonstrate the ability of MFC to aid with biosurfactants for the remediation of wastewater polluted with metals and organic compounds such as phenol.

5.4.4 Rhamnolipid concentration after MFC operation

After the operation in the MFC, the rhamnolipid concentrations were evaluated using the methylene blue method with a spectrophotometer; the results are reported in **Figure 5.7**. *S. oneidensis*, under standard conditions, has negligible rhamnolipid production. Moreover, rhamnolipid was consumed and used as a carbon source for the bacteria due to its 79.5 mg/L and 339.8 mg/L concentration in the wastewater treated at initial biosurfactant concentrations of 100 mg/L and 500 mg/L. Rhamnolipid and other surfactants at high concentrations have a bactericidal effect (Bailey et al., 2012; Bharali et al., 2013); however, in this study, at a concentration of 500 mg/L, growth was observed. Furthermore, rhamnolipid and SDS have been proven to enhance hydrocarbon remediation. In addition, surfactant degradation in MFC has also been reported by previous studies; SDS and rhamnolipid were used for enhancing hydrocarbon removal from soil and wastewater with results above 70.0% (Ambaye et al., 2022; Zeng et al., 2018; Y. Zhang, Zhao, et al., 2017). The removal of rhamnolipid with wastewater in the anode chamber was 20.5% and 32.0% for rhamnolipid concentrations of 100 mg/L and 500 mg/L, respectively. The results are lower than the removal of SDS of 88.0% and 70.0% from wastewater (I. Chakraborty et al., 2021; Radeef & Ismail, 2021).

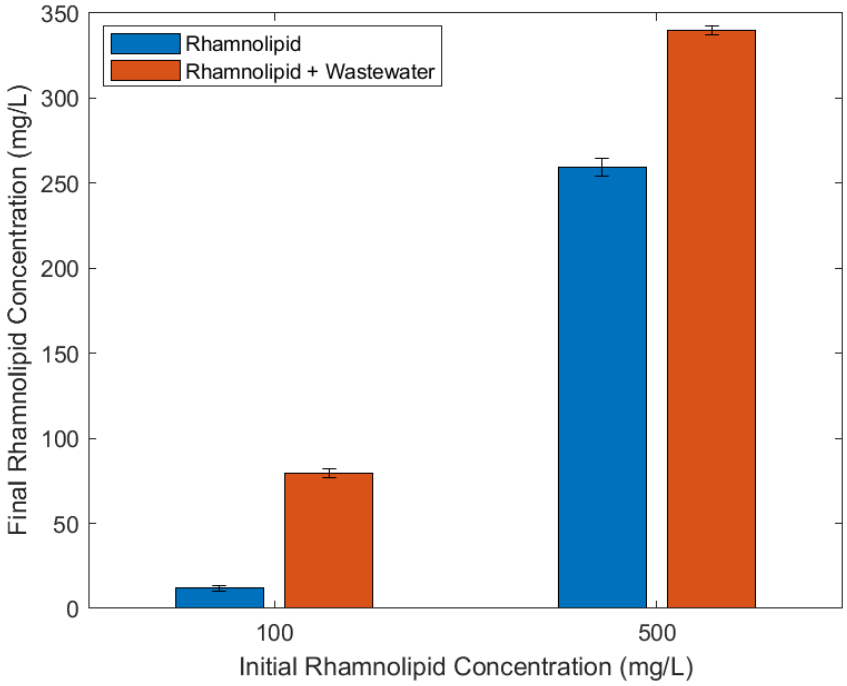


Figure 5.7 Final rhamnolipid concentrations after 140 h of MFC operation at initial concentrations of 100 mg/L and 500 mg/L.

5.5 Conclusions

In this study, the biofilm on the electrode and *S. oneidensis* growth revealed that at low rhamnolipid concentrations, the bacteria have similar specific growth rates to the control media. However, at a concentration of 500 mg/L, the bactericidal effect of the rhamnolipid impacts the growth and the biofilm formation on the electrode. The performance of the MFC was evaluated with the polarization curves and the OCV, showing higher power densities and voltage generated with rhamnolipid 100 mg/L. The results indicated that rhamnolipid at 100 mg/L performs better in the dual chamber MFC inoculated with *S. oneidensis* because of the excellent metal removal and higher power density generated. Moreover, metal removal was higher with the rhamnolipid at 500 mg/L due to the higher formation of micelles. Additionally, COD removal was negligible, and the rhamnolipid was demonstrated to be reduced after the MFC operation. The MFC and metal removal efficiencies can be enhanced increasing the redox potential between the anode and cathode chamber, which can be achieved increasing the conductivity of the electrodes using a higher area of them and reducing the internal resistance by enhancing the electron transport

by extracellular mediators such as methylene blue and riboflavin. In addition, a higher PEM is required for better proton transport which facilitates better electron transport.

5.6 References

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Chapter 6

6 Conclusions and recommendations

6.1 Conclusions

The scope of this work was to remove heavy metals from synthetic desalter effluent using a non-conventional method that is environmentally friendly and has a less chemically demanding process. At the initial phase, an ultrafiltration process aid with biosurfactant or MEUF with rhamnolipid was investigated for heavy metal remediation. Moreover, the use of metal-reducing bacteria such as *Shewanella oneidensis* MR-1 to remove heavy metals through a biological process without using chemicals was analyzed. A microbial fuel cell MFC with a dual-chamber configuration was studied to remove heavy metals from wastewater in the anode and cathode chambers. Furthermore, the final step investigated the performance and metal removal of MFC using the retentate from MEUF loaded with metals and rhamnolipid.

The overall conclusions from this study are:

- The critical review showed gaps in knowledge for the potential application of heavy metal remediation in refinery processes with low heavy metal concentrations in the wastewater. From the literature review, it was determined that a two-step approach would be beneficial to overcome problems associated with conventional heavy metal removal processes.

- It was found that heavy metal removal using MEUF was a reliable remediation process. The MEUF process with rhamnolipid biosurfactant demonstrated high removal efficiency with a simple operation. Rhamnolipid micelles effectively bind metals for the highest removal efficiency of 94%, 82%, 99%, 76%, and 42% for Zn, Mg, Cu, Mn, and Na, respectively. Additionally, the process in the ultrafiltration membrane indicated better results at low concentrations of rhamnolipid (100 mg/L), resulting in better permeate flux which reduced the cleaning cost associate with the ultrafiltration process.

- The investigation for metal remediation using MFC showed the viability of this process for bioremediation. The dual chamber MFC inoculated with *Shewanella oneidensis* MR-1 demonstrated the bacterial capacity for growing in synthetic desalter effluent. Moreover, the MFC efficiency with wastewater in the cathode chamber was analyzed. The anode chamber demonstrated metal removal efficiencies of 93%, 85%, 93%, 88%, and 42% for Cu, Mg, Mn, Zn, and Na, respectively; moreover, the maximum power production was 76.8 mW/m^2. The cathode chamber without bacteria showed removal efficiencies of 98%, 49%, 57%, 59%, and 36% for Cu, Mg, Mn, Zn, and Na, respectively; the maximum power density was 4.4 mW/m^2. The inoculated anode chamber presented higher efficiency in removal and higher power density generation. MFC systems showed better performance for the bioremediation of metals in the anode chamber due to the extracellular electron transport of *S. oneidensis*.

- The retentate with the complex rhamnolipid-metals was investigated in the anode chamber of the MFC inoculated with *S. oneidensis*. The MFC performance showed a maximum power density of 13.9 mW/m^2 for the concentration of 100 mg/L and better removal efficiencies at 500 mg/L of rhamnolipid with results of 60%, 90%, 84%, 91%, and 48% for Cu, Mg, Mn, Zn, and Na, respectively. The rhamnolipid concentration that kept a high MFC efficiency without affecting metal removal was 100 mg/L and it was found that the biosurfactant is bactericidal at high rhamnolipid concentrations, which significantly reduces microbial growth. This was demonstrated with the low specific growth rates and high double times at 100 mg/L.

- In general, the overall finding was that the two-step approach proposed in this study was efficient for heavy metal remediation from the wastewater. The study demonstrated that MEUF and MFC can be used together for higher removal efficiencies and sustainable by-products such as electricity.

- The metal remediation achieved by MEUF and MFC for metals from synthetic desalter effluent complies with the limits set by the Ontario regulation (**Appendix 7**). However, the sodium concentration remains above the permissible limits due to the high

concentration of this component in the wastewater. Additionally, the reduction in sodium concentration is still relatively close to the standard limits.

6.2 Recommendations

- The ultrafiltration process enhanced with rhamnolipid demonstrated high metal removal efficiency. However, the cost of rhamnolipid is one disadvantage of this biosurfactant. From the research, it is suggested that the viability of other biosurfactants with low costs, such as lecithin and saponin can be investigated. The experiments with new biosurfactants could be performed using the flat plate ultrafiltration membrane, and results can be analyzed by comparing the permeate flux, the heavy metal removal efficiency, and the membrane fouling.

- MEUF could be evaluated using different types of ultrafiltration membranes. The processes that can be evaluated are unstirred batch mode, stirred batch, and centrifugal. In addition, various ultrafiltration membranes such as polyether sulfone (PES) and tetramethylammonium hydroxide (TMAH) can be studied. The experiments with different processing characteristics and membrane types could enhance the permeate flux, which increases the membrane's capability for long-term operation.

- To avoid membrane cake formation, which could increase membrane fouling, the reduction of the processing time to 1 hour with a higher transmembrane pressure could be studied. However, the transmembrane pressure should be evaluated according to the maximum capacity of the membrane without reducing the metal removal from the wastewater.

- Fouling mitigation using different cleaning technologies can enhance the permeate flux. These technologies can include backwashing with a higher pressure, use of citric acid, and different changes in processing such as temperature.

- Increasing MFC performance without compromising metal removal efficiency could be evaluated by varying parameters.

- Use of gold or silver coating on the electrode surface increases electron transport due to the high conductivity of these elements. Moreover, modifying electrode geometry from square to brush can enhance biofilm distribution and formation on the electrode, which facilitates electron transport from the oxidized nutrients to the cathode chamber.

- Catalysts in the anode chamber increase electron oxidation; consequently, the activation losses and ohmic losses can be reduced, and the power and voltage generated can be increased. The experiments could be done by using low rhamnolipid concentrations and catalysts to increase the extracellular membrane's permeability and substrate oxidation.

- Increasing voltage generation can be reached using multiple dual-chamber MFCs in parallel. The parallel system increases the voltage generated and reduces the internal resistance of the system. The low internal resistance facilitates electron transport from the anode to the cathode.

- Large-scale application using the two-step approach proposed in this thesis could be performed using a pilot project with high volume capacity for the ultrafiltration system and the MFC. For the ultrafiltration phase, a connection between flat plate membranes reduces membrane fouling and increases metal removal due to increased rejection of metal-loaded micelles. Moreover, for MFC operation, a large volume with a larger area of cation exchange membrane allows for a greater proton exchange rate from the anode to the cathode, increasing the voltage generated in the MFC.

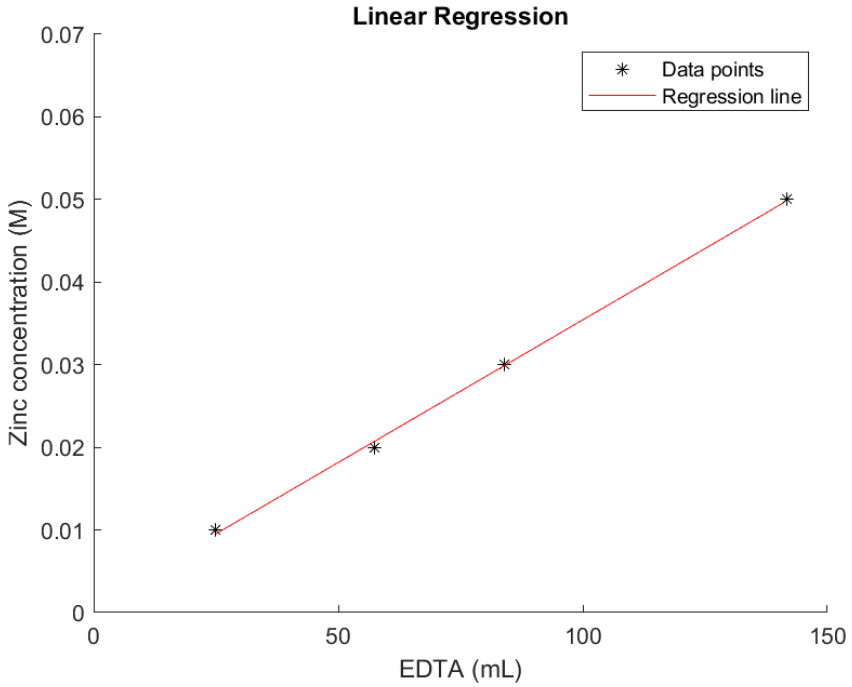
- The use of mixed cultures with metal reduction characteristics, such as *Shewanella* sp. and *Geobacter* sp., increases toxic resistance and metal removal. Furthermore, mixed cultures from natural environments such as lakes or soil assist faster bioremediation in fewer control conditions, expanding applicability on a large-scale.
- The limitations of scaling up this two-step approach are primarily associated with the cost of rhamnolipid for industrial applications and the relatively low power generated

by microbial fuel cells. The optimization of the limitations by improving biosurfactant extraction and optimizing the MFC performance.

Appendices

Appendix 1 Calibration curve zinc titration

The titration for zinc concentration in deionized water was performed using EDTA solution at 0.01 M with Eriochrome black T.



Appendix 2 Results obtained from ICP-OES for coker and avis brine wastewater provided by Imperial Oil

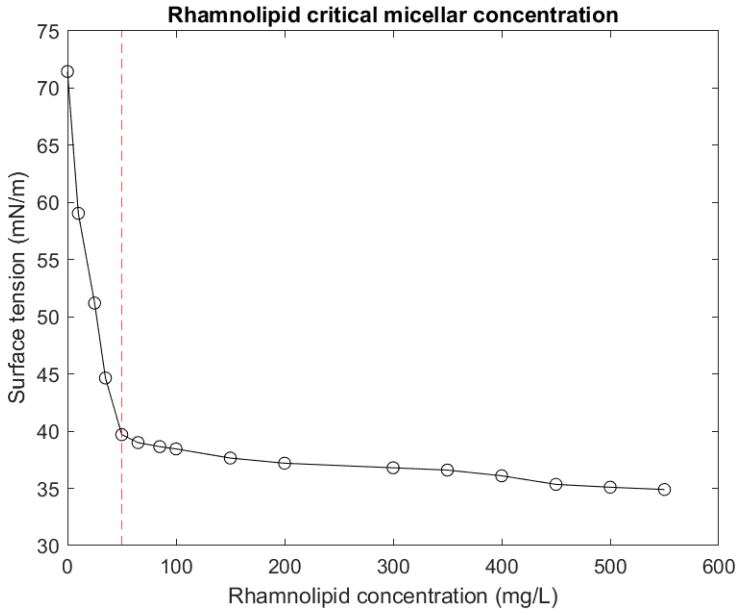
The samples proved by Imperial Oil were pretreated with acid digestion due to the high concentration of hydrocarbons. ICP-OES procedure consisted in the following steps:

- Blank was 2% Nitric Acid solution.
- Stock solutions of the metals shown in the table were diluted in 2% nitric acid solutions.
- Samples were analyzed after serial dilutions with stock metal solutions and 2% nitric acid.

	AVIS BRINE (mg/L)	COKER BRINE (mg/L)
Cd	0.029507	0.015445
Co	0.001090	0.000867
Cr	0.002754	0.004038
Cu	0.214712	0.063276
Fe	0.060535	0.255205
Li	0.116290	0.041810
Mg	8.304800	10.314940
Mn	0.149916	0.227616
Mo	0.003854	0.004570
Na	241.396667	76.355000
Ni	0.049075	0.049330
Pb	0.023753	0.019665
Sr	1.483200	0.516578
V	0.002276	0.012316
Zn	0.272577	0.626463

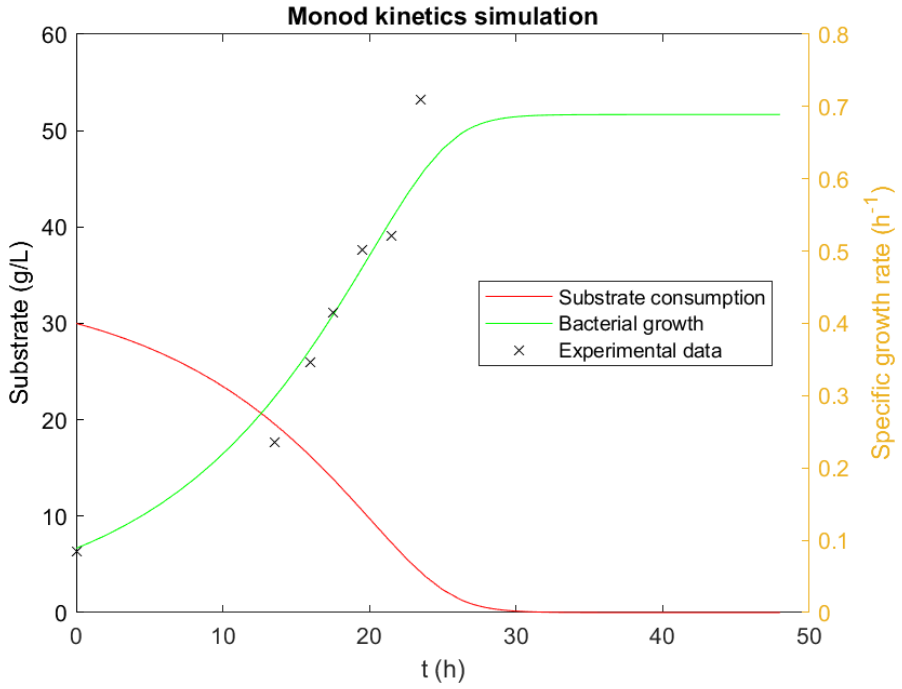
Appendix 3 Evaluation of rhamnolipid CMC by surface tension analysis

The surface tension analysis was elaborated using a tensiometer at different rhamnolipid concentrations in deionized water.



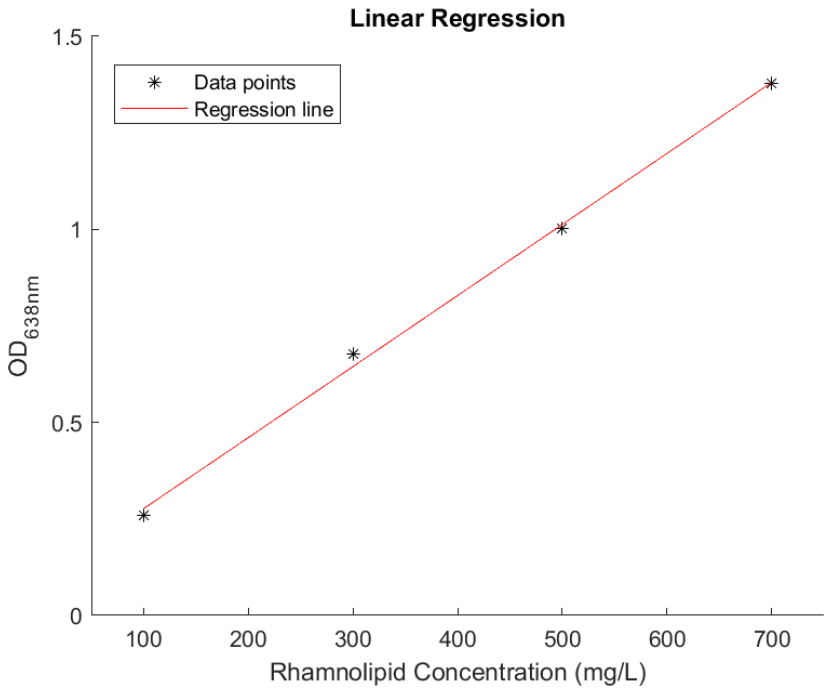
Appendix 4 Ultrafiltration membrane picture after processing demonstrating the membrane fouling.

Appendix 5 *Shewanella oneidensis* growth using monod kinetics model simulation in matlab



The following data was used for the simulation according to the experimental information with the conventional media or TSB. μ_{max} = 0.11 h^{-1}, K_d = 0.8, Y_{xs} = 1.5, and K_s = 5.9 g/L. Ks is the concentration of the substrate when μ/μ_{max} = 0.5.

Appendix 6 Linear regression for determination of rhamnolipid concentration after MFC operation.



Appendix 7 Metal removal in this study according to the Ontario regulation

The metal removal of the synthetic desalter effluent is compared with the regulation (Ontario Ministry of the Environment, Conservation and Parks under the Ontario Water Resources Act and Ontario Regulation 169/03).

Metal	Ontario Regulation	MEUF	MFC
Cu (mg/L)	0.5	0.03	0.2
Mg (mg/L)	200	76.5	13
Mn (mg/L)	1.9	1.7	0.2
Zn (mg/L)	1.5	0.7	0.5
Na (mg/L)	1000	1150	1400

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Munoz-Cupa, C., Hu, Y., Xu, C., Bassi, A. 2021. An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production. *Science of the Total Environment,* 754, 142429.

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Munoz-Cupa, C., Bassi, A. 2023. Investigation of rhamnolipid addition on the microbial fuel cell performance and heavy metal capture in metal laden wastewater. *Water Process Engineering.* Submitted after peer revision.

Conferences and Presentations:

Munoz-Cupa, C., Bassi, A. Investigation of current production and metal removal from synthetic desalter effluent using a dual chamber microbial fuel cell (MFC) with *Shewanella oneidensis* MR-1. AIChE Annual Meeting. 2022, Phoenix, AZ, *Oral Presentation.*

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Munoz-Cupa, C., Bassi, A. Investigation of heavy metal removal from synthetic desalter effluent using ultrafiltration and microbial fuel cell. Research Day - The Institute for Chemicals and Fuels from Alternative Resources, 2022, The University of Western Ontario, *Oral Presentation.*