

REMOVAL OF HEAVY METALS FROM INDUSTRIAL WASTEWATER
USING MICROBIAL FUEL CELL

by
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Declaration of Authorship

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Dedication

To my family...

Abstract

The development in science and technology has resulted in a steady increase in the growth rate of several industries, thereby resulting in an increased demand of precious metals as well, such as gold and platinum. This also results in the selling prices for these metals to increase. Hence, recovery of such metals from low grade ores and scraps plays an important role. Conventional methods of recovery of gold can result in the pollution of environment. Additionally, despite low concentrations of gold being released into the environment, the recovery of this metal can provide additional profit and help to reduce operating costs. The objective of this work is to use Microbial Fuel Cells (MFC) as an alternative green technology to simultaneously remove heavy metals and recover remaining gold found in the effluent samples taken from one of the gold refinery industries in the UAE. Removal efficiency of pure tetrachloroaurate ions was investigated first to ensure the feasibility of MFC in terms of removing gold ions and determining the optimum conditions for removal efficiency in terms of catholyte solution, as well as initial gold concentrations. Next, the effect of copper ions on the removal efficiency of the gold ions was investigated due to their high presence in the actual wastewater samples. Later, the gold removal efficiency was evaluated from the industrial effluent. The effect of pH and initial biomass concentration on the gold removal efficiency was also determined. Based on the successful results, after 5 hours, 95% of gold removal efficiency from the wastewater containing 250 ppm of initial gold ions at ambient temperature with the 80g/L yeast concentration was achieved. After 48 hours of the cell's operation under the same condition, 98.86% of AuCl_4^- ions were successfully removed from the solution. Based on the investigation of the effect of different factors, 100% removal efficiency of the gold was achieved at a pH of 2, initial yeast concentration of 80 g/L and the initial gold concentration in the waste solution being 250 ppm. On the other hand, the most suitable condition in terms of copper removal was found at a pH of 5.2 where 53% removal efficiency from the waste solution was accomplished.

Keywords: Microbial fuel cell, wastewater treatment, heavy metals, precious metals.

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List of Abbreviations

AAS	Atomic Absorption Spectroscopy
AC	Activated carbon
AOP	Advanced Oxidation Processes
CNT	Carbon Nanotubes
ED	Electrodialysis
LBMA	London Bullion Market Association
MCL	Maximum Contaminant Level
MF	Microfiltration
MFC	Microbial Fuel Cell
NF	Nanofiltration
OCP	Open Circuit Potential
PEM	Proton Exchange Membrane
PTFE	Polytetrafluoroethylene
RO	Reverse Osmosis
UF	Ultrafiltration

Chapter 1. Introduction

1.1. Introduction

In this chapter, a brief introduction about the application of microbial fuel cell and the reasons behind implementation of this technology in the wastewater treatment, as one of its main applications, will be discussed. Later, the main objectives of the study, thesis contribution, and organization will be presented.

1.2. Overview

The growth in population and increase in demand lead to an increase in the number of industries, such as gold mining, petroleum refinery, mining, textile, and batteries [1]. This has also resulted in an increase in the amount of effluent that must be discharged into the environment. This waste discharge may contain significant proportions of heavy metals [2]. Heavy metals refer to metallic elements that have high density (atomic weights between 63.5 and 200.6) and are also toxic even in low concentrations.

Heavy metals have high solubility in aquatic environment; hence they can easily be absorbed by living organisms. Their introduction into the food chain will result in accumulation in large quantities in human bodies. Heavy metals should have a maximum allowable limit in human body, which if exceeded will result in severe disorder and diseases [3]. Hence it is of utmost importance that the effluent from the industries is treated before discharged into the environment.

There are several methods that can be used to treat industrial effluents containing heavy metals, such as solvent extraction, filtration, ion exchange, coagulation, sedimentation, oxidation, and adsorption. However, these techniques have several disadvantages; for example, high cost, low removal efficiency, regeneration, and the problem of secondary contaminations [4]. Therefore, it is necessary to implement a new technique which will be more cost effective, have a higher removal efficiency and has reduced probability of secondary contamination.

One of the most important techniques that has been developed is the Microbial Fuel Cell (MFC) technology which is a bioelectrochemical process that has a wide range of applications. In other words, MFCs are converting the stored energy in the chemical

compound to electrical energy by using microorganisms. This electricity generation is one the most important application of MFCs which results in tremendous usage in different applications such as spacecraft and systems that require only low power to transmit signals. Additionally, hydrogen production can be achieved by MFC while applying external power. Because the generation of hydrogen from protons and produced electrons by metabolic reaction of microorganisms is thermodynamically unfavourable, thus, external potential is applied to increase the cathode potential and allow the reaction to become relatively more thermodynamically favourable [5]. MFCs also can be employed as a power source of implanted medical device by using glucose and oxygen from blood. Hence, the need of surgery to replace batteries in the conventional method is reduced [5]. Finally, yet importantly, another application of MFCs is to treat wastewater and industrial effluent. As compared to other renewable energy sources, MFC uses organic matter in order to treat wastewater while simultaneously producing energy [6].

1.3. Thesis Objectives

In order to treat wastewater effluent of different industries, although conventional methods are effective, however, further development is required to achieve more eco-friendly methods as well as a cost-effective system, which can be used as a versatile technique. Also, increase in world's demand for precious metals either as industrial demand or global demand, a sustainable method is required to remove even low concentration of precious metal such as gold from effluent of gold refinery industries. Microbial fuel cells can produce energy while removing heavy metals and treating wastewater to their allowable concentrations for the discharge into the environment. In addition, recovering precious metals such as gold from industrial effluent to provide further profit. Hence, they can be used as a source of renewable energy. However, there are some obstacles for scaling up MFCs such as low power generation, high cost associated with the materials used and low removal efficiency.

1.4. Research Contribution

The contributions of this research work can be summarized as follows:

- Investigate the feasibility of MFC for removing tetrachloroaurate ions from the solution with high removal efficiency. In addition, discover the most suitable

conditions in terms of initial gold concentration and catholyte solution for achieving the highest gold removal efficiency.

- Investigating the effect of the copper ions, as one of the main constituents in the effluent of gold refineries, on the gold removal efficiency.
- Evaluating the gold and copper removal efficiency from the effluent of one of the gold refineries in the UAE. Moreover, assessing the influence of different factors such as pH and initial yeast concentration on the removal efficiency.
- Suggesting the optimum conditions for removal of heavy metals, mainly gold ions, from the waste samples.

1.5. Thesis Organization

The rest of the thesis is organized as follows:

- Chapter 2 provides background about MFC and different methods for removal of heavy metals. Moreover, the application of MFC for removing heavy metals and the method for recovering gold metals that is using in the gold refineries are discussed.
- Chapter 3 gives the description of the proposed system along with the proposed biomass activation method.
- Chapter 4 discusses experimental setup and the experimental work carried out.
- Chapter 5 presents the obtained results and their interpretation within each stage of the experimental work is provided.
- Chapter 6, finally, concludes the thesis and outlines the future work.

Chapter 2. Background and Literature Review

In this chapter, the fundamentals of MFCs and the introduction to what is referred as heavy metals and why treatment of them before discharging to the environment is essential is explained. Next, different methods for treating and removing heavy metals from wastewater is discussed by summarizing the advantages and disadvantages associated with them. The performance of the MFC for treating different types of heavy metals is also reviewed using previous studies. Lastly, the current method that is being employed for recovering the gold metal by gold refineries is explained.

2.1. Fuel Cell

The current fuel cell is an electrochemical device which converts chemical energy stored in fuel to electrical energy in a single step, contrary to heat combustion engine which produces electrical energy from chemical energy via a multi-step process (i.e. chemical to thermal to mechanical to electrical energy) [7].

The major disadvantages of heat combustion engines over fuel cells are that, firstly, they depend on finite supplies of fossil fuels, which essentially would render the global population without a source of energy if they relied solely on fossil fuels. Secondly, and most importantly, these technologies cause severe damage to the environment, such as climate change, depleting the ozone layer, acidic rains, and hence, resulting in reduced vegetation cover.

On the contrary, compared to internal combustion engines, fuel cells can be environmentally friendly, since they utilize hydrogen which is considered as green source of energy for the electricity production. Additionally, their simple construction allows them to be used in various power generation applications where they can be utilized while being stationary, on transportation or as a portable device. Moreover, because of their static nature, they result in quiet operation and zero vibration (therefore, not resulting in mechanical damage and reducing maintenance costs) when compared to heat combustion engines. Lastly, in terms of efficiency, fuel cells are also reported to have a higher theoretical efficiency compared to the combustion engines that their efficiency is limited to the Carnot cycle. According to the work of Ziyad fuel cell ranges from 40 to 80% while in the case of internal heat combustion engines efficiencies are

typically in the order of 20% [8],[9]. Thus, fuel cells have a greater advantage compared to heat combustion heat engines.

Fuel cells are typically categorized based on the electrolyte materials, electrodes and cell configurations and arrangements. Hence, several different types of fuel cells exist each of which differ in their power outputs, operating temperature, electrical efficiencies, and applications. Table 2-1 presents a summary of the different types of fuel cells along with their advantages and disadvantages as well as the type of membrane used, material of electrodes and their efficiencies.

Table 2-1: Summary of different type of fuel cells

Fuel Cell Type	Typical Electrolyte	Typical Anode/Cathode Catalysts	Typical Interconnect Material	Typical Fuel	Efficiency	Specific Advantages	Specific Disadvantages	References
Proton exchange membrane	Perfluoro sulfonic acid	Anode: Platinum supported on carbon Cathode: Platinum supported on carbon	Graphite	Hydrogen	60 % for transportation, while 35 % stationary	<ul style="list-style-type: none"> • Solid electrolyte reduces corrosion and electrolyte management problems • Low temperature • Quick startup 	<ul style="list-style-type: none"> • Expensive catalyst • Low lifespan • Either availability of hydrogen fuel 	[10] [11] [12]
Molten carbonate	Liquid alkali carbonate (Li ₂ CO ₃ , Na ₂ CO ₃ , K ₂ CO ₃) in Lithium aluminate (LiAlO ₂)	Anode: Nickel Chromium (NiCr) Cathode: Lithiated nickel oxide(NiO)	Stainless steel	Methane	45–50 %	<ul style="list-style-type: none"> • High electrical efficiencies • High tolerance to contaminants • Possibility of internal reforming eliminated • Fuel flexibility • Inexpensive catalyst 	<ul style="list-style-type: none"> • Slow start-up • Low power density • Strict material requirements • High thermal stresses • Sealing issues • Durability issues • High manufacturing costs 	[13] [14]
Phosphoric acid	Concentrated liquid phosphoric acid (H ₃ PO ₄) in silicon carbide (SiC)	Anode: Platinum supported on carbon Cathode: Platinum supported on carbon	Graphite	Hydrogen	37–42 % (generation of power)	<ul style="list-style-type: none"> • Technologically mature and reliable • Simple water management • Good tolerance to contaminants • High-grade heat 	<ul style="list-style-type: none"> • Relatively slow start-up • Low power density • High sensitivity to contaminants • Expensive auxiliary systems • Low electrical efficiencies • Relatively large system size • Expensive catalyst • High cost 	[15] [16]
Alkaline	Potassium hydroxide (KOH) water solution. Anion exchange membrane (AEM)	Anode: Nickel Cathode: Silver supported on carbon	Metallic wires	Hydrogen	60 %	<ul style="list-style-type: none"> • High electric efficiency due to fast reduction reaction kinetics • Wide range of operation 	<ul style="list-style-type: none"> • Extremely high sensitivity to contaminants • Pure hydrogen and oxygen required for operation • Low power density 	[17] [18]

Fuel Cell Type	Typical Electrolyte	Typical Anode/Cathode Catalysts	Typical Interconnect Material	Typical Fuel	Efficiency	Specific Advantages	Specific Disadvantages	References
						temperature and pressure <ul style="list-style-type: none"> • Inexpensive catalyst • Catalyst flexibility • Relatively low costs 	<ul style="list-style-type: none"> • Highly corrosive electrolyte leads to sealing issues • Complex and expensive electrolyte management for mobile electrolyte systems 	
Microbial	Ion exchange membrane	Anode: Biocatalyst supported on carbon Cathode: Platinum supported on carbon	N/A	Any organic matter (e.g., glucose, acetate, waste-water)	H+	<ul style="list-style-type: none"> • Fuel flexibility • Biocatalyst flexibility • No need for enzymatic catalysts isolation, extraction, and preparation • Relatively higher lifetime for biocatalysts • Capacity for self-regeneration of enzymes 	<ul style="list-style-type: none"> • Electron transfer mechanisms from the metabolism in the microorganisms to the fuel cell anode is problematic • Relatively lower energy density due to using some of the energy for the microorganism activity • Very low power density • Low columbic yield • Inflexible operation conditions 	[19] [20]

2.2. Microbial Fuel Cell

2.2.1 History of MFCs

Microbial fuel cells were first reported by Potter in the early 20th century. He used live cultures of *E. coli* and *S. spp.* on platinum macroelectrodes to produce electricity in a setup similar to batteries, using sterile media [21]. Cohen [22] in 1931 further established the production of energy using bacterial fuel cells by producing a voltage of 35 V and a current of 0.2 mA. Although Potter and Cohen demonstrated the first establishment of using bacteria to produce electricity; the importance of this technology became apparent when NASA used bacteria to recycle and convert human waste to produce electricity on space flights [23]–[25].

In 1986, Habberman and Pommer [26] were the first to employ MFC on a long-term basis (i.e. for 5 years). During this period, the MFC used municipal wastewater as nutrient. Additionally, the cell required no maintenance and experienced no malfunction during its 5-year operation. Moreover, treatment of wastewater was reported for the first time in this study which also showed the usage of soluble mediators in form of sulphates/sulphides for indirect electron transfers by the bacteria. In 1999, it was found that mediators were not an essential component of the MFC and hence, allowed for the development of MFCs without the requirement of having expensive mediators in the configurations [27][28]. Thus, a significant increase in interest was noted in the research within the electromicrobiology field, resulting in over a thousand different types of MFCs.

2.2.2 Working principle of MFCs

In Figure 2-1, the MFCs consist of two chambers, i.e. anodic and cathodic chambers. Each chamber can be made of glass, polycarbonate, or Plexiglas, and each contains an electrode which can be carbon paper, carbon-cloth, graphite, graphite felt, Pt, Pt black, or reticulated vitreous carbon. The chambers are divided using a Proton Exchange Membrane (PEM) [29]. Protons and electrons are produced by the microorganisms in the anodic chamber by decomposing the organic matter; the electron transfers to the cathodic chamber from the anodic electrode to the cathodic electrode using external electrical circuit, while the proton transfers to the PEM membrane. From the thermodynamic viewpoint, the cathode compartment should always have higher

potential than anode compartment. Thus, allowing the electrons to spontaneously transfer from the cathode to the anode chamber without any requirement for external power. The cathodic chamber has a high potential electron acceptor where reduction reaction takes place in. The electron acceptor should ideally be non-toxic and not interfere with the microbes. An example of such an electron acceptor is oxygen, which is non-toxic and easily available. Additionally, besides oxygen, ferricyanide and heavy metals can also be used as an alternative electron acceptor [30].

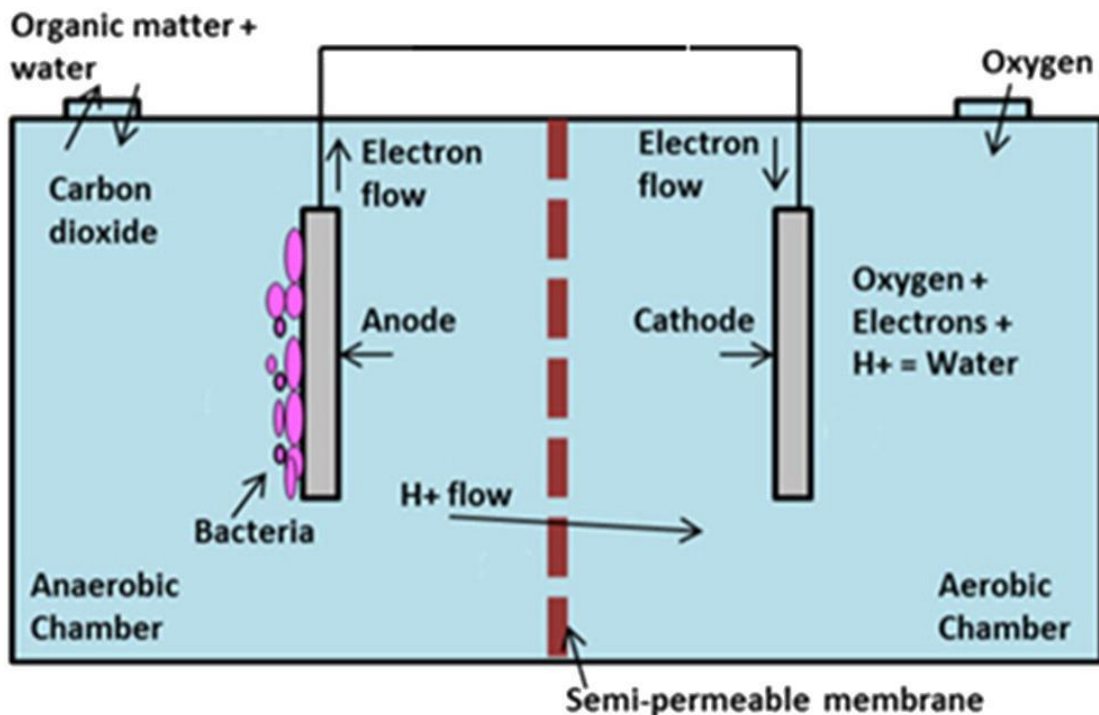


Figure 2-1: Schematic representation of MFC [31]

As a result of the ability of the microorganisms to liberate electrons and produce electricity, either as end products of anaerobic respiration or through intermediate reactions, the definition of MFC could be that of a device which converts chemical energy into electrical energy through the use of microbes as a catalyst [32]. These microbes are present as a biofilm on the surface of the anode in the MFC. This biofilm can be considered as a biocatalyst, which is used instead of precious chemical catalysts, employed to carry out the electrochemical redox reactions [33]. The types of redox reactions that can take place in the MFC are greatly dependent on the type of organic matter and the electron acceptor used in the MFC. A summary of the possible oxidation and reduction reactions for different substrates are displayed in Table 2-2.

Table 2-2: Possible oxidation and reduction reactions [34]

Oxidation Reaction at the Anode		
Substrate	Reaction	E (V)
Acetate	$CH_3COO^- + 3H_2O \rightarrow CO_2 + HCO_3^- + 8H^+ + 8e^-$	-0.3
Glucose	$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$	-0.429
Glycerol	$C_3H_8O_3 + 6H_2O \rightarrow 3HCO_3^- + 17H^+ + 14e^-$	-0.289
Domestic Wastewater	$C_{10}H_{19}O_3N + 18H_2O \rightarrow 9CO_2 + NH_4^+ + HCO_3^- + 50H^+ + 50e^-$	-----
Reduction Reaction at Cathode		
Substrate	Reaction	E (V)
Oxygen	$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	1.23
	$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.27
Nitrate	$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O$	0.43
	$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$	0.73
Ferric Ion	$Fe^{3+} + e^- + H^+ \rightarrow Fe^{2+} + 1/2 H_2O$	0.77

2.2.3 Classification of MFCs

In this section MFCs can be categorized based on many different parameters such as the type of material used for electrodes, design configuration, applications, etc. However, the MFC can be classified into two different categories depending on how the electrons are transferred, since the cost of mediators in the MFC is a significant factor. These are described, in the subsequent sections, below.

2.2.3.1. Mediator MFCs

In mediator MFCs, the microorganisms cannot transfer electrons to the anode surface, since they are unable to produce a protein which has active sites that allows for this process to be carried out. However, chemical mediators, also known as an electroactive

metabolite, are used as an intermediate step while anaerobic conditions exist in the cell. Anaerobic conditions are used in the MFC because the presence of oxygen tends to consume electrons and reduce the efficiency of the mediators. In the presence of a mediator, the electrons released by the microorganisms are accepted by the mediators and then released to the anode surface which is the final electron acceptor. Hence, after releasing the electrons the mediators are oxidized to their initial state [35]. Some examples of mediators used in MFC is thionine, methylene blue, neutral red, ferricyanide, methyl viologen or humic acid [36].

However, a major disadvantage of several mediators is that they are toxic simultaneously to both the environment and the microorganisms. Additionally, the mediators are also extremely expensive, especially when the MFC is designed to treat wastewater. Thus, research was carried out to investigate alternative ways to transfer electron without the need of mediators [37].

2.2.3.2. Mediator-free MFCs

Research carried out on mediator-free MFCs, have shown that most of the bacteria found in wastewater have the capability of producing appendages (i.e. nanowires) that allow for the electron transfer to the anodes. Such bacteria are known as “exoelectrogen” or “electrochemically active bacteria”. Table 2-3 provides example of different exoelectrogens.

Table 2-3: Examples of exoelectrogen bacteria used in MFC technology [38]

Year	Microorganism	Comment
1999	Shewanella putrefaciens Ir-1	Direct proof of electrical current generation in an MFC by a dissimilatory metal-reducing bacterium (gammaproteobacteria)
2002	Geobacter metallireducens	shown to generate electricity in a poised potential system (Deltaproteobacteria)
2003	Geobacter sulfurreducens ⁴⁰	generated current without poised electrode (Deltaproteobacteria)
2005	Geothrix fermentans	Produced an unidentified mediator (phylum Acidobacteria)
2008	Acidiphilium sp. 3.2 ^{sup}	Current at low pH and in the presence of oxygen in a poised potential system (Alphaproteobacteria)

Examples of exoelectrogen bacteria are, *Shewanella putrefaciens* that are found in earth soil, marine environments and *Geobacter* that exist in in deep earth soil and ocean sediments as represented in Table 2-3 [38]. Hence, the use of such bacteria makes the MFC less toxic and cost effective.

Moreover, other parameters also need to be taken into consideration, such as : External resistance of the circuit, pH imbalance due to the presence of the membrane, resulting low pH at the anodic chamber and reducing the microbial activity, and reduction of oxygen at the cathode [37].

2.2.4 Design of MFCs

In order, to increase and optimize the efficiency and reduce the limits of the cells, different combinations of the MFC have been investigated and developed. These can be based on the electrodes, wirings, glass cells and proton exchange membranes (PEM). This section discusses some of the mostly used and practical MFC configurations [5].

2.2.4.1. Single Chambered MFCs

In this case, the anode and cathode are located within the same compartment, thus sharing the same electrolyte. An important feature of such a cell is the cathode, which is exposed to air and is porous at the same time, hence, allowing the diffusion of the protons through them and causing them to be reduced on the side that is exposed to oxygen. The cathodes can be either manufactured using porous carbon electrodes or PEM bonded with flexible carbon cloth electrodes. The cathodes are prevented from drying by having them coated with graphite while electrolytes added steadily. These electrolytes, therefore, behave as catholytes. Hence, water or fluid management is an important issue in this cell, as the drying up of the cathode can greatly reduce their efficiency [39]. However, single chamber MFCs have significant disadvantages in the form of liquid leakage, evaporation of water, and oxygen diffusion into the anodic chamber. Such issues can be overcome by Polytetrafluoroethylene (PTFE), as an additional diffusion layer on the cathode. Moreover, the use of PTFE is also found to increase the columbic efficiency and maximum power density. Figure 2-2 shows a schematic of the single chambered MFC. Electricity generation while treating wastewater in the sewage industry is one of the common application of this configuration specially in a large scale [39].

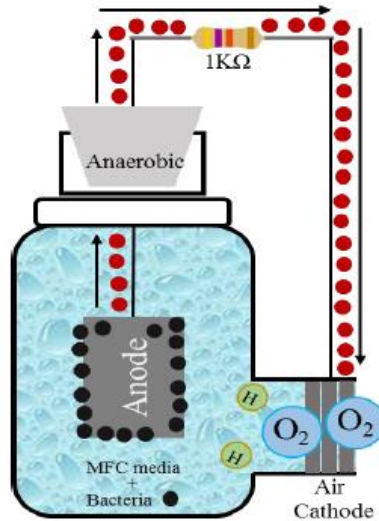


Figure 2-2: Single chamber MFC [37].

2.2.4.2. Double chambered MFCs

Contrary to the single chambered MFCs, these cells are made up of two different compartments: one for anode and the other for the cathode. The compartments are connected to each other, through the use of either PEM or a salt bridge. This connection functions mainly to provide a channel for the transfer of protons and to complete the circuit. Additionally, the PEM or the salt bridge also serves as a barrier to prevent oxygen from diffusing into the anodic chamber and limit crossover of solutions, thus having a higher Coulombic efficiency compared to single chambered MFCs. However, a significant disadvantage when comparing to the single chambered MFCs is that double chambered cells are difficult to scale up, hence, extensive research and utilization is conducted on single chambered MFCs [40].

The operation of double chamber MFCs take place in batch mode. In order, to achieve higher power energy generation, the growth medium suitable for the culture of microorganisms is defined. Also, this configuration is able to produce power in many inaccessible conditions as the compartment can take various practical shapes [33]. Another advantages of using double chamber is that the cathodic compartment shouldn't necessary contain catholyte and it can be used only with air supply [33]. The schematic representation of double chambered MFCs is presented in Figure 2-3. Removal of heavy metals which have positive redox potential (e.g. silver, gold, chromium and etc.) has been working sufficiently in this configuration [41].

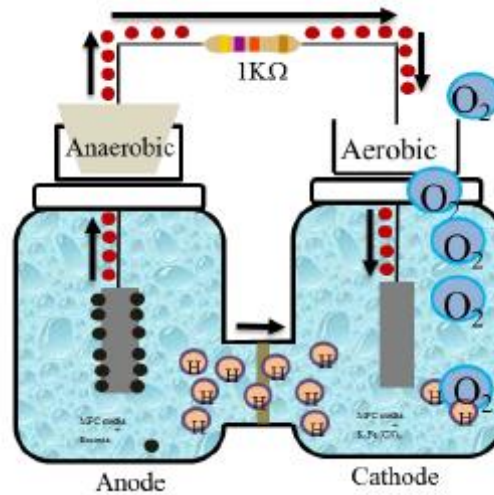


Figure 2-3: Double chambered MFCs [37].

2.2.4.3. Stacked MFCs

This category essentially utilizes a number of fuel cells to form a battery of fuel cells. Such a configuration can be done either by stacking the cells in series or in parallel and each have their own advantages. Figure 2-4 shows an example of a stacked MFC that is tubular and in series.

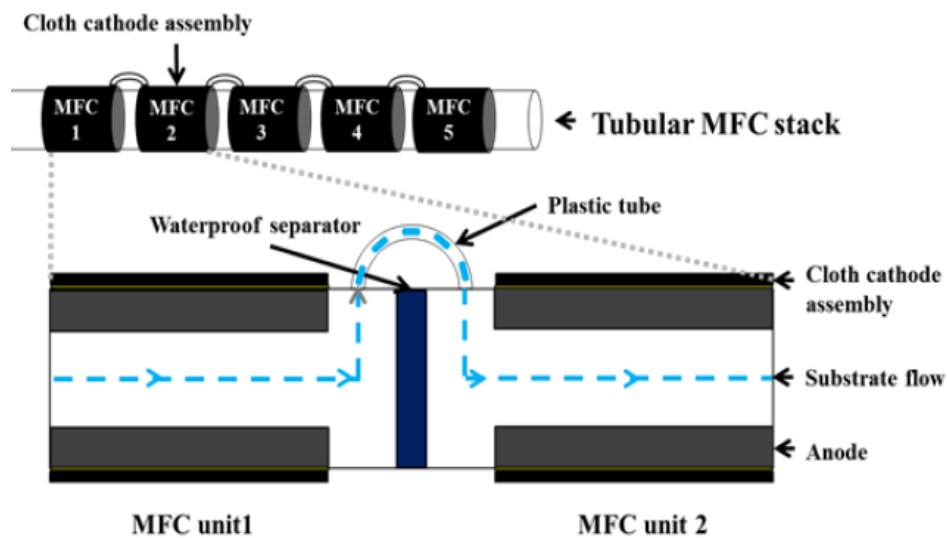


Figure 2-4: Tubular, air-cathode MFC horizontal stack [37].

The main benefit of such a configuration is attributed to the increase in the overall power output in order to match power output of the conventional power sources. Therefore, stacked MFCs can be used as an alternative power source [35]. Another example of using stacked MFCs is the self-power generation in robot EcoBot-II by using unrefined insect biomass. (i.e. 8 double chamber MFCs in series) [42].

2.3. Heavy Metals in Industrial Wastewater

Industries dealing with heavy metals are the most harmful amongst the chemical-intensive industries. Heavy metals are classified into three main groups as shown below[43]:

- Toxic metals (such as mercury, chromium, lead, copper, nickel, zinc, cadmium, cobalt, etc).
- precious metals (such as platinum, silver, gold, etc).
- radionuclides (such as uranium, thorium, radium, etc).

This is due to the large quantities of wastewater discharged from these industries into the environment. Also because of the high solubility of the heavy metals in the aquatic environments, they can be easily taken up by living organisms. In such a case where the metal pollutants enter the food chain, large quantities may end up accumulating in the human body in a process known as bioaccumulation. Some of the common diseases and disorders experienced due to heavy metal toxicity are presented in Table 2-4.

Table 2-4: Toxicities resulting from heavy metals exposure [44].

Heavy Metal	Toxicities
Arsenic	Skin manifestations, visceral cancer, vascular disease
Cadmium	Kidney damage, renal problem, human carcinogenic
Chromium	Carcinogenic, headache, diarrhea, nausea,
Copper	Liver damage, Wilson disease, insomnia
Nickel	Dermatitis, nausea, chronic asthma, coughing, human carcinogen
Zinc	Depression, lethargy, neurological signs, and increased thirst
Lead	Damage the fetal brain disease of the kidneys, circulatory system, and nervous system
Mercury	Rheumatoid arthritis, disease of kidneys, circulatory system, and nervous system

To prevent the harmful effects of heavy metals to the environment and ultimately to the organisms, environmental agencies set limits for their levels in different types of waters. These are based on the type of heavy metal and the maximum concentration allowed in the environment (i.e. maximum contaminant level, MCL) [3]. Table 2-5 presents the

MCLs for several heavy metal pollutants set by EPA for drinking water. Therefore, because of the hazardous nature of the heavy metals in the environment and their tendency to cause significant disorders to different organisms, including humans; it is imperative to treat the wastewater for heavy metals before it is discharged into the environment.

Table 2-5: EPA standard for maximum contaminant level, MCL

Heavy metal	Standards (mg/L)	Reference
Arsenic	0.01	[45]
Cadmium	0.003	[45]
Chromium	0.05	[45]
Copper	0.02	[45]
Lead	0.01	[45]
Mercury	0.006	[45]
Nickel	0.07	[45]
Silver	0.05	[43]
Zinc	5	[43]

Key industries that produce wastewater rich in heavy metals pollutants are the ones that deal in electroplating and metal surface treatment processes (for example, zinc, lead, copper, silver and gold). Such processes include electroplating, electrodeless depositions, conversion-coating, anodizing-cleaning, milling, and etching. Moreover, petroleum refineries are also considered to be a significant source of wastes containing heavy metals, with copper, lead and nickel being the most commonly found heavy metals in their waste effluents. Batteries are another source that result in effluents that are contaminated with heavy metals like nickel, lead, copper and chromium. The above mentioned industries result in massive amounts of wastewater, residues and sludge, that are categorized as hazardous waste and require intensive treatment before their release into the environment [46]. Tables 2-6 and 2-7 presents a summary of different heavy metals and their concentrations in the effluent discharged from different industries. It is important to note that gold refineries do not disclose the concentration of heavy metals in their effluent for the sake of confidentiality and hence, Table 2-7 provides minimal information for such cases.

Table 2-6: Heavy metal concentration in different industries

Heavy metal concentration in common wastewater (ppm)							
Heavy metal	Price USD/Kg	Painting [47]	Electro plating [48]	Battery industrial [49]	Paper and Pulp industry* [50]	Textile activity [51]	Gold mining [43]
Gold	48194 [52]	-	-	-	-	-	-
Silver	405.14 [52]	28.06	15.9	-	-	-	-
Arsenic	164.4 [53]	-	-	0.03	-	-	7.350
Mercury	58.82 [54]	-	-	-	-	-	-
Cadmium	2.6 [53]	16.82	16.6	-	8.75	23.14	-
Chromium	10.42 [55]	5.28	10998	1	187.3	-	-
Copper	4.69 [52]	189.16	598	1	144.4	-	5.063
Nickle	11.24 [52]	-	409	2	-	-	-
Lead	1.65 [52]	18.73	27.6	5	62.5	18.22	0.14
Zinc	1.86 [52]	-	8799	45	-	22.41	0.042

*The sludge was collected from wastewater treatment unit of a paper mill—Sri Bardari Kedar Papers Pvt., Nazibabad, Uttar Pradesh, India

Table 2-7: Heavy metal concentration in different industries

Heavy metal concentration in common wastewater (ppm)					
Heavy metal	Automobile [2]	Background Estuary * [56]	Discharge pipe by WWTP** [56]	Smelting and Pressing of Non -ferrous Metals [57]	Paper mill industry [58]
Gold	-	-	-	-	-
Silver	-	-	-	-	-
Arsenic	-	-	<0.05	0.86	-
Mercury	-	-	<0.02	0.002	-
Cadmium	6.09	-	<0.005	0.123	-
Chromium	0.46	0.19	0.3	0.036	-
Copper	14.50	38	36.8	0.003-0.38	212
Nickle	2.04	34	1.27	0.07-0.38	39.6
Lead	2.91	28	24.7	0.41	-
Zinc	19.38	100	3.98	0.6-17	28.4

*deep sediments in the Pearl River Delta Estuary, china

**pipe immediately behind the WWTP of the facility. Occasional wastewater

2.4. Methods for Removal Heavy Metals

To remove heavy metals from wastewater, different techniques exist that can be classified as either electrochemical treatment, physicochemical processes, or novel methods which developed recently to overcome obstacles of previous methods. This section details out the advantages and the limitations of such techniques, as well as brief description [59]. Flow diagram of different techniques is shown in Figure 2-5.

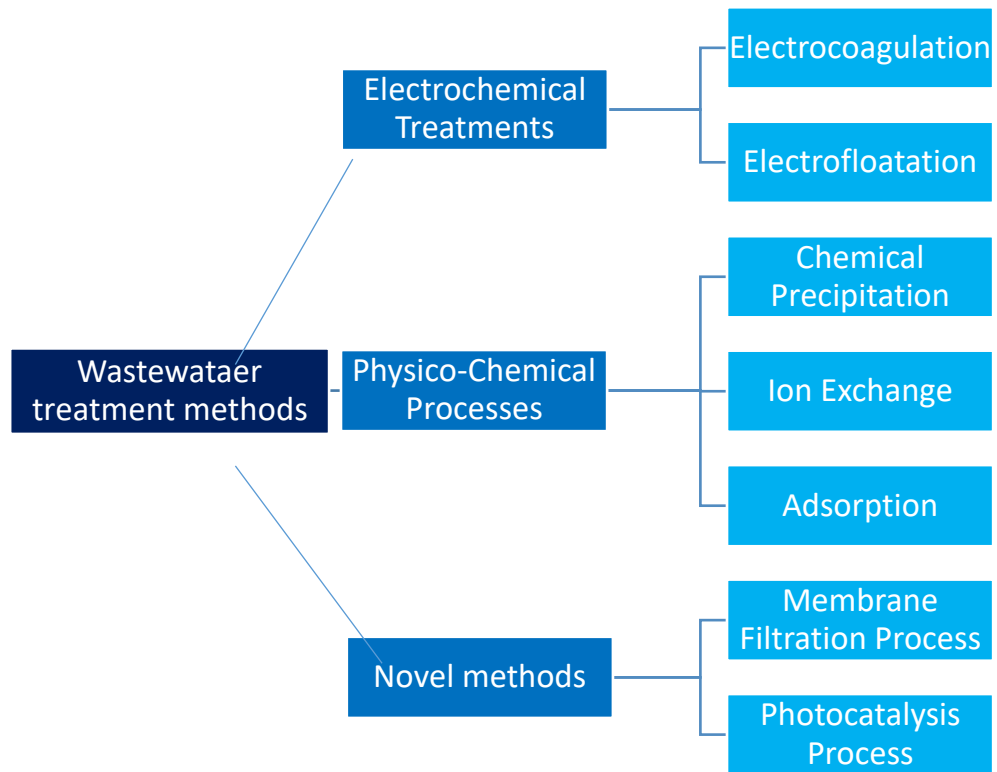


Figure 2-5: Tree diagram for wastewater treatment methods.

2.4.1. Electrochemical treatments

Recently, the electrochemical technologies are more comparable to other technologies not only in terms of capital cost, but also in terms of efficiency and are also considered to be more compact. Additionally, electrochemical treatment may form an important step in the treatment of wastewater that contain refractory pollutants. Two of the major techniques under electrochemical treatments are described below.

2.4.1.1. *Electrocoagulation*

In this process, a small electric current is applied to the wastewater solution. This electric field allows the electrostatic charges that hold the pollutants in a suspended or emulsified state to be neutralized. After this step, the pollutants will coagulate from the aqueous phase, forming what is known as the sludge or floc. The resulting flocs are produced in large quantities, which are more stable and contain less bounded water, when compared to other techniques [60]. Figure 2-6 displays the process of electrocoagulation. By this method, removal of multiple types of heavy metals (e.g., copper, chromium, nickel, etc.) is applicable according to the type and configuration of electrode. For instance, one can remove 96.7% of zinc by using aluminium electrode in parallel design [59].

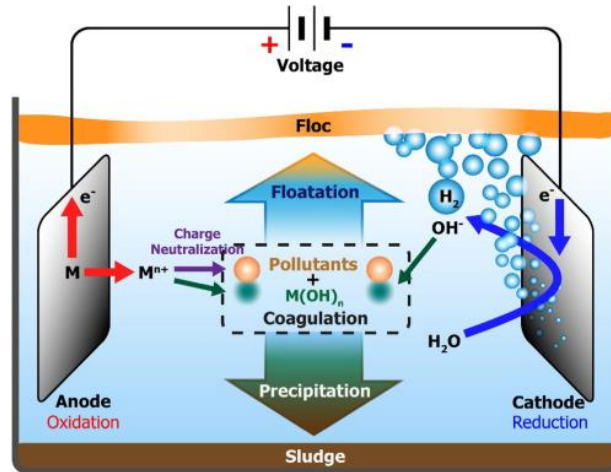


Figure 2-6: Schematic of how electrocoagulation-electrofloatation works [61].

2.4.1.2. *Electro-Floatation*

This technique separates pollutants by causing them to float to the surface of the liquid (see Figure 2-6). The separation of the pollutants takes place according to the following steps [62]:

- I. The reaction taking place at the electrode is electrolysis of water (see below), hence, oxygen and hydrogen are formed.



- II. The pollutants then adhere or adsorb on the oxygen and hydrogen molecules, thus resulting in destabilization of the emulsified particles
- III. After destabilization, the pollutants will then form foam and rise up or form flocs and settle down.
- IV. The final step is to remove the pollutants using filtration methods

Zink, copper, lead and nickel are common heavy metals that can be removed by electro floatation from different wastewater solutions [59].

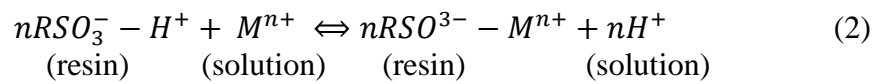
2.4.2. **Physiochemical process**

2.4.2.1. *Chemical precipitation*

In this case, chemicals are added to wastewater that cause heavy metal ions to convert into insoluble solid particles (hydroxide precipitation and sulfide precipitation). Thus, after the formation of the precipitates, the solid phase can be removed from the wastewater either by filtration or sedimentation [63]. Common heavy metals removed by the chemical precipitation are nickel, copper and chromium [64].

2.4.2.2. Ion exchange

The process of ion exchange operates by having an interchange of ions between the solid and liquid phases. This process utilizes resins that remove heavy metal ions from the wastewater solution and releases other ions that are having the same charge in a chemically equivalent amount. This also results in structural changes of the resin. The reaction below summarizes the process of ion exchange [65].



Mercury, arsenic, copper and lead are common heavy metals that can be removed by this method [64].

2.4.2.3. Adsorption

Adsorption is a mass transfer process between the liquid and solid phases (i.e. the adsorbent). The key steps involved in this process are:

- a. Entry of the pollutant from the bulk solution to the adsorbent surface
- b. Adsorption of the pollutant on the surface of the adsorbent
- c. Penetration in the adsorbent structure

A key advantage of adsorption is that the process is reversible (known as desorption), thus allowing for the regeneration of the adsorption and reducing costs. Activated carbon (AC), carbon nanotubes (CNT) and sawdust are some of the most commonly used adsorbents [66]. Removal efficiency of copper, cadmium by using CNT is greater than 99% and 80 % respectively, while by using AC as an adsorbent the efficiency is 100% and 96.4% [64].

2.4.3. Novel methods

2.4.3.1. Membrane filtration process

Membrane, porous or a nonporous structure can be used as a contact between two homogeneous phases in order to remove pollutants of different sizes. Different parameters affect the performance and efficiency of the membrane. These include the material of construction, pore size and composition [67]. The combination of such features can determine the efficiency and economics of separating pollutants. Membrane filtration technologies can be sub-categorized into five major treatment processes (as shown below) that are based on the pore structure (i.e. pore size, pore size

distribution, and porosity), membrane permeability, and applied operating pressures [68]. Namely, these are reverse osmosis (RO), ultrafiltration (UF), microfiltration (MF), nanofiltration (NF) and electro dialysis (ED). For example, by using RO membrane you can remove 95% of copper from the wastewater solution while removal efficiency of mercury by using UF membrane is 99%.

2.4.3.2. Photocatalysis process

Using a light source (such as ultraviolet) the pollutants in the wastewater can be degraded through photochemical reactions (as a kind of Advanced Oxidation Processes (AOP)) and ultimately removed from the solution. Silver, gold and platinum can be recovered by this method [59]. The mechanisms for such a process treatment involve the following steps:

- pollutants diffuse to the surface of the liquid phase,
- semi-conductors added to the liquid phase absorb the pollutants on their surface,
- photocatalytic reactions then take place in the absorbed phase, and
- the pollutants are then degraded and removed from the solution.

In the above steps, Step 3 is considered to be the most significant step, as the photocatalytic reactions take place in this step. This entire process uses light as an activator, as compared to thermal activation of old processes, thereby causing the photocatalysis process to consume less energy [69]. A summary of the above steps is provided in Figure 2-7 below [70].

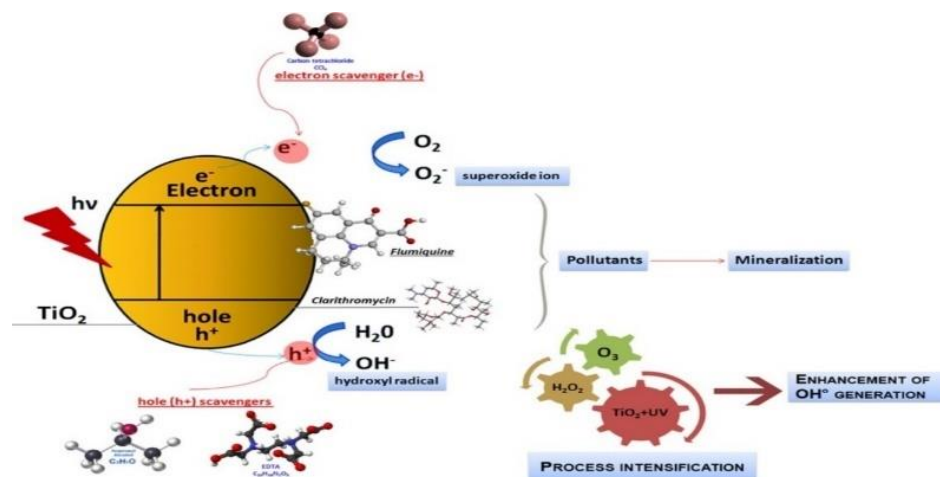


Figure 2-7: Schematic of photocatalytic process [70].

The advantages and disadvantages of the above-mentioned wastewater treatment techniques are presented in Table 2-8.

Table 2-8: Summary of advantages and disadvantages of wastewater treatment techniques [12], [61], [71], [72].

Method	Advantages	Disadvantages
Electrocoagulation	<ul style="list-style-type: none"> • Environment-friendly technique • Eliminate the need of adding new chemical additives • Removing even small colloidal particles 	<ul style="list-style-type: none"> • Cannot remove infinitely soluble particles • Extra operational cost for sludge disposal
Electro-Floatation	<ul style="list-style-type: none"> • Operate efficiently for very dilute solutions • Simplicity in design and operation • Low running costs • Small and compact units 	<ul style="list-style-type: none"> • Sensitive to operating condition • Power consumption • Subsequent treatments are required to improve the removal efficiency of heavy metal.
Chemical Precipitation	<ul style="list-style-type: none"> • Low capital cost, • Simple Operation 	<ul style="list-style-type: none"> • Sludge generation, • Extra operational cost for sludge disposal • Very sensitive to pH
Ion Exchange	<ul style="list-style-type: none"> • No sludge generation • Less time consuming • High selectivity 	<ul style="list-style-type: none"> • Not all ion exchange resins are suitable for metal removal • High capital cost
Adsorption	<ul style="list-style-type: none"> • Can be used for removal of toxic metals • High removal efficiency 	<ul style="list-style-type: none"> • Discharging lots amounts of adsorbents to water environment. • Small capacity • High cost
Membrane Filtration Process	<ul style="list-style-type: none"> • Higher removal efficiency • No pollution loads • Sometimes lower energy consumption than conventional methods 	<ul style="list-style-type: none"> • Establish an appropriate mathematical model for its operation • High cost • Complex process • High power consumption for RO (reverse osmosis) process
Photocatalysis Process	<ul style="list-style-type: none"> • Simple design • Low-cost operation • High stability • High removal efficiency 	<ul style="list-style-type: none"> • Designing effective reactor in scale-up use • Drawbacks in visible light activity

2.5. Removal Heavy Metals Using MFCs

As mentioned, MFC is a novel technique that can use the organic matter in wastewater to generate electricity and to remove pollutants, such as heavy metals, from their solutions. Metal pollutants, such as chromium, copper, vanadium and mercury, have been found to be removed using two chambered cells. This section discusses the performance of MFCs in removal of heavy metals, factors that can affect the efficiency and performance of MFCs and the challenges and their possible solutions when scaling up [73] [74].

2.5.1. MFC performance on heavy metals removal in wastewater

Heavy metals in MFCs are removed through the reduction of the cathode metal in the anaerobic cathodic chamber, while in the anodic chamber, organic matters are used as sources of carbon and electron donors [41]. It has been demonstrated that such processes as biosorption and precipitation reactions (i.e. sulfides and hydroxides) greatly aided in the removal of heavy metals from wastewater in the MFC system [75]. Table 2-9 presents a summary for the removal of different metals using MFC technologies along with the maximum removal and maximum power generation.

Table 2-9: Summary of heavy metals removal using MFCs [76]–[78].

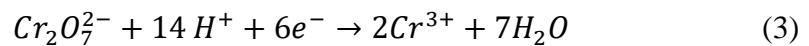
Metal	MFC Fabrication	Maximum removal recovery	Maximum power generation	Substrate
Cu (II)	Single-chamber MFC, Carbon brush for anode, Carbon cloth/Pt coated for cathode	98.3 %	10.2 W/m ³	Sludge
Cu (II)	Two-chamber MFC, Graphite plate for anode, Graphite foil for cathode	99.88 % (Anaerobic) 99.95 % (Aerobic)	0.43 W/m ² (Anaerobic) 0.80 W/m ² (Aerobic)	Acetate
Cr (VI)	Two-chamber MFC, Carbon felt for anode, Carbon cloth(a)/Carbon brush(b)/ Carbon felt(c) for cathode	100 % (a) 33.45 % (b) 12.72 % (c)	1221.91 mW/m ²	Acetate
Cd (II)	Single-chamber MFC; Carbon cloth for anode; Carbon cloth/Pt coated for cathode	90 %	3.6 W/m ²	Sewage sludge
Hg (II)	Two-chamber MFC, Graphite felt for anode, Carbon paper for cathode	99.54 % (for 100 mg/L Hg (II))	433.1 mW/m ²	Mixture of sludge with artificial wastewater
Ag (I)	Two-chamber MFC, Carbon brush for anode, Carbon cloth for cathode	99.91 % (for 50 ppm Ag (I))	109 mW/m ²	Mixture of sludge with artificial wastewater
Au (III)	Two-chamber MFC Carbon brush for anode, Carbon cloth for cathode	99.88 % (for 200 mg/L Au (III))	6.58 W/m ²	Mixture of sludge with artificial wastewater

2.5.2. Factors affecting removal of heavy metals from wastewater in MFCs

2.5.2.1. Effect of pH

Heavy metal removal is a strongly pH dependent process. However, dependence of removing these heavy metals to pH differ from each other based on their redox reaction that take place in cathode compartment.

Due to the presence of PEM, a transport barrier exists that prevents the protons to diffuse from the anodic chamber to cathodic chamber. During the earlier stages of MFC operation, the production rate in the anodic chamber is higher than the consumption rate in the cathodic chamber. This results in different pH in the two chambers. However, the driving force of protons between anode and cathode chamber will increase by pH difference. Thus, ultimately resulting in the formation of dynamic equilibrium, whereby the transportation rate of the protons from the anode chamber to the cathode chamber will equal to the sum of the consumption rate of the protons at the cathode and their production rate at the anode. Having the right pH in the MFC chambers is imperative for stability and efficiency of the cells, the performance of the microorganisms (bioelectrochemical performance) and recovery of metals in wastewater [79]. As a rule, a neutral pH is preferred in the anodic chamber as it allows for the existence of favorable conditions for the growth of the exoelectrogenic microorganisms. Whereas in the cathodic chamber, a lower pH is preferred as the reduction reactions of the metals greatly relies on acidic conditions [41]. For instance, typical reduction of Cr(VI) take place in MFC according to Eqn. 3. Increasing H^+ concentration (lower pH) provides enough reactant for reduction reaction. Hence metal recovery is increased. Decreasing pH can also result in increasing cell potential (according to Nernst equation (Eqn. 4) and definition of cell potential (Eqn. 5)).



$$E_{cat} = E_{cat}^0 - \frac{RT}{nF} \ln \left(\frac{[Cr^{3+}]^2}{[Cr_2O_7^{2-}][H^+]^{14}} \right) \quad (4)$$

$$E_{cell} = E_{cat} - E_{an} \quad (5)$$

pH in these chambers can be controlled through the use of phosphate buffers [80]. However, it is important to note that phosphate buffers are expensive and thus will

increase the overall cost of the MFC. Therefore, another solution could be to the use of a hybrid system in the form of stacked MFCs that results in self pH regulation [81].

2.5.2.2. Effect of cathode materials

The choice of the material of construction for the cathode can greatly determine the performance and efficiency of the MFC. A good material for the cathode should have the following properties:

- Excellent electrical conductivity
- Large surface area
- Good stability
- Cost effective

The most commonly used materials in the construction of cathode are graphite (such as graphite foil, graphite plate, graphite felt, graphite rod, etc.) and plain carbon (such as carbon cloth, carbon paper, carbon brush, carbon felt, carbon fiber, carbon rod, etc.). Other metals such as stainless-steel mesh, nickel foam and titanium sheet have also been used to make cathodes, as they have the advantages of having higher electrical conductivity, significant facilitation of microbial adhesion and durability. However, such materials also have the added disadvantage of being expensive, thus increasing the cost of the MFCs [82].

Real voltage output of a cell is lower than the voltage is predicted by thermodynamic. During MFCs operation, voltage of cell is reduced due to ohmic losses, mass transport losses and activation losses. Electrode materials play an important role in ohmic resistance and charge transfer resistance of MFCs. Hence changing removal efficiency and maximum power density of MFCs. In an experiment, removal efficiency of Cr(VI) in a MFC system using different cathode material was investigated (i.e. carbon cloth, carbon brush, and carbon felt). The charge transfer resistance of three electrode materials were 7.52, 73.86, and 113.66 Ω , respectively and the ohmic resistance were 5.6, 4.8, and 4.3 Ω , respectively. Ultimately, results showed a higher removal efficiency and maximum power density in MFC with carbon cloth cathode comparing to two other cathode materials [75]. Maximum power density of the MFC can be found according to polarization curve while implying different external resistance. The area under polarization curve can be used also for calculating the efficiency of the cell.

2.5.2.3. Bacterial culture

Research was carried out to understand which species of microorganisms produce the highest power and are efficient. Both mixed and pure cultures were studied, and it was observed that mixed cultures result in a higher generation of power when compared to pure cultures. For example, a mixed culture of bacteria (i.e. waste water or sludge that contain diverse source of bacteria) can produce 22% more electrical energy than a pure culture of *Geobacter sulfurreducens* [38]. However, in order to find suitable bacterial inoculum (pure culture or mixed culture) that is able to generate higher power density; similar MFCs performance with different inocula should be considered as few studies indicated that pure culture can also produce high current [83].

2.5.3. Challenges and solutions for the scale-up of MFCs

A key challenge in the scaling-up of the MFCs is the presence of thermodynamically favourable conditions for metals in the cathodic chamber. This implies that for the electron to be accepted directly by the metal without an external power supply, the metal has to have a positive standard potential. Metals with lower or similar redox potentials to that of the anode will not be able to readily accept electrons from the cathode, thus requiring the presence of an external power supply in order to “force” the electrons to the cathode and be ultimately accepted by the metals [41]. Examples of such metals are nickel, lead, cadmium, and zinc. A possible solution to overcome such a challenge (without the use of an external power supply) would be to use a hybrid system. For instance, two MFCs can be used where one is used as a power supply for the other (for example, one MFC for treating wastewater containing chromium powering another MFC containing cadmium) [84].

Heavy metals are known to be toxic to organisms. Hence, it is understood that their presence in wastewater will present a toxic environment for the microorganisms in the anodic chamber, thereby reducing the efficiency of the MFC and even resulting in its deterioration [76].

One of the major challenges faced during the operation of MFC is the phenomenon of membrane fouling. Membrane fouling often occurs in MFC due to the biofilm that grows on and inside the chambers during the long-term operation. This phenomenon has been extensively studied to understand the effects and consequences of uncontrolled

fouling, specifically biofouling [85]. It was demonstrated that poor cleaning of PEM can result in fouling on both the anodic and cathodic sides of the MFC. This would result in a decreased coulombic efficiency and reduced power density, as the protons are unable to move from the anodic chamber to the cathodic chamber due to the blockage of the membrane caused by fouling. A solution to fouling would be to clean the membrane every 6-7 months, as it takes about 6 months to results in fouling [86]. However, such a solution is only practical on the lab scale. Additionally, replacement of the membrane would also result in a high capital cost (38% of the cost of the MFC is due to the membrane), making replacement a poor option. The membranes generally used in MFC are based ion-exchange perfluorinated membranes, such as Nafion which are highly susceptible to biofouling and costly. An alternative to the use of Nafion membranes could be membranes that are based on polybenzimidazole. It was observed that polybenzimidazole based membranes are not only less susceptible to biofouling due to their polymer preventing adhesion of microbes, but also results in an increased power density, operating life, low cost and increased efficiency of the MFC during wastewater treatment when compared to Nafion membranes [87].

Lastly, although MFC provides relatively cheaper alternative for producing energy, a key drawback while scaling up the cell is the high costs associated with electrodes and membranes. It has been reported that for a MFC with a capacity of 250 ml; a platinum based electrode would cost around 250 USD for the 20 cm² surface area [83]. Hence, scaling up the cell would incur significant capital costs, thereby making it less desirable for industrial use.

2.6. Gold Refinery Process

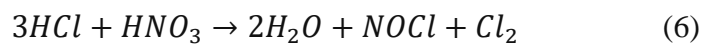
The process of refining gold from mined material, doré and recycled products (i.e. jewellery and electronics) generate large amount of wastewater include huge amount of heavy metals. Thus, treatment of this wastewater is necessary either to reduce their concentration to permissible values or precipitate more gold metals [88], [89].

There are several different methods for refining gold metals such as inquartation and parting, Miller Chlorination, Wohlwill Electrolyte and Aqua regia process. Each method can be chosen based on different parameters such as the amount of recovery, initial gold concentration and final cost and environmental effect [90]. One of the usual

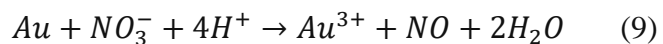
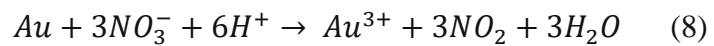
methods used in industry is Aqua regia process. The Aqua regia process refines gold from other metals by using a combination of acids and chemical products. The name of aqua regia comes from a Latin word meaning ‘royal water’ due to its property of dissolving notable metals such as gold and platinum. The aqua regia solution is produced by adding one part of nitric acid to three parts of hydrochloric acid. Hence, it fumes and it is corrosive in nature with yellow or red colour [91].

The process takes place according to the following steps [92], [93]:

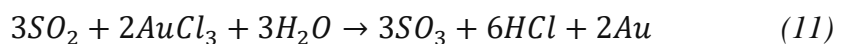
1. Aqua regia solution is prepared according to the reactions below:



2. Gold alloys is fed into aqueous solution to dissolve gold in the solution as shown below:



3. After allowing the acid to dissolve the gold; the solution will be filtered to participate gold metals.
4. Precipitating agents (such sodium metabisulfite and SO₂) will be added to solution to sediment gold from other metals and filter. Also, during this process urea is added to the solution to remove nitric acid and allowing precipitation reaction to take place.



5. The sludge is then washed and dried.
6. The gold is then melted into the required form.

However, the main disadvantage of this process is that hazardous by-products due to chemical reactions are formed and large quantities of wastewater containing heavy metals are produced. Hence, pre-treatment is required before the discharge into the environment [1].

Chapter 3. Methodology

In this chapter, an introduction of the problem along with the motivation of overcoming it is explained. Materials and instruments that was used during this experimental work is discussed in detail.

3.1. Introduction

This thesis aims to study the feasibility of recovering precious metals such as gold from metal containing solutions using microbial fuel cell. Moreover, it is also aimed to evaluate and assess the feasibility when other metals are present in the solution. One of the main possible applications of such research is the extraction of gold from refining effluents. One of the most prevalent heavy metals present in the gold refining industry effluents is copper; therefore, if an efficient method of extraction can be discovered, it would be highly beneficial. Recovering of even low concentration of precious metal such as gold has attracted lots of attention due to increasing demand in all sectors especially the rapid increase in industrial and individual demand. On the other hand, since heavy metals are highly soluble in the aquatic environment; hence, their introduction to the food chain will result in severe disorders and diseases. Thus, removal and recovery of them from the industrial effluent is required to ensure it meets global environmental standards. Several techniques have been used to treat heavy metals, and although some of them have been proven to remove heavy metals from industrial effluent to the limits required, however, they suffer from high costs, requirement of the regeneration processes and production of secondary contaminations. Therefore, implementation of a new technique which will be more cost effective and capable of simultaneous removal of other heavy metals and recovery of the precious ones and reduced chance of generation of the secondary contamination, is necessary.

MFC's are a source of renewable energy that has the capability of simultaneously reducing the concentration of heavy metals to an allowable limit, extracting precious metals, and producing energy. Hence, it can be considered as a self-dependent technique that does not require any external power for its performance. This research aims to investigate the effect of different parameters on the performance of MFC's mainly in terms of recovery of gold metal in addition to the removal efficiency of other heavy metals, such copper. Thereby offering the most suitable MFC conditions to

achieve the highest removal efficiency of gold and copper from the effluent of the gold refinery industry while producing maximum power. In 2020, the LBMA (which is a reference price and market-reflective benchmark for gold contract's price that broadly apply across industries) gold price (US\$/Oz) has increased by 27% in one year from 1393 US\$/Oz in 2019 to 1770 US\$/Oz in 2020 [94]. This has triggered the interest of golds recovery from industrial wastewater through an eco-friendly as well as cost-effective method.

In this study, initially, solutions of pure gold ions are tested to investigate the ability of MFC in removing and recovering them plus the power generation. Recovery of gold from the pure tetrachloroaurate ions from the artificial solution was previously investigated and a removal efficiency of 99.89% was reported for gold ions from a solution with Au(III) concentration of 200ppm [95]. This data was used to validate the experimental work at this stage.

Moreover, the most suitable condition in terms of catholyte solutions as well as initial gold concentration is discovered. The effect of copper, which is another common heavy metal present in the gold refinery effluent, on the gold removal efficiency was also investigated. The results of another previous study in this matter have been used to validate the experimental data in this section. In the research of Zhang et al [96] 95.4% of gold removal was reported where the initial concentration of Au (III) and Cu^{2+} were 100 ppm and 400 ppm respectively.

The removal efficiency of Au (III) ions from real industrial wastewater where other heavy metals such as copper, chromium, lead etc. may exist, was investigated for the first time. In addition, the removal of one other heavy metal (i.e., copper as the dominant heavy metal in the gold refinery effluent) was evaluated. Moreover, in this step, the effect of pH as well as initial biomass concentration was assessed to suggest the most suitable conditions.

3.2. Materials

The following materials have been utilized in this work:

- Preparing a buffer solution: 0.1 M of potassium phosphate buffer to control the pH difference of the chambers and prevent the detrimental effect of microbes due the fast proton diffusion at low pH. The desired buffer was prepared by

mixing 61.5 ml of potassium hydrogen phosphate (1M) with the 38.5 ml of potassium dihydrogen phosphate (1M) while making the solution up to 1 liter with deionized water [97].

- Preparing biomass: different concentrations of biomass (50, 80, 100 g/L) through the activation of dried yeast powder with the addition of glucose as nutrient. The yeast powder was purchased from a commercial market and it was manufactured by DCL.
- Preparing the mediator solution: 0.01 M methylene blue by dissolving 1.87g of it in a 500ml buffer solution while storing it at room temperature.
- Catholyte solution: different types of catholyte were used to for the purpose of finding the most efficient conditions as well as study the effect of existence of other types of heavy metals on the efficiency of the gold removal and recovery processes, as reported below:
 1. Preparing a 50 ml solution with a concentration of 500 ppm tetrachloroaurate with deionized water.
 2. Preparing 3 different 50 ml solutions of tetrachloroaurate ions with initial concentrations of 500 ppm, 250 ppm, and 125 ppm using a buffer.
 3. Preparing 50 ml solution containing a mixture of tetrachloroaurate and copper ions using a buffer to make the initial concentrations of Au (III) and copper as 250 ppm and 1000 ppm respectively.
 4. Collecting 50 ml samples from the wastewater effluent of one of the gold refineries in the UAE where the initial concentration of Au (III) and copper were 250 ppm and 1300 ppm separately.
- Preparing an anolyte solution (microbial medium): 25ml solution of the mixture of biomass, methylene blue as mediator, and buffer solution to control the pH.
- Anode and cathode: carbon cloth and carbon brushes were used as cathode and anode, respectively. Due to the limitation of time and budget, the carbon brushes were hand made using strips of carbon cloth braided on the steel wire.

- Membrane: Nafion membrane is used to separate the cathodic compartment from anodic compartment while allowing only proton diffusion between two compartments.
- MFC: the cell was constructed of rectangular blocks made of acrylic. To ensure no leakage is present rubber gaskets were used in between the rectangular blocks. The cell was held together using clamps.

3.3. Instrumentations

Different types of instruments have been used to control and examine the operating conditions along with the investigation of the gold and copper concentration within the samples during the performance of the MFC, as discussed below:

- pH and temperature meter: HANNA HI2020 benchtop pH meter was used to simultaneously assess and monitor the pH and temperature of the cell as time is passing during its performance of the MFC.
- Gold and Copper concentration: VARIAN Atomic Absorption Spectroscopy (AAS) was used where Au and Cu lamps, in addition to their standard solutions, were utilized to create the calibration curve and consequently, evaluate the concentration of the desired metals (Au, Cu) within each sample.
- Voltage: Open circuit potential was evaluated by using digital FLUKE 87-V-Eur industrial multimeter.
- Magnetic stirrer: a small magnetic chip is placed inside the anodic chamber to mix the microbial medium and force the microbes to work at their highest efficiency.
- Radwag weighing balance

3.4. Working Principle & Calibration Curves for AAS

In order to investigate and determine the concentration of certain metals inside the samples, AAS as an analytical technique has been utilized, since every specific atom/ion is only able to absorb light at a particular and unique wavelength. AAS uses this characteristic to evaluate the concentration of each metal. Once light with this specific wavelength has been used, the atoms are able to absorb this energy which results in the excitement of their electrons from ground level to an excited state. Hence, based on the amount of light absorption within the sample, the concentration of the

specific metal inside the sample can be evaluated. For each metal, a particular lamp is required to provide a unique wavelength suitable for that metal. In this study, since the concentration of Au and Cu are required, relevant lamps were chosen, and calibration curves generated accordingly.

The main purpose of the calibration curve is to determine the concentration of the desired metal (Au and Cu) in the sample based on the amounts of absorbents. To calibrate AAS for Au; initially a blank solution which determines the baseline measurement of the absorption can be used. This blank solution is free of Au elements and it is prepared using deionized water. Next, three different standard solutions with known amounts of Au concentration were prepared (2.5 ppm, 5 ppm, 7.5 ppm). After that, the amount of absorption for the standards was calculated and based on that the calibration curve was created. The AAS software will automatically create a different calibration curve every time since the absorption amounts vary depending on the operating conditions which were changed for every run. However, sometimes the software was unable to automatically create a calibration curve mainly due to the excessive curvature within the absorption of the standards. Figure 3-1 is the calibration curve that was constructed for the Au metal.

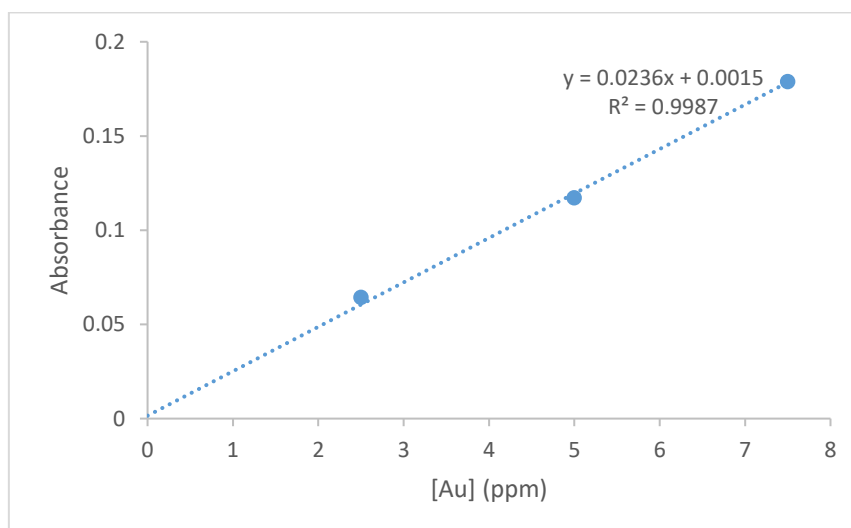


Figure 3-1: Au - Calibration curve

The same procedure was performed for the preparation of the calibration curve for Cu (Figure 3-2). However, in this case standards with the following concentrations were used: 2.5, 5, 7.5 and 10 ppm.

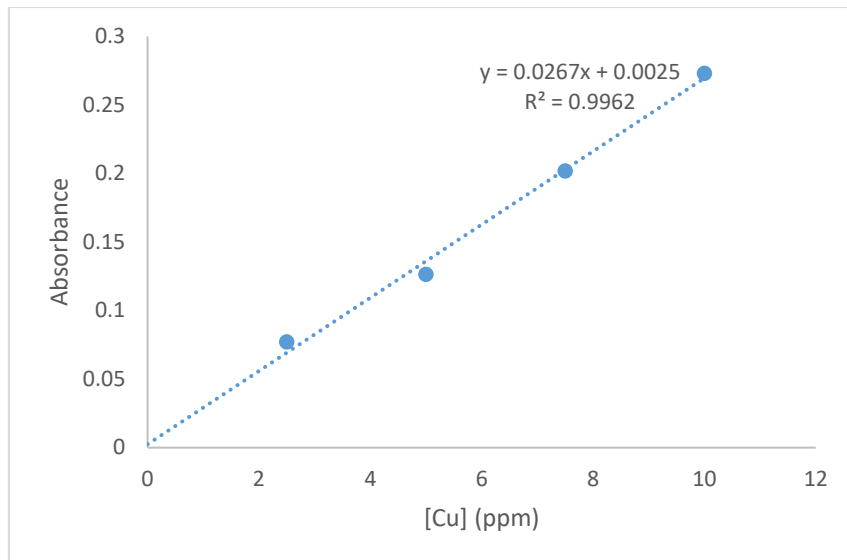


Figure 3-2: Cu - Calibration curve

Thus, by knowing the amount of absorbance, any Cu and Au concentration inside the unknown samples can be determined. Consequently, the percentage of removal efficiency as well as recovery efficiency can be calculated based on the Equations 12 and 13.

Chapter 4. Experimental Setup

In this chapter, the description for the proposed MFC is explained first. Next, the method that has been used for the activation of the biomass is discussed. Lastly, main stages involved during the experimental work is described

4.1. Description of Apparatus

In this study, a cubic dual chamber MFC from the acrylic rectangular blocks was implemented. This system is shown in Figure 4-1. The first chamber (on the left) is the anodic chamber containing the microbial medium and the anode where continuous mixing is required as mentioned in previous studies [95]. A small magnetic chip is placed inside the anode chamber while the whole cell is placed on the magnetic stirrer base to fulfill the aforementioned requirement. In addition, the carbon brush is used as an electrode due to its higher surface area in comparison to the carbon cloth electrodes as represented Figure 4-2.



Figure 4-1: Schematic of the cubic dual chamber of MFC.



Figure 4-2: Carbon brush as electrode for the anode chamber

The anionic chamber is split by the Nafion membrane while placing a gasket rubber sheet in both sides to prevent leakage. It's worth to mention that the pretreatment of the membrane was performed by keeping it initially for one hour in boiling deionized water and then soaking it in deionized water at room temperature for 24 hours before its usage inside the cell to allow hydration and expansion of the pores following the work of Wand et al along with the work of Ogawa [97][98]. The second chamber referred to as the heavy removal chamber is where the desired catholyte solutions will be fed. Also, the carbon cloth is utilized as an electrode.

The cell is operating under anaerobic conditions due to:

1. The type of the dried yeast that serves as biomass; they exhibit higher efficiency under anaerobic conditions. Consequently, higher electrons can be generated, and thus, higher power can be generated through an external circuit.
2. Since oxygen can also be assumed to act as an oxidizing agent, its existence will suppress the reduction reaction of the heavy metals. Therefore, it will reduce the removal efficiency of gold and copper. Hence, the cathode section must work under anaerobic conditions to prevent the reduction reaction of oxygen.

Therefore, all chambers were purged with nitrogen to remove dissolved oxygen inside each chamber and then the holes on top of each chamber were sealed with the aid of silicon sealant (Figure 4-3). The cathodic chamber held double the volume of the anodic chamber (50 ml in cathodic chamber and 25 ml in anodic chamber) according to the Wang et al study [99]. Lastly, by using clamps, the whole cell was held together.

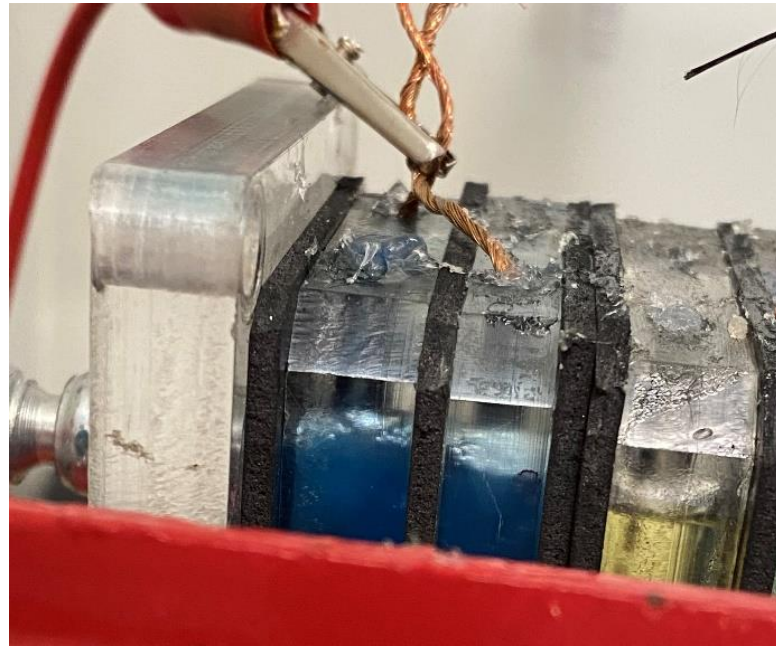


Figure 4-3: Sealing the holes with silicon sealant to prevent any oxygen diffusion inside each chamber.

4.2. Biomass (Yeast) Activation

Different biomass concentrations (50, 80, 100 g/L) were prepared during this study as follows:

4.2.1 Preparing 100 g/L activated yeast

2.5 g of DCL activated dried yeast was added to 25 ml of water at a temperature of 38 °C. 3.75 g of glucose was added to the mixture as a nutrient for the yeast. Next, the top of beaker was covered with the plastic cling foil while covering the whole beaker with a large cloth to allow resting while keeping the environment warm for the proper yeast activation. The solution was left for around 20 minutes until a layer of sludge with bubble was created on top of it. At this stage it is ensured that the yeast has been activated properly as shown in Figure 4-4.



Figure 4-4: Created sludge layer on top of solution as representation of proper yeast activation.

4.2.2 Preparing 50 and 80 g/L activated yeast:

In term of the activation process, it is similar to the aforementioned process (100 g/L yeast). The only difference is in terms of the amount of dried yeast and consequently, glucose used. For the preparation of 50 g/L yeast solution, 1.25 g of dried yeast were mixed with the 1.875 g of glucose while for the 80 g/L of yeast, 2 g of dried yeast were mixed with the 3g of glucose.

4.3. Sample Preparation

Each catholyte sample was diluted 100 times with the aid of nitric acid (1M) to create 10 ml volume. This was done to ensure the volume is sufficient for AAS to accurately analyze. Another reason behind the dilution of the solution is to ensure the sample concentrations exist within the range of the calibration curve.

4.4. Experimental Work

Experimental work will be divided into three main stages:

1. Removal efficiency of pure tetrachloroaurate ions.
2. Simultaneous removal of gold ions with the incorporation of copper ions.

3. Removal efficiency of gold ions from the industrial wastewater

Each stage will be illustrated in detail in the following sections.

4.4.1 Removal efficiency of pure tetrachloroaurate ions

In this stage, the performance of MFC for removing pure tetrachloroaurate ions is investigated under different catholyte solutions. After finding the proper catholyte solution, the effect of different initial Au (III) in removal efficiency was investigated. In terms of discovering the most proper catholyte solution two different catholytes were used. The first solution was prepared using Au (III) with a buffer solution; while the second solution was made with Au (III) with water, both having an initial concentration of 500 ppm Au (III) and total volume of 50 ml. On the other hand, the anodic compartments of both cells were same to each other. That means the 25 ml of anolyte solution was created by mixing 80 g/L of activated biomass with 0.33 ml of methylene blue and 2.5 ml of buffer (with the rest being the activated yeast) and was fed into the anodic compartment. After pretreatment of the membrane and purging the whole cell with nitrogen, the MFC was ready to run. The voltmeter was connected to the electrodes before filling the compartments with their respective solutions and starting the cell. Later, after the successful installation (in terms of leakage) and running the cell, 0.1 ml samples of the catholyte solutions were collected to analyze the gold concentration of the cell over time. This volume of sample was assigned to prevent any changes in operating conditions in terms of cathode chamber volume. Samples from the catholyte were taken every 5 minutes, 15 minutes, 25 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 24 hours, and 48 hours after starting the cell. During the collection of the samples the pH, temperature, and the OCP (open circuit potential) values were evaluated and monitored. After 24 hours of the cell operation a mixture of 1 ml yeast, 0.4 ml methylene blue, and 3ml buffer was added to the anolyte solution. This was done to prevent any reduction in the number of yeast cells.

Finally, the removal efficiency of the metal (either gold or copper) was calculated based on Equation 12.

$$\text{Metal removal efficiency (\%)} = \frac{C_0 - C_t}{C_0} * 100 \quad (12)$$

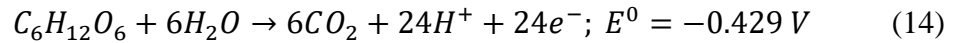
where the C_0 represent the initial gold concentration inside the catholyte (ppm) and C_t is the gold concentration inside the catholyte at time t (ppm) which in this study was only calculated at the end of experiment.

Another important parameter that was evaluated was the efficiency of gold recovery. It was calculated using the mass deposited on the electrode surface (Md) divided by the amount of mass removed from the catholyte. This can be seen in Equation 13 where V_{cat} is the volume of cathode chamber (L). The mass deposited on the electrode surface was determined by brushing the electrode surface as will be discussed in the results section.

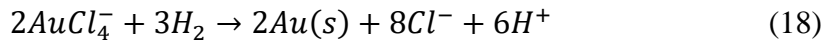
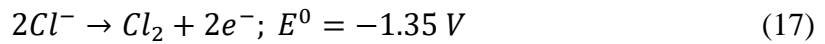
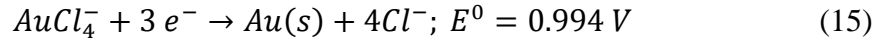
$$\text{Gold recovery efficiency (\%)} = \frac{Md}{(C_0 - C_t) * V_{cat}} * 100 \quad (13)$$

The possible reactions that can take place within this stage are:

- Anodic compartment



- Cathodic compartment



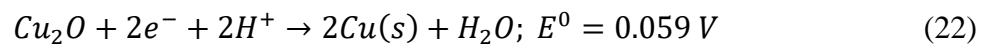
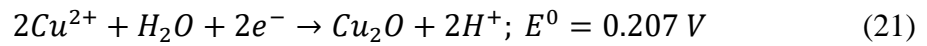
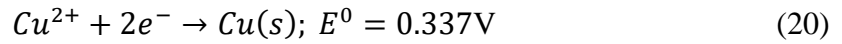
After that, different cells under different initial concentration of Au (III) with the similar anode compartment were operated to investigate the influence of initial concentration on the removal efficiency.

4.4.2 Simultaneous removal of gold ions with incorporation of copper ions

Similar MFC units were installed and operated with the only difference being in the catholyte solution. Initially at this stage, 50 ml of pure copper ions in the buffer solution with the initial concentration of 1000 ppm were fed to the cathode chamber where the performance of the MFC in terms of their removal was analyzed. Later on, a 50 ml solution of 250 ppm Au (III) mixed with 1000 ppm Cu^{2+} was fed to the cathode

chamber. Removal efficiency of both metals, Au and Cu, was calculated based on the Equation 12.

The additional possible reactions that take place in the cathode section during this stage are shown below:



4.4.3 Removal efficiency of gold ions from the industrial wastewater

In this stage, for the first time, the removal efficiency of Au (III) from actual industrial wastewater was evaluated where 50 ml of wastewater was fed inside the cell as catholyte. However, the effluent that was provided by the refinery were samples post-treatment with concentration of gold ions being less than 1 ppm. Thus, Au (III) was added to the solution to increase the gold concentration to 250 ppm. This concentration was chosen to ensure the proper operation of the cell and to have a solution that is more in line with concentrations found in pre-treatment.

The actual pH of the raw catholyte solution from the waste effluent was at 0.2. Hence, in order to investigate the influence of pH on the performance of the cell, sodium hydroxide flasks were added to the various solutions to change their pH levels to: 2, 2.65, 4.45, and 5.2. Finally, different initial biomass concentrations of 50 g/L, 80 g/L, and 100 g/L were used to evaluate their influence on the removal efficiency of both gold and copper ions from the wastewater solution.

Chapter 5. Results and Analysis

This chapter is dedicated to present the obtained results from the experimental work and analyze them to be able to draw proper conclusions and propose the most suitable conditions by investigating the effect of different parameters.

5.1. Removal Efficiency of Pure Tetrachloroaurate Ions

5.1.1 Effect of catholyte

Two different types of catholyte, water and buffer, were used to estimate the gold removal efficiency and consequently determine the most suitable catholyte solution. The cell was operated for 48 hours and the initial Au (III) concentration in both catholyte solution was at 500 ppm. Based on the Figure 5-1 it appears that once the buffer solution was used as catholyte the rate of Au removal was faster in comparison to water solution. This was expected since, by using the buffer as a catholyte solution, the conductivity increased due to the increase in the number of ions (i.e., combination of buffer ions and gold ions) which results in faster removal of Au ions when using a buffer rather than when using water solution. However, in terms of removal efficiency, changing the catholyte solution did not affect it significantly and in fact its influence was only in few decimals as it can be observed from Table 5-1. On the other hand, 83.09% of Au was recovered by brushing the electrode surface when the buffer solution was used as catholyte. However, for the water solution the recovery rate was found to be 80.78%.

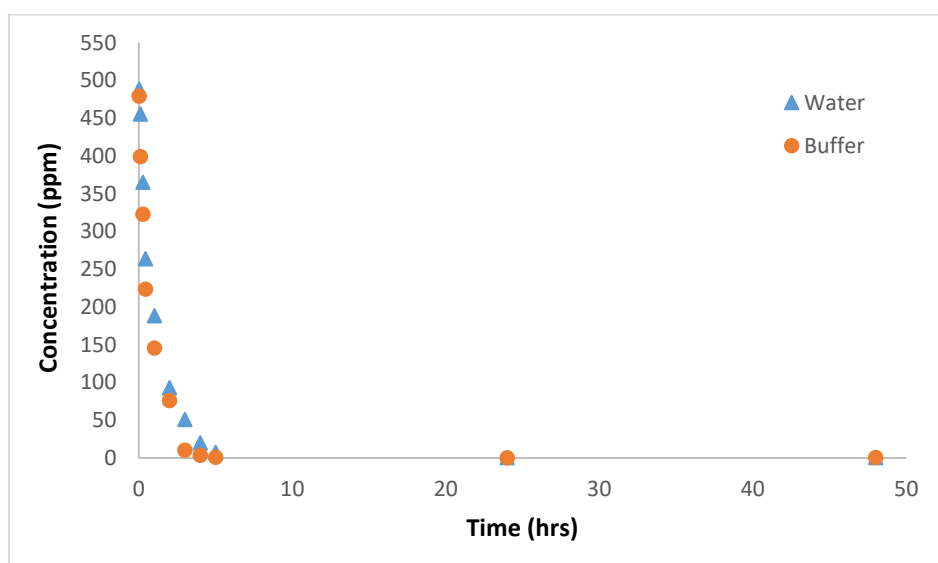


Figure 5-1: Concentration of $[\text{AuCl}_4]$ versus time (hrs) using water and buffer as catholyte solutions.

Table 5-1: Removal and recovery efficiency of gold ions inside two water and buffer as catholyte solutions.

	Water	Buffer
Removal %	99.90	99.90
Recovery %	80.87	83.10

Figure 5-2 represents the Open Circuit Potential of the cell vs. time during the operation of the cell.

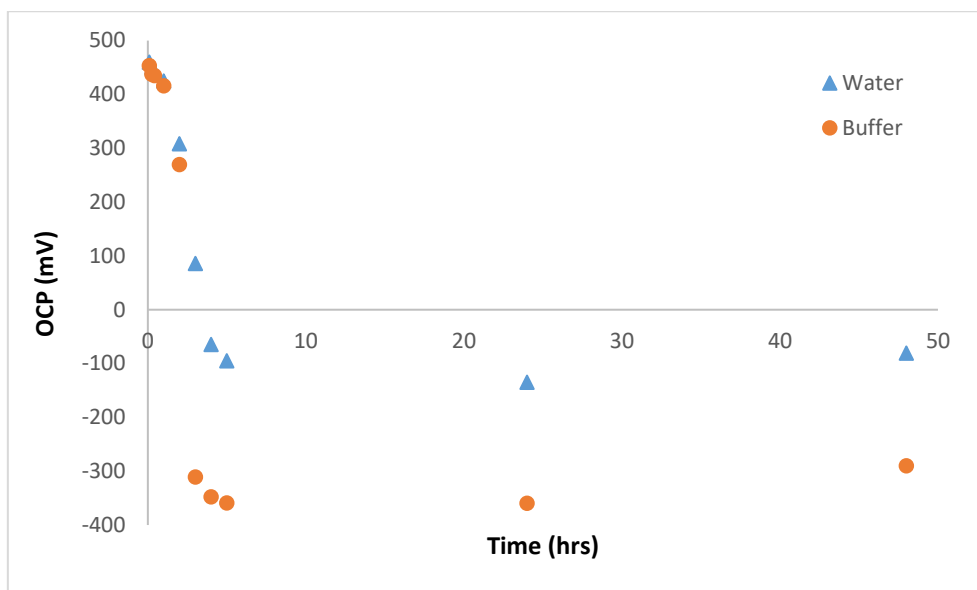


Figure 5-2:OCP vs Time for buffer and water as catholyte solution with 500ppm AuCl₄ initial concentration

As can be observed from Figure 5-2; when approximately 6 hours have passed, the OCP values became negative. In general, a negative value of OCP can be due to two factors which are:

1. The lack of biomass concentration which results in limited electron production. The occurrence of this problem was circumvented by using an appropriate initial concentration of the yeast and the refilling of the anolyte solution after 24h of the cell operation with fresh active yeast. Aside from that, the existence of bubbles in the anode compartment is an indication of the proper performance of the microorganisms. Hence, this cannot be the reason behind the negative OCP values.

2. The negative values of OCP can be assumed to be a result of the reduction reaction of Cl^- ions which is a reasonable assumption. As it can be observed from the Figure 5-1, once the gold concentration was reduced significantly (around 6 hours of the cell performance), considerable amounts of Cl^- were created due to the reaction as shown in Equation 15. Hence, the reaction as displayed in Equation 17 can take place. However, in this case since the potential of this reaction is less than the anodic reaction, the electrons will flow from the cathode to the anode chamber which results in the negative OCP. It is worth mentioning that the remaining electrons inside the cathode chamber will be utilized for further reduction of Au (III).

In terms of pH, since the hydrogen evolution reaction is thermodynamically favorable, the hydrogen gas can also be generated according to the Equation 16. Also, as the Cl_2 gas is generated in low Au concentration conditions, reaction as per Equation 19 is taking place which results in hydrochloric acid production. The existence of these gases can be identified by the presence of bubbles in the cathode compartment during the cell performance as represented in Figure 5-3.



Figure 5-3: Bubble creation in the cathode compartment during the performance of the cell.

In summary, the use of buffer as catholyte solution can increase the removal efficiency by neutralizing the H^+ ions. Consequently, the reduction reaction of protons is diminished which results in the higher and faster removal efficiency of Au ions as

discussed earlier. This is also demonstrated by the limited fluctuation in the buffer solution in comparison to the water solution (Figure 5-4). In conclusion, for further experiments, the buffer solution was utilized as catholyte.

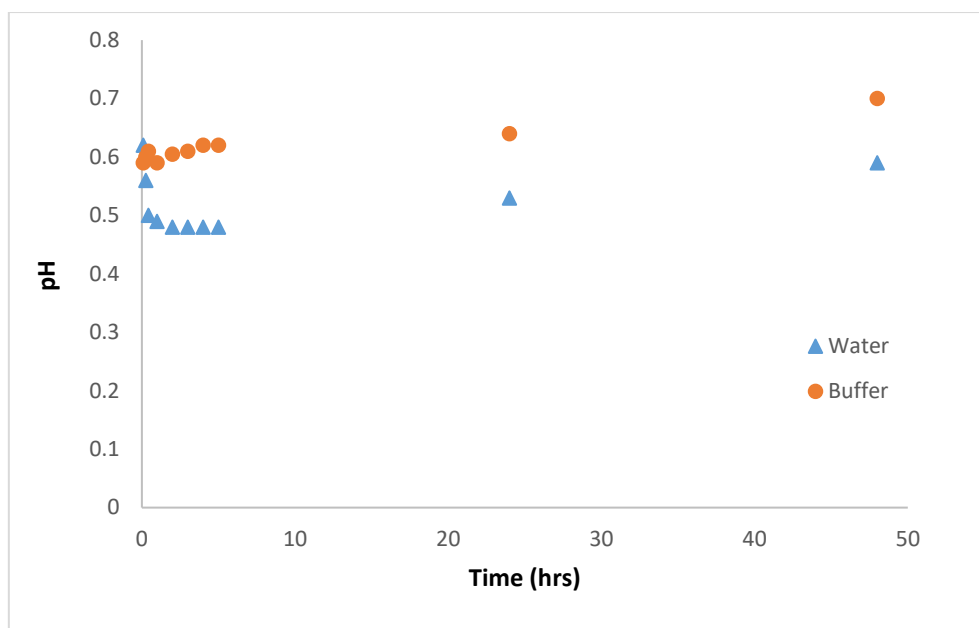


Figure 5-4: pH vs Time for water and buffer solution with 500 ppm initial AuCl_4^- concentration

5.1.2 Effect of initial gold concentration

The effect of different initial gold concentration on the performance of the cell in terms of removal efficiency percentage was investigated. Based on the results of the previous section, all solutions have been created with buffer. The experiment was performed using three different initial concentrations: 125, 250, 500 ppm. The removal efficiency as well as recovery efficiency of each run are presented in Table 5-2.

Table 5-2: Gold ions removal and recovery efficiency vs initial gold concentration within the solution.

	[Au]=500 ppm	[Au]=250 ppm	[Au]=125 ppm
Removal %	99.90	98.30	95.52
Recovery %	83.10	81.05	83.30

Figure 5-5 represents the removal efficiency of each run versus time. As the gold concentration increased the removal efficiency, as well as OCP, increased which is expected due to the increase in conductivity and equilibrium potential, based on the

Nernst equation. In addition, Figure 5-6 represent the amount of brushed gold on the cathode surface due to the gold deposition from the reduction reaction for different initial gold concentrations.

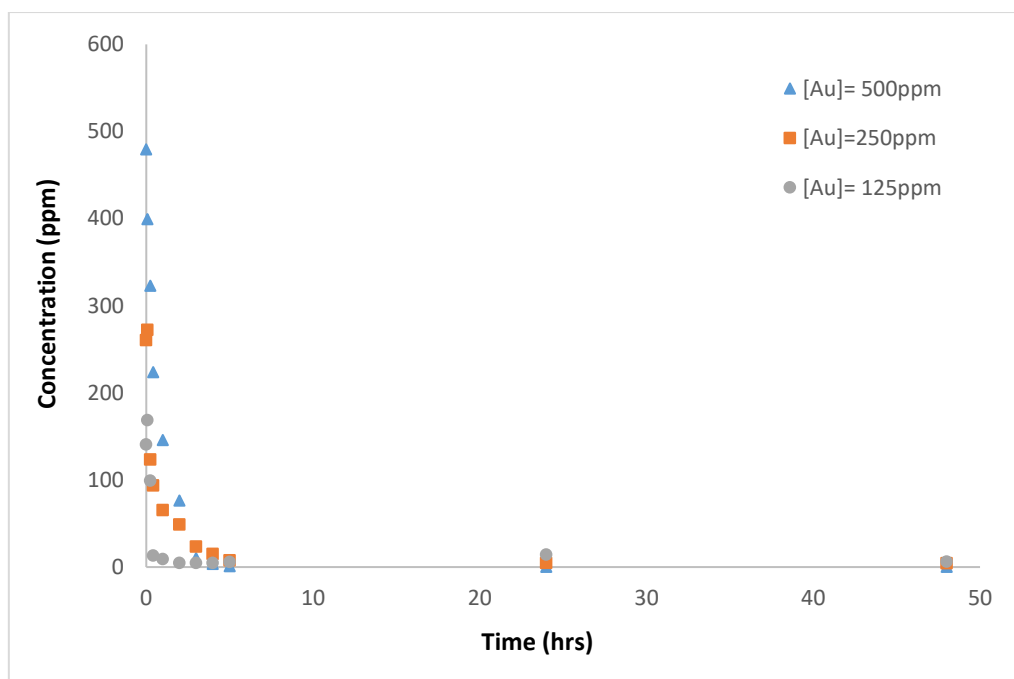


Figure 5-5: Concentration of Au ions versus time with different initial concentration of $[\text{AuCl}_4^-]$ inside the buffer solution.

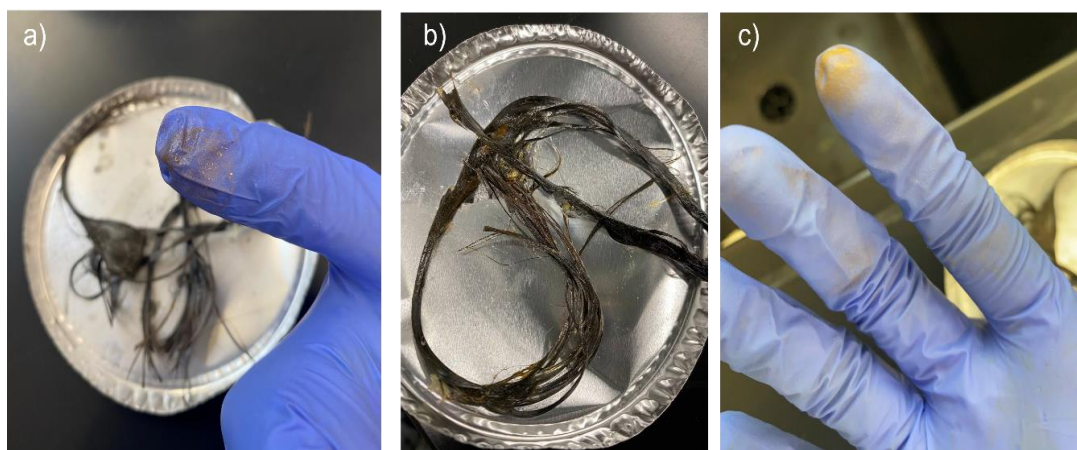


Figure 5-6: The amount of brushed gold deposited on the electrode surface for a) initial gold concentration= 125 ppm b) initial gold concentration= 250 ppm c) initial gold concentration= 500 ppm. Although in terms of removal efficiency higher concentration of Au (III) is better (99.9 % and 98.3 % for initial gold concentration of 500 ppm and 250 ppm respectively), 250 ppm was chosen for further experiments. This decision was made since the efficiency improvements are not sufficient to justify the use of more gold.

Lastly, Figure 5-7 shows the OCP and pH versus time for different initial gold concentration within the buffer solution.

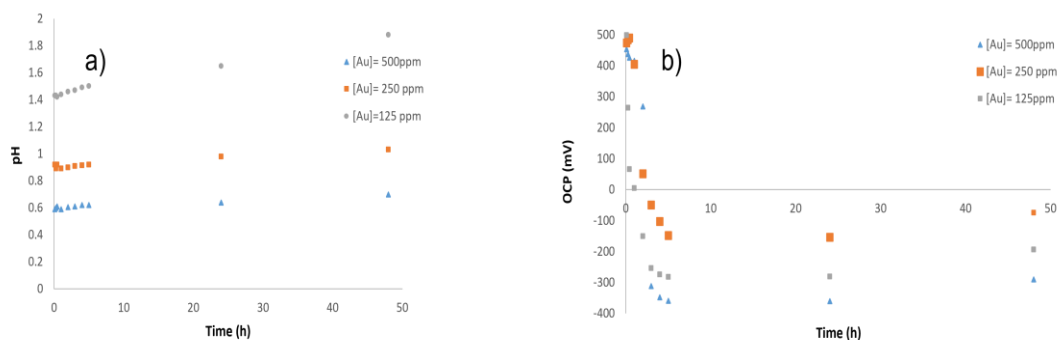


Figure 5-7: Changes of a) pH and b) OCP versus time for different initial AuCl_4 concentration within the buffer solution.

5.2. Simultaneous Removal of Gold Ions with Incorporation of Copper Ions

Since the effluent of gold refineries and mining has a high presence of copper ions [43], investigation of the effect of copper on the removal efficiency of gold ions is of utmost importance. The mixture of 250 ppm and 1000 ppm of the gold and copper ions was prepared inside the buffer solution. Same as the previous experiments, the cell was operated for 48 hours. Table 5-3 summarize the percentage of the removal efficiency in terms of both gold and copper ions. In Table 5-3, the results for the combination of gold and copper ions are presented in two different stages. The first stage presents the gold ions that is removed from the mixture as “Au (Au+Cu)” while the second stage shows the copper that is recovered from the mixture as “Cu (Au+Cu)”.

It is worth noting that in this and subsequent sections the calculation of recovery efficiency is not possible since other heavy metals will deposit with the gold atoms on the cathode surface. According to the study performed by Zhang et al [96], the Cu atoms were formed on the electrode surface after the Au atoms. This conclusion was reached since the different metals deposit in distinct layers on the surface. They also reported a 95.4% gold removal while the gold and copper ions coexisted. However, in this experiment the gold removal efficiency was found to be 99.81% after 48 hours. It is important to highlight that this removal efficiency level is higher than the one obtained from solutions containing only gold ions. The reason behind this increase in removal efficiency can be assumed to be due to the increase in the number of ions; as mentioned previously, this would also result in the increment of the conductivity.

Table 5-3: Removal efficiency % vs catholyte solution type

	Pure Au	Au (Au+Cu)	Pure Cu	Cu (Au+Cu)
Removal %	98.30	99.81	59.21	51.16

Figures 5-8 and 5-9 show the concentration for both Cu and Au vs time and pH vs time, respectively.

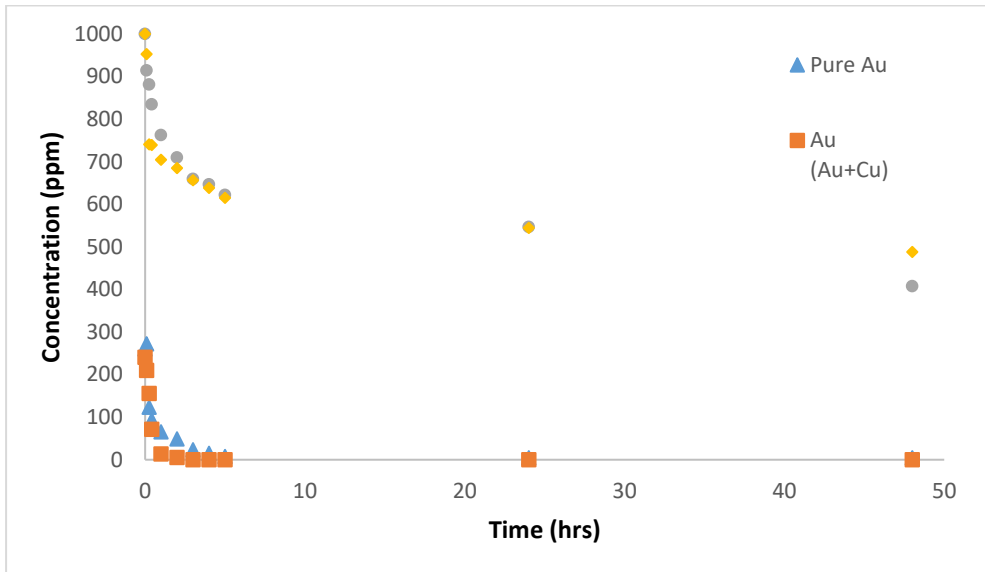


Figure 5-8: Concentration (ppm) vs Time for different catholyte solution

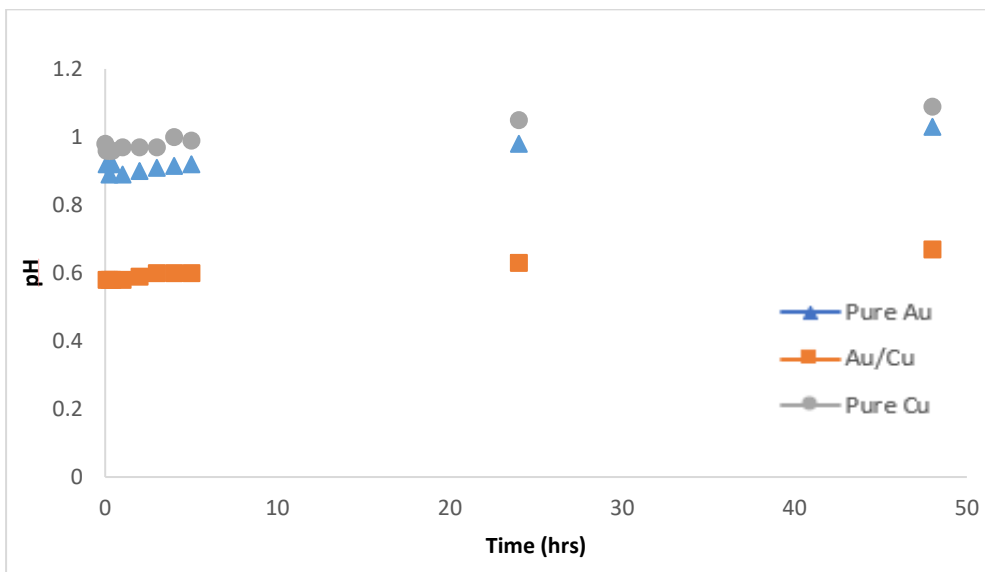


Figure 5-9: pH vs Time for different catholyte solution

In terms of OCP plots, as can be seen from Figure 5-10, for the pure Cu ions negative OCP cannot be observed since Cl^- ions cannot be generated in this case. In terms of the scenario when Cu ions coexist with the gold ions it can be assumed that the reaction in Equation 17 is not favorable one in comparison to other reactions. It's worth mentioning that the significant reduction in terms of OCP can be observed once the gold ions are coexistent with copper ions. It is unclear as to the reason behind such an observation.

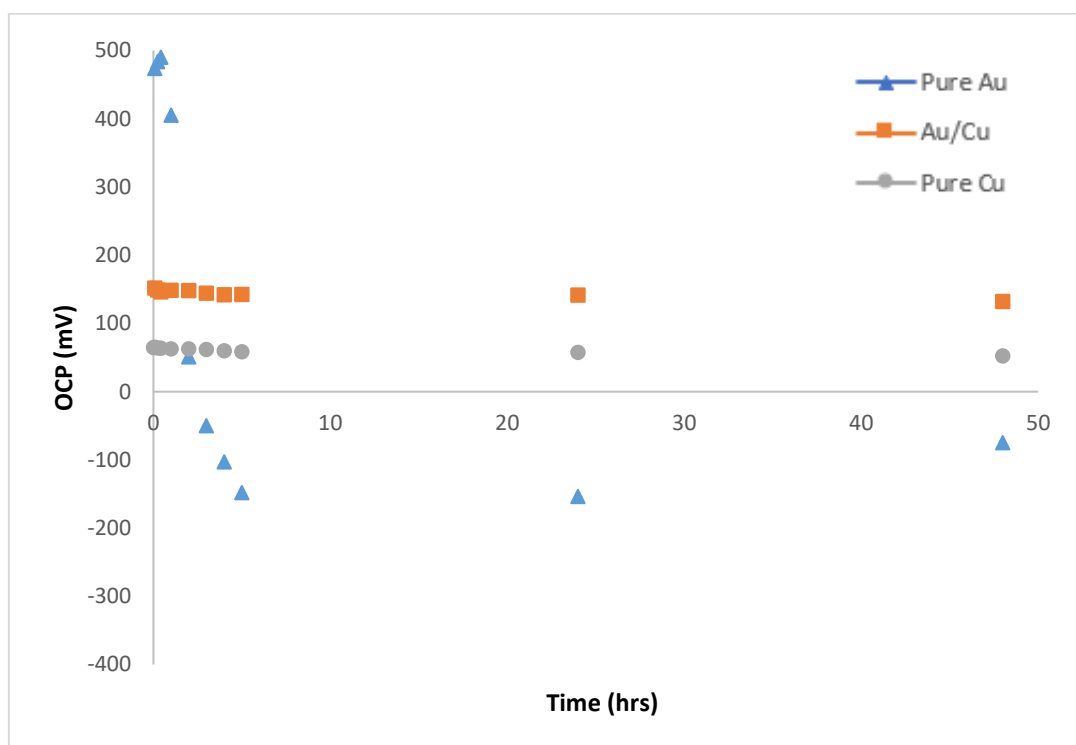


Figure 5-10: OCP (mV) vs Time for different catholyte solution.

5.3. Removal Efficiency of Gold Ions from the Industrial Wastewater

In this section the removal efficiency of gold ions while coexisting with other heavy metals from the industrial gold refinery waste is investigated for the first time based on the literature review presented earlier. According to Table 5-4, the removal efficiency of gold metals was slightly increased in comparison to the scenario where only Cu and Au ions were coexistent; from 99.81% to 98.86% as it is also represented in Figure 5-11. This can be due to the presence of the other heavy metals in the wastewater such as zinc, lead, etc. [42] Hence, a significant reduction in terms of copper removal efficiency is observed since the reduction reactions of other heavy metals were more favorable and took place first. However, by increasing the operating time to 72 hrs, higher removal efficiency of copper can be expected.

Table 5-4: Removal efficiency % for Au and Cu from the waste solution.

	Au (Au+Waste)	Cu (Au+Waste)
Removal %	98.86	31.60

The pH as an operating parameter plus OCP was monitored during the cell's operation as shown in Figures 5-12 and 5-13.

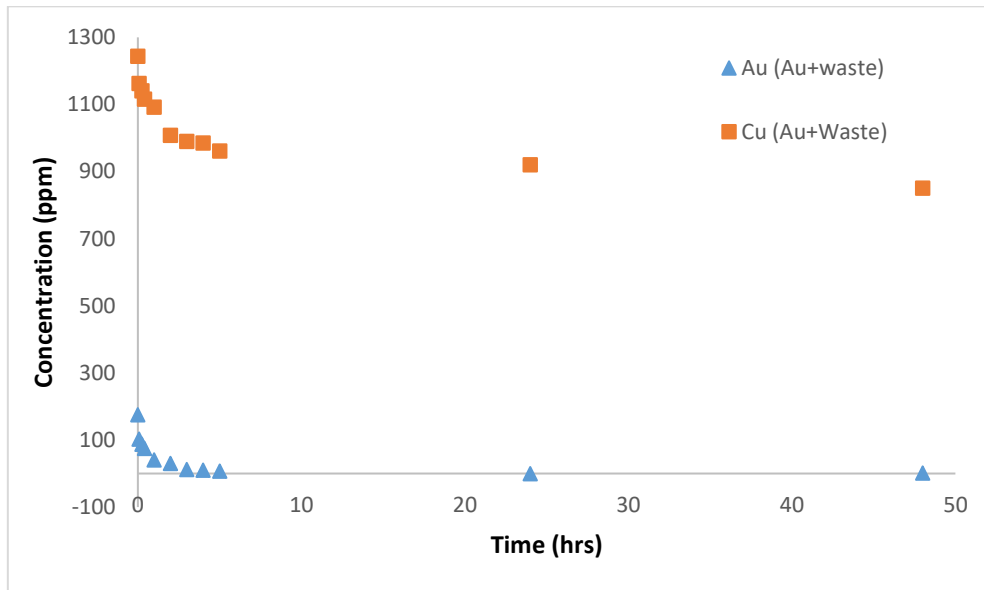


Figure 5-11: Concentration of Au and Cu vs Time within the waste solution.

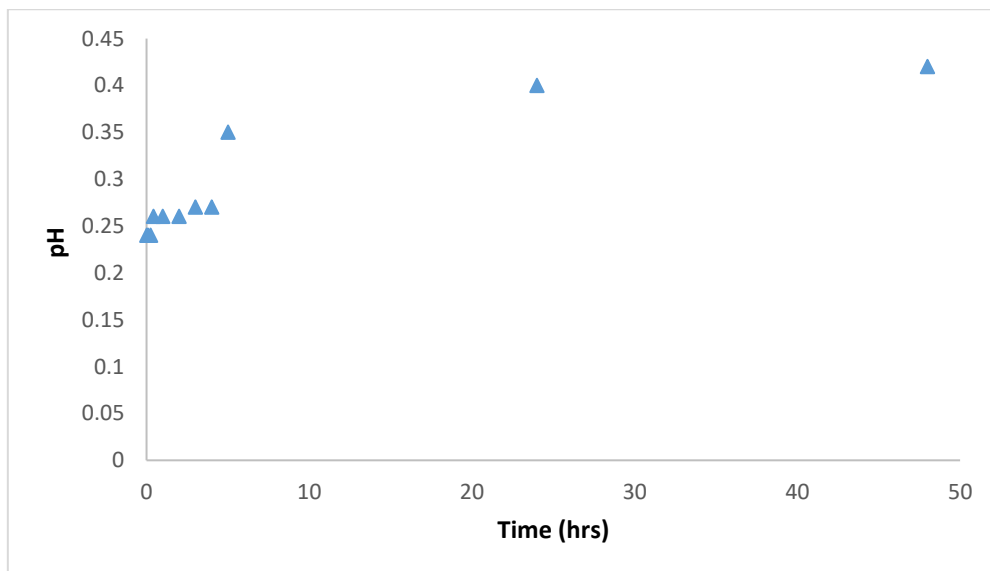


Figure 5-12: pH vs Time within the waste solution.

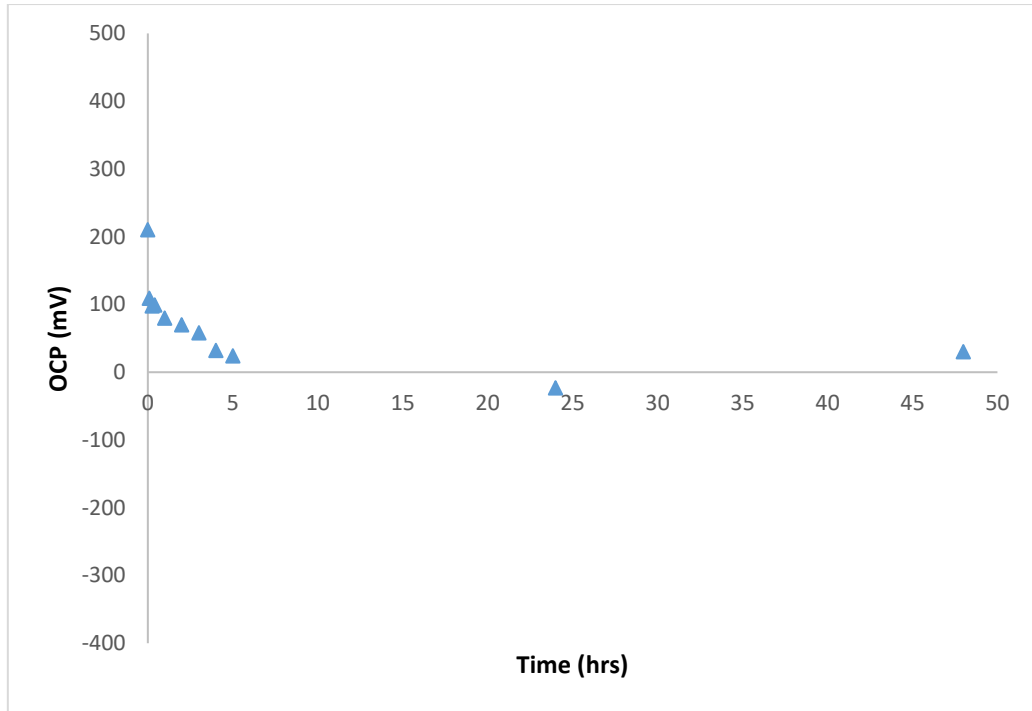


Figure 5-13: OCP (mV) vs Time within the waste solution.

Next, the effect of pH and initial yeast concentration on the removal efficiency is discussed below.

5.3.1 Effect of pH

Effect of pH on the removal efficiency of the Au ions and Cu ions are different from each other. The influence of the pH on each metal is discussed separately below.

5.3.1.1. Influence of the pH on the removal of the Au ions

pH can influence the removal efficiency of Au in two aspects:

1. Proton involvement: Since the reduction of gold ions protons is not involved (Equation 15), the effect of pH due to proton involvement on the removal efficiency is negligible.
2. Stability of $AuCl_4^-$ ions: According to the predominance diagram Figure 5-14 of the of $Au(III) - OH - Cl -$ species, there are two factors that can affect the solubility of the $AuCl_4^-$ ions inside the solution; pH and the Cl^- concentration. These two factors should be controlled to keep the $AuCl_4^-$ ions soluble. If these two factors are not controlled appropriately, the $AuCl_4^-$ ions will precipitate as $Au(OH)_3$ according to the predominance diagram. As the pH gets higher, the lower limit of Cl^- concentration will increase in order to keep the $AuCl_4^-$ ions

stable inside the solution (Figure 5-14). In this study, although all the samples were prepared with 250 ppm initial gold concentrations; the initial gold concentration value was lower for runs in the pH range of 4.46-5. It appears that the concentration of the Cl^- ions was not high enough to keep the $AuCl_4^-$ ion stable within this range of pH as represented in the Figure 5-14. A dark yellow color precipitate was also observed at the end of the experiments which can be reasonably assumed to be a precipitate of $Au(OH)_3$ (Figure 5-15).

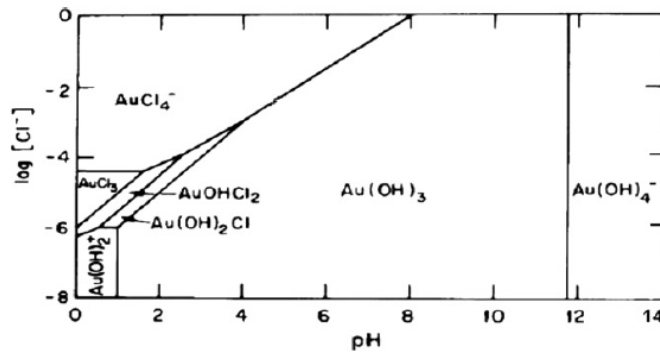


Figure 5-14: Predominance diagram of Au(III)- OH⁻-Cl⁻ species [100].



Figure 5-15: Precipitation of the Au(III) ions as $Au(OH)_3$ at the bottom of cathode chamber.

As can be concluded from Table 5-5, the highest and fastest gold removal efficiency is at pH=2. As the pH decreases, the rate of diffusion of the protons from cathode chamber to the anode chamber increases. This increase in diffusion rate has a

negative impact on the activity of the microorganisms. Hence, the electron production decreases as the microorganisms are being negatively impacted.

Table 5-5 : Au-Cu removal efficiency % vs pH

Au/Waste	pH=0.2	pH=2	pH=2.65	pH=4.45	pH=5.2
Au- Removal %	98.86	100.00	99.55	95.63	81.51
Cu- Removal %	31.60	34.06	34.48	44.44	52.98

On the other hand, for the pH range of 2 till 4.46, according to the predominance curve as mentioned earlier, the initial concentration of the Au ions is dropped mainly due to the precipitation. In addition, it seems that the reduction reaction of Au ions takes place first as long as a significant amount of gold ions are removed. After this step, the reduction reaction of other heavy metals is initiated. This hypothesis is supported by the low removal efficiency of the copper ions within 48 hours of the cell performance as well as OCP reduction within this pH range.

As the pH increases from 4.45 till 5, more gold ions precipitate. Hence, the initial concentration of gold is even less than before (i.e., 119 ppm). Nevertheless, it seems that in this case the reduction reaction of other heavy metals is taking place simultaneously as the reduction reaction of gold ions. This is the reason behind the higher OCP values as well as higher Cu removal efficiency at this specific pH range. The Figures 5-16 and 5-17 present the summary of the aforementioned explanation.

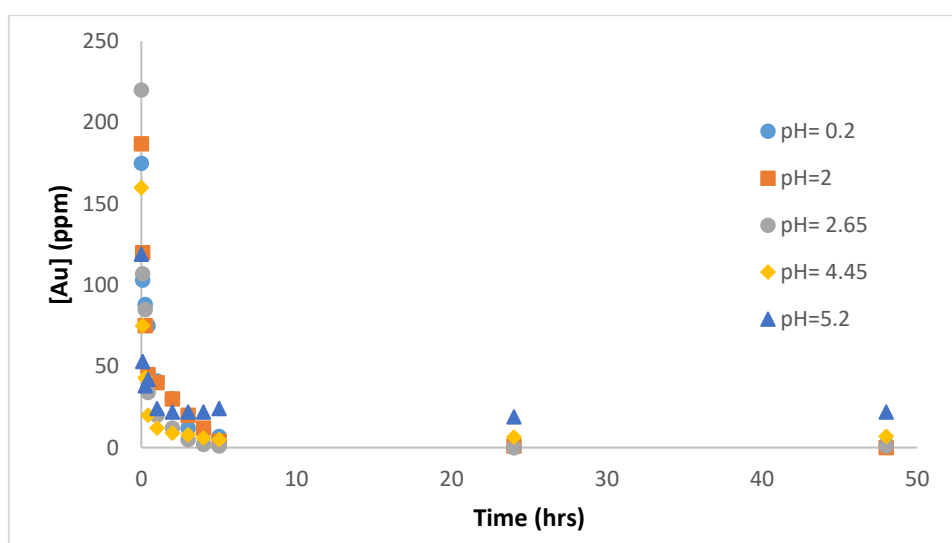


Figure 5-16: Au concentration vs time at different pH within the waste solution.

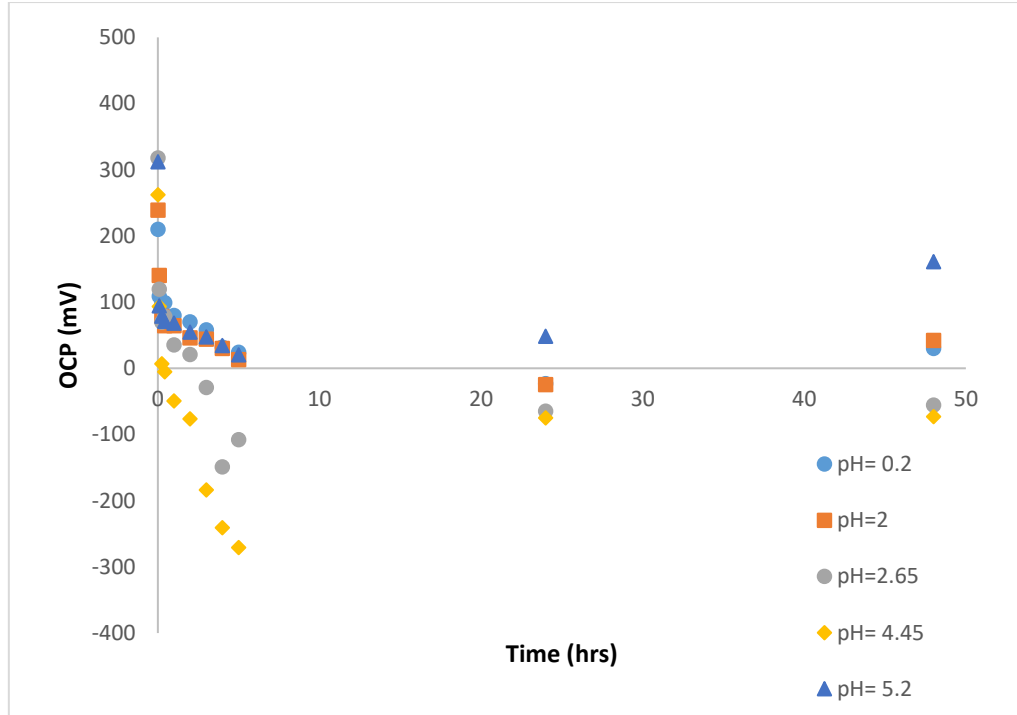


Figure 5-17: OCP (mV) vs time at different pH within the waste solution.

5.3.1.2. Influence of the pH on the removal of the Cu ions

In terms of proton involvement, pH cannot influence the reduction reaction of Cu^{2+} to Cu(s) reaction 23. This reaction is solely dependent on the concentration of Cu^{2+} . On the other hand, the reduction reaction of Cu^{2+} to Cu_2O is a function of not only the concentration of Cu^{2+} but also pH. Thus, increasing the pH causes the reaction in Equation 24 to take place more. Moreover, the reduction of Cu_2O to Cu(s) is only dependent on the pH and based on Equation 25 it is favored within the lower pH ranges.

Based on the Nernst equation, the equilibrium potential of the cathode for different reaction under various operating conditions such as pH, Cu^{2+} can be calculated. Consequently, the most favorable reaction under that specific condition can be determined. The Nernst equation for three different reactions is shown below:

$$E_{cat}(\text{Cu}^{2+}/\text{Cu}) = E_{cat}^0(\text{Cu}^{2+}/\text{Cu}) - \frac{RT}{nF} \ln\left(\frac{1}{[\text{Cu}^{2+}]}\right) \quad (23)$$

$$E_{cat}(\text{Cu}^{2+}/\text{Cu}_2\text{O}) = E_{cat}^0(\text{Cu}^{2+}/\text{Cu}_2\text{O}) - \frac{RT}{nF} \ln\left(\frac{[\text{H}^+]^2}{[\text{Cu}^{2+}]^2}\right) \quad (24)$$

$$E_{cat}(\text{Cu}_2\text{O}/\text{Cu}) = E_{cat}^0(\text{Cu}_2\text{O}/\text{Cu}) - \frac{RT}{nF} \ln\left(\frac{1}{[\text{H}^+]^2}\right) \quad (25)$$

Figure 5-18 indicates the relation between pH, Cu^{2+} concentration, and the cathode potential [101]. For the concentration of 0.02 M (initial concentration of Cu^{2+} in the waste samples), as the pH increases from 2 to 5.2, the potential for the Cu^{2+} to Cu_2O increases from 0.155 V to 0.395 V; keeping in mind that the potential for the Cu^{2+} to Cu(s) reaction is independent of the pH. At the concentration of 0.02M Cu^{2+} the cathode potential is equal to 0.285V. As a result, pH levels above 2.65, the reduction reaction of Cu^{2+} to Cu_2O becomes more favorable. However, the comparison of the dominance of different copper reactions at different pH is not very relevant to question addressed in this report. Rather, the main point that can be extracted from these observations, which is the one the objective of this study, is that by increasing the pH the removal efficiency of the Cu^{2+} increases as shown in Table 5-6. As it can be observed from the and Figure 5-19, the pH 5.2 is the most suitable condition in terms of copper removal. This suggests that the removal efficiency of the copper ions is more favorable at a relatively more basic media.

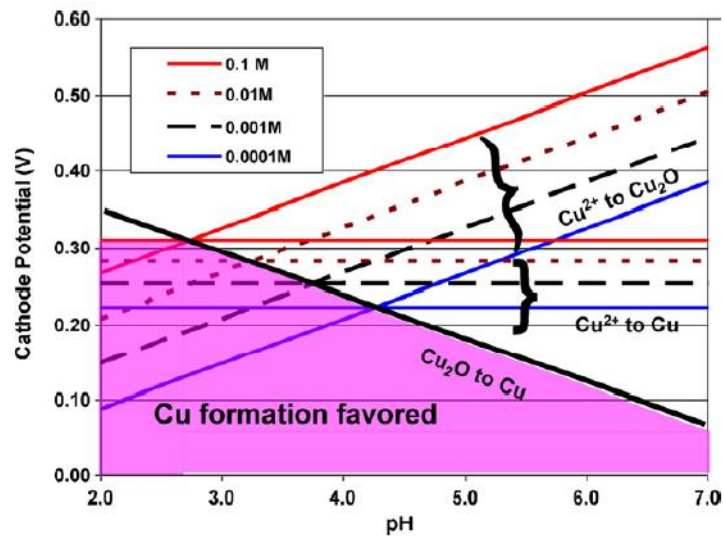


Figure 5-18: Relation between pH, Cu^{2+} concentration, and the cathode potential based on the Nernst equation [101].

Table 5-6: Au-Cu removal efficiency % vs pH.

Au/Waste	pH=0.2	pH=2	pH=2.65	pH=4.45	pH=5.2
Au- Removal %	98.86	100.00	99.55	95.63	81.51
Cu- Removal %	31.60	34.06	34.48	44.44	52.98

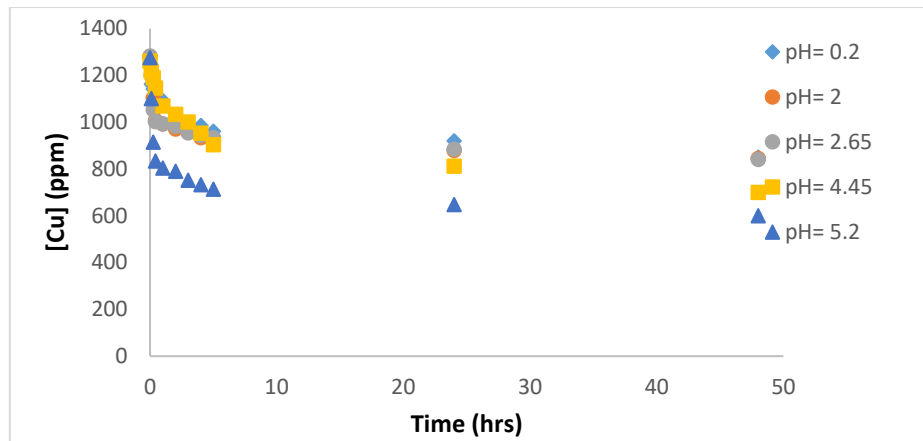


Figure 5-19: Cu concentration vs time at different pH within the waste solution.

5.3.2 Effect of initial yeast concentration

To explore the effect of initial yeast concentration on the gold removal efficiency, different concentration of yeast at 50, 80, 100 g/L were prepared. The results showed that the initial yeast concentration has a positive effect on both gold removal efficiency as well as OCP which was expected. More active yeast results in more electron production and consequently higher OCP and removal efficiency (Table 5-7; Figures 5-20, 5-21 and 5-22).

Table 5-7: Au-Cu removal efficiency % vs initial yeast concentration.

Au/Waste	Yeast= 100 g/L	Yeast= 80 g/L	Yeast= 50 g/L
Au- Removal %	99.44	98.86	92.25
Cu- Removal %	34.92	31.60	21.66

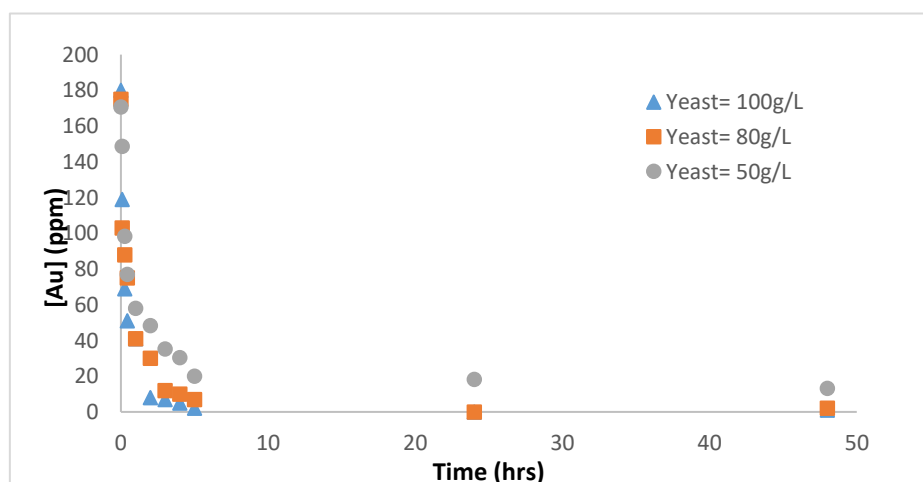


Figure 5-20: Au concentration vs time at different initial yeast concentration within the waste solution.

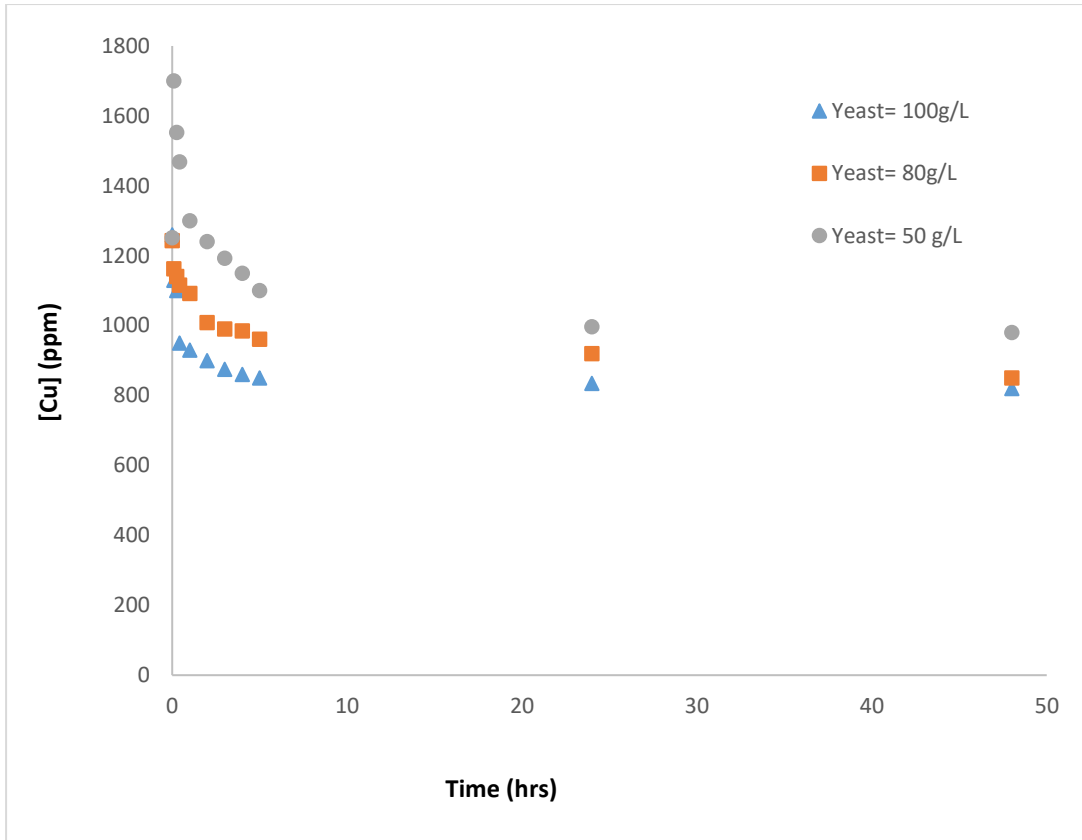


Figure 5-21: Cu concentration vs time at different initial yeast concentration within the waste solution.

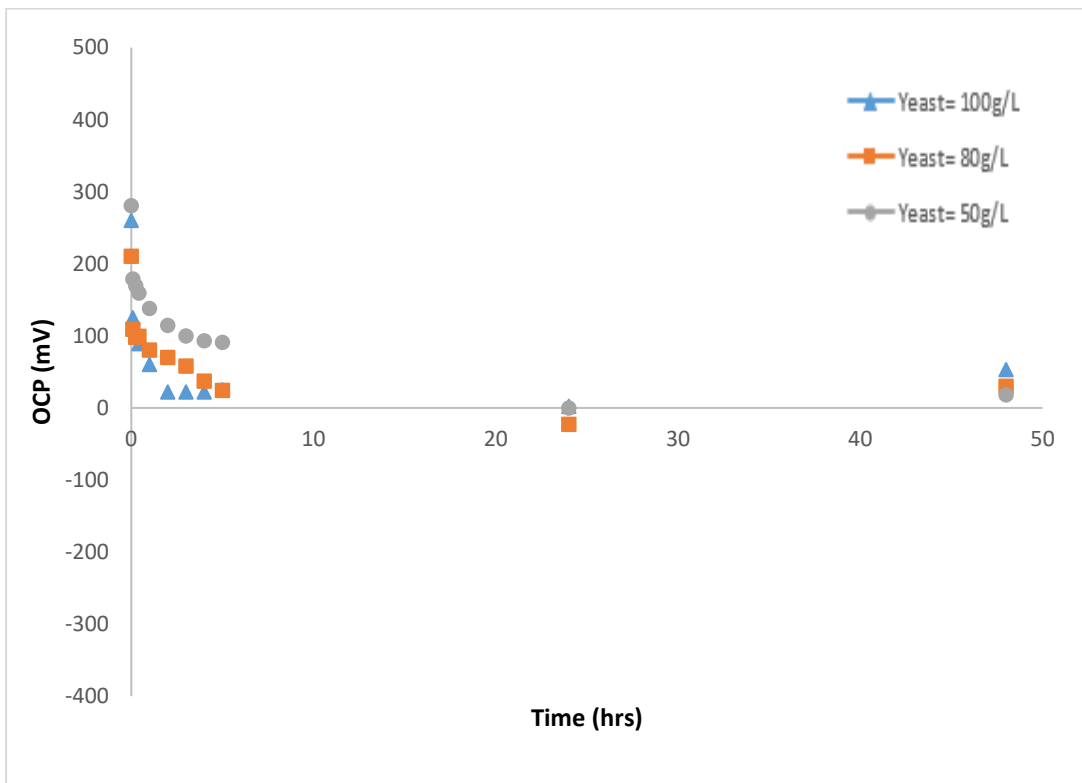


Figure 5-22: OCP (mV) vs time at different initial yeast concentration within the waste solution.

Chapter 6. Conclusion and Future Work

This work focuses on the feasibility of microbial fuel cell for recovering precious metals such as gold from metal containing solutions. One of the main applications of such research is the removal of gold ions from the effluent of gold refinery industries. Copper removal efficiency was also evaluated during this work due to its high presence in the gold refinery wastes. Because of the increase in the demand of precious metals globally as well as importance of treating waste before their discharge to the environment, the existence of the renewable source of energy that can overcome the aforementioned problems while being ecofriendly is of utmost importance. This research proves, for the first time, that microbial fuel cell can remove the gold ions from the wastewater while other heavy metal coexists simultaneously. Thereby, the existence of other heavy metals would not hinder the operation of MFC for removing gold ions. Hence, it can be assumed as one of the most beneficial method to fulfill the requirement for the precious metals while not generating any secondary effluent. Moreover, power can be generated during the operation of the cell.

The experimental work has shown above 95% of gold removal efficiency after 5 hours of cell's operation. This amount increases to 98.86% after 48 hours of operation from the waste sample at the 250 ppm gold initial concentration. The effect of different parameters (pH, initial yeast concentration, initial gold concentration, type of catholyte) on the performance of the MFC were assessed. Results demonstrated that the initial yeast concentration of 80 g/L at the pH=2 under room temperature is the most optimum condition in terms of removing gold metals from the real waste solution (at a 250 ppm initial concentration of gold). Under this condition 100% removal efficiency has been achieved.

On the other hand, scaling up the MFC operation to be applicable on the industrial level is facing some challenges. Therefore, future research can study the feasibility of the MFC for removing gold metals from the industrial waste on a large scale by performing various experiment to investigate the effect of other possible factors on the removal efficiency and consequently reducing the overall cost of the cell. These factors can be mentioned as the volume of each compartment, different type of biomass to increase the generated power during the cell operation and decrease the final cost by harnessing this power in other section. Moreover, another recommendation for the future work

would be allowing the cell running for longer time to enhance the removal efficiency of copper. Additionally, performing the impedance spectroscopy, potentiostat, and Pourbaix diagram would provide additional information about the electrochemical process that undergoes during its operation. Hence, this will allow for further improvement by knowing the dominant internal resistance within the cell. Finally, the investigation of the removing other possible type of heavy metals that may exists in the waste solution would be recommend.

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Appendix A: Magnified Plots of Concentration for the First Five Hours of Operation

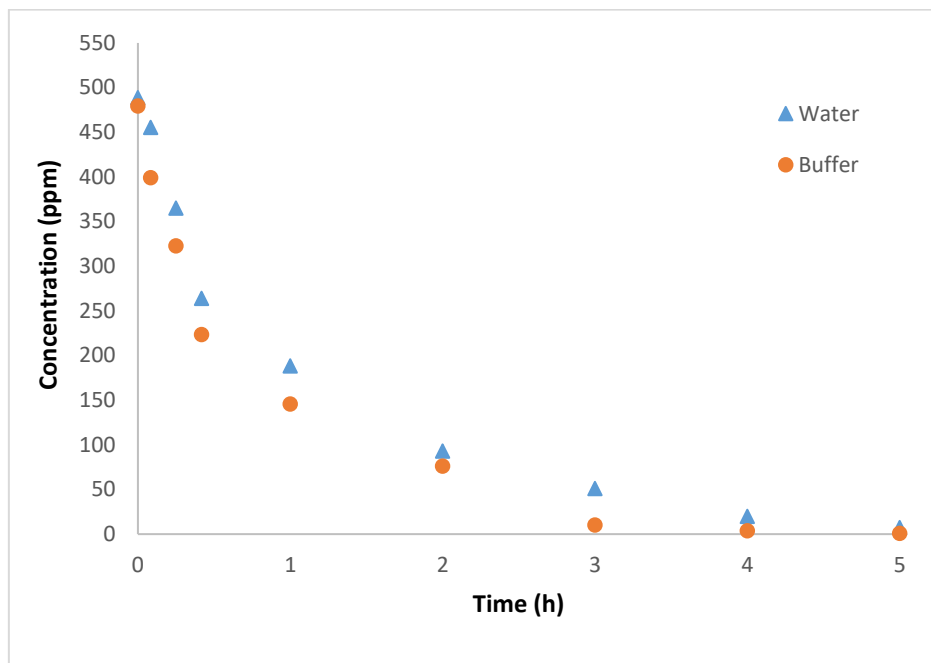


Figure A-1: Concentration of Au ions versus time using water and buffer as catholyte solutions.

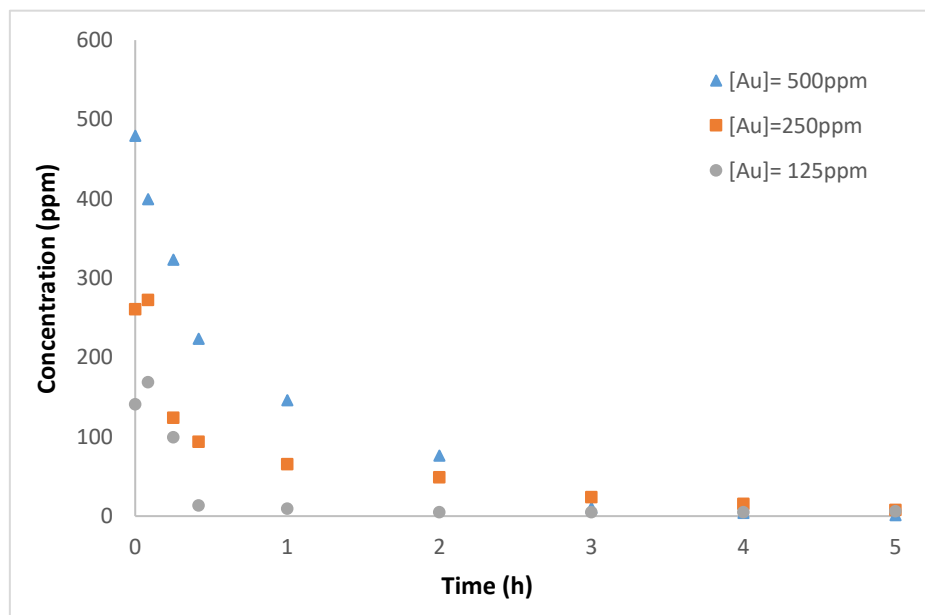


Figure A-2: Concentration of Au ions versus time with different initial concentration of gold ions inside the buffer solution.

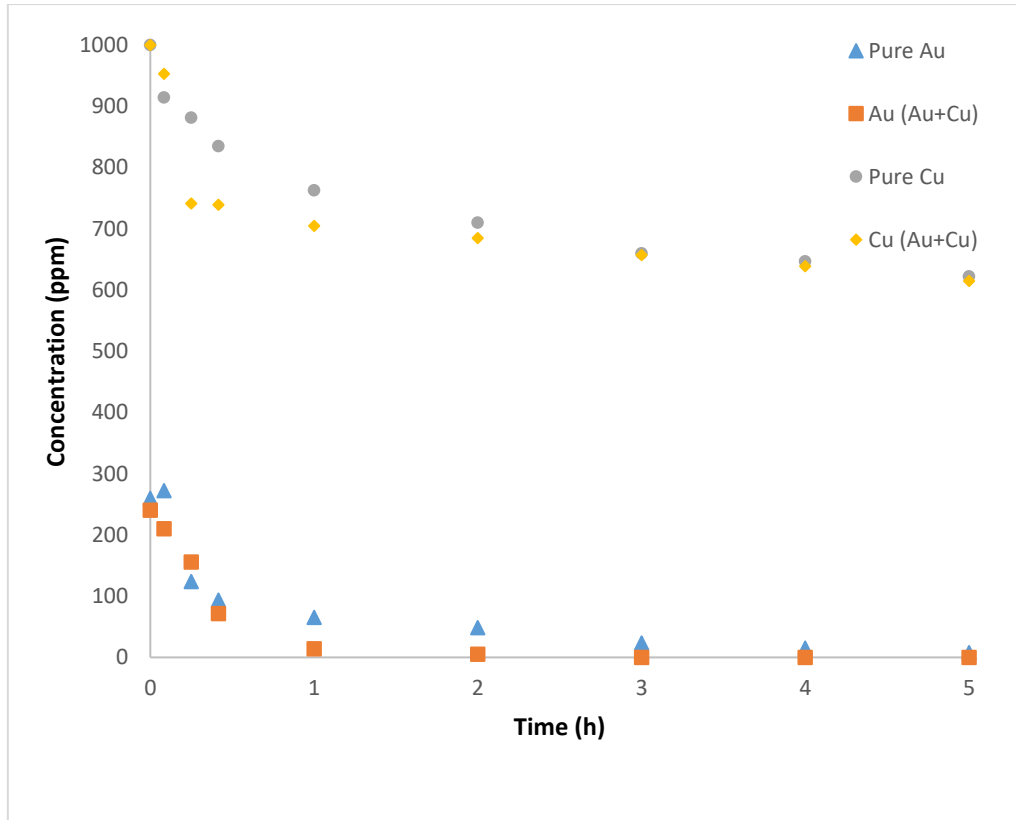


Figure A-3: Concentration (ppm) vs Time for different catholyte solution

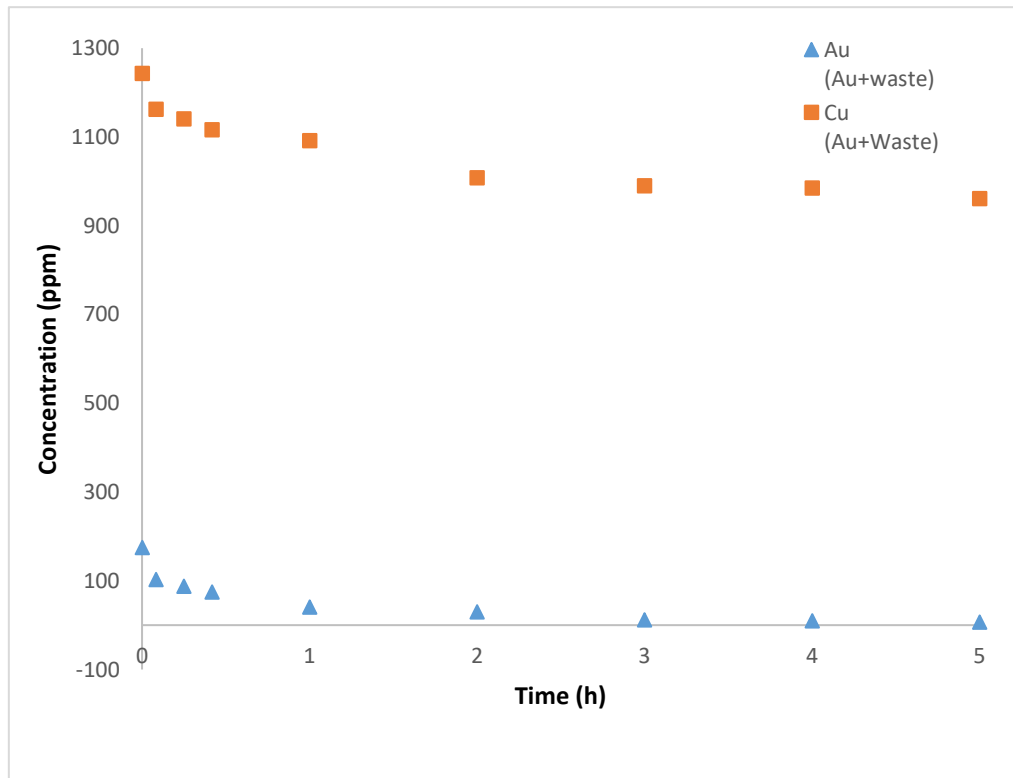


Figure A-4: Concentration of Au and Cu vs Time within the waste solution.

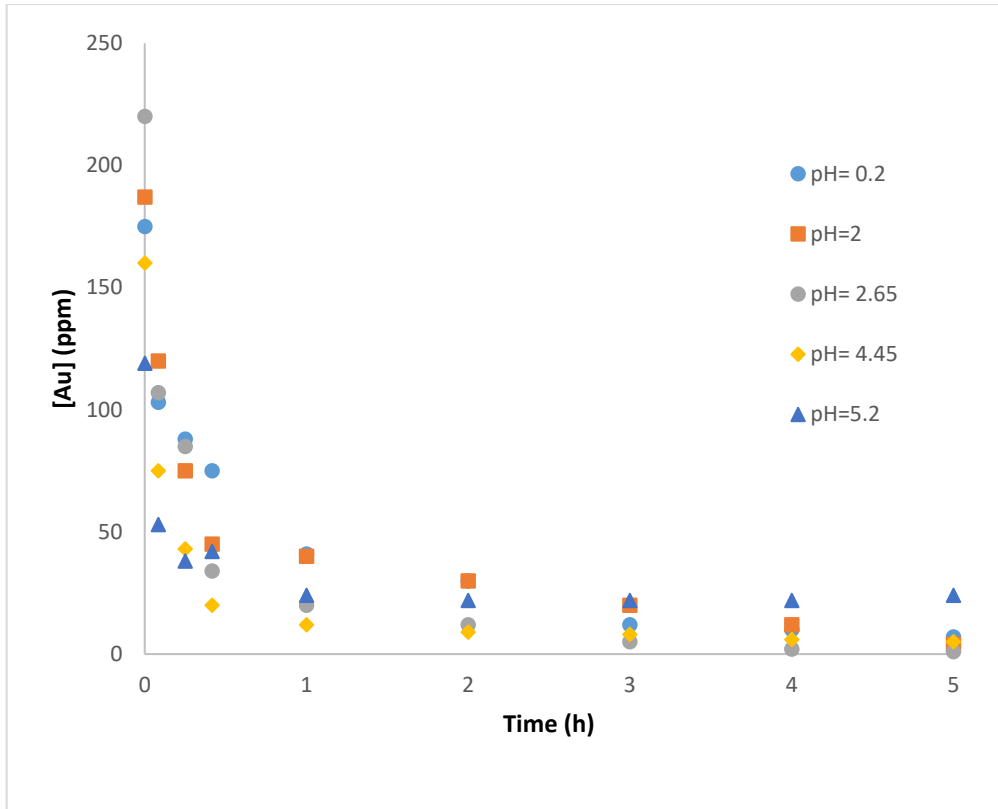


Figure A-5: Au concentration vs time at different pH within the waste solution.

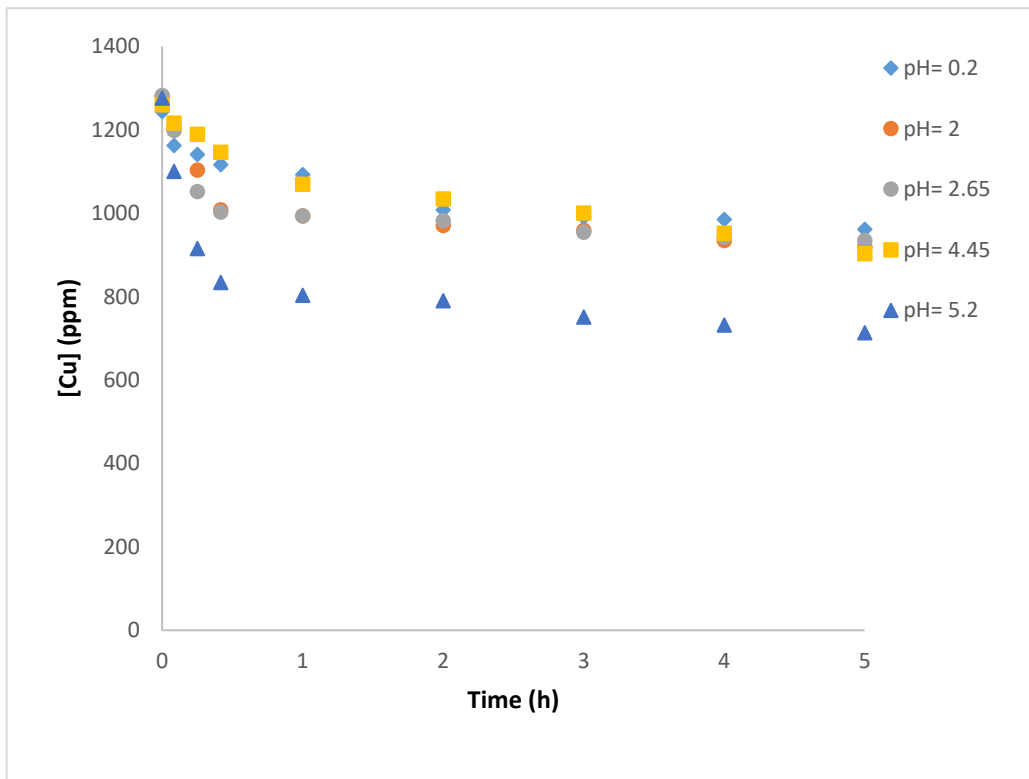


Figure A-6: Cu concentration vs time at different pH within the waste solution.

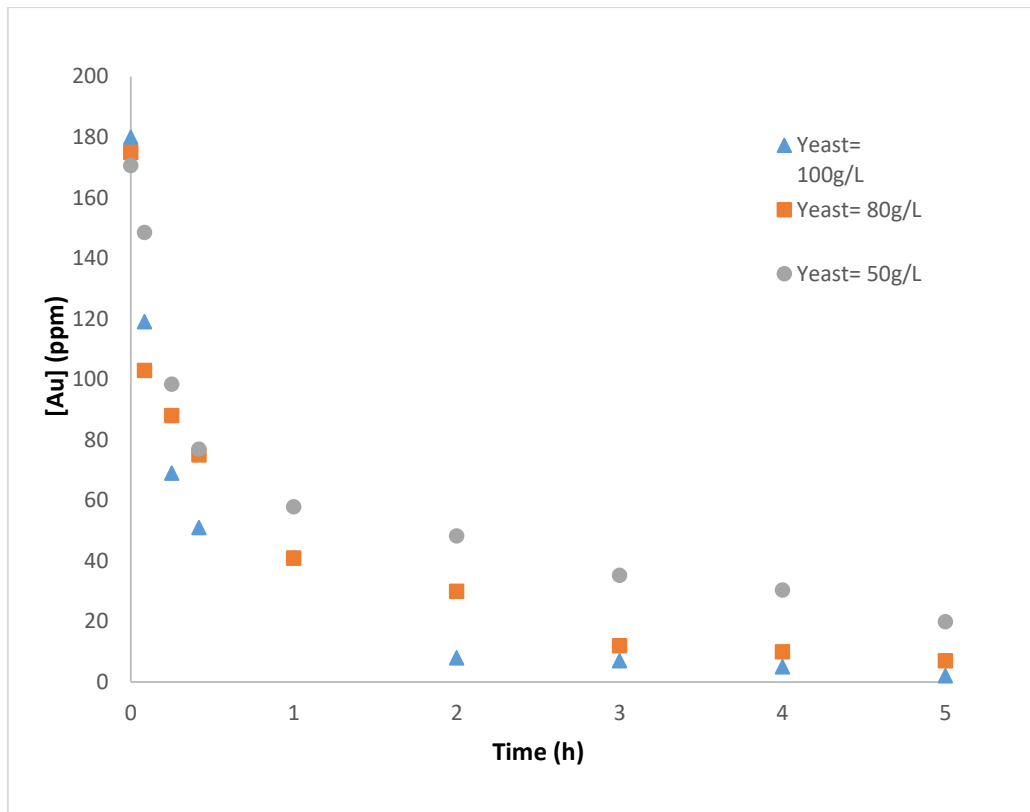


Figure A-7: Au concentration vs time at different initial yeast concentration within the waste solution.

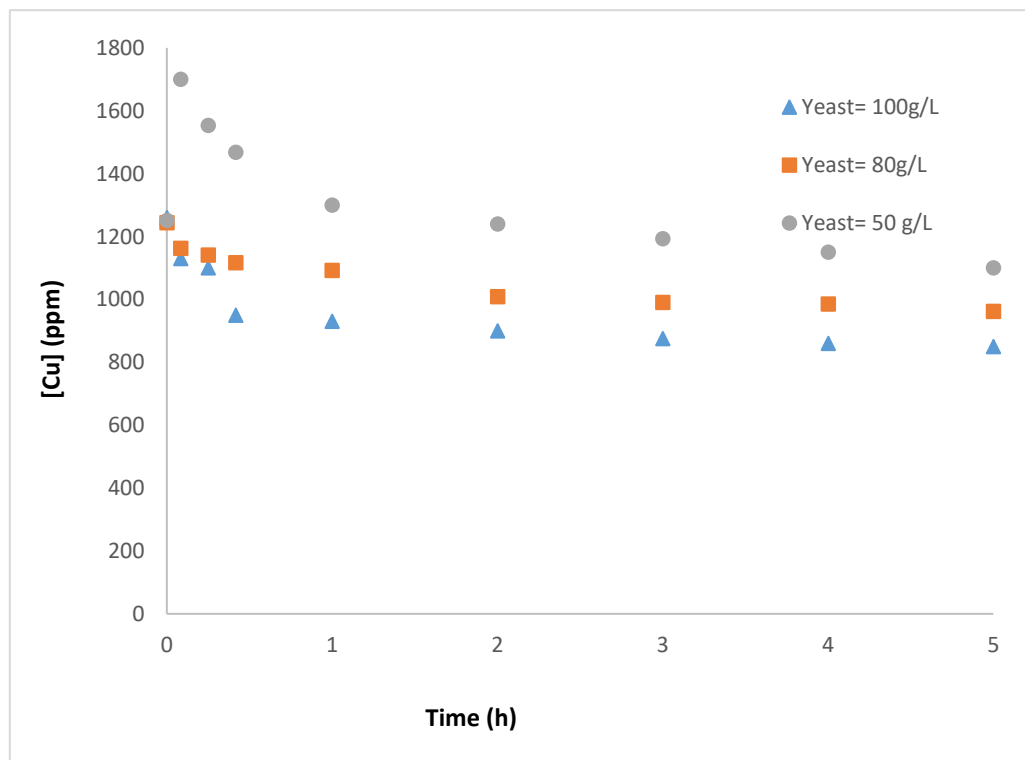


Figure A-8: Cu concentration vs time at different initial yeast concentration within the waste solution

Appendix B: Raw Data from the Experimental Work

Table B- 1: Raw concentration, pH, and OCP data for pure $[AuCl_4]$.using different catholyte solutions .

Concentration			pH			OCP		
Time	Water	Buffer	Time	Water	Buffer	Time	Water	Buffer
0	488.69	479.40	0	0.63	0.6	0	470	460
0.084	455.61	399.25	0.084	0.62	0.59	0.084	460	453
0.25	365.10	322.96	0.25	0.56	0.6	0.25	450	437
0.417	263.96	223.57	0.417	0.5	0.61	0.417	442	434.7
1	188.59	145.78	1	0.49	0.59	1	424.8	415.8
2	93.03	76.23	2	0.48	0.605	2	308	269.2
3	51.13	10.38	3	0.48	0.61	3	85.6	-311
4	20.07	3.74	4	0.48	0.62	4	-65	-348
5	7.24	1.05	5	0.48	0.62	5	-95	-359
24	0.69	0.26	24	0.53	0.64	24	-135	-360
48	0.47	0.46	48	0.59	0.7	48	-81	-290

Table B- 2: Raw concentration, pH, and OCP data for pure $[\text{AuCl}_4^-]$ using different initial $[\text{AuCl}_4^-]$.

Concentration				pH				OCP			
Time	500 ppm	250 ppm	125 ppm	Time	500 ppm	250 ppm	125 ppm	Time	500 ppm	250 ppm	125 ppm
0	479.40	260.57	168.49	0.00	0.60	0.90	1.44	0.00	460.00	480.00	497.00
0.084	399.25	272.14	140.76	0.084	0.59	0.92	1.43	0.084	453	473.5	499
0.25	322.96	123.57	99.16	0.25	0.6	0.89	1.43	0.25	437	483.4	264
0.417	223.57	93.57	13.03	0.417	0.61	0.92	1.42	0.417	425.7	490	66
1	145.78	65.25	9.00	1	0.59	0.89	1.44	1	415.8	405.2	4.5
2	76.23	48.76	4.62	2	0.605	0.9	1.46	2	269.2	50.7	-150
3	10.38	23.58	4.60	3	0.61	0.91	1.47	3	-311	-50	-254
4	3.74	15.00	4.62	4	0.62	0.915	1.49	4	-348	-103	-274
5	1.05	7.70	5.88	5	0.62	0.92	1.5	5	-359	-148	-282
24	0.26	4.59	14.29	24	0.64	0.98	1.65	24	-360	-154	-280.5
48	0.46	4.43	6.30	48	0.7	1.03	1.88	48	-290	-75	-193

Table B-3: Raw concentration, pH, and OCP data for different mixture of the [AuCl₄⁻] and copper ions.

Concentration					pH				OCP			
Time	Pure Au	Au (Au+Cu)	Pure Cu	Cu (Au+Cu)	Time	Pure Au	Pure Cu	Au/Cu	Time	Pure Au	Pure Cu	Au/Cu
0	260.57	240.557	1000	1000	0	0.9	0.98	0.58	0	480	64.4	152.9
0.084	272.14	210.11895	914.233	952.8097	0.084	0.92	0.96	0.58	0.084	473.5	65	151.7
0.25	123.57	155.64624	881.6487	741.1985	0.25	0.89	0.96	0.58	0.25	483.4	64	149
0.417	93.57	71.78178	834.8315	739.3266	0.417	0.92	0.96	0.58	0.417	490	63.6	146.2
1	65.25	13.78206	762.9205	704.8689	1	0.89	0.97	0.58	1	405.2	62.6	148.2
2	48.76	5.16048	710	685	2	0.9	0.97	0.59	2	50.7	62.7	147.9
3	23.58	0	659.5511	656.9295	3	0.91	0.97	0.6	3	-50	61.6	144.3
4	15.00	0	646.8165	638.5773	4	0.915	1	0.6	4	-103	59.6	141.7
5	7.70	0	622.0974	615	5	0.92	0.99	0.6	5	-148	58.4	142.1
24	4.59	0	547.1914	544.5693	24	0.98	1.05	0.63	24	-154	57.4	141.5
48	4.43	0.4578	407.8652	488.3895	48	1.03	1.09	0.67	48	-75	52.3	132.2

Table B-4: Raw concentration, pH, and OCP data for industrial effluent containing [AuCl₄] and copper ions.

Concentration			pH		OCP	
Time	Au (Au+Waste)	Cu(Au+Waste)	Time	Au+Waste	Time	Au+Waste
0	175	850.5617978	0	0.24	0	210
0.084	103	920	0.084	0.24	0.084	109
0.25	88	961.4241854	0.25	0.24	0.25	98
0.417	75	985.0187266	0.417	0.26	0.417	99
1	41	990.2611547	1	0.26	1	80
2	30	1008.238528	2	0.26	2	70
3	12	1092.135826	3	0.27	3	58
4	10	1116.103616	4	0.27	4	32
5	7	1140.82397	5	0.35	5	24
24	0	1162.547918	24	0.4	24	-23
48	2	1243.445693	48	0.42	48	30

Table B-5: Raw concentration, pH, and OCP data for industrial effluent containing [AuCl₄⁻] and copper ions using varied initial yeast concentration.

Au Concentration				Cu Concentration				OCP			
Time	100 g/L	80 g/L	50 g/L	Time	100 g/L	80 g/L	50 g/L	Time	100 g/L	80 g/L	50 g/L
0	180	175	170.6164	0	1260	1243.446	1251	0	260	210	280.3
0.084	119	103	148.57	0.084	1130	1162.548	1700	0.084	125	109	178.5
0.25	69	88	98.3532	0.25	1100	1140.824	1553	0.25	108	98	169.3
0.417	51	75	76.9192	0.417	950	1116.104	1468	0.417	89	99	159.1
1	41	41	57.9348	1	930	1092.136	1300	1	60	80	138
2	8	30	48.2604	2	900	1008.239	1240	2	22	70	114.2
3	7	12	35.276	3	875	990.2612	1193	3	22	58	99.8
4	5	10	30.4388	4	860	985.0187	1150	4	22	37	92.8
5	2	7	19.966	5	850	961.4242	1100	5	25	24	90.7
24	0	0	18.1288	24	835	920	997	24	2	-23	-0.3
48	1	2	13.2296	48	820	850.562	980	48	53	30	17.8

Table B-6: Raw concentration, pH, and OCP data for industrial effluent containing [AuCl₄] and copper ions using solutions at different pH.

Au Concentration						Cu concentration						OCP					
Time	pH=0.2	pH= 2	pH= 2.65	pH= 4.45	pH=5.2	Time	pH=0.2	pH= 2	pH= 2.65	pH= 4.45	pH=5.2	Time	pH=0.2	pH= 2	pH= 2.65	pH= 4.45	pH=5.2
0	175	187	220	160	119	0	1243.45	1280	1282	1260	1276	0	210	239	318	262.2	312.2
0.084	103	120	107	75	53	0.084	1162.55	1203	1198	1216	1100	0.084	109	140.5	119.5	93.5	94.5
0.25	88	75	85	43	38	0.25	1140.82	1103	1052	1189	915	0.25	98	78.5	69.6	7	78.7
0.417	75	45	34	20	42	0.417	1116.10	1008	1002.3	1146	834	0.417	99	64.3	78.3	-5.1	70.9
1	41	40	20	12	24	1	1092.14	993	993	1069	803	1	80	64.3	35.5	-49.5	68
2	30	30	12	9	22	2	1008.24	970	982	1034	790	2	70	45.8	20.8	-76.2	54.8
3	12	20	5	8	22	3	990.26	959	954	1000	751	3	58	44.2	-28.8	-183.8	47.2
4	10	12	2	6	22	4	985.02	934	943	952	732	4	32	29.8	-148.8	-240.8	34.2
5	7	4	1	5	24	5	961.42	920	934	903	713	5	24	13.3	-107.7	-270.7	20.3
24	0	1	0	6.5	19	24	920.00	879	884	812	647	24	-23	-24.6	-64.7	-74.8	48.3
48	2	0	1	7	22	48	850.56	844	840	700	600	48	30	42.2	-55.8	-72.8	161.2

Vita

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