TOWARDS OPTIMIZING THE OPERATION OF MICROBIAL ELECTROLYSIS CELLS FOR HEAVY METAL REMOVAL

TOWARDS OPTIMIZING THE OPERATION OF MICROBIAL ELECTROLYSIS CELLS FOR HEAVY METAL REMOVAL

By:

ERIN FULLER, B. Sc.

A Thesis Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements for
the Degree Master of Applied Science

McMaster University

McMaster University MASTER OF APPLIED SCIENCE (2018) Hamilton, Ontario
(Civil Engineering)

TITLE: Towards optimizing the operation of microbial electrolysis cells

for heavy metal removal

AUTHOR: Erin Fuller, B.Sc. (McMaster University)

SUPERVISOR: Dr. Younggy Kim

NUMBER OF PAGES: viii, 54

Abstract

Heavy metals are a growing environmental concern as they are unable to be metabolized in the environment, leading to bioaccumulation in the food chain and impacting human health. Treating heavy metals is difficult and expensive. Current methods include precipitation (which generates sludge that is costly to dispose of) or requires the use of a membrane, which fouls and requires regeneration.

Microbial electrolysis cells (MECs) represent an alternative for treating heavy metal contaminated wastewater. Reactor components are cheap, and operation requires only a small amount of electricity. The electrically active biofilm oxidizes organics in the wastewater while transferring electrons first to the anode, then to the cathode, where aqueous metals are reduced to a solid deposit, a mechanism called electrodeposition. Few studies have been conducted to investigate the best operational conditions for heavy metal removal in MECs. In this study, the effects of hydrodynamics, applied voltage, and initial metal concentration on heavy metal removal mechanisms are investigated, and the best operational practices are determined on a high level.

Mixing in the cathode chamber increased electrodeposition by 15%, decreased the cathode potential by -0.06 V, and increased current generation between 10-30%. Increasing the applied voltage from 0.6 V to 1.2 V increased electrodeposition by 22%. With both mixing and higher voltage applied, 93.35% of cadmium was removed from the catholyte in 24 hours. Although high voltage application maximized electrodeposition for short-term treatment, long-term treatment indicated lower applied voltage resulted in healthier MEC reactors, better overall metal recoveries, along with a more stable cathode potential.

Acknowledgements

I would like to thank my supervisor, Dr. Younggy Kim, for his guidance and support throughout my time in the master's program. I would also like to thank Monica Han for her help in the lab. I am grateful to Dr. Zoe Li and Dr. Charles de Lannoy for their knowledge and time as members of my examination committee.

Thank you to my fellow members in our research group; all your camaraderie, friendship and support transformed our office into a warm and productive place.

I would like to thank my mother, who imparted to me her strength and persistence, allowing me to stand where I am today. Thank you for all your encouragement over the years, and for always believing in me.

And finally, I would like to thank my fiancée, Kalyna, without whom this thesis would not have been completed. Thank you for being my fiercest supporter, and for all your invaluable help and advice.

This thesis is dedicated to the future, where graduate student mental health is fully understood and supported by the structure of academia.

Table of Contents

List of Figures

List of Tables

1 Introduction

1.1 Heavy metals: sources, danger, and treatment

Heavy metal contamination in groundwater, wastewater and soil is a prominent environmental risk factor to ecosystem damage, and damage to human health. The definition of a heavy metal used in this thesis will be "naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water" [1], [2]. This definition applies to transition and post-transition metals in the periodic table [3]. Thirteen of these elements are listed in the US EPA's priority pollutant list: antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium, and zinc [4].

The persistence of heavy metals in the environment is a major contribution to their harmful nature. Heavy metals cannot be metabolized in the environment or by humans, animals, and plants. Instead, heavy metals are prone to bioaccumulation where they occur in high levels in organisms high in the food chain, which is especially true for aquatic ecosystems [5].

This thesis focuses on the removal of cadmium, chromium, lead, and gold from wastewater. Cadmium is found in the earth's crust at concentrations around 0.1 mg/kg, although human exposure to cadmium is usually from anthropogenic activities, such as industrial emissions from mining, battery, and pigment manufacture. Main sources of everyday exposure are from cigarette smoke and consumption of food and tissue where cadmium builds up, such as liver, shellfish, mushrooms and seaweed [1]. Cadmium can cause vomiting, cramps and death at acute exposure ranging from 13 milligrams to several hundred milligrams [6]. Chromium as a contaminant is a mainly result of industries relating to metal processing, pigment production, and tanneries. It enters the environment in its hexavalent form, which is also the toxic form of chromium [1]. Hexavalent chromium can result in liver inflammation or necrosis, and death, and is considered a carcinogen [7]. Chromium also exists in the environment in its trivalent state, which is non-toxic [7]. Lead exposure results from fossil fuels, batteries, lead pipes, and paint that was commonly used for interior surfaces in homes in previous years before production of such paint was reduced [1]. Health complications from lead exposure include affected physical and mental development in fetuses and children, neurological damage, and renal disease [8].

Gold is not a danger to human health as it is chemically inert; however there has been recent interest in recovering dilute gold in wastewater and bodies of water [9]. Gold can end up in water from the frequent washing of hands while wearing jewelry and gold fillings used in dentistry. If a wastewater treatment technology could passively recover gold from continuously flowing effluent, it would potentially provide financial gain for municipalities to offset the cost of water treatment.

Often, heavy metals occur in dilute concentration in wastewater, leading to more complicated removal. Even with concentrated industrial effluents, effluent guidelines are stringent, and require treatment down to a very low level. The difficulty with removing dilute contaminants is the efficiency of treatment techniques for low concentrations.

The maximum acceptable concentrations of chemical contaminants in drinking water in Canada is 0.005 mg/L for cadmium, 0.05 mg/L for chromium, and 0.010 mg/L for lead [10]. These are all significantly lower than for a more recognizable toxic substance, cyanide, which may be present in drinking water at a maximum concentration of 0.2 mg/L [10]. These extremely low levels for heavy metals highlights the importance of reliable and energy efficient heavy metal treatment technologies for wastewater to prevent or at least properly treat contamination from runoff, point source, or other methods of heavy metals entering the environment and water table.

Commonly used wastewater treatment for heavy metal removal includes chemical precipitation, coagulation and flocculation, which all generate sludge that is costly to dispose of; ion exchange, which requires resin regeneration; adsorption, which can require either costly materials such as activated carbon, and requires separation of adsorption material from the wastewater stream; membrane filtration, which is very costly and requires membrane regeneration and managing membrane fouling; and traditional electrochemical heavy metal treatment, which has a high capital and operational cost [11]. Conventional wastewater treatment does not remove heavy metals, and implementing any of the previously listed technologies is expensive. This leaves a treatment void at the municipal and regional level.

1.2 Microbial electrolysis cells for heavy metal treatment

Microbial electrolysis cells (MECs) are a new technology that pairs wastewater treatment with energy recovery by the production of hydrogen gas which can be used in hydrogen fuel cells [12, 13, 14, 15]. MECs are also referred to as bioelectrochemical systems (BES), which is a term that also includes the parent technology of MECs, microbial fuel cells (MFCs).

MFCs are constructed and are operated the same as an MEC, though no external power is applied to the cell, and its cathode potential is more positive than the anode potential. MECs have external power applied to lower the cathode potential. Usually between 0.2 and 1.2 V is applied, which forms the lower boundary required to surpass the thermodynamic barrier for some MEC reactions, and the upper limit of what the biofilm is capable of being exposed to while remaining biologically active [14]. In addition, the reduction reaction $O_2(g) + 4H^+ + 4e^- \leftrightarrow 2H_2O(aq)$ $E^0 = 1.23$ V vs SHE begins to produce oxygen at the cathode if more than 1.2 V is applied. As exoelectrogens are anaerobic, some strictly anaerobic, any oxygen in the system will negatively impact MEC performance.

MFCs require the cathode to be exposed to air, as oxygen is the terminal electron acceptor, and they generate current directly and spontaneously. Alternatively, MECs are entirely anaerobic systems, and produce hydrogen gas, which can later be reclaimed as an energy source. Having an anaerobic wastewater treatment system is greatly beneficial from an economic perspective, as aeration in a conventional activated sludge (CAS) wastewater treatment plant comprises a significant portion of operational costs [16].

MECs are constructed usually with a bioanode (a biofilm grown on the anode) but can also be configured to have a biocathode [17]. Biocathodes were developed as an alternative to costly platinum cathodes, and both emphasize maximizing efficiency for hydrogen evolution. It is worth noting, however, that wastewater as an energy source is very dilute, and it is better to view energy recovery from MECs not as its main function, but a beneficial side effect to reduce overall cost of the system. The strength of MECs for wastewater treatment lies instead in more energy-efficient wastewater treatment while additionally being able to recover nutrients and heavy metals at the same time. In this thesis the focus of the MEC is taken to be organic contaminant oxidation and heavy metal removal, and so a bioanode is used.

1.2.1 Heavy metal removal mechanisms in an MEC

The defining feature of a BES is the biofilm, which is electrically active due to contained exoelectrogenic bacteria. These microorganisms oxidize organics and transfer the freed electrons outside of the cell. In the wild, the final electron acceptor is often a strong oxidizing agent, such as Fe(III) or Mn(III/IV) oxides [18]. In a MEC, however, the electrons are transferred to the electrode the biofilm is cultivated on, which can then be passed to the connected electrode. In this thesis, the bioanode transfers its electrons to the cathode, where electrons are used in reduction reactions facilitated by the sufficiently negative cathode potential. At the cathode, aqueous metals can be removed from the water via a mechanism called electrodeposition, where the reaction spontaneity is governed by the Nernst equation [19]:

$$E = E^0 - \frac{RT}{nF} ln \frac{1}{[Mn^{n+}]} \tag{1}$$

where E is the required electrode potential for spontaneous reduction, E^0 is the standard potential, R is the gas constant (8.314 J/mol·K), F is the Faraday constant (96485 C/mol), n is the number of electrons involved in the reduction reaction, and T is the temperature (298 K).

Each metal will be spontaneously reduced according to the general reaction if the cathode potential is more negative than the required potential, E:

$$M^{n+} + ne^- \rightarrow M \qquad E^0 \text{ [V vs. SHE]} \tag{2}$$

The required potential for reduction (assuming a concentration of 1 mg/L, and 22°C) is as follows: E = -0.547 V vs SHE for Cd^{2+}, E = -0.832 V vs SHE for Cr^{3+}, E = -0.281 V vs SHE for Pb^{2+}, and E = 1.383 V vs SHE for Au^{1+}. As can be seen, the required reduction potential varies by heavy metal. Species with a higher E value will reduce to a solid deposit on the cathode at a higher rate than a species with a more negative E value due to a greater thermodynamic driving force. Species with a sufficiently positive E value can even be reduced to a solid deposit in an MFC, where the cathode potential is more positive than in an MEC. However, for the majority of heavy metals an MEC is required to have the sufficiently negative cathode potential for electrodeposition to occur.

Other mechanisms to remove aqueous metals in an MEC system include precipitation at the cathode [20]; adsorption to the reactor materials, notably the anode if it is made of carbon fibre; bioadsorption into biosolids or the biofilm's extracellular matrix; and anodic reduction in the case of lead [21], as the anode potential can range from -0.2 to -0.3 V vs SHE during open and closed circuit conditions, which can therefore be more negative than the required potential of -0.281 V vs SHE for lead reduction at 1 mg/L and 22°C. These methods are not preferred, as precipitation requires the precipitants to be separated from the effluent, and precipitants can dissolve back into the effluent if the pH conditions at the cathode change [20], and adsorption does not allow for easy recovery of valuable metals through established electrochemical techniques such as reversing the electrodeposition reaction which requires a conductive electrode.

Bioadsorption has a unique removal mechanism for cadmium through the class of proteins called metallothioneins. This protein type has been determined to be in the genus

Pseudomonas spp., which is commonly found in wastewater [16], [22], and can exist in an anaerobic environment [23]. *Pseudomonas spp.* has been found in the biofilm of an MEC in previous studies [14]. Metallothioneins normally take up zinc, an essential trace mineral for bacterial growth. However, cadmium is in the same group as zinc in the periodic table, and only one row below. Thus, it is chemically similar, and can act as an inhibitor, being taken up instead of zinc [24].

1.2.2 Previous studies on heavy metal removal in MECs

Heavy metal removal in MECs have been demonstrated at a range of concentrations and catholyte compositions, some with a single metal contaminant and others with multiple metal species.

In a study by Modin et al. [25], it was demonstrated that copper, lead, cadmium and zinc could be selectively removed in a BES system from municipal waste ash leachate by controlling the cathode potential through the applied voltage. When operating the system as an MFC without applied voltage, copper was first removed due to its positive required potential for reduction. Lead was then extracted by controlling the cathode potential at -0.51 V vs SHE, operating as an MEC for the rest of the cycle. Cadmium was recovered next with a cathode potential of -0.66 V vs SHE, and finally zinc was recovered by maintaining the cathode at -1.00 V vs SHE. Initial concentrations of copper, lead, cadmium, and zinc were respectively 0.8 g/L, 0.4 g/L, 0.8 g/L, and 0.3 g/L, which is relatively concentrated. Concentrations of each element were close to zero at the end of the testing cycles.

Li et al. [26] demonstrated multiple heavy metal recoveries in a combined MFC-MEC system, where the three-part reactor contained the communal bioanode in the center. MFC and MEC cathode chambers were attached to the anode chamber, separated by Nafion membranes which allow movement of cations through the membrane pores. The MFC's generated electricity powered the MEC chamber, yielding a self-sustaining system. The study demonstrated the removal of chromium (VI), lead, and nickel with an initial concentration of 100 mg/L.

Chen et al. [27] recovered cadmium from an MEC system using a biocathode in a two-chamber reactor, separated by a cation exchange membrane, which allows the passage of cations. Initial cadmium concentrations of 20, 40, 50, and 60 mg/L were used, while applied voltage varied from 0.3, 0.5, 0.7 to 1.0 V. It was shown that there was greater removal efficiency at higher initial concentrations, which reflects the specific difficulties generally encountered when attempting to treat more dilute contaminants. They also found that using acetate as the carbon source for the biocathode resulted in improved cadmium removal rates (7.33 ± 0.37 mg-Cd/L/h) over using $NaHCO_3$ as the carbon source (6.56 ± 0.38 mg-Cd/L/h). Comparing these rates to their biotic open circuit control tests, whose cadmium removal rates were much lower with the same carbon sources (acetate: 2.04 ± 0.27 mg-Cd/L/h; $NaHCO_3$: 1.83 ± 0.44 mg-Cd/L/h), and to abiotic closed-circuit tests (both acetate and$NaHCO_3$: 3.51 ± 0.42 mg-Cd/L/h), it is clear that the presence of exoelectrogens and the availability of a sufficiently negative electrode potential to facilitate electrodeposition improved the removal of cadmium from the system. Cadmium metal deposits were confirmed on the electrode through subsequent SEM and XPS analysis.

Peiravi et al. [28] successfully removed aluminum, iron, lead, cadmium, zinc, manganese, and cobalt from acid mine drainage using their BES system over seven days. Initial concentrations ranged from 16.75 μg/L for cadmium to 315.92 mg/L for iron. A biocathode was used and was separated from the anode using an anion exchange membrane. Removal rates were >90% after 7 days for most of the metal species.

Colantonio and Kim [29] demonstrated the removal of cadmium from an MEC system at low concentrations, including 10, 50, and 100 μg/L, with applied voltage of 0.6 V, as well as an initial concentration of 2.5 mg/L, with an applied voltage of 0.9 V. The MEC system used a bioanode, and quantified the contribution of removal mechanisms (electrodeposition, precipitation, and bioadsorption) at different concentrations. It was shown that the three low concentrations had low metal recoveries, with electrodeposition accounting for between 7.8-10.2% of cadmium removal. Bioadsorption removed between 3-6 μg from the reactor regardless of concentration, and precipitation was not seen in the three low concentrations. At the higher concentration of 2.5 mg/L, a better cadmium recovery rate was seen (93% over 7 days), and electrodeposition accounted for 68% of cadmium removal, with 18% removed via precipitation, and 14% of the removed cadmium had been adsorbed [29].

Colantonio and Kim [20] also studied cadmium removal at 12.26 mg/L using a bioanode, and applied voltages of 0.4, 0.6, 0.8, and 1.0 V. Good removal of 50-67% was seen in just 24 hours. More importantly, it was determined that cadmium precipitation occurs at the cathode either as $Cd(OH)_2$, or $CdCO_3$, and that both products rely on ongoing electric generation from the bioanode. When the current dropped due to depleted organic substrate, the precipitants dissolved back into solution due to the pH at the cathode decreasing from slowed hydrogen evolution which is driven by the transfer of electrons from the anode.

Finally, Colantonio and Kim [21] demonstrated a novel mechanism for lead removal in an MEC system through reduction onto the anode, as the anode was sufficiently negative to facilitate lead reduction. An anion exchange membrane was employed to demonstrate the independence of the reduction from the cathode, and SEM and XPS analysis demonstrated lead deposits on the carbon fibres of the anode. Applied voltage ranged from 0, 0.3, 0.6, to 0.9 V, and initial lead concentration was 2.5 mg/L.

1.3 Study Objectives

This thesis aims to increase the understanding of operational effects on the removal efficiency of heavy metals in MECs. Though there are many studies that have varied applied voltage and initial concentration and their effect on total metal recovery, few studies have focused on other aspects of maintaining an MEC reactor for wastewater treatment of heavy metals. In particular, the effects of hydrodynamics and long-term operation were selected in order to determine macroscopically the best operational configuration. In the eventuality of MEC scale-up for industrial or municipal use, a better understanding of these operational conditions will be required to optimize a system intended to operate beyond short testing periods, more on the scale of years.

Additionally, this study focused on dilute concentrations of heavy metals, as levels in wastewater tend to be very low. Moreover, it would be beneficial to demonstrate the ability of MECs to reach stringent environmental regulations for effluent quality regarding heavy metal regulated limits.

The metals cadmium, chromium, and lead were chosen as they are common heavy metal contaminants from industrial processing and pose significant threat to human health and the environment. Gold was selected to investigate a proof of concept for using MECs for gold recovery from wastewater streams, as well as having a positive required reduction potential, which contrasts the negative required potentials for cadmium, chromium and lead.

In the first section of this study, the effects of hydrodynamics on the MEC and cadmium removal was investigated with the following objectives:

1) to determine the effects of mixing in the cathode chamber on cathode potential, electric current generation, total cadmium removal, and how much each removal mechanism contributed to overall removal; and
2) to examine the effects of a change in applied voltage to the previously listed MEC attributes.

In the second part of this study, long-term operation at dilute levels of four heavy metals was investigated with the following objectives:

1) to observe MEC behaviour over a long timeframe (14 months) as voltage and initial concentration was changed;
2) to investigate the ability of MECs to remove highly dilute concentrations of heavy metals; and
3) to quantify the contributions of multiple removal mechanisms to the total removal of each metal.

From the results from these examinations, a better understanding of the MEC system was gained, which will provide a clear basis for future work to further optimize the MEC system for heavy metal removal.

1.4 References

[1] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, "Heavy Metals Toxicity and the Environment," EXS, vol. 101, pp. 133–164, 2012.

[2] J. E. Fergusson, "The heavy elements: chemistry, environmental impact, and health effects," Pergamon Press, Oxford, 1990.

[3] S. J. Hawkes, "What Is a 'Heavy Metal'?," J. Chem. Educ., vol. 74, no. 11, p. 1374, 1997.

[4] US EPA, "Priority Pollutant List," 2014.

[5] T. M. Ansari, I. L. Marr, and N. Tariq, "Heavy Metals in Marine Pollution Perspective–A Mini Review," J. Appl. Sci., vol. 4, pp. 1–20, 2004.

[6] Health Canada, "Cadmium," Guidel. Can. Drink. Water Qual., no. 3, p. http://www.hc-gc.ca/ewh-semt/pubs/water-eau/cad, 1986.

[7] Health Canada, "Chromium," Guidel. Can. Drink. Water Qual., vol. 1979, no. January 1979, pp. 1–6, 1986.

[8] Health Canada, "Lead," Guidel. Can. Drink. Water Qual., vol. 1997, no. July, pp. 1–8, 1987.

[9] K. Kenison Falkner and J. M. Edmond, "Gold in seawater," Earth Planet. Sci. Lett., vol. 98, no. 2, pp. 208–221, 1990.

[10] Federal-Provincial-Territorial Committee on Drinking Water, "Guidelines for Canadian Drinking Water Quality Summary Table," Heal. Canada, 2014.

[11] F. Fu and Q. Wang, "Removal of heavy metal ions from wastewaters: A review," J. Environ. Manage., vol. 92, no. 3, pp. 407–418, 2011.

[12] H. Liu, S. Grot, and B. E. Logan, "E lectrochemically assisted microbial production of hydrogen from acetate," Environ. Sci. Technol., vol. 39, no. 11, pp. 4317–4320, 2005.

[13] R. A. Rozendal, H. V. M. Hamelers, G. J. W. Euverink, S. J. Metz, and C. J. N. Buisman, "Principle and perspectives of hydrogen production through biocatalyzed electrolysis," Int. J. Hydrogen Energy, vol. 31, no. 12, pp. 1632–1640, 2006.

[14] B. E. Logan, D. Call, S. Cheng, and H. V. M. Hamelers, "Critical Review Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter," Environ. Sci. Technol., vol. 42, no. 23, pp. 8630–8640, 2008.

[15] D. Call and B. E. Logan, "Hydrogen production in a single chamber microbial

electrolysis cell lacking a membrane," Environ. Sci. Technol., vol. 42, no. 9, pp. 3401–3406, 2008.

[16] C. P. L. Grady, G. T. Daigger, N. G. Love, and C. D. M. Filipe, Biological Wastewater Treatment, 3rd ed. Boca Raton: IWA Publishing - Co-Publication, 2011.

[17] E. Croese, M. A. Pereira, G. J. W. Euverink, A. J. M. Stams, and J. S. Geelhoed, "Analysis of the microbial community of the biocathode of a hydrogen-producing microbial electrolysis cell," Appl. Microbiol. Biotechnol., vol. 92, no. 5, pp. 1083–1093, 2011.

[18] R. S. Hartshorne et al., "Characterization of an electron conduit between bacteria and the extracellular environment," Proc. Natl. Acad. Sci., vol. 106, no. 52, pp. 22169–22174, 2009.

[19] A. J. Bard and L. R. Faulkner, Electrochemical Methods: Fundamentals and Applications. New York: Wiley, 2001.

[20] N. Colantonio and Y. Kim, "Cadmium (II) removal mechanisms in microbial electrolysis cells," J. Hazard. Mater., vol. 311, pp. 134–141, 2016.

[21] N. Colantonio and Y. Kim, "Lead(II) removal at the bioanode of microbial electrolysis cells," ChemistrySelect, vol. 1, no. 5743, 2016.

[22] C. A. Blindauer, "Bacterial metallothioneins: Past, present, and questions for the future," J. Biol. Inorg. Chem., vol. 16, no. 7, pp. 1011–1024, 2011.

[23] A. Zimmermann, C. Reimmann, M. Galimand, and D. Haas, "Anaerobic growth and cyanide synthesis of Pseudomonas aeruginosa depend on anr, a regulatory gene homologous with fnr of Escherichia coli.," Mol. Microbiol., vol. 5, no. 6, pp. 1483–1490, 1991.

[24] C. G. Elinder and M. Piscator, "Cadmium and zinc relationships," Environ. Health Perspect., vol. VOL. 25, no. August, pp. 129–132, 1978.

[25] O. Modin, X. Wang, X. Wu, S. Rauch, and K. K. Fedje, "Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute solutions," J. Hazard. Mater., vol. 235–236, pp. 291–297, 2012.

[26] Y. Li et al., "Self-sustained reduction of multiple metals in a microbial fuel cell-microbial electrolysis cell hybrid system," Bioresour. Technol., vol. 192, pp. 238–246, 2015.

[27] Y. Chen, J. Shen, L. Huang, Y. Pan, and X. Quan, "Enhanced Cd(II) removal with simultaneous hydrogen production in biocathode microbial electrolysis cells in the presence of acetate or NaHCO3," Int. J. Hydrogen Energy, vol. 41, no. 31, pp.

13368–13379, 2016.

[28] M. Peiravi, S. R. Mote, M. K. Mohanty, and J. Liu, "Bioelectrochemical treatment of acid mine drainage (AMD) from an abandoned coal mine under aerobic condition," J. Hazard. Mater., vol. 333, pp. 329–338, 2017.

[29] N. Colantonio, H. Guo, and Y. Kim, "Effect of Low Cadmium Concentration on the Removal Efficiency and Mechanisms in Microbial Electrolysis Cells," pp. 6920–6924, 2016.

2 The effect of hydrodynamics on cadmium removal in a microbial electrolysis cell

Abstract

The removal of heavy metals at low concentrations is expensive and operationally difficult, creating a need for low-cost treatment technique to remove metals from industrial effluent and contaminated water and soils. Microbial electrolysis cells are constructed from cheap materials and require only a small amount of electricity applied to have a system that can remove organics, recover nutrients, produce hydrogen gas, and remove concentrated and dilute heavy metals from wastewater. In this study, the effects of hydrodynamics and applied voltage on cadmium electrodeposition onto the cathode were investigated. Two voltage conditions were used in testing, 1.2 V and 0.6 V. Additionally, two hydrodynamic conditions were run, with mixing applied in the cathode chamber, or not. Rapid removal was seen under the optimal mixed, 1.2 V condition (93.35% in 24 h). Mixing was shown to increase electrodeposition of cadmium by approximately 15% compared to non-mixing, while increasing the voltage from 0.6 V to 1.2 V increased electrodeposition by approximately 22%. Cadmium precipitation at the cathode had an unpredictable effect with mixing, and further study is required to determine the true effects of hydrodynamics on precipitation.

2.1 Introduction

Cadmium is a toxic heavy metal, often found as a contaminant in groundwater, soil, and in the air. The danger to human health is significant as the body is incapable of metabolizing it, instead accumulating cadmium in the liver over a lifetime, leading to liver damage, cancer, and acting as a risk factor for osteoporosis [1]. Cadmium is also not metabolized in the environment. When it enters an ecosystem through industrial runoff and leaching from municipal dumps from improper disposal of nickel-cadmium batteries, both soil and groundwater become contaminated. Vegetation then takes up cadmium, especially in low pH soil, and bioaccumulation through the food chain occurs when contaminated plants are consumed [2]. Most human exposure to cadmium is from smoking cigarettes, and food ingestion, particularly shellfish [3].

Heavy metals are removed from wastewater streams using several established methods, including ion-exchange membranes, membrane filtration, chemical precipitation, and adsorption onto low-cost materials or activated carbon. Each of these has an operational drawback, usually related to high costs due to membrane fouling and regeneration, the cost of activated carbon material, or disposal of precipitant sludge. Additionally, many of these technologies are ineffective at low concentrations and are either incapable or costly to reach the environmental standards for effluent to be released into natural water bodies [4]. As many countries work to deploy contaminant prevention through better on-site effluent treatment, or remediation of contaminated groundwater, development of a low-cost, reliable method of heavy metal removal becomes pertinent.

Microbial electrolysis cells (MECs) are a novel wastewater treatment technology that utilizes exoelectrogenic bacteria [5, 6, 7, 8]. The bacteria oxidize organics, and transfers electrons to the anode the biofilm grows on, producing current as electrons travel to the connected cathode. A small voltage ($E_{ap} > 0.2$ V) is applied across the cell to drive the reaction [5]. MECs additionally are capable of energy recovery from wastewater through hydrogen production, and nutrient recovery from wastewater [5, 9]. Cadmium removal in MECs has been demonstrated in high concentrations (800 mg/L) [10], multi-metal solutions (50 mg/L) [11], and at low concentrations (12.26 mg/L, 10 µg/L) [12, 13], demonstrating the flexibility of MEC technology to wastewater treatment. By applying higher voltage (E_{ap} 0.4 – 1.2 V) across the MEC, the cathode potential is lowered sufficiently to cause electrodeposition, the spontaneous reduction of the cadmium ion to its solid state on the cathode:

$$Cd^{2+} + 2e^{-} \rightarrow Cd \qquad E^0 = -0.40 \text{ V vs. SHE} \qquad (1)$$

where E^0 is the standard potential for cadmium ion reduction. In this study, a low concentration of cadmium was selected (1.0 mg/L). The required cathode potential for cadmium electrodeposition at this concentration is -0.547 V vs SHE by the Nernst equation:

$$E = E^0 - \frac{RT}{2F} ln \frac{1}{[Cd^{2+}]} \qquad (2)$$

where E is the required potential for reduction, R is the gas constant (8.314 J/mol·K), F is Faraday's constant (96485 C/mol), and T is the temperature (298 K). With E_{ap} 0.6 to 1.2V applied across the cell, the cathode potential is typically 0.8 to 1.4 V vs SHE, respectively, which is enough to drive cadmium electrodeposition at 1 mg/L.

Currently, there is a lack of studies on the operational effects on heavy metal removal mechanisms in MECs, which aside from electrodeposition, includes bioadsorption and precipitation to $CdCO_3$ and $Cd(OH)_2$ [12]. It has been found that varying concentration will alter what removal mechanism dominates the system. For example, at higher concentrations, electrodeposition and precipitation are enhanced, and at lower concentrations, bioadsorption may dominate [13]. Additional operational conditions may affect removal mechanisms, such as pH and substrate conditions [11], and the hydrodynamic conditions, such as whether the effluent in the MEC is still or stirred.

If one considers the cathode (the active site for electrodeposition) submerged in aqueous effluent, the surface will have a diffusion boundary layer with thickness (δ) proportional to the turbulence in the reactor, represented by the Reynold's number (Re):

$$\delta \propto \sqrt{Re}^{-1} \tag{3}$$

As seen in Equation 3, as the Reynold's number increases (turbulence increases), the boundary layer decreases in thickness. The thicker the layer, the greater the mass transfer barrier for a cadmium ion to reach the reactive surface. It therefore follows that the thickness of the diffusion layer near the surface of the cathode will influence the rate of electrodeposition if the rate of reaction is faster than the rate of diffusion of cadmium ions across the boundary layer. This study seeks to quantify the effect of the hydrodynamic condition in an MEC on low-concentration cadmium removal, specifically on the electrodeposition removal mechanism.

2.2 Materials and Methods

2.2.1 Reactor Design

The two-chamber reactor was built from polypropylene with an internal cylindrical chamber 3 cm in diameter. Both the anode and cathode chambers were 20 mL each. The anode was a graphite fiber brush (2.54 cm in diameter, 2.54 cm long, from Mill-Rose, USA). The cathode was 99.00% pure nickel foil (SHOP-AID, Inc., USA). The reservoir was a sealed 250 mL Erlenmeyer flask with a stir bar. A peristaltic pump operated at 2 mL/min, taking catholyte from the top of the cathode chamber, through the pump, and to the reservoir. A connecting siphon tube replenished the cathode chamber from the reservoir. For each test, there was 20 mL total anolyte, and 250 mL total catholyte. Residence time for the catholyte was 10 minutes for a unit volume to move through the cathode chamber, and 125 minutes for a unit volume to move through the entire system, including the reservoir. A stir bar was placed in the cathode chamber to control the mixing condition. An Ag/AgCl reference electrode (Bioanalytical Systems, Inc., USA) was inserted in the cathode chamber. An anion exchange membrane (Selemion AMV, AGC Engineering, Japan) separated the two chambers, while a needle syringe was inserted into the anode chamber to relieve any osmotic pressure buildup from the catholyte.

A reservoir was used in the set up to increase the amount of cadmium in the system without increasing the concentration. More cadmium was beneficial for the final analysis of the cathodes in ICP-OES (inductive coupled plasma-optical emission spectrometry) (Vista Pro, Varian Inc., Australia) to prevent the cadmium deposits from being below the ICP-OES detection limit.

2.2.2 MEC Inoculation and Operation

The graphite brush was first pretreated by soaking in a 1% surfactant solution for 30 minutes, then rinsed in water before being inoculated with half active effluent from another healthy MEC (being used for heavy metal removal in dilute concentration), and half anolyte feed solution. The anolyte consisted of 1.0 g-acetate/L from $CH_3COONa(H_2O)_3$, trace vitamins and minerals [14] in 50 mM phosphate buffer solution (PBS; 1.145 g/L Na_2HPO_4, 0.613 g/L NaH_2PO_4-H_2O, 0.078 g/L NH_4Cl, 0.033 g/L KCl). The 250 mL catholyte was 10 mM PBS with 1.0 mg-Cd^{2+}/L from $CdCl_2$.

The reactor was operated as a fed batch system in 48-hour cycles. Samples were taken at 0, 1, 2, 4, 6, 24 and 48 hours from the stirred catholyte reservoir and immediately acidified with 0.5 mL of concentrated nitric acid and diluted with 3.5 mL of deionized water. A sample was taken from the anode chamber at 0 and 48 hours with the same acidification and dilution. The cathode was removed at the end of each test and rinsed in 10 mL deionized water to collect any precipitants. The cathode was then dissolved in 10 mL of concentrated nitric acid for at least 48 hours. Samples were filtered through a 0.45 µm syringe filter (polyethersulfone membrane, VWR International). All samples were analyzed using ICP-OES.

Both pH and conductivity were measured from both the cathode and anode chamber using a conductivity probe (METTLER TOLEDO, USA) and pH probe (METTLER TOLEDO, USA). The pH and conductivity in both the anode and cathode chambers remained stable throughout testing cycles, seen in Table 1.

Table 1. Values averaged across all tests of pH and conductivity in the catholyte and anolyte.

	Test Time (hr)	0	2	6	24	48
Anolyte	pH	7.08	7.15	7.20	7.10	7.05
	Conductivity (mS/cm)	9.20	9.26	9.33	9.58	9.49
Catholyte	pH	7.22	7.20	7.20	7.20	7.17
	Conductivity (mS/cm)	1.83	1.82	1.84	1.84	1.97

The reactor was run at three electric conditions: 0.6 V and 1.2 V applied across the cell, and an open-circuit condition. Current and electrode potential was monitored with a potentiostat (MGP-2, BioLogic, France) every ten minutes. Each electric condition was run at two different hydrodynamic conditions where mixing was either applied or not within the cathode chamber. The two 1.2 V and two 0.6 V tests were run consecutively for comparability, and the two open circuit experiments were run non-consecutively to prevent damaging the health of the bioanode. One open circuit voltage (OCV) condition was run between the 1.2 V and 0.6 V tests, while the other OCV test was run as the last test performed.

2.3 Results and Discussion

2.3.1 Overall Cadmium Removal

Rapid cadmium removal was seen in the E_{ap} = 1.2 V mixed condition test, where 47.60% of cadmium was removed from the catholyte after just six hours, and 93.35% of cadmium was removed after 24 hours, seen in Figure 1. It should be noted as well that for every 2.08 test hours, a unit volume of the catholyte was exposed to the cathode chamber for only ten minutes, where the cathode is the only area in the system where cadmium is able to be removed. Other test conditions saw slower removal rates, due to sub-optimal operational conditions, such as lower applied voltage, unmixed conditions in the cathode chamber, or a combination of both of those conditions. There was no significant difference in overall cadmium removal at the 0.6 V electric condition whether mixing was applied or not, with removal rates being nearly equivalent at each sampled time. Although the initial feed concentrations were different by 0.12 mg/L, the error makes this difference, and the overall difference insignificant.

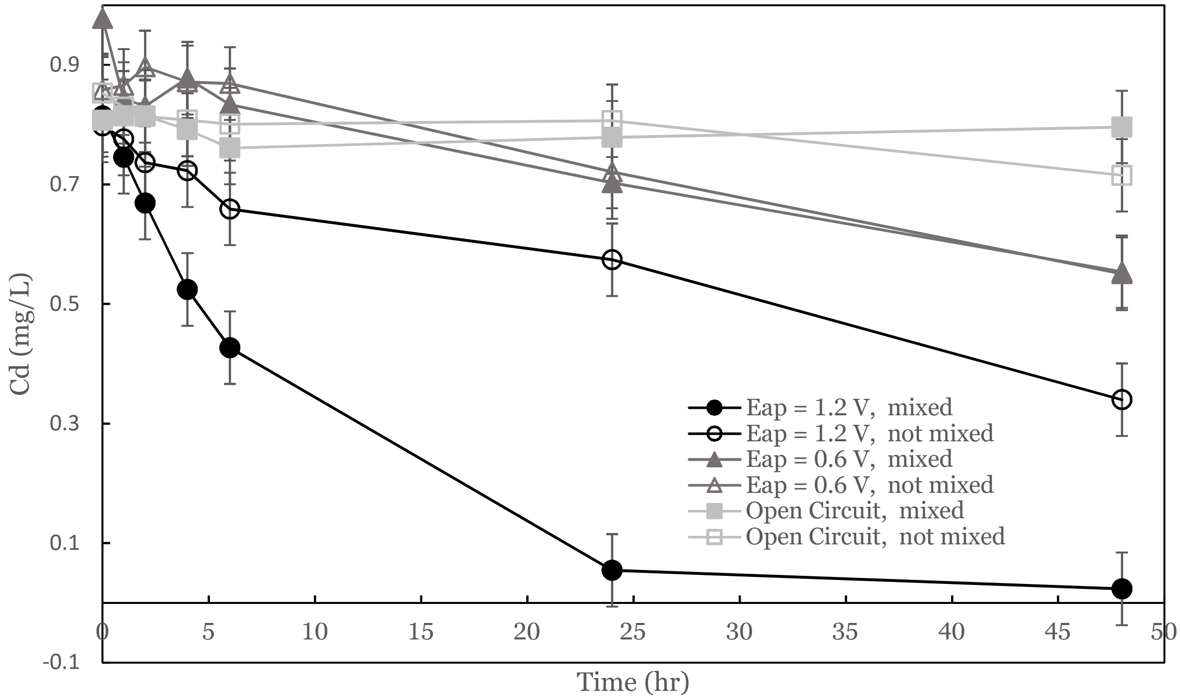

Figure 1. Cadmium removal in the catholyte over time. Error bars are the standard deviation of the feed concentration, and represent the error likely from ICP-OES measurements.

The OCV condition saw little removal, with 98.63% and 83.90% of cadmium remaining in the catholyte respectively for mixed and unmixed conditions. Acting as the control tests, the open circuit condition prevented any exoelectrogenic activity on the biofilm as the bioanode was unable to transfer electrons to the cathode without a direct connection, and without the artificially decreased cathode potential due to the applied voltage. The amount of cadmium deposited on the cathode and in the precipitants was very low for both OCV tests, with both mechanisms removing under 1% of the total cadmium in the system regardless of the hydrodynamic condition, as seen in Table 2.

The 14.73% increase in removal for the unmixed OCV test versus the mixed OCV test is likely due to experimental variation leading to more adsorption onto the anion exchange membrane (AEM). From all six tests run, 1.28 mg cadmium entered the system from all feed solutions, 0.39 mg cadmium was found on the six cathodes in total, 0.07 mg cadmium was found in the precipitants, 0.74 mg cadmium was found in the catholyte effluents, and 0.02 mg was found in the anolytes. Together, 96% of cadmium entering the system over the six tests is accounted for, leaving 0.07 mg unaccounted. The anion exchange membrane showed dark silvery-blue discolouration on the area exposed to the anolyte and catholyte. The AEM was acidified and the leachate analyzed in ICP-OES. A total of 0.06 mg cadmium was found to be on the anion exchange membrane, which is close to the unaccounted portion of cadmium. Including the cadmium from the AEM, 99.36% of the cadmium entering the six-test system was accounted for in the ICP-OES analysis. Therefore, the cadmium mass balance was maintained overall for all tests.

Additionally, all samples from the anode chamber had cadmium levels below the ICP-OES detection limit, therefore the anion exchange membrane functioned as expected to exclude cadmium from leaking into the anode chamber, as no cadmium was detected in the anode chamber before, or after the 48-hour test period.

2.3.2 Effect of Voltage Application on Cadmium Removal

As voltage was increased from 0.6 V to 1.2 V, there was a 21.34% increase in electrodeposition between tests with mixing applied, and a 22.32% increase in electrodeposition in tests without applied mixing. These similar results are expected: as the cathode potential decreases, there is more electrochemical driving force for the cadmium reduction reaction. Precipitation saw an increase in both hydrodynamic conditions, increasing by 16.45% and 4.26% respectively for mixing and non-mixing conditions. It is expected that decreasing the cathode potential (by increasing applied voltage) will yield more precipitants, as the cadmium precipitates either to $CdCO_3$, or $Cd(OH)_2$, both relying on hydroxide forming at the cathode from the hydrogen evolution reaction [12]. With more electrochemical driving force for that reaction, the hydroxide concentration will increase, and precipitation reactants are more readily available.

2.3.3 Effect of Hydrodynamics on Cadmium Removal

The electrodeposition of cadmium onto the cathode was increased with mixing applied in the cathode chamber by 15.12% for the 1.2V condition, and increased by 16.10% for the 0.6 V condition, seen by comparing the electrodeposition removal rates in Table 2Table 2. This result is within the reasonable range expected, as increasing turbulence in the reactor will decrease the boundary layer thickness, but not eliminate it. This result indicates that cadmium reduction in an MEC system is mass transfer limited.

Table 2. Distribution of where cadmium was found in the reactor after the 48 hour testing period.

Operational Conditions	Cd^{2+} accounted for	Cd^{2+} on cathode	Cd^{2+} in precipitants	Cd^{2+} in catholyte
E_{ap} = 1.2 V, mixed	86.17%	63.68%	19.62%	2.87%
E_{ap} = 1.2 V, not mixed	99.42%	48.56%	8.28%	42.58%
E_{ap} = 0.6 V, mixed	101.88%	42.34%	2.87%	56.67%
E_{ap} = 0.6 V, not mixed	94.41%	26.24%	4.02%	64.15%
Open Circuit, mixed	99.52%	0.20%	0.69%	98.63%
Open Circuit, not mixed	84.76%	0.53%	0.33%	83.90%

The most precipitation was seen in the 1.2 V mixed condition, accounting for 19.62% of removed cadmium. As this number is an order of magnitude higher than the other rates of precipitation removal, and considering the low concentration of cadmium in the system, it is likely due to normal variation between tests as no duplicates were performed.

The lowest amount of cadmium remaining in the catholyte was at the 1.2 V mixed condition (2.87% remaining), which is due as well to the high amount of precipitation seen in that test. The amount of cadmium left in the catholyte decreased as mixing was applied for both voltage conditions, with 7.48% and 39.71% more cadmium removed with mixing at the 0.6 V and 1.2 V conditions, respectively.

Increasing the turbulence in the cathode chamber was accompanied by a decrease in the average steady state cathode potential by -0.06 V for both applied voltage conditions, and by -0.01 V at the OCV condition (percent differences of 4.61%, 8.65%, and 3.87% respectively), as seen in Figure 2. The small difference at the OCV condition is expected, as the cathode potential is not artificially decreased by applying voltage across the cell. The exact same shift downwards in the cathode potential correlated to the increased cathode chamber turbulence, regardless of electric condition, is evidence for a reproducible effect the mixing condition has on the MEC system. By decreasing the cathode potential, the electrochemical driving force for cadmium reduction and hydrogen evolution are increased, providing additional means the hydrodynamic condition may affect cadmium electrodeposition beyond reducing the diffusion boundary layer thickness. It is worth mentioning that the negative slope seen at the beginning is linked to a lag in producing electric current by the biofilm, as it becomes acclimatised to new feed solution.

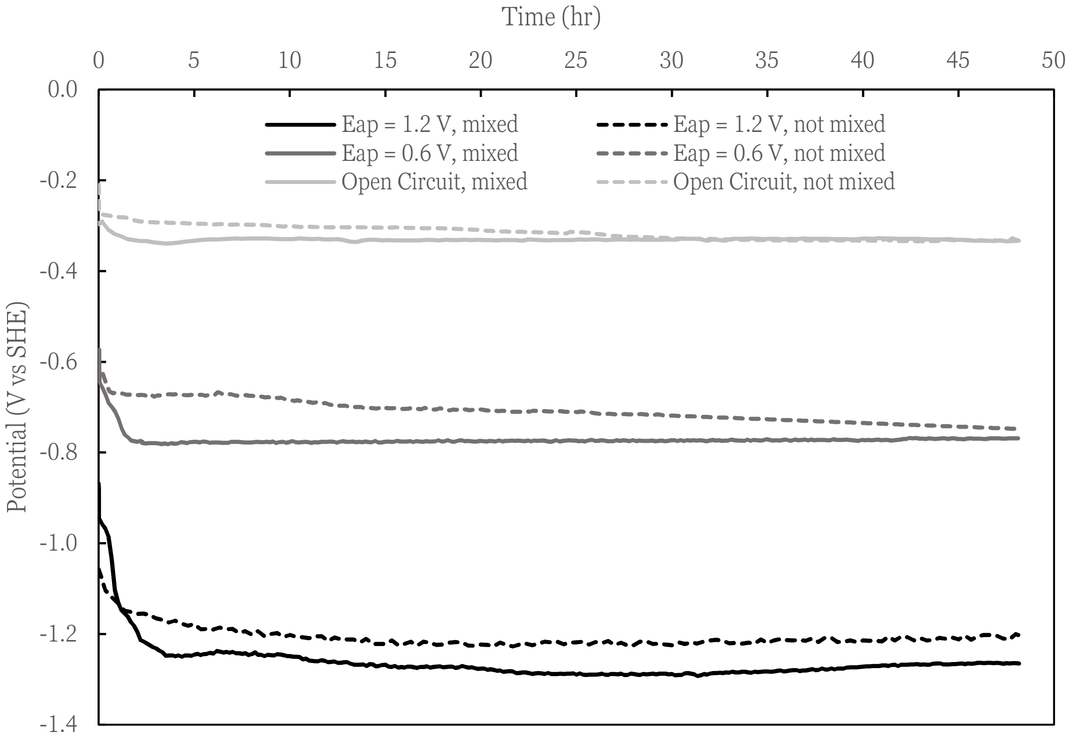

Figure 2. Electric potential at the cathode over time.

The electric current produced in the MEC system, averaged over the test period where the reactor reached an approximately steady state, was increased by 0.09 mA for E_{ap} = 1.2 V, and was increased by 0.11 mA for E_{ap} = 0.6 V when mixing was applied (percent differences of 10.52% and 30.46%, respectively), seen in Figure 3. As the cathode potential drops with mixing applied (Figure 2), electrons are more easily transferred between electrodes. As rate of transfer increases, the current will increase, as current is simply the rate of electron movement. Mixing was only applied in the cathode chamber, so it is important to note there was no change in the anode chamber, for example no mixing was available to increase the supply of substrate to the biofilm, and the biofilm diffusion boundary layer was not affected.

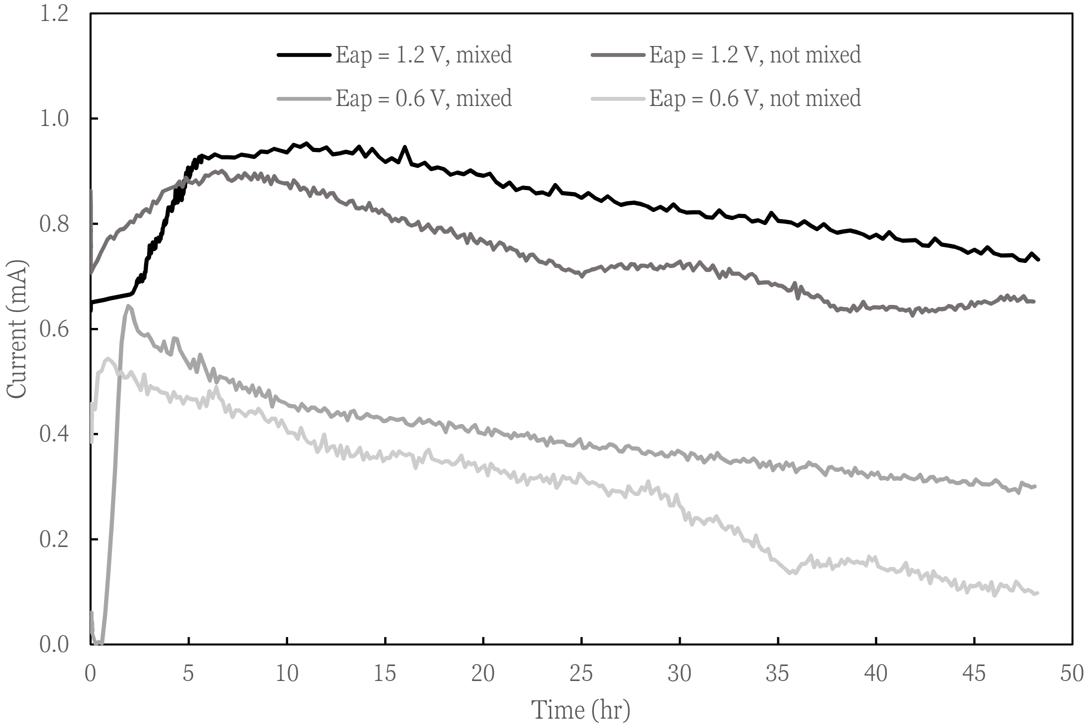

Figure 3. Electric current across the MEC over time.

2.4 Conclusions

In controlled lab-scale conditions, results suggest that electrodeposition was increased by approximately 15% with mixing applied in the MEC system, and an increase of approximately 22% by increasing the applied voltage from 0.6 V to 1.2 V. Overall, the test with both mixing and an applied voltage of 1.2 V saw the fastest rate of cadmium removal, and the lowest total cadmium remaining in the catholyte. Precipitation had mixed results with applied mixing, and may be an avenue of investigation for future studies. Increasing turbulence in the MEC reactor also influenced the cathode potential, lowering the potential in both electric conditions by -0.06 V. The electric current was also impacted by applied mixing, seeing an increase of 0.09 mA and 0.11 mA for mixing and unmixed, respectively, in the 40 mL MEC reactor used. In the future, a comparative analysis if the kinetic rates of electrodeposition, dissolution of metal deposits, and adsorption should be undertaken to further understand the relationships of these mechanisms.

Finally, considering the 10-minute residence time in the cathode chamber for every 115 minutes spent in the reservoir, the rate of cadmium removal could be more rapid than the rate seen in this study, where the set up was designed not to remove cadmium at the fastest possible rate, but to determine the effects of operational conditions on cadmium removal mechanisms.

2.5 References

[1] L. Järup, M. Berglund, and C. Elinder, "Health effects of cadmium exposure - a review of the literature and a risk estimate," *Scand. J. Work. Environ. Health*, vol. 24, no. 1, pp. 1–52, 1998.

[2] J. L. Gardea-Torresdey, J. R. Peralta-Videa, M. Montes, G. De La Rosa, and B. Corral-Diaz, "Bioaccumulation of cadmium, chromium and copper by Convolvulus arvensis L.: Impact on plant growth and uptake of nutritional elements," *Bioresour. Technol.*, vol. 92, no. 3, pp. 229–235, 2004.

[3] Health Canada, "Cadmium," *Guidel. Can. Drink. Water Qual.*, no. 3, p. http://www.hc-gc.ca/ewh-semt/pubs/water-eau/cad, 1986.

[4] F. Fu and Q. Wang, "Removal of heavy metal ions from wastewaters: A review," *J. Environ. Manage.*, vol. 92, no. 3, pp. 407–418, 2011.

[5] B. E. Logan, D. Call, S. Cheng, and H. V. M. Hamelers, "Critical Review Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter," *Environ. Sci. Technol.*, vol. 42, no. 23, pp. 8630–8640, 2008.

[6] B. E. Logan *et al.*, "Microbial fuel cells: Methodology and technology," *Environ. Sci. Technol.*, vol. 40, no. 17, 2006.

[7] B. E. Liu, H., Grot, S. and Logan, H. Liu, S. Grot, and B. E. Logan, "E lectrochemically assisted microbial production of hydrogen from acetate," *Environ. Sci. Technol.*, vol. 39, no. 11, pp. 4317–4320, 2005.

[8] R. A. Rozendal, H. V. M. Hamelers, G. J. W. Euverink, S. J. Metz, and C. J. N. Buisman, "Principle and perspectives of hydrogen production through biocatalyzed electrolysis," *Int. J. Hydrogen Energy*, vol. 31, no. 12, pp. 1632–1640, 2006.

[9] R. D. Cusick and B. E. Logan, "Phosphate recovery as struvite within a single chamber microbial electrolysis cell," *Bioresour. Technol.*, vol. 107, pp. 110–115, 2012.

[10] O. Modin, X. Wang, X. Wu, S. Rauch, and K. K. Fedje, "Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute solutions," *J. Hazard. Mater.*, vol. 235–236, pp. 291–297, 2012.

[11] Y. Zhang *et al.*, "Dependency of simultaneous Cr(VI), Cu(II) and Cd(II) reduction on the cathodes of microbial electrolysis cells self-driven by microbial fuel cells," *J. Power Sources*, vol. 273, pp. 1103–1113, 2015.

[12] N. Colantonio and Y. Kim, "Cadmium (II) removal mechanisms in microbial electrolysis cells," *J. Hazard. Mater.*, vol. 311, pp. 134–141, 2016.

[13] N. Colantonio, H. Guo, and Y. Kim, "Effect of Low Cadmium Concentration on the Removal Efficiency and Mechanisms in Microbial Electrolysis Cells," pp. 6920–6924, 2016.

[14] S. Cheng, D. Xing, D. F. Call, and B. E. Logan, "Direct biological conversion of electrical current into methane by electromethanogenesis," *Environ. Sci. Technol.*, vol. 43, no. 10, pp. 3953–3958, 2009.

3 Long term microbial electrolysis cell operation to remove dilute Au, Cd, Cr and Pb

Abstract

Heavy metals are important environmental contaminants and pose a hazard to human health when left untreated, even at dilute concentrations. Current technology is expensive and difficult to operate, and many techniques are incapable of meeting environmental effluent regulations. Microbial electrolysis cells are cheap to build and operate, and can clean wastewater of organics, nutrients and heavy metals. This study selected four heavy metals to study at dilute concentrations. Gold, cadmium, chromium and lead were removed in MECs at two concentrations, 0.05 µmol/L and 0.50 µmol/L over 14 months, and at two applied voltages, 1.2 V and 0.6 V. It was found that the lower voltage resulted in more reliable current generation, a more stable cathode potential and more metals recovered in the overall mass balance. Additionally, more metals were recovered from the more dilute conditions. Electrodeposition was unaffected by initial concentration or applied voltage, where the same mass was found on the cathode regardless of experimental condition.

3.1 Introduction

Heavy metals contamination in groundwater, surface water, and soil is one of the most prominent environmental risks to human health. Many heavy metals cannot be metabolized by the body, leading to disease, or metabolized in the environment, resulting in bioaccumulation in the food chain, which can also result in human exposure through consumption of contaminated vegetation or livestock [1].

Sources of heavy metals in the environment usually come from metal-centric industry, such as mining and ore processing, as well as commercial production and consumption of metal-containing products [2]. Human exposure has risen sharply in the past decades from industrial expansion, yet heavy metal treatment remains costly, and in many cases, ineffective at dilute, yet toxic contaminant levels.

Common methods for treating heavy metals include precipitation, ion exchange, membrane filtration, and adsorption. Precipitant sludge disposal, and membrane fouling and regeneration, and adsorption material such as activated carbon are high in cost, making heavy metal treatment inaccessible to municipalities. For industry, environmental regulations control the acceptable level of contaminants in effluent entering the water supply and are often stringent and costly to meet due to the difficult nature of removing dilute contaminants. The Guidelines for Canadian Drinking Water Quality limit the level of cadmium, chromium, and lead in water to 0.005, 0.05, and 0.01 mg/L respectively [3], while the Environmental Protection Agency limits drinking water levels at 0.005, 0.1, and 0.015 mg/L [4].

Microbial electrolysis cells (MECs) are a novel technology for water treatment, and are capable of removing organics, recovering nutrients, and removing heavy metals, even at dilute concentration [5, 6, 7]. MECs are constructed from low cost materials, and do not produce sludge to manage, nor requires a membrane for operation. MECs rely on exoelectrogenic bacteria that oxidize organics at the bioanode and drives hydrogen evolution at the cathode which can be captured for energy recovery from wastewater. They require relatively small power applied to the cell ($E_{ap} > 0.2$ V) [5] and can remove many heavy metals through higher voltage application.

This study chose four heavy metals to study in a long-term, dilute concentration MEC system. Metals chosen were lead (Pb^{2+}), cadmium (Cd^{2+}), chromium (Cr^{3+}), and gold (Ag^{1+}). Lead, cadmium and chromium are highly toxic and are all regulated contaminants in Canada and the United States [3, 4]. Chromium III was chosen, as this is the form found in surface and groundwaters, although chromium VI is the toxic species of chromium [8]. Recently, more attention has been paid to the recovery of dilute concentrations of gold in water sources from jewellery and dental fillings, and gold can be recovered in MECs. The two concentrations chosen for this study were 0.05 and 0.5 µmol/L for all elements.

Metal removal and recovery in an MEC is done mainly by electrodeposition, where the reaction spontaneity is governed by the Nernst equation:

$$E = E^0 - \frac{RT}{nF} ln \frac{1}{[M^{n+}]} \qquad (1)$$

where E is the required potential for reduction, E^0 is the standard potential, R is the gas constant (8.314 J/mol·K), F is the Faraday constant (96485 C/mol), n is the number of electrons involved in the reduction reaction, and T is temperature (298 K).

Each metal will be reduced according to the general reaction if the cathode potential is more negative than the required potential, E:

$$M^{n+} + ne^{-} \rightarrow M \qquad E^0 \text{ [V vs. SHE]} \qquad (2)$$

Both E and E^0 are given in Table 3.

Table 3. Standard and required potentials of selected metals [9].

Element	E^0 [V vs. SHE]	E (0.05 µmol/L) [V vs. SHE]	E (0.5 µmol/L) [V vs. SHE]
Pb^{2+}	-0.126	-0.339	-0.310
Cd^{2+}	-0.400	-0.613	-0.584
Cr^{3+}	-0.740	-0.882	-0.863
Au^{1+}	1.692	1.266	1.324

Other metal removal mechanisms in MECs include precipitation, bioadsorption, and in some cases, electrodeposition at the anode [10]. At low concentrations, precipitation is not expected [7].

This study was run for 14 months at dilute concentration of four heavy metals to demonstrate MEC capability of heavy metal treatment even at low concentration. As well, the long-term capabilities of MECs will be considered at both stressful and growth-optimal applied voltages.

3.2 Materials and Methods

3.2.1 Reactor Design

Four reactors were constructed from polypropylene with an internal cylindrical chamber (3 cm diameter, 40 mL volume). The bioanode was a graphite fiber brush (2.54 cm in diameter, 2.54 cm long, from Mill-Rose, OH) while the cathode was 99.00% pure nickel foil (SHOP-AID, Inc.). The system was open to the atmosphere to prevent pressurization from hydrogen evolution. Oxygen diffusion into the reactor was restricted by having a length of 20 cm of trailing 0.079375 cm ID tube as the pressure relief system. Each reactor had a small stir bar inside the chamber, which was on for the entire testing period. An Ag/AgCl references electrode (Bioanalytical Systems, Inc.) was used to test the cathode potential over two complete cycles in each reactor.

3.2.2 MEC Inoculation and Operation

The graphite brush was pretreated in an oven at 500°C for 30 minutes, cut to reduce the diameter across one direction to 1.27 cm from 2.54 cm (see Figure 4) to reduce bioadsorption, followed by soaking in 1% surfactant solution for 30 minutes before being rinsed.

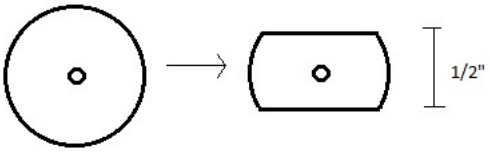

Figure 4. Anode dimensions.

Reactors were inoculated with effluent from another healthy MEC reactor. The feed solution was 10 mM phosphate buffer solution with 2.0 g-acetate/L from

$CH_3COONa(H_2O)_3$, with trace vitamins and minerals [11]. At every cycle, 200 µL of the metal solution was injected into each reactor. Two metal solution concentrations were prepared: 0.05 µmol/L per metal, and one at 0.5 µmol/L. The solution contained four heavy metals: Pb, Au, Cd, and Cr, and were prepared from standard solutions (Pb: Inorganic Ventures, USA, Au: Alfa Aesar, USA, Cd: VWR, USA, Cr: VWR, USA).

3.2.3 Experimental Design

The reactors were operated in batch mode where the effluent would be replaced with fresh feed solution every third cycle, and other cycles had 959 µL of effluent withdrawn to add in feed solution components (200 µL of metal solution, 667 µL of 120g-acetate/L, 63 µL of 10x mineral solution, 25 µL of 10x vitamin solution). Cycles were 2-4 days each, depending on electric current. Both pH and conductivity were measured every three cycles when the solution was changed for both the old effluent and the new feed solution using a conductivity probe (METTLER TOLEDO, USA) and a pH probe (METTLER TOLEDO, USA). Average pH in the feed solution was 7.06, while average pH in the effluent after three cycles was 7.77. Average conductivity in the feed solution was 3.72 mS/cm, while average conductivity in the effluent after three cycles was 5.93 mS/cm.

Two reactors had 1.2 V applied across the cell, and two reactors had 0.6 V applied. At each electric condition, two metal concentrations were tested, 0.05 µmol/L and 0.5 µmol/L. Voltage across a resistor was monitored using a multimeter (Keithley Instruments) and converted to current. Testing was done over 14 months for a total of 185 cycles.

3.2.4 Sample Analysis

At the end of the testing period the cathodes were removed and rinsed in 10 mL of deionized water to collect any precipitants formed on the surface. Each cathode was then digested in 10 mL of concentrated nitric acid for at least 48 hours. The anode was cut into thirds. One third was acidified in 10 mL of concentrated nitric acid for 24 hours. Another third was rinsed in ethanol for 24 hours to remove the biofilm, then washed and acidified in 10 mL of concentrated nitric acid for 24 hours. All samples were filtered in a 0.45 μm syringe filter (polyethersulfone membrane, VWR International) and analyzed using ICP-OES (inductive coupled plasma-optical emission spectrometry) (Vista Pro, Varian Inc., Australia).

3.3 Results and Discussion

3.3.1 Metal removal in the MEC system

Metal removal in the system was characterized by final metal location, quantifying what fraction of the metal entering the system over 14 months of operation ended up either as solid deposits on the cathode, adsorbed onto the carbon fibres of the anode, or adsorbed into the anodic biosolids. Removal of metals in an MEC is also possible through precipitation at the cathode [12], and electrodeposition at the anode [10], however no large amount of precipitant was seen at the end of the study when the cathodes were removed from the reactors.

Figure 5a compares percent of metal (on a mole basis) found at each location. The values reported are the total moles of metal from the location sample divided by the total moles of the metal added to the system over the study duration. The result is a mole balance for the entire testing period categorized by sample location to investigate how metals are removed in the MEC system.

Analyzing Figure 5a, more metals are recovered from the lower concentration reactors than the higher concentration reactors for all metal species except cadmium. The reason for this is not yet determined, and would be a good avenue for future study.

The fraction of removal by electrodeposition was highest in the low concentration reactors for Pb, Cr and Au. Electrodeposition was lowest for cadmium at both concentrations, and for the high concentration reactors for Au, Cr and Pb. This differs from the expected results of Au, Cd, and Pb removal at both voltages and concentrations being dominated by electrodeposition, and both concentrations of chromium removed at 1.2 V application, while a smaller fraction of removal by electrodeposition was expected for chromium. This is based on the required potentials for electrodeposition, and a redox tower visualizes the requirements versus cathode potentials later in Figure 7. Almost no cadmium was recovered from the cathode, which may be due to bioadsorption being the dominant removal mechanism for a single chamber, low concentration MEC system as indicated in additional tests run during this study in separate reactors. Additionally, there may be some biochemical reactions occurring specifically relating to cadmium removal in the system via bioadsorption. Some bacteria common to wastewater, including *Pseudomonas spp.*, possess a class of protein called metallothioneins, whose purpose is to take up zinc as an essential mineral for bacterial growth [13]. Cadmium and zinc are in the same group of elements in the periodic table, with cadmium positioned just one row below zinc. The two elements have very similar chemistry and cadmium can be taken up by metallothioneins instead of zinc due to this reason [14]. *Pseudomonas spp.* are typically aerobic, however some species do grow in anaerobic conditions [15]. Additionally, it is currently unknown how many microorganisms common to wastewater effluent possess metallothioneins, including exoelectrogens. It is possible that other bacteria also posses metallothioneins, not just *Pseudomonas spp.* This unique pathway for cadmium removal by bioadsorption may contribute to the low levels of cadmium seen on the cathode.

The amount of metal found adsorbed to both the carbon fibers and the biosolids is approximately the same fraction on a percent mole basis regardless of concentration, voltage application or metal species. The 12.23 mol% average removal by adsorption to the carbon fibres and 8.42 mol% average removal rates therefore could be the approximate fraction of metal to be removed through these pathways in any MEC system with heavy metals.

Figure 5b differs from Figure 5a in that it is not normalized by the total metal entering the system. Figure 5b compares the total mass of each metal found at each location. This representation reflects any upper limits each location might have for removal, or overarching patterns of removal on a mass basis. The total mass is just the total mass at each location.

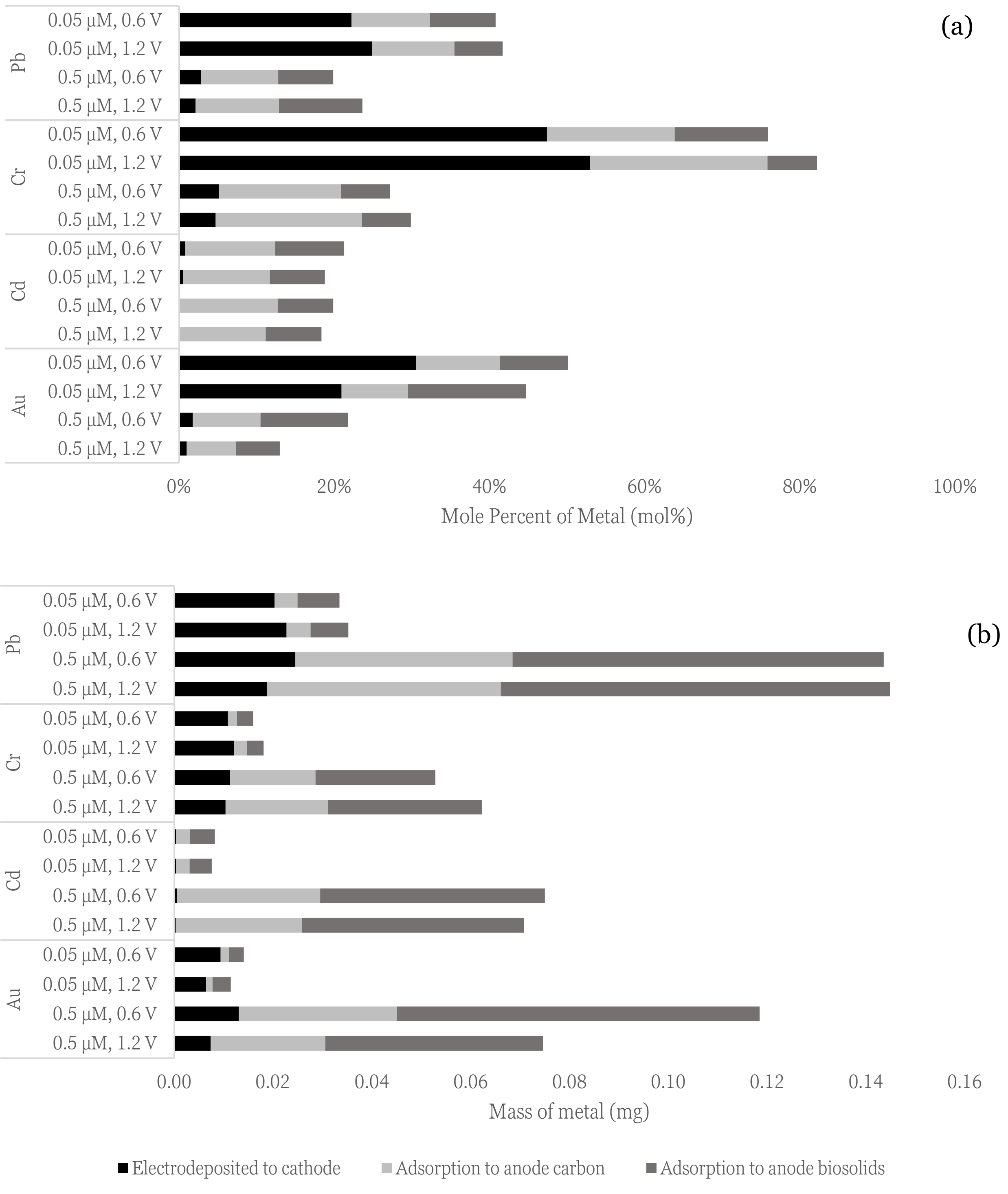

Figure 5 a, b. Final location of metal deposits after 14 months in mol% and total mass.

In Figure 5b, the fraction on the cathode is the same, regardless of operational conditions of voltage or concentration for each metal; however this was not seen in

Figure 5a for the mol% values. This may indicate an upper bound on electrodeposition, or be related to the kinetics of dissolution from the cathode deposits back into solution. Since each reactor spent some time with a low current and more positive cathode potential, perhaps this time allowing the reverse reaction equalized the mass of each metal on the cathode surface.

Approximately the same mass was found at each location regardless of voltage application for adsorption to the carbon fibres and biosolids, although this fraction differs significantly with respect to the operational difference in metal concentration.

Table 4 further analyzes the relationships seen from Figure 5b. Values in the table were calculated by dividing the mass at each location from the higher concentration by the mass at the lower concentration, minus 100%, leaving a percentage indicating how much mass had increased at that location by increasing concentration ten times.

There was no significant change in the mass deposited on the cathode by changing the feed metal concentration, nor the applied voltage. As seen in Table 4, all factors are relatively small and close to 0%, indicating minor change. Adsorption to the anode carbon fibres and biosolids was approximately 10 times greater (approximately 1000%) for Au at 1.2 V, and Cd, Cr and Pb for both voltage applications as concentration was increased from 0.05 µM to 0.5 µM. This is likely related to the 10 times increase in concentration and implies that bioadsorption to both the carbon fibres and biosolids do not have an upper limit below the total amount of metal added over the 14 months of operation. If this upper limit exists, it is not relevant for dilute metal solutions being treated by an MEC.

In contrast, Au saw nearly a 2000% increase in bioadsorption to both carbon fibres and biosolids for 0.6 V, and for adsorption to the carbon fibres at 1.2 V. This large increase is amplified due to the small mass of Au found on the carbon. The mass found in the biofilm is similar to the other metals for the higher concentration condition.

Table 4. Percent change in mass at each location from increasing the concentration from 0.05 µM fraction to 0.5 µM, based on total mass from Figure 5b.
i.e.) Au electrodeposition at 1.2 V was 14.25% higher at 0.5 µM than 0.05 µM.

		Electrodeposited to cathode	Adsorption to anode carbon	Adsorption to anode biosolids
Au	1.2 V	14.25%	1655.66%	1101.67%
	0.6 V	38.83%	1828.72%	2328.80%
Cd	1.2 V	-15.45%	838.44%	907.37%
	0.6 V	60.21%	922.95%	808.17%
Cr	1.2 V	-14.38%	691.41%	828.85%
	0.6 V	3.99%	817.75%	644.27%
Pb	1.2 V	-17.18%	876.52%	924.74%
	0.6 V	20.84%	850.36%	785.64%

3.3.2 Electric current production and MEC health over long term operation

Figure 6 demonstrates that applying a lower voltage (0.6 V) results in more stable MEC operation over a long period of operation. In fact, the electric current dropped faster in the 1.2 V reactors consistently, which is undesirable. While the 1.2 V reactors saw very high current produced, it was less predictable and especially for the high concentration/high voltage cell, current dropped below its 0.6 V counterpart after 350 days of operation, potentially signalling a decline in the anodic biofilm's health.

Over time, the 0.6 V cells increased the amount of current they generated from the same amount of organic substrate added, signalling an increase in either the fraction of healthy exoelectrogenic bacteria in the biofilm, or an increase in the electron transfer efficiency from organic oxidation to the anode.

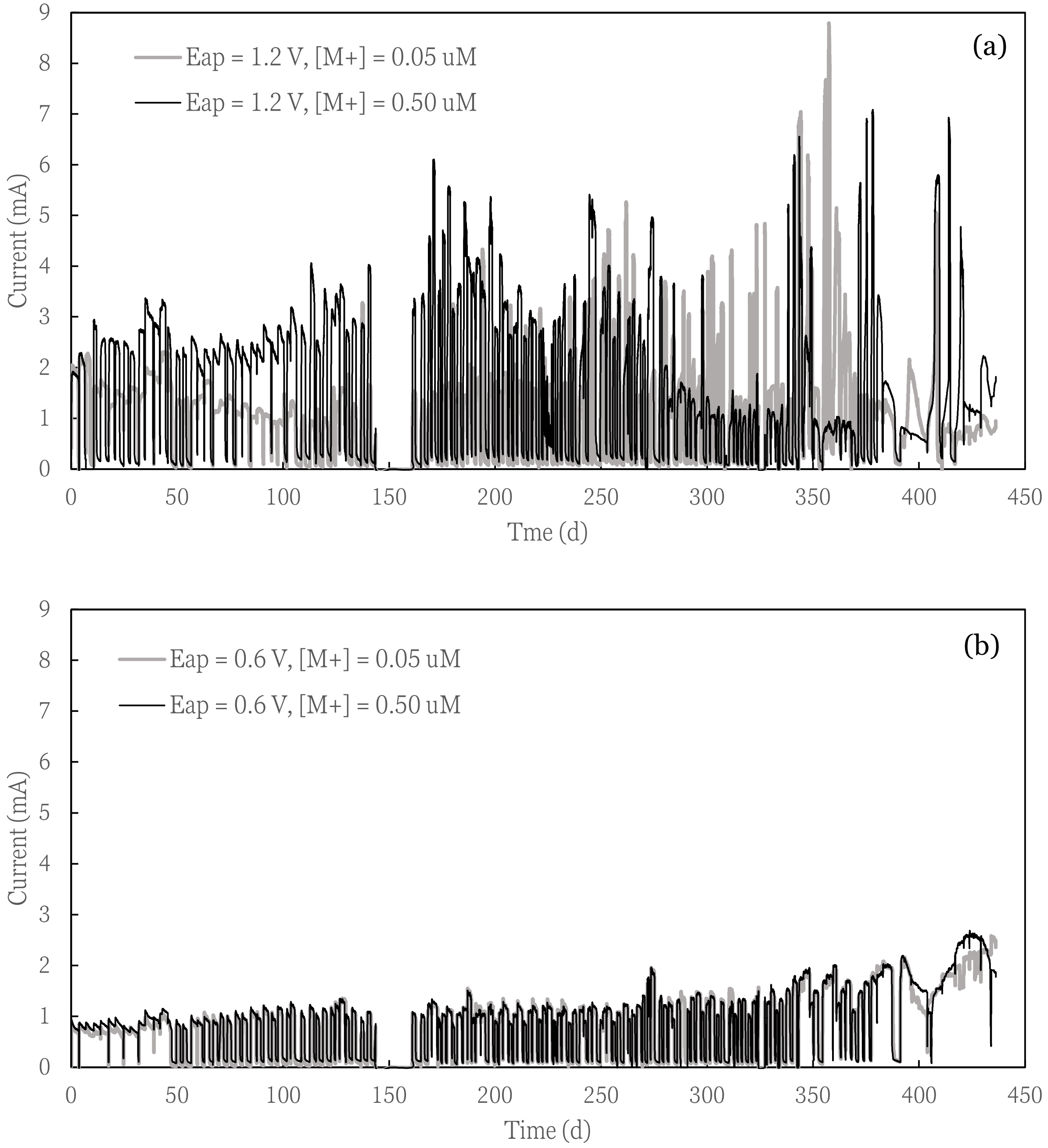

Figure 6 a, b. Electric current in the MEC reactors.
Top: current in the 0.5 µM reactors, bottom: current in the 0.05 µM reactors.

There exists the possibility of metal loss if the current drops and results in a more positive cathode potential than required by the metal reduction reaction calculated by the Nernst equation. This would reverse the electrodeposition reaction, and metals dissolve back into the effluent. For this study, the effluent is changed every three cycles, omitting any potential metals from the mass balance as the effluent from these changes was not analyzed in ICP-OES to determine metal concentrations.

Coulombic efficiency was calculated to investigate the high peaks in Figure 6a. Assuming 80% of the organic substrate was consumed over a cycle, all cycles had a coulombic efficiency below 100%. Therefore, the area under even the very high peaks do not violate the number of electrons possible to recover from the substrate in the reactor.

Finally, it should be noted that data from the Kiethley device recording electric current in the MECs was not captured over a two-week period, seen around 150 days in Figure 6a and b. Power was still applied to the cells during this period, and the reactors were still fed three times per week, with their effluent changed once out of the three weekly cycles. Therefore, analyses count those cycles as having properly occurred. The only impact is loss of the record of electric current.

3.3.3 The cathode potential compared to required potential for reduction

Figure 7 compares box plots for the cathode potential of the MEC reactors (over six cycles per voltage condition) to the required potential for the metal reduction reactions occurring in the system. Three complete cycles were measured per reactor, and the data for reactors sharing the same voltage condition were combined to get a good average representation of cathode potential behaviour.

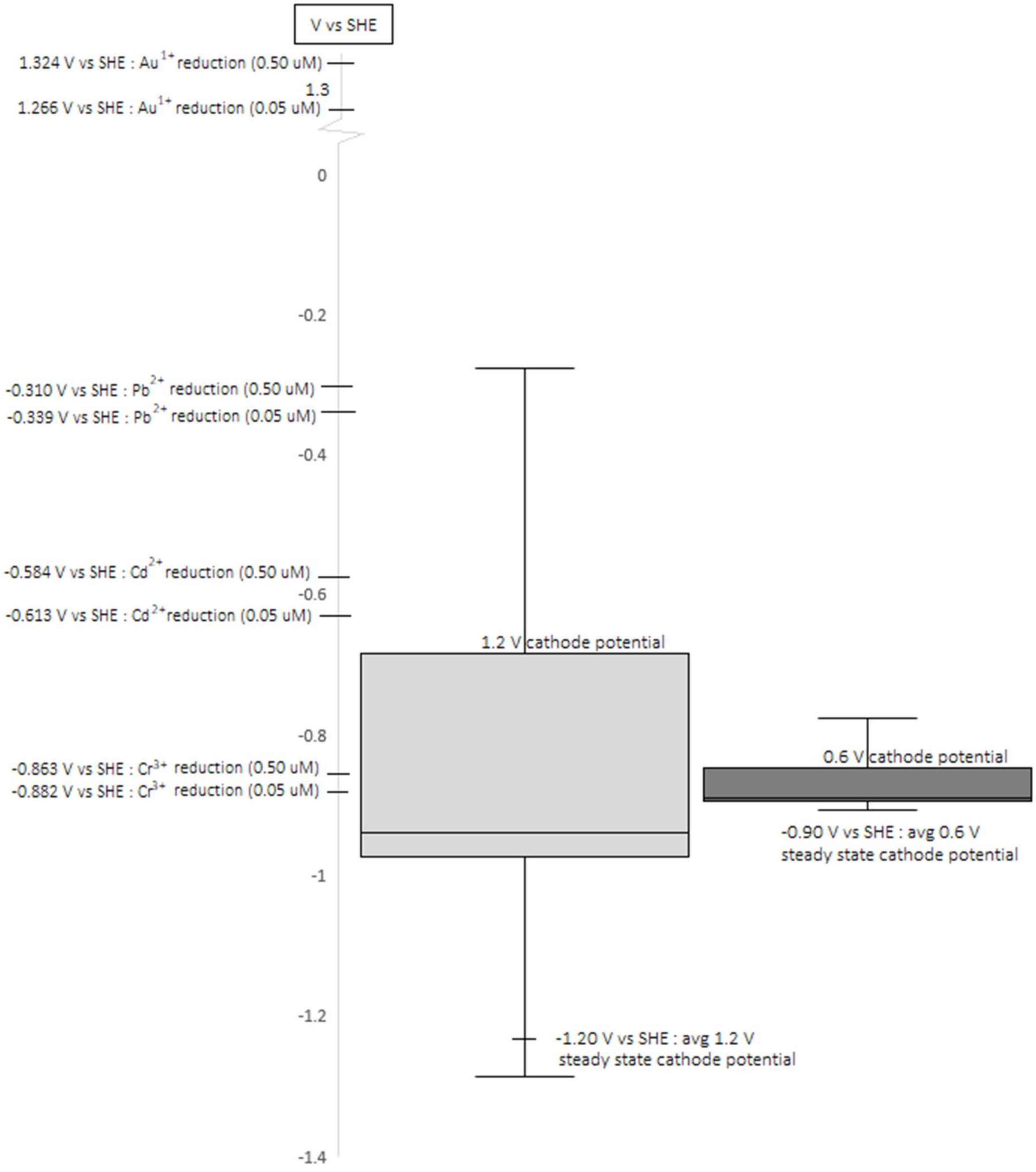

Figure 7. Redox tower comparing box plots for the cathode potentials over six tests per voltage condition to the required potentials for the metal reduction reactions. Reactions that require a potential for reduction to occur that is more positive than the cathode potential are spontaneous.

The box plot for the 1.2 V reactors shows a wider range of cathode potential values compared to the relatively tight box plot for the 0.6 V reactors. This indicates the value of the cathode potential was less stable in the 1.2 V reactors, which agrees with Figure 6, where current was shown to be stable in the 0.6 V reactors, and unstable in the 1.2 V reactors. Additionally, the average steady state cathode potential indicated in Figure 4 represents the potential the cathode was at when current was high and stable (during normal MEC operation, not during times of current drop). This value for the 1.2 V reactors is close to the end of the whisker, indicating the reactor spent approximately one quarter of the time in a cycle around this value. The steady state value for the 0.6 V reactor is quite close to the median value, as well as the bottom whisker, indicating that the 0.6 V MECs spent much more cycle time at its highest value. More time at this more negative cathode potential means more time for electrodeposition of metals to occur, and less time for the reverse metal deposit dissolution reaction to occur. This is likely why much more of the total metal entering the system was recovered from the 0.6 V reactors versus the 1.2 V reactors. The cathode potential was more positive for the 1.2 V cells than the expected -1.4 V vs SHE, while the cathode potentials for the 0.6 V cells was more negative than the expected -0.8 V vs SHE. These expected potentials are based an anode potential of -0.2 V vs SHE [16].

3.4 Conclusions and Future Work

It was seen that over long-term operation for controlled, lab-scale conditions, a lower voltage resulted in more metals being recovered from the system, a more reliable current output, and a more stable cathode potential. All of these are desirable for MEC systems built with the intention of removing heavy metals from wastewater.

Lead, chromium and gold had the highest amount recovered via electrodeposition at the low concentration condition on a mole percent basis in these tests. Cadmium showed almost no electrodeposition on the cathode, potentially due to uptake by bacterial metallothioneins. Whether these proteins exist in exoelectrogenic bacteria and contribute to the removal of cadmium could be determined in future studies.

About 12 mol% of metals were removed via adsorption to the anode carbon fibres and similarly, about 8 mol% of metals were adsorbed onto the anodic biofilm, regardless of applied voltage or metal concentration in the 40 mL MEC reactor using the cut carbon fibre brush anode. As well, it was seen that the mass% of each metal deposited onto the cathode was the same per metal, regardless of operational condition. This may have been due to an equalization effect from redissolution of the metal back into solution as the cathode potential became positive enough to facilitate this reaction. In the future, it should be noted that maintaining the cathode potential below the thermodynamic threshold is crucial for MECs applied to metal removal from wastewater. The extent of electrodeposition reversal when the cathode potential becomes too positive due to the electric current dropping from lack of organic substrate would require effluent samples to be taken at multiple points during a cycle. Quantifying this reversal would be a valuable avenue for future studies.

3.5 References

[1] J. L. Gardea-Torresdey, J. R. Peralta-Videa, M. Montes, G. De La Rosa, and B. Corral-Diaz, "Bioaccumulation of cadmium, chromium and copper by Convolvulus arvensis L.: Impact on plant growth and uptake of nutritional elements," *Bioresour. Technol.*, vol. 92, no. 3, pp. 229–235, 2004.

[2] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, "Heavy Metals Toxicity and the Environment," *EXS*, vol. 101, pp. 133–164, 2012.

[3] Federal-Provincial-Territorial Committee on Drinking Water, "Guidelines for Canadian Drinking Water Quality Summary Table," *Heal. Canada*, 2014.

[4] United States Environmental Protection Agency, "National Primary Drinking Water Regulations," 2018. [Online]. Available: www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations. [Accessed: 08-Apr-2017].

[5] B. E. Logan, D. Call, S. Cheng, and H. V. M. Hamelers, "Critical Review Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter," *Environ. Sci. Technol.*, vol. 42, no. 23, pp. 8630–8640, 2008.

[6] R. D. Cusick and B. E. Logan, "Phosphate recovery as struvite within a single chamber microbial electrolysis cell," *Bioresour. Technol.*, vol. 107, pp. 110–115, 2012.

[7] N. Colantonio, H. Guo, and Y. Kim, "Effect of Low Cadmium Concentration on the Removal Efficiency and Mechanisms in Microbial Electrolysis Cells," pp. 6920–6924, 2016.

[8] Health Canada, "Chromium," *Guidel. Can. Drink. Water Qual.*, vol. 1979, no. January 1979, pp. 1–6, 1986.

[9] J. R. Rumble, Ed., *CRC Handbook of Chemistry and Physics*, 98th ed. 2017.

[10] N. Colantonio and Y. Kim, "Lead(II) removal at the bioanode of microbial electrolysis cells," *ChemistrySelect*, vol. 1, 2016.

[11] S. Cheng, D. Xing, D. F. Call, and B. E. Logan, "Direct biological conversion of electrical current into methane by electromethanogenesis," *Environ. Sci. Technol.*, vol. 43, no. 10, pp. 3953–3958, 2009.

[12] N. Colantonio and Y. Kim, "Cadmium (II) removal mechanisms in microbial electrolysis cells," *J. Hazard. Mater.*, vol. 311, pp. 134–141, 2016.

[13] C. A. Blindauer, "Bacterial metallothioneins: Past, present, and questions for the future," *J. Biol. Inorg. Chem.*, vol. 16, no. 7, pp. 1011–1024, 2011.

[14] C. G. Elinder and M. Piscator, "Cadmium and zinc relationships," *Environ. Health Perspect.*, vol. VOL. 25, no. August, pp. 129–132, 1978.

[15] A. Zimmermann, C. Reimmann, M. Galimand, and D. Haas, "Anaerobic growth and cyanide synthesis of *Pseudomonas aeruginosa* depend on *anr*, a regulatory gene homologous with *fnr* of *Escherichia coli.*," *Mol. Microbiol.*, vol. 5, no. 6, pp. 1483–1490, 1991.

[16] B. E. Logan *et al.*, "Microbial fuel cells: Methodology and technology," *Environ. Sci. Technol.*, vol. 40, no. 17, 2006.

4 Overarching conclusions

4.1 Operational recommendations for heavy metal removal in MECs

This study systematically investigated the effects of different operational conditions in microbial electrolysis cells for heavy metal removal mechanisms.

Rapid cadmium removal was seen in tests with high applied voltage and mixing in the 20 mL cathode chamber, where 93.35% of cadmium was removed from the 250 mL of catholyte in 24 hours, where a unit of catholyte was being treated only 8% of the total testing time due to the reservoir set-up and pump rate of 2 mL/min.

It was found that mixing in the cathode chamber improved electrodeposition rates by approximately 15% in controlled, lab-scale conditions. Additionally, applying 1.2 V across the cell resulted in 22% more electrodeposition in short-term experiments, while over long-term operation it was preferable to apply only 0.6 V, which lead to better metal recoveries overall, more reliable current generation and more stable cathode potential in these series of tests. Over one year of operation, reactors where 1.2 V was applied had a wider range for the average cathode potential, which is undesirable when using the cathode potential to control heavy metal electrodeposition. Additionally, the 1.2 V reactors spent more time near zero current being produced, which can result in the cathode potential becoming too positive and facilitating the dissolution of deposited metals back into solution. It is recommended that systems intending to use MECs as part of a long-term wastewater treatment strategy use the minimum applied voltage to achieve the target metal removal, though an optimization study would be helpful to find the intersection of the improved rates of electrodeposition through a higher applied voltage with the better long-term reactor performance from a lower voltage which is less stressful on the exoelectrogenic biofilm.

Additionally, it was found that there was consistently 20% (mole basis) of metals adsorbed onto the bioanode regardless of applied voltage or initial metal concentration, with 12% being found on the carbon fibres, and 8% being found in the biofilm when using a graphite brush for the anode measuring 1" in diameter.

Specifically for cadmium removal, it is recommended to use an anion exchange membrane if the removal mechanism is preferred to be electrodeposition, as bioadsorption was indicated to play a major role in the removal of this metal in an MEC system.

4.2 Future work

This study found that increasing the turbulence in the cathode chamber decreased the cathode potential by -0.06 V at lab-scale, regardless of the applied voltage condition, and increased current generation between 10% and 30% in the tests ran in this study. The mechanism for this effect is not yet understood. One explanation could be mixing in the cathode chamber decreases the cathode potential, resulting in more potential energy between electrodes and thus facilitating more efficient electron transfer to the cathode, which manifests in increased current generation. Alternatively, mixing could increase current generation by affecting the MEC’s efficiency through better distribution of generated hydroxide ions and protons, which in turn lowers the cathode potential. Having a better understanding of the effects of hydrodynamics would be valuable for the eventual scale-up of MEC systems for heavy metal removal and wastewater treatment in general, especially as mixing represents increased operational cost, but may be justified by the increase heavy metal treatment rates.

In this study, the experimental parameters of mixing and voltage application were assumed to have independent effect on the rates of heavy metal removal mechanisms in the MEC system. In reality, these conditions are linked. For example, as mixing is applied in the cathode chamber, it affects the concentration of protons and hydroxide at the cathode surface, changing local pH conditions and shifting equilibrium of electrolysis reactions. Studies in the future should endeavor to link the effects of operational conditions on each other, and on MEC performance.

Additionally, it would be beneficial to investigate the effect of mixing and applied voltage on the rate of cadmium precipitation in an MEC system in order to decrease precipitation rates. Removal of metals through electrodeposition is preferable to precipitation, which is harder to control and requires precipitants to be filtered from the effluent.

Metallothioneins in exoelectrogens is also an interesting avenue for future studies. Determining the presence or absence of this class of proteins in the microorganisms found in an MEC biofilm would confirm or absolve the need for using an anion exchange membrane to separate the heavy metal contaminated water from the biofilm, where requiring a specialized membrane represents a higher capital and operational cost for MEC reactors.

Appendix

The methodology of chapter 2 was informed by approximately a year of failed experiments surrounding unexpected and significant open circuit removal of cadmium in the MEC system. Through trial and error, the mechanism of cadmium removal through bioadsorption was better understood at the end of these series of tests. The summarized methodologies and negative results of these tests follow in Table A-1.

Table A-1. The record of experiments with negative results that informed the methodology for the hydrodynamic effects on cadmium removal mechanisms paper.

Tests 1-10	Methods	• 1.0 V applied, 1.0 mg/L Cd^{2+}, 40 mL single chamber reactor • Tests were 3, 6, 9, 24, or 48 hours long, and either had mixing or not • Cut an already inoculated bioanode down from 1" diameter to 1" across, and ½" across in the perpendicular direction • The MEC reactors were angled so the cathode side was raised higher than the anode side to prevent hydrogen from collecting around the bioanode
	Notes on results	• The bioanode fibres came loose from the steel core holding them in place, leading to short circuiting in the system **Fix:** if the anode is to be cut down to reduce surface area for adsorption, a new anode should be used, and the fibres should be cut before inoculation. During this cutting, care had to be taken to keep the anode submerged in water to prevent death of strictly anaerobic *Geobacter* organisms in the biofilm, leading to less ideal conditions to ensure a clean cut that did not compromise the stability of the fibres in the steel core. • The cathode was not fully submerged for some portions of some tests as produced hydrogen gas could not escape the top corners of the reactor beyond the gas release tube. **Fix:** the reactor should be left flat to prevent hydrogen buildup either around the bioanode or the cathode. • There was a lag period in current production up to one hour for some tests **Fix:** the reactor should sit for one hour at OCV to acclimatize to new feed • Most of the cadmium was removed in under 6 hours
Tests 11-22	Methods	• 1.0 V applied (2 OCV tests), 1.0 mg/L Cd^{2+}, 40 mL single chamber reactor • Left the reactors at OCV for one hour before applying voltage • Tests were 0.25, 0.5, 1, 2, or 3 hours long, and either had mixing or not • Samples were taken from the feed before and after the 1 hour OCV
	Notes on results	• Feed concentrations before and after the 1 hour OCV were not consistent, and showed between 8-72% of Cd removed before voltage was applied. OCV tests showed between 49-67% removal of Cd. **Fix:** redo tests to validate OCV removal. Additionally run some tests in a 2-chamber reactor.

Tests 23-33	Methods	• 1.0 V applied (2 OCV tests), 1.0 mg/L Cd^{2+}, 40 mL single chamber reactor • Left the reactors at OCV for one hour before applying voltage • Tests were 0.5, 1, 2, or 3 hours long, and either had mixing or not • Samples were taken from the feed before and after the 1 hour OCV • Three 2-chamber tests run, same conditions, test lengths either 1, 2, or 26 hours. Used a 45 µm pore membrane.
	Notes on results	• Feed concentrations before and after the 1 hour OCV were not consistent, and showed between 20-48% of Cd removed before voltage was applied in single chamber tests. In the two-chamber tests, concentrations were also inconsistent around the 1 hour OCV, showing between 0-54% removal. **Fix:** sample location may affect results. Sample both from the middle of the reactor as before, and also extract remaining effluent and sample that as well. Ensure reactor and extra effluent both well mixed before sampling. Additional effluent may contain more precipitants. **Fix:** use a different cathode material in case nickel foil causes Cd removal
Tests 34-45	Methods	• 1.0 V applied, 1.0 mg/L Cd^{2+}, 40 mL single chamber reactor • Stainless steel (SS) cathodes used instead of nickel foil • Left the reactors at OCV for one hour before applying voltage • Tests were 1, 2, or 4 hours long, and either had mixing or not • Samples were taken from the feed before and after the 1 hour OCV • Duplicate tests were run for each condition • Samples at the end of the test were taken both from the middle of the reactor, and from the remaining effluent.
	Notes on results	• Feed concentrations before and after the 1 hour OCV were not consistent, and showed between 40-66% of Cd removed before voltage was applied. • The sample taken from the second location (the remaining effluent) contained on average 1.55 times more Cd than the sample taken from the middle of the reactor **Fix:** test several reactor conditions to see what affects OCV removal

Tests 46-50	Methods	• 46: 1 hour OCV, 1 hour 1.0 V, mixed, SS mesh cathode. Extra feed prepared and left in a beaker for 5 hours on the benchtop. • 47: 1 hour OCV, 1 hour 1.0 V, mixed, SS mesh cathode, no vit/mins • 48: 1 hour OCV, not mixed, MEC effluent + 1.0 mg/L Cd in beaker • 49: 1 hour OCV, not mixed, bioanode + 1.0 mg/L Cd in beaker • 50: 1 hour OCV, not mixed, SS foil foil + 1.0 mg/L Cd in beaker
	Notes on results	Percentage denotes % difference e in Cd concentration between initial feed concentration and the end of the test. • -0.32% Feed rests on benchtop 5 hours • 14.41% without vit/min • 80.34% Cd injected into MEC eff (pH 9.1), 4 mins after injection • 66.97% Cd injected into MEC eff (pH 9.1), 1 hour after injection • 40.07% only bioanode • 1.60% only SS foil cathode in beaker **<u>Fix:</u>** removal is seen in cases where Cd is in contact with the bioanode or with MEC effluent, which contains biosolids. Bioadsorption highly likely, though expected not to dominate when more Cd in the system as it is anticipated that bioadsorption may have an upper limit for how much can be taken up in the biosolids.
Tests 51-53	Methods	• 1.0 V applied, 1.0 mg/L Cd^{2+}, 40 mL single chamber reactor • 1000 mL feed prepared, resides in attached reservoir with peristaltic pump (2 mL/min pump rate). Reservoir is mixed. • Three tests: mixed 1.0 V, not mixed 1.0 V, mixed OCV • Each tests ran for 5 days. Sampled twice per day at least 6 hours apart. • Sampled from the reservoir
	Notes on results	• Mixed OCV removal exceeds mixed CC removal (CC mixed: 94.51% removed, CC not mixed: 93.07% removed, OCV mixed: 95.23% removed)
Tests 54-56	Methods	• Cut anode from previous tests into two sections. One half acidified in concentrated nitric acid, and one half washed clean of biosolids with ethanol, then acidified in concentrated nitric acid. • Tubes that connected the reactor to the reservoir were washed with ethanol then the wash acidified after ethanol evaporation as red algae grew inside the tubes.
	Notes on results	• 3.21% Cd found in the tubes • 21.45% found in the biosolids • 78.55% of Cd found on the carbon fibres **<u>Note:</u>** the washing of the carbon fibers was not thorough, as seen in SEM imaging, therefore this percentage is not solely due to abiotic adsorption. Additionally, the percentage of Cd found is based on the total Cd entering that particular reactor since inoculation, which includes thirteen 40 mL tests and three 1000 mL tests. • The cathodes from the three previous tests were washed (wash kept and analyzed for precipitants) and acidified. For each test, less than 1% of Cd was found in the wash, and between 0.03-0.14% of Cd was found deposited on the cathode.

Tests 57-62	Methods	• 1.0 mg/L Cd^{2+}, 40 mL single chamber reactor • 1000 mL feed prepared, resides in attached reservoir with peristaltic pump (2 mL/min pump rate). Reservoir is mixed. • Three tests: mixed 1.0 V, not mixed 1.0 V, mixed OCV • 4/6 tests ran for 2 days. Sampled at beginning and end of test. OCV • 1 test sampled at 0, 6, 24, 32, 48 hours. OCV • 1 test OCV 10 days, sampled at 0, 2, 4, 6, 8, 10 days • Sampled from the reservoir • Anode cut into halves, one washed with ethanol than acidified, one directly acidified • Some anode fibres kept from each half and analyzed with SEM/XPS
	Notes on results	• SEM/XPS analysis revealed little to no Cd on the fibres nor the biosolids • 79% removal in OCV reactor after 10 days • Four 2 day tests sampled at beginning and end showed between 13.37-46.26% Cd removed • Single 2 day test sampled at 0, 6, 24, 32, 48 hours shows linear decrease in Cd concentration over time, ending with 44.17% Cd removed • 18.14% of Cd from all 5 tests found on the anode: 93.89% of this was found on the fibres, and 6.11% found in the biosolids **Note:** the washing of the carbon fibers was not thorough, as seen in SEM imaging, therefore this percentage is not solely due to abiotic adsorption. **Note:** Washing the anode fibres did not clean them of biosolids entirely, so even if previous results saw most of the cadmium on the "fibres" and not in the washed off biosolids, it is not confirmed that cadmium was on the carbon fibres themselves, and not in the biosolids to a great degree. Cadmium not seen deposited on the anode fibres, so very likely bioadsorption the cause of OCV removal. At this time the information about metallothioneins was learned, offering a possible explanation for such significant bioadsorption of cadmium.
Tests 63-68 (the tests used in the Cd paper)	Methods	• 1.0 mg/L Cd^{2+}, 40 mL two chamber reactor separated with anion exchange membrane • 250 mL feed prepared, resides in attached reservoir with peristaltic pump (2 mL/min pump rate). Reservoir is mixed. • Tests are either mixed or not mixed, two are OCV, two have 1.0 V applied and two have 0.6 V applied
	Notes on results	• No OCV removal seen with the use of an anion exchange membrane. Before when the two chamber reactor was tested, a 45 μm pore membrane was used, which must have still allowed cadmium to pass through and allowed for bioadsorption. The AEM prevented cadmium from being exposed to biosolids, and therefore OCV had little to no removal. • Other results are discussed in detail in chapter 2 of this thesis.