

HEAVY METAL REMOVAL FROM WASTEWATER USING MICROBIAL
ELECTROLYSIS CELLS

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Abstract

Heavy metal contamination in water is a serious environmental and human health issue. Lead (Pb^{2+}) and cadmium (Cd^{2+}) are strictly regulated in wastewater effluent due to their high toxicity at low concentrations. Heavy metals are difficult to remove in conventional biological wastewater treatment because they are water soluble and non-biodegradable. Advanced treatment, such as tight membrane filtration and ion exchange, can be applied but they often require a high electrical energy input and a large amount of chemicals for pre- or post-treatment. Microbial electrolysis cells (MECs) can be used to treat wastewater while simultaneously recovering energy in the form of hydrogen gas. Additionally, MECs were proven to be effective for heavy metal removal. The commonly investigated removal mechanism for heavy metals in MECs is reduction at the cathode where heavy metal ions are reduced to metallic solids. The research presented in this thesis examined the effectiveness of cathodic reduction and other heavy metal removal mechanisms in MECs over a wide range of metal concentrations (10 $\mu\text{g/L}$ -12 mg/L). Lab-scale MEC operation demonstrated successful removal of both Pb^{2+} and Cd^{2+} under different electric conditions, operation times, and initial metal concentrations. In addition to cathodic reduction, heavy metal removal in MECs was demonstrated through chemical precipitation at the cathode and electrochemical reduction and biosorption at the bioanode. The results of this research also confirmed the importance of microbial activity at the bioanode to efficiently drive the removal mechanisms in MECs.

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1. Introduction

1.1 Heavy metal removal from wastewater

Heavy metal contamination in water is a serious environmental and human health hazard. Many toxic heavy metals, such as cadmium (Cd^{2+}) and lead (Pb^{2+}), are water soluble and non-biodegradable, so they are easily transported by water and can accumulate in soils and living organisms (Kurniawan et al., 2006). Both metals are found in various wastewaters and are included on the list of U.S. EPA priority pollutants (Nancharaiah et al., 2015). Various industrial processes, such as electroplating, battery manufacturing, and paper and pulp processing, can result in Cd^{2+} contamination in wastewater streams. Sources of Pb^{2+} in water are similar but more diverse, including paints with lead-based pigments, pesticides, and outdated lead-based plumbing (Lewinsky, 2007). Conventional biological wastewater treatment (e.g., activated sludge process) is ineffective for removing heavy metals. There are many physical and chemical treatment methods available for heavy metal removal in wastewater but they have several disadvantages. Chemical precipitation is commonly used, but it requires large amounts of chemicals. In addition, chemical treatment usually creates treatment by-products, which are costly to dispose of and require additional treatment (Kurniawan et al., 2006). Ion exchange is another method for heavy metal removal; however, it requires resin regeneration which uses strong acidic or saline reagent solutions, which needs additional treatment (Kurniawan et al., 2006). Tight membrane filtration, such as reverse osmosis or nanofiltration, is costly due to fouling problems and high energy consumption (Wang and Ren, 2014).

1.2 Microbial electrochemistry applications for heavy metal removal

Microbial electrolysis cells (MECs) are a type of bioelectrochemical systems that can be used for wastewater treatment and energy recovery (Logan et al., 2005; Rozendal et al., 2006; Logan et al., 2008; Call and Logan, 2008). With a relatively low applied voltage application ($E_{ap} > 0.2$ V), exoelectrogenic bacteria can oxidize organic matter at the bioanode of an MEC while water is simultaneously reduced to hydrogen gas at the cathode (Logan et al., 2008). Therefore, wastewater can be treated while energy is recovered as hydrogen gas, making it an energy-efficient technology. In addition to water reduction, other reduction reactions can take place at the cathode, such as the reduction of heavy metal ions into metallic solids. MECs and other bioelectrochemical systems (e.g., microbial fuel cells or MFCs) have been proven to be effective for heavy metal removal from aqueous solution.

1.3 Research Objectives

Although many previous studies have verified the capacity of MECs to remove heavy metals at relatively high concentration from aqueous solution, there is a lack of information on the mechanisms of their removal (Wang and Ren, 2014). It is commonly recognized that heavy metal removal in bioelectrochemical systems is induced at the cathode via cathode reduction; however, recent studies have also observed other removal mechanisms that require further investigation. Also, since many wastewaters contain relatively low concentrations of toxic metals, further studies need to be conducted in lower concentration ranges (10-100 $\mu\text{g/L}$). The overall research goal of this thesis is to provide a clear understanding of heavy metal removal mechanisms in bioelectrochemical systems. The metals Cd^{2+} and Pb^{2+} were chosen specifically

because they have not been explored extensively despite their serious health effects even at very low concentrations.

In the first part of this study (Chapter 2), we examined Pb^{2+} removal in MECs with the following specific objectives: 1) to achieve successful Pb^{2+} removal at low concentrations (1-2.5 mg/L) under a neutral pH condition; 2) to explain that mechanisms of removal not only at the cathode but also at the anode of the MEC; 3) to explore the effect of different electric conditions on Pb^{2+} removal in MECs; and 4) to emphasize the important of microbial activity at the anode for effective Pb^{2+} removal.

In the second part of this study (Chapter 3), we focused on Cd^{2+} removal mechanisms with the following objectives: (1) to demonstrate Cd^{2+} removal at a relatively high concentration (12.26 mg/L) under neutral pH conditions; (2) to examine of the effects of applied voltages (E_{ap}) on the rate of Cd^{2+} removal; (3) to clarify Cd^{2+} removal mechanisms by monitoring the cathode potential; and (4) to explain the potential dissolution of removed Cd^{2+} from the MEC cathode.

Finally, in Chapter 4, we continued studying Cd^{2+} removal mechanisms in MECs with different research objectives: 1) to quantify the amount of cadmium removed in MECs by electrodeposition and precipitation at neutral pH conditions; 2) to test the long term electrodeposition of cadmium at very low concentrations (10-100 $\mu\text{g/L}$); 3) to investigate the effects of concentration and reactor configuration on the mechanisms of cadmium removal; and 4) to emphasize the importance of microbial activity at the anode for both removal mechanisms.

With the knowledge obtained from these studies we can suggest optimal conditions for Pb^{2+} and Cd^{2+} removal in MECs. Also, we can provide a clear understanding of the different heavy metal removal mechanisms in MECs.

2. Literature Review

2.1 Heavy metal removal in bioelectrochemical systems

2.1.1 Microbial Electrolysis Cells

Microbial electrolysis cells (MECs) are an emerging technology for producing hydrogen while simultaneously treating wastewater (Liu et al., 2005; Rozendal and Buisman, 2005; Logan et al., 2008; Rozendal et al., 2008). Exoelectrogenic bacteria that grow on the bioanode have the ability to oxidize organic matter in the cell and transfer electrons to the anode. Through an external circuit, electrons can be transferred to the cathode where water can be reduced to hydrogen gas (Logan et al., 2008). A small voltage needs to be applied ($E_{ap} > 0.2$ V) because the formation of hydrogen from organic matter is not a spontaneous reaction (Call and Logan, 2008). Therefore, wastewater can be treated while energy is recovered in the form of hydrogen gas.

MECs can also be used to remove heavy metals from aqueous solution. In addition to the hydrogen evolution reaction, metal ions (for example, Cd^{2+}) in solution can be reduced to metallic solids at the cathode (Eq. 2-1.):

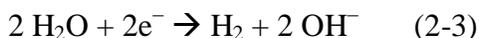


Electrodeposition can be a dominant removal mechanism with high concentrations of heavy metals and acidic conditions (Cai et al., 2016; Modin et al., 2012; Zhang et al., 2015). Experiments conducted with a typical applied voltage (0.6 V) result in a low cathode potential, which can explain recovery via electrodeposition. For example, the required reduction potential for Cd^{2+} can be calculated using the Nernst Equation (Eq. 2-2):

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

In the Nernst equation, E^0 is the standard potential for Cd^{2+} reduction (-0.40 V vs. SHE), T is the temperature (298 K), F is the Faraday's constant (96485 C/mol), and R is the gas constant (8.314 J/mol-K). From the above equation, it is clear that the reduction potential is dependent on the concentration of metal ions in solution. When the cathode potential is more negative than the required metal reduction potential, there is a substantial driving force for electrodeposition. Metals, such as Pb^{2+} , Cd^{2+} , Ni^{2+} , and Zn^{2+} , have negative reduction potentials so they require an applied voltage to be recovered in bioelectrochemical systems.

Chemical precipitation is a less common but still proved removal mechanism in MECs (Cai et al., 2016; Luo et al., 2014). For example, hydroxide precipitation at the cathode can occur because the hydrogen evolution reaction at the cathode results in the production of hydroxide ions (Eq. 2-3):



There are a number of studies that show removal of heavy metals in MECs through reduction at the cathode but also mention precipitation as another removal mechanism. In a study by Cai et al., (2016), it was found that removal of Ni^{2+} in a dual-chamber MEC was achieved with an applied voltage of 0.5 V through both electrodeposition on the cathode and precipitation as $\text{Ni}(\text{OH})_2$, although there was no quantification of each removal mechanism. It was emphasized that pH change of the catholyte from acidic (pH = 3) to neutral (pH of 7.76-8.26) resulted in the formation of $\text{Ni}(\text{OH})_2$ compounds on the cathode. The authors tested four different cathode materials (stainless steel mesh, copper sheet, graphite plate, and carbon cloth) and it was determined that a copper sheet cathode showed the greatest performance for metallic nickel recovery, even though the highest removal ratio achieved was only 40%.

Along with heavy metal removal many studies demonstrate the recovery of hydrogen or methane gas. In a study by Luo et al. (2014), recovery of both hydrogen gas and Cu^{2+} , Ni^{2+} , and Fe^{2+} from artificial acid metal-rich drainage was achieved. The metals were removed from acidic solution at high initial concentrations (500-600 mg/L) with an application of 1 V. The authors suggest that selective metal removal can be done based on retention time, as Cu^{2+} was removed first, followed by Ni^{2+} and Fe^{2+} . This study also investigated the relationship between heavy metal recovery and hydrogen gas production. The highest production rate of H_2 gas occurred when Cu^{2+} was dominant in the cathode chamber due to the increased surface area of the cathode from metallic Cu(s) deposits. The lowest H_2 gas production occurred when Fe^{2+} was dominant due to Fe(OH)_2 precipitants formed near the cathode, giving insight into how heavy metal removal mechanisms are interrelated with energy recovery.

A study by Jiang et al. (2014) demonstrated a successful recovery of Co^{2+} from a solution while producing hydrogen gas. A two-chamber MEC was used to test various applied voltages, pH values, temperatures, and buffer solutions on Co^{2+} reduction and hydrogen gas yields. 92.2% Co^{2+} removal was achieved from a starting concentration of 50 mg/L, compared to only 27% at open circuit, which was attributed to adsorption or diffusion into the anode chamber. Analysis of the cathode by both EDS (Energy-dispersive x-ray spectroscopy) and XRD (x-ray powder diffraction) show that pure metallic cobalt was the main product of electrodeposition. Recovery of Co^{2+} in MECs has also been investigated by Huang et al. (2014). With a low applied voltage (0.2 V), the authors were successful in removing 88% of Co^{2+} from an initial concentration of 20 mg/L while simultaneously producing methane gas. This previous study was unique because it employed the use of a “biocathode.” The authors stressed the importance of the role of

microorganisms at the cathode as in comparison only 36% of Co^{2+} was reduced on an abiotic cathode.

2.1.2 Microbial fuel cells

Microbial fuel cells (MFCs) are similar to MECs in that they both use exoelectrogenic bacteria to oxidize organic substrates at the anode but MFCs use the electrode reactions for electric energy production (Logan et al., 2009). Oxygen is the most commonly used electron acceptor at the cathode as it is easily available and it has a positive standard reduction potential. For heavy metal removal, metals with more positive standard reduction potentials, such as Hg^{2+} , Cu^{2+} , and Cr^{6+} , can act as electron acceptors in MFCs (Cai et al., 2016).

Many studies have demonstrated successful heavy metal removal in MFCs coupled with electricity generation. In a study by Wang et al. (2011), mercury (Hg^{2+}) was successfully removed from an acidic solution while electric power was generated in an MFC. Effects of initial Hg^{2+} concentration and pH on H^{2+} removal and power generation were assessed. It was found that the most acidic condition (pH = 2) resulted in the lowest effluent Hg^{2+} concentration. Deposits on the cathode were verified as elemental Hg using EDS analysis. The authors also observed white precipitants on the bottom of the cathode chamber, which were found to be Hg_2Cl_2 .

Similarly, recovery of Cu^{2+} in an MFC has been demonstrated by Tao et al. (2011). Power generation in the MFC was dependent on the external resistance and initial Cu^{2+} concentration, as the maximum power density was achieved when the starting Cu^{2+} concentration was extremely high (6.4 g/L). The products on the cathode shown mainly to be Cu_2O and $\text{Cu}_4(\text{OH})_6\text{SO}_4$ by XRD analysis. The authors also observed that some CuCO_3 precipitants were formed during the control experiment, as the anode medium contained NaHCO_3 .

Another study done by Wang et al. (2013) demonstrated successful silver (Ag^+) recovery from an ammonia chelated silver solution and electricity production in an MFC. From a high initial starting concentration (over 1 g/L), 99.9% of Ag^{2+} was removed from an alkaline solution (pH = 9.2). Analysis of the cathode using XRD and SEM-EDS show that Ag^+ was deposited on the graphite cathode as elemental silver.

Wang et al. (2008) tested the effect of pH and initial concentration of Cr(VI) removal and electricity generation in a two-chamber MFC. Since H^+ is consumed for the reduced of Cr(IV), it was found that low pH values (2-3) were effective for 100% removal of Cr(IV) at an initial concentration of 100 mg/L within 180 hours.

2.2 Pb^{2+} removal in bioelectrochemical systems

Only a few studies investigated Pb^{2+} removal in BESs. Bo et al. (2014) demonstrated successful Pb^{2+} removal in a BES from a relatively high initial concentration (40 mg/L) and under slightly acidic conditions (pH = 4). The experiment used a stainless steel cylinder as the cathode which was continuously supplied with oxygen as an electron acceptor. Pb^{2+} removal was achieved at a rate of 0.53 mg/L/hr and by XRD analysis it was proved that cathodic reduction of Pb^{2+} followed by $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ deposition on the cathode surface was the main removal mechanism.

Modin et al. demonstrated effective removal Cu^{2+} , Pb^{2+} , Cd^{2+} , and Zn^{2+} in an MEC at high initial concentrations (300-800 mg/L) using microbial electrolysis cells. The design involved a cathode and anode chamber separated by an anion exchange membrane, with the anode compartment being refreshed with a nutrient solution from a 1 L reservoir. The metals in

the cathode chamber were dissolved in a strong acid solution (2 M HCl) to simulate a municipal solid waste fly ash leachate. The metals were removed via electrodeposition by controlling the cathode potential. The authors found that Cu^{2+} could be removed at a cell voltage of 0 V due to the high reduction potential of Cu^{2+} . Subsequently, the cathode potential was controlled at -0.51 and -0.66 V vs. SHE to recover Pb^{2+} and Cd^{2+} , respectively. Finally, the anode potential was controlled at $+0.2$ V vs. SHE to recover Zn^{2+} . The cathodes were dissolved in concentrated acid, but only 44.2-84.3% of the metals removed from solution were recovered from the dissolved cathodes, which may be due to losses when removing the cathode or possibly additional removal mechanisms.

Studies have been done to combine the electricity production capabilities of MFC to metal removal in MECs. Li et al. (2015) recovered Pb^{2+} and Ni^{2+} at an MEC cathode driven by the spontaneous reduction of Cr(VI) in an MFC. Pb^{2+} was removed rapidly from an initial concentration of 100 mg/L to less than 2 mg/L in 3 days. Due to negligible removal at an open circuit condition, the reduction of both Pb^{2+} and Ni^{2+} was considered to be driven by electrode reactions. Analysis of the cathodes using EDS show that Pb^{2+} and Ni^{2+} were reduced as a combination of metallic solids and hydroxide compounds. Therefore, this previous study shows the capability of heavy metal removal in MECs to driven by separate metal removal in MFCs.

2.3 Cd^{2+} removal in bioelectrochemical systems

Similar to Pb^{2+} , there have only been some studies that examined Cd^{2+} removal in BESs. As discussed previously, Modin et al. recovered Cd^{2+} by controlling the cathode potential at -0.66 V vs. SHE. Another notable observation of the study was a decrease in Cd^{2+} concentration followed by a sudden increase after experiments were stopped for 4 days, where the authors acknowledged that Cd^{2+} may have formed precipitants that dissolved back into solution when the

reactors were unplugged, suggesting that precipitation may be a possible removal mechanism for Cd^{2+} .

Similar to the study by Li et al., Zhang et al. (2015) demonstrated how MFCs and MECs can be combined to remove several heavy metal with a self-driven system. Two MFCs, with one containing Cr^{3+} and the other Cu^{2+} , were stacked to power an MEC for Cd^{2+} removal from an acidic solution ($\text{pH} = 2$) and H_2 gas production. It was found that when the two MFCs were connected in series, the highest removal Cd^{2+} removal (26%) was achieved. The authors explain that the products that were formed on the cathodes depended on the pH condition and the amount of substrate available. Since the pH condition was low in the effluent (2.3-4.1), it was concluded that Cd^{2+} in the MEC was reduced to pure $\text{Cd}(\text{s})$ at the cathode.

Abourached et al. (2014) demonstrated power generation and effective Cd^{2+} and Zn^{2+} recovery in a single-chamber air-cathode MFC. Various metal concentrations ranging from 20-100 mg/L were added to individual MFCs to determine the toxicity effects of Cd^{2+} and Zn^{2+} on microorganisms for electricity generation. The authors explained both biosorption and precipitation as the two dominant removal mechanisms. Sulfate reducing bacteria converted sulfate in the medium solution into sulfide, which formed precipitants with both Cd^{2+} and Zn^{2+} . They suggested that a high local pH at the cathode may have resulted in cadmium hydroxide precipitation but concluded that hydroxide precipitation was not a dominant removal mechanism.

3. Is Pb(II) removed only via cathodic reduction in bioelectrochemical systems?

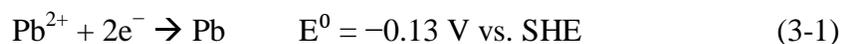
Abstract

Lead (Pb^{2+}) is strictly regulated in wastewater effluent due to its high toxicity even at low concentrations. Pb^{2+} is difficult to remove in conventional water and wastewater treatment as it is water soluble and non-biodegradable. Advanced treatment, such as tight membrane filtration and ion exchange, can be applied but they often require a large amount of electric energy and chemicals for pre- and post-treatment. Microbial electrolysis cells (MECs) can be used to treat wastewater, produce hydrogen gas, and remove heavy metals. Lab-scale MEC operation demonstrated successful Pb^{2+} removal (77-95% in 2 days) at various applied voltage conditions, including open circuit. Using an anion exchange membrane between the anode and cathode, independent Pb^{2+} removal in both anode and cathode chambers was observed at various voltage applications, including open circuit. By analyzing the anode potential and anode deposition, we confirmed that Pb^{2+} was removed by reduction at the anode. Inactivation of exoelectrogenic microorganisms using ethanol resulted in no Pb^{2+} removal, indicating the importance of microbial activity for heavy metal removal in MECs. These findings confirmed that heavy metal removal is not solely driven by the cathodic reduction and bioelectrochemical systems have the potential for broad applications on heavy metal removal.

3.1 Introduction

Lead (Pb^{2+}) contamination is a serious environmental concern. Exposure to Pb^{2+} is dangerous and can cause many serious health effects in humans, especially children (Bo et al., 2014). Sources of Pb^{2+} in the environment include paints with lead-based pigments, pesticides, outdated lead-based plumbing, mining operations, battery manufacturing, and various other industrial operations (Lewinski, 2007). Pb^{2+} is stable and non-biodegradable, making it difficult to remove in conventional water and wastewater treatment. Advanced treatment methods for Pb^{2+} removal have disadvantages, including high initial and operation cost, chemical and reagent requirement, and treatment by-product management (Babel and Kurniawan, 2003). Chemical precipitation is widely used, but it requires large amounts of chemicals and involves further management of concentrated sludge (Lewinsky, 2007). Ion exchange requires resin regeneration using strong acids or saline reagent solutions and the regeneration wastewater needs additional treatment (Kurniawan et al. 2006). Tight membrane filtration (reverse osmosis or nanofiltration) needs extensive pretreatment for fouling control as well as a large amount of electric energy for high pressure pumping (Wang and Ren, 2014). In this study, we aimed to demonstrate and explain energy-efficient removal of Pb^{2+} from aqueous solution without any chemical or intensive energy inputs.

Microbial electrolysis cells (MECs) are a type of bioelectrochemical systems that can simultaneously treat wastewater and recover energy in the form of hydrogen gas (Liu et al, 2005; Rozendal et al., 2006; Logan et al., 2008; Call and Logan, 2008). Exoelectrogenic bacteria grow on the bioanode of MECs and oxidize organic matter while water is reduced to hydrogen gas at the cathode (Logan et al., 2008). In addition to water reduction, other reduction reactions can take place at the cathode, such as the reduction of heavy metal ions into metallic solids (Eq. 3-1):



The cathodic reduction of heavy metals (including Pb^{2+}) has been demonstrated as the main metal removal mechanism in bioelectrochemical systems (BESs). For example, many previous studies have demonstrated utilization of BESs to recover cobalt from expired and dismantled lithium ion batteries (Huang et al., 2012; Huang et al., 2014; Jiang et al., 2014; Huang et al., 2012). Other heavy metals, such as Ni, Fe, and Cu, have also been successfully removed in BESs (Cai et al., 2016; Luo et al., 2014; Cheng et al., 2007; Cheng et al., 2013; Heijne et al., 2010; Li et al., 2014; Tao et al., 2011; Wang et al., 2010). Bo et al. demonstrated successful Pb^{2+} removal in BESs and they proved that cathodic reduction of Pb^{2+} followed by $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ deposition on the cathode surface was the main removal mechanism for a relatively high Pb^{2+} concentration (40 mg/L) and mildly acidic condition (pH = 4) (Bo et al., 2014). Modin et al. used an MEC to selectively remove highly concentrated Pb^{2+} , Cd^{2+} , Cu^{2+} , and Zn^{2+} from acidified incineration ash leachate (2 M HCl) by controlling the cathode potential (Modin et al. 2012). Li et al. used a coupled BES to recover Cr(VI) in a bioelectrochemical cell while spontaneously driving the reduction Pb^{2+} and Ni^{2+} at the cathode of another bioelectrochemical cell (Li et al., 2015).

In this study, we examined Pb^{2+} removal using MECs with the following particular research objectives: (1) to demonstrate successful Pb^{2+} removal from a neutral pH solution at relatively low concentrations (1-2.5 mg/L); (2) to explain Pb^{2+} removal mechanisms not only at the cathode but also at the anode; (3) to explore the effect of different electrical conditions on Pb^{2+} removal in MECs; and (4) to establish the importance of microbial activity at the anode for Pb^{2+} removal. Pb^{2+} was chosen to be investigated because it is highly toxic and can seriously damage the nervous and cardiovascular systems even at low concentrations (Bo et al., 2014).

Although many previous studies have demonstrated effective removal of various heavy metals in BESs, there is a lack of information on the mechanisms of their removal (Wang and Ren, 2014). It is generally accepted that metal removal in bioelectrochemical systems takes place at the cathode via cathodic reduction. However, recent studies have also found other heavy metal removal mechanisms, such as biosorption at the bioanode and precipitation at the cathode. Abourached et al. explained that biosorption by anode bacteria was potentially responsible for Cd^{2+} removal in BESs (Abourached et al., 2014). Precipitation at the cathode has also been discussed as another removal mechanism for Fe^{2+} and Zn^{2+} (Luo et al., 2014; Abourached et al., 2014). Colantonio and Kim demonstrated that Cd^{2+} removal from a neutral pH solution was achieved through a combination of cathode reduction and precipitation of $\text{Cd}(\text{OH})_2$ and CdCO_3 (Colantonio and Kim, 2016). However, Pb^{2+} removal in BESs has not been extensively investigated especially at low concentrations and neutral pH conditions. This study focused on Pb^{2+} removal mechanisms at relatively low Pb^{2+} concentrations (1-2.5 mg/L) and we also demonstrated the importance of microbial activity of exoelectrogenic bacteria for effective Pb^{2+} removal using MECs.

3.2 Methods

3.2.1 Single chamber MEC construction

The MEC was constructed using polypropylene blocks with an interior cylindrical chamber and a cross sectional area of 7 cm^2 . The chamber volume was 45 mL. The anode was a graphite fiber brush (2 cm in length and 2.5 cm in diameter; Mill-Rose, OH) which was pretreated in a muffle furnace at 450°C for one hour (Wang et al., 2009). The cathode was a

piece of stainless steel mesh (5.4 cm² area, AISI 304, 100 mesh; McMaster-Carr, OH) without any precious metal catalysts.

3.2.2 Single chamber MEC operation for Pb²⁺ removal

The MECs were inoculated with primary effluent from a local municipal wastewater treatment plant. The feed solution was prepared using sodium acetate (1 g/L NaCH₃COO) as an organic substrate along with trace vitamins and minerals (Cheng et al., 2009) in a 25 mM bicarbonate buffer solution (4.2 g/L NaHCO₃, 0.0434 g/L NaH₂PO₄-H₂O, 0.2 g/L NH₄Cl). Pb²⁺ was added to the feed solution as PbCl₂ at an initial concentration of 2.5 mg-Pb/L.

The duplicate reactors (MEC 1 and 2) were operated in fed-batch mode with a cycle duration of 96 h (4 days) at various voltage applications (E_{ap} = 0.3, 0.6, 0.9 V, and open circuit). The electric condition in each reactor was controlled and recorded by a multi-channel potentiostat instrument (MGP-2, Biologic, France). Electrode potential and electric current was recorded every 20 min.

A 1.5-mL sample was taken from the reactors at 20 min (as a time zero sample), 6 hr, 24 hr, and every 24 hr thereafter for Pb²⁺ measurement. The 1.5-mL sample was immediately diluted with 3 mL of deionized water, filtered through a syringe filter (pore size 0.45 μm, polyethersulfone membrane, VWR International, Canada), and acidified with 300 μL of 1 M nitric acid.

In addition to the 4-day fed-batch operation, four MECs (MEC 1, 2, 3, and 4) were operated at open circuit for 28 days to further verify results at open circuit. For this open circuit experiment, the samples were taken at 2, 4, 10, 21, and 28 days and pretreated for Pb²⁺ analysis.

3.2.3 Two-chamber MEC operation

After the single chamber MEC operations, an anion exchange membrane (Selemion AMV, AGC Engineering, Japan) was placed between the cathode and anode. The two-chamber MEC experiments were done in triplicate (MEC 1, 2, and 3). The feed solution for the anode chamber (25 mL) was identical to that of the single chamber experiments, but the cathode chamber (20 mL) was fed only with 2.5 mg-Pb/L in 25 mM bicarbonate buffer solution. The two-chamber MECs were operated at an applied voltage of 0.6 V, 0.9 V, or open circuit in a fed-batch mode with a cycle duration of 48 hours (2 days). The feed solution and the anode and cathode chamber effluents were acidified and filtered for Pb²⁺ measurement without dilution.

In a separate two-chamber MEC experiment, the anode potential was monitored for the applied electric conditions ($E_{ap} = 0.6$ V, 0.9 V, and open circuit) using a Ag/AgCl reference electrode (Bioanalytical Systems, Inc. IN).

3.2.4 Abiotic two-chamber MEC operation

After the two-chamber MEC experiments, the cathode and anode chambers were filled with ethanol (70% v/v) for 18 hours to inactivate microbes. After the ethanol treatment, the 2-day fed-batch operation was repeated at open circuit. The influent and effluent samples were acidified and filtered for Pb²⁺ analysis.

3.2.5 Anode material dissolution at high voltage oxidation

After the abiotic two-chamber MEC experiments, the two-chamber MEC was rinsed and refilled with the 25 mM bicarbonate buffer solution without PbCl₂ and NaCH₃COO. Using an

external power supplier, 10 V was applied to the MEC for 30 min to oxidize any materials that have been deposited at the anode. After 30 min, 20 mL of effluent was acidified and filtered before Pb^{2+} analysis.

3.2.6 Measurements

Pb^{2+} concentration of the samples was determined using ICP-OES (inductive coupled plasma-optimal emission spectrometry) (Vista Pro, Varian Inc., Australia). Conductivity and pH of the feed and effluent solutions were measured using a conductivity and pH meter, respectively (SevenMulti, Mettler-Toledo International Inc., OH). During the single-chamber MEC experiments, pH of the feed and effluent was 8.0 and 8.4, respectively. The conductivity was stable from 2.92 to 3.07 mS/cm. In the two-chamber MEC experiments, the anolyte pH decreased from 8.0 to 7.3 ($E_{ap} = 0.9$ V) and to 7.8 ($E_{ap} = 0.6$ V) while it remained at 8.0 (open circuit). The catholyte pH increased from 8.1 to 9.6 ($E_{ap} = 0.9$ V), to 9.0 ($E_{ap} = 0.6$ V), and to 8.2 (open circuit). The anolyte conductivity increased slightly from 2.98 to 3.02 mS/cm (0.9 V), to 3.26 mS/cm (0.6 V), and to 3.32 mS/cm (open circuit). The catholyte conductivity decreased slightly from 2.50 to 2.40 mS/cm (0.9 V), to 2.39 mS/cm (0.6 V), and to 2.38 mS/cm (open circuit). The temperature was stable at $22.7 \pm 0.6^\circ\text{C}$ as the experiment was conducted in an air-conditioned laboratory.

3.3 Results

3.3.1 Pb^{2+} removal in single chamber reactor

Pb^{2+} removal was successful regardless of the applied voltage conditions, including the open circuit test, with 77-95% removal achieved in two days (Fig. 3.1). It should be emphasized that the lowest Pb^{2+} concentration (0.095 mg/L) was observed at 3 days during the open circuit condition. The higher applied voltages (0.6 and 0.9 V) resulted in a higher magnitude of electric current, but the current dropped rapidly before the end of the fed-batch cycle (Fig. 3.1). This low electric current led to an increase in Pb^{2+} concentrations at the end of the fed-batch cycle, from 0.36 to 1.85 mg/L ($E_{ap} = 0.6$ V) and from 0.30 to 2.19 mg/L ($E_{ap} = 0.9$ V) in MEC-2 (Fig. 3.1). The drop in electric current occurred earlier in MEC-2 compared to that in MEC-1. As a result, the increase in Pb^{2+} concentration towards the end of the fed-batch cycle was greater in MEC-2 compared to that in MEC-1.

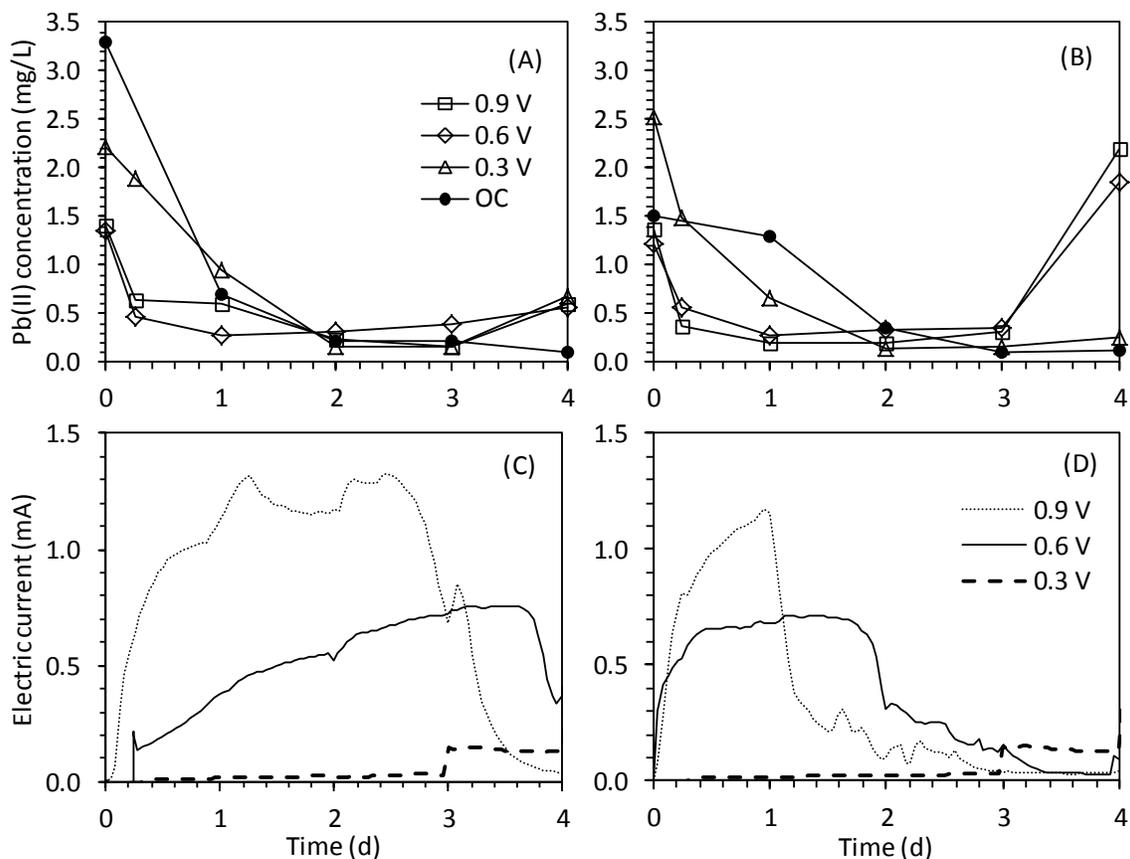


Figure 3.1: Single chamber MEC operation: (A) Pb^{2+} removal in MEC-1; (B) Pb^{2+} removal in MEC-2; (C) electric current in MEC-1; and (D) electric current in MEC-2 under various applied voltage and open circuit (OC) conditions.

In order to investigate Pb^{2+} removal without electric current, the MEC reactors were left open circuit for a period of 28 days. The results showed rapid removal at 4 days (up to 87% removal) with the lowest concentration achieved being 0.17 mg/L (Fig. 3.2). The low Pb^{2+} concentration was maintained at 10 days, but it increased substantially at 20 days to near or above the initial Pb^{2+} concentration (2.95-4.36 mg/L).

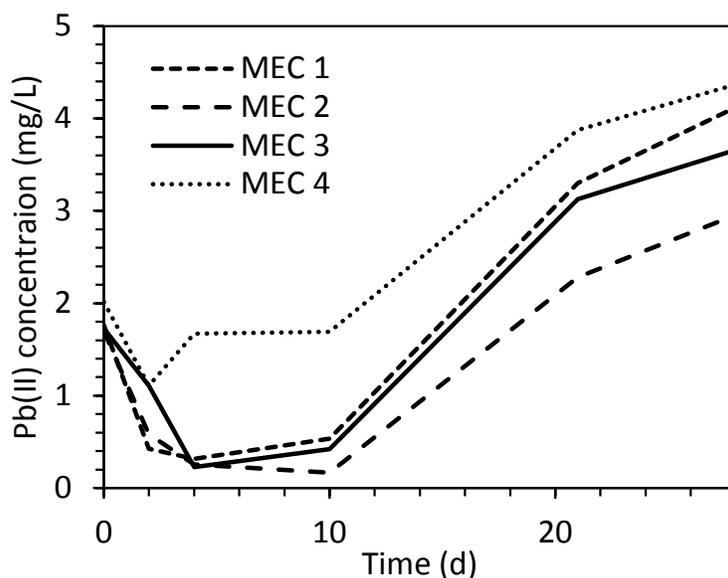


Figure 3.2: Pb^{2+} concentration in single chamber MECs over 28 days at open circuit.

3.3.2 Pb^{2+} removal in two chamber MECs with AEM

When the exoelectrogenic bacteria were alive at the anode, Pb^{2+} removal was observed both in the cathode and anode chambers regardless of the applied voltage (Fig. 3.3). For E_{ap} of 0.6 V, 57% of Pb^{2+} was removed in the anode chamber and 24% removal was achieved in the

cathode chamber. When the applied voltage was increased to 0.9 V, the percent removal in the cathode and anode chambers was similar at 64%. It should be emphasized that there was substantial removal of Pb^{2+} (83%) in the anode chamber and 62% removal in the cathode chamber during the open circuit experiment. After the exoelectrogenic bacteria were inactivated by filling the reactors with ethanol for 18 hours, there was no Pb^{2+} removal in either the cathode or anode chamber during the abiotic test at open circuit.

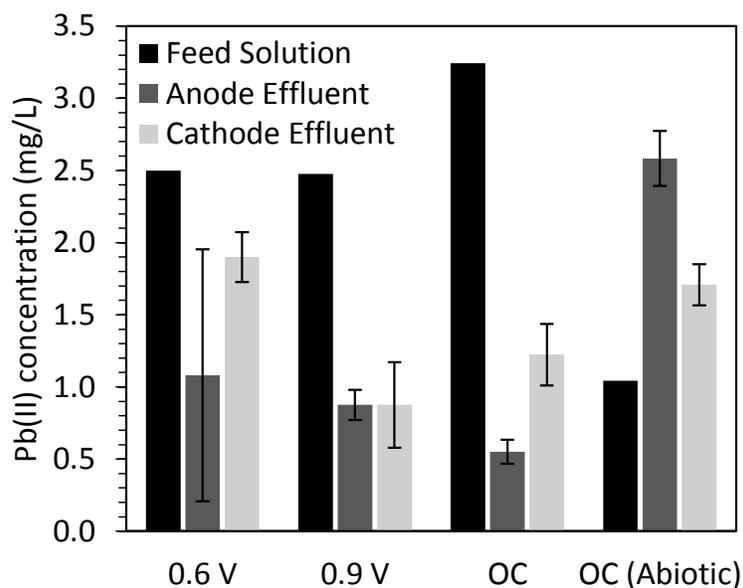


Figure 3.3: Pb^{2+} removal in two-chamber MECs under various applied voltage and open circuit (OC) conditions (n = 3). The abiotic results were obtained after the MECs were treated using ethanol for 18 hr.

3.3.3 Lead deposited on anode

After the abiotic test, the anode was oxidized by applying 10 V for 30 min in lead-free electrolyte. At this high voltage application, the anode materials, including deposited materials on the surface of the anode as well as graphite of the anode brush, were oxidized and dissolved

in the electrolyte. When the electrolyte was analyzed in ICP-OES, the amount of lead in the electrolyte was 0.10 ± 0.02 mg ($n = 3$) per anode.

3.3.4 Anode potential

The anode potential for each applied voltage condition in the AEM experiment is shown in Table 3.1. At the higher voltage application (0.9 V), the anode potential was less negative (i.e., more positive) at about -0.15 V vs. SHE. At 0.6 V application, the anode potential was more negative at about -0.24 V vs. SHE and it was substantially low at -0.33 V vs. SHE for the open circuit condition. This result confirms that the anode potential becomes more negative, indicating that the anode provides more reducing conditions with decreasing applied voltages or at open circuit conditions.

Table 3.2: Measured anode potentials at different electric conditions ($n = 3$).

Electric Condition	Anode Potential (V vs. SHE)
Open Circuit	-0.33 ± 0.01
$E_{ap} = 0.6$ V	-0.24 ± 0.01
$E_{ap} = 0.9$ V	-0.15 ± 0.01

3.4 Discussion

3.4.1 Pb^{2+} removal at the anode

It is evident that Pb^{2+} was removed at the anode according to the result from the two chamber MEC experiment (Fig. 3.3). The removal by the anode was achieved only if the exoelectrogenic microbes were active because no Pb^{2+} removal was observed when the anode was inactivated by ethanol (Fig. 3.3). Given the anode potential of -0.33 V vs. SHE during the open circuit operation, Pb^{2+} ions in solution were reduced to metallic Pb(s) at the anode (Eq. 3-

1). For Pb^{2+} concentration between 0.1 and 2.5 mg/L, the required potential for Pb^{2+} reduction ranges -0.317 to -0.267 V vs. SHE according to the Nernst equation (Eq. 3-2):

$$E = E^0 - \frac{RT}{2F} \ln \frac{1}{[Pb^{2+}]} \quad (3 - 2)$$

In the Nernst equation, E^0 is the standard potential for Pb^{2+} reduction (-0.13 V vs. SHE), T is the temperature (298 K), F is the Faraday's constant (96485 C/mol), and R is the gas constant (8.314 J/mol-K). The calculated required potential to reduce Pb^{2+} (-0.317 to -0.267 V vs. SHE) was more positive than the anode potential under open circuit (-0.33 V vs. SHE). As a result, exoelectrogenic bacteria gain energy as they transfer electrons to Pb^{2+} . Note that exoelectrogens cannot transfer electrons to the anode under open circuit conditions and this restricted electron transfer to the anode must have enhanced Pb^{2+} reduction at the anode during the open circuit experiment, resulting in better Pb^{2+} removal at open circuit compared to the applied voltages (Fig. 3.3). Since the majority of found exoelectrogenic bacteria (e.g., *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Shewanella oneidensis*) use ionic metals as the terminal electron acceptor in nature, Pb^{2+} removal in the anode chamber can be explained by Pb^{2+} reduction by exoelectrogens on the anode (Richter et al., 2012; Rotaru et al., 2011). The substantial amount of metallic Pb^{2+} deposited on the anode (0.10 ± 0.02 mg per anode) also confirms Pb^{2+} reduction at the anode as an important removal mechanism. Note that when the anode brush was treated using ethanol for 18 hours, the effluent ethanol contained a large amount of suspended biosolids as it dissolved microbial cells from the anode. Therefore, it is expected that Pb^{2+} removed by potential biosorption or bioaccumulation in anode bacteria cells was not included in the measured amount of Pb^{2+} deposited on the anode (0.10 ± 0.02 mg per anode).

The higher applied voltages (E_{ap} of 0.6 and 0.9 V) resulted in the anode potentials of –0.24 and –0.15 V vs. SHE, respectively (Table 3.1), which are more positive than –0.317 to –0.267 V vs. SHE for Pb^{2+} reduction. Thus, exoelectrogenic bacteria transfer electrons more favourably to the anode than Pb^{2+} ions. Note that more positive anode potential than Pb^{2+} reduction does not mean that all electrons are transferred to the anode. The electric potential of acetate oxidation (Eq. 3-3) is +0.32 V vs. SHE ($[\text{CH}_3\text{COO}^-] = 12 \text{ mM}$; $[\text{HCO}_3^-] = 25 \text{ mM}$; pH = 7).



Since the acetate oxidation potential is far more positive than the Pb^{2+} reduction potential, the exoelectrogenic bacteria on the anode can oxidize acetate and transfer electrons to Pb^{2+} even though they obtain a greater amount energy if they transfer electrons to the anode. As a result, Pb^{2+} removal in the anode chamber was smaller at the applied voltages (E_{ap} of 0.6 and 0.9 V) than that during the open circuit experiment.

Even though Pb^{2+} reduction coupled with acetate oxidation at the anode is thermodynamically spontaneous, the redox couple is not driven without exoelectrogenic microorganisms. Once the microorganisms at the anode were inactivated by the ethanol treatment, removal of Pb^{2+} at the anode during the open circuit was no longer observed (Fig. 3.3). This observation emphasizes the importance of the exoelectrogenic microbial activity at the anode for Pb^{2+} removal. The importance of active exoelectrogenic microorganisms is also confirmed by the result from the 28-day open circuit experiment (Fig. 3.2). When the reactors were not supplied with fresh feed with organic substrates for the extended period, the exoelectrogenic microbes became inactive due to insufficient substrate and lost Pb^{2+} reduction

capacity. MEC 4 in the experiment showed the least amount of Pb^{2+} removal and the highest increase in concentration towards the end of the 28 days (Fig. 3.2). After the 28-day open circuit experiment, electric current generation in MEC 4 was substantially low compared to that in the other MECs, indicating that the exoelectrogens in MEC 4 were not healthy and active. Therefore, the heavy metal removal capacity of MECs is linked to how healthy and active the microorganisms are at the anode.

3.4.2 Pb^{2+} removal at the cathode

Pb^{2+} was also removed at the cathode and its contribution was similar ($E_{ap} = 0.9$ V) or slightly smaller ($E_{ap} = 0.6$ V and open circuit) compared to the removal at the anode (Fig. 3.3), indicating that Pb^{2+} removal is more effectively achieved by exoelectrogenic bacteria at the anode. During the open circuit experiment, a substantial amount of Pb^{2+} (62.2%) was removed in the cathode chamber (Fig. 3.3). This removal was driven by biological activities because there was no Pb^{2+} removal after the MEC was treated with ethanol. In previous studies, exoelectrogenic bacteria were often found on the cathode (Croese et al., 2011; Shen et al., 2015; Siegert et al., 2014; Siegert et al., 2015). Therefore it is thought that exoelectrogenic bacteria attached on the cathode surface are responsible for Pb^{2+} removal during the open circuit experiment (Fig. 3.3) as they oxidize acetate and transfer electrons to Pb^{2+} for its reduction on the cathode.

The found Pb^{2+} removal mechanisms, in addition to cathodic reduction of Pb^{2+} as previously demonstrated, allow rapid removal of Pb^{2+} and support the potential of MEC applications for heavy metal removal (Modin et al, 2012; Wang et al., 2010;, Tao et al., 2011;

Cai et al., 2016). Many of the previous studies that proved cathodic reduction of heavy metal ions used acidic solutions in the cathode chamber; as a result, microbial activity was strictly inhibited (Luo et al., 2014; Abourached et al., 2014; Modin et al., 2012; Zhang et al., 2015). As demonstrated in this study, operation of MECs at a neutral pH allows additional Pb^{2+} mechanisms (i.e., reduction by exoelectrogens at the anode as well as cathode) and thus enhances the removal of the toxic heavy metal.

Acknowledgements

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4. Cadmium (II) removal mechanisms in microbial electrolysis cells

Abstract

Cadmium is a toxic heavy metal, causing serious environmental and human health problems. Conventional methods for removing cadmium from wastewater are expensive and inefficient for low concentrations. Microbial electrolysis cells (MECs) can simultaneously treat wastewater, produce hydrogen gas, and remove heavy metals with low energy requirements. Lab-scale MECs were operated to remove cadmium under various electric conditions: applied voltages of 0.4, 0.6, 0.8, and 1.0 V; and a fixed cathode potential of -1.0 V vs. Ag/AgCl. Regardless of the electric condition, rapid removal of cadmium was demonstrated (50-67% in 24 hr); however, cadmium concentration in solution increased after the electric current dropped with depleted organic substrate under applied voltage conditions. For the fixed cathode potential, the electric current was maintained even after substrate depletion and thus cadmium concentration did not increase. These results can be explained by three different removal mechanisms: cathodic reduction; $\text{Cd}(\text{OH})_2$ precipitation; and CdCO_3 precipitation. When the current decreased with depleted substrates, local pH at the cathode was no longer high due to slowed hydrogen evolution reaction ($2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$); thus, the precipitated $\text{Cd}(\text{OH})_2$ and CdCO_3 started dissolving. To prevent their dissolution, sufficient organic substrates should be provided when MECs are used for cadmium removal.

Keywords

Bioelectrochemical system; cadmium electrodeposition; cadmium precipitation; heavy metal recovery; industrial wastewater treatment

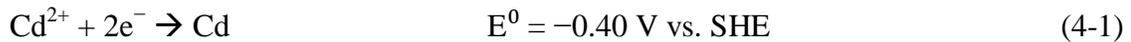
4.1 Introduction

Heavy metal pollution is a serious environmental and health hazard. As many toxic heavy metals are water soluble and non-biodegradable, they are transported by water and can accumulate in soils and living organisms (Kurniawan et al., 2006). Cadmium, in particular, is carcinogenic and toxic, and thus it is strictly regulated in wastewater effluent and composted wastewater biosolids. Various industrial processes, such as electroplating, inorganic pigment, and battery manufacturing, result in cadmium contamination in wastewater streams. Conventional biological wastewater treatment (e.g., activated sludge process) is ineffective for removing heavy metals, resulting in contamination of wastewater effluent high risks in land applications of digested wastewater biosolids and treated effluent (Kurniawan et al., 2006). Current methods for removing heavy metals from wastewater, such as ion exchange or tight membrane filtration, are expensive. Ion exchange requires a large amount of chemicals for resin regeneration and membrane processes are prone to fouling which leads to high operation costs (Kurniawan et al., 2006). In addition, these advanced treatment methods are not cost effective for relatively low concentrations of heavy metals (Abourached et al., 2014). Thus, in this study, we focused on demonstrating an efficient and inexpensive method for cadmium removal from wastewater to reduce potential risks of heavy metal contamination in treated wastewater effluent and wastewater biosolids.

Microbial electrolysis cells (MECs) are an emerging technology for wastewater treatment and simultaneous energy recovery (Liu et al., 2005; Rozendal et al., 2006; Logan et al., 2008; Call and Logan, 2008). With a relatively low applied voltage application ($E_{ap} > 0.2$ V), exoelectrogenic bacteria oxidize organic matter at the bioanode of the cell while water is

simultaneously reduced to hydrogen gas at the cathode (Logan et al., 2008). Thus, wastewater can be treated while energy is recovered in the form of hydrogen gas. MECs and other bioelectrochemical systems (e.g., microbial fuel cells or MFCs) can also be used to remove heavy metals from aqueous solution. In addition to the hydrogen evolution reaction, the MEC cathode can reduce heavy metal ions into metallic solids. For instance, the removal of tri-valent chromium cations as metallic chromium at the MEC/MFC cathode ($\text{Cr}^{3+} + 3\text{e}^- \rightarrow \text{Cr}$), has been extensively investigated in literature papers (Huang et al., 2010; Li et al., 2009; Xafenias et al., 2013; Li et al., 2008; Singhvi and Chharbra, 2013; Wang et al., 2008; Liu et al., 2011). Previous studies have also demonstrated removal of other heavy metals using bioelectrochemical systems. MECs were used to remove Ni^{2+} , Fe^{2+} , and Cu^{2+} from acid mine drainage (Luo et al., 2014). Recovery of copper has been demonstrated along with electricity production using MFCs (Heijne et al., 2010). In addition, removal of Hg^{2+} has also been reported by electric reduction at the MFC cathode (Wang et al., 2011). Recovery of cadmium has been demonstrated using multiple reactors of bioelectrochemical systems where one reactor is used to power the other reactor for the reduction of Cd^{2+} to Cd (Choi et al., 2014; Zhang et al., 2015). Modin et al. demonstrated successful removal of highly concentrated cadmium (800 mg/L) from a synthetic fly ash leachate solution (Modin et al., 2012). In this study, we further investigated cadmium removal using MECs with the following specific research objectives: (1) demonstration of cadmium removal at a relatively low concentration (12.26 mg/L) under neutral pH conditions; (2) investigation of the effects of applied voltages (E_{ap}) on the rate of cadmium removal; (3) clarification of cadmium removal mechanisms by monitoring the cathode potential; and (4) explanation of potential dissolution of removed cadmium from the MEC cathode. By achieving the objectives, we will be able to suggest optimal MEC operation conditions for cadmium removal.

We particularly focused on cadmium removal in this study because removal of cadmium has not been investigated as intensively as other metals, despite its toxic effects and the serious human health risks with repeated land applications of reclaimed wastewater or wastewater biosolids (Khan et al., 2007). Modin et al. controlled the cathode potential to demonstrate selective removal of cadmium from waste fly ash leachate in a recent study, where heavy metals were present at a relatively high concentration (800 mg/L) in a strong acid solution (2 M HCl) (Modin et al., 2012). Cadmium removal in MECs is known to be via the cathodic reduction or electrodeposition (Choi et al., 2014; Zhang et al., 2015; Modin et al., 2012):



This removal mechanism is especially dominant when the pH is substantially low in an acidic solution (e.g., 2 M HCl) or the solution contains substantially high buffering capacity (e.g., 100 mM phosphate) (Choi et al., 2014; Modin et al., 2012). In addition to this cathodic reduction, cadmium can also be removed by precipitation as cadmium hydroxide (Purkayastha et al., 2014; Stumm and Morgan, 2013):



This removal mechanism as the hydroxide salt can be dominant in MECs when the local pH near the cathode is increased as a result of the hydrogen evolution reaction ($2\text{H}_2\text{O} + 2\text{e}^{-} \rightarrow \text{H}_2 + 2\text{OH}^{-}$). Precipitation of cadmium as CdCO_3 is also expected to contribute to the cadmium removal because the high local pH near MEC cathodes converts bicarbonate ions (HCO_3^{-}) into carbonate ions (CO_3^{2-}) and the converted carbonate ions are likely to form precipitants with cadmium ions as (Stumm and Morgan, 2013):



Since bicarbonate is created as a byproduct of acetate oxidation at the bioanode, CdCO_3 precipitation can also be a dominant factor for cadmium separation.

Zhang et al. demonstrated that the removal mechanism at the cathode is dependent on substrate and pH conditions in MFCs (Zhang et al., 2015). If the formation of cadmium precipitants is the dominant pathway for cadmium removal, it is critical to maintain a high local pH near the cathode by continuously driving the hydrogen evolution reaction. Otherwise, the precipitation reactions in Eq (4-2) and Eq (4-3) will be reversed due to low hydroxide concentration. Thus, the amount of organic substrates can be an important factor that allows the hydrogen evolution reaction to occur at the MEC cathode. On the other hand, if the cathodic reduction of cadmium in Eq (4-1) is the dominant mechanism, the cadmium removal efficiency will not be affected by the amount of organic substrate as long as the cathode potential is favorable for cadmium reduction reaction in Eq (4-1). This study provides answers to these questions and suggests optimal MEC operation conditions for cadmium removal at a relatively low concentration (12.26 mg/L).

4.2 Materials and methods

4.2.1 Reactor construction

The single chamber MEC was constructed using a polypropylene block with an interior cylindrical chamber (cross sectional area of 7 cm^2) and a total volume of 45 mL as previously demonstrated. The bioanode was prepared using a graphite fiber brush (2.5 cm diameter and 2 cm in length; Mill-Rose, OH) which was pretreated in a muffle furnace at 450°C for one hour (Wang et al., 2009). The cathode was a piece of stainless steel mesh (5.4 cm^2 area, AISI 304, 100 mesh; McMaster-Carr, OH) without any precious metal catalyst application.

4.2.2 Reactor operation

The MECs were inoculated with effluent from an existing MEC reactor for digestion of waste activated sludge (Asztalos and Kim, 2015). The feed solution was prepared using sodium acetate (0.5 g/L NaCH₃COO) as the substrate along with trace vitamins and minerals (Cheng et al., 2009) in a 12.5 mM phosphate buffer solution (1.145 g/L Na₂HPO₄, 0.613 g/L NaH₂PO₄-H₂O, 0.078 g/L NH₄Cl, 0.033 g/L KCl). Note that alkalinity of the feed solution is approximately 313 mg/L as CaCO₃, which is still greater but reasonable considering the amount of alkalinity in typical domestic wastewater (City of Hamilton, 2011). Cadmium was added to the prepared medium solution using CdCl₂. The initial cadmium concentration was 12.26 mg/L as Cd. This initial Cd(II) concentration was chosen because we found in a separate experiment that Cd(II) concentration higher than 12.5 mg/L starts inhibiting the bioanode performance in generating electric current. The MEC reactors were operated in a fed batch mode with a cycle span of 96 hours (4 days) and all experiments were conducted in duplicate (MEC-1 and MEC-2).

The reactors were operated under 6 different electric conditions: four fixed voltage applications ($E_{ap} = 0.4, 0.6, 0.8, 1.0$ V); one fixed cathode potential (-1.0 V vs. Ag/AgCl); and open circuit. The electric condition in each reactor was controlled and recorded by a multi-channel potentiostat instrument (MGP-2, BioLogic, France). Individual electrode potentials and electric current were recorded every 20 minutes using the instrument. For individual electrode potential measurement or control, an Ag/AgCl reference electrode was placed between the bioanode and cathode (Bioanalytical Systems, Inc. IN). The reactors operated in an air-conditioned laboratory and temperature was stable 22.10 ± 0.74 °C.

4.2.3 Sample preparation and measurements

A 2-mL sample was taken from the MEC every 24 hours for the fixed applied voltage experiments ($E_{ap} = 0.4, 0.6, 0.8, 1.0$ V and open circuit). Even though the solution volume in the reactor decreased from 45 to 39 mL over the 96 hr experiment, the sample volume (2 mL) was necessary for a proper analysis of cadmium and chemical oxygen demand (COD). For the fixed cathode experiments, the solution volume decreased from 45 to 37 mL as 2-mL samples were taken at 18, 24, 48, 72 and 96 hr. The collected sample was immediately acidified with 8 mL of 1 M nitric acid. The acidified sample was filtered using a syringe filter (pore size 0.45 μm , polyethersulfone membrane, VWR International, Canada). Cadmium concentrations for the sample were measured using ICP-OES (Inductive Coupled Plasma-Optical Emission Spectrometry) (Vista Pro, Varian Inc., Australia).

The chemical oxygen demand (COD) was determined in spectrophotometry according to the standard methods (Hach Co., CO) (APHA, 2005). Conductivity and pH were also measured using a conductivity and pH meter (SevenMulti; Mettler-Toledo International Inc., OH). Conductivity of the feed and effluent solution was stable at 1.31 and 1.65 mS/cm and pH was 6.6 and 7.0, respectively.

After 9 months of MEC operation, the reactors were disassembled and precipitants on the cathode were analyzed in scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) (JEOL JSM-6610LV, Japan). The EDS result of the cathode precipitants was compared with that of $\text{Cd}(\text{OH})_2$ and CdCO_3 crystals. The $\text{Cd}(\text{OH})_2$ crystals were obtained by mixing 4.5 mM $\text{Cd}(\text{Cl})_2$ and 4.5 mM NaOH in deionized water. Note that Na^+ and Cl^- are soluble for the given concentrations; thus, the recovered crystals at the concentrations can only be $\text{Cd}(\text{OH})_2$. CdCO_3 was purchased from Alfa Aesar (99.998% purity).

After the SEM / EDS analysis, the cathode precipitants were scraped and dissolved in 0.01 M HCl. The HCl solution was then titrated using 0.025 M NaOH to find the amount of carbonate species in the cathode precipitants. Based on the amount of total carbonate species, the fraction of CdCO₃ in the cathode precipitants was determined.

4.2.4 Contribution of cadmium reduction to total charge transfer

To examine whether cadmium was removed solely by the cathodic reduction ($\text{Cd}^{2+} + 2\text{e}^- \rightarrow \text{Cd}$), the amount of cadmium removed during the first 24 hours was converted to the equivalent amount of electric charge as:

$$e_{\text{Cd}} = 2FV\Delta c_{\text{Cd}} \quad (4-4)$$

F is the Faraday constant, V is the volume of the reactor (0.045 L), Δc_{Cd} is the change in molar concentration of aqueous phase cadmium for the first 24 hours, and e_{Cd} is the equivalent amount of cadmium removed in Coulombs. The equivalent amount of cadmium removal was compared with the amount of charge transfer for the first 24 hours (e_i) by integrating electric current (I) for the total length of the cycle (t_{cycle}) as:

$$e_i = \int_0^{t_{\text{cycle}}} I dt \quad (4-5)$$

For instance, if e_{Cd} is greater than e_i , the cathodic reduction ($\text{Cd}^{2+} + 2\text{e}^- \rightarrow \text{Cd}$) is not the sole mechanism for cadmium removal, verifying another cadmium removal mechanism, such as precipitation as cadmium hydroxide ($\text{Cd}^{2+} + 2\text{OH}^- \rightarrow \text{Cd}(\text{OH})_2$) and/or cadmium carbonate ($\text{Cd}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CdCO}_3$). It should be emphasized that the majority of cadmium was removed in the first 24 hours of the experiment. Thus, we focused on comparing e_{Cd} and e_i for the first 24 hr to determine which mechanism was dominant when cadmium was actively separated in the MEC reactors.

4.3 Results and discussion

4.3.1 Cadmium removal under applied voltage operation

Cadmium removal was achieved rapidly as the majority of the initial cadmium was removed in two days (Fig. 4.1). However, the rate of the removal was not strongly dependent on the electric voltage applications (E_{ap}) between 0.4 and 1 V. For all examined applied voltage conditions, 50-67% of cadmium removal was demonstrated in 1 day and 71-91% in 2 days. The lowest concentration achieved was 0.46 mg/L with a removal of 95% occurring at $E_{ap} = 0.6$ V after a period of four days. The open circuit condition resulted in practically no removal of cadmium, indicating that the cadmium removal was driven by electrode reactions. The same trend was found in another duplicate MEC (Fig. 4.1).

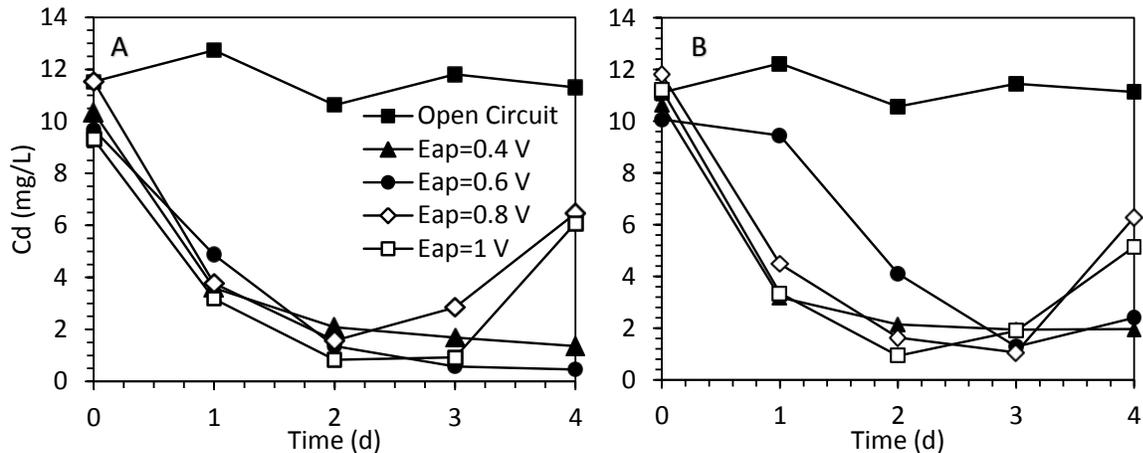


Figure 4.1 Cadmium removal in (A) MEC-1 and (B) MEC-2 under applied voltage operation.

The magnitude of the electric current was proportional to the applied voltage: the higher the applied voltage, the higher the magnitude of the electric current (Fig. 4.2). The magnitude of the current did not have a direct effect on the rate of cadmium removal as the majority of cadmium (>80%) was removed in two days regardless of the applied voltage condition except for the open circuit condition (Fig. 4.1). For the high applied voltages ($E_{ap} = 0.8$ and 1.0 V), the current dropped 2 days after the fed-batch cycle was started. The current drop between 2 and 3 days is consistent with the rapid increase in the monitored cathode potential (Fig. 4.3). As a result of the increase, the cathode potential became stable at -0.77 V vs. Ag/AgCl after 3 days. The low current (~ 0.02 mA) and cathode potential (-0.77 V vs. Ag/AgCl) between 3 and 4 days were caused by the low substrate conditions as more than 90% of COD was consumed at 3 days (Fig. 4.4)

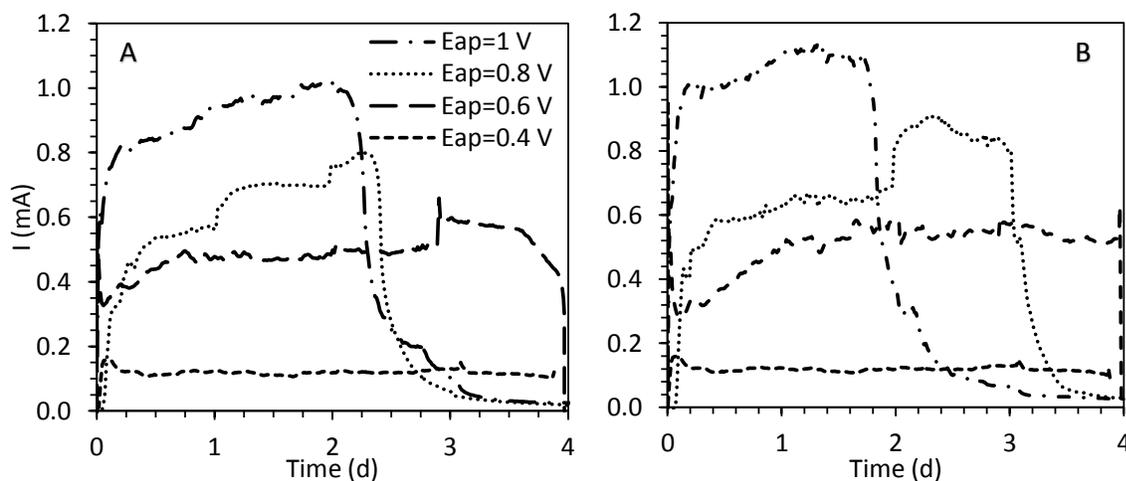


Figure 4.2: Electric current in (A) MEC-1 and (B) MEC-2 under applied voltage operation.

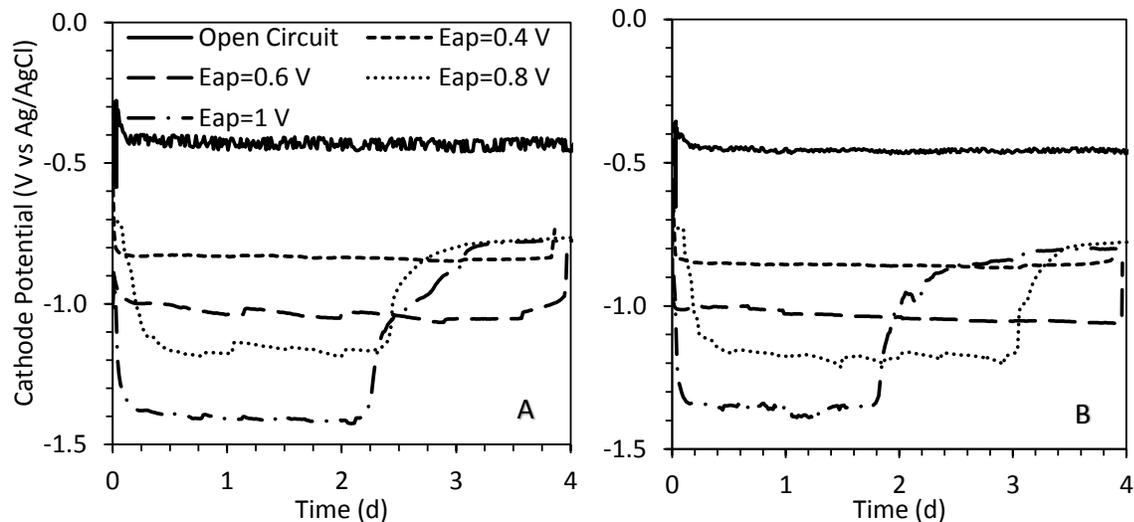


Figure 4.3: Cathode potential in (A) MEC-1 and (B) MEC-2 under applied voltage operation.

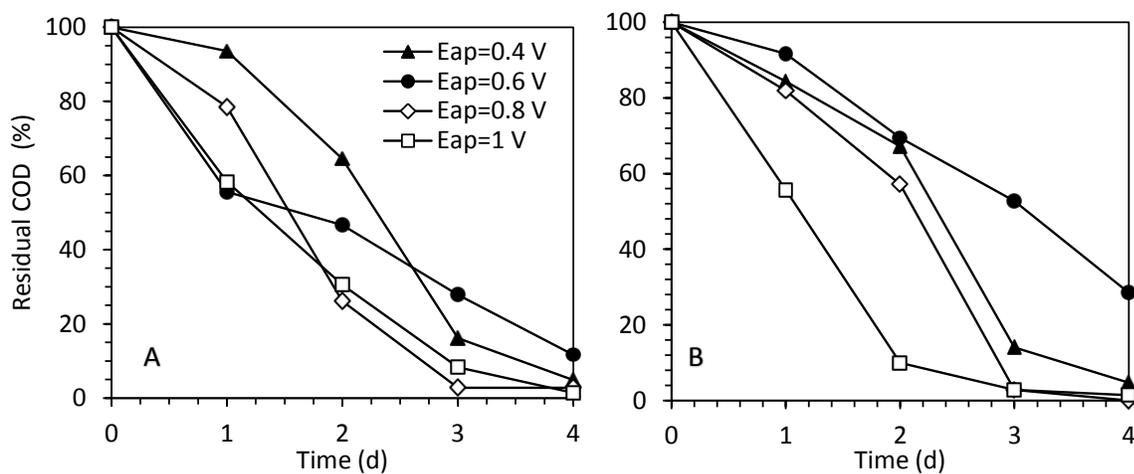


Figure 4.4: Residual COD in (A) MEC-1 and (B) MEC-2 under applied voltage operation.

4.3.2 Increase in cadmium concentration

After the current dropped and the cathode potential increased (between 3 and 4 days) for $E_{ap} = 0.8$ and 1 V, there was an increase in cadmium concentration (Fig. 4.1). For E_{ap} of 0.8 V, the cadmium concentration increased from 1.6 to 6.5 mg/L while it increased from 0.9 to 6.1 mg/L for E_{ap} of 1.0 V during the second half of the fed batch cycle. This increase in cadmium

concentration corresponds to the dropped current (Fig. 4.2), increased cathode potential (Fig. 4.3) and very low substrate concentration (Fig. 4.4).

For the relatively low voltage applications ($E_{ap} = 0.4$ and 0.6 V), the residual COD was always above 10% (Fig. 4.4), allowing stable current generation and consistently negative cathode potential below -0.8 V vs. Ag/AgCl. As a result, there was no increase of cadmium concentration at the end of the cycle. This result emphasizes the importance of the exoelectrogenic activity of the bioanode even though the cadmium removal occurs at the cathode. A higher level of residual COD at the end of the cycle indicates that there is still organic substrate available in the reactor to be utilized by the exoelectrogenic bacteria. When substrate was still available, the anode potential was consistently about -0.4 V vs. Ag/AgCl regardless of the applied voltage condition (except for the open-circuit control experiment), inducing a sufficient reduction condition at the cathode (i.e., below -0.8 V vs. Ag/AgCl) for the reduction of cadmium ions to metallic solid (Zhang et al., 2015; Modin et al., 2012). However, when the organic substrate was depleted for E_{ap} of 0.8 and 1.0 V, the anode potential increased above 0 V vs. Ag/AgCl due to inactive exoelectrogenic bacteria. As a result, the cathode potential increased to -0.77 V vs. Ag/Cl (Fig. 4.3). These experimental results imply that lower applied voltages (e.g., $E_{ap} = 0.4-0.6$ V) with slower utilization of organic substrate can be ideal for continuous removal of cadmium at a low electric energy requirement.

It should be noted that the cathode potential was stable at -0.77 V vs. Ag/AgCl when the organic substrate was depleted for E_{ap} of 0.8 and 1.0 V (Fig. 4.3). This cathode potential is far more negative than the calculated equilibrium potential for the cathodic reduction of cadmium ($\text{Cd}^{2+} + 2\text{e}^- \rightarrow \text{Cd}$) unless aqueous-phase cadmium concentration is extremely small below 0.1 ng/L (Fig. 4.5). This comparison confirms that the increased cadmium concentration between 3

and 4 days ($E_{ap} = 0.8$ and 1.0 V) was hardly due to dissolution of metallic cadmium ($\text{Cd} \rightarrow \text{Cd}^{2+} + 2e^{-}$).

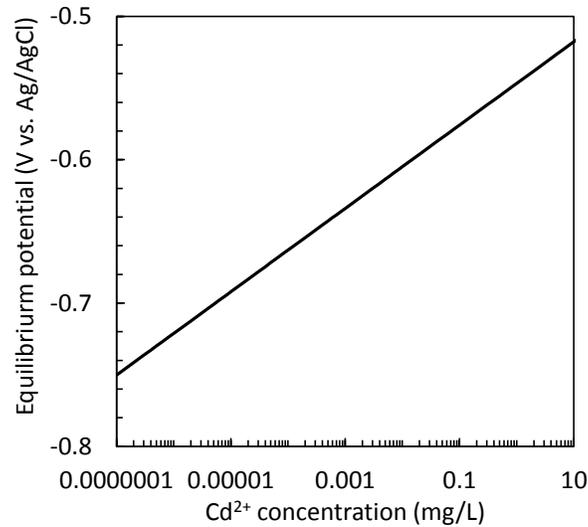


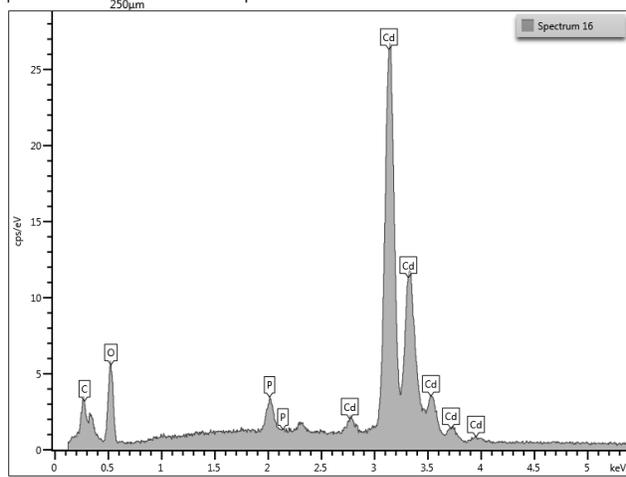
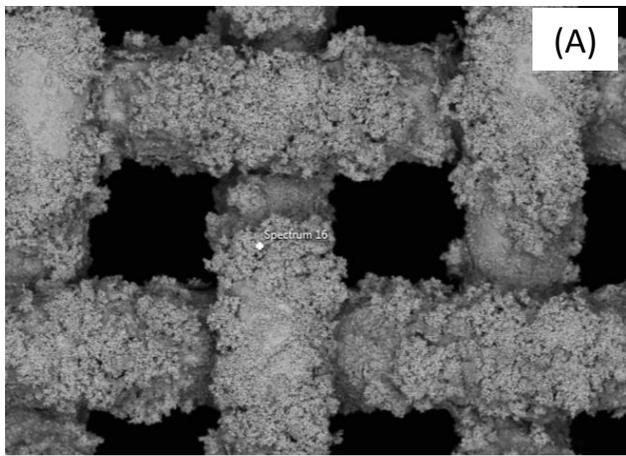
Figure 4.5: Effect of Cd^{2+} concentration on equilibrium potential for cathodic reduction of cadmium ($\text{Cd}^{2+} + 2e^{-} \rightarrow \text{Cd}$). (Calculated results using the Nernst equation at 20°C).

Therefore, the increased concentration can be explained by the dissolution of $\text{Cd}(\text{OH})_2$ and CdCO_3 as the local pH near the cathode decreased with the reduced electric current between 3 and 4 days. This finding implies that a certain fraction of cadmium (Cd^{2+}) in the MEC reactor was removed by cathodic reduction ($\text{Cd}^{2+} + 2e^{-} \rightarrow \text{Cd}$) while the remainder was removed as $\text{Cd}(\text{OH})_2$ and CdCO_3 because the hydrogen evolution reaction ($2\text{H}_2\text{O} + 2e^{-} \rightarrow \text{H}_2 + 2\text{OH}^{-}$) increases the local pH near the cathode surface. Even though the local pH at the cathode was not measured in this study, it has been reported in previous studies that pH near the MEC cathode is high enough to enhance salt precipitation (Bard and Faulkner, 2000; Cusick and Logan, 2012). While the electric current was high with active hydrogen evolution reaction (i.e., active production of OH^{-}), a high local pH was maintained and thus precipitation of $\text{Cd}(\text{OH})_2$ and

CdCO_3 was continuously induced. However, when the hydrogen evolution reaction was slowed down with reduced substrate levels, the precipitation reactions (Eq. 4-2 and Eq. 4-3) were reversed, increasing the aqueous phase cadmium concentration near the end of fed-batch cycle (Fig. 4.1).

4.3.3 Identification of cathode deposits

The scanning electron microscope (SEM) image shows that the stainless steel mesh cathode was covered with precipitants (Fig. 4.6A). The result from the energy dispersive X-ray spectroscopy (EDS) analysis indicates that the precipitants consist mainly of cadmium along with minor fractions of carbon and oxygen (Fig. 4.6B). It should be noted that hydrogen is not detected in EDS. This result confirms that the removal of cadmium was achieved by precipitation at the cathode. When the EDS result of the cathode precipitants was compared to that of $\text{Cd}(\text{OH})_2$ and CdCO_3 crystals (Fig. 4.6C and 4.6D, respectively), it was clear that the cathode deposits are either $\text{Cd}(\text{OH})_2$ or CdCO_3 or a combination of both. However, the precipitants could not be identified because the spectra of $\text{Cd}(\text{OH})_2$ and CdCO_3 were very similar to one another.



(B)

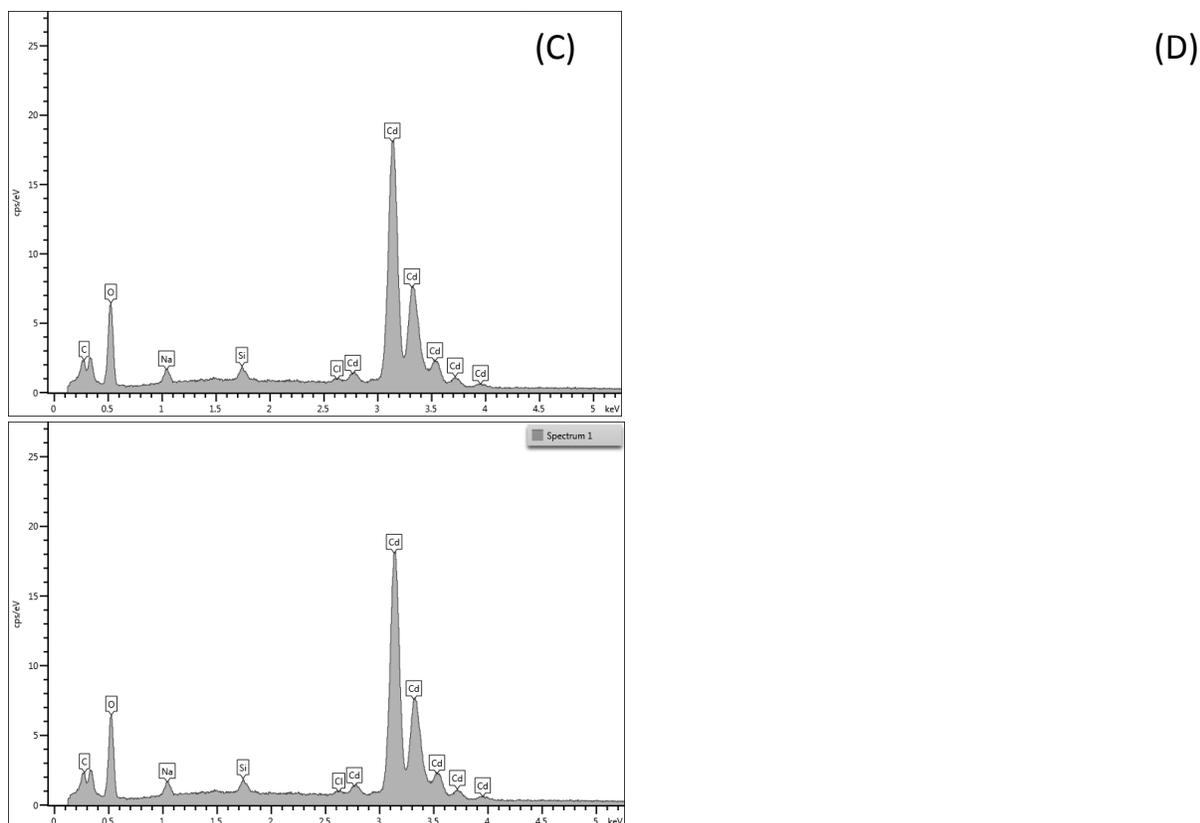


Figure 4.6: (A) Scanning electron microscope image of the cathode at 200x magnification; (B) Energy dispersive X-ray spectroscopy (EDS) result for cathode deposits; (C) EDS result for $\text{Cd}(\text{OH})_2$; and (D) EDS result for CdCO_3 .

To further identify the cathode deposits, they were scraped and dissolved in 0.01 M HCl solution. When the solution was titrated using 0.025 M NaOH, carbonate species were detected, indicating CdCO_3 is present in the cathode deposits. Using the titration test result, the total carbonate species was estimated and it was found that $22.5 \pm 9.4\%$ ($n = 3$) of the cathode deposit was CdCO_3 .

4.3.4 Cadmium removal under fixed cathode potential condition

The fixed cathode potential at -1.0 V vs. Ag/AgCl also resulted in rapid cadmium removal in the first 2 days and the rate of removal decreased gradually thereafter (Fig. 4.7A).

Approximately 50% of the initial amount of cadmium was removed in the first 19 hours and more than 70% removal was achieved in two days. Only an additional ~10% removal was achieved after 2 days. This trend of cadmium removal with time (rapid removal in the first two days and slow in the rest of the fed batch cycle) is similar to that in the experiments with the applied voltage conditions (Fig. 4.1).

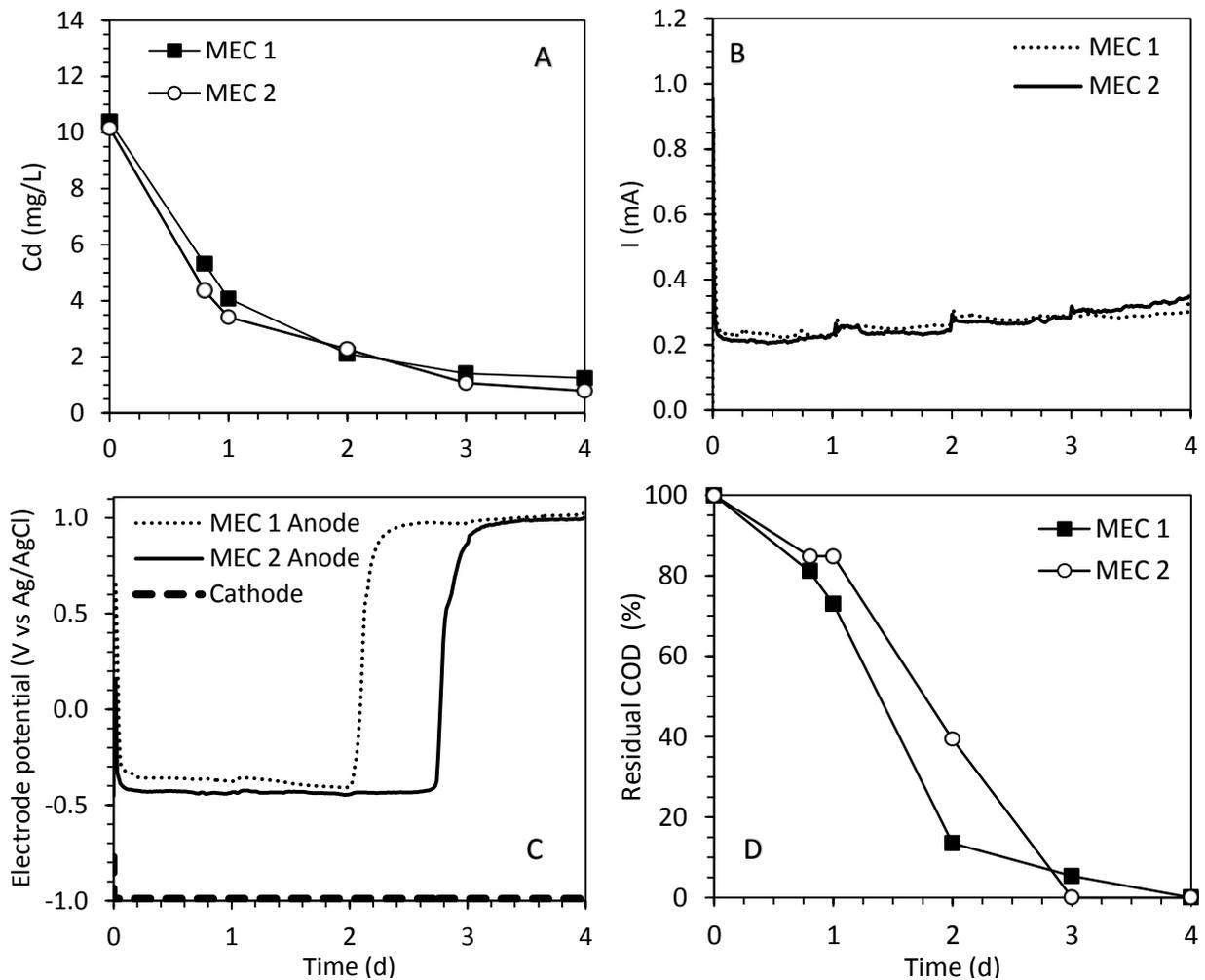


Figure 4.7 MEC operation results for the fixed cathode potential ($E_{\text{cathode}} = -1.0 \text{ V vs Ag/AgCl}$): (A) Cadmium removal; (B) Electric current; (C) Cathode and anode potentials; and (D) Residual COD.

For the fixed cathode potential condition at -1.0 V vs. Ag/AgCl, it should be emphasized that the cadmium concentration did not increase even after COD was depleted (Fig. 4.7D). During the MEC operation at the fixed cathode potential, the anode potential was also monitored throughout the cycle (Fig. 4.7C). The anode potential was stable at approximately -0.4 V vs. Ag/AgCl for the first two days, which corresponds to an overall applied voltage of 0.6 V (-0.4 V $- (-1.0$ V) = 0.6 V). However, when COD was depleted after two days, the anode potential was shifted to about $+1.0$ V for the fixed cathode potential at -1.0 V vs. Ag/AgCl. The anode potential corresponds to an overall applied voltage of about 2 V ($+1$ V $- (-1$ V) = 2 V). This result indicates that the electric energy requirement without organic substrate is substantially high to keep the cathode potential sufficiently negative to induce the hydrogen evolution reaction and cadmium removal. Thus, the electrically active bioanode with sufficient amounts of organic substrates is critical for energy-efficient cadmium removal, allowing rapid removals (e.g., 50% in one day) at only 0.4 V of the voltage application (Fig. 5.1). However, when the bioanode is inactive with depleted substrates, a large amount of electric energy (e.g., E_{ap} of about 2 V) is needed to keep removing cadmium from aqueous solution.

With the substantially high energy consumption (i.e., $E_{ap} \sim 2$ V), the electric current did not drop after the organic substrate was depleted (Fig. 5.7B). As a result, the hydrogen evolution reaction was continuously induced at the cathode and thus the high local pH was maintained near the cathode. The high local pH condition prevented potential dissolution of precipitated $\text{Cd}(\text{OH})_2$ and CdCO_3 during the fixed cathode potential experiment (Fig 7A).

4.3.5 Metal removal efficiency

The equivalent amount of cadmium removed in the first 24 hours (e_{Cd} , Eq. 4-4) was compared with the amount of electron transfer (e_i , Eq. 4-5) (Table 4.1).

Table 4.1. Coulomb-based quantification of cadmium removed (Eq. 4-4) and charge transferred (Eq. 4-5) for the first 24 hours.

Electric condition	Reactor	Cd removed, e_{Cd} (C)	Charge transferred, e_i (C)
$E_{ap} = 0.4$ V	MEC-1	25.03	10.30
	MEC-2	27.68	10.43
$E_{ap} = 0.6$ V	MEC-1	17.86	37.06
	MEC-2	2.31	34.25
$E_{ap} = 0.8$ V	MEC-1	28.82	38.93
	MEC-2	27.19	43.03
$E_{ap} = 1.0$ V	MEC-1	22.76	70.87
	MEC-2	29.22	84.61
$E_{cathode} = -1.0$ V vs. Ag/AgCl	MEC-1	23.44	21.37
	MEC-2	25.00	19.05

For relatively high voltage application conditions ($E_{ap} \geq 0.6$ V), e_i was greater than e_{Cd} . This result does not necessarily mean that the cathodic reduction (Eq. 4-1) is the sole pathway for cadmium removal because both of the hydrogen evolution and cadmium reduction reactions were included in the e_i calculation. For E_{ap} of 0.4 V and $E_{cathode}$ of -1.0 V vs. Ag/AgCl, the e_{Cd} was greater than e_i , indicating that the cadmium removal was achieved not only by the cathodic reduction of cadmium but also by the hydroxide precipitation. For E_{ap} of 0.4 V, the ratio between e_i and e_{Cd} is ~40%. Considering the hydrogen evolution reaction counted in calculation of e_i , this ratio indicates that the contribution of the cathodic reduction mechanism to the total cadmium removal was 40% at maximum. That is, cadmium precipitation was responsible for more than 60% of the total amount of cadmium removal for E_{ap} of 0.4 V. Since the hydrogen evolution reaction was not closely monitored in the experiment due to the difficulty in controlling hydrogenotrophic methanogens (Tice and Kim, 2014), the contribution of the two cadmium removal mechanisms cannot be precisely estimated. However, it is clear that the hydroxide and carbonate precipitation play an important role in cadmium removal in MECs.

4.4 Conclusions

This study provided a better understanding of the mechanisms and kinetics of cadmium removal in microbial electrolysis cells. It has been confirmed that MECs can successfully remove cadmium from solution at relatively low concentrations (12.26 mg/L) with a low energy input. In addition, the rate of cadmium removal was rapid and relatively independent of the electric condition as 50-67% removal was achieved in 24 hours for all of the examined applied voltages. While the cathodic reduction (Eq. 4-1) has been reported to be the major removal mechanism in previous studies (Choi et al., 2014; Zhang et al., 2015; Modin et al., 2012), we found that $\text{Cd}(\text{OH})_2$ and CdCO_3 precipitation are also important mechanisms for cadmium removal in MECs. To avoid potential dissolution of precipitated $\text{Cd}(\text{OH})_2$ and CdCO_3 , the bioanode has to be kept electrically active with sufficient amounts of organic substrates. Active bioanodes in MECs allow continuous hydrogen evolution reaction that keeps the local pH near the MEC cathode sufficiently high, inducing precipitation of $\text{Cd}(\text{OH})_2$ and CdCO_3 . We recommend that future studies on cadmium removal using MECs should focus on quantifying the contribution of the two cadmium removal mechanisms. Since high local pH conditions near the cathode play an important role in cadmium precipitation, the local pH needs to be investigated how it is affected by the electric current, cathode geometry, and mixing condition. Large scale experiments are also necessary for practical applications of MECs in heavy metal removal from actual wastewater. Development of energy-efficient methods for removing heavy metals from wastewater will allow safe applications of reclaimed wastewater and composted wastewater biosolids.

Acknowledgements

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5. Cd(II) recovery from low concentrations in microbial electrolysis cells via electrodeposition and precipitation

Abstract

Cadmium is a strictly regulated heavy metal due to its toxic and carcinogenic effects at low concentrations. Advanced treatment methods (tight membrane filtration, ion exchange) are necessary for effective cadmium removal; however, they are economically and environmentally expensive with intensive energy and chemical consumption. Microbial electrolysis cells (MECs) can remove cadmium on the cathode via electrodeposition (cathodic reduction) and chemical precipitation while treating wastewater and recovering energy simultaneously. Lab-scale MECs were operated to investigate cadmium removal at relatively high concentration (2-2.5 mg-Cd/L) and low concentrations (10-100 $\mu\text{g-Cd/L}$). Rapid removal of cadmium (88% removal in two days) was demonstrated during the high concentration experiment where electrodeposition of cadmium (67%) was dominant over chemical precipitation as cadmium crystals (33%). Due to the meaningful contribution of chemical precipitation, it was found that orientation and configuration of the cathode were important factors in cadmium removal efficiency in MECs. Another set of MECs was also operated at a low concentration over 18 weeks. After the long-term experiment, 8-10% of the total amount of cadmium fed to MECs was found on the cathode. Also, electrodeposition was the sole mechanism for cadmium removal at the cathode for the low concentrations. The rest of cadmium (90-92% or 0.008-0.04 mg) is thought to be removed by biosorption at the anode. Also, active exoelectrogens at the anode are essential for effective electrodeposition of cadmium at the cathode for the low concentration.

Keywords

Heavy metal removal; Microbial electrolysis cells, cadmium electrodeposition, cadmium precipitation

5.1 Introduction

Cadmium is a toxic and carcinogenic heavy metal that can be present in wastewater from many industrial operations, such as battery manufacturing and mining (Wang and Ren, 2014). Conventional biological wastewater treatment is ineffective for removing heavy metals, which can result in serious environmental contamination and human health risks as bioaccumulation of metals like cadmium can occur along the food chain in an aquatic ecosystem (Kurniawan et al., 2006). Heavy metal treatment processes such as chemical precipitation and ion exchange can be expensive and requires input of chemicals and can result with costly disposal problems (Wang and Ren, 2014). In addition, they are not effective for relatively low concentration of heavy metals. Tight membrane filtration (nanofiltration or reverse osmosis) is costly due to fouling control and intensive pretreatment.

Microbial electrolysis cells (MECs) are a developing technology for wastewater treatment and energy production (Liu et al., 2005; Rozendal et al., 2006; Logan et al., 2008; Call and Logan, 2008). With a small applied voltage (>0.3 V), organic matter is oxidized at the anode by exoelectrogenic bacteria and water is reduced at the cathode to create hydrogen gas (Logan et al., 2008). In addition to energy recovery, bioelectrochemical systems, including MECs, have proven to be effective for heavy metal removal (Modin et al., 2012; Luo et al., 2014; Heijne et al., 2010; Wang et al., 2011). The well-known removal mechanism is the cathodic reduction of ionic forms of heavy metals into metallic solids at the cathode, which is often called electrodeposition (Eq. 5-1):



Electrodeposition can be a dominant removal mechanism under acidic conditions (Cai et al., 2016; Modin et al., 2012; Zhang et al., 2015). For a typical applied voltage (0.4-1.0V), the cathode potential was low (i.e., negative) enough to reduce various ionic metals and thus drive their electrodeposition. For instance, the required reduction potential for cadmium reduction at a concentration of 2.5 mg/L is -0.54 V vs. SHE based on the Nernst Equation (Eq. 5-2):

$$E = E^0 - \frac{RT}{2F} \ln \frac{1}{[\text{Cd}^{2+}]} \quad (5-2)$$

E^0 is the standard potential for Cd^{2+} reduction (-0.40 V vs. SHE), T is the temperature (298 K), F is the Faraday's constant (96485 C/mol), and R is the gas constant (8.314 J/mol-K). Since the cathode potential typically ranges -0.6 to -1.2 V vs. SHE for the applied voltage of 0.4 to 1.0 V (Colantonio and Kim, 2016), there is a significant amount of driving force for electrodeposition of cadmium in MECs. For an even lower concentration (e.g., $10 \mu\text{g-Cd/L}$), cadmium reduction requires -0.61 V vs. SHE and thus the MEC cathode can provide sufficient driving force to reduce Cd^{2+} into metallic solids. One of the main objectives of this study is to demonstrate that MECs can remove Cd^{2+} even at substantially low concentrations (10 to 100 $\mu\text{g/L}$).

Chemical precipitation is also an important heavy metal removal mechanism in MECs because the water reduction at the cathode provides high local pH conditions (Cai et al., 2016; Luo et al., 2014; Colantonio and Kim, 2016). Visible precipitants were recovered on MEC cathodes and were identified as $\text{Cd}(\text{OH})_2$ and/or CdCO_3 (Colantonio and Kim, 2016). While both removal mechanisms (electrodeposition and chemical precipitation) have been verified, their contributions to cadmium removal were not quantified. Also, there is a lack of information on

heavy metal removal in MECs over longer periods of operation (Nancharaiah et al., 2015). With the limited understanding of cadmium removal in MECs, we focused on the following research objectives: 1) to quantify the amount of cadmium removed by electrodeposition and precipitation; 2) to explain the importance of cathode configuration and orientation depending on the removal mechanisms; 3) to demonstrate cadmium removal by electrodeposition at very low concentrations (10-100 $\mu\text{g/L}$); and 4) to emphasize the importance of microbial activity at the anode for proper cadmium removal at the very low concentrations. Cadmium is highly toxic even at very low concentrations; thus, demonstration of cadmium removal from the low concentration will bring impactful contributions to the development of MEC applications for sustainable wastewater treatment (Wang and Ren, 2014). In addition, clear understanding of the removal mechanisms not only encourages a progression towards an effective solution to heavy metal contamination but it also allows energy efficient recovery of heavy metals from various types of wastewater, including industrial wastewater and municipal sewage.

5.2 Methods

5.2.1 Reactor construction

One MEC for the high concentration experiment (MEC-HC) was constructed using a polypropylene block with an interior cylindrical chamber with a volume of 25 mL and a cross sectional area of 7 cm^2 . The cathode was a piece of pure nickel foil (7.0 cm^2 area, 99% purity, Shop-Aid, Inc) without any precious metal catalysts. For the low concentration experiment (MEC-LC), four MECs were constructed with the same cross sectional area (7 cm^2) but with a greater volume (45 mL). The cathode was the same material as the MEC-HC experiments but

with an area of 5.4 cm². The anode was a graphite fiber brush (2 cm long and 2.5 cm in diameter Mill-Rose, OH) and it was heat treated at 450⁰C for one hour (Wang et al., 2009).

5.2.2 MEC operation at high Cd concentration

The MEC was inoculated with effluent from existing lab scale MECs. The feed solution was prepared using sodium acetate (1.0 g/L NaCH₃COO) as substrate along with trace minerals and vitamins (Cheng et al., 2009) in 25 mM phosphate buffer solution (1.145 g/L Na₂HPO₄, 0.613 g/L NaH₂PO₄-H₂O, 0.078 g/L NH₄Cl, 0.033 g/L KCl). After the MEC start producing stable electric current, cadmium was added to the feed solution as CdCl₂ at an initial concentration of 2.5 mg/L-Cd (Table 5.1). The reactor was operated in a fed-batch mode over 7 consecutive cycles, including the first 7-day test cycle. After the initial test cycle, three 7-day cycles were repeated and followed by three 2-day cycles. The applied voltage was 0.9 V. The applied voltage and electric current in the reactor was controlled and measured by a multi-channel potentiostat instrument (MPG-2, BioLogic, France). The electric current in the reactor was measured every 20 min.

The influent and effluent samples were pretreated for cadmium analysis by acidifying them with 1 M nitric acid and filtering with syringe filters (pore size 0.45 µm, polyethersulfone membrane, VWR International, Canada).

Table 5.1: Operation conditions for MEC experiments

MEC Experiment	Initial Concentration (as Cd ²⁺)	Applied Voltage (V)	Experiment Duration (weeks)	Status of microorganisms at bioanode
MEC-HC	2.5 mg/L	0.9	5 (3 7-day cycles plus 3 2-day cycles)	Healthy
MEC-LC-100	100 µg/L	0.6	18 (7-day cycles)	Healthy
MEC-LC-50	50 µg/L	0.6	18 (7-day cycles)	Healthy
MEC-LC-10	10 µg/L	0.6	18 (7-day cycles)	Healthy

MEC-LC-10a	10 µg/L	0.6	18 (7-day cycles)	Unhealthy
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After the 7 fed-batch cycles with cadmium, the MEC-HC was disassembled and a substantial amount of precipitants were observed on the cathode similar to our recent study (Colantonio and Kim, 2016). The precipitants were dissolved in 21 mL 0.73 M nitric acid for 48 hours and the acid solution was filtered using the syringe filter. After the precipitants were removed, the nickel foil cathode, including electrodeposited cadmium on its surface, was completely dissolved in 5 mL of 70% (v/v) nitric acid for 48 hours. The acid solution was diluted (1:10) for cadmium analysis.

5.2.3 MEC operation at low Cd concentrations

The four MEC-LCs were inoculated with effluent from an existing MEC and operated at a fed-batch mode using the same feed solution as MEC-HC without cadmium. After the MEC-LCs started producing stable electric current, cadmium was added as CdCl₂ for the designated concentrations (Table 5.1). The applied voltage was 0.6 V and electric current was measured every 60 min using the potentiostat. The fed-batch operation with cadmium was repeated over 18 weeks.

After the 18-week operation, the MEC-LCs were disassembled and the cathodes were removed for further analysis. There were no visible precipitants on the cathode and the cathode surface was clean and shiny with a slight decolourization. The nickel foil cathode was completely dissolved in 5 mL of 70% (v/v) nitric acid for 30 hours and then diluted for cadmium analysis.

5.2.4 Measurements

Cadmium concentration of experimental samples was determined in ICP-OES (inductive coupled plasma-optical emission spectrometry) (Vista Pro, Varian Inc., Australia). The conductivity and pH of feed and effluent were measured during the MEC operation (SevenMulti, Mettler-Toledo International Inc., OH). During the MEC-HC operation, the conductivity decreased slightly from 4.98 to 4.53 mS/cm and pH was stable between 7.0 and 7.2. For the MEC-LC operation, the conductivity and pH were stable at 4.61 mS/cm and 7.4, respectively. All experiments were conducted in an air-conditioned laboratory and the temperature was stable at $22.7 \pm 0.6^\circ\text{C}$.

5.3 Results and discussion

5.3.1 Electrodeposition vs. precipitation at high Cd concentration

Rapid cadmium removal was achieved within two days (88%) and an extra 5 days of operation resulted in only a 5% increase in the percent removal (Fig. 5.1). The lowest effluent concentration was 0.072 mg/L after 2 days, indicating efficient cadmium removal in MECs. Based on the influent and effluent concentration, the MEC-HC removed a total of 0.34 mg of cadmium over the 6 fed-batch cycles (three 7-day cycles followed by three 2-day cycles).

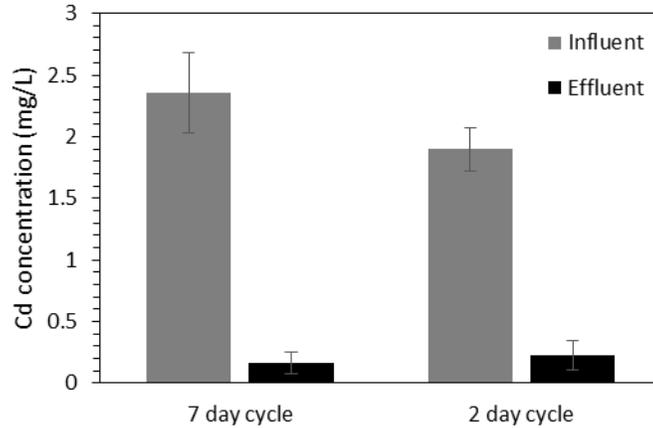


Figure 5.1: Cd²⁺ removal in 7-day and 2-day cycles in MEC-HC experiment

When the MEC-HC was disassembled after the 6 fed-batch cycles, a total of 0.044 mg of cadmium was recovered from the surface of the cathode as precipitants while 0.167 mg of cadmium was in the metallic form as a part of the cathode (Table 5.2). This result indicates that the majority of the removed cadmium was recovered on the cathode in the form of cadmium crystals and metallic cadmium (67%). Among the recovered cadmium from the cathode, 79% of the mass was electrodeposited on the cathode while the rest of 21% was present as cadmium crystals.

Table 5.2 Quantification of Cd²⁺ removal mechanisms in MEC-HC experiment

Cd ²⁺ precipitants on cathode (mg)	Cd ²⁺ electrodeposited on cathode (mg)	Fraction of Cd ²⁺ recovered by electrodeposition (%)	Fraction of Cd ²⁺ recovered by precipitation (%)	Total Cd ²⁺ Removed (mg)	Total Cd ²⁺ Recovery (%)
0.044	0.167	79.30	20.97	0.317	66.60

The electric current in the MEC-HC ranged from 0.51 to 0.98 mA with an applied voltage of 0.9 V (Fig. 5.2). During the 7 day fed-batch cycles, the current dropped after 6 days of operation while the current was constantly high for the 2-day fed-batch cycles. This consistently

high electric current condition ensures that there were no losses of cadmium precipitants, such as $\text{Cd}(\text{OH})_2$ and CdCO_3 , by dissolution as recently demonstrated (Colantonio and Kim, 2016). Therefore, the dominant cadmium removal by electrodeposition (79%) compared to precipitation (21%) was not affected by potential dissolution of precipitated cadmium crystals.

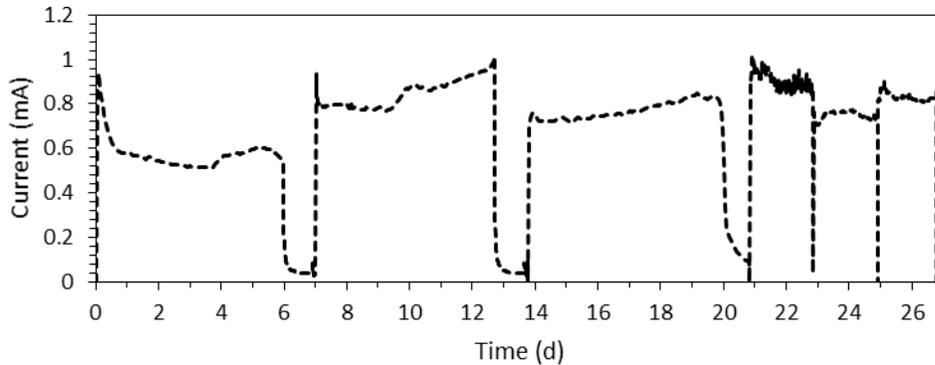


Figure 5.2: Electric current in MEC-HC with 0.9 V application

Approximately 33% (0.10 mg) of the cadmium removed in the experiment was not recovered through electrodeposition or precipitation. Biosorption of cadmium by anode bacteria can explain the missing 33% of cadmium removed as previously demonstrated (Abourached et al, 2014). The contribution by biosorption was not negligible in this study mainly because the experiment with cadmium was relatively short with 6 consecutive fed-batch cycles and one initial test cycle. Note that the HC-MEC was not exposed to cadmium before the 7 fed-batch cycles. If the experiment had been conducted for an extended period of time, potential contributions by biosorption would have been much smaller than 33%.

5.3.2 Importance of cathode configuration

Over the 6 fed-batch cycles during the HCMEC experiment, the average effluent cadmium concentration was 0.20 ± 0.10 mg/L, which is substantially smaller than 1.32 ± 0.78

mg/L observed in our recent study (averaged from 4 effluent concentrations for experiments without dissolution of cadmium precipitants) (Colantonio and Kim, 2016). This substantial difference in the effluent quality can be explained by the cathode material configuration and reactor orientation. In this study, the cathode was a plain piece of nickel foil and it was located horizontally below the anode brush on the bottom of the HC-MEC reactor. However, in the previous study, woven stainless steel mesh (100 mesh) was used as the cathode and located vertically in the MEC reactors (Colantonio and Kim, 2016); as a result, it is highly feasible that a certain fraction of precipitated cadmium crystals were detached from the cathode surface (where local pH is high) and dissolved into the aqueous solution, leading to relatively high cadmium concentration in the effluent. Thus, the orientation and material configuration of cathode materials are important design factors when precipitation is an important mechanism for toxic heavy metal removal using MECs.

While this study confirms that foil type cathodes without open spaces resulted in better cadmium removal, another previous study indicated that mesh type cathodes were more efficient than shim type cathodes for struvite precipitation (Cusick and Logan, 2012). This inconsistency implies that cadmium crystals (e.g., $\text{Cd}(\text{OH})_2$, CdCO_3) are not strongly bound to cathode surfaces and thus they are easily detached from the cathode. Thus, the cathode configuration and orientation need to be carefully designed not to lose precipitated crystals if MECs are used to remove cadmium.

5.3.3 Cd removal by electrodeposition at low concentration

The mass of cadmium recovered from the cathode in healthy LC-MECs (LC-MEC-100, LC-MEC-50, LC-MEC-10) ranged from 7.8-10.2% of cadmium added over the 128 days of operation (Table 5.3). No clear trends between the recovered cadmium amount and feed

concentration (10, 50, 100 µg-Cd/L) were found. Note that the recovered cadmium was obtained when the nickel foil cathode was completely dissolved in 70% (v/v) nitric acid. When the LC-MEC reactors were disassembled, there were no visible precipitants on the surface of the cathode, indicating that electrodeposition is responsible for the recovered cadmium at the cathode.

Table 5.3: Cd²⁺ recovered in MEC-LC Experiment

Reactor	Total Cd Added (mg)	Cd deposited on cathode (mg)	Cd recovered (%)
MEC-LC-50	0.0472	0.0037	7.75
MEC-LC-100	0.0945	0.0077	8.19
MEC-LC-10	0.00945	0.00096	10.17
MEC-LC-10a	0.00945	0.00027	2.85

The relatively low recovery from the cathode (7.8-10.2%) does not necessarily mean limited cadmium removal at the MEC cathode because biosorption is expected to have contributed to cadmium removal as previously demonstrated (Abourached et al, 2014). Note that the missing amount of cadmium in each of the healthy LC-MECs (LC-MEC-100, LC-MEC-50, LC-MEC-10) was 0.04, 0.09, 0.008 mg, respectively. In a previous study, a substantial amount of cadmium (0.64-0.87 mg) was removed only by biosorption on a 1.8-cm² carbon cloth anode (1.8 cm²) (Abourached et al., 2014), indicating that biosorption has substantial capacity for cadmium removal. Therefore, it is reasonably concluded that biosorption accounted for the missing amount of cadmium in our experiments.

5.3.4 Importance of exoelectrogenic activity

Cadmium recovery from the LC-MEC-10a cathode was substantially small (2.9%) compared to the recovery in LC-MEC-10 (10.2%) even though they were fed with the same

cadmium concentration (10 µg/L). During the MEC operation over 18 weeks, the electric current in LC-MEC-10 was consistently low (0.006 mA on average, 0.02 mA maximum) (Fig. 5.3). The substantially low magnitude of current led to a lower capacity for cadmium removal at the cathode (Table 5.3). Thus, high exoelectrogenic activity is necessary to remove cadmium at low concentration via electrodeposition on the cathode.

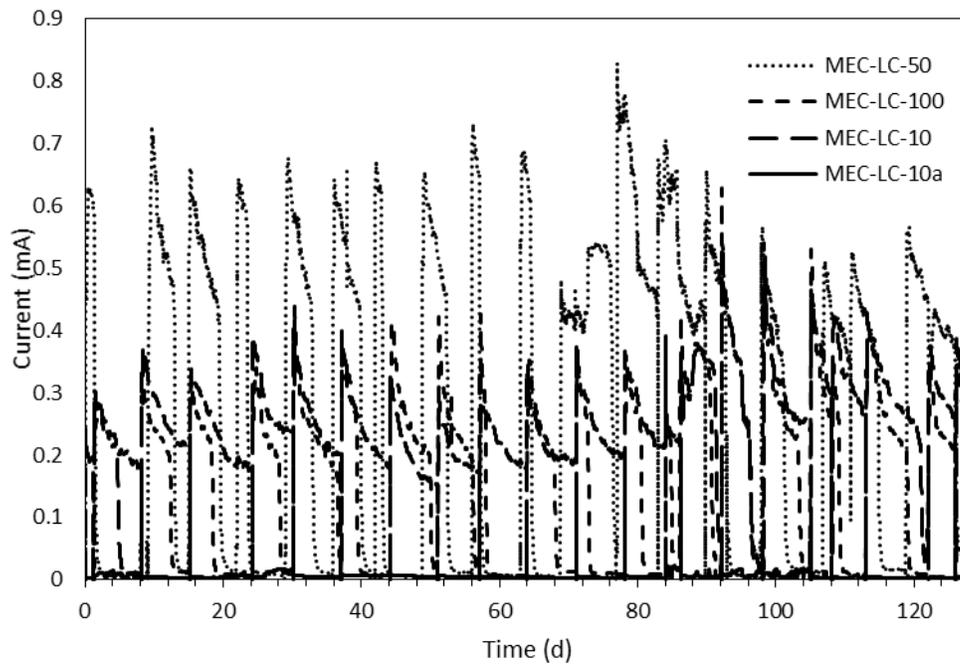


Figure 5.3 Electric current in MEC-LC experiment with 0.6 V application

5.4 Conclusions

This study provided a better understanding of cadmium removal at relatively low concentration and neutral pH conditions by quantifying cadmium chemically and electrochemically deposited on MEC cathodes. The experimental results also confirmed that cadmium removal is rapid in MECs, requiring only two days for 88% removal. At the relatively high concentration (2-2.5 mg/L), the cathode was responsible for 67% of removed cadmium.

Among the total amount cadmium removed at the cathode, we quantified the contribution by the two removal mechanisms: chemical precipitation by high local pH near the cathode; and electrodeposition by cathodic reduction to metallic cadmium. For the given experimental conditions, the majority of cadmium was removed by electrodeposition on the cathode (79%) while the rest (21%) was separated through cadmium precipitants on the cathode.

During the long term MEC operation (18 weeks) at low cadmium concentrations (10, 50, 100 $\mu\text{g/L}$), chemical precipitation of cadmium was not observed, indication that the removal at the cathode was achieved solely via electrodeposition. The fraction of electrodeposited cadmium at the cathode was 8-10% of the total amount cadmium fed in the reactor over 18 weeks. In addition, the cadmium removal at the cathode was sensitive to the exoelectrogenic activity of the anode.

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6. Conclusions

6.1 Pb²⁺ removal mechanisms in microbial electrolysis cells

Removal of Pb²⁺ was rapid (77-95% in 2 days) either with applied voltages or at open circuit conditions. It was found that Pb²⁺ was removed by electrochemical reduction at the anode, along with cathodic reduction as previously demonstrated. The multiple removal mechanisms allowed rapid heavy metal removal in MECs. Inactivation of microorganisms at the bioanode resulted in no Pb²⁺ removal, confirming the importance of active microbes for Pb²⁺ reduction at both the anode and cathode. Operation at a neutral pH condition allowed active microorganisms at the anode and cathode, enhancing the removal of Pb²⁺ in MECs.

6.2 Cd²⁺ removal mechanisms in microbial electrolysis cells

It was confirmed that MECs can effectively remove Cd²⁺ from solution at relatively low concentrations (12.26 mg/L) with low energy consumption. Rapid Cd²⁺ removal was achieved, from 50-67% removal in 24 hours to 88% removal in 2 days. Although cathodic reduction is reported to be the major Cd²⁺ removal mechanism in MECs, it was proved that Cd(OH)₂ and CdCO₃ precipitation at the cathode are also an important removal mechanisms. To ensure effective removal by precipitation, the bioanode must be kept electrically active with organic substrates which allow continuous production of hydrogen at the cathode because hydrogen evolution reaction keeps the local pH high near the cathode to induce precipitation of Cd(OH)₂ and CdCO₃.

For cadmium concentrations at 2-2.5 mg/L, the cathode was responsible for 67% of the total amount of Cd²⁺ fed in the reactor. We quantified the contribution of the two removal

mechanisms at the cathode: chemical precipitation induced by high local pH near the cathode; and electrodeposition by cathodic reduction to metallic Cd²⁺. For the given experimental conditions, the majority of Cd²⁺ was removed by electrodeposition on the cathode (79%) while the remainder (21%) was recovered as Cd²⁺ precipitants on the cathode.

A long term experiment for Cd²⁺ removal in MECs at low concentrations (10, 50, 100 µg/L) resulted in 8-10% of Cd²⁺ recovered on the cathode via electrodeposition, while chemical precipitation of Cd²⁺. In addition, the Cd²⁺ removal at the cathode was sensitive to the exoelectrogenic activity of the anode.

6.3 Significance

This research clearly demonstrates a better understanding of the removal mechanisms in MECs for both Pb²⁺ and Cd²⁺. All experiments in this study used neutral pH conditions with relatively low concentrations of metals (10 µg/L – 12 mg/L) to simulate a more realistic wastewater effluent. Active microorganisms at the bioanode were found to play a key role in driving all removal mechanisms. Development of energy-efficient methods for heavy metal removal from wastewater will allow for safer applications of reclaimed wastewater and wastewater biosolids for various purposes.

6.4 Future Work

Since this study only looked into the removal of two individual metals in MECs, future studies should focus on simultaneous removal of multiple metals. Competitive interactions among various metals may alter the mechanisms of removal. Additionally, since high local pH conditions near the cathode are necessary for efficient metal precipitation, various design and

operation factors, such as mixing conditions, electric current, and cathode geometry, should be systematically explored in future studies. Finally, large scale demonstration is required for practical applications of MECs for energy- and cost-effective heavy metal removal from various types of wastewater.

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