RECOVERY OF SILVER FROM AQUEOUS SOLUTION
BY USING BIO-ELECTROCHEMICAL SYSTEM (BES) TECHNOLOGY

BY

HO NGO ANH DAO

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (ENGINEERING AND TECHNOLOGY)
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Abstract

RECOVERY OF SILVER FROM AQUEOUS SOLUTION BY USING BIO-ELECTROCHEMICAL SYSTEM (BES) TECHNOLOGY

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Large amount of silver (Ag) from global supply has been used in many industrial applications. Accordingly, the Ag(I)-laden wastewater, either in acidic or alkaline conditions, are normally discharged from these processes. Generally, Ag(I) exists in different forms such as free silver ions (Ag⁺), silver complexes, and multi-metal solutions. Therefore, for both economic and environmental aspects, recovery of silver from effluents, is essential.

In this study, dual-chamber BES reactors are used to investigate the recovery of silver from different Ag(I)-containing solutions, accompanied with power generation and wastewater treatment. In each BES reactor, carbon brush serves as the anode inoculated by anaerobic sludge, while graphite plate works as the cathode. Depending on the target of experiments, either anion exchange membrane (i.e. AMI-7001S) or cation exchange membrane (i.e. CMI-7000S) is used as a separator. The research objectives are (i) to investigate the electrochemical reduction of Ag⁺ ions introduced as silver nitrate AgNO₃; (ii) to examine the migration and diffusion of electrolytes through AMI-7001S and CMI-7000S membrane; (iii) to characterize the anodic microbial communities and identify the dominant species along with exoelectrogen; (iv) and to investigate the reduction of Ag(I) complexes (i.e. diammine complex ([Ag(NH₃)₂]⁺), dithiosulfate complex ([Ag(S₂O₃)₂]³⁻), and mixed multi-metal solution at different initial Ag(I) concentration, to understand the difference in BES performance between them.

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Results and findings obtained in this study are summarized as following:

(i) The reduction of Ag$^+$ ions from AgNO$_3$ solution in the cathode chamber was a fast reaction, which almost completed within 24 hours of operation. A change in initial catholyte pH (2-7) and Ag$^+$ concentration (100-1,000 mg/L) had little effect on Ag removal but significantly affected the power generation. After 24 hours, a removal efficiency (RE) of 96.54-99.28% and a maximum power density ($P_{\text{max}}$) of 607-8,258 mW/m$^3$ along with a Columbic efficiency (CE) of 4.36-21.61% were achieved when the initial Ag$^+$ concentration was varied from 100 to 1,000 mg/L. These results did not take into account the diffusion of Ag$^+$ ions through CMI-7000S membrane.

(ii) Effects of AMI-7001S and CMI-7000S membranes on the overall performance in BES reactors operated with AgNO$_3$ solutions was demonstrated. A RE of 83.73-92.50%, and CE of 11.50-19.89% were obtained in CMI-based reactor after 24 hours with the initial Ag$^+$ concentration of 100-1,000 mg/L, although some diffusion of Ag$^+$ ions was observed. In contrast, the substrate loss through AMI-7001S caused low cell voltage production, and led to lower silver removal and power density in AMI-based reactor. This finding suggests that CMI-7000S showed better performance when compared to AEM-7001S at the same operational conditions.

(iii) After running BES reactors for 73 days with AgNO$_3$ solutions as the catholytes, the gene sequence results showed a considerable phylogenetic diversity in the anodic microbial communities, in which Firmulates, Proteobacteria, and Bacteroidetes were dominant phyla. Geobacter sp. and other reported exoelectrogenic bacteria could be detected while no Shewanella-like sequences were retrieved.

(iv) The reduction of silver complexes and mixed-metal solutions in BES reactors was also found in similar trends. However, as compared to free Ag$^+$ ions which has high standard reduction potential ((Ag$^+$/Ag$^0$, $E^0 = 0.799$ V versus the Standard Hydrogen Electrode (vs. SHE))) to facilitate the spontaneously electrochemical reduction in the cathode chamber, [Ag(NH$_3$)$_2$]$^+$ and [Ag(S$_2$O$_3$)$_2$]$^{3-}$ complexes showed a slower reduction rate due to their lower standard $E^0$ (i.e. [Ag(NH$_3$)$_2$]$^+/Ag^0$, $E^0 = 0.373$ V and [Ag(S$_2$O$_3$)$_2$]$^{3-}$/Ag$^0$, $E^0 = 0.016$ V vs. SHE)).

- The reduction of [Ag(NH$_3$)$_2$]$^+$ complex (1000-3000 mg/L) could be completed with RE > 96% when the duration was extended to 48 hours. Similarly to Ag$^+$ ions, [Ag(NH$_3$)$_2$]$^+$ complex also transported through CMI-7000S membrane, which led to
the formation of silver deposits at the membrane surface and Ag adsorption in the anodic biomass. This diffusion affected the internal resistance and decreased the electricity production performance (i.e. $P_{\text{max}}$) when the system was operated at high initial Ag(I) concentration (i.e. 3000 mg/L). The accumulated amount of silver in the biomass (i.e. mixture of suspended solid and the biofilm attached to the carbon brush anode) after operating BES reactor with $[\text{Ag(NH}_3\text{)}_2]^+$ solutions at the initial concentration of 1000 – 3000 mg Ag/L was 0.853 mg Ag/g biomass.

- In case of $[\text{Ag(S}_2\text{O}_3\text{)}_2]^3-$ solutions (10, 20, and 30 mM), although lower RE was found (i.e. 81.87, 95.17, and 93.22%, respectively) at the same operation time (48 hours), there was no loss of Ag as the diffusion of negatively charged $[\text{Ag(S}_2\text{O}_3\text{)}_2]^3-$ complex through CMI-7000S was avoidable. Electricity (i.e. a CE of 12.85 – 25.73%, a $P_{\text{max}}$ of 1500 – 2893 mW/m$^3$) was produced simultaneously without any supplying of external energy, indicating the reactions could occur spontaneously in spite of low cathodic reduction potential (i.e. $[\text{Ag(S}_2\text{O}_3\text{)}_2]^3-/\text{Ag}^0=0.016 \text{ V vs SHE}$).

- When synthetic mixed metal solution containing 10 mM Ag$^+$, 10-20 mM Fe$^{3+}$, and 1 mM Cu$^{2+}$ was investigated, the cathodic reduction occurred differently, due to the difference in the standard reduction potential among these metallic ions (i.e. $\text{Ag}^+/\text{Ag}^0=0.799 \text{ V}, \text{Cu}^{2+}/\text{Cu}^0=0.337 \text{ V}, \text{and Fe}^{3+}/\text{Fe}^0=-0.44 \text{ V vs. SHE}$). During the first 24 hours, Ag$^+$ concentration (10 mM) reduced rapidly, while Fe$^{3+}$ concentration (10 mM) slightly decreased, and Cu$^{2+}$ concentration (1 mM) was almost constant. A high Ag removal efficiency of 99.71% was obtained in this case. However, the diffusion of Ag$^+$, Fe$^{3+}$, and Cu$^{2+}$ through the membrane was observed, which is believed as the main reason for high removal efficiency of Cu (86.84%) and Fe (33.89%) after 48 hours. For electricity production, the increase of initial Fe$^{3+}$ concentration, from 10 to 20 mM, helped to improve OCV and $P_{\text{max}}$ value (i.e. OCV increased from 514 to 645 mV, $P_{\text{max}}$ increased from 1542 to 2472 mW/m$^3$).

(v) After BES operation with different Ag(I)-containing solutions, different morphologies of silver deposits formed at the cathode surfaces were found under SEM, in which dendrite and crystal were the main structures. High purity of silver in the cathodic deposits was confirmed by EDS and XRD analysis, indicating one-step Ag recovery by BES technology, as compared to traditional methods for silver recovery where further steps for refinement are required.
In conclusion, this study successfully demonstrated that BES technology can be employed for recovery of silver from different Ag(I)-containing solutions, coupled with wastewater treatment and electricity production without energy consumption. The vital roles of bacteria, especially exoelectrogen, in BES reactors was pointed out, indicating BES as a cost-effective method for the recovery of silver in particular and other metals in general.

**Keywords:** Bio-electrochemical System, Microbial Fuel Cell, Silver Recovery, Power Generation, Exoelectrogen
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Chapter 1
Introduction

1.1 Problem statement

Silver (Ag) is a naturally occurring element and a precious metal which is recognized as currency under ISO 4217. It is also used in large quantities for many purposes (e.g. industrial and decorative uses, photography, jewelry, silverware). However, world silver production was reported to be insufficient to meet the demand, which steadily increases annually. Statistics showed that the global silver demand in 2014 was 33,177 tons, which was higher than the supply capacity of mine production (27,293 tons) (GFMS, 2015). Therefore, silver recovered from secondary sources (i.e. wastes and wastewater) plays an important role in decreasing the primary sources which mainly depend on mine production. According to GFMS (2015), these secondary sources contributed 15.87% of total supply to the global silver market in 2014.

On the other hand, the effluents from the industrial processes, such as plating industry and photographic manufacturing, normally contain Ag with high concentration. Waste fixer solutions from photographic manufacturing, for instance, contain Ag in the range of 5,000-10,000 mg/L (Chen et al., 2012). Furthermore, the waste composition is also relatively complex, in which a variety of organic and inorganic substances, such as ammonia, EDTA, thiosulfate, usually exist (Chen et al., 2012; Wang et al., 2013; Yazici et al., 2011). Depending on the silver compound, and the medium in which it exists, the magnitude of Ag toxicity is different. The potential for effects in the water environment and living organisms, thus, is based not only on the total concentration of Ag, but more importantly on the chemical form of Ag. Free silver ion (Ag⁺) generally acknowledged to be the most toxic form of silver to aquatic organisms (Purcell and Peters, 1998). A study reported that the maximum acceptable toxicant concentration (MATC) of free silver ions (Ag⁺) in aquatic cladoceran species (i.e. Ceriodaphnia dubia, Daphniamagna) was a very low level (i.e. 9.61 and 3.00 µg/L, respectively) (Naddy et al., 2007). Accordingly, the discharge of silver-rich wastewater or the aqueous solutions into the environment is regulated stringently. The maximum daily discharged Ag concentration into surface water is 0.22 mg/L according to
Environmental Protection Agency (EPA) (USA, 2000). Moreover, silver is a valuable metal and is not considered as a waste requiring disposal, but as a recoverable material. Therefore, for both economic and environmental aspects, recovery of silver from the effluents is essential.

Metallic silver can be recovered from different silver-rich effluents, in which Ag ions exist in the form of either simple silver salts (e.g. silver nitrate $\text{AgNO}_3$) or complexes (e.g. silver(I) monothiosulfate $[\text{Ag}($$\text{S}_2\text{O}_3$)$$]^- \text{ or silver(I) dithiosulfate}$ $[\text{Ag}($$\text{S}_2\text{O}_3$)$_2$$]^3-$, silver(I) diammine $[\text{Ag}($$\text{NH}_3$)$_2$$]^+\text{). Several studies have investigated the recovery of Ag from mining wastewater (Koseoglu and Kitis, 2009), spent electrical and electronic wastes (Aktas, 2010), and photographic effluents (Bas et al., 2012; Chen et al., 2012; Petrova et al., 2013; Yazici et al., 2011). Among these waste sources, photography has been a major industry for silver consumption and silver recovery, although there is a decrease of silver-based imaging due to the growth of digital photography in the last decade. Statistic showed that about 18-20% of the world’s silver demand is supplied by recycling photographic wastes and wastewater (Petrova et al., 2013). However, most studies used traditional technologies for Ag recovery, such as membrane filtration, electrolysis, chemical precipitation, electrochemical, and cementation. Many disadvantages still exist with these technologies, such as high cost due to energy and chemical intensive, by-product generation, and requirement of further refinement processes. For instance, the electrochemical process required high energy consumption of 3.81 kWh per 1 kg of Ag recovered (Raju et al., 2009). Trimercapto-s-triazine (TMT) was demonstrated as an effective organic precipitant for Ag recovery, but its high cost was also a disadvantage since a large amount of TMT was consumed for precipitation (Ag: TMT molar ratio = 2.2: 6.6) (Yazici et al., 2011). Similarly, the cementation has the ability to recover silver, but sacrificial metals as metallic powders (e.g. Fe) are required, and the recovery efficiency obtained was low (35%) (Petrova et al., 2013). Besides, the extraction of pure Ag from deposits onto the iron surface is also a drawback of this method. Therefore, more effort has been urged to develop innovative and cost-effective methods for the recovery of Ag in particular and other metals in general.

Recently, BES technology has been demonstrated as an attractive approach in recovery of metals from aqueous solutions (Choi, 2014; Mathuriya and Yakhmi, 2014;
Most BES reactors consist of an anode and a cathode chamber, which are normally separated by an ion exchange membrane to prevent the cross-over of electrolytes. Proton, cation, and anion exchange membranes (PEMs, CEMs, and AEMs) are used frequently in BES reactors. In principle, BES employs microorganisms in the anode chamber to anaerobically oxidize the organic matter to produce electrons, which are then transferred through an electrical circuit to the cathode to supply for the reduction of metal ions. In a closed electrical circuit, in order to maintain the electro-neutrality, either negative charge equivalents (anions/hydroxide ions) migrate from the cathode to the anode through AEM or positive charged ions (cations/protons) migrate from the anode to the cathode through CEM or PEM. The organic substrates as electron donors in the anolyte can be either simple organic molecules (e.g. acetate, glucose) or complex wastewaters (e.g. protein-rich wastewater), whereas metal ions in the catholyte are terminal electron acceptors (TEAs). By acting as TEAs, metal ions are reduced electrochemically, and metallic deposits are formed at the cathode surface, which can be considered as a recovered product by BES technology.

Some studies have investigated the feasibility of BES technology for recovery of silver from AgNO$_3$ solution (Choi and Cui, 2012; Lim et al., 2015; Tao et al., 2012). This acidic solution containing free silver ions (Ag$^+$), was used as the model catholyte due to its positive standard reduction potential ($\text{Ag}^+/\text{Ag}^0$, $E^0 = 0.799$ V versus the Standard Hydrogen Electrode (vs. SHE) (Milazzo and Caroli, 1978)). This facilitate the spontaneous reduction of Ag$^+$ ions in the catholyte. The effects of operational parameters on BES performance, including initial pH (2.0 – 6.6) and Ag$^+$ concentration (50 – 4000 ppm), were investigated. However, these studies either focused on the pH or initial Ag$^+$ concentration, while both of them actually are influential parameters that need to be investigated simultaneously. Furthermore, in the above studies, either PEM or AEM was used as a separator in dual-chamber BES reactors. Due to the difference in ion transfer mechanism through different membranes, the BES performance, characterized by Ag recovery efficiency, power generation, and morphology of Ag deposits formed on the cathode surface, may be affected. The migration and diffusion of electrolytes through these separator and their effects on the overall efficiency, however, were not studied. Small-scaled BES reactors (i.e. 50-100 mL of working
volume for each chamber) operated in a short time duration (i.e. 8-10 hours) used in these studies, may not clearly indicate the influence of membranes and transport phenomenon.

Recovery of silver from complex solutions (i.e. [Ag(S₂O₃)]⁻, [Ag(NH₃)₂]⁺) were also examined by other researchers. Tao et al. (2012) investigated the reduction of [Ag(S₂O₃)]⁻ solution along with Ag⁺ solution in independent BES reactors, and found that the reduction rate of [Ag(S₂O₃)]⁻ ions was slower than that of Ag⁺ ions due to its lower reduction potential ([Ag(S₂O₃)]⁻/Ag⁰, E⁰ = 0.25 V vs. SHE). Meanwhile, Wang et al. (2013) used the alkaline chelated solution [Ag(NH₃)₂]⁺ ([Ag(NH₃)]⁺/Ag⁰, E⁰ = 0.373 V vs. SHE) as a catholyte in a bio-electrochemical cell to recover Ag and electricity production. However, in the above studies, the operation was carried out at only one level initial concentration of Ag (i.e. 1 mM [Ag(S₂O₃)]⁻, 10 mM [Ag(NH₃)₂]⁺), and the effect of initial [Ag(NH₃)₂]⁺ concentration was not considered. Also, the performance in electricity production found by Wang et al. (2013) was so low due to the use of high internal resistance materials in their reactor (e.g. bipolar membrane). In addition, the silver(I) dithiosulphate complex ([Ag(S₂O₃)₂]³⁻) holding a very low reduction potential ([Ag(S₂O₃)₂]³⁻/Ag⁰, E⁰ = 0.016 V vs. SHE (Milazzo and Caroli, 1978)), which may not be favorable for the spontaneous reduction in the cathode chamber, has not been studied yet. A detail comparison of BES performance among different Ag(I)-containing solutions, characterized by the power output, Ag(I) reduction rate, formation, and morphology of metallic silver deposits, is still missing.

Besides, real wastewater normally contains other metal ions, beside Ag⁺ ions, which may compete with Ag⁺ to accept electrons, thus, affect the Ag⁺ reduction rate. The reduction of other metallic ions in the catholyte may occur simultaneously or sequentially, depending on their reduction potential. Thus, further studies should be carried out with mixed multi-metal solutions containing different metallic salts to investigate their effects on the BES performance.

In addition, BES performance also depends on the microbial community, substrate conversion rate, and extracellular electron transfer (EET) in the anode chamber. Most BES studies normally use anaerobic sludge from digesters or sedimentation tanks of wastewater treatment plants to inoculate the bio-anodes. Compared to single strains, mixed cultures have attracted more attention due to their
higher adaptability, stability, and productivity (Mathuriya, 2013; Patil et al., 2009). The bacterial characterization of several MFC systems with different operational conditions (e.g. inoculum sources, substrate types), showed very different and diverse microbial communities (Bond et al., 2002; Choo et al., 2006; Holmes et al., 2004; Kim et al., 2004; Kim et al., 2007; Liu et al., 2007; Logan et al., 2005; Phung et al., 2004; Rabaey et al., 2004). Electrochemically active bacteria (EAB), participating in electricity generation, have been reported frequently as iron-reducing bacteria (i.e. Geobacter sp. and Shewanella sp.) and phototrophic bacteria (i.e. Rhodopseudomonas sp.) due to their prominent roles in most BES studies. However, there may be other unknown species, contributing to electricity generation through a variety of mechanisms, which need to be identified.

Consequently, it is essential to conduct further studies on BES technology for recovering of Ag from different aqueous solutions to tackle the above issues. Deeper understanding of vital factors, such as types of Ag(I) solution and initial Ag(I) concentration, types of membranes and ion transfer mechanisms, type of substrate and inoculum sources, microbial community and exoelectrogen, and their effects on BES performance, are expected to achieve.

1.2 Research objectives

The general objective of this study is to investigate the recovery of silver from different Ag(I)-containing solutions (i.e. Ag⁺ from silver nitrate AgNO₃, silver(I) diammine complex [Ag(NH₃)₂]⁺, silver(I) dithiosulfate complex [Ag(S₂O₃)₂]³⁻, mixed multi-metal solutions), accompanied with power generation and wastewater treatment by using dual-chamber BES reactors. This helps to clarify the differences in BES performance among different Ag(I)-containing solutions and contribute a better understanding of BES technology for recovery of silver from wastewater toward practical applicability.

Depending on types of Ag(I)-containing solution investigated, specific objectives are proposed as following:

(1) To understand the effect of initial pH, Ag concentration, substrate and inoculum, and separators on BES performance characterized in terms of silver removal efficiency, power generation, and electrodeposits composition when using solutions containing free Ag⁺ ions.
To investigate the electrochemical reduction and deposition of Ag at cathode from \([Ag(NH_3)_2]^+\), \([Ag(S_2O_3)_2]^{3-}\) complexes, and Ag\(^+\) in multi-metals solutions (i.e. Ag\(^+\), Fe\(^{3+}\), Cu\(^{2+}\)) at different initial Ag concentrations. The overall BES performance was observed through silver removal efficiency, cell voltage production, maximum power density, and internal resistance.

1.3 Scope of research

In order to obtain the above objectives, different lab-scale experiments were conducted in independent BES reactors, corresponding to types of Ag(I)-containing solution serving as catholytes. In all of experimental batches, the anolyte (i.e. substrate as either acetate or glucose) with initial COD of 1000 mg/L along with medium, was always refreshed each time the catholyte was replaced.

(1) With Ag\(^+\) solution:
- To investigate the correlation of initial pH and Ag\(^+\) concentration: the experiments are carried out at initial pH of 2.0-7.0 and Ag\(^+\) concentration of 100-1000 mg/L for 24h of operation time;
- To understand the effect of substrate and inoculum sources: the study uses 2 types of substrate (i.e. acetate and glucose) and 2 inoculum sources obtained from different wastewater treatment plants to clarify the difference in acclimation of the microbial community and the cell voltage progression during the inoculation stage of 2 independent BES reactors;
- To evaluate the effect of separators on BES performance Two common ion exchange membranes, including AEM as AMI-7001S and CEM as CMI-7000S, are employed in two reactors operated at the initial Ag concentration of 200-2000 mg/L, pH 2.0.

(2) With \([Ag(NH_3)_2]^+\) complex:
- To investigate the reduction of \([Ag(NH_3)_2]^+\) complex in the cathode chamber: the BES operation is conducted at different initial Ag concentrations (1000-3000 mg/L), and the influence of \([Ag(NH_3)_2]^+\) diffusion through CMI-7000S membrane on Ag removal efficiency and power output is also investigated;
- To investigate the improvement in electricity output, supporting electrolyte (i.e. NaNO\(_3\), 5-10 mM) is added to the \([Ag(NH_3)_2]^+\) solution containing 2000 mg Ag/L.
(3) With $[\text{Ag(S}_2\text{O}_3\text{)}_2]^{3-}$ complex
- To investigate the spontaneous reduction of $[\text{Ag(S}_2\text{O}_3\text{)}_2]^{3-}$ complex: the cathode chamber is fed with 10-30 mM $[\text{Ag(S}_2\text{O}_3\text{)}_2]^{3-}$ solutions. The non-diffusion of $[\text{Ag(S}_2\text{O}_3\text{)}_2]^{3-}$ through CMI-7000S membrane is clarified and demonstrated.

(4) With mixed multi-metal solution
- To demonstrate the highest priority of Ag$^+$ to be reduced spontaneously from mixed-metal solutions containing 10 mM Ag$^+$, 1 mM Cu$^{2+}$, and 10 mM Fe$^{3+}$
- The effects on power generation and Ag reduction rate is evaluated by increasing the Fe$^{3+}$ concentration from 10 mM to 20 mM.
Chapter 2
Literature review

2.1 Silver-laden wastewater sources

The industrial manufactures, such as electronic, plating, mining, jewelry, generate a large amount of silver-laden wastewater, which is normally separated from municipal wastewater for pre-treatment before discharging into the sewage system. Specific Ag(I)-containing wastewater and effluents from different processing are summarized in Table 2.1. Depending on types of industry, silver in the effluents exists in different forms (i.e. free silver ion Ag\(^+\), Ag complex) with a wide range of concentration.

Table 2.1 Sources of silver-laden wastewater

<table>
<thead>
<tr>
<th>Types of industry</th>
<th>Existing form</th>
<th>Quantities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mining industry</td>
<td>Cyanide complex</td>
<td>50 mg Ag/L</td>
<td>(Koseoglu and Kitis, 2009)</td>
</tr>
<tr>
<td></td>
<td>AgCN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photography processing</td>
<td>Thiosulfate complex</td>
<td>2700 - 3300 mg Ag/L</td>
<td>(Chen et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>in aqueous solutions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray film processing</td>
<td>[Ag(S(_2)O(_3))(_2)](^{3-})</td>
<td>1100 mg Ag/L</td>
<td>(Bas et al., 2012)</td>
</tr>
<tr>
<td>Button cell manufacturing</td>
<td>Solid waste Ag(_2)O</td>
<td>62.09 (wt.%) of cell powder</td>
<td>(Aktas, 2010)</td>
</tr>
</tbody>
</table>

Among industrial applications, photography has been a major silver consumption and generation of silver-rich solutions. In photographic processing, silver halides (e.g. AgBr, AgI) is used in the production of photographic films and X-ray films due to its prominent photosensitivity. When exposed to light, some part of silver-halides emulsion on the film will be reduced to metallic silver by light (Eq. 2.1), and this exposure results in the formation of a “latent image” (Wang et al., 2004). This nearly invisible latent image is then developed through a chemical process known as development to produce metallic silver image (Eq. 2.2). Subsequently, the remainder of the emulsion containing undeveloped silver halide is removed by complexing with excess sodium or ammonium thiosulphate fixing solution (Eq. 2.3).
Accordingly, the fixer waste solution contains silver at high concentration, which is considered as a silver-rich effluent. Depending on types of chemicals used, the silver amount found in fixer waste varies in a wide range of 5,000-10,000 mg/L and pH of 6.5-9.0 (Chen et al., 2012; Wang et al., 2004). Furthermore, the waste composition is also relatively complex, in which a variety of organic and inorganic substances, such as ammonia, EDTA, thiosulfate, usually exist. Basically, silver in photographic processing waste occurs in the form of tightly bound silver thiosulfate complex, predominantly as $[\text{Ag(S}_2\text{O}_3\text{)}_2]^{3-}$. It was reported that this complex is extremely stable, with a dissociation constant ($K_d$) of $5.0 \times 10^{-14}$, which makes it virtually impossible for free silver ions ($\text{Ag}^+$) to exist in the photographic effluents (Purcell and Peters, 1998; Wang et al., 2004). Unlike free silver ion ($\text{Ag}^+$), which is toxic to microorganisms and living things, $[\text{Ag(S}_2\text{O}_3\text{)}_2]^{3-}$ complex is relatively non-toxic and has no detrimental effect on the operation of wastewater treatment plant when it is discharged into the photographic effluent (Wang et al., 2004).

Another common source producing silver-rich effluent is mining industry, in which the cyanidation process has been the most important process in the extraction of silver from ores at mining sites. Due to insufficient leaching effectiveness of cyanidation process, a significant quantity of silver is present in the wastes of mining industry. Silver exists in the form of soluble cyanide complex ($\text{AgCN}$) and also low-soluble $\text{AgCl}$ particles.

### 2.2 Traditional technologies for Ag recovery and their limitations

Due to the precious feature and limited availability of $\text{Ag}$, a variety of technologies have been studied to recover $\text{Ag}$ from wastewater and aqueous solutions. Traditional methods, such as electrolysis, metallic replacement or cementation, and chemical precipitation, have been investigated by several researchers. Some recent studies are presented below.
2.2.1 Electrolysis

Electrolysis (EL) technology is a well-known process for removal and recovery of Ag from wastewater or aqueous wastes. In EL process, a direct current (DC) is applied between two electrodes (i.e. anode and cathode), at which different redox reactions occurs. The negative charged ions flow to the anode and will be oxidized, while the positive charged ions (i.e. Ag\(^+\)) flow to cathode and will be reduced. Metallic silver deposits form at the cathode surface, which then can be recovered. The EL process is suitable for Ag(I)-containing solutions with high concentration, and is capable of producing Ag with purity greater than 98%.

Chen et al. (2012) investigated the recovery of Ag from a waste fixer solution of a photographic shop (Kodak, Taiwan) by using EL technology. The waste fixer solution contain high Ag concentration (2700-3300 mg/l) at pH of 6.09. The EL experiment used a stainless steel as the cathode and graphite plate as the anode. A variation of operational parameters, such as pH (3-10), electric current (0.1, 0.3, 0.5, 0.7, 0.9 A), and electrolysis duration (0-8 h), were evaluated. Results showed that the optimum conditions of electrolysis for Ag recovery were electric current of 3 A, pH 8 and a duration of 2.5 hours. At these conditions, the highest Ag recovery efficiency of 99.55% was obtained.

However, high cost due to energy intensive is the main disadvantage of EL technology. Furthermore, EL is suitable only for silver-rich effluents and unable to reduce the silver levels below 100 mg/L. Therefore, further treatments to generate environmentally acceptable effluents (i.e. < 5 mg/L Ag) are required (Bas et al., 2012; Petrova et al., 2013; Yazici et al., 2011).

2.2.2 Metallic replacement or cementation

Basically, cementation bases on the use of an active solid metal, such as iron, zinc, and copper, to contact a solution containing a less active metal ion, normally noble or precious metal, such as silver, gold, rhodium. The reaction is generally composed of two redox half reactions, including reduction of the more active metal ion and oxidation of the less active metal. Ions of the more active metal are released into the solution, while atoms of the less one replace them in solid state.

Petrova et al. (2013) recovered Ag from a spent photographic fixer thiosulfate solution by using iron cementation in magnetically controlled particle beds. The
magnetically assisted fluidization is an efficient solid–fluid contacting technique for the performance of intensified mass and heat transfer operations in gas and liquid streams. However, the recovery efficiency obtained was low (i.e. 35%). In addition, the consumption of iron as a sacrificial metal is not an effective solution in term of cost. The product after cementation also needs further treatments to extract the pure silver, which leads to high cost.

### 2.2.3 Chemical precipitation

Chemical precipitation is one of the most traditional method for silver recovery. Several chemicals including sodium sulfide (Na$_2$S), sodium dithionate (Na$_2$S$_2$O$_4$), and 2,4,6-trimercapto-striazine (TMT; C$_3$N$_3$S$_3$) have been used as precipitating agents to recover silver from photo chemical processing wastes (Blais et al., 2008; Rivera et al., 2007; Yazici et al., 2011).

Recently, hydrogen peroxide (H$_2$O$_2$, 35%) was demonstrated as an effective precipitate for recovery of Ag from X-ray film processing effluent (Bas et al., 2012). Silver rich wastewater, collected from Farabi Hospital (Turkey), contained 1.1 g/l Ag$^+$ at pH 4.2. The experiments were conducted by using a full factorial design ($4^2$) to investigate the effects of H$_2$O$_2$ concentration (5.8–51.6 g/l) and pH (4.2–7) on the recovery efficiency. Results showed that high recovery ($\geq$ 95%) could be achieved at the H$_2$O$_2$ level of > 37.6 g/l and neutral/alkaline region. At lower concentration of H$_2$O$_2$, increasing pH was essential to obtain higher efficiency. The addition of ethylene glycol (0.5–10 ml) was also investigated to reduce the high consumption of H$_2$O$_2$. This could improve the efficiency from 1.3 to 18.7% at pH 4.2 and H$_2$O$_2$ of 22.4 g/l. However, the operational costs is so high. The cost of reagent (i.e. 37.6 g/L H$_2$O$_2$) was estimated to be ~$63/m^3$ of the effluent, which is corresponding to ~$61/kg of silver recovered. Further treatments of the silver product obtained in the peroxide process is also required to produce pure metallic silver.

### 2.2.4 Other technologies

The other unpopular technologies, such as membrane, adsorption, were also investigated for silver recovery from aqueous solutions (Kononova et al., 2007; Koseoglu and Kitis, 2009).

The hybrid cyanidation and high-pressure membrane process (NF or RO) was investigated to recover silver from aqueous waste (Koseoglu and Kitis, 2009). Synthetic
wastewater, in which the soluble AgCN complex was formed after cyanidation of low-soluble AgCl particles, was used. This solution simulate the leaching of Ag from real mining wastewater. Results showed that, the Ag recovery percentage obtained by this hybrid was 29-59\% and 54-62\% for NF and RO membrane, respectively. RO was thus considered as a favorable process because of higher Ag recovery, although a significant amount of Ag adsorbed on RO surface.

Kononova et al. (2007) investigated the recovery of silver from leach residues of lead–zinc sulfide concentrates using non-cyanide reagents — sodium thiosulphate and potassium thiocyanate. The adsorption of silver from these solutions was carried out with anion exchangers and carbon adsorbents, distinguished from each other by their physical structure and functional groups. Study showed that the strong base anion exchanger AV-17-10P and the carbon adsorbent LK-4 based on anthracite, possess the best sorption properties.

2.3 BES technology for metal recovery

2.3.1 Principle of BES technology

The bio-electrochemical treatment is a platform technology that offers a new approach for integrated waste treatment along with energy and resource recovery. Basically, BES consists of an anode and a cathode chamber separated by an ion exchange membrane (IEM), but differ in configuration which depends on target functions to be accomplished. In the anode chamber, the common principle is that biodegradable substrates, such as waste materials, are oxidized by microorganisms and generate electrons which are then transferred to the anode electrode through different extracellular electron transfer (EET) mechanisms (Nancharaih et al., 2015; Wang and Ren, 2013). The electron flow is then captured directly through an electrical circuit for electricity production or delivered to the cathode electrode to supply for the reduction of metal ions which acted as terminal electron acceptors (TEAs) (Mathuriya and Yakhmi, 2014; Nancharaih et al., 2015; Wang and Ren, 2014). All the BESs assisting metal recovery are associated with metal reduction in the cathode chamber. Depending on the reduction potential of recovered metal in the cathode, there are various approaches for recovering metals which can be classified into 4 categories of mechanisms (Figure 2.1) (Wang and Ren, 2014).
The membrane is optional

(A) Direct metal recovery using abiotic cathodes

(B) Metal recovery using abiotic cathodes supplemented by external power sources

(C) Metal conversion using bio-cathodes

(D) Metal conversion using bio-cathodes supplemented by external power sources

The first mechanism relates to direct reduction of metals on abiotic cathode, which have higher reduction potentials than that of organic compounds in anode chamber. The reduction of metal ions would occur spontaneously without external
power consumption. In the second mechanism, an extra power source would be supplied to promote the electrons flow from the anode to the abiotic cathode for reduction of low-potential metals. Due to the appearance of dissimilatory metal reducing bacteria species which use metal ions as the electron acceptors, the third mechanism involves microbial metal reduction on biotic cathode. The last mechanism is microbial assisted metal reduction with a poised potential in cathode chamber. Table 2.2 summarizes prominent studies on metal recovery based on these above mechanisms.

Accordingly, gold ion (Au\(^{3+}\)), vanadium ion (V\(^{5+}\)), chromium ion (Cr\(^{6+}\)), silver ion (Ag\(^{+}\)), copper ion (Cu\(^{2+}\)), iron ion (Fe\(^{3+}\)), and mercury ion (Hg\(^{2+}\)), with high reduction potential can be reduced spontaneously (Figure 2.1 A and Table 2.2). Most studies found that metallic deposits were formed on the cathode surfaces, except the case of Cr\(^{6+}\), in which Cr\(^{6+}\) was converted to a less toxic compound, as Cr\(^{3+}\). The BES reactor works as a microbial fuel cell (MFC) system. The reaction is thermodynamically favorable due higher reduction potential of metals in cathode, as compared to that of organic matter in anode. These metal ions can be directly used as the electron acceptor without any external power consumption.

On the other hand, metal species with negative reduction potentials, such as Ni\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\) and Zn\(^{2+}\) (Table 2.2), cannot accept electrons flow from the anode for the spontaneous reduction. Thus, the reduction requires an external power supply to forcibly drive the electrons travel from the high potential anode to the low potential cathode. The mechanism is based on microbial electrolysis cell (MEC) system (Figure 2.1 B). However, the addition to external power source for metal reduction, even though low voltage (< 2 V) used, conflicts with the purpose of energy savings according to BES principle. Basically, all metal ions with any reduction potential can be reduced to metals by using input voltage, but cost-benefit analysis including energy balance, recovery yield and rates should be taken into account before practical application. Unfortunately, no study until now, has conducted or discussed this consideration. Another problem of using external voltage is that when the cathode potential dropped below hydrogen evolution potential (-0.6 V vs NHE), the hydrogen (H\(_2\)) generation would occur which reduces the metal reduction efficiency (Modin et al., 2012).
## Table 2. Recent BES studies on removal and recovery of metals

<table>
<thead>
<tr>
<th>Metal</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
<th>Cathodic reactions</th>
<th>External power (V)</th>
<th>Time (h)</th>
<th>Recover efficiency (%)</th>
<th>Max Power density output</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct metal recovery using abiotic cathodes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>Acetate (1360 mg/L)</td>
<td>Cu(^{2+})</td>
<td>Cu(^{2+}) + 2e(^-) = Cu(^{0}), E(^0) = 0.337V</td>
<td>0</td>
<td>11</td>
<td>60.1-99.92</td>
<td>118-319 (mW/m(^2))</td>
<td>(Wang et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>Acetate (1640 mg/L)</td>
<td>Cu(^{2+})</td>
<td>Cu(^{2+}) + 2e(^-) = Cu(^{0}), E(^0) = 0.286V</td>
<td>0</td>
<td>7</td>
<td>99.88 (anaerobic)</td>
<td>430 (mW/m(^2))</td>
<td>(Heijne et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>Glucose (5000 mg/L)</td>
<td>Cu(^{2+})</td>
<td>Cu(^{2+}) + 2e(^-) = Cu(^{0}); Cu(^{2+}) + Cu(^{2+}) + 2H(^+) = 2Cu(^{0}) + H(_2)</td>
<td>0</td>
<td>144</td>
<td>&gt; 96%</td>
<td>314 mW/m(^3))</td>
<td>(Tao et al., 2011)</td>
</tr>
<tr>
<td>Cu</td>
<td>Acetate (1000 mg/L)</td>
<td>Cu(^{2+})</td>
<td>Cu(^{2+}) + 2e(^-) = Cu(^{0}), E(^0) = 0.337V</td>
<td>0</td>
<td>480</td>
<td>91.95 ± 0.11%</td>
<td>0.585 mW</td>
<td>(Tao et al., 2011)</td>
</tr>
<tr>
<td>Ag</td>
<td>Acetate (1000 mg/L)</td>
<td>Ag(^{0})</td>
<td>Ag(^{+}) + e(^-) = Ag(^{0}), E(^0) = 0.799V</td>
<td>0</td>
<td>8</td>
<td>99.91 ± 0.00%</td>
<td>4250 (mW/m(^2))</td>
<td>(Choi and Cui, 2012)</td>
</tr>
<tr>
<td>Ag</td>
<td>Acetate (COD = 500 -1500 mg/L)</td>
<td>AgNO(_3)</td>
<td>Ag(^{+}) + e(^-) = Ag(^{0}), E(^0) = 0.799V</td>
<td>0</td>
<td>36</td>
<td>96.74 (removal)</td>
<td>109 (mW/m(^2))</td>
<td>(Tao et al., 2012)</td>
</tr>
<tr>
<td>Au</td>
<td>Acetate (1000 mg/L)</td>
<td>AuCl(_4)</td>
<td>AuCl(_4) + 3e(^-) = Au(^{0}) + 4Cl(^-), E(^0) = 1.002 V</td>
<td>0</td>
<td>25</td>
<td>99.89 ± 0.00%</td>
<td>890 – 6580 (mW/m(^2))</td>
<td>(Choi and Hu, 2013)</td>
</tr>
<tr>
<td>Ag</td>
<td>Acetate (1600 mg/L)</td>
<td>5 mM Ag(_2)SO(_4), 50 mM NH(_4)OH</td>
<td>[Ag(NH(_3))(_2)(^{+}) + e(^-) = Ag(^{0}) + 2NH(_3), E(^0) = 0.373V</td>
<td>0</td>
<td>21</td>
<td>99.9%</td>
<td>317 (mW/m(^2))</td>
<td>(Wang et al., 2013)</td>
</tr>
<tr>
<td>Cd</td>
<td>Acetate (4920 mg/L)</td>
<td>Cd(^{2+})</td>
<td>Cd(^{2+}) + 2e(^-) = Cd(^{0}), E(^0) = -0.4V</td>
<td>0</td>
<td>50</td>
<td>90%</td>
<td>3600 (mW/m(^2))</td>
<td>(Abourached et al., 2014)</td>
</tr>
<tr>
<td>Zn</td>
<td>Acetate (3280 mg/L)</td>
<td>Zn(^{2+})</td>
<td>Zn(^{2+}) + 2e(^-) = Zn(^{0}), E(^0) = -0.764V</td>
<td>0</td>
<td>72</td>
<td>93 ± 4%</td>
<td>0.233 mW</td>
<td>(Fradlert et al., 2014)</td>
</tr>
</tbody>
</table>

Ref. code: 256057223032832300323QDG
### Metal reduction using bio-cathodes supplemented by external power sources

<table>
<thead>
<tr>
<th>Metal</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
<th>Cathodic reactions</th>
<th>External power (V)</th>
<th>Time (h)</th>
<th>Recovery efficiency (%)</th>
<th>Max Power density output</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>(800 mg/L)</td>
<td>Acetate (1640 mg/L)</td>
<td>2CrO$_4^-$ + 4H$^+$ + e$^-$ → 2Cr$^{3+}$ + Li$^+$ + 2H$_2$O</td>
<td>0.34</td>
<td>2-4 or</td>
<td>84.3%</td>
<td>0</td>
<td>(Modin et al., 2012)</td>
</tr>
<tr>
<td>Pb</td>
<td>(400 mg/L)</td>
<td>Acetate (1600 mg/L)</td>
<td>2CrO$_4^-$ + 4H$^+$ + e$^-$ → 2Cr$^{3+}$ + Li$^+$ + 2H$_2$O</td>
<td>0.34</td>
<td>6</td>
<td>47.5%</td>
<td>0</td>
<td>(Luo et al., 2014)</td>
</tr>
<tr>
<td>Cd</td>
<td>(800 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>CuO$_2$ + 2H$^+$ + e$^-$ → Cu$^{2+}$ + 2OH$^-$</td>
<td>0.34</td>
<td>6</td>
<td>0.4 W/m$^2$</td>
<td>(Huang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>(300 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>CuO$_2$ + 2H$^+$ + e$^-$ → Cu$^{2+}$ + 2OH$^-$</td>
<td>0.34</td>
<td>0.5</td>
<td>0.1 W/m$^2$</td>
<td>(Huang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>(1000 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>CuO$_2$ + 2H$^+$ + e$^-$ → Cu$^{2+}$ + 2OH$^-$</td>
<td>0.34</td>
<td>0.5</td>
<td>0.1 W/m$^2$</td>
<td>(Huang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>(50 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>CuO$_2$ + 2H$^+$ + e$^-$ → Cu$^{2+}$ + 2OH$^-$</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>(Jiang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>(50 – 200 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>CuO$_2$ + 2H$^+$ + e$^-$ → Cu$^{2+}$ + 2OH$^-$</td>
<td>0</td>
<td>60</td>
<td>93.6%</td>
<td>Cr(VI) reducing MFC</td>
<td>(Choi et al., 2014)</td>
</tr>
</tbody>
</table>

### Metal conversion using bio-cathodes

<table>
<thead>
<tr>
<th>Metal</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
<th>Cathodic reactions</th>
<th>External power (V)</th>
<th>Time (h)</th>
<th>Recovery efficiency (%)</th>
<th>Max Power density output</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>(22,31,40, 63 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>Cr2O$^-$ + 14H$^+$ + 6e$^-$ → 2Cr$^{3+}$ + 7H$_2$O, E$^0$ = 0.365V</td>
<td>2Cr$^{3+}$ + 7H$_2$O = 2Cr(OH)$_3$ + 6H$^+$ + H$_2$O</td>
<td>0</td>
<td>120-168</td>
<td>100%</td>
<td>0.46 mg Cr/mg VSS H</td>
</tr>
<tr>
<td>Cr</td>
<td>(39.2 mg/L)</td>
<td>Acetate (1600 mg/L)</td>
<td>Cr2O$^-$ + 14H$^+$ + 6e$^-$ → 2Cr$^{3+}$ + 7H$_2$O, E$^0$ = 0.365V</td>
<td>2Cr$^{3+}$ + 7H$_2$O = 2Cr(OH)$_3$ + 6H$^+$ + H$_2$O</td>
<td>0</td>
<td>7</td>
<td>99.2%</td>
<td>2.4 ± 0.1 W/m$^2$</td>
</tr>
<tr>
<td>Cr</td>
<td>(6 – 20 mg/L)</td>
<td>Glucose (1000 mg/L)</td>
<td>Cr2O$^-$ + 14H$^+$ + 6e$^-$ → 2Cr$^{3+}$ + 7H$_2$O, E$^0$ = 0.365V</td>
<td>2Cr$^{3+}$ + 7H$_2$O = 2Cr(OH)$_3$ + 6H$^+$ + H$_2$O</td>
<td>0</td>
<td>24</td>
<td>79.3%</td>
<td>9.7 ± 0.4 W/m$^2$</td>
</tr>
</tbody>
</table>

### Metal recovery using abiotic cathodes supplemented by external power sources

<table>
<thead>
<tr>
<th>Metal</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
<th>Cathodic reactions</th>
<th>External power (V)</th>
<th>Time (h)</th>
<th>Recovery efficiency (%)</th>
<th>Max Power density output</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>(800 mg/L)</td>
<td>Acetate (1640 mg/L)</td>
<td>Cu$^{2+}$ + 2e$^-$ → Cu, E$^0$ = 0.34V</td>
<td>0</td>
<td>88.1%</td>
<td>0</td>
<td>(Huang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>(400 mg/L)</td>
<td>Acetate (1600 mg/L)</td>
<td>Cu$^{2+}$ + 2e$^-$ → Pb$^{0}$, E$^0$ = -0.13V → E = -0.51V</td>
<td>0.34</td>
<td>2-4 or</td>
<td>84.3%</td>
<td>0</td>
<td>(Modin et al., 2012)</td>
</tr>
<tr>
<td>Cd</td>
<td>(800 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>Cu$^{2+}$ + 2e$^-$ → Cu$^{0}$</td>
<td>0.34</td>
<td>6</td>
<td>47.5%</td>
<td>0</td>
<td>(Luo et al., 2014)</td>
</tr>
<tr>
<td>Zn</td>
<td>(300 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>Cu$^{2+}$ + 2e$^-$ → Cu$^{0}$</td>
<td>0.34</td>
<td>0.5</td>
<td>0.4 W/m$^2$</td>
<td>(Huang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>(1000 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>Cu$^{2+}$ + 2e$^-$ → Cu$^{0}$</td>
<td>0.34</td>
<td>0.5</td>
<td>0.1 W/m$^2$</td>
<td>(Huang et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>(50 mg/L)</td>
<td>Acetate (1000 mg/L)</td>
<td>Cu$^{2+}$ + 2e$^-$ → Cu$^{0}$</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>(Jiang et al., 2014)</td>
<td></td>
</tr>
</tbody>
</table>

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Ref. code: 2560572230323QDG
In addition, the reducing environment in the cathode is an advantage for biological treatment technologies because it can be used for the dissimilatory process of oxidized pollutants, such as nitrate, bromate, chlorinated solvents, and heavy metals (Tandukar et al., 2009). Therefore, metal recovery in type (C) and (D) of BES mechanism relates to dissimilatory metal reduction, in which dissimilatory metal reducing bacteria (DMRB) species are employed (Figure 2.1 C and D). DMRBs respire metals outside the cell membrane for cellular energetic and they have been widely used in bioremediation of metal contaminated sites (Rahman et al., 2007; Shugaba et al., 2012) and metallic nano particles (NPs) production (Lengke et al., 2007; Yates et al., 2013).

However, few BES studies using bio-cathode for metal reduction have been conducted. Until now, only Cr and Co recovery were investigated in bio-cathode BESs (Table 2.2). Studies on bio-cathodic reduction of Cr, however, just investigated the conversion of Cr$^{6+}$ to Cr$^{3+}$ to mitigate the toxic impacts of Cr$^{6+}$, without recovery of pure Cr. Although the systems were demonstrated as a cost-effective reduction of Cr$^{6+}$ and Co$^{2+}$, the separation of metallic deposits from the biomass will be the major issue for the practical application. Furthermore, the retention of biomass and lower initial metal concentration used in the bio-cathode reactors, as compared to the abiotic system, are also limitations of bio-cathode systems.

2.3.2 Fundamentals of cell voltage generation

The reactions occurring at the anode and the cathode of a BES reactor can be considered as half-cell reactions or separate reactions (Eq. 2.4-2.6). When organic compound (e.g. acetate, glucose) is used as an electron donor, the thermodynamic anode potential ($E_{an}$) is generally poised at a low value, which is set by the respiratory enzymes of the electrochemically active bacteria (Liu et al., 2005; Logan et al., 2006). In contrast, depending on metal ions recovered in cathode (Eq. 2.6), the thermodynamic cathode potential can be computed by Nernst equation (Eq. 2.7), which varies with the types and concentrations of metal used in the catholyte (Wang and Ren, 2014).

1. In the anode chamber:
- with acetate: $2HO^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$, $E^0 = -0.289$ V  \hspace{1cm} (2.4)
- with glucose: $6\text{HCO}_3^- + 30\text{H}^+ + 24e^- \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 12\text{H}_2\text{O}$, $E^0 = -0.41 \text{ V}$ (2.5)

2. In the cathode chamber:

$$M^{n+}_{(\text{ox})} + ne^- \rightarrow M^0_{(\text{red})}, \quad E^0 \text{ depends on metal} \quad (2.6)$$

The thermodynamic cathode potential is determined as:

$$E_{ca} = E_{ca}^0 - \frac{RT}{nF} \ln \left( \frac{[M_{\text{red}}]}{[M_{\text{ox}}]} \right) \quad (2.7)$$

Where $M^{n+}$ is the metal ions in the catholyte,

- $E$ is the electrochemical redox potential in a specific condition,
- $E^0$ is the electrochemical redox potential at standard condition ($298 \text{ K}$, $\text{pH}_2 = 1$ bar, $[\text{H}^+] = 1\text{M}$)
- $R$ is the gas constant ($8.31447 \text{ J/mol/K}$), $T$ is the absolute temperature (K),
- $n$ is the number of electrons transferred, $F$ is Faraday’s constant ($96,485 \text{ C/mol}$),
- $[M_{\text{red}}]$ is the concentration of reduced metals (mg/L), and
- $[M_{\text{ox}}]$ is the concentration of oxidized metals (mg/L).

Accordingly, the thermodynamic cell voltage or ideal cell electromotive force ($E_{\text{emf}}$) is determined as the potential difference between the cathode and anode ($E_{\text{emf}} = E_{ca} - E_{an}$) (Logan et al., 2006). In practical, the actual cell voltage produced from the system is considerably lower due to a number of internal losses (i.e. activation loss, ohmic loss, and mass transport or concentration loss). These are referred as overvoltage and are shown in Eq. 2.8.

$$E_{\text{cell}} = E_{\text{emf}} - \left( \Sigma \eta_a + |\Sigma \eta_c| + IR_\Omega \right) \quad (2.8)$$

Where $\Sigma \eta_a$ and $\Sigma \eta_c$ are the overpotentials of the anode and cathode, respectively;

- $IR_\Omega$ is the sum of all ohmic losses which are proportional to the generated current ($I$) and ohmic resistance of the system ($R_\Omega$).

Most BES studies actually prefer to describe $E_{\text{cell}}$ in a relation to OCV (open circuit voltage) which is defined as the cell voltage measured after some time in the absence of current (Eq. 2.9) (Logan et al., 2006).

$$E_{\text{cell}} = \text{OCV} - IR_{\text{int}} \quad (2.9)$$
Where $IR_{\text{int}}$ is the sum of all the internal losses of system, which are proportional to the generated current ($I$) and internal resistance ($R_{\text{int}}$).

Theoretically, the OCV should approach the $E_{\text{emf}}$, however, it is practically lower than the $E_{\text{emf}}$ due to parasitic losses in the BES reactor. The parasitic losses arise from current leakage, gas crossover, and unwanted side reactions occurring in the system as IEM is normally used as a separator (Rismani-Yazdi et al., 2008; Wen et al., 2009). Although designed for a selective ion transfer, unselective permeability for the fuel (substrate) and other electrolytes is observed in any membrane process. These fluxes lead to parasitic processes that can result in lower fuel cell performance (Rabaey et al., 2009).

In general, the BES performance in term of $E_{\text{cell}}$ production can be assessed by either overpotentials and ohmic losses or OCV and internal losses ($R_{\text{int}}$ includes more than just $R_{\Omega}$). Herein, the ohmic losses are the most important factor that need to be overcome when designing the BES architecture. These losses come from the resistance of ion conduction due to the electrolyte and membrane, and the resistance of the electron flow through the electrodes and any relevant interconnections (Logan et al., 2006). The total resistance of BES reactors in operation is mostly ascribed to these internal resistances, therefore, they should be minimized to maximize the $E_{\text{cell}}$ production, which governs directly the reduction rate of metal ions in the cathode, and influences recovery efficiency as accordingly.

Recently, there has been a growing number of advances and improvements in reactor concepts, which focus on the development of functional components (e.g. electrode, separator, substrate) to enhance the long-term stability of all materials and increase the productivity of BES (Krieg et al., 2014; Li et al., 2011; Oliveira et al., 2013; Pant et al., 2010; Wei et al., 2011). The advantages and disadvantages of these materials along with their applications in BESs studies on metals recovery and simultaneous electricity generation, are presented in this thesis.

2.3.3 Architectural components of BES reactor

In general, three main and crucial components of BES reactor are the casing, the electrodes, and the separator (Krieg et al., 2014). Knowledge about these materials and their interaction with each other is essential to facilitate the BES fabrication. Table 2.3
shows the architectural designs used in recent BES studies for recovering of metals, in which the casing configuration, electrode material, and separator are described in detail.

### 2.3.3.1 BES configuration

A variety of BES reactors for metal recovery have been conducted at laboratory scale with different shapes and sizes (several milliliters up to 1 liter). They can be categorized by the reactor configuration. The widely used and inexpensive design is two-chamber MFC built in a traditional “H-shape” reactors, in which two bottles or two tubular chambers are connected by a tube containing a separator, normally an ion exchange membrane (Figure 2.2a). As long as the two chambers are kept separated, flat-plate chambers (Figure 2.2b) or cylindrical chamber (Figure 2.2c) can be pressed up onto either side of the membrane and clamped together by bolts to form a large surface. In addition, gastight condition in the anode and cathode chamber is always required strictly. In order to ensure the anaerobic environment, both chambers are normally purged by nitrogen gas in 15-30 minutes before continuous operation to eliminate the effect of dissolved oxygen which may compete with metal ions to accept the electrons.

Among these above designs, the H-shaped reactors showed less effective due to high ohmic losses caused by a distal electrode spacing (Krieg et al., 2014). The cubic-shape and tubular types have been constructed by various BES studies as these designs can reduce the internal resistance due to narrow electrode spacing. Furthermore, different electrode assemblies can be used in these systems, such as anode and cathode can be put in either single or dual-chamber reactor, stacked graphite disks served as the cathode while graphite felt was the anode (Abourached et al., 2014; Tao et al., 2011). The dual-chambers construction are normally preferred as the anolyte and catholyte are fed separately to implement different bio-electrochemical reactions in the systems (i.e. biological oxidation conducted by microorganisms in the anode chamber and electrochemical reduction of metal ions in the cathode chamber). In addition, the inhibition of bacterial metabolisms due to metal toxicity when using single-chamber BES is a limitation of this configuration.
<table>
<thead>
<tr>
<th>Metal</th>
<th>Reactor configuration</th>
<th>Electrode materials</th>
<th>Separator materials</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>Two chamber H-type MFCs constructed by two borosilicate glass bottles. The working volume: 230 mL/ each chamber</td>
<td>Graphite plates (5.4 cm × 2.5 cm × 0.6 cm) for anode and cathode</td>
<td>Proton exchange membrane PEM (Nafion 117; DuPont)</td>
<td>(Tandukar et al., 2009)</td>
</tr>
<tr>
<td>Cr</td>
<td>Two plastic (Plexiglas) cylindrical chambers (H15cm and D5cm; net volume of 250 ml each) are connected by a tube (inner diameter: 2.5 cm)</td>
<td>Both electrodes were made of graphite plates (4.5x2.5x1cm Shanghai, China)</td>
<td>Proton exchange membrane PEM (Nafion 117; DuPont)</td>
<td>(Wang et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anode was graphite plate (4.5x 2.5x1.0 cm); Cathode was the same size graphite plate submerged in granular graphite (diameter 1.5–4 mm, China)</td>
<td></td>
<td>(Huang et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>Flat-plates type (cube-shape design)</td>
<td>The anode was a rough graphite plate (Germany). The cathode was a piece of graphite foil (UK). Surface area was 22 cm²</td>
<td>Bipolar membrane (Fumasep Germany), 22 cm²</td>
<td>(Heijne et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>Two flow channels 10 × 2 × 1.5 cm (w × h × d) Reactor consisted of two plexiglass plates with a single flow channel. The two plates with a flow channel were separated by membrane. The other side of the flow channel faced the electrode</td>
<td>The cathode was graphite plate 14 x10 cm The anode was graphite plate or graphite felt with the same area of 294 cm²</td>
<td>No proton membrane</td>
<td>(Tao et al., 2011)</td>
</tr>
<tr>
<td>Cu</td>
<td>Two-chamber MFC was made of polycarbonate plates (1 L/each chamber). The middle channel between two chambers had a volume of 100 mL</td>
<td>Anode was graphite plates (14cm×10.5cm, 0.5cm) and graphite felt (China). Cathode was graphite plate 6x4.5 cm</td>
<td>Proton exchange membrane PEM (Nafion TM 212, DuPont Co., USA), a 121 cm²</td>
<td>(Tao et al., 2011)</td>
</tr>
<tr>
<td>Cu</td>
<td>A cubic-shape MFC with anode and cathode chamber was used. V_anode = V_cathode = 1 lit (working volume)</td>
<td>Anode was composed of three graphite plates (60x45x0.3 cm separated by two graphite felt (60x45x0.5 cm) (China). Cathode was a graphite plate (45 cm²)</td>
<td>An anion exchange membrane (AEM, AMI-7001, Membrane International, Inc. USA) 16 cm² (4x4cm).</td>
<td>(Choi and Cui, 2012)</td>
</tr>
<tr>
<td>Ag</td>
<td>The reactor was manufactured with an acrylic rectangle chamber. Each chamber holding a volume of 112 mL (length: 7 cm, width: 4 cm, height: 4 cm). The effective volumes were both 100 mL</td>
<td>Carbon brush anode (L 2.5 cm, D 2.5 cm) Carbon cloth with gas diffusion layers surface area of 2.2 cm² (1.7cm x 1.3 cm) was used as cathode</td>
<td>Proton exchange membrane PEM (Nafion 212, DuPont, USA) with a 22.5 cm²</td>
<td>(Tao et al., 2012)</td>
</tr>
<tr>
<td>Ag</td>
<td>Six identical sized dual-chamber cells consisting of an anode chamber (80 mL working volume) and a cathode chamber (50 mL working volume)</td>
<td>Anode was composed of three graphite plates (60x45x0.3 cm separated by two graphite felt (60x45x0.5 cm) (China). Cathode was a graphite plate (45 cm²)</td>
<td>Proton exchange membrane PEM (Nafion 212, DuPont, USA) with a 22.5 cm²</td>
<td>(Tao et al., 2012)</td>
</tr>
<tr>
<td>Metal</td>
<td>Reactor configuration</td>
<td>Electrode materials</td>
<td>Separator materials</td>
<td>Ref.</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>-----</td>
</tr>
<tr>
<td>Au</td>
<td>A cubical dual-chamber made from acrylic rectangle Both anode and cathode chamber held a volume of 72 mL (length: 4 cm, width: 4 cm, height: 4.5 cm)</td>
<td>The anode was carbon brush (L = 2.5 cm, D = 2.5 cm). The cathode was carbon cloth with surface area of 2.2 cm² (1.7x1.3 cm)</td>
<td>A cation exchange membrane (CEM, CMI-7000, Membrane International, Inc., USA) 18 cm²</td>
<td>(Choi and Hu, 2013)</td>
</tr>
<tr>
<td>Cr</td>
<td>The reactor was constructed from two plexiglass cubic chambers (volume of each chamber was 70 mL) Anode and cathode were sheets of graphite felt (5.0x5.0x0.5 cm; China)</td>
<td>Proton exchange membrane PEM (38.5 cm²; Nafion117, DuPont, USA)</td>
<td>(Wu et al., 2015)</td>
<td></td>
</tr>
</tbody>
</table>

**Cylindrical (tubular) type**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Reactor configuration</th>
<th>Electrode materials</th>
<th>Separator materials</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>A tubular dual-compartment MFC was fabricated by a plexi-glass tube (D = 5 cm, L = 7 cm) The effective volumes of anode and cathode were 120 mL and 100 mL, respectively.</td>
<td>Both anode and cathode were graphite felts 35.6 cm² (3.5 cm x 3 cm x 1.12 cm, USA).</td>
<td>Anion exchange membrane (AMI-7001, Membrane International, Inc., USA) 19.6 cm² (D = 5 cm).</td>
<td>(Wang et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>The reactor was made of polymethyl methacrylate column of ID 15 cm. Anode chamber was at the bottom, and the cathode chamber at the upper portion. V_anode = 6 L. V_cathode = 10 L. Five graphite disks (D= 14 cm, China) were cathodes. Graphite felt (73 cmx 25 cmx 1 cm, China) was anode.</td>
<td></td>
<td>A separator made from three polypropylene funnels located between two chambers.</td>
<td>(Tao et al., 2011)</td>
</tr>
<tr>
<td>Ag</td>
<td>Two same cylindrical chambers (ID = 3.2 cm, L = 3.0 cm) made of acrylic glass The anode is made of hydrophilic carbon cloth (HCN-030, China) while the cathode is made of graphite (0.5 mm, China).</td>
<td></td>
<td>A bipolar membrane (BPM-I, Beijing Ting Run Co., China).</td>
<td>(Wang et al., 2013)</td>
</tr>
<tr>
<td>Cu</td>
<td>Two-chamber MEC reactors were constructed using plexiglass plastic cylindrical. The volumes of anode and cathode chamber were 28 and 35 mL, respectively.</td>
<td>Anode was graphite brush Cathode was carbon cloth with a platinum catalyst layer (0.5 mg Pt cm⁻²)</td>
<td>Bipolar membrane (Fumasep FBM, Fumatech, Germany)</td>
<td>(Luo et al., 2014)</td>
</tr>
<tr>
<td>Ni</td>
<td>Single chamber air cathode MFC was consisted of a plastic (Plexiglas) cylindrical chamber (12 mL). The anode and cathode were placed in parallel on the opposite sides of the chamber with a spacing of 1.7 cm. The anode (1.8 cm²) was made from type A carbon cloth (no wet proofing) while the cathode (7 cm²) was made from type B carbon cloth (30% wet proofing; USA)</td>
<td></td>
<td>No separator</td>
<td>(Abourached et al., 2014)</td>
</tr>
<tr>
<td>Cd</td>
<td>The system consisted of a two-chamber tubular MFC and a two-chamber tubular MEC. The net working volume of each chamber was 25 mL</td>
<td>Graphite felt (China) was used as the anodes of MFCs and MECS, as well as the cathodes of MFCs whereas carbon rod was served as the cathodes of MECS</td>
<td>Cation exchange membrane (CMI-7000, Membranes International, NJ), D = 3.0 cm</td>
<td>(Huang et al., 2014)</td>
</tr>
<tr>
<td>Zn</td>
<td>A tubular two-chamber reactor was used. The networking volumes were 85 mL for the cathode chamber and 43 mL for the anode chamber</td>
<td>Graphite granules (China) were used for the cathode and a graphite brush was used for the anode.</td>
<td>Cation exchange membrane (Ultrex CMI7000, Membranes International Inc., USA)</td>
<td>(Huang et al., 2011)</td>
</tr>
</tbody>
</table>
A tubular two-chamber reactor was used. The networking volume in the anode and the cathode chambers were 25 mL and 40 mL, respectively. Graphite fiber and porous graphite felt (China) were used as the anodes and the cathodes. Cation exchange membrane (CMI-7000, Membranes International, NJ) (Huang et al., 2014)

(MFC: Microbial Fuel Cell, MEC: Microbial Electrolysis Cell)

Figure 2. 2 Common BES reactor configurations. (A) Two-chamber H-type, (B) Flat-plates type (cube-shape design), (C) Cylindrical design

Although there has been an ongoing development in BES design to improve the performance, a comparison of overall efficiency based on BES configurations is seemingly impossible. The influence of geometric and dimensional factors are difficult to assess (Krieg et al., 2014). Different setups and operational conditions show different outputs. Till date, most studies conducted experiments in pilot or lab scales, thus, the scale-up of BES reactors shows a challenges for industrial applicability. Several researchers tried to improve the power output by connecting a number of cell units to stacked reactors (Gálvez et al., 2009; Janicek et al., 2014; Zhuang et al., 2012). However, there has been few scale-up reactors using real wastewater for metal recovery.
2.3.3.2 Electrode

Electrodes including anode and cathode play an important role in BES performance as their presence involves in bio-electrochemical reactions and electron transfer. The requirement for all the types of electrodes is that their base materials must generally be of good conduction, good chemical stability, high mechanical strength, and low cost (Wei et al., 2011). Therefore, carbonaceous materials and non-corrosive metals (e.g. titanium, stainless steel) are normally chosen to fabricate electrodes in most BES reactors. Due to the bio-catalytic activities in the anode chamber, the anode functions not only as a conductor and but also as a supporter for bacterial attachment. Hence, some special surface characteristics, such as high surface roughness, good biocompatibility, and efficient electron transfer between bacteria and electrode are essential (Wei et al., 2011). Graphite plates, graphite felts, carbon fiber, and carbon brush have been used frequently as anodes and cathodes in many BES studies for metal recovery (Table 2.4). Before assembling, the electrodes are normally pretreated or pre-conditioned through different surface treatments with physical or chemical methods (e.g. heating, concentrated acid soaking) (Choi and Hu, 2013; Feng et al., 2010; Tao et al., 2012; Wang et al., 2010), and surface coating with gas diffusion or platinum catalyst layer (Choi and Cui, 2012; Luo et al., 2014) to enhance the efficiency.

In term of anode, graphite plates have been used more widely as they were reported to produce high power and had a defined surface area which facilitates the quantitative measurement of biomass formed at the anode surface (Wei et al., 2011). Graphite felts also show positive effects due to their larger surface area available to bacteria attachment to process the biological oxidation. Recently, studies reported that brush anode is an ideal electrode as it has high specific surface area and porosity for bacterial adhesion, coupled with high electrical conductivity and efficient current collection (Feng et al., 2010; Logan et al., 2007; Wei et al., 2011). Basically, more porous materials produce more power per geometric surface area compared to the smooth counterparts (Wei et al., 2011). The brushes are normally made of carbon fibers cut to a set length and wound into a twisted core consisting of two conductive but noncorrosive Ti wires. Compared with a packed structure electrode, the high porosity of brush electrodes can also effectively avoid the potential for clogging (Wei et al., 2011). Besides, brush structure can reduce the ohmic resistance which is known to be
linearly related to the path length of the electron flow. Study indicated that a major concern, which must be addressed in electrode configuration to minimize the ohmic losses, is the minimization of the travel distance of the electrons that have to flow through electrodes (Logan et al., 2007).

On the other hand, cathode materials and their designs are also a challenging aspect of BES reactor. Most carbonaceous materials mentioned above as anodes, have been also used as cathodes. Different from air-cathodes or aqueous cathodes with catalysts layer (e.g. Pt) used in BES reactors for only electricity production (i.e. MFC system), the cathodes used in BES for metal recovery need other specific requirements. As metallic deposits would form directly on cathode surface during the reduction of metal ions in the catholyte, the cathode therefore, should be in plane or flat structure to facilitate the separation of the deposits. Cathodes made of other carbon materials (e.g. carbon foil, carbon paper) face to many difficulties to separate the reduced deposits as they are so thin and relatively stiff, but slightly brittle (Wei et al., 2011). Carbon cloth and carbon felt are thicker than two above materials and have been used commonly in many studies to recover different metals (Table 2.4). However, the porous structure of these materials is better for the anode as they allow more surface area for bacterial growth, whereas when served as cathodes, it may cause obstacles to separate or purify the tiny metallic contents. Meanwhile, graphite plates or sheets with high strength are easy to handle. The smooth surface is also help to overcome these above difficulties.

Till date, although the feasibility of BES for metal recovery has been demonstrated by many studies, the separation of metallic deposits at cathode have still not been conducted completely. Physico-chemical methods, such as heating, acid extraction, and electrolysis, can be used for purification of metals. However, these methods may affect the cathode structure, especially when bio-cathodes are used, in which the live biofilms need to be kept intact for further operation. Therefore, more effective, durable, and tolerant cathode materials need to be discovered and investigated thoroughly.
Table 2. 4 Common electrode materials used in BES reactors for removal and recovery of metals

<table>
<thead>
<tr>
<th>Electrode material</th>
<th>Pictures</th>
<th>Applications</th>
<th>Advantages &amp; Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphite plate</td>
<td></td>
<td>Anode</td>
<td>(Heijne et al., 2010; Huang et al., 2011; Huang et al., 2010; Tandukar et al., 2009; Tao et al., 2011; Tao et al., 2012; Wang et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cathode</td>
<td>(Huang et al., 2011; Huang et al., 2010; Tandukar et al., 2009; Tao et al., 2011; Tao et al., 2012; Wang et al., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Has a defined surface area and a relatively smooth surface, which facilitate the</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>quantitative measurement of biomass per unit of surface area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Has a plane structure, easy to handle, easy to separate metallic deposits on the</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Roughened graphite electrodes have been reported to produce a higher power density</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>than flat graphite electrodes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Low specific area and high cost</td>
</tr>
<tr>
<td>Graphite felt</td>
<td></td>
<td>Anode</td>
<td>(Huang et al., 2014; Modin et al., 2012; Tao et al., 2011; Wang et al., 2010; Wu et al., 2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cathode</td>
<td>(Huang et al., 2014; Huang et al., 2014; Jiang et al., 2014; Tao et al., 2011; Tao et al., 2011; Wang et al., 2010; Wu et al., 2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Graphite or carbon felt is another fiber fabric that is much thicker than other</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>materials (graphite foil, carbon paper, carbon cloth)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Its loose texture confers more space for bacterial growth than carbon cloth and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>graphite sheets</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- The growth of bacteria is more likely to be restricted by the mass transfer of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>substrate and products on its inner surface.</td>
</tr>
<tr>
<td>Graphite foil</td>
<td></td>
<td>Cathode</td>
<td>(Heijne et al., 2010)</td>
</tr>
<tr>
<td>Carbon brush.</td>
<td></td>
<td>Anode</td>
<td>(Choi and Cui, 2012; Choi and Hu, 2013; Huang et al., 2011; Huang et al., 2014; Jiang et al., 2014; Luo et al., 2014)</td>
</tr>
<tr>
<td>Carbon cloth (fiber)</td>
<td></td>
<td>Anode</td>
<td>(Abourached et al., 2014; Wang et al., 2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cathode</td>
<td>(Abourached et al., 2014; Choi and Cui, 2012; Choi and Hu, 2013; Luo et al., 2014)</td>
</tr>
</tbody>
</table>
2.3.3.3 Separator

As anode and cathode chamber are separately fed by different bulk liquids (i.e. substrate and metal-rich solution), most BES reactors require a separator between them. It has to keep anolyte and catholyte separate from each other and prevent crossover processes and eliminate metal toxicity. It also has to be conductive for ions, since one ion has to be transferred between the anode and cathode compartment for every electron that crosses the external electric circuit (Krieg et al., 2014). Polymer ion exchange membranes (IEMs) are usually assigned for this task (Table 2.3).

The most-often used separator is proton exchange membrane (PEM), as Nafion 117 (Dupont Inc., USA), due to its good proton conductivity. Due to the presence of the negatively charged functional groups as sulfonate (SO_3^-) attached to the fluorocarbon backbone (-CF_2-CF_2-) of perfluorosulfonic acid membrane, Nafion also shows high conductivity to various cations (Li et al., 2011). Meanwhile, cation exchange membrane (CEM) as CMI-7000 (Membranes Inc., USA) is also sometimes used as it is less expensive and often structurally stronger than Nafion 117 (Kim et al., 2007). CMI-7000 is a strong acid polymer membrane with gel polystyrene and divinylbenzene (DVB) cross-link structure, which also contains a lot of sulphonic acid groups (R-SO_2OH). Compared to Nafion, it exhibits comparable cation conductivity and mechanical durability, but generally, has a high ohmic resistance (Harnisch et al., 2008). However, the biggest drawback of using Nafion or CEM is the pH splitting caused by the poor competition of proton (H^+) with other cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) existing in anolyte at higher concentration to transfer from the anode to the cathode chamber (Kokabian and Gude, 2015; Pandit et al., 2012). The anolyte gradually becomes more acidic and suppress oxidation activity of bacteria in anode chamber while the catholyte gradually becomes more alkaline and may affect the soluble state of metal ions in the cathode chamber. Another drawback is the undesired diffusion of metal ions from the catholyte to the anolyte through CEM. This may cause metal ions loss and further adverse effects on microbial community in the anode chamber.

The anion exchange membranes (AEMs), such as AMI-7001S (Membranes Inc., USA), have been considered to overcome these above disadvantages of Nafion and CEMs. With the positive charged quaternary ammonium group (-NH_4^+) attached to polymer structure of the membrane, AEMs allow the migration of anions between two
chambers. Metal ions thus, cannot transport directly through AEMs from the cathode to the anode, which presumably prevented any loss of metal. In addition, AEMs are found to facilitate proton transfer by using phosphate as the proton carrier and pH buffer solution, which produces a lower rate of pH spitting (Leong et al., 2013; Li et al., 2011). However, there is a possibility that few intermediate metabolites with a negative charge, such as acetate, butyrate, propionate occurring during the biological oxidation in the anode chamber, percolate from the anode to the cathode chamber (Pandit et al., 2012). This phenomenon leads to substrate loss which then affects the electron production and BES performance.

Figure 2.3 Schematic of ion transfer across different IEMs

Different from monopolar membranes (i.e. CEM, AEM), a bipolar membrane (BPM) consists of an anion and a cation exchange layer mounted together. Thus, unlike the CEM or AEM which is able to selectively transport only positively or negatively charged ions from one side to another, BPM allows the simultaneous migration of proton (H\(^+\)) and hydroxide (OH\(^-\)) ions generated from the water splitting reaction at the interface of CEM and AEM layer (Harnisch et al., 2008; Li et al., 2011). However, this transition area under equilibrium conditions always contains electrolyte salts due to the permeation through CEM and AEM layer. In electric field, the electrolyte anions migrate through the AEM toward the anode, whereas the cations pass through the CEM toward the cathode (Figure 2.3) (Harnisch et al., 2008).

Due to the difference in ion transfer mechanism through these above membranes, the power generation in BES reactors may be affected. The effect of separators, including cation, anion, and ultrafiltration membranes, on power density and columbic efficiency (CE) in different MFCs was investigated (Jung and Regan, 2007). Study
showed that the AEM-based MFC produced the largest power density (up to 610 mW/m²) and highest CE (72%) due to proton charge-transfer facilitated by phosphate anions and low internal resistance of the system. Higher performance in terms of voltage production (0.792 V) and power density (57.8 mW/m²) was obtained in another AEM-based MFC since the pH gradient associated potential losses were lower in the AEM, as compared to Nafion-based reactor (Pandit et al., 2012). However, study also indicated that a considerable voltage drop occurred in AEM-based reactor at longer period of operation because of metabolite losses at low external resistance.

Different types of membrane have an influence on current generation in BES reactors. Thus, the reduction rate of metallic ions in the catholyte, which depends on the intensity of electron flow, is accordingly affected. In electrochemical reactions of silver ions, for instance, current density is related to the reaction rate and therefore, it affects the nucleation and growth of silver particles (Liu et al., 2015). In other words, the silver recovery efficiency, and the formation of silver deposits on cathode surface along with the morphology of deposits may also differ when using different IEMs.

2.3.4 Functional components for BES operation

2.3.4.1 Inoculum

The BES performance generally depends on microbial activities, substrate conversion rate and electron transfer in the anode chamber. The electrochemically active bacteria (EAB), also called as exoelectrogen, are employed to process anodic reactions during their anaerobic respiration. Such microorganisms are able to transfer electrons out of their cell membranes to the anode through either direct electron transfer (DET) via outer membrane c-type cytochromes and conductive pili called nanowires, or indirect electron transfer via mobile electron shuttles as mediators (Nancharaiah et al., 2015; Wang and Ren, 2013). Therefore, the inoculation stage in which the anode is inoculated and colonized by bacteria is essential in BES operation.

There are a variety of inoculums which have been used in BES technology so far. They can be monocultures of bacteria (e.g. Geobacter sulfurreducens, Shewanella putrefaciens, Shewanella affinis) or mixed cultures (Mathuriya, 2013). Compared to single strains, mixed microbial communities have attracted more attention due to their higher adaptability, stability, and productivity (Mathuriya, 2013; Patil et al., 2009). Anaerobic sludge, activated sludge, domestic wastewater, and marine sediments have
successfully been used as inoculums in several MFCs for electricity production (Martin et al., 2010). Study also indicated that electricigenesis is widespread among microbial communities. Although Geobacter sp. and Shewanella sp. are the most prominent species reported in many MFCs, the dominance of these organism has not been found to support high electrical power outputs using mixed microbial culture (Rabaey et al., 2004). Several MFC systems revealed a diversity of bacteria in mixed cultures much greater than these model iron reducers persisting in the anodic biofilm communities (Logan and Regan, 2006). A diverse microbial community of hydrolytic, fermentative, acidogenic, and acetogenic microorganisms is a good inoculum choice, however, the significant methanogenic population in mixed cultures normally competes with the anodophilic microorganisms and thereby decreases the columbic efficiency (Logan, 2012; Martin et al., 2010)

<table>
<thead>
<tr>
<th>Metal</th>
<th>Inoculum source</th>
<th>Substrate</th>
<th>Metal-rich solution</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>Anaerobic sludge collected from the anaerobic digester (Okchen, Korea)</td>
<td>Sodium acetate</td>
<td>Synthetic wastewater was prepared by dissolving CuSO₄·5H₂O in DI water</td>
<td>(Wang et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>A mixed culture anolyte from operating MFCs running on acetate</td>
<td>Sodium acetate</td>
<td>Cu²⁺ solution was prepared with copper chloride dehydrate and the pH was lowered to 3 by addition of HCl</td>
<td>(Heinje et al., 2010)</td>
</tr>
<tr>
<td>Cu</td>
<td>Anaerobic sludge collected from an anaerobic digester (Shenzhen, China)</td>
<td>Glucose</td>
<td>CuSO₄ solution prepared with DI water</td>
<td>(Tao et al., 2011; Tao et al., 2011)</td>
</tr>
<tr>
<td>Cu</td>
<td>A mixed microbial culture scraping from anode of MFCs for copper removal (Tao et al., 2011)</td>
<td>Sodium acetate</td>
<td>CuSO₄ solution prepared with DI water and pH 2 was adjusted by HCl</td>
<td>(Tao et al., 2011)</td>
</tr>
<tr>
<td>Ag</td>
<td>Anaerobic sludge from a wastewater treatment plant (Okchen, Korea)</td>
<td>Sodium acetate</td>
<td>Ag⁺ solutions were prepared by dissolving AgNO₃ in distilled water</td>
<td>(Choi and Cui, 2012)</td>
</tr>
<tr>
<td>Ag</td>
<td>A mixed microbial culture obtained from a laboratory-scale BES (Tao et al., 2011)</td>
<td>Sodium acetate</td>
<td>Ag⁺ solutions were prepared by dissolving AgNO₃ in DI water; [Ag₂S₂O₃⁻] solutions were made by dissolving AgBr in Na₂S₂O₃ solution in DI water</td>
<td>(Tao et al., 2012)</td>
</tr>
<tr>
<td>Au</td>
<td>Anaerobic sludge from a wastewater treatment plant (Okchen, Korea)</td>
<td>Sodium acetate</td>
<td>The Au(III) solution (pH 2.8) was prepared by diluting 30 wt.% gold (III) chloride in dilute HCl solution and distilled water</td>
<td>(Choi and Hu, 2013)</td>
</tr>
<tr>
<td>Ag</td>
<td>Sludge obtained from an anaerobic reactor for fruit waste treatment.</td>
<td>Sodium acetate</td>
<td>Silver contaminated wastewater is prepared by dissolving Ag₂SO₄ in ammonia solution NH₃OH with DI water and contains</td>
<td>(Wang et al., 2013)</td>
</tr>
<tr>
<td>Cd</td>
<td>A mixed bacterial culture enriched from sewage sludge (Corvallis, OR)</td>
<td>Sodium acetate</td>
<td>Cadmium chloride and zinc sulfate were dissolved in the medium solution</td>
<td>(Abourached et al., 2014)</td>
</tr>
<tr>
<td>Zn</td>
<td>Anaerobic digestion sludge</td>
<td>Sodium acetate</td>
<td>Synthetic wastewater with Zn²⁺ as sulfate</td>
<td>(Fradler et al., 2014)</td>
</tr>
</tbody>
</table>

Table 2.5 Functional components in BES used for removal and recovery of metals

Ref. code: 25605722300323QDG
<table>
<thead>
<tr>
<th>Metal</th>
<th>Inoculum source</th>
<th>Substrate</th>
<th>Metal-rich solution</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>A mixture of aerobic and anaerobic sludge from a wastewater treatment plant</td>
<td>Sodium acetate</td>
<td>A simulated fly ash leachate solution was prepared by dissolving CuCl₂, 2H₂O, Pb(NO₃)₂, 2(CdCl₂)·5H₂O, and ZnCl₂ in 2M HCl either individually or in a mixture</td>
<td>(Modin et al., 2012)</td>
</tr>
<tr>
<td>Pb</td>
<td>Sodium acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>The anode electrodes of MEC reactors were pre-acclimated in the anode chambers of mature MFCs</td>
<td>Sodium acetate</td>
<td>An artificial acid mine drainage was prepared by dissolving FeSO₄·7H₂O, CuSO₄·5H₂O, and NiSO₄·6H₂O in a dilute H₂SO₄ solution</td>
<td>(Luo et al., 2014)</td>
</tr>
<tr>
<td>Zn</td>
<td>Wastewaters from primary sedimentation tank (Dalian, China)</td>
<td>Sodium acetate</td>
<td>A desired quantity of LiCoO₂ was uniformly spread and loaded on cathode surface of MFC</td>
<td>(Huang et al., 2014)</td>
</tr>
<tr>
<td>Co</td>
<td>Anodes were pre-acclimated in MFCs, which were originally inoculated by wastewater and an acetate-based medium (Huang et al., 2013)</td>
<td>Sodium acetate</td>
<td>A Co(II) concentration of 847 mM was used in the catholyte buffered with NaH₂PO₄ - Na₂HPO₄ (66.7 mM)</td>
<td>(Jiang et al., 2014)</td>
</tr>
<tr>
<td>Cr</td>
<td>The anode was inoculated by mixed anaerobic culture enriched from an anaerobic digester. The cathode inoculum was a mixture of denitrifying and anaerobic mixed cultures enriched in the presence of Cr⁶⁺.</td>
<td>Excess acetate for anode, and NaHCO₃ for cathode</td>
<td>Cr⁶⁺ solutions were prepared by dissolving K₂Cr₂O₇ in DI water</td>
<td>(Tandukar et al., 2009)</td>
</tr>
<tr>
<td>Cr</td>
<td>The anode was transferred from the previous one, which had been acclimated for 3 months (Wang et al., 2008). About 5 g of Cr (VI)-contaminated soil around a local electroplating plant (Dalian, China) was used as the sole inoculum for bio-cathode</td>
<td>Acetate for anode, and NaHCO₃ for cathode</td>
<td>The effluent from the local electroplating plant (Dalian, China) contained Cr⁶⁺ with the concentration about 40 mg/L at the time of sampling</td>
<td>(Huang et al., 2010)</td>
</tr>
<tr>
<td>Cr</td>
<td>The anode used in the reactor was developed in the anodic chamber of a previous MFC, which had been acclimatized by anaerobic digestion sludge for glucose oxidation and continuously operated for 8 months. The cathode inoculum was a mixed bacterial culture enriched in the presence of Cr(VI) with anaerobic digester sludge</td>
<td>Glucose</td>
<td>Cr⁶⁺ solutions were prepared by dissolving K₂Cr₂O₇ in DI water</td>
<td>(Wu et al., 2015)</td>
</tr>
<tr>
<td>Cr</td>
<td>The anode was developed in a separate MFC that was operated for 3 months with acetate and wastewater. The cathode was inoculated using primary clarifier effluent (Dalian, China)</td>
<td>Acetate for anode, and NaHCO₃ for cathode</td>
<td>Cr⁶⁺ solutions were prepared by dissolving K₂Cr₂O₇ in DI water</td>
<td>(Huang et al., 2011)</td>
</tr>
<tr>
<td>Co</td>
<td>The anode was pre-inoculated MFCs running on acetate. Seed sludge obtained from an anaerobic digester (Dalian, China) which received a combination of domestic and metalworking wastewaters, was inoculum for cathode</td>
<td>Acetate for anode, and Co²⁺ salt for cathode</td>
<td>Co²⁺ solutions were used.</td>
<td>(Huang et al., 2014)</td>
</tr>
</tbody>
</table>

Ref. code: 25605722300323QDG
In recent BES studies on metal recovery, mixed cultures are preferred to be as inoculums in the anode chamber (Table 2.5). Most studies employed anaerobic sludge from the digesters or sedimentation tanks of wastewater treatment plants to inoculate the bio-anodes. In order to shorten the start-up duration, several researchers used the anodes transferred from the previous anodic chambers in mature MFCs which were originally acclimated by the same substrate (Heijne et al., 2010; Jiang et al., 2014; Luo et al., 2014; Tao et al., 2011; Tao et al., 2012). This pre-acclimation of biological anodes is beneficial as it enhances the adaptability, obtains the stable state promptly, and reduces the operation costs.

When bio-cathodes are investigated, the metal recovery relates to microbial reduction, in which dissimilatory metal reducing bacteria (DMRB) species use metal ions as the terminal electron acceptor in the cathode chamber. Many DMRB species have been widely used in bioremediation of metal contaminated sites, metallic nanoparticles (NPs) production, and other applications (Wang and Ren, 2014). However, few BES studies using bio-cathode for metal reduction (Huang et al., 2011; Huang et al., 2010; Huang et al., 2014; Tandukar et al., 2009; Wu et al., 2015). In these studies, the cathodes were inoculated by mixed bacterial culture enriched in the presence of metal ions similar to the metal that need to be recovered. Metal ions in catholyte are reduced electrochemically or adsorbed into the biofilms of cathode and biologically reduced by microorganisms during their respiration (Wang and Ren, 2014). Although bio-cathode mechanism was demonstrated as a cost-effective reduction process for metal recovery in BES field, the separation of metallic deposits from the biomass is also a big barrier for the practical application. Furthermore, low initial concentration of metals and the retention of biomass in the bio-cathodes are also the limitations for designing a continuous-flow BES reactors (Tandukar et al., 2009).

2.3.4.2 Substrate

In BES technology, substrate is regarded as organic matters which are oxidized by microorganisms during their respiration in the anode chamber. Substrate is one of the most important biological factors because it serves as carbon (nutrient) and energy source. BESs can utilize a wide variety of substrates in anodic chamber, ranging from simple organic molecules to complex compounds. Complex substrates (e.g. protein-rich wastewater) help in establishing a diverse and electrochemically active microbial
community while a simple substrate (e.g. acetate, glucose) is easier to be degraded (Pant et al., 2010). Type of substrate also influences the integral composition of the bacterial community in the anode biofilm and the MFC performance in term of power density and CE (Chae et al., 2009). Study indicated that the category of substrates that Geobacter-like species can favorably utilize is limited to simple substances such as acetate (Chae et al., 2009). In contrast, Shewanella-like species normally prefer lactate to other substrate (Bond and Lovley, 2003).

Table 2.5 shows that acetate is the most common substrate used anodic chamber BES reactors. It has been the appropriate choice for different functions in BES process such as electricity generation, chemicals production and recovery of valuable materials (Pant et al., 2010). In most of new BES designs, acetate is frequently used due to its inertness towards alternative microbial conversions (e.g. fermentations, methanogenesis), which cannot produce electricity. Furthermore, it was considered as a simplest substance to induce electrochemical active bacteria (EAB) (Bond et al., 2002). The power yield generated varies with substrate used in the MFC, with the highest power densities typically produced from acetate (Logan and Rabaey, 2012). When compared with other substrates, the acetate-fed MFC showed the highest CE (72.3%), followed by butyrate (43.0%), propionate (36.0%) and glucose (15.0%) (Chae et al., 2009).

Glucose is another commonly used substrates in BES technology. However, when compared with acetate in the same system and operation conditions, glucose showed less effective. The energy conversion efficiency (ECE) of acetate and glucose found in a MFC was 42% and 3%, respectively (Lee et al., 2008). Low ECE with glucose led to low current and power density. This can be attributed to the fact that glucose is a fermentable substrate implying its consumption by diverse competing metabolic pathways which resulted in lower CE.

In terms of metal recovery, it is difficult from literature review to compare the efficiency between acetate and glucose due to the difference in types of recovered metal, BES configurations, and operating conditions. Till now, the effects of different substrates on recovery efficiency have not been investigated yet. As shown in Table 2.4, most studies use artificial wastewater containing pure substrate and medium (i.e. buffer solution, nutrients, trace elements) to process the biological conversion in the
anode chamber. However, the chemical intensive, even though low quantity is used, conflicts with the cost-effective concept according to BES principle. Therefore, further attempts should be made to investigate the possibility of real wastewater with high organic loading to serve as the carbon source for anodic bacterial community.

2.3.4.3 Metal-rich solution

Based on BES principle for metal recovery, metal ions is utilized as electron acceptors in the cathode chamber to get reduced through the electrochemical reaction. Depending on the standard reduction potential of metals, the recovery can be processed with or without supplying the external power source (section 2.2.1). As compared to other traditional methods, BES shows more flexibility since a wide range of initial metal concentration (i.e. from several mg/L to more than 1000 mg/L) can be used in recovery process (Table 2.2).

Till date, most studies have conducted in pilot models or lab scales and used synthetic aqueous solutions containing metal ions in the single form to investigate the BES performance. This may help to facilitate the BES operation and ensure the stability of system, which may require complicate and stringent controls when complex solutions or real wastewater are used. Thus, the metal-rich solutions acting as the catholyte were normally prepared from single and dissolvable metallic salts. High reduction potential metals such as \( \text{Cr}^{6+}/\text{Cr}^{3+} \) (1.33V), \( \text{Au}^{3+}/\text{Au}^{0} \) (1.00V), \( \text{Ag}^{+}/\text{Ag}^{0} \) (0.799V), and \( \text{Cu}^{2+}/\text{Cu}^{0} \) (0.28V) (Nancharaiah et al., 2015), have been selected as a pioneer in the early stage of BES development for metal recovery due to their spontaneous reduction without energy consumption.

During the operation, some studies maintained a low catholyte pH (e.g. pH 2-3) by adding HNO\(_3\) or HCl solution in order to keep metals in dissolved state (Choi and Hu, 2013; Heijne et al., 2010; Luo et al., 2014; Tao et al., 2011). However, the addition of concentrated acid into the catholyte may have an influence on the BES performance. The effects of initial catholyte pH on metal recovery efficiency and power generation have been investigated. The reduction of \( \text{Cr}^{6+} \) ions was shown to be strongly pH dependent as lower pH might make the reaction more favorable (Wang et al., 2008). In contrast, it was reported that the initial pH had less impacts on the reduction of \( \text{Ag}^{+} \) (Tao et al., 2012) and \( \text{Cu}^{2+} \) ions (Wang et al., 2010). Other researchers indicated that the low pH in the catholyte poses hydrogen (H\(_2\)) generation evolution in case of
supplying an external power source, which thus reduce the metal conversion efficiency (Wang and Ren, 2014). Besides, in order to enhance the ionic conductivity of catholyte, the supporting electrolytes such as KNO₃, KCl, and NaClO₄ in different amounts were added into the cathode chambers (Choi and Cui, 2012; Choi and Hu, 2013; Huang et al., 2013; Lim et al., 2015; Wang et al., 2010). This addition showed positive effects in cell voltage production and further increased the power generation, except in the case of AuCl₄⁻ serving as the catholyte, because more Cl⁻ ions in the cathode chamber may arrest the Au³⁺ reduction (Choi and Hu, 2013).

Practically, metals in complex forms, chelating agents, and multi-metal salts usually exist in real wastewater generated from different industrial processes. Therefore, BES researchers recently turn their attention to consider the recovery of metals from complex or simulated solutions. For instance, silver monothiosulfate complex [AgS₂O₃]⁻, silver(I) diaminem complex [Ag(NH₃)₂]⁺, and simulated photography wastewater were investigated to recover metallic silver in BES reactors (Tao et al., 2012; Wang et al., 2013). As compared to free silver ions (Ag⁺/Ag⁰, E⁰ = 0.79 V)), silver complexes had a slower reduction rate due to their lower standard reduction potential (i.e. [AgS₂O₃]⁻/Ag⁰, E⁰ = 0.25 V, [Ag(NH₃)₂]⁺/Ag⁰ = 0.373 V).

In addition, few studies tried to recover selectively metals from mixed or multi-metal solutions containing other metals with negative reduction potential (e.g. Cd, Zn, Pb, Fe) (Abourached et al., 2014; Luo et al., 2014; Modin et al., 2012). In this case, an external power supply was required to forcibly drive the electrons travel from the high potential anode to the low potential cathode of MEC reactors, so metals can still be reduced by accepting this electrons flow (Wang and Ren, 2014). By setting different input voltages at the cathode, four metals (i.e. Cu, Pb, Cd, and Zn) could sequentially be recovered from a mixed solution (Modin et al., 2012). The artificial acid mine drainage (AMD) containing Cu²⁺, Ni²⁺, Fe²⁺ was used as catholyte in a dual-chamber MEC to investigated the consecutive recovery of these metals. With an applied voltage of 1.0 V, Cu²⁺ in the AMD was prior to others to be recovered at the cathode, followed by Ni²⁺, and finally Fe²⁺ (Luo et al., 2014).

Overall, real waste solutions are complicate in chemical composition as they normally contain many types of metal. Basically, all metal ions with any reduction potential can be reduced by controlling the input voltages. However, energy
consumption along with recovery yield should be taken into account to ensure the cost-effective feature of BES technology.

### 2.4 Recent BES studies for Ag recovery and their gaps

Some recent studies investigated the feasibility of BES technology on recovery of Ag from different Ag(I)-containing solutions (Choi and Cui, 2012; Lim et al., 2015; Tao et al., 2012; Wang et al., 2013).

It was reported that when 200 ppm Ag⁺ as AgNO₃ solution was used in a batch-fed MFC with 1000 Ω resistance, high recovery efficiency (98.3%) was achieved along with an electrical energy output of 0.0143 kWh per kg of silver (Choi and Cui, 2012). When the initial Ag⁺ concentration increased in the range of 50-4000 ppm, the recovery efficiency was 98.2-92.3%, after operating for 10 hours (Lim et al., 2015). The reduction of Ag⁺ ions at the cathode was reported as a fast reaction in which higher removal rate was observed at higher initial Ag⁺ concentrations for the first 3 hours, and thereafter, it followed similar pattern at the initial Ag⁺ concentration investigated. However, the initial Ag⁺ concentration affected the power generation by both increasing the cathode potential and decreasing the internal resistance of the MFC (Choi and Cui, 2012; Lim et al., 2015). For the effects of initial catholyte pH, Tao et al. (2012) indicated that the adjustment of original pH of AgNO₃ solution, from 6.6 to 4.0, and 2.0, had little impact on the Ag⁺ reduction rate in a BES. According to this study, the decrease of pH led to higher concentration of proton (H⁺) present in the catholyte, which inhibited the transportation of H⁺ produced in the anode chamber through the membrane to finish closed loop current. The cell current thus, did not increase and the silver removal rate and efficiency appeared almost identical among investigated pH values. However, these above studies either examine separately the pH or initial Ag⁺ concentration. Both pH and initial Ag⁺ concentration are influential parameters that need to be investigated simultaneously in a BES reactor. Therefore, the current study will focus on both aspects.

The above studies used acidic solutions containing only simple free silver ions (Ag⁺) as the catholyte, except [Ag(S₂O₃)²⁻] solution was considered by Tao et al. (2012). Due to high standard reduction potential (i.e. Ag⁺/Ag₀, E⁰ = 0.799 V vs. SHE) (Nancharaiah et al., 2015), Ag⁺ ions can be reduced spontaneously in the cathode chamber. There are not many studies on recovery of silver from complex forms or from...
alkaline waste solutions. Silver(I) diammine contaminated wastewater containing \([\text{Ag(NH}_3\text{)}_2]^+\) complex was firstly studied by Wang et al. (2013) to recover Ag accompanied with electricity production. By using a dual chamber bio-electrochemical cell, a maximum electricity of 3.2 J and 160 C could be produced, and 1.6 g Ag could be recovered from \([\text{Ag(NH}_3\text{)}_2]^+\) solution after 9 hours of operation while 1 g COD was removed from the anode chamber. However, in Wang et al. (2013) research, the operation for silver recovery in the bio-electrochemical cell was carried out at one level concentration of \([\text{Ag(NH}_3\text{)}_2]^+\) (i.e. 10 mM), and the effect of initial \([\text{Ag(NH}_3\text{)}_2]^+\) concentration was not considered. Thus, further studies should be conducted to investigate thoroughly the reduction of \([\text{Ag(NH}_3\text{)}_2]^+\) complex.

Other Ag(I)-containing complexes and mixed multi-metal solutions, need to be investigated. One of the most important silver-laden wastewater sources is the fixer waste solution, which is released from the photographic processing. Silver in this effluent normally occur in the form of \([\text{Ag(S}_2\text{O}_3\text{)}_2]\text{)}^3^-\) complex, which has a very low standard reduction potential (i.e. \([\text{Ag(S}_2\text{O}_3\text{)}_2]\text{)}^3^-/\text{Ag}^0, E^0 = 0.016 \text{ V}). This may limit the reduction of \([\text{Ag(S}_2\text{O}_3\text{)}_2]\text{)}^3^-\) complex, as compared to that of \text{Ag}^+ ions. Also, different from the commonly positive charged \text{Ag}^+ ion, \([\text{Ag(S}_2\text{O}_3\text{)}_2]\text{)}^3^-\) complex is in the negative form, which holds a different mechanism in ionic transport through the membrane, and may affect the recovery efficiency and power generation. The above issues have not been considered by previous studies.

In most BES studies, proton exchange membrane (PEM) as Nafion 117 was used frequently to eliminate the transport of electrolytes due to its advantages in ionic conductivity (Tao et al., 2012). However, high cost of Nafion 117 is also a challenge. Although an anion exchange membrane (AEM) as AMI-7001S was a cost-effective alternative to replace Nafion 117, negative-charged ions, such as metabolites from the anaerobic oxidation and \text{PO}_4^{3-}, \text{Cl}^- from the medium of the anolyte, would migrate through the AEM. This caused substrate loss in the anode chamber and also formation of precipitants or insoluble compounds (e.g. \text{CH}_3\text{COOAg}, \text{Ag}_3\text{PO}_4) in the cathode chamber. These above phenomena were not considered by previous researchers during their studies (Choi and Cui, 2012; Lim et al., 2015).

On the other hand, the usage of cation exchange membrane (CEM), such as CMI-7000S, can prevent the silver precipitation and substrate loss in BES reactors, however,
cations in the electrolytes are also able to migrate or diffuse through CEM. Accordingly, the diffusion of Ag\(^{+}\) ions from the cathode to the anode chamber through the CEM, which causes Ag loss problem and further probably leads to negative effects on anodic biological activities, is unavoidable. Till now, there is no study using CEM in BES for Ag recovery. This needs to be investigated further.

Bipolar membrane as BPM-I (Beijing Ting Run Co., China) was used in the research of Wang et al. (2013). Basically, a BPM consists of an AEM and a CEM layer mounted together. BPM allows the simultaneous migration of H\(^{+}\) and OH\(^{-}\) ions generated from the water splitting reaction at the interface of CEM and AEM layer. However, due to the permeability of electrolyte, the transition area (or interface) always contains a certain concentration of electrolyte salts diffusing from both chambers. Under an electric field, there is also a migration of the anions through the AEM layer toward the anode, and the cations through the CEM layer toward the cathode, which is due to the ion exchange ability of membrane. These migrations increase polarization potential of the membrane and lead to an elevated internal resistance (Harnisch et al., 2008; Li et al., 2011). Furthermore, in bipolar-based system, the pH balance between the two chambers is severely affected due to the competition in migration of electrolyte at the interface (Li et al., 2011). These drawbacks of BPM were also pointed out by Wang et al. (2013), which caused low performance in electricity production in their research.

Therefore, it is essential to conduct further and deeper BES studies with focus on comparison of BES performance between different types of Ag(I)-containing solutions, types of membranes, types of substrate and inoculum sources. The findings from this study can help in selecting appropriate materials for recovery of Ag from aqueous solutions by using in BES technology.
The research methodology used in this study is summarized in Figure 3.1. The detail description are presented in following sections.

3.1 Design and fabrication of BES reactors

3.1.1 Structural description

Six cube-shaped BES reactors (R1-R6) made from acrylic plates of 10 mm thick were used in this study. Each reactor consisted of an anode and a cathode chamber (1.0 L working volume), which were separated by an IEM. Depending on experiment, either CEM as CMI-7000S or AEM as AMI-7001S (Membrane International, Inc. USA) was used. The membrane (264 cm² surface area) separated the anode and cathode chamber to prevent the cross-over of electrolytes. Rubber gaskets were also used to prevent leakage. A carbon brush (15 x 6 cm) prepared by twisting carbon fiber (Thai Carbonfiber Co., Thailand) with Ti wire of 1.0 mm in diameter (Prolog Titanium Co., Ltd) served as the anode, while the cathode was a graphite plate (15 x 6 x 0.4 cm, Fujian, China) (Figure 3.2). Holes of 2.0 mm diameter were firstly drilled at the cathode surface to insert Ti wire, and conductive epoxy was then used to make electrical connection.

![Main components of BES reactor](image)

(a) The casing (b) Carbon brush (c) Graphite plate (d) Membrane

Figure 3.2 Main components of BES reactor

3.1.2 Pretreatment of materials

Before making the electrical connection with Ti wire, all materials were pre-conditioned according to previous studies (Feng et al., 2010; Tao et al., 2012).
Design and fabrication of BES reactors

BES inoculation and operation for recovery of Ag and power generation from different Ag(I) solutions

- To understand the effect of substrate (i.e. acetate and glucose) and inoculum sources
- To investigate the effects of initial pH (2.6-7.0), initial Ag⁺ concentration (100-1000 mg/L), and operation time (24h)

With AgNO₃ solution

- To evaluate the effect of different separators (i.e. AMI-7001S, CM-7006S)
- To characterize the anodic microbial community and identify the dominant species along with exoelectrogens

With AgNH₃⁺ solution

- To investigate the reduction of AgNH₃⁺⁺ (1000-2000 mg Ag/L) and the influence of AgNH₃⁺⁺ diffusion through the membrane
- To demonstrate the improvement in electricity output as compared to study by Wang et al. (2013)

With Ag₂S(OH)₃⁻ solution

- To demonstrate the spontaneous reduction of Ag₂S(OH)₃⁻ (10-30 mM) without any external energy despite its very low redox potential
- To compare the difference among positively and negatively charged Ag(I) ions with different redox potential when serving as electron acceptors

With mixed multi-metal solution

- To investigate the advantage of Ag⁺ to be reduced spontaneously from a mixed multi-metal solution containing Ag⁺, Cu²⁺, and Fe²⁺

Working Volume of chamber: V_{water} = V_{cathode} = 1.0 L
- Anode: carbon brush (15.0 x 6.9 cm), Cathode: graphite plate (15.0 x 6.9 cm)
- Separator: CEM as CMI-7009S or AMI-7001S (246 cm²)
- Electrical circuit: Ti wire (1.0 mm diameter) with external resistor of 1000 Ohm or 5 Ohm

Reactor R1

- Inoculum: anaerobic sludge (0.1 L) obtained from WWTP of brewery factory (Thailand)
- Substrate as CIH-COOGNa & media (0.9 L)
- Refilled in each cycle (540 days/cycle) during the inoculation, and in each time catholyte was replaced during operation stage

- During inoculation stage: Buffer solution (1.0 L)
- e⁻ acceptor: O₂ supplied by aeration system
- Refilled in each cycle
- During operation stage: Ag⁺ solution 1000 mg/L, pH 5.0-6.7, Ag⁺ solutions 10×1000 mg/L, pH 2.0

Reactor R2

- Inoculum: anaerobic sludge (0.1 L) obtained from WWTP of brewery factory (Thailand)
- Substrate as Glucose & media (0.9 L)
- Refilled in each cycle (540 days/cycle) during the inoculation, and in each time catholyte was replaced during operation stage

- During inoculation stage: Buffer solution (1.0 L)
- e⁻ acceptor: O₂ supplied by aeration system
- Refilled in each cycle
- During operation stage: Ag⁺ solutions 250-2000 mg/L, pH 2.0

Reactor R3

- Inoculum: anaerobic sludge (0.1 L) obtained from WWTP of brewery factory (Thailand)
- Substrate as Glucose & media (0.9 L)
- Refilled in each cycle (540 days/cycle) during the inoculation, and in each time catholyte was replaced during operation stage

- During inoculation stage: Buffer solution (1.0 L)
- e⁻ acceptor: O₂ supplied by aeration system
- Refilled in each cycle
- During operation stage: Ag⁺ solutions 250-2000 mg/L, pH 2.0

Reactor R4

- Inoculum: anaerobic sludge (0.1 L) obtained a WWTP of brewery factory (Thailand)
- Substrate as CIH-COOGNa & media (0.9 L)
- Refilled in each cycle (540 days/cycle) during the inoculation, and in each time catholyte was replaced during operation stage

- During inoculation stage: Buffer solution (1.0 L)
- e⁻ acceptor: O₂ supplied by aeration system
- Refilled in each cycle
- During operation stage: Ag⁺ solution 100-2000 mg/L
- 5×10³ mM NaN₃ was added into Ag⁺/NH₃⁺ solution 2000 mg/L

Reactor R5

- Inoculum: anaerobic sludge (0.1 L) obtained a WWTP of brewery factory (Thailand)
- Substrate as CIH-COOGNa & media (0.9 L)
- Refilled in each cycle (540 days/cycle) during the inoculation, and in each time catholyte was replaced during operation stage

- During inoculation stage: Buffer solution (1.0 L)
- e⁻ acceptor: O₂ supplied by aeration system
- Refilled in each cycle
- During operation stage: Ag⁺ solutions 250-2000 mg/L, pH 2.0

Reactor R6

- Inoculum: anaerobic sludge (0.1 L) obtained a WWTP of brewery factory (Thailand)
- Substrate as CIH-COOGNa & media (0.9 L)
- Refilled in each cycle (540 days/cycle) during the inoculation, and in each time catholyte was replaced during operation stage

- During inoculation stage: Buffer solution (1.0 L)
- e⁻ acceptor: O₂ supplied by aeration system
- Refilled in each cycle
- During operation stage: Mixed multi-metal solutions containing 10 mM Ag⁺, 1 mM Cu²⁺, and 10×30 mM Fe²⁺

Figure 3.1 Summary of research methodology

Ref. code: 25607230323205G
The membrane (264 cm² surface area) was pre-treated by immersing in 5% NaCl solution for 12 hours to allow for membrane hydration and expansion (www.membranesinternational.com). After that, the membrane was washed thoroughly with DI water before being used.

For the carbon brush anode, it was first cleaned by soaking in pure acetone, overnight. After that, it was acid treated by soaking in a mixture of ammonium peroxodisulfate (200 g/L) and concentrated sulfuric acid (100 mL/L) for 15 min. The anode was then heat-treated in a muffle furnace at 450°C for 30 min and finally washed three times with distilled water. According to previous studies, the above procedure increased power generation by 25% due to the increase in specific area, which facilitated bacteria adhesion with less contaminants that interfere with charge transfer from bacteria to electrode (Feng et al., 2010; Wang et al., 2009; Wei et al., 2011).

For graphite plate cathode, it was immersed more than 24 h in 2 mM potassium permanganate solution (KMnO₄) and washed thoroughly with DI water before assembling, to eliminate all contaminants from the original materials. All components, including two chambers, rubber gaskets, electrodes, and membrane were fixed together and tightened by using 10 threaded rods and wing nuts. The detail description of BES reactors are also shown in Appendix A and B.

3.2 BES operation for Ag recovery and power generation

All experiments were conducted under batch fed conditions and room temperature (20-25°C). All chemicals used in this study are analytical grade and obtained from Fisher Chemical (Thermo Fisher Scientific, Belgium and Fisher Scientific, UK), Ajax Finechem Pty Ltd, Merck Millipore (Darmstadt, Germany), Laboratory Reagents and Fine Chemicals (Loba Chemie Pvt. Ltd, India), and Wako Pure Chemical Industries, Ltd. (Japan). The solutions and reagents were prepared by using deionized (DI) water or Milli-Q water.

Depending on types of Ag(I) solution investigated, which served as catholytes, BES operation was conducted accordingly in 6 different reactors (R1-R6) to achieve specific objectives (see Figure 3.1). In addition, several control reactors were also operated to confirm the results obtained in main reactors.

The detail operation in each BES reactor is described as below.
3.2.1 Catholyte as AgNO₃ solution (Ag⁺) (Reactor R1, R2, R3)

(1) To understand the effect of substrate and inoculum (Reactor R1 & R2)

The effects of substrate and inoculum sources on the anodic acclimation and \( E_{\text{cell}} \) progression were studied by comparing the performance of reactor R1 and R2 during the inoculation stage. Both reactors have a similar design, in which the CMI-7000S membrane was the separator. Although different inoculum and substrate sources were used in the anode chambers, the initial organic loading, referred as COD concentration of 1000 mg/L, was fed into the anode chambers of both reactors.

In reactor R1, the anode chamber was inoculated by anaerobic sludge (100 mL) collected from a brewery wastewater treatment plant (Pathum Thani, Thailand). For the anolyte (900 mL), the following medium (per liter) were used: \( \text{CH}_3\text{COONa} \) (1.28 g) as substrate, \( \text{NaHPO}_4\cdot2\text{H}_2\text{O} \) (5.18 g), \( \text{Na}_2\text{HPO}_4 \) (2.44 g), \( \text{NH}_4\text{Cl} \) (0.31 g), yeast extract (0.2 g), and trace elements.

On the other hand, reactor R2 was inoculated by anaerobic sludge (100 mL) from digester of a domestic wastewater treatment plant (Tokyo, Japan). The anolyte composition (900 mL) was changed to be (per liter) glucose \( \text{C}_6\text{H}_{12}\text{O}_6 \) (0.935 g) as substrate and other medium as \( \text{Na}_2\text{HPO}_4 \) (3.55 g), \( \text{KH}_2\text{PO}_4 \) (3.4 g), \( \text{NH}_4\text{OH} \) (0.2 g), and yeast extract (0.2 g).

During the inoculation, the cathode chambers in both reactors were filled by buffer solution (1.0 L) containing (per liter) \( \text{NaH}_2\text{PO}_4\cdot2\text{H}_2\text{O} \) (4.77 g), \( \text{Na}_2\text{HPO}_4 \) (2.75 g), \( \text{NaCl} \) (2.93 g) and purged continuously with air (80 mL/min) to supply \( \text{O}_2 \) as an electron acceptor.

The anodes and cathodes were connected through a closed electrical circuit with 1000 \( \Omega \) resistance as an external load, to build up full fuel cell and transport electron. This external resistance, as compared to lower levels, could help to shorten the lag phase of acclimation and development of exoelectrogenic biofilm in the anode chamber (Ahn and Logan, 2012). The anolytes and catholytes were refreshed every 5-6 days. The cell voltage (\( E_{\text{cell}} \)) produced was recorded automatically using a data logger to monitor the system’s behavior. After obtaining three repeatable stable voltages with air bubbling in the cathode chamber, BES reactors were considered to be successfully acclimated.
Polarization curve analysis was also conducted to compare the productivity between two reactors. The external resistance was varied in a range of 10,000 – 5 Ω. The stable \( E_{\text{cell}} \) was recorded, current density and power density were then calculated by Ohm’s law. The electrochemical parameters (e.g. OCV, \( R_{\text{int}} \), \( P_{\text{max}} \)) were determined.

(2) To investigate the effect of initial pH and Ag\(^+\) concentration (Reactor R1)

After successfully acclimating the anode chamber, air purging was stopped in the cathode chamber. Reactor R1 was operated for silver recovery as shown in Figure 3.3.

Figure 3.3 Schematic diagram of reactor R1 for silver recovery

The catholyte was replaced by AgNO\(_3\) solutions (1.0 L, initial Ag\(^+\) concentration of 100 mg/L) at different pH (2-7). The catholyte pH was adjusted by addition of either 1M HNO\(_3\) or 1M NaOH solution. The initial organic loading in the anolyte (i.e. COD of 1000 mg/L) was kept constant each time the catholyte was replaced. The half-cell reactions is shown in Eq. (3.1) and (3.2). If the catholyte is Ag\(^+\) of 1,000 mg/L (0.00926 M), the thermodynamic cathode potential \( E_{\text{ca}} \) calculated by the Nernst Equation is 0.679 V vs. SHE. The thermodynamic cell voltage \( E_{\text{therm}} \) is calculated as \( E_{\text{therm}} = E_{\text{ca}} - E_{\text{an}} = 0.679 \text{ V} - (-0.29 \text{ V}) = 0.969 \text{V}. \)

At the anode chamber: \[
\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_2^- + 9\text{H}^+ + 8\text{e}^- \quad E_{\text{an}}^0 = -0.29 \text{ V (3.1)}
\]

At the cathode chamber: \[
\text{Ag}^+ (\text{aq}) + \text{e}^- \rightarrow \text{Ag}_0 (s) \quad E_{\text{ca}}^0 = 0.799 \text{ V(3.2)}
\]

In order to avoid the effect of dissolved oxygen, the anode and cathode chamber were purged with N\(_2\) (80 mL/min) for 15 min and then sealed with septa during operation. Electrolytes (20 mL) in both chambers were withdrawn and analyzed for pH,
remaining Ag\(^+\) concentration, and soluble COD. Further experiments were to investigate the effect of initial Ag\(^+\) concentration by feeding the catholyte containing different Ag\(^+\) concentrations as 200, 500, and 1000 mg/L at pH 2. Similar sampling as described above was conducted. During the operation, a resistance of 5 \(\Omega\) was connected to maximize the current transferred from the anode to cathode for Ag\(^+\) reduction. The DC current in the closed circuit was calculated based on \(E_{\text{cell}}\) production, and Columbic efficiency (CE) was then determined.

Following these experiments, polarization curve analysis was also conducted with different Ag\(^+\) concentration levels (100-1000 mg/L) to characterize the microbial fuel cell of reactor. The analysis started after R1 was operated for 10 hours. The system was first kept in open circuit condition to obtain the stable open circuit voltage, and the \(R_{\text{ext}}\) was then varied gradually from 10,000 \(\Omega\) to 5 \(\Omega\). The \(E_{\text{cell}}\) was observed within 15 minutes and temporarily stable values were recorded to avoid the incidences caused by the change of substrate/product during the measurement. The current density (I) and power density (P) were then calculated at each external resistance.

(3) **To evaluate the effect of CMI-7000S and AMI-7001S as separators**

(Reactor R2 & R3)

In order to investigate the effect of CEM as CMI-7000S and AEM as AMI-7001S on BES performance, the experiments were conducted in reactor R2 and R3. The main components of reactor R3 was similar to that of R2 (e.g. the casing, volume, anode, and cathode), except the separator. The CMI-7000S membrane was used in R2 (CMI-based reactor), while AMI-7001S membrane was installed in R3 (AMI-based reactor).

The inoculum and substrate sources along with anolytes and catholytes in R2 and R3 were alike (i.e. the anaerobic sludge from digester of a domestic wastewater treatment plant in Tokyo (Japan) and glucose served as the substrate). The initial organic loading in the anolyte, as represented by total organic carbon (TOC) concentration, instead of COD, was 460 mg/L and also refreshed every 5-6 days during the inoculation stage. When the inoculation in R2 and R3 finished (i.e. stable \(E_{\text{cell}}\) was obtained with 1000 \(\Omega\) of external resistor), the BES operation for Ag recovery started (Figure 3.4).
The catholyte was replaced by AgNO₃ solutions (1.0 L, pH 2.0) containing 200, 500, 1000, and 2000 mg/l of Ag⁺. The initial catholyte pH was adjusted to pH 2.0 by the addition of concentrated HNO₃ solution, as high E\textsubscript{cell} production was found at pH 2.0 in the experiments of reactor R1. The initial organic loading in the anolyte (i.e. TOC of 460 mg/L) was kept constant each time the catholyte was replaced. A similar procedure of electrical connection and measurement was carried out, as described in R1. Both R2 and R3 were run for 24 hours in each batch. The anolyte and catholyte were sampled every 4 hours to determine the remaining TOC and Ag⁺ concentration.

For characterizing the electricity production, polarization curve analysis was conducted with different Ag⁺ concentration levels (200-2,000 mg/l). The external resistance was varied gradually from 10,000 to 5 Ω. A similar procedure, as described in R1, was conducted in R2 and R3.

In addition, two control reactors (i.e. R\textsubscript{02} and R\textsubscript{03}, corresponding to R2 and R3, respectively) without inoculation were operated under open circuit condition (i.e. no current transferred from the anode to the cathode) to investigate the transport of Ag⁺ ions through CMI-7000S and AMI-7001S, respectively. Anolytes containing glucose and medium along with catholytes (i.e. Ag⁺ solutions, 2000 mg/L) were fed into the anode and cathode chambers of R\textsubscript{02} and R\textsubscript{03}. The experiment was observed for 24 hours, and the Ag⁺ concentration in the anolyte and catholyte, were analyzed.
To characterize the anodic microbial community (Reactor R1, R2 & R3)

As bacterial species in the anode chamber play an important role in the anodic reactions, which affect directly on the BES performance, the characterization of bacterial communities is essential. Since different inoculums were used in R1, R2, and R3, the characterization was conducted as following:

In R1, the occurrence and the morphology of bacterial cells adhering on the anode surface were observed by using scanning electron microscope (SEM). After 59 days of inoculation and operation, R1 was emptied and the carbon brush anode was taken out of reactor. Prior to SEM observation, the brush was pre-treated with a buffer solution and paraformaldehyde 4% (4°C, 24h), to fix bacteria cells, and followed by washing and dehydration in ethanol solutions (10%, 30%, 50%, 70%, and 100%). Samples were then coated by gold (Au) before the SEM test.

For R2 and R3, a better understanding of the anodic microbial communities, dominant species, and exoelectrogen which contribute to electricity production, was achieved by using DNA extraction and gene sequence analysis. The analysis was conducted after running R2 and R3 for 73 days (i.e. 35 days of inoculation and 38 days of operation for silver recovery). For this, a sample taken from each reactor was a mixture of 10 ml of suspended biomass and biofilm attached to the carbon brush. The biofilm was collected by slicing small pieces of carbon brush (i.e. 1.34 g and 1.24 g of brush from R2 and R3, respectively) with sterilized scissors. Genomic DNA extraction was conducted according to the FastDNA® SPIN Kit for Soil Protocol (MP Biomedical, UK).

The extracted DNA was then amplified and purified by using the nested PCR technique to construct the 16S rRNA sequence library. For the first PCR cycle and purification, extracted DNA was amplified using 515F and 806R primers targeting constant regions, to amplify V4 hypervariable region of the 16S rRNA gene. For the thermal cycle (Veriti®, Applied Biosystems®, Thermo-Fisher Scientific, USA), the following conditions were applied: initial denaturation (94°C, 2 min); followed by 25 cycles of denaturation (94°C, 30 sec), annealing (50°C, 30 sec), and elongation (72°C, 30 sec); and a final elongation (72°C, 5 min). The PCR products were purified using a MinElute® PCR purification kit (Qiagen, Germany).
The purified PCR products were used as a template for the second round of PCR cycle with 519F and 802R primers linked with barcodes. Following purification with the same PCR purification kit, the second PCR products were then subjected to gel electrophoresis with 1.5% agarose. Bands corresponding to 450 bp were excised from the gel and eluted using the MinElute® Gel Extraction kit (Qiagen, Germany).

PCR quantification was conducted with a Quan-it™ Picogreen® dsDNA assay kit (Invitrogen, Thermo-Fisher Scientific, USA) and Nanodrop™ 3300 fluorospectrometer (Thermo Scientific, USA). The obtained PCR products were mixed and sent to Bioengineering lab. Co. (Japan) for sequence analysis. Sequencing results were classified into Operational Taxonomic Unit (OTU) using UCLUST (Edgar, 2010) and matched against the Green genes Database (McDonald et al., 2012).

3.2.2 Catholyte as [Ag(NH$_3$)$_2$]$^{+}$ solution (R4)

Reactor R4 was used to investigate the recovery of Ag coupled with electricity production and wastewater treatment from silver(I) diammine complex [Ag(NH$_3$)$_2$]$^{+}$. Besides, R4 was also used to confirm the improvement in electricity output. Before operation, R4 was designed and inoculated similarly to R1 (i.e. CMI-7001S as the separator, anaerobic sludge as inoculum, acetate as substrate) (Figure 3.5).

![Figure 3.5 Schematic diagram of reactor R4 for silver recovery](image)

The experiments were then conducted by feeding [Ag(NH$_3$)$_2$]$^{+}$ complex as electron acceptors (Figure 3.5). The complex was prepared by dissolving Ag$_2$SO$_4$ at
different Ag\(^+\) concentrations (1000-3000 mg/L) in the excess amount of 30% NH\(_4\)OH solution along with de-ionized (DI) water. The mixture was heated gently on a hotplate with occasional stirring until the solid Ag\(_2\)SO\(_4\) dissolved completely. Silver existed in the form of [Ag(NH\(_3\))\(_2\)]\(^+\) complex. The original catholyte pH was around 10.2.

In each batch of experiments, acetate as substrate (i.e. COD = 1000 mg/L), was biologically oxidized in the anode chamber, as shown in Eq. 3.1, while [Ag(NH\(_3\))\(_2\)]\(^+\) complex were reduced spontaneously in the cathode chamber, as shown in Eq. 3.3.

\[
[\text{Ag(NH}_3\text{)}_2]^+ + e^- \rightarrow \text{Ag}^0 + 2\text{NH}_3, \quad E_{ca}^0 = +0.373 \text{ V (vs. SHE)} \tag{3.3}
\]

The thermodynamic cell voltage (E\(_{\text{therm}}\)) is calculated as

\[
E_{\text{therm}} = E_{ca}^0 - E_{an}^0 = 0.373 \text{ V - (- 0.29 V) = 0.663 V.}
\]

The anolyte was refilled with fresh solutions each time the catholytes were replaced. A similar procedure of electrical connection and measurement was carried out, as described in reactor R1. The BES operation was conducted in a longer duration, as 48 hours for each batch of the experiments.

The effects of initial [Ag(NH\(_3\))\(_2\)]\(^+\) concentration (1000-3000 mg Ag/L) on the BES performance was investigated by considering (i) silver removal and E\(_{\text{cell}}\) production with time, (ii) COD removal and Columbic efficiency after 48 hours, and (iii) polarization and power curves. Besides, in order to investigate the effect of supporting electrolyte on power generation, different amounts of NaNO\(_3\) (5-10 mM) were added into the catholyte containing 2000 mg/L of Ag ions.

In addition, a control BES reactor (R\(_{04}\)) was employed to demonstrate the role of biologically anodic reactions and the feasibility of cathodic reduction. This experiment was also used to confirm the diffusion of [Ag(NH\(_3\))\(_2\)]\(^+\) complex through CMI-7000S membrane. R\(_{04}\) was not inoculated, and operated under the open-circuit condition. Anolyte containing acetate and medium along with catholyte (i.e. [Ag(NH\(_3\))\(_2\)]\(^+\) solution, 2000 mg Ag/L) were fed into the anode and cathode chamber, respectively. R\(_{04}\) was observed for 48 hours, and the Ag concentration in the anolyte and catholyte were analyzed.

### 3.2.3 Catholyte as [Ag(S\(_2\)O\(_3\))\(_2\)]\(^3-\) solution (Reactor R5)

The experiments with silver(I) dithiosulfate complex [Ag(S\(_2\)O\(_3\))\(_2\)]\(^3-\) were carried out in R5, which was designed and inoculated similarly to R1 and R4. After successfully acclimating the anode chamber, the operation was conducted at different
[Ag(S₂O₃)₂]³⁻ solutions as catholytes. During the operation, [Ag(S₂O₃)₂]³⁻ served as e⁻ acceptors, to be reduced electrochemically as shown in Eq (3.4).

\[ [\text{Ag(S}_2\text{O}_3]_2^{3-} + e^- \rightarrow \text{Ag}^0 + 2\text{S}_2\text{O}_3^{2-}, \quad E^0_{\text{ca}} = +0.016 \ \text{V (vs. SHE)} \] (3.4)

The anolyte in R5 was also refreshed each time the catholyte was replaced to keep the initial organic loading (i.e. COD 1000 mg/L) to be similar in each batch of experiments (i.e. 48 hours). Under the specific test conditions applied in this study (i.e. CH₃COO⁻ of 1.28 g/L, pH of 7.0 in the anolyte, and [Ag(S₂O₃)₂]³⁻ of 10 mM in the catholyte), the theoretical \( E_{\text{therm}} \) (i.e. \( E_{\text{therm}} = E_{\text{ca}} - E_{\text{an}} \)) is +0.313 V. A similar procedure of electrical connection and measurement was carried out, as described in R1.

**Figure 3.6 Schematic diagram of reactor R5 for silver recovery**

The [Ag(S₂O₃)₂]³⁻ complex was prepared as following: (1) mixed AgNO₃ (10-30 mM) with KBr (10-30 mM) to produce AgBr, (2) dissolved AgBr in 100 mM Na₂S₂O₃ solution. The original pH of complex was 7.39, 6.77, and 6.18, corresponding to the initial Ag(S₂O₃)₂³⁻ concentration of 10, 20, and 30 mM, and was not adjusted during
the operation. The catholyte as \([\text{Ag(S}_2\text{O}_3]_2]^{3-}\) solution used in R5 simulated the fixer waste solution normally generated from photographic processing.

As CMI-7000S was used, the diffusion of \([\text{Ag(S}_2\text{O}_3]_2]^{3-}\) was avoidable, which helped to prevent Ag loss in the system. In order to confirm the diffusion issue, a control BES reactor (i.e. R\(_{05}\)) was employed. R\(_{05}\) was designed similarly to R\(_5\), however, it was not inoculated. Instead, fresh anolyte and the catholyte of 20 mM \([\text{Ag(S}_2\text{O}_3]_2]^{3-}\) were fed into the anode and cathode chamber, respectively. R\(_{05}\) was operated for 24 hours, and the Ag concentration in both chambers were analyzed.

### 3.2.4 Catholyte as mixed multi-metal solutions (Reactor R6)

Real Ag(I)-containing wastewater normally contains other metals, beside Ag. Thus, reactor R6 was designed to investigate the reduction of Ag\(^+\) ions from a synthetic mixed metals solution, containing Ag\(^+\), Fe\(^{3+}\), and Cu\(^{2+}\) salts. These components based on the major metals found in the fixer waste solution of a local photographic shop (Pathum Thani, Thailand). For assembling the reactor, CMI-7000S membrane was used in R6. Other materials (e.g. the casing, the electrodes) were resemble to that used in reactor R1-R5. R6 was also inoculated similarly to R1.

For operation, the cathode was filled by mixed multi-metal solutions containing 10 mM AgNO\(_3\), 1 mM Cu(NO\(_3\))\(_2\), and 10-20 mM Fe(NO\(_3\))\(_3\), at pH 2-3. The anolyte was also replaced by fresh solutions each times of catholyte replacement. During the experiments, due to the difference in the standard reduction potential of metal ions (i.e. \(E^0(\text{Ag}^+/\text{Ag}^0) = + 0.799 \text{ V}, \ E^0(\text{Cu}^{2+/\text{Cu}^0}) = + 0.337 \text{ V}, \) and \(E^0(\text{Fe}^{3+/\text{Fe}^0}) = - 0.41 \text{ V})\), the reduction of metal ions would be different. The concentration of Ag\(^+\), Fe\(^{3+}\), and Cu\(^{2+}\) in the catholyte were determined by time of each batch to investigate the sequential reduction. The effects of Fe\(^{3+}\) and Cu\(^{2+}\) ions due to their competition to accept the electrons with Ag\(^+\) in the catholyte, were also considered.

For electrical measurement, a similar electrical circuit was connected, and \(E_{\text{cell}}\) progression was observed through a data logger, as described in R1. Polarization and power curves were also analyzed to evaluate the performance of electricity production.

### 3.3 Analytical methods and calculation

The analysis and calculation used in this study are summarized in Table 3.1. All measurements were performed in replicates, and the results were reported as means.
### Table 3.1 Analytical methods and calculations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reactor</th>
<th>Methods/ Equations/ Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrolytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>All reactors</td>
<td>A Mettler Toledo pH meter</td>
</tr>
<tr>
<td>Ag concentration (mg/l)</td>
<td>R1, R4, R5, R6</td>
<td>The catholytes were filtered through syringe filters NY 0.45 µm (Allpure), and Ag concentration was analyzed by an Inductively Coupled Plasma Spectrometer (ICP-OES Optima 8000, PerkinElmer)</td>
</tr>
<tr>
<td></td>
<td>R2, R3</td>
<td>Both anolytes and catholytes were filtered through syringe filters PTFE 0.45 µm (Advantec, Japan), and Ag concentration was measured by an ICP Spectrometer (ICP-MS Agilent 7500, Japan)</td>
</tr>
<tr>
<td>Silver removal efficiency RE (%)</td>
<td>R1, R4, R5, R6</td>
<td>$RE_{Ag} = \frac{C_0 - C_t}{C_0} \times 100%$ (3.5)</td>
</tr>
<tr>
<td></td>
<td>R2, R3</td>
<td>$RE_{Ag} = \frac{C_0 - C_t - C_{diff}}{C_0} \times 100%$ (3.6)</td>
</tr>
<tr>
<td>Silver removal rate r&lt;sub&gt;Ag&lt;/sub&gt; (%)</td>
<td>All reactors</td>
<td>$r_{Ag} = \frac{C_0 - C_t}{t}$ (3.7)</td>
</tr>
<tr>
<td>COD concentration (mg/L) and decrement of COD ($\Delta C_{COD}$)</td>
<td>R1, R4, R5, R6</td>
<td>Based on the Closed Reflux, Titrimetric method (APHA, 1998) $\Delta C_{COD} = COD_0 - COD_t$ (3.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COD&lt;sub&gt;0&lt;/sub&gt; is the initial COD concentration in the anolyte fed into the anode chamber (1000 mg/L), COD&lt;sub&gt;t&lt;/sub&gt; is the COD concentration in the anolyte at time t (mg/L)</td>
</tr>
<tr>
<td>Parameters</td>
<td>Reactor</td>
<td>Methods/ Equations/ Instruments</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>---------------------------------</td>
</tr>
</tbody>
</table>
| Total organic carbons (TOC) concentration (mg/L) and decrement of TOC (ΔC_TOC) | R2, R3 | Using the non-purgeable organic carbon (NPOC) method in a TOC analyzer (TNM-1, Shimadzu, Japan)  
\[
\Delta C_{TOC} = TOC_0 - TOC_t \quad (3.9)
\]  
TOC_0 is the initial TOC concentration of the anolyte fed into the anode chamber (460 mg/L). TOC_t is the remaining TOC concentration in the anolyte at time t (mg/L) |

### Electrical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reactor</th>
<th>Methods/ Equations/ Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell voltage, ( E_{\text{cell}} ) (mV)</td>
<td>All reactors</td>
<td>( E_{\text{cell}} ) was recorded by a data logger (Grant Instruments, Cambridge Ltd., Co), and analyzed by Squirrel software</td>
</tr>
<tr>
<td>Current density, ( I ) (mA/m(^3))</td>
<td>All reactors</td>
<td>Calculated by Ohm’s law ( I = E_{cell}/(R_{ext} \times V) ) (3.10)</td>
</tr>
<tr>
<td>Power density, ( P ) (mW/m(^3))</td>
<td>R1, R4, R5, R6</td>
<td>Calculated by ( P = 1000 \times (E_{cell} \times I)/R_{ext} ) (Logan, 2008) (3.11)</td>
</tr>
</tbody>
</table>
| Columbic efficiency, \( CE \) (%) | R2, R3 | \( CE = \frac{8000 \int_0^T I dt}{VF\Delta C_{COD}} \times 100\% \) (3.12)  
\( CE = \frac{M_s \int_0^T I dt}{Fb_{es}V\Delta C_{TOC}} \times 100\% \) (3.13)  
In Eq. 3.10-3.13, \( R_{ext} \) is the external resistance (\( \Omega \)), \( V \) is the working volume of anode chamber (1.0 L), \( F \) is the Faraday constant (96500 C/mole), \( M_s \) is the molecular mass of Glucose, \( b_{es} \) is the number of electrons produced from 1 mole of Glucose (24 mole e\(^-\)/mole glucose) |
<p>| SEM &amp; EDX analysis | R1 | Deposits on the cathode surface were characterized by using SEM equipped EDX detector (SU8030, Hitachi, USA) |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reactor</th>
<th>Methods/ Equations/ Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2, R3</td>
<td>Deposits on the surface of cathode and membranes were scraped to characterize by SEM (Keyence VE-8800, Japan, and S-3400N, Hitachi, USA) and EDX (Metek, Apollo XP 2060, USA).</td>
</tr>
<tr>
<td></td>
<td>R4, R5</td>
<td>Deposits on the surface of cathode and membranes were characterized by SEM (JSM-7800F, JEOL, USA) equipped with EDX detector (X-Max\textsuperscript{N}, Oxford Instruments, UK).</td>
</tr>
<tr>
<td></td>
<td>R6</td>
<td>Deposits on the surface of cathode and membranes were characterized by SEM (S-3400N, Hitachi, USA and JSM-5410LV, Oxford) and EDS (Metek, Apollo XP 2060, USA and LINK ISIS300, Oxford).</td>
</tr>
<tr>
<td>XRD analysis</td>
<td>R2, R3, R4, R5</td>
<td>Silver deposits on the cathode surface were analysed by XRD machine (PANalytical X’Pert PRO), operated at 40 kV, 30mA. Data were collected over a 20 range from 5° to 70°, at a scanning step of 0.02.</td>
</tr>
<tr>
<td>Anodic biomass digestion</td>
<td>R4, R5</td>
<td>Biomass digestion was conducted by Microwave synthesis (CEM, MARS 6, USA). The sample was a mixture of 0.5 dry sample (carbon brush + sludge) and 10 ml concentrated HNO\textsubscript{3} acid. The operated conditions are as follows: power of 1030-1800, ramp time of 20-25 min, hold time of 15 min, temperature of 200°C, and pressure of 406 psi</td>
</tr>
<tr>
<td>Anodic microbial community</td>
<td>R1, R2, R3</td>
<td>This analysis is described in Section 3.2.1 (4)</td>
</tr>
</tbody>
</table>
Chapter 4
Results and Discussion

This section presents results obtained in terms of recovery of silver using different types of Ag(I) solution as catholytes in different BES reactors. The effects on power generation through cell voltage production, power density, and columbic efficiency are also reported.

4.1 Catholyte as Ag\(^+\) solution

4.1.1 Effects of substrate and inoculum sources

The effect of substrate and inoculum sources on BES performance was investigated by considering the \(E_{\text{cell}}\) progression during the start-up stage of acetate-fed reactor (R1) and glucose-fed reactor (R2). Both reactor used CMI-7000S as separators. Figure 4.1a shows that the output voltage in both reactors increased in a similar pattern from 50 mV to 300-350 mV vs. SHE, which was stable after five consecutive feeding cycles (5-6 days per 1 cycle) corresponding to 720 hours of duration.

\[\text{Figure 4.1 (a) } E_{\text{cell}} \text{ progression (R}_{\text{ext}} = 1000 \Omega), \text{ and (b) Polarization curves (E1 and E2), power curves (P1 and P2) (R}_{\text{ext}} = 10000 - 5 \Omega) during the start-up stage in reactor R1 and R2, respectively}\]

In the 1st cycle, the system took longer (i.e. \(T_1 = 94\) hours for R1 and 120 hours for R2) for adaptation to reach the maximum \(E_{\text{cell}}\) and maintained this state within 11-12 hours (\(T_2\), Table 4.1). The \(E_{\text{cell}}\) thereafter dropped gradually due to the depletion of substrate. However, a stable \(E_{\text{cell}}\) was quickly obtained at the beginning of subsequent
cycles (i.e. \( T_1 = 3-8 \) hours) and maintained for longer duration \( T_2 \), indicating successful inoculation. The \( E_{\text{cell}} \) progression rate \( r \) (mV/h) of R1 and R2 in each cycle were also calculated to compare the performance of microbial activities during the inoculation (Table 4.1). Results showed that R1 provided faster rate of \( E_{\text{cell}} \) response when compared to that of R2. This finding indicated that inoculum sources and anolyte composition affected the start-up process. Acetate was used as a substrate in the anolyte of R1 while glucose was used in that of R2. Although the initial COD concentration was similar between two reactors, acetate was considered as the simplest substance and extensively used as the carbon source to induce electrochemical active bacteria (EAB) (Bond et al., 2002). In addition, acetate is the end product of several metabolic pathways for higher order carbon sources, such as glucose. This is the reason for a faster rate of \( E_{\text{cell}} \) production from reactor R1. It is suggested that the growth, adaptation, and exoelectrogenic activities have an important impact on BES performance.

### Table 4.1 \( E_{\text{cell}} \) progression during the inoculation stage (\( R_{\text{ext}} = 1000 \, \Omega \)) and electrochemical parameters obtained in polarization curves (\( R_{\text{ext}} = 10000 - 5 \, \Omega \)) of acetate-based reactor (R1) and glucose-based reactor (R2)

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Acetate-fed Reactor (R1)</th>
<th>Glucose-fed Reactor (R2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( T_1 ) (h)(^{(a)} )</td>
<td>( E_{\text{cell}} ) (mV)(^{(b)} )</td>
</tr>
<tr>
<td>1</td>
<td>94.00</td>
<td>320-325</td>
</tr>
<tr>
<td>2</td>
<td>3.00</td>
<td>325-330</td>
</tr>
<tr>
<td>3</td>
<td>3.50</td>
<td>340-345</td>
</tr>
<tr>
<td>4</td>
<td>6.00</td>
<td>340-350</td>
</tr>
<tr>
<td>5</td>
<td>6.50</td>
<td>345-350</td>
</tr>
</tbody>
</table>

\( \text{OCV} = 430.20 \, \text{mV}, \text{R}_{\text{int}} = 44.21 \, \Omega, \quad \text{P}_{\text{max}} = 708.12 \, \text{mW/m}^3 \)

\( \text{OCV} = 434.50 \, \text{mV}, \text{R}_{\text{int}} = 47.22 \, \Omega, \quad \text{P}_{\text{max}} = 641.84 \, \text{mW/m}^3 \)

\((a) \ T_1 \) (h) is the duration for cell voltage to reach a stable value
\((b) \ E_{\text{cell}} \) (mV) is the stable cell voltage (vs. SHE) obtained at \( R_{\text{ext}} \) of 1000 \( \Omega \)
\((c) \ r \) (mV/h) is the cell voltage progression rate during \( T_1 \)
\((d) \ T_2 \) (h) is the duration that the stable \( E_{\text{cell}} \) could be maintained
\((e) \text{OCV} \) (mV) is the open circuit voltage obtained from \( E_1, E_2 \) curves (Figure 4.1a)
\((f) \text{R}_{\text{int}} \) (\( \Omega \)) is the internal resistance of reactor determined as the slope of \( E_1, E_2 \) curves (Figure 4.1b)
\((g) \text{P}_{\text{max}} \) (mW/m\(^3\)) is the maximum power density obtained from the \( P_1, P_2 \) curves (Figure 4.1b)

Polarization curve analysis in inoculation stage was also conducted by varying the external resistance (\( R_{\text{ext}} \)) in a range of 10,000 - 5\( \Omega \). The stable \( E_{\text{cell}} \) obtained at each \( R_{\text{ext}} \) was recorded and current density was calculated by Ohm’s law. Figure 4.1b shows the current density in relation to \( E_{\text{cell}} \) (\( E_1, E_2 \)) and power density (\( P_1, P_2 \)) of reactor R1,
R2, respectively. The electrochemical parameters (e.g. OCV, R_{int}, P_{max}) were calculated based on these curves and summarized in Table 4.1. These results demonstrated the acclimation and effective activity of microbial communities in both reactors in terms of electron production. As compared to pure cultures, the higher diversity of microbial communities in mixed cultures can make bio-electrochemical processes more stable and efficient due to their higher adaptability, stability, and productivity (Mathuriya, 2013; Patil et al., 2009).

### 4.1.2 Effects of initial catholyte pH

The experiments were conducted in R1. To study the effect of pH, four kinds of catholyte with pH of 6.4, 4.0, 2.0, and 7.0 (pH 6.4 was the original pH of AgNO_{3} solution) containing 100 mg/L of Ag^{+} were put into cathode chamber. Fresh anolyte was always fed into the anode chamber each time of catholyte filling.

![Figure 4.2 Effect of initial catholyte pH on (a) Ag^{+} reduction and (b) E_{cell} production (R_{ext} = 5 \, \Omega) in reactor R1](image)

Figure 4.2a shows that the Ag^{+} concentration decreased rapidly to below 5 mg/L with > 95% removal efficiency after 24 hours of operation at all pH investigated. At the beginning of operation, the adjustment of initial pH of catholyte actually reduced the Ag^{+} removal rate, and the original pH of 6.4 accordingly showed the highest removal efficiency. The addition of either HNO_{3} or NaOH solution into the catholyte brought other electron acceptors (e.g. H^{+}, NO_{3}^{-}) to compete with Ag^{+} ions, which caused a decrease in the reduction rate. However, after 12 hours of operation, the change of initial catholyte pH (2.0-7.0) had little impact on Ag^{+} removal efficiency. The lower
and similar reduction rate at different pH values after 12 hours may be due to the lower concentration of remaining Ag\(^+\) in the catholyte (≤ 20 mg/L). Results are in line with other researchers (Tao et al., 2012). It was reported that the Ag\(^+\) removal rate and efficiency (> 89%) appeared almost identical after 5.5 hours when the catholyte pH was changed from 2.0 to 6.6.

In term of \(E_{\text{cell}}\) progression (Figure 4.2b), similar trends were found among different pH conditions, in which the voltage generally increased during the first 3 hours, and thereafter it dropped gradually due to the shortage of electron acceptors caused by the reduction of Ag\(^+\) ions. The \(E_{\text{cell}}\) production at pH 7.0 was better than that at pH 4.0 and 6.4 at times (i.e. from 3\(^{\text{rd}}\) to 12\(^{\text{th}}\) hour), which may be due to the reproductive effects of system because the experiment with pH 7.0 followed the three experiments with pH 6.4, 4.0, and 2.0. However, there was not much considerable difference in \(E_{\text{cell}}\) production among the investigated pH after 12 hours as the measured \(E_{\text{cell}}\) varied in a low range (i.e. 2-6 mV). During the operation, the highest \(E_{\text{cell}}\) was obtained with the catholyte at pH 2.0. This may be due to improved ionic conductivity at low catholyte pH. Similar results were reported in a MFC used for removal of Hg\(^{2+}\). When the catholyte pH was adjusted from 4.8 to 2.0, the ionic conductivity increased from 13.2 to 5,160 µS/cm which led to a decrease of internal resistance from 3816.6 to 126.7Ω (Wang et al., 2011). Consequently, this study suggests that the adjustment of the initial catholyte pH favored electricity production, but it did not affect much the silver removal efficiency.

The anolyte pH was also recorded to be stable in the range of 6.5 - 6.6 during 24h of operation. This pH value played an important role for bacterial activities to process biological conversion. This result was due to the role of phosphate buffer solution (NaH\(_2\)PO\(_4\) and Na\(_2\)HPO\(_4\)) in the anolyte. Buffer solution maintained the pH for optimum anaerobic bacterial activity and increased solution conductivity, and hence, can compensate for slow proton transfer (Leong et al., 2013). Therefore, it is believed that the change of initial catholyte pH did not influence the activity of bacteria growing in the anode chamber.

### 4.1.3 Effects of initial Ag\(^+\) concentration

According to the above results, the catholyte pH of 2.0 led to highest \(E_{\text{cell}}\) among evaluated pH values. Thus, the initial catholyte pH was adjusted to 2.0 when
investigating the effect of initial Ag$^+$ concentration (100; 200; 500; 1,000 mg/L) on silver recovery in reactor R1. The study considered the Ag removal efficiency (RE$_{Ag}$), E$_{cell}$ production, columbic efficiency (CE), and maximum power density.

### 4.1.3.1 Silver recovery and electricity production

Ag$^+$ concentration decreased rapidly even though the initial Ag$^+$ concentration increased from 100 to 1,000 mg/L (Figure 4.3a). After 24 hours of operation, the remaining Ag$^+$ concentration in the catholyte was 3.45-7.17 mg/L, which corresponds to the RE$_{Ag}$ of 96.54-99.28% without taking into consideration the diffusion of Ag$^+$ ions from the catholyte to anolyte through the CMI-7000S membrane. The remaining level of Ag$^+$ was higher when the initial Ag$^+$ concentration was higher. Compared to a study at the same initial Ag$^+$ concentration of 1,000 mg/L, a RE$_{Ag}$ of 97.1% was reported after 10 hours of operation (Lim et al., 2015). In this study, 99.28% reduction was observed, indicating that higher reduction of Ag$^+$ ions in the catholyte can be achieved when the operation time is extended. These findings suggest that a long operating time (> 24 h) should be applied in practical applications when silver needs to be reduced from high Ag$^+$ concentrations as normally found in real wastewater.

![Figure 4.3 Effect of initial Ag$^+$ concentrations on (a) Ag$^+$ reduction and (b) E$_{cell}$ production ($R_{ext} = 5 \, \Omega$) in reactor R1](image)

During experiments, the catholyte pH increased slowly from 2.0 to 5.0-6.0 due to the reduction of Ag$^+$ ions and slow transportation of H$^+$ from the anode through the CMI-7000S membrane. Generally, other cations (Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$), existing in anolyte at higher concentration, compete with proton H$^+$, to transfer through the...
membrane, which led to the reduction of H\(^+\) migrating to the cathode chamber (Kokabian and Gude, 2015; Pandit et al., 2012). However, silver precipitation does not occur due to the slightly acidic environment of the cathode chamber. In addition, other anions (e.g. CH\(_3\)COO\(^-\), Cl\(^-\), PO\(_4^{3-}\)) in the anolyte cannot migrate to the catholyte through the CMI-7000S membrane to form Ag precipitant. Consequently, when Ag\(^+\) diffusion from the cathode to the anode chamber was ignored, it can be inferred that Ag\(^+\) ions were reduced to form the metallic deposits on the cathode surface.

In terms of power generation, when the initial Ag\(^+\) concentration in the catholyte increased from 100 to 1,000 mg/L, the \(E_{\text{cell}}\) increased sharply (Figure 4.3b). Ag\(^+\) ions served as electron acceptors in the cathode chamber, thus, the increase in Ag\(^+\) concentration enhanced the cathodic potential, which further increased the \(E_{\text{cell}}\) generation. This explanation is confirmed by polarization curve results (Table 4.2). High \(E_{\text{cell}}\) output remained for a longer duration at high initial Ag\(^+\) concentration. Li et al. (2008) investigated the removal of of Cr\(^{6+}\) from wastewater by using a MFC, in which electrochemical deposition of Cr\(_2\)O\(_3\) on the surface of cathode was found. The study reported that the increase of electron acceptor concentration (i.e. Cr\(^{6+}\), 50 – 500 ppm) increased the cathode potential measured by a saturated calomel electrode, from 619 – 814 mV vs. SHE, and also decreased the internal resistance (i.e. \(R_{\text{int}}\), 300 – 100 \(\Omega\), respectively).

### 4.1.3.2 Columbic efficiency, polarization curves, and power curves

The coulombic efficiency (CE) was also used to evaluate the performance of electricity production. Theoretically, CE is defined as the fraction (or percent) of electrons recovered as current versus that in the starting organic matter, as shown in Eq. (3.12). Results showed that at a higher initial Ag\(^+\) concentration, higher CE was obtained (Table 4.2).

Specifically, CE increased from 4.36 % to 21.61 % with the increase of Ag\(^+\) concentration from 100 to 1,000 mg/L. As the same initial organic loading (COD 1,000 mg/L) was fed into the anode chamber for each batch of experiments, the improvement of CE was attributed to the increase of initial Ag\(^+\) concentration in the catholyte, which resulted in higher open circuit voltages (OCV) and electricity yield. The diversity of microorganisms in anaerobic sludge used as inoculum in the anode chamber may be responsible for the low CE values. Although a diverse microbial community of
hydrolytic, fermentative, acidogenic, and acetogenic microorganisms is a good inoculum choice, the significant methanogenic population in anaerobic sludge competes with the anodophilic microorganisms and thereby decreases the CE of power generation (Logan, 2012; Martin et al., 2010). Studies reported that the substrate in the anode chamber was consumed, not only by exoelectrogen for electricity production, but also by fermenters, methanogens, and unknown species to produce CH₄ and H₂ (Martin et al., 2010; Yu et al., 2015). In other words, the CE was influenced by different electron sinks which occurred during the oxidation of organic compounds. It was reported that the electron sink distribution (%) calculated by electron equivalents as soluble COD (mg) in the closed-circuit MFC was current (52.7%), biomass (20.8%), CH₄ gas (12.9%), unremoved COD (9.7%), and unknown sinks (3.9%) (Yu et al., 2015).

Table 4. 2 Electrochemical parameters obtained in reactor R1 at different initial Ag⁺ concentrations

<table>
<thead>
<tr>
<th>Initial Ag⁺ concentration (mg/L)</th>
<th>CE (%) (a)</th>
<th>E_ca (mV) (b)</th>
<th>E_therm (mV) (b)</th>
<th>OCV (mV) (d)</th>
<th>P_max (mW/m³) (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4.36</td>
<td>619</td>
<td>910</td>
<td>864.20</td>
<td>607</td>
</tr>
<tr>
<td>200</td>
<td>10.52</td>
<td>637</td>
<td>928</td>
<td>895.80</td>
<td>1,491</td>
</tr>
<tr>
<td>500</td>
<td>12.90</td>
<td>661</td>
<td>952</td>
<td>808.80</td>
<td>3,006</td>
</tr>
<tr>
<td>1,000</td>
<td>21.61</td>
<td>679</td>
<td>970</td>
<td>828.80</td>
<td>8,258</td>
</tr>
</tbody>
</table>

(a) Data was calculated based on Eq. (3.12)
(b) E_ca: Cathodic potential calculated by Nernst Eq., E_ca = E⁰ - RT/nF ln(1/[Ag⁺]), E⁰ = 0.799 V vs. SHE
(c) E_therm: Thermodynamic cell voltages defined as (E_ca – E_an), with E_an = -0.291 V
(d) OCV: Open circuit voltage obtained from polarization curves shown in Figure 4.4a
(e) P_max: Maximum power density obtained from power curves shown in Figure 4.4b

Power generation was also performed through polarization and power curves to determine the dependence on resistance of fuel cell performance. In this analysis, the stable E_cell were recorded at pseudo-steady state when the R_ext varied from 10,000 Ω to 5 Ω. The current density and power density were then calculated by Ohm’s law at each R_ext. Polarization curves represented the E_cell as a function of current density while power curves described the power density as a function of current density (Figure 4.4).
In the absence of current, the cell voltages, defined as OCV values, were obviously lower than the thermodynamic cell voltages ($E_{\text{therm}}$) which were defined as the theoretical potential difference between the cathode and anode (Table 4.2). These results indicated parasitic losses in the BES reactor due to current leakage, gas crossover, and unwanted side reactions occurring in the system (Rismani-Yazdi et al., 2008; Wen et al., 2009). Although designed for a selective ion transfer, unselective permeability for the fuel (substrate) and other electrolytes is observed in any membrane process. These fluxes lead to parasitic processes that can result in lower fuel cell performance (Rabaey et al., 2009). At high initial Ag$^+$ concentration (i.e. 500 and 1,000 mg/L), the OCV obtained from the system was slightly lower, probably due to more parasitic losses caused by higher solution conductivity.

When the current increased due to the decrease in $R_{\text{ext}}$, the $E_{\text{cell}}$ as measured dropped accordingly. Generally, the voltage decrease is fairly linear with current, and ohmic losses are dominant in MFC systems. Thus, linear polarization curves are most often encountered in MFC systems and the internal resistance can be determined easily from the slope of these curves (Logan et al., 2006). However, the polarization curves obtained in the current work were distorted and nonlinear (Figure 4.4a). This may be due to the influence of anode and cathode overpotentials which are generally current dependent and can be categorized as activation losses, bacterial metabolic losses, and
mass transport or concentration losses (Logan et al., 2006). Therefore, the decrease in measured \( E_{\text{cell}} \) of BES reactor during the polarization test was caused by both ohmic losses and electrode overpotentials. Among the polarization curves, the curve occurring at the highest initial \( \text{Ag}^+ \) concentration (i.e. 1,000 mg/L), showed the lowest slope, indicating the lowest ohmic resistance due to the increase in ion conductivity.

With respect to power curves, an improvement in power generation was found. The maximum power density \( P_{\text{max}} \) increased sharply with the increase of initial \( \text{Ag}^+ \) concentration from 100 to 1,000 mg/L. The highest \( P_{\text{max}} \) of 8,258 mW/m\(^3\) was achieved with the \( \text{Ag}^+ \) solution of 1,000 mg/L (Figure 4.4b and Table 4.2). The results suggested that the increase of initial \( \text{Ag}^+ \) concentration played an important role in power generation. This finding thus strengthened the practical applicability of BES technology in recovery of silver coupled with electricity production, as Ag is usually found at very high concentrations in real wastewater or aqueous solutions (e.g. waste fixer solutions from photographic processing contains Ag(I) of 2,700-3,300 mg/L (Chen et al., 2012)).

### 4.1.4 Effects of separators as CMI-7000S and AMI-7001S

The effects of separator on BES performance was investigated by examining the silver removal efficiency, power generation, and cathodic deposits obtained in two glucose-fed reactors, including CMI-based reactor (R2) and AMI-based reactor (R3). Two control reactor (i.e. \( R_{02} \) and \( R_{03} \)) without inoculation were also employed to confirm the transport of ions through CMI-7000S and AMI-7001S membranes.

#### 4.1.4.1 Silver recovery

The change of \( \text{Ag}^+ \) concentration with time in the catholytes and anolytes (i.e. \( C_t \), solid line and \( C_{\text{diff}} \), dashed line, respectively) is shown in Figure 4.5. When the initial \( \text{Ag}^+ \) concentration \( (C_0) \) increased from 200 to 2,000 mg/L, the Ag removal rate \( (r_{\text{Ag}}, \text{mg/L.h}) \) in each reactor increased correspondingly, although the remaining \( \text{Ag}^+ \) concentration in the catholyte after 24 hours \( (C_{24}) \) was higher when \( C_0 \) was higher. At the same \( C_0 \), the \( r_{\text{Ag}} \) in R2 was faster than that in R3. Specifically, the \( r_{\text{Ag}} \) was 7.83-76.95 and 7.61-31.49 mg/L.h when the \( C_0 \) increased from 200 to 2,000 mg/L in R2 and R3, respectively. In R3, the \( e^- \) produced during the anaerobic oxidation of exoelectrogen in the anode chamber was not sufficient to supply the fast reduction of \( \text{Ag}^+ \) at high concentration (1000, 2000 mg/L) and investigated time (24 hours), causing lower \( r_{\text{Ag}} \) in
R3. This may be due to the substrate loss occurring in R3, in which AMI-7001S was used as a separator.

Figure 4.5 Change of Ag⁺ concentration with time in the catholyte (solid line) and anolyte (dashed line) of (a) CMI-based reactor (R2) and (b) AMI-based reactor (R3) at different initial Ag⁺ concentrations. (c) Ag distribution in both BES reactors after 24 hours of operation.
The AMI-7001S membrane in R3, however, could prevent the cross-over of Ag\(^+\) ions from the catholyte to anolyte. During experiments, a negligible amount of Ag\(^+\) was detected in the anolyte of R3 (i.e. \(C_{\text{diff}} < 1.0\) mg/L), thus, the results are not presented in Figure 4.5b. A similar result was also found in the control reactor R\(_03\), as almost no Ag\(^+\) ions were detected in the anolyte (Figure 4.6). The decrease of Ag\(^+\) concentration in C3 of R\(_03\) was attributed to the precipitation of Ag\(^+\) with anions (e.g. PO\(_4^{3-}\), OH\(^-\)) migrated from the anolyte through AMI-7001S membrane.

In contrast, silver loss was found in R2 due to the transport of Ag\(^+\) ions from the catholyte to the anolyte through CMI-7000S. During the first 4 hours, only a small amount of Ag\(^+\) penetrated via the membrane. Thereafter, \(C_{\text{diff}}\) increased and reached a peak (\(C_{\text{diff}}^{\text{peak}}\)) at the 12\(^{th}\) hour of operation, except in the case of Ag\(^+\) 2000 mg/L, in which \(C_{\text{diff}}^{\text{peak}}\) was found at the 21\(^{st}\) hour (Figure 4.5a). The increase of C\(_0\) in the catholyte of R2 led to the increase of \(C_{\text{diff}}\) in the anolyte. After rising to the peak point, the \(C_{\text{diff}}\) declined gradually. This can be explained by the slow diffusion of Ag\(^+\) ions after 12 hours due to the accumulation of H\(^+\) from glucose degradation in the anode chamber and the decrease of the Ag\(^+\) concentration gradient between the catholyte and anolyte.

\[\text{Figure 4.6 Change of Ag}^{+}\text{ concentration with time in the catholytes (C2, C3) and anolytes (A2, A3) of control reactors (R} _{02}, \text{ R} _{03}\), respectively}\]

The diffusion of Ag\(^+\) ions through CMI-7000S was also found in the control reactor R\(_02\) fed with Ag\(^+\) solution (2000 mg/L), but a different trend was observed (Figure 4.6). \(C_{\text{diff}}^{\text{peak}}\) was quickly found in R\(_02\) at the 4\(^{th}\) hour, and then declined due to
the precipitation of Ag\(^+\) ions with other ions (e.g. \(\text{PO}_4^{3-}\), \(\text{OH}^-\)) in the anolyte. As \(\text{R}_0\) was operated without bio-reactions in the anode chamber and under open circuit condition, a faster diffusion of Ag\(^+\) ions in \(\text{R}_0\) was observed, as compared to that in \(\text{R}_2\). This was due to the concentration gradient between the two chambers and was also not hindered by the migration of other cations under the electrical field. The decrease of Ag\(^+\) concentration in C2 of \(\text{R}_0\) was mostly caused by the transport of Ag\(^+\) through membrane, as there was no precipitation or reduction observed in cathode of \(\text{R}_0\).

For \(\text{R}_2\) operated with anodic bio-reactions (Figure 4.5a), the presence of Ag\(^+\) ions in the anolyte and the adsorption of some Ag\(^+\) ions into the biomass may cause negative impacts on the anodic microbial community. However, as the anolyte in each reactor was refreshed every 24 hours for each batch of experiments, the accumulated impact was mitigated. Moreover, in this study, anaerobic sludge was used as inoculum. Mixed cultures were reported to possess high adaptability and resistance to stress (Mathuriya, 2013). In addition, Patil et al. (2010) also found that the wastewater-derived electroactive biofilms in a MFC was completely unaffected by the presence of antimicrobial compounds, including heavy metals (i.e. Ag\(^+\) of 0.02-1.0 mg/L, Pb\(^+\) of 0.41-12.48 mg/L, \(\text{Hg}^{2+}\) of 0.83-1.33 mg/L) and other biocides (i.e. antibiotics, disinfectant). They also indicated that a BES may possess a lower susceptibility to toxins than anaerobic digestion due to the high resistance of electroactive microbial biofilms. Nevertheless, the analysis of Ag\(^+\) adsorption in the anodic biomass and further studies on inhibitory effects of Ag on a bacterial community should be conducted, especially with high exposure concentration and longer time operation.

During the above experiments, the catholytes in both BES reactors were in acidic condition as the pH was in a range of 2.0-5.0. Therefore, it is believed that Ag\(^+\) precipitation does not occur. When the Ag\(^+\) attached on membrane surface and adsorbed into the biomass were ignored, the fate of Ag\(^+\) ions includes: (i) a significant amount reduced electrochemically to produce metallic deposits on the cathode surface, (ii) some remained in the catholyte, and (iii) some diffused to the anolyte. After 24 hours of operation, the Ag distribution in each BES reactor at different initial Ag\(^+\) concentrations is shown in Figure 4.5c. At low initial Ag\(^+\) concentrations (i.e. 200, 500 mg/L), higher removal efficiency (\(\text{RE}_{\text{Ag}}\)) was found in AMI-based reactor (R3), whereas CMI-based reactor (R2) was more effective at high initial Ag\(^+\) concentrations.
(i.e. 1000, 2000 mg/L). The highest $\text{RE}_{\text{Ag}}$ of 92.51% was obtained in R2 at $C_0$ of 1,000 mg/l. More $\text{Ag}^+$ ions diffusing through CMI-7000S caused lower $\text{RE}_{\text{Ag}}$ (i.e. 88.61%) in R2 when $C_0$ increased to 2000 mg/L. Although some transport of $\text{Ag}^+$ ions through CMI-7000S was observed, this was not considerable, as compared to the initial $\text{Ag}^+$ concentration in the catholyte. Based on $\text{RE}_{\text{Ag}}$, CMI-7000S is more favorable than AMI-7001S when serving as a separator in a BES reactor. This statement is confirmed by considering power generation and the formation of deposits at the cathode surfaces.

4.1.4.2 Substrate removal and electricity production

In the anode chamber, glucose served as the substrate for the anaerobic oxidation. For each batch of experiments at different initial $\text{Ag}^+$ concentrations, the initial organic loading ($\text{TOC}_0 = 460$ mg/L) was kept constant by feeding fresh anolyte. In order to investigate the substrate removal efficiency, the TOC concentration in the anolyte ($\text{TOC}_t$) was measured every 4 hours.

Figure 4.7a shows the change of TOC concentration with time in both reactors. The $\text{TOC}_t$ decreased in a similar pattern between different $\text{Ag}^+$ solutions, from 460 mg/L to 275-315 mg/L, and to 20-60 mg/L in R2 and R3, respectively. In both reactors, slightly higher TOC removal was observed at higher initial concentration of $\text{Ag}^+$ used.

Although higher TOC removal efficiency could be obtained in R3, this did not actually, contributed to produce electrons to reduce $\text{Ag}^+$ in the cathode chamber. Glucose is a fermentable substrate and is consumed by diverse competing metabolisms, such as fermentation and methanogenesis, which cannot produce electricity. Furthermore, low TOC concentration (< 10 mg/L) was also detected in the cathode chamber of R3 (dotted line, Figure 4.7a). This could be due to the transport of negatively charged organic compounds produced by anodic fermentation, such as butyrate, propionate, and acetate, across the AMI-7001S membrane to the catholyte. In a MFC using RALEX™ AEM membrane, the metabolite loss, as acetate (> 120 mg/L), was detected in the catholyte after 250 hours of operation (Pandit et al., 2012). In contrast, a very small amount of TOC (i.e. < 0.1 mg/L) was detected in the catholyte of R2 in the first batch of experiments (i.e. with $\text{Ag}^+$ 200 mg/L), which is negligible. This data, thus, is not presented in Figure 4.7a.
Figure 4.7 Substrate removal and power generation at different initial Ag$^+$ concentrations. (a) Change of TOC concentration with time. $E_{\text{cell}}$ production ($R_{\text{ext}} = 5 \, \Omega$) in (b) CMI-based reactor (R2), and (c) AMI-based reactor (R3)

The substrate loss in R3 was confirmed by considering the $E_{\text{cell}}$ progression during operation (Figure 4.7c). At the same initial Ag$^+$ concentration fed into the cathode chamber, $E_{\text{cell}}$ production in R3 was much lower than that in R2. In addition, with an increase of initial Ag$^+$ concentration, $E_{\text{cell}}$ in R3 was not improved significantly, as compared to that in R2. It even decreased slightly at high initial Ag$^+$ concentration (i.e. 2000 mg/L). This may be due to the change in the concentration gradient of anions and cations between two chambers. The increase of AgNO$_3$ concentration in the
catholyte accelerated \( \text{NO}_3^- \) diffusion of to the anolyte through AMI-7001S membrane, and also Ag precipitation with other anions migrating from the anolyte. More hindrance at the membrane surface due to the diffusion, migration, and precipitation, may affect the internal resistance of membrane, and thus lower \( E_{\text{cell}} \) production.

In each reactor, as the anolyte was refreshed for each batch of experiments, the difference in \( E_{\text{cell}} \) was due to changes in cathode potential, resulting from the change of initial \( \text{Ag}^+ \) concentration. This is inferred from the relationship with thermodynamic cell voltages \( (E_{\text{therm}}) \), defined as the theoretical difference between cathode and anode potential \( (E_{\text{ca}} - E_{\text{an}}) \) under open circuit condition, in which \( E_{\text{an}} \) (-0.41 V vs. SHE) was constant while \( E_{\text{ca}} \) varied depending on the initial \( \text{Ag}^+ \) concentration (see Appendix C).

The columnic efficiency was also determined to evaluate the electricity production and understand the effect of fermentable substrate (glucose) as an electron donor, and \( \text{Ag}^+ \) concentration as an electron acceptor. Based on the \( E_{\text{cell}} \) production with time in Figure 4.7b and c, the current was calculated by Ohm’s law. The CE obtained in R2 and R3 was in a range of 11.50-19.89% and 1.88-2.86% with the increase of initial \( \text{Ag}^+ \) concentration of 200-2,000 mg/L, respectively. Similar to R1, the diversity of microorganisms in anaerobic sludge used as inoculum in the anode chamber may have an influence on electricity production, and decrease CE. The difference in CE between the two reactors was due to the difference in current yield \( (\text{i.e. } \int_0^T I \, dt) \), which resulted from the conversion of organic matter, measured as the decrement of TOC concentration \( (\Delta C_s) \) in the anolyte. When compared to R2, R3 had lower CE as lower current (or \( E_{\text{cell}} \)) was produced, although higher \( \Delta C_s \) was obtained in R3 after 24 hours (Figure 4.7a). Higher TOC concentration was also detected in the catholyte of R3 as compared to that of R2. These results confirmed that TOC removal in R3 was not only due to the biologically anodic reactions, but also due to the substrate loss through AMI-7001S membrane. Nevertheless, further metabolic intermediate analysis in both chambers should be carried out to prove the migration of substrate.

CE was improved when the initial \( \text{Ag}^+ \) concentration increased. During operation, \( \text{Ag}^+ \) ion served as an \( e^- \) acceptor in the cathode chamber. It was reported that, an increase in the concentration of \( e^- \) acceptor enhanced the cathodic potential, which further increased the \( E_{\text{cell}} \) output, and thus, improve power production (Li et al., 2008).
4.1.4.3 Polarization curves and power curves

Polarization and power curves were used to determine the dependence on resistance of fuel cell performance. This analysis was conducted at the initial Ag$^+$ concentration of 2000 mg/L in both reactors, R2 and R3. The stable cell voltages were recorded at pseudo-steady state when the external resistance varied from 10,000 $\Omega$ to 5 $\Omega$. The current density and power density were then calculated by Ohm’s law at each resistance. Polarization curves (E2, E3) represent the cell voltage as a function of current density while power curves (P2, P3) describe the power density as a function of current density (Figure 4.8).

Figure 4.8 Polarization curves (E2, E3) and Power curves (P2, P3) ($R_{ext} = 10000 - 5 \Omega$) at initial Ag$^+$ concentration of 2,000 mg/L in reactors R2, R3, respectively

When the current increased due to the decrease in external resistance, the measured $E_{cell}$ dropped accordingly. The voltage decrease in E2 and E3 curves was almost linear with current, thus, ohmic losses are dominant in both reactors. The internal resistance ($R_{int}$) in R2 and R3 was determined as the slope of E2 and E3, respectively. Lower $R_{int}$ was found in R2 (i.e. 34.43 $\Omega$), compared to that in R3 (i.e. 77.64 $\Omega$). In addition, higher power density was also found in R2. Specifically, at the same initial Ag$^+$ concentration (i.e. 2,000 mg/L), the maximum power density ($P_{max}$), obtained from P2 and P3 curves, was 5396 and 3385 mW/m$^3$, corresponding to an external resistor ($R_{ext}$) of 60 and 90 $\Omega$ applied to R2 and R3 during polarization test. The measured $E_{cell}$ at the above $R_{ext}$ was 569 and 552 mV, respectively. Lower applied $R_{ext}$ and higher $E_{cell}$ obtained were the reason for higher $P_{max}$ found in reactor R2.
In general, this study suggests that a BES reactor using CMI-7000S as a separator is more effective than the one using AMI-7001S at the same operational conditions for silver recovery (i.e. type of substrate and organic loading in the anolyte, initial pH and Ag\(^+\) concentration in the catholyte, and operation time). Higher $E_{\text{cell}}$ could be produced from a CMI-based reactor supplying sufficient $e^-$ for the Ag\(^+\) reduction in the cathode chamber, which led to higher $R_{\text{EAg}}$. Therefore, CMI-7000S would be used in other BES reactors (i.e. R4, R5, and R6) to investigate the performance of other Ag(I) solutions.

### 4.1.5 Characterization of electrodeposits at the cathode surfaces

Prior to use, the graphite plate cathodes in three reactors (i.e. R1, R2, and R3) are the color of black-carbon. After BES operation for silver recovery (i.e. 59 days for R1, 72 days for each of R2 and R3), iridescent silver layer was found at the cathode surface of R1 and R2, whereas lustrous silver deposits could be observed at that of R3. Deposits were then scraped from the cathode surfaces and dried at room temperature before characterizing by SEM, EDX and XRD analysis (Figure 4.9 and 4.10). Under SEM, silver with crystalline particles and dendritic structures were detected in R1, while incoherently polyhedral crystalline silver particles were detected in R2. This polyhedral morphology was comparable to the crystal structure of silver atoms (i.e. faced-centered cubic silver). In contrast, thin paper-like silver films were detected mainly in R3.

The difference in morphology of silver deposits among these reactors was ascribed by the influence of Ag\(^+\) reduction rate, which was affected by the electron transfer from the anode through the electrical circuit. It was reported that in an electrochemical reaction, current density is related to the reaction rate, and therefore, it should influence the nucleation and growth of silver particles (Liu et al., 2015). It was also shown that dendritic structures are often produced in electrochemical deposition, especially in fine powder deposition. The formation of silver dendrites involves nucleation on the surface of electrodes first, followed by the growth of silver particles (Liu et al., 2015). In this study, as higher power density was produced from CMI-based reactors (i.e. R1 and R2), the formation of silver particles was observed on the cathode surfaces, while thin paper-like silver films were found in AMI-based reactor (i.e. R3), where a lower power density was produced.
Figure 4. Characterization of electrodeposits at the cathode surfaces of (a) CMI-based reactor (R1) after 59 days, (b) CMI-based reactor (R2), and (c) AMI-based reactor (R3) after 73 days, by SEM (a1, b1, c1) and EDX (a2, b2, c2).
In addition, the difference in the transport of anions and cations through the membranes may contribute to shaping the morphology of silver deposits. Ion transfer between two chambers is necessary to maintain the electro-neutrality in BES reactors, as the negatively charged electrons are always transferred from the anode to the cathode through the electrical circuit. Generally, ions, other than \( \text{OH}^- \) and \( \text{H}^+ \), are present at high concentration in the electrolytes. Thus the transport of non-hydroxide/non-proton ions is dominant (Pandit et al., 2012). In R1 and R2, cations, such as \( \text{H}^+ \), \( \text{Na}^+ \), and \( \text{K}^+ \), can migrate through CMI-7000S membrane due to the polarity, while \( \text{Ag}^+ \) can diffuse through the membrane due to the concentration gradient. Whereas, anions, such as \( \text{H}_2\text{PO}_4^- \), \( \text{HPO}_4^{2-} \), \( \text{NO}_3^- \), and negative-charged organic compounds can migrate through AMI-7001S membrane in R3. These transports cause different concentration gradients between the two chambers, which affects the \( \text{Ag}^+ \) reduction rate.

These results were verified by EDX spectra, in which a sharp peak occurred at an energy level of 2.98 keV, indicating silver was the main element in the deposits. The XRD patterns also confirm the EDX results. These XRD patterns in reactor R2 and R3 matched well with the standard patterns of silver (Figure 4.10). All of the peaks can be readily indexed to face-centered-cubic silver, where the diffraction peaks occur at 2θ values of 38°, 44°, and 64°. They also can be ascribed to the reflection of (111), (200), and (220) planes of the face-centered cubic silver, respectively.
4.1.6 Characterization of membrane surfaces

The morphology of CMI-7000S used in reactor R2 and AMI-7001S used in reactor R3 was characterized by SEM and EDX (Figure 4.11). The original membranes before experiments were relatively smooth without any fouling. After 73 days of operation, different deposits were found at membrane surfaces toward the cathode side.

In CMI-based reactor (R2), Ag in the form of thin films and crystalline particles covered the CMI-7000S surface. The appearance of Ag can be explained by the transport of Ag$^{+}$ ions through CMI-7000S, and the binding with sulphonic acid -SO$_2$OH groups present in CMI-7000S structure. The EDX spectrum confirmed Ag as the main element of the fouling at the CMI-7000S surface. Although the majority of Ag$^{+}$ ions in the catholyte was believed to be reduced electrochemically to form Ag deposits at the cathode surface, the detection of Ag$^{+}$ ions in the anolyte of R2 and the occurrence of Ag at the membrane surface were also considerable.

For AMI-based reactor (R3), rock-like silver particles were detected in some parts of the AMI-7001S surface. This may be due to the precipitation of Ag$^{+}$ ions with other anions from the anolyte (e.g. butyrate, propionate, and acetate, PO$_4^{3-}$). The negatively charged organic matter produced from the fermentation of glucose during the inoculation stage may bind with the positively charged functional groups (NH$_4^+$) present in the polymer backbone of the membrane. Therefore, precipitants (e.g. C$_3$H$_7$COOAg, C$_2$H$_5$COOAg, CH$_3$COOAg, and Ag$_3$PO$_4$) may be formed on membrane surface in the operation stage when Ag$^+$ solutions were fed into the cathode chamber. This phenomenon was also observed visually. These results were also verified by the EDX spectrum, in which other elements, such as C, O, and P, were also detected beside Ag. This finding suggested that besides the formation of reduced products as Ag$^0$ on graphite plate cathode, the formation of silver precipitants on the AMI-7001S surface slightly affected the Ag$^+$ removal in the catholyte of R3.

The membrane fouling at the cathode side due to Ag deposits may cause negative impacts on ion exchange capacity. The overall BES performance is thus negatively affected when the system is operated for long duration. This needs to be systematically studied to determine the comprehensive impacts of a separator. The replacement of a membrane, especially CMI-7000S, is essential for long-term operation, which may
increase the operational cost. Therefore, further study is required to investigate the frequency of membrane replacement over time.

Figure 4. 11 SEM images of (a) CMI-7000S and (b) AMI-7001S surface before operation (a1, b1) and after 73 days of operations (a2, b2). EDX spectra (a3, b3) characterize chemical composition of the membranes.
4.1.7 Characterization of anodic microbial communities

The characterization of anodic biofilms was conducted in three reactors, R1, R2, and R3 to achieve a better understanding of the bacterial community and dominant species contributing to electricity production.

In acetate-based reactor (R1) after 59 days of operation, the occurrence and the morphology of bacterial cells adhering on the anode surface were observed and detected using SEM. The SEM images (Figure 4.12) showed that the carbon brush was colonized by bacilliform bacteria. These microorganisms may be responsible for the oxidation of organic compounds in the anode chamber and electron transfer to the anode.

![Figure 4.12](image)

**Figure 4.12** Representative SEM images show (a) the formation of biofilm and (b) the presence of bacilliform bacteria at carbon brush surface of acetate-based reactor (R1) after 59 days of operation.

For R2 and R3 fed with glucose after 73 days of operation, DNA extraction and gene sequence analysis were conducted to classify the sequence into the OTU table, and all species were then identified by matching individual sequence against the Greengenes Database (McDonald et al., 2012). Sequencing results showed a considerable phylogenetic diversity in the anodic microbial communities. The taxonomy at the phylum level in both reactors is presented in Figure 4.13. There was a significant difference in the predominant bacteria between the two reactors although they were inoculated by the same inoculum and operated at similar conditions.

The anodic microbial community in R2 were dominated by *Firmicutes* (65.1%), followed by *Proteobacteria* (21.79%), *Bateroidetes* (8.66%), and others (5.60%). Within *Proteobacteria*, *Gammaproteobacteria* (16.80%) were the most abundant,
followed by *Deltaproteobacteria* (2.20%), *Alphaproteobacteria* (1.9%), and *Betaproteobacteria* (0.80%). On the other hand, R3 showed a different community, in which *Proteobacteria* (40.77%) were the most predominant, followed by *Firmicutes* (34.22%), *Bacteroidetes* (19.10%) and others (8.50%). The sequencing results in R3 showed a relatively different distribution among *Proteobacteria* when compared to that in R2. *Betaproteobacteria* (22.8%) were detected more than *Gammaproteobacteria* (13.3%), *Alphaproteobacteria* (2.50%), and *Deltaproteobacteria* (1.80%).

These results are consistent with previous studies which found *Gammaproteobacteria* and *Firmicutes* to be the most abundant sequences when artificial wastewater was used as a fuel source (Choo et al., 2006; Logan et al., 2005; Rabaey et al., 2004). Furthermore, *Gammaproteobacteria* was indicated as the possible species in electricity production (Kim et al., 2006), and *Shewanella* which belongs to class *Gammaproteobacteria*, is one of the most prevalent bacteria in BES field.

![Figure 4.13 Relative abundance of dominant bacterial taxonomy at phylum level in glucose-based reactors (R2 and R3), after 73 days of operation](image)

(*) Minor phyla, accounting for <0.05% of total sequences, are summarized in the group "Others"
In this study, however, no *Shewanella*-like sequences were retrieved in both reactors. It may be because glucose was added as the substrate and *Shewanella* sp. normally prefer lactate, as compared to other substrates (Chae et al., 2009). In contrast, *Geobacter* sp. or iron-reducing bacteria in the family *Geobacteraceae*, belonging to *Deltaproteobacteria*, could be detected in both reactors (i.e. relative abundance of 1.30% for R2 and 0.60% for R1). Similar results were also reported by previous studies, in which the dominance of various *Geobacter*-like strains could be detected, but there was no detection of *Shewanella*-like sequences in their MFCs (Chae et al., 2009; Jung and Regan, 2007). During the bio-electrochemical processes, *Geobacteraceae* can transfer electrons directly onto an electrode (Bond and Lovley, 2003). However, the substrates (as electron donors) that these bacteria can favorably utilize is limited to simple substances, such as acetate. Accordingly, in BES reactors fed with complex organic compounds (e.g. glucose), the fermentative bacteria or syntrophic microbial species are employed primarily to convert the higher order carbon sources into simpler substances to supply *Geobacter* sp. the electron donor. This is probably the reason that explains the lower dominance of *Geobacter* sp. and lower CE values found in the glucose-fed BES than in the others (Chae et al., 2009). *Streptococcus* sp., *Lactococcus* sp., *Bacillus* sp., *Clostrium* sp., *Lysobacter* sp., and *Rhodanobacter* sp. could be detected in both reactors. These bacteria have the ability to convert complex carbohydrates and other compounds into simpler substances, making easier for further utilization by exoelectrogens. *Streptococcus*, *Lactococcus*, and *Bacillus* are popularly known to produce lactic acid from glucose while *Clostridium* sp. ferment glucose to butyrate, acetate, CO₂, and H₂ gas.

Although *Geobacter* sp. and *Shewanella* sp. are the most prominent species reported in many MFCs, the dominance of these organisms in fact, has not been found to support high electrical power outputs using mixed microbial cultures (Rabaey et al., 2004). Several studies revealed a diversity of bacteria, much greater than these model iron reducers, persisting in the anodic biofilm communities (Logan and Regan, 2006). In this study, many bacterial sequences found in both reactors showed close affiliation with the species reported earlier in different MFCs, such as *Lysobacter* sp., *Planctomyces* sp. (Patil et al., 2009), *Dechloromonas* sp. (Kim et al., 2007), *Clostridium* sp., *Pseudomonas* sp. (Rabaey et al., 2004), *Enterococcus* sp. (Jung and Regan, 2007),
Burkholderia sp., Treponema sp. (ISHII et al., 2008), Desulfovibrio sp. (Kang et al., 2014), Klebsiella sp. (Deng et al., 2010), and Citrobacter sp. (Xu and Liu, 2011). These species were predicted as feasible exoelectrogens, involved in electricity generation.

In addition, many dominant sequences found in this study, such as Achromobacter sp., Telmatospirillum sp., Pseudoramibacter Eubacterium sp., Dysgonomonas sp., and Chryseobacterium sp. have not reported as exoelectrogens so far. Therefore, deeper studies need to be conducted to explore the roles of these bacteria to clarify their involvement in anodic electron transfer. This result indicates that electrochemically active bacteria, accounting for electrochemical activities, is not restricted to a few well-known phyla of bacteria, and other species may play a role.

4.2 Catholyte as [Ag(NH₃)₂]⁺ solution

4.2.1 Effect of initial [Ag(NH₃)₂]⁺ concentration

Ag(NH₃)₂⁺ solutions were investigated as catholytes in reactor R4 using CMI-7000S membrane. The effects of initial [Ag(NH₃)₂]⁺ concentration (C₀) were investigated by determining Ag removal efficiency in the cathode chamber, COD removal and columbic efficiency in the anode chamber, and maximum power density.

4.2.1.1 Silver recovery and electricity production

The reduction of [Ag(NH₃)₂]⁺ complex in the catholyte over time at different C₀ is shown in Figure 4.14a. With C₀ of 1000 and 2000 mg/L, the reaction occurred rapidly as Ag concentration decreased to < 50 mg/L, corresponding to RE > 96% after 24 hours of operation. The reduction seems to be completed when the experiment was extended to 48 hours (i.e. C₄₈ = 3-6 mg/L, RE > 99%). The removal rate of [Ag(NH₃)₂]⁺ (rAg) in the catholyte was considered to evaluate the silver removal efficiency. Specifically, the rAg calculated within the first 24 hours was 40.15, 81.32, and 74.88 mg/L.h when C₀ was 1000, 2000, and 3000 mg/L, respectively. Accordingly, a lower efficiency (i.e. RE = 59.9%) was found after 24 hours at C₀ of 3000 mg/L, as compared to that at C₀ of 1000, 2000 mg/L (i.e. RE > 96%). When the operation was extended to 48 hours, a comparable RE of 96.10% at C₀ of 3000 mg/L was obtained. Transport of positive charged ions, especially [Ag(NH₃)₂]⁺, through the CMI-7000S membrane was also found. During the first 12 hours in each batch of experiments, only the formation of sparkling deposits at the cathode surface was observed visually, but thereafter some deposits gradually occurred at the membrane surface. This may affect the ion transfer.
capacity of the CMI-7000S membrane as the membrane was not replaced after each cycle. Studies reported that besides the membrane bio-fouling which is an inevitable issue in the long-term operation of most BES reactors, the cation occupation of functional groups of CEMs and inorganic salt precipitations at the membrane surface are also detrimental issues for BES performance (Choi et al., 2011; Xu et al., 2012). This leads to a decrease in ion exchange capacity and ion conductivity; consequently, the BES performance may decrease with an increase in number of batch cycles.

Figure 4. 14 (a) Silver reduction and (b) E_{cell} production (R_{ext} = 5 \, \Omega) at different initial [Ag(NH_{3})_{2}]^{+} concentrations in CMI-based reactor (R4)

The diffusion of [Ag(NH_{3})_{2}]^{+} from the catholyte to the anolyte through the CMI-7000S membrane was confirmed by the results obtained in the control reactor (R_{04}), which was operated at C_{0} of 2000 mg/L without inoculation. This transport is due to the concentration gradient between the two chambers and is not under the effect of an electrical field as no E_{cell} production was found. The Ag concentration in the anolyte of the control reactor increased gradually from 0 to 12.61 mg/L after 48 hours. Some Ag deposits on the membrane surface were observed visually, which changed the original color of the CMI-7000S membrane (i.e. from brown to dark grey).

On the other hand, the transport of [Ag(NH_{3})_{2}]^{+} in the bio-anodic reactor (R4) was affected, not only by the diffusion due to the concentration gradient, but also by the electron flow through the electrical circuit, and the migration of other cations (e.g. H^{+}, Na^{+}, K^{+}) through the membrane. However, the Ag concentration in the anolyte of
R4 was not measured in each batch of experiments since most Ag ions presented in the anolyte was supposed to either precipitate or adsorb in the anodic biomass. Instead, the accumulated amount of Ag in the anodic biomass after 58 days of BES operation was determined to confirm the occurrence of Ag in the anode chamber (see Section 4.2.5).

In the bio-anodic reactor R4, along with the [Ag(NH$_3$)$_2$]$^+$ reduction in the catholyte, the $E_{cell}$ progression over time was found simultaneously (Figure 4.1b). When a $C_0$ of 1000 and 2000 mg/L was used, $E_{cell}$ increased rapidly during the first 8 hours and reached the maximum values of 51 mV and 85 mV, respectively. Thereafter, it dropped gradually due to the shortage of electron acceptors caused by the reduction of [Ag(NH$_3$)$_2$]$^+$ complex. The $E_{cell}$ production at $C_0$ of 2000 mg/L was higher than that at $C_0$ of 1000 mg/L during the first 24 hours, but the trends became similar after that as the measured voltage varied in a low range (i.e. 10-15 mV). However, when $C_0$ increased to 3000 mg/L, a quite different trend was found. It was observed that $E_{cell}$ increased slowly and reached a peak (i.e. 74.5 mV) at the 18$^{th}$ hour. Lower and slower $E_{cell}$ production at $C_0$ of 3000 mg/L can be attributed to the impacts of CMI-7000S. More bio-fouling and inorganic precipitation formed at the membrane surface after each cycle, leading to an increase of membrane internal resistance, which constituted a part of the total internal resistance of the BES. It was reported that the MFC with a fouled proton exchange membrane (PEM) after 90 days of operation had a much lower power output as the total internal resistance increased by 20%, the OCV reduced by 9.9%, and the maximum output power decreased by 32.3% (Xu et al., 2012). In this study, the $E_{cell}$ output at $C_0$ of 3000 mg/L after 18 hours, however, was better as compared to that at $C_0$ of 1000, 2000 mg/L. The slow rate of [Ag(NH$_3$)$_2$]$^+$ consumption at a $C_0$ of 3000 mg/L led to the high remaining [Ag(NH$_3$)$_2$]$^+$ concentration in the catholyte. This may help to retain high cathodic potential, and accordingly a high $E_{cell}$ output was recorded.

4.2.1.2 Substrate removal and Columbic efficiency

During the experiments with different initial [Ag(NH$_3$)$_2$]$^+$ concentrations in the cathode chamber, the performance in biological conversion of organic waste to produce electricity in the anode chamber was also evaluated by considering the substrate removal, referring as COD removal, and Columbic efficiency (CE).
When \( C_0 \) in the cathode chamber increased from 1000 to 2000 mg/L, COD removal efficiency in the anode chamber increased from 60 to 76.80% (Figure 4.15a). As the same initial organic loading (i.e. \( COD_0 = 1000 \) mg/L) was fed into the anode chamber for each batch of experiments, the increase in COD removal efficiency may be due to the improvement in biological activities, since reactor R4 was run for a long duration (i.e. 22 days of inoculation and 58 days of operation). It was reported that diverse microbial communities of hydrolytic, fermentative, acidogenic, and acetogenic microorganisms are normally found in BESs when the systems were inoculated by mixed cultures and operated for long duration (Logan and Rabaey, 2012; Martin et al., 2010; Yu et al., 2015). Thus, the biological adaptability and growth rate may improve, which led to a higher substrate consumption and an increase in COD removal. It was reported by Martin et al. (2010) that the combined activities of anodophilic and methanogenic bacteria in an MFC could provide high COD removal efficiency.

![Figure 4.15](image_url)

**Figure 4.15** Effects of initial \([Ag(NH_3)_2]^+\) concentration on power generation in CMI-based reactor (R4). (a) COD removal and Columbic efficiency after 48 hours, (b) Polarization curves (E1, E2, and E3) and Power curves (P1, P2, P3) \((R_{ext} = 10000 – 5 \, \Omega)\) at \( C_0 \) of 1000, 2000, and 3000 mg/L, respectively

However, a slight decrease in COD removal to 75.20% was found when \( C_0 \) of 3000 mg/L was used. A higher \( C_0 \) used in the catholyte caused a higher concentration gradient of electrolyte between the two chambers. The transport of cations through the CMI-70001S membrane, especially the diffusion of \([Ag(NH_3)_2]^+\) from the catholyte to the anolyte, was thus accelerated, as compared to that at \( C_0 \) of 1000 and 2000 mg/L.
Moreover, the experiment at $C_0$ of 3000 mg/L was the last batch of the operation. The increasing amount of Ag accumulated in the anode chamber after several batches of the experiments may affect the biological activities of anodic bacteria. The occurrence and accumulated amount of silver in the anode chamber was confirmed by quantitative analysis.

In term of CE, when $C_0$ was 1000-3000 mg/L, a CE of 8.55-14.69% was found, respectively (Figure 4.15a). Limited CE can be attributed to the competition of non-exoelectrogenic species in the anodic bacterial community with exoelectrogen, to consume substrate through different biological processes, which did not involve electricity production. However, the CE was improved when $C_0$ was increased in the catholyte. This resulted from the increase in current yield (i.e. $\int_0^T ld\tau$), calculated based on the $E_{cell}$ progression over 48 hours, shown in Figure 4.14b.

4.2.1.3 Polarization curves and power curves

The effect of initial $[\text{Ag(NH}_3)_2]^+$ concentration on power generation can be also evaluated by characterizing the polarization curves and power curves (Figure 4.15b). In the first two experiments with a $C_0$ of 1000 and 2000 mg/L, the voltage in $E_1$ and $E_2$ curves was almost linear with current. Accordingly, the $R_{int}$, determined simply as the slope of $E_1$ and $E_2$, was 32.83 and 26.24, respectively. An increase of $C_0$ from 1000 to 2000 mg/L led to an increase in the ionic conductivity and a decrease in $R_{int}$. However, $E_3$ obtained at $C_0$ of 3000 mg/L, was distorted and nonlinear as compared to $E_1$ and $E_2$. This may be attributed to cathodic potential loss after membrane fouling. Xu et al. (2012) found that the fouled layer on the PEM in the MFC system decreased the diffusion coefficients of cations through the membrane and heightened the cathodic potential loss. Thus, the $R_{int}$ in the system at $C_0$ of 3000 mg/L could not be simply determined as the slope of $E_3$, like for the case of $E_1$ or $E_2$ at a $C_0$ of 1000 or 2000 mg/L, respectively. Basically, the $R_{int}$ in bioelectrochemical systems includes other components than $R_\\Omega$, such as the activation (charge transfer) resistance ($R_{ct}$), and concentration resistance ($R_{diff}$) (He and Mansfeld, 2009; Sekar and Ramasamy, 2013). In order to determine the contribution of different internal resistances to the overall impedance of fuel cells, electrochemical impedance spectroscopic (EIS) analysis is suggested to be conducted.
The $P_{\text{max}}$ obtained in P1, P2, and P3 was also considered to understand the performance of electricity production. $P_{\text{max}}$ increased from 2984 to 3618 mW/m$^3$ when $C_0$ increased from 1000 to 2000 mg/L, but it slightly dropped to 3009 mW/m$^3$ when a $C_0$ of 3000 mg/L was used. This result confirmed the effect of fouling, as inorganic precipitation formed at the surface of CMI-7000S membrane, which caused higher internal resistance and lower performance in electricity production in the last cycle.

The results obtained in the current study were compared with that reported by Wang et al. (2013), in which $[\text{Ag(NH}_3\text{)}_2]^+$ complex was also investigated (Table 4. 3). A slightly higher performance in electricity production was found in this study. Specifically, at relatively similar conditions (i.e. $C_0$ of 1000 mg/L), $P_{\text{max}}$ obtained in this study and by Wang et al. (2013) was 331 and 317 mW/m$^2$, respectively. This was due to a decrease in $R_{\text{int}}$, as carbon brush and monopolar IEM (CMI-7000S) were used in current works, while carbon cloth and bipolar IEM (BPM-I) were employed in theirs.

**Table 4. 3 Summary of BES performance obtained with catholyte as Ag(NH$_3$)$_2^+$ solution in current study and study by Wang et al. (2013)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Current study</th>
<th>Study by Wang et al. (2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working volume of each chamber (mL)</td>
<td>1000</td>
<td>24</td>
</tr>
<tr>
<td>Operation time (hours)</td>
<td>48</td>
<td>21</td>
</tr>
<tr>
<td>Type of Ag solution</td>
<td>$[\text{Ag(NH}_3\text{)}_2]^+$</td>
<td>$[\text{Ag(NH}_3\text{)}_2]^+$</td>
</tr>
<tr>
<td>Initial Ag concentration (mg/L)</td>
<td>1000</td>
<td>2000, 3000</td>
</tr>
<tr>
<td>RE (%) (a)</td>
<td>&gt; 99.00</td>
<td>&gt; 99.00, 96.10</td>
</tr>
<tr>
<td>COD removal (%) (b)</td>
<td>60.00</td>
<td>76.80, 75.20</td>
</tr>
<tr>
<td>CE (%)</td>
<td>8.55</td>
<td>12.74, 14.69</td>
</tr>
<tr>
<td>$R_{\text{int}}$ (Ω)</td>
<td>32.83 (~ $R_{\Omega}$)</td>
<td>26.24 (~ $R_{\Omega}$)</td>
</tr>
<tr>
<td>$P_{\text{max}}$ (mW/m$^3$)</td>
<td>2984 at $V = 1.0$ L</td>
<td>3618 at $V = 1.0$ L</td>
</tr>
<tr>
<td>With $A = 90$ cm$^2$</td>
<td>at $R_{\text{ext}} = 40$ Ω</td>
<td>at $R_{\text{ext}} = 20$ Ω</td>
</tr>
</tbody>
</table>

(a) The amount of Ag diffused through the membrane was not considered in this calculation
(b) The initial COD concentration was 1000 mg/L in all cases
N/A: Not available
N/D: Not defined

Ref. code: 25605722300323QDG
Studies reported that carbon brush has high a surface area and porosity for bacterial adhesion, coupled with high electrical conductivity and efficient current collection (Feng et al., 2010; Wei et al., 2011). Before assembling, the carbon brush was pretreated (Section 3.1.2, Chapter 3). According to previous studies, this procedure could increase power generation by 25% due to an increase in specific area, which facilitated bacteria adhesion with less contaminants that interfere with charge transfer from bacteria to electrode (Feng et al., 2010; Wang et al., 2009; Wei et al., 2011). Harnisch et al. (2008) also indicated that monopolar IEMs (i.e. Nafion-117) yielded a linear polarization plot, whereas the polarization curve of bipolar IEMs (i.e. Fumasep-FBM) was sigmoidal-shaped and showed a considerably higher polarization than monopolar membranes. This can be explained that in a bipolar IEM- based reactor, the total ion flux is composed of the migration of electrolyte ions through AEM and CEM layers, and the migration of H+ and OH- ions generated from the water splitting reaction at the interface area between these layers.

4.2.2 Effects of supporting catholyte (NaNO₃) on power generation

In this experiment, [Ag(NH₃)₂]⁺ solutions containing 2000 mg Ag/L were added 5 or 10 mM NaNO₃ to investigate the effect on power generation and Ag removal. As a result, with a supplement of 5 mM NaNO₃, the Eₜₐₚₖ production was improved slightly during the first 16 hours. Thereafter, it dropped similarly to that obtained without NaNO₃ addition (Figure 4.16a). When the supplementary NaNO₃ level increased to 10 mM, the improvement in power generation was better, as high Eₜₐₚₖ remained for a longer time (i.e. 32 hours) before it became similar to the others. The addition of NaNO₃ improved the ionic conductivity, and accordingly decreased the resistance of the electrolyte. Thus, higher Eₜₐₚₖ could be produced. The Pₚₐₓ also increased by 4.66%, from 3,618 to 3,795 mW/m³, when 10 mM NaNO₃ was added to the catholyte (Figure 4.16b).

During experiments, the reduction of [Ag(NH₃)₂]⁺ complex in the catholyte was not affected by the addition of NaNO₃. The Na⁺ and NO₃⁻ ions, dissolved from a low amount of neutral salt NaNO₃ (5 or 10 mM), may not strongly compete with [Ag(NH₃)₂]⁺ complex (2000 mg/L), to accept the electrons in the cathode chamber. Results showed that the silver removal was over 99% in all of the cases.
Similar results were found by other researchers when KNO\(_3\) was used to improve the ionic conductivity of solution for copper (Cu) recovery and electricity production in a MFC (Wang et al., 2010). It was reported that Cu removal efficiency (\(> 99.6\%\)) was not affected by the addition of KNO\(_3\) (0-200 mM) within 11 hours, while the maximum power output was greatly improved from 96.2 to 143.6 mW/m\(^2\).

Figure 4. 16 Effects of NaNO\(_3\) addition on power generation in reactor R4. (a) \(E_{\text{cell}}\) production \((R_{\text{ext}} = 5 \, \Omega)\), and (b) \(P_{\text{max}}\) obtained from power curves \((R_{\text{ext}} = 10000-5 \, \Omega)\).

The improvement in power generation, however, was not significant when taking economic aspects into consideration. The addition of NaNO\(_3\) or KNO\(_3\) as a supporting electrolyte, even though a low quantity was used, conflicts with BES principles, in which chemical intensive should be avoided. On the other hand, other ions from metallic salts, beside silver ions, normally exist in real wastewater or aqueous solutions, which may also compete with Ag ions to accept electrons, thus, affecting the reduction rate. The reduction of other metallic ions in the catholyte may occur simultaneously or sequentially, depending on their reduction potential. Thus, it is recommended that further studies should be carried out with complex catholytes containing different metallic salts, to investigate their effects on the BES performance.

4.2.3 Characterization of electrodeposits at cathode surface

After 58 days of operation, the graphite plate cathode was taken out of R4 and air-dried at room temperature. The deposits on the cathode surface were scrapped and characterized by SEM, EDX, and XRD. Silver with dendritic structures was detected
These dendrites have long trunks and short branches which look like a fishbone. In contrast, Wang et al. (2013) found silver deposits in a compact layer structure at the cathode surface in their study.

Figure 4.17 Characterization of electrodeposits at the cathode surfaces of CMI-based reactor (R4) after 58 days of operation by (a) SEM, (b) EDX and (c) XRD

The difference in morphology of metallic silver deposits was due to the influence of operation time in each batch of experiments (see Table 4.3) and $E_{\text{cell}}$ output, which is related to the intensity of $e^-$ flow for the reduction of silver ions in the catholyte. Liu et al. (2015) investigated the formation of silver deposits by time and the effects of current density on the silver morphologies in a two-cell electrochemical reactor. It was reported that with progression of the reaction (i.e. 300 s), a spherical pattern composed of small crystals was firstly formed and followed by the growth of small particles on
the surface of the pattern. These small particles thereafter developed into dendritic structures. Also, the study found that a change in current density (i.e. 25-2000 µA) affected the reaction rate. Specifically, it affected the nucleation and growth of Ag deposits (i.e. from polyhedral aggregates to long thick rods, followed by thick dendrites structures, and mixture of dendrites and small cubic particles) (Liu et al., 2015).

The SEM result was verified by EDX (Figure 4.17b), in which a sharp peak at an energy level of 2.98 keV was found, indicating that the deposits were comprised of pure silver. Specifically, the elemental characterization at Point 1 of SEM image, had metallic silver of 98.16 % wt. The XRD pattern shown in Figure 4.17c also confirmed the EDX result. The XRD patterns matched well with the standard patterns of silver (i.e. JCPDS card No. 04-0783). All peaks can be readily indexed to face-centered-cubic silver, where the diffraction peaks occur at 20 values of 38.03°, 44.19°, 64.39°, 77.33°, and 81.45°. They also can be ascribed to the reflection of (111), (200), (220), (311), and (222) planes of the face-centered cubic silver, respectively. No peaks from other phases were detected, indicating the high purity of silver deposits.

**4.2.4 Characterization of membrane surface**

When the CMI-7000S membrane was used as a separator in BES reactor, transport of positive-charged ions through functional groups (i.e. sulphonic acid HSO\(_2\)OH) attached on the membrane structure, would occur. [Ag(NH\(_3\))\(_2\)]\(^+\) diffused from the catholyte to anolyte, which caused silver loss and affected the overall silver recovery. In order to verify the diffusion of [Ag(NH\(_3\))\(_2\)]\(^+\), the surface morphology of the CMI-7000S membrane was characterized by SEM and EDX analysis.

The original membrane, before assembling in the BES reactor, is relatively smooth without any fouling. After 58 days of operation with [Ag(NH\(_3\))\(_2\)]\(^+\) solutions, some bright deposits were observed visually in some parts of the membrane toward the cathode side. Under SEM observation, silver with bulk and dendritic structures were found in the deposits (Figure 4.18a). The EDX analysis confirmed that silver was the main element detected in deposits at the membrane surface (Figure 4.18b). The elemental characterization at Point 1 of SEM image had silver content of 96.97 % w.t. The majority of [Ag(NH\(_3\))\(_2\)]\(^+\) complex in the catholyte was believed to be reduced electrochemically to form silver deposits at the cathode surface. The occurrence of
metallic silver at the CMI-7000S surface, however, affected overall performance. Replacement of the CMI-7000S membrane is thus, essential for long-term operation.

Figure 4.18 Characterization of CMI-7000S surface of reactor R4 after 58 days of operations by (a) SEM (b) EDX

4.2.5 Adsorption and accumulation of silver in the anodic biomass

\[\text{[Ag(NH}_3\text{)}_2]^+\] complex diffused through CMI-7000S and may adsorbed on the biomass of the anode chamber. Silver gradually accumulated over time as the experiments were carried out with different concentration levels of \[\text{[Ag(NH}_3\text{)}_2]^+\] solutions. The anodic biomass, including suspended solid and the biofilm attached to the carbon brush, was collected after 58 days of BES operation. The biomass digestion and analysis of Ag content are described in Table 3.1 (Chapter 3). The original anaerobic sludge obtained from the wastewater treatment plant, before being used as inoculum in the BES reactor, served as a control sample.

Table 4.4 Silver accumulated in anodic biomass of CMI-based reactor (R4) after 58 days of operation

<table>
<thead>
<tr>
<th>Sample</th>
<th>(V_{\text{sample}}) (ml) (*)</th>
<th>(\text{Ag concentration (mg/L)}) (**)</th>
<th>(\text{Ag content present (mg Ag/ g sample)})</th>
<th>(\text{Ag accumulated (mg Ag/ g biomass)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample (Original anaerobic sludge)</td>
<td>8.2</td>
<td>0.00</td>
<td>0.00</td>
<td>(\Delta m = 0.853) (***)</td>
</tr>
<tr>
<td>Anodic biomass sample (Suspected solid + carbon brush)</td>
<td>8.5</td>
<td>50.17</td>
<td>0.853</td>
<td></td>
</tr>
</tbody>
</table>

(All measurements were conducted in duplicate and the means were reported)

(*) \(V_{\text{sample}}\) volume of liquid sample obtained from digestion

(***) \(\text{Ag concentration in liquid sample was analyzed by ICP Spectrometer}\)

(****) \(\Delta m\) is the accumulated amount of Ag in the biomass, calculated by the difference of Ag present in control sample before operation and biomass sample after operation
The results are shown in Table 4.4. No Ag was detected in the control sample. In contrast, a high concentration of Ag was found in the biomass sample, indicating the occurrence and accumulation of Ag in the anode chamber. The Ag accumulated due to adsorption was calculated as 0.853 mg Ag/ g biomass.

This leads to a concern about the toxic effects of Ag on microorganisms in the anode chamber. However, in the current study, mixed culture was employed in all reactors. As shown in reactor R2 and R3, mixed cultures could possess high adaptability and resistance to stress, which may help to overcome the toxic effects of silver. Nevertheless, further studies on the inhibitory effects of silver on a bacterial community should be conducted, especially with high exposure concentration and longer operation.

4.3 Catholyte as [Ag(S₂O₃)₂]³⁻ solution

The experiments with [Ag(S₂O₃)₂]³⁻ solution were conducted in reactor R5, in which CMI-7000S was also used. The spontaneous generation of electricity supplying for [Ag(S₂O₃)₂]³⁻ reduction was investigated at different [Ag(S₂O₃)₂]³⁻ concentrations.

4.3.1 Effects of initial [Ag(S₂O₃)₂]³⁻ concentration

4.3.1.1 Substrate removal and electricity production

During the BES operation, substrate (as acetate) was consumed by microorganisms in the anode chamber through biological reactions to produce electrons (Eq. 3.4). The performance of electricity production was characterized by substrate removal, measured in term of COD removal, and $E_{cell}$ production, which were then integrated into Columbic efficiency, an indicator to evaluate bioelectrochemical performance.

After 48 hours of operation at different $C_0$ levels in the cathode chamber (i.e. $C_0$ of 10, 20, and 30 mM), the COD removal efficiency in the anode chamber was 81.30, 77.40, and 79.00%, respectively (Figure 4.19a). As the same initial organic loading (i.e. COD$_0$ = 1000 mg/L) was fed into the anode chamber for each batch of experiments, slight fluctuation of COD removal efficiency resulted from the biological activities of anodic microbial community. It was reported that high phylogenetic diversity of bacteria is commonly found in electroactive microbial biofilms derived from mixed-culture inoculums, in which exoelectrogen and non-exoelectrogenic species co-exist and consume substrate through different biological processes (Ho et al., 2017; Logan and Regan, 2006; Mathuriya, 2013). In this study, a slight fluctuation of COD removal
in experimental cycles was believed to be due to the variation of bacterial species and their activities in the anode chamber over time, as the reactor was operated for long duration (i.e. 61 days).

Figure 4. 19 (a) Efficiency of biological conversion and (b) $E_{\text{cell}}$ production ($R_{\text{ext}} = 5 \, \Omega$) at different initial Ag(S$_2$O$_3$)$_2^{3-}$ concentrations in CMI-based reactor (R5)

An increase of $C_0$ from 10 mM to 30 mM in the cathode chamber may not influence the biological conversion in the anode chamber, as [Ag(S$_2$O$_3$)$_2$]$^{3-}$ was not able to transport through the CMI-7000S membrane. However, the increase of $C_0$ had a significant influence on $E_{\text{cell}}$ production (Figure 4.19b). In all of the cases, the $E_{\text{cell}}$ increased during the first hours of operation and decreased gradually thereafter due to the shortage of substrate in the anode chamber and the reduction of [Ag(S$_2$O$_3$)$_2$]$^{3-}$ complex in the cathode chamber. At higher initial [Ag(S$_2$O$_3$)$_2$]$^{3-}$ concentration (i.e. $C_0$ of 20, 30 mM), the $E_{\text{cell}}$ progression was better than that at $C_0$ of 10 mM. When $C_0$ was 10 mM, the system took a long time (i.e. 18 hours) to reach the maximum voltage (i.e. $E_{\text{cell}} = 96.3$ mV), but it quickly obtained higher values ($E_{\text{cell}} = 118.3 - 120$ mV) in a shorter period (i.e. 8-13 hours) when $C_0$ was 20 and 30 mM. The $E_{\text{cell}}$ progression rate $r$ (mV/h), calculated during this time, was 5.26, 14.04, and 8.97 mV/h with $C_0$ of 10, 20, and 30 mM, respectively. There was not much improvement in $E_{\text{cell}}$ production when $C_0$ increased to 30 mM, however, it was better than that at $C_0$ of 10, 20 mM after 24 hours. The improvement in $E_{\text{cell}}$ production was attributed to an increase of $C_0$ as it can enhance the cathodic potential and further increase the $E_{\text{cell}}$ output.
In term of CE, results showed that, a CE of 12.85-25.73% was obtained when \( C_0 \) was 10-30 mM, respectively. The improvement of CE resulted from the increase of current yield (i.e. \( \int_0^{T} I dt \)), calculated based on the \( E_{\text{cell}} \) progression over 48 hours shown in Figure 4.19b. This result indicated that BES technology was not only employed for silver recovery as expected, but also was accompanied with power generation from biodegradable organic matter. High diversity of anodic microbial community could accelerate the biological conversion, which resulted in high COD removal, whereas the competition of non-exoelectrogenic species with exoelectrogen to consume substrate would affect CE as the non-exoelectrogenic species are not involved in electricity production.

4.3.1.2 Ag recovery

The BES operation was conducted with \([\text{Ag(S}_2\text{O}_3)\text{)}_2]^3-\) complex serving as electron acceptors in the cathode chamber. The reduction of \([\text{Ag(S}_2\text{O}_3)\text{)}_2]^3-\) complex occurred spontaneously without any supply of external energy. Figure 4.20a shows the decrease of \([\text{Ag(S}_2\text{O}_3)\text{)}_2]^3-\) concentration with time.

During the first 8 hours, the \([\text{Ag(S}_2\text{O}_3)\text{)}_2]^3-\) removal rate (\( r_{\text{Ag}} \)) calculated based on Eq. (3.7) was relatively low, especially in the case of \( C_0 \) of 10 mM. Specifically, \( C_0 \) of 10 mM remained constant, corresponding to \( r_{\text{Ag}10} \sim 0 \) mM/h, while \( C_0 \) of 20 mM decreased slowly to 18.94 mM with \( r_{\text{Ag}20} = 0.13 \) mM/h, and \( C_0 \) of 30 mM decreased faster to 25.88 mM with \( r_{\text{Ag}30} = 0.52 \) mM/h. As the cathodic reduction depends on the energy exploited from organic matter through the anodic biological oxidation of microorganisms, low \( r_{\text{Ag}} \) occurred at the beginning of operation for each batch of experiments. The increase of \( r_{\text{Ag}} \) at higher \( C_0 \) was attributed to the improvement of cathodic potential which may result in higher \( E_{\text{cell}} \) output, boosting the electrochemical reduction of the \([\text{Ag(S}_2\text{O}_3)\text{)}_2]^3-\) complex. From the 8\(^{th}\) hour onwards, a fast decrease of Ag concentration was observed, in which \( r_{\text{Ag}} \) of 0.21, 0.45, and 0.60 mM/h was found, corresponding to \( C_0 \) of 10, 20, and 30 mM.

After 48 hours of operation, the remaining Ag concentration in the catholyte (\( C_t \)) was 1.82, 0.96, and 2.03 mM, along with a Ag removal efficiency (RE\(_{\text{Ag}}\)) of 81.7, 95.2, and 93.2\(\%\), respectively (Figure 3a). Although faster \( r_{\text{Ag}} \) and higher RE\(_{\text{Ag}}\) were observed at \( C_0 \) of 30 mM during the operation, a slow-rate reduction near the end of
experiment (i.e. from the 40th to 48th hour) caused the remaining high Ag concentration in the catholyte (i.e. C_t of 2.03 mM) and led to a drop of RE_{Ag} (i.e. 93.2%).

Figure 4. 20 (a) Reduction of [Ag(S_2O_3)_2]^{3-} with time and Ag removal efficiency at different C_0 levels (10-30 mM) in the bio-anodic reactor (R5). (b) Change of Ag concentration with time at C_0 of 20 mM in the control reactor (R_{05})

The catholyte pH was an important factor strongly affecting the reactivity of cathodic reactions. Thus, it was monitored regularly every 4 hours during the BES operation to verify the favorable conditions for electrochemical reduction of the [Ag(S_2O_3)_2]^{3-} complex. During experiments, the catholyte pH increased gradually in all of C_0 cases, from the original pH of 7.39 to 9.76, 6.77 to 8.57, and 6.18 to 8.02 at the 48th hour, corresponding to C_0 of 10, 20, and 30, respectively. The increase of catholyte pH was due to the cathodic reduction and slow transportation of H^+ produced from the anode through the CMI-7000S membrane. It was reported that other cations (i.e. Na^+, K^+) existing in the anolyte at higher concentration would compete with proton H^+ to transfer through the membrane, which led to the decrease of H^+ migrating to the cathode chamber and further increased catholyte pH (Kokabian and Gude, 2015; Pandit et al., 2012). Fortunately, this alkaline environment facilitated the electrochemically [Ag(S_2O_3)_2]^{3-} reduction and also avoided the decomposition of [Ag(S_2O_3)_2]^{3-} complex into sulfite ions (SO_3^{2-}) as well as colloidal sulfur (S). Therefore, it is believed that the deposition of S and the formation of other Ag precipitants or insoluble compounds (e.g. Ag_2S, Ag_2SO_3) would not occur in the cathode chamber during the operation.
Furthermore, in this study, silver(I) dithiosulfate solutions considered as artificial fixer-waste from photographic processing, were prepared by dissolving separately 10, 20, 30 mM AgBr in the excess amount of Na$_2$S$_2$O$_3$ solution (i.e. 100 mM). Thus, based on stoichiometric chemistry, the complexing reaction could be completed to directly produce soluble complex in the form of [Ag(S$_2$O$_3$)$_2$]$^3^-$, skipping intermediator as [Ag(S$_2$O$_3$)]$^-$. This helped to eliminate the formation of S and Ag$_2$S from the reduction of the intermediate [Ag(S$_2$O$_3$)]$^-$ complex at acidic pH, as reported by Tao et al. (2012).

In addition, the non-diffusion of [Ag(S$_2$O$_3$)$_2$]$^3^-$ complex through CMI-7000S membrane was demonstrated in the control reactor (R$_{05}$) (Figure 4.20b). Specifically, the initial Ag concentration fed to the catholyte of R$_{05}$ (i.e. 20 mM) did not change after 24 hours of observation. This result confirmed that the electrochemical reduction of [Ag(S$_2$O$_3$)$_2$]$^3^-$ did not occur in the cathode chamber of R$_{05}$ as there was no electron flow transferred from the abiotic anode (i.e. R$_{05}$ was not inoculated). On the other hand, there was also no Ag detected in the anolyte since Ag concentration was very low (i.e. < 0.05 mM, Figure 4.20 b). It means that the transport of negatively charged [Ag(S$_2$O$_3$)$_2$]$^3^-$ complex from the catholyte to the anolyte through CMI-7000S membrane was avoidable, which helped to prevent Ag loss.

Therefore, it can be concluded that the electrochemically cathodic reduction of the [Ag(S$_2$O$_3$)$_2$]$^3^-$ complex to produce metallic Ag$^0$ on the cathode surface was favorable and predominant during the BES operation. The Ag removal efficiency, shown in Figure 4.20a, was thus interpreted as Ag recovery efficiency.

### 4.3.1.3 Polarization curves and Power curves

The polarization curve analysis at different initial [Ag(S$_2$O$_3$)$_2$]$^3^-$ concentrations was also conducted to characterize the fuel cell of reactor The $R_{ext}$ was varied in a range of 10,000 – 5 Ω and the stable $E_{cell}$ was recorded at each $R_{ext}$, from which current density (I) and power density (P) were obtained.

For the polarization curves (E1-E3, Figure 4.21), the $E_{cell}$ obtained at $R_{ext}$ of 10000Ω, corresponding to I ~ 0 mA/m$^3$, was defined as the open circuit voltage (OCV). Theoretically, the OCV should approach the $E_{therm}$, which is considered as the ideal cell electromotive force ($E_{emf}$, Section 2.3.2) for anodic and cathodic reactions. Practically, it is lower than the $E_{therm}$. At the specific initial conditions used in this study (i.e. $C_0$ of 10 mM, CH$_3$COONa of 1.28 g/L at pH 7.0), the calculated $E_{therm}$ was + 0.313 V (or 313
mV, Section 3.2.3), while the measured OCV was 241 mV (Figure 4.21). This may be due to the parasitic losses, which normally occur in systems using membranes, as presented in the review of BES fundamentals (Section 2.3.2).

**Figure 4.21** Polarization curves (E1-E3), power curves (P1-P3) (R_{ext} = 10000 – 5 \ \Omega) at different initial [Ag(S_2O_3)_2]^2\textsuperscript{−} concentrations (10-30 mM) in reactor R5

The P_{max}, defined as the peaks of P1-P3 curves, confirmed the performance in electricity production. Specifically, P_{max} increased sharply from 1500 to 2647 mW/m\textsuperscript{3} when C_0 increased from 10 to 20 mM, however, it slightly raised to 2893 mW/m\textsuperscript{3} when C_0 was 30 mM. This increase of C_0 may lead to an increase in the ionic conductivity, and help to reduce the R_{int} of the electrolyte, which resulted in an improved P_{max} yield. However, the R_{int} could not be determined in each C_0 case by polarization-slope method, as E1-E3 curves were distorted and nonlinear (Figure 4.21). Thus, EIS analysis is again recommended to be conducted to determine the actual R_{int}.

**4.3.2 Characterization of electrodeposits at the cathode surface**

To demonstrate the formation of silver deposits on the cathode surface, the characterization of cathode surface was carried out. Prior to use, the graphite plate cathode is black-carbon color (Figure 4.22a). After 61 days of operation for silver recovery, cathode was taken out of reactor and air-dried at room temperature. Bright deposits of silver color were observed at the cathode surface (Figure 4.22b).
Figure 4.22 Visual images of graphite plate cathode of reactor R5 (a) before and (b) after 61 days of operation. Characterization of electrodeposits at the cathode surface by (c) SEM, (d) EDX, and (e) XRD

The deposits were scrapped and characterized by SEM analysis, in which different-sized silver cluster were detected (Figure 4.22c). This result was also verified by the EDX spectrum (Figure 4.22d), where the sharp peak at an energy level of 2.98 keV was found indicating the deposits comprised of pure silver. Specifically, the elemental characterization at point 1 of SEM image, had metallic Ag of 99.38 % wt. The XRD pattern shown in Figure 4.22e also confirmed the EDX result. These XRD pattern matched well with the standard patterns of silver (i.e. JCPDS card No. 04-0783). All of the peaks can be readily indexed to face-centered-cubic silver, where the diffraction peaks occur at 2θ values of 38.09°, 44.27°, 64.41°, 77.37°, and 81.53°. They also can be ascribed to the reflection of (111), (200), (220), (311), and (222) planes of the face-centered cubic silver, respectively.
4.3.3 Characterization of membrane surface

Compared to different CMI-based reactors investigated before (i.e. R1, R2, and R4), reactor R5 had no loss of Ag through CMI-7000S membrane (section 4.3.1.2). By visual observation, there were no deposits formed at the membrane surface of reactor R5 during the experiments.

![Image](image.png)

**Figure 4.23** (a) Characterization of CMI-7000S surface of reactor R5 after 61 days of operations by (a) SEM (b) EDX, and (c) XRD

After 61 days of operation, the CMI-7000S membrane, towards the cathode side, was characterized by SEM and EDX (Figure 4.23) to verify the occurrence of Ag at the membrane surface. Under SEM, no deposits, precipitants, and uncommon fouling were detected at the CMI-7000S surface, which confirmed the visual observation. The EDX element characterization at point 1 of SEM image also showed that C (61.03%), F...
(30.93%), and S (5.14%) were the main elements, which may come from membrane structure. The occurrence of other cations (i.e. Na, K) can be explained due to some transport through CMI-7000S membrane. A small amount of Ag (1.29%) detected can be attributed to either unselective permeability or diffusion of the catholyte, which is normally observed in any membrane process, but it did not relate to ion exchange mechanism. Under XRD characterization, only polypropylene was detected as the diffraction peaks occur at 2θ values of 14.16°, 17.16°, 18.72°, 21.3°, and 25.6°, corresponding to the reflection of (110), (040), (130), (140), and (150) planes in ICDD database (card # 00-054-1936).

The occurrence of polypropylene can be ascribed to the structural materials of the membrane as CMI-7000S is essentially a polymer membrane. There was no XRD peaks matching Ag compounds, indicating that [Ag(S\(_2\)O\(_3\))\(_2\)]\(^3-\) did not transport through the membrane. Therefore, it is believed that with [Ag(S\(_2\)O\(_3\))\(_2\)]\(^3-\) solutions, CMI-based reactor R5 could prevent effectively the loss of Ag.

4.4 Catholyte as mixed multi-metal solutions

Recovery of Ag from a synthetic mixed metals solution, containing Ag\(^+\), Fe\(^{3+}\), and Cu\(^{2+}\) ions, was investigated in CMI-based reactor R6. Due to the difference in the standard reduction potential among these metallic ions (i.e. Ag\(/\)Ag\(^0\); \(E^0 = 0.799\) V, Cu\(^{2+}\)/Cu\(^0\); \(E^0 = 0.337\) V, and Fe\(^{3+}\)/Fe\(^0\); \(E^0 = -0.44\) V vs. SHE), the cathodic reduction occurred differently.

4.4.1 Effect of mixed metal solutions

4.4.1.1 Metals removal

When the catholyte containing 10 mM Ag\(^+\), 10 mM Fe\(^{3+}\), and 1 mM Cu\(^{2+}\) was employed, the reduction of metals is shown in Figure 4.24a. The reduction of Ag\(^+\) occurred rapidly as Ag\(^+\) concentration decreased significantly from the initial level of 10 mM to 0.032 mM after 24 hours. On the other hand, Fe\(^{3+}\) concentration slightly decreased with time, from 10 mM to 2.68 mM, whereas Cu\(^{2+}\) concentration (i.e. 1.0 mM) was almost constant during the first 24 hours. Rapid reduction of Ag\(/\)Ag\(^0\) was explained by its highest reduction potential (\(E^0 = 0.799\) V vs. SHE), as compared to Cu\(^{2+}\)/Cu\(^0\); \(E^0 = 0.337\) V, and Fe\(^{3+}\)/Fe\(^0\); \(E^0 = -0.44\) V vs. SHE). The removal efficiency (RE) of Ag, Fe, and Cu in the BES reactor after 24 hours of operation was 95.79%, 35.71%, and 25.47%, respectively. When the operation was extended to 48 hours, the
corresponding RE was 99.68%, 73.19%, and 90.49%. However, in CMI-based reactor, the diffusion of Ag\(^{+}\), Fe\(^{3+}\), and Cu\(^{2+}\) through the membrane was observed, which is believed as the main reason for high RE of Fe and Cu after 48 hours.

When the initial concentration of Fe\(^{3+}\) was increased to 20 mM, while the others were kept constant (i.e. 10 mM Ag\(^{2+}\) and 1 mM Cu\(^{2+}\)), the reduction rate of Fe\(^{3+}\) \(r_{Fe}\) decreased accordingly (Figure 4.2b). The negatively reduction potential and non-priority for electrochemical reduction of Fe\(^{3+}\) were the reason for the decrease of \(r_{Fe}\) when the initial concentration of Fe\(^{3+}\) was increased to 20 mM. Also, the increase of Fe\(^{3+}\) concentration slightly affected the reduction rate of Ag\(^{+}\) ions \(r_{Ag}\). Specifically, the \(r_{Ag}\) decreased from 0.21 mM/h to 0.16 mM/h along with the increase of Fe\(^{3+}\) concentration from 10 mM to 20 mM, respectively. It can be explained that large amount of Fe\(^{3+}\) ions in the catholyte may compete with Ag\(^{+}\) ions to accept the electrons transferred from the anode. The increase of Fe\(^{3+}\) concentration, however, did not affect much the Cu\(^{2+}\) reduction rate (i.e. \(r_{Cu} = 0.02\) mM/h). The RE of Ag, Fe, and Cu in this case was 99.71%, 33.89%, and 86.84%, respectively. Nevertheless, higher initial concentration of Fe\(^{3+}\) in the catholyte increased the concentration gradient between two chambers and the mobility of ions, which accelerated the transport of metallic ions through CMI-7000S membrane. This led to the loss of metals, which may affect the recovery efficiency in BES reactor.

Figure 4.24 Reduction of metallic ions in the catholyte (a) C1 and (b) C2 of CMI-based reactor (R6)
In order to confirm the occurrence of metal ions in the anode chamber due to the diffusion from the cathode chamber, anodic biomass was digested to analyze the Ag amount. As a result, after 53 days of BES operation, the metallic contents in the anode chamber was 0.073 mM Ag⁺, 3.283 mM Fe³⁺, and 0.403 mM Cu²⁺, which were higher than that found in the original sludge before being used as inoculum (i.e. 0.0016 mM Ag⁺, 2.182 mM Fe³⁺, and 0.190 mM Cu²⁺).

### 4.4.1.2 Electricity production

The $E_{\text{cell}}$ produced during the operation of reactor R6 were recorded to examine the power generation. In the case of catholyte C1 (i.e. 10 mM Ag⁺, 10 mM Fe³⁺, and 1 mM Cu²⁺), $E_{\text{cell}}$ increased during the first 12 hours, and declined gradually after that due to the shortage of substrate as electron donor and the decrease of metal concentration. When the initial Fe³⁺ concentration of 20 mM was used in the catholyte C2, higher $E_{\text{cell}}$ was quickly obtained in the first hours, but this state just remained for a short time (Figure 4.25a). It can be explained by increase of Fe³⁺ concentration, which may help to increase the cathodic potential and improve $E_{\text{cell}}$ output at the beginning of operation.

![Figure 4.25 Performance in power generation. (a) $E_{\text{cell}}$ production ($R_{\text{ext}} = 5 \, \Omega$), (b) Polarization curves (E1, E2) and Power curves (P1, P2) ($R_{\text{ext}} = 10000 - 5\Omega$) with different catholytes (C1, C2) of CMI-based reactor (R6)](image-url)
determined as the slopes of E1 and E2, were almost similar (i.e. $R_{\text{int}}$ of 40$\Omega$). However, electrochemical parameters obtained in the C2 case (i.e. OCV of 645 mV and $P_{\text{max}}$ of 2472 mW/m$^3$) was higher than that of C1 case (i.e. OCV of 514 and $P_{\text{max}}$ of 1542 mW/m$^3$). This was due to the increase of initial concentration of Fe$^{3+}$, as a trivalent positively-charged ion, which may help to improve the ionic conductivity. Basically, the charge and valence of ions existing in the electrolyte affect significantly its conductivity. Thus, the increase of initial concentration of catholyte was conducted with Fe$^{3+}$, to show the effects of ionic conductivity on power generation.

4.4.2 Characterization of electodeposits at cathode and membrane surface

Prior to use, the graphite plate was black-carbon color. During the experiments, bright and lustrous deposits grew from small particles to thin layer on the surface when the reaction time elapsed. After 53 days of operation, the cathode was taken out of reactor R6 for SEM-EDX analysis. Under the SEM analysis, silver with dendritical and thin-debris structures was detected (Figure 4.26a). These results were then confirmed by the EDX spectrum, in which the sharp peak at an energy level of 2.98 keV indicated that the deposits on the cathode surfaces were comprised of pure silver (Figure 4.26b).

![Figure 4.26 Characterization of electodeposits at the cathode surface of CMI-based reactor (R6) after 53 days of operation by (a) SEM and (b) EDX](image)

The CMI-7000S membrane after operation were also characterized by using SEM-EDX analysis to observe the morphology and clarify the deposits on the membrane surface. SEM showed that the deposits was in dendritically film-like structure (Figure 4.27a). EDX analysis confirmed the chemical composition of the
deposits, in which Ag, Fe, and Cu were detected (Figure 4.27b). This result confirmed the transport of metallic ions through the CMI-7000S membrane.

![Figure 4.27 Characterization of CMI-7000S surface of reactor R6 after 53 days of operation by (a) SEM and (b) EDX](image)

**Figure 4.27** Characterization of CMI-7000S surface of reactor R6 after 53 days of operation by (a) SEM and (b) EDX

### 4.5 Comparison of BES performance with different catholytes

As the study investigated the recovery of silver from different Ag(I)-containing solutions (i.e. Ag⁺, [Ag(NH₃)₂]⁺, [Ag(S₂O₃)₂]³⁻, and multi-metal solution) in different BES reactors, a comparison of BES performance is carried out to understand their effects. At the same conditions (i.e. initial organic loading, measured in term of COD 1000 mg/L, initial Ag concentration of 1000 mg/L), a considerable difference in RE₄Ag, power generation, and morphology of electrodeposits, was found (Table 4.5). The limitations in each reactor with corresponding Ag(I) solution are also presented.

Overall, the reduction rate of Ag⁺ ions was faster than that of other Ag(I) ions, as the experiments with Ag⁺ solutions almost completed (i.e. RE₄Ag > 90%) in a shorter duration (i.e. 24 hours). This can be explained by the highest reduction potential of Ag⁺ as compared to the others. But the original pH of Ag⁺ solutions need to be adjusted to pH 2.0 to obtain high E₄cell output. Highest OCV (i.e. > 800 mV), was mostly found in the reactors operated with Ag⁺ solutions (i.e. R1, R2, R3) (Table 4.5).
Table 4.5 Comparison of BES performance using different Ag(I)-containing solutions as catholytes in BES reactors

<table>
<thead>
<tr>
<th>Types of catholyte</th>
<th>E° vs. SHE</th>
<th>Reactor/membrane</th>
<th>Initial Ag conc. (mg/L), pH</th>
<th>Time (h)</th>
<th>BES performance</th>
<th>Morphology of Ag deposits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag⁺</td>
<td>E°(Ag⁺/Ag⁰) = + 0.799 V</td>
<td>R1/ CMI-7000S</td>
<td>1000 mg/L pH = 2.0 (adjusted)</td>
<td>24</td>
<td>RE (%)</td>
<td>CE (%)</td>
<td>OCV (mV)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.28</td>
<td>21.1</td>
<td>828.8</td>
</tr>
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<td></td>
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<td></td>
<td></td>
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<td>(*)</td>
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<tr>
<td></td>
<td></td>
<td>R2/ CMI-7000S</td>
<td>1000 mg/L pH = 2.0 (adjusted)</td>
<td>24</td>
<td>92.51</td>
<td>18.5</td>
<td>858.9</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>(*)</td>
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<tr>
<td></td>
<td></td>
<td>R3/ AMI-7001S</td>
<td>1000 mg/L pH = 2.0 (adjusted)</td>
<td>24</td>
<td>50.00</td>
<td>2.1</td>
<td>805.5</td>
</tr>
<tr>
<td>[Ag(NH₃)₂]⁺</td>
<td>E°([Ag(NH₃)₂]⁺/Ag⁰) = + 0.373 V</td>
<td>R4/ CMI-7000S</td>
<td>1000 mg/L pH = 10.2 (original)</td>
<td>48</td>
<td>&gt; 99</td>
<td>8.55</td>
<td>664</td>
</tr>
</tbody>
</table>

Ref. code: 2560572230323QDG
<table>
<thead>
<tr>
<th>Types of catholyte</th>
<th>$E^0$ vs. SHE</th>
<th>Reactor/membrane</th>
<th>Initial Ag conc. (mg/L), pH</th>
<th>Time (h)</th>
<th>BES performance</th>
<th>Morphology of Ag deposits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ag(S₂O₃)₂]⁻³</td>
<td>$E^0$([Ag(S₂O₃)₂]⁻³ /Ag⁰) =: + 0.016 V</td>
<td>R5/ CMI-7000S</td>
<td>10 mM Ag⁺ pH = 7.39 (original)</td>
<td>48</td>
<td>RE (78.13)</td>
<td>CE (12.85)</td>
<td>OCV (241 mV)</td>
</tr>
<tr>
<td>Multi-metal solution (Ag⁺, Fe³⁺, Cu²⁺)</td>
<td>$E^0$(Ag⁺/Ag⁰) = +0.799 V $E^0$(Cu²⁺/Cu⁰) = +0.337 V $E^0$(Fe³⁺/Fe⁰) = -0.41 V</td>
<td>R6/ CMI-7000S</td>
<td>10 mM Ag⁺ 10 mM Fe³⁺ 1 mM Cu²⁺ pH = 2-3 (original)</td>
<td>48</td>
<td>RE (99.68)</td>
<td>CE (13.33)</td>
<td>OCV (514 mV)</td>
</tr>
</tbody>
</table>

(*) The diffusion of Ag⁺ ions through CMI-7000S membrane was considered in this calculation
For silver removal, a very high $\text{RE}_{\text{Ag}}$ (i.e. 99.28\%) was obtained in R1. However, in this case, the diffusion of $\text{Ag}^+$ ions through CMI-7000S membrane, which led to Ag loss, was not considered, and not taken into RE calculation. In R2, when the crossover of $\text{Ag}^+$ ions through membrane was included, a decrease of RE (i.e. 92.51\%) was found. CE obtained in R2 (18.5\%) was also lower than that in R1 (21.61\%). However, lower CE in reactor R2 was actually caused by the difference in electron donors and inoculum used in the anode chamber. In R1 and R2, although the initial organic loading was similar (i.e. COD of 1,000 mg/L), acetate was the substrate in R1 while glucose was used in R2. Compared to acetate as a simple substance, glucose is a fermentable substrate, implying its consumption by diverse competing metabolic pathways, which resulted in lower CE. Nevertheless, higher OCV (i.e. 858.9 mV) obtained in R2 helped to produce higher $P_{\text{max}}$ (i.e. 11252 mW/m$^3$) during the polarization curve experiments.

When AMI-7001S membrane was investigated in R3, a sharp decrease in RE (i.e. 50\%) was found after 24 hours of operation, although Ag loss was avoidable as $\text{Ag}^+$ ions cannot transport through AMI-7001S membrane. Instead, substrate loss due to the migration of negatively charged metabolites through AMI-7001S membrane, was believed as the main reason for low BES performance in R3 (i.e. a CE of 2.1\% and $P_{\text{max}}$ of 2289 mW/m$^3$). This statement was based on the occurrence of organic matters in the catholyte, which was measured in term of TOC concentration. However, in this study, the metabolites could not be analyzed to prove this migration.

In term of $[\text{Ag(NH}_3\text{)}_2]^+$ solutions used in R4, as it has a lower reduction potential (i.e. $E^0$ ([Ag(NH$_3$)$_2$]$^+$/Ag$^0$) = 0.373 V vs SHE), compared to Ag$^+$ (i.e. $E^0$ = 0.799 V vs SHE), a lower performance in power generation was observed (i.e. OCV of 664 mV, $P_{\text{max}}$ of 2984 mW/m$^3$). Meanwhile, the reduction of $[\text{Ag(NH}_3\text{)}_2]^+$ occurred spontaneously at alkaline condition (i.e. original pH of 10.2) without pH adjustment. However, at longer duration (i.e. 48 hours), $[\text{Ag(NH}_3\text{)}_2]^+$ concentration in the catholyte decreased to < 5 mg/L, which is corresponding to a RE of above 99\%. Nevertheless, the diffusion of $[\text{Ag(NH}_3\text{)}_2]^+$ complex through CMI-7000S was also observed in R4, like for the case in R1 and R2, which caused Ag loss problem. A better understanding of $[\text{Ag(NH}_3\text{)}_2]^+$ diffusion and its effects on BES performance was obtained in R4, as compared to that in R2, by characterizing the internal resistance at each level of initial $[\text{Ag(NH}_3\text{)}_2]^+$ concentration and the adsorption of Ag in anodic biomass.
In reactor R5, where [Ag(S\textsubscript{2}O\textsubscript{3})\textsubscript{2}]\textsuperscript{3-} solutions were investigated, the OCV of 241 mV and P\textsubscript{max} of 1500 mW/m\textsuperscript{3} were much lower than that found in other reactors due to the lowest standard reduction potential (i.e. E\textsuperscript{0}([Ag(S\textsubscript{2}O\textsubscript{3})\textsubscript{2}]\textsuperscript{3-}/Ag\textsuperscript{0} = 0.016 V vs SHE). High CE obtained in R5 (i.e. 12.85%) was attributed to high COD removal and E\textsubscript{cell} produced during the experiments, which partially relied on biological activities of anodic microorganism. A RE\textsubscript{Ag} of 81.73\% obtained after 48 hours of operation was attributed to the electrochemical reduction of [Ag(S\textsubscript{2}O\textsubscript{3})\textsubscript{2}]\textsuperscript{3-} complex to form the metallic deposits on the cathode surface, without any diffusion (i.e. [Ag(S\textsubscript{2}O\textsubscript{3})\textsubscript{2}]\textsuperscript{3-} are not able to transport through CMI-7000S membrane). The Ag loss was avoidable, and the Ag removal efficiency can be considered as recover efficiency. This is an advantageous feature of [Ag(S\textsubscript{2}O\textsubscript{3})\textsubscript{2}]\textsuperscript{3-} as compared to other Ag(I)-containing solutions, when being used as a catholyte in CMI-based-reactors.

When mixed multi-metal solutions containing Ag\textsuperscript{+}, Fe\textsuperscript{3+}, and Cu\textsuperscript{2+} were studied in R6, the performance in terms of power generation was still low (i.e. OCV of 514 mV, P\textsubscript{max} of 1542 mW/m\textsuperscript{3}), as compared to R1 and R2, in which the catholytes contained only Ag\textsuperscript{+} salt. Basically, the addition of other metallic salts to simulate the real wastewater is expected to increase the power generation due to the increase of ionic conductivity. However, the occurrence of Fe\textsuperscript{3+} and Cu\textsuperscript{2+} salts in the catholytes also increases the concentration gradient between the two chambers, which accelerated the transport of metallic ions through CMI-7000S membrane and led to the increase of membrane-internal resistance. On the other hand, the reduction of Ag\textsuperscript{+} ions in mixed solutions was not almost affected by Fe\textsuperscript{3+} and Cu\textsuperscript{2+}. The decrease of Ag\textsuperscript{+} concentration in the catholyte occurred more rapidly than that of Fe\textsuperscript{3+} and Cu\textsuperscript{2+}, which was ascribed to the highest reduction potential of Ag\textsuperscript{+}, as compared to Fe\textsuperscript{3+} and Cu\textsuperscript{2+}. Accordingly, a high silver removal (i.e. RE\textsubscript{Ag} > 99\%) was also obtained in reactor R6, similarly to that in R1, R2. The electrochemical reduction of Fe\textsuperscript{3+} and Cu\textsuperscript{2+} did not occur in R6 as SEM and EDX characterization could not detect Fe and Cu in the electrodeposits scrapped from the cathode surface. The decrease of Fe\textsuperscript{3+} and Cu\textsuperscript{2+} concentration was explained by the diffusion of Fe\textsuperscript{3+} and Cu\textsuperscript{2+} ions through CMI-7000S membrane (i.e. the deposits on membrane surface contained Fe and Cu under SEM and EDX characterization). However, further measurements, such as cathode potential
monitoring and controlling, need to be conducted to have a deeper understanding of electrochemically reductive mechanism of different metals in a mixed solution.

As a whole, in all BES reactors, metallic silver deposits were found at the cathode surface, which demonstrated that BES technology can be used successfully for recovery of silver from different aqueous solutions. Depending on Ag(I)-containing solution used as the catholyte in each reactor, different morphologies of silver deposits were observed, in which dendrite and crystal were the main structures. Crystalline structure are closed to the standard structure of Ag atom (i.e. faced-centered cubic silver). The current density produced in the system also affected the formation and growth of silver deposits. High $E_{\text{cell}}$ and $P_{\text{max}}$ obtained in reactor R1, R2, and R4 may help to produce dendritical and crystalline silver deposits, while other non-common structures (i.e. thin films, thin debris, and cluster) were detected in reactor R3, R5, and R6 due to their low $E_{\text{cell}}$ and $P_{\text{max}}$ production.

However, in all of the cases, the mass balance of Ag in BES reactors was not determined due to several obstacles. After BES operation in CMI-based reactor, silver could exist in following forms (i) metallic electrodeposits on the cathode surface, (ii) remaining dissolved Ag in the catholyte, (iii) deposits or precipitant at the membrane surface, (iv) dissolved Ag in the anolyte due to the diffusion through membrane, and (v) accumulated Ag in the anodic biomass. The quantification of Ag in the (i), (iii) forms, however, cannot be obtained under SEM, EDX, and XRD characterization. Further analysis to decompose the metallic deposits are required to determine the mass of Ag. In addition, the experiments were conducted in batch-fed condition, but the cathode and membrane were not renewed in each batch of the experiments. This would lead to the overlapping results if the quantification of Ag deposited on cathode and membrane surface is carried out. Lack of silver mass balance in BES reactors is one of the limitations of this study, which needs to be tackled in further studies.

Besides, due to the different bio-electrochemical processes occurring in BES reactors, the overall impedance of fuel cell consists of many individual resistance (i.e. activation (charge transfer) resistance, ohmic resistance representing the resistance from electrolyte, electrode materials and membrane, and concentration (diffusion) resistance (He and Mansfeld, 2009; Sekar and Ramasamy, 2013)). In this study, polarization slope method was used to determine the internal resistance in all reactors.

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This method, however, is preferable in a simple resistive fuel cells (that obeys Ohm’s law), in which ohmic resistance is dominant. Lack of characterization of different internal resistance in BES reactors is also a limitation of this research. Thus, electrochemical impedance spectroscopic (EIS) analysis, a useful tool to measure the impedance of a fuel cell, is suggested to be conducted in further works.
Chapter 5
Conclusions and Recommendations

5.1. Conclusions

This study successfully demonstrated the recovery of silver from different Ag(I)-
containing solutions, including AgNO₃ solution containing free Ag⁺ ions, [Ag(NH₃)₂]⁺,
[Ag(S₂O₃)₂]³⁻ complex, and mixed multi-metal solutions containing Ag⁺, Fe³⁺, and Cu²⁺
ions using bioelectrochemical systems and compare the performance for each solution.
Recovered product as metallic silver and simultaneous by-product as electricity,
accompanied with wastewater treatment, without supplying of external power and
further refinement, are significant achievements, as compared to other traditional
treatment techniques. A summary of results achieved in this study is shown as below.

- With Ag⁺ solution
  - Source of inoculum and anolyte composition had effects on the adaptation
    and Eₜₜ progressions during the inoculation stage, and also on the
    performance of power generation, which was characterized by CE. Compared to acetate, glucose is a fermentable substrate which was
    consumed by different competing metabolic pathways, resulting in a
    slower rate of voltage production and lower CE.
  - A change in initial pH (2-7) and Ag⁺ concentration (100-1,000 mg/L) in
    the catholyte had little effect on Ag recovery but affected considerably the
    power generation. A RE₁₅₅ of 96.54-99.28% and a Pₘₐₓ of 607-8,258
    mW/m³ along with CE of 4.36-21.61% were achieved when the initial
    Ag⁺ concentration was varied from 100-1,000 mg/L.
  - As a separator in BES reactors, CMI-7000S was better than AMI-7001S
    membrane at the same operational conditions. High RE₁₅₅ (83.73-92.50%)
    and CE (11.50-19.89%) were obtained in CMI-based reactor after 24
    hours, although, some diffusion of Ag⁺ ions was observed. In contrast,
    substrate loss was found in AMI-based reactor, which caused low overall
    performance. At an initial Ag⁺ concentration 2,000 mg/L, a Pₘₐₓ of 5,396
    mW/m³ and 3,385 mW/m³ were obtained in CMI and AMI-based reactor,
    respectively.
High diversity of anodic microbial communities were found, in which *Firmulates*, *Proteobacteria*, and *Bacteroidetes* were dominant phyla. *Geobacter* sp. and other exoelectrogen could be detected while no *Shewanella*-like sequences were retrieved.

**With [Ag(NH$_3$)$_2$]$^+$ solution**

- High Ag removal efficiency (> 96%) and COD removal (> 60%) could be obtained at different initial Ag(NH$_3$)$_2$+ concentration (1000 – 3000 mg Ag/L), while an encouraging CE (8.55-14.69%) was found. A comparable $P_{\text{max}}$ (2984 – 3618 mW/m$^3$) was also achieved in the experiments.

- $P_{\text{max}}$ slightly increased by 4.66%, from 3618 to 3795 mW/m$^3$, when 10 mM NaNO$_3$ was added to the catholyte containing 2000 mg Ag/L.

- Some transport of positive charged [Ag(NH$_3$)$_2$]$^+$ ions through the CMI-7000S membrane was observed, which affected the performance. This diffusion was confirmed by characterizing the membrane surface and quantifying the accumulated mass of silver in anodic biomass (i.e. 0.853 mg Ag/g biomass) after 58 days of operation.

**With [Ag(S$_2$O$_3$)$_2$]$_3^-$ solution**

- A RE$_{\text{Ag}}$ of 81.87-95.17% was obtained with the initial [Ag(S$_2$O$_3$)$_2$]$_3^-$ concentration of 10-20 mM, respectively. There was no [Ag(S$_2$O$_3$)$_2$]$_3^-$ migration through the membrane due to their negatively charged form, which avoided Ag loss.

- In terms of power generation, a columbic efficiency of 12.85-25.73% was achieved, although the COD removal efficiency in the anode chamber slightly fluctuated.

- When the initial [Ag(S$_2$O$_3$)$_2$]$_3^-$ concentration increased to 30 mM, power did not improve significantly, and a small decrease in Ag removal efficiency was also found.

**With mixed multi-metal solution containing 10 mM Ag$^+$, 1 mM Cu$^{2+}$, 10-20 mM Fe$^{3+}$**

- Due to the highest standard reduction potential of Ag$^+$ vs SHE, the reduction of Ag$^+$ in the cathode chamber occurred rapidly, as compared to that of Cu$^{2+}$ and Fe$^{3+}$. Higher RE$_{\text{Ag}}$ (> 99%) was found, which resulted
from electrochemical reduction at the cathode surface, but some diffusion of Ag⁺, Fe³⁺, and Cu²⁺ ions through CMI-7000S membrane were observed.

- Removal of Cu²⁺ and Fe³⁺ in the cathode chamber was attributed to the diffusion through the membrane, as there was no Cu and Fe detected in the deposits at the cathode surface. This diffusion was also confirmed by characterizing the membrane surface and anodic biomass, in which Ag⁺, Cu²⁺, and Fe³⁺ were found.

- Increasing the initial Fe³⁺ concentration (10-20 mM) had little effect on the Ag⁺ reduction rate in this reactor, but improved significantly the E_{cell} production due to the increasing ionic conductivity of the catholyte.

In all of cases, after BES operation, pure silver deposits with crystalline and dendritic morphology as the main structures, were detected the cathode surface by SEM and confirmed by EDX, and XRD analysis. The difference in morphology of silver deposits among BES reactors was ascribed by the influence of current density produced in each reactor, which is related to the reaction rate and the nucleation and growth of silver deposits at the cathode surface.

Overall, as compared to other traditional technologies for recovery of silver from aqueous solutions, the BES technology shows a great possibility due to its remarkable features, such as high Ag removal efficiency, high purity of silver products, no energy consumption, and integrated wastewater treatment (i.e. silver-rich wastewater and organic-contaminated wastewater). However, much more consideration should be done to confirm the applicability of BES technology for real silver-rich wastewater. Here, some recommendations are suggested, which require deeper investigations.

5.2. Recommendations

- Further experiments are needed to test real Ag-containing wastewater serving as the catholyte in BES reactor. The complexity of real conditions may not facilitate the spontaneous reduction of Ag ions in the cathode chamber. Subsidiary processes or pre-treatment, which may be required during BES operation with real wastewater, need to be investigated.

- In this study, pure substrates (i.e. acetate or glucose) and synthetic medium, were used as the anolyte to supply C sources and nutrients for biological
activities in the anode chamber. Other mixed substrate or high organic-loading real wastewater should be studied to mitigate the chemical intensive and also decrease the environmental impacts due to the discharge of organic-contaminated wastewater.

- In BES reactor, Ag ions are expected to be reduced electrochemically in the catholyte, to produce silver deposits on cathode surface. However, some amounts of Ag are also detected in the anolyte, anodic biomass, and membrane surface due to the diffusion through the separator (if CEMs are used). Thus, the Ag mass balance should be done to determine actual recovery efficiency.

- The characterization of different internal resistances contributing to the overall impedance of fuel cells, should be conducted by electrochemical impedance spectroscopic. This helps to understand complicate impacts of internal resistance on BES performances.
References

Books

Articles

Ref. code: 25605722300323QDG


microbial community in the anode chamber. *Bioresource Technology, 100*(21), 5132-5139.


Appendices
Appendix A

BES reactor explosion view
Appendix B
BES reactor – Detail sections
Appendix C
Calculation of redox potential and thermodynamic cell voltages

In BES reactors, the half-cell reactions written in the direction of chemical reduction according to IUPAC convention, are shown in Eq. (S1) and (S2) – (S3) – (S4)

At the anode chamber: \( \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^- \) (S1)

At the cathode chamber:
- with Ag\(^+\) ions: \( \text{Ag}^{+} + \text{e}^- \rightarrow \text{Ag}^0 \) (S2)
- with [Ag(NH\(_3\))]\(^2+\) complex: \( \text{[Ag(NH}_3\text{)]}^{+} + \text{e}^- \rightarrow \text{Ag}^0 + 2\text{NH}_3 \) (S3)
- with [Ag(S\(_2\)O\(_3\))]\(^3+\) complex: \( \text{[Ag(S}_2\text{O}_3\text{)]}^{3} + \text{e}^- \leftrightarrow \text{Ag}^0 + 2\text{S}_2\text{O}_3^{2-} \) (S4)

The thermodynamic anode and cathode potential (\( \text{E}_{an} \) and \( \text{E}_{ca} \)) are calculated by the Nernst equation as below.

\[
\text{E}_{an} = \text{E}_{an}^0 - \frac{RT}{nF} \ln \left( \frac{[\text{CH}_3\text{COO}^-]}{[\text{HCO}_3^-][\text{H}^+]^9} \right),
\]

Where, \( \text{E}_{an}^0 \) is the electrochemical redox potential at standard conditions (298 K, 1atm, \([\text{H}^+] = 1\text{M}) (0.187 \text{V versus} \text{the Standard Hydrogen Electrode (vs. SHE)} \) (Logan, 2008)), \( R \) is the gas constant (8.31447 J/mol/K), \( T \) is the absolute temperature (298 K), \( n \) is the number of electrons transferred (8 \text{e}^-), \( F \) is Faraday’s constant (96,485 C/mol), \([\text{H}^+]\) is the concentration of protons in the anolyte (10\(^{-7}\) M as pH = 7), [HCO\(_3\)] is the concentration of HCO\(_3^-\) in the anolyte (5 mM), [CH\(_3\)COO\(^-\)] is the concentration of CH\(_3\)COO\(^-\) in the anolyte (1.28 g/L, corresponding to ).

\( \text{E}_{an} \) was calculated as -0.292 \text{V vs SHE}.

\[
\text{E}_{ca} = \text{E}_{ca}^0 - \frac{RT}{nF} \ln \left( \frac{[\text{products}]}{[\text{reactants}]} \right)
\]

- with Ag\(^+\) solution: \( \text{E}_{ca} = \text{E}_{ca}^0 - \frac{RT}{nF} \ln \left( \frac{1}{[\text{Ag}^+]} \right) \) (S6a)
- with [Ag(NH\(_3\))]\(^2+\) solutions: \( \text{E}_{ca} = \text{E}_{ca}^0 - \frac{RT}{nF} \ln \left( \frac{1}{[\text{Ag(NH}_3\text{)]}^{+}} \right) \) (S6b)
- with [Ag(S\(_2\)O\(_3\))]\(^3+\) solutions: \( \text{E}_{ca} = \text{E}_{ca}^0 - \frac{RT}{nF} \ln \left( \frac{[\text{S}_2\text{O}_3^{2-}]}{[\text{Ag(S}_2\text{O}_3\text{)]}^{3}} \right) \) (S6c)

Where, \( \text{E}_{ca}^0 \) is the electrochemical cathode potential at standard conditions (298 K, 1atm, \([\text{Ag]} = 1\text{M})). Depending on type of Ag ions, \( \text{E}_{ca}^0 \) vs SHE is different (i.e. 0.799 \text{V for Ag}^+, 0.373 \text{V for} [\text{Ag(NH}_3\text{)]}^{+}, \text{and} 0.016 \text{V for} [\text{Ag(S}_2\text{O}_3\text{)]}^{3-} \). In addition, with different initial Ag concentration used in the experiments (i.e. [Ag\(^+\)], [Ag(NH\(_3\))]\(^2+\)], and
[Ag(S_2O_3)_2]^-), the E_{ca} accordingly varied. Thus, the thermodynamic cell voltages (E_{therm} = E_{ca} – E_{an}) are different and are summarized in Table S1.

Table S1 Electrode redox potential and thermodynamic cell voltages

<table>
<thead>
<tr>
<th>Type of Ag(I)-containing solution</th>
<th>Initial Ag concentration</th>
<th>E^{0}_{ca} (V) vs SHE</th>
<th>E_{ca} (V) vs SHE</th>
<th>E_{an} (V) vs. SHE</th>
<th>E_{therm} (V)</th>
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</thead>
<tbody>
<tr>
<td>Ag^+ solution</td>
<td>200 mg/L → 1.852 mM</td>
<td>+ 0.799</td>
<td>+ 0.637</td>
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<td>+ 0.929</td>
</tr>
<tr>
<td></td>
<td>500 mg/L → 4.63 mM</td>
<td></td>
<td>+ 0.661</td>
<td></td>
<td>+ 0.953</td>
</tr>
<tr>
<td></td>
<td>1000 mg/L → 9.26 mM</td>
<td></td>
<td>+ 0.679</td>
<td></td>
<td>+ 0.971</td>
</tr>
<tr>
<td></td>
<td>2000 mg/L → 18.52 mM</td>
<td></td>
<td>+ 0.696</td>
<td></td>
<td>+ 0.989</td>
</tr>
<tr>
<td>[Ag(NH_3)_2]^+ solution</td>
<td>1000 mg Ag/L → 9.26 mM</td>
<td>+ 0.373</td>
<td>+ 0.253</td>
<td></td>
<td>+ 0.545</td>
</tr>
<tr>
<td></td>
<td>2000 mg Ag/L → 18.52 mM</td>
<td></td>
<td>+ 0.270</td>
<td></td>
<td>+ 0.562</td>
</tr>
<tr>
<td></td>
<td>3000 mg Ag/L → 27.78 mM</td>
<td></td>
<td>+ 0.281</td>
<td></td>
<td>+ 0.573</td>
</tr>
<tr>
<td>[Ag(S_2O_3)_2]^- solution</td>
<td>10 mM</td>
<td>+ 0.016</td>
<td>+ 0.0217</td>
<td></td>
<td>+ 0.313</td>
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<tr>
<td></td>
<td>20 mM</td>
<td></td>
<td>+ 0.0306</td>
<td></td>
<td>+ 0.322</td>
</tr>
<tr>
<td></td>
<td>30 mM</td>
<td></td>
<td>+ 0.0358</td>
<td></td>
<td>+ 0.328</td>
</tr>
</tbody>
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Appendix D
Preparation of standard $\text{Ag}^+$ solutions to build ICP standard curve for determination of Ag concentration

In order to determine Ag concentration in electrolyte samples obtained in each batch of experiments, a series of standard $\text{Ag}^+$ solutions for ICP analysis was prepared according to Standard Methods for Examination of Water and Wastewater, 20th ed. section 3.120B. The following is a representative procedure conducted in a specific experiment of reactor R1.

1. At first, for preparation of stock solution (i.e. $\text{Ag}^+$ of 100 mg/L) (section 3111B.3 j22): Dissolve 157.5 mg silver nitrate $\text{AgNO}_3$ in 100 mL DI water, add 10 mL concentrated $\text{HNO}_3$, and then make up to 1000 mL by DI water in a volume metric; 1.00 mL = 100 µg Ag. The stock solution was kept in fridge for use within 1 month.

2. For preparation a series of standard $\text{Ag}^+$ solutions in the optimum concentration range (i.e. 1, 5, 10, 50 mg/L), an appropriate dilution of the stock $\text{Ag}^+$ solution (i.e. 100 mg/L) with DI water containing concentrated $\text{HNO}_3$ (1.5 mL $\text{HNO}_3$/1.0 L of DI water) was conducted in each batch of experiments.

3. The ICP calibration was carried out with a blank sample (i.e. DI water) and these above standard $\text{Ag}^+$ solutions. At each calibration step, a triplicate measurement was set up, and the average intensity corresponding to standard $\text{Ag}^+$ concentration identified by ICP spectrometer was recorded.

4. ICP standard curve was obtained based on intensity and standard $\text{Ag}^+$ concentration with a $R^2$ co-efficiency of 0.999758, as shown in the Calibration summary of data sheet below.
Method: Dao Ag

Analysis Begun
Logged In Analyst: Stiff ICP9000
Spectrometer: Optima 8000
Sample Information File:
Batch ID:
Results Data Set:
Results Library:

Method Loaded
Method Name: Dao_Ag
Method Last Saved: 21/10/2059 14:05:05
Method Description: Dao_Ag

Nebulizer Parameters: Calib Blank 1

<table>
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<tr>
<th>Analyte</th>
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<th>Flow</th>
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</thead>
<tbody>
<tr>
<td>All</td>
<td>282.0 kPa</td>
<td>0.55 L/min</td>
</tr>
</tbody>
</table>

Replicate Data: Calib Blank 1

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<thead>
<tr>
<th>Repl#</th>
<th>Analyte</th>
<th>Net Intensity</th>
<th>Corrected Intensity</th>
<th>Conc. Units</th>
<th>Analysis Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ag 328.068</td>
<td>-2397.2</td>
<td>-2397.2</td>
<td>(0.00) mg/L</td>
<td>15:30:38</td>
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<tr>
<td>2</td>
<td>Ag 328.068</td>
<td>-2527.1</td>
<td>-2527.1</td>
<td>(0.00) mg/L</td>
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<tr>
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<td>-3032.7</td>
<td>-3032.7</td>
<td>(0.00) mg/L</td>
<td>15:30:50</td>
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Mean Data: Calib Blank 1

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<th>Std.Dev.</th>
<th>RSD</th>
<th>Conc. Units</th>
</tr>
</thead>
<tbody>
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<td>-2525.2</td>
<td>335.75</td>
<td>12.6%</td>
<td>(0.00) mg/L</td>
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Sequen No.: 2
Sample ID: Ag 1
Date Collected: 6/4/2016 15:30:58

Nebulizer Parameters: Ag 1

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</thead>
<tbody>
<tr>
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Replicate Data: Ag 1

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<th>Corrected Intensity</th>
<th>Conc. Units</th>
<th>Analysis Time</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1156209.6</td>
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<tr>
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<td>Ag 328.068</td>
<td>1119505.9</td>
<td>1122158.2</td>
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<td>1142057.7</td>
<td>1144710.1</td>
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<td>15:31:48</td>
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Mean Data: Ag 1
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<tr>
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<th>Page 2</th>
<th>Date: 6/4/2560 16:21:56</th>
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<td>Ref. code: 25605722300323QDG</td>
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### Sequence No. 1: Ag 5

**Sample ID:** Ag 5  
**Date Collected:** 6/4/2560 15:31:58  
**Data Type:** Original  
**Initial Sample Vol:**  
**Sample Prep Vol:**  
**Wash Time:**  

**Nebulizer Parameters:** Ag 5  
**Back Pressure:** 284.0 kPa  
**Flow:** 0.55 L/min  

### Replicate Data: Ag 5

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<th>Corrected Intensity</th>
<th>Calib</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ag 328.068</td>
<td>539459.1</td>
<td>5397248.5</td>
<td>(5) mg/L</td>
<td>15:32:42</td>
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<tr>
<td>2</td>
<td>Ag 328.068</td>
<td>5478573.6</td>
<td>5481225.9</td>
<td>(5) mg/L</td>
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<td>Ag 328.068</td>
<td>5403246.5</td>
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### Mean Data: Ag 5

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<th>RSD</th>
<th>Calib</th>
<th>Conc. Units</th>
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</thead>
<tbody>
<tr>
<td>Ag 328.068</td>
<td>5428124.4</td>
<td>46190.19</td>
<td>0.85%</td>
<td>[5] mg/L</td>
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### Sequence No. 2: Ag 10

**Sample ID:** Ag 10  
**Date Collected:** 6/4/2560 15:33:08  
**Data Type:** Original  
**Initial Sample Vol:**  
**Sample Prep Vol:**  
**Wash Time:**  

**Nebulizer Parameters:** Ag 10  
**Back Pressure:** 284.0 kPa  
**Flow:** 0.55 L/min  

### Replicate Data: Ag 10

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<tbody>
<tr>
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<td>Ag 328.068</td>
<td>10641214.2</td>
<td>10643886.6</td>
<td>(10) mg/L</td>
<td>15:33:51</td>
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<tr>
<td>2</td>
<td>Ag 328.068</td>
<td>10741043.3</td>
<td>10743695.7</td>
<td>(10) mg/L</td>
<td>15:33:55</td>
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<tr>
<td>3</td>
<td>Ag 328.068</td>
<td>10587136.3</td>
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<td>(10) mg/L</td>
<td>15:33:58</td>
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### Mean Data: Ag 10

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<th>RSD</th>
<th>Calib</th>
<th>Conc. Units</th>
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<tbody>
<tr>
<td>Ag 328.068</td>
<td>10659117.0</td>
<td>78078.62</td>
<td>0.73%</td>
<td>(10) mg/L</td>
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### Sequence No. 3: Ag 50

**Sample ID:** Ag 50  
**Date Collected:** 6/4/2560 15:34:22  
**Data Type:** Original  
**Initial Sample Vol:**  
**Sample Prep Vol:**  
**Wash Time:**  

**Nebulizer Parameters:** Ag 50  
**Back Pressure**  
**Flow**
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<th>Corrected Intensity</th>
<th>Calib. Conc.</th>
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</thead>
<tbody>
<tr>
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<td>Ag 328.068</td>
<td>599988568.8</td>
<td>600015091.1</td>
<td>[50] mg/L</td>
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<tr>
<td>2</td>
<td>Ag 328.068</td>
<td>59859141.4</td>
<td>59861793.7</td>
<td>[50] mg/L</td>
<td>15:35:11</td>
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<td>Ag 328.068</td>
<td>59386732.7</td>
<td>59389385.0</td>
<td>[50] mg/L</td>
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**Mean Data: Ag 50**

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<th>Units</th>
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<tr>
<td>Ag 328.068</td>
<td>59817562.6</td>
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<td>0.35%</td>
<td>(50) mg/L</td>
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**Calibration Summary**

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</thead>
<tbody>
<tr>
<td>Ag 328.068</td>
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<td>Lin, Calc Int</td>
<td>-453034.4</td>
<td>1202000</td>
<td>0.00008</td>
<td>0.999758</td>
<td></td>
</tr>
</tbody>
</table>
Appendix E

Preparation of standard TOC solutions for determination of TOC concentration

In order to determine TOC concentration in anolyte samples obtained in each batch of experiments in reactor R2 and R3, a series of standard TOC solutions for TOC analysis was prepared according to the non-purgeable organic carbon (NPOC) method for a TOC analyzer (TNM-1, Shimadzu, Japan), as below.

1. At first, stock solution (i.e. TOC of 1000 mg/L) was prepared from Potassium hydrogen phthalate $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$
   - Dry an appropriate amount of $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH})$ in oven 1 hour at $110^\circ\text{C}$
   - Weight 2.125 g of $\text{C}_6\text{H}_4(\text{COOK})(\text{COOH}$, dissolve in MiliQ water and then make up to 1000 mL by MiliQ water in a volume metric
   - Keep stock solution in a fridge and use within 2 months

2. For preparation a series of standard TOC solutions in the optimum concentration range (i.e. 1, 5, 10, 50, 100 mg/L), an appropriate dilution of the stock TOC solution (i.e. 1000 mg/L) with MiliQ water was conducted. A blank sample (i.e. MiliQ water) was also used as a standard solution (i.e. TOC of 0 mg/L).

3. All of electrolyte samples obtained from experiments after appropriate dilution and standard TOC solutions were transferred to TOC vials for analysis by a TOC analyzer (TNM-1, Shimadzu, Japan).

4. A representative calibration curve obtained in a TOC analysis is shown in Figure S1.

![Figure S1. A representative TOC calibration curve](image_url)

\[y = 4.2825x - 2.4021\]
\[R^2 = 0.9997\]