Acid Water Neutralization Using Microbial Fuel Cells: An Alternative for Acid Mine Drainage Treatment

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Abstract: Acid mine drainage (AMD) is a complex environmental problem, which has adverse effects on surface and ground waters due to low pH, high toxic metals, and dissolved salts. New bioremediation approach based on microbial fuel cells (MFC) can be a novel and sustainable alternative for AMD treatment. We studied the potential of MFC for acidic synthetic water treatment through pH neutralization in batch-mode and continuous-flow operation. We observed a marked pH increase, from ~3.7 to ~7.9 under batch conditions and to ~5.8 under continuous-flow operation. Likewise, batch reactors (non-MFC) inoculated with different MFC-enriched biofilms showed a very similar pH increase, suggesting that the neutralization observed for batch operation was due to a synergistic influence of these communities. These preliminary results support the idea of using MFC technologies for AMD remediation, which could help to reduce costs associated with conventional technologies. Advances in this configuration could even be extrapolated to the recovery of heavy metals by precipitation or adsorption processes due to the acid neutralization.

Keywords: acid mine drainage; microbial fuel cell; acid water; low pH; air-cathode

1. Introduction

Acid mine drainage (AMD) is a worldwide environmental concern that produces acidic waters (pH < 4) with high metals and sulfate concentrations that pose a serious pollution threat to surface and ground waters [1]. Therefore, AMD treatment has been one of the major focus of study in recent decades [2–5]. Unfortunately, conventional treatment technologies for AMD remediation have several limitations, both economic and operational [6]. Nevertheless, new bioremediation systems based on microbial fuel cells (MFC) might appear as a new and sustainable alternative [7,8].

MFC is an emerging technology for electricity generation, using electrochemically active microorganisms to catalyze the oxidation of organic/inorganic electron donors and transfer electrons towards an electrode (anode) [9,10]. Recent advances in MFC have enabled the development of biotechnological applications that allow for the removal of various pollutants (e.g., heavy metals, organic compounds, sulfur, and perchlorate) [11–15].

In this context, AMD treatment was investigated using fuel cell technology to generate abiotic electricity with simultaneous iron removal from acidic waters [16]. Similarly, removal of other heavy metals such as silver [17], gold [18], cobalt [19] and copper [20] have also been studied using MFC. For example, Hai et al. [21] investigated AMD remediation using a coupled membrane-free MFC with
a permeable reactive barrier inoculated with sulfate-reducing bacteria. They observed significant removal of sulfate (33%) and heavy metals (Cu\(^{2+}\), Zn\(^{2+}\), and Pb\(^{2+}\)) (99%) with influent pH values between 4 and 5 and sulfate concentrations higher than 1 g·L\(^{-1}\). Even though the efforts that have been conducted to apply MFC technology to AMD remediation, very few studies have focused in pH neutralization of AMD waters. To our knowledge, only Lefebvre et al. [22] has shown, in a double-chamber MFC that the pH of a synthetic AMD solution may rise from 2.5 to values higher than 7.

In this study, single-chamber air-cathode MFC (SC-MFC) were used to attempt the pH neutralization of acidic waters. The reactors, inoculated with consortia coming from an AMD-affected site, were operated under batch and continuous-flow modes. Additionally, batch cultures inoculated with microorganisms extracted from MFC were used to evaluate their ability for acid neutralization in non-MFC systems. The purpose of this article is to demonstrate the potential of microorganisms enriched in MFC systems to raise the pH and therefore gain knowledge of the key processes for the remediation of AMD.

2. Materials and Methods

2.1. Single-Chamber MFC Start-Up and Operation

Experiments were performed using two SC-MFC (Reactors “a” and “b”) (Figure 1). Carbon felt was used as anode material, while carbon cloth (Type B-1B, E-TEK) was used as the air-cathode, prepared as previously described [23]. The cathode used 0.5 mg/cm\(^2\) platinum catalyst applied to its water-facing side. MFC were initially operated as batch reactors in a controlled-temperature room (~20 °C).

![Figure 1](image-url)

Figure 1. (a) Photograph of a single-chamber air-cathode microbial fuel cell (SC-MFC) and conceptual model used in the study; (b) SEM micrographs from surface of biofilm attached to the anode (bottom left image) and cathode (bottom right image) of the MFC reactor.
The reactors were inoculated with an enrichment culture from an AMD-affected sediment, located in the upper section of the Azufre River sub-basin, Northern Chile [24]. Acidic synthetic water, with pyruvate as electron donor, was used as the electrolyte during MFC operation. This solution contained the following (per liter): sodium pyruvate (1.3 g); NH₄Cl (0.31 g); KCl (0.13 g); vitamins (5 mL); metal salts (12.5 mL). The pH of the medium was adjusted to 3.7 ± 0.1 with 0.5 M HCl. During the first months of operation, one of the cells was operated with the same electrolyte but including sulfate (1.95 ± 0.29 g L⁻¹). Afterwards, operation continued for both reactors with the medium previously described (sulfate concentration less than 0.025 g L⁻¹).

The anode and cathode were connected by a 100 Ω external resistor. Cell voltage was measured every 10 min using a data acquisition system (2700; Keithley, Solon, OH, USA). Batch-operation cycles lasted ~2 days, according to soluble chemical oxygen demand (sCOD) consumption. An abiotic control was operated to check that cell voltage generation occurs because of biological catalysis at the anode. After every batch cycle, MFC effluents were collected for measurement of pH, electrical conductivity, and concentrations of sulfate and sCOD.

Additionally, the performance of the reactor during batch operation was characterized through the calculation of coulombic efficiency, from sCOD values according to Logan et al. [10], and power density, obtained from the peaks of observed current cycles and Ohm’s law.

2.2. Continuous-Flow Experiments

Continuous-flow experiments were conducted in SC-MFC reactors using inlet and outlet flows of 0.6 mL min⁻¹ for ~1 day. The corresponding hydraulic retention time (HRT) was estimated in approximately 35 min. Electrolyte utilized in these experiments was identical to the one used during batch operation, except for pyruvate concentration (0.03 g L⁻¹). For the first experiment (only continuous-flow), the concentration was set at 0.03 g L⁻¹, which was set proportional to sCOD consumption observed for batch-mode HRT (~2.5 days).

Additionally, intermittent continuous-flow experiments (a sequential continuous-flow period is followed by a batch-mode one) were conducted. Different concentrations of the electron donor were used: 0.03 g L⁻¹, 0.06 g L⁻¹, and 0.12 g L⁻¹. Batch intervals were set as 1.5, 3.0, and 6.0 h, respectively, in order to promote the consumption of almost all the electron donor. About the continuous-flow periods, an interval of 6 h was set, according to the results of the first continuous-flow experiment.

For all the experiments, the corresponding effluent was collected for pH measurement, every 15 min. Additionally, cell voltage was registered every 10 min (100 Ω external resistor), and sCOD was measured at the beginning and the end of the experiments.

2.3. Batch (Non-MFC) Experiments

At the end of the MFC operation, reactors (“a” and “b”) were disassembled and biofilm samples from anodes, cathodes and reactor walls were collected in 5 mL sterile tubes (BD Biosciences, Mountain View, CA, USA). These samples were used as inocula for new batch cultures in order to separate the influence of each microbial community in pH behavior. Culture medium was the same synthetic acidic water used during batch operation.

Cultures were performed in 50 mL sterile centrifuge tubes at 37 °C with continuous agitation for five days. The initial cell concentration was set at ~5.5 × 10⁸ cells mL⁻¹. The pH of these cultures was monitored every 12 h. The experiment also included an abiotic control and a culture inoculated with the environmental sample used as the inoculum for the first enrichments, before the MFC operation.

3. Results and Discussion

3.1. pH Neutralization by MFC Reactors Operated in Batch-Mode

The performance of MFC reactors during batch operation was characterized by a power density of 3.8 ± 4.9 mW m⁻² (calculated from current peaks, Figure A1) and a coulombic efficiency of
4.6% ± 3.9%. This performance did not differ significantly in the presence of sulfate (data not shown). Furthermore, since sulfate concentrations were practically equal at the beginning and the end of a cycle, sulfate reduction was ruled out in both conditions.

Acid neutralization was observed simultaneously with voltage generation in both SC-MFC reactors. After ~50 h current cycles (Figure A1), pH rose from 3.7 ± 0.1 (influent culture medium) to 7.9 ± 0.1 (reactor effluent). It was previously observed that pH of synthetic AMD water dominated with iron rose from 2.5 to 7.9 in the abiotic cathodic chamber of a MFC, when a charge of 662 coulombs was transferred between the electrodes [22]. Similarly, a batch-operated SC-MFC, using initial moderately-acidic conditions (pH = 5.0) evidenced a pH increase of ~1 unit [25]. However, they used an inoculum from a wastewater treatment plant and, according to their results, showed a much better performance under higher-pH initial conditions. Thus, the inoculum used in this study, extracted from an AMD-affected environment, was expected to have an important role in the neutralization observed inside the MFC.

3.2. pH Neutralization and Continuous-Flow Operation Mode

Under continuous-flow operation a clear rise in pH was observed, condition that was maintained for approximatively 4 h (Figure 2). The increase in the pH was associated with the removal of sCOD from synthetic acidic water. However, after this period, pH decreased and the neutralization was not stable over time, suggesting that the acid neutralization is slower than the tested HRT (~35 min). This is consistent with what was previously proposed by He et al. [25], who hypothesized that biological neutralization fulfilled in batch-operated MFC may be reduced in a continuous-flow operated one due to the impact of a constant flow with low pH on neutralizing bacterial activity.

![Figure 2. pH increase and sCOD removal in continuous-flow assays in SC MFC reactors.](image)

According to the first continuous-flow experiment results, pH neutralization was assessed alternating batch and continuous-flow modes (Figure 3). At closed circuit conditions (Figure 3a), it was observed that the pH after 1.5, 3.0, and 6.0 h of batch operation rose by 0.46 (up to 4.2 ± 0.1), 0.66 (up to 4.6 ± 0.1), and 2 pH units (up to 5.7 ± 0.1), respectively. However, as occurred at the first continuous-flow experiment, neutralization was not stable over the continuous-flow operation.
period. These results suggest that pH neutralization is related to the length of batch condition periods, and therefore, the time during the acidic water is in contact with the biofilms. However, as the length of batch periods is proportional to the electron donor concentration, this latter variable may also have an effect on the neutralization magnitude.

Even more, under open circuit conditions (Figure 3b), a behavior very similar to the one observed under closed circuit conditions was achieved, with the highest pH peak of 5.8 ± 0.1 obtained after the 6.0 h batch operation period. A similar performance was observed at 1.5 h and 3.0 h compared to closed circuit conditions. One possible explanation for pH neutralization is the proton consumption carried out by the oxygen reduction reaction at the cathode [26,27], but the similarity observed for results obtained under both circuit conditions precludes this possibility. Therefore, the pH neutralization would not be related to redox reactions involved in voltage generation, but other microbial reactions, or the accumulation of their products in the biofilms, could be responsible for it.

Figure 3. pH neutralization in experiments with alternated batch and continuous-flow operation mode under (a) closed-circuit conditions (100 Ω resistor) and (b) open-circuit conditions. The experiment was performed with different concentrations of electron donor (pyruvate). Batch-periods are shown by grey stripes (1.5, 3.0, and 6.0 h). Data sets are representative of multiple replicates of each run.
He et al. [25] has previously proposed that microorganisms might be able to promote pH changes inside MFC reactors in order to make the environment more favorable for microbial growth. Furthermore, some proton-consuming biological reactions have been reported in the literature, supporting the idea of the potential role of microbial activity in pH neutralization inside MFC. Gregory et al. [28] showed that Geobacter species were able to retrieve electrons directly from a graphite electrode and use them to reduce nitrate to nitrite. Additionally, Rodenas et al. [29] reported proton consumption associated with biological sulfate reduction in the presence of acetate and sulfate inside the anode chamber of a MFC.

Thus, there is evidence of biological processes inside the MFC that can cause a rise in pH. However, previous studies are based on indirect associations under moderately acidic conditions (pH > 5.0), while the results indicate that an acid-tolerant microbial metabolism is relevant, and the MFC could be decisive for the selection of these microorganisms.

3.3. MFC as an Enrichment Method for Acid-Neutralizing Microorganisms

Considering the outcomes of the previous experiments, we proceeded to evaluate whether the microorganisms could keep their neutralizing ability outside the MFC reactors and to confirm the hypothesis that the neutralizing mechanism is not related to voltage generation. Furthermore, the experiment also aimed to assess the neutralizing ability for the different MFC communities separately. For this purpose, batch cultures (non-MFC reactors) were performed using the different MFC biofilms as inoculum sources.

Figure 4 shows that the three types of cultures, from both studied reactors, accomplished acid neutralization of the culture medium (final pH values between 6.7 and 7.2). According to the figure, neutralization achieved in reactor “b” cultures was slightly slower than the one obtained in reactor “a” cultures. Additionally, it could be also observed that pH increases were achieved by the three types of culture with a very similar velocity, for both reactors “a” and “b”. Hence, since pH increases were achieved by every culture, it is probable that the neutralization observed inside the MFC, during batch-mode and continuous-flow operation, was due to a synergistic effect between the influences of the three different biofilms.

![Figure 4](image_url)

**Figure 4.** pH evolution in batch cultures of microorganisms present at electrodes and reactor walls from reactor “a” (a) and reactor “b” (b). Biofilms were used as inocula for cultures under initial acidic conditions (pH = 3.7, 1.3 g L⁻¹ Pyruvate). Batch experiments were performed at room temperature (~20 °C), with an initial cell concentration of ~5.5 × 10⁸ cells mL⁻¹. The data are presented as mean ± SD (error bars) (n = 2). Abiotic control and environmental sample culture were also included in the experiment.

Interestingly, the rise in pH value observed for cultures inoculated with MFC microorganisms did not occur for the cultures inoculated with the environmental sample (Figure 4). This result suggests that the neutralizing ability of the microbial communities is associated with the species enriched inside the MFC during its operation period. If so, MFC operation would have a very important role in selecting microorganisms involved in some proton-consuming reactions.
4. Conclusions

This study revealed that pH neutralization of a synthetic acidic medium occurred in SC-MFC reactors during batch-mode and continuous-flow operation. The results showed that pH neutralization was mediated by microbial activity, but not associated with voltage generation. Additionally, batch conditions were shown to favor acid neutralization, suggesting that flow conditions may significantly limit neutralizing microbial capacity. Therefore, the neutralizing ability of this acid-tolerant microbial community enriched inside the MFC could be useful in the development of new AMD treatment technologies.

These findings might also contribute to a better understanding of biogeochemical processes of acid neutralization in MFC systems through the comprehension of the proper interaction between acid-tolerant and exoelectrogenic microorganisms. Furthermore, these advances could be an initial step towards improving the performance of traditional MFC and could expand their use towards the biological treatment of other AMD pollutants, such as heavy metals or sulfate.

Additional efforts are necessary to describe the complex mechanisms involved in pH neutralization and the specific selection of neutralizing microorganisms, as well as their interactions with exoelectrogenic bacteria. Likewise, an analysis of bacteria and archaeal species present in biofilms could allow us to understand the mechanisms that are relevant to pH neutralization and AMD treatment. Future research should include the study of these microorganisms and the impact of pH neutralization in the recovery and removal of dissolved heavy metals.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Current Cycles of ~50 h of Duration in SC-MFC Operated under Initial Acidic Conditions (pH = 3.7)

![Figure A1](image)

Figure A1. Current generation during batch operation. Typical shape is observed for the current behavior, which reaches a peak and then descend gradually over every cycle. Culture medium replenishments (1.3 g.L⁻¹ Pyruvate, pH = 3.7 ± 0.1) are shown by the arrows. The data set shows four current cycles representative of the entire operation period.
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