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Electricity generation from tetrathionate in microbial fuel cells by acidophiles

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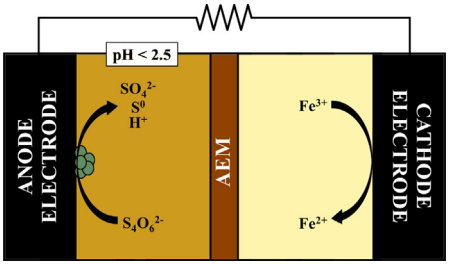
HIGHLIGHTS

- Electricity can be generated from tetrathionate in MFCs at pH below 2.5.
- Tetrathionate disproportionated to sulfate and elemental sulfur.
- Biohydrometallurgical process waters contained electrochemically active bacteria.
- *Acidithiobacillus* spp. and *Ferroplasma* spp. were identified from the MFCs.

GRAPHICAL ABSTRACT



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ABSTRACT

Inorganic sulfur compounds, such as tetrathionate, are often present in mining process and waste waters. The biodegradation of tetrathionate was studied under acidic conditions in aerobic batch cultivations and in anaerobic anodes of two-chamber flow-through microbial fuel cells (MFCs). All four cultures originating from biohydrometallurgical process waters from multimetal ore heap bioleaching oxidized tetrathionate aerobically at pH below 3 with sulfate as the main soluble metabolite. In addition, all cultures generated electricity from tetrathionate in MFCs at pH below 2.5 with ferric iron as the terminal cathodic electron acceptor. The maximum current and power densities during MFC operation and in the performance analysis were 79.6 mA m^{-2} and 13.9 mW m^{-2} and 433 mA m^{-2} and 17.6 mW m^{-2}, respectively. However, the low coulombic efficiency (below 5%) indicates that most of the electrons were directed to other processes, such as aerobic oxidation of tetrathionate and unmeasured intermediates. The microbial community analysis revealed that the dominant species both in the anolyte and on the anode electrode surface of the MFCs were *Acidithiobacillus* spp. and *Ferroplasma* spp. This study provides a proof of concept that tetrathionate serves as electron donor for biological electricity production in the pH range of 1.2–2.5.

1. Introduction

Processing of sulfide minerals often releases metals and reduced inorganic sulfur compounds (RISCs), such as thiosulfate ($S_2O_3^{2-}$) and tetrathionate ($S_4O_6^{2-}$), to the mining process and waste waters [1]. The sulfur compound containing water streams require treatment, because the naturally occurring biocatalyzed oxidation of RISCs releases protons and may cause acidification of aquatic environments [2]. The increased acidity further enhances the dissolution of heavy metals from minerals increasing also the metal concentrations in such environments [3,4]. Several organisms require metals as trace elements, whilst high metal concentrations are often toxic [5]. Therefore, the untreated acidic metal-rich water may seriously harm the environment.

The biochemistry of the RISC oxidation process is often complex, and the high reciprocal reactivity of the intermediary sulfur

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compounds impede their analysis [6]. Several acidophilic microorganisms – such as *Acidiphilium acidophilum* and *Acidithiobacillus thiooxidans* – degrade tetrathionate anaerobically via disproportionation (Eq. (1)) [7,8]. The intermediary sulfur compounds formed in the reaction, such as thiosulfate and elemental sulfur, can be further oxidized to sulfate [8–10].

$$S_4O_6^{2-} + H_2O \rightarrow S^0 + S_2O_3^{2-} + SO_4^{2-} + 2H^+ \quad (1)$$

Electricity production in microbial fuel cells (MFCs) is based on the bioelectrochemical conversion of the chemically bound energy into electrical energy. Most MFC studies report operation at near-to-neutral conditions with organic substrates such as acetate and glucose [11]. Only Borole et al. [12] have reported electricity generation at pH below 4. They used an acidophilic bacterium, *Acidiphilium cryptum*, as biocatalyst and reached the maximum power density of 12.7 mW m^{-2} with glucose as the substrate and nitrilotriacetic acid and phenosafranin as mediators [12]. Only few studies have examined sulfur compounds as substrates in MFCs. Rabaey et al. [13] oxidized dissolved sulfide to sulfur at pH 6–7.5 and reached the maximum power output of 101 W m^{-3}. Zhao et al. [14] used sulfate reducing bacteria to reduce sulfite and thiosulfate to sulfide, which was then separately oxidized in a MFC at pH 7.5 with the maximum power density of 0.19 W m^{-2}. Recently, Zhang et al. [15] reported electricity production from elemental sulfur with *Desulfuromonas* strain TZ1.

In addition to electricity production, MFC technology can also be used for the treatment of wastewaters [16]. The oxidation of organic compounds lowers the chemical oxygen demand (COD) of the waste streams [17]. Moreover, ter Heijne et al. [18] reported that MFCs can also be used for the recovery of metals from water streams. They showed that copper ions can serve as the electron acceptors at the cathode, leading to electrodeposition of solid elemental copper on the cathode electrode surface. Several other metals, including lead and zinc, have been subsequently deposited [18–21].

The process and waste waters of sulfide ore processing facilities usually contain reduced inorganic sulfur compounds but no organic matter. The use of RISCs as substrates in MFCs would enable treatment of these waste streams with simultaneous utilization of the energy bound in RISCs for electricity production. By combining the RISC degrading anode with Cu^{2+} reducing cathode, copper could be recovered from mining waters with an electron donor present at the same site. Moreover, by using acidic streams as the substrate source, the pH gradient observed with organic copper reducing MFCs could be eliminated [18]. However, tetrathionate degradation in MFCs has not been reported earlier.

The objective of this study was to examine tetrathionate as an anodic electron donor in MFCs in highly acidic conditions (pH < 3) typical for mining environments. First, tetrathionate oxidation by microorganisms present in biohydrometallurgical process waters of multimetal ore heap bioleaching was screened in aerobic shake flask cultivations. The possibility to utilize these cultures for tetrathionate oxidation in anaerobic anodes was then studied in simple two-chamber flow-through MFCs. Different catholytes (dissolved oxygen in phosphate buffer, Cu^{2+} ions, and Fe^{3+} ions) were tested, and degradation of tetrathionate and formation of sulfur-containing degradation products were monitored. Finally, the microbial communities from the original cultures and from the anolyte solutions and anode biofilms were analyzed. This is the first study to demonstrate electricity production from tetrathionate in MFCs at anodic pH below 2, which would enable the utilization and treatment of acidic mining process and waste waters without costly pH adjustment.

2. Materials and methods

2.1. Microbial cultures

All four studied microbial cultures (En1–En4) originated from hydrometallurgical mining process waters (pH 2.7–3.6), which had been previously shown to contain several acidophilic sulfur- and iron-oxidizing microorganisms [22–24]. En1 had been aerobically enriched on sulfur (10 g L^{-1}) at 26 °C and pH 2 for a year, while the rest of the cultures were fresh samples of mining process waters.

2.2. Aerobic batch bottle experiments

Two parallel aerobic batch bottle cultivations were inoculated with 5% (v/v) of the enrichment culture pre-grown on sulfur (En1) or 10% (v/v) of the fresh process water sample (En2–En4). The growth medium contained 10% (v/v) of mineral salts medium solution (MSM; 1 L contained 3 g $(NH_4)SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, and 0.013 g $Ca(NO_3)_2{\cdot}4H_2O$) and 1% (v/v) of trace element solution (TES; 1 L contained 11 mg $FeCl_3{\cdot}6H_2O$, 0.5 mg $CuSO_4{\cdot}5H_2O$, 2 mg H_3BO_3, 2 mg $MnSO_4{\cdot}H_2O$, 0.8 mg $Na_2MoO_4{\cdot}2H_2O$, 0.6 mg $CoCl{\cdot}6H_2O$, 0.9 mg $ZnSO_4{\cdot}7H_2O$, and 0.1 mg Na_2SeO_4). The cultures were grown in 250 mL Erlenmeyer bottles with the working volume of 100 mL either at 20 ± 2 °C or at 26 ± 2 °C with mixing of 150–155 rpm. The initial tetrathionate ($S_4O_6^{2-}$) concentration was 7.4– 12.5g L^{-1} and pH 2.5–3.1. Culture En1 was enriched for tetrathionate utilization (nine steps) by transferring an aliquot of culture (10% (v/v)) to fresh medium after all of the tetrathionate was degraded. With En1, the original substrate (5 g L^{-1} sulfur) was added during the first two enrichment steps to alleviate culture adaptation. Cultures En2–En4 were grown aerobically on tetrathionate once. Samples were taken from the aerobic cultivations at 1–3 days intervals and filtered (0.2 μm) before the analysis of pH, tetrathionate, and sulfate.

2.3. MFC construction and operation

Two-chamber flow-through MFCs previously described by ter Heijne et al. [25] with 33 cm^3 anode and cathode chambers separated by an anion exchange membrane (AMI-7001, Membrane International, USA) were used in the experiments. Graphite plate (MR Graphite, Germany) and graphite plate covered with a carbon paper (Coidan graphite products, USA) were used as the cathode and anode electrode, respectively. The effective area of both electrodes was 22 cm^2. The anolyte and catholyte solutions (total volume of each 0.625 L) were continuously recirculated over a recirculation bottle at a rate of 166–170 mL min^{-1} [18]. Anode and cathode potentials were measured against Ag/AgCl reference electrodes (Sentek, UK; estimated standard potential 205 mV vs. normal hydrogen electrode (NHE)) placed in 3 M KCl and connected to the anolyte/catholyte with a glass capillary (QiS, the Netherlands).

The anolyte consisted of 10% (v/v) MSM, 1% (v/v) TES, and phosphate buffer (20 mM K_2HPO_4). To remove oxygen, anolyte and catholyte solutions were purged with nitrogen (15 min) prior to the inoculation. After inoculation, 1% (v/v) of 1 M sodium bicarbonate ($NaHCO_3$) was added to the anolyte. In acidic conditions, sodium bicarbonate reacts forming carbon dioxide (CO_2), which most chemolithotrophic microorganisms can utilize as a carbon source [2]. The En1 MFC was inoculated with 10% (v/v) of aerobic enrichment culture (En1). With the other cultures, the MFCs were inoculated with the original process water samples (En2–En4). Culture En3 was also grown in two parallel MFCs (En3_2A and En3_2B), in which 5% (v/v) of original process water sample was added twice during the operation after removing an equal amount of the anolyte to increase the microbial cell densities at the anode. Moreover, two control MFCs – one with tetrathionate but without inoculum and