

# Reduced Inorganic Sulfur Compounds of Simulated Mining Waters Support Bioelectrochemical and Electrochemical Current Generation

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**Abstract** Tetrathionate ( $S_4O_6^{2-}$ ) was removed from simulated acidic mining water ( $pH < 2.5$ ) while simultaneously generating electrical current in (bio)electrochemical systems. The current density in bioelectrochemical system was improved by optimizing the external resistance and the long-term stability was monitored for over 700 days. The electricity production efficiency improved over time and microbial cultures were dominated by *Acidithiobacillus* sp. and *Ferroplasma* sp. With bioelectrochemical systems, current was generated at lower anode potentials ( $\geq 0.3$  V vs. Ag/AgCl) and tetrathionate was degraded at higher rate ( $\geq 110$  mg  $L^{-1}$  d $^{-1}$ ) than in the electrochemical system ( $\geq 0.5$  V vs. Ag/AgCl and  $\leq 35$  mg  $L^{-1}$  d $^{-1}$ ).

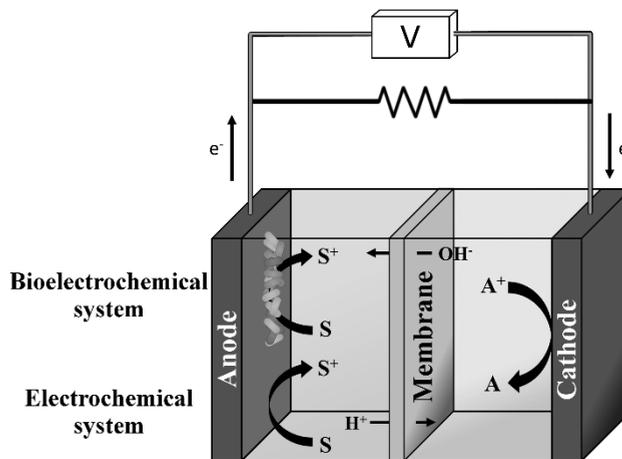
**Keywords** reduced inorganic sulphur compounds, tetrathionate, bioelectrochemical, electrochemical

## Introduction

The processing of sulphide minerals often releases metals and reduced inorganic sulphur compounds, such as thiosulfate ( $S_2O_3^{2-}$ ) and tetrathionate ( $S_4O_6^{2-}$ ), to mining water streams. In the environment, elevated metal concentrations can limit the growth of several organisms (Nies 1999). Sulphur-oxidizing microorganisms degrade RISCs producing sulphuric acid in the reaction (Johnson and Hallberg 2008). This causes acidification of the environment and promotes the formation of acidic metal rich water known as acid mine drainage (AMD). Therefore, both RISCs and metals should be removed from mining water streams before their release to the environment.

In electrochemical systems, electrical current is produced via oxidation and reduction reactions. A substrate is oxidized on an anode electrode and the electrons released in the oxidation reaction flow through an electrical circuit to a cathode electrode, where an electron acceptor accepts the electrons and becomes reduced (Figure 1). The reduction potentials of the anodic electron donor and the cathodic electron acceptor define whether the system is spontaneously producing electrical energy or whether external energy is required to run the oxidation and reduction reactions. Electrochemical systems can be used, for example, to recover metals from water streams. Certain metal ions can be reduced on the cathode electrode and the metals will electrodeposit on the surface of the electrode, from where they can be recovered in pure elemental form (Modin et al. 2012, ter Heijne et al. 2010). The current required to run the electrochemical metal reduction is usually drawn from oxidation of water. The theoretical reduction potentials of metals are usually lower than the reduction potential of water (Table 1) and, therefore, external energy is required for combining water oxidation to metal reduction.

Certain microorganisms can donate electrons to and/or accept electrons from a solid electron acceptor, such as an electrode (Kumar et al. 2015). Therefore, microorganisms can catalyse the oxidation and/or reduction reactions in the electrochemical systems. Electrochemical systems with a microbial catalyst are called bioelectrochemical systems (BESs). BESs producing electrical current are microbial fuel cells (MFCs) and BESs with applied external energy are microbial electrolysis cells (MECs) (Rozendal et al. 2008). With BESs, several biodegradable organic compounds, which have low theoretical reduction potentials (e.g. acetate, glucose), can be utilized as the electron donors for electricity production. Therefore, no or less external energy is required for metal recovery in BESs than in water oxidizing electrochemical systems (Modin et al. 2012, ter Heijne et al. 2010). Besides pure organic compounds, also wastewaters, which are rich in organic compounds, can be used the substrate source (Pandey et al. 2016).



**Figure 1:** Schematic illustration of (bio)electrochemical system. The substrate (S) is oxidized on the anode electrode electrochemically or with the assist of electroactive microorganisms. The electrons flow through an electrical circuit to a cathode electrode, where an electron acceptor (A<sup>+</sup>) is reduced. Ions (e.g. H<sup>+</sup>, OH<sup>-</sup>) transfer through the membrane separating the anode and cathode chambers to maintain the charge balance. If the oxidation and reduction reactions occur spontaneously, the system is producing electrical energy. Alternatively, external energy (V) can be applied to realise the oxidation and reduction reactions

Mining waters usually contain no organic compounds. External organic substrate source would thus be required for the organic bioelectrochemical recovery of metals from mining waters. However, processing of sulphide minerals often releases RISCs to mining waters. If RISCs could be used as the substrate for bioelectrochemical and/or electrochemical recovery of metals, both substrate and electron donor could be found from the same streams. In addition, with the (bio) electrochemical treatment both metals and RISCs could be simultaneously removed from the water streams. Moreover, sulphur-oxidizing microorganisms are often acidophilic, and thus both anode and cathode can be operated in acidic conditions, which lowers the pH gradient over the membrane and thus also the energy requirements of the process. The use of RISCs as the substrate for bioelectrochemical systems, however, has not been previously studied.

**Table 1:** Theoretical reduction potentials in standard conditions (25 °C, pH 7, concentration 1 M) for selected reactions.

Reaction	E° (V vs. NHE)	E° (V vs. Ag/AgCl)
$O_2 + 4 H^+ + 4 e^- \leftrightarrow 2 H_2O$	+1.23	+1.03
$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	+0.77	+0.57
$Cu^{2+} + 2 e^- \leftrightarrow Cu$	+0.34	+0.14
$S_4O_6^{2-} + 10 H_2O \rightarrow 4 SO_4^{2-} + 20 H^+ + 14 e^-$	+0.31	+0.11
$S_4O_6^{2-} + 6 H_2O \rightarrow S^0 + 3 SO_4^{2-} + 12 H^+ + 8 e^-$	+0.27	+0.07
$Ni^{2+} + 2 e^- \leftrightarrow Ni$	-0.25	-0.45
$CH_3COO^- + 2 H_2O \rightarrow 2 CO_2 + 7 H^+ + 8 e^-$	-0.29	-0.49
$C_6H_{12}O_6 + 6 H_2O \rightarrow 6 CO_2 + 12 H^+ + 24 e^-$	-0.43	-0.63
$Zn^{2+} + 2 e^- \leftrightarrow Zn$	-0.76	-0.96

This paper provides compilation of research done by our research group and demonstrates the use of tetrathionate as the anodic electron donor for bioelectrochemical and electrochemical current generation. Bioelectrochemical tetrathionate degradation was first studied in MFCs (Sulonen et al. 2015, Sulonen et al. 2016). The current generation was enhanced by optimizing the external resistance and the long-term stability of a MFC was monitored for over two years (Sulonen et al. 2016). The effect of anode potential on tetrathionate degradation and current generation was studied in bioelectrochemical and electrochemical systems (Sulonen et al. 2017).

## Methods

### Performed experiments and the cell configuration

The electricity production was first studied in MFCs (MFC A and MFC B). The long-term stability was monitored in MFC LT. The effect of anode potential on tetrathionate degradation and current generation was studied in bioelectrochemical system (MEC) and electrochemical systems (EC). The experiments were conducted using two-chamber flow-through systems, which have been previously described by (ter Heijne et al. 2008). Graphite electrodes covered with carbon paper were used as both anode and the cathode. The effective surface area of the electrodes was 22 cm<sup>2</sup>. The anode and cathode chambers were separated with an anion exchange membrane (MFC A, MFC B, MEC and EC) or a monovalent cation exchange membrane (MFC LT). The volume of both chambers was 33 mL.

### Solutions and inoculum

The anolyte solution of the BESs consisted of phosphate buffered (20 mM K<sub>2</sub>HPO<sub>4</sub>) mineral salts medium (MSM, 10%(v/v)) with trace elements solution (TES, 1%(v/v)). In abiotic electrochemical systems, phosphate buffered (20 mM K<sub>2</sub>HPO<sub>4</sub>) MQ-water was used as the anolyte. The initial tetrathionate concentration was 2 g L<sup>-1</sup> and tetrathionate was added to the fed-batch systems from a stock solution (125 g L<sup>-1</sup> S<sub>4</sub>O<sub>6</sub><sup>2-</sup>) after the concentration decreased

below  $0.5 \text{ g L}^{-1}$ . Ferric iron ( $2 \text{ g L}^{-1} \text{ Fe}^{3+}$ , added as  $\text{FeCl}_3$ ) was used as the electron acceptor at the cathode. The anolyte and catholyte solutions were constantly recirculated ( $166 - 170 \text{ mL min}^{-1}$ ) over a recirculation bottle, the total volume of anolyte and catholyte being  $0.625 \text{ L}$ . MFCs A and B were inoculated with biohydrometallurgical process waters from a multimetal ore heap bioleaching operation. MFC LT was inoculated with a sample taken from the anolyte of MFC B and MEC was inoculated with a sample taken from the anolyte of MFC LT. After inoculation, sodium bicarbonate ( $1 \text{ M NaHCO}_3$ ,  $1\% \text{ (v/v)}$ ) was added to the anolyte of the BESs to provide a carbon source for the microorganisms. All the cells were operated in room temperature ( $22 \pm 5 \text{ }^\circ\text{C}$ ).

### Chemical and electrochemical analyses

Samples were taken from the anolyte and the catholyte every one to seven days. The tetrathionate concentrations were analysed with modified cyanolysis (Kelly et al. 1969, Sulonen et al. 2015). The sulfate and thiosulfate concentrations were analysed using ion chromatography. Ferrous iron was measured using a spectrophotometric method (1,10-phenantroline method) and total iron with an atomic absorption spectrophotometer (AAS). The analyses were performed as previously described (Sulonen et al. 2015, Sulonen et al. 2016).

Cell voltage, anode potential and cathode potential were constantly monitored with a data logger (Agilent 34970A Data Acquisition/Switch Unit, Agilent Technologies, USA). The anode and cathode potentials were measured against  $\text{Ag/AgCl}$  –reference electrodes (Sentek, UK). In MEC and EC, the anode potential was controlled with  $\mu\text{Stat 8000P}$  Multi Potentiostat (DropSens, Spain). Performance analysis was conducted by decreasing the external resistance gradually from  $5000 \text{ } \Omega$  to  $10 \text{ } \Omega$  every 30 min (MFC A, MFC B, MFC LT until day 284) or by linear sweep voltammetry (LSV) with scanning rate of  $1 \text{ mV s}^{-1}$  (MFC LT, days 665 and 711).

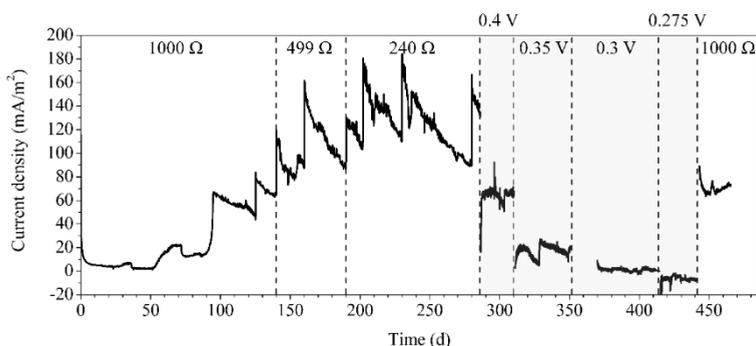
## Results and discussion

### Electricity production in microbial fuel cells and long-term stability

In MFCs inoculated with the mining process waters, the electricity generation remained low for 25 to 50 days, but then the voltage increased to a maximum of  $0.175 \text{ V}$  (MFC A), which was obtained after 100 days of operation (Figure 2) (Sulonen et al. 2015). The maximum current and power densities during the performance analysis in MFC B were  $433 \text{ mA m}^{-2}$  and  $17.6 \text{ mW m}^{-2}$  (Sulonen et al. 2015).

The current generation was successfully improved by optimizing the external resistance. The MFC was let to stabilize at each resistance for at least 60 days before decreasing the resistance. The current density obtained in the performance analysis increased up to  $1120 \text{ mA m}^{-2}$  and power density up to  $44.4 \text{ mW m}^{-2}$  in MFC LT (Table 2) (Sulonen et al. 2016). After optimizing the external resistance, MFC LT was operated with a resistor of  $100 \text{ } \Omega$  up to a total run time of 740 days. Current was constantly generated, the average current density being  $150 \text{ mA m}^{-2}$  (Sulonen et al. 2016). In long-term operated MFCs fed with organic substrates, formation of biofilm on the membrane or on to the surface of the cathode

electrode has been observed to limit the electricity production (Xu et al. 2012, Zhang et al. 2011). In MFC LT, no performance limiting biofilm formation was observed even after 2 years of operation. With inorganic substrates, the chemolithotrophic microorganisms consume significant amount of the energy they obtain from the degradation of the substrate to synthesisation of cellular components and thus less energy is available for growth (McCollom and Amend 2005). In addition, no reaction products ( $H^+$ ,  $SO_4^{2-}$ ,  $S^0$ ) were limiting the bioelectrochemical electricity production.



**Figure 2:** The current density in MFC A with varying external resistances and applied anode potentials (grey area). The applied anode potential values are presented as V vs. Ag/AgCl (Sulonen et al. 2017, Sulonen et al. 2016). The peak current densities were obtained after replacement of the cathodic ferric iron solution.

**Table 2:** The electricity production in the performance analysis of the long-term operated MFC LT (Sulonen et al. 2016).

Day	$R_{Ext}^a$ Ω	OCV <sup>b</sup> V	CD <sub>Max</sub> <sup>c</sup> mA/m <sup>2</sup>	PD <sub>Max</sub> <sup>d</sup> mW/m <sup>2</sup>	$R_{Int}^e$ Ω
74	1000	0.122	145	6.5	420
134	499	0.137	220	10.2	280
284	240	0.132	315	14.1	190
665	100	0.142	735	29.4	90
711	100	0.145	1120	44.4	50

<sup>a</sup> external resistance before performance analysis, <sup>b</sup> open circuit voltage, <sup>c</sup> maximum current density, <sup>d</sup> maximum power density, <sup>e</sup> internal resistance calculated from the slope of the linear region of the voltage-current curve

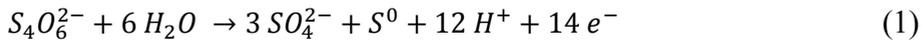
### The effect of anode potential

In MFCs, the minimum anode potential reached was 0.363 V vs. Ag/AgCl (Sulonen et al. 2015). To determine, if current generation could be obtained with lower anode potential in bioelectrochemical or electrochemical systems, the anode potential was gradually decreased

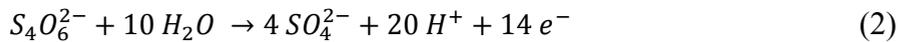
from 0.4 V vs. Ag/AgCl to 0.275 V vs. Ag/AgCl (Sulonen et al. 2017). Electricity was generated in MEC with anode potential of 0.3 V vs. Ag/AgCl and above. In abiotic electrochemical systems, anode potential of 0.5 V vs. Ag/AgCl or above was required for current generation. Both tetrathionate-fed systems produced higher current density than the water oxidizing control reactor.

### Tetrathionate degradation

In bioelectrochemical systems, the reaction products of tetrathionate degradation were sulphate and elemental sulphur. The tetrathionate was, therefore, presumed to be degraded via disproportionation following Equation 1.



In electrochemical systems, the only observed reaction product was sulphate, suggesting that tetrathionate was degraded following Equation 2.



Higher tetrathionate degradation rates were obtained in the bioelectrochemical system (110 – 240 mg L<sup>-1</sup> d<sup>-1</sup>) than in the abiotic electrochemical system (15 – 35 mg L<sup>-1</sup> d<sup>-1</sup>). The coulombic efficiency – calculated as the relation of the electric charge generated in coulombs to the electric charge theoretically releasable from the degraded tetrathionate – remained higher in the electrochemical system (≤48%) than in the bioelectrochemical system (≤10%). The chemical energy was thus converted to electrical energy more efficiently in the electrochemical system than in the bioelectrochemical systems.

### Microbial communities

Biometallurgical mining waters often contain several sulphur- and iron-oxidizing microorganisms (Halinen et al. 2012, Sulonen et al. 2015). With mining process waters as the inoculum, the current generation improved after 100 days of operation. When the anolyte of a previously operated MFC was used as the inoculum, the electricity production started after one to 20 days of operation. The mining waters thus contained tetrathionate degrading microorganisms capable of electricity production. After enrichment, the microorganisms were able to start the electricity production quite rapidly.

The microbial communities were analysed from samples taken from the anolyte solution and from the surface of the anode electrode. The dominant genera in the anodic microbial communities inoculated with mining process waters were *Acidithiobacillus* sp. and *Ferroplasma* sp (Sulonen et al. 2015). In the long-term operated MFC LT, which was inoculated with the anolyte of MFC B, *Acidithiobacillus* was observed to dominate the microbial culture throughout the experiment (Sulonen et al. 2016). The low microbial variation pre-

sumably results from the extreme operation conditions; only sulphur-oxidizing mesophilic chemolithotrophs can grow in the used operational conditions ( $22\pm 5$  °C,  $\text{pH} \leq 2.5$ , inorganic substrate).

Both *Acidithiobacillus* and *Ferroplasma* sp. are often found from mining environments. *Acidithiobacillus* sp. are acidophilic bacteria, some species of which are known to degrade sulphur compounds (Bobadilla Fazzini et al. 2013, Mangold et al. 2011, Osorio et al. 2013). *Ferroplasma* sp. are acidophilic archaea and the species have not been reported to degrade sulphur compounds (Dopson et al. 2004, Golyshina et al. 2000). *Ferroplasma* sp. was thus presumably growing by utilizing the excreted products of other microorganisms as the source of energy.

### Conclusions

With (bio)electrochemical systems, the chemical energy stored in acidic mining waters can be converted to electrical energy while simultaneously removing reduced inorganic sulphur compounds from the mining waters. With bioelectrochemical systems, tetrathionate is degraded and electrical current is generated spontaneously with ferric iron as the cathodic electron acceptor. Biofouling or reaction product inhibition were not observed to limit the electricity production even after two years of operation. With electrochemical systems, an external power source is required and the tetrathionate was degraded with lower rate than in the bioelectrochemical systems, but the chemical energy was converted to electrical energy more efficiently. Tetrathionate is thus a promising substrate for both bioelectrochemical and electrochemical metal recovery.

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