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Abstract

The effect of poised anode potential on electricity production and tetrathionate degradation was studied in two-chamber flow-through electrochemical (ES) and bioelectrochemical systems (BES). The minimum anode potential (vs. Ag/AgCl) for positive current generation was 0.3 V in BES and 0.5 V in the abiotic ES. The anode potential required to obtain average current density above 70 mA m^{-2} was 0.4 V in BES and above 0.7 V in ES. ES provided higher coulombic efficiency, but the average tetrathionate degradation rate remained significantly higher in BES (above 110 mg L^{-1} d^{-1}) than in the abiotic ES (below 35 mg L^{-1} d^{-1}). This study shows that at anode potentials below 0.7 V, the electrochemical tetrathionate degradation is only efficient with microbial catalyst and that significantly higher tetrathionate degradation rates can be obtained with bioelectrochemical systems than with electrochemical systems at the tested anode potentials.

Keywords Bioelectrochemical cell; electrochemical cell; tetrathionate; anode potential; current generation

1. Introduction

Uncontrolled biological degradation of reduced inorganic sulfur compounds (RISCs) often found in mining and mineral processing process and waste waters contribute to formation of acidic metal-rich waters in the environment. Bioelectrochemical treatment enables the integration of controlled removal of RICSs to production of electrical energy. Bioelectricity production via RISC degradation in acidic conditions was first demonstrated by Sulonen et al. (2015). Recently, Ni et al. (2016) showed that also sulfide mineral flotation process water can be used as the substrate for bioelectricity production and Sulonen et al. (2016) reported stable electricity production from tetrathionate ($S_4O_6^{2-}$) for over 2 years with average current density of 150 mA m^{-2}.

In microbial fuel cells (MFCs), the cell voltage is a result of the potential difference of the anode and cathode electrodes. The minimum anode potential is determined by the reduction potential of the final electron donor, which can be, for example, a cytochrome on the microbial cell surface (direct electron transfer) or an electrochemically oxidizable mediator compound (mediated electron transfer). The energy gained for microbial growth is defined by the difference in the reduction potential of the electron donor and the potential of the anode electrode. Therefore, high anode potential leads to high microbial energy gain, but to low cell voltage. To optimize the electrical energy output, the potential of the anode electrode should remain close to the minimum value still supporting the growth and current generation of the electroactive microorganisms.

Besides electricity production, electrochemical systems can be used to run the desired oxidation and reduction reactions, for example for remediation or synthesis purposes (Li et al., 2015; Li et al., 2016; Qin et al., 2012; Villano et al., 2011). By applying external voltage, thermodynamically unfavorable reactions can be realized, i.e. substrate with high reduction potential can be oxidized at the anode and/or electron acceptor at low reduction potential can be reduced at the cathode. Microbial catalyst on the anode side enables the utilization of biodegradable compounds as a partial source of energy, thus decreasing the total external energy need. Applied external voltage has been used, for example, to recover metals (Colantonio and Kim, 2016) and to synthesize commercially valuable organic compounds such as acetate (Batlle-Vilanova et al., 2016) and butyrate (Ganigué et al., 2015) at the cathode of bioelectrochemical systems.

Anode potential of bioelectrochemical systems can be externally controlled to selectively enrich for electroactive microorganisms (Finkelstein et al., 2006; Kokko et al., 2015) or to enhance the current generation efficiency (Sleutels et al., 2011; Wang et al., 2009), but reports on the optimal potential for enrichment contradict. Some studies report faster start-up and higher current, coulombic efficiency (CE) and chemical oxygen demand (COD) removal rate with more positive anode potentials (Ishii et al., 2008; Wang et al., 2009), while in other studies higher power densities and biofilm densities were obtained with lower anode potentials (Aelterman et al., 2006; Sun et al., 2012; Torres et al., 2009; Zhang et al., 2013). The optimum anode potential for enrichment of anodic cultures and electricity production depends on the used substrate, the composition of the used inoculum, operation

conditions and the electrochemical cell configuration (Kumar et al., 2012; Wagner et al., 2010).

Tetrathionate has been shown to be bioelectrochemically degradable (Ni et al., 2016; Sulonen et al., 2015; Sulonen et al., 2016), but the effect of anode potential on current generation in tetrathionate-degrading bioelectrochemical systems has not been previously studied. In addition, no studies have addressed the abiotic electrochemical degradation of tetrathionate. The objective of this study was to examine the effect of anode potential on current generation in tetrathionate-fed bioelectrochemical and electrochemical systems under highly-acidic conditions (pH<2.5) typical for mining environments. The adaptation of the acidophilic culture originating from multimetal mining process waters to lower anode potentials than previously reported with tetrathionate-fed MFCs was studied by poising the anode electrode. With the abiotic systems, the minimum anode potential required for electrochemical tetrathionate degradation was determined by gradually adjusting the anode potential. The current generation and tetrathionate degradation rates were also studied in electrochemical systems at selected constant anode potentials.

2. Materials and methods

2.1. Electrochemical cell configuration

The electrochemical system used for abiotic and biological experiments has been previously described (ter Heijne et al., 2008). The anode and cathode chambers (33 cm^3 each) were separated with an anion exchange membrane (AMI-7001, Membrane International, USA). The anolyte and catholyte solutions were recirculated (0.166 L min^{-1}) over a recirculation

bottle, the total volume of each solution being 0.625 L. Graphite plates (MR Graphite, Germany) covered with carbon paper (Graphite foil, Coidan graphite products, USA) were used as the anode and cathode electrodes. The effective area of all electrodes was 22 cm^2. Anode and cathode potentials were measured against Ag/AgCl reference electrodes (Sentek, UK; estimated standard potential 0.205 V vs. Normal Hydrogen Electrode (NHE)) placed in 3 M KCl and connected to the anolyte or catholyte with a glass capillary (QiS, the Netherlands). The potential values reported are against Ag/AgCl -reference electrode, if not otherwise stated.

2.2. Bioelectrochemical system

The solution compositions, the start-up and the operational conditions of the bioelectrochemical system (BES) have been previously reported (MFC A) (Sulonen et al., 2016). BES was inoculated with hydrometallurgical mining process water. With this inoculum, the microbial culture in the anolyte of tetrathionate-fed MFCs has been dominated by *Acidithiobacillus* sp. and *Ferroplasma* sp. (Sulonen et al., 2015). The initial tetrathionate concentration in the anolyte was 2 g L^{-1} and pH 2.5. The BES was operated at room temperature (20 °C ± 2 °C) in a fed-batch mode. The system was fed by adding medium solution containing 125 g L^{-1} $S_4O_6^{2-}$ (final concentration 2 - 2.5 g L^{-1} $S_4O_6^{2-}$) after the tetrathionate concentration of the anolyte decreased below 0.5 g L^{-1}. Ferric iron (2 g L^{-1} Fe^{3+}, added as $FeCl_3$) was used as the terminal electron acceptor at the cathode and the solution pH was adjusted to 1.5 with HCl. The catholyte solution was replaced after the ferrous iron (Fe^{2+}) concentration of the solution increased above 1 g L^{-1} and every time

before changing the applied anode potential. Both anolyte and catholyte were purged with nitrogen for 15 min prior the experiment to remove oxygen from the solution.

The external resistance of BES was first gradually decreased from 1000 Ω to 240 Ω (days 0 - 286) to enhance the current generation and to study the effect of external resistance on anode potential. The effects of the decreasing of the external resistance on current density and tetrathionate degradation rates have been previously reported (Sulonen et al., 2016). After 286 days of operation, the anode potential of the bioelectrochemical cell was gradually lowered from 0.4 V to 0.275 V with a potentiostat to study the effect of lower anode potentials on the current generation and tetrathionate degradation efficiency. The operation time at each anode potential varied from 20 to 60 days, the operation time increasing with decreasing anode potential.

The rate of non-electrochemical degradation of tetrathionate was studied in a control reactor operated in open circuit. The cell was started up as an MFC connected to a resistor of 1000 Ω and inoculated with the anolyte of an MFC that had been operated on tetrathionate for 8 months. After 12 days, the reactor was disconnected and the tetrathionate degradation was followed in open circuit for 14 days.

2.3. Abiotic electrochemical system

The abiotic electrochemical systems (ES) contained tetrathionate (initial concentration 2 g L^{-1} $S_4O_6^{2-}$) in acidic phosphate buffered MilliQ (20 mM K_2HPO_4, pH 2.5) as the anolyte and ferric iron (2 g L^{-1} Fe^{3+}) as the catholyte. Oxygen was removed from both solutions by

purging with nitrogen for 15 min. The operational conditions were otherwise the same as for the bioelectrochemical systems. To study the effect of anode potential on the abiotic electrochemical degradation of tetrathionate, the anode potential was first gradually increased from 0 V to 1.2 V. The cell was let to stabilize at each potential for 2 hours before reading the current. The current density values are presented as the average of two independent measurements conducted in separate reactors. The rate of electrochemical tetrathionate degradation and the formation of reaction products was additionally studied with abiotic cells operated with constant anode potentials of 0.6 V, 0.8 V and 0.9 V.

The rate of electrochemical tetrathionate degradation without anode potential control was studied with an abiotic electrochemical fuel cell. The cell was connected to a resistor of 1000 Ω and the tetrathionate degradation and current generation were followed for 12 days. The non-electrochemical degradation of tetrathionate was studied with a reactor operated in open circuit for 14 days. The operation conditions for both reactors were otherwise the same as for ESs.

An electrochemical control reactor without tetrathionate was also included in the study to determine the rate of current generation via water oxidation at different anode potentials. The anode potential of the control reactor was increased in similar manner as in ES and it was operated with only phosphate buffer (20 mM K_2HPO_4) as the anolyte and ferric iron (2 g L^{-1} Fe^{3+}) as the catholyte.

2.4. Electrochemical measurements

The anode potentials were applied with μStat 8000P Multi Potentiostat (DropSens, Spain) using anode electrode as the working electrode, Ag/AgCl –electrode connected to the anolyte with a glass caplillary as the reference electrode and cathode electrode as the counter electrode. The current was measured every 2 minutes and the data is presented as the average of the values obtained in five consecutive measurements. For the gradual increase of the anode potential of ES, the anode potential was controlled with a PalmSens 3 potentiostat/galvanostat (PalmSens BV, the Netherlands). The cell voltage and the potentials of the anode and cathode electrodes against the reference electrodes were measured with Agilent 34970A Data Acquisition/Switch Unit (Agilent, Canada) every two minutes, and the data is presented as the average of the values obtained in five consecutive measurements. The power and current densities were calculated against the effective surface area of the anode electrode (22 cm^2).

2.5. Sampling and chemical analyses

Samples were taken from the anolytes of BES with the intervals of three to seven days. With ESs with constant anode potentials, anolyte samples were taken every 24 to 48 hours. The anodic samples were filtered (0.2 μm) before the analysis of tetrathionate concentration (modified cyanolysis), sulfate and thiosulfate concentration (ion chromatography), ferrous iron concentration (1,10-phenantroline method), total iron concentration (atomic absorption spectroscopy) and pH. Cathodic ferric iron reduction was monitored by measuring the ferrous iron concentration of the catholyte every two to seven days. The analyses were performed as previously described (Sulonen et al., 2015).

3. Results and discussion

3.1. Gradual decrease of the anode potential in BES

In tetrathionate-fed BESs, the anode potential has been reported to remain above 0.36 V when operated with external resistance of 1000 Ω (Ni et al., 2016; Sulonen et al., 2015). Before starting the anode potential control, the external resistance of BES was gradually decreased from 1000 Ω to 240 Ω to enhance the current generation (Sulonen et al., 2016). When the external resistance was decreased, the average anode potential increased, the values being 0.375 V, 0.400 V and 0.427 V with external resistances of 1000 Ω (days 100-140), 499 Ω and 240 Ω, respectively. The average tetrathionate degradation rates were 150 mg L^{-1} d^{-1} (1000 Ω), 180 mg L^{-1} d^{-1} (499 Ω) and 240 mg L^{-1} d^{-1} (240 Ω). Before the anode potential control was started, the average current density with external resistance of 240 Ω was 125 mA m^{-2} (Sulonen et al., 2016).

After gradually decreasing the external resistance, the anode potential of BES was gradually decreased until no positive current was observed to determine the minimum anode potential, where current generation was still possible. With all the tested anode potentials, the current density decreased right after the anode potential was decreased and remained relatively stable. The average current densities with anode potentials of 0.4 V, 0.35 V, and 0.3 V were 70 mA m^{-2}, 25 mA m^{-2} and 3 mA m^{-2}, respectively (Figure 1). With the anode potential of 0.275 V the current density was negative (-6 mA m^{-2}), indicating that only anode potentials above 0.3 V were high enough for bioelectricity generation through tetrathionate degradation with the used microbial culture and electrochemical cell configuration.

The proposed reaction for tetrathionate degradation in bioelectrochemical systems is disproportionation to sulfate and elemental sulfur (Equation 1), as both sulfate and elemental sulfur have been detected as reaction products (Sulonen et al., 2015).

$$S_4O_6^{2-} + 6\,H_2O \rightarrow S^0 + 3\,SO_4^{2-} + 12\,H^+ + 8\,e^- \quad (1)$$

The theoretical reduction potential of this reaction is 0.069 V vs. Ag/AgCl in standard conditions (pH 7, 25 ºC) and -0.15 V vs. Ag/AgCl at pH 2.5 and 20ºC. However, besides direct conversion of tetrathionate to electrical energy, the current generation may occur via intermediate reaction products, such as sulfide (S^{2-}) or thiosulfate ($S_2O_3^{2-}$), that may remain undetected due to their immediate consumption or degradation. The reaction leading to electricity production in tetrathionate degrading bioelectrochemical systems is yet to be confirmed.

Despite the changes in the anode potential and the decreasing current density, the average tetrathionate degradation rates remained between 110 and 180 mg L^{-1} d^{-1} with all the tested anode potentials. The anolytic pH remained at 0.9 - 1.1 and sulfate concentration at 18 - 50 g L^{-1} throughout the experiment (Figure 2). CE was calculated as the relation of the electric charge generated in coulombs to the electric charge theoretically releasable from the degraded tetrathionate (Equation 1). The CE values decreased with the decreasing anode potential, being 7.1%, 5.7% and 0.3% for anode potentials 0.4 V, 0.35 V, and 0.3 V, respectively. The electrons were thus mainly lost to the alternative electron acceptors and unmeasured metabolites or consumed for biomass growth with all the tested anode potentials. In addition, if the electricity production occurred through unidentified intermediate

compounds, such as hydrogen sulfide, the anode potentials might not have been high enough to support the abiotic electrochemical oxidation of these intermediate compounds.

Fe^{3+} used as the electron acceptor at the cathode was observed to leak from the cathode to the anode through the membrane. In BESs with Fe^{3+} as the cathodic electroron acceptor, the anodic ferric iron concentrations have been reported to increase up to 600 mg L^{-1} (Sulonen et al., 2015). Before the anode potential control and with the applied anode potentials of 0.4 V and 0.35 V, the ferric iron concentrations of the anolyte – calculated as the difference in the total iron concentration and the ferrous iron concentration – ranged from 250 mg L^{-1} to 625 mg L^{-1}. With lower anode potentials (0.3 V and 0.275 V), the ferric iron concentration remained below 100 mg L^{-1}. After the anode potential control was released and the cell was connected to a resistor (1000 Ω), the ferric iron concentration of the anolyte again increased, reaching values up to 310 mg L^{-1}. The results indicate that ferric iron was acting as an electron acceptor for tetrathionate degradation. This may have happened also with the high anode potentials (> 0.3 V), but with low anode potentials (≤ 0.3 V) the ferric iron reduction rates were significantly higher. This presumably caused the tetrathionate degradation rates to remain at the same range with all the tested anode potentials. Besides Fe^{3+}, oxygen leaked to the anode through the tubes and connectors (O_2) may have acted as an alternative electron acceptor.

Biotic degradation of tetrathionate in the absence of current flow was tested by disconnecting the electrodes of a bioelectrochemical system started up as an MFC connected to an external resistor of 1000 Ω. During the first feeding cycle (9 days) in open circuit, the average

tetrathionate degradation rate remained at 150 mg L^{-1} d^{-1}, which is only slightly lower than what was obtained in the connected MFC (180 mg L^{-1} d^{-1}). However, after more tetrathionate was added to the system, tetrathionate degradation was slower, the average tetrathionate degradation rate being 100 mg L^{-1} d^{-1}. This is lower than what was obtained with the tested poised anode potentials. The total iron concentration of the anolyte remained below 5 mg L^{-1} throughout the experiment, showing that no significant amounts of iron were leaking from the catholyte to the anolyte during the experiment and providing an electron acceptor. In systems operated for longer time periods, the anodic ferric iron concentrations can increase to significantly higher values, as was observed in the BES (up to 625 mg/L). In open circuit, the tetrathionate degradation rate in the bioelectrochemical cells was thus presumably decreasing due to the shortage of electron acceptors. In similarly started tetrathionate-fed MFCs, the tetrathionate degradation has been reported to continue with high rate (150 mg L^{-1} d^{-1}) for multiple feeding cycles (Sulonen et al., 2016). The results thus indicate that a current flow is required for maintaining the tetrathionate degradation at high rate. However, further studies are required to determine the effect of the anode electrode on the tetrathionate degradation pathway.

After the anode potential control was released and the BES was again connected to a resistor of 1000 Ω, the anode potential increased back to above 0.4 V and the current density and CE increased up to 70 mA m^{-2} and 7.7%, respectively, close to the values obtained before starting the anode potential control. The results indicate that the culture could not reach the same current generation efficiency at lower anode potentials within the given operation time (20 - 60 days), but was able to resume the electricity production when the anode potential again

increased after the external potential control was released and the system was connected to a resistor (1000 Ω).

The direct electron transfer from microorganisms to the electrode has been shown to occur through membrane-bound c-type cytochromes in several electroactive species (Kumar et al., 2016). In tetrathionate-fed MFCs, one of the dominant genus has been observed to be *Acidithiobacillus* sp. (Sulonen et al., 2015), which is also known to possess several c-type cytochromes involved in the anaerobic growth (Ohmura et al., 2002) and even to form conductive nanowires (Li and Li, 2014). The c-type cytochromes of *Acidithiobacillus* sp. have been shown to operate with relatively high midpoint reduction potentials, the values varying from 0.225 V vs. Ag/AgCl (0.43 V vs. NHE) (Giudici-Orticoni et al., 2000) to 0.455 V vs. Ag/AgCl (0.66 V vs. NHE) (Ohmura et al., 2002) in acidic conditions. To obtain microbial growth through electrode respiration, the potential of the anode electrode should remain higher than the reduction potential of the final electron donor in the metabolic pathway, e.g. the c-type cytochrome. Therefore, with anode potentials below the reduction potential of the final cellular electron donor, the microorganisms are incapable of utilizing the anode electrode as the terminal electron acceptor neither directly nor via a mediator. BES used in this study had been operated as an MFC for 286 days before the anode potential adjustment, and, therefore, the enriched microorganisms in the system might have adapted to the high anode potential (above 0.4 V) and thus be unable to utilize the anode electrode as the electron acceptor at lower anode potentials. Starting up the system with fresh diverse microbial culture and anode poised at lower potential could enrich for alternative microorganisms capable of current production also at lower anode potentials.

3.2. Abiotic current generation from tetrathionate

The anode potential of an abiotic electrochemical cell was gradually increased from 0 V to 1.2 V to determine the anode potential required to produce current and to efficiently degrade tetrathionate electrochemically without microorganisms. With anode potentials below 0.5 V, the current density remained below 1 mA m^{-2} in both the tetrathionate degrading ES and water oxidizing control reactor (Figure 3). With anode potentials above 0.7 V, ES provided significantly higher current density than the electrochemical control reactor. Theoretically, water oxidation occurs at 1.02 V vs. Ag/AgCl in standard conditions (pH 7, 25 °C) and 0.88 V vs. Ag/AgCl at the used operational conditions (pH 2.5, 20 °C). Therefore, with high anode potentials the water oxidation may have contributed to the electricity production also in the tetrathionate-containing ES. However, with anode potential of 0.9 V, the current density in ES was 590 mA m^{-2}, while in the control reactor it remained below 50 mA m^{-2}, showing that the generated current mainly resulted from electrochemical tetrathionate degradation. With anode potential of 1.2 V, the current density of ES was above 4500 mA m^{-2} while only 220 mA m^{-2} was obtained in the water oxidizing control.

The step-wise increase of the anode potential can lead to overestimation of the current due to insufficient stabilization time. Therefore, the rate of electrochemical current generation and tetrathionate degradation was also studied at constant anode potentials of 0.6 V, 0.8 V and 0.9 V. The average current densities were 4 mA m^{-2}, 115 mA m^{-2} and 315 mA m^{-2} for anode potentials of 0.6 V, 0.8 V and 0.9 V, respectively (Figure 4).

Tetrathionate was consumed and sulfate was formed with all the tested potentials (Figure 5) and the tetrathionate degradation rate increased when the applied potential was increased, but the values remained relatively low at all potentials. At anode potential of 0.6 V, the tetrathionate degradation rate was 20 mg L^{-1} d^{-1}, at 0.8 V 30 mg L^{-1} d^{-1} and at 0.9 V 35 mg L^{-1} d^{-1}. At all potentials, 89% - 98% of the molar amount of sulfur in degraded tetrathionate was present in the formed sulfate, indicating that tetrathionate was directly degraded to sulfate (Equation 2). No sulfide or thiosulfate were detected.

$$S_4O_6^{2-} + 10\ H_2O \rightarrow 4\ SO_4^{2-} + 20\ H^+ + 14\ e^- \quad (2)$$

Abiotic tetrathionate degradation in the absence of current flow was tested in a control reactor operated in open circuit. The anode potential was decreasing throughout the experiment and reached a value of 0.381 V by day 14. The anodic tetrathionate concentration was slowly decreasing with the average rate of 15 mg L^{-1} d^{-1}. The results indicate that tetrathionate degrades also non-electrochemically. However, in open circuit the molar amount of sulfur in formed sulfate covered only 12% of the sulfur available in the removed tetrathionate and no thiosulfate was detected. Therefore, the decrease in tetrathionate concentration in open circuit presumably mainly resulted from the diffusion of tetrathionate ions through the anion exchange membrane from anolyte to catholyte. This diffusion may have occurred also in the other electrochemical systems, but the measured sulfate concentrations indicate that with poised anode potentials tetrathionate was utilized efficiently for current generation. When current is flowing, the anode electrode becomes positively charged, as electrons are transferred from anode to cathode, and is thus attracting the negative ions from the solution.

This may have lowered the rate of the diffusion of tetrathionate from anolyte to catholyte in ESs.

3.3. Comparison of bioelectrochemical and electrochemical tetrathionate degradation

The results show that with low anode potentials (below 0.5 V) electrochemical tetrathionate degradation requires a microbial catalyst. At anode potential of 0.4 V, bioelectrochemical system provided current density of 70 mA m^{-2}, while in the abiotic ES the current remained negative at this anode potential. To obtain a corresponding current density (70 mA m^{-2}) in the abiotic electrochemical system, anode potential of above 0.75 V was required.

For abiotic ES, the CE values (calculated based on tetrathionate degradation according to Equation 2) were 1%, 20% and 48% at anode potentials of 0.6 V, 0.8 V and 0.9 V, while in bioelectrochemical system the CE remained below 8% with all the tested anode potentials (0.275 - 0.4 V). This shows that in the electrochemical system, the chemical energy of tetrathionate was utilized more efficiently for current generation than in the bioelectrochemical system. However, the tetrathionate degradation rates were significantly higher in the bioelectrochemical reactor (110 - 240 mg L^{-1} d^{-1}) than in the electrochemical reactor (20 - 35 mg L^{-1} d^{-1}). Thus, electrochemical treatment enables more efficient utilization of the chemical energy of tetrathionate, but bioelectrochemical treatment provides faster tetrathionate removal.

To produce electricity in a fuel cell, a higher cathode than anode potential is required. The theoretical reduction potentials of oxygen and ferric iron – two commonly used cathodic

electron acceptors due to their high reduction potential – are 0.898 V and 0.640 V, respectively, at the used operational conditions (pH 2, 20°C). However, the actual cathode potential values usually remain lower than the theoretical potentials due to activation and concentration losses (Clauwaert et al., 2008). Current generation from tetrathionate has been obtained in MFCs with ferric iron as the cathodic electron acceptor (Ni et al., 2016; Sulonen et al., 2015; Sulonen et al., 2016). Abiotic current generation from tetrathionate was tested with a fuel cell connected to an external resistor of 1000 Ω with ferric iron as the cathodic electron acceptor. In this cell, the average anode potential was 0.57 V, which is higher than the measured minimum anode potential for current generation (0.5 V). However, the average current density remained below 10 mA m^{-2}, indicating that only very low current densities can be obtained with abiotic electrochemical systems with ferric iron, oxygen or other electron acceptor with lower reduction potential as the cathodic electron acceptor. Nevertheless, tetrathionate can be utilized as partial source of energy to run desired reduction reactions (e.g. hydrogen production or metal reduction), with less external energy than is required for electrochemical systems relying, for example, on anodic water oxidation (theoretical reduction potential 0.88 V vs. Ag/AgCl).

Even though bioelectrochemical tetrathionate degrading systems operate at lower potentials and degrade tetrathionate more efficiently than electrochemical systems, the start-up time of ESs is usually shorter than in BES, as in bioelectrochemical systems biofilm formation time can vary from a few days (Wang et al., 2010) to several weeks (Sulonen et al., 2015; Wang et al., 2009). As microorganisms require specific operational conditions (pH, temperature) and nutrients for growth, bioelectrochemical systems are also more vulnerable for

performance limiting disturbances than electrochemical systems (Jadhav and Ghangrekar, 2009). For example, significant increase in the operation temperature could not only prevent the microbial growth, but also lead to microbial cell lysis disabling the system recovery. However, tetrathionate degrading bioelectrochemical systems have been shown to be able to maintain average current density above 100 mA m^{-2} for over two years without the need for significant maintenance (Sulonen et al., 2016).

To estimate the differences in the electrochemical and bioelectrochemical processes in the larger scale, the energy efficiencies were calculated for the treatment of 1000 L waste water containing 0.5 g L^{-1} of tetrathionate in 10 electrochemical reactors each with a volume of 5 L and anode electrode area of 0.05 m^2 (see Supplementary material for additional information on the system and the calculations). In this study, the anode potential required to produce current density of above 70 mA m^{-2} was approximately 0.4 V in bioelectrochemical system and 0.75 V in electrochemical system. The theoretical reduction potential for ferric iron in the given conditions (pH 2.5, 20 °C) is approximately 0.64 V and the cathode potential was measured to remain at around 0.55 V both in the bioelectrochemical system and in the electrochemical systems with controlled anode potential. Therefore, the BES would produce a voltage of 0.15 V, while 0.2 V of applied voltage would be required for ES. BES would thus be producing electrical energy, while 33% more external energy than what would be obtained from BES would be required to operate the ES.

Ferric iron is good electron acceptor due to its high reduction potential, but as it is consumed in the reduction reaction, the catholyte needs to be regularly replaced. Alternatively, the iron

catholyte can be regenerated with the assist of iron-oxidizing microorganisms. Ter Heijne et al. (2007) demonstrated that *At. ferrooxidans* oxidized electrochemically generated ferrous iron back to ferric iron on the cathode of an electrochemical system. Similar iron regeneration in the cathode has been observed to occur also in tetrathionate-degrading bioelectrochemical system, in which *At. ferrooxidans* was detected from the anodic microbial culture and had likely shifted also to the catholyte through the membrane separating the chambers (Sulonen et al., 2016).

If no ferric iron or other attractive electron acceptors are available, hydrogen can be produced from water at the cathode. The theoretical reduction potential for hydrogen in the given conditions (pH 2.5, 20 °C) is -0.35 V vs. Ag/AgCl. The external voltages required to combine tetrathionate degradation to hydrogen production were thus estimated to be 0.75 V in BES, 1.1 V in ES, and 1.35 V for the electrochemical hydrogen production via water oxidation. Therefore, the electrochemical system would require over 45% and water oxidizing system approximately 80% more external power than the bioelectrochemical system to generate the same amount of hydrogen.

Assuming average tetrathionate degradation rates of 150 mg L^{-1} d^{-1} for BES and 30 mg L^{-1} d^{-1} for ES, all the tetrathionate would be degraded in 67 days in the bioelectrochemical system and in 333 days in an electrochemical system. Due to the longer treating time and higher applied voltage, complete tetrathionate degradation would require over seven times more external power in the electrochemical system than in the bioelectrochemical system. As normalized against the amount of tetrathionate degraded, bioelectrochemical system would

require only 0.08 kWh kg^{-1} $S_4O_6^{2}$ of external energy to degrade all the tetrathionate, while in the electrochemical system the external energy need would be 0.62 kWh kg^{-1} $S_4O_6^{2-}$. For the calculations, the bioelectrochemical system was assumed to be already enriched with an active tetrathionate-degrading microbial community. The reactor configuration, operational parameters and the energy consumed by the system (e.g. pumping) were assumed to be equal in all systems, and were thus not taken in to account in the calculations.

4. Conclusions

Current generation through bioelectrochemical and electrochemical tetrathionate degradation was observed to depend highly on the anode potential. In tetrathionate-fed BES, the minimum anode potential for current production was lower (0.3 V vs. Ag/AgCl) than in abiotic electrochemical system (0.5 V vs. Ag/AgCl). Even though higher coulombic efficiencies were obtained in the electrochemical system, the tetrathionate degradation rate remained significantly lower in electrochemical (15-35 mg L^{-1} d^{-1}) than in bioelectrochemical systems (110-240 mg L^{-1} d^{-1}). This study shows that with a microbial catalyst tetrathionate can be degraded faster and at lower anode potentials than with abiotic cells.

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Figure captions

Figure 1: Current densities obtained at the different anode potentials studied in BES. The anode potential control was started on day 286 and released on day 441, before and after which the BES was connected to an external resistor. The current density data between days 350 and 370 was lost due to an equipment malfunction.

Figure 2: Sulfate (SO_4^{2-}) concentration, tetrathionate ($S_4O_6^{2-}$) concentration and pH in BES when gradually decreasing the poised anode potential. The solid lines indicate the time points when the anode potential was changed (anode potential values are presented above the chart). The anolyte solution was partly replaced on days 321 and 429 for inoculation purposes (marked in the figure with dashed lines), which decreased the anodic sulfate concentration.

Figure 3: The current density in relation to anode potential in electrochemical tetrathionate degrading reactor (ES) and water oxidizing control reactor (Control).

Figure 4: Current density over time with constant anode potentials of 0.6 V, 0.8 V and 0.9 V vs. Ag/AgCl in abiotic tetrathionate degrading ES.

Figure 5: Sulfate (SO_4^{2-}) concentration, tetrathionate ($S_4O_6^{2-}$) concentration and pH in ESs with constant poised anode potentials of 0.6 V, 0.8 V and 0.9 V vs. Ag/AgCl.

Figure 1

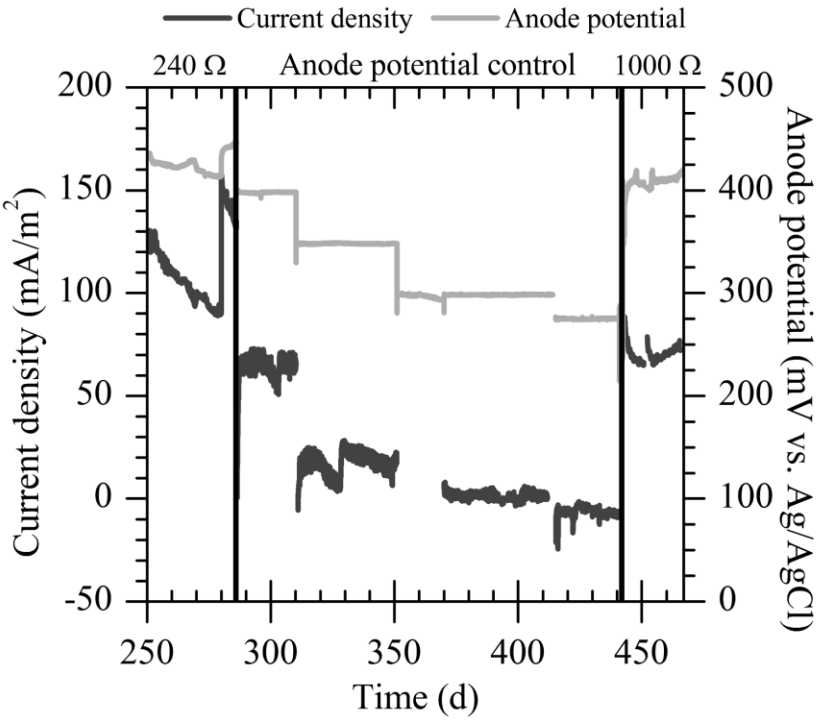
Current density
Anode potential
240 Ω
Anode potential control
1000 Ω
Current density (mA/m2)
Anode potential (mV vs. Ag/AgCl)
Time (d)
200
150
100
50
0
-50
500
400
300
200
100
0
250
300
350
400
450

Figure 2

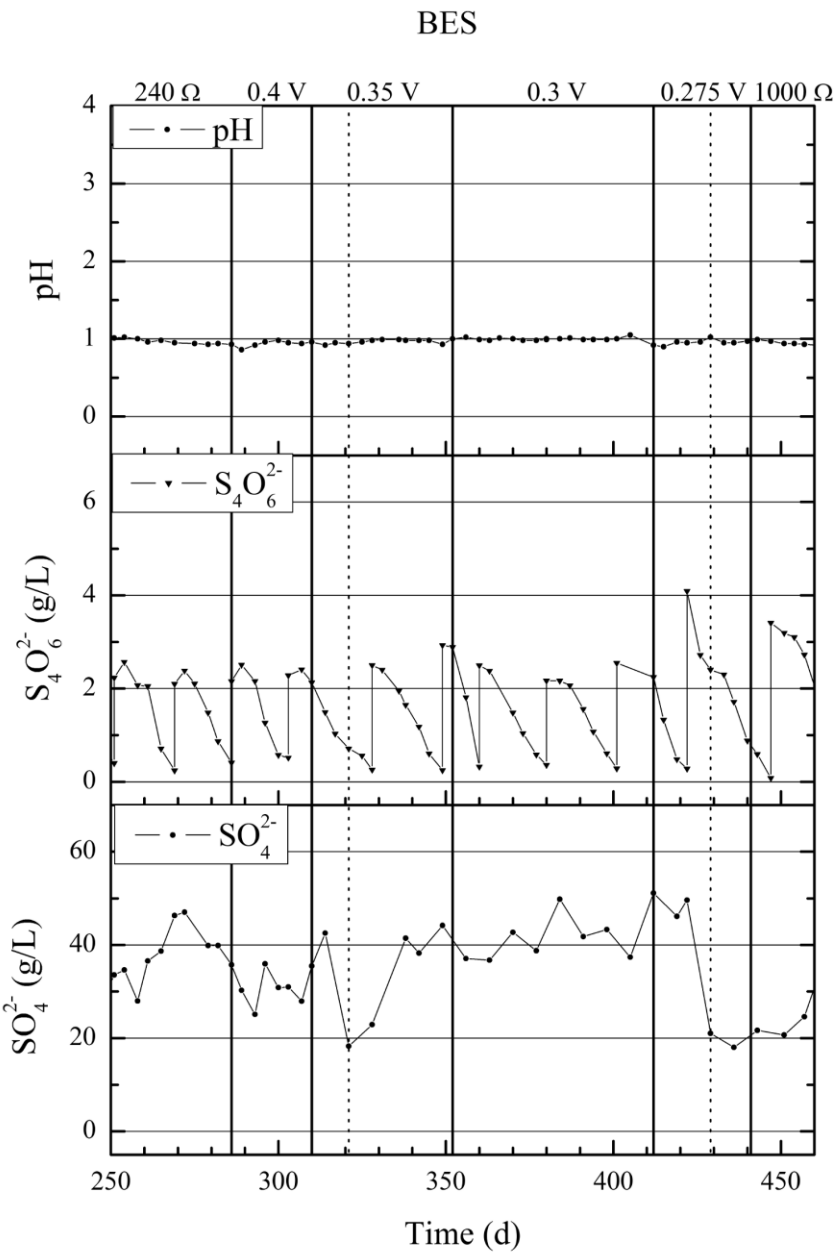
BES
240 Ω
0.4 V
0.35 V
0.3 V
0.275 V
1000 Ω
pH
$S_4O_6^{2-}$
SO_4^{2-}
$S_4O_6^{2-}$ (g/L)
SO_4^{2-} (g/L)
Time (d)

Figure 3

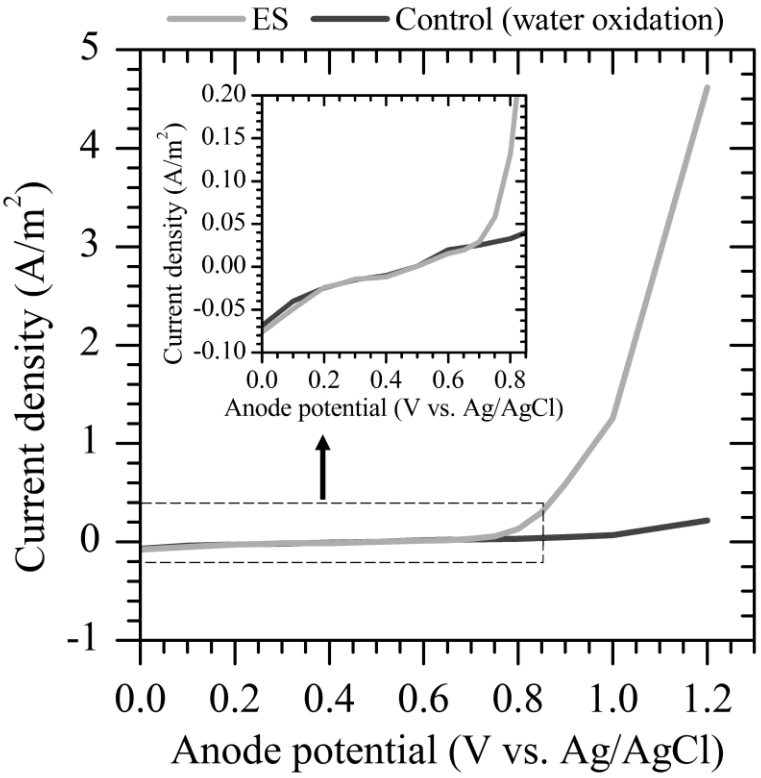
ES
Control (water oxidation)
Current density (A/m^2)
Anode potential (V vs. Ag/AgCl)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
-1
0
1
2
3
4
5
Current density (A/m^2)
Anode potential (V vs. Ag/AgCl)
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
0.0
0.2
0.4
0.6
0.8

Figure 4

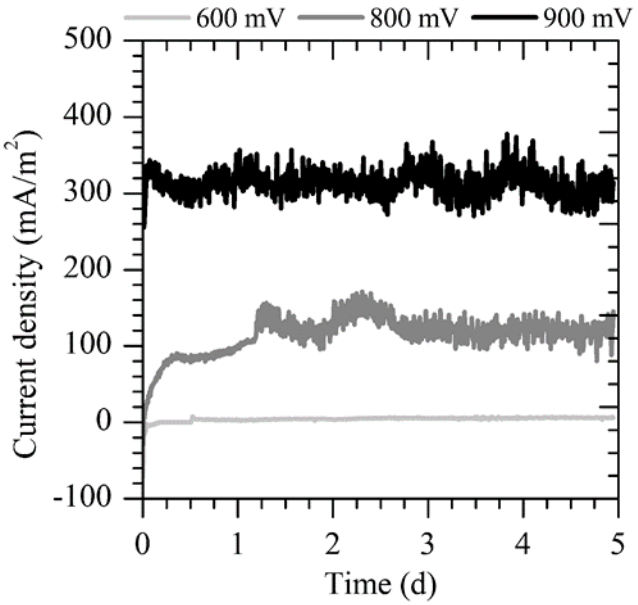
600 mV
800 mV
900 mV
500
400
300
200
100
0
-100
Current density (mA/m^2)
0
1
2
3
4
5
Time (d)

Figure 5