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Electricity generation from an inorganic sulfur compound containing mining wastewater by acidophilic microorganisms

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Abstract

Sulfide mineral processing often produces large quantities of wastewaters containing acid-generating inorganic sulfur compounds. If released untreated, these wastewaters can cause catastrophic environmental damage. In this study, microbial fuel cells were inoculated with acidophilic microorganisms to investigate whether inorganic sulfur compound oxidation can generate an electrical current. Cyclic voltammetry suggested that acidophilic microorganisms mediated electron transfer to the anode, and that electricity generation was catalyzed by microorganisms. A cation exchange membrane microbial fuel cell, fed with artificial wastewater containing tetrathionate as electron donor, reached a maximum whole cell voltage of 72 ± 9 mV. Stepwise replacement of the artificial anolyte with real mining process wastewater had no adverse effect on bioelectrochemical performance and generated a maximum voltage of 105 ± 42 mV. 16S rRNA gene sequencing of the microbial consortia resulted in sequences that aligned within the genera *Thermoplasma*, *Ferroplasma*, *Leptospirillum*, *Sulfobacillus* and *Acidithiobacillus*. This study opens up possibilities for bioremediate mining wastewater using microbial fuel cell technology.

Keywords: Microbial fuel cell; Electricity generation; Acidophile; Mining; Wastewater

1. Introduction

The world's demand for metals requires the continued mining and processing of metal-bearing ores. A common treatment process for extracting valuable metals from metal-sulfide minerals involves crushing and subsequent flotation to create a mineral concentrate suitable for further (bio)hydrometallurgical treatment. In addition to the valuable metal-containing phases, the low economic value mineral pyrite (FeS_2) is also usually present. Pyrite is oxidized during ore crushing and flotation [1,2], during which time the inorganic sulfur compound (ISC) thiosulfate ($S_2O_3^{2-}$) is produced (Eq. (1)).

$$2FeS_2 + 3.5O_2 + 4OH^- + H_2O \Rightarrow 2Fe(OH)_3 + 2S_2O_3^{2-} \quad (1)$$

Additional sources of ISCs in sulfide mineral processing include hydrogen sulfide from flotation of molybdenum and from ammoniacal thiosulfate leaching for gold recovery. The generated ISCs are contained in the processed waters that carry waste (gangue) minerals to tailings ponds, where the solid waste metal sulfides sink to the bottom of the pond. The tailings are covered in water to impair ingress of oxidation that, in turn, retards the release of acid and metals (reviewed in [3]). In the tailings pond, acidophilic microorganisms can subsequently oxidize thiosulfate to tetrathionate ($S_4O_6^{2-}$; Eq.

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(2)) and ultimately, the sulfur moiety will end up as sulfuric acid (Eq. (3)), lowering the pH to values typically between 1 and 3. This provides requisite conditions for the growth of extremely acidophilic microorganisms that have an optimum growth pH < 3 [4].

$$2S_2O_3^{2-} + 0.5O_2 + 2H^+ \Rightarrow 2S_4O_6^{2-} + H_2O \quad (2)$$

$$S_4O_6^{2-} + 3.5O_2 + 3H_2O \Rightarrow 4SO_4^{2-} + 6H^+ \quad (3)$$

If released to recipient water bodies, these ISCs can pose environmental risks, including microbial oxidation to sulfuric acid and the depletion of dissolved oxygen (reviewed in [5]). The most common treatment method for ISC-containing mining wastewaters is to raise the pH with 'lime' (CaO) before release of the water. A drawback of this technique is the production of a metal-containing sludge that requires safe disposal. Further investigated biotechnological solutions include metal precipitation with biologically produced hydrogen sulfide at near neutral [6] or acidic [7,8] pH, as well as biological oxidation of the ISCs to sulfuric acid prior to neutralization with lime and release to downstream water bodies [1]. However, neither of these methods has been extensively implemented at mining sites around the world.

Microbial fuel cells (MFCs) are bioelectrochemical systems capable of sustainable microbial oxidation of a substrate in the anode compartment, while reducing electron acceptors in the cathode, with the flow of electrons forming an electrical current (reviewed in [9]). The general principles of MFCs (Fig. 1) are that the electron donor is oxidized in the anode compartment, often by microorganisms attached as a biofilm to the anode surface and, in the absence of competing electron acceptors, they pass their electrons to the anode. Microorganisms able to donate electrons at the anode can be termed 'electricigens' and the most commonly investigated species come from the genera *Geobacter* and *Shewanella* [10,11]. Electrons donated to the anode are chemically or biologically reduced at the cathode. Research into MFCs has intensified in the past decade, and they have been extensively tested for treatment of organic carbon containing wastewaters at neutral pH to simultaneously generate a current and remove the waste product (reviewed in [12]). Most MFCs use microbial communities from 'non-extreme' environments such as municipal wastewater, activated sludge or sediment [13–16], while MFCs using extremophilic microorganisms are less common. Several studies have documented the use acidophiles in MFCs fed with organic carbon and energy sources [17–21]. However, to treat ISCs in mining wastewaters at acidic pH values, it is required to utilize microorganisms that donate electrons to the anode during growth using carbon dioxide and inorganic compounds as carbon and energy sources, respectively. However, research into the use of ISCs in MFCs is rare. A single study has shown that a mixed culture of acidophilic microorganisms is capable of generating electricity from tetrathionate, with *Acidithiobacillus ferrooxidans* and *Ferroplasma* spp. being the dominant populations present in the anolyte and on the bioanode surface [22]. The maximum current and power densities achieved during operation were 79.6 mA m^{-2} and 13.9 mW m^{-2}, respectively, but the low coulombic efficiency of 5% suggested that processes other than donation of the electrons to the anode also occurred [22].

In this study, the feasibility of using acidophilic microorganisms in MFCs fed with wastewater from an industrial sulfide mineral flotation process was investigated. If successful, the acid-generating ISCs would be removed before pH neutralization of water and release to recipient water bodies. The specific aims were to select suitable acidophilic microbes to remove the ISC compounds while simultaneously producing an electrical current in MFCs.

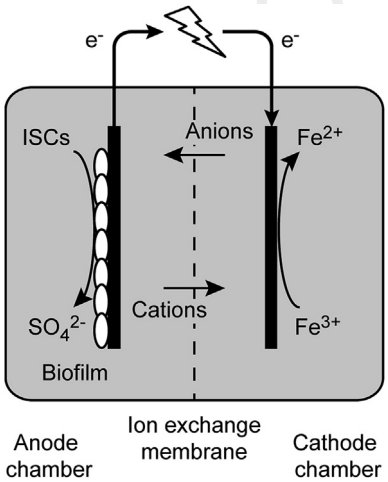

Fig. 1. Schematic diagram of a MFC showing the ISC oxidizing biofilm on the anode, the ferric iron-reducing cathode, with the two compartments separated by an ion exchange membrane. Horizontal arrows denote the exchange of ions across the membrane.

2. Materials and methods

2.1. Inoculum and growth conditions

Acidophilic microorganisms were enriched from a pH 2.5 to 2.7 underground acid mine stream sediment from a polymetal sulfide mineral mine located in Kristineberg, Sweden [23] and an acid sulfate soil containing metastable iron sulfides from Vaasa, Finland [24]. These environments were chosen as they were likely to contain populations able to grow anaerobically at low pH while utilizing ISCs [23–25].

Initial selection of the microbial consortium was carried out in MFCs containing an anolyte of autoclaved mineral salts medium (adjusted to pH 2 using sulfuric acid) plus sterile filtered (0.22 μm membrane filter; Sarstedt, Nümbrecht, Germany) trace elements solution [26] and various concentrations of filter-sterilized potassium tetrathionate and/or sodium thiosulfate. Additional ISC substrate (raising the ISC concentrations to the original values) was added to the anolyte when the concentration dropped below 1 mM in the fed batch system. Inorganic carbon for autotrophic growth was provided either by flushing the anolyte with CO_2 gas, or when the anolyte was changed by the addition of sterile filtered 10 mM (final concentration) sodium bicarbonate. The catholyte

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