

Microbial Fuel Cells for Sulfide Removal[†]

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Thus far, microbial fuel cells (MFCs) have been used to convert carbon-based substrates to electricity. However, sulfur compounds are ubiquitously present in organic waste and wastewater. In this study, a MFC with a hexacyanoferrate cathodic electrolyte was used to convert dissolved sulfide to elemental sulfur. Two types of MFCs were used, a square type closed to the air and a tubular type in which the cathode compartment was open to the air. The square-type MFCs demonstrated a potential-dependent conversion of sulfide to sulfur. In the tubular system, up to 514 mg sulfide L⁻¹ net anodic compartment (NAC) day⁻¹ (241 mg L⁻¹ day⁻¹ total anodic compartment, TAC) was removed. The sulfide oxidation in the anodic compartment resulted in electricity generation with power outputs up to 101 mW L⁻¹ NAC (47 W m⁻³ TAC). Microbial fuel cells were coupled to an anaerobic upflow anaerobic sludge blanket reactor, providing total removals of up to 98% and 46% of the sulfide and acetate, respectively. The MFCs were capable of simultaneously removing sulfate via sulfide. This demonstrates that digester effluents can be polished by a MFC for both residual carbon and sulfur compounds. The recovery of electrons from sulfides implies a recovery of energy otherwise lost in the methane digester.

Introduction

Interest in microbial fuel cells (MFCs) is gaining momentum worldwide. This technology enables the direct capture of the energy contained in biodegradable organic matter in the form of electricity. The basis of the technology lies within the fact that electron transfer is inherent to the nature of microbial metabolism, as bacteria derive their energy from the transfer of electrons from a substrate to an electron acceptor at a

higher redox potential. In a microbial fuel cell, the bacteria are stimulated to transfer their electrons to an electrode, from which the electrons then depart to the external electrical circuitry (1). On the basis of this principle, MFCs have been developed that can convert a wide variety of organic compounds such as acetate, glucose, and even complex organics in wastewater (2–5). Configurations of MFCs vary from cylindrical and tubular (3, 4, 6, 7) to stacked systems (8) and even to open sedimentary electrode combinations (9).

In “reactor”-type microbial fuel cells, attention has been focused on the removal of carbon-based compounds. However, complex substrates supplied to MFCs often contain sulfurous and nitrogenous compounds besides the carbohydrates. The conversion of these compounds leads to the release of sulfides, which are toxic and odorous. Sulfides can also function as a mobile carrier of electrons from bacteria to electron acceptors such as Fe(III) (hydr)oxides (10). Sulfides can be oxidized to various sulfur species. Depending on the redox potential and on the specific reaction conditions, over 30 different species can be produced (11). Sulfide is oxidized under standard conditions to elemental sulfur at potentials at least higher than -0.274 V versus standard hydrogen electrode (SHE). Increasing the potential can further oxidize elemental sulfur. At higher redox potentials, more oxidized forms of sulfur such as sulfite and sulfate will be the reaction products. Also polysulfides can be generated, which is often the result upon the presence of both sulfides and (microbiologically formed) elemental sulfur (12, 13).

One of the first MFC systems described encompassed the use of microorganisms to reduce sulfate to sulfide, which was catalytically reoxidized at an anode within a reactor (14). Sulfide oxidation also plays a key role in sedimentary microbial fuel cells (15, 16). A sedimentary MFC system consists of an anodic electrode placed in the anoxic sediment, which is connected to a cathodic electrode at higher potential in the oxic water body. This connection increases the anode potential and enables oxidation of the reduced species present in the sediment (9). It appears that sulfide oxidation is one of the key players in electricity generation in sediment systems (16).

Reactor-type MFCs could constitute an interesting technology for the removal of sulfides and, by extension, also sulfate from wastewaters. Reduction of the sulfate to sulfide in anaerobic methane digester systems represents both an energy loss, as substrate is used to form sulfide instead of methane, which is the core process of the reactor, as well as a treatment cost to abate the emission of sulfurous compounds in the biogas. A MFC could partially recover the energy comprised in the sulfide through its reoxidation at the anode. Indeed, the energy loss due to the lower methane gas production can be compensated for by electricity generation from sulfide. In this study, we operated MFCs on both specific sulfide- and sulfate-containing solutions and digester effluents. Our aim was to determine (i) the electricity production based on sulfide oxidation in a reactor system, (ii) the operational context in which this sulfide oxidation and removal can occur in a MFC system, and (iii) the involvement of microorganisms within this process.

Methods

Microbial Fuel Cell Construction. Two types of microbial fuel cells were used in this study. Square-type MFCs (17) were used for both continuous and batch-fed experiments in which the influent contained only sulfides and no organic carbon source. The anode internal volume was 320 mL (total

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anode compartment, TAC). Granular graphite (type 00514, diameter 1.5–5 mm, estimated projected surface between 817 and 2720 m² m⁻³, Le Carbone, Belgium) was used as the anodic electrode, which caused a decrease of the anode internal liquid volume to 170 mL (net anodic compartment, NAC). The cathode compartment had a volume of 320 mL and contained the same granular graphite. The hexacyano-ferrate-based cathodic electrolyte was prepared according to Rabaey et al. (4) and recycled over the cathodic compartment using a 1000 mL external buffer vessel, except in the first experiments in which no external vessel was foreseen. The tubular microbial fuel cells were constructed according to Rabaey et al. (4). The MFC (TAC 390 mL) was filled with graphite granules (as above), resulting in a NAC of 210 mL. The external contact was provided through a graphite rod (Morgan, Belgium, 5 mm diameter). The cathode consisted of a high surface area woven graphite felt (Alfa Aesar, Belgium) tightly matched around the membrane and open to the air. The hexacyanoferrate-based cathodic electrolyte was the same as above, was sprinkled (with recycling) over the cathode at a rate of 6 L h⁻¹, and was replaced before it was depleted (4). The latter was observed through a decrease of the cathodic potential over time and a concomitant discoloration (18). For all experiments, the notation “open circuit” implies that no current is allowed to flow while the notation “closed circuit” indicates that the MFC is connected to an electrical circuitry and current can flow over a resistor. All experiments were performed at room temperature. Only the methane reactors were heated to 35 °C. In all cases, an Ag/AgCl electrode was used as the reference. Its potential was estimated at +0.197 V versus SHE. Due to varying chloride concentrations this may differ slightly.

Experiments with Square Microbial Fuel Cells. In a first series of experiments, modified M9 medium (17) spiked with sulfide (0.1 g L⁻¹ S as Na₂S·xH₂O 60–62%) was recirculated over the MFC anode with a 2000 mL external buffer vessel. The anode was inoculated with a mixed aerobic sulfide-oxidizing culture and allowed to develop for several days. A fixed external resistor of 50 Ω was applied for one MFC (reactor A); the other MFC was placed in open circuit (reactor B). At regular time intervals, granules were removed from MFC reactor A and analyzed using scanning electron microscopy (see below). In a second phase, the MFC systems, supplied with new graphite granules but inoculated with approximately 10% granules from the first phase, were operated in a potentiostatic controlled mode. For this, two potentiostatic channels were used in a stepwise potentiostatic procedure in which the initial anode potential was set at -0.300 V vs SHE. Every 24 h the potential was increased stepwise to a maximal anodic potential of +0.200 V vs SHE. At regular intervals, sulfur species analyses were performed (see below). In a third phase, the graphite granules were replaced again (10% granules from the previous system). A fixed resistor of 50 Ω was applied, and medium (composition as before, with 0.1 g L⁻¹ S as Na₂S·xH₂O 60–62%) was continuously supplied at a flow rate of 0.720 L day⁻¹. This corresponded to a sulfide loading of 0.60 g S L⁻¹ NAC day⁻¹ (0.28 g S L⁻¹ TAC day⁻¹). In a later stage, this sulfide loading rate was increased to 1.98 g S L⁻¹ NAC day⁻¹ (0.93 g S L⁻¹ TAC day⁻¹).

Methanogenic Upflow Anaerobic Sludge Blanket Reactor Coupled to a Tubular MFC. Two anaerobic digesters of the upflow anaerobic sludge blanket (UASB) type were constructed (internal volume 2.5 L, 35 °C). The UASB was inoculated with aggregated sludge from a full-scale UASB at a potato processing company (Primeur, Waregem, Belgium). The influent, modified M9 medium (pH 7) (4), supplemented with 19.3 g L⁻¹ sodium acetate and 2.5 g L⁻¹ MgSO₄·7H₂O, was fed to the reactor at an organic loading rate of 4 g acetate L⁻¹ day⁻¹. A recirculation was foreseen to achieve a liquid

upflow velocity of 1.5 m h⁻¹ in the UASB. Both the liquid and the gaseous effluent of the reactor were transferred to a tubular-type MFC for reoxidation. The MFC was not inoculated; the bacteria present in the UASB effluent were allowed to inoculate the anode matrix. The sulfide and acetate concentrations were measured before and after passage through the MFC. The external resistance over MFC reactor C was set at 10 Ω; a control reactor was operated in open circuit (MFC reactor D).

Experiments with Tubular MFCs. The tubular MFC originating from the UASB/MFC combined setup was disconnected and used to directly treat a synthetic wastewater containing glucose and sulfate (composition as before, modified with 1 g L⁻¹ glucose and 4.1 g L⁻¹ MgSO₄·7H₂O). The external resistance was set at 100 Ω in a first phase (MFC reactor E). After 3 days of operation, the system was branched to the potentiostat, and the anodic potential was controlled at -0.150 V vs SHE (first 2 days) and subsequently at -0.100 V vs SHE. A control reactor was operated in open circuit (MFC reactor F).

Electrochemical Monitoring and Data Representation. Measurements were performed according to Rabaey et al. (4) and Logan et al. (18). Potentiostatic measurements and controls were performed using a PAR Bi-Stat Potentiostat (Princeton Applied Research, USA). An Ag/AgCl reference electrode (BAS, USA) was used as the reference within the anode compartment. Unless indicated otherwise, the calculations concerning loading and power output per unit volume refer to the NAC. The *Coulombic* efficiency expresses the efficiency of transferring electrons available in the substrate to an electrode (substrate is expressed as *chemical oxygen demand* (COD), mg O₂ L⁻¹) (5). This efficiency can be calculated as (18)

$$\epsilon_{\text{Coulombic}} = \frac{I t_{\text{exp}} M}{Fn \times \text{COD}_{\text{added}}}$$

with I the current (A), F Faraday's constant (96 485 C mol⁻¹), t_{exp} the experiment time (s), $\text{COD}_{\text{added}}$ the amount of COD added (g), n the number of electrons exchanged per mole of oxygen (4), and M the molar mass of oxygen (32 g mol⁻¹). In cases where reactors were operated in duplicate, standard deviations are calculated based on both reactors. For single reactor tests, standard deviations indicate the variability over time.

Chemical Analysis. For analysis of the volatile fatty acids, an extraction in diethyl ether was performed (19). The samples were analyzed with a capillary flame ionization detector (FID) gas chromatograph, GC 8000 Carlo Erba Instruments (Wigan, U. K.), connected to a computer. The column used was an Alltech (Deerfield, USA) EC-1000 (30 m, i.d. 0.32 mm, d_f 0.25 μm). The temperature was controlled at 135 °C for the isotherm oven and 200 °C for the detector and the injector. Nitrogen gas was used as the carrier gas at 3 mL min⁻¹. Gas chromatography (Shimadzu, Belgium) was used for determination of CO₂ and CH₄ in the headspace (1). Chemical oxygen demand measurements were made according to the dichromate method (19). Samples were filtered through a syringe 0.22 μm filter unit (Millex, USA), and SO₄²⁻ was determined using a Dionex ion chromatograph with an AS9HC column (Dionex, USA); a 9 mM Na₂CO₃ solution was used as eluent at a flow of 1 mL min⁻¹. A sample loop of 100 μL was used; the detector was an electrochemical conductivity detector. Sulfide was determined according to the methylene blue method (20) and using a silver/sulfide electrode 27502-41 (Cole-Parmer Instruments, Illinois). The indication “sulfide” describes all species (H₂S, HS⁻, and S²⁻). Elemental sulfur was determined on dried graphite granules with a CE Instruments EA 1110 element analyzer equipped with a

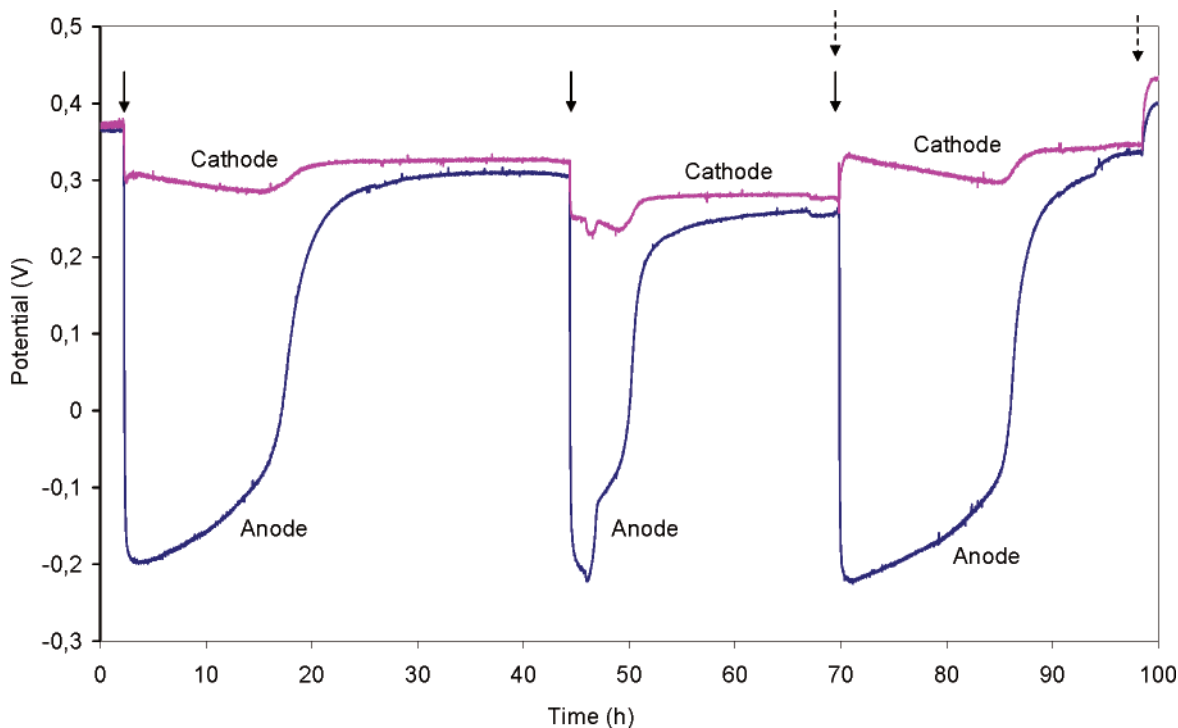


FIGURE 1. Evolution of the redox potential of the anode and the cathode for a sulfide-fed square MFC A (spiked $0.1 \text{ g S}^{2-} \text{ L}^{-1}$) (potentials calculated vs SHE). Arrows in full indicate the sulfide replenishment; the dashed arrows indicate a cathodic electrolyte replenishment. The decrease of the cathode due to electrochemical losses (step decrease at times 4, 44, and 70 h) and the linear degeneration of the cathodic electrolyte (both directly related to the current density through the MFC) can be discerned on the cathode curves. After 98 h, the hexacyanoferrate-based cathodic electrolyte was replenished without a concomitant sulfide spike.

thermal conductivity detector (Interscience, The Netherlands).

Isolation and Identification of Sulfide-Oxidizing Bacteria. Samples obtained from the continuous square MFCs were plated on a modified M9 medium (12) in which $0.2 \text{ g S}^{2-} \text{ L}^{-1}$ was present as Na_2S . The buffer was replaced by a 100 mM carbonate buffer; no other carbon source was added. These plates were incubated aerobically at 27°C . Molecular analysis was performed according to Aelterman et al. (8). In parallel, the isolates from the agar plates were grown in medium according to Siller et al. (21), in which the acetone was replaced by Na_2S (3.9 g L^{-1}) and nitrate served as the electron acceptor.

Scanning Electron Microscopy. The microscopic images of the graphite granule surfaces were made using a Philips XL30 scanning electron microscope (Philips, Eindhoven, The Netherlands). The backscattered electron (BSE) detector provides images with compositional contrast, which readily distinguishes graphite and deposits. For the images of granules with attached bacteria, an environmental scanning electron microscope XL30 of the same manufacturer was used in wet mode. Both apparatuses are equipped with energy-dispersive X-ray detectors from EDAX for elemental analysis.

Results

Electricity Generation Based on Sulfide in Square-Type MFCs. Upon addition of 0.1 g L^{-1} sulfide to the adapted reactor, operated in batch mode, a rapid decline of the anode potential was noted (Figure 1). The drop in anode potential was reproducible, although the degeneration of the cathodic electrolyte influenced this potential pattern during the first and second run. After 1 day of operation, all sulfides were removed by the MFC. The current obtained reached a maximum value of 11 mA, corresponding to a power generation of 37 mW L^{-1} NAC (20 mW L^{-1} TAC). The removal of sulfide was almost complete in the MFC reactor A

connected to an electrical circuitry. In the control MFC reactor B, with open circuit, no substantial amount of the sulfides was removed. The anodic potential of MFC B remained at $-0.310 \pm 0.045 \text{ V}$ vs SHE, a potential at which sulfide oxidation is indeed unlikely. This resulted in an open circuit potential of $0.704 \pm 0.049 \text{ V}$. Assuming that sulfide is converted to elemental sulfur (MFC reactor A), the charge generated during the first and third sulfide oxidation peak corresponded to $50\% \pm 4\%$ of the delivered charge by the sulfide. Scanning electron microscopy elucidated the formation of elemental sulfur grains onto the surfaces of the graphite granules (Figures 2a and 2b). Sulfur grains of the type seen in Figure 2c contained up to 85% sulfur on an elemental basis. The other 15% consisted of O, Na, and K. Figure 2d type grains contained only up to 8% sulfur and had a more diverse composition encompassing among others N, O, P, K, Ca, Na, and Fe. The carbon content was omitted from the elemental analyses as the measurement includes also part of the graphite matrix beneath the sulfur grains.

Potential-Dependent Oxidation of Sulfide within the Square-Type MFCs. A potentiostatic test in batch mode was performed in which the redox potential of the anode was increased with 0.100 V day^{-1} (Figure 3). Sulfide-containing medium was recirculated over the MFCs. At a potential of -0.300 V vs SHE, no sulfide was oxidized. At -0.200 V vs SHE all sulfide disappeared within the step interval of 24 h. In the following stepwise increases of the potential, the current generation was considerably lower. The total current generated to an anodic potential of -0.200 V vs SHE corresponded to the generation of a cumulative charge of $614 \pm 54 \text{ C}$ (Figure 3). The sulfide added corresponded to a possible charge generation of 1929 C. Hence, on an elemental sulfur basis, the recovery as current was $32\% \pm 3\%$.

Continuous Sulfide Removal in the Square-Type MFCs from Synthetic Influent. The MFCs were operated for 2 weeks, supplied with 100 or $330 \text{ mg S}^{2-} \text{ L}^{-1}$. The effluent sulfide concentrations of the MFCs decreased to 1.3 ± 1.7

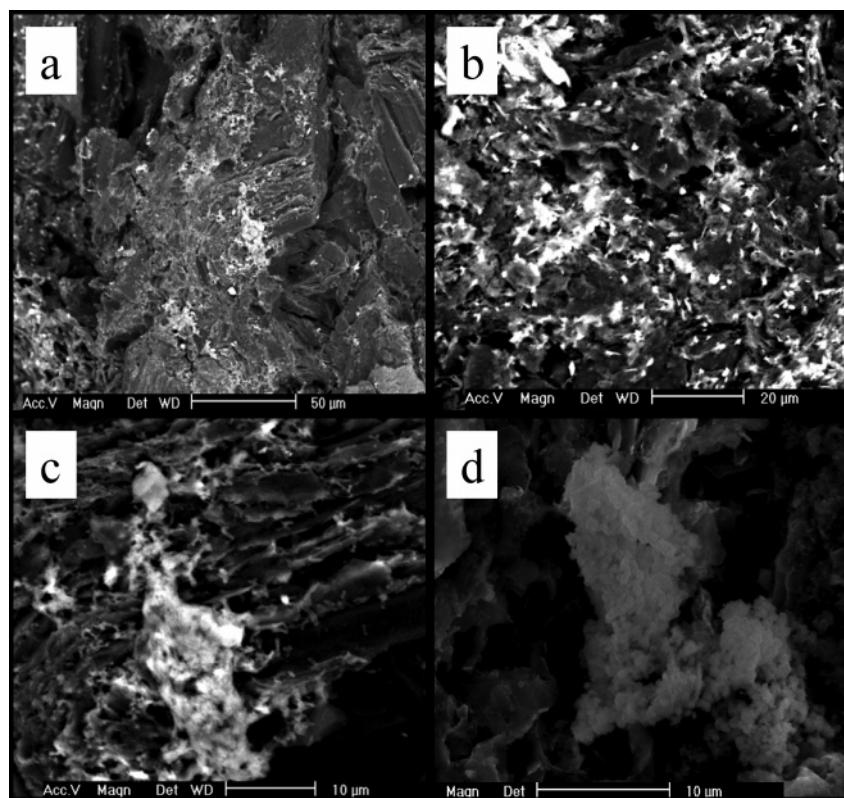


FIGURE 2. Scanning electron micrographs of white sulfur deposits on the black graphite anode of square-type MFCs fed with sulfide (MFC A): (a) large precipitates on graphite (500 \times); (b) fine grained precipitates (1000 \times); (c) detail of deposit, with sulfur contents up to 85% (balance-corrected for carbon); (d) crystallized deposits containing up to 8% sulfur (balance-corrected for carbon).

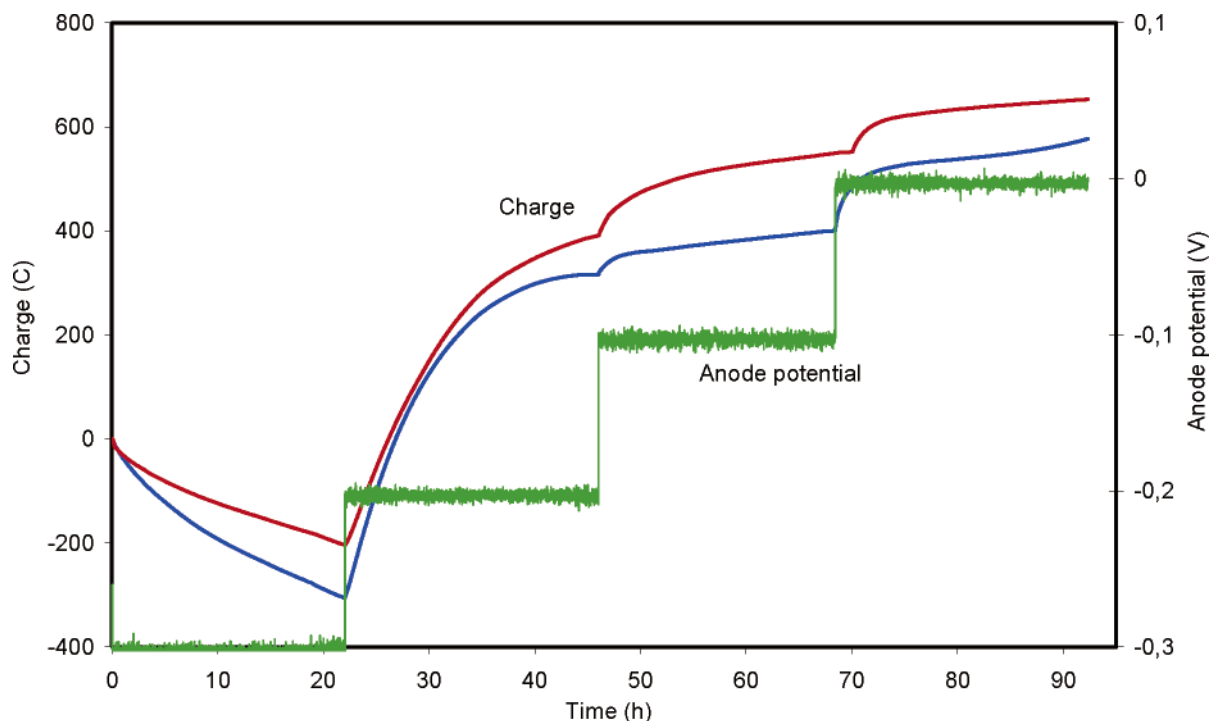


FIGURE 3. Charge (C) produced by sulfide-fed square-type MFCs as a function of time for a stepwise anode redox potential increase (calculated vs SHE) (times 24, 48, and 72 h). Two reactors were operated simultaneously.

and $6.1 \pm 7.9 \text{ mg L}^{-1}$ for the low and high levels, respectively. This resulted in a continuous power generation of 1.1 ± 0.6 and $3.3 \pm 6.2 \text{ mW L}^{-1}$ NAC (0.5 ± 0.3 and $1.6 \pm 2.9 \text{ mW L}^{-1}$ TAC) for the low and high concentration, respectively (maximum 3.5 and 27.8 mW L^{-1} NAC, respectively). The Coulombic efficiency of the conversion, calculated based on

a sulfide to sulfur oxidation, was $29.5 \pm 7.9\%$ for the low loading rate and $14.6 \pm 4.4\%$ for the high loading rate. At the end of the experiment, the granules contained $190 \pm 94 \text{ mg}$ of precipitated sulfur, which corresponds to a recovery of $9 \pm 4\%$ based on the sulfide dose and assuming sulfur deposits are homogeneously distributed in the MFC. Samples were

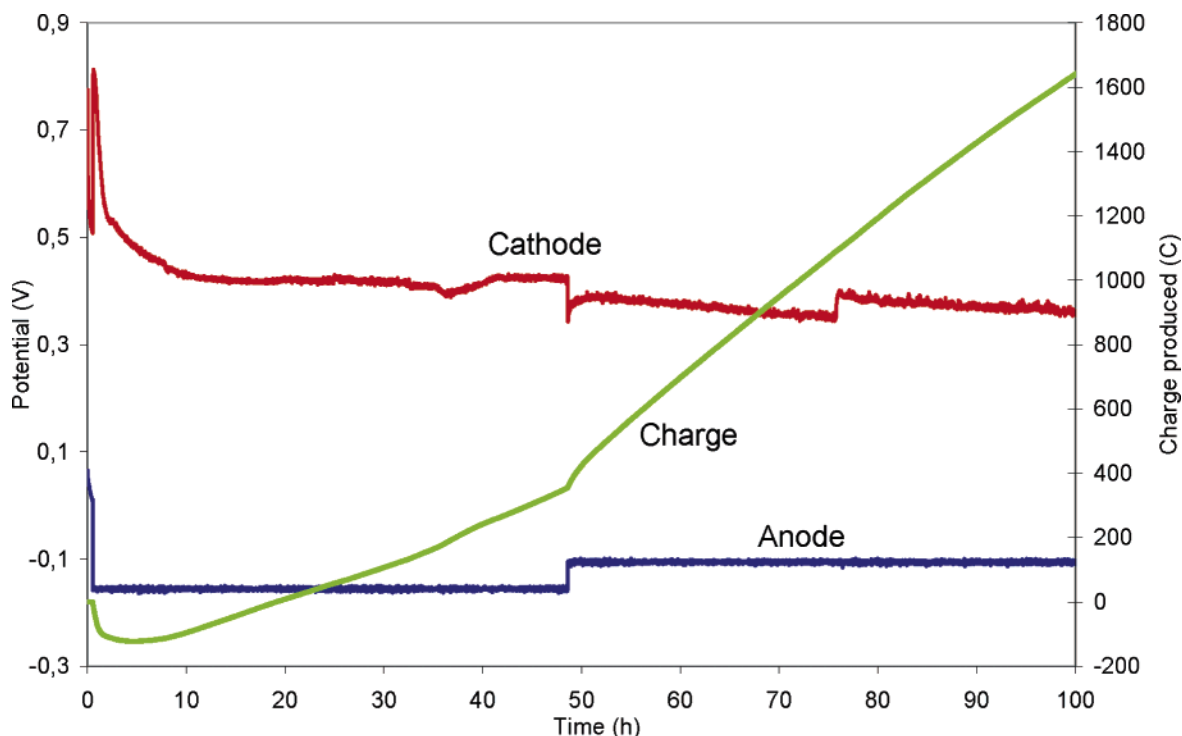


FIGURE 4. Evolution of the anodic and cathodic redox potential (calculated vs SHE) and the produced charge through time for a MFC fed with glucose and sulfate (reactor E). The anodic potential was controlled at -0.150 V vs SHE until 48 h, after which it was increased to -0.100 V vs SHE.

taken from the anodes of the MFC and incubated on sulfide-containing agar plates. Individual bacterial colonies were identified as either *Alcaligenes* sp. or *Paracoccus* sp. The latter bacterium was found to be able to grow with sulfides as the electron donor and nitrate as the electron acceptor.

Removal of Sulfide from Digester Effluent in Tubular-Type MFCs. UASB-type digesters were operated with acetate as the carbon source, and the effluent was connected to MFCs operated either in closed circuit (MFC reactor C) or in open circuit (MFC reactor D). The anaerobic digester removed $78\% \pm 15\%$ of the acetate. The digester effluent acetate concentrations were 3013 ± 2108 mg L⁻¹. No other short chain fatty acids were present in significant concentrations during the whole experimental period. The passage through the MFC reactor C (closed circuit) caused a decrease in acetate concentration of 1391 ± 139 mg L⁻¹ in the UASB effluent during an 8 day stable period, which implies the removal of 4769 ± 477 mg acetate L⁻¹ NAC day⁻¹ (2241 ± 224 mg acetate L⁻¹ TAC day⁻¹). The power output of the MFC was 26 ± 24 mW L⁻¹ (maximum of 101 mW L⁻¹ NAC, 47 mW L⁻¹ TAC). The substantial power and removal fluctuations were due to the rapid depletion of the hexacyanoferrate-based cathodic electrolyte. The sulfide concentrations in the effluent decreased from 115 ± 35 to 36 ± 49 mg S L⁻¹. Hence, the removal was 278 ± 181 mg S L⁻¹ NAC day⁻¹ (131 ± 85 mg S L⁻¹ TAC day⁻¹, maximum 514 mg S L⁻¹ day⁻¹ NAC). In the MFC reactor D (open circuit), the acetate concentrations decreased by 558 ± 446 mg L⁻¹ during a period of stable operation. The sulfide concentrations did not decrease significantly, i.e., from 68 ± 38 to 51 ± 35 mg S L⁻¹. The open circuit potential of MFC reactor D was 0.721 ± 0.131 V, with a maximum of 0.899 V. Analysis of graphite granules at the end of the experimental period yielded 432 mg of precipitated sulfur for MFC reactor C. In MFC reactor D, 136 mg of sulfur was found. In total, MFC C removed 729 mg of sulfide, so the sulfur found represented a recovery of 59%.

The total sulfide removed corresponded to the generation of a 4396 C of charge (sulfide to sulfur basis). The total charge

delivered by the system over this measurement period was $36\,537$ C; hence sulfide removal toward elemental sulfur can account for 12% of the delivered charge. Other oxidation products of sulfur (e.g., $S_2O_3^{2-}$ and SO_3^{2-}) were not detected. The remainder, approximately $32\,141$ C, can be delivered by the oxidation of residual acetate and other carbon compounds to CH_4 and CO_2 . The total acetate removed accounted for a charge generation of $101\,366$ C, demonstrating that the Coulombic efficiency of the MFC for acetate oxidation was approximately 32%.

Combined Sulfate Removal and Sulfur Formation in Tubular-Type MFCs. The tubular MFCs previously used in combination with the UASB were supplied with sulfate and glucose (MFC reactors E and F). The sulfate concentrations decreased from 533 to 35 ± 29 mg SO_4^{2-} -S L⁻¹ for the MFC with closed circuit (MFC E) and to 61 ± 33 mg SO_4^{2-} -S L⁻¹ for the MFC with open circuit (MFC F). For the first 3 days, an external resistance of 100Ω was applied for MFC reactor E. In a second phase the anodic potential was controlled at -0.150 V vs SHE first and subsequently at -0.100 V vs SHE (Figure 4).

The total chemical oxygen demand (influent 1060 mg COD L⁻¹) decreased to 647 ± 339 (MFC E) and 686 ± 327 (MFC F) mg COD L⁻¹. The sulfide concentrations in the effluent of MFC F continued to increase to 32 ± 17 mg sulfide L⁻¹ while MFC reactor E removed the sulfides to a concentration of 2 ± 2 mg S L⁻¹ (data for potentiostatic control at -0.100 V vs SHE). The average power outputs of the MFC reactor E were, in the potentiostatically controlled period, 16 and 36 mW L⁻¹ NAC (8 and 18 mW L⁻¹ TAC) for anodic potentials of -0.150 and -0.100 V vs SHE, respectively. The positive charge produced was 1773 C, accounting for a removal of sulfide as elemental sulfur of 336 mg S²⁻ L⁻¹ NAC day⁻¹ or a removal of 98 mg sulfide L⁻¹ influent.

Discussion

In this paper, we demonstrate that sulfides can be oxidized with the generation of electricity in reactor-type MFCs, even

in the presence of other electron donors such as acetate. This enables the recovery of energy that is otherwise lost in anaerobic digesters and allows the removal of sulfides out of waste streams.

Sulfide Oxidized in MFCs with Generation of Electricity.

Upon the addition of sulfide to the square MFC, the potential of the anode decreased, and current was generated, representing electron recoveries of up to $50\% \pm 4\%$ and power outputs of up to 39 mW L^{-1} (18 mW L^{-1} TAC). Controlling the anodic potential demonstrated that the sulfide is oxidized between -0.300 and -0.200 V vs SHE (Figure 3). The potential of the dissolved sulfide depends, according to the Nernst equation, on the pH of the solution, the temperature, the molar ratio between sulfides and oxidized sulfur species present, and the reaction type. As such, the exact potential at which the sulfides are oxidized in a MFC cannot be extrapolated from the data set as it will be case-specific. At higher redox potentials, no further oxidation appeared to occur in the potentiostatically controlled experiments (Figure 3). In tubular MFCs, up to 514 mg of sulfide was removed $\text{L}^{-1} \text{ NAC day}^{-1}$ ($241 \text{ mg sulfide L}^{-1} \text{ TAC day}^{-1}$), corresponding to a decrease in concentration from 155 to $5 \text{ mg sulfide L}^{-1}$.

Balance of Sulfur Compounds. There was a discrepancy in the balance of sulfur compounds between the amount of sulfide removed and the amount of current registered. This can have several reasons. First, part of the sulfides may diffuse toward the cathodic compartment. At the pH levels in the anode compartment ($6-7.5$), an equilibrium exists between HS^- and H_2S . The latter can, as a gas or dissolved, diffuse through the membrane separating the anode and cathode. In the tubular designs, the cathodic electrolyte-air interface facilitates volatilization. Hence, part of the discrepancy can be explained by such losses. Reliable sulfide detection in the cathode compartment failed, due to the interference of the hexacyanoferrate with the measurements. Second, other sulfur species may be formed. Polysulfides can be formed during the sulfide oxidation (11), or precipitated biogenic sulfur can react with sulfide in solution to form soluble polysulfides (13). These compounds have a composition of S_n^{2-} , and their formation can affect the overall Coulombic efficiency. Also thiosulfate and other oxidized species can be formed, but they were not detected by the equipment used in this study. From the potentiostat-driven experiment, it can be observed that, although the potential of the anode is increased, this does not result in a further substantial recovery of electrons. It is clear that rigorous control of the anode potential and current will be essential to increase the elemental sulfur yield and alleviate the formation of adverse and soluble sulfur species such as polysulfides. Third, oxygen can, particularly in the case of tubular systems, migrate from the cathode to the anode compartment, thereby causing the formation of soluble, oxidized sulfur species. Likewise, also the Coulombic efficiencies for acetate were only on the order of 32% . Such low recoveries have been observed previously (3, 5, 22). Possible explanations are particularly the oxygen influx through the membrane (3) and some biomass growth (23).

In this study hexacyanoferrate was the cathodic electron acceptor. In the case of the square-type MFC, this enabled oxygen-free operation of the cathode compartment. In future applications, open air cathodes need to be developed that operate in a more sustainable way. In case metal-based cathode catalysts are used, considerable attention toward the formation of metal sulfides at the cathode will be required.

Coupling Anaerobic Digestion and MFC Technology.

Anaerobic digestion can generate sulfide, which brings about additional costs due to the installation of units for sulfide removal. One needs to take into account that at the pH values used in anaerobic reactors (pH $7-8$) the major fraction of the sulfides remains in the liquid phase. The sulfide removal

from the active MFC gradually evolved to become almost complete. The cathode compartment from the tubular MFC was exposed to the air, contrarily to the square-type reactors. For that reason, the sulfide removal, as observed in the open circuit tubular MFC, decreases due to oxygen intrusion from the cathode to the anode compartment. Liu et al. (2) also examined a combination of anaerobic digestion and MFCs and reported values of $25-30\%$ for acetate removal. Our levels of acetate removal were 46% . This low removal was likely caused by an overloading of the MFC. Indeed, the effluent of the UASB, provided an influx of $17.9 \text{ g COD L}^{-1} \text{ NAC day}^{-1}$, while normal MFC loading rates are on the order of $1-3 \text{ g COD L}^{-1} \text{ day}^{-1}$.

Bacteria and Sulfide Oxidation in MFCs. The involvement of bacteria in the anode in the sulfide oxidation process is supported by the fact that a sulfide-oxidizing organism was isolated. The anaerobic sulfide oxidations of *Paracoccus denitrificans* (with nitrate as the electron acceptor) and *P. pantotrophus* (24) are well described. It is a challenging question how these bacteria shift from nitrate as an electron acceptor to an insoluble electron acceptor. Sulfide is a well-known redox shuttle between bacteria and insoluble electron acceptors (10). The *Paracoccus* species possess a membrane-bound complex that allows sulfide oxidation by the respiratory chain (24).

Sulfate Removal Via Sulfide Oxidation. This study demonstrates that a MFC has the special capability of combining sulfate reduction and sulfide oxidation. Sulfate reduction in a MFC fed with carbohydrates has been described previously (4). This study demonstrated that controlling the anode potential can decrease the corresponding efflux of sulfide. This will require a balance between maximizing power generation (lower anode potentials, e.g., -0.280 V vs SHE , are beneficial) and maximizing sulfide oxidation (higher anode potentials, e.g., -0.150 V vs SHE , are beneficial). Low and variable substrate removal efficiencies were observed, which are considered to be due to the high glucose loading rates applied ($3.5 \text{ g L}^{-1} \text{ day}^{-1}$) (23).

Applications. The results of this study provide a proof of principle that MFCs can be used to simultaneously remove carbon compounds, sulfide, and sulfate from wastewaters, with concomitant electricity generation. Sulfide production presents a possible economic loss as well as an environmental hazard. A major advantage of MFCs is the fact that the operator is in direct control through the current and the potential. This enables the determination of a potential frame in which sulfide is oxidized to elemental sulfur. It also allows for monitoring and control of the current toward quantitative control of the removed sulfide. As such, it is feasible to construct systems in which the potential is varied to obtain a specific current, corresponding to a desired amount of sulfides to be removed. Microbial fuel cells can thus be applied for the polishing of effluents originating from anaerobic digesters and other waste streams. The concomitant electricity generation can be an advantage, certainly in the case of anaerobic digester effluents, as the overall efficiency of the installation can increase and follow-up treatment becomes less complicated. It is necessary to fine-tune the oxidation to maximize elemental sulfur generation. Further research is needed to elucidate the stability of this process on the longer term and to assess the influence of the precipitated sulfur on the electron transfer toward the anode.

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microscopy. For more information, we refer to <http://www.microbialfuelcell.org>.

Supporting Information Available

Proposed biofilm model for sulfate reduction with simultaneous sulfide oxidation as well as a possible configuration in which a MFC is installed on top of an UASB reactor for effluent polishing and a table with all measurement data and additional experimental results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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