

Microbial Fuel Cell Operation with Continuous Biological Ferrous Iron Oxidation of the Catholyte

ANNEMIEK TER HEIJNE,^{†,‡}
HUBERTUS V. M. HAMELERS,^{*,†} AND
CEES J. N. BUISMAN^{†,‡}

Sub-Department of Environmental Technology,
Wageningen University, Bomenweg 2, P.O. Box 8129, 6700 EV
Wageningen, The Netherlands, and Wetsus, Centre for
Sustainable Water Technology, Agora 1, P.O. Box 1113,
8900 CC Leeuwarden, The Netherlands

The oxygen reduction rate at the cathode is a limiting factor in microbial fuel cell (MFC) performance. In our previous study, we showed the performance of an MFC with ferric iron (Fe³⁺) reduction at the cathode. Instead of oxygen, ferric iron was reduced to ferrous iron (Fe²⁺) at the cathode with a bipolar membrane between the anode and cathode compartment. This resulted in a higher cathode potential than is usually obtained with oxygen on metal-based chemical catalysts in MFCs. In this study, we investigated the operation of the same MFC with ferric iron reduction at the cathode and simultaneous biological ferrous iron oxidation of the catholyte. We show that the immobilized microorganism *Acidithiobacillus ferrooxidans* is capable of oxidizing ferrous iron to ferric iron at a rate high enough to ensure an MFC power output of 1.2 W/m² and a current of 4.4 A/m². This power output was 38% higher than in our previous study at a similar current density without ferrous iron oxidation. The bipolar membrane is shown to split water into 65–76% of the needed protons and hydroxides. The other part of the protons was supplied as H₂SO₄ to the cathode compartment. The remaining charge was transported by K⁺ and HSO₄⁻/SO₄²⁻ from the one compartment to the other. This resulted in increased salt concentrations in the cathode. The increased salt concentrations reduced the ohmic losses and enabled the improved MFC power output. Iron could be reversibly removed from the bipolar membrane by exchange with protons.

Introduction

The increasing threat of global warming by greenhouse gases requires further development of renewable energy sources. The microbial fuel cell (MFC) is a new energy technology in which microorganisms produce electricity directly from renewable biodegradable materials (1–3). The MFC consists of an anode and a cathode, separated by a membrane. At the anode, microorganisms convert organic material under anoxic conditions. These microorganisms use the electrode as the electron acceptor (4). The electrons flow through an external circuit to the cathode, where oxygen is reduced to water. Oxygen is superior to other electron acceptors for its unlimited availability and its high redox potential (5).

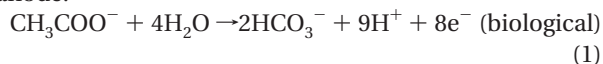
Oxygen reduction at the cathode, however, is kinetically limited, especially on carbon or graphite electrodes, and therefore needs a catalyst or electron mediator (6). Platinum is a chemical catalyst that is often used in MFCs, but it has several disadvantages like its cost and its instability for oxygen reduction (7). Biologically catalyzed cathodes have the advantage of being cheaper and more renewable (8).

In our previous study (9), we investigated an alternative cathode reaction, namely, the reduction of ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) with a bipolar membrane between anode and cathode compartments. This bipolar membrane was needed to maintain the low catholyte pH (<2.5) required to keep ferric iron soluble, whereas the conventionally used cation exchange membrane failed to maintain the pH difference between the anolyte and the catholyte. A bipolar membrane consists of a cation and an anion exchange layer. At the junction, water is split into protons and hydroxides when a certain voltage is applied (10). The protons migrate to the cathode, while the hydroxides move to the anode. The reduction of ferric iron results in a higher cathode potential than is obtained with oxygen on a metal-based catalyst in MFCs (5, 11).

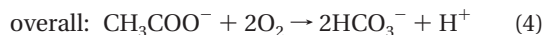
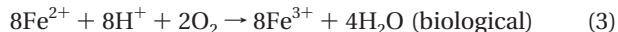
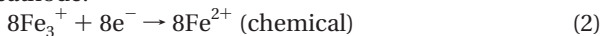
In this study, we investigate the performance of the MFC with a bipolar membrane and continuous ferrous iron oxidation of the catholyte by an acidophilic chemolithoautotrophic microorganism (Figure 1). We used the microorganism *Acidithiobacillus ferrooxidans*, which can be found in acid mine drainage and is a dominant organism in the process of ore bioleaching (12). This microorganism derives its energy from the oxidation of Fe²⁺, using CO₂ as a carbon source and O₂ as the electron acceptor.

The following reactions occur in the MFC with continuous ferrous iron oxidation:

anode:



cathode:



The oxidation of ferrous iron using oxygen as an electron acceptor yields only a relatively small amount of energy for microbial growth. The Gibb's free energy of iron oxidation has been estimated to be between -42 and -27 kJ/mol (13, 14). The biomass yield of iron oxidizing microorganisms is consequently low: approximately 20 mol of ferrous iron is needed to produce 1 mol of biomass-C (12, 13). The low energy consumption and low excess biomass production makes the couple ferric/ferrous iron an attractive electron mediator for oxygen reduction.

Our objective was to study the performance of the MFC with continuous ferrous iron oxidation. The MFC was operated with low iron concentrations in the catholyte, as these lower the chance of precipitation (15). At the same time, the initial ferrous iron oxidation rate should be sufficiently high for the investigation of the MFC performance. Because of the low biomass yield of the iron oxidizing microorganisms, however, it takes time before a high conversion rate is obtained in a solution with low iron concentrations. The iron oxidizing microorganisms were therefore first grown on biomass support particles (BSPs) in a medium with a high ferrous iron concentration. Thereafter,

* Corresponding author phone: +31 317 483447; fax: +31 317 482108; e-mail: Bert.Hamelers@wur.nl.

[†] Wageningen University.

[‡] Wetsus.

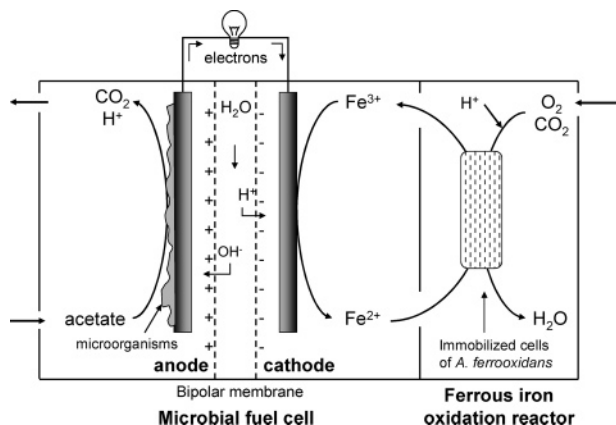


FIGURE 1. MFC with continuous ferrous iron oxidation.

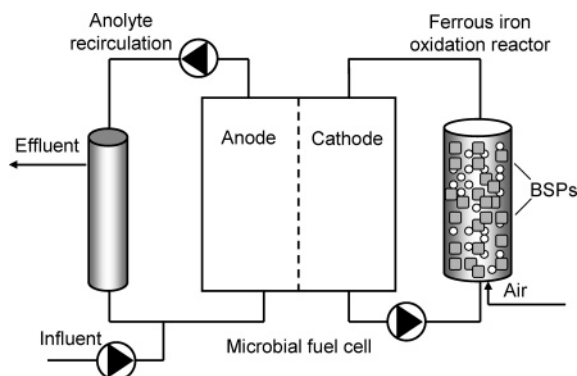


FIGURE 2. Process scheme of the MFC with continuous ferrous iron oxidation.

we placed this immobilized culture of *A. ferrooxidans* in a separate reactor and combined it with a ferric iron reducing cathode in an MFC (Figure 2). In this study, we investigate the operation of the MFC with ferric iron reduction at the cathode and simultaneous biological ferrous iron oxidation.

Materials and Methods

MFC Setup. The flat plate MFC used for the experiments is described in more detail in ref 9. The effective projected surface area of both anode and cathode was 290 cm², and each compartment had a liquid volume of 0.62 L. The electrodes were made of graphite felt (thickness: 3 mm, FMI Composites Ltd., Galashiels, Scotland). Both the cathode and the anode compartments contained reference electrodes (Ag/AgCl, 3 M KCl, +205 mV vs NHE, ProSense QiS, Oosterhout, The Netherlands). The anode and the cathode compartments were separated by a bipolar membrane (fumasep FBM, FuMa-tech GmbH, St. Ingbert, Germany) with the cation exchange side facing the cathode and the anion exchange side facing the anode. The anode and cathode were connected via a resistor with a range of 0–50 Ω. Both anolyte and catholyte were recirculated at a rate of approximately 10 L/h.

Anode Microorganisms and Medium. The anode compartment was inoculated with effluent from another MFC running on acetate. The anode was continuously supplied with medium, containing potassium acetate and a 20–30 mM potassium phosphate buffer (pH = 7) at a rate of 0.4 L/day. The acetate concentration was different for each current density and was at a level so that no acetate depletion occurred. Every fifth day, the anode was provided with macronutrients (10 mL of a solution containing 4.31 g/L NH₄Cl, 5.39 g/L CaCl₂·2H₂O, and 4.31 g/L MgSO₄·4H₂O), micronutrients (1 mL of a solution containing 0.72 g/L FeCl₂·4H₂O, 0.30 g/L CoCl₂·6H₂O, 0.18 g/L MnCl₂·4H₂O, 0.18 g/L ZnCl₂,

0.034 g/L H₃BO₃, 0.18 g/L Na₂MoO₄·2H₂O, and 0.50 g/L EDTA), and vitamins (1 mL of a solution containing 1 g/L pyridoxine·HCl, 0.5 g/L nicotinic acid, 0.25 g/L riboflavin, 0.25 g/L thiamine·HCl, 0.2 g/L biotin, 0.2 g/L folic acid, and 0.01 g/L vitamin B12).

Ferrous Iron Oxidizing Microorganisms and Medium.

A. ferrooxidans strain 583 (DSMZ, Braunschweig, Germany) was first grown in ferrous sulfate medium (33.3 g/L FeSO₄·5H₂O and standard nutrients: 0.4 g/L (NH₄)₂SO₄, 0.4 g/L KH₂PO₄, and 0.4 g/L MgSO₄ in H₂SO₄ at pH 2) and thereafter immobilized on 1 cm³ polyurethane foam BSPs to ensure a high volumetric ferrous iron oxidation rate from the start of the experiments. The immobilization procedure was based on Nemati and Webb (16). The first immobilization step consisted of inoculating 10% (v/v) of the culture into Erlenmeyer flasks containing 200 mL of ferrous sulfate medium and 25 BSPs each. The flasks were incubated on a rotary shaker at 175 rpm at 30 °C. Before complete conversion of the ferrous iron, determined from the dark yellow-brown color of the solution, the medium was refreshed. After repeating this procedure 5 times, 75 BSPs were placed in the ferrous iron oxidation reactor together with 475 mL of medium. The ferrous iron oxidation reactor was operated first batch-wise with the same medium but with a lower ferrous iron concentration equal to our previous study (0.95 g/L). The medium was recirculated and aerated with compressed air. Before all ferrous iron was converted, the medium was replaced. After this procedure was repeated 8 times, the ferrous iron oxidation reactor was connected to the MFC.

MFC Operation. The MFC was operated in a constant temperature chamber at 30 °C. The anode and cathode potential were logged every 60 s on a PC via a FieldPoint FP-AI-110 module. Data were collected using LabVIEW.

The MFC was first characterized without ferrous iron oxidation with 0.95 g/L ferric iron (sulfate) at pH 2 as the catholyte by decreasing the external resistance from 50 to 5 Ω. Thereafter, the MFC was connected to the ferrous iron oxidation reactor containing BSPs with immobilized cells of *A. ferrooxidans*. The standard nutrients were supplied to the cathode compartment only at the beginning of the experiment. The total catholyte volume was kept at 1.35 L. The catholyte pH was controlled at 2.5 with 1.8 M H₂SO₄, and the amount of acid dosed was monitored. The current density of the MFC was increased stepwise by decreasing the external resistance from 20 to 2.1 Ω. The MFC was operated for at least 1 week at each current density. Four resistances were tested (run 1: 20 Ω; run 2: 8.2 Ω; run 3: 4.0 Ω; and run 4: 2.1 Ω), resulting in current densities similar to our previous study. Samples from the anode and the cathode compartment were taken at least 5 times at each current density.

When the anolyte pH decreased below 6, it was manually adjusted to 6.6 with 2 M KOH. The acetate concentration in the anode was measured regularly to ensure that no depletion of acetate occurred.

Activity Tests. To determine the activity of *A. ferrooxidans*, activity tests were performed at the end of each run. Three BSPs were randomly taken from the ferrous iron oxidation reactor, and each BSP was placed in an Erlenmeyer flask containing 25 mL of medium (0.95 g/L ferrous sulfate and standard nutrients). The same was done with 1 mL of catholyte (*n* = 3). Blanks with BSPs without *A. ferrooxidans* in medium and blanks with medium alone were also tested (*n* = 2). Samples were taken twice within 10 h and analyzed for their ferric and total iron concentration according to the method described by Karamanev et al. (17). The maximum activity was determined from the steepest part of the curves. After the activity test, the BSPs were placed back in the ferrous iron oxidation reactor.

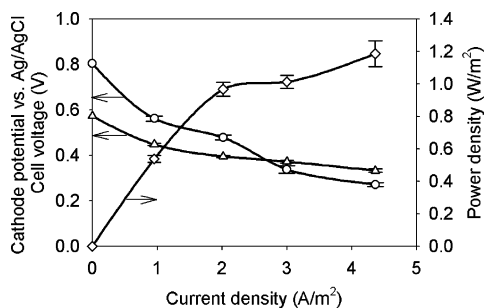


FIGURE 3. Performance of the MFC with continuous ferrous iron oxidation by *A. ferrooxidans*: power density (◇), cell voltage (○), and cathode potential (△). Average values and standard deviations were calculated over a period of at least 7 days (except for open circuit conditions).

Analyses. Anode and cathode potential were measured versus their reference electrodes. The cell voltage was measured as the difference between anode and cathode. The difference between the cathode and the anode reference electrode was defined as the voltage across the membrane.

Current density j (A/m²) and power density P (W/m²) were calculated from the cell voltage E (V), the circuit load R (Ω), and the electrode surface area A_{el} (m²) according to $j = E/RA_{el}$ and $P = E^2/RA_{el}$.

Ferric iron and total iron concentrations were measured according to ref 17. K⁺, total S, total P, and total Fe concentrations were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES; Vista-MPX, Varian, Inc., Palo Alto, CA).

The transport number t was calculated for H⁺, K⁺, and S. This transport number indicates the charge carried by this species (Q^+) as compared to the charge carried by the electrons in the electrical current (Q^-). The detailed calculations of the transport numbers as based on mass balances can be found in the Supporting Information.

The bipolar membrane was stored in demineralized water after the MFC was stopped. For 24 h before elemental analysis, one sample of the membrane was stored in 1 M NaCl, and one sample was stored in 1 M HCl to test whether iron could be reversibly removed from the bipolar membrane. The membrane samples were washed with MilliQ water and then analyzed with energy dispersive X-ray spectrometry (EDX) to determine the element content of the outside layers (1–2 μm). For the EDX analysis, a JEOL JSM-6480LV scanning electron microscope (SEM) equipped with a NORAN System SIX model 300 X-ray microanalysis system (Thermo Electron Corporation, Waltham, MA) was used. Measurements were done at an acceleration voltage of 20 kV.

Results and Discussion

MFC with Continuous Ferrous Iron Oxidation Showed a 38% Higher Power Output than Obtained in Our Previous Study. The polarization curve of the MFC directly after ferric iron addition without ferrous iron oxidation showed power and current densities in the same range as in our previous study (9).

When connecting the MFC to the ferrous iron oxidation reactor, the circuit load was set at a fixed resistance ($R = 20.2$ Ω). This resulted in a current density of 0.96 ± 0.02 A/m². After 1 week, the external resistance was decreased stepwise to yield 2.0 ± 0.05 A/m² ($R = 8.2$ Ω), 3.0 ± 0.95 A/m² ($R = 4.0$ Ω), and 4.4 ± 0.15 A/m² ($R = 2.1$ Ω). The MFC was operated at each current density for at least 7 days. The average values and standard deviations were calculated over the full period. Figure 3 shows the performance of the MFC with continuous ferrous iron oxidation. The average power density increased to 1.2 W/m² (55 W/m³) at a current density of 4.4 A/m², which

is 38% higher than the power density found in the previous study (9) at a similar current density. The cathode potential decreased upon increased current density but was nearly constant during each run (run 1: 0.448 ± 0.004 V; run 2: 0.396 ± 0.005 V; run 3: 0.372 ± 0.007 V; and run 4: 0.333 ± 0.007 V, all values vs Ag/AgCl). This indicates a constant ratio of ferric/ferrous iron. The decrease in cathode potential upon increased current was 1.6 times higher than in our previous study (9) in the current density range of 0.96 – 2 A/m². Part of the electrode might have been blocked by precipitates and deposition of extracellular polymeric substances (EPS) produced by *A. ferrooxidans* on the electrode (18). A decreased surface area results in a higher current density at the electrode as compared to a situation where no precipitates are formed. As the cathode potential decreases with increasing current density, a lower cathode potential would result in case part of the electrode is blocked.

In all runs, the ferrous iron concentration was below 6% of the total iron concentration, which indicates that the system's microbial ferrous iron oxidation capability was large enough for the MFC current in all runs. From this, we can conclude that the ferrous iron oxidation reactor had an overcapacity. The activity tests showed that the microbial ferrous iron oxidation activity was mainly in the BSPs at the start of run 1: the fraction of the activity in the BSPs was 0.95 ± 0.03 of the total activity, versus 0.05 ± 0.03 in the catholyte. After run 4, the microbial ferrous iron oxidation activity in the catholyte turned out to be considerable as well: the fraction of the activity in the BSPs was 0.62 ± 0.06 of the total activity, versus 0.38 ± 0.06 in the catholyte. No ferric iron was found in the blanks of the activity tests, indicating the absence of chemical ferrous iron oxidation.

Ion Transport through the Bipolar Membrane. Rozendal et al. (19) showed that a cation exchange membrane in an MFC does not only transport protons, but also other cations are transported that are present in higher concentrations. We show that this bipolar membrane in an MFC, similarly, allows transport of both positively and negatively charged species other than protons and hydroxides. The migration of charged species through the membrane was analyzed by their transport numbers, indicating the charge carried by a species (Q^+) as compared to the charge carried by electrons (Q^-) in the electrical current. Electroneutrality requires that these charges should be equal. The proton transport number t_{H^+} is defined as the charge carried by protons relative to the total charge and is equal to the hydroxide transport number t_{OH^-} .

For each mole of electrons arriving at the cathode, 1 mol of ferric iron is reduced, and 1 mol of protons is consumed by the microorganisms for ferrous iron oxidation (reactions 2 and 3). The catholyte pH would increase for a proton transport number lower than 1, as the protons produced by the water splitting reaction in the membrane would be less than the protons consumed by the ferrous iron oxidizing microorganisms. We indeed observed a pH increase in the catholyte. The amount of acid (H₂SO₄) dosed for pH control was used to calculate the proton transport number.

The dominant species present in the MFC, besides H⁺ and OH⁻, are K⁺, S_{total} (SO₄²⁻/HSO₄⁻), Fe_{total} (Fe²⁺/Fe³⁺), and P_{total} (HPO₄²⁻/H₂PO₄⁻). Other positive ions, supplied to the anode compartment through the nutrient solutions, were present in concentrations less than 1% of the K⁺ concentration. K⁺ was therefore assumed to be responsible for the charge transport from the anode compartment to the cathode compartment. The direction of the current mainly determines the transport of these species: a positive charge migrates from the anode to the cathode compartment and/or a negative charge migrates from the cathode to the anode compartment (all contributing to Q^+). We measured an increase in K⁺ concentration in the catholyte, from which

TABLE 1. Transport Numbers for H⁺, K⁺, and S

average current density (A/m ²)	transport number H ⁺	transport number K ⁺	transport number S
0.96	0.74	0.15	n.a. ^a
2.0	0.65	0.31	0.08
3.0	0.76	0.17	0.13
4.4	0.71	0.19	0.08

^a n.a. = not analyzed.

the K⁺ transport number was calculated. With the dosage of H₂SO₄ for pH control, S was added to the system as well. We measured an increase in S concentration in the catholyte, but this increase was lower than the increase we expected from the amount of acid dosed. Therefore, we concluded that S (in the form of SO₄²⁻ and HSO₄⁻) was transported from the cathode to the anode compartment. The concentrations of P and Fe in the catholyte were fairly constant, which is in accordance with the direction of the current. A typical concentration profile in the catholyte (Figure S1) as well as the detailed calculation procedure for the transport numbers can be found in the Supporting Information.

The transport numbers for H⁺, K⁺, and S for each run are shown in Table 1.

The proton transport number, varying between 0.65 in run 2 and 0.76 in run 3, is in the same range as found in our previous study (9): 0.6–0.7. This proton transport number not only had an effect on catholyte pH but also on anolyte pH. Per mole of electrons (and protons) produced at the anode, only 0.65–0.76 mol of hydroxide migrated from the membrane to the anode. Despite the buffer capacity in the influent, the anolyte pH decreased considerably, especially at high current densities, and a higher base dosage was required. There was a considerable transport of K⁺ from the anode to the cathode compartment ($t_{K^+} = 0.15–0.31$). The transport of species other than protons and hydroxides thus resulted in a pH increase in the catholyte and a pH decrease in the anolyte and increased salt concentrations in the catholyte. The occurrence of salt ion transport through bipolar membranes has previously been studied in, for example, refs 20 and 21. The experimental setup and conditions, however, were different from our MFC (high current densities, a six-compartment setup with several membranes, and high salt concentrations), and therefore, no quantitative comparison can be made with these studies.

The bipolar membrane should have a water splitting effectiveness of >98% at a current density of 1000 A/m² according to the supplier FuMa-tech GmbH. A proton transport number below 1 can be a result of the relatively low current densities in an MFC as compared to industrial applications. Although no trend can be seen from Figure 3, with current densities considerably lower than 1000 A/m², we expect that increased current densities could reduce the problem of pH changes in the catholyte and anolyte and the problem of increasing salt concentrations in the catholyte.

Low Iron Concentrations in the Catholyte Still Enabled a High Power Output, While High Salt Concentrations Positively Influenced Power Output. Most iron was in the ferric form as observed by measurements and the stable cathode potential. After 2 weeks of operation, a sudden decrease in total iron concentration from 0.95 to 0.5 g/L was observed. This concentration decrease occurred at the moment that pH 2.5 was reached, the point where the pH control began. Iron is indeed subjected to precipitation at pH values around 2.5 (15). Furthermore, the decrease in iron concentration was not likely to be a result of iron migration through the membrane. On average, the iron concentration in the anolyte effluent was lower than or equal to the iron concentration in the micronutrient solution, and the trans-

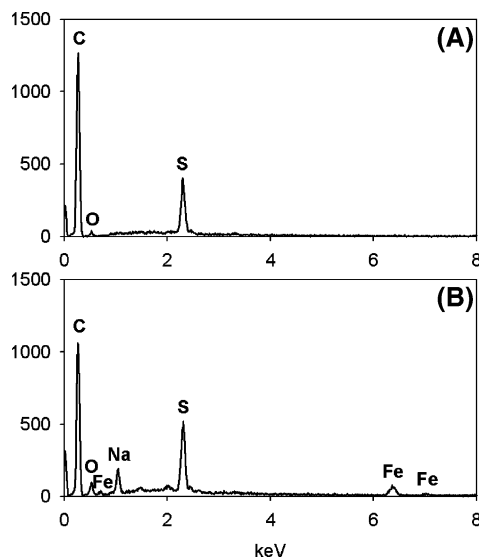


FIGURE 4. EDX spectrum for the cation exchange side of the bipolar membrane stored in 1 M HCl (A) and in 1 M NaCl (B).

port of iron from the cathode to the anode compartment would be against the direction of the current. No decrease in MFC performance was found with a lower iron concentration down to 0.5 g/L. Low iron concentrations are beneficial, as these lower the chance of iron precipitation (15).

The power density of the MFC with continuous ferrous iron oxidation was found to be 38% higher than the power output of the same MFC in our previous study (9) at a similar current density. This increase in power density is a result of a higher cell voltage at the same current density. The higher cell voltage (0.271 V vs 0.186 V at $j = 4.4$ and 4.5 A/m², respectively) could be explained by the higher salt concentrations in the catholyte (conductivity up to 44 mS/cm vs 10 mS/cm) and the resulting decreased ohmic losses. The ohmic losses were calculated using the equation $E_{ohmic} = dj/\sigma$ (9), where d is the distance between membrane and electrode (cm), j is the current density (A/cm²), and σ is the conductivity (S/cm). The higher conductivity was calculated to contribute to decreased ohmic losses of 0.074 V, which is 86% of the difference in the cell voltage.

Iron Could Be Reversibly Removed from the Membrane by Protons. Although no decrease in membrane performance was observed, we found that the membrane had an orange-brown color at the end of operation. Irreversible bounding of iron to the bipolar membrane could lead to decreased membrane and MFC performance. The outside layer of the bipolar membrane was exposed to the catholyte and was therefore expected to contain the highest iron concentrations. Therefore, the outside layers of the membrane samples were analyzed on the cation exchange side for their elements. For 24 h before analysis, one sample was stored in 1 M NaCl, and one sample was stored in 1 M HCl to test whether iron could be reversibly removed from the membrane. A quantitative analysis of the spectrum identified 2.0 and 1.7% of the atoms as S for the cation exchange side of the samples stored in NaCl and HCl, respectively. These S groups represent the negatively charged sulfonate groups in the cation exchange side of the bipolar membrane. No iron was detected on the membrane stored in HCl (Figure 4A). Iron was detected on the cation exchange side of the membrane stored in NaCl (0.59 atom %) and occupied 30% of the S groups (Figure 4B). The other S groups were mainly occupied by Na⁺ (69%) for the membrane stored in NaCl. The sample stored in demineralized water showed a similar spectrum to the one stored in NaCl, except that it contained K⁺ instead of Na⁺

(72% of the S groups contained K⁺, and 20% contained iron, data not shown). These findings show that iron can be reversibly removed from the membrane by exchange with protons.

Future Perspectives. Continuous ferrous iron oxidation in the catholyte by *A. ferrooxidans* was successfully achieved. Ferrous iron was oxidized at a rate high enough to ensure an MFC power output of 1.2 W/m² throughout more than a week. The ferrous iron oxidation reactor had an over capacity, as less than 6% of the iron was found to be in the ferrous form during all runs. The MFC current density of 4.4 A/m² requires a ferrous iron oxidation rate of 13.4 g Fe²⁺/L/day, normalized to the liquid volume of the ferrous iron oxidation reactor. This is indeed considerably lower than the conversion rate in other research (e.g., 194 g/L/day (22) and 521 g/L/day (23)). It is therefore likely that a 10-fold higher current can still be achieved in the MFC with the same ferrous iron oxidation reactor size as used in our study (0.475 L vs 0.62 L MFC cathode compartment).

For an MFC to be competitive with existing technologies, it should have a high power density as well as little or no use of chemicals and a low cost renewable catalyst for oxygen reduction. The power and current densities obtained in this study for the MFC with continuous ferrous iron oxidation are promising. To achieve higher power densities, a new MFC design is required. A new reactor design with decreased ohmic losses and consequentially higher power and current densities could increase the proton transport number in the MFC, but further tests are necessary to verify this. As a result, relatively less acid and base will be needed. The performance of *A. ferrooxidans* and/or other iron oxidizing microorganisms in the catholyte of a newly designed MFC will be further studied. Thereafter, the MFC power output should be balanced with the power needed for aeration so that optimizing strategies can be investigated in more detail.

Acknowledgments

We thank Janneke Tempel for her help with the SEM/EDX analyses and Nienke Stein and Vinnie de Wilde for their help with the experiments. This work was supported by Wetsus. Wetsus is funded by the city of Leeuwarden, the Province of Fryslân, the European Union European Regional Development Fund, and the EZ/KOMPAS program of the "Samenwerkingsverband Noord-Nederland".

Supporting Information Available

Detailed calculation procedure for transport numbers of H⁺, K⁺, and S and Figure S1 of typical concentration profiles in the catholyte. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Literature Cited

- (1) Chaudhuri, S. K.; Lovley, D. R. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat. Biotechnol.* **2003**, *21*, 1229–1232.
- (2) Rabaey, K.; Verstraete, W. Microbial fuel cells: Novel biotechnology for energy generation. *Trends Biotechnol.* **2005**, *23*, 291–298.
- (3) Logan, B. E.; Hamelers, B.; Rozendal, R.; Schröder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40*, 5181–5192.

- (4) Bond, D. R.; Lovley, D. R. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* **2003**, *69*, 1548–1555.
- (5) Zhao, F.; Harnisch, F.; Schröder, U.; Scholz, F.; Bogdanoff, P.; Herrmann, I. Challenges and constraints of using oxygen cathodes in microbial fuel cells. *Environ. Sci. Technol.* **2006**, *40*, 5193–5199.
- (6) Shukla, A. K.; Suresh, P.; Berchmans, S.; Rajendran, A. Biological fuel cells and their applications. *Curr. Sci.* **2004**, *87*, 455–468.
- (7) Zhang, J.; Sasaki, K.; Sutter, E.; Adzic, R. R. Stabilization of platinum oxygen–reduction electrocatalysts using gold clusters. *Science* **2007**, *315*, 220–222.
- (8) He, Z.; Angenent, L. T. Application of bacterial biocathodes in microbial fuel cells. *Electroanalysis* **2006**, *18*, 2009–2015.
- (9) Terheijne, A.; Hamelers, H. V. M.; De Wilde, V.; Rozendal, R. A.; Buisman, C. J. N. A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells. *Environ. Sci. Technol.* **2006**, *40*, 5200–5205.
- (10) Nagasubramanian, K.; Chlanda, F. P.; Liu, K. J. Use of bipolar membranes for generation of acid and base—Engineering and economic analysis. *J. Membr. Sci.* **1977**, *2*, 109–124.
- (11) Cheng, S.; Liu, H.; Logan, B. E. Power densities using different cathode catalysts (Pt and CoTMP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. *Environ. Sci. Technol.* **2006**, *40*, 364–369.
- (12) Jensen, A. B.; Webb, C. Ferrous sulfate oxidation using *Thiobacillus ferrooxidans*—A review. *Process Biochem. (Oxford, U.K.)* **1995**, *30*, 225–236.
- (13) Leduc, L. G.; Ferroni, G. D. The chemolithotrophic bacterium *Thiobacillus ferrooxidans*. *FEMS Microbiol. Rev.* **1994**, *14*, 103–119.
- (14) Rohwerder, T.; Gehrke, T.; Kinzler, K.; Sand, W. Bioleaching review part A: Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. *Appl. Microbiol. Biotechnol.* **2003**, *63*, 239–248.
- (15) Stumm, W.; Morgan, J. J. *Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters*, 2nd ed.; John Wiley and Sons: New York, 1981.
- (16) Nemati, M.; Webb, C. Effect of ferrous iron concentration on the catalytic activity of immobilized cells of *Thiobacillus ferrooxidans*. *Appl. Microbiol. Biotechnol.* **1996**, *46*, 250–255.
- (17) Karamanev, D. G.; Nikolov, L. N.; Mamartarkova, V. Rapid simultaneous quantitative determination of ferric and ferrous ions in drainage waters and similar solutions. *Miner. Eng.* **2002**, *15*, 341–346.
- (18) Gehrke, T.; Telegdi, J.; Thierry, D.; Sand, W. Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. *Appl. Environ. Microbiol.* **1998**, *64*, 2743–2747.
- (19) Rozendal, R. A.; Hamelers, H. V. M.; Buisman, C. J. N. Effects of membrane cation transport on pH and microbial fuel cell performance. *Environ. Sci. Technol.* **2006**, *40*, 5206–5211.
- (20) Elmoussaoui, R.; Pourcelly, G.; Maeck, M.; Hurwitz, H. D.; Gavach, C. Co-ion leakage through bipolar membranes. Influence on I–V responses and water-splitting efficiency. *J. Membr. Sci.* **1994**, *90*, 283–292.
- (21) Wilhelm, F. G.; Punt, I.; van der Vegt, N. F. A.; Wessling, M.; Strathmann, H. Optimization strategies for the preparation of bipolar membranes with reduced salt ion leakage in acid–base electrodialysis. *J. Membr. Sci.* **2001**, *182*, 13–28.
- (22) Ebrahimi, S.; Morales, F. J. F.; Kleerebezem, R.; Heijnen, J. J.; van Loosdrecht, M. C. M. High-rate acidophilic ferrous iron oxidation in a biofilm airlift reactor and the role of the carrier material. *Biotechnol. Bioeng.* **2005**, *90*, 462–472.
- (23) Park, D.; Lee, D. S.; Park, J. M. Continuous biological ferrous iron oxidation in a submerged membrane bioreactor. *Water Sci. Technol.* **2005**, *51*, 59–68.

Received for review February 5, 2007. Revised manuscript received March 13, 2007. Accepted March 14, 2007.

ES0702824