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Open Air Biocathode Enables Effective Electricity Generation with Microbial Fuel Cells

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The reduction of oxygen at the cathode is one of the major bottlenecks of microbial fuel cells (MFCs). While research so far has mainly focused on chemical catalysis of this oxygen reduction, here we present a continuously wetted cathode with microorganisms that act as biocatalysts for oxygen reduction. We combined the anode of an acetate oxidizing tubular microbial fuel cell with an open air biocathode for electricity production. The maximum power production was 83 ± 11 W m^{-3} MFC (0.183 L MFC) for batch-fed systems (20−40% Coulombic yield) and 65 ± 5 W m^{-3} MFC for a continuous system with an acetate loading rate of 1.5 kg COD m^{-3} day^{-1} (90 ± 3% Coulombic yield). Electrochemical precipitation of manganese oxides on the cathodic graphite felt decreased the start-up period with approximately 30% versus a non-treated graphite felt. After the start-up period, the cell performance was similar for the pretreated and non-treated cathodic electrodes. Several reactor designs were tested, and it was found that enlargement of the 0.183 L MFC reactor by a factor 2.9−3.8 reduced the volumetric power output by 60−67%. Biocathodes alleviate the need to use noble or non-noble catalysts for the reduction of oxygen, which increases substantially the viability and sustainability of MFCs.

Introduction

A microbial fuel cell (MFC) is a fuel cell in which at least the anodic reaction is catalyzed by microorganisms. If only the cathode contains microorganisms, or energy generation is not the objective of the system as a whole, these systems should rather be designated as bioelectrochemical systems (BES) (*1*). In conventional MFCs, only the anodic compartment is colonized by bacteria that oxidize hydrogen gas (*2*), sulfide (*3*), or an organic substrate (*4*, *5*). The singularity of these anodophilic microorganisms is their ability to use an inert electrode as an electron acceptor. Two bacterial strategies to efficiently conduct electrons to an anodic electrode have been described: the direct contact by outer membrane cytochromes and conductive pili or pilus-like structures (*2*, *6*) and the shuttling of electrons by mediating molecules, either produced by the microbial consortium (*7*) or introduced to the system (*8*). The working potential of the anodic electrode typically ranges from −0.250 to −0.100 V versus a standard hydrogen electrode (SHE), depending on the type and concentration of the substrate that is being oxidized (*5*, *9*, *10*). The electron flow is conducted over an external resistance that harvests electrical energy. Finally, the electrons are diverted to the cathodic electrode where an oxidant such as oxygen, nitrate, or iron is reduced. For every electron that flows through the electrical circuit, a cation is being transferred from the anode to the cathode, or an anion in the opposite direction. To enable this, an ion exchange membrane needs to be placed between the two electrode compartments (*5*, *11*). A membraneless system is also possible and has a lower resistance toward the ion transport, but the mixing of anodic and cathodic reagents can cause a low Coulombic efficiency (*9*). Recently, the use of ultrafiltration membranes has successfully been tested in microbial fuel cells, although a large fraction of unidentified losses was observed (*11*). Also, the use of bipolar membranes is promising (*12*).

In conventional MFCs, an abiotic cathode is used. The use of platinum as a catalyst for oxygen reduction is popular but expensive and unsustainable as it suffers from poisoning compounds in a bacterial solution. Moreover, the production of platinum is an environmentally detrimental process, which directly conflicts with the sustainable nature of MFCs. Other metals have been tested, and the use of pyrolyzed iron (II) phthalocyanine (FePc) and cobalt tetramethylphenylporphyrin (CoTMPP) as cathodic catalysts appears to be very promising (*13*, *14*). Ferricyanide and permanganate as a cathodic electron sink have successfully been used to investigate the anodic system with less cathodic constraints (*15*, *16*), but these cathodic systems are not considered to be sustainable since, generally, these chemicals need to be replaced.

Recent studies with biological cathodes have aroused interest for an inexpensive and sustainable alternative for chemical cathodes. Gregory et al. (*17*) have demonstrated that bacteria can take up electrons from a graphite electrode without hydrogen as an intermediate electron shuttle for nitrate and fumarate reduction. Recently, it has been shown that a bioanode oxidizing acetate could be combined with a biocathode, reducing nitrate to nitrogen gas (*18*). Aerobic biocathodes have been tested in seawater with a stainless steel cathode by Bergel et al. (*19*) and in freshwater with manganese as an electron shuttle between graphite electrode and *Leptothrix discophora* (*20*). The maximum power outputs were 0.13 and 0.32 W m^{-2}, respectively. These values were already competitive as compared to chemical cathodes at that time (for an overview of biocathodes, see He and Angenent (*21*)).

In this study, it was our objective to combine acetate oxidation in the anodic compartment with oxygen reduction by an open air biocathode in freshwater conditions. We determined (i) the attainable electricity and power production and (ii) the operational context in which this oxygen reduction occurs.

Experimental Procedures

Microbial Fuel Cells. The microbial fuel cells were constructed by gluing a cation exchange membrane (Ultrex

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TABLE 1. Overview of Dimensions, Cathodic Start-Up Catalyst, and Ohmic Resistance Measured[a] of Different Reactor Types When a Biological Cathode Was Used

	start-up catalyst	membrane length (m)	membrane diameter (m)	anodic volume (L TAC[b])	cathodic volume (L TCC[b])	membrane surface (m^2)	ohmic resistance ($N = 10$) (Ω)
R1	Mn	0.120	0.080	0.603	0.096	0.0302	2.0 ± 0.7
R2	Mn	0.260	0.045	0.414	0.117	0.0368	2.5 ± 0.7
R3	Mn	0.090	0.045	0.143	0.040	0.0127	6.4 ± 1.2
R4	none	0.090	0.045	0.143	0.040	0.0127	5.9 ± 1.7

[a] Current interrupt method, 10 times in 7 months. [b] TAC = total anodic compartment and TCC = total cathodic compartment = electrode surface × 3.18 mm.

CMI7000, Membranes International Inc.) into a cylinder with superglue according to Rabaey et al. (*5*). The tube was filled with granular graphite (type 00514; diameter 1.5−5 mm, estimated projected surface in between 817 and 2720 m^2 m^{-3}), and rubber stoppers were used to air tighten the inside of the tube, which functioned as the anode. The porosity of the graphite bed was 0.55, and the density of the granules was 1.83 kg L^{-1}. Carbon felt (3.18 mm thick, Alfa Aesar) was used as a cathodic electrode (*5*). The contacts were provided through graphite rods (5 mm diameter). The cathodic felt and graphite rod were connected to the tubular reactor with cable ties (3M). Some of the graphite felts were electrochemically pretreated to contain manganese oxide. For this purpose, the graphite felt was submerged in a 1 L vessel (0.5 M $MnSO_4$, 1 M H_2SO_4, and trace amounts of MnO_2, Mn_2O_3, and Mn_3O_4) and connected to a titanium electrode. A dc power supply provided 2.3 V for 20 min, with the graphite felt functioning as the anode and a titanium counter electrode producing hydrogen gas. Before using these felts in a microbial fuel cell, the felts were rinsed thoroughly with distilled water.

Operational Conditions. The anodic liquid stream was recirculated in an upstream mode through the anodic compartment with a peristaltic pump (6 L h^{-1}) out of a recirculation vessel (1 L) for the batch tests. The medium (4.4 g KH_2PO_4 L^{-1}, 3.4 g K_2HPO_4 L^{-1}, 2 g $NaHCO_3$ L^{-1}, 0.5 g NaCl L^{-1}, 0.2 g $MgSO_4 \cdot 7H_2O$ L^{-1}, 0.0146 g $CaCl_2$ L^{-1}, and trace elements as previously described (*22*)) was supplemented with 2 g of sodium acetate per liter upon depletion for the batch test. During these tests, the reactor performance was evaluated as a function of the reactor design. For the continuous tests, an 80 mL anodic recirculation vessel was used (6 L h^{-1} recirculation flow rate), and concentrated acetate was continuously added with a syringe pump. In the latter case, the modified M9 medium was continuously added to the recirculation vessel at a rate of 0.650 L day^{-1}. Effluent originating from highly competent MFCs (*23*) was used as an anodic inoculum. The cathodic liquid was always the same medium as the anodic liquid but without acetate. It was recirculated (6 L h^{-1}) from a recirculation vessel (2 L) to humidify the graphite felt. For a schematic overview, see Rabaey et al. (*5*). Different types of sludge and sediment were mixed to obtain a cathodic inoculum with sufficient microbial diversity.

Electrochemical Monitoring. The graphite rod contacts of both the anodic and the cathodic electrode were connected to an external resistor (5−100 Ω) or to a Bi-Stat potentiostat (PAR Bi-Stat Potentiostat, Princeton Applied Research). Measurements and determination of the ohmic resistance were performed according to the current interrupt method (10 min stabilization) (*23*). During potentiostatic or potentiodynamic measurements, the working electrode was connected with the cathodic electrode and the counter electrode with the anodic electrode. The potential of the cathodic electrode was monitored with a Ag/AgCl reference electrode (assumed to be +0.197 V vs SHE) (model RE-5B, BASi).

Calculations. A data acquisition unit (HP 34970A, Agilent) recorded the voltage difference every minute. Only hourly averaged values with a standard deviation and the value of the external resistance were then used for further calculations. The current and power production was calculated according to Logan et al. (*13*). The total reactor volume is the sum of the total anodic compartment (TAC) and the total cathodic compartment (TCC) (TAC + TCC = MFC). The Coulombic yield expresses the recovery of electrons as a current over the amount of electrons dosed as acetate (*5*). A total of 1 kg COD m^{-3} MFC day^{-1} equals 140 A m^{-3} MFC (96485 C mol^{-1} electrons; 4 mol electrons mol^{-1} COD).

Microbial Community Analysis. Biofilm samples of the cathodic electrode were taken by scraping the surface of the felt. Total DNA extraction from the biomass samples and PCR conditions were based on the protocols described previously (*24*). The 16S rRNA genes for all bacteria were amplified by PCR using the forward primer P338F and the reverse primer P518r, and a GC-clamp of 40 bp was added to the forward primer. PCR denaturing gradient gel electrophoresis (PCR-DGGE) was performed as described previously (*25*). The normalization and analysis of DGGE gel patterns was performed with the BioNumerics 9 software 2.0 (Applied Maths). Clone libraries were made from samples R3 m.o. and R3 a.o. (Figure S1 of the Supporting Information) by amplifying 16S rDNA gene fragments with primers P63F and R1378r (*25*). They were cloned by using the TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. DNA sequencing of the clones was carried out by ITT Biotech-Bioservice. Analysis of DNA sequences and homology searches was performed with the Ribosomal Database Project−-II (*26*). Nucleotide sequences from organisms O1 to O9 have been deposited in the GenBank database under accession numbers EU088406 to EU088414.

Microscopy. Sample preparation for SEM was performed according to Bond and Lovley (*2*). The samples were then treated with a Baltec SCD005 Sputter Coater. The thin Au layer was applied by operating the equipment at a pressure of 0.1 mbar for 60 s with a current of 40 mA. The samples were subsequently studied in a FEI XL30 SEM microscope equipped with a tungsten filament. DNA staining microscopy was performed with a LIVE/DEAD staining kit (Invitrogen) in accordance with the manufacturer's instructions.

Results

Start-Up Procedure. Three MFC reactor types (R1−3) were constructed (Table 1) with manganese treated graphite felt as the cathodic electrode. R4 was equipped with a cathode that did not receive manganese pretreatment. The reactors with the precipitated manganese oxides (R1−3-type reactors) needed a start-up period between 10 and 20 days to become fully active upon inoculation, whereas the reactors without manganese treatment (R4-type reactors) could obtain a similar activity after 20−30 days. This was verified by operating three more reactors in the same way as R4. Another 30 days was generally required to obtain the maximum power

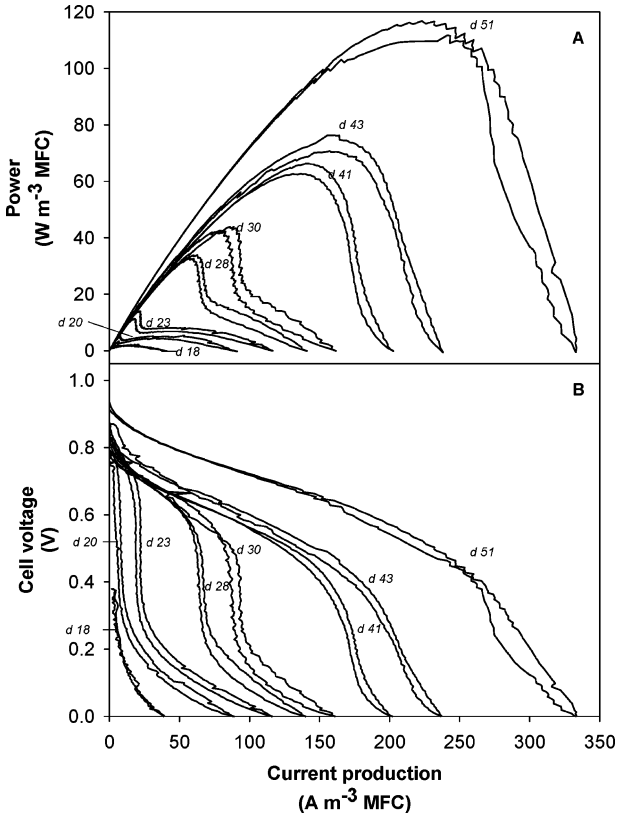

FIGURE 1. Polarization curves (current vs power (A) and cell voltage (B): scan rate: 1 mV s^{-1}) during the first 51 days of a graphite biocathode without manganese pretreatment (R4-type MFC).

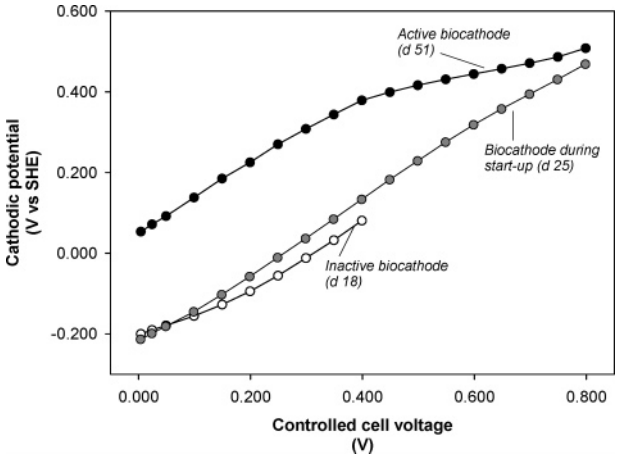

FIGURE 2. Potential of the cathodic electrode of an inactive and an active biocathode and a cell with a biocathode during start-up (R4-type MFC). Cell voltage was fixed with a potentiostat, and every cell voltage was maintained for 1 h (error bars were too small to be visualized).

output for all reactor types (Figure 1). During the start-up period, the open circuit voltage (OCV) was approximately 0.450−0.500 V after 15 min of stabilization. The cell voltage was typically below 0.200 V when operated with a 100 Ω external resistor, and the cathodic electrode potential ranged from −0.200 to +0.080 V versus SHE depending on the cell voltage (Figure 2). During the polarization curve, the maximum power production was 3 W m^{-3} complete MFC volume (m^{-3} MFC), and the maximum current production was between 40 and 60 A m^{-3} MFC (for a R3/R4-type reactor) (Figure 1).

The MFCs described in this study were considered to have a cathode that was biologically activated when the connected

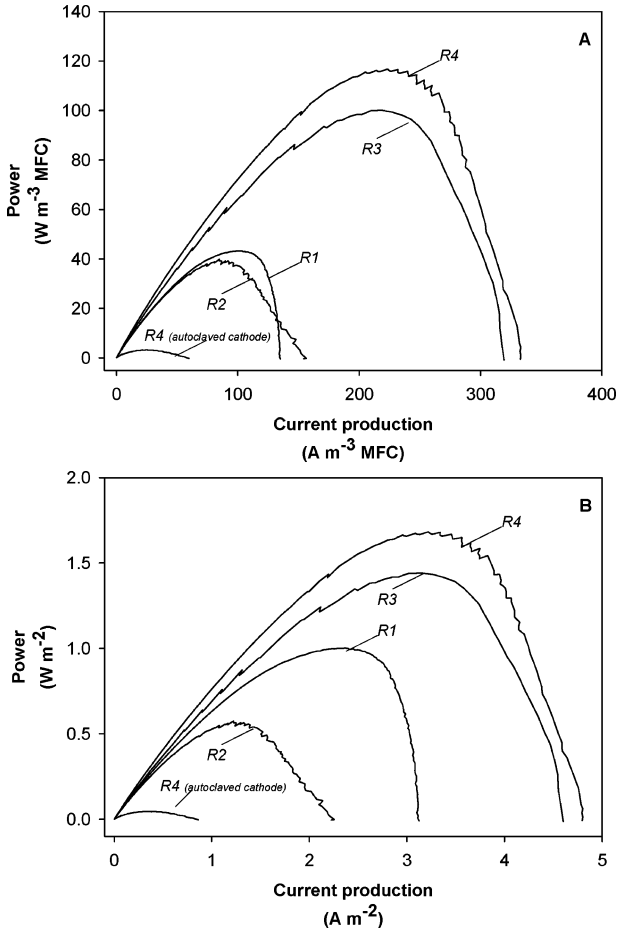

FIGURE 3. Effect of reactor design during forward polarization (1 mV s^{-1}) expressed per cubic meter MFC (A) or per square meter membrane surface (B).

cell had a cell voltage higher than 0.6 V with an external resistance of 100 Ω and the disconnected cell an OCV between 0.790 and 0.930 V (after 15 min of stabilization) (Figure 1) with a cathodic electrode potential higher than +0.400 V versus SHE (Figure 2). The addition of 0.2 g NH_4^+-N L^{-1} to the cathodic recirculation medium did not decrease the start-up time (data not shown). When two cathodic graphite felts were autoclaved (R3-type and R4-type) and reinstalled in the reactor, the activity of both reactors was similar to the activity during the start-up period (Figure 3). The biological involvement was also confirmed by the fact that the activity of the felt could irreversibly be hampered by letting the felt dry out for 3 days (data not shown). Epifluorescence microscopy revealed active cells without dense biofilm formation (Figure S1 of the Supporting Information). Moreover, the presence of bacteria on the different cathodic felts was confirmed by PCR-DGGE. The microbial cathode community facing the membrane was different from that facing the aerobic outside (Figure S1 of the Supporting Information).

Effect of the Design. Reactor type R2 had the same diameter as the R3 and R4 reactor types but had a longer length (Table 1). Increasing the reactor volume by a factor 2.9 by an extension in length (R2 versus R3/R4) resulted in a decrease of the maximum volumetric power output of 60−67% (Figures 3 and 4). Reactor R1, with a diameter of 8 cm, had a volume 3.8 times larger than the *R*3/R4-type reactors and 1.3 times larger than reactor type R2. The volumetric power and current output of reactor type R1 were similar to reactor R2 (Figures 3A and 4). When the performance was expressed based on the membrane surface, it appeared that the larger design R1 (43 m^2 membrane m^{-3} MFC) was

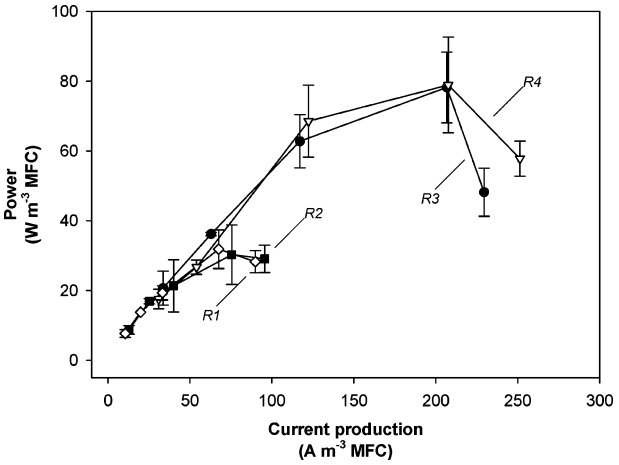

FIGURE 4. Power density curves with a fixed external resistance (5−100 Ω) for the different batch-fed reactor designs (R1−4). Average values and standard deviations were calculated from the stabilized cell voltages of three subsequent feeding cycles.

performing better than the R2 design (69 m^2 membrane m^{-3} MFC), although this was not the case when expressed on a volumetric basis (Figure 3B). These findings could also be verified by using ferricyanide as the cathodic electron sink (Figure S2 of the Supporting Information). The ohmic losses generally decreased when the reactor size was enlarged (Table 1).

Operational Parameters for Batch-Fed and Continuous Anode Systems. After spike feeding of acetate, the cell was active at its highest cell voltage for several hours to days (depending on the current production), and then the cell voltage gradually decreased to values below 0.010 V. The pH decrease in the anodic electrolyte was adjusted daily to pH 7 with a 1 M NaOH solution if the pH was lower than 6. If not, the current production was drastically hampered at pH levels lower than 5.8 (data not shown). The anodic liquid was replaced weekly to keep the sodium concentration below 0.15 M. Replacing the medium of the anodic recirculation vessel did not significantly enhance nor hamper the cell performance. During operation, the pH of the cathodic medium increased up to 9.5, while the cell voltage dropped to values below 0.100 V. A 1 M HCl solution was used daily to adjust the pH to 7. The cathodic medium was renewed weekly to maintain the chloride concentration below 0.15 M. Also, in this case, refreshing the medium did not affect the cell performance. The Coulombic yield was typically between 20 and 40% for the batch-fed reactors. To increase the Coulombic yield, the anodic system of R4 was operated in a continuous mode. With acetate loading rates between 0.5 and 1.5 kg COD m^{-3} MFC day^{-1}, the average Coulombic yields varied between 88 and 96%, and the average power production varied between 8 and 65 W m^{-3} MFC (Table 2).

A test was performed to evaluate the effect of low pH by the addition of HCl in the cathodic electrolyte. The anode of a R4-type reactor was operated in continuous mode (R_{ext} = 10 Ω and 1.5 kg COD m^{-3} MFC day^{-1}) and produced up to 64 W m^{-3} MFC when the pH of the cathodic electrolyte was between 7.0 and 7.5. When the pH was between 5 and 7, the power output decreased to 18−39 W m^{-3} MFC, and for pH values lower than 5, the power output was only 1−5 W m^{-3} MFC; here, the OCV was between 0.470 and 0.512 V after 15 min of stabilization.

The addition of ammonium (0.2 g NH_4^+-N L^{-1}) in the anode and cathode did not significantly improve the reactor performance. When an ammonium concentration of 1 g NH_4^+-N L^{-1} was added to the anode and the cathode, the cell performance and the potential of the cathodic electrode was similar to a reactor during the start-up phase (data not shown). Hence, the bacterial process at the cathode was severely impaired upon the addition of substantial ammonium quantities. It was verified that the anode of a MFC was not inhibited, and even improved, by an ammonium concentration of 1 g NH_4^+-N L^{-1} by using a ferricyanide solution as a chemical cathodic electron sink (Figure S2 of the Supporting Information). The effect of the reactor design on the performance between reactor types 1, 2, and 3/4 was confirmed with ferricyanide as an abiotic cathode system. The power output was slightly higher than when a biological cathode was used, but the maximum current production was almost twice as high (Figure S2 of the Supporting Information).

Discussion

Manganese Oxides Enhance the Start-Up of an Open Air Biocathode. The reactors described in this article had a low performance during the start-up period, which could be derived from the OCV (lower than 0.600 V) and a low current and power production. It was clear that the biological activity of the cathode was the limiting factor since it generally takes only 60−100 h to start up a MFC with an abiotic cathode when an acclimatized anodic consortium is used (*4*, *5*, *9*). Rhoads et al. (*20*) described a biological manganese shuttling mechanism in the cathodic biofilm where *Leptothrix discophora* used Mn^{2+} as the electron donor and oxygen as the electron acceptor. The oxidized manganese was then chemically reduced at the electrode, and the system had a lower power output without biomineralized manganese (*20*). In the system described here, where a mixed culture was used as inoculum, the only difference between a graphite felt that was chemically pretreated with manganese and an untreated graphite felt was the duration of the start-up period. Modification of an anodic electrode has also been described as a way to enhance the start-up (*27*). From Figure S1 of the Supporting Information, it was clear that the different cathodic felts had a similar microbial composition after 5 months of operation. Besides TM7, only α- and γ-proteobacteria were detected with the clone libraries. Two species of the genera *Pseudomonas* and *Novosphigobium* were more enriched at the air oriented side of the cathodic felt (Figure S1 and Table S1 of the Supporting Information). The difference in oxygen solubility might have affected the microbial composition on both sides of the cathodic felt.

Besides a long start-up period, the biological involvement in the cathodic process was demonstrated by inhibiting the process with a high ammonium concentration (1 g NH_4^+-N L^{-1}) (likely due to ammonia formation), a high or low pH, autoclaving, or water activity stress by letting the cathodic felt dry out. The difference between a cell with an active biological cathode and a cell with an inactive cathode (during start-up) was also visualized by the potential of the cathodic electrode (Figure 2). This potential expresses the energy content of the electrons that are withdrawn from the cathodic electrode. High cathodic potentials were also reported by Rhoads et al. (*20*) and Bergel et al. (*19*) for biological cathodes operating under different conditions and in another reactor design. The power and current production could be improved in our study without the need for saline water and without biomineralized manganese precipitations, which can be lost in a continuous system. However, the mechanisms of electron transport between the cathodic electrode and the bacteria remain unknown. Gregory et al. (*17*) suggested that *Geobacter* species have a direct electron-transfer pathway to obtain electrons from the cathode. It is likely that similar processes occur in a microbial community as described for MFC anodes, although community analyses and proteomic analyses are needed to confirm this.

Reactor Design Greatly Influences the Power Output of Microbial Fuel Cells. Increasing the reactor volume with a

TABLE 2. Performance of a Microbial Fuel Cell with a Biological Open Air Cathode (R4-Type Reactor) Operated in Continuous Mode with Different Acetate Loading Rates[a]

loading rate (kg COD m^{-3} MFC day^{-1})	cell voltage (V)	current production (A m^{-3} MFC)	Coulombic yield (%)	power production (W m^{-3} MFC)
0.5	0.123 ± 0.003	67 ± 2	96 ± 2	8 ± 0
1.0	0.224 ± 0.012	122 ± 6	88 ± 5	27 ± 3
1.5	0.344 ± 0.013	188 ± 7	90 ± 3	65 ± 5

[a] Averages and standard deviations calculated from a period of 3 days for each loading rate; R_{ext} = 10 Ω, 0.183 L MFC.

factor of 2.9−3.8 (R1 and R2 vs R3/4) had a negative effect on the current and power production (Figure 4). The effect of an increased volume on the power production was similar for a R2-type reactor as for a R1-type reactor when expressed on a volumetric basis (Figure 3A). When these results were expressed based on the membrane surface, the R1-type reactor seemed to perform better than the R2-type reactor. From these data, it seems that the enlargement of MFCs as such is not a good upscaling approach and that compartmentalization in small units appears to be necessary. The ohmic resistance was lower for the larger reactors because of the larger electrode surface (Table 1).

In this research, we obtained a maximum power output for batch tests of 117 W m^{-3} MFC during polarization (1 mV s^{-1}, Figure 3A) and 83 ± 11 W m^{-3} MFC with a fixed external resistance for R3/R4 batch-fed reactor types (Figure 4). When this type of reactor was operated in a continuous way with a loading rate of 1.5 kg COD m^{-3} MFC day^{-1}, we obtained a performance of 65 ± 5 W m^{-3} MFC. The difference in performance between the batch and the continuous operated cells can be attributed to the difference in acetate concentration. Power density curves obtained by polarization at a 1 mV s^{-1} scan rate overestimate the cell performance. However, these polarization data provide very valuable data in a short time span (*23*). The best volumetric performance in the literature, 115 W m^{-3} MFC, was obtained by Cheng and Logan (*27*) in a 14 mL batch-fed MFC with an ammonium treated anodic electrode and platinum as the cathodic catalyst. Therefore, we conclude that biological cathodes have interesting possibilities for sustainable and low cost microbial fuel cells. Nevertheless, further research is needed to minimize the start-up period for biological cathodes, and power outputs need to be enhanced by a factor of 5−10 to obtain an economically valuable technology for sustainable power production. Besides investigating microbial processes and interactions, technological improvements are still needed to achieve this goal.

All the anodes and cathodes could be operated for 7 months without ammonium or nitrate input. It is thus possible to use MFCs for organic carbon removal with energy recovery in streams that contain no ammonium. Organisms with the capacity to fixate nitrogen have been found in abundance in anodes of microbial fuel cells (*28*, *29*), and ammonium can diffuse and migrate through a cation exchange membrane (*30*). Small amounts of ammonium can be trapped from ambient air. It is also possible that nitrogen fixation occurred in the cathodic biofilm. Further research is needed to address nitrogen fixation in bioanodes and biocathodes.

The use of a phosphate buffer and pH correction proved to be necessary for pH control in both the anode and the cathode, which means that the proton flux through the membrane is too slow in our experiments. This has also been reported for NAFION membranes (*30*). In this research, there was a continuous recirculation of the anodic and cathodic fluids to provide a good distribution of the substrate and removal of products. However, further research is needed to evaluate the minimal fluid distribution rate since it is an energy cost. One of the challenges for microbial fuel cells remains the proton balance, as a sustainable MFC should also work at high rates without the need to add buffers——or control the pH through chemical addition. Three promising strategies have been brought forward: the use of ultrafiltration membranes (*11*), the use of bipolar membranes (*12*), and using the effluent of the anode in the cathode (*31*, *32*).

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Supporting Information Available

Table with names of sequenced clones, epifluorescence microscopy and secondary electron images, DGGE patterns of biocathodes of different reactor designs, and power density curves of different reactor designs with ferricyanide as catholyte. This material is available free of charge via the Internet at http://pubs.acs.org.

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