Hydrogen Production with a Microbial Biocathode

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This paper, for the first time, describes the development of a microbial biocathode for hydrogen production that is based on a naturally selected mixed culture of electrochemically active micro-organisms. This is achieved through a three-phase biocathode startup procedure that effectively turned an acetate- and hydrogen-oxidizing bioanode into a hydrogen-producing biocathode by reversing the polarity of the electrode. The microbial biocathode that was obtained in this way had a current density of about −1.2 A/m^2 at a potential of −0.7 V. This was 3.6 times higher than that of a control electrode (−0.3 A/m^2). Furthermore, the microbial biocathode produced about 0.63 m^3 H_2/m^3 cathode liquid volume/day at a cathodic hydrogen efficiency of 49% during hydrogen yield tests, whereas the control electrode produced 0.08 m^3 H_2/m^3 cathode liquid volume/day at a cathodic hydrogen efficiency of 25%. The effluent of the biocathode chamber could be used to inoculate another electrochemical cell that subsequently also developed an identical hydrogen-producing biocathode (−1.1 A/m^2 at a potential of −0.7 V). Scanning electron micrographs of both microbial biocathodes showed a well-developed biofilm on the electrode surface.

Introduction

Since the discovery of direct electron transfer by electrochemically active micro-organisms on electrodes at the end of the last century (*1*), bioelectrochemical treatment of wastewaters has become a rapidly emerging research field (*2*). The most studied bioelectrochemical treatment technologies so far are (i) microbial fuel cells (MFCs) for electricity production (*3*) and (ii) biocatalyzed electrolysis (or BEAMR process) for hydrogen production (*4–6*). Many research challenges, however, still remain before the bioelectrochemical treatment of wastewater can be considered to be a mature wastewater treatment technology. One of the largest research challenges in this respect is the cathode catalyst.

Laboratory MFC or biocatalyzed electrolysis systems typically apply platinum as the cathode catalyst, as platinum has proven to be an effective cathode catalyst in conventional fuel cells and electrolyzers. However, conventional fuel cells and electrolyzers typically operate at current densities (~10^3 to 10^4 A/m^2) that are orders of magnitude higher than those of MFCs and biocatalyzed electrolysis systems (~1 to 10 A/m^2). In fact, as a result of the low current densities, MFCs and biocatalyzed electrolysis systems produce too little electricity or hydrogen per amount of platinum to justify the use of such an expensive material as the cathode catalyst. This has encouraged researchers to look for alternative cathode catalysts in MFCs and biocatalyzed electrolysis systems.

Microbial biocathodes hold great promise as an alternative, as they can apply inexpensive electrode materials (e.g., graphite) and they are self-regenerating (*7*). Several interesting microbial biocathode concepts have already been implemented successfully for catalyzing cathodic oxygen reduction. These concepts include cathode systems that are based on redox cycling of transition metals (e.g., Mn and Fe) between the cathode and metal-oxidizing bacteria (*8–10*) and systems that are based on direct electron transfer by electrochemically active micro-organisms (*11*). However, biocathode concepts that so far were developed for catalyzing cathodic hydrogen production have mostly been enzymatic (*12, 13*) and not microbial. Enzymatic biocathodes have the important drawback that they are relatively instable and that they are not self-regenerating (*14*).

The only microbial biocathode concept that was developed so far for catalyzing cathodic hydrogen production was based on an immobilized pure culture of *Desulfovibrio vulgaris* with methyl viologen as a redox mediator (*15, 16*). However, with respect to the stability of operation of biocatalyzed electrolysis systems, a mediator-less microbial biocathode based on a naturally selected mixed culture would be much more desirable.

The objective of this study, therefore, was to develop a novel microbial biocathode system for hydrogen production that is based on a naturally selected mixed culture of electrochemically active micro-organisms. Our strategy for achieving such a microbial biocathode system was based on the well-known reversibility of hydrogenases (*17*). On the basis of this reversibility of hydrogenases, we developed the following three-phase biocathode startup procedure (Figure 1): (A) startup of an acetate- and hydrogen-oxidizing bioanode after inoculation with a mixed culture of electrochemically active micro-organisms, (B) adaptation to hydrogen oxidation only, and (C) polarity reversal to a hydrogen-producing biocathode and adaptation. In phase A of this procedure, we aim to quickly establish a well-performing bioanode. Subsequently, in transition from phase A to B, we aim to select for hydrogen-oxidizing electrochemically active micro-organisms. Finally, in transition from phase B to C, we aim to reverse the microbial metabolism and select for hydrogen-producing electrochemically active micro-organisms.

Materials and Methods

Biocatalyzed Electrolysis Cell. The experiments were performed in two identical electrochemical cells. The electrochemical cells consisted of four Plexiglas plates of 22 × 32 cm^2 (see Figure S1, Supporting Information) of which the two outer plates served as the heating jacket for temperature control (303 K). The two inner plates served as the electrode chambers and were separated from each other by a cation-selective membrane (Fumasep FKE, FuMA-Tech GmbH, St. Ingbert, Germany). The electrode chambers consisted of vertically orientated channels (width 1.5 cm; depth 1 cm) for liquid transport (volume 0.25 L), and a headspace for gas

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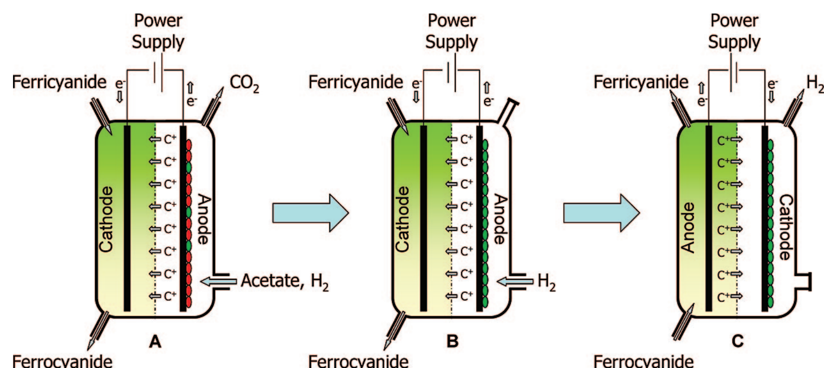

FIGURE 1. Overview of the three-phase biocathode startup procedure (C^+ = cations): (A) startup of an acetate- and hydrogen-oxidizing bioanode after inoculation with a mixed culture of electrochemically active micro-organisms, (B) adaptation to hydrogen oxidation only, and (C) polarity reversal to a hydrogen-producing biocathode and adaptation.

TABLE 1. Experimental Conditions during the Three-Phase Biocathode Startup Procedure

	phase A	phase B	phase C
medium supplement	10 mM $NaCH_3COO$	10 mM $NaHCO_3$	10 mM $NaHCO_3$
headspace flushing	H_2	H_2	N_2
bioelectrode potential (V)	0.1/−0.2	−0.2	−0.7

collection (volume 0.03 L). Both electrode chambers contained graphite felt (effective surface area, 250 cm^2; thickness, 6.5 mm; National Electrical Carbon BV, Hoorn, The Netherlands) as the electrode material. Three gold wires were pressed onto the graphite felt electrodes for current collection. Both electrode chambers were equipped with a Haber-Luggin capillary that was connected to an Ag/AgCl reference electrode (QM710X, ProSense BV, Oosterhout, The Netherlands). The electrochemical cells were each connected to a potentiostat (Wenking Potentiostat/Galvanostat KP5V3A, Bank IC, Pohlheim, Germany) and operated as a three-electrode setup (*18*). The working electrode of one of the electrochemical cells was inoculated and subjected to the three-phase biocathode startup procedure (see below). This electrode is referred to as the bioelectrode (during biocathode startup) or biocathode (after biocathode startup). The working electrode of the other electrochemical cell, which was not inoculated and not subjected to the three-phase biocathode startup procedure, is referred to as the control electrode.

Experimental Setup. A schematic overview of the experimental setup can be found in Figure S2, Supporting Information. The bioelectrode and the control electrode chamber were operated in continuous mode by supplying a microbial nutrient medium (1.3 mL/min). Prior to entering the bioelectrode chamber, the microbial nutrient medium was flushed with nitrogen from a nitrogen generator (purity >99.9%). The standard microbial nutrient medium was without a carbon source and contained (in deionized water) the following: 0.74 g/L KCl, 0.58 g/L NaCl, 0.68 g/L KH_2PO_4, 0.87 g/L K_2HPO_4, 0.28 g/L NH_4Cl, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, and 1 mL/L of a trace element mixture (*19*). During the biocathode startup, the standard microbial nutrient medium was supplemented with 10 mM sodium acetate or 10 mM sodium bicarbonate (Table 1). The counter electrode chamber contained a 100 mM potassium ferricyanide/ferrocyanide solution that was recycled (80 mL/min) over a 10 L buffer vessel.

The bioelectrode chamber solution was recycled (250 mL/min) over a gas washing bottle (640 mL). Liquid effluent left the gas washing bottle via a water lock to prevent the diffusion of oxygen into the bioelectrode chamber. The headspace of the bioelectrode and control electrode chamber could be flushed with H_2 (>99.9992%), N_2 (>99.9992%), or CO (>99.997%) from a cylinder. Excess gas, either supplied or produced, left the system via the gas washing bottle and a gas flow meter (Milligascounter, Dr.-Ing. Ritter Apparatebau GmbH & Co. KG, Bochum, Germany). The gas washing bottle contained a pH electrode for controlling the anode chamber at pH 7 by dosing with 1 M NaOH or 1 M HCl (Liquisys M CPM 253, Endress + Hauser, Naarden, The Netherlands). The electrochemical cells were connected to a data logger (Ecograph T, Endress + Hauser, Naarden, The Netherlands), which continuously logged the applied voltage, current, anode potential, cathode potential, pH, temperature, and gas production. All potentials are reported against the normal hydrogen electrode.

Experimental Procedures. The bioelectrode chamber was inoculated with a mixed culture of electrochemically active micro-organisms by adding 100 mL of effluent taken from an active bioelectrochemical cell. Subsequently, the bioelectrode was subjected to the three-phase biocathode startup procedure (Figure 1). Table 1 shows the differences in the experimental conditions during the three phases of the biocathode startup procedure.

After inoculation (phase A), the bioelectrode was started up as a bioanode. The bioelectrode chamber was initially operated in batch mode (standard microbial nutrient medium supplemented with 17 mM sodium acetate). This was done to allow the electrochemically active micro-organisms to adapt to the bioelectrode chamber without being washed out immediately. At t = 50 h, anodic current generation started and the operation was switched from batch to continuous mode by starting the supply of microbial nutrient medium. When the headspace of the bioelectrode chamber was flushed with hydrogen gas (phase A and B), the gas phase of the gas washing bottle was recycled (10 L/min) over the liquid phase of the gas washing bottle using a vacuum pump to achieve a high level of hydrogen saturation in the recycled bioelectrode chamber solution.

In between phases B and C of the biocathode startup procedure, the proper bioelectrode potential for biocathode operation was determined by means of a polarity reversal scan. During this scan, the bioelectrode potential was lowered from −0.2 to −0.8 V at a scan rate of 0.025 mV/s using a potentiostat (Autolab PGSTAT12, Eco Chemie BV, Utrecht, The Netherlands).

After startup, the biocathode was compared to the control electrode on the basis of polarization curves, which were

obtained by means of chronoamperometry. For this purpose, current generation was logged every 5 min for 1 h at −0.8, −0.75, −0.7, −0.65, −0.6, and −0.55 V. The last five data points at every potential were averaged and plotted in the polarization curve. Subsequently, the biocathode was compared to the control electrode on the basis of hydrogen yield tests. The hydrogen yield tests (duplicate) were performed in batch mode at −0.7 V and lasted for 48 h. At the start of the experiment, the headspace of the biocathode and the control electrode chamber contained only nitrogen. During the hydrogen yield tests, the headspace of the biocathode chamber was sampled seven times and analyzed for its hydrogen fraction with a gas chromatograph (Shimadzu GC-2010, Shimadzu Benelux, 's-Hertogenbosch, The Netherlands). Due to the low gas production of the control electrode, the headspace of the control electrode chamber could only be sampled/analyzed twice (at the beginning and at the end of the hydrogen yield test). The hydrogen production was calculated from the total gas production and the measured hydrogen fractions by means of a mass balance equation as described in ref *20*.

Next, both the biocathode and the control electrode were subjected to an inhibition test. During the inhibition test, the biocathode and the control electrode chamber of both electrochemical cells were flushed with carbon monoxide. After a carbon monoxide exposure period of 20 h, the headspace of both reactors was flushed again with nitrogen to remove the carbon monoxide.

Scanning Electron Microscopy (SEM). Electrode samples were fixed for 2 h in 3% glutaraldehyde, washed twice for 15 min in a PBS buffer (10 mM, pH 7.4), dehydrated in graded series of ethanol (10%, 25%, 50%, 75%, 90%, and 2 × 100% with 20 min for each stage), and dried in a desiccator. The samples were coated with gold and observed with a JEOL JSM-6480LV SEM [JEOL (Europe) BV, Nieuw-Vennep, The Netherlands] at an acceleration voltage of 6 kV (HV-mode, SEI detector).

Results and Discussion

Biocathode Startup. The current development during the three phases (Figure 1; Table 1) of the biocathode startup procedure is shown in Figure 2. During phase A, the bioelectrode was first started up as an acetate- and hydrogen-oxidizing bioanode at a potential of 0.1 V (Figure 2A). During this phase, the headspace of the bioelectrode chamber was continuously flushed with hydrogen gas. At t = 167 h, anodic current generation stabilized (~7 A/m^2) and the bioelectrode potential was lowered to −0.2 V.

Phase B of the biocathode startup procedure was started at t = 197 h (anodic current density: 5 A/m^2) by removing the 10 mM sodium acetate from the microbial nutrient medium and replacing it with 10 mM sodium bicarbonate. Hydrogen gas remained as the only available electron donor for anodic current generation. Two tests were done to investigate whether the anode was indeed oxidizing hydrogen: (i) the supply of hydrogen to the bioelectrode chamber was twice increased by changing the liquid recycling rate over the gas washing bottle from 250 to 500 mL/min to see whether anodic current generation would increase, and (ii) the hydrogen flushing was twice replaced by nitrogen flushing to see whether anodic current generation would decrease (Figure 2B). Both tests indeed confirmed that hydrogen was oxidized and suggested that the electrochemically active micro-organisms had an active hydrogen metabolism.

Next, in between phases B and C, the proper bioelectrode potential for biocathode operation was determined by means of a polarity reversal scan (see Figure S3, Supporting Information). During the polarity reversal scan, anodic current generation stopped at about −0.3 V, which is a typical open circuit potential for a bioanode (*3*). Cathodic current

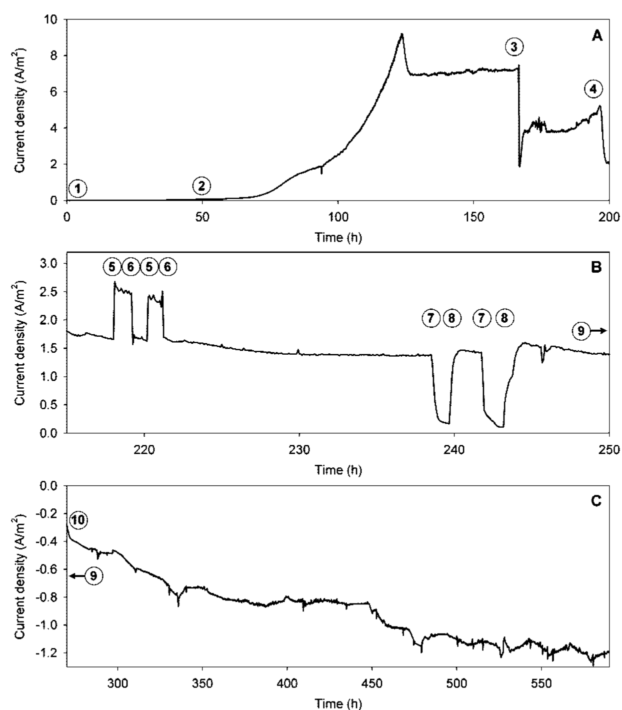

FIGURE 2. Current development during the three-phase biocathode startup procedure: (A) startup of an acetate- and hydrogen-oxidizing bioanode after inoculation with a mixed culture of electrochemically active micro-organisms, (B) adaptation to hydrogen oxidation only, and (C) polarity reversal to a hydrogen-producing biocathode and adaptation. (1) Inoculation of the reactor at a bioelectrode potential of 0.1 V; (2) operation is switched from batch to continuous mode; (3) bioelectrode potential is lowered from +0.1 to −0.2 V; (4) sodium acetate is removed from the microbial nutrient medium and replaced by sodium bicarbonate; (5) liquid recycling rate over the gas washing bottle is increased from 250 to 500 mL/min; (6) liquid recycling rate over the gas washing bottle is decreased from 500 to 250 mL/min; (7) hydrogen flushing is replaced by nitrogen flushing; (8) nitrogen flushing is replaced by hydrogen flushing; (9) polarity reversal scan (see Figure S3, Supporting Information); (10) bioelectrode operation is controlled at a potential of −0.7 V, and hydrogen flushing is replaced by nitrogen flushing.

generation started at a bioelectrode potential of about −0.65 V, which is 0.23 V below the theoretical potential for hydrogen formation at pH 7 (−0.42 V). To stimulate the development of a hydrogen-producing biocathode, therefore, the bioelectrode potential was controlled at a slightly lower value of −0.7 V during phase C of the biocathode startup procedure. Hydrogen flushing was replaced by nitrogen flushing. From t = 270 h until t = 590 h, the cathodic current increased from −0.3 to about −1.2 A/m^2 (Figure 2C), which suggested that the consortium of electrochemically active micro-organisms was adapting to cathodic current generation.

Polarization Curves. After the biocathode startup, the performance of the biocathode was compared to that of a control electrode on the basis of polarization curves (Figure 3). The biocathode outperformed the control electrode with respect to current generation over the complete measuring range of the polarization curves. Furthermore, at a potential of −0.7 V, the biocathode even outperformed a platinum-coated titanium electrode that was used in previous experiments (*6*) under comparable conditions. At −0.7 V, the current density of the biocathode was about −1.1 A/m^2, which was about 3.6 times that of the control electrode (−0.3 A/m^2) and 2.4 times that of the platinum-coated titanium electrode (−0.47 A/m^2) used previously. The cathodic current density achieved by the platinum-coated titanium electrode at a

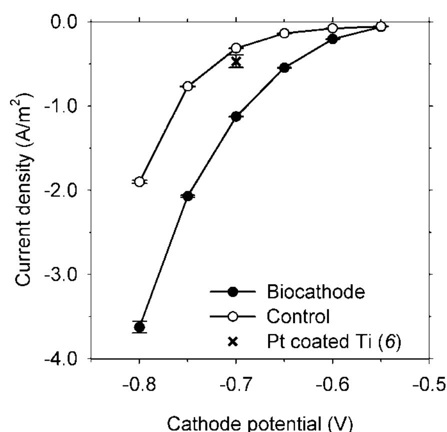

FIGURE 3. Polarization curves of the biocathode and the control electrode. The cross indicates the performance of a platinum-coated titanium electrode that was used in previous experiments under comparable conditions (*6*).

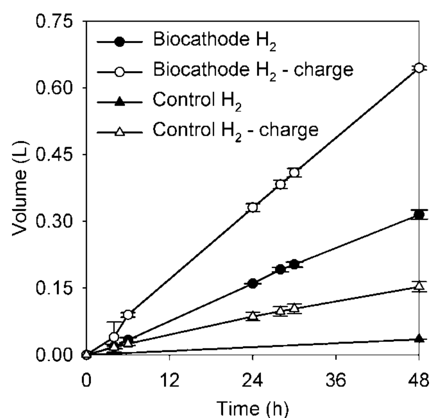

FIGURE 4. Measured hydrogen production (H_2) and expected hydrogen production based on the cumulative charge production (H_2, charge) of the biocathode and the control electrode during 48 h hydrogen yield tests at a cathode potential of −0.7 V.

potential of −0.7 V (−0.47 A/m^2) was already achieved by the biocathode at a potential above −0.65 V. This shows that under comparable conditions the biocathode suffers less overpotential than the platinum-coated titanium electrode used previously.

Hydrogen Yield Tests. After the biocathode startup, initially only methane was produced and no hydrogen could be detected. Presumably, the produced hydrogen was consumed directly by hydrogenotrophic methanogens that were naturally selected from the inoculum due to the presence of bicarbonate as a carbon source during the biocathode startup. Therefore, from about 150 h prior to performing the hydrogen yield tests, bicarbonate was removed from the microbial nutrient medium. This was a successful strategy to prevent hydrogenotrophic methanogenic consumption of hydrogen gas. Remarkably, these carbon-limited conditions have remained for over 1000 h and did not influence current generation of the biocathode (see Future Research and Outlook).

The results of the hydrogen yield tests are shown in Figure 4. During the hydrogen yield tests at a cathode potential of −0.7 V, the average current generation of the biocathode was about −1.2 A/m^2, while that of the control electrode was about −0.3 A/m^2.

During the hydrogen yield tests, the biocathode produced about 0.31 L of hydrogen gas, which was over 8 times the hydrogen production of the control electrode (0.04 L). This corresponds to an average volumetric hydrogen production rate during the hydrogen yield tests of about 0.63 m^3 H_2/m^3 cathode liquid volume/day for the biocathode chamber and 0.08 m^3 H_2/m^3 cathode liquid volume/day for the control electrode chamber. Furthermore, the cathodic hydrogen efficiency (i.e., the measured hydrogen production compared to the expected hydrogen production based on the cumulative charge production) of the biocathode was about 49% (i.e., $\sim 4e^- \rightarrow H_2$), whereas that of the control electrode was about 25% (i.e., $\sim 8e^- \rightarrow H_2$).

From the difference between the measured hydrogen production and the expected hydrogen production based on the cumulative charge production in Figure 4, it can be calculated that during the hydrogen yield tests about 0.33 L of hydrogen was lost from the biocathode chamber. Most of this hydrogen loss can be explained from the diffusional loss of hydrogen through the membrane (*4, 6*). The cation-selective membrane (Fumasep FKE) used in this experiment was over 3 times thinner than the Nafion 117 membrane we have used previously (*6*). Using an average hydrogen head-space concentration during the hydrogen yield tests of about 28.5% and assuming that the diffusion coefficient and solubility of hydrogen in the Fumasep FKE membrane are similar to those in Nafion (*21*), we estimate (*22*) that between 0.22 to 0.31 L of the produced hydrogen was lost through diffusion from the biocathode chamber into the anode chamber (see the Supporting Information). This amount of hydrogen explains about 67−94% of the hydrogen loss in the electrochemical cell with the biocathode. In future experiments, the use of thicker membranes and other membrane materials can significantly reduce the diffusional hydrogen loss. Furthermore, as reported previously (*6, 20*), the relative importance of the diffusional hydrogen loss becomes smaller at higher current densities as more hydrogen is produced at higher current densities, while the diffusional hydrogen loss remains about constant.

Indications for the Microbial Origin of the Biocathode. The experiments so far gave a strong indication that the developed biocathode for hydrogen production was indeed of microbial origin. During phase C of the biocathode startup procedure, cathodic current generation (Figure 2C) increased to about −1.2 A/m^2. This cathodic current generation was significantly higher than that of the control electrode (−0.3 A/m^2) and remained stable over long periods of time (>2000 h). These observations are in line with what would be expected from a growing, adapting, and regenerating consortium of electrochemically active micro-organisms. However, this indication does not yet unequivocally prove that the biocathode is of microbial origin.

Therefore, a carbon monoxide inhibition test was performed. Carbon monoxide is a well-known inhibitor for iron-hydrogenases, that is, the type of hydrogenases that are most associated with microbial hydrogen production (*23, 24*). The carbon monoxide inhibition test showed that the performance of the biocathode was indeed negatively affected by the presence of carbon monoxide and that this effect could be completely reversed by removing the carbon monoxide through nitrogen flushing (see Figure S4, Supporting Information). This agrees with the expected behavior of a microbial biocathode.

Subsequently, we investigated whether the effluent of the biocathode chamber could be used to inoculate and start up another biocathode. For this purpose, we inoculated the control electrode chamber by connecting the medium outlet of the biocathode chamber to the medium inlet of the control electrode chamber for a period of about 100 h (see Figure S5, Supporting Information). At first, no significant increase of the cathodic current generation of the control electrode could be observed, but after supplementing the microbial nutrient medium with a carbon source (10 mM sodium bicarbonate), cathodic current generation increased to about −1.1 A/m^2, effectively turning the control electrode into

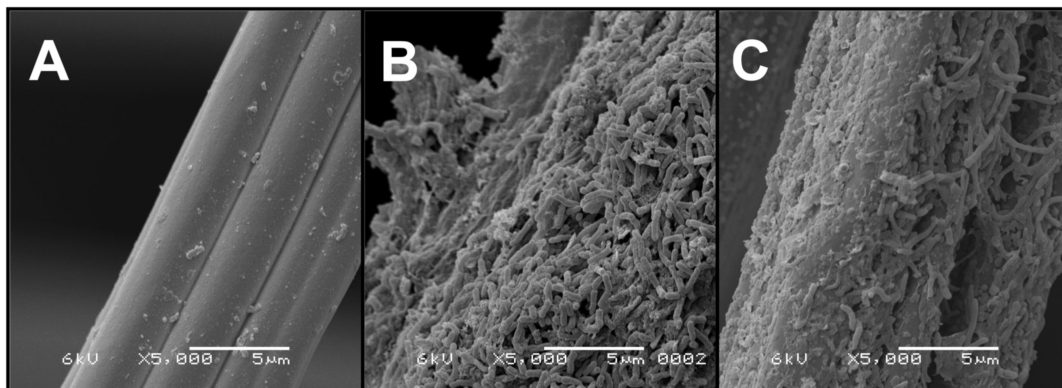

FIGURE 5. Scanning electron micrographs of fibers of graphite felt electrode samples: (A) unused electrode, (B) original biocathode that had been operated as a biocathode for over 2000 h, and (C) former control electrode that had been operated as a biocathode for less than 600 h.

another biocathode. After removal of the carbon source from the microbial nutrient medium, cathodic current generation remained stable and hydrogen production could be detected. The fact that the effluent of the biocathode could be used for the inoculation and startup of another biocathode is an extra indication that the biocathode is of microbial origin.

Finally, the electrochemical cells were disassembled, and samples of both biocathodes, that is, the original biocathode and former control electrode, were investigated with SEM (Figure 5). A well-developed biofilm could be observed on all electrode samples. This again indicated that the biocathode was of a microbial origin. Furthermore, the original biocathode (Figure 5B) generally had a thicker biofilm layer than the former control electrode (Figure 5C). This was not unexpected, as the original biocathode had also been operated as a bioanode during the biocathode startup and had been operated as a biocathode for over 2000 h, while the former control electrode had only been operated as a biocathode for less than 600 h.

Future Research and Outlook. In a previous study (*20*), we estimated that the volumetric production rate of biocatalyzed electrolysis can be improved to about 10 m^3 H_2/m^3 reactor liquid volume/day at an energy input of below 1 kWh/m^3 H_2. For the cathode performance, this objective implies that the cathode overpotential needs to be reduced to about 0.05 to 0.10 V at a current density of −5 to −10 A/m^2, which means that at pH 7 the cathode potential needs to be in the range −0.47 to −0.52 V at a current density of −5 to −10 A/m^2. The microbial biocathode of this study had a current density of about −1.2 A/m^2 at a potential of −0.7 V (i.e., cathode overpotential of 0.28 V), which shows that the performance of the microbial cathode needs to be improved. Nevertheless, the performance of the microbial biocathode of this study is already a significant improvement compared to our previous study with a platinum-coated titanium electrode as the cathode (*6*). Furthermore, there is no reason to assume that the microbial biocathode cannot be improved much further. In the past decade, power production of MFCs has increased by several orders of magnitude (*25*), partly due to a better understanding of the bioanode. To get a better understanding of the microbial biocathode, future research work among others should focus on identifying the responsible microbial species that catalyze cathodic hydrogen production, elucidating their electron transfer mechanisms (electron shuttles, direct contact, and nanowires (*2*)), and understanding their ATP generation mechanisms. As just after the startup procedure the biocathode produced only methane, an interesting topic for further investigation is whether methanogens were also responsible for the hydrogen production and whether they were capable of accepting electrons from the cathode directly. Up till now, methanogens have never been demonstrated to be electrochemically active. Alternatively, the methanogens could have produced the methane from hydrogen that was produced by nonmethanogenic hydrogen producing electrochemically active microorganisms.

Another important topic that needs a better understanding is the role and the effects of the carbon-limited conditions that were applied in this study to prevent hydrogenotrophic methanogenic consumption of the produced hydrogen gas. These carbon-limited conditions were maintained for over 1000 h without any significant loss of performance. As it seems unlikely that micro-organisms survive that long without any carbon source, it is probable that there has been an alternative carbon source present in the system. The electrochemically active micro-organisms might have used decaying biomass as a carbon source that was likely present after the biocathode startup, or they might have derived the carbon from polymeric storage products that were stored during the biocathode startup (*26*). Furthermore, some carbon dioxide might still have been present in the influent (although it was flushed with nitrogen) or might have come through the membrane from the counter electrode chamber. It is important to investigate what the optimal carbon source availability should be for maintaining a well-performing microbial biocathode as the removal of the bicarbonate from the microbial nutrient medium was a successful strategy to prevent the hydrogenotrophic methanogenic consumption of hydrogen.

Nevertheless, the results described in this paper comply with the objective of a microbial biocathode system for hydrogen production that is based on a naturally selected mixed culture of electrochemically active micro-organisms. This is an important finding, as it allows for the use of inexpensive electrode materials and holds great promise for the cost-effective production of hydrogen gas from wastewaters through biocatalyzed electrolysis.

Acknowledgments

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Supporting Information Available

Design of the electrochemical cells, schematic overview of the experimental setup, figure of the bioelectrode polarity reversal scan, calculation of the diffusional hydrogen loss through the membrane during the hydrogen yield tests, figure of the current development of the biocathode and the control electrode in response to carbon monoxide flushing, and a figure of the cathodic current development of the control electrode after inoculation. This material is available free of charge via the Internet at http://pubs.acs.org.

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