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# A Hybrid Microbial Fuel Cell Membrane Bioreactor with a Conductive Ultrafiltration Membrane Biocathode for Wastewater Treatment

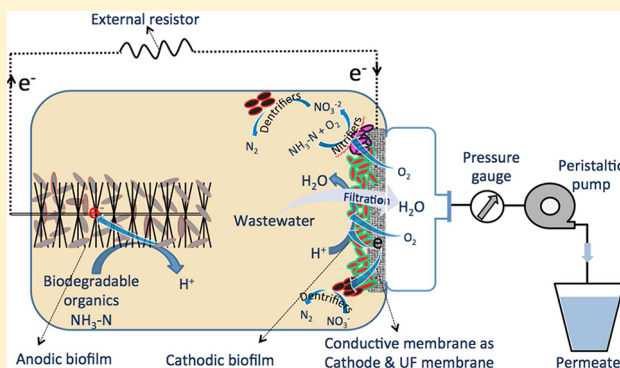
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## Supporting Information

**ABSTRACT:** A new hybrid, air-biocathode microbial fuel cell-membrane bioreactor (MFC-MBR) system was developed to achieve simultaneous wastewater treatment and ultrafiltration to produce water for direct reclamation. The combined advantages of this system were achieved by using an electrically conductive ultrafiltration membrane as both the cathode and the membrane for wastewater filtration. The MFC-MBR used an air-biocathode, and it was shown to have good performance relative to an otherwise identical cathode containing a platinum catalyst. With 0.1 mm prefiltered domestic wastewater as the feed, the maximum power density was 0.38 W/m<sup>2</sup> (6.8 W/m<sup>3</sup>) with the biocathode, compared to 0.82 W/m<sup>2</sup> (14.5 W/m<sup>3</sup>) using the platinum cathode. The permeate quality from the biocathode reactor was comparable to that of a conventional MBR, with removals of 97% of the soluble chemical oxygen demand, 97% NH<sub>3</sub>-N, and 91% of total bacteria (based on flow cytometry). The permeate turbidity was <0.1 nephelometric turbidity units. These results show that a biocathode MFC-MBR system can achieve high levels of wastewater treatment with a low energy input due to the lack of a need for wastewater aeration.



## INTRODUCTION

Rising demands for clean water supplies continue to drive the search for new and sustainable water sources worldwide. Wastewater reclamation and reuse is essential to avoid water shortages and degradation of the environment. Sustainable and low-energy demanding technologies are especially important for achieving these goals. A microbial fuel cell (MFC) is a technology that uses exoelectrogenic biofilms on the anode to degrade organic matter and produce renewable energy in the form of electricity.<sup>1</sup> However, MFCs may not be sufficient as a stand-alone wastewater treatment technology to achieve high effluent quality. Several methods have been proposed to improve treatment, such as integrating the MFC with a nitrification step,<sup>2</sup> submerging the MFC reactor in the aeration tank of an activated sludge process,<sup>3</sup> combining the MFC with a sequencing batch reactor<sup>4</sup> or a membrane-aerated biofilm process,<sup>5</sup> or integrating the MFC into a rotating biological contactor.<sup>6,7</sup> The main problem with these combined systems, however, is that in all cases the effluent quality is poor without subsequent sedimentation or filtration to remove particulates, and also that some of these systems require wastewater aeration.

Treatment technologies such as membrane bioreactors (MBRs) have gained increasing popularity in the context of

wastewater reclamation and reuse,<sup>8</sup> and are of special interest due to their ability to retain and degrade particulate biomass. MBRs use membrane modules for solids and liquid separation, and biological reactions for organic carbon and nitrogen removal. Compared to conventional technologies (e.g., activated sludge processes), MBRs have a smaller footprint, and produce a higher-quality effluent and less sludge. The idea of combining MFCs with MBRs for wastewater treatment has recently been considered,<sup>9–11</sup> but so far these systems have not truly integrated the two processes (i.e., the two processes were separated in the same reactor), they have failed to achieve nitrogen removal, and most designs have required energy-demanding aeration. In one approach, a 40- $\mu$ m pore-diameter stainless steel mesh was used in a tubular MFC to filter the treated wastewater, but wastewater aeration was needed.<sup>9</sup> In another approach, the wastewater flowed into the MFC anode chamber and then out into a surrounding chamber that contained an MBR, but aeration was again required to provide oxygen to the cathode and for scouring the membranes.<sup>10</sup> In a

Received: March 11, 2013

Revised: August 30, 2013

Accepted: September 9, 2013

Published: September 9, 2013



third design, a hollow-fiber membrane module was inserted into an air-cathode tubular MFC reactor,<sup>11</sup> but complete nitrification was not achieved.

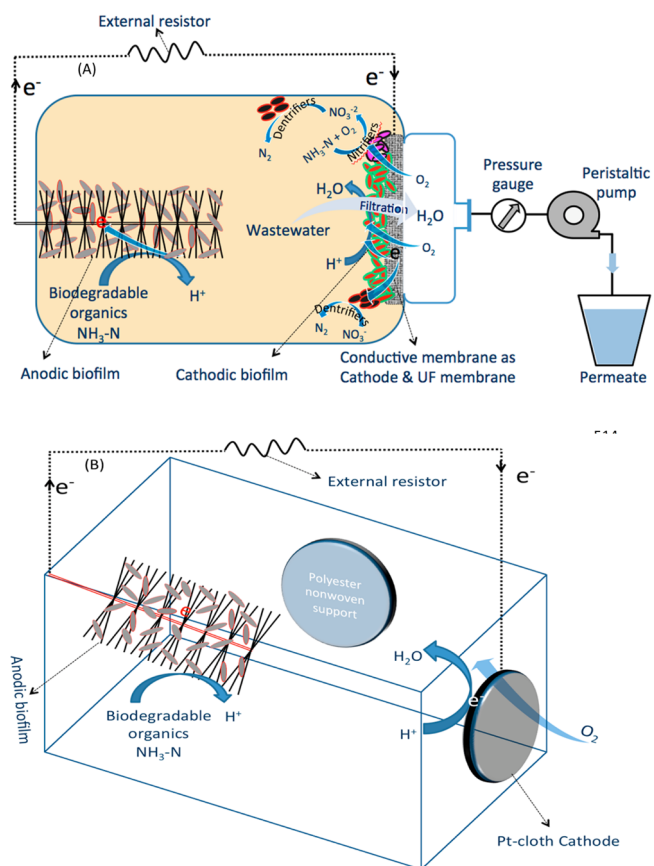
In this study we designed a truly integrated and effective single-chambered, air-biocathode MFC-MBR that avoided the need for wastewater aeration, and it achieved simultaneous chemical oxygen demand (COD) and nitrogen removal. The system consisted of an electrically conductive ultrafiltration (UF) membrane that functioned as both a biocathode for electricity generation, with passive oxygen transfer to the cathode, and as a membrane for wastewater filtration. This combination avoided the need for wastewater aeration or separate membrane modules.

## MATERIALS AND METHODS

**Preparation of Conductive UF Membranes.** The conductive UF membranes used as biocathodes (BCs) were prepared by dispersing multiwalled carbon nanotubes (MCNTs, Heji Inc., Hong Kong, China) as a multilayer on top of a 7 cm<sup>2</sup> polyester nonwoven (Sojitz Europe, Germany) membrane base (40 μm average pore size). According to the manufacturer, the MCNTs have a specific surface area of 117 m<sup>2</sup>/g, an average diameter of 8–15 nm, a length of 30 μm, and an electrical conductivity of 100 S/cm. The MCNTs (22 mg, 3.15 mg MCNT/cm<sup>2</sup>) were suspended in *N*-methyl-2-pyrrolidone (Sigma-Aldrich, St. Louis, MO) at a concentration of 1 mg/mL, vortexed and then sonicated (Branson, Probe Sonifier 450) for five cycles of 4 min at 120 W and 40 kHz, followed by cooling for 2 min. The slurry was then transferred onto the polyester nonwoven material and deposited slowly through vacuum filtration. The resulting UF membrane was then washed by filtration using ethanol (50 mL) followed by deionized water (500 mL).

Platinum-based MCNT/polyester UF membrane cathodes (PtC; 7 cm<sup>2</sup>) used as Pt-catalyzed controls were prepared in a similar fashion by mixing 4.66 mg of 60% Pt/Vulcan carbon with 22 mg MCNTs (0.4 mg/cm<sup>2</sup> of Pt, and 3.15 mg/cm<sup>2</sup> of MCNT).

**Reactor Construction and Operation.** The cube-shaped MFC-MBR reactors (BC and PtC reactors) had a total working volume of ~40 mL (5 cm long cylinder, internal diameter of 3 cm cut within a 5 cm cubic of plexi-glass column), and were constructed as previously described<sup>12</sup> except for an additional hole used in some tests to remove fluid. The graphite brush anode (2.5 cm diameter × 2.5 cm length; fiber type PANEX 33 160 K, ZOLTEK) was positioned in the center of the reactor facing the cathode (Figure 1A). The distance between the edge of the anodic brush and the cathode was 1 cm. The cathodes were sandwiched between the main reactor body and a plexiglass end plate, sealed with a rubber “O ring” gasket, and bolted together. The cathode end plate had a centered threaded hole (3.0 cm in diameter) that was fitted with threaded male connection to enable application of a transmembrane pressure (TMP) to remove the fluid. A piece of titanium mesh (0.02 mm thickness, Goodfellow, UK) was used as the current collector and cathode connection. Both BC and PtC reactors were operated in duplicate and in closed circuit mode. Duplicate reactors were also constructed and operated the same way as the BC reactors but in open circuit mode (no electrical connection). A third reactor (NC) was similarly prepared (i.e., configuration and anode similar to closed-circuit BC reactors) but no fluid was removed through the cathode. Instead, a 40 μm pore size filter placed on the side of this



**Figure 1.** Schematic representation of (A) the BC reactor with conductive UF membrane used as air-biocathode. (B) NC reactor with Pt and carbon cloth cathode.

reactor over the side hole was used to pump out the water (Figure 1B). The cathode in NC reactor was prepared using Pt (0.4 mg/cm<sup>2</sup>) and carbon cloth (30% wet-proofing). Reactor NC was operated in duplicate and in closed circuit mode.

Domestic wastewater was collected from a local wastewater treatment plant (Al-Rwais, Jeddah, KSA) and used as the fuel after being prefiltered through a 106 μm sieve (ASTM, model 140, Germany). The composition of the wastewater is shown in Table 1. Due to lack of sanitary sewage collection systems in many areas in the city of Jeddah, wastewater is usually collected

**Table 1.** Feed and Permeate Chemical Quality for the Two Reactor Configurations

| parameter                                 | feed <sup>a</sup> | permeate <sup>a</sup> |             |
|---|-------------------|-----------------------|-------------|
|   |                   | BC reactor            | PtC reactor |
| NH <sub>3</sub> -N (mg/L)                 | 32 ± 4.28         | 0.8 ± 0.41            | 1.4 ± 0.73  |
| NH <sub>3</sub> -N removal (%)            |                   | 97.3 ± 1.6            | 95.2 ± 2.9  |
| TN removal (%) <sup>b</sup>               |                   | 97.7 ± 1.9            | 96.0 ± 3.3  |
| nitrification efficiency (%) <sup>c</sup> |                   | 97.3 ± 2.3            | 95.2 ± 4.1  |
| SCOD (mg/L)                               | 1080 ± 160        | 27 ± 7.1              | 33 ± 1.5    |
| SCOD removal (%)                          |                   | 97.3 ± 1.1            | 96.9 ± 0.6  |

<sup>a</sup>Values are averages ± standard errors of feed permeate collected in the last three reproducible cycles of voltage generation (Filled round circles in Figure 3). <sup>b</sup>TN removal (inorganic) = 100 - {[(NH<sub>4</sub><sup>+</sup>-N) + (NO<sub>2</sub><sup>-</sup>-N) + (NO<sub>3</sub><sup>-</sup>-N)]<sub>effluent</sub> / [(NH<sub>4</sub><sup>+</sup>-N) + (NO<sub>3</sub><sup>-</sup>-N)]<sub>influent</sub>} × 100%. <sup>c</sup>Nitrification efficiency = 100 - {[(NH<sub>4</sub><sup>+</sup>-N)<sub>effluent</sub> + (NO<sub>2</sub><sup>-</sup>-N)<sub>effluent</sub>] / [(NH<sub>4</sub><sup>+</sup>-N)<sub>influent</sub>] × 100%.<sup>7</sup>

from septic tanks by tanker trucks and emptied directly to this treatment plant. This explains the high SCOD of the wastewater used in this study. At the end of each fed-batch cycle, when the voltage decreased to  $<0.3$  mA, the solution (40 mL) was pulled through the membrane (BC and PtC reactors) or through the filter (NC reactor) using a pump (Masterflex L/S, Cole-Parmer, Vernon Hills, IL). Open-circuit control reactors were operated under the same conditions. All reactors were operated at room temperature ( $23 \pm 3$  °C). The TMP was measured using a pressure sensor (model 68075, Cole-Parmer) attached to the filtrate line, and recorded using a data acquisition device (LabJack U6, LabJack Corporation, Lakewood, CO) connected to a personal computer, and converted into pressure using a standardized calibration curve (<http://labjack.com/support/software>).

**Electrochemical Analyses.** The cell voltage ( $E$ ) under 10  $\Omega$  external resistance was recorded using a data acquisition system (2700, Keithley, Cleveland, OH) connected to a personal computer via high resolution custom built analog cable. The current ( $I$ , in A) was calculated from the cell voltage according to Ohm's law ( $I = E/R$ ) where  $R$  ( $\Omega$ ) is the external resistor used. The Coulombic efficiency (CE, %) was calculated based on starting and final soluble CODs (SCOD) as given in literature.<sup>13</sup>

Linear sweep voltammetry (1 mV/s) was used to obtain polarization and power density data, with the power and current normalized by the cathode projected area. The redox behavior of the anodic and cathodic biofilms was studied using cyclic voltammetry (CV, CH Instrument, Austin, TX). CV analysis with a standard three-electrode arrangement was performed with the anode or cathode as working electrode, a platinum mesh (10 cm<sup>2</sup>) as counter electrode, and a Ag/AgCl reference electrode (3 M KCl, 0.205 V vs a normal hydrogen electrode, NHE). CV was performed at 1 mV/s scan rate. The voltammograms were potentially checked between  $-1.0$  to  $0.5$  V vs Ag/AgCl. However, the potential windows were fixed based on redox process observed from the individual electrodes. The same wastewater feed was used as the electrolyte during analysis. Before starting the experiment, the electrochemical response of the prepared membranes was also studied using CV probing with potassium ferricyanide (50 mM in 100 mM sodium phosphate buffer, pH 7.0).

**Chemical and Microscopic Analyses.** All wastewater samples were filtered through a 0.2  $\mu$ m pore diameter syringe filters (Grace Alltech, Deerfield, IL) prior to chemical analysis. SCOD, NH<sub>3</sub>-N (Amver, catalog no. 26069-45, HACH) and NO<sub>3</sub><sup>-</sup> (NitraverX, catalog no. 26053-45, HACH) were analyzed using a spectrophotometer (HACH DR/2010, HACH Co., Loveland, CO). The turbidity, in nephelometric turbidity units (NTU), was measured using turbidimeter (HACH 2100AN).

Bacterial cell counts in the feed and permeate were measured by flow cytometry (BD Accuri C6 flow cytometer, BD Biosciences, Franklin Lakes, NJ). Samples (700  $\mu$ L) were transferred to a sterile Eppendorf tube and incubated at 35 °C for 10 min prior to staining with SYBR Green I (7  $\mu$ L of 100 $\times$  stock solution in 700  $\mu$ L sample), vortexed, and then incubated again at 35 °C for 10 min.<sup>14</sup> Samples (200  $\mu$ L) were then transferred to a 96-well plate for cell counting.

The morphology and MCNT distribution and biofilms on the UF membranes were examined using a scanning electron microscope (SEM) and a transmission electron microscope (TEM). Prior to SEM imaging, new and biofilm covered membranes were dehydrated in a series of graded alcohol

solutions and oven-dried (2 h at 30 °C). The samples were plunged into liquid nitrogen, sectioned, and mounted either flat onto an aluminum stub using thin aluminum tape or vertically inside a machined slot aluminum stub using carbon paste. SEM imaging (Quanta 200D, FEI, The Netherlands) was performed using an accelerating voltage of 25 kV and working distance of 5 mm, after sputter-coating the samples with gold palladium for 90 s at 5 mA current in an argon atmosphere. For TEM analysis, membranes were embedded in low-viscosity epoxy resin (Agar R1165) and cured at 60 °C for 24 h. Ultrathin sections (80 nm) were prepared using an Ultramicrotome Leica EM UC6 (Leica Microsystems GmbH, Germany) and then placed on a carbon-coated copper grid. The grids were imaged using a FEI Tecnai 12 TEM (FEI Company) operating at 120 keV.

**Pore Size Distribution of the Conductive UF Membrane.** A Porolux 1000 porometer (IB-FT GmbH, Germany) was used to determine the mean pore size distribution of the conductive UF membranes, by the wet-up/dry-up method with the analysis done using an automated capillary flow porometer system software. A 20 mm diameter membrane sample was fully wetted with porefil liquid (fluorinated hydrocarbon with surface tension 16 dyn/cm) and sealed in the chamber. Pure nitrogen gas was added up to a point where liquid dislocation was detected to identify the first bubble point. The pressure was then continuously increased and the nitrogen permeation flow rate was measured until all pores were empty of porefil, and the sample was considered dry. Nitrogen pressure and permeation flow rates through the dry sample were also recorded. Based on the nitrogen flow rates through the wet and dry membranes, the mean flow pore size and the pore size distribution were calculated.

## RESULTS AND DISCUSSION

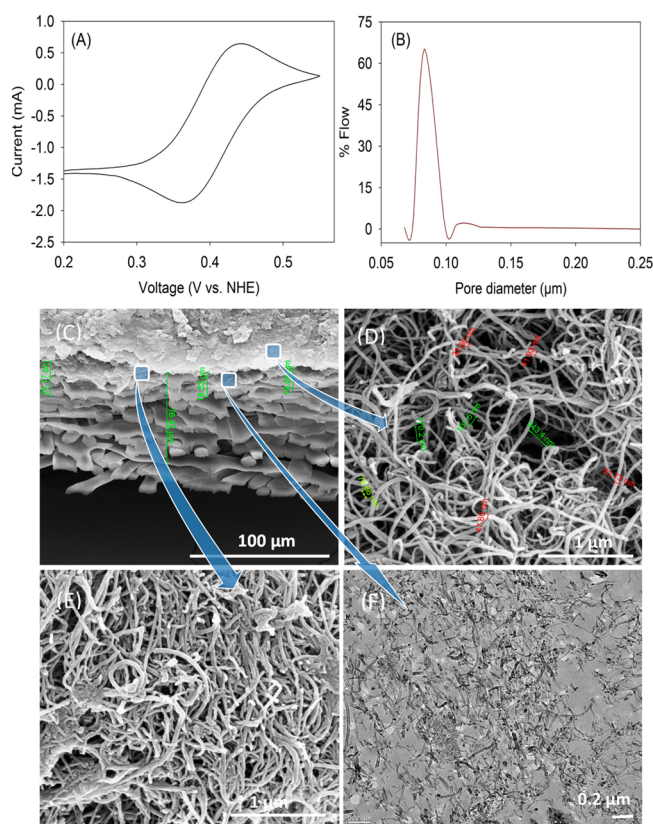
### Characterization of the Conductive UF Membranes.

The characteristic electrochemical response from CV probing (Figure 2A) coupled with the results of pore size distribution analysis (average pore size of  $\sim 65$  nm) (Figure 2B) revealed that the manufactured membranes of the BC reactors could serve as a conductive electrode material and as a UF membrane. SEM and TEM images of MCNTs in the membranes (Figures 2C–F) showed densely packed thin fiber, forming a tridimensional porous structure. The fiber arrangement led to a relatively broad pore size distribution, and membrane thickness varied between 16 to 28  $\mu$ m (Figures 2D–F).

### Performance of the Conductive UF Membranes as Air-Cathodes.

Reactors with BC or PtC cathodes were examined over multiple fed-batch cycles (Figure 3). Current generation occurred much later for the BC reactors ( $83 \pm 7$  h; Figure 3A) than for the PtC reactors ( $6.9 \pm 0.7$  h; Figure 3B). The current of the BC reactors reached a maximum at  $1.5 \pm 0.1$  mA after the ninth cycle (Figure 3A). The PtC reactor had a higher peak current ( $2.0 \pm 0.2$  mA, Figure 3B) than the BC reactor, but the current was highly variable during start up, as shown by inconsistent results for the duplicate reactors during cycles 3 to 6. This instability was likely due to biofouling, as replacement of the cathodes improved performance (Figure 3B). Pt cathode biofouling over time has previously been identified as a problem in MFCs.<sup>15</sup> During the startup phase, the low current in the PtC reactor is attributed to both fouling of the cathode and to the time required to establish the anodic biofilm. At later stages (e.g., after replacement of biofouled



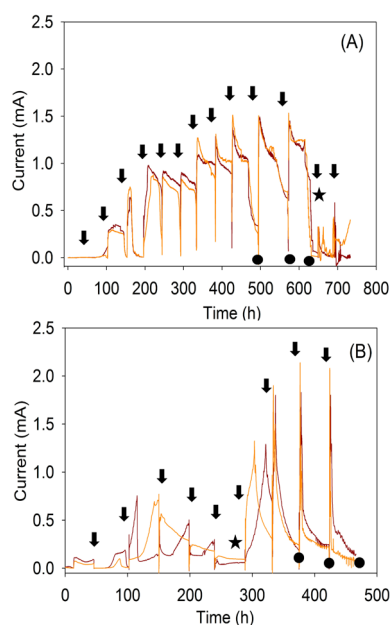


**Figure 2.** (A) Cyclic voltammogram (1 mV/s) response of conductive UF membrane in 100 mM sodium phosphate buffer (pH 7.0) containing 50 mM potassium ferricyanide. (B) Pore size analysis of the conductive UF membrane. (C) SEM image of MCNT filter cross section. (D) SEM image of top view of MCNT filter at high magnification. (E) SEM image of MCNT filter cross section at high magnification. (F) TEM image of MCNT filter cross section at high magnification.

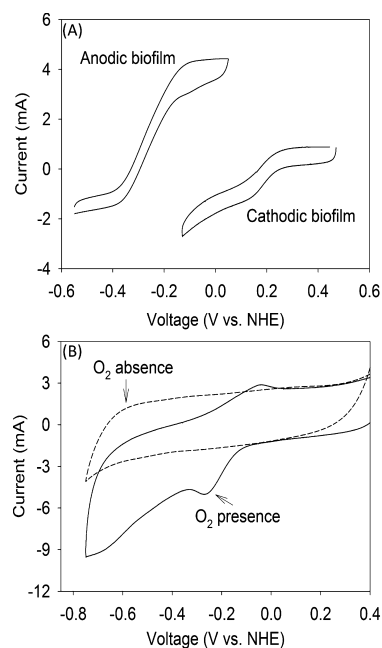
cathode), the drop in current was less, since the anodic biofilm has already been established.

The reactor was operated as an MBR at the end of each fed-batch cycle. Suction was applied to the cathode membrane to collect permeate, and a new cycle was started upon adding fresh medium into the reactor. The performance of the cathode did not appear to be disturbed by this process as there was no substantial change in cell voltages over subsequent cycles. The average cycle time or hydraulic retention time (HRT) varied between 2 and 3 days since the current drop to  $<0.3$  mA varied from batch to batch (Figure 3). The HRT was considered to be similar to the cycle time as a small amount of settled sludge ( $\sim 10\%$  of reactor volume) of undetermined solid content was retained in the reactor at the end of each cycle.

The cathode was shown to be functioning as a biocathode using two approaches. First, when the BC was replaced after 12 cycles with a new cathode, the maximum cell voltage decreased by  $\sim 87\%$  to  $3.3 \pm 0.2$  mV (Figure 3A). Second, an in situ CV analysis was conducted to compare the BC and new cathodes (Figure 4). The CV spectra of the BC showed redox bioprocesses for oxygen reduction (Figure 4) with a characteristic sigmoid shape coupled with electron turnover at  $\sim 0.2$  V vs NHE, indicating a role of microorganisms in voltage generation. The onset of the oxygen reduction reaction (ORR) using the biocathode began at  $\sim 0.2$  V (NHE) (Figure 4), which is  $\sim 0.4$  V earlier than that of the clean cathode. These observations

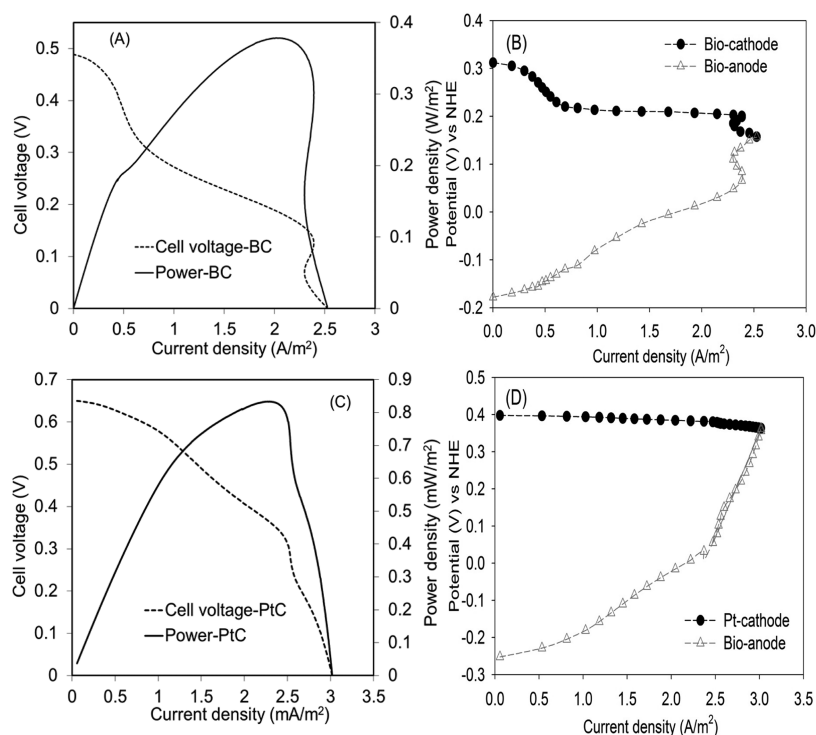


**Figure 3.** Profiles of current generation over time under  $10 \Omega$  external resistor: (A) reactor BC and (B) reactor PtC. Filled round circles represent the time intervals of permeate subjected for chemical/microbial analysis. Asterisks in figures A and B indicate the time when the bio/chemical cathodes were replaced with new cathodes. Arrows represent batch feeding.



**Figure 4.** In-situ CV (1 mV/s) analysis of (A) biofouled and (B) new cathode. Domestic wastewater was used as electrolyte with the new cathode.

show that the biofilms enriched on the MCNT conductive UF membrane cathode were catalytically active and thus contributing to a reduction of the cathode overpotential. While not as effective as new platinum in lowering the cathodic overpotential, the performance was maintained by the biocathode compared to the PtC cathode, which had to be replaced to obtain good performance.



**Figure 5.** Polarization behavior of BC and PtC reactors. Potentiodynamic sweep (1 mV/s) technique was used to generate the power curves. (A) Polarization curves for BC reactor. (B) polarization behavior of individual bioelectrodes of BC reactor during potentiodynamic sweep voltammetry analysis. Figures C and D represent the polarization curve of PtC reactor and respective electrodes behavior. Cathode projected area ( $7 \text{ cm}^2$ ) was used for current and power density calculations. Prior to polarizing the cells, a new batch of domestic wastewater ( $\sim 1000 \text{ mg COD/L}$ ) was added to the reactors.

CV analysis of the anodic biofilms that developed in the BC reactor produced voltammograms similar to those reported for *Geobacter sulfurreducens*,<sup>16–19</sup> based on a redox potential centered at  $\sim -0.27 \text{ V}$  vs NHE (Figure 4), as well as other mixed consortia anodic biofilms.<sup>20,21</sup>

**Maximum Power Densities.** Based on polarization data, the maximum power density for the BC reactor was  $0.38 \text{ W/m}^2$  ( $6.8 \text{ W/m}^3$ ,  $2.0 \text{ A/m}^2$ ; OCV of  $0.49 \text{ V}$ ) (Figure 5A). When the biocathode was replaced with a new conductive UF cathode, the maximum power production was greatly reduced ( $0.014 \text{ W/m}^2$ , Supporting Information (SI) Figure S1). The PtC reactor had a higher maximum power density of  $0.82 \text{ W/m}^2$  ( $14.5 \text{ W/m}^3$ ) (Figure 5C), as did the MFC with the Pt and carbon cloth air-cathode (NC,  $1.04 \text{ W/m}^2$ ,  $18.6 \text{ W/m}^3$ ) (SI Figure S2).

The maximum power density obtained with the BC reactor ( $6.8 \text{ W/m}^3$ ) was comparable to those obtained in other types of integrated MFC-MBRs ( $4.35 \text{ W/m}^3$  and  $6.0 \text{ W/m}^3$ ),<sup>9,10</sup> even though domestic wastewater was used here compared to acetate in previous studies. It is well-known that much higher power densities can be produced using acetate than with domestic wastewater.<sup>22</sup> Power densities in these previous MFC-MBR studies were obtained using aqueous phase (aerated) biocathodes, not direct air cathodes as done here. Compared to MFCs that use air-biocathodes (e.g.,  $554 \text{ mW/m}^2$ ),<sup>23</sup> power densities here were slightly lower ( $380 \text{ mW/m}^2$ ) due to the use of domestic wastewater. However, the power density obtained in this study was higher compared to studies that use domestic wastewater (e.g.,  $72\text{--}240 \text{ mW/m}^2$ ).<sup>24,25</sup>

**Potential Power Limitations.** Electrode potentials measured during polarization tests (Figure 5B) and in situ CV analysis of the biocathode (SI Figure S3) revealed some

possible factors limiting overall power generation in the BC reactor. During cell polarization, the cathode potential curve for BC ( $0.55 \text{ A/m}^2$ , Figure 5B) showed a faster drop in current density than for the PtC reactors ( $2.35 \text{ A/m}^2$ , Figure 5D), which significantly reduced the power yield due to increased cathode over potentials. The cathode potentials were generally quite stable, with the greatest changes observed in the anode potentials. The biocathode potentials ( $150\text{--}300 \text{ mV}$ ) remained positive, and were all much lower than the potential of  $350\text{--}600 \text{ mV}$  that has recently been shown to limit increases in current densities of biocathode MFCs.<sup>23</sup> This lower potential is likely due to using real wastewater in our case as well as to different biocathodic enrichment procedures (e.g., using resistors in our study versus potentiostatic control<sup>23,26</sup>). Results from in situ CV analysis (SI Figure S3) suggest that the biocathode in the BC reactor was limited by a lack of sufficient oxygen availability in the biofilm matrix. A better conductive membrane fabrication with a more extended three-dimensional pore network and lower thickness would presumably allow the biocathode to overcome limitations associated with carbon/nutrient and oxygen fluctuations in the ORR. For PtC reactor, the anode polarization (Figure 5D) exhibited a limiting current at higher current densities rather than a cathodic potential drop since the cathode had only a small contribution to the cell polarization due to the presence of Pt catalyst. The profile of individual electrode potentials (nearly constant cathode potentials; Figures 5B, D) observed in this study was different from previous reports<sup>23,26</sup> using air-cathode MFCs. This difference could be attributed to (1) the rate of fermentation of fermentative substrates in real wastewater slows the kinetics of exoelectrogenic catalysis compared to nonfermentative substrates (acetate), which was used as the sole carbon source

in previous studies;<sup>23,26</sup> and (2) in the BC and PtC reactors, the presence of MCNT (in addition to Pt/biofilm catalysis) promotes the cathodic reaction by providing indigenous ORR active sites, high surface area and high electrical conductivity.

**Coulombic Efficiencies.** The Coulombic efficiencies of the BC reactor ( $8.5\% \pm 4.5$ ) were much less than the PtC reactor ( $47.3\% \pm 1.5$ ), with both results showing that processes other than current generation were predominant in the reactors. However, the low CE produced by the BC reactor here is in general agreement with other MFC tests using real wastewater.<sup>22</sup> In addition, the MFC-MBR system used by Wang et al.,<sup>10</sup> produced only a CE of 1.5% using acetate.

**Effectiveness of Wastewater Treatment.** At the end of several fed-batch cycles (filled round circles in Figure 3), the permeate from the UF cathode was collected and examined for chemical and bacterial analysis. The removals measured for the BC reactors were  $97.3 \pm 1.1\%$  for SCOD,  $97.3 \pm 1.6\%$  for  $\text{NH}_3\text{-N}$ , and  $97.7 \pm 1.9$  for TN, which were similar to those obtained for the PtC reactor (Table 1). No significant differences in removal efficiencies for these chemical species were measured between the reactor effluent (samples collected from the reactor chamber) and permeate. This level of performance of chemical removal efficiencies is typical of MBRs that do not produce electricity.<sup>27</sup>

Much lower SCOD ( $64 \pm 5\%$ ) and  $\text{NH}_3\text{-N}$  ( $57 \pm 4.2\%$ ) removal efficiencies were obtained for the BC reactors when the biocathodes were replaced with new conductive UF cathodes lacking a biofilm (asterisks in Figure 3A). This observation indicates that biocathode had an important role in COD and  $\text{NH}_3\text{-N}$  removal over the course of a fed-batch cycle. In open-circuit reactors (i.e., no current flow), the average SCOD ( $52 \pm 3.5\%$ ) and  $\text{NH}_3\text{-N}$  ( $39 \pm 9\%$ ) removals were much less than those of the BC and PtC reactors, indicating that current generation increased performance, as reported in previous studies.<sup>2,7,28</sup> Nitrogen removal in BC and PtC reactors could have been due to (i) simultaneous nitrification-denitrification due to the microaerobic environments of the air-cathode;<sup>2,28</sup> (ii) through direct  $\text{NH}_3$  oxidation by the anodic biofilm,<sup>29</sup> although previously reported CEs by this route are very low ( $<0.4\%$ ); and/or (iii) reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  by denitrifiers using the cathode as the electron donor.<sup>2,7,28</sup> It is possible that the improved TN removal observed in the BC and PtC reactors could be due to the reduction in competition between denitrifiers and other heterotrophs. This stems from the fact that the electron donor for denitrification in the BC and PtC reactors was provided from the cathode and from the oxidation of organic matter present in wastewater, whereas in the open-circuit reactors the electron donor for denitrification was provided only from the oxidation of organic matter present in wastewater where denitrifiers and aerobic heterotrophs competed for the available COD. Although it is not known which of the above processes contributed to nitrogen removal, it is clear that the MFC-MBR system was effective at nitrogen removal.

**Microorganisms Removal.** High permeate quality was obtained for all reactor tests based on an effluent turbidity  $<0.1$  NTU, consistent with previous studies.<sup>9–11</sup> Flow cytometry, a culture-independent molecular biology technique, was also used here to evaluate the microbiological permeate quality. The percentage of bacterial cells removed due to filtration through the cathode with the BC reactor ( $91 \pm 3\%$ ) was larger than that obtained using the PtC reactor ( $80 \pm 7\%$ ) (SI Figure S4). This

was likely due to the formation of the cathodic biofilm, which acted as an additional filtration layer.

The microbiological permeate quality of MBRs is normally assessed by turbidity measurements and culture-dependent methods. The concentrations of bacteria reported can vary over wide ranges<sup>30–32</sup> in MBR effluents when using culture-based methods. However, culture-based methods do not detect cells that are injured or that have entered a viable but nonculturable (VBNC) state, thus leading to false-negative results.<sup>33</sup> Flow cytometry measures both culturable and nonculturable bacterial cells,<sup>34</sup> thus giving a more comprehensive assessment of the microbiological permeate quality. The permeates of the MFC-MBRs contained  $1.8 \pm 0.66 \times 10^5$  cells/mL (BC) or  $3.8 \pm 0.13 \times 10^5$  cells/mL (PtC). Since data on MBR permeate quality using culture-independent methods such as flow cytometry are limited, permeates from two local full-scale MBR plants were also analyzed using flow cytometry. Results from the two full-scale MBR plants showed similar amounts of bacteria in the effluent (MBR plant 1:  $7.7 \times 10^5$ ; MBR plant 2:  $2.1 \times 10^5$ ) with turbidities  $<0.1$  NTU. Thus, measurements for the full-scale MBRs confirmed the relative numbers of bacteria in the MBR and MFC-MBR effluents, and the importance of culture-independent methods to assess overall microbiological permeate quality.

#### Trans-Membrane Pressure and Membrane Imaging.

The TMPs measured under constant flux conditions over the period of operation of the reactors increased from  $2.7 \pm 0.6$  kPa (BC) and  $3.1 \pm 0.4$  kPa (PtC) at the beginning of the experiments, to  $11.9 \pm 1.2$  kPa (BC) and  $14.5 \pm 1$  kPa (PtC) at the end of the experiments. These increases in TMP are common in MBRs when no steps are taken to reduce membrane biofouling. At the end of the experiments, the biocathodes (i.e., fouled membrane surfaces) were examined using SEM and TEM (SI Figure S5) in order to visually determine if the shear stress affected the biofilm or MCNT filter structures. SEM images of the fouled membranes showed that the membrane surfaces were covered with a film of microorganisms (SI Figures S5B, D). In the BC reactor, the biofilms reached a thickness of  $\sim 23 \pm 3$   $\mu\text{m}$  (SI Figure S5D) on the conductive UF membrane. TEM images showed that the MCNT distribution pattern remained the same as observed in the virgin membranes, and there was no apparent biofilm damage or cell rupturing (SI Figures S5C, F).

**Implications.** The hybrid MFC-MBR described in this study represents a true integration of these two biotechnologies into a single, simple design. Bioanodes were coupled with electrically conductive membranes that could be used as both air-biocathodes and filters to produce high quality treated wastewater. One of the major challenges in MBRs is membrane biofouling, which requires further advances to produce a long-term solution.<sup>35–37</sup> Unlike the Pt catalysts used for oxygen reduction, the biocathode biofilms are self-regenerative and sustainable. The use of direct air-cathodes also avoids the need for wastewater aeration, which often has been necessary in previous MFC-MBR designs.<sup>9,10</sup> Simultaneous COD and  $\text{NH}_3\text{-N}$  removal were also achieved here using these bioelectrodes, which has not been previously observed in these previous combined systems. The power required for permeate pumping in this system is in the range of  $0.0014$  kWh/ $\text{m}^3$  (details in the SI). The energy required for large-scale MBRs is typically in the range<sup>36</sup> of  $0.5\text{--}1$  kWh/ $\text{m}^3$  and is in large part attributed to aeration, which is avoided in this design. The process configuration used in this study has not been



optimized for energy considerations and there is still lot of room for improvement before retrofitting to existing designs. The main objective of this study was to provide a proof-of-concept of the air-cathode MFC/MBR with special focus on wastewater effluent characteristics rather than on energy generation. Future studies that consider continuous modes of filtration as well as the effects of operational and design parameters (e.g., HRT, SRT, permeate flux, and reactor geometry) would provide the basis for system optimization and energetic performance. Improvements and further optimization of this MFC-MBR system will result in a more sustainable biological treatment technology that has decreased energy requirements compared to traditional MBRs.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Supporting Information is available on in situ CV analyses of the conductive UF membranes, polarization behavior of BC reactors after replacing the biocathodes, polarization behavior of the NC reactors, in situ CV analysis of air-biocathodes (with and without O<sub>2</sub> sparging), flow cytometry analyses, and SEM/TEM images of biofilm layer on the conductive UF membrane. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported by SABIC Fellowship (K.K.) and discretionary investigator funds (P.S.), and award KUS-I1-003-13 (B.E.L.) from the King Abdullah University of Science and Technology (KAUST). Dr. Cyril Aubry and Dr. Rachid Sougrat are acknowledged for their assistance with SEM and TEM analysis, respectively.

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