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Graphene-coated hollow fiber membrane as the cathode in anaerobic electrochemical membrane bioreactors – Effect of configuration and applied voltage on performance and membrane fouling

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ABSTRACT

Electrically conductive, graphene-coated hollow-fiber porous membranes were used as cathodes in anaerobic electrochemical membrane bioreactors (AnEMBRs) operated at different applied voltages (0.7 V and 0.9 V) using a new rectangular reactor configuration, compared to a previous tubular design (0.7 V). The onset of biofouling was delayed and minimized in rectangular reactors operated at 0.9 V, compared to those at 0.7 V due to higher rates of hydrogen production. Maximum transmembrane pressures for the rectangular reactor were only 0.10 bar (0.7 V) or 0.05 bar (0.9 V) after 56 days of operation, compared to 0.46 bar (0.7 V) for the tubular reactor after 52 days. The thickness of the membrane biofouling layer was approximately 0.4 μm for rectangular reactors and 4 μm for the tubular reactor. Higher permeate quality (TSS = 0.05 mg/L) was achieved in the rectangular AnEMBR than the tubular AnEMBR (TSS = 17 mg/L), likely due to higher current densities that minimized the accumulation of cells in

suspension. These results show that the new rectangular reactor design, which had increased rates of hydrogen production, successfully delayed the onset of cathode biofouling and improved reactor performance.

TOC Art

INTRODUCTION

Wastewater treatment based on conventional activated sludge (CAS) processes consumes relatively large amounts of energy ($\approx 0.6 \text{ kWh/m}^3$),¹ with about half of that energy used for wastewater aeration. These systems also require large and efficient clarifiers to meet stringent water quality standards for discharge. Aerobic membrane bioreactors (MBRs) have significantly smaller footprints than CAS systems because they can achieve biological wastewater treatment and solids separation in the same tank using a membrane to separate solids from treated water. MBRs produce treated water of excellent quality suitable for reuse, but at a considerable energy penalty ($1\text{--}2 \text{ kWh/m}^3$).² A large fraction of the energy used to operate MBRs is due to aeration, which is required to supply oxygen for bacterial respiration as well as to scour the membranes to minimize fouling.³

Microbial electrochemical technologies (METs), such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), offer an alternative approach to effectively treat domestic wastewater with recovery of the inherent energy as electricity (MFCs)⁴ or hydrogen (MECs).⁵ These systems use electroactive bacteria that oxidize organic matter in waste streams using an electrode as the terminal electron acceptor. This electrode (anode) is electrically connected to a cathode electrode where a reduction reaction occurs. By integrating MFCs or MECs with MBRs it is possible to reclaim water for reuse purposes and significantly reduce energy consumption for wastewater treatment.⁶⁻¹⁹ These systems are either truly integrated^{6, 7, 9, 12-14, 19} or the treatment and water reclamation processes are separated within single-stage^{11, 17, 18} or two-stage systems^{8, 10, 15, 16} (Table S1). In truly integrated systems the cathode served the dual function for filtration and cathodic reaction. The advantage of using dual function cathodes is that it reduces cost and footprint. However, the majority of the truly integrated systems incorporated electrically

conductive flat-sheet membranes as the cathode, which lowers the specific surface area of the cathode (i.e. cathode surface area per reactor volume). Katuri *et al.* recently developed a novel tubular anaerobic electrochemical membrane bioreactor (AnEMBR) incorporating electrically conductive nickel based hollow fiber membrane (Ni-HFM) as a cathode in a MEC reactor to produce biogas at the cathode electrode surface.⁷ This cathode design increased the surface area of the cathode per reactor volume, which is a critical aspect of the design of METs for practical applications for wastewater treatment.⁷

Membrane fouling control is a considerable challenge in both aerobic and anaerobic membrane-based bioreactors.¹⁵ The primary fouling control strategies for MFC-MBR systems use aeration or granular activated carbon (GAC) (Table S1). However, neither approach can be used in the AnEMBR system as it is a single-stage anaerobic system. An alternative suggested for an MEC-based system was that increased hydrogen production rates might reduce membrane fouling in the AnEMBR system due to the scouring effect of hydrogen gas bubbles on the membrane surface.⁷ This alternative approach was based on an observation by Katuri *et al.* where they increased the applied voltage from 0.5 V to 0.9 V in a tubular AnEMBR and observed a significant drop in transmembrane pressure (TMP) at 0.9 V due to increased hydrogen production rates.⁷ However, it was a one-time observation and their AnEMBR system was designed only for “proof of concept” and thus was not optimized for performance. Several factors including reactor configuration (e.g. specific cathode surface area and electrode spacing) and operation (e.g. applied voltage) can affect hydrogen production rates²⁰ and thus fouling propensity in the AnEMBR system. Therefore, the objective of this study was to evaluate the effect of reactor configuration (specific cathode surface area and electrode spacing) and applied voltage on the performance of the AnEMBR system.

To address this objective, two AnEMBR configurations (rectangular vs. tubular) were evaluated in terms of hydrogen production rates, electrochemical performance, membrane fouling propensity (biofilm formation and transmembrane pressure), and energy balances. Microscopic and next-generation sequencing techniques were used to characterize the biofouling layer on the cathode hollow fiber membranes (HFMs). The electrode spacing and specific cathode surface area was different in both configurations. For the rectangular configuration, the anode was placed directly opposite the vertically-aligned cathode HFMs to minimize electrode spacing (~ 1.5 cm) and reduce ohmic (solution) resistance (Figure 1), whereas in the tubular AnEMBR the anode was placed below the complete cathode bundle with ~ 2 cm between the bottom of the cathode bundle and the top of the anode. The specific surface area of the cathode for the rectangular reactor was $8.6 \text{ m}^2/\text{m}^3$, compared to $4 \text{ m}^2/\text{m}^3$ in the tubular AnEMBR. The cathode in both configurations was made of graphene HFMs (Gr-HFMs) fabricated using an earth abundant and inexpensive Ni skeleton as a support material in order to study the potential of hydrogen evolution by carbon-catalyzed cathodes as an alternative to metal-catalyzed cathodes in these type of systems. The key drivers to test graphene as the cathode catalyst in this study were sustainability and cost. Carbon based catalysts can be made sustainably and at a fraction of the cost of metal catalysts. To evaluate the effect of applied voltage, the rectangular AnEMBRs were operated at two different applied voltages (0.7 V and 0.9 V) commonly used in MEC studies²¹⁻²³, whereas to evaluate the effect of electrode spacing and cathode specific area, the performance of the tubular AnEMBR operated at only 0.7 V was compared to the rectangular AnEMBR operated at the same applied voltage.

MATERIALS AND METHODS

Preparation of Cathodes. The porous Ni-HFM skeleton was fabricated using a combined phase-inversion/sintering method as previously described.⁷ Graphene layers were synthesized directly on Ni-HFMs through a chemical vapor deposition (CVD) technique by exposing Ni-HFMs to a mixture of acetylene (22 mL/min) and H₂ (495 mL/min) gases at 1000 °C for 5 min, and then cooled in a tube furnace in argon gas.

Reactor Construction and Operation. A schematic representation of the rectangular AnEMBR configuration is shown in Figure 1; the design of the tubular AnEMBR was previously described.⁷ Four single chamber, rectangular AnEMBRs were constructed from plexiglass. A single tubular AnEMBR was constructed and operated in parallel in order to compare reactor performance and fouling propensity of the Gr-HFMs. The high reproducibility of duplicate tubular AnEMBRs was demonstrated previously.⁷ The rectangular AnEMBRs had a central housing ($14.5 \times 5 \times 5$ cm) and two solid end plates (14.5×5 cm), with a final working liquid volume of 165 mL, compared to 350 mL for the tubular AnEMBR. All chambers were sealed with silicon gaskets. The anode electrode of the rectangular AnEMBRs was prepared from non-wet proofed carbon cloth (CC-B1DBN40, Clean Fuel Cell Energy, USA) cut to fit on one side of the reactor (projected surface area of 35 cm²). The anode was held in position using a silicone adhesive spread around the edge of the reactor housing. The titanium foil ($2.5 \times 1.5 \times 0.1$ cm) current collector was positioned on one edge midway along the reactor housing. To ensure a good electrical connection, the area below the titanium current collector was not coated with silicone adhesive. The anode in the tubular AnEMBR was a graphite fiber brush (10 cm $\times$ 5 cm, PANEX 33 160 K, ZOLTEK) containing a titanium core that was heat treated at 450°C for 15 min.

Each reactor contained five Gr-HFMs bundled together using copper wire and conductive

silver epoxy. The wire was fed through a port on the top of the reactor and connected to the cathode bundle. Individual cathode fibers were 10 cm long with an outer diameter of 0.09 cm (projected surface area of 2.8 cm²). Each fiber was sealed at the bottom end with a small drop of epoxy. Silicone tubing was placed over the top of the fibers up to the point of connection, and the juncture was covered with epoxy to create a water tight membrane module and to seal the copper wire from the solution. The ends of the fibers located within the silicone tubing were left open to allow for filtration, and the tubes were connected to a peristaltic pump. The silicone tube at the top of the cathode was fixed in place using epoxy glue. In the rectangular AnEMBR, the cathode was placed ~1.5 cm from the anode, whereas in the tubular AnEMBR the anode was placed below the complete cathode bundle with ~2 cm between the bottom of the cathode bundle and the top of the anode.

A gas collection bag was connected to the top of the reactor using black tubing (10 cm) connected through a 10 mm diameter threaded port on the top of the housing. A reference electrode (Ag/AgCl, MF-2052, BASi) was placed between the anode and cathode in a vertical orientation through a hole on the top of the housing to measure the anode potential. All connections were sealed with non-conductive epoxy.

Duplicate rectangular AnEMBRs were started up at an applied voltage of 0.7 V or 0.9 V and fed with 10 mM acetate in a bicarbonate buffered medium containing (g/L): NH₄Cl, 1.5; Na₂HPO₄, 0.6, KCl, 0.1 and 2.5, Na₂HCO₃; and trace minerals and vitamin solutions (pH 7.0, conductivity = 2.9 mS/cm).⁷ Anaerobic digester sludge (10 % v/v, Manfouha Wastewater Treatment Plant, Riyadh, KSA) was used as the inoculum. After steady current production, the acetate concentration was reduced to 5 mM (320 mg/L COD) to provide a COD concentration more representative of domestic wastewater. Voltage was applied using an external power source

(3645A; Circuit Specialists, Inc., AZ), with the voltage monitored using a data logger (ADC 24, PicoLog, UK) across an external resistor (R_{ex} 10 Ω). A tubular single AnEMBR⁷ was started up in the same manner at an applied voltage of 0.7 V. All reactors were operated under fed-batch conditions at room temperature (25°C). Fresh medium was added at the end of each batch cycle, when the current density dropped to within 10% of the peak current density [1 day (0.9 V); 2 days (0.7 V); 4 days for the tubular design (0.7 V)]. The rectangular AnEMBRs were operated for a period of 72 days, whereas the experiments in the tubular AnEMBR were terminated on day 52 due to severe cathode fouling.

The reactor medium was not filtered at the end of each batch cycle due to time constraints. Instead, filtration was conducted at the end of alternate batch cycles or within at least two batch cycles of the previous filtration event. The anolyte was filtered through the cathode using a peristaltic pump (Masterflex L/S, Cole-Parmer, Vernon Hills, IL.) at a permeate flux of 6.9 L/m²-h (LMH). The transmembrane pressure (TMP) of the membrane filters was measured using a pressure transducer (68075-32, Cole-Parmer Instrument Company), and recorded using a data acquisition system (LabVIEW, National Instruments) connected to a computer.

Membrane Chemical Cleaning. The rectangular AnEMBRs were disassembled on day 64 and the cathodes removed for cleaning. Each set of fibers was placed into a sodium hypochlorite solution (1%) for 15 minutes. The fibers were next immersed into concentrated hydrochloric acid (HCl) for 5-10 s, before rinsing (5-10 s at 40 KHz) in a beaker of deionized water in a sonication bath (Branson, M1800, USA). The fibers were then dipped five times in the concentrated HCl before being rinsed with distilled water and replaced into the reactor.

Analyses. Acetate and gas (H₂, N₂, CH₄ and CO₂) measurements were done at the end of each batch cycle. Details on gas measurements, calculations of coulombic efficiency (CE) and

internal resistance (R_{int}), and analysis methods for linear sweep voltammetry (LSV), scanning electron microscopy (SEM), and Raman spectroscopy are provided in the SI. All potentials are reported versus Ag/AgCl.

To characterize the microbial communities, at the end of the experiment (i.e. day 72 for the rectangular AnEMBRs and day 52 for the tubular AnEMBR), a small piece of a single fiber (~5 mm) was cut for analyses. Genomic DNA was extracted using the PowerSoil™ DNA extraction kit (MO BIO Laboratories Inc., USA) as per the manufacturer's instructions. The quality (A260/A280) and quantity (A260) of the extracted genomic DNA was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). The bacterial and archaeal community composition on the cathode was determined using 16S rRNA gene pyrosequencing. Quantitative polymerase chain reaction (qPCR) was used to quantify the abundance of total Bacteria and Archaea on the cathode. Details of qPCR and pyrosequencing are presented in the Supporting Information.

RESULTS AND DISCUSSION

Characterization of the Cathodes. The Gr-HFM had an inner diameter of ~750 μm (Figure 2A) and an average pore size of 1 μm (Figure 2B). The graphene covering on Ni-HFM is evident from the dark gray coloring with the characteristic wrinkled topography (Figure 2B) due to variations in the thermal expansion coefficients of graphene and Ni during the cooling process of CVD synthesis.²⁴ The thickness of the graphene layer was ~135 nm based on SEM of virgin Gr-HFM (Figure S1). The microscale (laser spot size / of 1.5 μm) Raman spectroscopic analysis of the Gr-HFMs (Figure 2C) at random locations exhibited the characteristic G (1580 cm^{-1}) and

symmetric 2D (2730 cm^{-1}) bands with the 2D to G band (I_{2D}/I_G) intensity ratio of 0.3 which clearly indicated the formation of multi-layered graphene. The multi-layering of the graphene can be distinguished by analysing the I_{2D}/I_G ratio of Raman spectra. A I_{2D}/I_G ratio of 2 with a FWHM of $\sim 30\text{ cm}^{-1}$ (for 2D band) suggests a defect free single layer graphene. The I_{2D}/I_G ratio decreases with increase in graphene layer thickness, due to variations in both G and 2D band frequencies.²⁵ The absence of a D band (at 1350 cm^{-1}) in the Raman spectrum signify that the synthesized graphene layers on Ni-HFM skeleton were of high crystallinity, which is essential for good electrical conductivity. The differences in Raman shifts between the Gr-HFM and Ni-HFM skeleton clearly showed that the graphene layers completely covered the Ni surface during the gaseous CVD process (Figure 2C). As a result, the characteristic peaks of Ni were not found in the analysis of the Gr-HFM.

LSVs showed that Gr-HFM cathode had similar overpotentials compared to Ni-HFM ($\sim -0.5\text{ V}$), but both were more positive than stainless steel ($\sim -0.8\text{ V}$) or graphite cathode ($\sim -1.2\text{ V}$) (Figure 2D). Gr-HFM had much better catalytic activity than SS and graphite with regards to the onset of hydrogen evolution reaction (HER) potential. At -1.0 V , the maximum current density of the Gr-HFM was only 20% that of the Ni-HFM (Figure 2D). The low Tafel slope (0.100 V/dec) and large exchange current density of Ni-HFM (0.002 mA/m^2) compared to Gr-HFM (0.130 V/dec and 0.00084 mA/m^2) from the Tafel analysis (Figure S2) confirms the fact that Ni is a better catalyst for HER than graphene even though both materials had the same onset potential for HER.

Performance of AnEMBRs. Initial cycles following stable current production, up to day 30, produced the highest hydrogen recoveries. After ~ 50 days of operation, prior to membrane cleaning, methanogenesis was well established with little hydrogen gas recovery. The final stage

up to 72 d shows performance for the rectangular AnEMBR following membrane cleaning on day 64. The slight drop in average current density observed at 40 d for reactors at 0.9 V was due to experimental error during a filtration cycle that affected anode performance. The reactors recovered within a few days to produce current densities similar to those measured before the error.

The rectangular AnEMBRs produced an average current density of $29 \pm 2 \text{ A/m}^3$ ($3.4 \pm 0.2 \text{ A/m}^2$; based on cathode surface area) at an applied voltage of 0.7 V, and $49 \pm 5 \text{ A/m}^3$ ($5.7 \pm 0.5 \text{ A/m}^2$) at 0.9 V over the first 50 d of operation (Figures 3A and B) compared to $10 \pm 1 \text{ A/m}^3$ ($2.6 \pm 0.2 \text{ A/m}^2$) at 0.7 V in the tubular AnEMBR (Figure 3C). The volumetric current density of the rectangular reactor at 0.7 V was nearly three times that of the tubular design, mainly due to reduced electrode spacing and increased specific cathode surface area.²⁶ The internal resistance of the rectangular AnEMBR was $21.2 \pm 0.4 \Omega$ compared to $34.2 \pm 0.3 \Omega$ for the tubular AnEMBR. The current density remained stable following chemical cleaning of the rectangular AnEMBR after 64 d of operation (Figures 3A and B) suggesting that the Gr-HFMs maintained their integrity and catalytic activity after chemical treatment, and demonstrated the robustness of the system.

Coulombic efficiency (CE) in the rectangular AnEMBRs was >100% during the first 30 d of operation at both applied voltages (Table 1). Coulombic efficiencies of >100% have been reported previously due to hydrogen recycling by exoelectrogens using hydrogen as the electron donor.^{27, 28} However, when methanogenesis was observed in the system after 30 d of operation (Figure 3) the CE was reduced to <100%, likely due to efficient conversion of hydrogen to methane. The CEs decreased to 83% (0.7 V) and 79% (0.9 V) at 50 d, but increased to 87% (0.7 V) and 95% (0.9V) after 72 d of operation (Table 1). This increase in CE could be due to the

cleaning of the membrane. After cleaning the cathode fibers of the rectangular reactors, the hydrogen content of the biogas increased (Figures 3A and 3B) and possibly some hydrogen recycling occurred resulting in increased CE. The CEs of the tubular reactor were always <60%.

Hydrogen production rates in the rectangular AnEMBRs were the highest for the six days following stable current generation, and reached $0.21 \pm 0.05 \text{ m}^3 \text{ H}_2/\text{m}^3 \text{ d}$ at 0.7 V, and $0.41 \pm 0.08 \text{ m}^3 \text{ H}_2/\text{m}^3 \text{ d}$ at 0.9 V at 30 d (Table 1). Hydrogen recoveries decreased with time to $0.02 \pm 0.03 \text{ m}^3 \text{ H}_2/\text{m}^3 \text{ d}$ (0.7 V) and $0.28 \pm 0.15 \text{ m}^3 \text{ H}_2/\text{m}^3 \text{ d}$ (0.9 V) at 72 d. Rates of hydrogen production for the tubular AnEMBR were always very low, averaging $0.01 \pm 0.01 \text{ m}^3 \text{ H}_2/\text{m}^3 \text{ d}$, likely due to the longer cycles that allowed more time for hydrogen conversion to methane.

The cathodic recovery of biogas (r_{Cat}) was highly variable in all tests. The highest recoveries of 75 to 90% were obtained using an applied voltage of 0.9 V for the rectangular reactors with the highest H_2 production rates. Lower recoveries of 55 to 69% resulted for these reactors at 0.7 V, and recoveries of 38-57% were obtained for the tubular reactor with the lowest H_2 production rates (Table 1).

Methane was detected at very low concentrations after 20 d (0.7 V) and 23 d (0.9 V), and concentrations increased steadily with successive cycles. Methane generation increased more rapidly at 0.7 V compared to 0.9 V (Figures 3A and 3B) and it became the predominant gas after 42 d of operation at 0.7 V, except when the applied voltage was increased from 0.7 V to 0.9 V (Figure 3A). In contrast, hydrogen was dominant in the biogas at 0.9 V for the duration of the experiment (Figure 3B). The longer cycle time of approximately 2 d at 0.7 V, compared to 1 d at 0.9 V, likely allowed for more time for conversion of H_2 to methane. Methane generation was well established after 15 d of operation in the tubular AnEMBR (Figure 3C). After cleaning the cathode fibers of the rectangular reactors, the hydrogen content of the biogas increased (Figures

3A and 3B). However, methane generation was quickly re-established to previous levels within 2-3 d. It is likely that hydrogenotrophic methanogens on the walls of the reactor (as detected by qPCR, data not shown) acted as an inoculum source for rapid establishment of hydrogenotrophic methanogenesis on the membrane surface. The electron balances for the tubular AnEMBR and rectangular AnEMBs revealed that a higher proportion of electrons were lost at the cathode to other sinks (e.g. biomass, soluble microbial products)²⁹ in reactors operated at 0.7 V versus those operated at 0.9 V (Figures S3, S4 and S5).

The AnEMBRs showed a net positive energy gain over the course of the experiment except for the tubular AnEMBR that showed a negative energy balance after 30 d of operation (Table S5). After 50 d of operation, however, more energy was recovered as biogas than was supplied as electricity in the tubular AnEMBR (Table S5). The composition of the recovered gas shifted from predominantly hydrogen (>80%) to a mixture of hydrogen and methane after 50 d of operation of the rectangular AnEMBRs (Figures 3A and 3B). A gas comprised of a mixture of H₂ and CH₄ results in less energy due to the low conversion efficiencies of methane combustion. For example, after 50 d the methane fraction of the biogas in the rectangular AnEMBRs was 68% (0.7 V) and 25 % (0.9 V) (Figure 3) and the net process energy was negative (i.e. energy demanding) (details provided in the energy balance section of SI). However, the energy required to operate the AnEMBR at 0.7 V (0.19 kWh/m³) and 0.9 V (0.13 kWh/m³) after deducting the maximum electrical energy that could be recovered are still much lower than what is required for large-scale aerobic MBRs (1-2 kWh/m³).²

Analysis of Microbial Communities. Generation of methane is well documented in single-chamber MEC systems,²² but the microbial community on methane-producing biocathode is less well studied. Analysis of archaeal 16S rRNA gene pyrosequencing data revealed that

hydrogenotrophic methanogens belonging to the genus *Methanobrevibacter* (family *Methanobacteriaceae*) were the dominant methanogens (89%-99%) in the rectangular AnEMBRs regardless of the applied voltage tested. Two previous studies reported a predominance of members of the family *Methanobacteriaceae* on MEC cathodes.^{30, 31} Hydrogenotrophic methanogens belonging to the genus *Methanobrevibacter* (27%), and *Methanobacterium* (30%), were the dominant methanogens in the tubular AnEMBR (Figure 4, Table S3). In addition, members of unclassified *Methanobacteriaceae* (43%) were detected on the cathode of the tubular AnEMBR (Figure 4). The difference in the methanogenic community composition between the tubular and rectangular AnEMBR could be due to differences in the rates of hydrogen production. Acetoclastic methanogens were not detected in any of the AnEMBRs, based on 16S rRNA gene pyrosequencing, despite the use of acetate as a fuel.

Pyrosequencing analysis of bacterial 16S rRNA gene revealed that members of the genus *Desulfovibrio* were the dominant bacterial communities (24%-50%) on the cathodes of the rectangular AnEMBRs at both applied voltages (Figure 4, Table S4). Members of the genus *Desulfovibrio* are sulfate-reducing bacteria and it has been reported that under electron acceptor limited conditions several members of *Desulfovibrio* are able to catalyze hydrogen production at low cathode potentials ≤ -0.44 V vs SHE using the cathode as the sole source of electrons and energy.³¹⁻³³ Therefore, it is possible that members of the genus *Desulfovibrio* were selectively enriched on the Gr-HFMs due to the low cathode potentials (-0.89 V vs SHE at 0.7 V and -0.99 V vs SHE at 0.9 V) and electron donor availability. Also, *Desulfovibrio* sp. might have contributed to bioelectrochemical methane production in the AnEMBR system through production of hydrogen, which was then consumed by hydrogenotrophic methanogens.³⁰

Fouling of AnEMBR. Filtration and TMP measurements were started after 24 d of operation for the rectangular AnEMBRs, and 15 d for the tubular AnEMBR. Rectangular AnEMBRs operated at 0.9 V showed lower TMPs than those operated at 0.7 V over the course of the experiment (Figures 3A and B). From day 24 to 40 the TMP for reactors at 0.9 V was approximately half that of reactors at 0.7 V, with a TMP of 0.05 bar at 72 d at 0.9 V, compared to 0.10 bar at 0.7 V. This reduction in cathode fouling at 0.9 V was likely a result of the greater hydrogen production rates of this reactor. We hypothesize that biofouling and TMP in the AnEMBR are directly related to rates of reactions. Data from this study shows a correlation between current density, hydrogen production rates and TMP (Figure S6). Since the hydrogen evolution rate at the cathode membrane surface is directly proportional to the current density, any change in the configuration, such as increasing the specific cathode surface area and reducing electrode spacing, that increases the current density should lead to reduced fouling and lower TMP.

Katuri *et al.* similarly observed a noticeable drop in TMP in a tubular AnEMBR after increasing the applied voltage from 0.5 V to 0.9 V.⁷ It was suggested that this reduction in TMP was due to the scouring effect of the increased rate of hydrogen bubble formation at the membrane surface. However, increasing the applied voltage from 0.7 V to 0.9 V on day 59 (after the cathode had already developed a higher TMP) did not reduce the TMP (Figure 3A). The biofouling layer that had developed on these cathodes (0.4 - 0.5 μm) (Figures 5A and 5B) likely limited the effectiveness of this approach. The biofilm was significantly thicker (4 μm) in the report by Katuri *et al.*⁷ and scouring a biofilm of such a thickness would result in a more noticeable drop in the TMP.

After chemically cleaning the membranes (day 64), the TMP decreased significantly to 0.05 bar for the rectangular reactor at 0.7 V, and 0.02 bar at 0.9 V. After further operation for 8 d, the TMP of reactors at 0.7 V increased to 0.13 bar, compared to 0.06 bar for reactors at 0.9 V (Figures 3A and 3B). Although the TMP increased again with time, the final TMP values (72 days of operation) were 72% (0.7 V) and 87% (0.9 V) lower than the TMP of the tubular design (0.46 bar, 50 d of operation). These results indicate that the rectangular configuration reduced biofouling resulting in a lower TMP (Figure 3). It should be noted that chemical cleaning of the Gr-HFM did not result in any structural changes of the graphene layers as revealed by Raman analysis (data not shown). Graphene adheres strongly to a metal support such as nickel due to strong bonding with the metal lattice that occurs during the preparation process.^{24, 34} Therefore it is unlikely to be damaged easily or separate from the nickel support unless the graphene corrodes. However, corrosion is unlikely since the cathode potential in these systems is lower than the equilibrium potential for carbon oxidation (>1.2 V vs NHE). In addition, graphene has been shown to be very resistant to corrosion under both acidic and basic conditions.³⁵

SEM images indicated that the membrane fouling layer from reactors of the rectangular design were relatively flat and thin after 64 days of operation (Figures 5A and 5B). The fouling layer showed an irregular scattering of cells and a glue-like coating, presumed to be extracellular polymeric substances (EPS).³⁶ There were no clear differences in the morphologies or coverages of the fouling layer that might explain the differences in TMPs. However, the morphology of the fouling layer that developed after cleaning was different for reactors at 0.7 V and 0.9 V (Figures 5C and 5D). The fouling layer at 0.7 V reactors appeared to contain larger quantities of EPS whereas the fouling layer at 0.9 V appeared more sponge-like with less EPS (Figures 5C and 5D). More open pores were visible in the membranes from reactors at 0.9 V compared to

membranes from reactors at 0.7 V. The presence of more open pores could explain the lower TMP measured for reactors at 0.9 V after 72 d of operation. The formation of hydrogen contributed to the formation of pores in the membrane fouling layer and these pores may have affected the morphology of this layer.

The fouling layers were approximately 0.4 - 0.5 μm thick in the rectangular AnEMBRs (Figures 5A-D). This is approximately one tenth the thickness of the fouling layer observed on the cathode membranes of the tubular AnEMBR design ($\sim 4 \mu\text{m}$) (Figures 5E and 5F). Also, the biofouling layer of cathode from the tubular reactor showed higher abundance of prokaryotes (bacteria and archaea), expressed as copies/ng DNA, compared to the rectangular reactors (Figure S7). These support other evidence that improving the reactor performance, in terms of current densities, improved the hydrogen production rates, resulting in reduced fouling. In addition to the *in situ* cleaning effect due to hydrogen bubble formation at the membrane surface, other factors such as the low cathode potential³⁷, the localized high pH at the cathode surface due to proton consumption³⁷ and the electrostatic repulsion of negatively charged foulants away from the negatively charged cathode membrane surface^{12, 13, 38} may have collectively contributed to fouling control in the AnEMBR system.

Both pyrosequencing and qPCR of 16S rRNA gene revealed that members of the domain Bacteria and Archaea contributed to biofouling in the tubular and the rectangular AnEMBRs (Figures 4 and S7). However, the composition and abundance of the two domains varied according to applied voltage and reactor configuration (Figures 4 and S7). Also, the abundance of methanogenic archaea on the cathodes of the rectangular reactors were significantly lower compared to the tubular reactor (Figure S7). This noticeable difference in abundance is also due to the rates of hydrogen production. Due to the low solubility ($0.74 \mu\text{M}$)³⁹ and density

(0.0000089 g/mL) of H_2 , the hydrogenotrophic methanogens on the cathode of the rectangular reactor were not capable of consuming the H_2 as fast as its rate of generation on the membrane surface. This phenomenon resulted in lower abundance of hydrogenotrophic methanogens in the rectangular reactor compared to the tubular reactor (Figure S7). The opposite is true for the tubular configuration, as hydrogenotrophic methanogens were capable of consuming the H_2 generated on the cathode due to lower rates of hydrogen production compared to the rectangular reactors.

Permeate Water Quality. Acetate removal in the rectangular AnEMBRs after 72 d of operation was >83% at both applied voltages compared to >99% in the tubular AnEMBR. The higher acetate removal in the tubular AnEMBR was likely due to the longer cycle time (4 days compared to 1 or 2 days for the other reactors). It is known that higher current densities result in faster COD removal rate,²⁰ and hence a shorter cycle time is required to remove COD. The TSS in the permeate was very low in the rectangular AnEMBRs (0.05 mg/L). However, the TSS was 17 mg/L in the tubular AnEMBR effluent despite similar pore size ($\sim 1\mu\text{m}$). The lower TSS may be due to higher current densities that resulted in faster acetate removal rates at the anode⁴⁰ for electricity generation (CE = 85%) and shorter cycle time and may have minimized the accumulation of cells in suspension. Improvements in membrane fabrication to reduce the pore size ($< 0.1\mu\text{m}$) would help to improve permeate water quality.⁶

In conclusion, the graphene layer on the cathode served as both a catalyst for HER and as a separate membrane for filtration. The improved performance of the graphene coated cathodes demonstrated that graphene-catalyzed cathodes could be a feasible alternative to traditional

metal-catalyzed cathodes. Graphene is particularly attractive as an electrode material as its specific surface area is at least 1000 fold higher than conventional carbon-based materials.⁴¹

Membrane cleaning or replacement is a significant operational expense in aerobic and anaerobic MBRs and poses a constant challenge.⁴² Here we demonstrated that modifications in reactor configuration improved the rates of hydrogen evolution and hence *in-situ* cleaning of the membranes, which enabled operation of the rectangular AnEMBRs for two months without replacement or cleaning (e.g. backwashing or chemical treatment) of the membranes.

Although energy-neutrality was not achieved in our system, the energy balance values for the AnEMBRs were much lower than those required for large-scale aerobic MBRs.

Hydrogenotrophic methanogenesis is unavoidable in single chamber MECs and presents an alternative direction for the development of AnEMBRs. Understanding the ecophysiology of methane producing biocathodes in AnEMBRs and how it can be tailored to maximize methane production would be beneficial to achieve energy-neutral AnEMBR system.

The results obtained here were only for an acetate-based medium, and thus the performance with actual domestic wastewaters still needs to be investigated. Future studies that test the performance of AnEMBRs with real domestic wastewater are needed to further develop this technology and move it towards implementation.

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ASSOCIATED CONTENT

Supporting Information Available

Supporting information includes: Summary table of studies on integrating MET with membrane filtration processes, details on chemical, electrochemical, SEM, Raman spectroscopy, qPCR and pyrosequencing analysis, energy calculations, SEM-EDX analysis of virgin Gr-HFM, Tafel plot, and electron balance. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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FIGURE CAPTIONS

Figure 1. Schematic diagram of the rectangular AnEMBR used in this study. (1) Gr-HFM bundle, (2) bioanode, (3) power source, (4) external resistor, (5) gas bag, (6) pressure sensor, (7) pump and (8) permeate. Bubbles represent different gases: CO₂ (light blue), H₂ (green) and CH₄ (yellow).

Figure 2. Scanning electron micrographs of Gr-HFM (A and B), Raman spectra of Gr-HFM and Ni-HFM (C) and linear sweep voltammetry (D).

Figure 3. Peak volumetric current densities, TMP and gas composition for rectangular AnEMBRs operated at (A) 0.7 V, (B) 0.9 V and (C) the tubular AnEMBR operated at 0.7 V. Values represent averages of duplicate reactors. Section highlighted with gray color (A) indicates cycles at which 0.7 V reactors were switched to 0.9 V (day 59 to 63). Gray arrows represent the time at which the Gr-HFMs were sampled for SEM imaging and black arrows represent the time when biomass samples were collected for microbial community analysis.

Figure 4. Pyrosequencing results of the bacterial and archaeal community on the cathode from the rectangular and tubular configuration. Samples from the tubular AnEMBR failed to amplify with bacterial primers. The asterisk denotes the results for the tubular configuration. Class: C; Order: O; Family: F; Genus: G. Taxa that represent less than 1% of the total bacterial community composition were classified as “others”.

Figure 5. Scanning electron micrographs of Gr-HFMs after 64 d (A – 0.7V and B – 0.9V) and 72 d (C - 0.7V, D - 0.9V) of operation. Also shown are SEM images of Gr-HFMs from the tubular reactor after 52 d of operation at 0.7 V (E and F).

Table 1. Performance of the rectangular AnEMBRs acclimated at different applied voltages and the tubular AnEMBR operated at 0.7 V

0.7 V					
Experimental stage	Acetate removed (%)	CE (%)	Q_{H_2} (m ³ /m ³ /d)	Q_{CH_4} (m ³ /m ³ /d)	r_{Cat} % (biogas)
30 d	83.4 ±4.1	104 ±16	0.21 ±0.05	0.01 ±0.01	69 ±13
50 d	89.5 ±2.9	83 ±21	0.07 ±0.06	0.04 ±0.02	60 ± 9
72 d	85.0 ±11.3	87.2 ±6	0.02 ±0.03	0.04 ±0.03	55 ±34
0.9 V					
30 d	62.1 ±3.6	114 ±20	0.42 ±0.08	0.01 ±0.004	76 ±10
50 d	83.4 ±7.7	79 ±30	0.17 ±0.08	0.07 ±0.028	75 ±14
72 d	83.3 ±3.7	95 ±2	0.28 ±0.15	0.09 ±0.033	90 ±29
Tubular 0.7 V					
30 d	99.9 ±0.04	63 ±21	0.01 ±0.01	0.01 ±0.001	38 ±18
50 d	99.7 ±0.5	57 ±7	0.001 ±0.001	0.02 ±0.004	57 ±8

Figure 1

Figure 2

Figure 3

Figure 4

645 **Figure 5**

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