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Methane Production in Microbial Reverse-Electrodialysis Methanogenesis Cells (MRMCs) Using Thermolytic Solutions

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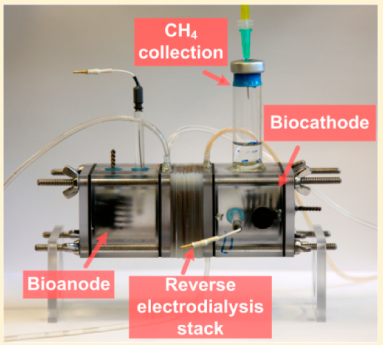
Supporting Information

ABSTRACT: The utilization of bioelectrochemical systems for methane production has attracted increasing attention, but producing methane in these systems requires additional voltage to overcome large cathode overpotentials. To eliminate the need for electrical grid energy, we constructed a microbial reverse-electrodialysis methanogenesis cell (MRMC) by placing a reverse electrodialysis (RED) stack between an anode with exoelectrogenic microorganisms and a methanogenic biocathode. In the MRMC, renewable salinity gradient energy was converted to electrical energy, thus providing the added potential needed for methane evolution from the cathode. The feasibility of the MRMC was examined using three different cathode materials (stainless steel mesh coated with platinum, SS/Pt; carbon cloth coated with carbon black, CC/CB; or a plain graphite fiber brush, GFB) and a thermolytic solution (ammonium bicarbonate) in the RED stack. A maximum methane yield of 0.60 ± 0.01 mol-CH_4/mol-acetate was obtained using the SS/Pt biocathode, with a Coulombic recovery of 75 ± 2% and energy efficiency of 7.0 ± 0.3%. The CC/CB biocathode MRMC had a lower methane yield of 0.55 ± 0.02 mol-CH_4/mol-acetate, which was twice that of the GFB biocathode MRMC. COD removals (89−91%) and Coulombic efficiencies (74−81%) were similar for all cathode materials. Linear sweep voltammetry and electrochemical impedance spectroscopy tests demonstrated that cathodic microorganisms enhanced electron transfer from the cathode compared to abiotic controls. These results show that the MRMC has significant potential for production of nearly pure methane using low-grade waste heat and a source of waste organic matter at the anode.



INTRODUCTION

Bioelectrochemical systems (BESs), which use microorganisms attached to the electrodes to reduce overpotentials for diverse electrochemical reactions, are promising technologies for capturing the energy in waste biomass as electricity or biofuels.[1,2] The most widely studied BESs are microbial fuel cells (MFCs), which can be used to directly extract electrical power from different organic or inorganic wastes.[3] Applying additional electrical power to a BES can increase current densities or enable otherwise nonspontaneous reactions for the production of biofuels or valuable chemicals, such as hydrogen,[4−6] ethanol,[7] acetate,[8,9] or hydrogen peroxide.[10] This approach of adding energy into a BES has greatly expanded the range of their potential applications, particularly for biofuel production.

Methane is commonly produced in anaerobic digesters by methanogens using specific organic substrates, such as acetate and formate, or hydrogen gas.[11] The rate of methane gas production can be limited by slow hydrolysis and fermentation kinetics of the complex sources of organic matter typically used in these systems. In addition, the product gas can require cleaning and further treatment prior to use to remove chemicals such as H_2S. The use of BESs for clean methane gas production has been recently proposed.[12−15] In methanogenic BESs, methane is produced from the reduction of carbon dioxide by methanogens indirectly, using abiotically produced hydrogen gas, or directly using electrons from the cathode.[16] Current production from the anode can be abiotic (through water splitting) or biotic using exoelectrogenic microorganisms and organic matter as the fuel. Abiotic methane production has the advantage of not needing a source of organic matter, and in this process there is net fixation of CO_2 at the cathode. When organics are used as the source of electrons for the anode, and one or more membranes are used to separate the electrode compartments, waste oxidation on the anode side is kept separated from the methane production on the cathode side. This two-chamber configuration can protect methanogens from inhibitory compounds in wastewater, and no additional treatment is needed for the product gas produced at the cathode as it is essentially pure methane (with traces of H_2 and CO_2).[13,14] In addition, any H_2S gas produced in the anode

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chamber would be oxidized on the anode.[2] Methanogenic BESs can be particularly effective for the treatment of wastewaters having relatively low substrate concentrations (compared to high concentrations needed for anaerobic digesters), which could enable their use to directly treat low-strength domestic wastewater or to further treat effluent from an anaerobic digester.[11,12]

The main disadvantage of methane production using BESs is that a power source is needed either to split water (abiotic anode) or provide sufficient voltage to overcome large electrode overpotentials (biotic anode). Appreciable methane production has been achieved through the use of setting cathode potentials more negative than −0.7 V (vs standard hydrogen electrode, SHE).[12,13] This potential is more negative than the theoretical potential of −0.244 V (vs SHE) needed for direct use of electrons from the cathode, or −0.410 V (vs SHE) required for H_2 gas evolution under standard conditions. Thus, to make BESs a sustainable method for methane production, green methods are needed to provide the energy for producing these electrode potentials. This energy could be provided through technologies based on solar, wind, hydropower, or salinity gradients.

Reverse electrodialysis (RED) is a process for direct electricity generation from the mixing of two solutions with different salinities.[17−19] A RED stack is composed of alternately stacked cation (CEMs) and anion exchange membranes (AEMs). When the chambers between the membranes are fed alternately with high concentration (HC) and low concentration (LC) solutions, an electrical potential difference will be created across the membranes.[20,21] It was recently shown that the RED stack can be placed between the anode and cathode of an MFC or a microbial electrolysis cell (MEC) for hydrogen production, establishing a hybrid system that was more effective at energy capture than either system alone.[22−25] Renewable salinity gradient energy production by the RED stack enhances power production by the MFC or drives hydrogen evolution by the MEC without the need for an external power source. When thermolytic solutions[26] such as ammonium bicarbonate are used for the RED stack, the ionic species can be volatilized at very low temperatures (∼60 °C) and then easily condensed to form the HC solution. Waste heat can therefore be used to regenerate the salinity gradients (HC and LC solutions) using conventional distillation technologies.[24,27,28] Thus, the use of a RED stack as a power source is not limited to coastal applications where the HC and LC solutions are seawater and river water.

We propose here a novel system for methane production using a RED stack and a methanogenic biocathode. When the anode is biotic, this approach has the advantage that less voltage (fewer membranes) is needed compared to an abiotic anode (water splitting). In this study, we therefore examined the use of a biotic anode, in a system called a microbial reverse-electrodialysis methanogenesis cell (MRMC). The feasibility of the MRMC for methane production was examined utilizing ammonium bicarbonate (NH_4HCO_3) as the regenerable salt for producing the HC and LC solutions and three different cathode materials. The electrochemical performance of the biocathodes was characterized using several electrochemical techniques, including galvanostatic polarization, linear sweep voltammetry (LSV), and electrochemical impedance spectroscopy (EIS).

MATERIALS AND METHODS

Methanogenic Biocathodes Acclimation. Three, two-chamber bioelectrochemical cells used to develop methanogenic biocathodes were each constructed from two 100 mL media bottles (Figure S1). The anode and cathode chambers (152 mL each) were separated by a pretreated proton exchange membrane (Nafion 117, Dupont Co., Newmark, DE). Three different types of cathodes were used: a heat treated graphite fiber brush (projected area: 6.25 cm^2; 25 mm diameter × 25 mm length; Mill-Rose Lab Inc., OH; GFB);[29] non wet-proofed carbon cloth (projected area: 7 cm^2; CCP 20, Fuel Cell Earth, USA) coated with a carbon black layer (5 mg cm^{-2}) on one side (CC/CB);[12] and stainless steel mesh (projected area: 7 cm^2; Type 304, #60 mesh, McMaster-Carr) coated with a platinum catalyst (0.5 mg cm^{-2}) on one side and a carbon black layer (5 mg cm^{-2}) on the other side (SS/Pt).[22] These cathode materials were chosen to provide a diverse set of materials that would differ in adhesion properties for methanogens, as well as have different catalytic activities for H_2 evolution. Abiotic and heat treated GFBs were used as counter electrodes (anodes) for the acclimation of GFB and CC/CB biocathodes, while Pt mesh (10 mm × 10 mm; #100 mesh, Sigma-Aldrich) was used as the counter electrode for SS/Pt biocathode acclimation. Anodes and cathodes were immersed in electrolytes and connected to the external circuits through titanium wires. Reference electrodes (Ag/AgCl, RE-5B, BASi; +211 mV vs SHE) were placed in cathode chambers to control cathode potentials. All potentials are reported here versus Ag/AgCl reference electrode. Plastic syringes (10 mL) were inserted into the cathode chambers for gas collection.

Cathodes were inoculated with the cell suspensions obtained from existing single-chamber MECs which were producing methane. Each reactor chamber was filled with an anaerobic basal medium containing $NaHCO_3$, 8.4 g L^{-1}; NH_4Cl, 0.31 g L^{-1}; KCl, 0.13 g L^{-1}; Na_2HPO_4, 0.05 g L^{-1}; $NaH_2PO_4{\cdot}H_2O$, 0.03 g L^{-1}; and trace minerals (12.5 mL L^{-1}) and vitamins (5 mL L^{-1}).[12] The final pH of the medium was adjusted to 7.0 using 1 M HCl in an anaerobic glovebox (Coy, Laboratory Products, Grass Lake, MI). The headspace of all reactor chambers (32 mL) was purged with a N_2/CO_2-gas mix (80:20, v/v) for 20 min before the two-chamber bioelectrochemical cells were connected to a potentiostat (VMP3, BioLogic, USA). During the start-up period, the cathode potential was set at −0.8 V for SS/Pt. However, little current or methane were generated for the GFB and CC/CB electrodes at −0.8 V, and therefore the cathode potentials were set at −1.0 V for studies with these two electrodes. For the first three batch cycles, anolytes and catholytes were replaced in the glovebox when the methane production rate significantly decreased (after ∼22 d). Afterward, the medium was replaced every 5 days. Reference electrodes were checked and recalibrated (as needed) after each cycle to ensure a potential drift <5 mV. After repeatable methane production was obtained for at least three successive cycles (Figure S2), biocathodes were transferred to MRMCs.

MRMC Construction and Operation. The MRMC consisted of an anode chamber, a cathode chamber, and a RED membrane stack (Figure 1). Two cubic-shaped Lexan blocks were drilled to produce cylindrical anode and cathode chambers (4 cm long and 3 cm in diameter). A glass tube glued to the top of the cathode chamber and a gasbag (100 mL capacity; Cali-5-Bond, Calibrated Instruments Inc.) was used for gas collection.[30] All anodes were heat treated GFBs. Two

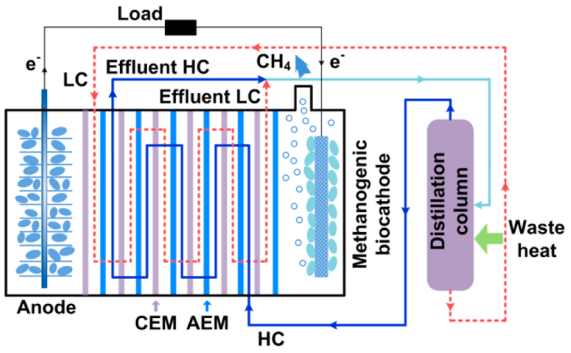

Figure 1. Schematic of the microbial reverse-electrodialysis methanogenesis cell and the regeneration of HC and LC solutions (AEM, anion exchange membrane; CEM, cation exchange membrane; HC, high concentration solution; LC, low concentration solution).

Ag/AgCl reference electrodes were separately inserted into the anode and cathode chambers to measure stack voltages and electrode potentials. All potentials were reported versus Ag/AgCl. The RED stack, sandwiched between the anode and cathode chambers, comprised six pairs of CEMs and AEMs (Selemion CMVs and AMVs, Asashi glass, Japan), creating five HC and six LC chambers (Figure 1).[22] Each chamber had an 8 cm^2 (4 cm × 2 cm) rectangular cross section. The intermembrane distance was maintained using polyethylene woven spacers and silicone gaskets with a thickness of 1.3 mm.

The anolyte in the MRMC contained 1 g L^{-1} of sodium acetate in a 100 mM bicarbonate buffer solution with minerals (12.5 mL L^{-1}) and vitamins (5 mL L^{-1}).[27] The catholyte was the same anaerobic basal medium used in two-chamber bioelectrochemical cells. A 1.7 M NH_4HCO_3 solution was used as the HC solution.[22] The LC solution was prepared to produce a salinity ratio of 75 by diluting HC solution with deionized water.[28] The MRMC was operated in fed-batch mode with a 10 Ω external resistor in the circuit to calculate the current. The cathode headspace was sparged with a N_2/CO_2-gas mix (80:20, v/v) for 15 min before each batch cycle. Anolyte and catholyte were replaced when the produced current was <0.2 mA. HC and LC solutions were continuously supplied to the stack at a flow rate of 0.8 mL min^{-1} during one cycle. The HC solution flowed serially through the HC chambers from the cathode to anode side (Figure 1). The LC solution had a similar flow path but in the opposite direction to HC solution. All experiments were conducted at 30 °C.

Analyses. The voltage drop across the 10 Ω external resistor was recorded every 10 min using a data acquisition system (Keitheley Instruments, OH). Current was then calculated using Ohm's law.

The internal resistance of the MRMC was determined by galvanostatic polarization using a potentiostat. Before the tests, the MRMC was left with an open circuit for 3 h. Galvanostatic polarization was conducted with the anode as the working electrode and the cathode as the counter and reference electrode. Different current steps were used for the three biocathodes based on the current production range in the batch cycle. GFB and CC/CB biocathodes: 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mA. SS/Pt biocathode: 0.5, 0.6, 0.7, 0.8, 1, 1.2, and 1.3 mA. The current was set for 50 min for the first point, and at 20 min intervals afterward.[22] Electrode potentials and stack voltage were also measured in polarization tests.

Linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS) were carried out to characterize the electrochemical activities of the biocathodes using a potentiostat. All tests were conducted in the same two-chamber reactors used for starting up the biocathodes, with the cathode as the working electrode, a Pt mesh (10 mm × 10 mm) as the counter electrode, and an Ag/AgCl reference electrode placed in the cathode chamber. Each reactor chamber was filled with the same anaerobic basal medium used for acclimating biocathodes. The LSV tests were performed in the potential range from −0.5 to −1.1 V at a scan rate of 1 mV s^{-1} for three cycles. Only the third cycle was reported due to the good agreement between the last two cycles. EIS tests were conducted at cathode potentials of −0.8 and −1.0 V, as these were similar to stable cathode potentials produced in the MRMC in each fed-batch cycle (−0.86 V for the SS/Pt biocathode, −1.02 V for the GFB biocathode, and −1.04 V for CC/CB biocathode), over a frequency range of 100 kHz to 5 mHz with a sinusoidal perturbation of 14.2 mV amplitude. Charge transfer resistances were obtained by fitting spectra to an equivalent circuit (Figure S3). Two charge transfer elements were used here to calculate the total charge transfer resistance ($R_{ct} = R_{ct1} + R_{ct2}$).

The gas composition in the cathode headspace of the MRMC and two-chamber bioelectrochemical cells was analyzed using gas chromatographs (models 8610B and 310, SRI Instruments, CA) as previously described.[31] Produced gas volume was determined based on the nitrogen gas content and initial nitrogen gas volume in the cathode headspace.[30] Methane production rate was calculated from a linear regression over the entire fed-batch cycle and normalized by catholyte volume.[32] The anolyte chemical oxygen demand (COD) was measured before and after each cycle (COD Reagent, HACH Company). A conductivity-pH meter (SevenMulti, Mettler Toledo, OH) was used to determine the conductivity and pH of anolyte, catholyte, HC, and LC solutions. A second order calibration curve was used to convert the conductivities of the HC and LC solutions into concentrations as previously described.[22]

Methods for calculating Coulombic efficiency (CE, %) and methane yield (Y, mol-CH_4/mol-acetate) were similar to those described for the tests of hydrogen gas production,[25] except that 8 mol of electrons were used in the calculation for the production of 1 mol of methane. Coulombic recovery (CR) was defined as the ratio of the charge recovered as methane to the total charge transferred at the electrodes. Energy efficiency (η_E) was calculated as the ratio of the combustion energy of methane produced to the total supplied energy extracted by the MRMC, as[27]

$$\eta_E = \frac{\Delta H_m n_m}{\Delta H_s (n_s^{in} - n_s^{out}) + X^{in} - X^{out}} \quad (1)$$

where n is the moles of methane or substrate (mol), ΔH the heat of combustion (J mol^{-1}), and X the free energy obtained by totally mixing the HC and LC solutions (J). The subscripts m and s denote methane and substrate, respectively. The superscripts in and out indicate influent and effluent, respectively. X^{in} and X^{out} were calculated as previously described.[24]

RESULTS AND DISCUSSION

MRMC Performance with Different Cathodes. Current was generated, and nearly pure methane (0−0.8% H_2, 0.7−2.6% CO_2) was produced in all MRMCs using the three

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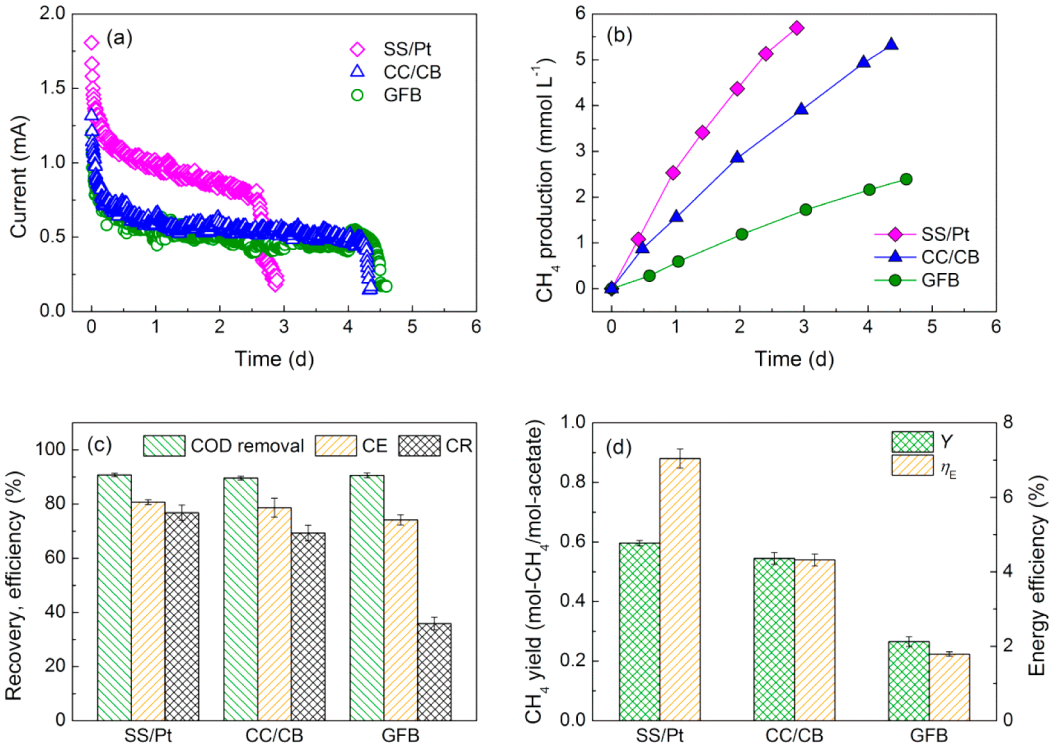

Figure 2. Effect of the different types of cathodes on (a) current and (b) methane gas production (per catholyte volume) over time for a representative cycle; (c) COD removal, Coulombic efficiency (CE), and Coulombic recovery (CR); and (d) methane yield (Y) relative to energy efficiency (η_E). Error bars are standard deviations based on data from three repeatable batch cycles. [SS/Pt, stainless steel (SS) mesh coated with Pt; CC/CB, carbon cloth (CC) coated with carbon black (CB); GFB, plain graphite fiber brush.]

different cathode materials (Figure 2a and b; see Figure S4 in Supporting Information for additional cycles). The current declined gradually over time in each fed-batch cycle (Figure 2a), mainly due to the loss of substrate in the anode chamber. The MRMC with the SS/Pt biocathode produced the highest current, while similar current profiles were obtained for the CC/CB and GFB biocathode MRMCs. The peak current for the MRMC with SS/Pt biocathode (1.5 mA) was nearly 50% higher than that for the MRMC with CC/CB (1.1 mA) or GFB biocathodes (1.0 mA). As a higher current resulted in faster oxidation of the substrate, the cycle time with the SS/Pt biocathode was only 2.9 days, which was much shorter than that for MRMCs with the CC/CB (4.4 days) or GFB (4.7 days) biocathodes (Figure 2a). Although the current profiles for CC/CB and GFB biocathodes were similar, the methane production rate for the GFB biocathode (0.55 ± 0.01 mmol L^{-1} day^{-1}) was much lower than that for the CC/CB (1.2 ± 0.07 mmol L^{-1} day^{-1}) or SS/Pt (2.3 ± 0.1 mmol L^{-1} day^{-1}) biocathodes (Figure 2b).

COD removals (89−91%; Figure 2c) and the final pHs (6.7−7.0) in the anode chamber were similar for all reactors with different biocathodes. The reduction in COD was only slightly lower for the GFB biocathode, with a CE of 74 ± 2% compared to 81 ± 1% for the SS/Pt and 79 ± 3% for the CC/CB biocathodes (Figure 2c). Thus, anode performance, on the basis of COD removal and CE, was relatively unaffected by the use of the three different cathodes or the different cycle times.[22,23]

The largest volume of methane recovered from the biocathode was obtained with the SS/Pt biocathode (5.5 ± 0.07 mL, Y = 0.60 ± 0.01 mol-CH_4/mol-acetate; Figure 2d). Despite the presence of the Pt catalyst, hydrogen gas recovery was minimal for this cathode, as well as the other two biocathodes (<0.2 mL). Methane production was slightly lower with the CC/CB biocathode (4.9 ± 0.1 mL, Y = 0.55 ± 0.02 mol-CH_4/mol-acetate) despite the absence of a Pt catalyst. However, methane recovery by the GFB biocathode (2.4 ± 0.1 mL, Y = 0.27 ± 0.02 mol-CH_4/mol-acetate) was 51% lower than that produced with CC/CB biocathode.

On the basis of methane and current generation, the highest Coulombic recovery was obtained with the SS/Pt biocathode MRMC (CR = 75 ± 2%). The CC/CB biocathode MRMC had a slightly lower CR of 69 ± 3%, but the GFB biocathode had a CR of only 36 ± 2% (Figure 2c). CRs less than 100% indicate a relative absence of cathode corrosion.[32] The differences in CRs were primarily due to the different methane recoveries, as the CEs were similar for all three biocathodes. The much lower CR for the GFB biocathode, and a lack of H_2 gas recovery, suggests that electrical current most probably resulted in the formation of soluble organics. The presence of different organics in solution was not examined here as the focus was on methane production. Previous studies also have shown that both methane and soluble organics can be simultaneously produced in BESs using biocathodes under anaerobic conditions, with carbon dioxide as the only carbon source.[33,34]

The variations in methane production and CRs likely resulted from how electrons are conveyed to and disposed of by methanogens. Pt is an excellent catalyst for hydrogen evolution, and it has previously been shown that the volume of methane production from a Pt-catalyzed biocathode is consistent with the amount of hydrogen gas that is evolved under abiotic conditions. However, the two other cathode materials examined here are much poorer catalysts for hydrogen production and have low abiotic rates of H_2 gas

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production.[32] Thus, it is likely that methane was produced primarily from H_2 gas for the SS/Pt cathode, while direct electron transfer was more important for the other two cathode materials. The GFB cathode, which had the poorest performance here for methane production, has previously been shown to be effective for production of soluble organics in BESs.[35] This suggests that the ineffective conversion of electrical current to methane with the GFB most likely resulted from the production of products (soluble organics) other than methane or H_2 gases.

Energy Efficiency. The highest energy efficiency of 7.0 ± 0.3% was obtained for the MRMC with SS/Pt biocathode, compared to 4.3 ± 0.2% for the CC/CB biocathode MRMC and 1.8 ± 0.06% for the MRMC with the GFB biocathode (Figure 2d). The energy extracted from the substrate [$\Delta H_s(n_s^{in} - n_s^{out})$] was similar for all biocathodes (~317 J) due to similar COD removals. The salinity gradient energy extracted ($X^{in} - X^{out}$) using the SS/Pt biocathode (2552 ± 60 J) was smaller than that obtained using the CC/CB (3722 ± 66 J) or GFB (4502 ± 283 J) biocathodes. Thus, the highest energy efficiency with the SS/Pt biocathode resulted in part from the slightly higher methane production, but mostly it was a result of the smallest energy input due to the utilization of Pt as a catalyst.

Anode, cathode, and stack resistances were estimated from the slopes of the polarization data. Stack voltages and anode potentials were very similar in all MRMCs, but the cathode potentials appreciably varied (Figure 3a and b), demonstrating that the changes in current generation of the MRMC were only due to the cathode. The minimum cathode resistance was obtained with a SS/Pt biocathode (121 ± 7 Ω; Figure 4), consistent with the energy analysis that showed the SS/Pt biocathode required less energy for methane production than

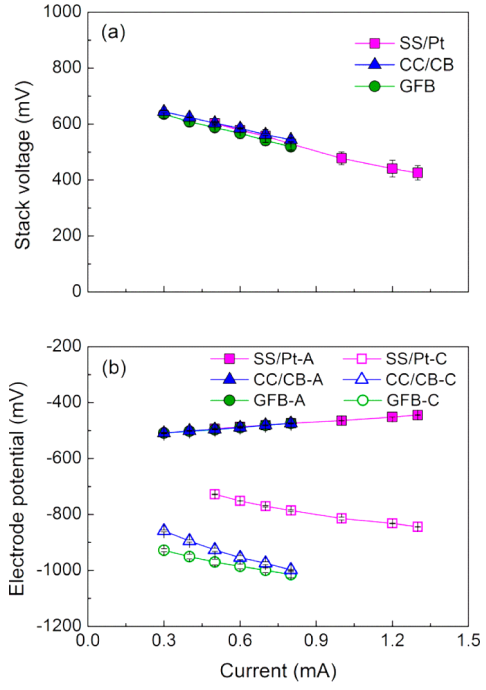

Figure 3. (a) RED stack voltage and (b) anode (A) and cathode (C) potentials (vs Ag/AgCl) as a function of current for the MRMC with different biocathodes. Error bars are standard deviations of duplicate tests. [SS/Pt, stainless steel (SS) mesh coated with Pt; CC/CB, carbon cloth (CC) coated with carbon black (CB); GFB, plain graphite fiber brush.]

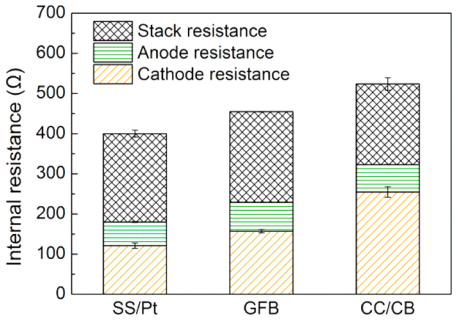

Figure 4. Internal resistance distribution of the MRMC using different biocathodes. Error bars are standard deviations of duplicate tests. [SS/Pt, stainless steel (SS) mesh coated with Pt; CC/CB, carbon cloth (CC) coated with carbon black (CB); GFB, plain graphite fiber brush.]

the other biocathodes. The biocathode resistance for GFB (157 ± 4 Ω) was slightly larger than that for SS/Pt but 38% lower than that for CC/CB (255 ± 13 Ω). Much smaller variations were observed for stack (from 201 ± 15 Ω to 225 ± 1 Ω) and anode resistances (59 ± 1 Ω to 72 ± 0 Ω) than those of the biocathodes.

The energy efficiencies obtained here are relatively low compared to those obtained in previous tests using a microbial reverse-electrodialysis electrolysis cell (MREC) for hydrogen,[22,27] rather than methane production. This lower energy efficiency was mainly due to the larger amount of salinity gradient energy input ($X^{in} - X^{out}$) into the MRMC (2552 ± 60 J to 4502 ± 283 J) than the MREC (650 to 1000 J), due to the much longer cycle times here for the MRMC (2.9 to 4.7 d) than the MREC (0.9 to 1.5 d). The conductivity of the catholyte (9 mS/cm) was lower here for the MRMC due to the use of biocathodes, compared to that used for the MREC (51 mS/cm) with abiotic cathodes.[22,27] This lower conductivity resulted in larger internal resistances (400−524 Ω for the MRMC compared to 200 Ω for the MREC) and lower current densities (peak current of 1.0−1.5 mA for the MRMC compared to peak current of 3.2 mA for the MREC), which overall increased the cycle duration. Since the stack resistance accounted for 38% to 55% of the total internal resistance, energy efficiencies of the MRMCs could be improved by reducing the stack resistance, for example by using thinner spacers[18,19] or profiled membranes,[36] or by using a commercially available RED stack that is known to have a lower stack resistance.[28]

Linear Sweep Voltammetry Analysis of Biocathodes. LSV tests were conducted to further examine the ability of the cathodic microorganisms to directly accept electrons from the electrodes. Quite different results were obtained for the SS/Pt biocathodes compared to abiotic cathodes, than those obtained for the other two cathode materials. The current produced with the SS/Pt biocathode in LSV tests was much lower than that produced with the abiotic SS/Pt cathode (Figure 5). As Pt is a very effective catalyst for hydrogen evolution,[32] this lower current for the SS/Pt biocathode resulted from biofilm formation on the electrode. However, this response cannot provide information on whether electrons are directly used or whether hydrogen gas was first produced. To further examine the impact of the biofilm, the SS/Pt biocathode was exposed to the air for 3 days to kill methanogens and other obligate anaerobes on the electrode that may have been involved in electron uptake or H_2 gas utilization. The current produced in

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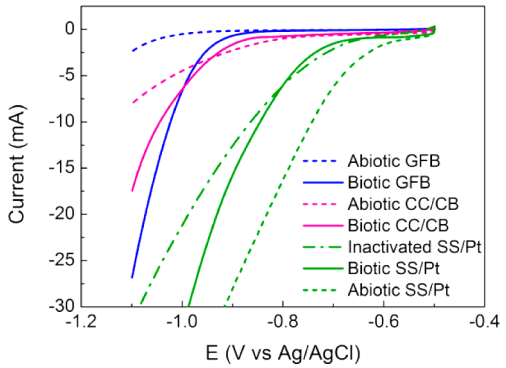

Figure 5. Linear sweep voltammetry of cathodes in the presence (biotic) and absence (abiotic) of a biofilm, and with a killed biofilm (inactivated). [SS/Pt, stainless steel (SS) mesh coated with Pt; CC/CB, carbon cloth (CC) coated with carbon black (CB); GFB, plain graphite fiber brush.]

LSV tests using this inactivated SS/Pt biocathode was significantly lower than that for the original SS/Pt biocathode when the potential was more negative than −0.8 V (Figure 5). This reduction in current suggests that microorganisms on the SS/Pt biocathode were involved in direct electron transfer from the electrode.

For the CC/CB and GFB electrodes, currents produced by the biocathodes were significantly higher than those for the abiotic ones when the potential was more negative than −1.0 V (Figure 5). This increased current with the biofilm provides strong evidence to support direct electron transfer, facilitated by autotrophic microorganisms on these biocathodes.

The current produced with the abiotic CC/CB cathode was much higher than that with the abiotic GFB cathode, despite a much smaller total surface area (Figure 5). In order to show that the carbon black enhanced H_2 evolution, LSV tests were conducted using abiotic carbon cloth cathodes with and without a carbon black layer. Very little current was produced by the abiotic cathode lacking a carbon black layer (−0.02 mA) compared to the cathode with carbon black (−4.5 mA) at −1.0 V (Figure S5). At more negative potentials (−1.0 V) than those used here, however, the current produced by the GFB biocathode improved and could surpass that of the CC/CB biocathodes.

These LSV results demonstrate that the autotrophic microorganisms on the biocathodes used in the MRMC enhanced electron transfer from the electrodes that were poor relative to hydrogen evolution (CC/CB and GFB) compared to the SS/Pt cathodes. Thus, methane was generated in the MRMC via both the direct electron transfer and indirect electron transfer (intermediate production of hydrogen), consistent with previous electromethanogensis studies,[13,33,34] to an extent dependent on the cathode materials.[32]

Electrochemical Impedance Spectroscopy. EIS analysis showed that the biocathodes had smaller charge transfer resistances than the abiotic ones at both −0.8 and −1.0 V, further demonstrating that the microorganisms on these biocathodes catalyzed the release of electrons from the electrodes. At a set potential of −0.8 V, the SS/Pt biocathode had a charge transfer resistance of 21.2 ± 1.3 Ω, compared to 23.5 ± 1.4 Ω with the inactivated SS biocathode (Figure 6a and d). (See Figure S6 in the Supporting Information for Bode

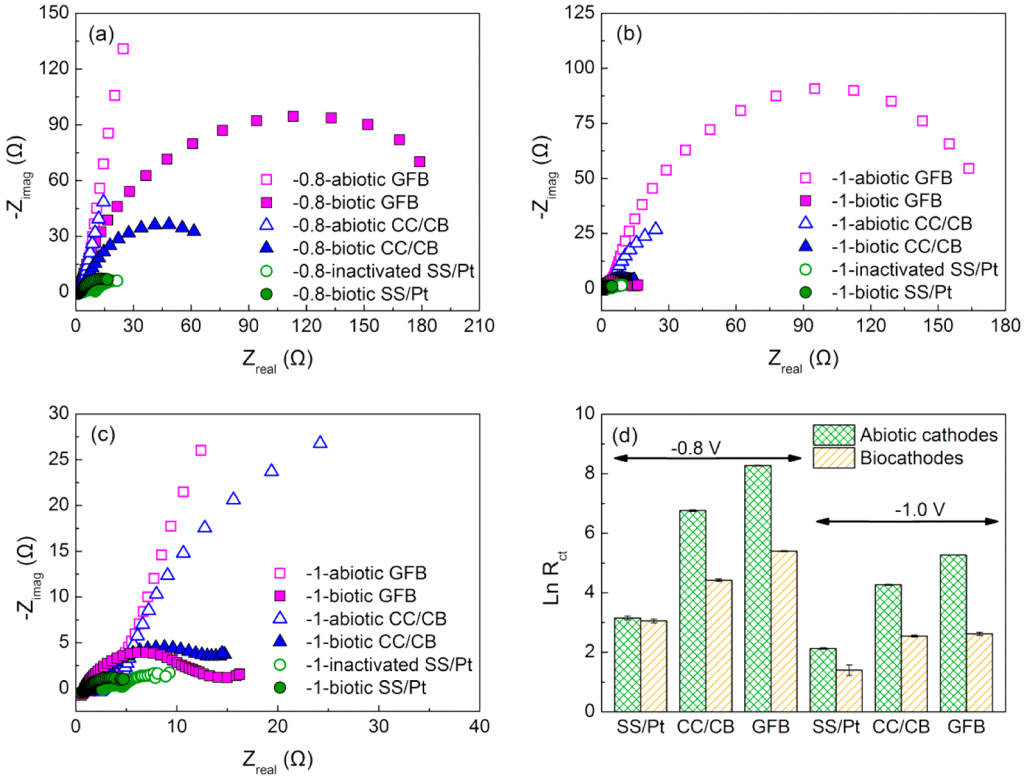

Figure 6. Nyquist plots of impedance data for biocathodes and abiotic controls [SS/Pt, stainless steel (SS) mesh coated with Pt; CC/CB, carbon cloth (CC) coated with carbon black (CB); GFB, plain graphite fiber brush] at different potentials (vs Ag/AgCl) of (a) −0.8 V (only a part of the spectra for abiotic GFB at −0.8 V was shown due to the large impedance) and (b) −1.0 V. (c) Enlargement of impedance data at −1.0 V. (d) Charge transfer resistances (R_{ct}) of biocathodes and abiotic controls at −0.8 and −1.0 V. Error bars are standard deviations of duplicate tests.

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plots.) Much larger charge transfer resistances were obtained with the CC/CB biocathode (84 ± 4 Ω), although this was 90% lower than that obtained with the abiotic CC/CB cathode (864 ± 28 Ω). The charge transfer resistance of the GFB biocathode (222 ± 4 Ω) was the largest among the biocathodes but still significantly smaller than that of the abiotic GFB cathode (3916 ± 40 Ω).

At −1.0 V, the charge transfer resistances for different electrodes decreased significantly (Figure 6b−d), as shown by the much smaller semicircles in Nyquist plots, mainly due to the increased kinetic driving force by the larger overpotential. The charge transfer resistances of the biocathodes were generally 51−93% lower than those of the abiotic ones. The CC/CB biocathode had a similar charge transfer resistance with the GFB biocathode, indicating a more significant decrease of charge transfer resistance for the GFB biocathode when lowering the potential from −0.8 to −1.0 V, which was in accordance with the faster increase of current for the GFB biocathode in the LSV tests.

Outlook. The least amount of energy used to produce the largest volume of methane was obtained using SS/Pt cathodes. However, when considering both performance and cost of materials, the CC/CB cathode would be the most useful cathode for the MRMC due to a lack of a precious metal, and considering its better performance than the GFB for methane production. The use of the RED stack enabled effective current generation needed to sustain methane production in the cathode chamber of this system, resulting in a sustainable method for producing relatively pure methane gas. The use of the thermolytic NH_4HCO_3 solutions could enable energy capture from renewable low-grade waste heat. The amount of waste heat has been estimated to be 204 GW in the United States,[37] and such waste heat is normally available in a wastewater treatment plant.[27] The high COD removals obtained for the MRMC (89−91%) may also enable effective wastewater treatment, although further COD reductions might be needed.[38] Thus, the MRMC coupled with NH_4HCO_3 solutions holds great promise for use as a sustainable method for methane production from waste biomass and waste heat.

■ ASSOCIATED CONTENT

Supporting Information

Photograph of the two-chamber bioelectrochemical cells used to start up the methanogenic biocathodes, methane gas production (per catholyte volume) of the two-chamber bioelectrochemical cell using different biocathodes, equivalent circuit for impedance data fitting, current generation and methane gas production of the MRMC using different biocathodes, linear sweep voltammetry of the abiotic carbon cloth cathode in the presence and absence of a carbon black layer, and Bode plots of impedance data for biocathodes and abiotic controls. 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.

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