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Mass Transport through a Proton Exchange Membrane (Nafion) in Microbial Fuel Cells†

Kyu Jung Chae, Mijin Choi, Folusho F. Ajayi, Wooshin Park, In Seop Chang, and In S. Kim*

Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261, Cheomdan-gwagiro Buk-gu, Gwangju 500-712, South Korea

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Proton exchange membranes (PEMs) are one of the most important components in microbial fuel cells (MFCs), since PEMs physically separate the anode and cathode compartments while allowing protons to transport to the cathode in order to sustain an electrical current. The Nafion 117 membrane used in this study is generally regarded as having excellent proton conductivity, though many problems for its application in MFCs remain. We investigated problems associated with Nafion including: oxygen leakage from cathode to anode, substrate loss, cation transport and accumulation rather than protons, and biofouling. It was found that Nafion was quite permeable to oxygen. The oxygen mass transfer coefficient (K_O) and the oxygen diffusion coefficient (D_O) for Nafion was estimated as $K_O = 2.80 \times 10^{-4}$ cm/s and $D_O = 5.35 \times 10^{-6}$ cm^2/s, respectively when a 50 mM phosphate buffer was used as the catholyte. The MFC with distilled water instead of phosphate buffer showed similar values ($K_O = 2.77 \times 10^{-4}$ cm/s, $D_O = 5.27 \times 10^{-6}$ cm^2/s), indicating that the catholyte shows no significant effects on oxygen diffusion. Nafion was also found to be permeable to acetate, but this seems to be negligible. Cations in the anolyte, presenting in high concentration due to their supply to optimize the anodic bacterial growth condition, were rapidly transported through the Nafion membrane. They occupied negatively charged sulfonate groups of Nafion, which consequently reduced contact chance of protons due to their competition. According to energy dispersive X-ray (EDX) analysis, the relative atomic percentage of carbon (30.9%) and fluoride (59.7%), the basic backbone materials of Nafion, in used Nafion was lowered, compared to that obtained with new Nafion (C = 32.8%, F = 60.1%); whereas sodium and iron, which do not exist in new Nafion, increased by up to 1.16% and 0.24% of the atoms, respectively. This indicates that these two cation species already occupied an important fraction of the negatively charged sulfonate groups, which can cause the hindrance of proton migration. Nafion operated over a period of 50 days was contaminated with biofilm causing adverse effects on mass transport through the membrane. Bacteria growing on Nafion were much more heterogeneous compared to those observed on the anode carbon felt surface. The densest biofilm was observed in the outermost few millimeters of anode carbon felt, and the biofilm density gradually decreased with inward depth.

Introduction

Renewable energy generation and waste disposal are two key challenges for the sustainability of future societies. Microbial fuel cells (MFCs) have been considered as a promising solution by linking both tasks at the same time.[1–3] MFCs are devices that convert chemical energy contained in the bonds of organic or inorganic compounds to electrical energy with the aid of bacteria as biocatalysts.[1,3–7] MFCs can also be modified to produce hydrogen gas by maintaining the cathode in an oxygen-free condition and adding in an external small voltage.[8,9] The generation of electricity in MFCs is based on the fact that bacteria derive energy from the oxidation of organic matter linked to the reduction of another compound, typically oxygen. Bacteria in the anode chamber, diversely referred to as electrocigens,[3] anodophilic bacteria,[4] and electrochemically active bacteria,[10] oxidize the substrate, separating electrons from protons. These electrons and protons travel to the cathode, the former via an external circuit and the latter diffusing through electrolyte and a proton exchange membranes (PEM). The protons and electrons subsequently combine at the cathode with oxygen, aided by a catalyst such as platinum, to form water.[3–6,11–15] Chemical oxidizers, such as ferricyanide or Mn (IV), can also be used, but these must be replaced or regenerated as they are not self-sustaining.[15–19]

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* Corresponding author. E-mail: iskim@gist.ac.kr. Phone: +82 62 970 2436. Fax: +82 62 970 2434.

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For each electron that is produced, an equivalent proton must be transported to the cathode through the electrolyte to sustain the current. Therefore, PEMs are one of the most important components in MFCs, as they physically separate the anode and cathode compartments while allowing protons to pass trough to the cathode. The Nafion 117 membrane (Dupont Co., USA) is one of the most commonly used PEMs in MFCs, though a number of problems associated with Nafion membranes still exist, including: oxygen leakage from cathode to anode, substrate loss, cation transport and accumulation rather than protons, and biofouling. Of these, oxygen leakage into the anode chamber can either lower energy recovery due to the substrate loss from aerobic respiration by facultative bacteria, or inhibit the growth of obligate anaerobes.[9,13,20,21] To overcome this problem, Bond and Lovley[6] suggested maintaining aeration to a minimum to prevent excessive transfer of oxygen across PEMs. Kim et al.[22] further reported that Nafion was the most permeable membrane to oxygen from among the commercially available membranes tested in their study.

Nafion, a sulfonated tetrafluorethylene copolymer, consists of a hydrophobic fluorocarbon backbone (–CF_2–CF_2–) to which hydrophilic sulfonate groups (SO_3^-) are attached.[23] The presence of negatively charged sulfonate groups in the membrane explains the high level of proton conductivity of Nafion, while also showing a significant undesirable affinity for other cations rather than protons. Most MFCs are operated at a neutral pH in order to optimize bacterial growth in the anode chamber, while other cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+) contained in growth medium are typically present at a 10^5 times higher concentration than protons.[24] Consequently, these cations combine with the sulfonate groups of Nafion and inhibit the migration of protons produced during substrate degradation, causing a decrease in MFC performance due to the pH reduction in the anode chamber, with a corresponding pH increase in the cathode chamber.[24] In addition, the frequent replacement of the buffer solution as a catholyte reduced the economic viability of MFCs. Biofilm development in Nafion has not been discussed in previous research, even though it is inevitable during the long term operation of MFCs.

Up to now, the above problems have not been systemically examined in MFCs for their effect on power generation. Therefore, the focus of this study is to examine the transports of cations, oxygen, and substrates through Nafion at various operational conditions and their effects on MFC performance. Biofouling of the Nafion membrane is also investigated.

Materials and Methods

Experimental Setup. Mass transport studies (e.g., cations, substrate, oxygen) through Nafion were conducted with three uninoculated MFCs, each having the same operational conditions only without inoculums in the anode compartment. However, normal inoculated MFCs were used to investigate the effects of oxygen diffusion through Nafion and catholyte types on MFC performance and to examine biofouling in Nafion.

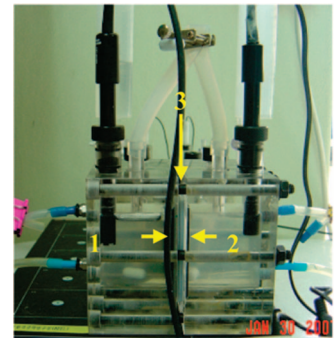

Figure 1. Two-chamber MFC used in this experiment: (1) anode, (2) cathode, and (3) Nafion.

Anolyte, Catholyte, and Microorganisms. The anode chamber was filled with an autoclaved anaerobic nutrient mineral buffer (NMB, pH 7.0) solution containing (milligrams per liter in deionized water): NH_4Cl (530), $CaCl_2$ (150), $MgCl_2 \cdot 6H_2O$ (200), NaH_2PO_4 (6000), KH_2PO_4 (140), $CoCl_2 \cdot 6H_2O$ (2.5), $NaMoO \cdot 2H_2O$ (0.05), $FeCl_2 \cdot 4H_2O$ (20), $NiCl_2 \cdot 4H_2O$ (0.25), $MnCl_2 \cdot 4H_2O$ (0.5), Na_2SeO_4 (0.25), $NaVO_3 \cdot 4H_2O$ (0.05), $ZnCl_2$ (0.25), and $CuCl_2$ (0.15). The anode chamber was inoculated using anaerobic digester sludge (20% v/v) from Gwangju sewage treatment plant in South Korea under an oxygen-free condition at 30 °C, and acetate (0.5 mM, unless stated otherwise) was used as a substrate in all tests with inoculated MFCs. The cathode chamber normally contained a phosphate buffer (50 mM, pH 7.0) of NaH_2PO_4 6000 mg/L and was continuously sparged with air, though deionized water was sometimes used for specific purposes. Both chambers were initially purged with nitrogen gas to remove oxygen.

MFC Construction and Operation. MFCs consisted of two plastic rectangular chambers (180 mL each) separated with a Nafion 117 proton exchange membrane (a projected area of 25 cm^2, Dupont Co., USA), as shown in Figure 1. The anode consisted of a carbon felt electrode (5 × 5 cm, 6 mm thickness, Morgan, UK) which was adhered to the perforated stainless steel plate with conductive glue. The cathode was made of a square-shaped perforated titanium plate electrode, with a 0.5 mg/cm^2 platinum coating (5 × 5 cm, 1.5 mm thickness, Labco Co., South Korea) as a catalyst. To minimize internal resistance, both electrodes were placed in direct contact with the Nafion membrane with a 3-mm-electrode spacing. The projected area of the Nafion membrane was designed to be as large as possible (identical in size to both the anode and cathode) in order to facilitate proton migration through the membrane. The membrane was held in place with a rubber gasket on each side to prevent leakage. Copper wire was used to connect the circuit with a 1000 Ω resistor. For MFC operations, the anode chamber was sterilized with 70% ethanol, flushed with nitrogen gas to prevent anaerobic bacteria from oxygen damage, and filled with an anaerobic NMB solution. The cathode chamber was then filled with a phosphate buffer (50 mM, pH 7.0). Both chambers were mixed slowly with a magnetic stir bar at 200 rpm, and the cathode was sparged with air. All MFC tests were conducted in a temperature-controlled room at 30 °C.

Electrode and Nafion Membrane Pretreatment. New electrodes were soaked in 100% ethanol for 30 min and in 1 M HCl for 1 h. After each use, the electrodes were washed in 1.0 M HCl followed by 1.0 M NaOH, each for 1 h, to remove possible metal and organic contamination, and stored in distilled water before use. Nafion was pretreated by boiling in H_2O_2 (30% v/v) and deionized

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water, followed by soaking in 0.5 M H_2SO_4 and then deionized water, each for 1 h. To prevent membrane swelling when placed in the MFC compartment, membranes were stored in deionized water prior to being used.

Oxygen Transfer Coefficients. Oxygen mass transfer coefficients for different experimental conditions were determined as previously described,[22] using uninoculated MFC reactors. A dissolved oxygen (DO) probe was placed in the anode chamber, and the water was sparged with nitrogen gas to remove DO. The cathode chamber was continuously aerated to maintain saturated DO conditions. The mass transfer coefficient of oxygen in the membrane, K_O, was determined by measuring the DO concentration over time and using the mass balances (eq 1).

$$K_O = -v/At \ln[(C_O - C)/C_O] \quad (1)$$

where v is the liquid volume in the anode chamber, A is the membrane cross-sectional area, C_O is the saturated oxygen concentration in the cathode chamber, and C is the DO in the anode chamber at time t. The diffusion coefficient (D, cm^2/s) for each chemical was calculated as $D_O = k_O L_t$, where L_t is the membrane thickness reported by the manufacturer.

Calculations and Analyses. The voltage across a 1000 Ω resistor in the external circuit in the MFC was monitored at 10 min intervals using a multimeter (Keithley, OH) connected to a personal computer. Current (I), power (P), power density (PD), and Coulombic efficiency (CE) were calculated as previously described.[14,15] Cation concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+) were determined using an ion chromatograph (Dionex 120, USA) equipped with a conductivity detector (cation column: IonPac CS12A). Acetate was measured using a gas chromatograph (Hewlett Packard 6890 plus series) equipped with a flame ionization detector (FID) and an EC-1000 capillary column (Alltech, USA) with helium as the carrier gas. The pH, conductivity, and dissolved oxygen were measured with a pH meter (Orion, USA), a conductivity meter (Oakton, Singapore), and a DO meter (Orion, USA), respectively.

Scanning Electron Microscope and Energy Dispersive X-ray Spectrometry Analyses. For scanning electron microscope (SEM) analysis, parts of the carbon felt were removed from the anode chambers, rinsed with a sterile medium, and immediately fixed using an anaerobic solution of 2% glutaraldehyde and 1% formaldehyde. After immersion in 1% osmium tetroxide for 24 h, samples were carefully rinsed three times in a HEPES buffer (pH 6.8) and once in deionized water. Samples were then subjected to a serial dehydration protocol using increasing concentrations of ethanol (10, 25, 50, 75, 90, 100, 100, and 100; 30 min for each stage with very gentle periodic agitation) and then dried completely at room temperature. Desiccated samples were coated with gold and observed using a Hitachi S-4700 cold field emission SEM at 15 kV. To evaluate the cation accumulation on the used Nafion membrane, a new membrane and membrane samples from MFC after a 50 day run were taken; they were first washed with MilliQ water and then analyzed with energy dispersive X-ray spectrometry (EDX) using a Hitachi S-4700 SEM at an acceleration voltage of 10 kV.

DAPI Staining. In order to examine bacterial growth on the Nafion membrane, biofilm grown on Nafion was detached and DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) staining was performed. After DAPI staining, a confocal laser scanning microscope (CLSM, Carl Zeiss, LSM 5 PASCAL), equipped with an inverted microscope, an argon laser (458/488/514 nm, 25 mW), a green helium/neon laser (543 nm, 1 mW), and a red helium/neon laser (633 nm, 5 mW) was used to visualize the biofilm.

Results and Discussion

MFC Integrity. In order to determine the integrity (leakage-free condition) of assembled MFCs, a pressure decay test and an air permeation test were conducted prior to use. The purpose of these tests was to determine any possible leakages, since anode bacteria require strict anaerobic conditions and outside air penetration can cause false results in MFCs with respect to the oxygen diffusion coefficient and a loss of Coulombic efficiency (CE) due to aerobic bacterial respiration. As the first step in the test, a pressure decay test was conducted by pressurizing both MFC chambers with nitrogen gas and then by measuring the pressure drop over time; the chambers contained 50% (v/v) of deionized water to prevent Nafion membrane drying, which causes swelling. This test was conducted at a constant temperature condition to avoid water vapor pressure interfering with the total nitrogen gas pressure. As can be seen in Figure 2, the anode and cathode chambers maintained a constant pressure of 81 and 80 mbar for over 150 min, respectively.

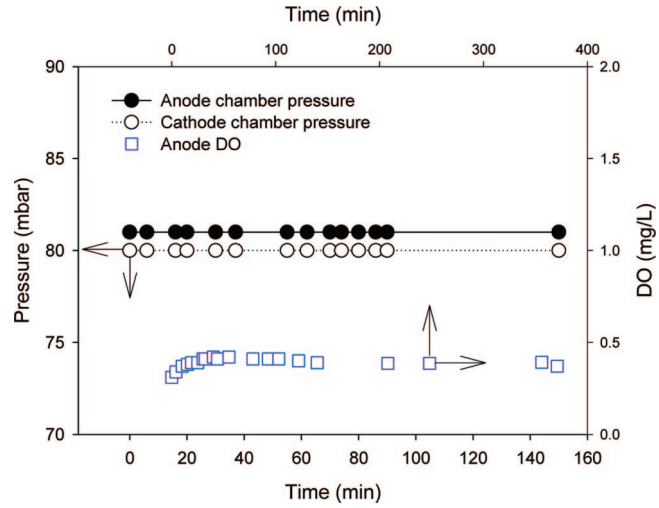

Figure 2. MFC integrity result. Pressure decay and air permeation test results confirm the completely gas-leakage-free condition of MFCs.

To verify the pressure decay test, the following air permeation test was conducted. Both chambers were sparged with nitrogen gas for 4 h to remove any dissolved oxygen (DO), and then, the change of anode DO concentration was monitored using a DO electrode installed on the top of the chambers. The DO level increased from 0.31 to 0.41 mg/L in about 30 min, due to diffusion of residual DO in the cathode chamber, but it showed no increase from 30 to over 340 min, indicating that there was no oxygen penetration from outside the MFC reactor (Figure 2). All MFC tests were only performed after the confirmation of leak-free conditions in the reactor. According to our previous experiences, a simple gas bubbling test for determining leakages by observing the stream of pressurized gas bubbles in the immersion tank is not sufficient, because DO penetration still occurs, even in the case of no gas bubbling.

Oxygen Transport through Nafion Membrane. The oxygen flux from cathode to anode through the Nafion membrane was evaluated using uninoculated MFC reactors with different catholytes (anolyte NMB; catholyte phosphate buffer of 50 mM or distilled water) by measuring DO accumulation in the substrate-free NMB solution of the anode chamber over time. DO probes were placed in both the anode and cathode chambers. The NMB solution in the anode chamber was sparged with nitrogen gas to remove DO prior to measuring DO concentration, but the cathode chamber was continuously aerated to maintain saturated DO conditions. Each chamber of the reactor was continuously stirred during these tests using a magnetic stir bar. The DO concentration of the anode chamber increased from 0.38 to 1.36 mg/L within 655 min due to oxygen transfer across the Nafion membrane, as shown in Figure 3. When a 50 mM phosphate buffer was used as the catholyte, the oxygen mass transfer coefficient (K_O) and the oxygen diffusion coefficient (D_O) for Nafion were estimated to be $K_O = 2.80 \times 10^{-4}$ cm/s and $D_O = 5.35 \times 10^{-6}$ cm^2/s, respectively. The MFC with

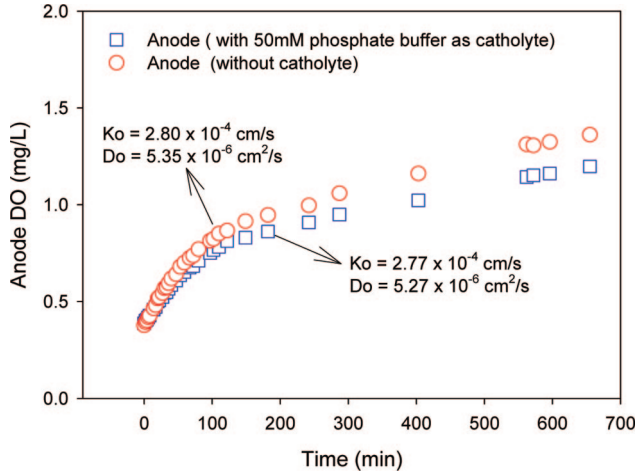

Figure 3. Oxygen diffusion into the anode chamber through a Nafion membrane in an uninoculated MFC (K_O = 2.82 × 10^{-4} cm/s, D_O = 5.36 × 10^{-6} cm^2/s).

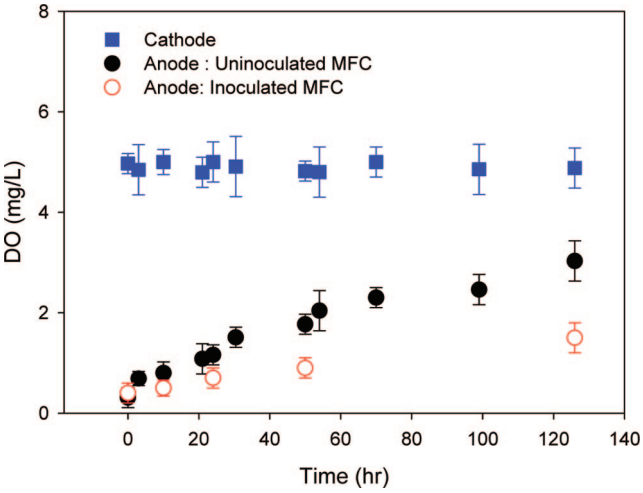

Figure 4. Comparison of the increase of anode chamber DO concentration between inoculated (1 mM acetate) and uninoculated MFC. The bars indicate standard deviations (n = 3).

distilled water instead of the phosphate buffer showed similar values (K_O = 2.77 × 10^{-4} cm/s, D_O = 5.27 × 10^{-6} cm^2/s), indicating that the catholyte shows no significant effects on oxygen diffusion.

According to previous reports,[22,25] the value of the oxygen diffusion coefficient was in the range of 1–6 × 10^{-6} cm^2/s; our estimate was about 2.2 times larger than that reported by Kim et al.[22] This high value of D_O implies that Nafion is quite permeable to oxygen, resulting in the diffusion of oxygen into the anode chamber. Consequently, this could lower the performance of MFCs, either by damaging anaerobic bacteria or loss of substrate due to aerobic bacterial respiration. Supporting this view, Liu and Logan[25] reported that up to 28% of the glucose added to an MFC was lost through aerobic bacterial respiration due to the oxygen diffusion via the Nafion membrane. Figure 4 represents the anode DO increase in an inoculated MFC, reflecting biological effects on oxygen transport. Inoculated MFC showed an approximately 2 times slower increase in anode DO concentration compared to the uninoculated MFC, due to the fact that the large amount of diffused oxygen was continuously consumed by aerobic bacteria.

Oxygen Effects on MFC Performance. The effects of oxygen diffusion into the anode compartment on power generation were evaluated under two different conditions: nitrogen gas sparged and nonsparged. Results of this study indicate that a nitrogen gas sparged MFC displayed a much higher power output than the nonsparged one due to the continuous removal of diffused oxygen from the cathode (Figures 5a and b). During the first voltage evolution after inoculation, the voltage output of an MFC sparged with nitrogen gas (272 mV, PD = 29.4 mW/m^2) was 1.7 times larger than that obtained using a nonsparged MFC (160 mV, PD = 10.3 mW/m^2), producing CE values of 20.3% and 12.8%, respectively. With respect to time, the voltage output increased in both cases, but the difference between the two MFCs gradually decreased. The relatively plentiful existence of facultative or aerobic bacteria at the start might have resulted in the significant difference between the two MFCs, due to substrate loss in the nonsparged MFC, though their gradual suppression over time, causing the dominance of other electrocigens, lowered the difference. However, at the third substrate feeding, the voltage generated (325 mV, 41.2 mW/m^2) by the nitrogen gas sparged MFC was still larger than that obtained with the nonsparged MFC (272 mV, 31.4 mW/m^2). The CEs in both cases were quite low because they were obtained in the early stages of an MFC run. The anode DO concentrations of the sparged and nonsparged MFCs were 0.8 and 1.2 mg/L, respectively, when the voltage dropped to zero.

In many studies, the two electrodes have the same cross-sectional area, but the area of PEM is often smaller than that of the electrodes.[12,15] However, Nafion installed in our MFC was designed to have a maximum possible size so as to facilitate proton transport and as such had the same cross-sectional size as both electrodes with only a 3-mm-electrode spacing. This design reduced the internal resistance but at the same time facilitated oxygen diffusion into the anode, which is one reason for the low CE in our study. Therefore, further consideration of PEM size is required, even though Oh and Logan[26] have previously suggested that an increase in PEM size enhances power output.

Cation Transport through Nafion Membrane. As shown in Figure 6, dominantly present cation species in the anolyte were actively transported through the Nafion membrane in the uninoculated MFC, resulting in an increased concentration of these cation species in the catholyte. The increase in conductivity in the catholyte, therefore, was due to the cation transport effect. In fact, the cation transport rates were actually slower than that previously reported using an inoculated MFC.[24] In the inoculated MFC, to sustain a proper reaction ($O_2 + 4H^+ + 4e \rightarrow 2H_2O$ in the cathode), the transport of electrons produced during the biological substrate degradation to the cathode needs to be compensated for by the transport of an equal amount of positive charge to the cathode compartment in order to maintain electroneutrality. This transport works to accelerate cation migration through Nafion. In contrast, the physicochemical concentration gradient of a cation species is the sole driving force in the uninoculated MFC tested in our research because of the absence of bacteria in the anode compartment, which explains the relatively slow transport rate of cations. Most cation species were transported from the anode to the cathode direction due to the concentration gradient, except for sodium. In the case of sodium, NaH_2PO_4 was used as the catholyte, which resulted in an increase of the sodium concentration in the cathode chamber; consequently, reverse transport occurred. The active transport of cation species through Nafion is thus seen to reduce a proton's chance for contact with the sulfonate groups in Nafion due to their competition for available carriers.

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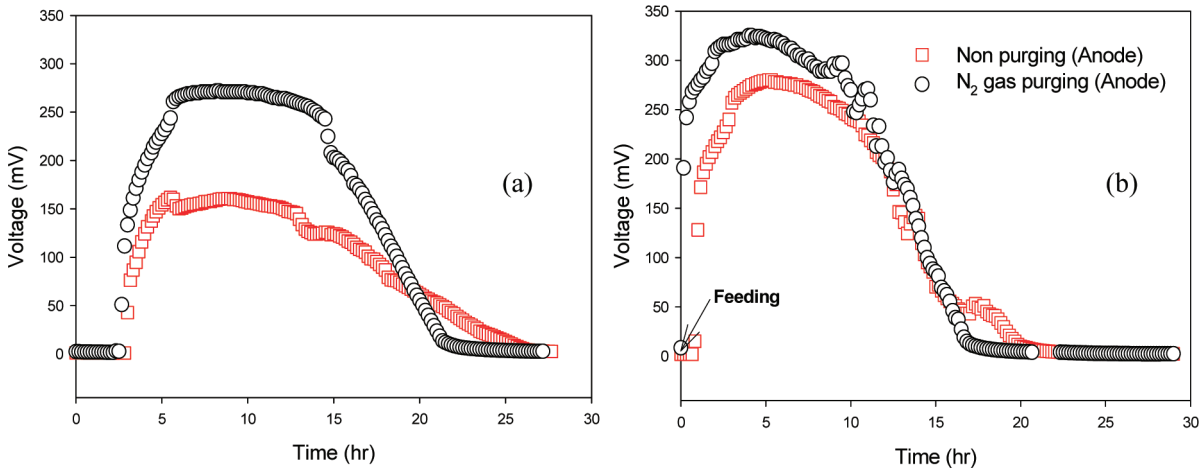

Figure 5. Comparison of voltage evolution between a nitrogen gas spared MFC and a nonsparged MFC when 0.5 mM acetate was used as the substrate: (a) first acetate feeding (first voltage evolution after inoculation) and (b) third acetate feeding.

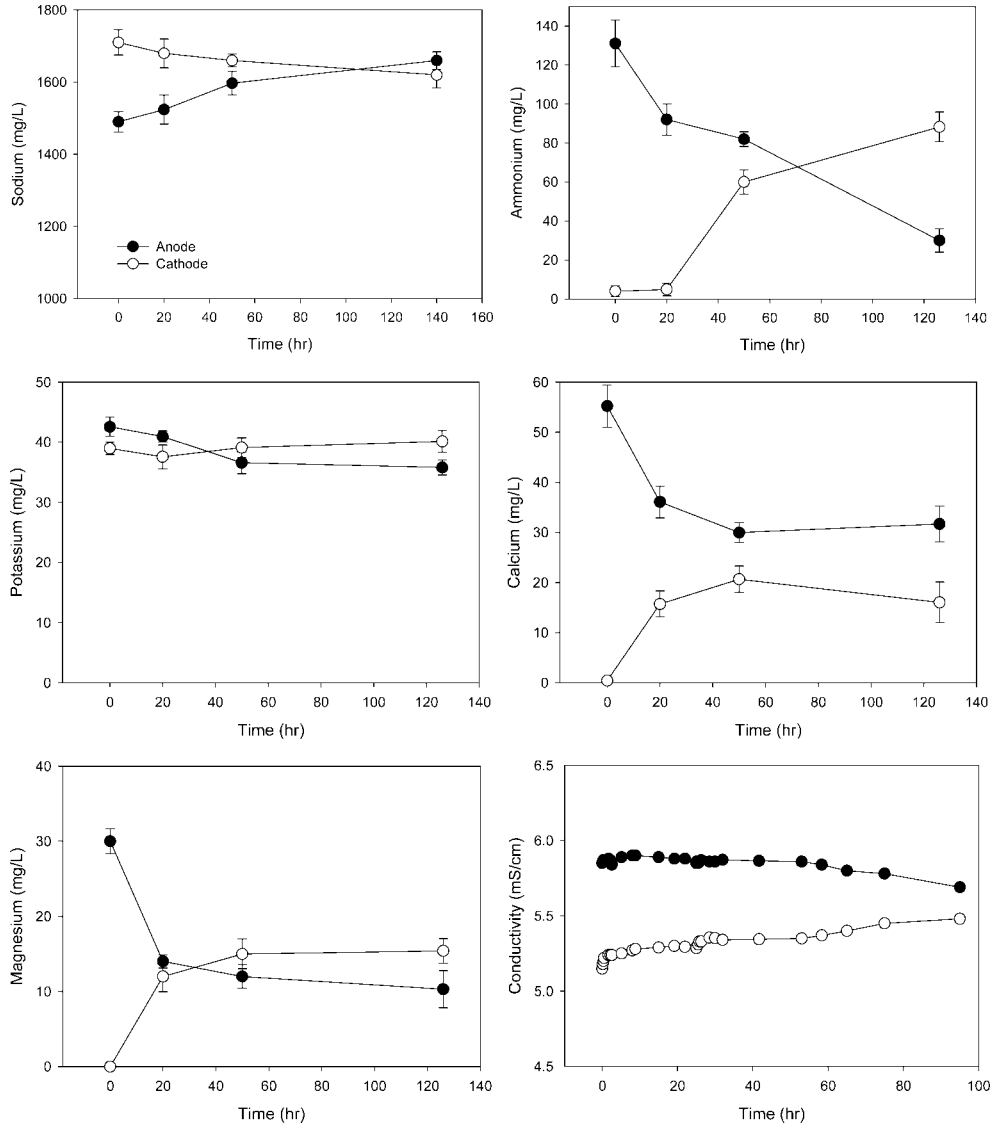

Figure 6. Sodium, ammonium, potassium, calcium, magnesium, and conductivity change in both anode and cathode chambers of uninoculated MFCs: (•) anode (anolyte NMB solution); (○) cathode (catholyte 50 mM phosphate buffer). Data indicate mean ± SD (n = 3).

Rozendal et al.[24] reported that over 99.999% of the sulfonate groups were occupied by cations other than protons, based on the fact that sulfonate groups in Nafion have a higher affinity for most other cation species. In addition, the concentrations of other cation species in an anolyte are significantly higher than that of protons, thus making proton transport negligibly small compared to the transport of other cations, even with a relatively higher diffusion coefficient of protons in Nafion than that of cations.

In order to verify the occupation of sulfonate groups of Nafion membrane by other cations, three samples of Nafion (new

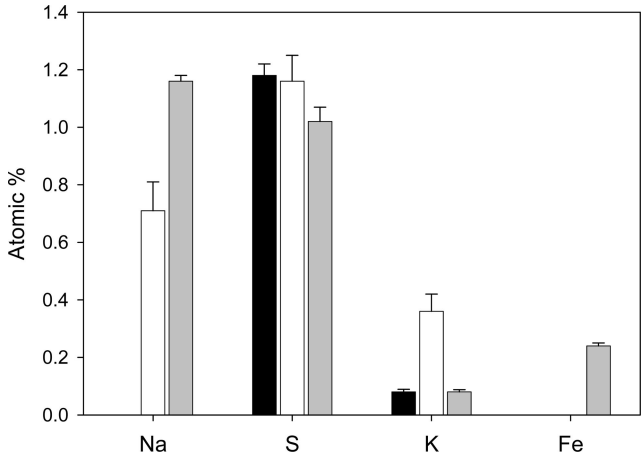

Figure 7. Cations occupation of sulfonate groups on three Nafion membranes: (black box) new Nafion; (grey box) projected area of used Nafion from a 50 day run in inoculated MFC; (white box) outside of the projected area of used Nafion. Data indicate mean ± SD ($n = 3$).

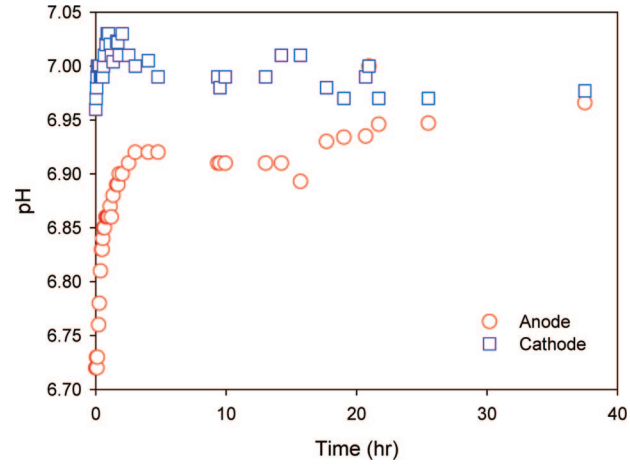

Figure 8. pH changes of the anode and cathode chambers in the uninoculated MFC (anolyte NMB, catholyte 50 mM phosphate buffer).

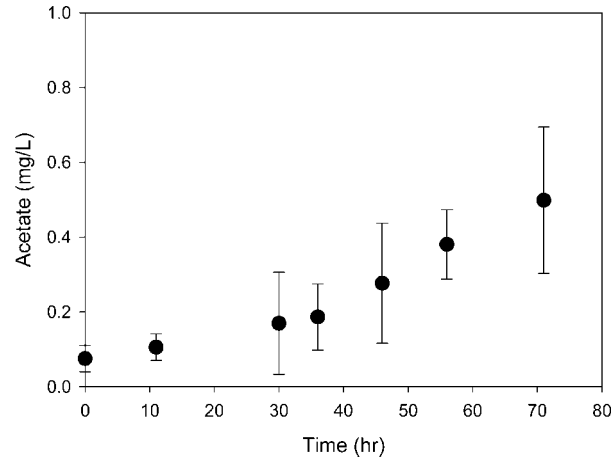

Figure 9. Increase of acetate concentration in the cathode chamber due to diffusion from anode to cathode in the uninoculated MFC. Initially, a 67 mg/L of acetate was fed in the anode chamber only. Bars indicate standard deviations ($n = 3$).

Nafion, a projected area of used Nafion, and outside of the projected area of used Nafion) were analyzed with energy dispersive X-ray spectrometry (EDX). Figure 7 shows the cation's occupation on sulfonate groups in Nafion from a 50 day run in an inoculated MFC fed with a 1 mM of acetate. The relative atomic percentages of carbon (C, 30.9 %) and fluoride (F, 59.7 %), the basic backbone materials of Nafion, in used Nafion were lower compared to results obtained with new Nafion (C = 32.8%, F = 60.1%), indicating an increase of other materials such as cation species. Additionally, the sulfur (S) content for used Nafion was 1.02%, which originated from the negatively charged sulfonate groups in Nafion but also decreased by 15% compared to new Nafion. However, sodium (Na) and iron (Fe), which do not exist in new Nafion, increased up to 1.16% and 0.24% of the atoms, respectively, which indicates that these two cation species already occupied an important fraction of the negatively charged sulfonate groups. The outside of the projected area of used Nafion has a similar increasing trend of cations species, though somewhat less than the projected area of the used Nafion.

pH Changes in an Uninoculated MFC. As predicted, the pH differences between the anode and cathode compartments of an uninoculated MFC gradually decreased and ultimately converged; the initial pH values of the anode and cathode compartments were 6.70 and 6.96, respectively. To reach equilibrium, the anode pH value increased sharply from 6.70 to 6.92 in 3 h and then gradually increased to 6.97, whereas the cathode pH was shown to decrease (Figure 8). The slow increase of pH after the rapid increase during the startup period might be explained not only by the decreased proton gradient between both chambers, but also by the prior occupation of sulfonate groups of Nafion by other cation species. However, in the case of the inoculated MFC operation without a buffer supply to the anode for a long period of time, cations such as sodium and calcium rather than protons already occupied an important fraction of the negatively charged sulfonate groups of the Nafion membrane. This prior occupation resulted in the hindrance of proton migration produced in the anode chamber, and consequently, the pH decreased in the anode chamber (data not shown). This result agrees with previous observations, a decreasing pH in the anode chamber and an increasing pH in the cathode chamber, because proton transport through the Nafion seemed to be slower than both the proton production rate in the anode chamber and the proton consumption rate in the cathode chamber.[24,27]

Substrate Loss through Nafion Membrane. During MFC operation, the substrate was lost not only by aerobic bacterial respiration in the anode chamber sustained by oxygen diffusion through the Nafion membrane but also by substrate diffusion itself into the cathode chamber across Nafion. According to the study of acetate diffusion using the uninoculated MFC, Nafion was found to be permeable to acetate, even at a low concentration as shown in Figure 9. Approximately 0.8% of acetate diffused from the anode to cathode through Nafion within 71 h when the anode chamber was fed with acetate at the concentration of 67 mg/L. This value is quite negligible. In the case of normal inoculated MFC operation, this direct substrate diffusion is further reduced as a result of substrate degradation by anodic bacteria which results in gradual lowering of the substrate concentration gradient between the anode and cathode chamber over time.

Catholyte Effects on MFC Performance. During the prolonged operation of MFCs, periodic replacement of catholyte (normally a phosphate buffer) is common because the buffer offers a supply of protons, thus compensating for the slow proton transport rate through Nafion.[24,27] Protons are equimolarly

(27) Gil, G. C.; Chang, I. S.; Kim, B. H.; Kim, M.; Jang, J. K.; Park, H. S.; Kim, H. J. *Biosens. Bioelectron.* **2003**, *18*, 327–334.

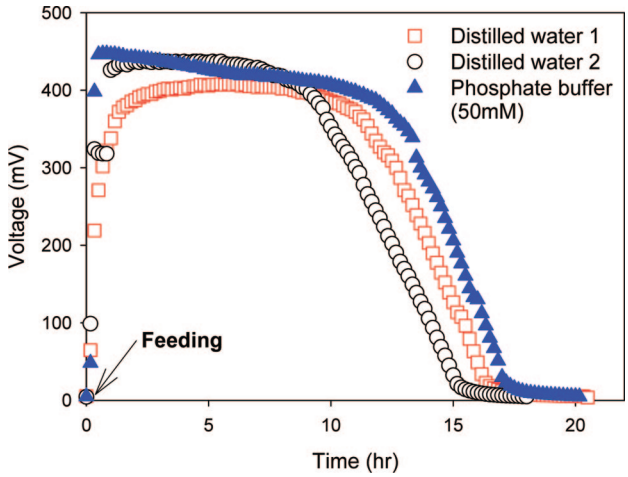

Figure 10. Catholyte effects on MFC performance. Voltage evolutions were obtained at the sixth acetate feeding (0.5 mM) after inoculation.

consumed with electrons in the oxygen reduction reaction in the cathode chamber, thus a pH increase is expected if protons are not supplied through Nafion. However, this periodic buffer replacement evidently reduces the economic viability of MFCs. Therefore, we investigated the feasibility of distilled water usage as an alternative catholyte, instead of a phosphate buffer, using inoculated MFCs fed with 0.5 mM of acetate. The pH drops in the anode chambers of both MFCs were negligible due to the buffering capacity of the NMB solution (anolyte). Conversely, the pH of the cathode chamber with deionized water severely increased from 6.51 to 8.3, compared to that obtained by the phosphate buffer applied MFC (from pH 7.01 to 7.10). However, as shown in Figure 10, the cell voltage and CE showed no significant difference between the MFC with phosphate buffer as a catholyte (410 mV) and the MFC with distilled water (400 mV), which contradicts previous studies.[27] Anolyte and catholyte were replaced every 150 h of operation, and the applied acetate concentration was relatively low (0.5 mM) in our test compared to other studies, which might have resulted in the limited pH suffering in MFCs with distilled water—a decrease in the anode causing an unfavorable bacterial growth condition and an increase in the cathode with a corresponding shortage of available protons for oxygen reduction. However, if experiments take longer than a few weeks or if the substrate was applied in high concentration, a severe, negative pH effect is expected. Therefore, the cathode chamber requires a buffer to compensate for the possible lack of proton transport.

Biofilms on the Anode and Biofouling of the Nafion Membrane. Bacteria growing on the anode appeared to be uniform in morphology, as shown in Figure 11, with the dominant rod type species (e.g., *Bacilius*) forming a multilayer biofilm. When the medium in the anode chamber was removed, indicating the removal of the suspended bacteria, and replaced with fresh acetate medium, current production rapidly returned to levels observed before the replacement of the medium. This rapid return shows that bacteria attached to the surfaces of electrodes were primarily responsible for current generation (data not shown). In our MFC reactors, the anode carbon felt (6 mm thickness) is bonded to the rubber gasket assembled perforated stainless steel plate, which directly faces the Nafion membrane. This stainless steel plate was connected to the external circuit wire in order to transfer electrons from the carbon felt to the cathode. To examine the depth stratification, samples were taken from three points of anode carbon felt: the surface (contacting bulk liquid), middle, and bottom (contacting perforate stainless plate). The densest biofilm was observed in the outermost few millimeters of the carbon felt (the outer interface of carbon felt/bulk liquid), and the biofilm density was found to gradually decrease with respect to the depth towards the perforate stainless steel plate. This stratification resulted from the limited opportunities for contact between the substrate and the bacteria deep inside the felt due to the quite densely entwined structure of carbon felt, with a corresponding small amount of void space or pores.

After a 50 day run, Nafion was contaminated with biofilm as shown in Figure 12. The bacteria growing on Nafion showed a significant morphological difference (much more heterogeneous) compared to those observed on the anode carbon felt surface (Figure 12c). At present, there is not a certain explanation for this heterogeneity. Nevertheless, a cohabitation of both aerobic bacteria and anaerobic bacteria on the Nafion surface is probably considered to be the reason. Biofilm on Nafion could cause adverse effects on the mass transport through the membrane. For instance, if aerobic bacteria grow on the anodic side of the Nafion membrane, they could continuously consume the oxygen diffused from cathode. This in turn could increase the oxygen concentration gradient across the membrane, resulting in the acceleration of oxygen diffusion. Furthermore, reduced proton migration would be also expected due to the physical barrier of the biofilm. However, little study has been conducted regarding the problem of biofilm in Nafion to date.

Conclusion

The Nafion membrane used as a PEM in this study has excellent proton conductivity but still has many problems, including: oxygen leakage from cathode to anode, substrate losses, cation transport and accumulation rather than protons, and biofouling of the membrane. Nafion was quite permeable to oxygen, which caused significant substrate losses due to

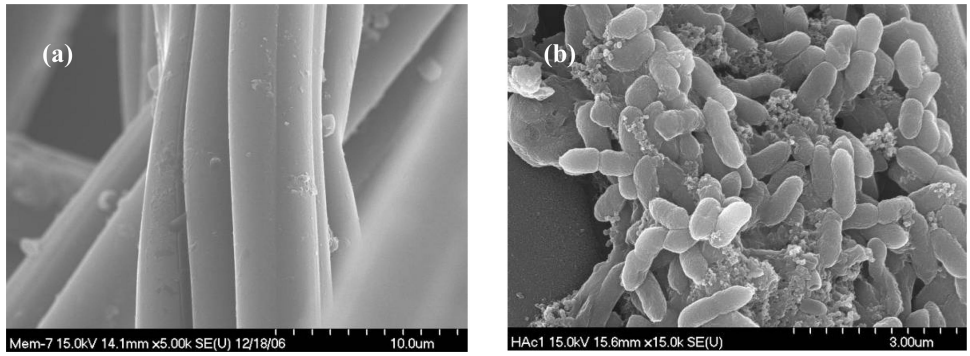

Figure 11. SEM images: (a) new carbon felt and (b) bacteria growing on the anode carbon felt surface in the MFC fed with acetate (0.5 mM) as an electron source for over 50 days.

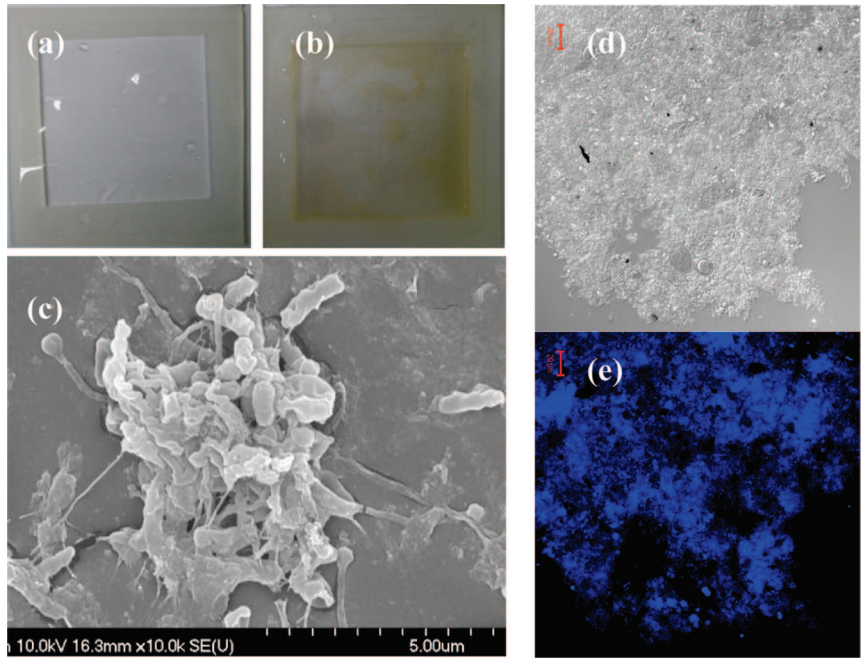

Figure 12. (a) Photograph of new Nafion. (b) Photograph of Nafion used for over 50 days in an acetate fed MFC. (c) SEM image of bacteria growing on the anodic side of the used Nafion in part b. (d) Differential interference contrast (DIC) image. (e) DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) image for the biofilm taken out of the used Nafion in part b.

aerobic bacterial respiration. The substrate loss via direct diffusion through the Nafion membrane seemed to be negligible. Cations existing in higher concentrations compared to protons in the anolyte were rapidly transported through the Nafion membrane and occupied the negatively charged sulfonate groups of Nafion, which consequently inhibited proton exchanges through Nafion. During the evaluation of deionized water as an alternative for the phosphate buffer in the cathode chamber, little difference was revealed in both the oxygen diffusion and cation transport rates when compared to the phosphate buffer catholyte. Even though deionized water displayed a similar cell voltage and columbic efficiency to the phosphate buffer during the MFC operation with a relatively low concentration of acetate (0.5 mM), the usage of phosphate buffer as a catholyte is still recommended to prevent pH suffering in both the anode and cathode chamber for the normal long term operation with a high substrate concentration. The biofouling of Nafion has the potential to cause adverse effects on mass transport through the membrane, though not enough study has been performed regarding this problem until now. Further studies, therefore, are required to determine the effects of biofouling in the Nafion membrane if better performances of MFCs are to be achieved.

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