

Analysis of Ammonia Loss Mechanisms in Microbial Fuel Cells Treating Animal Wastewater

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ABSTRACT: Ammonia losses during swine wastewater treatment were examined using single- and two-chambered microbial fuel cells (MFCs). Ammonia removal was 60% over 5 days for a single-chamber MFC with the cathode exposed to air (air-cathode), versus 69% over 13 days from the anode chamber in a two-chamber MFC with a ferricyanide catholyte. In both types of systems, ammonia losses were accelerated with electricity generation. For the air-cathode system, our results suggest that nitrogen losses during electricity generation were increased due to ammonia volatilization with conversion of ammonium ion to the more volatile ammonia species as a result of an elevated pH near the cathode (where protons are consumed). This loss mechanism was supported by abiotic tests (applied voltage of 1.1 V). In a two-chamber MFC, nitrogen losses were primarily due to ammonium ion diffusion through the membrane connecting the anode and cathode chambers. This loss was higher with electricity generation as the rate of ammonium transport was increased by charge transfer across the membrane. Ammonia was not found to be used as a substrate for electricity generation, as intermittent ammonia injections did not produce power. The ammonia-oxidizing bacterium *Nitrosomonas europaea* was found on the cathode electrode of the single-chamber system, supporting evidence of biological nitrification, but anaerobic ammonia-oxidizing bacteria were not detected by molecular analyses. It is concluded that ammonia losses from the anode chamber were driven primarily by physical-chemical factors that are increased with electricity generation, although some losses may occur through biological nitrification and denitrification.

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Introduction

Wastewater treatment using microbial fuel cells (MFCs) has recently been suggested as a sustainable method of simultaneous wastewater treatment and bioenergy generation (Kim et al., 2007a; Liu et al., 2004; Logan, 2004; Rabaey and Verstraete, 2005). Although electricity generation using pure substrates and wastewater has been widely studied, the effect of MFC treatment on ammonia has received relatively little attention. Nitrogen removal from wastewater is an important component of treatment, particularly for high-strength animal wastewaters. Nitrogen removal has been difficult to achieve in single-process systems, and usually multiple reactors are needed (Choi et al., 2004; Rittmann and McCarty, 2001). Two-stage and modified oxic/anoxic processes using sequencing batch reactors can be used for treatment of ammonia-rich swine wastewaters (Angenent et al., 2002; Chen et al., 2004; Kim and Yang, 2004), but these processes have high operational costs.

While it was recently shown that MFCs could be used to generate power and treat swine wastewater, it was also observed that COD removal was accompanied by a high level of ammonia removal (Min et al., 2005). Four potential nitrogen removal mechanisms were suggested, all with a focus on a biological reaction. These four mechanisms were: ammonia oxidation by nitrifying bacteria (using oxygen that diffused through the cathode) coupled to denitrification; ammonia oxidation by ammonia-oxidizing bacteria (AOB) coupled with ammonia oxidation and nitrite reduction by anaerobic ammonia oxidation (ANAMMOX) bacteria; ammonia oxidation and nitrite reduction by AOB (Rot-

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thauwe et al., 1997); and ammonia oxidation directly coupled with anode reduction by a novel unidentified community member. An additional explanation for ammonia loss in an MFC is that it results from chemical/physical processes, but this has not been previously explored for an actual wastewater despite evidence in the literature that this can occur. For example, it was recently shown in MFC tests that many different cations (not just protons)—including ammonium—are transported through a cation exchange membrane (CEM) in an MFC (Kim et al., 2007b; Rozendal et al., 2006), presenting a possible abiotic mechanism of ammonium loss from the anode chamber. This physical removal mechanism, however, was not previously examined to determine its importance for ammonia removal relative to the other four biological processes for an ammonia rich wastewater in MFCs. Also, the loss of protons near the cathode can result in a locally elevated pH, enhancing the potential for nitrogen losses due to ammonia volatilization at the cathode. Nitrogen losses through volatilization have also not been previously considered or examined in MFC tests using wastewater.

In this study, we investigated these different mechanisms of possible nitrogen losses in two types of MFCs: single-chamber MFCs that use an air–cathode, and two-chamber MFCs that have a CEM and use either dissolved oxygen (DO) or ferricyanide (FCN) as catholytes. Ammonia losses were measured in these MFCs and compared to losses occurring in the same reactors operated in open-circuit mode (no electricity generation). Additional tests were also conducted in the absence of microorganisms, with a voltage applied to the circuit, to determine if nitrogen losses occurred in the system due solely to the potential between the electrodes. The possibility of biological ammonia oxidation with current generation was investigated by intermittent dosing of reactors with ammonia and by conducting cyclic voltametry. The potential for nitrification and ANAMMOX occurring in the reactor was assessed using molecular methods targeting the specific bacteria associated with these processes.

Materials and Methods

Swine Wastewater

Wastewater was obtained from the Swine Research Facility at Penn State University (University Park, PA). Raw manure was collected from a mixed underground concrete swine slurry pit, and stored at 4°C prior to use. To remove large particles, the raw wastewater was sieved (0.25 mm mesh) before use. Microorganisms already present in the wastewater were used as the inoculum (full strength wastewater) without any modifications such as pH adjustment or addition of nutrients or trace metals. Tests were also conducted using wastewater diluted using ultrapure water (Milli-Q system; Millipore Corp., New Bedford, MA) in

order to adjust organic and nitrogen loading rates, and using a phosphate buffer (50 mM, pH 7.0) as indicated.

MFC Configurations and Operation

Tests were conducted using single- and two-chamber MFCs constructed as previously described (Min et al., 2005). The single-chamber, air–cathode reactor was 4 cm long and 3 cm in diameter (28 mL empty volume) (Liu and Logan, 2004). Anodes (3 cm diameter) were made of a carbon paper (E-Tek, Inc., Somerset, NJ) and connected with the cathode via an external circuit containing a resistor ($R = 470 \Omega$, closed-circuit operation). The cathodes were made of the same material, with the solution side coated with a catalyst (0.35 mg-Pt/cm^2), and the air side coated with a single layer of polytetrafluoroethylene (PTFE) as previously described (Cheng et al., 2006).

Two-chamber aqueous-cathode MFCs made of two bottles (Kim et al., 2005) or cubes (Kim et al., 2005, 2007c) were used to assess losses of ammonium from the anode chamber due to diffusion through the membrane (Nafion 117, Dupont Co., Wilmington, DE). The electrodes were the same as those described above, except the cathode did not contain a PTFE layer. The catholyte was 50 mM phosphate buffer (pH 7.0) with either oxygen maintained by air sparging or FCN (100 mM).

A single-chamber MFC was also used without being inoculated (abiotic control) to evaluate the effect of current generation on ammonia losses due to localized pH changes. A DC power supply (3645A, Circuit Specialists, Inc., Mesa, AZ) was used to apply a constant potential of 1.1 V between the electrodes to a current similar to that produced by the microorganisms. The reactor was filled with a 200 mg/L NH_4Cl solution in phosphate buffer (50 mM, pH 7.0) or sodium chloride (0.6% NaCl) to investigate the effect of the buffer on ammonia removal under constant-conductivity conditions (10 mS/cm).

MFCs were operated as indicated in either closed-circuit (electricity generation) or open-circuit (no external circuit) mode to investigate the effect of current generation on the concentrations of chemical components. Reactors were operated in fed-batch mode, with the wastewater added to the anode chamber when the voltage decreased to $<10 \text{ mV}$. An additional control was conducted by placing a wastewater sample in a sealed serum bottle (165 mL, no head space) to investigate ammonia loss in the absence of membrane or cathode. All MFCs and controls were operated in batch mode and continuously mixed with a magnetic stirrer in a temperature-controlled room at 30°C.

Analyses

Current was calculated by monitoring the voltage across the resistor in the circuit (30-min intervals) using a multimeter (Keithley Instruments, Cleveland, OH) connected to a personal computer. Power density, $P \text{ (W/m}^2\text{)}$, was obtained using $P = IV/A$, where $I \text{ (A)}$ is the current, $V \text{ (V)}$ is the

voltage, and A (m^2) is the projected surface area of the anode (Min et al., 2005).

All samples for chemical analysis were filtered through a 0.45 μm pore diameter syringe filter to remove particles. COD was measured in duplicate using Standard Methods (method 5220; HACH COD system, HACH Company, Loveland, CO). The sum of ammonia and ammonium (herein referred to as ammonia unless making specific reference to the ammonium ion) was measured by Standard Methods (Salicylate method, HACH; APHA, 1995). Nitrite and nitrate concentrations were quantified using ion chromatography (Dionex DX-100, Dionex Co., Sunnyvale, CA) and an AS4 column with a 1.8 mM $Na_2CO_3/1.7$ mM $NaHCO_3$ eluent.

Cyclic voltammetry (PC 4/750 potentiostat, Gamry) was used to investigate the possibility of electrochemical oxidation of ammonia and acetate. Two-bottle MFCs using a FCN catholyte (100 mM, Sigma–Aldrich, St. Louis, MO) were tested in an anaerobic glove box (Coy Scientific Products, Grass Lake, MI). The potential was varied from -600 to 600 mV at a scan rate of 20 mV/s while monitoring the current response as previously described (Kim et al., 2007c).

Bacterial Analyses in MFC by PCR

The polymerase chain reaction (PCR) was used to detect AOB and ANAMMOX bacteria in MFC samples using primers targeting fragments of the gene coding for subunit A of ammonia monooxygenase (*amoA*) and the 16S rRNA gene (16S rDNA), respectively (Table I). For ANAMMOX-targeted PCR, separate reactions were performed using forward primer Planc-0046 paired with each of the other three ANAMMOX primers listed in Table I. The AOB assay was also quantitative, using real-time PCR with primers *amoA*-1F and *amoA*-2R to generate *amoA* products and TaqMan probes designed to differentiate *amoA* products from three distinct AOB groups (Regan et al., 2007). Bacterial samples were collected from the anode, the cathode, and the solution of a single-chamber MFC operated in fed-batch mode with swine wastewater for 3 months. Genomic DNA was extracted and purified as previously described (Kim et al., 2007c), and the PCR conditions were identical to those reported for the respective primer pairs

(Jetten et al., 2005; Regan et al., 2007). PCR products were separated and visualized using agarose gel electrophoresis and ethidium bromide staining.

Results

Ammonia Losses in Single-Chamber MFCs

Ammonia removal from swine wastewater was faster in the single chamber MFC with electricity generation (closed circuit) than it was in the same type of MFC with an open circuit or in a sealed bottle (control) (Fig. 1). After 120 h of operation, the ammonia concentration was reduced from 188 ± 6 to 76 ± 8 mg-N/L (Fig. 1A), and sCOD was reduced from 1820 ± 83 to 250 ± 29 mg/L (Fig. 1B). The concentrations of nitrite and nitrate, which are products of bacterial nitrification, were small and unchanged in the solution (<1.5 ppm) in the open- and closed-circuit MFCs over the duration of the experiment (data not shown). Ammonia losses during the first 48-h of operation following replacement of the wastewater (i.e., a fed batch cycle) for selected cycles showed that the rate of ammonia removal in the reactor increased with successive batches over the 9-week period, along with an increase in power density (Fig. 2). This shows that ammonia removal was enhanced by microbial degradation of ammonia or as a result of current generation.

It was hypothesized that localized pH increases near the cathode would produce a shift of ammonium ions to ammonia, resulting in nitrogen losses due to ammonia volatilization through the cathode. To examine the possibility of ammonia volatilization occurring by this mechanism, ammonia concentrations were monitored in uninoculated single-chamber MFCs in the presence and absence of a pH buffer (50 mM, pH 7.0), at a fixed applied potential that produced a current (0.18 ± 0.06 mA) similar to that in an electricity-generating MFC (Fig. 3). The ammonia concentration decreased from 212 to 60 mg N/L after 7 days in the unbuffered reactor. In contrast, there was no significant change in the ammonia concentration in the well-buffered reactor at the same voltage, or a control without an applied voltage. These results show that in the absence of a buffer, where pH could vary near the cathode, the ammonia was lost as a result of ammonia volatilization due to elevated pH levels near the cathode. The pH at the

Table I. Oligonucleotide probe and primer sequences and their respective target groups.

Probe/primer	Target group (specificity)	Sequence 5'–3'	References
<i>amoA</i> -1F	<i>β-Proteobacteria</i> ammonia-oxidizing bacteria	GGGGTTTCTACTGGTGGT	Rotthauwe et al. (1997)
<i>amoA</i> -2R	<i>β-Proteobacteria</i> ammonia-oxidizing bacteria	CCCCTCKGSAAAGCCTTCTTC	Rotthauwe et al. (1997)
<i>amoA</i> -Nm3	<i>Nitrosomonas europaea</i> group	TGTCGATGGCTGAYTACATGGG	Regan et al. (2007)
<i>amoA</i> -Nm4	<i>Nitrosomonas oligotropha</i>	ATCATGTTGCTGACCGGTAAGTGGC	Regan et al. (2007)
<i>amoA</i> -Ns	<i>Nitrospira</i> group	CCGACSCACCTGCCGCTGG	Regan et al. (2007)
Planc-0046	<i>Planctomycetales</i>	GACTTGCATGCCTAATCC	Jetten et al. (2005)
Amx-0368	All ANAMMOX organisms	CCTTTCGGGCATTGCGAA	Jetten et al. (2005)
Amx-0820	Genera <i>Candidatus</i> “Brocadia” and <i>Candidatus</i> “Kuenenia”	AAAACCCCTCTACTTAGCGCCC	Jetten et al. (2005)
Scabr-1114	<i>Candidatus</i> “ <i>Scalindua brodae</i> ”	CCCCTGTTAACTAAAAACAAG	Jetten et al. (2005)

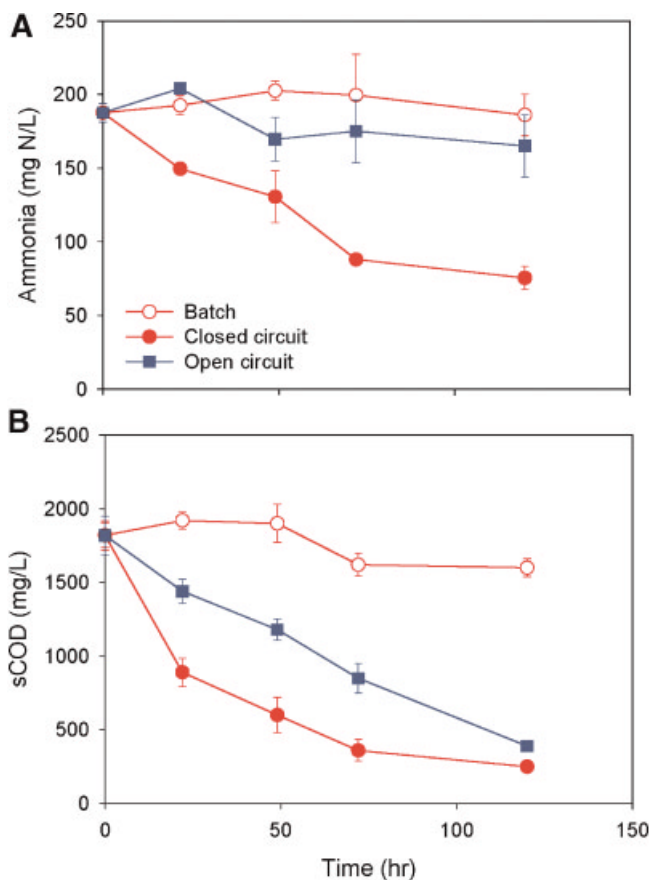


Figure 1. Nitrogen and organic carbon removal in swine wastewater (1:10 diluted) in single-chamber MFCs with the cathode exposed to air. **A:** Ammonia. **B:** Soluble COD. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

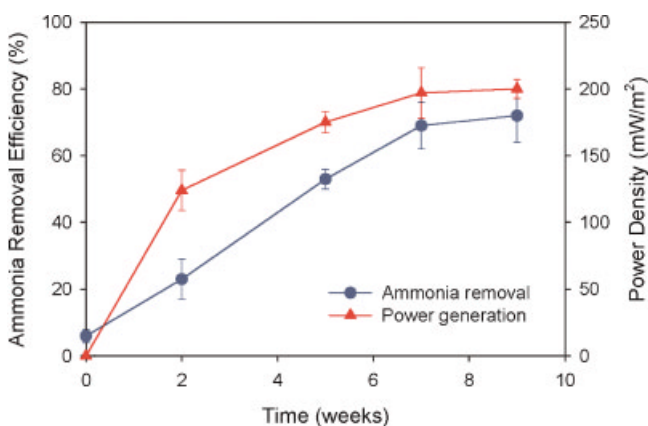


Figure 2. Percent of ammonia removal and power density produced for the first 48-h period of operation in an MFC operated in fed-batch mode over a 9-week period. Each point represents initial ammonia removal and power density of each fed-batch cycle, with the wastewater replaced every week. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

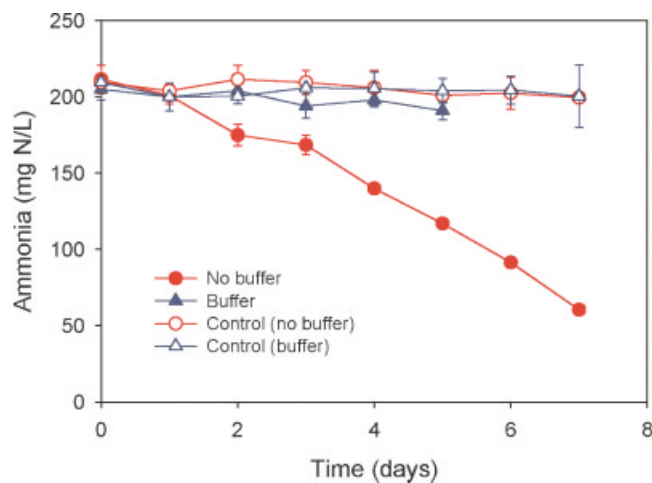


Figure 3. Ammonia removal in single-chamber MFC (abiotic conditions) with an air-cathode without (control) or with a fixed applied potential of 1.1 V using phosphate buffer (50 mM, pH 7.0) or a NaCl solution (0.6%) at the same solution conductivity (10 mS/cm). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

cathode was not directly measured in this reactor, but pH changes have been widely observed in other MFC systems even in the presence of a buffer (Gil et al., 2003; Kim et al., 2007b; Rozendal et al., 2006; Zhao et al., 2006).

Ammonium Losses From the Anode Chamber in a Two-Chamber MFCs

Ammonium ions can be transported through the membrane in a two-chamber MFC to maintain charge balances between the anode, where protons are produced, and the cathode, where protons are consumed (Rozendal et al., 2006). In order to further investigate ammonium ion diffusion through membrane (Nafion), ammonia was monitored over time in the anode and cathode chambers of MFCs using either dissolved oxygen (DO) or FCN as catholytes (Fig. 4). Ammonia decreased in the anode chamber by 68% (from 219 ± 2 to 69 ± 10 mg N/L) over the first 6 days, and by 89% over 22 days in the reactor using DO with air sparging. There was little ammonia measured in the cathode chamber over the same time period, suggesting either nitrogen loss due to volatilization from air sparging or nitrogen biodegradation.

The same experiment was conducted using FCN as the catholyte in order to determine the effect of sparging in the sealed system. With FCN and no air sparging, the ammonia concentration decreased by 60% (from 219 ± 2 to 87 ± 4 mg N/L) over the first 6 days, and 64% over 22 days (Fig. 4). This is slightly less than losses observed in the presence of sparging. However, ammonia accumulated in the cathode chamber using FCN (132 mg N/L loss in anode and essentially an equal recovery of 120 mg N/L in the cathode after 6 days). This result with FCN suggests that volatilization (and possibly biodegradation) was the key factor in

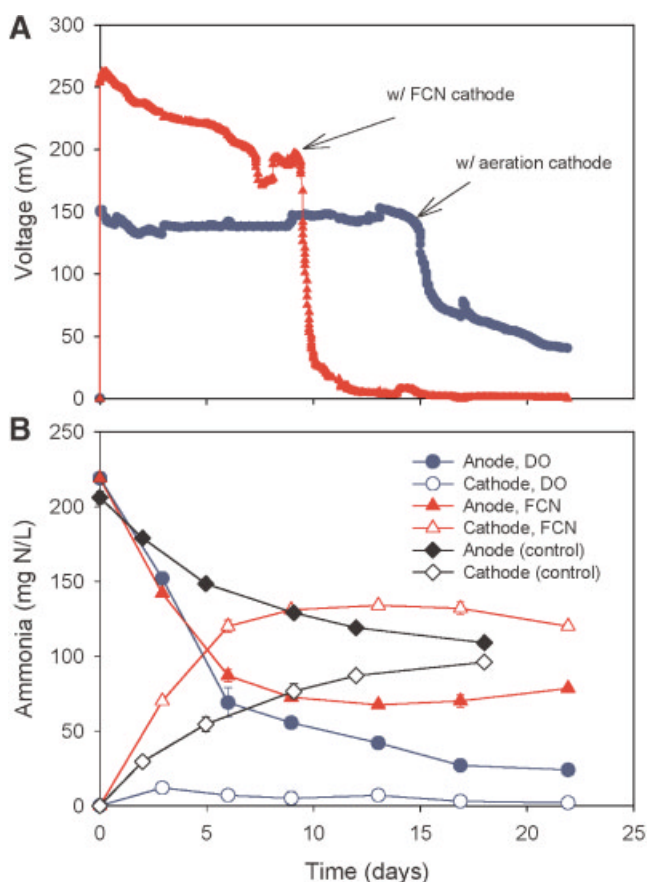


Figure 4. Voltage generation and ammonium concentrations in two-bottle MFCs with air sparging providing dissolved oxygen (DO) or without sparging using ferricyanide (FCN). **A:** Voltage generation. **B:** Ammonia concentration in anode and cathode chamber (the control lacks bacteria but contains a 50 mM phosphate buffer in each chamber). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

nitrogen losses from the cathode chamber when using DO (150 mg N/L loss in anode and 7 mg N/L accumulation in cathode). Ammonium ion was continuously transported from the anode chamber to the cathode chamber when there was electricity production (for the first 13 days; FCN reactor). The loss of ammonia from the anode chamber cannot be due solely to diffusion, because after 6 days the concentrations in the two chambers were equal, and thus there would be no concentration gradient driving diffusion. However, we can see that from day 6 to 13, ammonia continued to be transported against the concentration gradient into the cathode chamber. Once the power generation essentially ceased (<8 mV after 13 days in the FCN reactor), the ammonia concentration gradient between the two chambers resulted in the slow diffusion of ammonium ions back into the anode chamber (Fig. 4). This redistribution of chemical species following a decrease in the power output has previously been observed in a two-chamber MFC (Kim et al., 2007b).

In the absence of electricity generation, air sparging, and microorganisms (abiotic control, sealed anode and cathode; Fig. 4), the ammonia concentration decreased more slowly in the anode chamber compared to that observed with electricity generation. The ammonia loss over the initial period (first 6 days) was only 29% (47% over 18 days) without electricity generation (from 206 ± 4 to 146 mg N/L over 6 days). Based on this initial rate, a mass transfer coefficient for ammonium ion diffusion through the membrane was calculated as $k_A = 6.2 \times 10^{-5}$ cm/s using a mass balance equation presented elsewhere (Kim et al., 2007b).

The observation that ammonia lost from the anode chamber was increased in reactors producing power indicates that ammonium is being transported faster across the membrane (than by diffusion) to maintain charge balance. The fact that ammonium transport was greater with the air-sparged reactor than with the FCN reactor shows that the driving concentration gradient produced by removing ammonia in the cathode enhanced ammonia losses in the anode chamber. Air sparging (or biodegradation) removed ammonia from the cathode chamber, increasing the ammonia concentration gradient across the membrane and therefore the rate of mass transfer (Logan, 1999). The FCN reactor generated more current than the DO reactor, but ammonia was lost more slowly. Thus, the presence of oxygen or gas sparging in the cathode chamber were responsible for the faster rates of ammonium ion loss from the anode chamber than in the other cases due possibly to both physical and biological removal mechanisms.

Examination of the Possibility for Electricity Generation Directly From Ammonia Oxidation

It is possible that ammonia was used directly as a substrate by microorganisms for power generation. To test this hypothesis, the voltage produced in an MFC was monitored following intermittent injections of ammonia as the sole electron donor into a single-chamber reactor (Fig. 5). There was no noticeable voltage increase with ammonia addition, while voltage increases were consistently observed with acetate addition. Similar results were obtained with other types of MFCs (two-chamber bottle reactor, and two-chamber cube) using FCN at the cathode in an anaerobic glove box to exclude oxygen and to maintain a low redox potential (data not shown).

In order to further examine the possibility of direct ammonia oxidation, cyclic voltammetry tests were conducted with a fully enriched anode electrode (Fig. 6). There were no additional oxidation peaks when ammonia was added into the system, similar to that observed with the control (no ammonia), indicating a lack of direct ammonia oxidation in the system. As expected, two oxidation peaks (−315 and −379 mV) were observed when acetate was added to the reactor as the acetate was a source for electricity generation. Thus, these results further suggest that ammonia

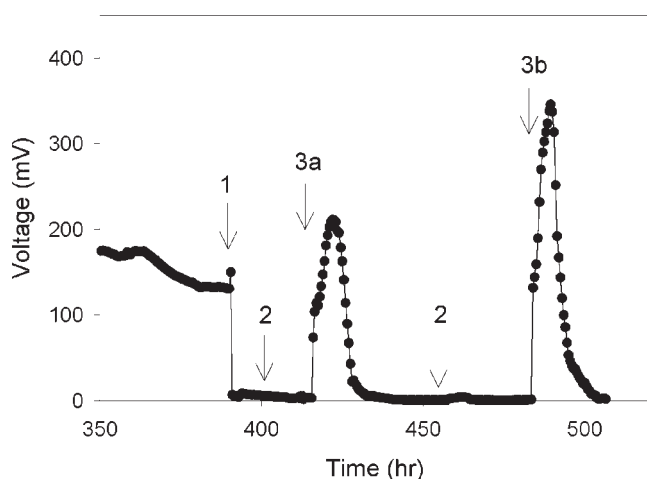


Figure 5. The effect of ammonia and acetate additions on voltage generation by a single-chamber MFC: 1, electrolyte replaced with nutrient medium lacking acetate and ammonia; 2, ammonia injections (54 mg N/L); 3, acetate injections of 300 mg/L (3a) and 600 mg/L (3b).

was not a source for electricity generation by direct oxidation in the anode chamber.

Detection of Microbes Involved in Ammonia Removal Process

Another reason for ammonia loss without a commensurate increase in nitrite and nitrate could be biodegradation due to nitrification and denitrification, or to ANAMMOX (Choi et al., 2004; Jetten et al., 2005; Rittmann and McCarty, 2001). To investigate whether bacteria capable of nitrification or ANAMMOX were present in the system, biofilms from the electrodes (anode and cathode) and the suspension of a

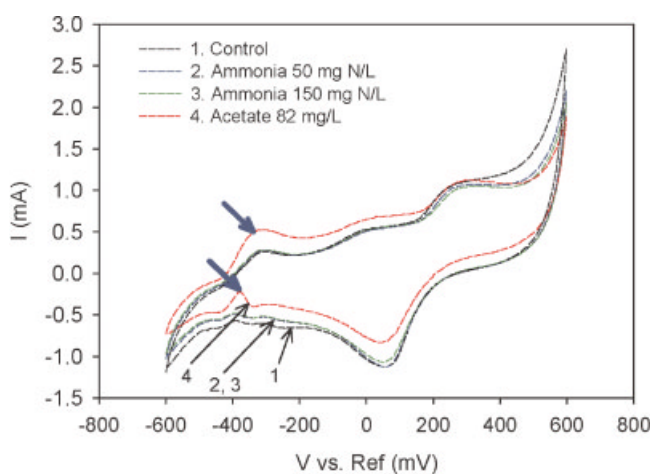


Figure 6. Cyclic voltammetry of an anode containing an established biofilm. Arrows indicate the observed oxidation peaks: 1, no ammonia (control); 2, 50 mg N/L; 3, 150 mg N/L; 4, 150 mg N/L and 82 mg/L acetate. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

single-chamber MFC were analyzed by AOB- and ANA-MMOX-targeted PCR. *Nitrosomonas europaea* was detected on the cathode at a density of 2040 ± 720 amoA copies/cm² and in suspension at 220 ± 70 amoA copies/mL, but not on the anode (quantitation level 200 amoA copies). These results suggest that nitrification could be occurring by AOB on the cathode, supported by oxygen diffusion through the cathode electrode. It is unlikely that AOB contributed directly to current generation. There were no AOB on the anode, and the addition of a nitrification inhibitor (50 or 150 ppm of trichloromethyl pyridine; data not shown) did not affect voltage. Thus, the cathode in the single- and two-chamber MFCs seems to provide a suitable microenvironment for both biological ammonia oxidation as well as for volatilization of ammonia.

Discussion

Ammonia losses occurred primarily by physical-chemical mechanisms in both single- and two-chamber MFC systems, with removal rates increased due to electricity generation. In a single-chamber MFC lacking a membrane, nitrogen losses appear to be primarily due to volatilization of ammonia at the cathode. Ammonia has a $pK_a = 9.3$ (Snoeyink and Jenkins, 1980), so an increase in pH at the cathode would result in a re-distribution of ammonium ions to the more volatile ammonia form. We hypothesize that the development of a biofilm at the cathode, coupled with proton losses due to chemical reaction with electrons and oxygen, created a localized high pH within the biofilm resulting in the conversion of ammonium ion to ammonia. Although we did not have the capability to measure such pH changes in our system (using for example pH microprobes), experimental evidence here suggests that this elevated pH and volatilization was occurring. While we measured some loss of nitrogen in the same system without electricity generation (i.e., with no pH change occurring as a result of the open circuit), it was relatively small compared to the case with power generation. Thus, while nitrification and denitrification could be occurring on the cathode biofilm in both systems, nitrification alone can not fully explain the observed nitrogen losses in the closed-circuit MFC since the open- and closed-circuit MFCs would have the same rate of oxygen transfer through cathode ($k_O = 2.3 \times 10^{-3}$ cm/s; Cheng et al., 2006).

In the case of the two-chamber MFC, ammonia was lost due to ammonium ion diffusion through the membrane into the cathode chamber as well as through charge transfer resulting from electricity generation. The loss of ammonium ion was increased by current generation above the rate due to diffusion alone due to a charge imbalance created by proton generation in the anode chamber and loss at the cathode. The transport of ionic species through Nafion was recently investigated in detail in MFCs (Kim et al., 2007c; Rozendal et al., 2006). Cationic exchange membranes such as Nafion have negatively charged sulfonate functional

groups on the side chain which mediate the transport of cationic species, including ammonium. Ammonium was therefore preferentially transported (compared to protons) across the membrane due to its higher concentration when high nutrient organic wastewater (e.g., swine wastewater) was used, resulting in an ammonia concentration gradient (against a spontaneous process minimizing the difference) between the cathode and anode chambers (Fig. 4). Once power generation ceased, the concentration gradient was reduced due to passive diffusion of ammonium across the membrane accompanied by transfer of other charged species.

These results demonstrate that if MFC-based processes are used for wastewater treatment that both biological and physical nitrogen removal mechanisms will have to be considered in nitrogen balances. If air-cathode MFCs are used volatilization will be an important removal mechanism and may substantially reduce the amount of ammonia removal needed by other methods. The volatilization of ammonia into the air might be a concern for some locations, however, and therefore further control of the off gas could be required for the MFC in the same way it is for air stripping systems. If ammonia can first be converted to nitrate, then a combined biological nitrification/denitrification MFC-based process may be possible as recently demonstrated by Clauwaert et al. (2007). The use of these MFC-based technologies could potentially reduce high operational costs currently needed for aeration-based treatment systems, and could create conditions whereby ammonia removal is accomplished while simultaneously generating electricity.

Further tests are needed with higher-strength wastewaters and continuous flow systems. Diluted swine wastewater was used in this study in order to adjust organic and nutrient loading and to achieve reasonable times for fed batch cycles. In continuous flow systems, effluent recycle can be used to dilute the influent wastewater to an organic loading that can be treated at an acceptable hydraulic retention time (Lew and Guiot, 2003). The retention times needed in current MFC systems will likely be reduced in the future in MFCs as many recent developments have improved MFC current densities, thus decreasing needed treatment times (Cheng and Logan, 2007; Logan et al., 2007). These new reactor designs will need to be evaluated not only in terms of carbonaceous removal, but also relative to ammonia losses using actual wastewaters. The current study has clearly established that both physical and biological mechanisms can affect ammonia losses in MFC systems, and thus both of these mechanisms of nitrogen loss will need to be considered in future studies.

Conclusions

- Ammonia losses occurred mainly as a result of volatilization enhanced by localized pH increases at the cathode in a single chamber MFC. Abiotic tests using an

applied potential of 1.1 V produced an ammonia loss equivalent to that observed in the MFC producing electricity, supporting the voltage-induced mechanism of nitrogen loss.

- In two bottle type MFCs using ion exchange membranes, ammonia was lost from the anode chamber due to diffusion into the cathode chamber ($k_A = 6.2 \times 10^{-5}$ cm/s). The transport rate was increased with electricity generation with both DO or FCN catholytes as a result of ammonium transport used to maintain charge balance between the chambers.
- There was no evidence for electricity generation supported by direct ammonia oxidation. Cyclic voltammetry tests similarly did not support evidence of direct ammonia oxidation due to a lack of oxidation peaks in the scans.
- Ammonia removal by nitrification likely occurred in reactors using oxygen, supported by oxygen diffusing through the membrane (if present) or cathode. *N. europaea* was detected on the cathode electrode but it was not on the anode.
- Anaerobic ammonia oxidizing bacteria (ANAMMOX) were not detected in the MFC.

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References

- Angenent LT, Sung S, Raskin L. 2002. Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. *Water Res* 36(18):4648–4654.
- APHA. 1995. Standard methods for the examination of water and wastewater. 19th edn. Washington, DC: American Public Health Association, American Water Works Association. Water Pollution Control Federation.
- Chen M, Kim J-H, Kishida N, Nishimura O, Sudo R. 2004. Enhanced nitrogen removal using C/N load adjustment and real-time control strategy in sequencing batch reactors for swine wastewater treatment. *Water Sci Technol* 49(5–6):309–314.
- Cheng S, Logan BE. 2007. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. *Electrochem Commun* 9(3):492–496.
- Cheng S, Liu H, Logan BE. 2006. Increased performance of single-chamber microbial fuel cells using an improved cathode structure. *Electrochem Commun* 8(3):489–494.
- Choi E, Yun Z, Chung TH. 2004. Strong nitrogenous and agro-wastewater: Current technological overview and future direction. *Water Sci Technol* 49(5–6):1–5.
- Clauwaert P, Rabaey K, Aelterman P, DeSchampheleire L, Pham TH, Boeckx P, Boon N, Verstraete W. 2007. Biological denitrification in microbial fuel cells. *Environ Sci Technol* 41(9):3354–3360.
- Gil G-C, Chang I-S, Kim BH, Kim M, Jang J-K, Park HS, Kim HJ. 2003. Operational parameters affecting the performance of a mediator-less microbial fuel cell. *Biosens Bioelectron* 18(4):327–334.

- Jetten M, Schmid M, van de Pas-Schoonen K, Sinninghe Damste J, Strous M. 2005. Anammox organisms: Enrichment, cultivation, and environmental analysis. *Methods Enzymol* 397:34–57.
- Kim SJ, Yang PY. 2004. Two-stage entrapped mixed microbial cell process for simultaneous removal of organics and nitrogen for rural domestic sewage application. *Water Sci Technol* 49(5–6):281–288.
- Kim JR, Min B, Logan BE. 2005. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. *Appl Microbiol Biotechnol* 68(1):23–30.
- Kim B, Chang I, Gadd G. 2007a. Challenges in microbial fuel cell development and operation. *Appl Microbiol Biotechnol* 76(3):485–494.
- Kim JR, Cheng S, Oh SE, Logan BE. 2007b. Power generation using different cation, anion and ultrafiltration membranes in microbial fuel cells. *Environ Sci Technol* 41(3):1004–1009.
- Kim JR, Jung SH, Regan JM, Logan BE. 2007c. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. *Bioresour Technol* 98(13):2568–2577.
- Liu H, Logan BE. 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environ Sci Technol* 38(14):4040–4046.
- Liu H, Ramnarayanan R, Logan BE. 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ Sci Technol* 38(7):2281–2285.
- Logan BE. 1999. *Environmental transport processes*. New York: Wiley InterScience.
- Logan BE. 2004. Extracting hydrogen and electricity from renewable resources. *Environ Sci Technol* 38(9):160A–167A.
- Logan B, Cheng S, Watson V, Estadt G. 2007. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ Sci Technol* 41(9):3341–3346.
- Lyew D, Guiot S. 2003. Effects of aeration and organic loading rates on degradation of trichloroethylene in a methanogenic-methanotrophic coupled reactor. *Appl Microbiol Biotechnol* 61(3):206–213.
- Min B, Kim JR, Oh S, Regan JM, Logan BE. 2005. Electricity generation from swine wastewater using microbial fuel cells. *Water Res* 39(20):4961–4968.
- Rabaey K, Verstraete W. 2005. Microbial fuel cells: Novel biotechnology for energy generation. *Trends Biotechnol* 23(6):291–298.
- Regan JM, Cho A-Y, Kim S, Smith CD. 2007. *Monitoring ammonia-oxidizing bacteria in chloraminated distribution systems*. Denver, CO: AWWA Research Foundation.
- Rittmann BE, McCarty PL. 2001. *Environmental biotechnology: Principles and applications*. Boston: McGraw-Hill.
- Rotthauwe J-H, Witzel K-P, Liesack W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol* 63(12):4704–4712.
- Rozendal RA, Hamelers HVM, Buisman CJN. 2006. Effects of membrane cation transport on pH and microbial fuel cell performance. *Environ Sci Technol* 40(17):5206–5211.
- Snoeyink VL, Jenkins D. 1980. *Water chemistry*. New York, NY: Wiley.
- Zhao F, Harnisch F, Schroder U, Scholz F, Bogdanoff P, Herrmann I. 2006. Challenges and constraints of using oxygen cathodes in microbial fuel cells. *Environ Sci Technol* 40(17):5193–5199.