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Ammonium as a Sustainable Proton Shuttle in Bioelectrochemical Systems

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Abstract

This work examines a pH control method using ammonium (NH_4^+) as a sustainable proton shuttle in a CEM-equipped BES. Current generation was sustained by adding NH_3 or ammonium hydroxide (NH_4OH) to the anolyte, controlling its pH at 7. Ammonium ion migration maintained the catholyte pH at approximately 9.25. Such NH_4^+/ NH_3 migration accounted for 90±10 % of the ionic flux in the BES. Reintroducing the volatilized NH_3 from the cathode into the anolyte maintained a suitable anolyte pH for sustained microbial-driven current generation. Hence, NH_4^+/ NH_3 acted as a proton shuttle that is not consumed in the process.

Keywords: microbial fuel cell; proton gradient; pH split; cation exchange membrane; optimization

1. Introduction

Microbial fuel cells (MFCs) convert chemical energy in organic compounds to electrical energy (Rabaey and Verstraete, 2005). Bacteria developing at the anode conserve energy for growth and cell maintenance by transferring electrons from an organic electron donor with a low redox potential to the anode at a higher potential. Via an external circuit, electrons flow from the anode to the cathode (typically at a higher potential than the anode) through a resistor to produce electricity. At the cathode, an electron acceptor such as molecular oxygen is being reduced. During current generation, protons are continuously produced at the anode by bacterial oxidation of organic substrate and consumed at the cathode for oxygen reduction. Ideally, for every electron that is transferred to the cathode via the external circuit, a proton (H^+) should migrate from the anolyte to the catholyte or a hydroxide ion (OH^-) should migrate to the opposite direction to conserve electroneutrality (Harnisch et al., 2008).

The anolyte of MFC always contains mineral salts and buffer at concentrations manifold higher (about 10^{-3} to 10^{-1} mol L^{-1}) than that of protons (10^{-7} mol L^{-1} at pH 7). Hence, those salt cations (e.g. Na^+) rather than protons will become the dominant charge balancing species, resulting in a severe pH gradient between anolyte and catholyte (Rozendal et a., 2006). The decrease in pH of the anolyte is more detrimental than the pH increase in the catholyte (Cheng et al., 2010; Harnisch et al., 2008). This is because lowering pH from 7 towards 5 typically stifles microbial activity and hence the current flow. At the cathode compartment, H^+ depletion decreases the oxygen reduction reaction rate. Freguia et al. (2007) reported that even with a decrease in oxygen overpotential, the oxygen reduction reaction was still limited by the lack of proton diffusion from the anolyte to the catholyte.

To date, most two-chamber MFCs are operated on pH-static control with continuous addition of bases into the anolyte and acids to the catholyte. For every mole of cation

migrating to the catholyte, one mole of base and acid has to be added to the anolyte and catholyte respectively. The most commonly used, NaOH and HCl are manufactured from electrolysis of NaCl with an electricity input of 1.35 MJ $mole^{-1}$ of NaCl (Steen and Borg, 2002), representing arguably more power input than what is produced by the MFC. Buffering agents have also been used to resist the pH gradient (Rozendal et al., 2008). However, buffer can only sustain the pH for a limited period of operation time until the buffering capacity is depleted. Alternatively, the alkalinity build-up at the cathode could be exploited as a method of caustic soda production (valuable commodity) to offset cost of the wastewater treatment (Rabaey et al., 2010). Nonetheless, operating a BES with regular dosing of pH controlling chemicals imposes a technical and operational cost that will further hinder the real-world application of BES and MFCs.

To address the pH limitation, Torres et al. (2008) have demonstrated a novel concept of using carbonate species (CO_3^{2-} and HCO_3^-) as a hydroxide ion (OH^-) shuttle in an anion-exchange membrane (AEM) equipped-MFC. The addition of carbon dioxide (CO_2) into the cathodic compartment of the AEM-MFC, helped to neutralize the alkalinity build-up from the cathodic oxygen reduction. The resulting bicarbonate (from the reaction between CO_2 and OH^-) could migrate across the AEM towards the anode for maintaining electroneutrality and neutralizing the anodic acidity (Torres et al., 2008). This concept has been taken further by Fornero et al. (2010), who demonstrated in an air-cathode MFC, CO_2 addition resulted in a stable catholyte pH of 6.6 and a 152% increase in power output.

For cation exchange membrane (CEM) based MFC with an air cathode or oxygenated catholyte, an effective and self-sustaining method to shuttle protons from the anolyte to the catholyte needs to be developed to overcome current limitations and facilitate further development of the technology. To sustainably shuttle protons across a CEM, a chemical species should posses four properties: (1) it has to be an alkaline compound that can associate

with excess protons when added to the anolyte to neutralize anode acidity; (2) upon association with proton it becomes a cation, which migrates across the CEM to the catholyte to maintain charge balance during current generation; (3) the cation should readily dissociate to release the proton in the catholyte and hence replenish the protons consumed in the cathodic reaction; (4) upon releasing the proton, the shuttling species must be recoverable from the cathode and then recycled back to the system (i.e. anode).

In this study, ammonia is tested as a possible species that fulfills the above criteria to sustain proton shuttling in a two-chamber bioelectrochemical cell equipped with a CEM. The significance of the study is that with future generations of more effective MFC cathodes and higher current densities, effective proton transfer will be critical.

2. Materials and Methods

2.1. Bioelectrochemical Cell Construction and Monitoring

Experimental runs were performed in a two-chamber bioelectrochemical cell made of transparent Perspex as described in (Cheng et al., 2008). The two chambers were physically separated by a cation exchange membrane (CMI-7000, Membrane International Inc.) with a surface area of 168 cm^2 and were identical in volume and dimension (350 mL (14 cm x 12.5 cm x 2 cm)). Two reticulated vitreous carbon (RVC) blocks (ERG, Oakland, CA) with 45 pore per inch (PPI) (surface area 27.9 cm^2/ cm^3) and 80 pore per inch (PPI) (surface area 49.2 cm^2/ cm^3) were used as the anode and the cathode respectively. They were of equal dimension (13.3 cm x 11.4 cm x 1.3 cm). Graphite rods (5 mm diameter) were inserted 2 cm into the RVC electrodes to allow contacts between the electrodes and the external circuit. Unless otherwise stated, the BES was operated in a fed-batch mode. Both anolyte and catholyte were of equal working volume (0.5 L). They were continuously re-circulating through the anode and the cathode, respectively, at a rate of 6.5 L h^{-1} to minimize mass transfer limitation. The BES was initially operated as a MFC and then as a potentiostat-assisted BES (details specified as follow).

Process control and monitoring of the BES were partially automated by using LabVIEW™ 7.1 software interfaced with a National Instrument™ data acquisition card (DAQ). The following parameters were online-monitored: cell voltage, oxidation-reduction potential (Eh) of the anolyte, anolyte and catholyte pH and catholyte dissolved oxygen (DO). The anodic potential was measured against a silver/ silver chloride (Ag/AgCl) reference electrode (saturated KCl), which was placed in the anodic recirculation loop. The cathodic potential was obtained from the difference between the cell voltage and the anodic potential. All reported electrode potentials in this paper refer to values against Ag/AgCl reference electrode (ca. +197 mV vs. standard hydrogen electrode (Bard and Faulkner, 2001)).

2.2. *Process Startup and Operation*

To start up the BES, the anode chamber was inoculated with an activated sludge collected from a local domestic wastewater treatment plant (Woodman Point, Perth, WA, Australia) with a biomass concentration of ca. 2.0 g L^{-1}. It was mixed with a synthetic wastewater (10 %, v/v) as described in Cheng et al. (2008). Initially, 50 mM potassium ferricyanide $K_3Fe(CN)_6$ (Sigma-Aldrich, Inc., Purity ca. 99 %) solution supplemented with 100 mM phosphate buffer (pH 7) was used as the catholyte to maintain a stable and efficient cathodic reaction. The anolyte was controlled at pH 7 $\pm$ 0.3 by feedback dosing of NaOH (1 M). After the first 30 days using ferricyanide as the catholyte, an effective electrochemically active biofilm had developed on the anode. The ferricyanide catholyte was then replaced with a dissolved oxygen (DO)-based catholyte (100 mM phosphate buffer, pH 7). DO in the catholyte was maintained at near saturation of 8 $\pm$ 0.2 mg L^{-1} by continuously purging the catholyte with humidified air (flow rate 50 L h^{-1}) in a 1 L glass recirculation bottle.

Acetate was used as a sole electron donor for the established electrochemical active biofilm. During the experimental runs, acetate concentration in the anolyte was always maintained at 2-5 mM to avoid substrate limitation. This was done by automated acetate addition using a computer-feedback (either set at a predetermined time interval or have the anolyte Eh as the set point (from -440 to -480 mV). Unless stated otherwise, the process was operated at 30 ^{o}C. During the initial start-up period, the MFC was operated with a 5 ohm external resistance to encourage the bacteria to use the electrode as electron acceptor. Regular polarization curve analyses were performed to quantify the performance of the MFC over the start-up period (Logan et al., 2006).

2.3. *Experimental Procedures*

In Microbial Fuel Cell Mode. To test whether ammonium ion could be used as a sustainable proton carrier, the automated NaOH dosing was replaced with NH_4OH (1 M) to control the anolyte pH at pH 7 $\pm$ 0.3. Each dosing event was registered by the LabVIEW^{TM} program to determine the NH_4OH application rate. This information is useful to establish mass balance of ammonium in the MFC as specified below. To study the effect of pH split in a highly active MFC a small external resistor of 5 ohms was used, which facilitated the current flow. Ammonium concentration in both the anolyte and the catholyte were quantified overtime. In all instances whereby the effect of ammonium shuttling was examined, 100 mM NaCl was used instead of phosphate buffer to provide electrical conductivity to the catholyte.

In Potentiostat-Assisted BES Mode. Since the use of a DO-based cathode had limited the oxidation of the electron donor (here acetate) and hence the activity of the anodophilic biofilm, a potentiostat (Model no. 362, EG&G, Princeton Applied Research, Instruments Pty. Ltd.) was used to apply additional voltage (from 0.2 to 0.9 V) to the circuit in order to maintain a higher and hence an anodic potential more representative of high power output MFC (-0.35 to -0.45 V). This also allowed a higher current and hence enabled the study of the effect of ammonium in a more difficult to control condition with a more pronounced tendency of forming a pH split between anode and cathode. This was done by connecting the positive pole of the potenstiostat to the anode, while the negative pole and the reference were both connected to the cathode (Logan et al., 2006). The cathodic potential was not controlled and varied from -0.6 to -1.3 V.

The catholyte was re-circulated through an external N_2 gas stripping vessel (humidified N_2 gas at a flow rate of 10 L h^{-1}), and was consistently stirred with a magnetic stirrer to strip off any free ammonia in the catholyte. This created an anaerobic condition within the cathode chamber, in favor of cathodic hydrogen production. The off-gas from the stripping chamber was allowed to pass through a 250 mL of 2 M sulfuric acid trap to collect the ammonia.

In some experiments, the anodic chamber was operated in a continuous mode. A synthetic wastewater consisting of 0.1 g L^{-1} yeast extract, 100 mM NH_4OH and 50 mM sodium acetate was continuously fed into the anode chamber using a peristaltic pump giving an acetate loading rate of 11 mmole L^{-1} day^{-1} (dilution rate of 0.0076 h^{-1} = hydraulic retention time of approx. 132 h). The cathode was operated in batch mode. Ammonium at the outflow of the anode chamber was quantified to determine the ammonium removal efficiency.

In this study, a separate bioelectrochemical cell with a much higher (>10-fold) anodic current producing activity was used to demonstrate the effectiveness of recycling the ammonia containing off-gas from the cathode to the anodic half cell for sustained current generation. The need of an effective pH control would be more conspicuous in a high-current BES. The reactor was identical as the one described above, except that graphite granules (3-6 mm diameter) was used as both the anode and cathode instead of RVC. A highly active anodic biofilm was also established from activated sludge. The system was operated without aeration at the catholyte and the anode was potentiostatically controlled at -0.3V to sustain the anodic biofilm activity. NH_3 recycling was done by stripping the catholyte with a pure nitrogen gas stream (~1 L/min) which was then introduced back into the anolyte, completing an NH_3-recycling loop. Current, anolyte pH (pH sensor, TPS Pty. Ltd., Australia) and acetate concentration in the anolyte were recorded over a period of two days with or without NH_3 looping.

2.4. Analysis and Calculation

Chemical Analysis. Ammonium was measured colorimetrically at 425 nm using a 1240 Shidmazu UV-Vis spectrophotometer as described in APHA (1992). This method measures the total ammonium/ammonia (both NH_4^+ and NH_3) in the sample. Prior to analysis, the samples were centrifuged at 13,000 rpm for 5 minutes to spin down any suspended solids

from the liquid phase. The supernatant was diluted with deionised (DI) water to a concentration ranging from 0.05 mM to 0.3 mM. Acetate in the filtered samples (0.8/0.2 µm Supor® Membrane, PALL® Life Sciences) was quantified by using a Dionex ICS-3000 reagent free ion chromatography (RFIC) system equipped with a IonPac® AS18 4 x 250 mm column. Potassium hydroxide was used as an eluent at a flow rate of 1 mL min^{-1}. The eluent concentration was 12-44 mM from 0-5 min and 44-52 mM from 8-10 min. The temperature of the column was maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppressioin® recycle mode).

Determination of Current. Voltage (*V*) was measured across a known resistor (*R*). Current production (*I*) in the BES setup was calculated according to Ohm's Law, $I = V/R$ where *V* is the measured voltage and *R* (Ω) is the external resistance. Current production (*I*) in the potentiostat assisted BES was directly obtained from the potentiostat.

Ammonium Removal Efficiency Calculation for Mass Balance Purpose. To establish mass balance of ammonium in the BES, the amount of ammonia volatilized from the catholyte was calculated. Such ammonia volatilization was interpreted as ammonia removal, and the removal efficiency in the fed-batch operated BES was calculated as follow:

$$\text{Ammonia Removal Efficiency}\,(\%) = \frac{NH_4^{+\,\text{added}} - [(NH_4^{+\,\text{An t2}} - NH_4^{+\,\text{An t1}}) + (NH_4^{+\,\text{Cath t2}} - NH_4^{+\,\text{cath t1}})]}{NH_4^{+\,\text{added}}} \times 100\%$$

Where NH_4^{+added} is the total ammonium added to the anolyte as ammonium hydroxide during the time interval of (t2 – t1); $NH_4^{+\,An\,t1}$ and $NH_4^{+\,An\,t2}$ are the total ammonium in the anolyte at time t1 and t2, respectively; and $NH_4^{+\,cath\,t1}$ and $NH_4^{+\,cath\,t2}$ are the total ammonium in the

catholyte at time t1 and t2, respectively. The total amounts of ammonium were all expressed in mmoles N.

The ammonium removal efficiency was only quantified for systems without reintroducing the ammonia from the cathode to the anode. In these experiments, an acid trap was used to collect ammonia that was volatilized from the catholyte, the total amount of ammonia collected was used to calculate the cumulative ammonia removal efficiency as follow:

$$\text{Cumulative ammonia removal efficiency (\%)} = \frac{\sum NH_4^{+\,\text{acid trap}}}{\sum NH_4^{+\,\text{added}}} \times 100\%$$

Where $\Sigma\ NH_4^{+\ \text{acid trap}}$ is the cumulative ammonia collected by the acid trap from the catholyte gas outlet expressed in mmoles N and $\Sigma\ NH_4^{+\ \text{added}}$ is the cumulative ammonia added as ammonium hydroxide expressed in mmoles N.

Charge (Q^- and Q^+) Calculation. It is expected that for each mole of electron flowing from the anode to the cathode, a mole of positive charge has to be transferred from the anolyte to the catholyte to maintain electroneutrality. By correlating the amount of electron flow as negatively charged species (Q^-) with the amount of positively charged ammonium ion (Q^+) migrated from anolyte to catholyte, one could determine whether ammonium can out-compete other cation species in the anolyte to serve as the dominant charge balancing species. Both positive and negative charges were expressed in coulombs (C). The negative charges (Q^-) in the form of electrons transferred through the electrical circuit during current generation were determined by integrating current over time. Positive charges (Q^+) migrated

through the CEM in the form of ammonium ion (NH_4^+) were determined based on the following equation:

$$Q^+(t) = (NH_4^{+\,added} + NH_4^{+\,t1} - NH_4^{+\,t2})z\,F$$

Where $NH_4^{+\,added}$ is the total ammonium added to the anolyte as ammonium hydroxide during the time interval of (t2 – t1); $NH_4^{+\,t1}$ and $NH_4^{+\,t\,2}$ are the initial (t1) and final (t2) amounts of ammonium in the anolyte, respectively; z the valence of ammonium, and *F* the Faraday constant (96485 coulombs/mole). Unit of ammonium is moles N.

3. Results and Discussion

3.1. Effect of pH Gradient between the Anolyte and Catholyte on Current

A two-chamber BES was set up as described above. Both cathode and anode chambers were controlled at pH 7 over 30 days to establish the cell. To demonstrate the need for the pH control system, it was stopped, resulting in a pH split between anode and cathode (Figure 1). The anolyte acidified over time whereas an alkaline pH was build up in the catholyte. Within 48 hours, the pH split had reduced the BES performance to 20 % of its original, a finding that is in line with the literature (Cheng et al., 2010; Harnisch et al., 2008).

Since the anodic electron transfer was the only microbial process in our tested BES and it was more susceptible to a local pH change, it was decided to control the anolyte pH at 7 to maintain a suitable activity of the anodic biofilm.

3.2. Sustaining Current Production by using Ammonium as the dominant cation migration across the CEM

Ammonia (NH_3) is a base that forms ammonium hydroxide (NH_4OH) in water (Reaction 1). When pH adjustment was required, adding NH_3 in the form of NH_4OH into the anolyte consumed the excess protons produced from the anodic oxidation reaction. The addition of ammonium hydroxide to the anode chamber of the BES, which was previously impeded by the build-up of a pH split, restored the original current completely within minutes (Figure 1, at ~45 h). As expected the current and pH could be maintained by continued automated dosing of ammonium hydroxide.

$$NH_3 + H_2O \longleftrightarrow NH_4OH \qquad \text{(Reaction 1)}$$

$$NH_4OH \longleftrightarrow NH_4^+ + OH^- \qquad \text{(Reaction 2)}$$

When ammonium hydroxide was continuously dosed into the anolyte for pH control, the OH^- neutralized the anolyte and the resulting NH_4^+ became a dominant cation to transfer positive

charges across the CEM to the cathode. At pH 7, the NH_4^+ concentration (15-20 mmole N/L) was about 10^5 times higher than that of protons (Reaction 2). Here, we observed that despite the continuous addition of ammonium to the anolyte, the ammonium concentration in the anolyte remained steady at around 15 mmole N/L (after 30 h), whilst the ammonium concentration in the catholyte had increased to a significantly higher level (Figure 2a).

Ammonium served as the dominant cation and migrated steadily (Figure 2b) via the CEM to the cathode, even against the concentration gradient. Since the continuous cathodic reaction (here oxygen reduction) had increased the catholyte pH to a level that was higher than the pKa value of ammonia, the concentration of free ammonia in the catholyte became more than twice that of the total ammonium/ammonia, facilitating ammonia volatilizing from the liquid to the gaseous phase. This was supported by the strong ammonia smell in the headspace of the cathode chamber.

Volatilization of ammonia provides a means to remove ammonia from the catholyte, prevents ammonium accumulation in the catholyte and facilitates continuous ammonium migration. Unlike other cation species (e.g. Na^+, K^+, Mg^{2+}, Ca^{2+}) which tend to accumulate in the catholyte after migrating from the anolyte across the CEM, the ammonia in the catholyte can be collected and recycled to shuttle protons (here in the form of NH_4^+) within the system (Figure 3), sustaining the microbial-driven current production. These properties distinguish ammonia/ammonium from other currently used pH control systems or buffers as a potentially sustainable pH control species.

To test the principle whether by recycling the ammonia from the cathode back to the anolyte could sustain current generation in a bioelectrochemical cell, pure nitrogen gas stream was used to strip off the ammonia from the catholyte. Then, the ammonia-containing gas stream was introduced back to the anolyte (Figure 4). For this test, we used a voltage

controlled BES (see section 2.3) to demonstrate the principle for higher current producing conditions. The results showed that reintroducing the volatile ammonia gas from cathode back to the anode could resume the microbial-driven anodic current generation, preventing the system from stalling.

3.3. The Effect of Ammonium Shuttling on the Catholyte pH and mass-balance of ammonium

In the anode chamber, the automated pH control using sodium hydroxide was now replaced by using ammonium hydroxide to investigate whether ammonium can act as the charge transferring species and at the same time as a proton carrier. Replacing the NaOH with NH_4OH resulted in a gradual decrease in the catholyte pH from 13.9 to 12.8 (Figure 5c). This pH drop can only be explained by some ammonia volatilization from the gas stripped catholyte leaving protons behind. However, mass balance analysis indicated that not all, but only 47 % of the ammonium added into the anolyte was removed from the catholyte via gas stripping.

Replenishment of protons in the catholyte by NH_4^+ migration from the anode is dependent on NH_3 removal since ammonium dissociates and releases protons upon volatilisation and stripping. To increase the ammonia removal from the cathode by increased volatilization, the catholyte temperature was increased from 25 to 55 ^oC while keeping the anode at about 35 ^oC. The catholyte pH decreased further to pH 11.2 despite an increase in current and hence cathodic proton consumption (Figure 5).

To verify whether increased migration of NH_4^+ from anolyte to catholyte and NH_3 volatilization had indeed replenished protons at the cathode, all ammonia escaping via the N_2 gas stream through the catholyte was collected with an acid trap. The mass balance showed that at the end of the experiment over 90% of the ammonium added as NH_4OH could be

retrieved as NH_3 gas captured from the exhaust N_2 of the cathode (Figure 5b). Also the electron balance showed that ammonia was the key charge transferring species with close to one mole of ammonium migrating from anolyte to catholyte for each mole of electrons transferred as electrical current (Figure 5a and insert).

3.4. Ammonium as the Main Positive Charge Carrier to Sustain Current Production under Prolonged BES Operation

To test whether during prolonged operation, NH_4^+ becomes the only positive charge carrier migrating to the cathode chamber, a mixture of ammonium chloride (100 mM) and sodium chloride (50 mM) was continuously fed into the anode chamber. Initially, both Na^+ and NH_4^+ migrated to the cathode as charge balancing species with the ammonium transfer only accounting for 50 % of the charges. As the Na^+ concentration kept increasing in the catholyte to 1 M, this further stimulated the selective migration of NH_4^+ by counteracting further Na^+ migration from the anode resulting in an immediate drop in ammonium concentration in the outflow from the anode (Figure 6). All of the positive charges were calculated (e.g. average 2819 coulombs (Q+) / 2637 coulombs (Q-)) to have been transported as ammonium to maintain electro-neutrality of the system. Ammonium transfer was slightly higher than the electron flow, presumably due to ion exchange from back-diffusion of Na^+. This experiment demonstrated that in a long-term operated system with an excess alkali ion build-up in the catholyte (i.e. caused by Na^+ rather than H^+ migration at pH7), all charge transfer is carried out by ammonia.

3.5. Practical Implication

In principle, all ammonia that is removed from the catholyte can be recycled back into the anolyte, allowing the ammonium shuttling process to be self-sustaining (Figures 3 and 4). If

the ammonia containing gas exiting from the catholyte is transferred directly into the acidic anolyte, then a complete ammonia loop is completed with the ammonium becoming a sustainable proton carrier for the long term operation of BES without the need of replacing the electrolytes.

In the present study, the feasibility of this concept has been demonstrated by using a clean nitrogen gas stream to recycle the ammonia from the cathode to the anode (Figure 4). However, this approach incurs extra energy expenses and hence it only serves as proof of concept that the principle of using ammonia as a proton shuttle in BES works. A more practical way of recycling the ammonia without using an extra nitrogen stream has been evaluated and will be documented in a separate report.

4. Conclusions

Overall, this study demonstrates the principle of using ammonium/ammonia to shuttle protons from the anolyte to the catholyte. The proposed pH control method could reduce the "pH split" between anolyte and catholyte from about 6 to 2 pH units in BES operation without using conventional pH controlling methods. Under the condition of increased current, high efficiency ammonia removal from the catholyte could also prevent further increase in catholyte pH which would shut down the cell if conventional NaOH type pH control was used.

5. Acknowledgements

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Figure Captions

Figure 1. Effect of anolyte pH adjustment with an automated ammonium hydroxide (NH_4OH) dosing on current generation of a non-buffered two-chamber BES (operated in MFC mode) after 48 hours of pH gradient development in the cathode and anode chambers under acetate saturated condition. Under pH controlled condition, the BES was operated at steady state for 20 days with an average current output of 10 mA.

Figure 2. BES with automated ammonium hydroxide (ammonia) addition after 45 hours of pH split. (a) Average total ammonium (ammonium and ammonia) present in the anolyte and the catholyte of a BES and cumulative ammonium hydroxide (ammonia) addition to maintain the anolyte pH at 7. (b) Percent of ammonia removal and current produced. Each point represents total ammonia loss compared to total ammonia loaded into the system at each time interval. The BES was operated in MFC mode.

Figure 3. Utilizing ammonium/ammonia (NH_4^+/NH_3) to shuttle protons from the anode chamber across a cation exchange membrane to the cathode chamber of a BES. Stoichiometric balance is normalized based on the transfer of one reducing-equivalent (i.e. one electron). PS: power supply; CEM: cation exchange membrane

Figure 4. Effect of recycling ammonia via a gas stream from catholyte to anolyte on current generation of a bioelectrochemical cell in batch mode. Anode potential was poised at -0.3 V vs. Ag/AgCl and sodium acetate was added to the anolyte at 1, 23, 29 h. Legend: (1) acetate addition; (2) purging N_2 through the catholyte with the off-gas through the anolyte (N_2 flow rate: 1 L/min); (3) acetate addition; (4) pausing of N_2

purging; (5) acetate addition; (6) N_2 purging resumed; (7) Doubling N_2 purging rate to 2 L/min. Note: data presented here were obtained from a separate BES reactor with a completely different electrochemically active biofilm (Refer to materials and methods section).

Figure 5. The effect of increase in catholyte temperature (55 ^{o}C) on (a) current flow (electron transfer rate) and ammonium migration rate from the anode chamber to the cathode chamber (insert: linear correlation between electron flow rate and ammonium migration rate), (b) cumulative ammonia added (into the anolyte) and collected (in the acid trap) and (c) the catholyte pH of a BES driven by 0.9 V applied voltage from the potentiostat.

Figure 6. Effect of NaCl addition (to 1 M Na^+ concentration) to the catholyte on the concentration of ammonium in the outflow and on ammonium migration from the anolyte to the catholyte of a potentiostat-assisted BES with continuous inflow of high ammonium wastewater to the anolyte. Cathode was operated in fed-batch mode at 75 ^{o}C. Average current generation was 18 mA.

Figure 1

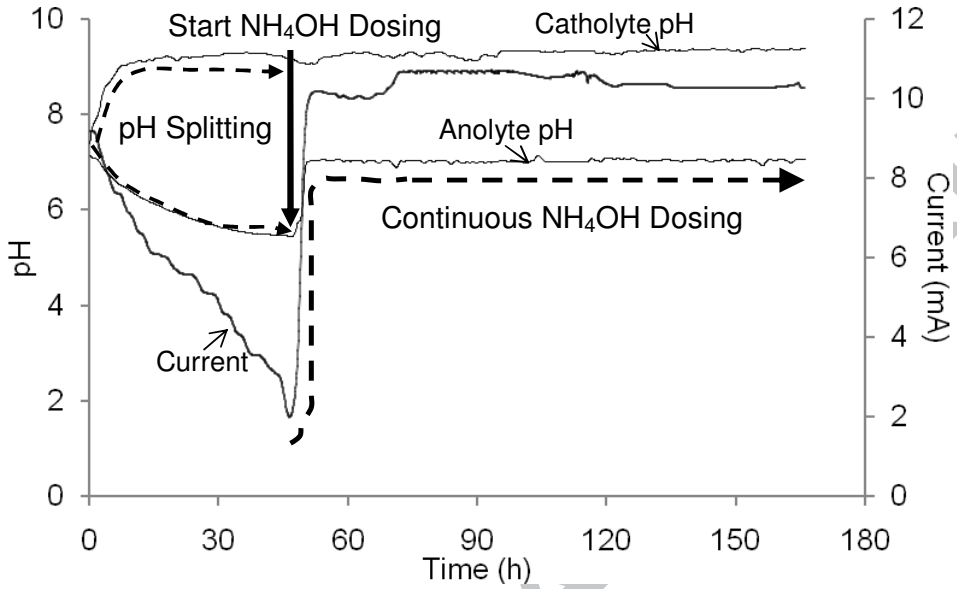


Figure 2

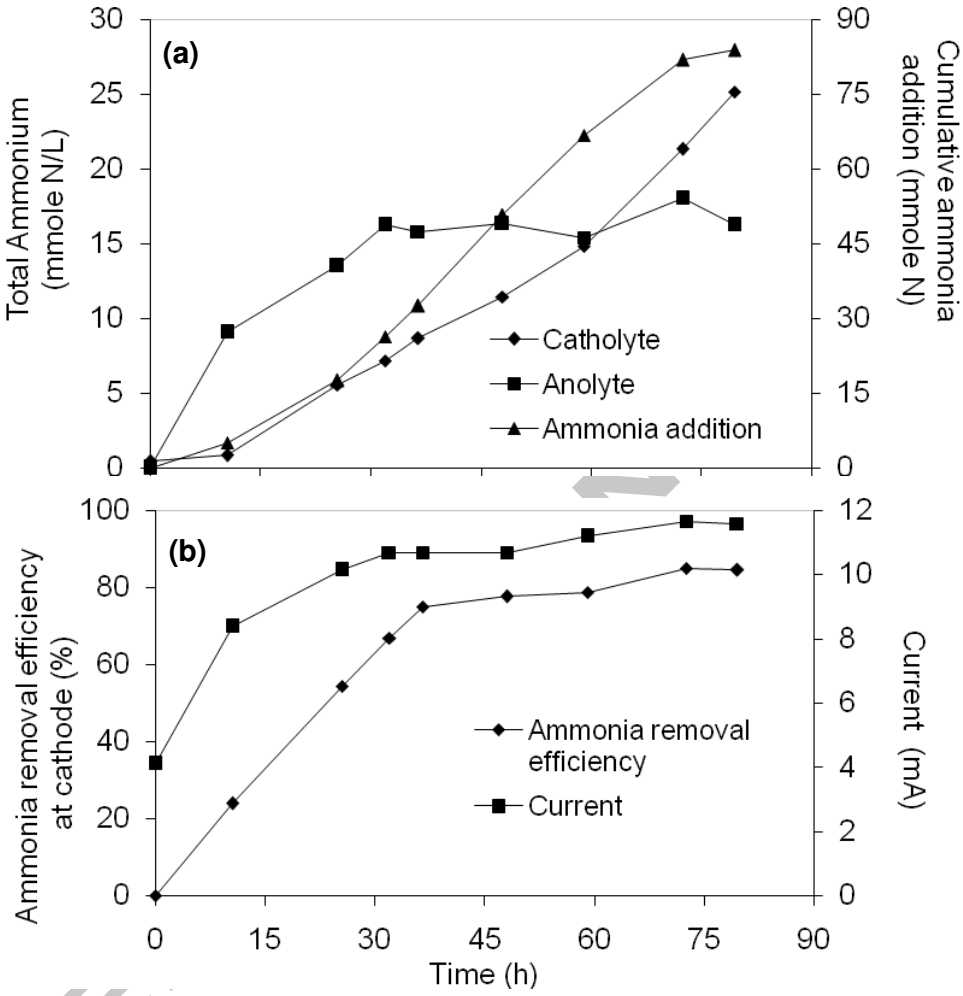


Figure 3

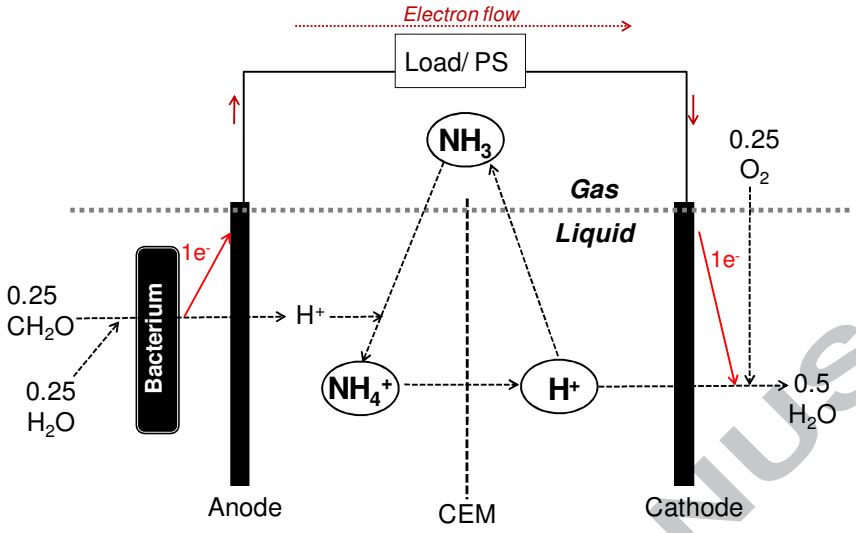


Figure 4

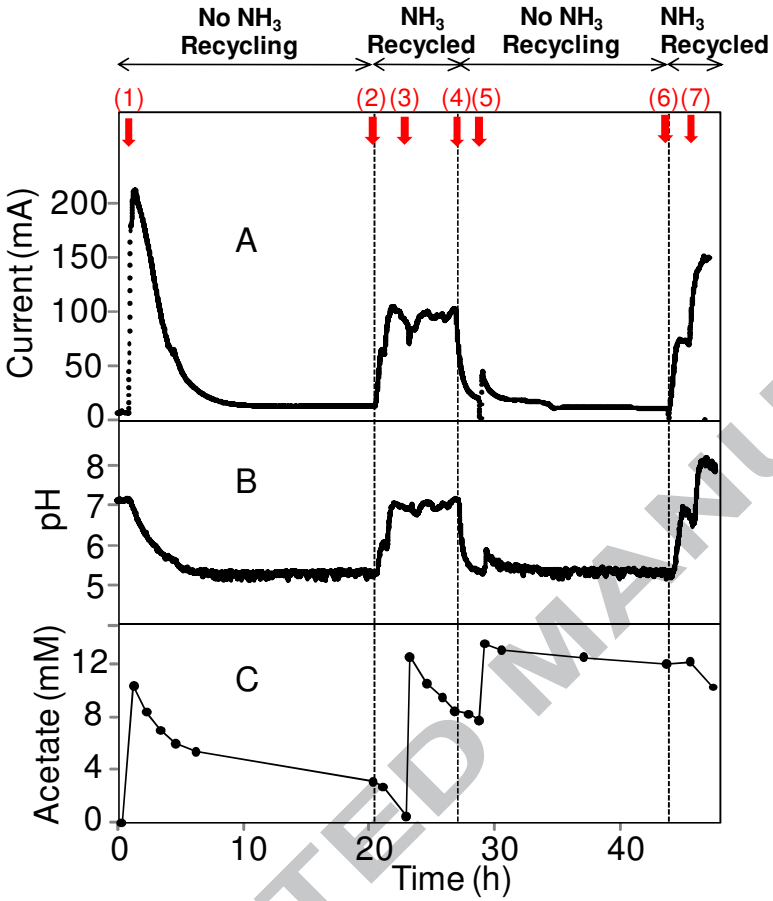


Figure 5

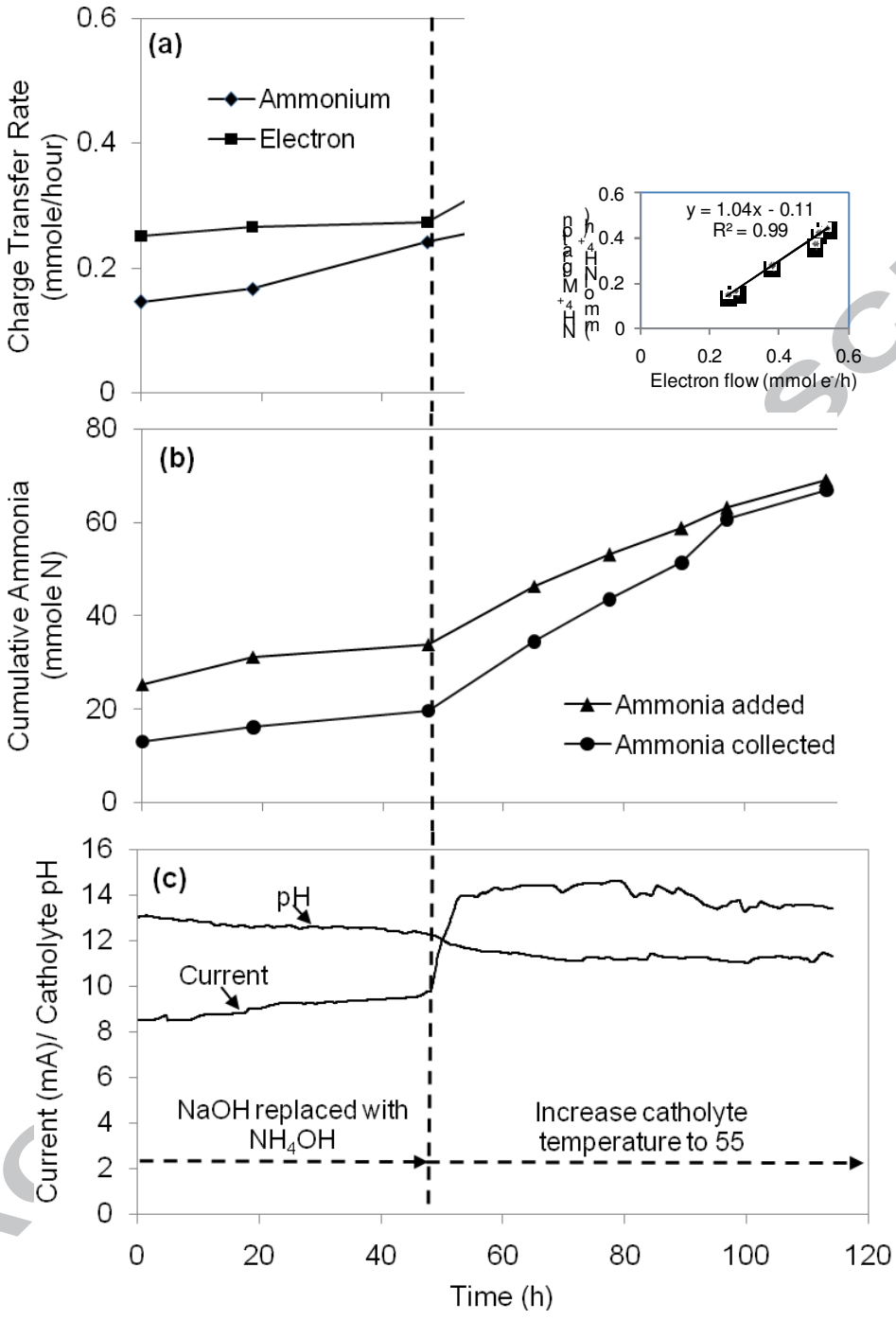
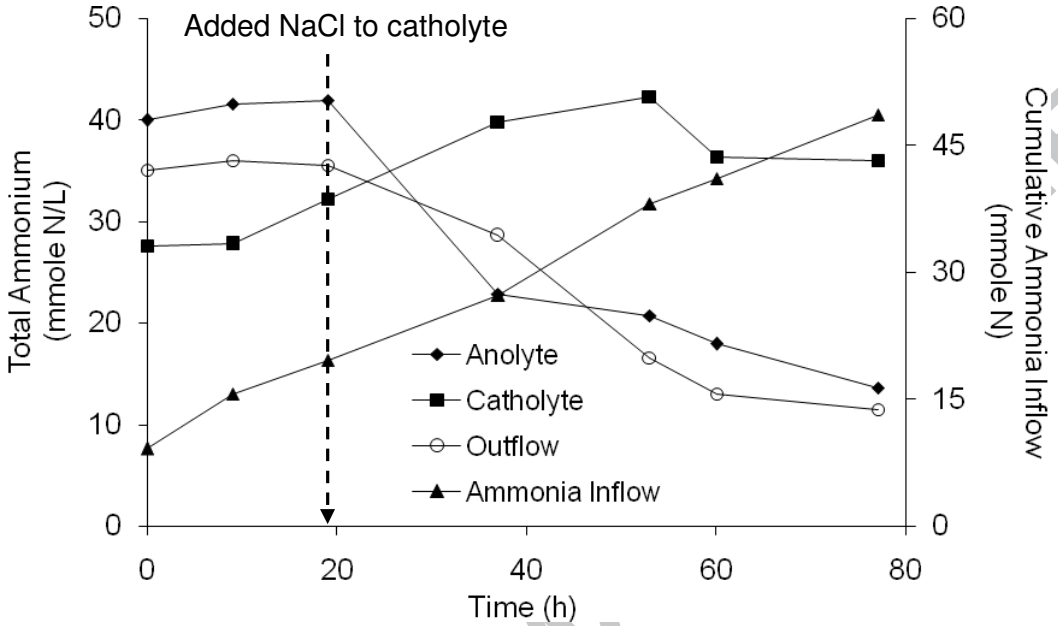


Figure 6



Highlights

* A novel way of overcoming the bottleneck of slow proton flux in BES is described.
* Ammonia addition to the anode prevented acidification and enabled proton transfer.
* Ammonia could be recovered from the cathode and continuously recycled to the anode.
* Ammonia recycle allowed long term sustainable operation of the cell.
* Using ammonium as proton shuttle prevented cation accumulation in the catholyte.
