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ARTICLE TYPE

Simultaneous Current Generation and Ammonium Recovery from Real Urine Using Nitrogen-purged Bioelectrochemical Systems

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Bioelectrochemical systems (BESs) offer a strategy to treat source-separated urine with current generation, whereas high content of ammonia is still a challenge for sustainable maintenance of BESs due to ammonia inhibition. Therefore, an integrated BES setup was developed to overcome this problem by ammonia recovery. This setup, working in closed circuit mode with nitrogen purging (CN), allowed for the produced ammonia continuously channeled to an absorption bottle. In addition, the control reactors in closed circuit (CC) or in open circuit mode (OC) were also run for comparison. A maximum power density of 310.9 ± 1.0 mW/m^2 was obtained for the CN reactor, and 127.1 ± 0.9 mW/m^2 for the CC reactor. Total nitrogen (TN) removal efficiency (84.9 ± 2.2 %) from urine was much higher in the CN reactor than it was in the CC (29.7 ± 6.7 %) or OC (30.0 ± 8.2 %) reactor. As part of TN in the CN reactor, 52.8 ± 3.6 % of TN was recovered in the form of NH_3-N, with a NH_3 recovery rate of 435.7 ± 29.6 gN m^{-3} d^{-1}. The improved performance of the CN reactor was contributed to the mitigation of ammonia inhibition to the anode electro-activities. 16S rDNA sequencing showed that no nitrifiers or anammox and denitrifiers were detected on the anodes and cathodes. Overall, nitrogen purging provides the urine-fed BESs with a useful approach to maintain system performance by ammonia recovery.

Introduction

In municipal wastewater treatment plant (WWTP), approximately 80 % of nitrogen (N) and 50 % of phosphorous (P) come from urine, although it contributes only 1 % to the total wastewater volume[1, 2]. In another aspect, nitrogen and phosphorus are two of the main elements leading to eutrophication in natural water bodies. Therefore, necessary process, either physical/chemical (e.g., activation carbon, flocculation and membrane technologies) or biological (e.g., nitrification/denitrification and Anammox) should be applied to remove them from wastewater[3, 4]. These practices have their own disadvantages which include the high operation costs of chemical addition or the requirement of large volume reactors to create the desired operating conditions[5]. Besides N and P, there exist a large amount of other nutrients and organics in urine. Supposing that urine is separated at source instead of being directly discharged into WWTPs or natural water bodies, it can be used for different purposes, such as nutrient recovery[6-8]. Among them, phosphorous and nitrogen recoveries have received more attention, because both elements are not only essential nutrients for plant growth, but also offer great potential for fertilizer production.

Struvite precipitation offers a strategy for simultaneous recovery of nitrogen and phosphorus from source-separated urine. In natural conditions, urea hydrolysis can readily occur and then reach a NH_4^+/NH_3 equilibrium. This will cause an increase in pH and trigger the precipitation of ammonium, phosphate and magnesium in the form of struvite. Being an effective slow-release fertilizer, struvite is of great value to fertilizer market. Its production can be achieved by the following chemical reaction:

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4{\cdot}6H_2O\downarrow \quad (1)$$

Where the molar ratio of NH_4^+ : PO_4^{3-} : Mg^{2+} is 1 : 1: 1 in theory. In hydrolyzed urine, ammonium is sufficient for struvite production with the ratio of 260 : 6 : 1 (NH_4^+ : PO_4^{3-} : Mg^{2+}) [6]. So, if more struvite is expected to be produced, additional phosphate and magnesium (e.g., MgO and $MgCl_2$) are needed[9, 10].

By comparison, the technologies for nitrogen recovery are more economically attractive, since the practice for phosphate or magnesium addition can be obviated. It has been reported that various technologies such as selective ion exchange and ammonia stripping, which are usually used to recover nitrogen from high-strength ammonium wastewater, can also be applicable to urine treatment[11, 12]. Even so, these technologies have to take into account the extra energy input for cation transport or the addition of caustics for pH increase, whereas these challenges can be circumvented by applying

bioelectrochemical systems (BESs). Recently, BESs have been intensively investigated as an alternative to ammonia recovery. In a BES equipped with a cation exchange membrane (CEM), ammonium can be transported from the anode to the cathode chamber, a process driven by electro-migration and diffusion. It has been reported that the NH_4^+/NH_3 migration could account for about 90 % of the ionic flux[13]. With the pH increasing, the concentrated ammonium in the cathode chamber is readily transformed into volatile ammonia, which can be subsequently removed through NH_3 stripping[14]. Following up on the proof-of-concept for BESs to achieve ammonia recovery, the feasibility of recovering ammonia from real urine was first demonstrated and evaluated using a two-chamber microbial fuel cell (MFC) with a gas diffusion layer cathode, from which volatile ammonia was carried away with the gas stream and absorbed by acid solution[15].

Although ammonia recovery from urine is a promising concept for application of BESs, effects of ammonia on sustainable maintenance of BESs remain controversial. Some reports show that ammonia was oxidized with anode as an electron acceptor in BESs[16-18], while others support adverse effects of ammonia on anode electro-activity[19]. These contradictory conclusions are believed to be dependent on the types of dominant bacterial species enriched on the anodes. Generally, the production of electricity from ammonium is only found on anodes enriched with nitrifiers such as *Nitrosomonas europaea*[18], whereas in most cases, they are rarely detected. Therefore, it is critical to identify and circumvent the potential challenges if a BES is expected to recover ammonia from urine.

In this research paper, a hypothesis was raised that if urine, being treated inside a BES, was continuously purged with nitrogen gas (purity, 99.999 %), which was then channeled to an absorption bottle, we not only recovered the desired ammonia, but also alleviated the possible ammonia inhibition to the BES performance. Therefore, we developed an integrated BES setup and its performance was examined in terms of current generation, nitrogen conversion, and compared to that of controls in closed and open circuit modes. The difference in performance between the setup and the controls was further evaluated from the perspective of the electrochemical characteristics of the electrode. In addition, the possibilities for nitrification and denitrification in these reactors were also assessed using 16S rDNA pyrosequencing to target specific bacteria associated with these biological processes.

Materials and Methods

Experimental Setup Construction

The integrated BES reactor (Fig.1), operated in closed circuit mode with nitrogen purging (CN), was constructed based on an air-cathode MFC as previously described[20]. Graphite fiber was chosen as anode, which was wound into two twisted titanium wires and heat treated according to previous reports[21]. The cathode was carbon cloth, with one side as the catalytic layer and the other side as the diffusion layer[22]. Anode was connected to cathode via an external circuit containing a resistor of 1000 Ω. There were two ports on the top of the CN reactor, which were sealed with rubber stoppers. Two custom-made needles (N-1, N-2) were vertically inserted through the rubber stopper (close to the cathode) into the reactor chamber at different depths, allowing for the nitrogen gas in (N-1) and out (N-2) of the chamber. The N-1 needle hub was connected via a peristaltic pump (LongerPump BT100-1L, Baoding) to the washing bottle, from which moist nitrogen gas streams was pumped (at a flow rate of 1.35 mL/min) into the chamber of the CN reactor. The N-2 needle hub was connected via a reflux tube to the absorption bottle, where ammonia was absorbed by 0.4 M sulfuric acid. For the washing bottle, there were also two needles as inlet and outlet. When the nitrogen gas was continuously pumped into the chamber of the CN reactor, it was necessary to keep the pressure in the washing bottle slightly positive compared to that of the atmosphere. Another two sets of identical air-cathode MFCs as controls, one operating in open circuit mode (OC), and the others in closed circuit mode (CC), were also used to compare the differences in performance or microbial community with the CN reactors.

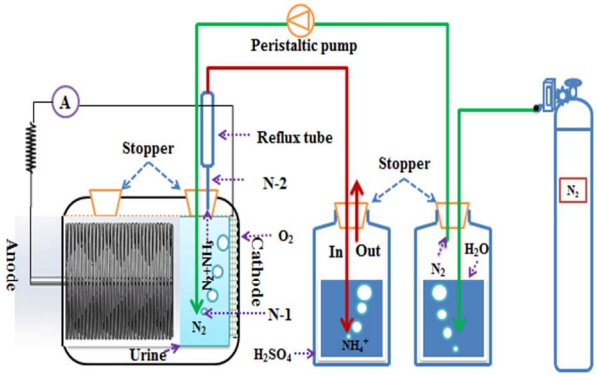

Fig. 1 Schematic diagram of the integrated BES (CN) for recovery of ammonia from human urine

Inoculation and Operation

All the reactors (in triplicate) were inoculated with a 30:70 (v/v) mixture of domestic wastewater and fresh urine. After 3 batch cycles, all reactors except for the OC reactors produced maximum currents more than 0.2 mA. Subsequently, the influents were completely changed to urine. Urine was provided by a healthy male volunteer. The reactors were operated in batch mode and fed with fresh urine once the voltage decreased to 50 mV. The CN reactors were connected with the needles when the peak currents increased up to 0.35 mA. Similar enrichment was also conducted in our early research, where electrochemical characteristics of electrodes over time in air-cathode MFCs were investigated based on polarization data, whereas the urine used in each cycle was same. All reactors were operated in a temperature-controlled chamber (30 ^{o}C). All experiments were performed in triplicate and mean values or typical results were presented.

Physical and Chemical Analyses

In order to compare the maximum power densities as well as the electrochemical characteristics of the electrodes, power density

and polarization curves were made for the nitrogen-purged and non-purged reactors following the single-cycle method[23]. In our early research, to investigate the impact of real urine on MFC performance during long-term operation, power density and polarization curves at different times were also conducted on single-chamber air-cathode MFCs. The external resistance was varied from OCV to 30 Ω, with each resistor being connected for 20 min. The oxygen flux into the chamber over one full batch cycle was monitored for the CN and CC reactors using a non-consumptive oxygen probe (NeoFox, Ocean Optics, Inc., Dunedin, FL). The rubber stopper, next to the air-cathode, was used to fix the probe. All urine samples for chemical analysis were centrifuged (Eppendorf Centrifuge 5418; 14,000 × g, 5 min) to remove suspended solids. TN was determined using a HACH T-N kit (10 – 150 mg/L). Total ammonia nitrogen (TAN, the sum of NH_4^+-N and NH_3-N) was measured by the salicylate method using a Test N' Tube kit (HACH, Loveland, CO, USA). Nitrite and nitrate concentrations were measured using ion chromatography (Dionex ICS-3000 system with an IonPac AS11-HC Analytical column, 4 × 250 mm). Na, K, Mg, and P were analyzed using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Perkin Elmer Optima 5300DV, Waltham, Massachusetts) and presented in Table S2. Precipitates on graphite fiber anodes were characterized by X-ray diffraction (XRD) using a diffractometer (Bruker D 8 ADVANCE) operated at 40 kV and 30 mA. Qualitative analysis was performed using PDXL software.

Electrochemical analysis

Cyclic voltammetry (CV) was performed on the anode of the CC and CN reactors with cathode as the counter electrode and Ag/AgCl electrode as the reference electrode. The reference electrode was inserted into the port close to the anode side. Both reactors were fed with fresh urine. When the current output approached to the maximum (based on averge values) in either of the reactors, circuits were disconnected from the data acquisition system and connected to the potentiostat/galvanostat (Autolab, Metrohm, model PGSTAT 128N). For the CN reactor, nitrogen purging was stopped prior to the CV test. Subsequently, the potential was scanned positively from -0.7 V to 0.2 V at a rate of 5 mV/s. The catalytic feature of the biofilms was characterized by plotting the first derivative of each sweep as a function of potential [24].

Microbial Community Analysis

To compare the microbial communities on anode and cathode, six samples were collected from the three types of reactors for 16S rDNA pyrosequencing after 152 days of operation. Total genomic DNA was extracted from biofilms using a Bacteriag DNA Mini Kit (Watson Biotechnologies, Inc., Shanghai) according to the manufacturer's instructions and assessed by electrophoresis in 1 % agarose gels. The V3 and V4 regions of 16S rDNA genes were amplified by PCR using the fusion primers: 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) [25]. Amplification was carried out as previously described[26]. After PCR amplification, amplicons were purified with the Agarose Gel DNA purification kit, and cloned using the T-carrier cloning kit (Sangon Biotech Shanghai Co., Ltd., China) following the manufacturer's instructions. Pyrosequencing of amplicons was performed by Sangon Biotech (Shanghai) Co., Ltd. using a 3730 type DNA sequencing system. Low-quality sequences (<25) or those with lengths lower than 200 base pairs (bp) were removed according to standard protocols. Representative sequences from each OTU were phylogenetically assigned to a taxonomic identity using the RDP Naïve Bayesian rRNA classifier at a confidence threshold of 80 %[27].

Calculations

Current was calculated by monitoring the voltage (U) across the external resistor (R) in the circuit using a data acquisition system (PISO-813, ICP DAS Co., Ltd). Power density, P (mW/m^2), was calculated from the measured voltage as $P = U^2/A{\cdot}R$, where A is the projected surface area of the cathode (7 cm^2).

NH_3 concentration in urine was estimated from the following equation [28]:

$$\frac{[NH_3]}{[TAN]} = \left(1 + \frac{10^{-pH}}{10^{-(0.09018 + (2729.92/T))}}\right)^{-1} \quad (2)$$

Where $[NH_3]$ is the concentration of free ammonia, [TAN] is the total ammonia concentration including the ammonia (NH_3) and the ammonium ion (NH_4^+), and T is the temperature (Kelvin).

The ammonia recovery rate (gN m^{-3} d^{-1}) was calculated as

$$\text{Ammonia recovery rate} = \frac{\varpi}{V} \quad (3)$$

Where ϖ is the amount of ammonia recovered daily from urine and V is the volume of liquid in the reactor.

Results

Current and Power Generation

After 50 days of operation, all the reactors began to produce stable current, showing that MFCs are applicable to high-strength human urine. As can be seen in four successive feeding cycles (Fig. 2), the peak current reached to 0.43 mA for the CN reactor, and 0.32 mA for the CC reactor. The difference in peak current between both systems was observed within a stable range of 0.10 ± 0.02 mA.

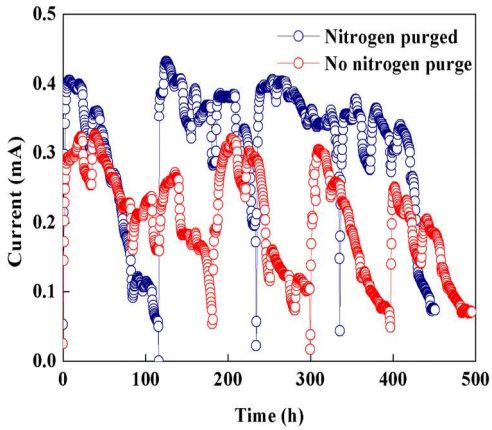

Fig. 2 The current generation over four successive feeding cycles in the CC and CN reactors fed with fresh urine after 50 days of operation.

On day 62, the performances of these reactors were evaluated by the polarization and power density curves. A

maximum power density of 310.9 ± 1.0 mW/m^2 was obtained for the CN reactor, and 127.1 ± 0.9 mW/m^2 for the CC reactor (Fig. 3A). Accordingly, the electrode potentials varied differentially along the resistance decrease. When the anode (or cathode) potential increased (or decreased) to the final potentials (30 Ω), the current density was 0.22 ± 0.02 mA for the CN reactor, but 0.11 ± 0 mA for the CC reactor (Fig. 3B). The sharp convergence of electrode potentials with the increasing current density suggests that limitation or inhibition occurred especially on the anodes in the CC reactors.

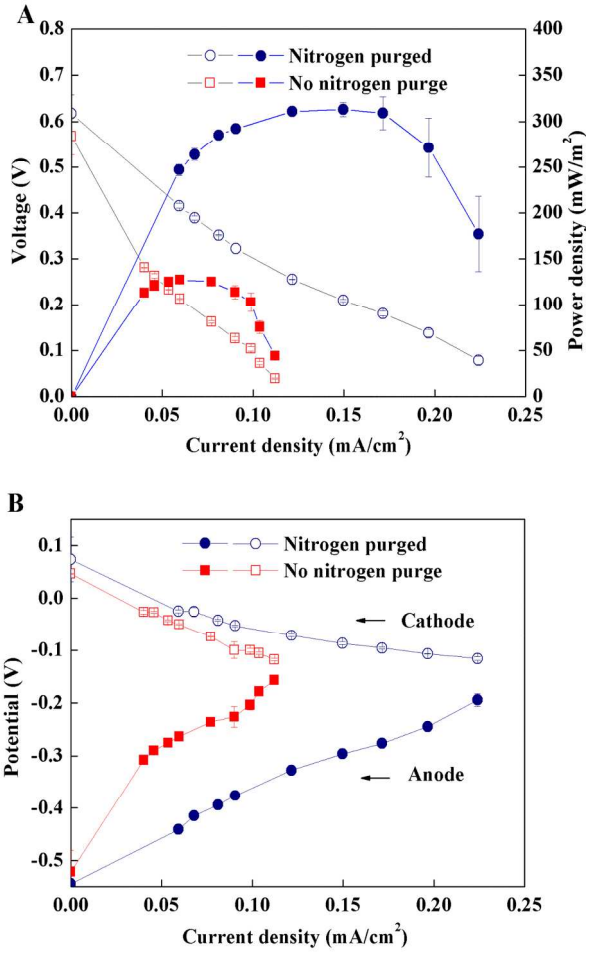

Fig. 3 (A) Power density and (B) electrode potential curves for the CC and CN reactors.

Nitrogen Conversion and Recovery

In one of the complete feeding cycles, the urine samples were taken for chemical analysis (Table 1). The initial concentration of TAN was 270 mg N/L, but the finial concentration increased to 3020 ± 214 mg N/L (10 days) for the OC reactor, 2890 ± 156 mg N/L (10 days) for the CC reactor, and 570 ± 87 mg N/L (6.4 days) for the CN reactor. In fresh urine, most of the nitrogen existed in the form of urea, and the increase of TAN was attributed to urea hydrolysis, whereas the slight difference in TAN concentration between the OC and CC reactors was possibly due to ammonium transport to the cathode with current generation in the CC but not in the OC reactor. It is assumed that ammonium ion was subsequently converted to volatile ammonia at the elevated pH[14]. Although the increase of TAN was found in all the reactors, it was not followed by the increase of NO_2^--N or NO_3^--N. On the contrary, NO_2^--N was undetectable in both the influent and effluent, and NO_3^--N (initial concentration, 0.58 mg/L) decreased by one order of magnitude, with 0.02 ± 0 mg/L remaining in the CC and CN reactors. Nitrate might be reduced by denitrifying bacteria or at the cathode, as nitrate is also an alternative electron acceptor or oxidant [29].

Table 1. Key parameters measured for real urine before and after treatment.

	Reactor type	TN	TAN	NO_2^--N	NO_3^--N	TOC	COD
Unit		(mg N/L)					
Fresh urine		5273	270	-	0.58	3613	8500
Treated urine	OC	3688 ± 432	3020 ± 214	-	-	1158 ± 130	1500 ± 100
	CC	3706 ± 360	2890 ± 156	-	0.02	538 ± 20	1400 ± 141
	CN	708 ± 230	570 ± 87	-	0.02	325 ± 59	1600 ± 182

TN removal efficiency (84.9 ± 2.2 %) from urine was much higher in the CN reactor than it was in the CC (29.7 ± 6.7 %) or OC (30.0 ± 8.2 %) reactor. As part of TN in the CN reactor, 52.8 ± 3.6 % of TN was recovered in the form of NH_3-N, suggesting that about 32 % of TN was probably lost through ammonia volatilization or bacterial nitrification/denitrification. The NH_3-N recovery rate for the CN reactor was 435.7 ± 29.6 g N m^{-3} d^{-1}, 2.3 times higher than that (131.6 gN m^{-3} d^{-1}) achieved in another research, where ammonia was recovered from the air-cathode via volatilization and subsequent absorption into an acid solution[15]. Furthermore, COD removal rates of these reactors were also compared, with 1078.0 ± 40.2 g m^{-3} d^{-1} for the CN reactor, 710.0 ± 19.9 g m^{-3} d^{-1} for the CC reactor and 700.0 ± 14.1 g m^{-3} d^{-1} for the OC reactor.

Microbial Community Analysis

The microbial communities at genus level were much diverse (Table S1) on the anodes (A-OC; A-CC; A-CN), with the species relatively evenly distributed (Fig. 4A). Generally, the composition and structure of microbial community are closely related to the substrates used in MFCs. Different from the non-fermentable substrates such as acetate, the composition or content of urine is more complex. According to the Urine Metabolome database (www.urinemetabolome.ca), the most abundant organic constituents (based on average values) of urine are urea (22.5 ± 4.4 mM/mM creatinine), creatinine (10.4 ± 0.2 mM), hippuric acid (298.5 ± 276.8 μM/mM creatinine) and citric acid (280.6 ± 115.2 μM/mM creatinine). As members of *Clostridia*, *Tissierella* (25.0 %; 13.4 %; 12.8 %), *Finegoldia* (8.1 %; 17.6 %; 4.8 %), *Anaerosphaera* (2.1 %; 6.2 %; 2.9 %) and *Clostridium XII* (5.3 %; 4.4 %; 4.0 %) were four dominant genera detected on the anodes, and most of them are responsible for fermentation of various organic acids, proteins and their hydrolytic products[30-33]. Among them, *Tissierella* species have been known to ferment urine creatinine to acetate, ammonia and other products[34]. *Atopostipes* (18.3 %; 15.5 %; 9.5 %) and *Facklamia* (6.8 %; 10.9 %; 5.4 %) were two dominant genera belonging to class *Bacilli*,

which can use carbohydrates to produce various fatty acids as well as urease[35, 36]. They, therefore, are believed to be related to urea hydrolysis. *Petrimonas*, was the main genus belonging to class *Bacteroidia* on the anodes (8.8 %; 1.2 %; 0.8 %). It can ferment carbohydrates and some organic acids, and reduce nitrate to ammonium[37]. Another two genera *Oligella* and *Serpens* were found with the proportion increasing in the following sequence: A-OC (0; 0.5 %), A-CC (2.0 %; 9.7 %), A-CN (12.1 %; 23.1 %). *Oligella* is a member of class *Betaproteobacteria* and has been reported capable of hydrolyzing urea[38]. *Serpens*, belonging to class of *Gammaproteobacteria*, is considered to be the most likely exoelectrogens, since most bacterial species affiliated to class *Gammaproteobacteria* are electrochemically active bacteria. This may explain which bacteria further consumed the end products of fermentation or hydrolysis for current generation.

Most genera present on the anodes were also found on the cathodes, but at least five dominant genera were unique on the cathodes (C-OC; C-CC; C-CN) including: *Pusillimonas* (1.5 %; 8.4 %; 2.8 %), *Azorhizophilus* (4.9 %; 1.9 %; 2.2 %), *Erysipelothrix* (6.2 %; 2.1 %; 1.4 %), *Dokdonella* (1.9 %; 3.0 %; 6.6 %), *Sporanaerobacter* (5.4 %; 4.9 %; 1.2 %) (Fig. 4B). Most of them show an aerobic respiratory metabolism, and none of them have been reported to be related to nitrification or denitrification.

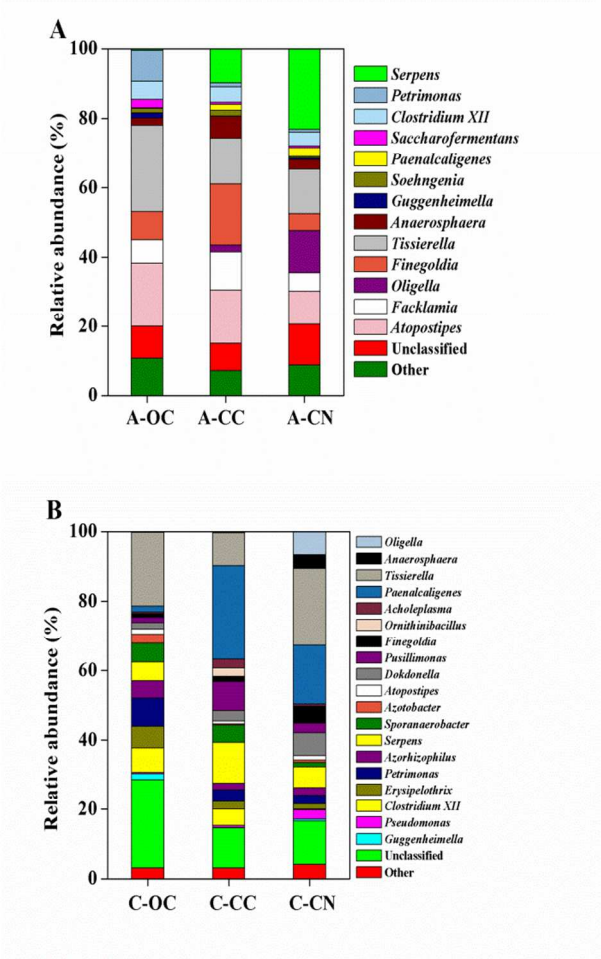

Fig. 4 Microbial community distribution for biofilms developed on electrodes (anode; cathode) at genus levels (A;B) of these three types of reactors (OC, open circuit; CC, closed circuit; CN, closed circuit and nitrogen purged). "A" and "C" represent the anode and cathode samples.

Discussion

Without nitrogen purging, the impact of real urine on MFC performance during long-term operation was investigated in our early research, with the maximum power density and polarization curves measured at different times. The maximum power density decreased from 143.5 ± 4.9 mW/m^2 on day 32 to 51.1 ± 1.0 mW/m^2 on day 118 (Fig. 5A). Correspondingly, the anode potential at specific resistance shifted toward more positive values over time (e.g., from - 0.38± 0.01 V on day 32 to - 0.28 ± 0.01 V on day 118, 1000 Ω) with simultaneous decrease of current density, suggesting that remarkable polarization occurred on anode. However, there was no appreciable change in cathode potential (Fig. 5B). Given the ammonia accumulating in the chamber due to urea hydrolysis and its toxicity to bacterial activity, the decrease of maximum power density should be associated with long-term exposure of anodic biofilms to high concentration of ammonia, leading to the decline of anodic electro-activity over time. Hence, the anodic electro-activity was the major limitation leading to the decline in performance. In this study, the enhanced performance exhibited on the CN reactors was believed to be attributed to nitrogen purging, leaving less ammonia in the chamber and allowing for higher biological activity.

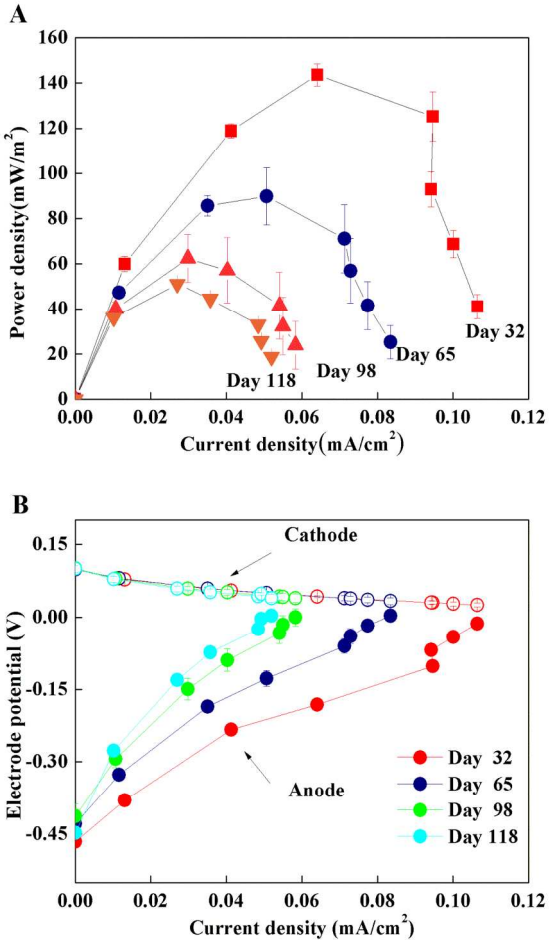

Fig. 5 (A) Power density and (B) electrode potential curves over

time for urine-fed air-cathode MFCs.

The CVs and their first derivatives indicated the differences in bacterial electro-activity between the nitrogen-purged and non-purged reactors (Fig. S1). CV revealed that the anodic current from the CN reactor was much higher than that from the CC reactor over a broad potential range (i.e., from - 0.70 V to - 0.05 V). The increase in anodic current from the CN reactor was consistent with the polarization data that purging urine being treated inside the chamber with nitrogen could improve system performance (Fig. 3). First derivative analysis of the CV data showed that with nitrogen purging the peak intensity was more evident especially at - 0.67 V compared to that without nitrogen purging, suggesting that redox activity of anodic biofilms in the CN reactor was enhanced due to the involvement of nitrogen purging.

The improved performance of the CN reactor was believed to be independent of dissolved oxygen (DO), since DO concentration in the CN reactor was slightly higher than in the CC reactor over time (Fig. 6). When the chamber was continuously purged with nitrogen gas, a portion of DO would be carried away with volatile ammonia, causing a difference of oxygen partial pressure between inside and outside. In this case, oxygen would continuously diffuse through the air-cathode into the chamber. The turbulent flow was believed to intensify oxygen diffusion. Furthermore, since the microbial community on the cathode of the CC reactor was dominated by aerobes (Fig. 4B), more oxygen was likely to be consumed before entering the non-purged chamber in comparison to that in the nitrogen-purged reactor. These may explain the difference of DO in both reactors.

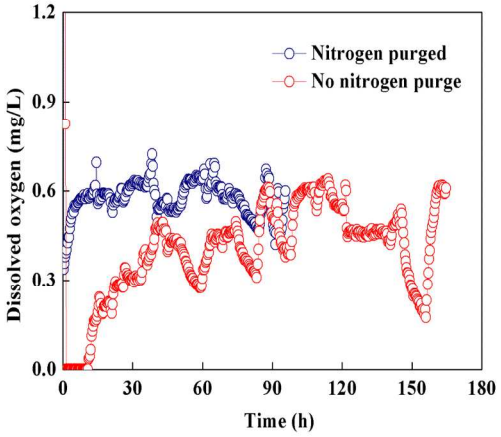

Fig. 6 DO evolution over time in the CC and CN reactors in one complete batch cycle.

Generally, weak acids and bases are toxic for bacterial growth, because their undissociated forms dissipate proton motive force, leading to disturbance of energy metabolism[39]. Acting as a base, a certain fraction of ammonia remains undissociated depending on pH at constant temperature and pressure. In this study, the pH of the effluent from the CN reactor reached up to 9.1, followed by the CC (8.9) or OC (8.9) reactor, but this doesn't mean that the treated urine in the CN reactor had a NH_3-N concentration (214 mg/L) much higher than that in the CC (822 mg/L) or OC (1087 mg/L) reactor. Although the threshold ammonia concentrations that cause inhibitory effect are not in agreement in published papers, usually ranging from 500 – 3500 mg N/L[15, 40], the inhibition of exoelectrogenic activities is often observed in BESs [41]. According to Eq. (2), the ratio (δ) of NH_4^+-N to NH_3-N could be simplified as a function (Eq. (4)) of pH:

$$\delta = \frac{[NH_4^+]}{[NH_3]} = 10^{9.1\text{-pH}} \quad (4)$$

The ratio (δ_{CN} =1.00) for the CN reactor was much smaller than that ($\delta_{CC} = \delta_{OC}$ =1.58) for the CC and OC reactors. The significant difference in ratio suggests that the equilibrium of urea hydrolysis (Eq.(5),(6)) in the CN reactor accelerated the conversion of urea by continuously carrying volatile ammonia out of the chamber in comparison to that in the OC or CC reactor. Thus, the lower ammonia content was the reason for the improved electro-activity or performance in the CN reactor.

$$(NH_2)_2CO + H_2O \xrightarrow{\text{urease}} NH_3 + H_2NCOOH \rightarrow 2NH_{3(gas)} + CO_{2(gas)} \quad (5)$$

$$NH_{3(gas)} + H_2O \leftrightarrows NH_4^+ + OH^- \quad (6)$$

In addition, the nitrogen removed from the OC and CC reactors as well that lost in the CN reactor is believed to be mainly related to volatilization through the air-cathode[42]. As noted above, the TN removal efficiency for the OC reactor was similar to that of the CC reactor. It seems that the CC reactor failed to enhance ammonia volatilization through air-cathode, although current generation from BESs could increase NH_4^+/NH_3 transport to cathode, where the conversion of NH_4^+ to volatile NH_3 occurs as a result of an elevated pH near the cathode [43]. There was possibility that after long-term operation the ammonium migration process driven by current generation was undermined for the non-purged (CC) reactors, since the biofilm activities were reduced over time. In another aspect, although NH_3/NH_4^+ transport in the OC reactor just depended on diffusion, a process slower than ammonium migration with current generation[15], the enrichment of functional bacteria on the anode without current generation might facilitate urea hydrolysis, leading to a high ammonia concentration gradient and hence the increase of ammonia transport. As can be seen in Fig. 4A, the genera such as *Tissierella, Atopostipes and Petrimonas* made up a large proportion of the microbial community on the anode of the OC reactor, which contrasted with that on the anode of the CC reactor. This may explain why the TN removal efficiencies in both types of reactors were comparable to each other. Given the salinity of real urine, there existed another possibility that ammonium compared to other ions might contribute little to the charge balance in the CC reactor and therefore the NH_4^+/NH_3 transport, making the ammonia lost through air-cathode comparable to that in the OC reactor. Furthermore, the TN lost through the air-cathode of the CN reactor also showed similar removal efficiency with other two types of reactors, but the cause remains unclear. In the CN reactor, nitrogen purging not only carried away a great part of volatile ammonia, but also improved bacterial activities, especially of exoelectrogens (e.g., the uncharacterized *Serpens*). The combination of BES with nitrogen purging offered a strategy for simultaneous recovery of ammonia and maintenance of BESs. These may explain why large amounts of nitrogen were removed from the CN reactor through nitrogen purging.

Besides ammonia content, salt precipitation might be another limiting factor responsible for the decreased anode electro-activities. The XRD analysis showed that the precipitates on the

OC or CC reactors were mainly comprised of $KMgPO_4 \cdot 6H_2O$ and $MgNH_4PO_4 \cdot 6H_2O$ (Fig. 7). By contrast, few precipitates were formed on the anodes of the CN reactors. On average, the molar ratios in urine are 50 - 100 : 10 : 1 for N : P : Mg, whereas the formation of $MgNH_4PO_4 \cdot 6H_2O$ needs equimolar amounts of Mg, NH_4^+ and PO_4^{3-} [10]. In this study, the dramatic decrease in NH_4^+-N might not facilitate salt precipitation.

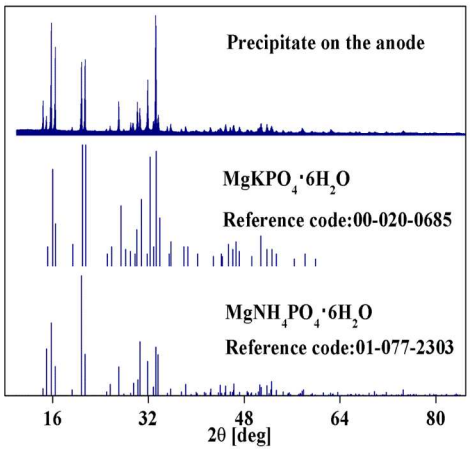

Fig. 7 X-ray diffraction patterns of precipitates deposited on the graphite fiber anode of the OC or CC reactors.

As the major product of urea hydrolysis, ammonia was not further converted into nitrite or nitrate. This was reflected by the microbial community analysis that no nitrifiers or anammox bacteria were found on the anodes. Generally, nitrification process only occurs on anodes enriched with some nitrifiers such as *Nitrosomonas europaea*. The lack of nitrifiers in this study might be related to the inoculums. Actually, biological hydrolysis of urea is an unfavourable process for nitrification[44]. There is more evidence supporting that ammonia is a major factor limiting the activity of nitrifiers[45, 46]. Generally, nitrite oxidizing bacteria are sensitive to NH_3-N in the range of 0.1 mg/L to 1.0 mg/L, while ammonia oxidizing bacteria are inhibited in the range of 10 mg/L to 150 mg/L[47]. Here the NH_3-N concentration in the CN reactor remained at 214 mg/L, although more ammonia had been recovered in the absorption bottle. This may explain why no nitrifiers were detected on the electrode samples. The decrease of nitrate should be associated with chemical reduction reaction, since no denitrifying bacteria were detected on the cathodes.

Conclusions

This study presents a possibility that ammonia inhibition of anode electro-activities could be mitigated concomitantly with ammonia recovery from urine in a nitrogen-purged BES. With nitrogen purging, the peak current from the CN reactor reached up to 0.43 mA, which contrasted to that (0.32 mA) of the CC reactor. Correspondingly, TN removal efficiency was 84.9 ± 2.2 % for the CN reactor but was 29.7 ± 6.7 % for the CC reactor. As part of TN in the CN reactor, 52.8 ± 3.6 % of TN was recovered in the form of NH_3-N, with a NH_3 recovery rate of 435.7 ± 29.6 gN m^{-3} d^{-1}. Ammonia content in hydrolyzed urine was the determining element affecting anode electro-activities or performance of urine-fed BESs. The results show that nitrogen-purging offers a strategy for sustainable maintenance of BESs with simultaneous ammonia recovery.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [estimators for evaluation of community diversity and richness; Electrochemical analysis; Key components measured in real urine]. See DOI: 10.1039/b000000x/

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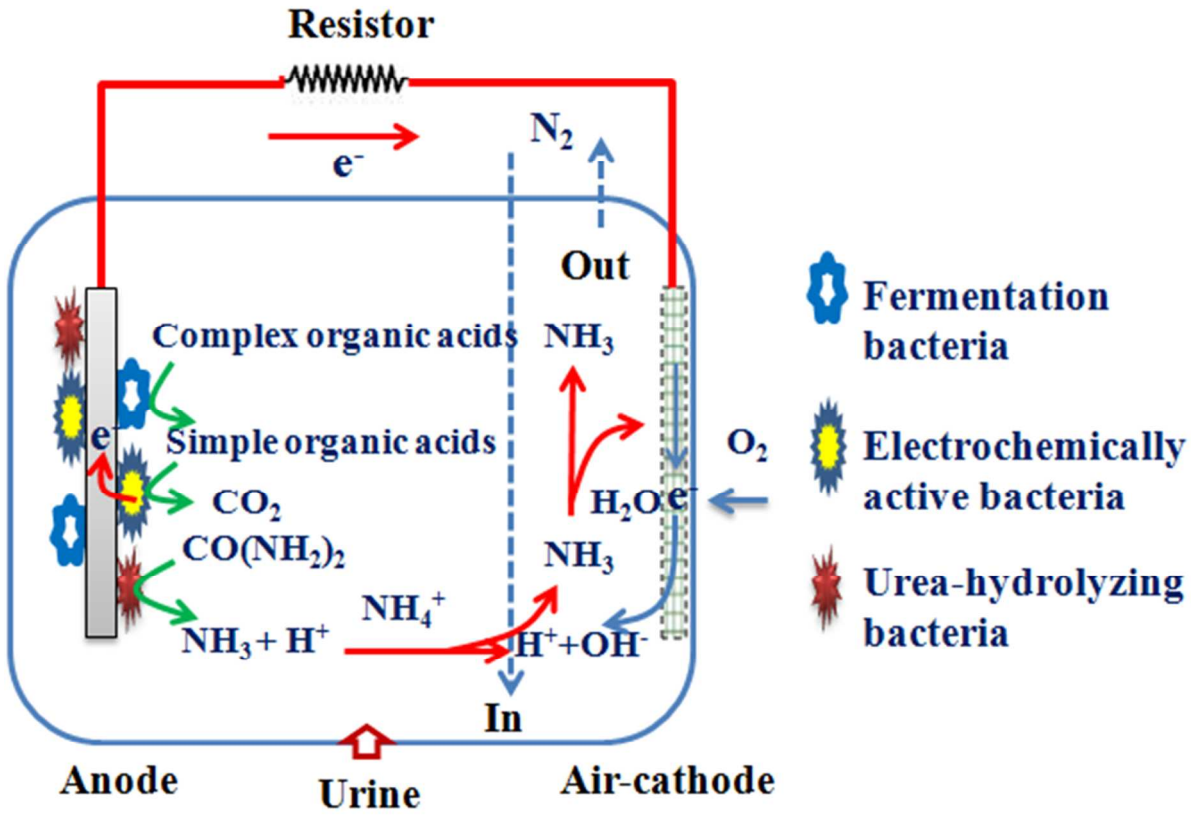
Image and text for graphical Table of Contents



Ammonia could be recovered from human urine through combination of bioelectrochemical systems and nitrogen purging, with concomitant mitigation of ammonia inhibition on anode electroactivities. Nitrogen conversion and carbon removal were also accelerated with simultaneous current generation.