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Ammonia inhibition of electricity generation in single-chambered microbial fuel cells

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ABSTRACT

Batch experiments are conducted at various concentrations of initial total ammonia nitrogen (TAN) with acetate as an electron donor to examine the effects of free ammonia (NH_3) inhibition on electricity production in single-chambered microbial fuel cells (MFCs). This research demonstrates that initial TAN concentrations of over 500 mg N L^{-1} significantly inhibit electricity generation in MFCs. The maximum power density of 4240 mW m^{-3} at 500 mg N L^{-1} drastically decreases to 1700 mW m^{-3} as the initial TAN increases up to 4000 mg N L^{-1}. Nitrite and nitrate analysis confirms that nitrification after complete acetate removal consumes some TAN. Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are also inhibited by increasing the initial TAN concentrations. Another batch experiment verifies the strong inhibitory effect of TAN with only small differences between the half-maximum effective concentration (EC_{50}) for TAN (894 mg N L^{-1} equivalent to 10 mg N L^{-1} as NH_3) and optimum TAN conditions; it requires careful monitoring of the TAN for MFCs. In addition, abiotic control experiments reveal that granular activated carbon, which is used as an auxiliary anode material, adsorbs a significant amount of ammonia at each TAN concentration in batch MFCs.

1. Introduction

Various bioelectrochemical systems are being developed to produce clean and renewable energies such as electricity [1], hydrogen [2], and methane [3]. Most of these systems rely on the generation of electrons from various organic substrates by electrochemically active bacteria on anodes. These systems could offer high energy production if wastewater could act as a feasible electron donor. To this end, several attempts have been made to produce electricity using wastewater in microbial fuel cells (MFCs). Various types of wastewater have been examined, e.g., beer brewery wastewater [4], leachate [5], swine wastewater [6], paper recycling wastewater [7], and fermented wastewater [8]. Most systems, however, have performed poorly due to physicochemical and biological limitations that prevent the efficient transfer of electrons. Excess amounts of total ammonia nitrogen (TAN), which are produced by the hydrolysis of proteinous organic materials, can be one limiting factor in a microbial metabolism. Widespread research efforts have been undertaken to develop cost-effective biological wastewater treatment since most wastewaters, produced by human activities, contain high concentrations of TAN.

The effect of TAN or free ammonia (NH_3) on the electricity generation in MFCs has received little attention. Recent approaches have reported TAN removal in a single-chambered MFC, namely: nitrification by diffused oxygen through air cathodes [5,9]; volatilization achieved by increasing the pH of cathode surfaces; application that combines nitrification and denitrification [10]. Biocathodes are attracting increased attention because of their ability to treat wastewater by reducing nitrate compounds rather than the oxygen in a cathode chamber, thereby replacing precious metal catalysts in MFCs [11]. This approach has led to the simultaneous removal of organics and nitrogen using a novel nitrifying biocathode MFC [12,13]. Electron transfer, however, could be inhibited if oxidized nitrogen species (nitrite and nitrate) are present in a single-chambered MFC system because oxidized species will compete with the MFC anode for electrons [14,15]. It could be argued that the ammonia in MFCs is not the main substrate for electricity generation. Nonetheless, the availability of ammonia in an anode chamber could be involved in electricity generation by directly acting as a building block for anode-attached microorganisms, or by indirectly serving as a substrate for nitrifiers to produce organic compounds for heterotrophs [16].

While ammonium (NH_4^+) is the dominant species when dissolved in aqueous phase, the formation of NH_3 depends on TAN concentration, pH, and temperature. It has been suggested that this NH_3 concentration is the active component that causes ammo-

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Table 1
Ammonia conversion, removal efficiency and substrate removal rates in batch microbial fuel cells (MFCs).

Total ammonia nitrogen (TAN) (mg N L^{-1})	Free ammonia nitrogen (FAN) (mg N L^{-1})	Conductivity (mS cm^{-1})	k ($\times 10^{-4}$ h^{-1})	Total nitrogen removal (%)
84	0.9	8.1	1.75	63.4
500	5.6	11.2	2.33	49.6
1000	11.1	15.3	1.25	52.9
1500	16.7	19.1	0.92	43.0
2000	22.2	21.6	0.96	28.3
2500	27.8	25.5	1.00	21.6
3000	33.3	28.4	0.79	11.7
3500	38.9	32.2	0.79	7.6
4000	44.5	37.1	0.58	4.4

nia inhibition in biological processes [17,18]. The following two mechanisms of ammonia toxicity have been postulated: (1) un-ionized NH_3 can directly inhibit the activity of cytosolic enzymes; (2) hydrophobic NH_3 molecules that diffuse passively into the cell are rapidly converted to NH_4^+ due to intracellular pH conditions. The NH_4^+ ions that accumulate inside a cell may cause inhibition by altering intracellular pH conditions [19,20]. Furthermore, high ammonia salt levels can cause bacterial dehydration because of altered osmotic pressure [21]. The inhibitory effects of NH_3 have been well studied in terms of methanogens [17,18,22,23]. Reported inhibitory concentrations of TAN ranged from 1.5 to 2.5 g N L^{-1} for un-adapted microbial cultures [18]. On the other hand, an adaptive tolerance to TAN levels exceeding 4 g N L^{-1} has been demonstrated [17,24]. There is no relevant literature that evaluates ammonia inhibition of electricity generation in MFCs.

In this study, batch experiments using single-chambered MFCs are performed with various concentrations of ammonium chloride (NH_4Cl) to investigate the inhibitory effects of TAN on the generation of electricity. As TAN concentrations are increased, the conductivity (affects the ohmic resistance) and current generation increase in MFCs. To provide a quantitative comparison, the conductivity of the control reactor is adjusted to eliminate the effects of NH_4Cl concentration on the conductivity. The main goals of this research are to verify the effect of ammonia inhibition on MFC performance and to discuss whether wastewater containing strong nitrogen levels is applicable to MFCs.

2. Materials and methods

2.1. Experimental set-up

Single-chambered MFCs were designed using square bottles (2016, ALGENE) with a working volume of 250 mL. Air cathodes (projected surface area = 27 cm^2), which consisted of wet-proofed carbon cloth (Type B, E-TEK) and 0.5 mg platinum (Pt) cm^{-2}, were prepared as described elsewhere [8]. The anodes, which consisted of 40 g of granular activated carbon (GAC) (SGW-200, SHIN KI Chemical, Republic of Korea) and a non-wet-proofed carbon cloth (12.5 cm^2), were inserted into the pile of the GAC to collect electrons efficiently. Each cathode and anode was connected with a titanium wire and equipped with pluggable bulkhead connectors. These cathodes and anodes were wired to an external resistance (1000 Ω) to create a closed electrical circuit.

2.2. Batch experiments

Batch experiments were conducted using various concentrations of NH_4Cl to verify the inhibitory effects of ammonia on bioelectricity generation in MFCs. The design of the batch experiment is described in Table 1. The growth medium contained 32.4 mM of acetate (2 g chemical oxygen demand (COD) L^{-1}), 50 mM of phosphate buffer, 5.8 mM of NH_4Cl, 1.7 mM of KCl, and trace metal salts and vitamins [23]. The TAN concentration in the fresh medium ranged from 84 to 4000 mg N L^{-1}. The MFCs were inoculated using activated sludge from a wastewater treatment plant that had a volatile suspended solids (VSS) concentration of 2.3 g L^{-1}. The initial pH of the MFC was buffered to seven using NaOH and HCl solutions. Before beginning the experiment, all reactors were sparged with nitrogen gas (N_2) to remove oxygen. All experiments were performed at 35 °C. A second batch of experiments was conducted with 84 mg N L^{-1} of TAN to verify that the increase in conductivity was caused by NH_4Cl and to confirm its effect on the performance of the MFC (NH4Cl-MFC). The conductivity of the medium in the second batch was adjusted using potassium chloride (KCl) (KCl-MFC) (Table 1). NH_4Cl-MFCs from the first batch experiment were used for a comparison study.

A separate test was conducted with the same MFCs described in Section 2.1 and with abiotic open-circuit MFCs to verify ammonia adsorption to GAC. Inoculums were not added to the abiotic open-circuit MFCs and circuits were not closed to prevent the removal of ammonia from volatilization on the cathode surface [10]. The effects of different TAN concentrations were tested at 500, 1500 and 3000 mg N L^{-1}.

2.3. Analysis

The current (I, A) was calculated by $I = E/R$, where R is the resistance (Ω) and E is the voltage (V). The power output of cells (P, W) was calculated as $P = IV$. The variable external resistance (10–10^6 Ω) was used to obtain polarization curves. The power density (mW m^{-3}) and current density (mA m^{-3}) were based on the reactor working volume (m^3). Following filtration with a 0.45 μm membrane filter, acetate was analyzed by high-performance liquid chromatography (HPLC, Spectra SYSTEM P2000) equipped with an ultraviolet (210 nm) detector and a 300 m × 7.8 mm Aminex HPX-97H column. Sulfuric acid (0.005 M) was used as the mobile phase at a flow rate of 0.6 mL min^{-1}.

Non-linear regressions of acetate removal (S_{HAc}) were conducted as a function of time (t) and by assuming exponential decay as follows:

$$\frac{dS_{HAc}}{dt} = -k \cdot S_{HAc},\ S_{HAc}(t) = S_{HAc,0}e^{-k \cdot t} \quad (1)$$

where k is the removal rate constant (h^{-1}), t is time (h), $S_{HAc,0}$ is the initial acetate concentration (mg COD L^{-1}), and S_{HAc} is the acetate concentration (mg COD L^{-1}) at time t.

The pH and conductivity were monitored by sampling with a pH meter (Orion model 720A, Thermo Scientific) and a conductivity meter (HI8633, Hanna). The dissolved oxygen (DO) was measured using a digital DO meter that included a 083010MD probe (Orion 3-star plus, Thermo Scientific). Concentrations of TAN ($NH_3 + NH_4^+$), NO_3^--N, and NO_2^--N were determined with a UV visible spectrophotometer (DR/2010, HACH Company) at wavelengths of 415, 540, and 410 nm, respectively. The concentration of NH_3 was cal-

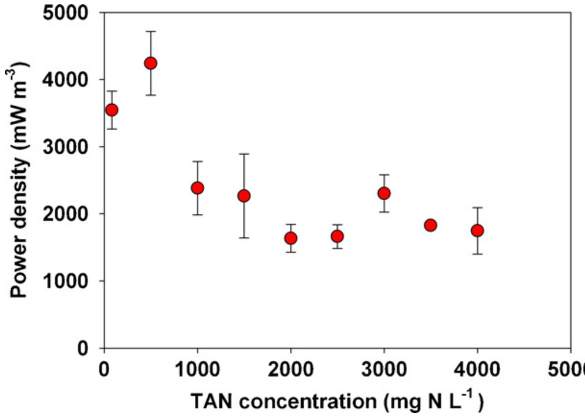

Fig. 1. Maximum power density in each batch experiment at initial TAN concentration.

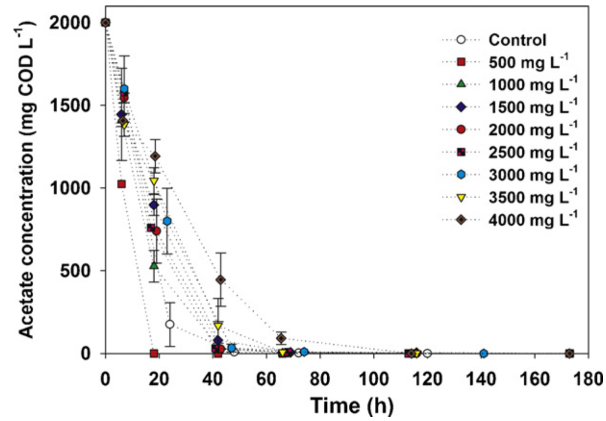

Fig. 2. Acetate removal in batch experiments at each initial TAN concentration.

culated from the following equation suggested by Hansen et al. [18]:

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

where $[NH_3]$ is the NH_3 concentration (mg N L^{-1}), [TAN] is the total ammonia nitrogen concentration (mg N L^{-1}), and T is the temperature (K).

The half-maximum effective concentration (EC_{50}) of TAN (i.e., the TAN concentration that causes a 50% inhibition of power generation in a single-chambered MFC) was determined to evaluate the quantitative toxicity of TAN levels, as well as the corresponding NH_3 nitrogen (FAN) levels and conductivity. In the case of KCl-MFC, only the EC_{50} of conductivity was estimated since no excess TAN was added.

3. Results and discussion

3.1. Inhibition of electricity generation

To study the inhibitory effect of ammonia, batch experiments were performed by adjusting the amount of NH_4Cl used. Initial TAN concentrations in MFCs ranged from 84 to 4000 mg N L^{-1}, and the corresponding NH_3 concentrations (estimated using Eq. (2)) ranged from 0.9 to 44.5 mg N L^{-1} (Table 1). After inoculating all MFCs, the lag periods lasted for about 48 h and the voltage increased drastically to 540–570 mV.

The highest power density is 4240 mW m^{-3} at 500 mg N L^{-1}. The power density decreases by approximately 59% when the TAN is increased eight-fold to 4000 mg N L^{-1} (Fig. 1). This relationship suggests an optimum TAN concentration of around 500 mg N L^{-1}. In general, methanogens are known to be more tolerant of elevated ammonia concentrations than electricity-producing bacteria. The optimum ammonia concentration reported for fermentative hydrogen production is 0.1 g N L^{-1} [25], which is comparable with the results obtained in this research.

Increasing TAN levels negatively affects acetate consumption in MFCs (Fig. 2). Rapid acetate consumption within 1–3 days corresponds to electricity generation trends in each MFC, which is indicative of anode-attached growth of electrochemically active microorganisms. There are significant differences in the slope of the decrease in acetate that correspond to different TAN concentrations. At the end of the batch experiment, the acetate in all MFCs is completely removed. The estimated decay constants by regression analysis (Table 1) show the negative impacts of TAN on the acetate consumption of anode-attached bacteria. The highest acetate consumption rate is attained with TAN concentrations of 500 mg N L^{-1} and a corresponding rate constant (k) of 2.33×10^{-4} h^{-1} (Table 1). Mitigation of the slope by applying increasing TAN concentration clearly reveals that high TAN concentrations inhibit the utilization of acetate by anode-attached bacteria.

It has been reported [26] that FAN could cause not only biological inhibition at the anode but also physicochemical inhibition of catalytic activity of the Pt-coated cathode [26]. Hinz et al. [27] reported that the catalytic activity of Pt could decrease in the presence of 3 ppm NH_3. Although the testing range of FAN was 0.9–44.5 mg L^{-1}, the cathode of the single-chambered MFC was difficult to be inhibited by NH_3 due to the hydrophobic nature of the Nafion polymer that surrounded the Pt on the surface of the cathode [28]. This observation indicated that the effect of ammonia inhibition on the activity of the anode is more significant than that on the cathode. Overall, this research demonstrates that TAN concentrations greater than 1000 mg N L^{-1} (11 mg N L^{-1} as FAN) are critical for optimizing the performance of anode-attached microorganisms.

3.2. Nitrogen removal

The efficiency of total nitrogen removal in all MFCs is summarized in Table 1. Results were determined based on differences between the initial and final samples of MFCs during batch experiments. As the initial TAN concentration is increased, the pattern of nitrogen removal efficiency shows a gradual decrease. The highest removal efficiency is 63.4% at 84 mg N L^{-1}, but is significantly decreased to 4.4% at 4000 mg N L^{-1}. Quantitative comparisons of the total nitrogen that is removed and the proportion of inorganic nitrogen species are described in Fig. 3. The total nitrogen removed

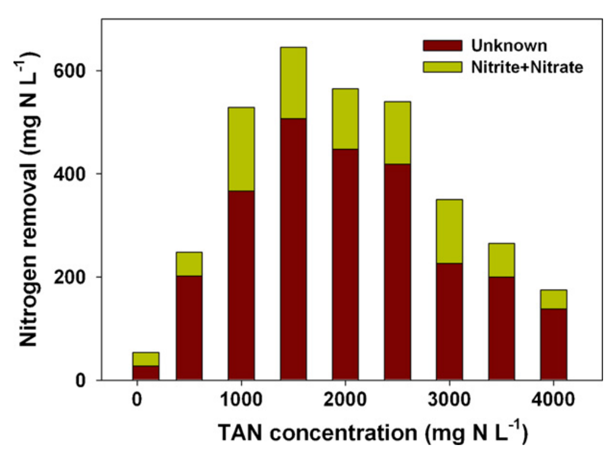

Fig. 3. Total nitrogen removal at the end of batch experiments, and composition analyses of nitrogen species.

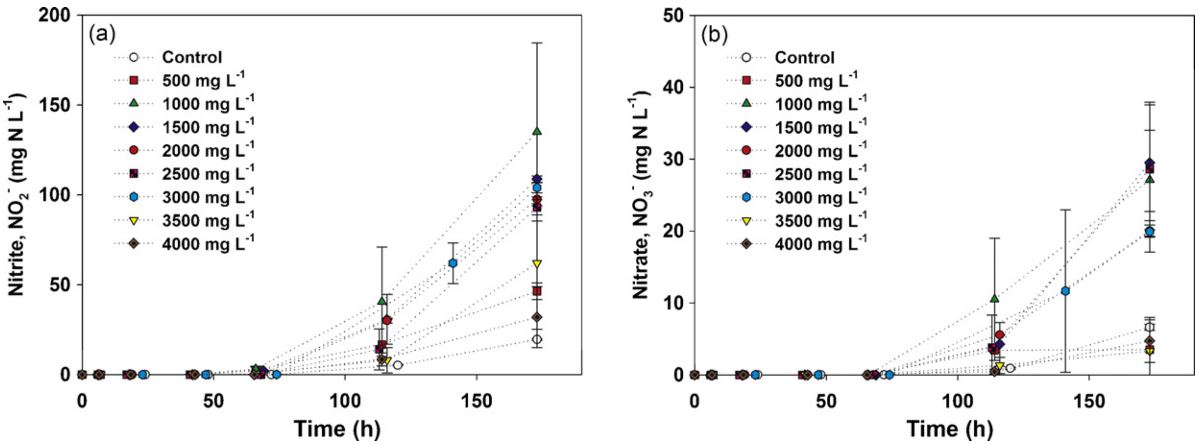

Fig. 4. Nitrification in MFCs at different TAN concentrations: (a) nitrite production; and (b) nitrate production.

was significantly increased up to 645 mg N L^{-1} with an increase in the initial TAN (∼1500 mg N L^{-1}). It gradually decreases, however, with further increases in TAN (2000–4000 mg N L^{-1}).

The removal of nitrogen in MFCs has been reported by processes such as the volatilization of ammonia by an increase in local pH values on the cathode surface and biological nitrification/denitrification [9,10]. Among these processes, this study suggests that one probable mechanism of nitrogen removal is ammonia oxidation by air diffusion through cathodes [5,9]. Oxygen that is diffused over time from the cathode reacts with TAN, which leads to nitrite and nitrate production (Fig. 4). Oxygen diffusion through the air cathode allows approximately 2 ppm of DO into solutions in MFCs. This oxygen diffusion results in an accumulation of nitrite and nitrate as a consequence of biological ammonia oxidation by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). In addition, this research confirms that low DO limits nitrite oxidation more significantly than ammonia oxidation and thereby results in nitrite accumulation (∼135 mg N L^{-1}) in the reactors [29]. This supports that AOB and NOB become active following the inactivation of heterotrophs since acetate depletion is followed by nitrite and nitrate accumulation [30]. This line of evidence suggests that partial nitrification takes place readily in most single-chambered MFCs.

It is also found that variations in the initial TAN concentration have an impact on the accumulation of nitrite in MFCs. The conversion rate of NH_4^+ to nitrite increases as the TAN is increased to 1000 mg N L^{-1}, resulting in nitrite accumulations of 135 mg N L^{-1} (Fig 4a). A further increase in the TAN concentration from 1500 to 4000 mg N L^{-1}, however, causes a decrease in nitrite concentrations from 109 to 5 mg N L^{-1}, indicating that AOB are inhibited. This outcome suggests that the overall inhibitory impact of TAN (caused by FAN) is stronger than that of nitrite in MFCs [31]. Elevated nitrite concentrations may, however, inhibit anode-attached bacteria because nitrite has been indicated as an inhibitor of the bacteria metabolism [32–34]. The literature also indicates that nitrite inhibition can be significant for anaerobes and facultative anaerobes [33,34]. In this research, the highest nitrite concentration at 1000 mg N L^{-1} of initial TAN corresponds to a drastic decrease in power density and a reduced acetate removal rate. Therefore, simultaneous ammonia inhibition and nitrite production (11 mg N L^{-1} of the FAN and 125 mg N L^{-1} of nitrite) are the likely reasons for the reduced performance of the MFC. Elevated TAN concentrations appear to be the main cause of decreased bacterial activity because TAN governs electricity generation and the efficiency of substrate removal. Conversely, nitrite inhibition decreases significantly at more than 1500 mg N L^{-1} of TAN concentrations. Due to low DO concentrations, the nitrate concentration in MFCs remains low. A significant inhibition of nitrate formation occurs when the TAN concentration is greater than 3000 mg N L^{-1} (Fig. 4b). This finding confirms that NOB are more tolerant than AOB. It has also been reported in other studies [35] that the inhibitory TAN concentration is higher for *Nitrosomonas* species than for *Nitrobacter* species [35].

3.3. Effect of conductivity

The conductivity of the medium solution significantly affects ohmic resistance and current generation in MFCs [36,37]. Thus, a second batch of experiments using KCl was conducted to estimate the net inhibitory effects and to rule out the influence of conductivity. The results show that excess addition of NH_4Cl increases conductivity from 8 to 37 mS cm^{-1} (Table 1). Power densities under the same level of conductivity for NH_4Cl and KCl indicated that NH_4Cl-MFC yields a lower power density than KCl-MFC at the same level of conductivity (Fig. 5). For low conductivity MFCs, an increase in conductivity from 8.1 to 11.2 mS cm^{-1} leads to an increase in power density of 13.8% for a KCl-MFC and 19.6% for a NH_4Cl-MFC. As the conductivity is increased from 11.2 to 37.1 mS cm^{-1} by the addition of NH_4Cl, the maximum power density of the MFC (4240 mW m^{-3} at 11.2 mS cm^{-1}) is drastically decreased by 58.8% (1746 mW m^{-3} at 37.1 mS cm^{-1}) as a consequence of ammonia inhibition. Conversely, the addition of KCl always yields power densities that are 24.2–91.6% higher than those of the NH_4Cl-MFC.

Non-linear regressions for TAN, FAN and conductivity measurements can determine EC_{50} values of TAN (Table 2). These

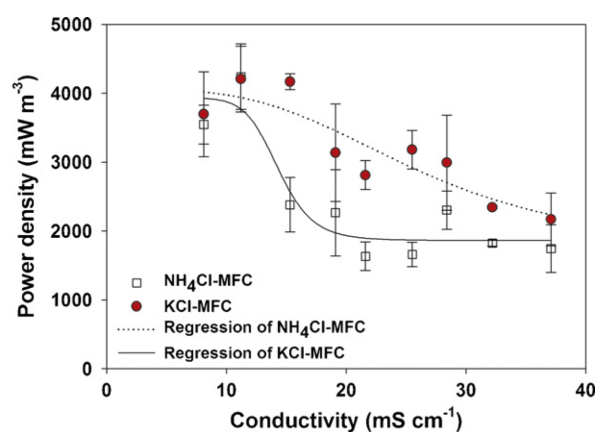

Fig. 5. Power densities from NH_4Cl-MFCs and KCl-MFCs as a function of conductivity.

Table 2
Half-maximum effective concentration (EC_{50}) of TAN, FAN and conductivity in batch experiments.

Division	Item	Unit	Value
KCl-MFC[a]	EC_{50} of conductivity	$mS\,cm^{-1}$	26
NH_4Cl-MFC	EC_{50} of TAN	$mg\,N\,L^{-1}$	894
	EC_{50} of FAN	$mg\,N\,L^{-1}$	10
	EC_{50} of conductivity	$mS\,cm^{-1}$	14

[a] For KCl-MFC, only EC_{50} of conductivity is estimated because no excess TAN is added.

parameters are important for the quantitative evaluation of TAN toxicity and to consider conductivity effects (Fig. 5). The EC_{50} of the NH_4Cl-MFC for conductivity is 54% (EC_{50} = 14 mS cm^{-1}), which is less than that of the KCl-MFC (EC_{50} = 26 mS cm^{-1}). The corresponding EC_{50} values for the TAN and the FAN are 894 and 10 mg N L^{-1}, respectively. The steeper slope of the NH_4Cl-MFC also indicates that TAN levels have a more significant impact on the electrochemical function of anode-attached bacteria. While excessive KCl displays an inhibitory effect, it is expected that KCl and other dissolved salts would be toxic to microbial biota by causing osmotic stress [38]. It is evident that increased TAN levels (>500 mg N L^{-1}) enable a dominant and rapid decrease in power during the generation of electrochemical electricity in MFCs, even when conductivity effects are ruled out. The small difference between optimum and EC_{50} values for TAN concentration (394 mg N L^{-1}) provides evidence that TAN has a stronger inhibitory effect on electrochemically active microorganisms than other anaerobes.

3.4. Nitrogen absorption in anode

To verify the unknown portion of TAN removal, it is hypothesized that the adsorption of TAN by GAC significantly contributes to TAN removal because GAC has been reported [39] as a suitable adsorbent for the removal of ammonia. A third batch of experiments was conducted in which TAN concentrations were varied while keeping other parameters the same. As a control MFC, an abiotic open-circuit MFC, which contained no inoculums, was operated to estimate the net decrease in TAN levels due to GAC adsorption.

Similar amounts of TAN disappear within 1–3 days in both normal MFCs and abiotic open-circuit MFCs regardless of the TAN concentration, although the NH_4Cl-MFC shows slightly lower values due to the cellular metabolism (Fig. 6). These results confirm that GAC, which is used as an auxiliary anode in this study, can adsorb 14–51% of TAN levels before AOB and NOB become active. Consequently, denitrification and ammonia volatilization [10], as well as GAC adsorption, alleviates TAN removals and can explain the unknown proportion of TAN removed (Fig. 3).

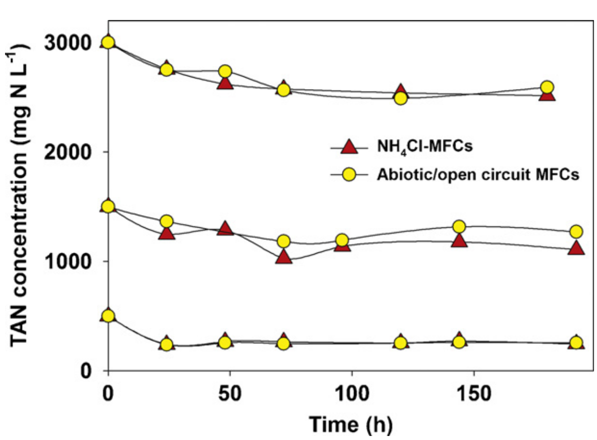

Fig. 6. TAN removal in NH_4Cl-MFCs and abiotic open-circuit MFCs at 500, 1500, and 3000 mg N L^{-1}.

4. Conclusions

A high TAN concentration of >500 mg N L^{-1} for a batch MFC can result in severe inhibition of electricity generation, and thereby prevents efficient substrate removal. The maximum power density (4240 mW m^{-3}) at 500 mg N L^{-1} drastically decreases to 1700 mW m^{-3} as TAN concentrations increase to 4000 mg N L^{-1}. EC_{50} analysis quantifies the net effect of TAN concentration on MFC performance. It is found that the EC_{50} value of the TAN (894 mg N L^{-1}), which corresponds to 10 mg N L^{-1} as FAN, is relatively close to the optimum TAN concentration. Therefore, control of TAN concentration seems essential for MFCs that use wastewater with elevated nitrogen as an electron donor. Nitrification activity can be detected by measuring the accumulation of nitrite and nitrate concentrations by continuously diffused oxygen from the air cathode. GAC significantly contributes to the removal of TAN along with previous removal mechanisms such as ammonia volatilization and denitrification.

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