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Short communication

Ammonia inhibition and microbial adaptation in continuous single-chamber microbial fuel cells

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ABSTRACT

Here, we report that a continuous single-chamber microbial fuel cell (MFC) is applicable to wastewaters containing a high nitrogen concentration using a process of adaptation. Continuous experiments are conducted to investigate the inhibitory effect of total ammonia nitrogen (TAN) on the MFC using influents with various concentrations of TAN ranged from 84 to 10,000 mg N L^{-1}. As the TAN concentration increases up to 3500 mg N L^{-1}, the maximum power density remains at 6.1 W m^{-3}. However, as the concentration further increases, TAN significantly inhibits the maximum power density, which is reduced at saturation to 1.4 W m^{-3} at 10,000 mg N L^{-1}. We confirm that the adapted electrical performance of a continuous MFC can generate approximately 44% higher power density than the conductivity control. A comparative study reveals that the power densities obtained from a continuous MFC can sustain 7-fold higher TAN concentration than that of previous batch MFCs. TAN removal efficiencies are limited to less than 10%, whereas acetate removal efficiencies remain as high as 93–99%. The increased threshold TAN of the continuous MFC suggests that microbial acclimation in a continuous MFC can allow the electrochemical functioning of the anode-attached bacteria to resist ammonia inhibition.

1. Introduction

A single-chamber microbial fuel cell (MFC) has received substantial attention in recent years because it not only provides electrical energy while removing organic matter from wastewater [1–3], but also it presents several advantages over conventional two-chamber MFCs due to the economic benefits of a simplified design, a reduced total volume, and improved power output [4,5]. The featured step in single-chamber MFCs is extracellular electron transfer to the anode by electrochemically active microorganisms without a proton exchange membrane, and this electron flow then contributes to reduction reactions in the air cathode, allowing for passive diffusion of oxygen [4]. Hence, a long-term enrichment and cultivation of microorganisms involved in electron transfer is a crucial factor for the success of single-chamber MFC technologies [6].

Recent studies have evaluated various substrates for anode-attached bacteria to determine whether a microbial limitation exists. It has been shown that most electroconvertible organics are utilized during electricity generation, and the corresponding metabolic activity of the anode-attached bacteria influences MFC electrical efficiency and process stability [7–11]. Prior researchers have demonstrated that a variety of environmental biotic and abiotic factors, such as nutrients, temperature, toxic substances, operating conditions, reactor configuration, and inoculum, influence MFCs [11–13]. Among nutrient factors, total ammonia-nitrogen (TAN), which includes ammonia (NH_3) and the ammonium ion (NH_4^+), is a common and problematic substance, especially in a wastewater stream. Moreover, NH_3, for which the concentration mainly depends on TAN, pH, and temperature, has been suggested to be the active component that causes inhibition in anaerobic biological processes [14–18].

The disruption of enzymatic activity, alterations in the intracellular pH and dehydration due to osmotic stress has been characterized as mechanisms of ammonia inhibition [19–21]. It has also been reported that this inhibition occurs at TAN concentrations of 1.5–2.5 g N L^{-1} for unadapted anaerobic microbial cultures [15]. However, tolerance to more than 4 g N L^{-1} as TAN has been demonstrated by adaptation to ammonia [14,18].

Various MFCs have demonstrated negative responses based on the amount of nitrogen loading because significant TAN concentrations in wastewater may contribute to a reduction in electrical performance and organic contaminant treatment by MFCs [8,16,22,23]. However, these adverse effects of TAN on the

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Table 1
Nitrogen loading rates (NLR) and medium conductivities at each TAN concentration.

TAN (mg N L^{-1})	Free ammonia (mg N L^{-1})	Nitrogen loading (g N L^{-1} d^{-1})	Conductivity (mS cm^{-1})
84	0.9	0.2	8.1
500	5.6	1.2	10.4
750	8.3	1.8	11.5
1000	11.1	2.4	14.4
1500	16.7	3.6	18.5
2000	22.2	4.8	21.9
2500	27.8	6.0	27.2
3500	38.9	8.4	34.6
4500	50.0	10.8	41.1
6000	66.7	14.4	49.3
10,000	111.0	24.0	76.2

electricity generation have mainly been evaluated in batch mode MFCs. Little data has been reported on the long-term inhibition of TAN in continuous single-chamber MFCs, which can lead to poor effluent quality in a continuous wastewater treatment system. Furthermore, the success of continuous MFC systems is attributed to an acclimation of the electrochemically active microorganisms growing on the anode surface because the microorganisms must be viable at all stages of development and must function as the dominant community. Thus, the threshold levels of TAN and the adaptation capabilities of a continuous MFC must be confirmed before considering the practical application of a continuous single-chamber MFC system, but no relevant reports were found in the literature.

Here, we investigate the inhibitory effects of ammonia nitrogen on electricity generation in a single-chamber MFC using a continuous operation mode. Control experiments on conductivity, which affects ohmic resistance and current generation in MFCs, were performed to evaluate the net inhibition by TAN. We also evaluated nitrogen and organic removal efficiencies to allow for a quantitative comparison of the test and control cells.

2. Materials and methods

2.1. Operating procedures

Continuous experiments with single-chamber MFCs were conducted using various TAN concentrations, expressed as NH_4Cl, to determine how ammonia affects electricity generation in MFCs. Inocula were return activated sludge, with a volatile suspended solids (VSS) concentration of 2.3 g L^{-1}, that was obtained from a wastewater treatment plant (Daejeon, Korea). The growth medium contained 50 mM phosphate buffer, 5.8 mM NH_4Cl, 1.7 mM KCl, and a trace metal salt and vitamin solution [24]. The initial pH of the medium was adjusted to 7. For an initial acclimation, 32.4 mM of acetate (2 g COD L^{-1}) was injected as an electron donor. After observing stable current, the MFCs were continuously fed with 16.2 mM (1 g COD L^{-1}) of acetate in the same medium. The hydraulic retention time (HRT) was set to 10 h, which corresponds to an organic loading rate (OLR) of 2.4 g L^{-1} d^{-1}. The TAN concentration in continuous operation was varied from 84 to 10,000 mg N L^{-1} and increased in a stepwise manner. The reactors were operated for 4–5 days at each TAN concentration, and polarization curves were obtained at each steady-state condition; all of the continuous tests were performed in duplicate at 35 °C.

Table 1 shows the nitrogen loading rates (NLR) and medium conductivities at each TAN concentration. The feed and operating conditions were held constant for more than 40 days until the data acquisition of steady-state polarization curves were obtained in triplicate. Another set of continuous MFCs was operated with 84 mg N L^{-1} of TAN to offset the performance improvement due to the increased conductivity imparted by NH_4Cl addition and to confirm the sole effect of TAN on the performance of a continuous single-chamber MFC. The conductivity of the medium was adjusted using potassium chloride (KCl) for these control MFCs (KCl-MFCs), whereas the original continuous MFCs (NH_4Cl-MFCs) were used for a comparison study.

2.2. Experimental setup

The design of the single-chamber MFCs was reported elsewhere [11]; the reactors were based on modified square media bottles (Nalgene® 2016) with a working volume of 250 mL. The air cathode (projected surface area = 27 cm^2), which consists of wet-proofed carbon cloth (Type B, E-TEK) and 0.5 mg Pt cm^{-2}, was prepared as described previously [16]. The anode compartment consists of 40 g of granular activated carbon (GAC) (SGW-200, SHIN KI Chemical, Republic of Korea) as an auxiliary anode material and a non-wet-proofed carbon cloth (12.5 cm^2). The carbon cloth was inserted into the randomly packed GAC to efficiently collect the generated electrons. Each cathode and anode were connected with a titanium wire and equipped with pluggable connectors, and the anodes were wired to an external resistance (1 kΩ) to create a closed electrical circuit.

2.3. Analyses

The current (I, A) was computed from $I = VR^{-1}$ where R is the resistance (Ω) and V is the voltage potential (V) across the resistor. The power output of the cells (P, W) was calculated as $P = IV$. To obtain polarization curves at a steady state, we changed the external resistance in a stepwise manner (from 10 to 10^6 Ω). After triplicate determination of the power output, the power density (mW m^{-3}) and current density (mA m^{-3}) were estimated based on the reactor working volume (m^3). Acetate (HAc) levels were analysed using a high-performance liquid chromatography (HPLC) with a 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 a mobile phase at a flow rate of 0.6 mL min^{-1}. All of the samples were filtered with a 0.45 μm membrane filter and stored at 4 °C before use.

The pH and conductivity of samples were monitored with a pH meter (Orion model 720A, Thermo scientific) and a conductivity meter (HI8633, Hanna), respectively. We measured dissolved oxygen (DO) using a digital DO meter equipped with an 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 spectrophotometer (DR2010, HACH) at the wavelengths of 415, 540, and 410 nm, respectively. The NH_3 concentration was esti-

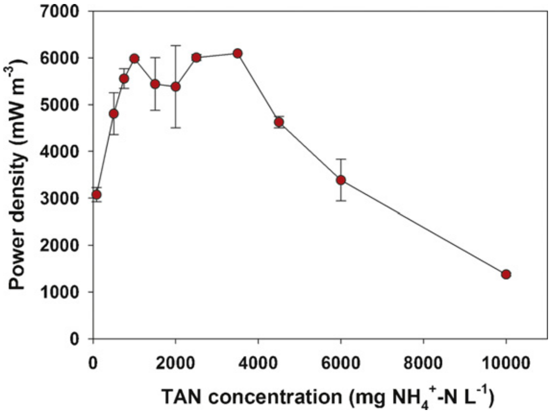

Fig. 1. Maximum power densities of continuous single-chambered MFCs at each TAN concentration.

mated from the following equation, according to Hansen et al. [15]:

$$[\mathrm{NH_3}] = \frac{[\mathrm{TAN}]}{1 + 10^{-\mathrm{pH}+0.09+2730/T}} \qquad (1)$$

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

We also evaluated the half-maximum effective concentration (EC_{50}) based on TAN and conductivity [16] to determine the quantitative toxicities of TAN and total conductivity; the EC_{50} was defined as the concentration that causes a 50% inhibition of power generation in a continuous single-chamber MFC.

3. Results and discussion

3.1. Inhibition of electricity generation in continuous MFCs

Single-chamber MFCs were operated continuously at a constant HRT (10 h) and organic loading rate ($2.4\,\mathrm{g\,COD\,L^{-1}\,d^{-1}}$) to investigate the inhibitory effect of high concentrations of ammonia nitrogen. The total amounts of NH_4Cl in the influent solution were adjusted as shown in Table 1. The corresponding nitrogen loading rate ranged from 0.2 to $24.0\,\mathrm{g\,N\,L^{-1}\,d^{-1}}$.

Fig. 1 shows the maximum power densities of the continuous single-chamber MFCs at each TAN concentration. Increasing the influent TAN from 84 to $1000\,\mathrm{mg\,N\,L^{-1}}$ results in an increase in steady-state power density from 3.1 to $6.0\,\mathrm{W\,m^{-3}}$. A further increase to $3500\,\mathrm{mg\,N\,L^{-1}}$ leads a saturation of power density. The highest power density recorded is $6.1\,\mathrm{W\,m^{-3}}$ at $3500\,\mathrm{mg\,N\,L^{-1}}$, and the corresponding FAN concentration is $38.9\,\mathrm{mg\,N\,L^{-1}}$ (Table 2). Upon applying higher TAN concentrations ranging from 3500 to $10{,}000\,\mathrm{mg\,N\,L^{-1}}$, the power density rapidly decreases to $1.4\,\mathrm{W\,m^{-3}}$.

Previously, it has been reported that single-chamber batch MFCs can generate $1.7–4.1\,\mathrm{W\,m^{-3}}$ at $84–4500\,\mathrm{mg\,N\,L^{-1}}$ of initial TAN, with the maximum power density ($4.1\,\mathrm{W\,m^{-3}}$) obtained at $500\,\mathrm{mg\,N\,L^{-1}}$ [16]. It appears that continuous single-chamber MFCs can generate 1.1–3.3-fold higher power densities than those of batch MFCs within the same TAN concentrations tested ($500–4500\,\mathrm{mg\,N\,L^{-1}}$).

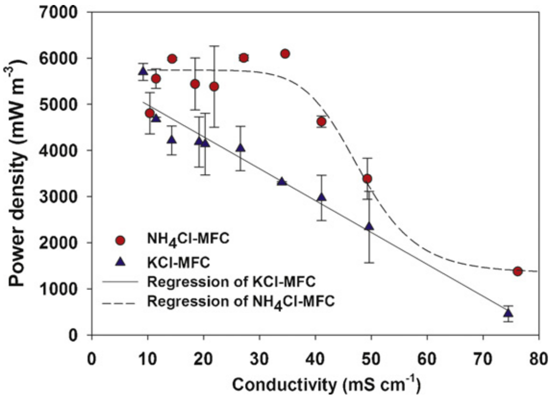

Fig. 2. Power densities from NH_4Cl-MFCs and KCl-MFCs as a function of conductivity ($>10\,\mathrm{mS\,cm^{-1}}$).

We hypothesized that the anode-attached bacteria grown in continuous MFCs are more capable of tolerating high TAN concentration because continuous exposure may naturally adapt the bacteria to a high-nitrogen environment. The results of this study clearly support the hypothesis, suggesting that the adaptation of anode-attached bacteria can allow their electricity generating mechanism to remain operational in any continuous influent that contains variable TAN concentrations up to $3500\,\mathrm{mg\,N\,L^{-1}}$.

3.2. Effect of conductivity on continuous MFCs

Previous studies have reported that medium conductivity significantly affects the performance of MFCs [16,25,26]. In this study, medium conductivity ranged from 8 to $76.2\,\mathrm{mS\,cm^{-1}}$. Therefore, we sought to exclude the influence of conductivity by conducting control experiments to estimate the net TAN inhibition. As a conductivity control, KCl-MFCs were operated in the same continuous mode as the NH_4Cl-MFCs.

Fig. 2 shows the power densities obtained from NH_4Cl-MFCs and KCl-MFCs at each operating condition. KCl-MFCs yields lower power densities than NH_4Cl-MFCs at the same level of conductivity. At the two lowest conductivities, 8.1 and $10.4\,\mathrm{mS\,cm^{-1}}$, the KCl-MFCs shows 20% and 18% (3.7 and $5.7\,\mathrm{W\,m^{-3}}$) higher power compared to those of the NH_4Cl-MFCs (3.1 and $4.8\,\mathrm{W\,m^{-3}}$). However, for higher conductivities ($11.5–76.2\,\mathrm{mS\,cm^{-1}}$), KCl-MFCs generates 16–67% less power than the NH_4Cl-MFCs.

The power density of the KCl-MFCs decreased gradually at a ratio of $69\,\mathrm{mW\,m^{-3}}$ per unit $\mathrm{mS\,cm^{-1}}$ as the conductivity was increased, indicating a stronger inhibitory effect of osmotic stress by K^+ than that by TAN. However, continuous operation with TAN led to a power density plateau. The highest power density of the NH_4Cl-MFCs ($6.1\,\mathrm{W\,m^{-3}}$) was obtained at $34.6\,\mathrm{mS\,cm^{-1}}$. This more effective electrical performance seems to be attributed to the availability of protons due to the presence of NH_4^+, which can contribute to current generation by speciation between NH_4^+ and NH_3. This result indicates that the electrochemically active bacteria became tolerant of the inhibitory stress due to high TAN concentrations. In addition, the probability that the bacteria are less adaptable to the high osmotic stress induced by K^+ for long-term electricity

Table 2
Half-maximum effective concentration (EC_{50}) of TAN, FAN, and conductivity in continuous single-chambered MFCs.

Division	Item	Unit	Value	Reference
Batch NH_4Cl-MFC	EC_{50} of TAN	$\mathrm{mg\,N\,L^{-1}}$	894	[16]
Continuous NH_4Cl-MFC	EC_{50} of conductivity	$\mathrm{mS\,cm^{-1}}$	48	This study
	EC_{50} of TAN	$\mathrm{mg\,N\,L^{-1}}$	5826	
	EC_{50} of FAN	$\mathrm{mg\,N\,L^{-1}}$	65	

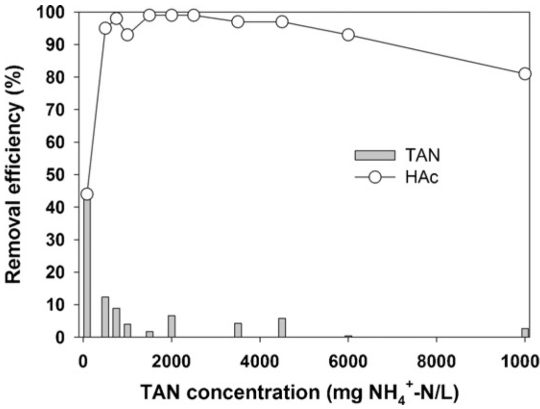

Fig. 3. TAN and HAc removal efficiencies in continuous MFCs at each TAN concentration.

generation in continuous MFCs is comparable to previous studies that reported that methanogenic anaerobes could be acclimated to 6 g K^+ L^{-1} without a significant loss of microbial activity [27,28]. This different tolerance explains the decrease of power density because the osmotic stress can selectively make methanogens, competing with exoelectrogens for acetate, active.

As summarized in Table 2, we conducted a nonlinear regression for TAN and conductivity to evaluate the half-maximum effective concentrations. The continuous NH_4Cl-MFC shows 6.5-fold higher EC_{50} for TAN (5826 mg N L^{-1}) than that of the batch NH_4Cl-MFC (894 mg N L^{-1}) as previously reported [16]. The corresponding EC_{50} of FAN for the continuous NH_4Cl-MFC is also as high as 65 mg N L^{-1}. This difference between the batch and continuous MFCs evidences that microbial acclimation makes the electrochemical function of anode-attached bacteria sustainable, overcoming the inhibitory effects of TAN on enzymatic activity and intracellular pH.

3.3. Nitrogen and acetate removal efficiency

Fig. 3 shows the TAN and acetate removal efficiencies for continuous single-chamber MFCs at each OLR and NLR. When the TAN of the influent is as low as 84 mg N L^{-1} (NLR: 0.4 g N L^{-1} d^{-1}), 44% of the nitrogen can be removed. An increase in the NLR leads to a decrease in the removal efficiency. Based on the data from the batch MFCs [16], most of the nitrogen appears to be removed by GAC adsorption; however, it appears that the adsorption capacity of GAC in continuous MFCs must have been saturated by successive nitrogen loadings, and thus, the adsorption effect was considered negligible.

Nitrate and nitrite were not detected in the effluents of most continuous MFCs, suggesting that heterotrophic bacteria were dominant over ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) because heterotrophs grow faster relative to AOB and NOB in the presence of an organic carbon source [29,30]. In addition, a previous study confirmed that AOB and NOB begin to grow after the depletion of acetate in the medium [16]. We clearly detected acetate remaining in the effluent because its removal efficiency did not exceed 99% in most cases. In this study, possible ammonia loss in the continuous single chamber MFCs may not be biological denitrification after nitrification due to air diffusion, but either adsorption by GAC or local ammonia volatilization due to the high pH near the cathode [23].

Overall, these results verify that continuous single-chamber MFCs can achieve high acetate removal efficiencies (93–99%) even at extreme TAN or FAN concentrations due to the dominance of electrochemically active microorganisms resulting from microbial adaptation.

4. Conclusions

The results of this study demonstrate that electrochemically active microorganisms can acclimate themselves to high ammonia nitrogen concentrations in a continuous single-chamber microbial fuel cell system. In continuous operation mode (an HRT of 10 h and an OLR of 2.4 g COD L^{-1} d^{-1}), bacterial adaptation to high TAN makes continuous single-chamber MFCs produce stable electrical energy, which is as high as 6.1 W m^{-2} at 3.5 g N L^{-1} as TAN, while simultaneously removing 99% of the organic load. A considerable time, more than 40 days, was required to obtain continuous electricity generation above a desirable level. Once acclimated; however, the electrical performance of the continuous MFCs can generate approximately 44% higher maximum power density than the control MFCs at 3.5 g N L^{-1}. The negligible nitrification observed confirms the predominance of electrochemically active microorganisms on the anodes of the continuous MFCs. In most cases, ammonia removal efficiencies are limited to less than 10%, but acetate removal efficiencies remains as high as 93–99%. When considering the practical application of MFCs, these experimental results suggest that high-nitrogen wastewater can be applicable to continuous MFC system after a proper acclimation period, and the threshold guideline for maximum TAN in wastewater should be 3.5 g N L^{-1} or less.

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