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Influence of substrate concentration and feed frequency on ammonia inhibition in microbial fuel cells

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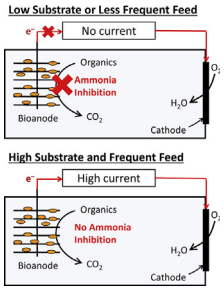
HIGHLIGHTS

- Ammonia inhibition was found to be dependent on substrate feed conditions in MFC.
- High substrates allow stable current generation at high ammonia concentration.
- Frequent substrate feed also makes bioanodes resistive against ammonia inhibition.
- Power density curves can predict ammonia inhibition before bioanodes are damaged.
- Continuously monitored current does not show inhibition until bioanodes are damaged.

GRAPHICAL ABSTRACT



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ABSTRACT

Excessive amounts of ammonia are known to inhibit exoelectrogenic activities in microbial fuel cells (MFCs). However, the threshold ammonia concentration that triggers toxic effects is not consistent among literature papers, indicating that ammonia inhibition can be affected by other operational factors. Here, we examined the effect of substrate concentration and feed frequency on the capacity of exoelectrogenic bacteria to resist against ammonia inhibition. The high substrate condition (2 g L^{-1} sodium acetate, 2-day feed) maintained high electricity generation (between 1.1 and 1.9 W m^{-2}) for total ammonia concentration up to 4000 mg-N L^{-1}. The less frequent feed condition (2 g L^{-1} sodium acetate, 6-day feed) and the low substrate condition (0.67 g L^{-1} sodium acetate, 2-day feed) resulted in substantial decreases in electricity generation at total ammonia concentration of 2500 and 3000 mg-N L^{-1}, respectively. It was determined that the power density curve serves as a better indicator than continuously monitored electric current for predicting ammonia inhibition in MFCs. The chemical oxygen demand (COD) removal gradually decreased at high ammonia concentration even without ammonia inhibition in electricity generation. The experimental results demonstrated that high substrate concentration and frequent feed substantially enhance the capacity of exoelectrogenic bacteria to resist against ammonia inhibition.

1. Introduction

Bioelectrochemical systems can be used to effectively treat wastewater and produce renewable energy. Microbial fuel cells (MFCs) are a type of bioelectrochemical system that can remove organics in wastewater and simultaneously produce electrical

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energy. Recent developments in MFC design (e.g., air cathodes [1]; sandwiched electrode assemblies [2]; inexpensive cathode catalysts [3]) along with pilot-scale demonstrations [4–7], make MFCs an attractive alternative for sustainable wastewater treatment and energy recovery [8]. Electric energy generation in MFCs relies on electrochemically active bacteria that form a biofilm on the anode. These exoelectrogenic bacteria oxidize organic substrates present in wastewater and transfer electrons to the anode, creating electric current. At the cathode of MFCs, water is formed through the reduction of oxygen that can be provided directly from the atmosphere using air cathodes [9]. The performance of MFCs as a wastewater treatment and energy recovery process centers on the activity of exoelectrogenic bacteria to transfer electrons to the anode; hence, the sensitivity of exoelectrogenic bacteria to various wastewater treatment conditions (e.g., ammonia, salinity, oxygen, etc.) needs to be investigated.

Recent studies have examined MFC performance with various sources of wastewater, including swine wastewater, anaerobic digester supernatant and human urine [10–12]. These high strength wastewaters contain excessive amounts of ammonia that can inhibit microbial metabolism and thus prevent exoelectrogenic bacteria from generating electric current in MFCs. In this study, we primarily focused on the effect of TAN (total ammonia nitrogen) on MFC performance under various substrate feed conditions.

Ammonia is well known for its cytotoxic effects on microorganisms [13,14]. Specifically, ammonia inhibition mechanisms include enzymatic activity disruption, alteration in the intracellular pH, and dehydration due to osmotic water loss [15–17]. Previous experimental studies consistently demonstrated negative responses of MFC bioanodes to significantly high TAN levels beyond 4000 mg-N L^{-1} [18–20]. However, the previous studies showed inconsistent results on the threshold TAN level that triggers ammonia inhibition effects in bioelectrochemical systems. For instance, Nam et al. reported that TAN concentrations exceeding 500 mg-N L^{-1} at neutral pH of 7 can result in severe inhibition of electricity generation in MFCs, implying that a relatively low ammonia concentration can trigger limited performance of bioanodes [18]. In another study using a continuously operated MFC, electric current generation gradually increased with increasing TAN concentrations up to 3500 mg-N L^{-1} [19]. Similarly, Kuntke et al. found no cytotoxic effects of ammonia up to 4000 mg-N L^{-1} [20]. It should be noted that experiments in these previous studies were performed under neutral pH conditions using phosphate buffer, indicating that equilibrium between free ammonia (NH_3) and ammonium ions (NH_4^+) did not affect the degree of ammonia inhibition as free ammonia is known to be more toxic to microorganisms than ammonium ions in biological wastewater treatment [14,21]. Thus, here we aim to explain the reported inconsistent TAN concentrations that trigger the ammonia inhibition effects in MFCs.

To further investigate the effects of ammonia inhibition in MFCs, we hypothesized that the substrate concentration and feed frequency can affect the capability of exoelectrogenic bacteria to generate electric current under high ammonia conditions. To our knowledge, none of the previous studies have investigated potential effects of the level and frequency of substrate feed on the ammonia inhibition effects in MFCs. MFC performance is often described with continually monitored current results as well as power density curves developed using various external resistors. Between the two performance indicators, we also investigated which can better predict ammonia inhibition before MFC bioanodes are completely damaged by high ammonia conditions.

2. Material and methods

2.1. MFC configuration and operation

Five single-chamber MFCs were constructed using polypropylene blocks with an inner cylindrical chamber (23 mL; 7 cm^2 in cross section). Graphite fiber brushes (2 cm diameter and 2.5 cm in length; Mill-Rose, OH) were pretreated in a muffle furnace at 450 °C for 30 min [22] and used as the bioanode. The bioanodes were inoculated with primary clarifier effluent and digested sludge collected from a domestic wastewater treatment plant. All MFCs underwent an enrichment period of about 5 months. The air cathodes (7 cm^2 in cross section) were prepared using wet proofed carbon cloth (Fuel Cell Earth, MA) with a Pt/C catalyst (0.5 mg cm^{-2}), as previously described [1]. Constructed MFCs were operated under fed-batch mode with an external resistance (100 Ω).

To study the inhibitory effect of ammonia at different substrate levels and feed frequencies, two MFCs were fed with 2 g L^{-1} sodium acetate every two days (high acetate and short cycle, HASC-1 and -2); one with 0.67 g L^{-1} sodium acetate every two days (low acetate and short cycle, LASC); and two with 2 g L^{-1} sodium acetate every 6 days (high acetate and long cycle, HALC-1 and -2) (Table 1). The feed medium was prepared with sodium acetate (according to Table 1) in 50 mM phosphate buffer solution (4.7 g L^{-1} Na_2HPO_4; 0.6 g L^{-1} $NH_4H_2PO_4$; 1.6 g L^{-1} KH_2PO_4; 0.4 g L^{-1} $NaHCO_3$), and trace amounts of vitamins and minerals [23]. The total amount of ammonia was adjusted by adding NH_4Cl in the feed medium and was gradually increased from 100 to 4000 mg-N L^{-1}. For each ammonia condition, MFCs were operated for 6 days (i.e., 3 short fed-batch cycles or one long fed-batch cycle) except for the very high ammonia concentrations (3500 and 4000 mg-N L^{-1}). At the end of each fed-batch cycle, the solution in the MFCs was completely removed from the reactor and the reactor was refilled with the fresh medium solution.

2.2. Experimental measurements

Electric current in the reactors was determined by measuring the voltage drop every 20 min across an external resistor of 100 Ω using a multimeter and data acquisition system (Model 2700, Keithley Instruments, OH). After each fed-batch cycle, effluent was analyzed for conductivity and pH (SevenMulti; Mettler-Toledo International Inc., OH). The fresh medium pH was stable due to the sufficient phosphate buffer capacity (50 mM), slightly decreasing from 7.1 to 6.7 with increasing TAN concentration. The effluent from the MFC was also neutral between pH 6 and 7 for the TAN concentration 500 mg-N L^{-1} or higher. This stable pH condition indicates that the speciation between free ammonia (NH_3) and ammonium ion (NH_4^+) was kept constant during the experiment. The chemical oxygen demand (COD) was determined according to standard methods (Hach Co., CO) [24]. All experiments were performed in an air-conditioned laboratory and temperature was stationary over the course of MFC operation at 23.2 ± 0.8 °C. Note that temperature affects the ammonia speciation between free ammonia and ammonium ion. For instance, the amount of free

Table 1
Three different operation conditions in fed-batch experiments.

MFC operation condition	Substrate level (g L^{-1} sodium acetate)	Batch cycle length (days)
HASC (high acetate, short cycle)	2.0	2
LASC (low acetate, short cycle)	0.67	2
HALC (high acetate, long cycle)	2.0	6