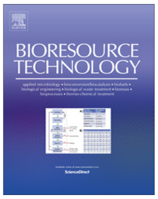
Bioresource Technology 161 (2014) 208–214

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech



Substrates and pathway of electricity generation in a nitrification-based microbial fuel cell

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HIGHLIGHTS

- Nitrogen removal in N-MFC was shown to be coupled with electricity generation.
- Roles of original substrate, intermediates and end product of nitrification in N-MFC were examined.
- Besides ammonium, the intermediates (hydroxylamine and nitrite) of nitrification could also serve as fuel.
- Pathway of electricity generation in N-MFC was proposed to be a combination of biological reaction and electrochemical reaction.

ARTICLE INFO

Article history:
Received 2 January 2014
Received in revised form 21 February 2014
Accepted 22 February 2014
Available online 3 March 2014

Keywords:
Nitrogen removal
Microbial fuel cell
Nitrification
Electrogenesis pathway

ABSTRACT

Nitrification-based microbial fuel cell (N-MFC) is a novel inorganic microbial fuel cell based on nitrification in the anode compartment. So far, little information is available on the substrates and pathway of N-MFC. The results of this study indicated that apart from the primary nitrification substrate (ammonium), the intermediates (hydroxylamine and nitrite) could also serve as anodic fuel to generate current, and the end product nitrate showed an inhibitory effect on electricity generation. Based on the research, a pathway of electricity generation was proposed for N-MFC: ammonium was oxidized first to nitrite by ammonia-oxidizing bacteria (AOB), then the nitrite in anolyte and the potassium permanganate in catholyte constituted a chemical cell to generate current. In other words, the electricity generation in N-MFC was not only supported by microbial reaction as we expected, but both biological and electrochemical reactions contributed.

1. Introduction

In industries such as synthetic fiber, plastic, explosives, pharmaceutical, rubber, preservatives, petroleum, ammonia has been used substantially as a raw material and it is also an essential ingredient in fertilizer widely used in agriculture sector. The excessive industrial use of ammonia and the ample consumption of nitrogenous compounds in the daily life are the major drivers of eutrophication in the receiving waters (Duce et al., 2008). Ammonia contamination of water is a long existing problem over the world, and therefore has attracted extensive attention.

Microbial fuel cell (MFCs) is a bioelectrochemical device that uses bacteria as the catalysts to convert chemical energy (stored in organic/inorganic matter in the wastewater) to electrical energy (Khera and Chandra, 2012; Logan et al., 2006). By means of MFCs, the dual purposes of pollution control and resources recycle can be achieved.

So far, most of the research works focused on the organics-based MFCs, and a few research efforts were conducted on the inorganics-based MFCs (Cai et al., 2012; He et al., 2009; Pant et al., 2010; Sun et al., 2009; Xie et al., 2013). Min et al. (2005) for the first time presented the high removal of ammonium in MFC under anaerobic conditions, but they did not ensure whether the ammonia oxidation was coupled to electricity generation. Later, Kim et al. (2008) examined the electricity generation from ammonia oxidation by intermittently injecting ammonia into the anaerobic anode chamber as the sole electron donor and no power was produced, they concluded that ammonia could not serve as a substrate for electricity generation under anaerobic conditions. On the contrary, He et al. (2009) demonstrated that ammonium could serve as a substrate for electricity generation as it was either used directly as the anodic fuel or used indirectly as the substrate for nitrifiers to produce organic compounds for heterotrophs in the

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http://dx.doi.org/10.1016/j.biortech.2014.02.081

rotating-cathode MFC. It appears that no unanimous agreement has been achieved on whether ammonium is the substrate for electricity generation, let alone the pathway associated with it.

Nitrification is the basis of N-MFC. It has been demonstrated that nitrification involves three successive enzymatic reactions and two intermediates (Michael et al., 2010):

$$\underbrace{NH_4^+ \xrightarrow{AMO} NH_2OH \xrightarrow{HAO} NO_2^-}_{\text{Ammonia-oxidizing bacteria}} \underbrace{\xrightarrow{NXR} NO_3^-}_{\text{Nitrite-oxidizing bacteria}}$$

where, AMO is ammonia monooxygenase; HAO is hydroxylamine oxidoreductase; NXR is nitrite oxidoreductase.

Apparently, ammonium is the original substrate of N-MFC. The intermediates (hydroxylamine and nitrite) of nitrification, which can donate electrons in view of its redox state, may also possibly serve as fuel. Until now, no studies have been conducted to investigate the effects the intermediates and end product of nitrification on the performance of N-MFC. Currently, it is believed that the pathway of electricity generation in N-MFC is similar to that in organics-based MFCs. In other words, the electricity generation in N-MFC is supposed to be the outcome of electron transfer from the nitrifying cells to the anode. But whether the pathway of electricity generation in N-MFC is the same as described above or not is still an enigma. The objective of this study is to make clear the effects of substrate, intermediates and end product of nitrification on electricity generation and to elucidate the pathway of electricity generation in N-MFC.

2. Methods

2.1. Inoculum and medium

The inoculum in the anode compartment of N-MFC was collected from our laboratory nitrifying reactor which has been in operation for 4 years, the main functional microorganisms are Planctomycetes, *Nitrosomonas*, and *Nitrospira*. (Wang et al., 2012). The total solid (TS) and volatile suspended solid (VSS) of the inoculum were 15.5 g L^{-1} and 13.7 g L^{-1} respectively. The functional bacteria were grown under aerobic condition in a phosphate buffered, defined medium (Xie et al., 2013) as described previously, and 100 mg L^{-1} NH_4^+–N was dosed as basic substrate.

2.2. Nitrification-based microbial fuel cell

A double-compartment N-MFC with an anode and a cathode compartment having each capacity of 230 mL was constructed as described previously (Xie et al., 2013). The two compartments were separated by a cation exchange membrane (CEM) (40 × 40 mm). A microporous aerator tube was installed at the bottom of anode compartment, which was connected to an external air pump, providing oxygen to the anode compartment. The anode was of graphite felt (2 × 2.5 cm × 2 cm, 10 cm^2 net surface area), the cathode was of carbon rod (8 cm × φ0.5 cm, 12.76 cm^2 net surface area), and they were connected by wires and external resistance to form a closed circuit. Electrical data were recorded every 5 min using a data collection system (Agilent 34970A, Agilent Technologies, USA). 10 mM potassium permanganate solution mixed with phosphate buffer (50 mM, pH 7.0), serving as the oxidant, was fed into the cathode compartment at a rate of 5 mL/min using a peristaltic pump.

2.3. Intermediates and end product

In order to investigate the effect of intermediates and end product on nitrification and electricity generation, three groups of batch tests were set up by adding intermediates (hydroxylamine and nitrite) and end product (nitrate) into inorganic medium respectively. An MFC with nitrifying bacteria was designated as the biotic reactor (referred to N-MFC), whereas the other MFC without nitrifying bacteria was designated as an abiotic reactor or a control. In each batch test, both the biotic and abiotic reactors were operated at 30 °C for 24 h.

2.4. Analysis and calculation

Standard methods were employed to quantify ammonium, nitrite, nitrate, TS and VSS (APHA, 1998) and hydroxylamine was determined by spectrophotometric method (Frear and Burrell, 1955). Conductivity was measured by a conductivity meter (FE30, Mettler Toledo, Switzerland). Dissolved oxygen concentration was determined using a dissolved oxygen meter (SG9, Mettler Toledo, Switzerland), pH was measured by a pH meter (FE20, Mettler Toledo, Switzerland).

Voltage across the 1 KΩ was continuously recorded with the data collection system. The measured voltage was used to calculate the current; the power density was normalized by the net surface area of CEM (Logan, 2012) and coulomb yield, expressed as total coulombs (amperes × seconds) passed through the fuel cell, was calculated by integrating the current over time. The amount of generated electron was expressed both as coulombs and electron mols, and the relationship between these two is $n = Q/(e{\cdot}N_A) = 1.04 \times 10^{-5}Q$, where e is the charge that elementary charge brings, $e = 1.60 \times 10^{-19}$ C; N_A is Avogadro's constant, $N_A = 6.02 \times 10^{23}$ mol^{-1}.

3. Results and discussion

3.1. Nitrogen removal coupled with electricity generation in N-MFC

When 100 ± 0.36 mg L^{-1} NH_4^+–N was dosed, ammonium concentration in the abiotic reactor decreased slightly to 81.55 ± 0.52 mg L^{-1} (22 h). The ammonium removal ratio was 18.4%, with no detection of nitrite or nitrate in the anolyte (Fig. 1). It is reported that ammonium can migrate from the anode compartment to the cathode compartment through CEM (Kim et al., 2008; Kuntke et al., 2012). Therefore, the loss of ammonium in the abiotic reactor could be inferred to the diffusion of ammonium ion through CEM. No analysis of ammonium in the catholyte was conducted since the catholyte potassium permanganate has color which interferes the detection of ammonium. In the biotic reactor, ammonium rapidly went down to 15.57 mg L^{-1} during the first 13 h, while nitrite and nitrate went up to 41.89 ± 0.24 mg L^{-1} and 31.74 mg L^{-1} respectively. Ammonium decreased gradually to 0.69 ± 0.13 mg L^{-1} after 22 h, while nitrate increased to 94.23 mg L^{-1}, but no nitrite was detected. The total nitrogen loss in the biotic reactor was 5.64 mg L^{-1}, which was less than that in the abiotic reactor (18.79 mg L^{-1}).

Fig. 1a illustrates the profiles of power density in the two reactors during the batch test. The circuit was open in the first 0.5 h, and then was closed at 0.5 h. As the profile of power density shows, the process of electricity generation can be divided into three phases: prompt-drop phase, plateau phase, and slow-descent phase. In the abiotic reactor, the power density dropped rapidly from 313.78 ± 8.54 to 0.43 mW m^{-2} in the prompt-drop phase (0.5–3 h); no plateau phase showed; and it decreased slowly to 0.22 mW m^{-2} in the slow-descent phase (3–24 h). In the biotic reactor, the power density dropped rapidly from 114.83 ± 8.34 to 2.02 mW m^{-2} in the prompt-drop phase (0.5–1 h); increased slightly in the plateau phase (1–13 h); and later declined slowly to 0.12 ± 0.01 mW m^{-2} in the slow-descent phase (13–24 h). The similar results were obtained in three repeated batch tests (data not shown). The above results suggested that ammonium could serve as anodic fuel to generate current in N-MFC.