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Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells

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ABSTRACT

Microbial fuel cells (MFCs) can use nitrate as a cathodic electron acceptor, allowing for simultaneous removal of carbon (at the anode) and nitrogen (at the cathode). In this study, we supplemented the cathodic process with *in situ* nitrification through specific aeration, and thus obtained simultaneous nitrification and denitrification (SND) in the one half-cell. Synthetic wastewater containing acetate and ammonium was supplied to the anode; the effluent was subsequently directed to the cathode. The influence of oxygen levels and carbon/nitrogen concentrations and ratios on the system performances was investigated. Denitrification occurred simultaneously with nitrification at the cathode, producing an effluent with levels of nitrate and ammonium as low as $1.0 \pm 0.5\ mg\,N\,L^{-1}$ and $2.13 \pm 0.05\ mg\,N\,L^{-1}$, respectively, resulting in a nitrogen removal efficiency of $94.1 \pm 0.9\%$. The integration of the nitrification process into the cathode solves the drawback of ammonium losses due to diffusion between compartments in the MFC, as previously reported in a system operating with external nitrification stage. This work represents the first successful attempt to combine SND and organics oxidation while producing electricity in an MFC.

1. Introduction

Increasing concerns arise regarding the treatment of nitrogen containing wastewater streams, due to the severe impacts they have on regulating the eutrophic level of water bodies and on human and animal health (Ghafari et al., 2008). Nitrogen is present in domestic and industrial wastewater mainly in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Tchobanoglous et al., 2003). Biological nitrogen removal is generally preferred over physicochemical methods such as ion exchange, adsorption, and chemical treatment due to its lower cost and chemical/energy requirements (Park and Yoo, 2009). Conventional biological nitrogen removal is achieved using a two-step process involving nitrification, which is the aerobic oxidation of ammonia to nitrite (NO_2^-) and nitrate, and denitrification, which is the dissimilatory reduction of nitrate to nitrogen gas (Ahn, 2006). The latter requires an electron donor, typically organic compounds, to enable the bacteria to gain energy from the nitrate/nitrite reduction reaction. Although being a well-established technology, the costs associated with external carbon addition for wastewaters lacking sufficient organics for complete denitrification, as well as the problems associated with the handling of the large volumes of sludge produced during denitrification, have driven the development of alternative processes.

Inorganic compounds such as hydrogen and elemental sulfur can provide the electrons needed for nitrate reduction instead of organics (Park and Yoo, 2009). Besides gas-sparging, hydrogen can be delivered *in situ* through water electrolysis (Sakakibara and Kuroda, 1993), with the advantages of simplified control and improved dissolution of H_2 (Ghafari et al., 2008). However, generating H_2 electrochemically is not

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usually economic given the high voltage and hence power consumption needed.

The electricity required to provide the necessary reducing power for denitrification can be drastically reduced if bacteria use the cathodic electrode directly as the electron donor. Gregory et al. (2004) firstly reported of *Geobacter* species capable of using a graphite electrode as direct electron donor during nitrate reduction to nitrite. More recently, the cathodic denitrification without intermediate H_2 production was coupled with anodic oxidation of organic carbon using microbial fuel cells (MFCs) (Clauwaert et al., 2007; Virdis et al., 2008). Microbial fuel cells are bioelectrochemical systems (BESs) that couple the oxidation of a substrate at a low redox potential with the reduction of an electron acceptor at a higher redox potential, thus producing electricity. Microorganisms are used to catalyse one or both reactions at the electrode surface.

We previously reported the simultaneous removal of carbon and nitrogen using an MFC-based process where a synthetic wastewater containing acetic acid and ammonium was sequentially fed to the anode compartment for carbon oxidation, then to an external aerated vessel allowing for nitrification, and finally to the cathode for denitrification, in a so-called ''loop configuration'' (Virdis et al., 2008). Even though the system was successfully coupling denitrification with energy recovery from acetate, it was unable to achieve low nitrogen levels in the effluent. This was largely due to the crossover of ammonia through the cation exchange membrane separating anode and cathode compartments.

In this study, we have successfully resolved this shortcoming by integrating the nitrification stage into the cathodic compartment, thus promoting simultaneous nitrification and denitrification (SND) in the same compartment. SND has been well described in literature (Masuda et al., 1991; Munch et al., 1996; Rittmann and Langeland, 1985; Zeng et al., 2003). We have investigated various important operational parameters for SND including the COD/N ratio of the influent, the dissolved oxygen level and the loading rate (Chiu et al., 2007; Park and Yoo, 2009). We have optimised the performance of the system to primarily achieve a high N removal capacity while still enabling a spontaneous electrochemical reaction, hence having a net power output from the MFC (not including pumping and aeration power requirements). To our best knowledge, this work represents the first comprehensive study of SND in microbial fuel cells.

2. Materials and methods

2.1. Microbial fuel cell construction, enrichment, and operation

A microbial fuel cell consisting of two compartments of 336 cm^3 each (Total Compartment Volume, TCV) was assembled and filled with granular graphite as the anode and cathode material (2–6 mm diameter, El Carb 100, Graphite Sales, Inc., USA), leaving a liquid volume per each compartment of approximately 200 cm^3. Graphite rods were used to create electric contact between the granules and the outside of the cell. A cation exchange membrane (CEM) (Ultrex CMI-7000, Membrane International, USA) was used to separate the MFC compartments. Ag/AgCl reference electrodes (MF-2052 Bioanalytical Systems, USA) were placed in both compartments. Their potential was assumed to be +0.197 V *vs* Standard Hydrogen Electrode (SHE).

The anode and cathode rods as well as the reference electrodes were connected to a data acquisition unit (Agilent 34970A), which recorded the voltage of the MFC (measured as the voltage difference between the operating electrodes) and the half-cell voltage values (measured as the voltage difference between reference electrode and the operating electrode of each compartment) every minute. A variable resistor (set at 5 Ohms) connected anode and cathode and the current was determined using Ohm's law.

Anode and cathode were inoculated with a microbial consortium previously operating in an MFC performing carbon and nitrogen removal. The microbial fuel cell was fed with autoclaved growth medium containing 6 g L^{-1} Na_2HPO_4, 3 g L^{-1} KH_2PO_4, 0.5 g L^{-1} NaCl, 0.1 g L^{-1} $MgSO_4$ $7H_2O$, 0.015 g L^{-1} $CaCl_2$ $7H_2O$, and 1 mL L^{-1} trace nutrients solution as described in Lu et al. (2006). During the enrichment, acetate and ammonia, two common constituent of domestic and industrial wastewater, were supplied to the medium in the form of sodium acetate ad ammonium chloride in concentrations of 0.393 g L^{-1} CH_3COONa and 0.407 g L^{-1} NH_4Cl. Aeration of the catholyte was provided and the system was operated for a period of approximately 6 months to promote the establishment of a microbial community performing SND. Following the enrichment procedure, sodium acetate and ammonium chloride were supplied to the medium in concentrations depending on the conditions applied (detailed below). Each feed had a pH of 7 and a conductivity of 11 mS cm^{-1} and was continuously pumped into the compartments at a flow rate of approximately 0.7 L d^{-1}, resulting in a hydraulic retention time (HRT) of 6.86 h. Mixed conditions were guaranteed by continuous recirculation of the anodic and cathodic liquids at a rate of 200 mL min^{-1} (Fig. 1).

2.2. Liquid- and gas-phase analysis

Samples obtained from the anode and cathode compartments were immediately filtered through 0.22 μm sterile filters. The acetate content was determined using a High-Performance Liquid Chromatograph (HPLC; Shimadzu, Japan), which was equipped with a refractive index detector (RID-10AVP), a diode array detector (SPD-M10AVP) and an HPX-87H ion exclusion column (300 mm × 7.8 mm; BioRad). Sulphuric acid 0.008 N was used as the eluent at a flow rate of 0.4–0.6 ml min^{-1}, injection volume of 20 μL and 65 °C column temperature.

Ammonium (NH_4^+-N), nitrite (NO_2^--N), and nitrogenous oxides (NO_x-N, i.e., sum of NO_2^--N and NO_3^--N) were measured using a Lachat QuikChem 8000 Flow Injection Analyzer (Lachat Instruments, Milwaukee, USA). Dissolved oxygen (DO) was measured on-line with a DO probe placed in the cathode recirculation system (Fig. 1). N_2 and N_2O production rates were measured with the Titration and Off-Gas Analysis (TOGA) sensor, developed by Pratt et al. (2003), which works by stripping the gases produced in the reactor