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Nitrogen removal in a two-chambered microbial fuel cell: Establishment of a nitrifying–denitrifying microbial community on an intermittent aerated cathode

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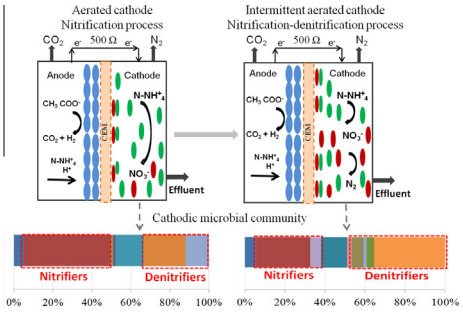
HIGHLIGHTS

- Diffused ammonium is being mostly nitrified at the cathode compartment.
- *Nitrosomonas* sp. and *Nitrobacter* sp. were the main cathode's nitrifying bacteria.
- Heterotrophic acetate-dependent denitrification from cathode biomass was confirmed.
- Intermittent aerated cathode established a nitrifying–denitrifying community.

GRAPHICAL ABSTRACT



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ABSTRACT

A microbial fuel cell (MFC) was used to study nitrogen dynamics and its feasibility for high strength wastewater treatment. Intermittent aeration was applied on the cathode chamber accomplishing the establishment of a simultaneous nitrifying–denitrifying microbial community. A total of 30.4% of the $N\text{-}NH_4^+$ migrated through the ion exchange membrane being primarily nitrified at the cathode chamber. When intermittent aeration was applied in the cathode, denitrification also occurred achieving 17.8% of nitrate removal without acetate addition, and 41.2% with acetate addition. The microbial community analysis revealed that the nitrification process at the cathode chamber could be explained due to a high predominance of *Nitrosomonas* sp. as ammonia-oxidising bacteria and other *Comamonadaceae* phylotypes as potential denitrifiers. Parallel batch denitrification assays, carried out outside the MFC using the cathode effluent, confirmed the existence of heterotrophic denitrification processes with other well known denitrifying dominant phylotypes enrichment (*Burkholderiadaceae*, *Comamonadaceae*, *Alcaligenaceae*).

1. Introduction

Nitrogen removal from wastewater is increasingly becoming more relevant as a cause for serious environmental problems such as eutrophication of rivers, the deterioration of water sources, and as a serious hazard for human and animal health [1]. Ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are the most important forms of reactive nitrogen found in the environment, and nitrate in particular (NO_3^-), is one of the most problematic compounds found in water and wastewater. Therefore, efforts to improve the removal of nitrogen have intensified in the last decades. Nitrification/denitrification is a well known process applied to remove nitrogen from wastewaters. Nevertheless, as different microbial populations are involved with different requirements

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of oxygen, temperature, etc., it is often fairly complicated and expensive to implement. Finding new treatment methods to achieve an effective and less expensive nitrogen removal is still an issue to be adequately solved.

Bioelectrochemical systems (BESs) offer a promising technology for nutrient removal while at the same time recovering bioenergy [2]. These systems are capable of converting the chemical energy of organic wastes into electricity. Among all BES, microbial fuel cells (MFCs) are the most widely researched. Microbial fuel cells (MFCs) use bacteria as catalysts to oxidise organic and inorganic matter and generate electrical current since the electrons derived from these metabolic reactions are transferred from the anode (negative terminal) to the cathode (positive terminal) producing a current flow running through an external circuit [3]. A two-chambered MFC, consisting of an anode and a cathode separated by an ion exchange membrane, is the most common configuration found where, at least one of the anodic or cathodic reactions, is microbiologically catalysed [4].

So far, nitrogen removal by MFCs has focused on two different strategies: MFC ammonium removal under anaerobic conditions [5] or, since that ammonia can be diffused from anode to cathode through the cation exchange membrane [6], it can be stripped and subsequently absorbed [7]. Instead of recovering ammonia at the cathode chamber, another strategy is to remove it by external nitrification and a subsequent denitrification accomplished by microorganisms in the cathode chamber [8,9], or by simultaneous cathodic nitrification–denitrification [10]. So far, very few studies referring to nitrogen removal via simultaneous nitrification and denitrification (SND) processes as an alternative of using an external nitrifying bioreactor, which is known to be more difficult and expensive to scale up, have been reported. Afterwards, to simplify the reactor structure and reduce the costs associated caused by using an external nitrifying bioreactor, others MFC designs were investigated in order to carry out SND in these systems. However, to date majority of studies are performed using groundwater or synthetic wastewater, and to contrary the use of high strength animal wastewater, particularly pig slurries, has received little attention so far. Thus, there is a lack of knowledge about the feasibility of using a MFC-SND, and its potential application for treating high strength animal wastewater to accomplish the requirements for agricultural uses.

Nitrification is the biological oxidation of ammonia (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-) (Eq. (1)). It is an aerobic process performed by autotrophic ammonia-oxidising bacteria (AOB), ammonia-oxidising archaea (AOA) and nitrite-oxidising bacteria (NOB). The first step of nitrification is the oxidation of ammonia to nitrite catalysed by bacteria containing the ammonia monooxygenase enzyme (*amo*A), being the most studied AOB belonging to the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira* and *Nitrosolobus*. There is less information about AOA, but currently two genera, *Nitrosopumilus* and *Nitrososphaera*, have been isolated. The second step is the oxidation of nitrite to nitrate catalysed by bacteria containing the nitrite oxidoreductase enzyme (*nxr*) such as bacteria belonging to the genera *Nitrobacter*, *Nitrococcus*, *Nitrospina*, and *Nitrospira*. Denitrification is an anaerobic respiration pathway for diverse facultative anaerobic bacteria and archaea [11]. It is a sequencing reductive process which involves four steps, from nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and finally resulting in the production of di-nitrogen gas (N_2) (Eq. (1)). The reduction of nitrous oxide that occurs during the last step of the denitrification pathway involves the *nosZ* enzyme (encoding nitrous oxide reductase), which has received most of the attention in molecular microbial ecology studies in the environment [12]. Heterotrophic bacteria, such as *Paracoccus* and *Pseudomonas*, are the most common denitrifier bacteria, although autotrophic denitrifiers (e.g. *Thiobacillus*) have also been identified.

$$\underset{\text{Nitrification}}{\underbrace{NH_4^+ \xrightarrow{amoA} NO_2^- \xrightarrow{nxr} NO_3^-}} \underset{\text{Denitrification}}{\underbrace{\xrightarrow{\substack{napA\\narG}} NO_2^- \xrightarrow{\substack{nirK\\nirS}} NO \xrightarrow{\substack{norB\\norZ}} N_2O \xrightarrow{nosZ} N_2}} \quad (1)$$

Previous studies focussing on microbial communities' enrichment on bio-cathodes have shown a predominance of members belonging to *Proteobacteria*, *Firmicutes*, and *Chloroflexi* phyla [13]. Taking into account the non-correspondence between changes in predominant microbial populations on the MFC and the reactor's performance, the complex bacteria community harboured on MFCs electrodes, and the fact that occasionally changes in the predominant members of the bacterial community did not correspond with changes in reactor operation [13], it is suggested that more information about functional groups is needed for a better understanding on potential nitrogen transformation mechanisms that could take place in a MFC fed with wastewater.

This study aims to evaluate nitrogen dynamics and microbial community structure in a two-chambered microbial fuel cell operating with an intermittently aerated cathode, and its feasibility as a treatment for high strength (organic and nitrogen) wastewater simulating a liquid fraction of pig slurry. This work focuses on three main goals: (i) to study nitrogen dynamics in a MFC harbouring active microbial biomass both in the anode and cathode chambers; (ii) to enhance the nitrification–denitrification process at the cathode chamber, and (iii) to assess the microbial community enriched both in the anode and in the cathode compartment.

2. Materials and methods

2.1. Experimental set-up

A methacrylate two-chambered MFC reactor was built with the anode and cathode compartments ($0.14 \times 0.12 \times 0.02\ m^3$) separated by a cation exchange membrane (CEM) (14×12 cm) (Ultrex CMI-7000, Membranes International Inc., Ringwood, NJ, USA). Granular graphite rods with a diameter ranging from 2 to 6 mm (El Carb 100, Graphite Sales Inc., U.S.A.), and stainless steel mesh were used as anode and cathode respectively, resting in 165 mL of net anodic volume (NAV) and 250 mL of net cathodic volume (NCV). Prior to its use, the granular graphite was sequentially soaked in 1 M of HCl, and 1 M of NaOH, in each case for 24 h, and finally rinsed in deionised water. Copper wires were used to connect the electrodes to a 500 Ω external resistance.

2.2. MFC operation

The anodic chamber was inoculated with 1 mL of digestate from a bench-scale mesophilic methanogenic continuously stirred tank reactor fed with slaughterhouse waste harbouring a high content of $N\text{-}NH_4^+$.

The feed solutions were prepared containing (per litre of distilled water): $CaCl_2$, 0.0147 g; KH_2PO_4, 3 g; Na_2HPO_4, 6 g; $MgSO_4$, 0.246 g; and 1 mL L^{-1} trace elements solution as described in Lu et al. [14]. Additionally the anode feed solution contained 2.9 g L^{-1} CH_3COONa, as carbon source, and 3.82 g L^{-1} NH_4Cl; accordingly, the COD:N ratio of the medium was 2.23. The feed solution for the cathode chamber contained KH_2PO_4, 3 g L^{-1}; Na_2HPO_4, 6 g L^{-1}. The MFC was operated at a room temperature of ~23 °C and in a continuous mode with a flow rate of 0.628 L d^{-1}, resulting in organic and nitrogen loading rates of 1.7 g COD L^{-1} d^{-1} and 4.2 g $N\text{-}NH_4^+$ L^{-1} d^{-1} respectively. The operated hydraulic retention time (HRT) was of 6.3 and 9.4 h at the anode and cathode respectively (Table 1). To keep the cathode under aerobic conditions, air was supplied at a flow rate of 2 L min^{-1}. Then, a side