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Identification of hotspots for NO and N_2O production and consumption in counter- and co-diffusion biofilms for simultaneous nitrification and denitrification

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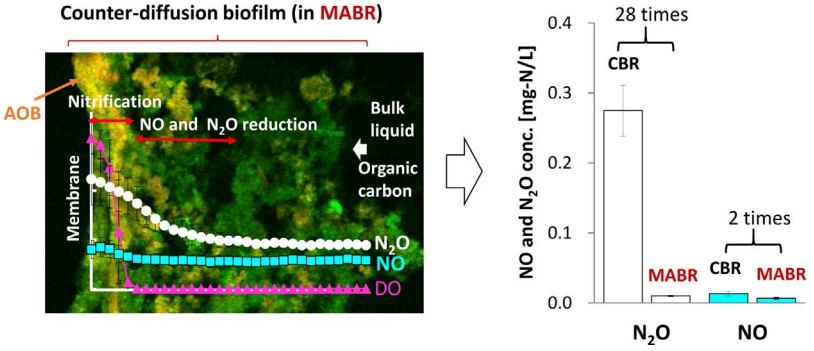
GRAPHICAL ABSTRACT



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ABSTRACT

A membrane-aerated biofilm reactor (MABR) provides a counter-current substrate diffusion geometry in which oxygen is supplied from a gas-permeable membrane on which a biofilm is grown. This study hypothesized that an MABR would mitigate NO and N_2O emissions compared with those from a conventional biofilm reactor (CBR). Two laboratory-scale reactors, representing an MABR and CBR, were operated by feeding synthetic industrial wastewater. The surficial nitrogen removal rate for the MABR [4.51 ± 0.52 g-N/(m^2 day)] was higher than that for the CBR [3.56 ± 0.81 g-N/(m^2 day)] ($p < 0.05$). The abundance of β-proteobacterial ammonia-oxidizing bacteria in the MABR biofilm aerobic zone was high. The NO and N_2O concentrations at the biofilm–liquid interface in the MABR were 0.0066 ± 0.0014 and 0.01 ± 0.0009 mg-N/L, respectively, two and 28 times lower than those in the CBR. The NO and N_2O production hotspots were closely located in the MABR aerobic zone.

1. Introduction

The increasing NO and N_2O concentrations in the atmosphere are of environmental concern because they are ozone-depleting gases (IPCC, 2007). Emissions of N_2O, which is a highly potent greenhouse gas, from wastewater were 0.2 Tg N_2O-N/year in 2010, or 3% of total gross

Abbreviations: NO, nitric oxide; N_2O, nitrous oxide; MABR, membrane-aerated biofilm reactor; CBR, conventional biofilm reactor; WWTPs, wastewater treatment plants; DO, dissolved oxygen; CO_2, carbon dioxide; NH_4^+, ammonium; NH_2OH, hydroxylamine; NO_2^-, nitrite; NO_3^-, nitrate; AOB, ammonia-oxidizing bacteria; SND, simultaneous nitrification and denitrification; Nir, nitrite reductase; Nor, nitric oxide reductase; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; OUR, oxygen utilization rate; OUE, oxygen utilization efficiency; FISH, fluorescence *in situ* hybridization; FITC, fluorescein isothiocyanate

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anthropogenic emissions (Davidson and Kanter, 2014), and therefore need mitigation. However, N_2O emission has yet to be successfully mitigated in biological nitrogen removal (Desloover et al., 2012). The amount of N_2O emitted from wastewater treatment plants (WWTPs) is expected to increase by approximately 13% between 2005 and 2020 (Law et al., 2012), and its impact accounts for up to 78.4% of the total CO_2 footprint in WWTPs (Daelman et al., 2013). In comparison, the level of NO emissions from biological nitrogen removal is limited (Kampschreur et al., 2009; Schreiber et al., 2012); further research on this is needed.

NO and N_2O are produced as by-products of nitrification and intermediates of denitrification (Schreiber et al., 2012; Stein, 2011b). N_2O production in biological nitrogen removal is mainly mediated by ammonia-oxidizing bacteria (AOB) via several metabolic pathways, i.e., nitrifier denitrification (Ji et al., 2015; Stein, 2011a), hydroxylamine oxidation (Sutka et al., 2006), and N-nitrosation hybrid reactions (Frame et al., 2016; Terada et al., 2017). Similarly, N_2O is produced and reduced by heterotrophic denitrifying bacteria using sequential denitrifying enzymes. NO is produced as a by-product via hydroxylamine oxidation by AOB (Chandran et al., 2011; Kampschreur et al., 2008; Schreiber et al., 2012; Stein, 2011b) and as an intermediate by the enzyme nitrite reductase (Nir), followed by consumption to produce N_2O by the enzyme nitric oxide reductase (Nor) (Schreiber et al., 2012; Zumft, 1997). These multiple enzymatic reactions mainly involving AOB and heterotrophic denitrifiers determine NO and N_2O release from WWTPs. An understanding of the mechanisms and development of mitigation strategies is therefore important (Desloover et al., 2012; Law et al., 2012; Lu and Chandran, 2010).

Membrane-aerated biofilm reactors (MABRs), which use counter-current supplies of electron acceptors and donors, provide a promising method for achieving small-footprint nitrogen removal from wastewater streams (Syron and Casey, 2008; Terada et al., 2003) and minimization of N_2O emissions (Kinh et al., in press; Pellicer-Nacher et al., 2010). A gas-permeable membrane, on which a biofilm is grown, is used to precisely supply oxygen from the bottom of the biofilm, and organic carbon and NH_4^+ from the outermost surface of the biofilm (Nerenberg, 2016; Syron and Casey, 2013; Terada et al., 2003). Because of the counter-diffusion biofilm geometry, NH_4^+ encounters oxygen at the bottom of the biofilm, with minimum contact with organic carbon, enhancing nitrification. In addition, the middle part of the biofilm provides a suitable environment for denitrification, in which organic carbon and oxidized nitrogen compounds, including NO and N_2O, are present without oxygen (Cole et al., 2004; Nerenberg, 2016; Syron and Casey, 2013; Terada et al., 2003), enabling simultaneous nitrification and denitrification (SND). This unique biofilm geometry solves the inherent challenge presented by a conventional biofilm, i.e., a co-diffusion biofilm, where organic carbon for denitrification becomes limited. This is because the co-diffusion biofilm geometry provides an environment for oxidation of organic carbon at the biofilm exterior.

The N_2O concentrations in the bulk liquid and biofilm depend on NO and N_2O reduction, therefore investigation of not only N_2O but also NO production/consumption in the biofilm could improve the understanding of the degree of N_2O production. However, the spatial distribution of NO for N_2O production in a counter-diffusion biofilm has yet to be investigated. This study was therefore undertaken to (1) compare the depth profiles of dissolved NO and N_2O concentrations in counter- and co-diffusion biofilms and (2) identify hotspots for NO and N_2O production/consumption in both types of biofilm. To this end, two biofilm reactors with correspondent two biofilm geometries were operated, followed by determination of the dissolved oxygen (DO), NO, and N_2O concentrations at various depths in the biofilms using microelectrodes, and the spatial distributions of AOB in both biofilms.

2. Materials and methods

2.1. Reactor setup

Two laboratory-scale flow-cell reactors of volume 200 mL, with counter-diffusion and co-diffusion biofilm geometries, representing an MABR and a conventional biofilm reactor (CBR), respectively, were operated at 30 °C. Each reactor consisted of a liquid (200 mL) and gas (20 mL) compartment, with a gas-permeable, flat silicone membrane (Rubber Co., Tempe, AZ, USA) inserted between them, ensuring a specific surface area of 20.8 m^2/m^3. Air was supplied to the MABR in flow-through mode from the gas compartment at an air flow rate and pressure of 20 mL/min and 15 kPa, respectively. The CBR system mounted a non-permeable plate beneath the silicone membrane, deterring oxygen entry. Air was supplied by a bundle of hollow fibers (96 fibers; MHF3504, Mitsubishi Rayon Co., Ltd., Tokyo, Japan) in flow-through mode at an air flow rate and pressure of 20 mL/min and 10 kPa, respectively. These settings provided comparable oxygen loading rates to both biofilms, based on estimation of an oxygen transfer rate. The bundle in the CBR was cleaned regularly to avoid biofilm formation. Both reactors recycled the bulk liquid at a velocity of 1.4 cm/s using recirculation pumps (Model 7553-50, Tokyo, Japan). The reactors were operated at influent total dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations of about 170 mg-C/L and 170 mg-N/L; and the DOC concentration was increased by a factor of three, to give an influent DOC/TDN ratio of 3:1, for days 95–132.

A synthetic medium was continuously supplied at 25.2 mL/h, ensuring a hydraulic retention time of 13 h, using a tubing pump (ISMATEC, ISM 930, Wertheim, Germany). The medium component concentrations mimicked those in food-processing wastewater: CH_3COONa (1.80 g/L), $(NH_4)_2SO_4$ (0.81 g/L), and aliquots of a mineral solution consisting of (mg/L): $MgSO_4{\cdot}7H_2O$ (280), KH_2PO_4 (27), $CaCl_2{\cdot}2H_2O$ (120), NaCl (600), $FeSO_4{\cdot}7H_2O$ (3.3), $MnSO_4{\cdot}H_2O$ (3.3), $CuCl_2{\cdot}2H_2O$ (0.8), $ZnSO_4{\cdot}7H_2O$ (1.7), and $NiSO_4{\cdot}6H_2O$ (0.3). The medium was sterilized prior to supply to the two biofilm reactors. Biomass from a partial nitrifying sequencing-batch reactor supplying only an inorganic synthetic medium was inoculated.

2.2. Chemical analyses of influent and effluent wastewaters

The influent and effluent concentrations of organic carbon and nitrogen compounds were monitored three times per week. The DOC and TDN were measured using a TOC analyzer (TOC 5000A, Shimadzu, Kyoto, Japan). The NH_4^+, NO_2^-, and NO_3^- concentrations were determined using a flow injection analyzer (PE-230, Human Manufacture Engineering, Japan). *t*-Test was performed to compare the effluent concentrations of DOC and dissolved nitrogen constituents of the two reactors, using SPSS 13.0 statistical software (IBM Co., Armonk, NY, USA).

2.3. Calculation of oxygen transfer and utilization rates

The oxygen utilization rate (OUR) was evaluated assuming SND via NO_2^- (Eq. (1)) and NO_3^- (Eq. (2)):

$$OUR = \frac{Q_{in}[3.43(S_{NH4,in} - S_{NH4,eff})]}{A} \quad (1)$$

$$OUR = \frac{Q_{in}[4.54(S_{NH4,in} - S_{NH4,eff})]}{A} \quad (2)$$

where A is the surface area of the biofilm, Q_{in} is the influent flow rate, and $S_{NH4,in}$ and $S_{NH4,eff}$ are the NH_4^+ concentrations in the influent and effluent (mg-N/L), respectively. The oxygen utilization efficiency (OUE) for nitrification is defined as