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Influence of biofilm thickness on nitrous oxide (N_2O) emissions from denitrifying fluidized bed bioreactors (DFBBRs)

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ABSTRACT

Nitrous oxide (N_2O) is a significant anthropogenic greenhouse gas emitted from biological nutrient removal (BNR) processes. This study tries to get a deeper insight into N_2O emissions from denitrifying fluidized bed bioreactors (DFBBRs) and its relationship to the biofilm thickness, diffusivity, and reaction rates. The DFBBR was operated at two different organic and nitrogen loading rates of 5.9–7 kg COD/(m^3 d) and 1.2–2 kg N/(m^3 d), respectively. Results showed that the N_2O conversion rate from the DFBBR at a biofilm thickness of 680 μm was 0.53% of the total influent nitrogen loading while at the limited COD and a biofilm thickness of 230 μm, the N_2O conversion rate increased by 196–1.57% of the influent nitrogen loading concomitant with a sevenfold increase in liquid nitrite concentration. Comparing the N_2O emissions at different biofilm thickness showed that the N_2O emission decreased exponentially with biofilm thickness due to the retention of slow growth denitrifiers and the limited diffusivity of N_2O.

1. Introduction

Anthropogenic greenhouse gases (AnGHGs) emissions are globally recognized by the United Nation framework Convention on Climate Change (UNFCCC) (UNFCCC, 2007). GHGs include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). N_2O, the dominant ozone-depleting substance, is the third most important GHGs with a global warming potential (GWP) of 310 times that of CO_2 (IPCC, 2007; Ravishankara et al., 2009). Wastewater treatment plants (WWTP) employing biological nutrient removal (BNR) including nitrification and denitrification are an important anthropogenic source of N_2O emissions and are estimated to account for 3.2% of the global anthropogenic N_2O emission (Sahely et al., 2006; Kampschreur et al., 2009). Considering the widespread use of BNR processes due to the rigorous effluent water quality standards, there is a potential that N_2O emissions from WWTP will increase (Ahn et al., 2010; Park et al., 2000).

During nitrification, N_2O can be produced through the aerobic hydroxylamine oxidation by ammonia oxidizing bacteria (AOB). This hydroxylamine, generated during ammonia oxidation, is oxidized to NO directly under the catalysis of hydroxylamine oxidoreductase (HAO) encoded by the heoAB genes, and reduced to N_2O under the catalysis of c554 cytochrome (Cyt c554) (Chandran et al., 2011). N_2O also can be produced through chemical decomposition of intermediate from the oxidation of NH_4 to NO_2 (Ritchie and Nicholas, 1972).

Generally, conditions that are conducive to incomplete denitrification and consequently accumulation of nitrites in the liquid phase, such as limited carbon, high dissolved oxygen (DO), competition for carbon by other microbial groups, and cold temperatures result in increased N_2O emissions. All the above parameters differ significantly in biofilm processes as compared with suspended growth systems. During denitrification, N_2O is one of the obligatory intermediates in the biochemical reaction by heterotrophic denitrifying bacteria (HDN) where NO_3-N is reduced to NO_2-N, NO_2 and N_2O, with N_2O finally reduced to N_2 gas (Hu et al., 2013). The reduction of NO_3 to N_2 involves six enzymes and reductase using five electrons (Desloover et al., 2012; Stein, 2011). First, NO_3 is reduced to NO_2 by periplasmatic nitrate reductase (NAP) and membrane-bound nitrate reductase (NAR). Second, the reduction of NO_2 to NO involving Cu-containing nitrite reductase (NirK) and cytochrome *cd1* nitrite reductase (NirS) occurs. Using the nitric oxide reductase (NOR), the NO is reduced to N_2O. Finally, N_2O is reduced to N_2 with help of nitrous oxide reductase (NOS) as shown in Fig. 1a (Desloover et al., 2012).

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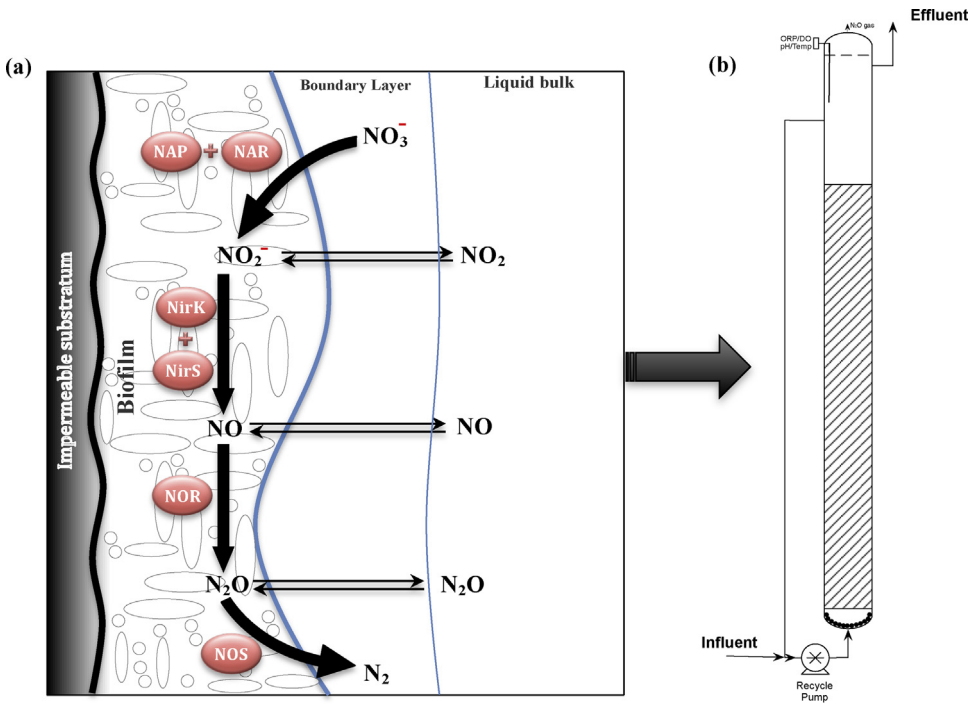

Fig. 1. (a) Conceptual overview of the N_2O production and consumption pathways of biofilm during denitrification process and (b) schematic diagram of DFBBRs.

During the denitrification process, the emissions of N_2O from BNR processes have been reported to be reduced by the changing the operation conditions in a step-feed sequencing batch reactor (SBR) and maximizing the anoxic time by decreasing the aerobic carbon breakdown to increase the carbon-to-nitrogen ratio (Hu et al., 2011). Additionally, Park et al. (2007) reported that the addition of immobilized *Alcaligenes faecalis* to the intermittent aeration-activated sludge process would enhance denitrification and mitigate the emissions of N_2O. Furthermore, Manconi et al. (2006) found that the addition of copper (Cu^{2+}) in a range of 10–100 μg/L as a catalyst increased the nitrous oxide reductase (NOS) activity and accelerated the bio-reductions of both nitrite to nitric oxide and nitrous oxide to nitrogen gas (Manconi et al., 2006).

Most of the studies on N_2O emissions during denitrification from BNR systems were conducted predominantly for suspended growth system, i.e. activated sludge system and sequencing batch reactors (SBR), and few studies have been conducted to investigate N_2O emissions from particulate bioparticles systems. Gaëlle et al. (2006) studied the N_2O emissions from biofilters during nitrification (Biostyr®) and denitrification (Biofor®) processes at a nitrogen loading rate of 2.2 kg TN/m^3 d and found that 0.4–1% of the oxidized ammonium was emitted as N_2O during nitrification and 0.2–1.3% of the nitrate removed during the denitrification stage. They found that the denitrification rates and N_2O emissions are directly correlated with the quantities of added carbon source (COD/N ratios ranged from 1.9 to 5), without any correlation to the biofilm thickness. In biofilm processes, nitrification and denitrification are maintained predominantly in the attached biomass (Eldyasti et al., 2010), which would hypothetically contribute to N_2O emissions. During nitrification, N_2O may be produced during the oxidation of hydroxylamine and/or the reduction of nitrites. As shown in Fig. 1a, NO_3-N is reduced to NO_2-N and N_2O, with N_2O finally reduced to N_2 gas. Since the aforementioned processes area microbially mediated and strongly influenced by substrates diffusion in and out of the biofilm, understanding the contribution of biofilm thickness to the N_2O emissions would help reduce the N_2O emissions from biofilm processes.

Among the biological processes for the municipal and industrial wastewater, the denitrifying fluidized bed bioreactor (DFBBR) system is a promising particulate bioreactor for the biological nutrient removal (BNR) and proves to be economic and efficient, due to a large specific surface area (SSA) ranging from 2000 m^2/m^3 to 4000 m^2/m^3 which sustains a very high biomass (biofilm) concentrations of up to 40,000 mg VSS/L (Eldyasti et al., 2010, 2012). As a result of much higher bioparticle density per unit reactor volume and smaller media size, the DFBBR exhibits very different bioreactor properties i.e. biofilm thickness, detachment rates, and attrition rates than conventional biofilm processes (Eldyasti et al., 2010, 2012).

Since literature studies explored N_2O emissions from bioparticles system without correlating the N_2O emissions to the biofilm thickness at high specific surface area, the primary goal of this study is to investigate N_2O emissions from a DFBBR characterized by high SSA of 2880 m^2/m^3 with a focus on the contribution of biofilm thicknesses to N_2O emissions from biofilm. This work also investigates the relationship of maximum reaction rates and diffusivity through denitrification biofilm and conducts a comprehensive nitrogen mass balance. This work for the first time provides an insight into N_2O emission and conversion rates in DFBBR, which will help improve the design and operation of such systems. This study also aimed at examining the relationship between the N_2O emission and nitrite concentrations during denitrification at two different carbon to nitrogen ratios. Furthermore, after the limited carbon phase, the DFBBR was tested at chemical oxygen demand (COD)-nitrogen ratio (COD/N) of 5 again for 50 days to investigate the dynamics of N_2O emissions for the particulate biofilm. Additionally, N_2O emissions from DFBBR were further compared to those in other BNR systems.

2. Materials and methods

2.1. System description

An anoxic fluidized bed bioreactor (Fig. 1b) comprising a plexiglass reactor with a total working volume of 507 ml, height of