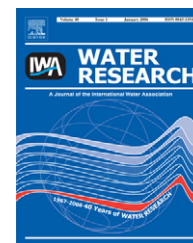


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# Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: Results of a modeling study

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## ABSTRACT

A 1-d multi-population biofilm model was constructed to study the effect of heterotrophic activity on completely autotrophic ammonium ( $\text{NH}_4^+$ ) removal in membrane-aerated (counter-diffusion) versus conventional biofilm systems (co-diffusion). Growth of heterotrophic bacteria (HB) was supported either solely by biomass decay products or by organic carbon (as chemical oxygen demand (COD)) in the influent. Three scenarios were considered: influence of HB growing on biomass decay products on steady-state performance (total nitrogen (TN) removal efficiency); influence of the influent COD/N ratio on steady-state performance (supplying COD in the influent); and impact of dynamic changes in the influent COD/N ratio on TN removal efficiency. The results revealed that the TN removal efficiency in the counter-diffusion biofilm was significantly different when HB were included in the simulations at  $\text{NH}_4^+$  surface loads of  $L_{\text{NH}_4^+} > 2.7 \text{ g-N m}^{-2} \text{ d}^{-1}$ . Influent COD significantly altered the microbial community composition in the counter-diffusion biofilm and anaerobic  $\text{NH}_4^+$  oxidation could not be sustained at  $\text{COD/N} > 2$ . The co-diffusion system, however, was less affected and more than 50% of the TN removal originated from anaerobic  $\text{NH}_4^+$  oxidation at those ratios. Perturbation experiments showed that step increases to influent COD/N ratios of 2 or higher over a period of 50 d or longer caused a loss of anaerobic  $\text{NH}_4^+$  oxidation capacity which could not be regained within a reasonable time frame ( $> 1000 \text{ d}$ ) in the counter-diffusion system. In contrast, simulating a 1-d sloughing event only caused a disturbance of 200 d although a maximum biofilm loss of 90–95% occurred. These results clearly indicate the importance of heterotrophic activity in autotrophic N removal biofilms, especially in counter-diffusion systems where they may compromise N removal capacity.

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Abbreviations:  $a$ , specific membrane surface area ( $\text{m}^2 \text{ m}^{-3}$ ); AnAOB, anaerobic ammonium oxidizing bacteria; AOB, ammonium oxidizing bacteria; COD, chemical oxygen demand ( $\text{g-COD m}^{-3}$ );  $d$ , detachment intensity coefficient; HB, heterotrophic bacteria;  $J_{\text{O}_2}$ , oxygen flux ( $\text{g-O}_2 \text{ m}^{-2} \text{ d}^{-1}$ );  $k_{\text{tot}}$ , total membrane mass transfer coefficient;  $L_f$ , biofilm thickness (m);  $L_{\text{NH}_4^+}$ , ammonium surface load ( $\text{g-N m}^{-2} \text{ d}^{-1}$ ); MABR, membrane-aerated biofilm reactor; N, nitrogen;  $\text{N}_2$ , nitrogen gas;  $\text{NH}_4^+$ , ammonium ( $\text{g-N m}^{-3}$ );  $\text{NO}_2^-$ , nitrite ( $\text{g-N m}^{-3}$ );  $\text{NO}_3^-$ , nitrate ( $\text{g-N m}^{-3}$ ); NOB, nitrite oxidizing bacteria;  $\text{S}_{\text{O}_2, \text{g}}$ , oxygen concentration in the gas phase;  $\text{S}_{\text{O}_2, \text{bb}}$ , oxygen concentration at the biofilm; TN, total nitrogen ( $\text{g-N m}^{-3}$ );  $u_{\text{de}}$ , detachment velocity ( $\text{m d}^{-1}$ );  $u_f$ , growth velocity of the biofilm ( $\text{m d}^{-1}$ )

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## 1. Introduction

Nitrogen (N) removal is one of the crucial steps in wastewater treatment today. Effective treatment technologies become especially important when handling high-strength nitrogenous wastewater with N concentrations from 100 to 1000 g-N m<sup>-3</sup>, such as sludge digester supernatants (Fux et al., 2002; van Loosdrecht and Salem, 2006), which can account for up to 30% of the ammonium (NH<sub>4</sub><sup>+</sup>) load for a typical wastewater treatment plant. Other sources of high-strength wastewaters are manure, piggery wastewaters (Terada et al., 2003) and several industrial wastewaters (Carrera et al., 2004; Kalyuzhnyi et al., 2006).

Traditionally, NH<sub>4</sub><sup>+</sup> is biologically removed via the nitrification/denitrification route, where NH<sub>4</sub><sup>+</sup> is aerobically converted into nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) and then anoxically to nitrogen gas (N<sub>2</sub>). However, with the discovery of biological conversion of NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> to N<sub>2</sub>, termed anaerobic NH<sub>4</sub><sup>+</sup> oxidation (Jetten et al., 1997; Mulder et al., 1995), new options for wastewater treatment arose. The potential benefits of N removal via this new route vs. the traditional nitrification/denitrification route are enormous: the requirements for oxygen are reduced 2-fold, and organic substrate is no longer required. Furthermore, the production of biosolids and alkalinity consumption are reduced approximately 10-fold per gram of N removed (Jetten et al., 2001; Schmidt et al., 2002; Strous et al., 1999).

However, the application of this N removal process can be limited by its inhibition by certain compounds. One of the most important inhibitors is oxygen, which reversibly inhibits anaerobic NH<sub>4</sub><sup>+</sup> oxidizing bacteria (AnAOB), but recovery after disturbance with oxygen can be achieved relatively fast (Jetten et al., 1999; Third et al., 2005). Also, elevated concentrations of NO<sub>2</sub><sup>-</sup> (100 g-N m<sup>-3</sup>) negatively impact anaerobic NH<sub>4</sub><sup>+</sup> oxidation (Strous et al., 1999). The inhibitory effect of NO<sub>2</sub><sup>-</sup> might be overcome only by addition of one of the process intermediates (Strous et al., 1999) and full recovery is observed only several weeks after NO<sub>2</sub><sup>-</sup> concentrations have returned below the detection limit (Third et al., 2005). Another important limitation is the low specific growth rate of these bacteria; it is estimated to be between 0.001 and 0.003 h<sup>-1</sup> (Jetten et al., 2001; van de Graaf et al., 1996), with few reports of higher rates (Isaka et al., 2005; Tsushima et al., 2007).

Application of anaerobic NH<sub>4</sub><sup>+</sup> oxidation for enhanced N removal requires a combination of aerobic and anaerobic conditions and has been studied in several different reactor systems: different combinations of two-stage processes (Kalyuzhnyi et al., 2006; van Loosdrecht and Salem, 2006; Wyffels et al., 2004), but also single-reactor systems, like sequencing batch reactors (Dapena-Mora et al., 2004; Jetten et al., 2001). Biomass retention is crucial due to the low growth rates of AnAOB, and biofilm reactors such as rotating biological contactors (Egli et al., 2003; Nielsen et al., 2005; Pynaert et al., 2004) have been successfully used for N removal via the anaerobic NH<sub>4</sub><sup>+</sup> oxidation route.

A new biofilm reactor concept, the membrane-aerated biofilm reactor (MABR), has evolved in recent years, and is being studied for its applicability to wastewater treatment. In such a system, oxygen is supplied through a gas-permeable

membrane that also serves as biofilm support. Applying this counter-diffusion concept, oxygen is provided to the base of the biofilm, whereas the substrate, in our case NH<sub>4</sub><sup>+</sup> and carbon, is supplied from the bulk liquid phase. The merits of such a system lie in the high and efficient oxygen transfer through the membrane (Ahmed et al., 2004) and also in the potential for a more amenable control strategy due to separation of oxygen and nutrient fluxes. Several studies have already employed such reactors for N removal via nitrification/denitrification (Satoh et al., 2004; Semmens et al., 2003; Terada et al., 2003).

Most previous studies of anaerobic NH<sub>4</sub><sup>+</sup> oxidation have focused on the autotrophic performance only. However, it has been shown experimentally that in autotrophic biofilms (even without any external carbon source) up to 50% of the biomass can be heterotrophic, supported by microbial decay products (Kindaichi et al., 2004; Okabe et al., 2005). In addition, very few researchers have tried to specify the effect of (influent) organic carbon on completely autotrophic NH<sub>4</sub><sup>+</sup> removal efficiency. A modeling study on completely autotrophic N removal in a biofilm reactor (Hao and van Loosdrecht, 2004) reports no negative effect of influent COD on removal performance. However, this study did not explicitly consider autotrophic decay products as a carbon source for heterotrophs.

Nevertheless, the presence of COD either from decay product hydrolysis or from organic carbon in the influent wastewater may significantly influence the microbial community composition in an otherwise autotrophic biofilm. Indeed, the competition between heterotrophs and autotrophs for terminal electron acceptors needs to be considered when the latter become limiting (Okabe et al., 1996). Knowledge is sparse on the effect of COD and heterotrophic activity, particularly on membrane-aerated biofilm systems for completely autotrophic N removal. This prompted our study, which had three aims: (i) to study the influence and importance of COD originating from autotrophic decay products on performance and community structure of a membrane-aerated biofilm vs. a conventional biofilm system; (ii) to clarify the impact of influent COD (COD/N ratio) on steady-state performance (removal efficiency); (iii) to investigate the influence of the extent and duration of perturbations of the influent COD/N ratio on performance.

## 2. Model development

A 1-D multipopulation biofilm model was constructed using the simulation software Aquasim 2.1d (Reichert, 1998). The model considered a completely mixed biofilm compartment and a completely mixed gas compartment that was connected to either the bottom (counter-diffusion geometry) or the bulk liquid (co-diffusion geometry) of the biofilm compartment by a diffusive mass transfer link (Terada et al., 2007). The liquid volume was 2.5 L, the volume of the gas compartment 0.1 L. The biofilm support had a cylindrical geometry and a specific surface area  $a$  of 250 m<sup>2</sup> m<sup>-3</sup>, intended to mimic the geometries of a lab reactor system. The total membrane oxygen mass transfer coefficient was set at 1.5 m d<sup>-1</sup> (typical for a silicone membrane (Casey et al.,

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