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## Membrane-aerated Nitrifying Biofilms: Continuous versus Intermittent Aeration

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### ABSTRACT

This study evaluated the process performance of a lab-scale membrane-aerated nitrifying biofilm under continuous versus intermittent aeration regimes. Effects of intermittent aeration on the competition between individual microbial communities and the emission of nitrous oxide (N<sub>2</sub>O) were specifically studied. The principle observation under continuous aeration was more efficient ammonium removal (4.3 gNH<sub>4</sub><sup>+</sup>-N/m<sup>2</sup>/day) but also higher N<sub>2</sub>O emission (2.9% of the N loading) and minor anaerobic ammonium oxidizer (AMX) activity compared to intermittent aeration (3.1 gNH<sub>4</sub><sup>+</sup>-N/m<sup>2</sup>/day, 0.3% of the N loading). AMX activity increased at the expense of decreasing nitrite oxidizer (NOB) activity with intermittent aeration. Dissolved oxygen and pH microprofiles under each aeration regimes revealed that the dynamic variation of pH relevant effects could be the potential causes to these different performances. A high intermittency in aeration favors the suppression of NOB with positive effects on N<sub>2</sub>O emission reduction.

### MATERIALS & METHODS

#### Reactor operation and N mass balance analysis

The lab-scale membrane-aerated biofilm reactor (MABR) used PDMS membranes (3100506, Labmarket, Germany) and was inoculated with nitrifier-enriched biomass. Synthetic wastewater was fed with an influent NH<sub>4</sub><sup>+</sup> concentration of 75 mgNH<sub>4</sub><sup>+</sup>-N/L providing a surface loading of 9 gN/m<sup>2</sup>/day. The reactor was operated with continuous aeration (100% air) until effluent N concentrations remained stable for several HRTs (hydraulic retention time). Then MABR was switched to intermittent aeration consisting of a 6-hour aeration (100% air) followed by a 6-hour non-aeration (100% N<sub>2</sub>). Bulk N species were recorded; N<sub>2</sub>O was measured in the bulk liquid phase, in the lumen gas phase and within the biofilm. Bulk DO and pH were monitored with electrodes, and profiles of pH, DO, and N<sub>2</sub>O within the biofilm under each aeration regime were measured with micro-electrodes.

Consumption/production of individual N species was calculated using mass balance equations. Anammox process was assumed to follow reported stoichiometry (Strous, 1998). Heterotrophic denitrification was assumed to be supported solely by organic carbon derived from endogenous biomass decay (no external COD in the influent) and solely with NO<sub>3</sub>-N as electron acceptor.

$$\text{NH}_4^+_{\text{AOB}} + \text{NH}_4^+_{\text{AMX}} = \text{NH}_4^+_{\text{in}} - \text{NH}_4^+_{\text{out}} \quad (1)$$

$$\text{NH}_4^+_{\text{AOB}} - \text{NO}_2^-_{\text{NOB}} - 1.32 \text{NH}_4^+_{\text{AMX}} = \text{NO}_2^-_{\text{out}} \quad (2)$$

$$\text{NO}_2^-_{\text{NOB}} + 0.26 \text{NH}_4^+_{\text{AMX}} - \text{NO}_3^-_{\text{HB}} = \text{NO}_3^-_{\text{out}} \quad (3)$$

$$\text{NH}_4^+_{\text{AOB}} * Y_{\text{AOB}} + \text{NO}_2^-_{\text{NOB}} * Y_{\text{NOB}} + \text{NH}_4^+_{\text{AMX}} * Y_{\text{AMX}} = 2.86 \text{NO}_3^-_{\text{HB}} / (1 - Y_{\text{HB}}) \quad (4)$$

where NH<sub>4</sub><sup>+</sup><sub>AOB</sub>, NH<sub>4</sub><sup>+</sup><sub>AMX</sub>, NO<sub>2</sub><sup>-</sup><sub>NOB</sub>, NO<sub>3</sub><sup>-</sup><sub>HB</sub> (mgN/L) are NH<sub>4</sub><sup>+</sup>-N consumption by AOB, NH<sub>4</sub><sup>+</sup>-N consumption by AMX, NO<sub>2</sub><sup>-</sup>-N consumption by NOB, NO<sub>3</sub><sup>-</sup>-N consumption by HB, respectively.

## Microprofiling measurements and quantitative PCR

Under each aeration regimes commercially available DO, pH and N<sub>2</sub>O microsensors (Unisense, Denmark) were used for replicate (n > 3) *in situ* profiling within the biofilm at steady state. Biomass samples were collected at the end. Fractions of different phylogenetic groups (approximating functional guilds) in the inoculum and biofilm were measured using quantitative PCR assays.

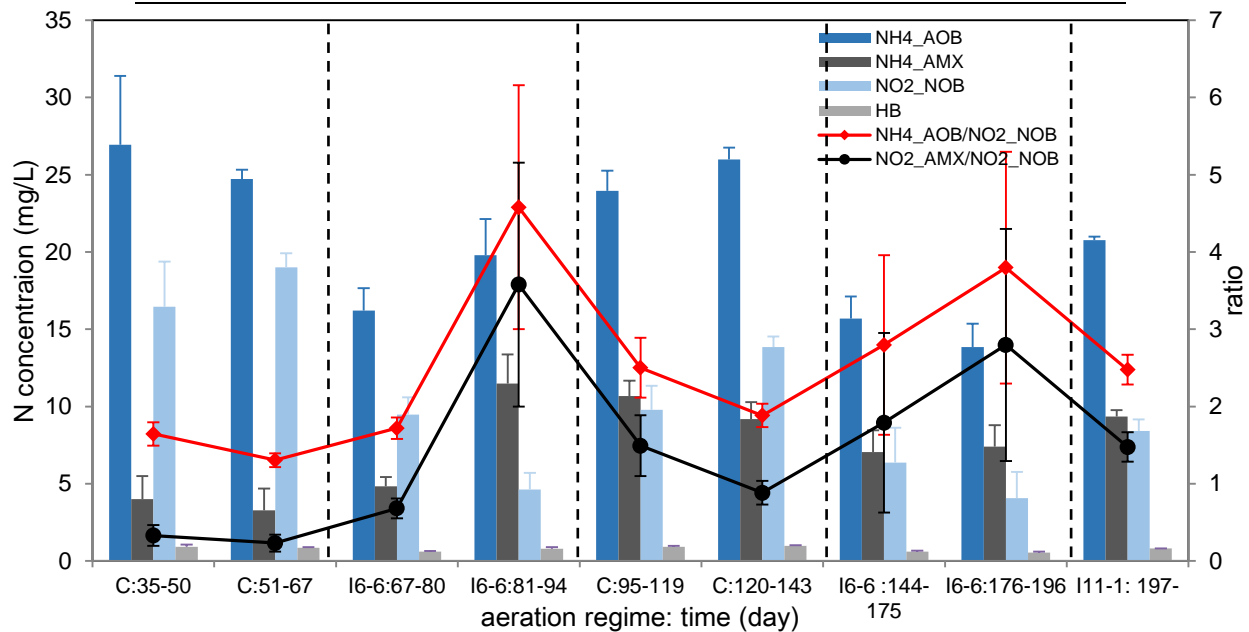
## RESULTS & DISCUSSION

### Process performance and N consumption under different aeration regimes

A nitrifying biofilm readily developed when the MABR was initially operated under continuous aeration: NO<sub>3</sub><sup>-</sup> was the dominant NO<sub>x</sub><sup>-</sup>-N species in the effluent and NO<sub>2</sub><sup>-</sup> was at or below 1 mg/L after 2 months operation. N consumptions by individual microbial groups were calculated to estimate relative microbial activities (Figure 1). The ratio of NH<sub>4</sub><sup>+</sup>-AOB to NO<sub>2</sub><sup>-</sup>-NOB reflects the oxygen competition between AOB and NOB: it was 1.31 at day 50-67 suggesting active NOB activity with continuous aeration. The ratio of NO<sub>2</sub><sup>-</sup>-AMX to NO<sub>2</sub><sup>-</sup>-NOB reflects the NO<sub>2</sub><sup>-</sup> competition between NOB and AMX: it was 0.26 showing minor AMX activity. Total N<sub>2</sub>O emission in the gas plus liquid phase under continuous aeration was ca. 2.91% of the total N loading (Table 1).

**Table 1.** Aeration changes during MABR operation and N<sub>2</sub>O emission

time (days)	Aeration regime	N <sub>2</sub> O emission (% of N loading)	
		Bulk phase	Gas phase
50-67	Continuous	1.54	1.37
68-95	Intermittent 6+6	0.01	0.34
96-143	Continuous	0.22	Not measured
144-196	Intermittent 6+6	0.07	0.11
197-	Intermittent 11+1	Not measured	Not measured



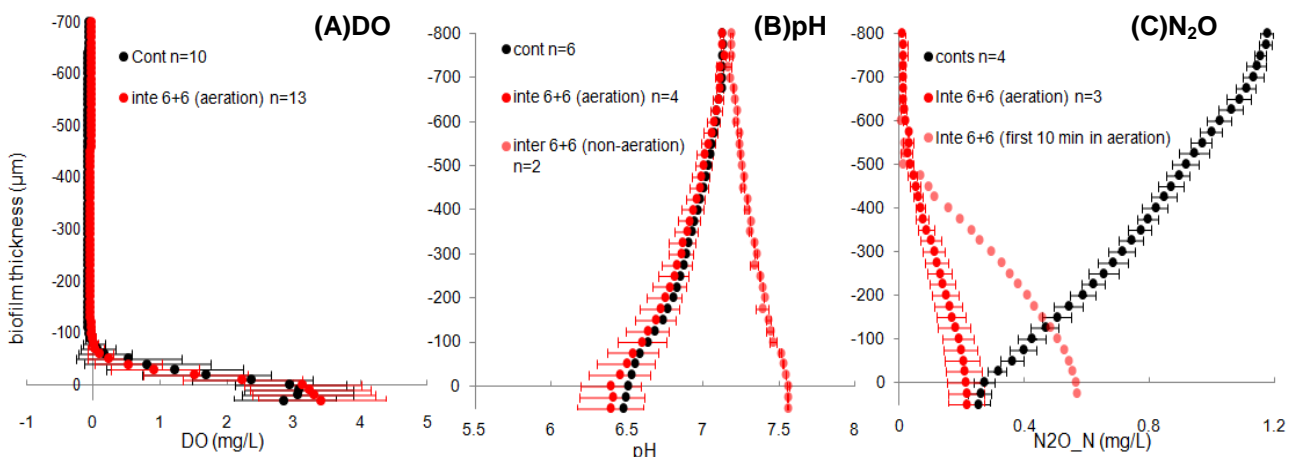
**Figure 1.** Calculated N consumption by individual microbial groups under different aeration regimes.

At day 67, MABR was switched to intermittent aeration (6-hour aeration and 6-hour non-aeration).

NOB activity was suppressed significantly after 2 weeks with increasing ratios of  $\text{NH}_4^+$ \_AOB/ $\text{NO}_2^-$ \_NOB and  $\text{NO}_2^-$ \_AMX/ $\text{NO}_2^-$ \_NOB. While the oxygen loading was reduced 50% under intermittent aeration,  $\text{NH}_4^+$ \_AOB reduced only by 46~23%, which is probably due to the gradually activated AMX thus prompting AOB activity and enhancing oxygen transfer rate. Moreover,  $\text{N}_2\text{O}$  emission decreased to 0.35% of the total N loading (Table 1, Figure 2C).

### Potential causes of NOB suppression and $\text{N}_2\text{O}$ mitigation in intermittent aeration

Competition between AOB and NOB can be caused by DO and pH conditions, involving such phenomena as DO limitation, pH effects on enzyme activities and pH effects on substrate speciation (free ammonia, FA and free nitrous acid, FNA) (Park, 2015). Therefore, DO and pH microprofiles in the biofilm were compared between continuous and intermittent aeration to further evaluate variations of each influencing factors under. The main observations are: 1. DO profiles in the biofilms were insignificantly different between continuous aeration and during aeration phases in intermittent aeration, in terms of interface oxygen concentrations and oxygen penetration depths (Figure 2A). After aeration transitions in intermittent aeration, DO profiles in the biofilm became time-invariant within 3 minutes (data not shown); 2. pH profiles during the aeration phases in intermittent aeration were similar to profiles in continuous aeration (Figure 2B). However, during non-aeration pH upshifts at the deeper biofilm depths were significant and pH profiles became steady after 30 minutes in the following aeration phase (data not shown). We conclude that the pH shifts, and especially its effect on FA/FNA speciation, was the key factor leading to NOB suppression in intermittent aeration. Once NOB were suppressed at the biofilm base,  $\text{NO}_2^-$  became available for AMX growth in the outer anoxic biofilm layers. Bulk  $\text{NO}_2^-$  concentrations were below detection limit once intermittent aeration started, which suggested that the  $\text{N}_2\text{O}$  mitigation was related to  $\text{NO}_2^-$  absence. Effects of various patterns of intermittent aeration on NOB suppression,  $\text{N}_2\text{O}$  emission, and community composition are being tested.



**Figure 2.** Comparison of microprofiles under continuous (day 51-67) and intermittent (day 81-94) aeration regimes.

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