Membrane aerated biofilm reactors for mainstream partial nitritation/anammox: Experiences using real municipal wastewater

Philipp Bunse*, Laura Orschler, Shelesh Agrawal, Susanne Lackner

Department of Civil and Environmental Engineering Sciences, Institute IWAR, Chair of Wastewater Engineering, Technical University of Darmstadt, Germany

**Abstract**

This study investigated the potential of Membrane-Aerated Biofilm Reactors (MABRs) for mainstream nitrogen removal via partial nitritation/anaerobic ammonium oxidation (anammox). Four laboratory-scale MABRs were operated with real municipal wastewater characterized by low concentrations of nitrogen (varying between 31 and 120 mg-NH4-N L-1) and the presence of biodegradable organic carbon (soluble COD (sCOD) between 7 and 230 mg-O2 L-1). Two reactors were operated with different aeration strategies (intermittent vs. continuous), the other two with differences in biomass retention (recirculation or removal of detached biomass). Keeping a constant HRT caused instabilities due to difficulties with setting the optimal oxygen flux for the respective surface loadings (1.6–6 g-NH4-N m-2 d-1). Operating the MABRs with a constant surface loading (2 g-NH4-N m-2 d-1) resulted in higher and more stable total nitrogen (TN) removal independent of the aeration strategy. The intermittently aerated MABR improved from an average TN removal of 23%–69%, the continuously aerated MABR from 20% to 50% TN removal. Independent of the feeding strategy, the continuously aerated reactor removed slightly more ammonium (80–95%) compared to the intermittently aerated reactor (74–93%).

Limiting the oxygen supply by intermittent aeration proofed successful to favor partial nitritation and anammox. Continuous aeration did not achieve stable suppression of nitrite oxidizing bacteria (NOB). Of the removed ammonium, approx. 26% were left in the effluent as nitrate (only 10% with intermittent aeration).

Recirculation of the detached biomass resulted in reattachment onto the biofilm or membrane surface. This recirculation led to significantly higher biomass retention times and thus to better performance. Removing detached biofilm from the reactor caused a slightly lower TN removal of 33% compared to 45% with reattachment, while average ammonium removal was 58% compared to 63%, respectively. Scouring events had a significant impact on the overall operation, resulting in short term losses of TN removal capacities of 50–100%.

The microbial community composition was different depending on the aeration strategy and biomass retention. The continuously aerated reactor contained significantly more AOB than the intermittently aerated MABR. The reactor with biomass retention contained less ammonium oxidizing bacteria (AOB), compared to the reactor with low biomass retention. In all MABRs, anammox bacteria established in the biofilm after an initial drop in abundance.

© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**1. Introduction**

The membrane-aerated biofilm reactor (MABR) concept relies on biofilms growing on a permeable membrane and oxygen supply at the bottom of the biofilm, whereas the other substrates, i.e. ammonium or organic carbon are supplied from the liquid bulk phase to the top of the biofilm. This so called counter-diffusion concept has already been successfully implemented for Nitritification and Denitritification (N/DN) (Hibiya et al., 2003; Satoh et al., 2004) and for Partial Nitritation and Anammox (PN/A) in lab-scale with synthetic feed and without organic carbon (Gilmore et al., 2013; Pellicer-Nacher et al., 2010). However, there are no studies yet which have explored MABRs for PN/A using real municipal wastewater and test these systems under mainstream conditions, i.e. treating municipal wastewater with low ammonium concentrations.
Lackner et al. (2008) revealed that for COD:N ratios of 2 or higher, AnAOB was additionally equipped with 200 and 80 LMT-55 tubes (ID 2.29 mm) provided the feed. Complete mixing of lab-scale MABRs supplied by SUEZ (SUEZ Water Technologies & Solutions, Oakville, ON, Canada) with a height of 1 m including one bundle of ZeeLung® membrane cords were used in this study. The cords with an effective length of 1 m and a diameter of 1.2 mm were made of oxygen-permeable hollow fiber membranes distributed around the circumference of a yarn reinforcement core. A schematic of the reactor setup is provided in Fig. 1. Reactors MABR 1 and MABR 2 had an operating volume of 0.91 L and a surface area of 0.034 m², reactors MABR 3 and MABR 4 had a volume of 4.1 L and a surface area of 0.151 m², resulting in a specific surface area of 37 m² m⁻³ for all four reactors.

A peristaltic pump (MABR 1 and MABR 2: Ismatec REGLO Digital; MABR 3 and MABR 4: Ismatec REGLO Analog, Cole-Parmer GmbH, Wertheim, Germany) equipped with Ismatec Tygon® LMT-55 tubes (ID 2.29 mm) provided the feed. Complete mixing of the bulk liquid was ensured by external recirculation pumps (Eheim Universal pump 1200, EHEIM GmbH & Co. KG, Deizisau, Germany) to reduce substrate gradients in the liquid phase.

Aeration of the membrane lumen was performed with compressed air and controlled by pressure limiters. Dinitrogen gas was used to remove residual oxygen from the membrane lumen at the start of every non-aerated phase. Aerated and non-aerated phases were controlled by solenoid valves connected to a timer.

MABR 1 and MABR 2 were continuously monitored with ion-selective electrodes (ISE) for ammonium and nitrate including a pH and temperature reference (Endress+Hauser, ISEmax CAS40D, Endress+Hauser Messtechnik GmbH+Co. KG, Weil am Rhein, Germany), MABR 3 and MABR 4 were equipped with pH sensors (Endress+Hauser, Memosens CPS171D, Endress+Hauser Messtechnik GmbH+Co. KG, Weil am Rhein, Germany). DO was alternately checked on a regular base in all reactors with amperometric sensors (Endress+Hauser, Oxymax COS22D, Endress+Hauser Messtechnik GmbH+Co. KG, Weil am Rhein, Germany). MABR 4 was additionally equipped with 200 and 80 µm sieves in the recirculation line to remove suspended biomass for sludge retention control.

For the reactor startup and initial biofilm formation, MABR 1 and MABR 2 were inoculated with 150 mL activated sludge (total suspended solids (TSS) = 1.06 g L⁻¹) from a local municipal WWTP in Germany; MABR 3 and MABR 4 were inoculated with 200 mL suspended biomass (TSS = 5.63 g L⁻¹) from a DEMON® sidestream sequencing batch reactor in Germany.
the feed was cooled to ~6 °C. To prevent biological activity in the storage tank, sieves with mesh sizes of 160 and 100 µm were used to reduce the load of particulate matter and protect the lab equipment. Aeration was controlled with magnetic valves driven by a timer and pressure adjustments.

2.1.2. Feed origin

Raw wastewater (only mechanical pre-treatment without primary settling) from the local municipal WWTP was collected regularly from a buffer tank (HRT 0.5 h) and pretreated in the laboratory prior to use. Sieves with mesh sizes of 160 and 100 µm were used to reduce the load of particulate matter and protect the lab equipment. To prevent biological activity in the storage tank, the feed was cooled to −6 °C and renewed two times per week. An overview over the feed concentration and sCOD:N ratio is given in Fig. 2.

2.1.3. Reactor operation

Table 1 summarizes the operational settings for all four MABRs. The reactors operated at room temperature and no pH control was applied. In the first 183 days of operation, the hydraulic retention time (HRT) of MABR 1 and MABR 2 was kept constant at 0.5 days comparable to Wei et al. (2012). After 183 days, the feeding strategy was switched to a constant ammonium surface load of approximately 2 g−NH4−N m⁻² d⁻¹. This surface load was adjusted manually with each fresh feed batch by measuring the ammonium concentration of the batch and setting the feed pump to the desired flow rate. MABR 1 operated with intermittent aeration while MABR 2 operated with continuous aeration.

MABR 3 and MABR 4 already started with a constant load of approximately 2 g−NH4−N m⁻² d⁻¹ and ran for more than 183 days. Both reactors were aerated intermittently, identical to the aeration pattern and pressure of MABR 1. The operation of MABRs 3 and 4 started when MABRs 1 and 2 had already been operated for 183 days. From this time onwards, all four reactors were fed with the same feed composition and surface load. Aeration of all intermittently aerated reactors (MABR 1, 3 and 4) was adjusted to the same intervals and pressures.

Scouring events were induced after taking biomass samples for molecular analysis. Shear force was increased by blowing dinitrogen gas into the bottom of the reactor. Dinitrogen gas was used instead of air to prevent large amounts of dissolved oxygen entering the bulk liquid phase. The biomass detached during scouring was not removed intentionally in MABRs 1−3 and only left the system via the effluent. In MABR 4, the detached biomass was almost directly removed with the sieves in the recirculation line (see section 2.2.1).

2.2. Oxygen transfer rate (OTR)

The oxygen transfer rates of the clean membranes were determined with tap water in MABR 1 prior to inoculation with biomass. Sodium sulfate (Na₂SO₃) was used to remove the oxygen from the water bulk before pressurizing the membrane with compressed air at different inlet pressures. Experiments were carried out with a backpressure of half the inlet pressure or with an open-end configuration (ambient pressure at gas exhaust). DO was measured with amperometric sensors (Endress + Hauser, Oxymax COS22D, Endress + Hauser Messtechnik GmbH + Co. KG, Weil am Rhein, Germany).

Further, the OTR was estimated after some time of operation with existing biofilm in the membranes in all reactors. To measure the OTR with biofilm on the membrane the outflow oxygen concentration and airflow volume were measured and the OTR was calculated from the measured values. The oxygen concentration in the gas phase was measured with an oxygen partial pressure sensor (Greisinger GMH3695 with Greisinger GGO381 sensor, GHM Messtechnik GmbH, Regenstauf, Germany). Detailed calculations of the OTR are included in the supplementary information.

2.3. Physicochemical analyses (offline)

Physicochemical analyses of the feed and effluents were conducted to determine concentrations of the relevant parameters. Cuvette tests (Hach Lange GmbH, Berlin, Germany) were used for sCOD, Ion Chromatography (Metrohm 930 Compact IC Flex, Metrohm AG, Herisau, Switzerland) was used for nitrite and nitrate, and the DIN 38406-5 standard (German Institute for Standardization, 1983) was followed for ammonium. Every sample was filtered with 0.45 µm syringe filters before further analysis. Samples were stored in the fridge at −5 °C if not analyzed directly. Samples for ammonium were acidified (20 µl nitric acid (HNO₃) per 10 ml sample) to stabilize the ammonium before storage. Suspended solids were determined by filtration of the sample through glass fiber filters according to EN 872:2005 (British Standards Institution, 2005).

2.4. Molecular analyses of the biomass

Samples for qPCR were taken regularly at three positions over the complete height of the fiber bundles (top, center and bottom). Biomass was carefully scraped off from the fibers after removing them from the reactors. Biomass samples were stored at −80 °C prior to analysis. Total genomic DNA was extracted from the samples using the Fast DNA Spin kit for soil (MP Biomedicals, USA). The DNA concentration and its integrity were analyzed with the Qubit
3.0 Fluorometer with the Qubit dsDNA HS kit (Thermo Fisher Scientific). Each qPCR reaction mixture contained 12.5 μL PerfeCTa SYBR® Green SuperMix 2X (QuantaBio), 5 μL DNA template (10 ng μL⁻¹), water (PCR grade) and 0.5 μL of each primer (10 μM) as shown in Table SI1.

3. Results and discussion

3.1. Oxygen transfer

To achieve PN/A through oxygen flux control (limiting oxygen availability), effective OTR control is required. The total oxygen transfer through the membrane at defined pressures was first determined without biofilm and in tap water to estimate the OTR of the reactor systems. The applied inlet pressure ranged from 0 to 0.4 bar relative pressure and the results of these OTR experiments are shown in Fig. SI 1, A (given relative to the membrane surface area). With clean membranes and tap water, the OTR reached values of up to 8 g-O₂ m⁻² d⁻¹.

However, the experiments with clean membranes are problematic because several researchers already determined much higher OTRs when biofilm was present on the membrane (Casey et al., 2000; Jacome et al., 2006; Lackner et al., 2010; Shanahan and Semmens, 2006). Under operating conditions with biofilm on the membrane, the oxygen transfer rate reached values between 15 and 25 g-O₂ m⁻² d⁻¹. Even with low input pressure (0.1 bar), the OTR was almost double the OTR with high pressure (0.6 bar) in the clean system (Fig. SI 1, B).

OTR tests with backpressure showed a linear increase of the OTR. The open-end configuration showed higher OTRs with low pressure compared to the operation with backpressure. The relationship between pressure and airflow was proportional. The open-end configuration showed a significantly higher airflow and a lower oxygen gradient in the gas phase (0.1 bar: 5.7 ml min⁻¹, ΔO₂: 4%; 0.2 bar: 11.9 ml min⁻¹, ΔO₂: 2.25%) compared to the operation with backpressure (0.1 bar: 1.43 ml min⁻¹, ΔO₂: 8.75%; 0.2 bar: 2.7 ml min⁻¹, ΔO₂: 8.25%). This behavior showed that, especially with low pressure, the airflow had a significant influence on the oxygen transfer rate.

Important to note is, that the reactors did not operate with the maximum possible oxygen surface load of up to 25 g-O₂ m⁻² d⁻¹. The oxygen surface load was lower due to the non-aerated phases in the intermittently aerated reactors and low input pressure in the continuously aerated reactor (MABR 2). The values were adjusted to fit the respective substrate surface load while keeping the liquid bulk phase anoxic. The applied oxygen surface loads for the intermittently, as well as the continuously aerated reactors are provided in Table SI2.

3.2. Reactor startup and operation

Startup times of ~30 days with activated sludge and ~20 days with PN/A sludge were achieved to reach ammonium removal rates above 80%, and this is consistent with other studies (Augusto et al.,...
Using real municipal wastewater resulted in fluctuations of the feed composition. The ammonium concentration varied between 31 and 120 mg NH₄⁺-N L⁻¹ (average 57 mg NH₄⁺-N L⁻¹) while the soluble chemical oxygen demand (scCOD) varied between 7 and 230 mg O₂ L⁻¹ (average 82 mg O₂ L⁻¹) as shown in Fig. 2. The scCOD:N ratio varied between 0.13 and 5.5 (average 1.44). The readily biodegradable COD (rbCOD) accounted for approximately 60%, estimated by comparing the influent scCOD concentrations to data of the final effluent of the WWTP (assuming the scCOD leaving the activated sludge tank is the non-biodegradable fraction). Overall, the average TN removal per surface area and day of 0.5–1.4 g-N m⁻² d⁻¹ and the maximum TN removal rates of 2.2 g-N m⁻² d⁻¹ (3.4 g-N m⁻² d⁻¹ with high surface loads in Phase 1) reached values comparable to other MABR systems (Gilmore et al., 2013; Hibiya et al., 2003).

The theoretical TN removal via the anammox pathway was estimated considering either aerobic Heterotrophs (aHet), Nitrification-Denitrification (N/DN) or Nitritation-Denitrification (Ni/DNi) as the sink for scCOD (calculations are provided in the supplementary information). These estimates allow some insight into the possible TN removal activity via anammox with the maximum potential sCOD removal rates decreased. Thus, the estimates of the potential anammox activity (Fig. SI8, Fig. SI9) and less TN removal occurred via N/DN (MABR 1: 11%, MABR 2: 12%) or Ni/DNi (MABR 1: 18%, MABR 2: 21%). This data, however, only allows a rough estimation about the potential anammox activity in the MABRs, as the calculated removal pathways are influenced by the actual scCOD removal.

3.2.2. MABR 1 and MABR 2: intermittent vs. continuous aeration

MABR 1 and MABR 2 operated with different aeration patterns, which resulted in different behavior and reactor performance. MABR 1 was aerated intermittently employing a cycling pattern with a short aeration phase (5–10 min, 0.4 bar aeration pressure), followed by a nitrogen flush (1 min) and a longer non-aerated phase (20–25 min). The length of the aerated and non-aerated phase varied depending on the feed composition to convert as much ammonium as possible and to suppress NOB growth and therefore nitrate production. MABR 2 was aerated continuously with low pressure (0.05–0.2 bar) to limit the oxygen availability. For both aeration strategies, the aim was a maximum TN removal and with a low use of oxygen. Therefore, the pressure and timings were adjusted to the surface loads resulting in 4–10 g-O₂ m⁻² d⁻¹ for intermittent aeration and 8–16 g-O₂ m⁻² d⁻¹ for continuous aeration (Fig. SI2).

The oxygen to substrate ratio was calculated based on the aeration settings and ammonium load (Fig. 3, B). Due to the variability in the feed composition, the ratio of oxygen to nitrogen fluxes in MABR 1 and MABR 2 was between 0.7 and 5 g-O₂ per g NH₄⁺-N in the first phase (days 0–183). From day 183 onwards, the nitrogen load was kept constant at approximately 2 g NH₄⁺-N m⁻² d⁻¹. Therefore, the need of oxygen for ammonium conversion was more stable and this made a more sustainable adjustment of aeration pressure and timings possible.

Intermittent aeration resulted in a cyclic change between high oxygen availability and low/no oxygen availability at the base of the biofilm which is representative shown by the off gas oxygen concentration (Fig. SI3) measured directly at the exhaust of the membrane bundle. With an immature biofilm, these conditions can be monitored in the bulk liquid (with iSE) showing a cycling pattern of ammonium reduction and accumulation depending on the oxygen availability (Fig. SI4). This, conversely, also applies to nitrate. With a mature biofilm, the direct effects of aeration were no longer visible in the bulk liquid (Fig. SI5).
Intermittent aeration seemed to be an effective tool for higher nutrient removal rates in MABR 1 because average nutrient removal rates exceeded the nutrient removal rates of MABR 2. After longer operation (>270 days), the average nitrogen removal of MABR 1 (~70%) was twice as high as the one in MABR 2 (~32%). The absence of anoxic conditions in the majority of the biofilm caused by continuous aeration might have been one possible explanation for this observation. The lack of anoxic phases caused limited potentials for denitrification, denitrification and anammox activity and led to an uncontrolled growth of NOB, and after all unwanted nitrate production. With the intermittent aeration, the biofilm was completely anoxic at certain times, which favored either denitrification, denitrification or anammox and at the same time limited NOB activity. This result coincides with the observations of Ma (2018) who operated MABRs with synthetic feed without organic carbon and found that NOB suppression occurred under intermittent aeration but not under continuous aeration.

Overall, the ammonium removal in the continuously aerated reactor was slightly higher (MABR 2: 80–92%) than in the intermittently aerated reactor (MABR 1: 74–89%) (Table 2). In Phase 1, the TN removal of MABR 1 and MABR 2 was not significantly different (0.4–1.4 g-N m⁻²d⁻¹ and 0.3–1.5 g-N m⁻²d⁻¹, respectively). In Phase 2, MABR 1 (1.4–1.8 g-N m⁻²d⁻¹) outperformed MABR 2 (0.2–1.0 g-N m⁻²d⁻¹) in terms of TN removal (Table 2). In terms of sCOD removal, no significant difference between intermittent and continuous aeration was observable as summarized in Fig. 4: A-B, except a longer period for regaining sCOD removal performance for MABR 2 after scouring on day 257.

Scouring events had a big impact on the performance as shown by the loss of TN removal activity (Fig. 3: C–F). Especially after the scouring event on day 257, the nitrogen removal performance of MABR 2 could not be restored within a short period. The performance of MABR 2 only started to recover towards the end of the experimental phase around day 313.

### 3.2.3. MABR 3 and MABR 4: influence of biofilm age on reactor performance

The operation of MABR 3 and 4 started only after MABR 1 and MABR 2 had already been operating for 183 days. The feed was provided with a constant load of approximately 2 g-NH₄-N m⁻² d⁻¹. With MABR 3 and 4, the focus was on biomass retention, and due to the positive experience with MABR 1, these systems were also operated with intermittent aeration employing the same patterns and pressures as in MABR 1 at the respective times. The setup, i.e. inoculum biomass and operating time, differed from the other reactors, which made a direct comparison somewhat difficult. MABR 3 operated with a higher biofilm age accomplished by the biomass retention in the system and reattachment of detached biomass. In MABR 4 the biofilm age was lower due to sieving and effective removal of detached biomass, which prevented reattachment or entrapment of the biomass.

While the ammonium removal was almost equal in both reactors with 49–79% in MABR 3 and 49–75% in MABR 4, both reactors could not keep up with the performance of MABR 1 (78–89%) in the same operating phase (Table 3). The TN removal rate was slightly higher with higher biofilm age of 0.6–1.2 g-N m⁻²d⁻¹ in MABR 3 compared to MABR 4 with 0.3–1.1 g-N m⁻²d⁻¹. Further, the average TN removal was higher (40–50%) than with continuous aeration in MABR 2, but lower than in MABR 1. After the scouring event on day 257, MABR 4 needed more time to regain its TN and sCOD removal performance. In terms of sCOD removal, there was no further difference between both reactors as summarized in Fig. 4: C-D.

Calculating the potential TN removal via the anammox pathway in MABR 3 and MABR 4, the results show a clear difference between both reactors (Fig. 8). MABR 3 showed higher TN removal rates through the anammox pathway from the start (~0.9 g-N m⁻²d⁻¹) compared to MABR 4 (~0.7 g-N m⁻²d⁻¹) (Fig. 9, 183–270 days). The differences became clearer after prolonged operation, because potential TN removal through the anammox pathway MABR 3 increased to ~1.0 g-N m⁻²d⁻¹ while MABR 4 remained at the same level (Fig. SI 9, 270–360 days). Estimating the percentage share of anammox on the TN removal, the values indicated potentially high anammox activity and an almost equal percentage of TN removal via N/DN (MABR 3: 14%; MABR 4: 15%) or Ni/DNi (MABR 3: 23%; MABR 4: 25%).

The amount of biomass in the biofilm was estimated assuming an average biofilm thickness of 500 μm and a biomass density of 40 kg m⁻³. This resulted in 4.17 g of total biomass in MABR 3 and 4.27 g in MABR 4. With the measured values of effluent total suspended solids (TSS) and sieved solids of MABR 4, the sludge removal (~200 mg-TSS d⁻¹) and approximate biofilm age (~120 days) were calculated (Fig. 5). Within the first weeks after inoculation the effluent TSS concentrations were higher due to detachment and washout of inoculum biomass, with approximately 38 mg TSS L⁻¹ for both reactors (Fig. SI 7). From day 230 onwards, the effluent TSS reduced to ~4–5 mg-TSS L⁻¹. The reactor TSS was the same as the effluent TSS due to good mixing of the liquid phase and low settling ability of the solids. On average, the daily-sieved solids of MABR 4 had a mass of ~0.16 g (Fig. SI 7). With these values, the sludge ages in MABR 3 and 4 were estimated to maximum values of 148 and 22 days, respectively (Fig. 5).

The observed difference between the system with high biomass retention (MABR 3) and the system with low biomass retention (MABR 4) were small. Ammonium and TN removal of both systems were almost equal (~60–70%) with slightly higher values for MABR 3 compared to MABR 4. This slightly better performance of MABR 3 could be attributed to the higher biomass age and amount of biomass in the reactor, because of the reattachment of detached biofilm. Additionally, MABR 3 showed shorter recovery periods after scouring events, which can be attributed to the higher biomass age as well as the reattachment of biomass. Theoretically, systems with suspended biomass retention should have higher TN removal potential, as detached biomass can remain in the anoxic
Fig. 3. Summary of Operational Parameters and Results. A: NH$_4^+$–N and COD surface load of MABRs. B: NH$_4^+$–N related Oxygen Flux of the two different aeration strategies. C–F: Surface NH$_4^+$–N removal and percentage TN removal of MABR 1–4. Red lines indicate the scouring events and sampling points for qPCR analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
bulk liquid, performing denitrification, denitriﬁcation and/or anammo. Divided from the temporarily aerobic bioﬁlm, the suspended biomass is not inhibited by oxygen. To examine the difference between a hybrid system and a pure bioﬁlm system in more detail, the bioﬁlm reactor should be coupled with a suspended sludge reactor in the recirculation line to provide a higher volume for the suspended biomass vs. the membrane surface.

3.3. Bioﬁlm composition

The abundance of total eubacteria (EUB), AOB, AnAOB and NOB (i.e. *Nitrobacter* and *Nitrospira*) was monitored using qPCR in all four reactors. The microbial community composition in MABR 1 and MABR 2 (intermittent vs. continuous aeration) as well as in MABR 3 and MABR 4 (with and without biomass retention) evolved similarly. In MABR 1 and MABR 2 the abundance of AOB and AnAOB recovered (i.e. reaching values similar to the inoculum) after

---

**Table 3**

Performance of MABR 3 and 4 as average (25th – 75th percentile) and maximum surface specific nitrogen removal rates.

|                  | **NH₄⁻N removal** |                  | **TN removal** |
|------------------|-------------------|------------------|----------------|
|                  | **Average**       | **Maximum**      | **Average**    | **Maximum**    |
|                  | g–NH₄–Nm⁻² d⁻¹ % | g–NH₄–Nm⁻² d⁻¹ % | g–Nm⁻² d⁻¹ %  | g–Nm⁻² d⁻¹ %  |
| MABR 3 Phase 2   | 1.1 – 1.6         | 49 – 79          | 2.7            | 99.8           |
|                  |                   |                   | 0.6 – 1.2      | 26 – 59        |
| MABR 4 Phase 2   | 0.9 – 1.5         | 49 – 75          | 2.6            | 98.8           |
|                  |                   |                   | 0.3 – 1.1      | 17 – 56        |

---

Fig. 4. sCOD surface load (black dots) and removal (circles) of MABR 1–4 combined with the percentage removal (grey bars). Red lines indicate the scouring events and sampling points for qPCR analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
approximately 260 days from their initial drop after inoculation (Fig. 6). *Nitrobacter* showed no clear trend whereas the concentration of *Nitrospira* increased over time in both, MABR 1 and MABR 2 (Data provided in Table SI2). The AnAOB populations of MABR 3 and MABR 4 almost recovered to their initial abundance after around 100 days (Fig. 7). In MABR 3, the abundance of *Nitrobacter* and *Nitrospira* decreased during the reactor operation. In MABR 4, *Nitrobacter* and *Nitrospira* behaved differently — *Nitrobacter* decreased, and *Nitrospira* increased. Although in all four reactors an initial loss of AnAOB was observed, the operational conditions supported the growth of AnAOB and allowed the establishment of AnAOB in the MABR biofilm.

Pellicer-Nacher et al. (2014) observed significant shifts in the microbial community after switching the MABR (high nitrogen load without COD) from continuous aeration (with almost full ammonium to nitrate conversion) to sequential aeration, resulting in an overall decrease of NOB and increase of AnAOB abundance in the biofilm. In this study, the different aeration strategies did not affect the abundance of NOB and AnAOB significantly, which might have been caused by the oxygen limitation also when aerating continuously but with low pressures. The microbial community analysis in this study did not give any information about the spatial distribution of the bacteria in the biofilm. Analyzing the distribution of the bacteria in the different layers, however, might provide further insight about the effects of the aeration strategy.

The detached biomass of MABR 4 was sieved out of the recirculation line and the biomass was analyzed with qPCR on operation day 230 and 357 to compare the microbial community of the detached biomass with the one of the biofilm at the same sampling day. The abundance of all analyzed species in the suspended biomass was lower as in the biofilm but with a comparable distribution (Fig. 8) with the exception of the abundance of AnAOB. The AnAOB abundance was under the detection limit on day 230. On day 357, the AnAOB abundance was measureable, but significantly lower.

**Fig. 5.** Sludge age of MABR 3 (black dots) and MABR 4 (grey dots).

**Fig. 6.** Abundance of Ammonium Oxidizing Bacteria (AOB) and Anaerobic Ammonium Oxidizing Bacteria (AnAOB) in MABR 1 and 2. After inoculation (Day 0), an initial loss of both AOB and AnAOB species was observed in both reactors. AOB was significantly higher in MABR 2 due to continuous aeration. In both reactors, AnAOB was able to re-establish in the biofilm.
lower compared to the value in the biofilm. This suggests that AnAOB did not primarily grow on the bulk liquid side of the membrane-aerated biofilm, but must have been present also in deeper layers (closer to the membrane) and thereby protected from detachment. This observation contradicts earlier simulation results by Lackner et al. (2008), who experienced high losses of AnAOB due to the theoretical stratification of MABR biofilms, with the AnAOB mostly on the bulk side. The intermittent aeration allowed for an expansion of the anoxic zones to deeper layers of the biofilm during non-aerated phases, and thereby enabled AnAOB to establish themselves in these layers and to remain in the biofilm.

4. Conclusions

This study evaluated the feasibility of MABRs for short-cut nitrogen removal under mainstream conditions with four lab scale reactors. Nitrogen removal was affected by several operational parameters in these MABRs. Our main findings were:

- The achieved ammonium removal was between 70 and 90% and TN removal between 60 and 80% with average TN removal rates of approximately 1.2 g-N m$^{-2}$ d$^{-1}$ and maximum TN removal rates of ~2 g-N m$^{-2}$ d$^{-1}$.

**Fig. 7.** Abundance of AOB and AnAOB in MABR 3 and 4. After inoculation (Day 183), an initial loss of both AOB and AnAOB species was observed in both reactors. Meantime, AOB was much higher in MABR 4 compared to MABR 3. In both reactors, AnAOB was able to re-establish in the biofilm within 100 days.

**Fig. 8.** Abundance of AOB, AnAOB, Nitrobacter and Nitrospira of MABR 4 in the biofilm on the membrane surface compared to the suspended biomass caught in the sieves. The numbers of AOB, Nitrobacter and Nitrospira show a proportional distribution in the biofilm and the suspended biomass. Only AnAOB shows no proportional distribution, as it is significant lower in the suspended biomass (day 230: below detection limit).
Operating the systems with constant surface load of 2 g-N m⁻² d⁻¹ resulted in a higher and more stable nitrogen removal independent of the aeration strategy. Operating with constant HRT caused difficulties in the adjustment of the oxygen flux in relation to the substrate surface loads.

Limiting the oxygen supply with intermittent aeration was a powerful tool to reduce nitrate production and to favor short-cut nitrogen removal. Under continuous aeration, ammonium removal but no stable NOB suppression was achieved.

Removing detached biofilm from the reactor caused a slightly lower overall turnover. Recirculation of the detached biomass resulted in reattachment to the biofilm surface. With recirculation, the biomass age in the MABR was significantly higher resulting in a more stable operation.

The microbial community composition was different in dependence of the aeration strategy. Although in all four reactors initial loss of AnAOB was observed, the operational conditions favored the growth of AnAOB and allowed the establishment of AnAOB in the MABR bioreactors.

Scouring events had a significant impact on the overall operation, causing a loss of nitrogen removal activity. The short- and long-term effects in dependency of strength and frequency of the scouring events require further research.

The results of this study show that mainstream PN/A is possible with MABRs, if the oxygen to substrate ratio can be controlled effectively. Future research should address the long-term effects in dependency of strength and frequency of the aeration pattern and residual ammonium concentration in a membrane-aerated biofilm reactor under continuous aeration: a demonstration. Environ. Eng. Sci. 30 (1), 38–45. https://doi.org/10.1089/ees.2012.0222.

Hibiya, K., Terada, A., Tsuneda, S., Hirata, A., 2003. Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane-aerated biofilm reactor. J. Biotechnol. 100 (1), 23–32. https://doi.org/10.1016/S0168-1656(02)00227-4.

Jäcome, J., Molina, J., López, J., Monzón, L., 2006. Simultaneous removal of organic matter and nitrogen compounds in autoaerobed biofilms. J. Environ. Eng. 132 https://doi.org/10.1061/(ASCE)0733-9372(2006)132:10(1255).

Jenni, S., Vlaeminck, S.E., Morgenroth, E., Uedt, K.M., 2014. Successful application of nitrogen/ammonium to wastewater with elevated organic carbon to ammonia ratios. Water Res. 49, 315–326. https://doi.org/10.1016/j.watres.2013.10.073.

Joss, A., Salzgeber, D., Eugster, J., Konig, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J., Siegrist, H., 2009. Full-scale nitrogen removal from digester liquid with partial nitrification and anammox in a SBR. Environ. Sci. Technol. 43 (14), 5301–5306. https://doi.org/10.1021/es000107w.

Kornaros, M., Dokianakis, S.N., Lyberatos, G., 2010. Partial nitrification/denitrification can be attributed to the slow response of nitrite oxidizing bacteria to periodic anoxic disturbances. Environ. Sci. Technol. 44 (19), 7245–7253. https://doi.org/10.1021/es100564j.

Lackner, S., Terada, A., Smets, B.F., 2008. Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: results of a modeling study. Water Res. 42 (4–5), 1102–1112. https://doi.org/10.1016/j.watres.2007.08.025.

Lackner, S., Terada, A., Horn, H., Henze, M., Smets, B.F., 2010. Nitrification performance in membrane bioreactors depends on the choice of conventional bioreactors. Water Res. 44 (20), 6073–6084. https://doi.org/10.1016/j.watres.2010.07.074.

Lackner, S., Thoma, K., Gilbert, E.M., Gander, W., Schrefl, D., Horn, H., 2015. Start-up of a full-scale denitriification SBR-treating effluent from digested sludge dewatering. Water Sci. Technol. 71 (4), 553–559. https://doi.org/10.2166/wst.2014.421.

Lotti, T., Kleerebezem, R., Hu, Z., Kartal, B., de Kreuk, M.K., van Ep Taalman Kip, C., Kruit, J., Hendrickx, T.L., van Loosdrecht, M.C.M., 2015. Pilot-scale evaluation of anammox-based mainstream nitrogen removal from municipal wastewater. Environ. Technol. 36 (9–12), 1167–1177. https://doi.org/10.1080/09593330.2015.1027227.

Ma, Y., Peng, Y.Z., Wang, S.Y., Yuan, Z.G., Wang, X.L., 2009. Achieving nitrogen removal via nitrite in a pilot-scale continuous pre-denitrification plant. Water Res. 43 (3), 563–572. https://doi.org/10.1016/j.watres.2008.08.025.

Ma, Y., 2018. Monitoring and Modeling of Nitrogen Conversions in Membrane-Aerated Biofilm Reactors: Effects of Intermittent Aeration. Kgs. Lyngby: Department of Environmental Engineering, Technical University of Denmark (DTU) Thesis.

Pellicer-Nacher, C., Sun, S.P., Lackner, S., Terada, A., Schreiber, F., Zhou, Q., Smets, B.F., 2010. Sequential aeriation of membrane-aerated biofilm reactors for high-rate autotrophic nitrogen removal: experimental demonstration. Environ. Sci. Technol. 44 (19), 7628–7634. https://doi.org/10.1021/es101467.

Pellicer-Nacher, C., Franck, S., Guay, A., Racusenda, M., Terada, A., Al-Soud, W.A., Hansen, M.A., Sørensen, S.J., Smets, B.F., 2014. Sequentially aerated membrane biofilm reactors for autotrophic nitrogen removal: microbial community composition and dynamics. Microb. Biotechnol. 7 (1), 32–43. https://doi.org/10.1111/1751-7915.12079.

Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., Bort, C.B., 2014. Control of aeration, aerobic SRT and COD input for mainstream nitrification/denitrification. Water Res. 57, 162–171. https://doi.org/10.1016/j.watres.2014.03.035.

Sarohn, M., Aziz, H., Rulin, B., Heng, J., Okabe, S., Fukushima, K.-L., 2004. Macroscale and microscale analyses of nitrification and denitrification in biofilms attached on membrane aerated biofilm reactors. Water Res. 38 (6), 1633–1641. https://doi.org/10.1016/j.watres.2003.12.029.

Semmens, M.J., Dahn, K., Shanahan, J., Christianson, A., 2003. COD and nitrogen removal by biofilms growing on gas permeable membranes. Water Res. 37 (18), 4343–4350. https://doi.org/10.1016/S0043-1354(03)00416-6.

Seuntjens, D., Carvajal-Arroyo, J.M., Ruopp, M., Bunse, P., De Mulder, C.P., Lochmatter, S., Agraywal, S., Boon, N., Lackner, S., Vlaeminck, S.E., 2018. High-
resolution mapping and modeling of anammox recovery from recurrent oxygen exposure. Water Res. 144, 522–531. https://doi.org/10.1016/j.watres.2018.07.024.

Shanahan, J.W., Semmens, M.J., 2006. Influence of a nitrifying biofilm on local oxygen fluxes across a micro-porous flat sheet membrane. J. Membr. Sci. 277 (1), 65–74. https://doi.org/10.1016/j.memsci.2005.10.010.

Wei, X., Li, B., Zhao, S., Qiang, C., Zhang, H., Wang, S., 2012. COD and nitrogen removal in facilitated transfer membrane-aerated biofilm reactor (FT-MABR). J. Membr. Sci. 389, 257–264. https://doi.org/10.1016/j.memsci.2011.10.038.

Yang, J.J., Trela, J., Zubrowska-Sudol, M., Plaza, E., 2015. Intermittent aeration in one-stage partial nitritation/anammox process. Ecol. Eng. 75, 413–420. https://doi.org/10.1016/j.ecoleng.2014.11.016.