Simultaneous nitrogen and organics removal using membrane aeration and effluent ultrafiltration in an anaerobic fluidized membrane bioreactor

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ARTICLE INFO

ABSTRACT

Dissolved methane and a lack of nutrient removal are two concerns for treatment of wastewater using anaerobic fluidized bed membrane bioreactors (AFMBRs). Membrane aerators were integrated into an AFMBR to form an aeration membrane fluidized bed membrane bioreactor (AeMFMBR) capable of simultaneous removal of organic matter and ammonia without production of dissolved methane. Good effluent quality was obtained with no detectable suspended solids, $93 \pm 5\%$ of chemical oxygen demand (COD) removal to 14 ± 11 mg/L, and $74 \pm 8\%$ of total ammonia (TA) removal to 12 ± 3 mg-N/L for domestic wastewater (COD of 193 ± 23 mg/L and TA of 49 ± 5 mg-N/L) treatment. Nitrate and nitrite concentrations were always low (< 1 mg-N/L) during continuous flow treatment. Membrane fouling was well controlled by fluidization of the granular activated carbon (GAC) particles (transmembrane pressures maintained < 3 kPa). Analysis of the microbial communities suggested that nitrogen removal was due to nitrification and denitrification based on the presence of microorganisms associated with these processes.

1. Introduction

An anaerobic fluidized bed membrane bioreactor (AFMBR) was first developed as a post-treatment method for an anaerobic fluidized bed bioreactor (AFBR), achieving 87% removal of chemical oxygen demand (COD), 82% of soluble COD (SCOD), and ~100% of total suspended solid (TSS) (Kim et al., 2011). In addition, a low energy demand of 0.028 kWh/m³ was estimated for the process, which is 10 times lower than that needed for treatment using anaerobic membrane bioreactor (0.25–1 kWh/m³) (Liao et al., 2006). Membrane fouling is well controlled in an AFMBR by mechanical scouring due to fluidization of granular activated carbon (GAC) particles. Effective treatment has also been obtained using AFMBRs as a second process that followed treatment by other types of bioreactors. For example, the effluent of a microbial fuel cell (MFC) treating domestic wastewater was reduced to a COD of 16 ± 3 mg/L and TSS of < 1 mg/L, at an AFMBR hydraulic retention time (HRT) of 1 h (Ren et al., 2014). Low effluent COD (11 mg/L) and negligible TSS were also achieved at an HRT of ~1 h for effluent from an anaerobic baffled bioreactor (ARB) (Lee et al., 2015). The combined AFBR and AFMBR process was found to have an additional advantage of effective removal of pharmaceuticals from wastewater (86–100%) (Dutta et al., 2014). A disadvantage of AFMBR treatment, however, is that the effluent contains dissolved methane (16 mL CH₄/L) which would need to be removed prior to discharge (Yoo et al., 2012). In addition, total nitrogen has not been reported to be reduced during AFMBR treatment, since a combination of anoxic and anaerobic conditions are required to achieve nitrification and denitrification.

Membrane-aerated bioreactors (MABRs) were developed to obtain efficient nitrogen removal through the growth of a biofilm on the aeration membranes. Oxygen is added by bubbleless gas transport through the membrane to the biofilm. Nitrification can occur in the stratified biofilm on the membrane, and the nitrate produced can reduce organics concentration to a low levels by denitrification, which in return favors nitrification in the biofilm (Gilmore et al., 2013). Ammonia-oxidizing bacteria (AOB) have been identified in the deep biofilm layer near the membrane, while denitrifiers and heterotrophic bacteria grow on the outer layer (Terada et al., 2003). Stratified biofilm growth of nitrifiers and denitrifiers has also been confirmed using fluorescence in situ hybridization (Gilmore et al., 2013). Typically the biofilms on the membranes are 50–200 μm thick (Casey et al., 1999a), which is usually deep enough to prevent oxygen transfer into the bulk liquid, thus maintaining anaerobic conditions in the solution (Casey et al., 1999b). Membrane aerators immobilized with microorganisms

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were first tested using synthetic wastewater (total organic carbon, TOC, 1000 mg/L and total nitrogen, TN, 58.5 mg-N/L) in batch mode (24 h), achieving a removal efficiency of 97.9% for TOC and 98.3% for TN with a lumen pressure of 245 kPa (pure oxygen) (Hirasa et al., 1991). When treating organic-free synthetic wastewater (217 mg-N/L of ammonium) in continuous-flow mode, separate arrays of hollow fiber membranes (HFMs) that supplied pure bubbleless hydrogen and oxygen in a redox controlled membrane bioreactor obtained a high ammonia removal flux (AR) of 5.8 g-N/m^2 membrane-d, with a nitrate and nitrite removal flux of 4.4 g-N/m^2-d, at a pressure of 861 kPa (Smith et al., 2008). A total nitrogen removal flux (NRF) of 1.7 g-N/m^2-d was achieved using an MABR supplied with air to treat COD-free wastewater (47.1 mM NH_3-N), with 75% removal of the influent nitrogen (Gilmore et al., 2013). Nitrogen and carbonaceous compounds in synthetic wastewater (TOC of 100 mg/L and TN of 25 g-N/m^3) were simultaneously removed using an MABR supplied with air, showing a carbon removal flux (CRF) of 7.4 g-C/m^2-d and NRF of 2.8 g-N/m^2-d (Hibiya et al., 2003). One disadvantage of using MABRs is that they require relatively long hydraulic retention times (HRTs) compared to other processes. HRTs can be as long as several days using air, for example 1 day (Smith et al., 2008), 4–6 days (Gilmore et al., 2013), 1.2–12 days (Gilmore et al., 2009) and 15 days (Terada et al., 2003). However, HRTs can be reduced to only ~1 to several hours by using pure oxygen, for example 0.6 h (Pankhania et al., 1994), 6 h (Hibiya et al., 2003) and 1–10 h (Brindle et al., 1998).

In order to achieve effective ammonia removal in an AFMBR, it was hypothesized that adding a membrane aerator module into the AFMBR could enable simultaneous removal of both carbonaceous and nitrogen compounds in a single aeration membrane fluidized bed membrane bioreactor (AeMFMBR). By infusing oxygen into the system, nitrogen could be removed through nitrification on aeration membranes, and denitrification by microorganisms on the aeration membranes or on GAC and in the mixed liquor. In addition, it was hypothesized that production of methane could be avoided through introducing a membrane aerator, which allows the production of nitrate via nitrification, resulting in an anoxic environment. A bench-scale AeMFMBR was constructed by integrating two different modules, the membrane aerators and the membranes used for ultrafiltration of the effluent, into a single reactor containing fluidized GAC. The performance of the AeMFMBR was initially examined using synthetic influent in fed-batch mode, and then by using synthetic or diluted domestic wastewaters in continuous flow mode. The mechanism of nitrogen removal was investigated through a microbial community analysis of the suspended biomass and the biomass on membrane aerators, and GAC.

2. Material and methods

2.1. Reactor setup

The AeMFMBR made of polyvinyl chloride (PVC, McMaster Carr) contained two chambers, one for filtration (lower section) and the other for aeration (upper section), with a total volume of 4.5 L (Fig. 1). The aeration membrane module contained 135 polyvinylidene fluoride (PVDF) HFMs (pore size of 0.1 μm, Kolon Inc., South Korea) that were sealed at one end. The ultrafiltration membrane module used to filter the wastewater had 54 PVDF HFMs. The total surface area was estimated to be 0.08 m^2 for the aeration membrane module (18 m^2/m^3), and 0.03 m^2 for the filtration membranes (7 m^2/m^3). A magnetic water pump (50 px-x, 1100 GPH, Pan World, Japan) was used to keep the mixed liquor recirculated at a constant flowrate of 4.3 ± 0.9 L/min. Two peristaltic pumps (model no. 7523-90, Masterflex, Vernon Hills, IL) were used for influent and effluent pumping. A mass flow controller (0–10 LPM, Air/He/Ar, Cole-Parmer, US) was used to measure the air flowrate, and a pressure gauge (type1490, Ashcroft, Stratford, CT) was used to measure the air pressure. GAC particles (45 g/L; DARCO MRX, 10 × 30 mesh, Norit Activated Carbon, Cabot, GA) were added into the
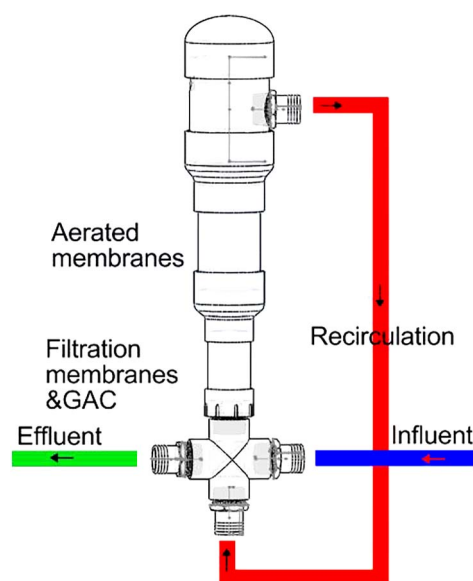

Fig. 1. Schematic of the AeMFMBR showing locations of the aeration and filtration membranes.

filtration chamber for biofilm growth and to control membrane fouling.

2.2. Operation

AeMFMBR operation was separated into six phases, with each phase used to sequentially examine the different aspects of the AeMFMBR components and test conditions, for example operation only with aeration membranes compared to operation with GAC and organic carbon in the feed, to identify the impact of the organic carbon on nitrogen removal. Each of these phases are identified with notation to indicate the specific aspects of operation, as follows (see Supplemental information): B for fed batch operation, or C for continuous flow operation; HN for high (~240 mg-N/L) and LN for low (50–80 mg-N/L) nitrogen concentrations; S for synthetic wastewater, and W for actual domestic wastewater; G for operation with GAC particles added to the reactor; U for operation with ultrafiltration of the effluent; and P for tests with a higher air pressure used in the aeration module compared to other tests (Table 1). For example, phase 3B-SG indicates phase 3 operation with fed batch conditions, a synthetic wastewater feed, and GAC fluidization (but no ultrafiltration of the effluent).

The membrane aerator (air flowrate of 1 mL/min) was inoculated with sludge from a nitrification tank (Pennsylvania State University Wastewater Treatment Plant) and feed solution (40 mM NH_4HCO_3, 14.3 mM NaCl, 3.7 mM $KHCO_3$, 0.8 mM $KHSO_4$, 1.25 mM KH_2PO_4, 0.83 mM $MgSO_4$, 1.23 mM $CaCl_2$, and 0.11 mM $FeCl_3$) (Gilmore et al., 2013) in a column with stirring for 50 days prior to phase 1B-HN. Each time the operational conditions were changed the reactor was operated for at least one week under the new conditions for reactor acclimation. The AeMFMBR was operated at a constant temperature room with 20 °C (minimum light source to avoid phototrophic growth).

In phase 1B-HN, the membrane aerator module alone was tested for ammonia removal with the reactor operated in batch mode (two repeated cycles), using a COD-free medium with a high concentration of ammonia (HN), same as the feed solution for acclimation of the biofilm for nitrification (236 ± 9 mg-N/L, Table 1). In all subsequent phases (2–6), lower nitrogen concentrations were used in the range of 49–79 mg-N/L, as indicated in Table 1. In phase 2B-LN, the AeMFMBR was therefore operated under the same conditions as phase 1 except the total ammonia concentration in the feed solution was reduced to 79 ± 11 mg-N/L.

In phases 3 through 6, GAC particles were added into the filtration

Table 1
Operational conditions of the reactors for the six phases in terms of duration, HRT, operational mode (batch mode, B and continuous flow mode, C), type of wastewater, air flow, influent COD, sCOD and TA.

Phase	Duration (day)	HRT (d)	Mode	Wastewater	Air flow (mL/min)	COD (mg/L)	SCOD (mg/L)	TA (mg-N/L)
1B-HN	45	24	B	COD free	1.0 ± 0.2	nm[a]	nm[a]	236 ± 9
2B-LN	25	13	B	COD free	1.0 ± 0.3	nm[a]	nm[a]	79 ± 11
3B-SG	24	9	B	Synthetic	1.0 ± 0.2	162 ± 3	162 ± 3	73 ± 7
4C-SGU	29	0.9/1.7	C	Synthetic	1.0 ± 0.1	154 ± 12	154 ± 12	68 ± 6
5C-WGU	38	1.7	C	Domestic	1.2 ± 0.4	202 ± 9	127 ± 8	52 ± 3
6C-WGUP	34	1.7	C	Domestic	2.2 ± 0.2	193 ± 23	107 ± 17	49 ± 5

[a] nm, not measured.

chamber and only lower nitrogen concentrations were tested. In phases 3 and 4, glucose (0.075 g/L) and acetate (0.1 g/L) were added into the feed solution as a source of COD, producing a TCOD of ~150 mg/L. In phase 3B-SG, the reactor was therefore operated in batch mode (three repeated cycles) to examine the impact of a defined, synthetic wastewater on nitrogen removal, compared to no COD in the influent in phase 2. The addition of GAC was used to provide a large surface area for biofilm growth.

In phases 4 through 6, the ultrafiltration membrane module was placed in the reactor operated under controlled flux, and the operation was switched to continuous flow, producing the combined conditions for complete AeMFMBR operation in all subsequent tests. In phase 4C-SGU, the reactor was fed synthetic wastewater at two different flow-rates, with a 10 min on and 1 min off for membrane relaxation; 3.6 mL/min (7.2 L/m^{2-} h), producing a net HRT of 20.5 h; and 1.8 mL/min (3.6 L/m^{2-} h), to produce a longer HRT of 41 h.

For tests in phases 5 and 6, the feed was switched to a domestic wastewater obtained from the primary clarifier of the Pennsylvania State University Wastewater Treatment Plant and operated at the longer HRT of 41 h. The filtration membrane was operated with the same relaxation cycle used in phase 4C-SGU. The wastewater was diluted to a TCOD of ~200 mg/L using distilled water, and the total ammonia (TA) concentration was adjusted to ~50 mg-N/L by adding ammonium bicarbonate, to simulate COD removal by an upstream process (such as an MFC) with no nitrogen removal. For phase 5C-WGU, the reactor operation was therefore the same as that in phase 4 except the synthetic wastewater was replaced by a diluted wastewater with a similar TA concentration. In phase 6C-WGUP, the lumen pressure (P) was increased to 4–5 kPa from 2–3 kPa in the first 5 phases to increase air flowrate in order to try to increase the ammonia removal rate. A low lumen pressure was applied in this study to make sure an axoic environment was maintained in the AeMFMBR.

2.3. Analytical methods

TCOD and SCOD were measured using commercial kits (COD digestion vials, low range and high range, Hach). Three-day headspace biochemical oxygen demand (HBOD) tests were used to analyze the reactor effluent and mixed liquor organic concentrations, where the HBOD$_3$ is approximately equal to a BOD$_5$ measured using standard methods (Logan and Patnaik, 1997). Total ammonia (Nitrogen-ammonia reagent Set, high range, Hach), nitrite (NitraVer X nitrogen-nitrate reagent set, high range, Hach) and nitrate (NitriVer 3 TNT reagent set, nitrogen-nitrite, low range, Hach) concentrations were measured for the effluent and the liquid inside the AeMFMBR. Mix liquor suspended solids (MLSS) was quantified based on total suspended solid (TSS) following standard methods (method 2540D, with filters having with a pore size of 1.5 μm; GE Whatman) using a sample volume of ~50 mL. Dissolved oxygen (DO) was monitored using an oxygen meter (NeoFox oxygen monitoring kit with probe, Ocean optics, US). Dissolved methane for the effluent and mixed liquor were measured based on gases desorbed from solution as previously described (Ren et al., 2014), except 4 mL of headspace was left for air while transferring the

sample to serum bottle (10 mL) instead of filling serum bottle without leaving a headspace. Transmembrane pressure (TMP) of the ultra-filtration membrane was monitored using a pressure transducer (TDH 31, Transducer Direct, US). The pH and conductivity of the diluted wastewater were measured using a probe and meter (Seven-Multi, Mettler-Toledo International Inc.).

Microorganisms were sampled from the aeration membranes at the end of phase 2B-LN and 4C-SGU, and from GAC particles and the suspended biomass in 4C-SGU. DNA was extracted from the samples following the Power Soil DNA isolation kit protocol with some modifications to improve DNA extraction (Mo Bio Laboratories, Inc) (Ye et al., 2016). Bead tubes with 0.1 mm glass beads were used instead of the garnet bead-beating tube in the original kit. The sample (GAC, centrifuged suspended solids from mixed liquor sample or aeration membranes cut into small pieces) and 750 μL bead solution were added to the bead tube, and the tubes were mixed using a bead mill (Bead Ruptor 12 Homogenizer, Kennesaw, GA) for 45 s instead of using a vortexer, followed by centrifugation at 10,000 × g for 1 min, and incubation at 4 °C for 10 min. The extracted DNA was then sequenced by DNASense (Denmark). Briefly, DNA samples were amplified by polymerase chain reaction (PCR). The forward 515F (5′-GTGYCAGCMGCCGCGGTA-3′) and reverse 805R (5′-GACTACHVGGGTATCTAATCC-3′) tailed primers were designed for targeting the V4 region of bacterial and archaeal 16S rRNA gene (Ye et al., 2016). The resulting amplicons were then purified, and single read sequenced (251 bp) on MiSeq (Illumina). Taxonomy was assigned using the RDP classifier in QIIME (Caporaso et al., 2010), using the MiDAS database v.2.1.2 (McIlroy et al., 2017). Principle component analysis (PCA) was generated based on the relative abundance of operational taxonomic units (OTUs) with OTUs (unit vector) > 5% shown as axes. Square-root transformed Bray-Curtis similarities (BCS) were calculated between samples.

The oxygen transfer efficiency (OTE) was calculated assuming complete nitrification (total ammonium converted to nitrate) using the set air flowrate and measured AR as:

$$OTE = \frac{nAR_f Am_{O_2}}{0.23 m_N \rho Q_{air}} \qquad (1)$$

where n the stoichiometric ratio of ammonia and oxygen (4), AR_f is ammonia removal flux (g-N/m^2 d), A the membrane aerator area (0.03 m^2), m_{O_2} the molecular weight of oxygen gas (32 g/mol), 0.23 the mass fraction of oxygen in air, m_N is the molecular weight of nitrogen (14 g/mol), ρ the density of air at 20 °C (1.205 × 10^3 g/m^3) and Q_{air} the airflow rate (m^3/d). The Student-T test was used to assess differences in COD and ammonia removals among phases, with the differences considered to be significant for p ≤ 0.05.

3. Results and discussion

3.1. Nitrogen removal with COD-free synthetic wastewater

In the initial operation of the reactor (phase 1B-HN) with a high initial ammonia concentration of 236 ± 9 mg-N/L, the AR was 0.4 ± 0.02 g/m^2-d (Fig. 2 and Supplemental information), resulting in
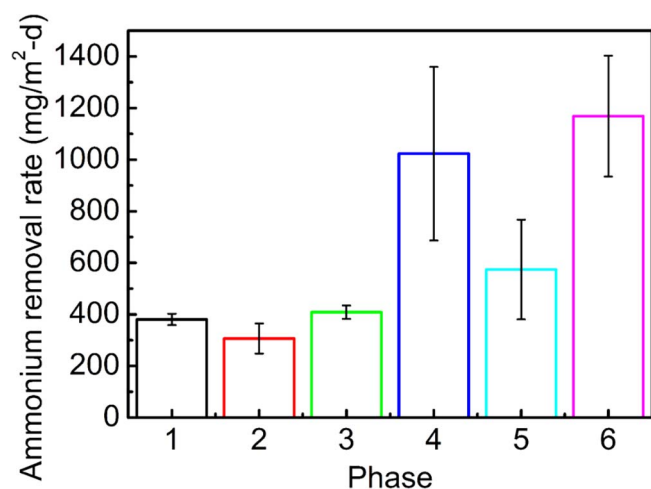
Fig. 2. Comparison of ammonia removal rate (AR) in each of the six phases of operation (phase 1B-HN, 2B-LN, 3B-SG, 4C-SGU, 5C-WGU and 6C-WGUP).

a total nitrogen removal efficiency of 72 ± 2% over a period of 20 days. When the ammonia concentration was reduced to 79 ± 11 mg-N/L in the next phase (2B-LN) the rate was only slightly lower at 0.3 ± 0.06 g-N/m^2-d (Fig. 2). The TN concentration at the end of cycle in phase 2B-LN was < 5 mg-N/L, indicating the membrane aeration could effectively reduce TN to a low concentration. These two ARs were lower than those previously reported for MABRs treating inorganic ammonium using air, which ranged around 1.7 g-N/m^2-d (∼30–50 kPa) (Gilmore et al., 2013) to 2 g-N/m^2-d (Semmens et al., 2003). The lower rate here was probably due to the low lumen pressure (2 kPa) and the low ambient temperature (20 °C) compared to these previous studies.

The nitrite concentrations during the operation were always low (see Supplemental information), with 0.6 ± 0.6 mg-N/L for phase 1B-HN (Table 2) and 0.8 ± 0.7 mg-N/L for phase 2B-LN (Table 2). The nitrate concentrations were also low, with 1.8 ± 1.4 mg-N/L for phase 1B-HN and 1.6 ± 1.4 mg-N/L for phase 2B-LN (Table 2). The low nitrite and nitrate accumulated indicated that ammonium oxidation to nitrite was the limiting step in nitrification. Although the feed did not contain any appreciable COD, the measured TCOD in mixed liquor in phase 2B-LN was 41 ± 23 mg/L. The COD was believed to be generated by autotrophic nitrifiers, consistent with a previous study treating COD-free synthetic wastewater (Rezania et al., 2007), where COD was also found to be produced, likely by the generation and release of soluble microbial products (SMP) into the solution.

3.2. COD and nitrogen removals using synthetic wastewater

In phase 3B-SG when COD was added to a synthetic wastewater (162 ± 3 mg-COD/L) and GAC particles were used, COD and nitrogen were degraded simultaneously with membrane aeration. The AR of
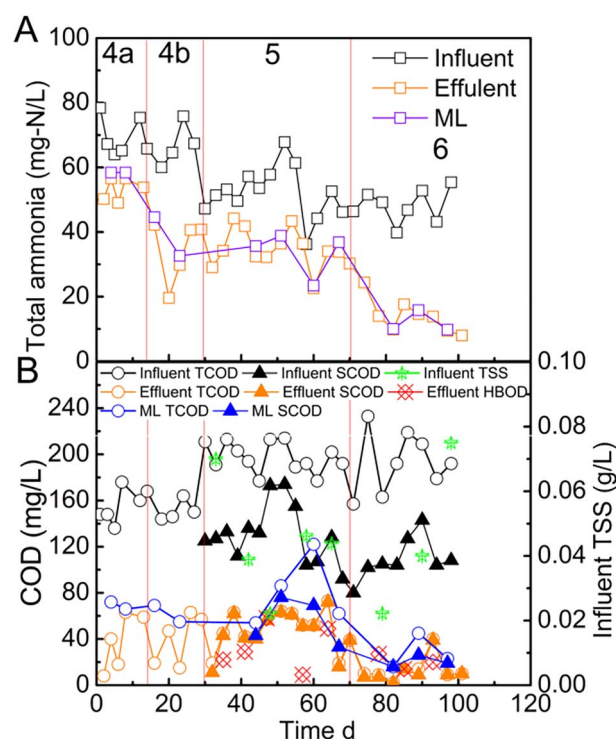

Fig. 3. (A) Total ammonia (TA) concentration of the influent, effluent, and the mixed liquor during continuous mode operation (phase 4C-SGU, 5C-WGU and 6C-WGUP); (B) TCOD, SCOD, HBOD, and TSS of the influent, effluent and the mixed liquor (ML). Two different HRTs were used in phase 4C-SGU with 4a (20.5 h) and 4b (41 h).

0.4 ± 0.03 g/m^2-d (Fig. 2) obtained in phase 3B-SG was similar to that in phase 1B-HN and 2B-LN, despite the addition of the organic matter into the feed solution. Nitrate and nitrate concentrations remained low (< 1 mg-N/L) (see Supplemental information), consistent with phases 1B-HN and 2B-LN. Organic matter was degraded to a low concentration of 25 ± 19 mg-COD/L (see Supplemental information). However, compared with the nearly linear decrease in ammonia in the first three phases, the COD removal rate decreased over time, with a very rapid initial decrease in COD followed by a slower rate of removal over time.

When the filtration membrane was introduced into the system in phase 4C-SGU, and the AeMFMBR was switched from fed-batch mode to continuous flow operation at an HRT of 20.5 h (4C-SGU1), the average AR increased by 1.5 times to 1.0 ± 0.3 g/m^2-d (Fig. 2). The TA concentration inside the AeMFMBR was similar to that in the effluent (Fig. 3), indicating the filtration membrane did not impact retention of soluble ammonia. An average of 25 ± 8% removal of nitrogen was obtained, with effluent TN of 51 ± 11 mg-N/L (Fig. 3). In order to increase nitrogen removal, the HRT was increased to 41 h (4C-SGU2), leading to 55 ± 11% of TA removal, with an effluent TA of 37 ± 11 mg-N/L. The ARs obtained in 4C-SGU at the two different HRTs (1.0 ± 0.4 g/m^2-d at 20.5 h, and 1.1 ± 0.2 g-N/m^2-d at 41 h)

Table 2
Effluent nitrate and nitrite concentration, total ammonia removal, and TCOD/SCOD removal during the six phases of operation.

Phase	Nitrite (mg-N/L)	Nitrate (mg-N/L)	Removal efficiency (%)			
			TA	TN	TCOD	SCOD
1B-HN	0.6 ± 0.6	1.8 ± 1.4	72 ± 2	68 ± 2	nm[a]	nm[a]
2B-LN	0.8 ± 0.7	1.6 ± 1.4	94 ± 1	93 ± 2	nm[a]	nm[a]
3B-SG	0.7 ± 0.9	0.9 ± 0.3	81 ± 12	77 ± 14	85 ± 12	nm[a]
4C-SGU	0.08 ± 0.06	0.6 ± 0.4	36 ± 16	35 ± 16	75 ± 12	nm[a]
5C-WGU	0.02 ± 0.01	0.4 ± 0.1	33 ± 9	32 ± 9	75 ± 8	64 ± 14
6C-WGUP	0.02 ± 0.01	0.5 ± 0.1	74 ± 8	71 ± 8	93 ± 5	89 ± 7

[a] nm, not measured.

were not significantly different (T-test, p = 0.6), indicating that the AR was not dependent on the HRT.

There was no significant increase in COD removal efficiency (T-test, p = 0.4) when the HRT was increased in phase 4C-SGU from 20.5 h ($77 \pm 12\%$) to 41 h ($70 \pm 11\%$). The average effluent TCOD was 39 ± 19 mg/L, which would be below the standard discharge standard for BOD_5 of 30 mg/L assuming a typical ratio of 2:1 $COD:BOD_5$ ratio (Hays et al., 2011).

3.3. COD and nitrogen removal using diluted domestic wastewater

When domestic wastewater was treated instead of the synthetic wastewater in phase 5C-WGU, the AR was reduced by 40% to 0.6 ± 0.2 g/m²-d (Fig. 2). The difference was likely due to the form of nitrogen, which was only NH_4HCO_3 in phases 1B-HN to 4C-SGU, but a mixture of organic nitrogen and this ammonium salt ($\sim 40\%$) in 5C-WGU. In addition, domestic wastewater may contain inhibitors for nitrifying bacteria. The overall nitrogen removal was $33 \pm 9\%$, with an effluent TA concentration of 36 ± 5 mg-N/L (Fig. 3). Only small concentrations of nitrate (0.02 ± 0.01 mg-N/L) and nitrite (0.4 ± 0.1 mg-N/L) were measured in the treated effluent.

When the aeration pressure in phase 6C-WGUP was increased from 2 to 4–5 kPa, the AR (1.2 ± 0.2 g/m²-d, Fig. 2) was twice that obtained in 5C-WGU (0.6 ± 0.2 g-N/m²-d, T-test, p = 0.002). A lower effluent TA of 12 ± 3 mg-N/L (Fig. 3) was obtained, with a TA removal efficiency of $74 \pm 8\%$ that was significantly higher than that in 5C-WGU (T-test, p < 0.001). The effluent nitrate (0.02 ± 0.01 mg-N/L) and nitrite (0.4 ± 0.1 mg-N/L) concentrations remained low and were not significantly different from those in 5C-WGU (T-test, p > 0.4), indicating the ammonia oxidation was still the limiting step for nitrification in 6C-WGUP. The TA concentration inside the AeMFMBR was similar to that in the effluent in both phases 5C-WGU and 6C-WGUP (Fig. 3), while the nitrate and nitrite concentration inside the AeMFMBR were slightly higher than those in the effluent (see Supplemental information).

COD was effectively degraded, with an effluent TCOD of 49 ± 16 mg/L and SCOD of 47 ± 17 mg/L in phase 5C-WGU (Fig. 3), resulting in removal efficiency of $75 \pm 8\%$ for TCOD and $64 \pm 14\%$ for SCOD. In phase 6C-WGUP with the higher aeration rate, COD removal was further improved with an effluent TCOD of 14 ± 11 mg/L and SCOD of 13 ± 11 mg/L, indicating that the increase in air flux enhanced both COD removal and nitrogen removal. The removal efficiencies reached $93 \pm 5\%$ for TCOD, and $89 \pm 7\%$ based on SCOD in 6C-WGUP (Table 2). The similar effluent TCOD and SCOD in 5C-WGU and 6C-WGUP (Fig. 3) indicated good solids removal with the filtration membrane. This was further confirmed by TSS tests, where no TSS were detected in the effluent, with influent TSS of diluted domestic wastewater ranging from 20 mg/L to 80 mg/L (Fig. 3).

3.4. Transmembrane pressure, DO and dissolved methane

Membrane fouling was well mitigated in the AeMFMBR by using fluidized GAC particles, with a maximum TMP of 3 kPa (Fig. 4) during continuous-mode of operation. There was a decrease in TMP in phase 4C-SGU with a longer HRT, indicating that a longer HRT reduced fouling due to the decreased flux. This result was consistent with a previous study where shorter HRTs led to larger membrane fouling (Huang et al., 2011).

In phase 5C-WGU when the synthetic wastewater was switched to actual wastewater, the TMP was still low (0.4 ± 0.3 kPa). However, the TMP increased to 1.1 ± 0.4 kPa when air flowrate was doubled in phase 6C-WGUP. The MLSS of the AeMFMBR in 6C-WGUP (70 ± 3 mg-TSS/L) (Fig. 4) was significantly higher than that in 5C-WGU (30 ± 10 mg-TSS/L) (T-test, p = 0.01). The small standard deviation in MLSS concentrations indicates that the limit in biomass concentration was likely reached in phase 6C-WGUP. The increase in
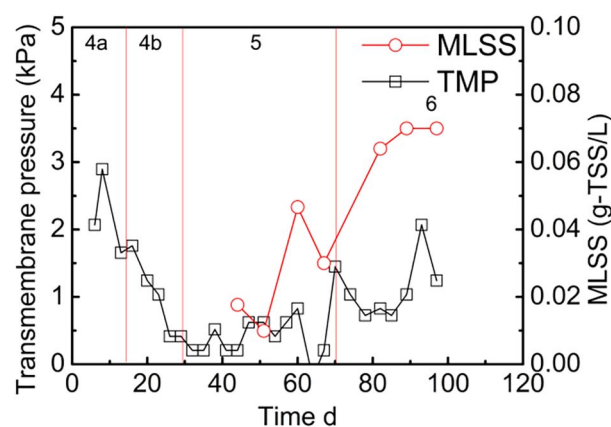

Fig. 4. Transmembrane pressure and mixed liquor suspended solids in phase 4C-SGU, 5C-WGU and 6C-WGUP. Two different HRTs were used in phase 4C-SGU with 4a (20.5 h) and 4b (41 h).

MLSS could likely explain the increase in TMP in phase 6C-WGUP, although the impact of MLSS on membrane fouling is controversial as it may have no impact or even a positive impact on fouling (Drews et al., 2006). The TCOD and SCOD inside the reactor were higher than those in the effluent (Fig. 3), suggesting that a cake layer formed on the filtration membrane that could have contributed to the removal of COD, as suggested by others (Smith et al., 2012; Ye et al., 2016).

There was no measurable DO (0 mg/L) in the AeMFMBR during continuous mode operation period, indicating the fluid environment in the AeMFMBR was anoxic. Although the DO in the mixed liquor of the AeMFMBR remained zero, dissolved methane was never detected in either the mixed liquor or effluent samples possibly because methanogenic archaea (relative abundance < 0.01%) were not enriched in the system. This result is different from that obtained in a previous AFMBR study where a COD to methane conversion of 10% resulted in a dissolved methane concentration of 1.5 mL/L (Ren et al., 2014).

The OTE during continuous flow mode operation was calculated using Eq. (1) and the calculated ammonia flux and air flow to be high, with $82 \pm 16\%$ for phase 5C-WGU, and $74 \pm 9\%$ for 6C-WGUP when treating diluted domestic wastewater. These high OTEs indicated that most of the oxygen was consumed for nitrogen removal even with the introduction of organic matter. The rest of the oxygen in the air supply was believed to be consumed by heterotrophs on the membrane aerator surface, since the DO was maintained near zero.

3.5. Microbial community analyses

The microbial community samples formed four distinct groups as they did not cluster with each other based on PCA analysis (Fig.5A). The BCS of the membrane aerator samples in phases 2B-LN and 4C-SGU was 46%, indicating the community changed after organics were introduced. The BCS between the membrane aerator and GAC in 4C-SGU was 57%, higher than that between membrane aerator and the mixed liquor (37%).

In phase 2B-LN, the ammonia removal for membrane aerators treating COD-free synthetic wastewater was likely due to biological nitrification and denitrification, based on the microbial community analysis of the membrane aerator. *Nitrosomonas* (6%) was one of the dominant genera (Fig.5B) on the membrane aerator in phase 2B-LN. Members of this genus are commonly found in activated sludge process (Wagner et al., 2002; Zhang et al., 2012), and were reported to be present in the biofilm on membrane aerator in other several studies (Gilmore et al., 2013; Hu et al., 2009) as the AOB converting ammonia to nitrite. In addition, *OTU_28* was shown to have a relative abundance of 6% (*Rhodocyclaceae* family), with 96% sequence similarity to *Dechloromonas denitrificans* which was reported as denitrifying bacteria
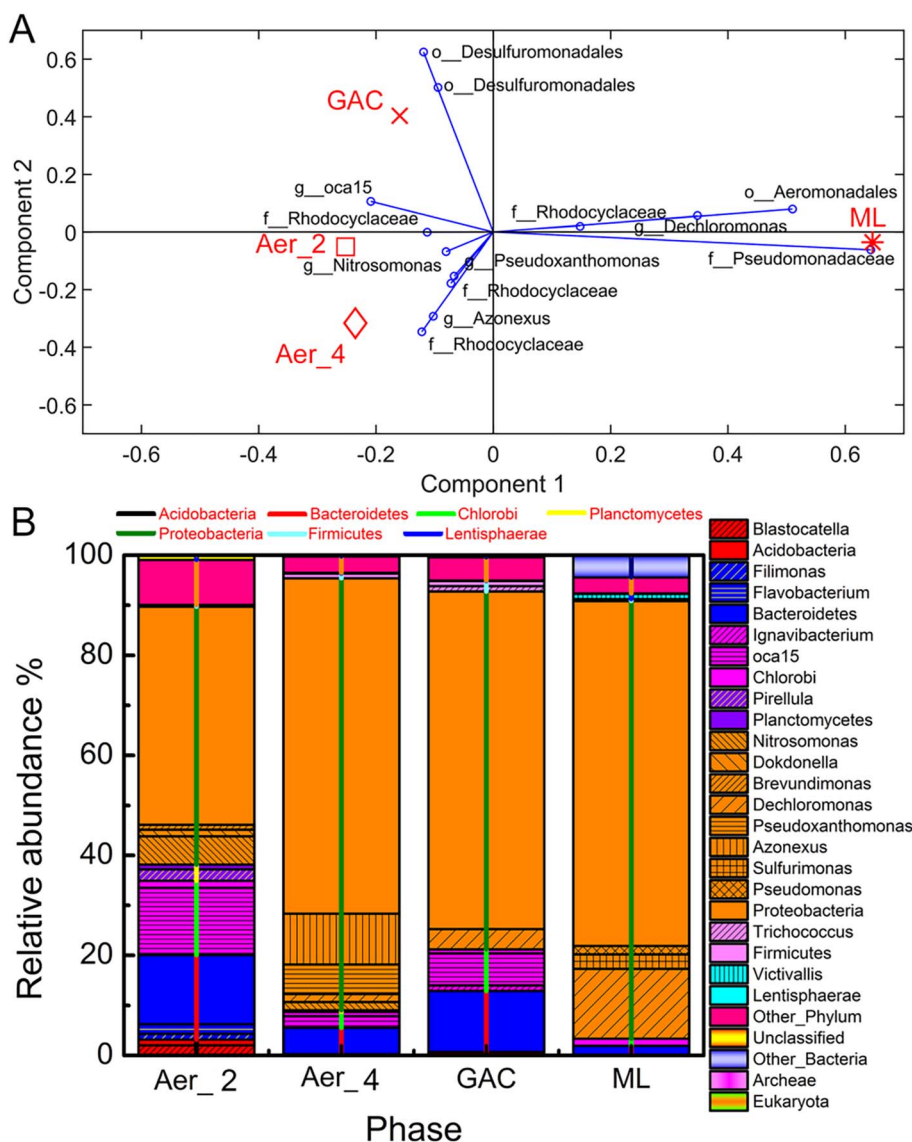
Fig. 5. Community analysis of membrane aerator in phase 2B-LN (Aer_2) and GAC, mixed liquor (ML), and the membrane aerator in phase 4C-SGU (Aer_4). (A) Principal component analysis based on OTUs relative abundance. Microbial community presented with first two principal components with OTUs relative abundance > 5% shown as the axes. The axes pointing at the sample indicates the high relative abundance; (B) microbial community analysis based on the relative abundance on the genus level. Only the genera with a relative abundance higher than 1% were shown (with pattern), while the other genera were included based on phylum level (pure color and vertical lines). Archaea (< 0.01%) and Eukaryota were shown at the Kingdom level.

(Hu et al., 2009). No anammox genera were found in the membrane aerator sample.

The microbial community on the membrane aerator in phase 4C-SGU with synthetic wastewater changed compared with that in phase 2-LN/B with no COD in the feed, although ammonia removal was still occurring by nitrification and denitrification. The *Rhodocyclaceae* became the dominant biofilm family (47%) on the membrane aerator when the organics were introduced. Within the *Rhodocyclaceae* family, *Azonexus* (10%) (Fig. 5B) was the dominant genus, which includes denitrifying bacteria species such as *Azonexus caeni* (Lee et al., 2006) and *Azonexus hydrophilus* (Chou et al., 2008). *Nitrosomonas* were also dominant on membrane aerator in 4C-SGU (2%). In contrast, members of the genus *Nitrosomonas* were not detected in the mixed liquor and were present at very low relative abundance (0.2%) on GAC. The family of *Comamonadaceae*, whose members were reported as denitrifying bacteria (Adav et al., 2010), also had a high relative abundance of 6%. In addition, *Pseudoxanthomonas* was shown to have a high relative abundance of 6%, and bacteria in this genus have been reported to stabilize the sludge structure in a sequencing batch reactor achieving partial nitrification (Wan et al., 2013).

Both the mixed liquor and GAC probably also contributed to denitrification, indicated by the high relative abundance of the denitrifying genus *Dechloromonas* on the GAC (5%) and in the mixed liquor (14%)

samples (Fig.5B) (Hu et al., 2009; Tago et al., 2011). Other than *Dechloromonas*, other OTUs belonging to the *Rhodocyclaceae* family were found with a high relative abundance of 11% in GAC and 12% in ML. Obligate or facultative anaerobes, such as *Geobacter metallireducens* (OTU_19, similarity of 99%) (Schleinitz et al., 2009), *Geobacter hydrogenophilus* (OTU_136, similarity of 99%) (Kerin et al., 2006) and *Aeromonas rivipollensis* (OTU_17, similarity of 99%) (Marti and Balcázar, 2015), were shown to have a significant relative abundance (> 13%) on the GAC and mixed liquor samples, indicating an anoxic environment was maintained in the AeMFMBR, consistent with the result of the DO measurements.

The genus *Oca15* belonging to the order *Ignavibacteriales* was found to colonize with a relatively high abundance the membrane aerator in phase 2B-LN (14%) and 4C-SGU (2%) tests, and the GAC in phase 4C-SGU (6%). *Oca*15 was previously found in a wastewater treatment plant treating textile wastewater (Meerbergen et al., 2017), but it has not been well studied. The colonization of *Oca*15 on the growth media was quite interesting and could be worth investigating in a future study.

3.6. Overall assessment and future studies

Based on the microbial community analysis, ammonia removal was most likely due to biological nitrification and denitrification. The lack

of any dissolved oxygen or methane, or visible gas bubbles, suggests that air stripping was not a factor in ammonia removal in the AeMFMBR. In order to rule out the effect of air stripping for ammonia removal, an abiotic test was conducted using membrane aerators to strip an ammonium bicarbonate solution (80 mg-N/L). No noticeable ammonia removal was shown during the first 20-day stripping (bubbleless addition of the air) (see Supplementary information). In addition, there was no decrease in TN even when sparging tests were conducted with a gas diffuser using air.

Although the HRT of the AeMFMBR was shorter than those reported in some other MABR tests (several days using air) (Gilmore et al., 2013, 2009; Smith et al., 2008; Terada et al., 2003), and comparable with a hybrid systems combining anaerobic baffled reactor with membrane aerator (40 h) (Hu et al., 2009), the HRT was still a little too long to be feasible as a post-treatment technique. According to the COD and nitrogen removal in the AeMFMBR, it is indicated that the nitrogen removal had a slower rate than COD removal. The slow nitrogen removal rate was likely due to the low lumen pressure applied in this study for maintaining an anaerobic environment for mixed liquor, supported by the DO measurement and microbial community analysis. In order to reduce the HRT, increased lumen pressure or larger surface area should be used in future tests to increase the rates of nitrification using membrane aerators. In these future studies, a balance could be found between maintaining an anaerobic environment and applied lumen pressure to maximize the AR.

4. Conclusions

The operation of the AeMFMBR at an HRT of 41 h produced a good effluent quality with a TN of 12 ± 3 mg-N/L and COD of 14 ± 11 mg/L, and non-detectable dissolved oxygen or methane. Nitrate and nitrite concentrations in the effluent were < 1 mg-N/L. High removal efficiencies were obtained for both COD ($93 \pm 5\%$) and ammonia ($74 \pm 8\%$). Membrane fouling was well mitigated with a TMP < 3 kPa. Analysis of the microbial communities supported a mechanism of ammonia removal in the AeMFMBR based on nitrification and denitrification in the presence or absence of added COD.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.07.183.

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