

Single-Stage Biofilm-Based Total Nitrogen Removal in a Membrane Aerated Biofilm Reactor: Impact of Aeration Mode, HRT and Scouring Intensity

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Research Article

Keywords: Membrane aerated biofilm reactor, aeration mode, ammonia removal, biofilm-based total nitrogen removal, simultaneous nitrification-denitrification, membrane scouring

Posted Date: May 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-400202/v1>

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Abstract

High energy costs, organic carbon availability, and space limitation are some of the barriers faced by wastewater treatment processes. This research investigates the impact of membrane aeration mode, scouring intensity, and loading rate in a single-stage total nitrogen removal process in a membrane aerated biofilm reactor (MABR). Under ammonia loading of 2.7 g N/m².d, continuous process aeration led to 1.7 g NH₄-N/m².d and 0.8 g TN/m².d removal, respectively. Conversely, intermittent (5/12 min on/off) aeration resulted in 35% less ammonia removal but 34% higher total nitrogen (TN) removal. The MABR under ammonia load of 1.6 g N/m².d showed an enhanced effluent quality with an average of 2.5 mg/L effluent ammonia concentration. This finding highlights the nitrification potential of a flow-through MABR as a standalone treatment step without any downstream process. Also, slough-off, a common issue in the biofilm process and was hypothesized to reduce the removal efficiency, showed increased ammonia removal rates by 20%. The microbial analysis indicated the dominant AOB and NOB species as *Nitrosomonas spp.* and *Nitrospira spp.*, respectively. Moreover, the relative abundance of denitrifying bacteria (40.5%) were found twice in intermittently-aerated MABR compared to the continuously-aerated one (20.5%). However, NOB and denitrifying bacteria relative abundances were comparable where continuous air was supplied.

1. Introduction

The extent of energy consumption in the biological wastewater treatment industry has become the center of attention in recent years. Population growth and the resulting higher contaminant loading is the motivation to establish energy-efficient approaches. In biological nitrogen removal processes, nitrification and denitrification pathways, the process's energy efficiency can be assessed through aeration in nitrification and carbon demand in denitrification. Total nitrogen removal using a conventional treatment requires substantial biological aeration (4.57 g O₂/g NH₄-N) and a carbon demand of 3.71 g organic carbon/g NO₃-N (Tchobanoglous et al., 2014). Furthermore, processes such as carbon oxidation by aerobic heterotrophs and the use of low-efficiency blowers for oxygen supply influence the energy balance negatively. Alternatively, membrane aerated biofilm reactor (MABR) can be considered to overcome the high energy demand associated with bubble aeration (Houweling et al., 2017b; Martin et al., 2012). MABR technology uses a gas transfer selective membrane to deliver oxygen to a biofilm attached to a membrane surface while other substrates like ammonia and carbon diffuse from the bulk liquid into the biofilm layers (Fig. 1).

Oxygen mass transfer rate (OTR) across the membrane is one of the most critical parameters characterized in MABRs. When the membrane is immersed in clean water with no biofilm grown on, gas flux through the membrane depends on the membrane mass transfer coefficient and the oxygen concentration gradient, influenced by oxygen-water Henry's constant (Cote et al., 1989). However, in a wastewater treatment process where biofilm grows on the membranes' surface, biofilm plays a significant role in determining the oxygen concentration gradient. Biofilm oxygen demand, which is a

function of biofilm characteristics and operational parameters such as other substrates concentration (nitrogen and carbon), influences the oxygen gradient's extent within the membrane wall in a treatment process (Pellicer-Nàcher et al., 2013; Shanahan and Semmens, 2006). The OTR and OTE are calculated as per equations 1 and 2 (Côté et al., 2015):

$$\text{OTR} = \frac{24 \cdot M_o}{V_m} (Q_{PF} \cdot X_F - Q_{PE} \cdot X_E) \quad \text{Eqn. (1)}$$

$$\text{OTE} = \frac{J}{24} \frac{V_m}{Q_{PF} \cdot M_o \cdot X_F} \quad \text{Eqn. (2)}$$

Where OTR is in g/m².d, M_o is oxygen molecular weight (32 g/mol), Q_{PF} and Q_{PE} are process gas feed and exhaust specific flow rates (Nm³/m².h), V_m is the standard gas volume at standard temperature and pressure (STP) (0.0224 m³/mol), and X_F and X_E are molar fractions of oxygen in feed and exhaust gas.

MABR systems are becoming popular in biological carbon and nitrogen removal processes because of their capability of hosting a higher amount of biomass and, consequently, achieving higher removal rates while saving a considerable amount of oxygen (up to 70%) compared to conventional systems as well as smaller footprint (Houweling et al., 2017a). Several studies have shown the potential of MABR to address carbon removal, nitrification (municipal and ammonium-rich industrial wastewater) (Da Silva et al., 2018; Downing et al., 2010; Gilmore et al., 2013; Gong et al., 2007; Kunlasubpreedee and Visvanathan, 2020; Pankhania et al., 1999; Premarathna and Visvanathan, 2019; Quan et al., 2018; Semmens, 2005; Stamatopoulou, 2019; Wei et al., 2012), reverse osmosis concentrate, and other chemicals (Lan et al., 2018; Liu et al., 2020; Mei et al., 2019; Potvin et al., 2012; Tian et al., 2019). However, despite the advantages of the simultaneous nitrification-denitrification (SND) process, such as minimizing the cost of extra tanks and recycle streams, research on one-stage biofilm-based SND in MABR is limited. As Table 1 demonstrates, the few MABR studies implementing SND either have obtained low removal efficiencies or have applied longer HRTs (12–28 h) as well as higher C:N (5–18) ratios. However, the C:N ratios that were studied are much higher than the C:N ratio (< 3) that can be achieved in post-carbon redirection processes, hence further studies at lower C:N ratios are required to evaluate the feasibility of using chemically enhanced primary treated (CEPT) wastewater in denitrification without adding any external carbon source. In addition, the longer HRTs considered in previous studies results in larger reactor volume; establishing a robust SND process at relatively lower HRTs (approximately 6–8 times lower than typical HRTs applied in the literature) is of great interest. Another challenge that is supposed to be addressed in this study is comparing MABR performance under intermittent versus continuous aeration mode. Although MABR has been proved as an energy saver in terms of aeration, no investigation has been conducted to examine mainstream SND efficiency in MABR under intermittent aeration condition except the few sidestream (ammonium-rich wastewater) nitritation studies (Ma et al., 2017; Pellicer-Nàcher et al., 2010).

The counter-diffusional mass transfer pathway occurring within MABR biofilm layers is hypothesized to establish a robust SND system using CEPT wastewater at short HRTs. Overall, in this study, MABR's potential is explored to establish a simultaneous nitrification-denitrification system in the shape of a "single-stage biofilm-based total nitrogen removal system" low-strength municipal wastewater characterized by lower C:N ratio. The specific objectives of this study include: 1. Implementing one-stage biofilm-based SND in MABR a) using low-carbon CEPT feed without adding any external carbon source and b) under quite short HRTs (2.5 h), 2. Identifying the maximum nitrogen removal efficiency using intermittent aeration and characterizing the trade-off between energy consumption and removal efficiency

Table 1
Recent mainstream nitrogen removal studies in MABR

Process	sC:N	HRT (h)	Influent NH ₄ -N (mg/L)	NH ₄ -N removal rate (g/m ² .d)	Biofilm-based TN removal rate (g/m ² .d)	Ref.
COD & N removal	15	25.5	16.4	0.5	0	(Semmens, 2005)
COD & N removal*	2.8	21	25	0.27	0	(Downing et al., 2010)
COD & N removal	3	12	65	0.55	0.18	(Sun et al., 2015)
	5			0.53	0.37	
	7			0.51	0.28	
SND	10	24	30	0.24	0.22	(Lin et al., 2015)
			70	0.46	0.44	
			120	0.29	0.28	
SND	10	24	50	0.39	0.34	(Lin et al., 2016)
	8			0.4	0.36	
	5			0.41	0.35	
	3			0.38	0.23	
SND ^a	5	7.6	24	1.2	0	(Peeters et al., 2017)
SND	8	12	25	1.02	0.45	(Li and Zhang, 2018)
SND	2.7	28	57	0.94	0.94	(Tian et al., 2019)
COD & N removal	5	12	50	2.22	1.85	(Wu et al., 2019)
		20		1.85	1.44	
Nitrification	0	2.13	60	0.7–1.4	0	(Castrillo et al., 2019)
COD & N removal	18.3	24	37	1.55	0.3	(Gong et al., 2020)
			75	3	0.9	

^aMABR-Activated sludge hybrid system

2. Materials And Methods

2.1. MABR Pilot setup and operation

The study was conducted using four membrane-aerated biofilm reactor pilots with automatic control capability. Each pilot consisted of a 2L membrane tank and a junior Zeelung® membrane module (surface area of 0.14 m²). Variable-speed peristaltic pumps (capacity range of 30–450 mL/min) were used to pump feed wastewater into the reactors. Compressed air was used as the process air source, and high-purity (> 99.998%) compressed nitrogen gas was used as the mixing and scouring gas throughout the study. The process air (15 mL/min) was supplied intermittently through the membranes, where it diffused into the biofilm and was used as an electron acceptor during the carbon and ammonia oxidation processes. The intermittent aeration sequence used was 5 min on and 12 min off operation. The mixing gas (170 mL/min) was directly supplied to the membrane tank continuously to replenish the media with a fresh substrate, whereas the scouring nitrogen gas (2000 mL/min) was supplied to the tank intermittently as according to the experimental design. Figure 2 represents a schematic view of the MABR pilot.

Two of the reactors, henceforth referred to as R1 and R2, were operated under the normal scouring condition and HRT of 2.5 h, while one reactor was supplied by intermittent aeration and one by continuous aeration. Normal scouring refers to performing scouring every four hours, with each scouring lasting for one minute. The second set of reactors, R3 and R4, were operated under no scouring condition and HRT of 4 h, one intermittently and one continuously aerated. Aeration, mixing, scouring, and feed-flow control sequences were programmed using Factory Talk® View software (Site Edition, CPR 9 SR 6). Each pilot was equipped with two correlated flowmeters to measure gas flow rates and two pressure gauges to monitor inlet and outlet process air pressures.

2.2. Wastewater characteristics

The reactors were seeded with a nitrifying sludge obtained from an activated sludge plant (Vauxhall pollution control plant (PCP), London, Ontario, Canada). Sludge was screened using a 0.2 mm sieve and then seeded into the MABRs. The reactors were fed with CEPT wastewater from the Vauxhall PCP. 500 L of feed wastewater was delivered every week, and the feed was stored at room temperature over one week. The average feed ammonia concentration, sCOD concentration, and sCOD:N ratio were 16.9 ± 4.3 mg NH₄-N/L, 33 ± 11 mg COD/L, and 2.2 ± 1.1 , respectively.

2.3. Sample Analysis

Samples were collected twice a week from each MABR reactor and the feed wastewater. All samples were analyzed using Hach methods for NH₄-N (method #10031), NO₃-N (method #10020), NO₂-N (method #10019), TCOD, and sCOD (method #8000). Influent TSS and effluent ML(V)SS were measured following standard methods (Rice et al., 2012). In addition, bulk liquid DO and pH (Orion 087003, Thermo Scientific) were monitored twice per week. The off-gas oxygen fraction was monitored by connecting the oxygen analyzer device (MaxO₂ + AE, Maxtec) and the AML oxygen analyzer (model 70R1) directly to the exhaust

side of the membrane (Fig. 2). At the end of the study, biofilm samples were collected from each reactor to identify the relative abundance of microbial species on the biofilm (Molecular Research, MRDNA, Shallowater, TX, U.S.). Single-factor analysis of variance (ANOVA) was carried out in Excel to discern a significant difference between mean values, and a P-value < 0.05 is considered significant.

2.4. Batch Test

Two batch experiments were conducted to estimate the maximum nitrification and denitrification rates of a well-developed biofilm and compare the maximum total nitrogen removal vs. nitrification rate under intermittent aeration vs. continuous aeration. The batch tests were conducted using CEPT effluent and the MABR pilot that had been operating for over eight months. Before a batch test was initiated, each MABR was drained entirely and loaded with 2 L of feed. For nitrification batch tests, the feed stream was the CEPT wastewater similar to the one used in the long term study, whereas the CEPT wastewater was spiked with NaNO_2 and NaNO_3 to achieve 15–20 mg/L of nitrite and nitrate for the nitrite and nitrate denitrification batch tests, respectively. All batch experiments were carried out at 22°C, and samples were collected every 30 minutes for $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, sCOD, and DO analysis.

2.5. Microbial analysis

At the end of the experiments, biofilm samples grown on the membrane fibers were shipped to Molecular Research DNA LAB (Shallowater, TX, USA) for microbial analysis. The samples were analyzed using the MiSeq sequencing method under bTEFAP. The 16S rRNA gene V4 variable-region PCR primers 515/806 were used in a 30–35 PCR with the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 minute after final elongation at 72°C for 5 minutes. Sequencing was performed on an Ion Torrent PGM following the manufacturer's guidelines. Sequence data were processed using a proprietary analysis pipeline (MR DNA, Shallowater, TX, USA).

3. Results And Discussion

3.1 Impact of aeration mode on ammonia and total nitrogen removal: HRT of 2.5 h with membrane scouring

Two MABRs were operated for 230 days, under the normally-scoured condition to minimize ammonia diffusion limitation to AOB. Scouring is a typical membrane operation strategy to keep biofilm thickness within an optimum range and minimize mass transfer resistance. Using scouring, ammonia diffusion within the biofilm layers will face less resistance, and consequently, higher ammonia removal efficiency is supposed to occur. In this work, the normal mode of scouring refers to 1 min long of scouring (2000 mL/min) with four times per day frequency. The ammonia loading rate in previous MABR mainstream wastewater studies ranged between 0.3-7 g/m².d (Gilmore et al., 2013; Semmens, 2005). In this experiment, the ammonia loading rate was selected as 2.7 g/m².d resulting in a short HRT of 2.5 h. Although the loading rate of 2.7 is within the typical range for nitrification systems, no studies have been

found at a shorter HRT of 2.5 h. The shortest HRT used in the previous co-diffusional or counter-diffusional biofilm studies was 7.6 h, while the typical range was 12–28 h (Table 1). Two different aeration modes, intermittent (5 min On/12 min Off) and continuous aeration, were applied to R1 and R2.

Figure 3a represents the removal and accumulation of nitrogen compounds in intermittently- (R1) and continuously- (R2) aerated MABRs. Ammonia removal of 62% ($1.7 \text{ g/m}^2\cdot\text{d}$) has been achieved in the continuously-aerated MABR (R2) that is significantly higher than the 40% ($1.1 \text{ g/m}^2\cdot\text{d}$) removal achieved in R1 ($P < 0.05$). Having used an average C:N ratio of 2.2, TN removal efficiencies of 64% and 47% were achieved (removal rates of 0.7 and $0.8 \text{ g/m}^2\cdot\text{d}$) in R1 and R2, respectively. However, at lower C:N ratios ($< 4-5$), unstable and low denitrification efficiency has been reported in conventional (co-diffusional) biofilm systems like moving bed biofilm reactor (MBBR), and integrated fixed-film activated sludge (IFAS) (Gu et al., 2018; Iannacone et al., 2019; Liao, 2017). It is important to point out that the ammonia and total nitrogen were both removed using the same biofilm. Thus, the biofilm's overall capacity can be estimated by adding the ammonia oxidation and the nitrate or nitrite reduction rate that yields 1.9 and $2.5 \text{ gN/m}^2\cdot\text{d}$ for the intermittently and continuously-aerated MABRs, respectively. Previous studies have looked at biofilm processes for total nitrogen removal from mainstream wastewater using IFAS mode. Nitrification rates of $0.3-1.3 \text{ g/m}^2\cdot\text{d}$ were reported within the biofilm while the suspended growth bacteria supported the denitrification (Liao, 2017; Onnis-Hayden et al., 2007; Regmi et al., 2011). Regmi et al. (2011) reported a 50% denitrification rate while using C:N ratio of 7; however, the denitrification occurred in bulk liquid by suspended growth, not by attached growth bacteria (Regmi et al., 2011). A few numbers of investigations using hybrid biofilm and activated sludge systems observed denitrification activity and hypothesized that part of denitrifying activity might belong to the biofilm, but the report lacked further analysis to confirm the hypothesis (Germain et al., 2018; Ito et al., 2019). A study by Peeters et al. (2017) reported relatively higher nitrification rates of 1.2 and $2.6 \text{ g/m}^2\cdot\text{d}$ in a hybrid MABR-activated sludge system where denitrification occurred in the suspended sludge zone rather than the biofilm zone (Peeters et al., 2017).

One of the challenges reported regarding TN removal efficiency is outcompeting denitrifiers by aerobic heterotrophs when C:N ratio of 5 and higher is applied (Iannacone et al., 2019). Using intermittent aeration mode in MABR, it is hypothesized that developing an environment for the denitrifiers to outcompete the aerobic heterotrophic bacteria for the limited available carbon is a possible counter-diffusional pathway that would limit oxygen availability to the most exterior biofilm layer where the aerobic carbon oxidizing bacteria typically grows. Therefore, the significance of combining counter-diffusional biofilm and supplying intermittent aeration comes to play when dramatically decreased nitrification and denitrification rates can be seen in an MBBR (co-diffusional biofilm) under C:N ratio of 5.6, which caused aerobic heterotrophs overgrowth and consequently out-competing both nitrifiers for oxygen and denitrifiers for carbon (Iannacone et al., 2019). Table 2 summarizes the recent co-diffusional biofilm studies conducted to remove total nitrogen from municipal wastewater.

Table 2
Summary of recent biofilm studies in nitrogen removal

Process	Reactor	HRT (h)	Inf. NH ₄ (mg/L)	Nitrification rate	Denit. in biofilm	Ref.
Nit/denit.	IFAS train	30	40	0.35 g/m ² .d	None	(Regmi et al., 2011)
Nit/denit	IFAS	N.A.	35	1.12 g/m ² .d	None	(Onnis-Hayden et al., 2007)
SND	Hybrid RBC ^a -AS ^b	8	38	1.63 g/m ² .d	1.2 g/m ² .d	(Ito et al., 2019)
		12		1.13 g/m ² .d (in AS)	0.86 g/m ² .d (in biofilm)	
Nitrification	IFAS	10	25	0.095 g/m ² .d	None	(Liao, 2017)
Nitrification	RS ^c	16	35	0.6 kg/m ³ /d	None	(Hewawasam et al., 2017)
Nit/denit.	IFAS	6	27	0.8 g/m ² .d	0.35 g/m ² .d	(Germain et al., 2018)
SND	MBBR	48	62	0.16 g/m ² .d	0.05 g/m ² .d	(Iannacone et al., 2019)
SND	MABR	2.5	16.9	1.1–1.7 g/m ² .d	0.7–0.8 g/m ² .d	This study
		4		0.9–1.4 g/m ² .d	0.4–0.6 g/m ² .d	

^a Rotating Biological Contactor

^b Activated Sludge

^c Rotational Sponge

In this study, the average oxygen transfer rate (OTR) and average bulk liquid DO was measured as 5.2 g/m².d and 0.6 mg/L, respectively. The OTR values were 50% lower than the one observed in continuous aeration (11.7 g/m².d); however, the bulk liquid DO was slightly lower, where the latter had an average DO of 0.8 mg/L. As a pathway indicator, the ratio of g O₂ consumed/g NH₄-N removed was calculated as 4.7 and 6.9 g/m².d in R1 and R2, respectively. In continuously-aerated MABR, the ratio of 6.9 is relatively higher than the stoichiometric oxygen demand for nitrification (4.57 g O₂ consumed/g NH₄ removed). As a result, although the amount of available carbon was relatively low, the carbon removal pathway included aerobic oxidation and denitrification in continuously-aerated MABR.

It is also worth noting that, compared to intermittent aeration, a significant proportion ($P < 0.05$) of the oxidized ammonia remained as nitrate in the continuously-aerated MABR (0.4 and 1 g/m².d for R1 and R2, respectively) (Figure 3a). Figures 3b and 3c also present ammonia and TN removal efficiency profiles over time. The results demonstrate that 27% more of the oxidized ammonia was removed as total nitrogen in R1 compared to R2. Remarkably, this correlation between ammonia removal and nitrate production explains the trade-off between intermittent vs. continuous operation: (i) with intermittent aeration: less ammonia removal and having nitrogen gas as the dominant end-product with a saving of 70% aeration intensity or (ii) with continuous aeration: higher ammonia removal rate with more nitrate as the end-product. This result has further strengthened the conviction that MABR can be used as an adaptable technology depending on the WWTP's treatment goal(s). In WWTPs, which consider ammonia removal as the primary target, and there is no restriction to meet effluent nitrate criteria, continuous aeration will be a wise choice since it can lead to higher ammonia removal efficiency. However, when total nitrogen removal is emphasized in a treatment plant, intermittent aeration can be a suitable strategy to achieve higher total nitrogen removal efficiency.

3.2 Impact of aeration mode on ammonia and total nitrogen removal: HRT of 4 h associated with no membrane scouring

The ammonia and total nitrogen removal performance under a lower loading rate of 1.6 g/m².d, resulting in HRT of 4 h, were characterized using a second pair of MABR pilots (R3 and R4). The MABRs were continued from previous research, where they had been running under non-scoured and intermittent aeration mode for 365 d (Mehrabi et al., 2020). In this study, both MABRs were continued under non-scoured mode; however, continuous aeration was supplied to R4. Since the ammonia loading rate has been a parameter of interest in characterizing nitrification efficiency, a lower ammonia loading rate was selected in this experiment, comparable to the loading rate used in previous studies that reported higher ammonia removal rates (Lin et al., 2016; Martin et al., 2012). At ammonia loading rate of 1 g/m².d, nitrification efficiency was reported as 98% in MBBR; however, at higher loadings, decreased nitrification rates were reported (Andreottola et al., 2000). In agreement with the high ammonia removal efficiency at lower ammonia loadings, Lin et al. (2016) reported 0.27 g/m².d of ammonia removal rate in MABR when loading was 0.29 g/m².d (Lin et al., 2016). On the other hand, Peeters et al. (2017) observed 2.6 and 1.2 g/m².d of ammonia removal rates in MABR when ammonia loading was 7 and 3.1 g/m².d, respectively (Peeters et al., 2017). In this study, it was hypothesized that using a combination of lower ammonia loading rate and a lower C:N (< 3) wastewater, the MABR effluent that does not require downstream polishing can be generated.

Figure 4a presents the removal and accumulation rates for different nitrogen compounds in R3 (intermittent aeration) and R4 (continuous aeration) as well as Figs. 4b and 4c which demonstrate ammonia and TN removal efficiency profiles. On day 40 of the 230-day operation period, R4 faced a slough-off phenomenon, and consequently, a significant portion of sludge/biomass was washed out

from the reactor, and denitrifying activity reached almost zero (Fig. 4c). The collapsed denitrification efficiency due to slough-off proved that the denitrifying population was developed on the biofilm's outer layers. In addition, the fact that the slough-off occurred in the continuously-aerated reactor confirms the overgrowth of heterotrophs and the significance of scouring to control the biofilm thickness. Under the loading of $1.6 \text{ g/m}^2\cdot\text{d}$, continuously-aerated non-scoured MABR (R4) achieved ammonia removal rate of $1.4 \text{ g/m}^2\cdot\text{d}$ (86%), which resulted in an average effluent ammonia concentration of 2.5 mg/L that is aligned with discharge limits. While continuously-aerated non-scoured MABR showed the full capability of removing ammonia with no need for downstream processes, intermittently-aerated MABR (R3) achieved $0.9 \text{ g/m}^2\cdot\text{d}$ (56%) ammonia removal rate. In terms of total nitrogen removal, R3 showed a higher TN removal capability of 70% with 30% nitrate accumulation, while a much higher nitrate accumulation (87%) was observed in R4. It should be noted that all the R4 data discussed so far include the whole experiment containing before and after the slough-off phenomenon.

Regarding the performance of R4, ammonia removal efficiency can be divided into two different periods, before and after the slough-off phenomenon. Before slough-off occurred, the ammonia removal efficiency was 75% in R4, whereas 90% efficiency was observed after the slough-off. Regarding denitrifying activity, TN removal efficiency dropped from 65–14%, and consequently, residual nitrate increased from 35–85%. These findings indicated that although denitrifying activity was destroyed due to slough-off, a 20% increase in ammonia removal efficiency was seen, which might be the result of decreased diffusion resistance for ammonia into a thinner biofilm after the slough-off. Slough-off is a common issue in any biofilm process. It is typically accompanied by loss of nitrification activity; however, this study's finding showed that it does not reduce nitrification activity and enhanced ammonia oxidation rate. Increased nitrification rate after the slough-off can confirm the reliability of MABR in terms of ammonia removal.

As discussed earlier, although extensive research has been carried out on SND in conventional biofilm systems, very few numbers of MABR studies explored the possibility of implementing a biofilm-based SND process, particularly for carbon-captured municipal wastewater. Among such MABR studies, some used hybrid systems (which do not support a biofilm-based process), and others have been restricted to apply very long HRTs, higher influent ammonia concentration, and higher C:N ratios (Table 1). For example, Lin et al. (2016) conducted a study that examined the potential of MABR in establishing SND. They reported a 62% TN removal efficiency at C:N of 3 while HRT was 24 h. However, the same TN removal efficiency was achieved in this study by the HRT of 2.5 h, which translates into a faster process by almost ten times. Hence, we hope that this study's results could eliminate the research gap in using MABR to establish a robust one-stage TN removal system.

Table 3 and Fig. 5 summarized the whole performance of the four MABRs operated under different aeration modes, scouring modes, and HRTs. Comparing ammonia and total nitrogen removal rates and efficiencies in two continuously-aerated MABRs (R2 and R4) but under normal and non-scoured conditions showed a 20% more ammonia removal in R2 compared to R4. This agrees with the finding obtained in (Mehrabi et al., 2020), where normal scouring led to a higher ammonia removal efficiency than the non-scoured MABRs. The higher ammonia removal corresponding with normal scouring is

expected to relate with the scouring of the outer heterotrophic carbon oxidizing biofilm layer resulting in a reduced mass transfer resistance for ammonia diffusion through biofilm layers. Another interesting point that came off this experiment is scouring versus non-scouring in TN removal efficiency of the continuously-aerated MABRs. Under continuous aeration condition, the TN removal efficiency remained comparable despite the difference in scouring modes. This observation is quite different from the results reported in the intermittently-aerated reactors (R1 and R3) and also reported by Mehrabi et al. (2020) that indicated a higher TN removal rate of non-scoured MABRs compared to normally-scoured MABRs when intermittent aeration was supplied. That might be justified as when continuous air is introduced to the process, the scouring has no impact on total nitrogen removal efficiency due to the excess air available within the biofilm layers. This unlimited oxygen availability challenges developing an extensive anoxic biofilm layer on the MABR. Typically, the oxygen transfer rate is controlled by the substrate (ammonia and carbon) availability and biomass demand. However, the observation in this study indicates that the nature of aeration supply mode (intermittent versus continuous) through the membrane also dictates the biochemical environment. Continuous aeration encourages the nitrifying organisms and limits the anoxic zone's availability as a desirable environment for denitrifying activity; however, further biofilm micro-level profiling is required to confirm this observation.

Table 3
Summary of operating conditions and obtained results

Operating condition		NH ₄ -N (g/m ² .d)		OTR (g/m ² .d)	TN removal (g/m ² .d)	NO _x -N residue (g/m ² .d)	Effluent NH ₄ -N (mg/L)	Effluent TN (mg/L)
Aeration mode	HRT (h)	Loading	Removal					
Intermittent	2.5	2.7	1.1	5.2	0.7	0.4	10.3	13
	4	1.6	0.9	5.6	0.6	0.37	7.4	11.5
Continuous	2.5	2.7	1.7	11.7	0.8	1	6.3	12.4
	4	1.6	1.4	10.6	0.4	1.08	2.5	14.2

3.3. Determination of maximum specific substrate removal rate

Batch experiments were carried out to characterize the performance of the MABRs to remove ammonia and total nitrogen and identify the maximum removal potential of the system. The ammonia oxidation and denitrification rate studies were carried out using CEPT wastewater and spiked CEPT wastewater, respectively (as described in Sect. 2.3). Figures 6a and b present the ammonia profile data obtained during the nitrification batch tests. The batch test showed ammonia removal rates of 3.5 and 5.4 g/m².d in R1 and R2 (intermittent and continuously-aerated reactors at HRT of 2.5 h), respectively. As can be seen, the maximum potential of both MABRs is 3.2 times higher than the rates observed in the long-term experiment, representing the substantial nitrification capability of the MABRs if they were run at higher loading rates in a batch mode using a similar HRT of 2.5 h. In non-scoured MABRs (Fig. 6b) where the

biofilm was developed on a long-term ammonia loading of 1.6 g/m².d, ammonia removal rate obtained in batch tests were 2.3 and 2.9 g/m².d in intermittently and continuously-aerated MABRs, respectively. Again, the maximum capability of the non-scoured MABRs is higher than the long-term results by 2-2.5 times; however, it is relatively lower than the maximum capability of the normally-scoured MABRs.

Denitrification rates were also identified in R1 and R2. Both nitrite and nitrate removal rates have been identified as the denitrification capacity of the system. The nitrite removal rate (Fig. 6c) was measured as 5 and 3.5 g/m².d under intermittent and continuous aeration. However, the corresponding nitrate removal rates were lower (2 and 1.8 g/m².d, respectively) (Fig. 6d). The exciting finding is the substantially higher potential (two times) of the system to remove nitrite compared to the nitrate in both reactors. This observation might be due to the concept of biofilm-based SND, which grows both nitrifiers and denitrifiers on the same medium. When denitrifiers grow adjacent to the nitrifiers, they might have the better capability to compete for the NOB for nitrite compared to the systems that denitrifiers mostly grow in suspended activated sludge. As a result, in long-term treatment, the denitrifying population might selectively improve its nitrite reduction capability rather than nitrate. However, due to the high amount of available air in R2, the denitrification rate (including both nitrite and nitrate) is relatively lower than R1, which shows the potential of intermittent aeration in MABR to enhance denitrification activity via nitrite over NOB.

3.4. Impact of aeration on the microbial community of biofilm

To further characterize the biological process, the microbial population grown on the surface of membrane fibres were analyzed. The bacterial population are presented in the phylum, class, and species-level in Fig. 7. At the phylum level (Fig. 7a), the dominant phylum, which accounted for more than 50% of the population, is *Proteobacteria*, including *Betaproteobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria* and *Epsilonproteobacteria* classes, which was reported as the dominant phylum in many WWTPs involved with biological nitrogen and carbon removal processes. *Betaproteobacteria* and *Gammaproteobacteria* classes were identified as related bacteria to nitrogen and organic matter removal. Besides, the AOB genera of *Nitrosomonas* belongs to *Betaproteobacteria*, which is the dominant AOB genera in both R1 and R2. *Bacteroidetes* and *Chloroflexi*, which were identified as two of the most popular phyla in both reactors, were observed as subdominant phyla in several WWTPs (Cyzdik-Kwiatkowska and Zielińska, 2016; Gong et al., 2020; Tchobanoglous et al., 2014). The aerobic phylum of *Nitrospirae* grew in a significantly higher amount under continuous aeration rather than intermittent, which is consistent with the species result reporting *Nitrospira sp* as the most dominant species in R2 (Fig. 7c). Since unlimited oxygen was supplied to the reaction zone in continuous aeration, it justifies the higher ammonia removal efficiency and higher residual nitrate in R2. Therefore, at the species level, respective AOB and NOB relative abundance (RA) were 10.3% and 20.7% under continuous aeration (R2); however, the relative abundance of AOB and NOB were 1.3% and 1.8% under intermittent aeration (R1). This finding highlights the fact intermittent aeration significantly limits nitrifiers' growth, which might have simultaneous positive and negative impacts on the process efficiency, as previously

discussed. The lower NOB relative abundance in intermittently-aerated MABR was in agreement with previous works that reported a decreased nitrite-oxidizing bacterial (NOB) activity and growth rate under intermittent aeration. The authors reported *Nitrospira* as the dominant NOB species rather than the *Nitrobacter* that is typical for continuous aeration systems (Mota et al., 2005; Yang and Yang, 2011). Similarly, another study indicated that in the biofilm regions with oxygen scarcity, *Nitrospira* was found as the dominant species, which has a lower substrate utilization rate than *Nitrobacter* (Downing and Nerenberg, 2008).

The other striking result of the microbial analysis data is the significantly different relative abundance of nitrifiers vs. denitrifiers in intermittently-aerated MABR vs. the continuously-aerated one. The relative abundance of denitrifying bacteria was measured as 40.5% in R1, which is two times higher than R2 (20.5%). It is worth mentioning that 16% and 6% of denitrifying bacteria comprised of sulphur-oxidizing bacteria such as *Sulfurisoma sediminicola* in R1 and R2, respectively, can use nitrate as an electron acceptor and convert it to nitrogen gas. In general, R2 hosted a narrower range of microbial populations, mostly aerobic microorganisms, while R1 grew a vast range of different species, mostly anoxic/anaerobic with denitrifying capability as *Sulfurisoma sediminicola*, *Trichococcus pasteurii*, *Thiothrix disciformis*, and *Methyloversatilis*. The higher percentage of denitrifiers and higher total nitrogen removal efficiency observed in R1 confirms the hypothesis of a well-developed anoxic region within the intermittently-aerated biofilm. In other words, denitrifiers could outcompete the NOB population for nitrite due to the favourable anoxic condition; however, NOB and denitrifiers relative abundances were almost equal in R2. Besides, while none of the reactors were seeded with anammox bacteria, it was interesting to find 0.67% and 0.9% of anammox bacteria in R1 and R2, respectively. Figure 8 shows the relative abundance of different functional populations in each MABR.

3.4.1. Impact of biofilm sample extraction method

For microbial analysis, three biofilm extraction methods from the membrane were utilized. The methods include Qiagen Powersoil DNA extraction kit, Ultrasonic bath, and Vortex Mixer were examined. In both ultrasonic bath (30 min, 70 kHz) and vortex mixer (3 min, 2500 rpm), distilled water was used as the solvent. According to the analysis results, significant differences were reported in the relative abundance of microbial populations using three different extraction methods. The relative abundance of AOB, NOB, and denitrifying bacteria for ultrasonic bath and vortex mixer methods was detected as approximately 50% lower than using the Qiagen Powersoil DNA extraction kit. The relative abundance of denitrifying bacteria using vortex mixer in R1 (Fig. 9). Regardless of the negative or positive error percentage, both ultrasonic bath and vortex mixers were found highly unreliable methods to extract the biofilm sample from the media comparing to the Qiagen Powersoil DNA extraction kit.

4. Conclusion

In this study, a single-stage biofilm-based total nitrogen removal was established to treat CEPT wastewater using MABR at short HRT of up to 2.5 h. The obtained results underlined the importance of

aeration mode and HRT on different nitrogen compounds removal and accumulation rates through the biofilm. Continuous aeration led to higher ammonia removal as well as accumulating nitrate as the end-product. On the other hand, although intermittent aeration resulted in less ammonia removal, TN removal was identified as the dominant pathway under this aeration mode, which showed the capability of establishing a single-stage biofilm-based total nitrogen removal process. Based on the ammonia loading vs. removal rate observed in the MABR pilot, ammonia removal achieved the discharge limit (2.5 mg/L), highlighting the possibility of operating flow-through MABR as the only treatment step without any downstream process. Another highlighted finding was a 20% increase in ammonia removal rate after the slough-off occurred in R4, most probably, due to the higher ammonia flux into the biofilm resulting from reduced diffusion resistance for ammonia molecules that can address the concerns regarding the common slough-off issue in MABR.

Taken together, the results in this study stand for the fact that the unique counter-diffusional biofilm formed in MABR facilitates one-stage biofilm-based total nitrogen removal by hiring different strategies such as aeration mode and HRT. Therefore, MABR would enable different wastewater treatment plants to fulfill the treatment targets. In plants that aim to remove only ammonia, a combination of non-scoured conditions and continuous aeration might be a proper alternative to achieve higher ammonia removal efficiency while no energy is consumed to scour the biofilm. This strategy might have a high risk of slough-off, which can be considered a benefit since it increases the removal efficiency when the main target is ammonia removal. However, for the WWTPs requiring total nitrogen removal, intermittent aeration should be considered as one of the necessary design parameters.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

Authors' contribution: Material preparation, data collection and analysis were performed by Sadaf Mehrabi. Study conception and experiments design were carried out by Sadaf Mehrabi, Martha Dagneu and Dwight Houweling. The first draft of the manuscript was written by Sadaf Mehrabi and Martha Dagneu commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This research is funded by the Natural Sciences and Engineering Research Council of Canada and Ontario Centre of Excellence.

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Figures

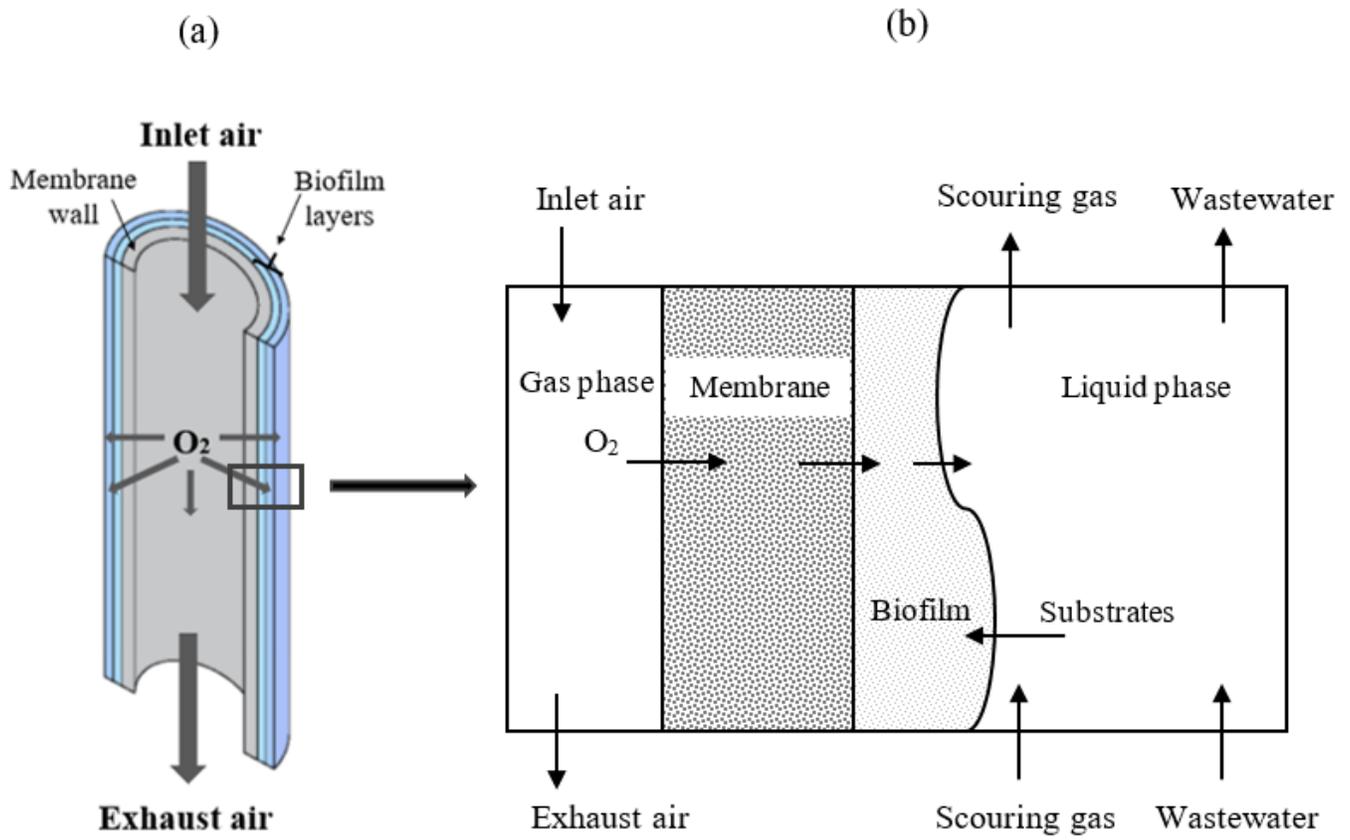


Figure 1

a) Schematic of a cross-sectional view of the membrane fiber, b) mass transfer within membrane and biofilm

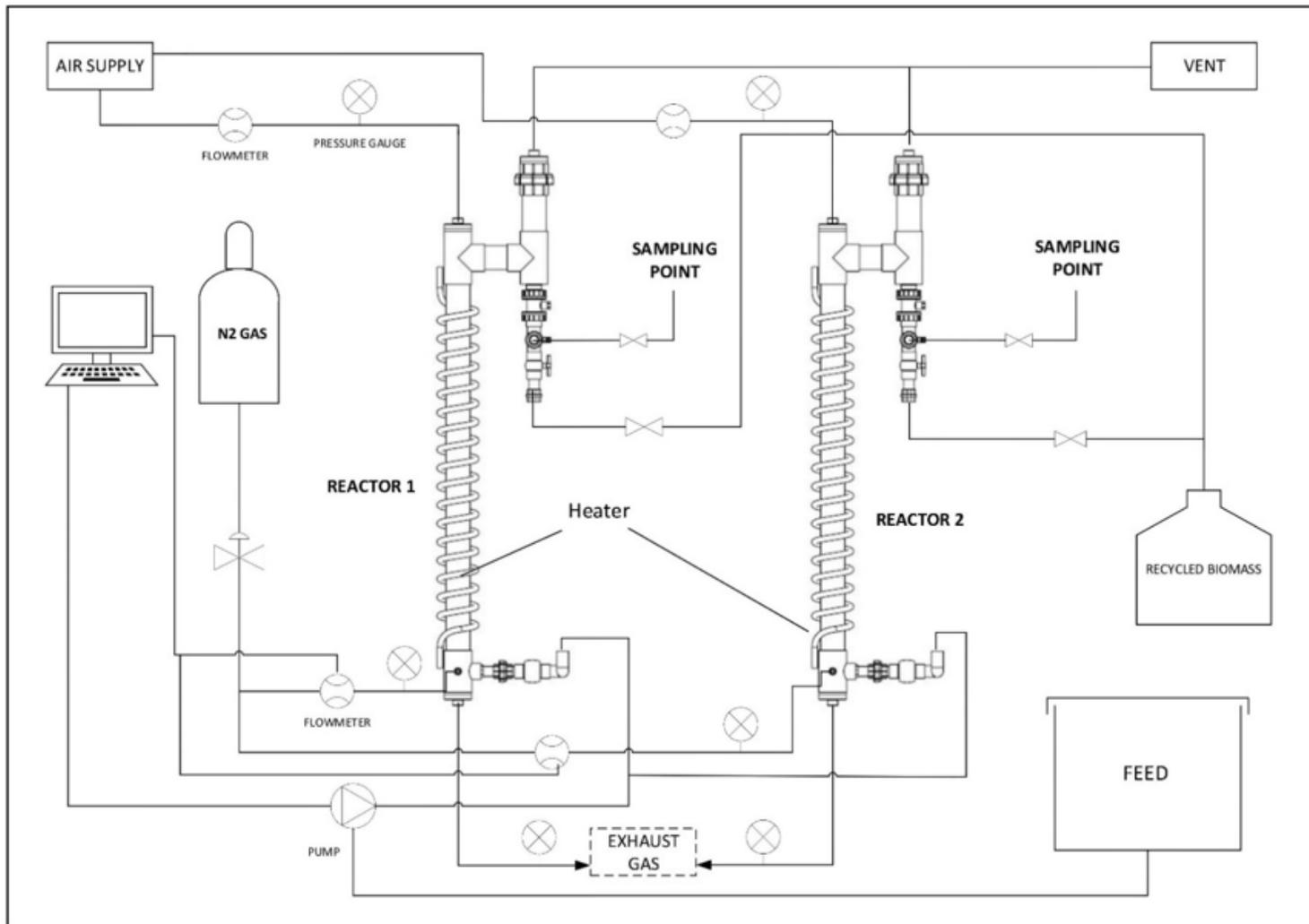


Figure 2

MABR pilot layout (Stamatopoulou, 2019)

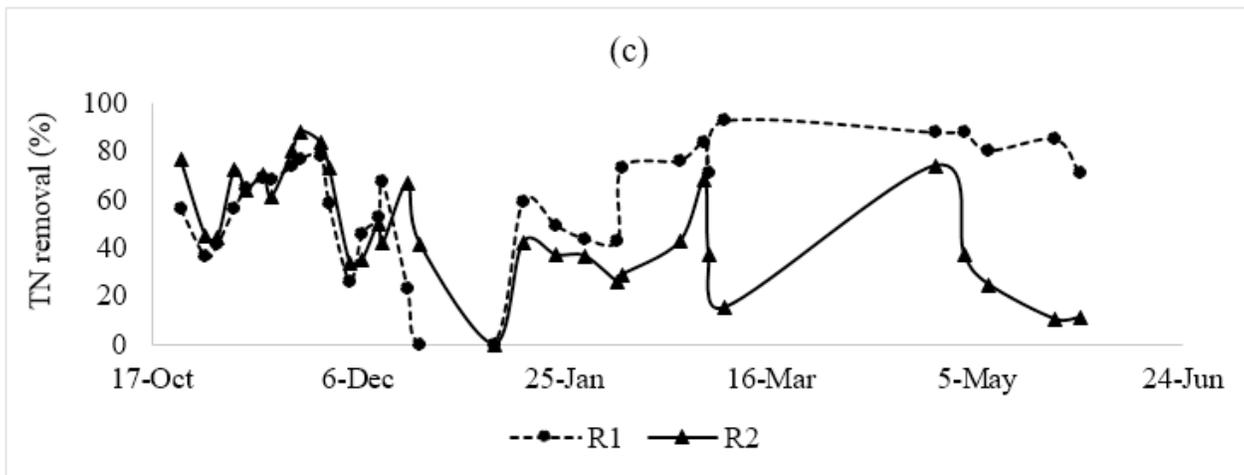
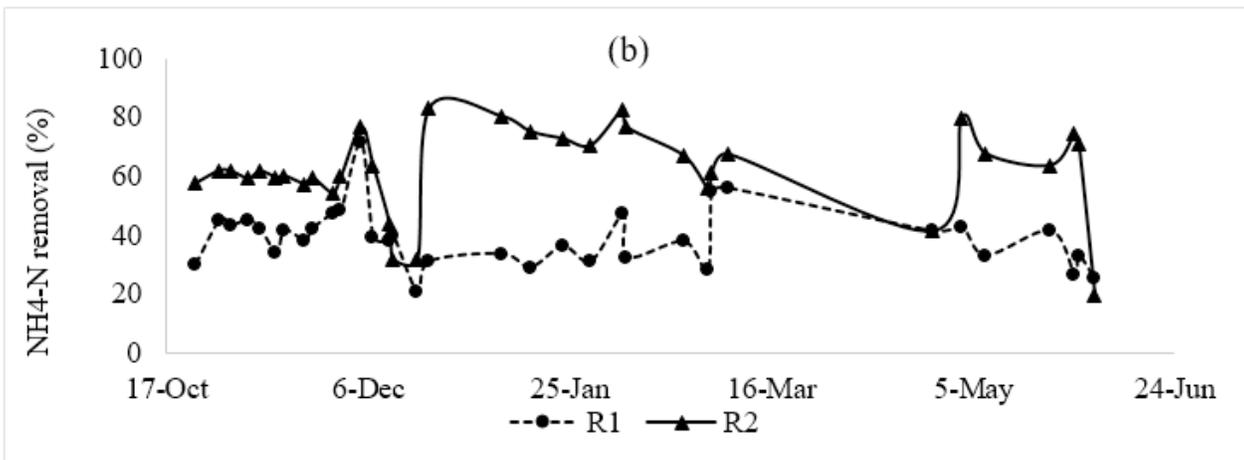
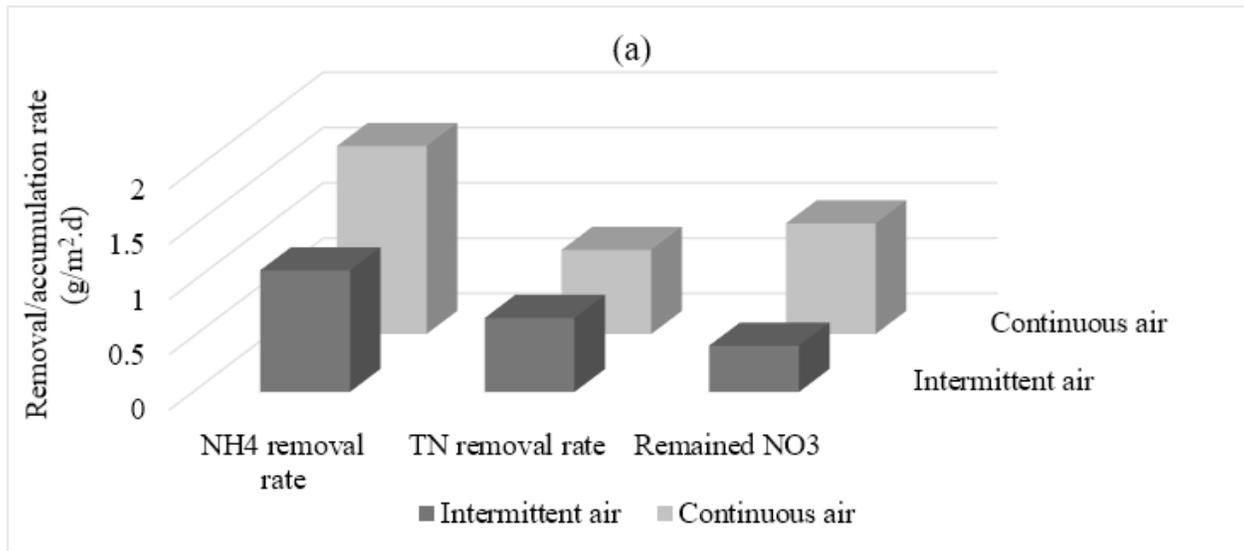


Figure 3

a) Nitrogen compounds removal/accumulation rates in intermittent (R1) and continuous (R2) aeration modes, b) Ammonia removal percentage, c) Total nitrogen removal percentage in R1 and R2

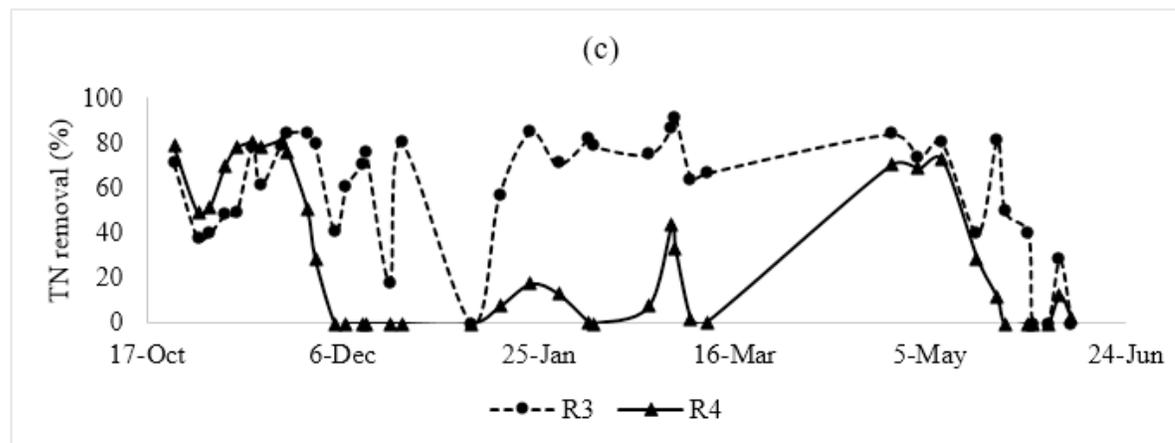
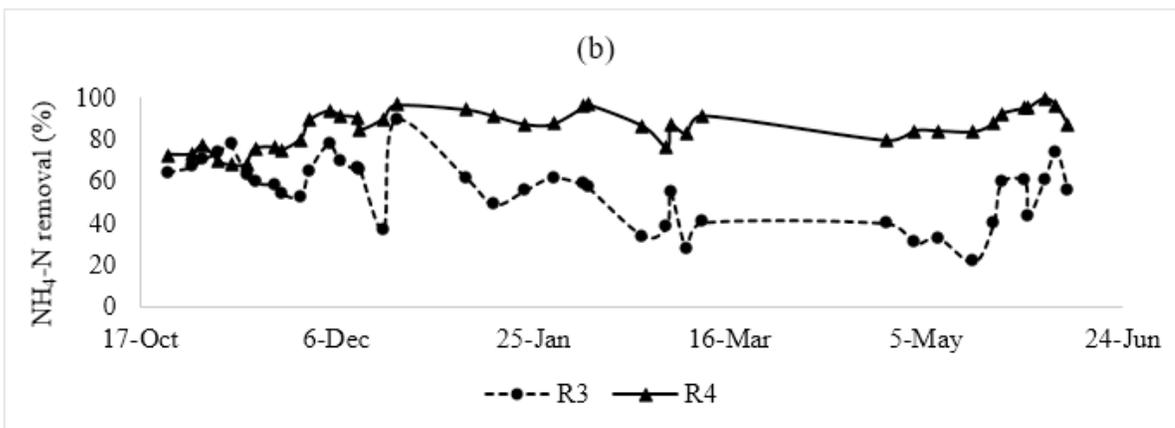
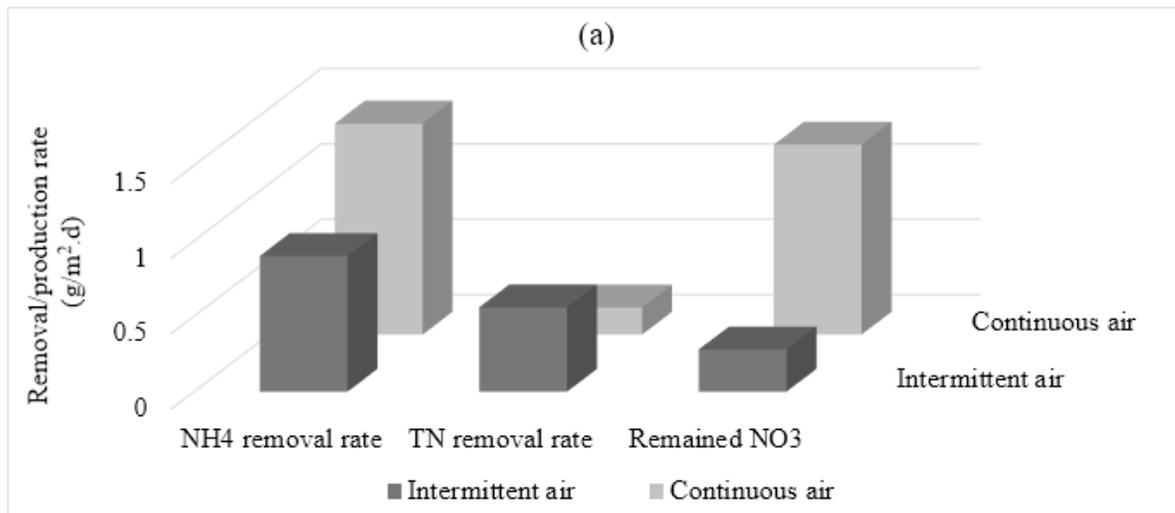


Figure 4

a) Nitrogen compounds removal/accumulation rates in intermittent (R3) and continuous (R4) aeration modes, b) Ammonia removal percentage, c) Total nitrogen removal percentage in R3 and R4

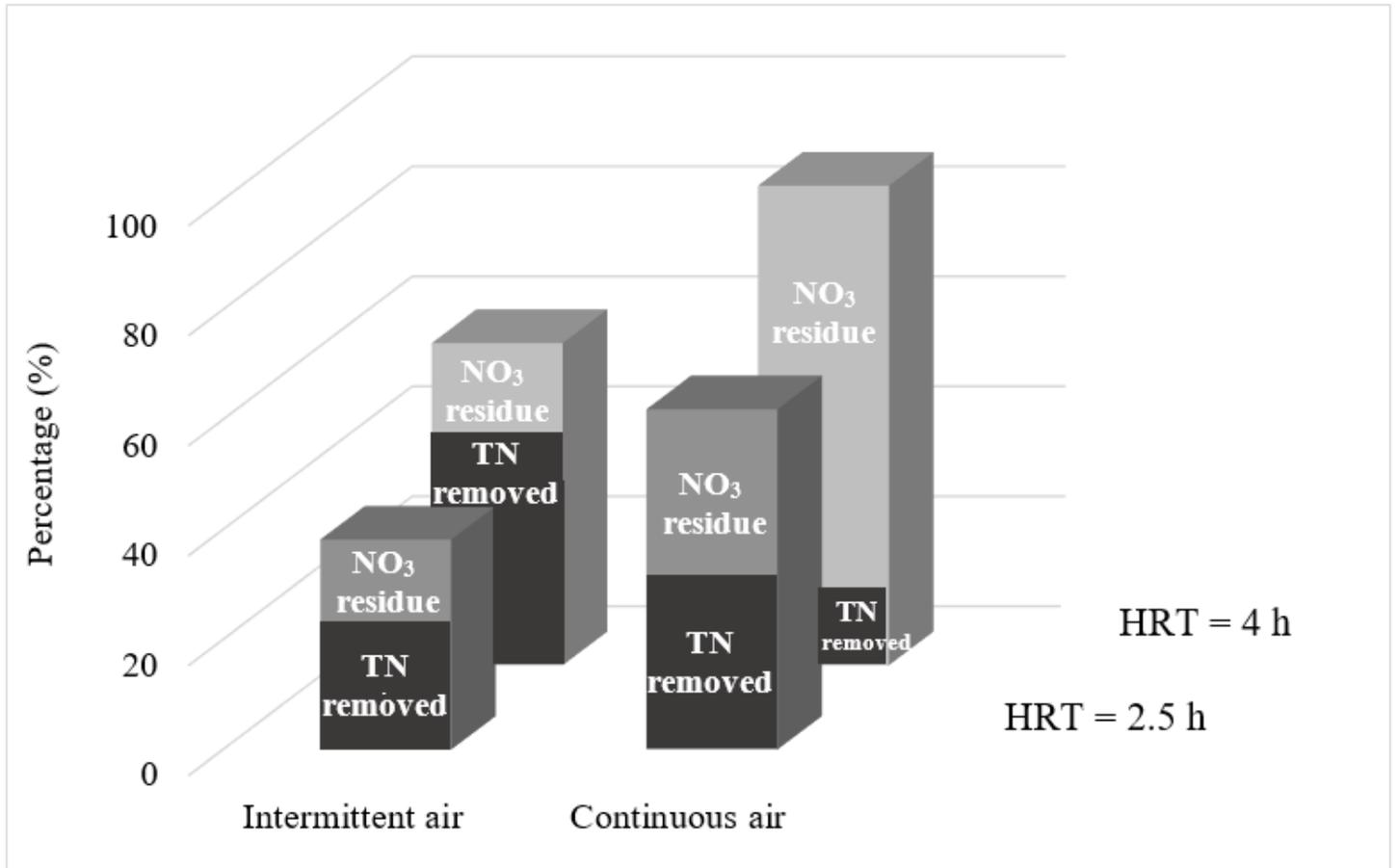


Figure 5

The fate of removed ammonia in MABRs operated under varying aeration modes and HRTs

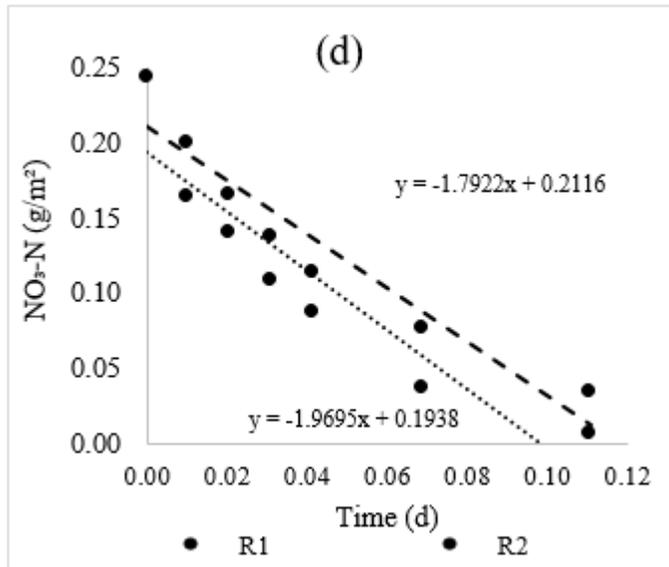
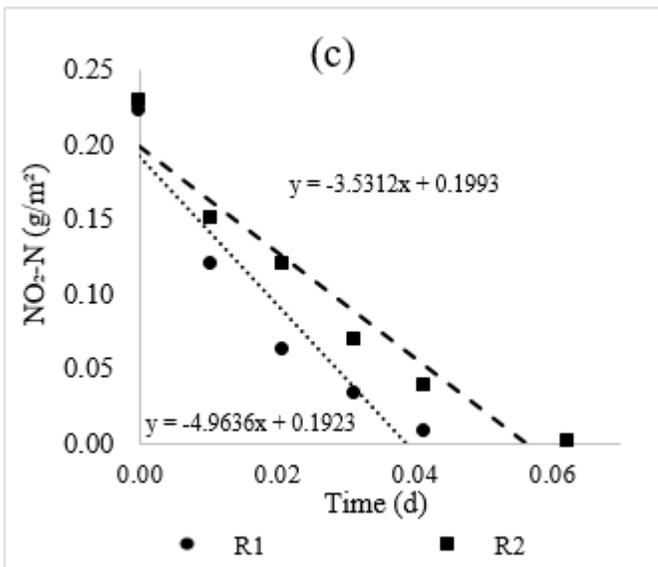
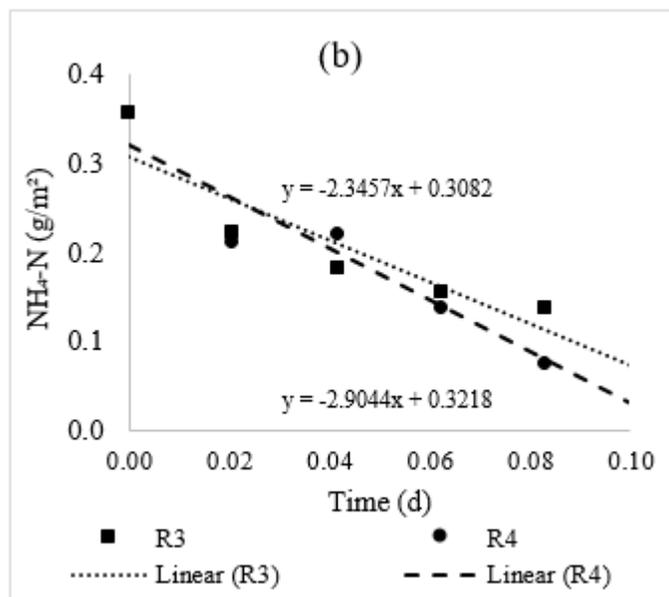
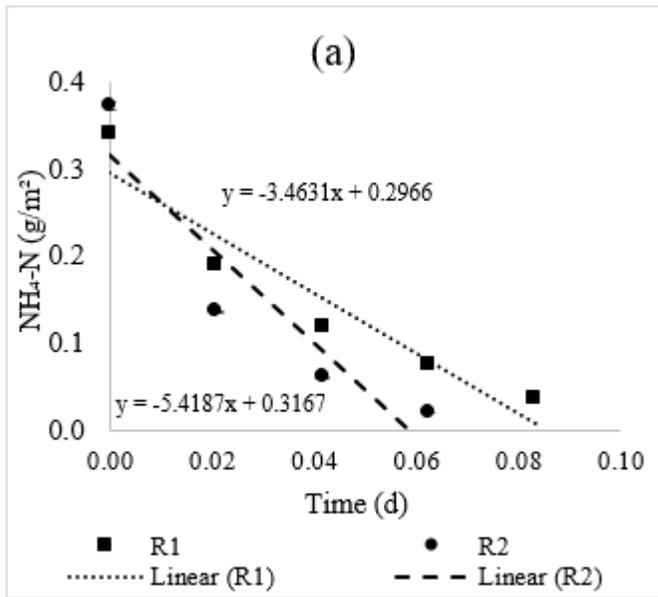


Figure 6

Batch tests: a) Ammonia removal rate in R1 and R2, b) Ammonia removal rate in R3 and R4, c) Nitrite removal rate in R1 and R2, d) Nitrate removal rate in R1 and R2

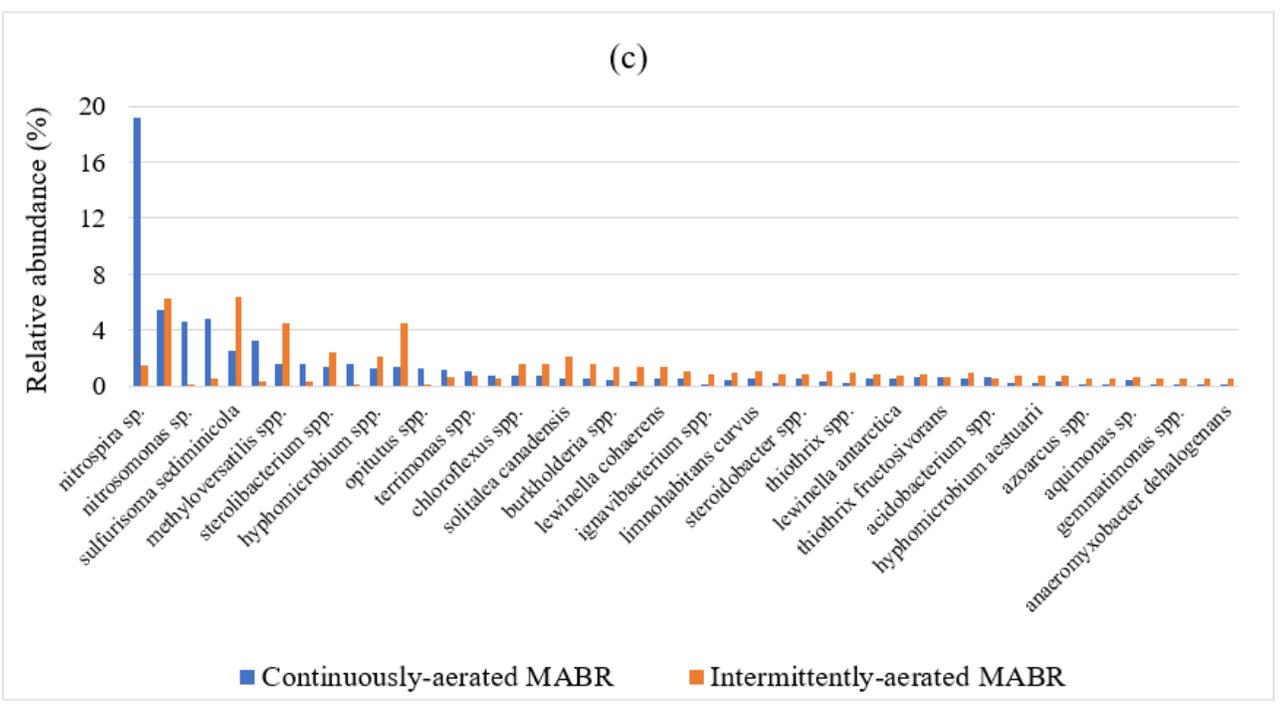
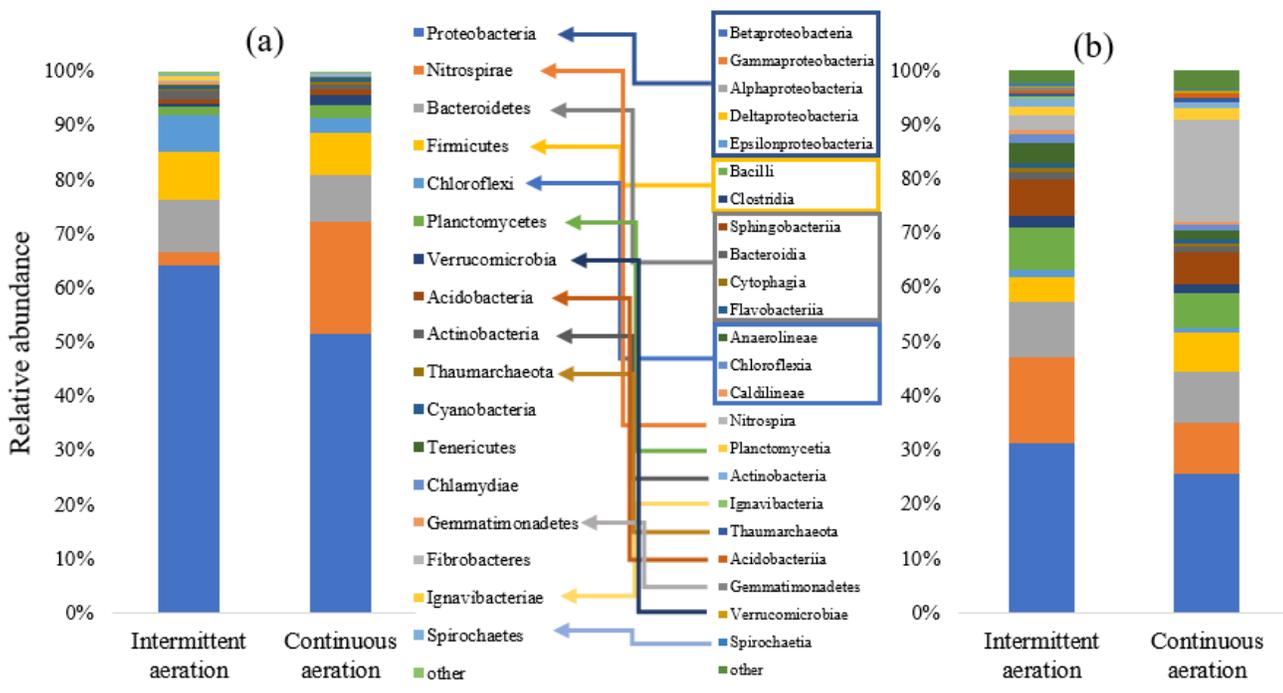


Figure 7

Function microbial population a) phylum, b) class, and c) species level relative abundance in intermittently-aerated and continuously-aerated MABRs

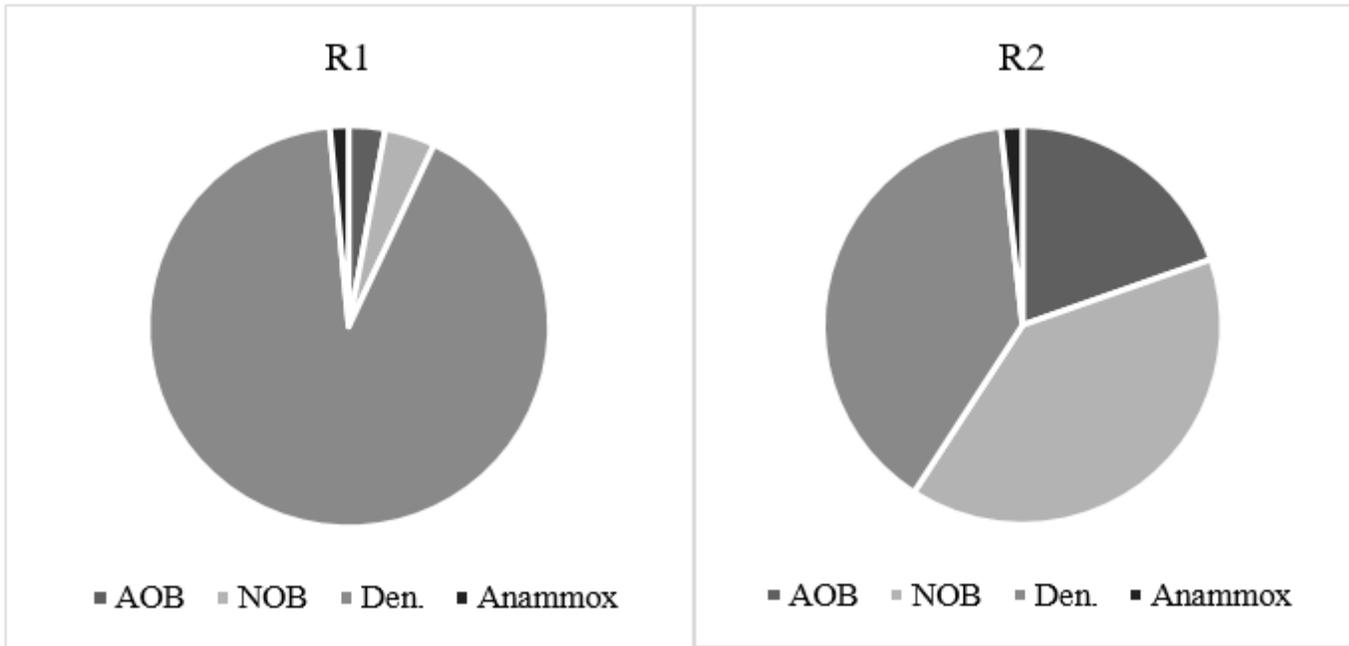


Figure 8

The relative abundance of the different microbial population in R1 and R2

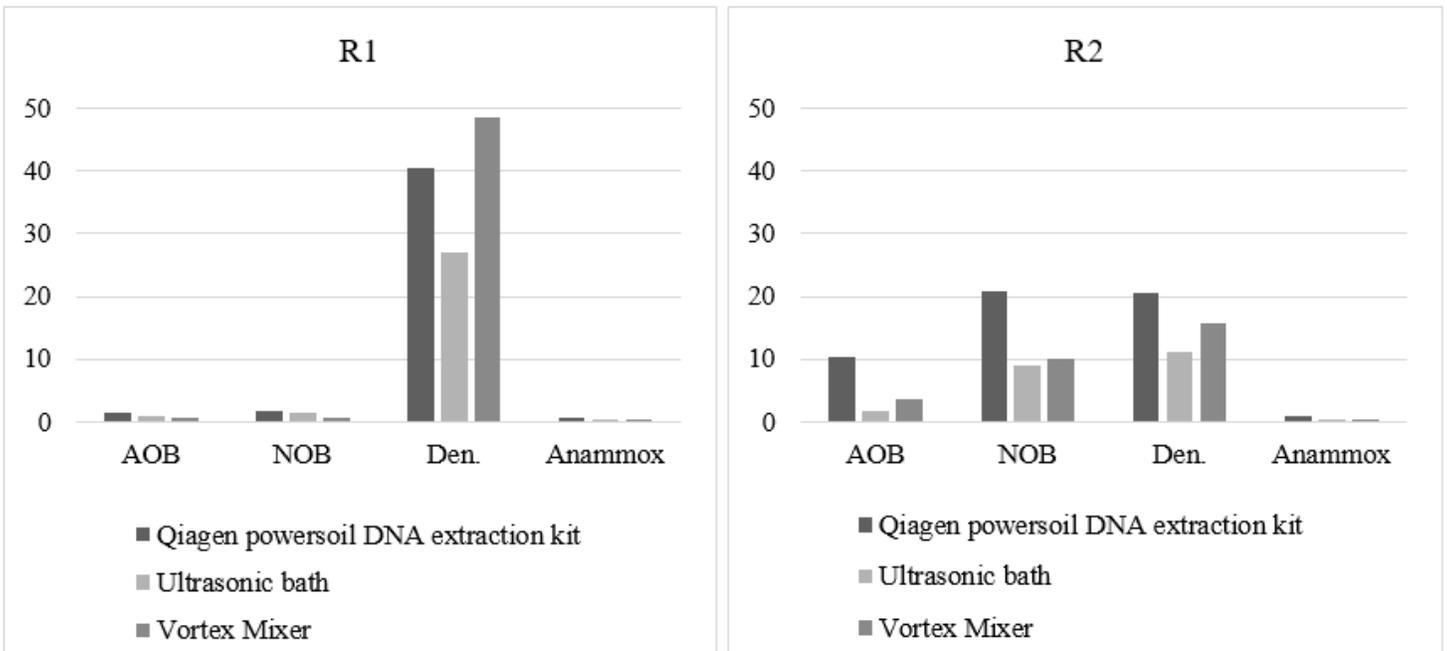


Figure 9

The relative abundance of the functional microbial population using different biofilm extraction methods in R1 and R2