Environmental Technology & Innovation 27 (2022) 102387

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Biological treatment of municipal wastewater using fixed rope media technology: Impact of aeration scheme

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ARTICLE INFO

Article history:
Received 25 October 2021
Received in revised form 25 January 2022
Accepted 31 January 2022
Available online 7 February 2022

Keywords:
Aeration
Ammonia removal
Rope-media biofilm
Primary effluent treatment

ABSTRACT

This paper characterized a new fixed rope media system to upgrade decentralized and small-scale wastewater treatment plants. A primary effluent of municipal wastewater was treated using two pilot-scale reactors equipped with full-scale sized fixed rope media technology, one of the reactors was aerated using a coarse bubble tube and the other using a custom fine bubble aeration system. The study examined the impact of the aeration scheme and intensities and the COD/NH_3-N ratio on ammonia and COD removal rates, excessive biofilm growth, slough-off, and microbial communities' composition. The average biofilm ammonia and COD removal rates ranged from 0.23 ± 0.15 to 0.38 ± 0.26 gNH_3-N/m^2.d and 1.35 ± 0.95 to 3.05 ± 1.21 gCOD/m^2.d, respectively. The fine and coarse bubble reactors showed comparable carbon oxidation rates; however, the fine bubble reactor showed a higher nitrification rate than the coarse bubble reactor at lower aeration intensities despite the similar dissolved oxygen concentration. Correspondingly, an increase in COD/NH_3-N and excessive biofilm growth decreased the NH_3-N removal performance but did not affect the COD removal efficiency. Further analysis of the microbial communities composition revealed that the reactors supported a relatively substantial amount of AOB (55 and 63%) and denitrifying bacteria (36 and 21%) with a relatively lower NOB (7 and 8%) and anammox (1 and 8%) species in the fine and coarse bubble aeration reactors, respectively. Overall this study demonstrated the feasibility of one stage fixed rope media to treat COD and ammonia and meet treatment objectives, thus providing an alternative solution to decentralized and smaller plant upgrades.

1. Introduction

Demand for fixed-film biological wastewater treatment is growing for conventional plant upgrades to attain nitrification and subsequent secondary effluent standards and increase plant capacity through increased biomass inventory per plant footprint. Furthermore, fixed-film technologies for decentralized or onsite nutrient removal are increasingly considered to meet future realistic effluent nutrient regulations (Rout et al., 2021). Fixed-film treatment systems include trickling filters, rotating biological contactors, fluidized bed, moving bed biofilm reactors, biological granular activated carbon, membrane aerated biofilm and immobilized cell reactors. The advantages of fixed-film reactors compared to conventional activated sludge systems include (i) simple operation; (ii) handling shock loads; (iii) better process stability

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https://doi.org/10.1016/j.eti.2022.102387

and resiliency; (iv) lower sludge production and (v) reduced footprint (WEF, 2011; Waqas et al., 2020; Nowrouzi and Abyar, 2021; Xu et al., 2021; Mehrabi et al., 2020; Mannina et al., 2020).

The disadvantages of fixed-film processes are more specific to each technology. However, some general concerns associated with fixed-film processes include (i) potential clogging of the media system as a result of inadequate screening; (ii) excessive growth, which could plug the media system or cause free-floating media to sink, (iii) high energy requirement and (iv) inadequate mixing or short-circuiting resulting in inefficient use of the media (WEF, 2011).

In recent years, smaller and passive wastewater treatment operations, including decentralized systems and lagoons initially designed for carbon removal, are required to meet ammonia removal objectives. This requirement has brought attention to developing robust, resilient, and cheaper biofilm systems for decentralized and small-scale wastewater treatment systems. So far, different types of advanced low-cost fixed-film media systems have been developed to enhance treatment capacity and nutrient-removal capabilities that include (1) Looped-cord or strand media: a rope-type media, (2) AccuWeb: a mesh-type media, (3) BioWeb: a woven media that has a mesh-like pattern and (4) Sponge media (Captor or Linpor) (Sen et al., 2006; Bushey et al., 2009; Borchert et al., 2011; WEF, 2011). Bushey et al. (2009) performed an integrated fixed-film activated sludge (IFAS) pilot study using Bioweb fixed media to enhance tank capacity and cold weather nitrification. The addition of BioWeb allowed for the conversion of 25% of the aeration basin to an anoxic basin, providing additional denitrification capacity at a facility with no room for expansion. Borchert et al. (2011) demonstrated a design nitrification rate of 0.039 g/m^2 of BioWeb media at a minimum wastewater temperature of 9 °C. The previous studies on these advanced fixed-biofilm systems focused mainly on evaluating the treatment efficiency under a given operating condition. The reported nitrification rates were on the lower side, requiring further process optimization and characterization of biofilm performance under diverse wastewater characteristics. Besides the impact of different aeration schemes on the media system's performance and microbial growth, aeration efficiency and modeling of these fixed-film wastewater technologies to improve performance were not considered.

This study used fixed rope media in a flow-through mode to assess how such fixed-media performs in different aeration environments and wastewater characteristics. The operating conditions considered in the current study were representative of small communities or distributed systems. The specific objectives of this study were to (i) evaluate the impact of different aeration schemes and aeration intensities on the fixed-film system performance, and the dynamics of microbial communities developed and (ii) examine the impact of COD/NH_3–N ratio and biofilm excessive growth and slough off on NH_3–N and COD removal rates.

2. Materials and methods

2.1. Reactor setup and operation

The type of media used in this study was a fixed rope media called BioCord (Fig. S1, Supplementary material). BioCord is a solid woven material made of polypropylene and developed by Bishop Water Technologies (Renfrew, Ontario, Canada) (Gan et al., 2018). The study was conducted using two pilot systems consisting of two full-scale BioCord cassettes individually housed in 2 m^3 reactors (Fig. 1). The pilot reactors were installed at a test bay located in the Greenway Pollution Control Plant (PCP) (London, ON, Canada). Reactor 1 (referred herein as R1) is a biofilm reactor equipped with a coarse bubble tube and a blower (Model No. DBMX300, Diann Bao Inc., Changhua, Taiwan) aeration system. Reactor 2 (referred herein as R2) is equipped with a custom fine bubble and compressor (Canadian pond CSP14.50, Québec, Canada) aeration system. The system also had a third tank that serves as a holding tank and helps to equalize the flow to each reactor. All the reactors were seeded with waste-activated sludge (WAS) from the Greenway PCP. The reactors were continuously fed with a primary effluent (PE) from the break tank using two dedicated peristaltic pumps (WT600-4F, Longer Precision Pump Co., Ltd., Hebei, China) per the design flow.

Reactors R1 and R2 were operated in parallel under similar process and operating conditions, including hydraulic retention time (HRT), influent characteristics, and specific surface area of the media. The surface area of the fixed rope media cassette in each reactor was 140–145 m^2. All the reactors have a working volume of 1890 L and were operated at a flow rate of 2 L/min. The diffusers were mounted at the bottom of each media cassette, and cleaning of the air pipes and diffusers was done whenever necessary using bio-purge liquid (Anjou Technologies Inc., Marieville, Canada). The bio-purge liquid eliminates the biofilm and calcium deposits that may be hindering the efficiency of the aeration system.

This study was conducted at design and reduced airflows; the design airflows for reactors R1 and R2 were 96 L/min and 50 L/min, respectively. The reduced flows were 50 and 30 L/min for reactor R1 and 30 L/min for reactor R2. The experimental design is summarized in Table S1 (in the supporting document). The changeover of the airflows was made once a steady-state condition was achieved.

2.2. Influent characteristics

The pilots were fed authentic municipal PE with an average tCOD and NH_3–N concentrations of 210.1 $\pm$ 68.6 and 23.9 $\pm$ 6.9 mg/L, respectively. The pH for reactors R1 and R2 were 7.68 $\pm$ 0.22 and 7.59 $\pm$ 0.15, respectively. The average PE characteristics are shown in Table 1. The pilot was operated in a flow-through mode, and the performance was monitored over 200 days for NH_3–N and COD removal. A variation in feed NH_3–N and COD concentration was observed during the duration of this study. The COD/NH_3–N ratio observed in the present study ranges from 5 to 20, which allowed characterizing the system performance under dynamic loading and wastewater composition.

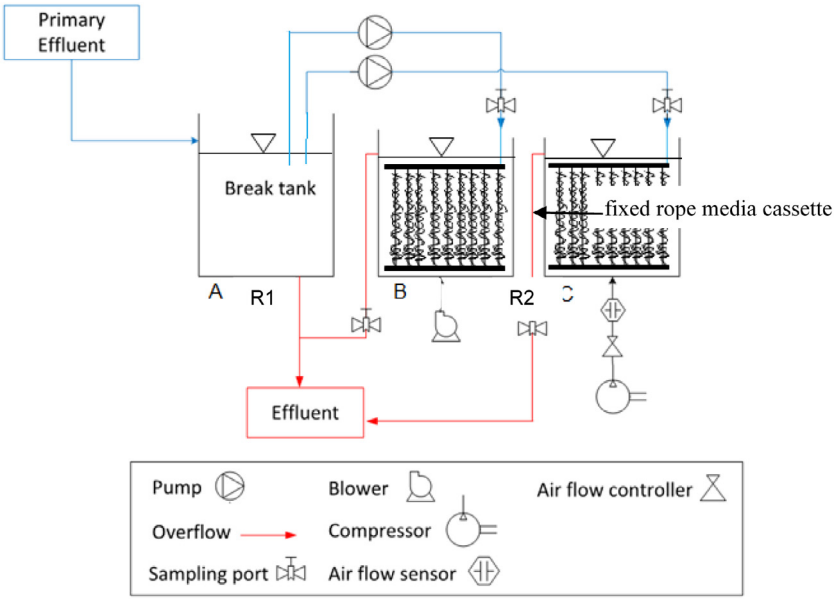

Fig. 1. Schematic of reactor operation.

Table 1
Characteristics of primary effluent.

Parameter	Average ± SD
pH	7.2 ± 0.2
Alkalinity as (CaCO3), mg/L	330.5 ± 46.6
tCOD (mg/L)	210.1 ± 68.6
TSS (mg/L)	109.3 ± 44.1
TKN (mg/L)	34.1 ± 5.0
NO_2–N (mg/L)	1.8 ± 1.27
NO_3–N (mg/L)	0.5 ± 0.05
NH_3–N (mg/L)	23.9 ± 6.9

2.3. Analytical schedule and methods

Bioreactor temperature, dissolved oxygen (DO), and pH were monitored daily. Influent and effluent samples were collected twice per week for total and soluble COD, NH_3–N, NO_2–N, NO_3–N, alkalinity, TSS, and VSS analysis. The samples were measured for BOD_5 and TKN once a week. The DO concentration, pH and temperature were measured using a YSI portable meter (YSI, Xylem). COD, NH_3–N, NO_2–N, NO_3–N, and TKN were measured using HACH kits and HACH DR/2000 photometer (HACH, Loveland, CO). Alkalinity (APHA 2320B), TSS (APHA 2540 B), VSS (APHA 2540 E), and BOD_5 (APHA 5210 B) were measured using the standard method of APHA (APHA et al., 2005).

2.4. Microbial analysis

The biofilm and mixed liquor microbial analysis was done at a commercial molecular research DNA LAB. The mixed liquor samples were centrifuged at 4500 rpm for 5 min, and the pellets were shipped for analysis. The mixed liquor and the biofilm samples were frozen before being sent for analysis. The DNA analysis was conducted utilizing 16S rRNA gene V4 variable region PCR primers 515/806 in a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). The analysis was conducted under the following PCR program conditions: 94 °C for 3 min, followed by 30 cycles (5 cycles used on PCR products) of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. 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). In summary, sequences were depleted of barcodes and primers, then sequences <150bp, ambiguous base calls, and homopolymer run exceeding 6bp were removed. The sequences were denoised, operational taxonomic units (OTUs) generated, and chimeras removed. OTUs were defined by clustering at a 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a database derived from RDPII (http://rdp.cme.msu.edu) and NCBI (www.ncbi.nlm.nih.gov).

2.5. Off-gas oxygen concentration analyzer setup

A simplified off-gas analyzer was constructed and installed for measuring the off-gas oxygen fraction twice per week, following a method proposed by Redmon et al. (1983). In the present study, the collected oxygen fraction data was used to determine the oxygen transfer efficiency (OTE) and evaluate each aeration system's dynamic oxygen transfer rates and their relationship to microbial oxygen consumption. The off-gas analyzer consists of a hood (95 cm × 95 cm × 10 cm) made of acrylic, oxygen analyzer (AMI model 70R1, Huntington Beach, CA, USA), diaphragm pump, Drierite gas drying column (Xenia, OH, USA) and a column (2.5 cm × 20 cm) filled with NaOH pellets (Sigma-Aldrich) (Fig. S2, Supplementary material)

2.6. Oxygen transfer rate analysis and calculations

Biofilm systems are primarily diffusion-limited, contrary to suspended growth systems, which are kinetically limited (Boltz and Daigger, 2010); hence characterizing mass transfer phenomena in biofilm systems is necessary. Oxygen transfer is often the rate-limiting step in aerobic biofilm processes due to the low solubility of oxygen in the medium (Garcia-Ochoa and Gomez, 2009). The mass balance for the dissolved oxygen in a complete mixed system was established, as shown in Eq. (1) (Garcia-Ochoa and Gomez, 2009; Garcia-Ochoa et al., 2010). These equations relate two critical nonlinear time-varying parameters that characterize the DO concentration dynamics, including the oxygen uptake rate (OUR) related to microorganism activity and the volumetric oxygen mass transfer function, represented by the oxygen transfer rate (OTR) (Pittoors et al., 2014). This study determined the OTR based on the dynamic OTE values using the measured off-gas oxygen concentration (Eq. (2)) and compared it with the actual oxygen requirement determined as per Eq. (3). The OTR and OUR comparison identify the fraction of oxygen transferred to the system and consumed by the biomass. Fundamentally, if OTR is equal to OUR, there will be no excess or deficit oxygen to increase/decrease dissolved oxygen levels in the reactor (Trillo et al., 2004).

$$dC/dt = OTR - OUR \tag{1}$$

$$OTR = Q * OTE * O_{2^{air}} \tag{2a}$$

$$OTE = \frac{O_{2\ in} - O_{2\ out}}{O_{2\ in}} \tag{2b}$$

$$OUR = OUR_N + OUR_C + OUR_D \tag{3}$$

where dC/dt = oxygen accumulation rate in the liquid phase (mgO_2/L h), OTR = the oxygen transfer rate from the gas to the liquid (mgO_2/L h), OUR = the oxygen uptake rate by the microorganism (mgO_2/L h), OTE = oxygen transfer efficiency, Q = airflow rate (L/h), $O_{2^{air}}$ = oxygen concentration entering the system (mg/L), $O_{2^{in}}$ = Oxygen fraction entering the system, $O_{2^{out}}$ = Oxygen fraction leaving the system (measured by the off-gas analyzer, Section 2.4), AOR = actual oxygen requirement (kg/h); OUR_N = oxygen uptake rate of nitrification (kg/m^2 h), OUR_C = oxygen uptake rate of carbon oxidation (kg/m^2 h), OUR_D = oxygen uptake rate for endogenous respiration (kg/m^2 h).

2.7. Statistical analysis

Data analysis was done using MINITAB 16 (Minitab Inc., State College, PA). The level of statistical significance was determined using a t-test, and correlations were considered statistically significant at the 95% confidence interval ($p < 0.05$).

3. Results and discussions

3.1. Evaluation of aeration efficiency and oxygen uptake rate

In most water and resource recovery facilities (WRRFs) in Europe and North America, aeration is still the most energy-intensive process (up to 50%–80%) of the total facility energy cost and corresponds to 1% of these countries' total electricity usage (Amaral et al., 2018). One of the key objectives of this study was to discern the minimum energy expenditure of the aeration system necessary to treat the PE using fixed media and reduce ammonia levels below regulatory limits. Several factors related to the aerator system and biological performance were considered to identify the optimal energy expenditure. Table 2 shows a summary of the average bulk liquid dissolved oxygen (DO) level, the oxygen transfer rate (OTR) and oxygen uptake rate (OUR under different airflow conditions and aeration systems.

Comparing the bulk liquid DO levels under the two aeration schemes at different airflow rates, it can be seen that the DO was not impacted significantly by the airflow rates (Table 2, Fig. S3 (Supporting material)). Table 2 also shows no significant difference in the bulk liquid DO for a custom fine bubble aeration scheme when the gas flow rate was reduced

Table 2
Average aeration efficiency, oxygen uptake rate, and process performance.

Parameters	Fine bubble (R2) airflow rate (L/min)		Coarse bubble (R1)airflow rate (L/min)		
	50	30	96	50	30
Days of operation	110	130	110	100	30
Bulk liquid DO (mg/L)	5.45	5.12	6.47	4.90	4.29
OUR (kg/h)	0.016	0.013	NA	0.016	0.011
OTR (kg/h)	0.017	0.017	NA	0.018	0.015

from 50 L/min (average DO = 5.45 mg/L) to 30 L/min (average DO = 5.12 mg/L). Similarly, air flow change from the design airflow (96 L/min) to a reduced airflow rate (50 and 30 L/min) did not significantly impact the bulk liquid DO for the coarse bubble aeration scheme (Table 2 and Fig. S3 (Supporting material)). At all airflow rates, the fine bubble aeration maintained a sufficient bulk liquid DO (Table 2) and was comparable to the optimal 5 mg/L bulk liquid DO concentration required for stable nitrification in biofilm systems (Park et al., 2008; Hwang et al., 2009). The 5 mg/L optimal bulk liquid DO level is higher than that in suspended processes (DO = 2 mg/L). It is important to note that the actual DO at the biofilm would be less than the bulk liquid DO due to oxygen transfer resistances from the bulk liquid to the biofilm.

The results from the pilot study were compared with the airflow requirements of the full-scale Greenway activated sludge plant equipped with fine bubble aeration. The theoretical airflow requirement was calculated for an operating condition of 15 h. hT and considering the past/historical wastewater characteristics of Greenway PCP (average influent BOD_5 and TKN concentration of 55 and 27.7 mg/L, respectively) was 44 L/min (Table S2, Supporting material). The calculated airflow is lower than the one used for coarse bubble aeration but higher than the one obtained for fine bubble aeration in this study. This study showed that 30 L/min and 50 L/min for the fine bubble (reactor R2) and coarse bubble (reactor R1) aeration systems were the lower thresholds specific air flowrates resulting in corresponding DO levels 5.12 mg/L and 4.19 mg/L, respectively. These lower threshold flowrates help achieve lower energy consumption and lower operating costs.

Airflow typically is governed by process oxygen requirements in carbon oxidation and combined carbon oxidation and nitrification systems. A study on airflow rate on MBBR indicated that the minimum specific airflow rate required to distribute plastic biofilm carriers uniformly is 120 to 240 m^3/m^2 d, with a typical design value in the range of 144 to 192 m^3/m^2 d (McQuarrie and Boltz, 2011). In this study, the specific airflow rates for the coarse bubble (reactor R1) and the fine bubble (reactor R2) aeration systems at design airflow were 0.984 m^3/m^2 d and 0.48 m^3/m^2 d, respectively. The meager-specific airflow rate compared to MBBR is an advantage and result in reduced operating costs. Note that MBBRs require more air for moving or mixing the plastic carriers, whereas, in the case of BioCord, there is no need to move the media.

Further, the fraction of oxygen transferred to the system (OTR) and oxygen consumed by the biomass (OUR) was compared to assess the aeration efficiency (Table 2, Fig. S4 (Supporting material)). An aeration system is considered efficient if the system transfers oxygen to meet the demand if the AOTR and AOR values are comparable. Overall, no significant difference was observed between the OTR and OUR at the 50 LPM airflow rates; however, the observed OUR was lower than the OTR at reduced (30 LPM) airflow rates indicating a less efficient oxygen transfer process in the latter test condition.

3.2. Comparison of reactor performance

3.2.1. Nitrogen removal performance

Table 3 shows the average NH_3–N and tCOD loading and removal rates noted for each system and the corresponding bulk liquid DO concentration during the specific aeration flow rates employed in this study. Comparable removals up to 99% for NH_3–N were achieved in the reactors operated with the fine bubble and coarse bubble aeration systems at design airflow rates of 50 L/min and 96 L/min, respectively, with an average NH_3–N removal rate of 0.38 and 0.37 g/m^2 d, respectively (Table 3). The average ammonia removals obtained in this study were one order of magnitude higher than the 0.039 g/m^2 d reported earlier using low-cost fixed film media systems such as BioWeb media (Borchert et al., 2011). The maximum NH_3–N removal rate achieved was 0.75 and 0.72 g/m^2 d for fine bubble and coarse bubble aeration systems. In addition, the *t*-test also indicated that the mean effluent NH_3–N concentration for both aeration systems was not statistically different ($p = 0.22$) at these design airflow rates. Therefore, comparable ammonia removal rates can be achieved with a lower airflow rate (50 L/min) using the custom fine bubble aeration and a higher airflow rate (96 L/min) using the coarse bubble tube aeration. The considered design air flowrates were based on Bishop Water Technology's recommendation and current design practice. Upon reducing the airflow rates to 30 L/min, the observed ammonia removal rate in R2 remained the same at 0.38 g/m^2-d. However, the NH_3–N removal achieved with the coarse bubble aeration dropped to 0.23 g/m^2-d (less than 60%), which could be attributed to mass transfer resistance related to mixing and/or biofilm scouring intensity as discussed below (Table 3).

Aeration provides scouring (for biofilm thickness control) and mixing (for replenishing the media with a new substrate) capability beyond satisfying the biological process aeration demand. In this study, the NH_3–N performance of R2 (coarse

Table 3
Comparison of fine and coarse bubble aeration systems at different airflow rates.

Reactor type	Airflow[b] rate (L/min)	DO[a] (mg/L) mean (std)	NH_3–N mean (std)			tCOD mean (std)		
			Removal (%)	Loading[b] rate (g/m^2 d)	Removal rate (g/m^2 d)	Removal (%)	Loading rate (g/m^2 d)	Removal rate (g/m^2 d)
R2	50	5.45 (1.69)	89.9	0.42 (0.24)	0.38 (0.26)	82.3	2.90 (1.20)	2.39 (1.21)
	30	5.12 (1.05)	85.2	0.45 (0.35)	0.38 (0.27)	64.8	2.27 (1.29)	1.47 (1.08)
R1	96	6.47 (1.67)	79.6	0.47 (0.13)	0.37 (0.13)	71.7	4.25 (1.43)	3.05 (1.21)
	50	4.90 (1.00)	88.0	0.44 (0.26)	0.38 (0.24)	81.2	2.51 (1.15)	2.04 (1.10)
	30	4.29 (1.05)	58.0	0.40 (0.35)	0.23 (0.15)	59.5	2.27 (1.29)	1.35 (0.95)

[a]DO = bulk liquid dissolved oxygen concentration.
[b]Design airflow rates and ammonia loading rates were based on Bishop Water Technology's recommendation and current design practice.

bubble aerator reactor), when operated at air flows of 50 L/min and 30 L/min, were 88% and 58%, respectively. Note that the DO at these air flows (4.90 and 4.29 mg/L, respectively) were comparable. Therefore, although the 70% process airflow decrease in R1 (coarse bubble aeration) did not decrease the bulk liquid DO level, the impact on the ammonia removal performance was detrimental (a corresponding 40% decrease). This impact could have been caused by the lack of scouring and/or mixing at the reduced airflow rate. The nitrifiers (ammonia and nitrite-oxidizing bacteria) typically grow in the most inside layer of the biofilm, whereas the other heterotrophic bacteria (aerobic carbon oxidizers) grow on the outer side. The lower ammonia removal performance at the lower airflow but similar bulk liquid DO concentration indicates poor biofilm control leading to potential overgrowth of heterotrophic bacteria that limited the ammonia and oxygen diffusion from the bulk liquid to the biofilm. Another reason could be a limitation in substrate mixing that limits substrate diffusion from the bulk liquid into the biofilm. The observation from the coarse-bubble reactor was consistent with previous studies. For example, experimental results from nitrification kinetics of biofilm studies conducted by Chen et al. (2006) indicated that mixing caused by aeration significantly impacted the nitrification rate in fixed film reactors.

3.2.2. COD removal performance

The fixed-film reactors run with design 50 L/min and reduced 30 L/min airflows using custom fine bubble aeration (Reactor R2) achieved tCOD removal rates of 2.39 and 1.47 g COD/m^2 d, respectively. The corresponding tCOD removal rates found with the coarse bubble tube aeration were 2.04 and 1.35 g COD/m^2 d at 50 L/min and 30 L/min airflows, respectively. Therefore, the COD removal capacity of the fixed rope media is higher for custom fine bubble aeration compared to the coarse bubble aeration by about 15% (for 50 L/min airflows) and by 8% (for 30 L/min airflows). The highest COD removal rate was observed in R1 (coarse bubble aeration) operated at the highest 96 L/min; however, the corresponding removal was 72% lower than the 50 L/min airflow operation. The discrepancy comes from the difference in the loading rate, which was 4.25 (at 96 L/min) versus 3.19 (at 50 LPM). Generally, removal rates are proportional to loading rates, where high removal rates correspond to higher loading rates and vice versa (Jianlong et al., 2000; Mehrabi et al., 2020). Therefore, these results should be interpreted carefully.

The results from the current study were also compared with the previous similar type of biofilm studies primarily designed for COD removal (Table 4). Comparing the fixed rope media in the current study with other fixed-film reactors may be a challenge because of the variation in the specific area of the fixed media, process configuration such as biofilm-based (this study) versus IFAS (biofilm + suspended biomass-based) type treatment (Table 4), and the strength of wastewater treated. The COD removal performance achieved by this study (82.3 and 82.1 for the fine bubble and coarse bubble tube aeration, respectively) is comparable to those reported in other IFAS studies except the loop knitted polypropylene fabric that achieved a COD removal of 92% (Table 4). It should be noted that the loop knitted polypropylene fabric IFAS processes were operated at a higher COD loading rate of 1786 g/m^3 d compared to this study, which was operated at 200–225 g/m^3 d.

3.2.3. Influence of COD/ammonia ratio on treatment efficiency

Another parameter that was investigated in this study is how the tCOD/ammonia nitrogen (COD/N) ratio influences the reactor performance relative to carbon and ammonia removal. Note that a study conducted by Mannina et al. (2020) on an IFAS-MBR system confirmed that the COD/N ratio is a critical variable affecting treatment efficiency. The COD/NH_3–N ratio observed in the present study ranges from 5 to 20 (the average being 9.8) (Fig. 2). An increase in COD and corresponding sudden increase in COD/N ratio occurred on the 8th, 17th, 56th, 77th, 128th, 140th and 185th day of pilot operation (Figs. 2 and 3) that allowed assessment of the COD/N ratio on the pilot performance. The increase in COD had never affected the dynamic COD removal efficiency of the reactors that remained above 71% irrespective of the COD load. However, the ammonia removal was affected, often taking 2 to 3 days to recover and come to the optimum NH_3–N removal (Fig. 2).

Table 4
Average COD loading and removal rates of different fixed-film bioreactors.

Type of fixed-film media	COD loading (g/m^3 d)	COD loading (g/m^2 d)	% Removal	Reference
Looped-cord	NA	NA	78–90[b]	Haley III (2001)
Loofa Sponge	400–2400		81.3–93.4	Nabizadeh et al. (2008)
Loop knitted polypropylene fabric	1786	NA	92	Singh and Kazmi (2016)
Submerged ceramic tiles	NA	5–20[a]	67.8–73.6	Hamoda and Al-Sharekh (1999)
Polyurethane foam	NA	NA	85–90	Jou and Huang (2003)
Plexiglas sheets	110–1300	0.31–3.71	80–92.5	Eslami et al. (2018)
Fixed rope media (custom fine bubble aeration)	202.94	2.90	82.3	This study
Fixed rope media (tube aeration)	223.46	3.19	81.2	This study

[a] g $BOD/m^2/d$.
[b] % BOD removal.

For example, for the custom fine bubble aeration up to 110th day (airflow = 50 l/min), the average influent and effluent NH_3–N concentrations were 23.31 and 4.74 mg/L, respectively, resulting in an 80% ammonia removal. However, the NH_3–N removal decreased to 52% and 47% on the 17th and 56th day, respectively, when the COD spike was observed. The decline shows that carbon oxidation prioritizes nitrification with the co-diffusion biofilm systems like fixed rope media, where all substrates diffuse from the bulk liquid to the biofilm. In these systems, the carbon oxidizers grow on the exterior of the biofilm, where it is rich in both oxygen and carbon substrates. The increase of organic loading rate (OLR) promotes competition among heterotrophic species with ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) to access the available DO and organic matter (Nowrouzi and Abyar, 2021). Therefore, when there is an abundant organic carbon source, the heterotrophic bacteria proliferate rapidly, compete for oxygen with nitrifying bacteria, and inhibit nitrification activity (Lin et al., 2016; Mahto and Das, 2022).

In contrast, the nitrifiers grow inside the biofilm, where there is a potential deficit in oxygen. While the system could recover over shock loads, long-term exposure to higher COD concentrations will favor the development of faster-growing heterotrophs over autotrophs, leading to a loss in nitrification. A decrease in nitrification at higher C/N ratios was also reported by Kocaturk and Erguder (2016) and Hwang et al. (2009). A similar experimental study conducted under different COD: *N* ratios by Zhang et al. (2021) verified a similar trend in ammonia removal efficiencies. To minimize the impact of COD load spikes in the nitrification performance, chemically enhanced primary treatment (Shewa et al., 2020) or tank in a series approach (Lin et al., 2020) can be considered to increase the nitrogen removal efficiency. In addition, if CEPT is employed, the aeration units could be operated at airflow rates less than the lower threshold airflow rates suggested by this study.

3.3. Impact of biofilm detachment on ammonia and COD removal

Developing biofilms in fixed-film media is a multistage process (Wang and Zhang, 2010). First, free-floating planktonic bacterial cells land on a damp surface and are later attached. After a sufficiently high cell density is reached, cells begin excreting an extracellular polymeric substance (EPS) and forming an EPS matrix. The EPS function as cementation agents (“glue”), aiding the fixation of the microorganisms to the support medium and each other (Lippel and Cerqueira, 2018). The bacteria population inside the matrix grows as well by consuming nutritious substrates supplied by the surrounding environment. As a result, the biofilm begins to grow. In this study, the biofilm formation and detachment of the fixed rope media were assessed for the R2 reactor by visually observing the biofilm module and comparing it to the removal performance for a selected duration (Day 56 to Day 116). Most importantly, an emphasis was given to characterize the biofilm’s ammonia and COD removal performance after observing excessive growth followed by biofilm/sludge detachment. Fig. 4a shows the analytical data points in orange color where biofilm images were taken before and after sloughing off, and Fig. 4b shows the corresponding images of the biofilm modules.

Fig. 4b clearly shows the occurrence of sloughing off excessive biofilm growth from the fixed rope media. It can visually be observed that there is a variation in the biofilm/sludge thickness on the media. It can be seen in Fig. 4b that there is excessive growth observed on Day 84, and there was a decrease in the dynamic NH_3–N removal rate (from 0.31 to 0.15 g/m^2 d) on this day compared to the other days where there is no excessive biofilm growth. Correspondingly, the NH_3–N removal was 23.2% compared to the different days, which achieved 89 to 100% removal efficiency (Fig. 4a). However, the tCOD removal rates are comparable and not affected by the biofilm’s excessive growth or slough-off (Fig. 4a and b). This study shows that slough off is not a concern for the type of media studied here; during slough off, it appears a significant portion of the biomass remained attached to the media. The slough-off for the media studies here works favorably as a self-cleaning, thereby recovering ammonia removal performance that was reduced due to thicker biofilm formation. From the observation of this study, it was also noted that carbon oxidation is not significantly affected by biofilm thickness. The lower ammonia removal in thicker biofilms could be attributed to reduced diffusion of oxygen to AOB and NOBs mainly populated on the rope media that are adapted to the wastewater. Several studies investigating biofilm thickness have revealed similar observations regarding biofilm thickness and ammonia removal relationship (Torresi et al., 2016; Alpkvist et al., 2007). It should also be noted that a study conducted by Piculell et al. (2016) on

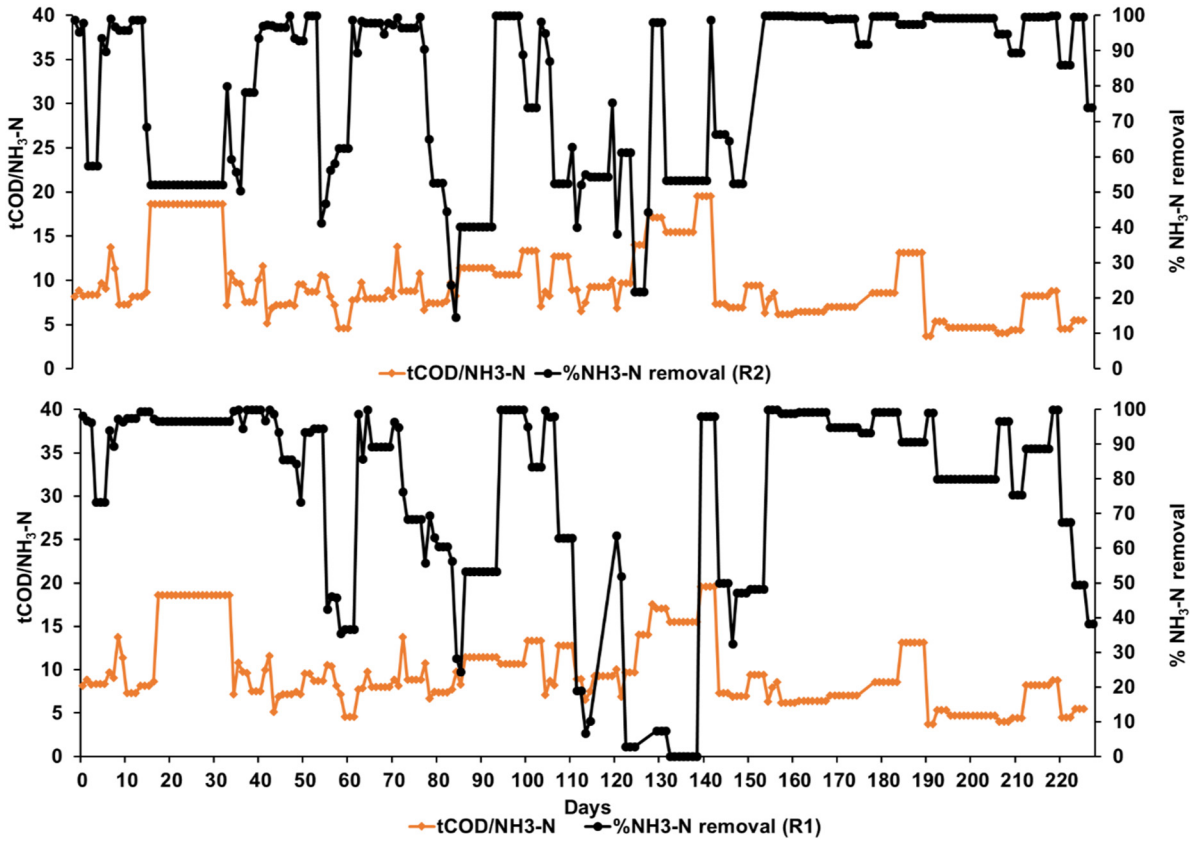

Fig. 2. Effect of C/N ratio on NH_3–N removal (a) Reactor R1 (b) Reactor R2.

moving bed biofilm reactors indicated that biofilm thickness alone does not significantly affect the ammonium. However, Piculell et al. (2016) conducted the study with a specific maximum biofilm thickness range (200–500 μm).

Self-sloughing off of excessive biofilm avoids the cleaning of the BioCord. Therefore, BioCord has additional advantages: no need for sludge recirculation, no potential clogging, and self-cleaning capacity. Due to the excessive biofilm growth sloughing, suspended biomass could be observed in the reactors.

3.4. Comparison of microbial community

Different bacteria in the biofilm respond to their specific microenvironmental conditions with different growth patterns, and a structurally complex mature biofilm gradually develops (Costerton et al., 1995). The identification and quantification of members of particular microbial communities and a clear understanding of the functional relationship between members are required to appreciate and possibly manage these communities' complex processes fully. In nitrifying activated sludge plants, the principal genera, *Nitrosomonas* and *Nitrobacter* are responsible for the oxidation of ammonium to nitrite and of nitrite to nitrate, respectively.

In this study, we examined the microbial community structure in the biofilms and investigated the composition of AOB and NOB in the biofilm. The abundance of other microbial communities, including anammox, denitrifies, and other heterotrophs, were also investigated. Heterotrophs constitute the larger portion of the microbial communities in both biofilms (95% in Reactor R2 and 91% in reactor R1). The microbial community analysis indicated that *Proteobacteria*, *Firmicutes*, *Bacteroidetes,* and *Actinobacteria* were the predominant phyla in the fixed rope media biofilm. The relative percentages of *Firmicutes*, *Bacteroidetes* and *Actinobacteria* were higher on the biofilms of Reactor R2 (custom fine bubble aeration) compared to that of Reactor R1 (coarse bubble tube aeration) (*Firmicutes*: 4.3% (Reactor R2), 2.1% (Reactor R1); *Bacteroidetes*: 3.1% (Reactor R2), 2.6% (Reactor R1); and *Actinobacteria*: 2.2% (Reactor R2), 1.4% (Reactor R1)). Generally, the dominant bacterial genera for fixed-film processes are very similar to those found in activated sludge (Lessard and Bihan, 2003). Studies conducted on IFAS biofilms by Bai et al. (2016) and Huang et al. (2017) reported similar observations about the dominant phyla. Bai et al. (2016) reported that *Proteobacteria, Nitrospirae, Chloroflexi,* and *Firmicutes* were the predominant phyla in the IFAS system. In contrast, Huang et al. (2017) identified are *Proteobacteria, Nitrospirae, Acidobacteria,* and *Bacteroidetes*.

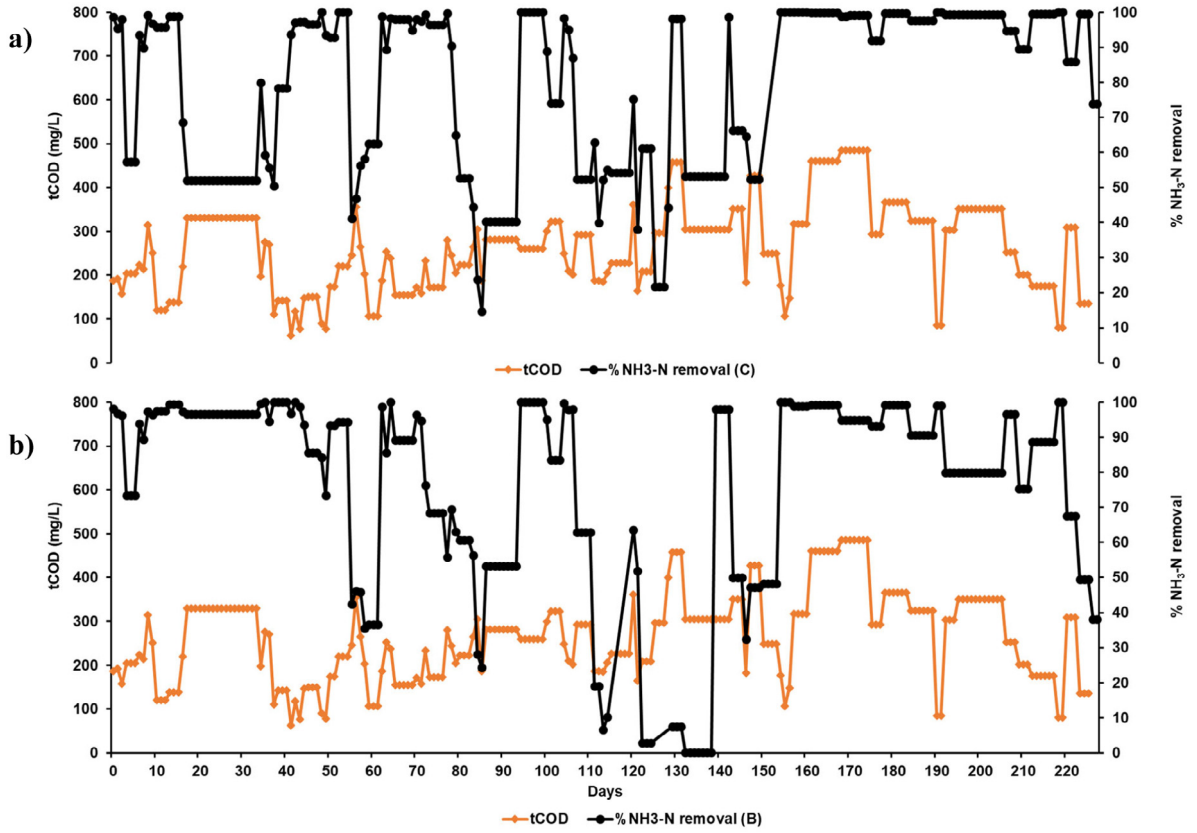

Fig. 3. Effect of COD loading on NH_3–N removal (a) Reactor R1 (Coarse bubble aeration) (b) Reactor R2 (fine bubble aeration).

The relative abundance of AOB, NOB, anammox, and denitrifying bacteria in Reactors R1 and R2 are shown in Fig. 5. It is also important to note that the fine bubble biofilm reactor supported a relatively larger fraction of denitrifying bacteria, indicating the development of an anoxic environment rich in carbon. Whereas the inside of the coarse bubble biofilm reactor may have created a more anaerobic and carbon-limited environment hence supporting a larger fraction of anammox bacteria. This information can be used for the further development of second-generation nitrogen removal processes.

Out of the AOB species identified in the biofilms of the fixed rope media *nitrosococcus, nitrosospira, nitrosomonas, nitrosovibrio* species were identified in both biofilms of Reactors R1 and R2, and *nitrosomonas* is the dominant species. Previous studies have also indicated that *Nitrosomonas* species is the most common ammonium-oxidizing bacteria in conventional activated sludge-based wastewater treatment systems (Winkler et al., 2012). The NOB species found in the fixed rope media biofilms were *nitrospira and nitrobacter* species. In addition to the nitrifiers, the DNA analysis showed an abundance of denitrifying and anammox bacteria, including *Paracoccus denitrificans* and *Candidatus kuenenia sp.*, respectively. This was not expected as the system was fully aerated; however, their presence shows that some sections deep in the biofilm layer could be anoxic and anaerobic.

3.5. Future prospects and application

Recently, with climate change, areas initially required to meet only carbon removal are now being mandated to meet nitrogen removal. Most of these regions and several underserved regions worldwide share the same characteristics, including remote sites, smaller plants, decentralized systems, lack of skilled power, often requiring passive treatment systems. This created a challenge for environmental engineers to develop and re-visit technologies and optimize them, making them attractive to address the problem at hand. Thus, the objective of this research was to assess a new type of fixed rope media and characterize its performance under varying aeration schemes and other process operating conditions such as loading and varying carbon to nitrogen ratio. The work was conducted using a full-scale-sized rope-type media cassette at a test bay located in a wastewater treatment plant. The research conditions were challenging due to the high degree of variability; however, the research had successfully generated detailed information, including ammonia and COD loading and removal rates, aeration intensity and schemes and footprint requirements that can be applied for the design of these systems. In the subsequent study, the potential of the technology on total nitrogen removal will be assessed.

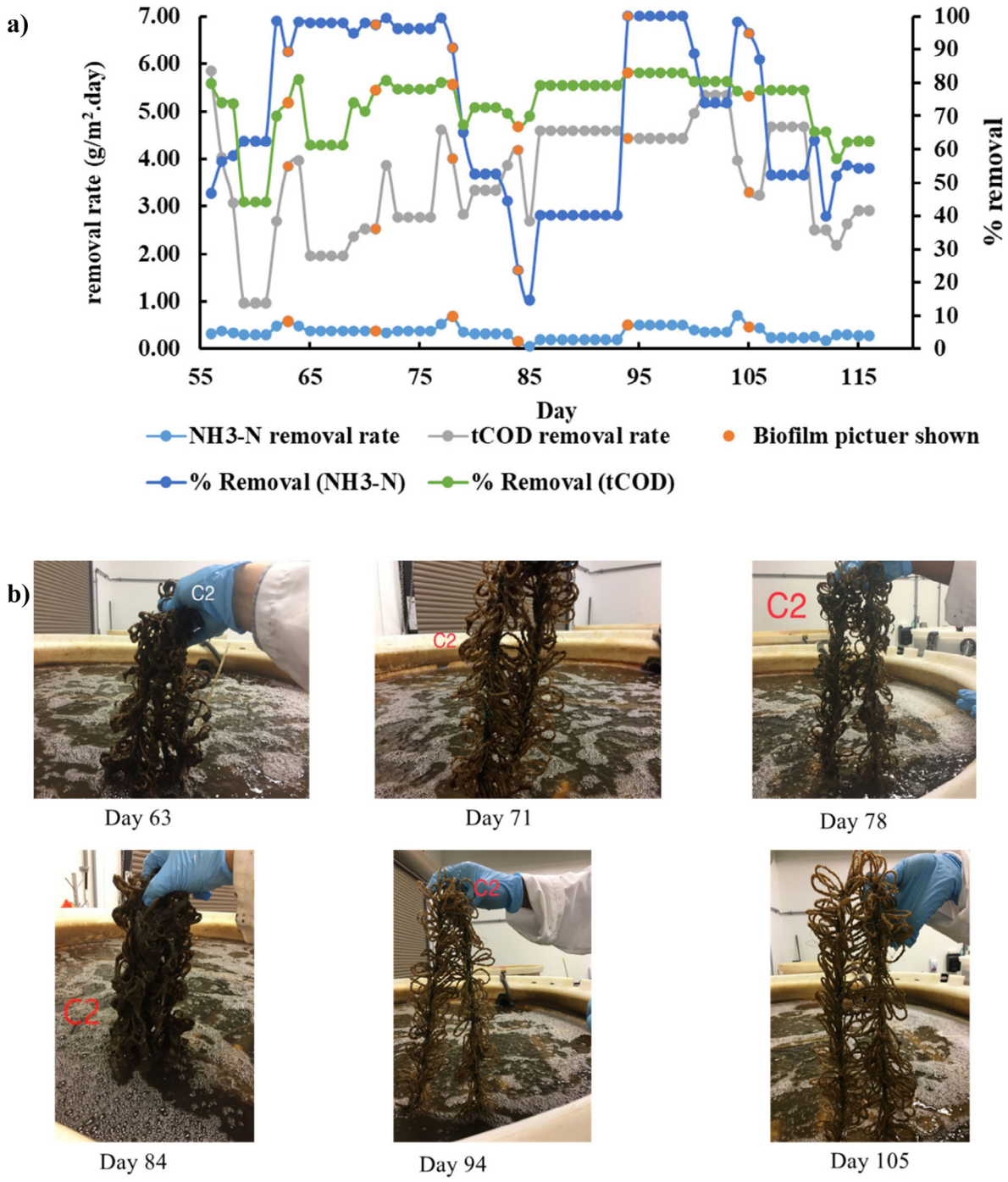

Fig. 4. (a) Performance of reactor R2 (Day 56 to Day 116) (b) Representative digital images of biofilm formation. . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

This work characterized a fixed rope media bioreactor performance under different aeration environments and NH_3–N/COD ratios. Fixed rope media technology is feasible for treating PE under fine and coarse bubble aeration systems handling 0.2–0.9 NH_3–N g/m^2 d and as high as 10 g tCOD/m^2 d loadings with up to 99% NH_3–N removals. No significant variation in bulk liquid DO was observed at design and reduced air flows in both aeration methods. However, lower airflow

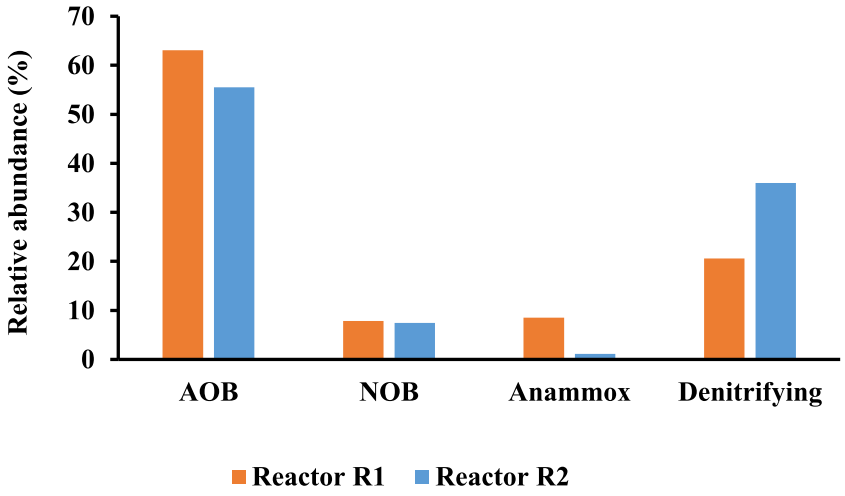

Fig. 5. Relative abundance of AOB, NOB, anammox, and denitrifying bacteria in biofilm samples.

rates resulted in lower removal efficiencies because of the impact on the scouring of excess biofilm and turbulence of the bulk liquid that enhanced the transfer of substrates to the nitrifying microbial population. Lower ammonia removal was also observed during higher COD loading and thicker biofilms. Subsequently, sloughing off excessive biofilm growth from the fixed rope media was observed, which can also be considered a self-cleaning fixed-film media, recovering the ammonia removal performance.

CRediT authorship contribution statement

Wudneh Ayele Shewa: Conceived and designed the experiments, Analyzed the data, Wrote the draft paper. **Lin Sun:** Performed the experiments and analyzed the data. **Christine Gan:** Supervised the project and assisted in data analysis. **Kevin Bossy:** Project administration, Funding acquisition. **Martha Dagnew:** Conceived and designed the experiments, Reviewed and edited the paper, Funding acquisition, Overall supervision of the project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by South Ontario Water Consortium (SOWC) [grant number SUB02730] and NSERC-CRD [543948-19]. In addition, the authors thank Admiro Ndala and Rinujan Kamalachandran, who assisted with the wastewater analysis and daily monitoring of the reactors.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102387.See the uploaded supplementary material.

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