Research Paper

Performance evaluation of anoxic–oxic–anoxic processes in illuminated biofilm reactor (AOA-IBR) treating septic tank effluent

Sittikorn Kamngam, Thammarat Koottatep, Nawatch Surinkul, Chawalit Chaiwong and Chongrak Polprasert

ABSTRACT

This study was conducted to evaluate the treatment performance of the anoxic–oxic–anoxic processes in illuminated biofilm reactor (AOA-IBR) in removing organics and nitrogen contained in septic tank effluent. The 27 L of the AOA-IBR was illuminated with red light-emitting diode (LED) lamps (peak wavelength of 635 nm, intensity of 100 μmol/(m^2 s)). Three types of biofilm media, namely ball ring®, plastic sheets and zeolite beads, were placed in the anoxic, oxic and anoxic zones, respectively, of the reactor to support the growth of microalgal–bacterial biofilm. The AOA-IBR was continuously fed with septic tank effluent and operated at hydraulic retention times (HRTs) of 24, 48 and 72 h. The experimental results found the increases in chemical oxygen demand (COD), total nitrogen (TN) and ammonia nitrogen (NH$_4$-N) removal efficiencies with increasing HRTs in which the HRT of 72 h resulted in 78.6, 72.8 and 90.6% removals of COD, TN and NH$_4$-N, respectively. The effluent quality of the AOA-IBR could meet the ISO 30500 effluent standards for Non-Sewered Sanitation Systems. The predominant microalgal biofilm species was observed to be *Oscillatoria* sp., while *Proteobacteria* was the predominant bacterial phylum found in the biofilm growing in the reactor. The above results suggested the applicability of the AOA-IBR in improving septic tank treatment performance which should result in better water pollution control.

Key words | adsorption, kinetic model, microalgal–bacterial symbiosis, on-site sanitation system, photobioreactor, septic tank effluent

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HIGHLIGHTS

- The anoxic–oxic–anoxic processes in illuminated biofilm reactor (AOA-IBR) as an on-site post-treatment system could effectively treat chemical oxygen demand (COD) and total nitrogen (TN) concentrations in septic tank effluent.
- The Stover–Kincannon model could be applicable to determine the kinetic values for COD and TN removal.
- The observed major microalgal and bacterial groups were corresponding to the results of COD and TN removal.

INTRODUCTION

Decentralized wastewater treatment systems or on-site systems such as septic tanks are widely used in several areas of developing countries to treat blackwater (Taweesan *et al.* 2015). In Thailand, about 84% of the on-site systems are septic tanks, but their effluent characteristics were found to contain high concentrations of chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) and still do not meet the national effluent standards (Taweesan *et al.* 2015). Although leaching or drainage fields are employed to further treat the effluent of the on-site systems in most developed countries, due to land limitation and other constraints, the effluent of these on-site systems in developing countries is usually discharged directly into nearby environment, resulting in contamination of surrounding soil, surface and ground water (Richards *et al.* 2017). Therefore, for urban areas of developing countries, there is a need to develop a compact system to further treat the on-site effluent to reduce organics, nutrients and fecal microorganisms to meet the effluent discharge standards. The illuminated biofilm reactor (IBR) or photobioreactor (PBR) is a system that involves microalgal–bacterial symbiotic reactions in which oxygen produced by microalgae during photosynthesis is utilized by bacteria to decompose the organic compounds (Posadas *et al.* 2013; Boelee *et al.* 2014). Moreover, in the PBR, ammonia nitrogen (NH_4-N) compound can be removed by biomass uptake as well as nitrification/denitrification reactions. The microalgal–bacterial PBR system has been applied to remove organic and nutrient matters from blackwater, but it was not effective in removing nutrient (TN and TP) concentrations because of the fluctuation in climatic conditions which resulted in limited natural sunlight for microalgal growth, high nutrient loading rates as well as short hydraulic retention times (HRTs) of the treatment systems (Surinkul *et al.* 2017). Therefore, there is a need to modify the PBR to promote the efficient growth of microalgae and nitrification–denitrification reactions for better nutrient removal.

Due to its high biomass density and promising treatment performance, the biofilm wastewater treatment reactor has been widely used as a post-treatment unit (Chaiwong *et al.* 2018). Several biofilm materials such as polyethylene and polyethylene terephthalate were employed to provide the surface area for the growth of biofilm microorganisms (Barwal & Chaudhary 2014; Surinkul *et al.* 2017). Furthermore, due to its high surface area for bacterial growth and rich adsorptive capacity, porous media, namely zeolite, has been applied to enhance TN removal in biofilm treatment systems (Yang *et al.* 2017). Moreover, to overcome the uncontrolled lighting conditions of the natural sunlight in the IBR, red light-emitting diode (LED) light (having wavelengths of 600–700 nm) was selected as an artificial light source to increase the growth rate of microalgal cultivation to achieve maximum rates of photosynthesis (Chaiwong *et al.* 2018).

In this study, it was hypothesized that effective TN removal in the anoxic–oxic–anoxic processes in illuminated biofilm reactor (AOA-IBR) could be achieved by enhancing nitrification/denitrification reactions, biomass uptake and increased adsorption by zeolite. The AOA-IBR was divided into three zones, i.e. anoxic, oxic and anoxic in series. The first zone received combined influent and recycled effluent from the 3rd zone. The nitrification reactions were designed to occur in the aerobic zone (2nd zone), while the denitrification reactions were supported to occur in the first and third zones. The experiments aimed to evaluate the performance of the AOA-IBR system in removing both COD and TN from a septic tank effluent and to determine the related kinetic constants applicable for design and operation of the AOA-IBR system.

METHODS

Wastewater characteristics

The septic tank effluent used as influent wastewater in this study was collected three times a week from septic tanks of some households located near the AIT campus, Pathumthani, Thailand, and preserved in the refrigeration room (under temperature of 5 °C) before feeding to the AOA-IBR. The septic tank effluent characteristics (Table 1) had average total suspended solids (TSS), COD, TN,

Table 1 | Characteristics of the influent wastewater

Parameters	Units	Value
pH	–	7.63 ± 0.17
DO	mg/L	0.20 ± 0.4
Temperature	°C	28 ± 1.2
TSS	mg/L	1,065 ± 862
COD	mg/L	693 ± 130
TN[a]	mg/L	169 ± 23
NH_4-N	mg/L	132 ± 19
TP	mg/L	24 ± 13

Number of samples (n) = 33.
[a]NO_X-N less than 1.0 mg/L.

NH_4-N and TP concentrations of about 1,065, 693, 169, 132 and 24 mg/L, respectively. The N:P ratio of the wastewater was observed to be 8:1 which was in the recommended range of 8–45 for microalgal–bacterial systems (Cuellar-Bermudez et al. 2017). The average NO_X-N (nitrate (NO_3-N) and nitrite (NO_2-N)) concentration was less than 1.0 mg/L.

Experimental setup

The AOA-IBR used in the experiment was made of acrylic material with a dimension of $0.15 \times 0.40 \times 0.60$ m (width:

depth:length) and a working volume of 27 L (Figure 1). It included an A_{NX1} (anoxic$_1$) of 3 L, oxic or A_{OX} (aerobic) of 18 L and A_{NX2} (anoxic$_2$) of 6 L, in series (Metcalf & Eddy 2014). Due to their commercial availability, affordability and technical characteristics, three types of biofilm media including ball ring®, plastic strip and zeolite beads, as illustrated in Table 2, were selected. Two types of the biofilm media, namely ball ring® and plastic strip, were placed in the A_{NX1} and A_{OX} zones, respectively, while the A_{NX2} zone was filled with zeolite beads for biofilm growth and simultaneous adsorption. The A_{OX} zone was operated with four submerged red LED lamps (Bogdan® J&Y Trading, Thailand) having a peak wavelength of 635 nm and light intensity of 100 μmol/(m^2 s), suitable for the growth of microalgae (Chaiwong et al. 2018). Aeration with a flow rate of 7.6 L/h was also provided in the A_{OX} zone to enhance mixing condition and maintaining dissolved oxygen (DO) concentrations higher than 2 mg/L, suitable for nitrification reactions (Metcalf & Eddy 2014).

Microalgal–bacterial biofilm cultivation

The A_{OX} zone of the AOA-IBR system was inoculated with 14 L of a microalgal consortium collected from an algal
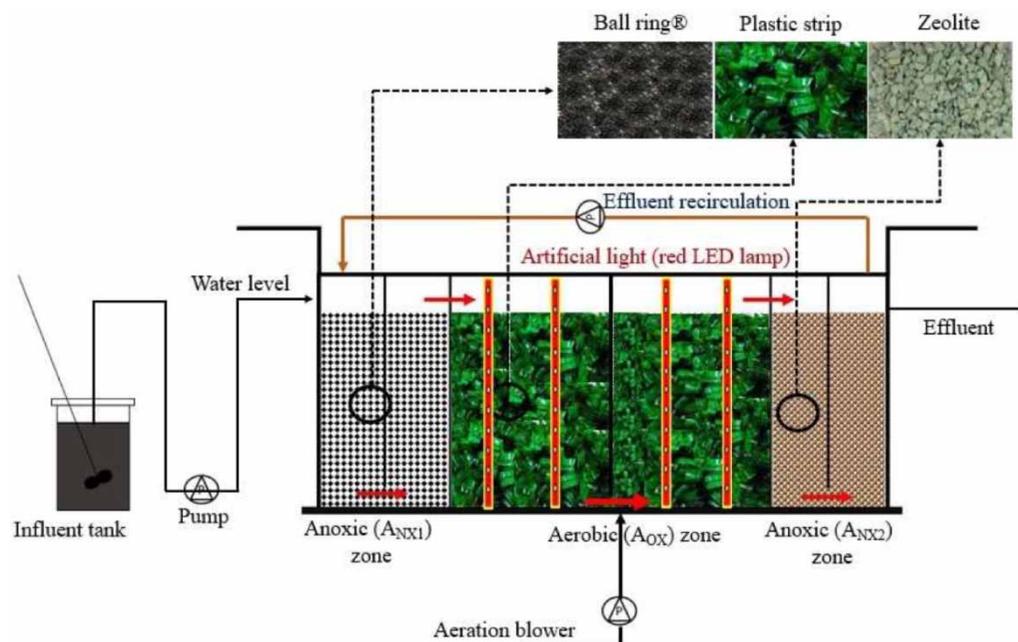

Figure 1 | Schematic diagrams of the AOA-IBR experimental setup.

Table 2 | Characteristics of biofilm media used in the experiments

Properties	Configurations		
	A_{NX1} zone	A_{OX} zone	A_{NX2} Zone
Types	Ball ring®a	Plastic strip	Zeolite
Biofilm media	Polyethylene	Polyethylene terephthalate	Zeolite
Size (mm)	35×35 (Length × Height)	120 (Ø)	1.80 (Ø)
Specific area (m^2/m^3)	200	210	43
Porosity (%)	93	92	47
Photo			

Ø, diameter (mm).
[a]Bio Media and Water System Co., Ltd.

pond water in AIT campus, and 10 L of activated sludge samples (MLSS 0.9 g/L) taken from the AIT wastewater treatment plant (Chaiwong *et al.* 2018). The microalgal–bacterial cultivation was conducted under red LED lamps (14.4 Watt) and aeration, similar to those described in the 'Experimental setup' section. The cultivation was carried out for 30 days to achieve the biofilm biomass concentration of about 0.6 g/L ($2.8 \text{ g/m}^2_{\text{media}}$), sufficient for the growth of the microalgal–bacterial biofilm on the plastic strip media before start-up of the AOA-IBR experiments (Chaiwong *et al.* 2018).

Operating conditions

The AOA-IBR, continuously fed with the septic tank effluent (24 h/day), was operated under various HRTs of 24, 48 and 72 h for 72 days with 100% internal recirculation from A_{NX2} to A_{NX1} (Yan *et al.* 2016), as shown in Table 3. The organic loading rate (OLR) and the nitrogen loading rate (NLR) were varied from 0.69 to 0.25 gCOD/(L d) and 0.19 to 0.05 gN/(L d), respectively. Additionally, the A_{OX} zone of the AOA-IBR was continuously illuminated (24 h/day) by the red LED light in order to result in better photosynthetic oxygen production for bacterial utilization in wastewater treatment efficiency and higher microalgal biomass productivity.

Table 3 | Operational conditions of the AOA-IBR experiment

HRT (h)	Operation period (day)	OLRs (gCOD/(L d))	NLRs (gN/(L d))
24	12	0.69	0.19
48	22	0.34	0.08
72	38	0.25	0.05

Analytical methods

Wastewater analysis

Influent and effluent samples of the AOA-IBR were collected three times a week to measure DO, pH and temperature using a multiparameter portable meter (HATCH-HQ40d, USA). Analysis of TSS, volatile suspended solids (VSS), COD, total Kjeldahl nitrogen (TKN), NH_4-N and TP concentrations were according to Standard Methods for the Examination of Water and Wastewater (APHA 2017) (see Supplementary Material, Table S1). Meanwhile, the collected samples were filtered with GF/C filters with a pore size of 0.45 μm before the analysis of NO_3-N and NO_2-N concentrations by a portable analyzer (HACH DR-2700, Germany) using the reagents of NitraVer®6 Nitrate and NitriVer2 Nitrite Reagent Powder Pillows, respectively, according to the manufacturer procedures (©Hach Company/Hach Lange GmbH, USA). Accordingly,

the TN concentration in the wastewater was equal to combined TKN, NO_3-N and NO_2-N concentrations. In addition, in order to determine TN and NH_4-N removal efficiencies of the $A_{NX1+}A_{OX}$ and A_{NX2}, the effluent from each zone of the AOA-IBR (see sampling points in Supplementary Material, Figure S1) was also sampled for the wastewater analysis according to the above-mentioned methods. Light intensity of the red LED lamps was measured by a quantum meter (Model MQ-200, USA). The laboratory analysis was undertaken at the Environmental Engineering and Management laboratory, AIT, Pathumthani, Thailand.

Microalgal–bacterial species analysis

At the end of the AOA-IBR operation at the HRT of 72 h, the suspended and attached-growth biomass samples were collected from the A_{OX} and A_{NX2} zones of the AOA-IBR for analysis of the microalgal–bacterial species. The biofilm biomass was randomly scraped from biofilm media at 5, 25 and 40 cm depths of the A_{OX} and A_{NX2} zones. The collected biomass samples from the A_{OX} were then diluted in 1 L of distilled water, fixed with Lugol acid and stored at 4 °C before analysis of microalgal species at the Thailand Institute of Scientific and Technology Research (TISTR) laboratory, Pathumthani, Thailand, by using the microscopic method (microalgal strain identification and microalgal cell count under a microscope) according to APHA (2017) (see Supplementary Material, Table S1). The bacterial species of the suspended and biofilm biomass samples were analyzed by next-generation sequencing (NGS) platform (Illumina's Hiseq) (Ibekwe et al. 2017). Briefly, the samples were firstly centrifuged prior to total DNA extraction by using a CTAB/SDS method (Demeke et al. 2010). Polymerase chain reactions (PCRs) (targeting bacterial genes of 16S rRNA) were amplified by using specific primer, e.g. 16S V4: 515F-806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R (Ibekwe et al. 2017), with the Phusion® high-fidelity PCR master mix (New England Biolabs, UK). Sequencing analysis was performed on Illumina platform (Hiseq) with similarity more than 97% and was assigned to the same operational taxonomic units (OTUs) via Uparse software (Uparse v7.0.1001 http://drive5.com/uparse/) (see Supplementary Material, Figures S2 and S3).

Kinetic model

The Stover–Kincannon model is commonly used for determining kinetic constants based on organic and nutrients removal rates against OLR in the AOA-IBR as shown in Equation (1). This kinetic model was originated for determining kinetic constants of a rotating biological contactor. Later, the model has been applied to determine kinetic constants of both the suspended and biofilm microorganism in other types of the bioreactor (Yang et al. 2015; Derakhshan et al. 2018).

$$\frac{V}{Q(S_o - S_e)} = \frac{K_B}{U_{max}} \times \frac{V}{QS_o} + \frac{1}{U_{max}} \tag{1}$$

in which, S_o and S_e are the influent and the effluent substrate concentrations (mg/L), U_{max} is the maximum utilization rate (g/(L d)), K_B is the saturation value constant (g/(L d)), V is reactor volume (L), Q is the flow rate (L/d). The experimental data obtained from this study were used to determine the K_B and U_{max} values of the AOA-IBR system for comparison with other biofilm systems and application of the results.

Statistical analysis

The statistical comparisons among data obtained from the experiments of the AOA-IBR were analyzed by using a variance analysis (ANOVA) in SPSS software (V.16.0).

RESULTS AND DISCUSSION

At the steady-state conditions (relatively constant effluent COD and TN concentrations for 4 weeks), the average pH values in the A_{NX1}, A_{OX} and A_{NX2} zones were observed to be 7.7 ± 0.18, 8.1 ± 0.08 and 8.1 ± 0.26, respectively, while the DO concentrations were 0.2 ± 0.03, 3.7 ± 1.17 and 0.4 ± 0.12 mg/L, respectively ($n = 33$) (see Supplementary Material, Table S2). The A_{OX} zone with the DO concentrations more than 2 mg/L was considered suitable for growth of aerobic and nitrifying bacteria (Metcalf & Eddy 2014), while the DO concentrations of less than 1.0 mg/L in the A_{NX1} and A_{NX2} could support denitrifying bacteria

to remove NO_3-N and NO_2-N in the wastewater via denitrification reactions (Shen *et al.* 2009). Accordingly, the average pH value and DO concentration of the effluent samples were found to be 8.0 ± 0.18 and 0.94 ± 0.32 mg/L, respectively, (see Supplementary Material, Table S2). Probably due to continuous lighting and aeration, there were no statistical differences among pH value and DO concentrations in the AOA-IBR under various HRTs. The average temperature of 28 ± 0.91 °C was recorded during the experimental period.

Treatment performance of the AOA-IBR system

As shown in Table 4, the TSS, COD, TN and NH_4-N removal efficiencies were used to evaluate the treatment performance of the AOA-IBR system operating at HRTs of 24, 48 and 72 h.

Because there was no significant difference ($P < 0.05$), the average TSS removal efficiency of three experimental conditions of the AOA-IBR was found to be $95.7 \pm 2.3\%$, resulting in the average effluent TSS concentration of 18.1 ± 7.8 mg/L which could meet the type B effluent standard (≤ 30 mg/L) of the ISO 30500: Non-Sewered Sanitation Systems (ISO 2018) and the domestic effluent standard of Thailand (≤ 50 mg/L) (PCD Thailand 2017). Because the last zone (A_{NX2}) of the AOA-IBR was filled with the zeolite beads, the TSS was retained in the reactor by the filtration of the zeolite beads.

The highest COD removal efficiency of $79 \pm 6\%$ was achieved in the AOA-IBR operating under the HRT and OLR of 72 h and 0.25 gCOD/(L d), respectively, corresponding to the average effluent COD concentration of 145 ± 47 mg/L which also comply with type B effluent standard (≤ 150 mg/L) of the ISO 30500. However, the lower COD removal efficiencies of $76 \pm 14\%$ and $74 \pm 2\%$ ($P < 0.05$)

were found in the AOA-IBR operating under the HRT and OLR of 48 and 24 h and 0.34 and 0.69 gCOD/(L d), respectively. Due to the relatively stable influent COD concentration of 693 ± 130 mg/L during these three experimental stages, increasing HRT (or decreasing OLR) resulted in increased COD removal efficiencies in the AOA-IBR ($R^2 = 0.98$). The COD concentrations in the influent wastewater were mainly degraded by aerobic heterotrophs in the A_{OX} zone, while some of the COD concentrations were unutilized in denitrification processes by denitrifiers in the A_{NX1} and A_{NX2} zones (Posadas *et al.* 2013; Boelee *et al.* 2014) corresponding to the results of the bacterial community (Figure 5).

Similarly, the highest TN removal efficiency of $73 \pm 12\%$ was observed in the AOA-IBR operating under the HRT and NLR of 72 h and 52 gN/(L d), respectively (Table 4), whereas the lower TN removal efficiencies of $67 \pm 5\%$ and $60 \pm 17\%$ ($P < 0.05$) were found with the HRT of 48 and 24 h, respectively, similar to the previous study of Karapinar-Kapdan & Aslan (2008). TN removal of the AOA-IBR was also found to meet the ISO 30500 ($\geq 70\%$ more than TN removal efficiency). The NH_4-N removal efficiencies were found to be 91 ± 4, 79 ± 4 and $75 \pm 5\%$ under the HRT of 72, 48 and 24 h, respectively (Table 4), indicating effective nitrification reactions and microalgal uptake of NH_4-N in the A_{OX} zone. Moreover, the average NO_3-N concentration in the A_{OX} zone was found to increase from less 1 than to 29 mg/L, indicating the occurrence of nitrification processes, corresponding to the results of the bacterial community as revealed in the 'Bacterial community in the A_{OX} and the A_{NX2} zones' section. Because the pH value of about 8 was not high enough, the NH_4-N removal via NH_3 volatilization in the AOA-IBR was considerably negligible (Valero & Mara 2007). Additionally, the TN and NH_4-N removal efficiencies of 49 and 81%, respectively,

Table 4 | Treatment performance of the AOA-IBR system

HRT (h)	Parameters							
	TSS ($n = 33$)		COD ($n = 33$)		TN ($n = 33$)		NH_4-N ($n = 33$)	
	Removal (%)	Effluent (mg/L)	Removal (%)	Effluent (mg/L)	Removal (%)	Effluent (mg/L)	Removal (%)	Effluent (mg/L)
24	98.3 ± 0.7	12.6 ± 5.7	74 ± 1.9	176.3 ± 18.1	60 ± 14.6	75.6 ± 24.6	75 ± 3.9	34.5 ± 3.7
48	94.4 ± 5.1	27 ± 15.1	76 ± 9.2	164.3 ± 46.1	67 ± 4.3	54.2 ± 11.5	79 ± 3.6	26.8 ± 5.1
72	94.4 ± 3.4	14.8 ± 4.1	79 ± 6.9	145.5 ± 47.3	73 ± 2.3	42.2 ± 15.7	91 ± 3.4	11.1 ± 4.4

were found in A_{NX1} and A_{OX} zones of the AOA-IBR at the HRT of 72 h, while 24 and 10% were removed in the A_{NX2} zone (Figure 2). Because of the suitable condition (DO < 2 mg/L) in the A_{NX1} zone, some TN could be removed via denitrification processes corresponding to the data of bacterial community as described in the 'Bacterial community in the A_{OX} and the A_{NX2} zones' section. The bacterial community in the A_{OX} and the A_{NX2} zones, while some TN was assimilated into the microalgal–bacterial biofilm in the A_{OX} zones (Boelee *et al.* 2014). Additionally, some TN could be removed by denitrification in the inner layers of the biofilm, and further investigation in this respect is recommended. Some previous studies have reported that the red LED light is effective for the growth of microalgae in the microalgal based treatment systems (Zhang *et al.* 2017; Chaiwong *et al.* 2018). Moreover, because about 24 and 10% of TN and NH_4-N were found

to be removed in the A_{NX2} zone containing the zeolite media (Figure 2), it could be hypothesized the denitrification reaction (Yang *et al.* 2017).

Kinetics model of the AOA-IBR system and its application

The Stover–Kincannon model was applied to determine the maximum utilization rate (U_{max}) and saturation value constant (K_B) for COD and TN removal in the AOA-IBR by considering the linear plot of $VQ/(S_o - S_e)$ against $V/(QS_o)$, as shown in Figure 3(a) and 3(b), respectively. From Figure 3(a), the kinetic values of the U_{max} and K_B for COD removal in the AOA-IBR were calculated to be 5.00 and 3.56 gCOD/(L d), respectively ($R^2 = 0.94$), which were relatively higher than other biofilm reactors, namely the biofilm photobioreactor (BPBR) (Surinkul *et al.* 2017; Chaiwong
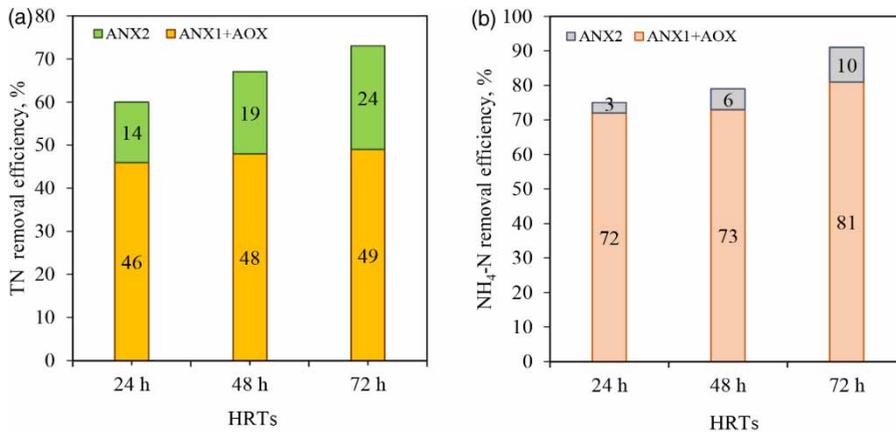
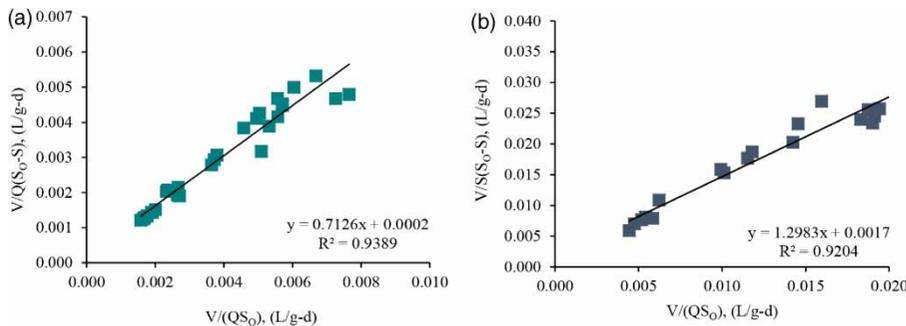**Figure 2** | (a) TN and (b) NH_4-N removals in the AOA-IBR system.

Figure 3 | Stover–Kincannon model of COD (a) and TN (b) removal in the AOA-IBR system.

et al. 2018), the hybrid microalgal membrane photobioreactors (HMPBR) and the microalgal membrane photobioreactors (MPBR) (Derakhshan et al. 2018; Table 5). The high kinetic values for COD removal in the AOA-IBR could be due to the combination of the aerobic (A_{OX}) and anoxic (A_{NX1} and A_{NX2}) zones which enhanced organic biodegradation and denitrification reactions (Zhou et al. 2017), respectively. In addition, the U_{max} and K_B values for TN removal in the AOA-IBR were calculated to be 0.59 and 0.76 gN/(L d), respectively, with R^2 of 0.92 (Figure 3(b)), higher than the U_{max} and K_B values of the BPBRs treating synthetic wastewater (Karapinar-Kapdan & Aslan 2008) and septic tank effluent (Chaiwong et al. 2018), the bio-diatomite biofilm reactor (BBR) treating polluted raw water (Yang et al. 2015), as revealed in Table 5. The high U_{max} and K_B values indicated the high efficiencies of the AOA-IBR system in recovery COD and TN concentration from wastewater.

Hence, the obtained kinetic values for COD and TN removal in the AOA-IBR could be substituted into Equation (1) to become Equations (2) and (3), respectively, which could be used to estimate the effluent COD and TN concentrations of the AOA-IBR as well as to design size of the AOA-IBR for treatment of septic tank effluent.

COD removal:

$$S_0 = S_0 - \frac{5.00xS_o}{3.56 + \frac{QS_o}{V}} \qquad (2)$$

TN removal:

$$S_0 = S_0 - \frac{0.59S_o}{0.76 + \frac{QS_o}{V}} \qquad (3)$$

Microalgal community in the A_{OX} zone of the AOA-IBR system

Due to the presence of both suspended and biofilm biomass in the A_{OX} zone, its samples were collected for analysis of the major microalgal species. The predominant biofilm microalgal species was *Oscillatoria* sp. (83.3% or 1.27×10^4 filaments/mL), while *Scenedesmus* sp. (85.2% or 1.94×10^4 colony/mL) was predominant in suspended microalgal species, as shown in Figure 4. Other microalgal species densities are detailed in Supplementary Material, Table S3. These two microalgal species were also found to

Table 5 | Comparison of kinetic constants of Stover–Kincannon model for COD and TN removals

| System	Type of wastewater	Substrate	Kinetic constant	Unit	Values	R^2	References
AOA-IBR	Septic tank effluent	COD	U_{max}	gCOD/(L d)	5.00	0.94	This study
			K_B	gCOD/(L d)	3.56		
		TN	U_{max}	gN/(L d)	0.59	0.92	
			K_B	gN/(L d)	0.76		
BPBR	Blackwater	COD	U_{max}	gCOD/(L d)	0.17	0.93	Surinkul et al. (2017)
			K_B	gCOD/(L d)	0.19		
BPBR	Septic tank effluent	COD	U_{max}	gCOD/(L d)	0.41	0.95	Chaiwong et al. (2018)
			K_B	gCOD/(L d)	0.58		
		TN	U_{max}	gN/(L d)	0.03	0.85	
			K_B	gN/(L d)	0.04		
HMPBR	Secondary-treated effluent	COD	U_{max}	gCOD/(L d)	3.31	0.99	Derakhshan et al. (2018)
			K_B	gCOD/(L d)	3.39		
MPBR	Secondary-treated effluent	COD	U_{max}	gCOD/(L d)	1.70	0.99	Derakhshan et al. (2018)
			K_B	gCOD/(L d)	1.99		
BBR	Polluted raw water	TN	U_{max}	gN/(L d)	0.33	0.94	Yang et al. (2015)
			K_B	gN/(L d)	0.41		
BPBR	Synthetic wastewater	TN	U_{max}	gN/(L d)	0.01	0.93	Karapinar-Kapdan & Aslan (2008)
			K_B	gN/(L d)	0.01		
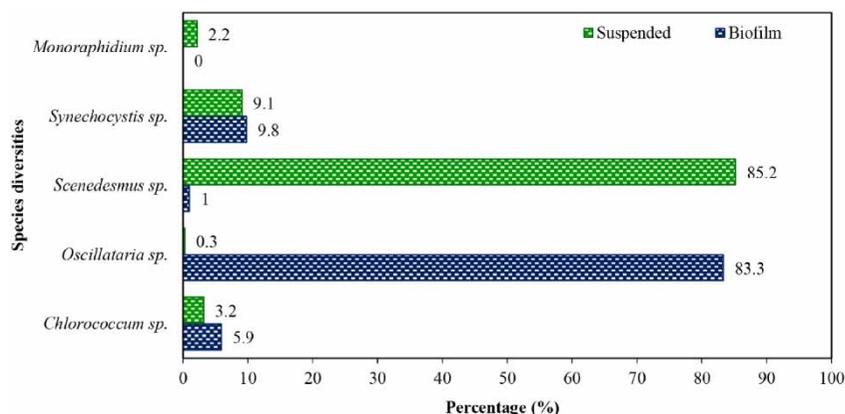
Figure 4 | Microalgal communities in the AOA-IBR system.

be predominant in other microalgal systems used for waste-water treatment, especially for TN removal via the microalgal biomass assimilation as well as producing oxygen (Boelee *et al.* 2014; Rasheedy *et al.* 2017).

Bacterial community in the A_{OX} and the A_{NX2} zones

Both suspended and biofilm biomass samples in the A_{OX} and the A_{NX2} zones were collected for examination of the predominant bacterial species and the results are shown in Figure 5. Additionally, the species diversity curves reveal about 40,000 of the sequence number which was retrieved from the NGS technique (see Supplementary Material, Figure S4).

In the A_{OX} zone, the phylum of *Proteobacteria* (∼62%) was found to be the predominant suspended bacterial
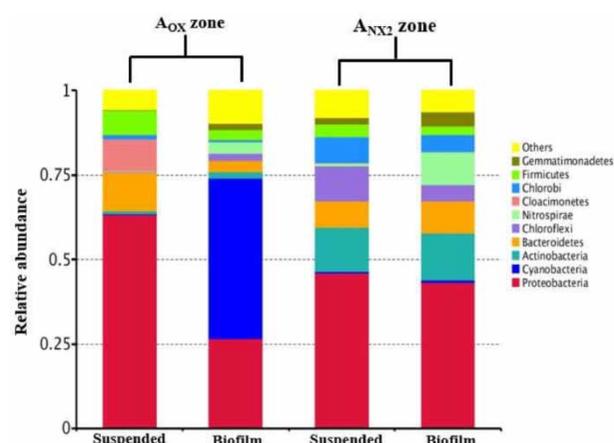

Figure 5 | Phylum of the bacterial group in the AOA-IBR system.

group, followed by *Bacteroidetes* (∼12%), *Cloacimonetes* (∼10%) and *Firmicutes* (∼8%), whereas the phylum of *Cyanobacteria* (∼52%) was observed to be the predominant biofilm bacterial group followed by the phylum of *Proteobacteria* (∼29%), *Bacteroidetes* (∼3%) and *Nitrospirae* (∼3%) (Figure 5). The cyanobacteria or blue-green algae were responsible for photosynthetic oxygen production for the heterotrophic bacteria to biodegrade and taking up TN in the wastewater (Rasheedy *et al.* 2017). Meanwhile, in the A_{NX2} zone, the phylum of *Proteobacteria* (∼50%) was found to be the predominant suspended bacterial group, followed by the phylum of *Actinobacteria* (∼14%), *Choroflexi* (∼11%), *Chorobi* (∼9%) and *Bacteroidetes* (∼8%), as illustrated in Figure 5; similarly, the predominant biofilm bacterial group in the A_{NX2} zone were observed to be the phylum of *Proteobacteria* (∼47%) followed by the phylum of *Actinobacteria* (∼15%), *Bacteroidetes* (∼10%) and *Nitrospirae* (∼9%). Hence, it could be hypothesized that the observed bacterial phylum of *Proteobacteria*, *Bacteroidetes* and *Firmicutes*, the typical heterotrophic bacteria (Ibekwe *et al.* 2017), were responsible for COD degradation (Ibekwe *et al.* 2017), while the phylum of *Nitrospirae* was responsible for nitrification processes in the biofilm AOA-IBR system (Wongkiew *et al.* 2017). Some of the *Proteobacteria* consisting of denitrifying bacteria (Wongkiew *et al.* 2017) were hypothesized to be responsible for denitrification processes for TN removal in the A_{NX2} zone.

The overall results of this study suggest the technical feasibility of applying the AOA-IBR system for treating septic tank effluent to meet the ISO 30500 effluent

standards for Non-Sewered Sanitation Systems. However, more pilot and full-scale experiments should be further conducted to validate these experimental results and determining the system's cost-effectiveness.

CONCLUSIONS

Based on the experimental results obtained from this study, the following conclusion is made:

- The AOA-IBR could be applied as an on-site post-treatment system to effectively treat COD and TN concentrations in septic tank effluent via combined microalgal and bacterial reactions such as aerobic biodegradation, biomass assimilation and nitrification/denitrification as well as adsorption by zeolite media.
- The highest of COD and TN removal efficiencies were achieved in the AOA-IBR system operating at the HRT of 72 h, OLR of 0.25 gCOD/(L d) and NLR 0.05 gN/(L d).
- The Stover–Kincannon model was applied to determine the kinetic values for COD and TN removal in the AOA-IBR for use in designing a suitable size of the AOA-IBR treating septic tank effluent.
- The major microalgal groups in the AOA-IBR were found to be *Oscillatoria* sp. and *Scenedesmus* sp., while *Proteobacteria* and *Nitrospirae* were the predominant bacterial phylum responsible COD and TN removal, respectively.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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