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Comparison of an integrated short-cut biological nitrogen removal process with magnetic coagulation treating swine wastewater and food waste digestate

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HIGHLIGHTS

G R A P H I C A L A B S T R A C T

- Integrated MC and SMBR showed a new pathway for high strength wastewater treatment.
- MC showed high removals of TSS, turbidity, TP, and phosphate for both wastewaters.
- \bullet SMBR achieved stable and excellent removals of COD, TN, NH_4^+-N for both wastewaters.
- NAR achieved 74.54% and 76.02% for swine wastewater and FW digestate, respectively.
- *Nitrosomonas* and *Diaphorobactor* & *Thaurea* dominant in nitritation and denitritation.

ARTICLE INFO

Keywords: Food waste digestate Swine wastewater Magnetic coagulation Short-cut nitritation and denitritation



ABSTRACT

An integration of two processes, magnetic coagulation (MC) and short-cut biological nitrogen removal (SBNR), coupled with a sequencing batch membrane bioreactor (SMBR) controlled by an automatic real-time control strategy (RTC), was developed to treat different characteristics of high strength wastewater. The treatment efficiency and microbial community-diversity of the proposed method was evaluated and investigated using swine

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SMBR Stable nitritation wastewater and food waste (FW) digestate. The MC showed high removal of TSS (89.1 \pm 1.5%, 92.21 \pm 1.8%), turbidity (90.58 \pm 2.1%, 95.1 \pm 2.1%), TP (88.5 \pm 1.9%, 92.1 \pm 1.5%), phosphate (87.76 \pm 1.6%, 91.22 \pm 1.5%), and SMBR achieved stable and excellent removal of COD (96.05 \pm 0.2%, 97.39 \pm 0.2%), TN (97.30 \pm 0.3%, 97.44 \pm 0.3%) and NH⁺₄ – N (99.07 \pm 0.2%, 98.54 \pm 0.2%) for swine wastewater and FW digestate, respectively. The effluent COD and NH⁺₄ – N concentrations were found to meet their discharge standards. The microbial community comparison showed similar diversity and richness, and genus *Diaphorobacter* and *Thaurea* were *dominant* in denitritation, and *Nitrosomonas* was dominant in nitritation treating both swine wastewater and FW digestate.

1. Introduction

China is the largest pork consumer in the world, and the rising pork demand has increased swine production from smaller farms to larger industrialized operations, which produce a large quantity of livestock manure with high concentrations of pollutants with hostile ecological impact (Ministry of Ecology and Environment of People's Republic of China, 2020). As a result, the second national pollution source survey of China in 2017 showed that the animal industry was one of the main sources of water pollutants discharging to the environment, and the swine industry accounts for a large percentage (60%) (Ministry of Ecology and Environment of People's Republic of China, 2020). Besides, FW is a global issue and accounts for the largest volume of municipal solid waste (MSW) sending to landfills. Since 2019, China has been spreading the source separation practice of municipal solid wastes nationwide (National Bureau of Statistics People's Republic of National Statistical Report on Ecology and Environment, 2019). It is well known that anaerobic digestion (AD) has been increasingly practiced worldwide as such alternative waste management process and ultimately served as a market source for secondary raw materials, e.g., a nutrientrich digestate as a fertilizer and renewable energy (biogas or biomethane) (Ren et al., 2018).

In line with such increasing AD worldwide, the treatment of high strength industrial wastewater like anaerobic digesters supernatant (digestate) and swine wastewater has become a global challenge in terms of stringent discharge standards, capital and operation & maintenance cost (Xia and Murphy, 2016) due to high concentrations of organic matters, nutrients, suspended solids, oil content and pathogens (Scaglione et al., 2017). As shown in the supplementary materials, the swine wastewater and FW digestate have different wastewater characteristics. Mainly, FW digestate analysis proved a higher concentration of chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4^+-N) , total suspended solids (TSS) compared with swine wastewater. Conversely, the total phosphorous (TP) and phosphate (PO_4^{3-}) concentrations were relatively higher in swine wastewater than FW digestate. High electric conductivity (EC) of the raw wastewater causes the inactivation of the microbial community in the biological treatment system. Also, wastewater with high EC has been known as a challenge for conventional biological treatment processes (Lotti et al., 2019b; Scaglione et al., 2017). It was expected from the EC and C/N vales (Table. 1) that FW digestate would be more difficult for conventional treatment than swine wastewater. When comparing the EC, it varies from 12 to 35 mS/cm for FW digestate and 2-6 mS/cm for swine

Table 1

Characteristics of the s	source of swine	wastewater and	FW dige	estate
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Description	Swine wastewater	FW Digestate
COD (mg/L)	9000 ± 2100	15000 ± 3200
TN (mg/L)	1100 ± 225	2100 ± 300
NH ₄ ⁺ -N (mg/L)	700 ± 350	1600 ± 205
TSS (mg/L)	2200 ± 600	1500 ± 800
EC (mS/cm)	6.0 ± 2.1	22 ± 6.0
pH	7.2 ± 0.8	$\textbf{7.8} \pm \textbf{0.4}$
TP (mg/L)	160 ± 26	125 ± 16
PO ₄ ³⁻ (mg/L	130 ± 18	90 ± 15

wastewater. The C/N ratio ranges from 6 to 9 and 5–7 for swine wastewater and FW digestate, respectively. Besides, a swine wastewater composition fluctuation with a variation on manure management practices is the main challenge in China on meeting stricter discharge standards (GB18596-2001 and GB/T 31962–2015 belong to swine wastewater and FW digestate, respectively) (Sui et al., 2018). The challenge could also extend to have a determinantal effect on the application/spreading of AD technology. Such a difference in discharge standards makes different requirements for treating these wastewaters. As a result, there has been considerable interest in alleviating the above bottlenecks by designing a treatment approach satisfactory for both.

Sequence batch reactor (SBR) has been identified (Gonzalez-Tineo et al., 2020; Sui et al., 2018) and suggested as the best approach to treat swine wastewater and liquid fraction of the digestate. Other studies prefered an integrated SBR process such as partial-nitrification, comammox, anammox processes (Zuriaga-Agustí et al., 2016), aerobic granular sludge batch reactor (GSBR)(Świątczak and Cydzik-Kwiatkowska, 2018), and integrated fixed-biofilm activated sludge sequencing batch reactor (IFAS-SBR) (Yang et al., 2019) for the treatment of digestate. Nevertheless, the SBR shows some main disadvantages, such as poor clarification due to insufficient sedimentation rate, and resulting in high turbid effluent. However, the effluent quality has been proved to be improved by submerging membranes in a sequencing batch reactor (SMBR) due to its capacity of handling a high concentration of mixed liquor suspended solids (MLSS) and better liquid-solid separation (Sui et al., 2018; Yan et al., 2019). Besides, the introduction of aerobic and anoxic conditions into the SMBR enable high removal of carbon and nitrogen simultaneously compared with conventional MBR (Xu et al., 2019), because short-cut biological nitrogen removal (SBNR) based on nitritation and denitritation processes saves aeration costs during the nitrification phase (Soliman and Eldyasti, 2016; Sui et al., 2018) and carbon source during the denitrification phase. In addition, SBNR has been reported to significantly decrease sludge production in nitritation and denitritation processes by 35% and 55%, respectively, compared to the full nitrification and denitrification processes (Peng and Zhu, 2006).

Nevertheless, the presence of a high amount of TSS, COD, $NH_4^+ - N$, TP, and EC humpers the effectiveness of the SBNR process but would be improved by employing efficient pre-treatment technologies, like coagulation. However, owing to the problem of polyaluminium chloride (PAC) flocs depolymerization (Demissie et al., 2021, 2020), magnetic seeds and polyelectrolytes injection into the coagulation process is proved to enhance the floc settlement and the pre-treatment efficiency (Ritigala et al., 2021). Therefore, combining magnetic coagulation (MC) and SBNR with a membrane-coupled real-time control sequencing batch reactor is hypothesized to meet the discharge standard and satisfy the current need for a cost-effective approach.

This study was carried out based on the advantage of coagulationbased pre-treatment, SBNR, and a membrane-coupled real-time control sequencing batch reactor to mitigate the above main bottleneck and to minimize the operational cost by making use of the following key points: (1) MC with the commercial PAC can effectively improve the floc structure and strength, thereby improving the solid–liquid separation efficiency (Ritigala et al., 2021). (2) SBR is thought to be cost-effective, efficient, and flexible technology that simply can upgrade and intensify the process by adopting a submerged membrane. SMBR is particularly suitable to SBNR because of its unique advantages, such as less footprint, high concentration of biomass, high-quality effluent (Sui et al., 2018), short hydraulic retention time (HRT), and longer sludge retention times (SRT) (Chon et al., 2012). (3) Real-time control (RTC) strategies enhance accuracy, adaptability, and flexibility (Yang et al., 2007; Sui et al., 2018). Further, RTC which was applied to switch nitrification and denitrification reaction phases would improve biological nitrogen removal efficiency and save energy (Gao et al., 2003; Peng et al., 2006). The efficiency of the approach was evaluated using the most known and typical high strength wastewater, FW digestate and Swine wastewater.

Though SMBR was successfully developed recently for swine wastewater treatment, the study of the SMBR for treatment of FW digestate was scarce in the literature. Therefore, the main objective of this investigation was to carry out a feasibility study of combined process (the MC and SMBR) to treat FW digestate, and compare the performance and microbial community diversity of such an integrated process for treating swine wastewater and FW digestate.

2. Materials and methods

2.1. Wastewater characteristics

The swine wastewater was collected from a large-scale swine farm (Changping District, Beijing). The FW digestate was taken from one of the largest municipal FW anaerobic digester (Dongcun Biogas Company, Beijing, China). Both swine wastewater and FW digestate were filtered using a 0.9 mm mesh size (GB/T60031-2012) to coarse particles removal. Table 1 shows the detailed characteristics of both raw water quality.

2.2. Experimental setup

The schematic diagram of the experimental setup is shown in Fig. 1, and it was divided into two separate units, the magnetic coagulation unit (for pre-treatment) and the SMBR unit (for subsequent biological treatment). The magnetic coagulation unit was a cylinder made of plexiglass with an effective volume of 4 L (Diameter \times Height = 200

 $mm \times 190 mm$), and a mechanical mixing device was installed in the middle of the reactor. The SMBR was a rectangular tank with an effective volume of 30 L (Length imes Width imes Height = 260 mm imes 260 mm imes450 mm) and operated at a temperature between 25 °C and 30 °C using a water bath (Xin Yin, China). The polyvinylidene fluoride (PVDF) flat sheet microfiltration membrane module (0.1 μ m, 0.14 m², SINAP, China) was fixed in a frame and mounted in the reactor as shown in Fig. 1. The air pump I (1-2 L/min) was connected to the fine bubble diffuser fixed at the bottom for oxygen supply and mixing. The perforated pipe was fixed under the membrane element and connected to the air pump II (5 L/min) for membrane flushing. It was equipped with two gas flow meters to control aeration and a pressure gauge to monitor the transmembrane pressure (TMP). There were three pumps used for influent feeding (BT300-2 J, Longer), effluent discharging (BT100, Longer), and the supply of sodium acetate as an external carbon source (BT300-2 J, Longer).

2.3. Magnetic coagulation process

The commercial PAC (Al_2O_3 content 30%), magnetic seeds (MS) (main component: Fe_3O_4 , average particle size: 280–300 mesh), and polyelectrolyte (polyacrylamide, PAM) were used in this study. 4L of swine wastewater/FW digestate were each transferred into the magnetic coagulation device at room temperature. Firstly, MS was added simultaneously with the start of rapid mixing (250 rpm) and stirred for 40 s, then PAC was added to the system while keeping the same mixing speed for 60 s. Finally, PAM was added after 30 s of the slow mixing (50 rpm) and kept for 180 s. The supernatant was transferred to the intermediate tank after 30 min of settling time. The optimization details of the magnetic coagulation process were reported in our previous study (Ritigala et al., 2021).

2.4. Startup and operations of the SMBR

The reactor was started with swine wastewater, which is designated as Phase I. Later, swine wastewater diluted with FW digestate was gradually introduced (Transition Phase), and finally, the system was run only with FW digestate (Phase II). Detailed operational parameters of



Fig. 1. Schematic digram of lab scale experimental setup.

SMBR are shown in Table 2. The inoculum (6.0 g MLSS/L) was taken from the existing conventional swine wastewater treatment plant (Changping District, Beijing, China) and introduced to the SMBR. The SMBR operation was adapted to five operation modes of sequential cycles and consisted of feeding, anoxic, oxic, discharging and idle as shown in the supplementary material. The fixed time control (FTC) was used for influent feeding, effluent discharging and idle phases. The RTC and fixed time delay were adopted for the anoxic and oxic phases. The "Ammonia valley (dpH/dt = 0.0)" and "Nitrate knee (dORP/dt = $\langle -5 \rangle$ " were used as RTC points, as per reported by Sui et al., 2018. The anoxic and oxic phases length was monitored by online sensors of ORP (HBM-102A, TOA-DKK), DO (SC100, HACH) and pH (HBM-102A, TOA-DKK) through the RTC. In every single cycle, 1 L of pre-treated influent was pumped into the reactor and pumped out before the next cycle at the exchange ratio of 1/30. The TMP was maintained at 30 kPa, and when the effluent suction pump exceeded 30 kPa, a clean membrane of the same type was replaced until the cleaning of the used membrane. The ex-situ membrane cleaning was carried out physically with clean water (Tap water) and followed chemically by submerging in hydrochloric acid (HCl) at 0.5 M and sodium hypochlorite (NaClO) solution at 0.5 M for 24 h to recover the membrane flux. A 10 mL of sodium acetate at 104 mg/L was added as an external carbon source during the anoxic phase to achieve complete denitritation based on the RTC strategies. The SRT was kept as 15-18 days, and the total operation time of this study was 257 days.

Once the reactor had stabilized, e.g., over 90% removals of both COD and TN, the operation was then continued further for 60 days (Day 192 – 257), which was followed by the end of the transition phase. The "Ammonia valley (dpH/dt = -0.2)" and "Nitrate knee (dORP/dt = <-5)" control points were changed accordingly, but other operational parameters were kept similar to the swine wastewater treatment conditions. The operation parameters were optimized until the system showed stable performance and further monitored for 60 days under the same conditions.

2.5. Real-Time control strategy

Bending-points detection was employed to control the lengths of the anoxic and oxic phases in the SBR in order to save footprint, e.g., energy and process capacity (Sui et al., 2018). In closed-loop control, the changes (bending points) of the pH, DO, and ORP profiles used to identify the length of anoxic and oxic phases. These bending-points are used to determine the end-point of each reaction phase and reduce the excess aeration costs and reaction time (Jaramillo et al., 2018). ORP and pH are the most common parameters in the control strategy, and reflect the actual microbial activity condition in the reactor system and can be detected reliably. Furthermore, it can be divided into two categories, one based on the changes of the bending points in the pH and ORP profile (maximum value, minimum value), and the other based on

Table	2
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Operational	parameters	of the	three	phases.
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Description	Parameters	Phase I (Day 1–121)	Transition Phase (Day 122–191)	Phase II (Day192- 257)
Operational	HRT (days)	$\textbf{5.0} \pm \textbf{0.1}$	$\textbf{4.4} \pm \textbf{0.1}$	$\textbf{4.4} \pm \textbf{0.1}$
Parameters	SRT (days)	15–18	15-18	15–18
	OLR (kg·kg/	0.28 \pm	$\textbf{0.22} \pm \textbf{0.08}$	0.36 ± 0.02
	VSS·d)	0.02		
	NLR (kg·kg/	0.05 \pm	0.05 ± 0.01	0.09 ± 0.01
	VSS·d)	0.01		
	MLSS (g/L)	$\textbf{4.8} \pm \textbf{0.6}$	5.9 ± 0.3	6.1 ± 0.8
	Temperature	25–30	25-30	25–30
	(°C)			
	pH	7.2 \pm	$\textbf{7.3} \pm \textbf{0.22}$	7.6 + 0.28
		0.14		
	EC (mS/cm)	$4.2 \pm$	$\textbf{7.2} \pm \textbf{1.52}$	11.9 ± 3.24
		0.81		

differential signals of pH and ORP such as dpH/dt, d^2 pH/dt², dORP/dt, d^2 ORP/dt². In this study, dpH/dt, dORP/dt differential signals were used in the change point in the pH and ORP curve. The total control logic was illustrated in the supplementary material.

2.6. Analytical methods

The COD was measured by HACH tube reagent (HACH, USA) using a HACH DR2800. Total nitrogen (TN), NH_4^+ – N, NO_3^- –N and NO_2^- –N were determined by spectrophotometry methods (TU-1901, China). pH and conductivity were measured using pH/conductivity meter (Multi 3420 WTW, Germany). The standard methods (APHA, 2005) were used to determine the MLSS and mixed liquor volatile suspended solid (MLVSS) concentrations.

The nitrite accumulation ratio (*NAR*), free ammonia (*FA*), and free nitrite (*FNA*) concentrations inside the SMBR were calculated based on equations (1), 2, and 3, respectively (Soliman and Eldyasti, 2016; Yan et al., 2019).

$$NAR = \frac{NO_2^- - N}{NO_3^- - N + NO_2^- - N}$$
(1)

$$FA = \frac{17}{14} \times \frac{\text{TN} \times 10^{\text{P.H.}}}{e^{\left(\frac{6344}{273 + T}\right)} + 10^{\text{P.H.}}}$$
(2)

$$FNA = \frac{46}{14} \times \frac{\text{TNO}_2}{e^{\left(\frac{-2300}{273+T}\right)} + 10^{\text{P.H.}}}$$
(3)

Where TN is total $NH_4^+ - N$ concentration (mg/L), TNO_2 is total $NO_2^- - N$ concentration (mg/L), T is the temperature in °C, pH is the measured value in the SMBR reactor, and $NO_2^- - N$ and $NO_3^- - N$ are the concentrations of the SMBR at the end of the oxic phase (mg/L).

2.7. DNA extraction and microbial community analysis

DNA of the sludge samples were extracted by using a FAST DNA Spin Kit for Soil (M.P. Biomedicals, Solon, OH, USA) to evaluate the microbial community dynamics in the SMBR system according to the manufacturer's instructions. The bacterial community was assessed by polymerase chain reaction (PCR) amplification of 16S rRNA genes using the 515F/806R primers (Chen et al., 2021; Sui et al., 2018), and sequencing was conducted at the Sangon Co., Ltd. (Shanghai, China).

2.8. Data analysis

The SPSS 20 statistical software (IBM, USA) was used statistical analysis, and figures were plotted by OriginPro 9.0 (OriginLab, USA). Redundancy analysis (RDA), principal component analysis (PCA) was performed using Canoco 5.0 (Microcomputer Power, USA). Heml software (http://hemi.biocuckoo.org/) was used to plot the heatmap.

3. Results and discussions

3.1. Performance comparison of magnetic coagulation process

The performance of MC with commercial PAC was optimized via a series of jar tests for Phase I and Phase II, and the optimum conditions were shown in the supplementary material. Transition Phase was a dilution of swine wastewater and FW digestate, where the dilution procedures and ratios are attached in supporting information. Pretreatment (MC) was done according to optimum conditions illustrated in the supplementary material for each wastewater. MC was mainly used to remove suspended solids and oxygen-consuming organics, but it was not performed in the Transition Phase. MC is also thought to have a significant removal effect on TP and phosphate. The detailed performance of the MC process was shown in Table 3. Accordingly, in Phase I (Day 1–121), the removals of COD, TSS, turbidity, TP, phosphate and ammonium by MC were 40.25%, 89.10%, 90.58%, 88.50%, 86.90%, and 7.82%, respectively. Whereas, in Phase II (Day 192–257) their corresponding removals were, 33.64%, 92.21%, 95.10%, 92.10%, 89.30%, 13.28% respectively. The COD/TN ratios were decreased from 7.98 to 5.14 for swine wastewater, from 7.51 to 4.27 for FW digestate, respectively. Consistent with previous studies (Ren et al., 2019; Ritigala et al., 2021), the dissolved nitrogen removal was not as effective as other components. Hence such MC pre-treatment is useful for the following SBNR process, and dissolved nitrogen is expected to be removed by the subsequent SBNR process.

3.2. Performance comparison of the SMBR

3.2.1. Removals of COD, NH_4^+ – N, and TN

It could be identified from Fig. 2 that the SMBR demonstrated excellent removals of COD, NH_4^+ –N and TN throughout the study period and effluent water quality in three phases has met both the discharge standard of pollutants for livestock and poultry breeding (GB18026-2001) and the discharged standards for discharge to municipal sewers (GB/T 31962–2015) of China, respectively.

3.2.1.1. Phase I. In Phase I, the total nitrogen loading rate (NLR) was ranged from 0.033 kg·kg⁻¹·VSS·d⁻¹ to 0.070 kg·kg⁻¹·VSS·d⁻¹, 5.0 \pm 0.1 day of HRT and 4.8 \pm 0.6 g/L of MLSS concentration were well maintained. The average TN concentrations of influent and effluent of Phase I were 1087 \pm 177 mg/L and 33.58 \pm 5.5 mg/L, respectively, while as the average concentrations of NH⁺₄ – N in the influent and effluent were 936 \pm 144 mg/L and 9.43 \pm 2.2 mg/L, respectively. The corresponding TN and NH⁺₄ – N removal efficiencies were consistently averaged to be 96.85 \pm 0.60% and 98.97 \pm 0.3%, respectively. Besides, the organic loading rate (OLR) was ranged from 0.22 kg·kg⁻¹·VSS·d⁻¹ –0.34 kg·kg⁻¹·VSS·d⁻¹ with the average COD removal rate of 94.42 \pm 1.02%. The average TP concentration of influent and effluent was 21 \pm 6.0 mg/L and 6.87 \pm 1.3 mg/L, respectively and its average removal efficiency

Table 3

Performance	of magnetic	coagulation	under	optimum	conditions.
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Description	Parameters	Phase I (Day 1–121)	Phase II (Day192- 257)
Raw Water	COD (mg/L)	8782 ± 2100	13520 ± 3200
	TSS (mg/L)	2300 ± 600	1530 ± 800
	Turbidity	1660 ± 400	1311 ± 400
	(NTU)		
	TP (mg/L)	161 ± 26	147 ± 21
	PO ₄ ⁻³ -P (mg/L)	139 ± 18	114 ± 18
	NH ₄ ⁺ -N (mg/L)	1018 ± 350	1613 ± 350
	COD/TN	$\textbf{7.98} \pm \textbf{1.1}$	7.51 ± 0.6
Pre-treated water	COD (mg/L)	5247 ± 512	8971 ± 541
	TSS (mg/L)	250 ± 60	120 ± 40
	Turbidity	156 ± 77	64 ± 41
	(NTU)		
	TP (mg/L)	18 ± 4.0	11 ± 6.0
	PO ₄ ⁻³ -P (mg/L)	17 ± 6.0	10 ± 4.0
	NH ₄ ⁺ -N (mg/L)	938 ± 350	1613 ± 350
	COD/TN	5.14 ± 0.4	4.27 ± 0.8
Removal Rate	COD	40.25 ± 4.2	33.64 ± 3.6
(%)	TSS	89.10 ± 1.5	92.21 ± 1.8
	Turbidity	90.58 ± 2.1	95.10 ± 2.1
	TP	88.50 ± 1.9	92.10 ± 1.5
	PO ₄ -3-P	87.76 ± 1.6	91.22 ± 1.5
	NH ₄ ⁺ -N	$\textbf{7.82} \pm \textbf{2.1}$	13.28 ± 1.8

***Transition Phase performance was not included here; Transition Phase was a dilution of pre-treated swine wastewater and FW digestate. Pre-treatment was done according to the optimum condition shown in the supplementary information.

was 67.5 \pm 0.50%.

3.2.1.2. Transition Phase. Pre-treated swine wastewater and FW digestate were mixed according to the mixing ratio shown in the supplementary material and gradually introduced to the SMBR. The dilution ratio of the FW digestate was increased week by week while reducing the swine wastewater. The SMBR operated from 0.031 kg·kg⁻¹·VSS·d⁻¹ to 0.083 kg·kg⁻¹·VSS·d⁻¹ of NLR, 5.9 \pm 0.3 g/L of MLSS concentration and 4.4 ± 0.1 day of HRT were kept. The average concentrations of TN and NH_{4}^{+} –N in the influent were at 1170 \pm 294 mg/L and 1001 \pm 152 mg/L, respectively and effluent TN and NH_4^+ –N concentrations were at 62.92 \pm 57 mg/L and 29.32 \pm 44 mg/L. The corresponding TN and NH⁺₄ –N removal efficiencies were consistently averaged at 94.85 \pm 4.16% and 97.18 \pm 4.20%, respectively. When the reactor was fed with OLR ranged from $0.08 - 0.35 \text{ kg} \cdot \text{kg}^{-1} \cdot \text{VSS} \cdot \text{d}^{-1}$, the average COD removal rate was 92.40 \pm 2.50%. TP removal rate was averaged at 67.25 \pm 0.30% when the influent and effluent concentration averaged at 16 \pm 2.8 mg/L and 12 ± 4.0 mg/L, respectively. From day 167 to 177 of the period, effluent TN and NH_{4}^{+} –N concentrations were suddenly increased due to insufficient aeration time for the nitritation process. Hence aeration time was increased to maintain the required DO concentration (Soliman and Eldyasti, 2016).

3.2.1.3. Phase II. The reactor was started to run entirely with FW digestate from the 191st day onwards. The NLR was ranged from 0.071 to 0.099 kg·kg^{-1.}VSS·d⁻¹ while keeping at the 4.4 ± 0.1 day of HRT and 6.1 ± 0.8 g/L of MLSS. In this phase, the average influent TN and NH₄⁺ –N concentrations were at 2077 ± 184 mg/L and 1621 ± 166 mg/L with their average effluent concentrations of 51.87 ± 5.5 mg/L and 25.06 ± 6.3 mg/L, respectively. Accordingly, the system was back to the normal condition and average removal efficiencies of TN and NH₄⁺ –N were consistently at 97.42 ± 0.30%, and 98.47 ± 0.32%, respectively. Throughout the period (Day 192–257), the OLR changed 0.27 kg·kg^{-1.}VSS·d⁻¹ to 0.39 kg·kg^{-1.}VSS·d⁻¹. Here, the average COD removal rate was at 95.62 ± 0.40% with fluctuating feeding and organic load rate, and TP removal rate was averaged at 51.66 ± 0.6% with 12 ± 4.0 mg/L and 5.80 ± 1.2 mg/L of influent and effluent concentration, respectively.

Since EC is recognized as one of the major inhibitory factors for the microbial community when treating industrial wastewaters with the biological treatment system (Lotti et al., 2019b; Scaglione et al., 2017). Throughout the experiment period, the averaged influent EC was $6.0 \pm 2.0 \text{ mS/cm}$, $9.0 \pm 3.6 \text{ mS/cm}$ and $16 \pm 3.2 \text{ mS/cm}$ for Phase I, Transition Phase, and Phase II, respectively. Though high EC industrial effluent is difficult to treat using a biological treatment system (Lotti et al., 2019b), the SMBR integrated with SBNR and RTC in this study was successfully adopted and demonstrated significant removal performance with significant differences (p < 0.05) of EC in Phase I (swine wastewater) and PhaseII (FW digestate). The *t*-test was used to compare the performance of Phase I and Phase II. Statistical analysis of COD, TN and NH⁴₄ – N removal rates were significant in swine wastewater (Phase I) and FW digestate (Phase II), respectively.

The summary of the SMBR performance is shown in Table 4 and comparable results were reported in previous studies where SBR was fed with ammonium rich wastewater (Liu et al., 2017). For instance, while treating swine wastewater using SMBR with RTC control strategies (Sui et al., 2018) resulted in a COD removal rate of 95%, conventional SBR (Kornboonraksa and Lee, 2009) and other activated sludge systems reported much less (70%-80%) COD removal rate (Meng et al., 2015). There have been reported different digestate treatment strategies elsewhere, which include treating digestate using two-stage reactors by SBR (Lotti et al., 2019a; Zuriaga-Agustí et al., 2016), high-rate anaerobic fixed-film reactors (AFFRs) (Demirer et al., 2019), and continuously stirred tank reactor (CSTR) (Chini et al., 2019) with removal rate ranged



Fig. 2. Reactor performance during the operation time: (a) influent and effluent COD concentrations Vs the COD removal efficiencies; (b) influent and effluent TN concentrations Vs the TN removal efficiencies; (c) influent and effluent NH_4^+ -N concentrations Vs the NH_4^+ -N removal efficiencies;

Table 4

1	Perr	orma	ince	or	SMBR.	
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Description	Parameters	Phase I (Day 1–121)	Transition Phase (Day 122–191)	Phase II (Day192- 257)
Influent	COD (mg/ L)	$\begin{array}{c} 6261 \pm \\ 512 \end{array}$	5036 ± 1902	7994 ± 541
	TSS (mg/L)	250 ± 60	240 ± 66	280 ± 42
	TN (mg/L)	$\begin{array}{c} 1087 \pm \\ 177 \end{array}$	1170 ± 294	2077 ± 184
	NH4-N (mg/L)	936 ± 144	1001 ± 152	1621 ± 166
	TP (mg/L)	21 ± 6.0	16 ± 2.8	12 ± 4.0
	pН	7.2 ± 0.8	7.4 ± 1.1	$\textbf{7.6} \pm \textbf{0.9}$
	EC (mS/cm)	$\textbf{6.0} \pm \textbf{2.0}$	9.0 ± 3.6	16 ± 3.2
	COD/TN	$\textbf{5.9} \pm \textbf{1.12}$	$\textbf{4.2} \pm \textbf{1.02}$	$\textbf{3.97} \pm \textbf{0.2}$
Effluent	COD (mg/ L)	374 ± 57	359 ± 143	348 ± 19
	TN (mg/L)	$\begin{array}{c} 33.58 \pm \\ 5.5 \end{array}$	62.92 ± 57	51.87 ± 5.5
	NH ₄ ⁺ -N (mg/L)	$\textbf{9.43} \pm \textbf{2.2}$	29.32 ± 44	25.06 ± 6.3
	NO_2^-N (mg/L)	14.28 ± 3.0	16.73 ± 4.1	$\textbf{17.47} \pm \textbf{6.4}$
	NO_3^-N (mg/L)	$\textbf{4.83} \pm \textbf{1.2}$	$\textbf{4.02} \pm \textbf{1.1}$	$\textbf{5.30} \pm \textbf{1.6}$
	TP (mg/L)	6.87 ± 1.3	5.24 ± 1.2	5.80 ± 1.2
SMBR Removal Efficiency (%)	COD	$\begin{array}{c} 94.42 \pm \\ 1.02 \end{array}$	$\textbf{92.40} \pm \textbf{2.50}$	95.62 ± 0.40
-	TN	$\begin{array}{c} 96.85 \pm \\ 0.60 \end{array}$	$\textbf{94.85} \pm \textbf{4.16}$	$\textbf{97.42}\pm\textbf{0.30}$
	NH ₄ ⁺ -N	$\begin{array}{c} \textbf{98.97} \pm \\ \textbf{0.3} \end{array}$	$\textbf{97.18} \pm \textbf{4.20}$	$\textbf{98.47} \pm \textbf{0.32}$
	TP	67.5 ± 0.5	67.25 ± 0.3	51.66 ± 0.6
	NAR (%)	$\begin{array}{c} \textbf{74.54} \pm \\ \textbf{5.10} \end{array}$	$\textbf{64.90} \pm \textbf{18.72}$	$\textbf{76.02} \pm \textbf{4.54}$

from 65% - 80%. Other recent studies reported that a single-stage reactor could achieve the removal of COD efficiency ranging from 75% to 85% for treating high strength digested lagoon supernatant by IFAS-SBR (Zou et al., 2020). This study showed higher efficiency than other studies treating wastewater with similar characteristics due to the membrane separation process.

3.2.2. Stable nitritation

The SBNR shortened the nitrification process until nitrite accumulation, which is followed by subsequent denitrification of the nitrite. The most critical condition for the success of the SBNR process is the accumulation of nitrite to suppress nitrite oxidation without excessively hindering the ammonia oxidation rate (Soliman and Eldyasti, 2016; Sui et al., 2016a). In our study, the nitrite accumulation was well built up throughout the study period with an average of 74.54 \pm 5.10%, 64.90 \pm 18.72% and 76.02 \pm 4.54% for Phase I, Transition Phase and Phase II, respectively (Fig. 3a), which represents the success of nitrite accumulation and NOB suppression throughout the study. The NAR was statistically not significant (p > 0.05) in swine wastewater and FW digestate. From Day 167 to 177 (Transition Phase), the NAR was dropped down due to insufficient DO for the nitritation process and significantly different(p < 0.05) compared with other phases. AOB activity was measured three times per each phase and averaged values were used to calculate the activity of AOB. As shown in Fig. 3b, 3c and 3d, AOB activity was 25.20 mg/L/h,16.82 mg/L/h and 40.80 mg/L/h, respectively, for Phase I, Transition Phase and phase II, conversely, NOB activity was not detected in all three phases.

According to the literature, AOB (Nitrosomonas) and NOB (Nitrobacters) were inhibited when the FA concentration reached 10–150 mg/L and 0.1–1 mg/L, respectively (Guo et al., 2009; Soliman and Eldyasti, 2016; Sui et al., 2018). Accordingly, the calculated average FA and FNA concentrations within each cycle are shown in the supplementary



Fig. 3. Nitritation performance of the reactor: (a) Nitrite accumulation ratio during the operation period. AOB activity at (b) 77th day, (c) 162nd day and (d) 232nd day for Phase I, Transition Phase and Phase II, respectively.

material, and FA concentration was ranged from 0.74 mg/L – 3.18 mg/L, 0.35 mg/L – 1.62 mg/L, and 0.19 – 0.72 mg/L for Phase I, Transition Phase, and Phase II, respectively, resulting in inhibition of the *Nitrobacter* under this operating condition. The maximum FNA values, e. g., 6.22×10^{-7} mg/L, 1.49×10^{-6} mg/L and 1.60×10^{-6} mg/L for Phase I, Transition Phase, Phase II, respectively, in this study, and it was lower than 0.1 mg/L, thus FNA might not be the main inhibition factor for *Nitrobacter*. Moreover, it could be considered that the FA and DO were the factors affecting for inhibition of the NOB and accumulation of nitrite. As shown in Fig. S3, the relatively high FA concentration in the mixed liquor resulted due to high pH and residue ammonia (Winkler and Straka, 2019). The DO was within the range of 0 mg/L to 4.0 mg/L through the control of aeration duration, which was favourable for nitrite accumulation (Ciudad et al., 2005; Yan et al., 2019). The aeration

control was simply and effectively applied to achieve the simultaneous inhibition of FA and DO. Furthermore, the AOB shows a higher growth rate than NOB, and short SRT (15–18 days in this study) might be washed out the NOB from the system. Hence, this might be another factor for NOB suppression (Jubany et al., 2009).

As the concentration of suspension and solid organic matter in wastewater were sufficiently reduced due to the magnetic coagulation pre-treatment, the supernatant of pre-treated wastewater contained mostly soluble organic matter. However, although the removal of colloidal and suspended particles is beneficial to improve the utilization efficiency of microorganisms for organic matters (reduce toxicity), the low carbon to nitrogen (C/N) ratio of influent was not enough to ensure the carbon source needed for denitrification, e.g. the averaged C/N ratios of this study were 5.9 ± 1.12 , 4.2 ± 1.02 and 3.97 ± 0.2 for Phase I,

Transition Phase and Phase II, respectively. Hence it was found necessary to add an additional carbon source to maintain a high removal efficiency of total nitrogen. Therefore sodium acetate solution was added as an external carbon source during the anoxic condition to enhance denitritation (Ji et al., 2018; She et al., 2016; Wang et al., 2013).

3.2.3. Pollutants removal within the SMBR cycle

Fig. 4a shows the variation of $NH_4^+ - N, NO_2^- - N, NO_3^- - N$, pH, DO, and ORP in one typical cycle on the 76th, 161st and 231st day corresponding to Phase I, Transition Phase and Phase II in the SMBR. The SMBR consisted of four cycles staring from feeding, anoxic, oxic and discharging. The first 5 min was feeding time, and it was controlled with FTC in all phases. Then, to achieve biological denitrification, anoxic mixing in which the microorganism use the organic matter in the influent water as the electron donor to reduce the nitrite nitrogen and nitrate nitrogen to nitrogen gas.

In Phase I, the concentrations of $NO_2^- - N$ and $NO_3^- - N$ were

decreased sharply and then slightly changed, respectively, until the oxic condition started in the first 15 min of the cycle. Half reduction in the concentration of NH_4^+ –Nwas achieved at 180th min in the oxic stage. The concentrations of NO_2^- –Nand NO_3^- –N increased to 13.7 mg/L and 3.95 mg/L, respectively at the end of the cycle, and the accumulation rate of nitrite was 77.62%.

In the Transition Phase, the concentrations of NO₂⁻ –N and NO₃⁻ –N were decreased sharply and slightly changed for the first 45 min, respectively similar to the Phase I and gradually increased starting with the oxic condition. Half reduction in the concentration of NH₄⁺ –Nwas achieved at 165th min, where the concentrations of NO₂⁻ –Nand NO₃⁻ –N increased to 13.7 mg/L and 3.95 mg/L, respectively at the end of the cycle, and 80.02% of NAR was achieved.

Whereas, in the first 60 min of the cycle for Phase II, the concentrations of $NO_2^- -N$ and $NO_3^- -N$ were decreased sharply and slightly changed until the start of oxic condition. Half reduction of $NH_4^+ -N$ was achieved at 195th min in the oxic phase. The concentration of $NO_2^- -N$



Fig. 4a. Variation of NH_4^+ -N, NO_2^- -N and NO_3^- -N concentrations during the cycle test: (a) cycle test carried out directly in the reactor at (A) 76th day, (B)161st , (C) and 231st day for Phase I, Transition Phase and Phase II, respectively.

and NO_3^- –N increased to 20.30 mg/L and 3.65 mg/L, respectively at the end of the cycle, and the accumulation rate of nitrite was 84.72%.

Generally, in all phases, TN concentration was sharply increased during the feeding and slowly decreasing during the anoxic (denitritation) stage, and gradually decreased with the starting of the oxic stage and again increased at the end of the cycle. The COD concentration was rapidly increased with the addition of influent and continuously decreased from anoxic to end of the oxic stage. The high biodegradation rate was shown during the anoxic stage, and the low biodegradation rate was shown in the oxic stage.

In all three phases, the discharge time was 40 min, and it was controlled with the FTC. The details of other variables are as follows. The total cycle time was 240 min and 210 min for Phases I and II,

respectively. The DO gradually increased from 0.56 mg/L – 3.88 mg/L in Phase I, 0.31 mg/L – 0.82 mg/L in Transition Phase and 0.33 mg/L – 3.03 mg/L in Phase II, the pH gradually decreased from 8.19 – 7.89, 7.94 – 7.58 and 7.57–7.26 in Phase I, Transition Phase and Phase II, respectively. Whereas the "ammonia valley point" appeared between 200th – 210th minutes in all phases, and delayed aeration enhanced the removal of organic matters in wastewater. The ORP value in the reactor gradually increased from – 330 mV to 75 mV in Phase I, –314 mV – 67 mV in Transition Phase and –288 to 33 mV in Phase II in the aeration process due to the reduction of reducing substances and the increase of oxidizing substances.



Fig. 4b. The variation of pH, ORP and DO in typical cycles at (A) 76th day, (B) 161st , (C) and 231st day for Phase I, Transition Phase and Phase II, respectively; Bending-points detection, The ORP breakpoint was around at -300 mV to -350 mV (Phase I and Transition Phase), and -250 mV to -300 mV (Phase II) related to the end of denitrification (Anoxic condition), The nitritation was stopped at the observation of the ammonia valley (Oxic condition) to stimulate the SBNR process.

3.2.4. Real-Time control point calibration and determination

The variation of pH, ORP, and DO within a cycle are shown in Fig. 4b. The ORP breakpoint was around at -300 mV to -350 mV (for both Phase I and Transition Phase) and -250 mV to -300 mV (Phase II), which was related to the end of denitritation. The pH breakpoint indicated the ammonia valley and slope of pH linked well with nitritation process in the oxic phase (Fig. 4b). The oxic phase was stopped with the observation of the ammonia valley to stimulate the SBNR process. Besides, 0–4.0 mg/L of DO concentration was maintained within all phases, which was favorable for nitrite accumulation (Antileo et al., 2013; Sui et al., 2016a,b). The use of pH and ORP control strategy demonstrating the applicability of the biological oxidation–reduction process to treat municipal wastewater, swine wastewater, and landfill leachate, which has been successfully applied by other studies (Jubany et al., 2009; Liu et al., 2017; Sui et al., 2018).

3.3. Performance comparison of integrated process

As per the results, the removal rates of COD, TSS, TN, NH_4^+ –N and TP in the integrated treatment process were 96.05 \pm 0.2%, 99.99 \pm 0.01%, 97.30 \pm 0.3%, 99.07 \pm 0.2% and 96.25 \pm 0.5%, respectively, for Phase I. Their corresponding removal rates were 94.12 \pm 0.2%, 99.99 \pm 0.01%, 95.80 \pm 0.3%, 97.31 \pm 0.2%, 94.61 \pm 0.5% respectively for Transition Phase, but there were 97.39 \pm 0.2%, 99.99 \pm 0.01%, 97.44 \pm 0.3%, 98.54 \pm 0.2% and 95.59 \pm 0.5% respectively for Phase II. In this study, the FW digestate (22 \pm 6.0 mS/cm) has higher EC than swine wastewater (6.1 \pm 2.0 mS/cm), and both effluent water quality could successfully meet the relevant discharge standards (GB18596-2001for swine wastewater, GB/T 31962-2015 for FW digestate). The experimental results clearly showed that the proposed integrated process could be applicable for treatment of industrial effluent with a wide range of EC (4-22 mS/cm). As the MC process had effectively reduced the loading of COD load, TSS, TP and phosphate from the raw wastewater, the effectiveness of the SMBR was thus improved, particularly the biological denitritation step was enhanced. It should be related to the improved performance of the short-cut nitrification and denitrification process of SMBR via the nitrite pathway. Though, influent having relatively low C/ N (~4-6 in this study), suggesting the successfulness of RTC strategy to enhance nitrogen removal under fluctuation of high COD and ammonia concentration. Furthermore, the membrane separation process could have greatly improved effluent water quality.

Efficiency and cost-effectiveness are considered essential basics for the sustainable wastewater treatment process (Xia and Murphy, 2016). The MC process increases the sedimentation rate of suspended solids and reduces the sedimentation tank volume, and saving the footprint of the treatment process. In addition, magnetic seeds can be recovered and reused at approximately 99%; hence it causes the reduction of magnetic seeds dosage and the total chemical costs to make the cost-effective alternative process which suitable for limited space area or highly developed areas (Ritigala et al., 2021)

The SBNR saves aeration cost 25%, reduces external carbon source by 40%, and decreases the sludge production compared with the conventional full biological nitrogen removal process (Ji et al., 2018). Moreover, SMBR has the following advantages, e.g., a single reactor having a small footprint, and it's flexible for handling and saving the investment cost (Chen et al., 2021). Hence, the integrated treatment process shortened the HRT and improved the performance of organic and nitrogen removal from both swine wastewater and FW digestate with a relatively low footprint than currently using treatment processes, which include a series of a membrane separation process (UF + NF + RO), AD process, incineration and evaporation (Chiumenti et al., 2013; Demirer et al., 2019; Świątczak et al., 2019)

3.4. Evolution of microbial community

3.4.1. The biodiversity and community structures

The microbial community of the SMBR reactor was assessed using seven activated sludge samples labeled as SW-1, SW-2, SW-3, TS-1, TS-2, FW-1, FW-2 corresponding to sampling on days 31, 76, 118, 144, 161, 207 and 231, respectively. The identified microbial communities at the phylum level were summarized in Fig. 5a. The most representative bacterial phyla present in all samples were Proteobacteria, Bacteroidetes, Patescibacteria, Actinobacteria, Firmicutes, Chloroflexi and Planctomycetota. The Proteobacteria is the largest and most diverse bacterial phylum observed in the activated sludge system, other dominant phyla followed by different proportions of Bacteroidetes, Chloroflexi, Actinobacteria, Planctomycetes and Firmicutes (Sui et al., 2018; Zou et al., 2020). Therefore, the ammonia oxidation and nitrite/nitrate reduction in the SMBR could be related to the presence of Proteobacteria and Bacteroidetes. Moreover, Bacteroidetes and Chloroflexi can degrade a variety of organic compounds and improve nitrogen removal (Sui et al., 2018; Zou et al., 2020).

As EC showed a higher inhibition potential to the microbial community (Lotti et al., 2019b; Scaglione et al., 2017), hence the SMBR operation was carefully monitored. In Phase I, the average EC was $6.0 \pm$ 2.0 mS/cm. At this phase, the *Proteobacteria* (35.25%, 30.31%, 23.51%), *Bacteroidetes* (25.71%, 29.93%, 26.30%), and *Patescibacteria* (19.22%, 14.73%, 11.25%) were identified as the top three phyla and their activities were steady during the whole phase. However, *Actinobacteria* (6.17%, 6.18%, 11.42%), *Firmicutes* (5.62%, 6.73%, 9.80%) and *Chloroflexi* (1.87%, 2.41%, 5.29%) were increased gradually at the end of Phase I. Furthermore, homogeneous microbial community distribution was observed throughout the operation time in Phase I. The results clearly showed that microbial community structure was not affected by the influent EC.

In Transition Phase and Phase II, Proteobacteria (40.32%, 40.39%, 48.53%, 52.79%), Bacteroidetes (9.28%, 20.71%, 16.28%, 9.56%) and Chloroflexi (14.33%, 9.07%, 4.74%, 9.30%) were the dominant top three phyla observed. However, the extent of their dominance seemed to be correlated with wastewater type. For instance, Proteobacteria gradually increased in all four samples (TS-1, TS-2, FW-1 and FW-2); Bacteroidetes increased in sample TS-2; FW-1 and decreased in FW-2, Chloroflexi decreased in TS-2, FW-1 and increased in FW-2, Firmicutes suddenly increased in TS-2, FW-1 and very less amount count were observed in FW-2. The microbial community data in Transition Phase and Phase II revealed that Proteobacteria and Bacteroidetes were successfully adopted with an increase of influent EC (from 6.0 \pm 2.0 to 16 \pm 3.2 mS/cm). Chloroflexi was decreased with the influent EC at 9.0 \pm 3.6 mS/cm during the Transition Phase, and its abundance was increased later on. Firmicutes are more dominant in the effluent of anaerobic digesters. The gradual increase of FW digestate might have caused the increase of Firmicutes count in TS-2 and FW-1 (Wang et al., 2020), and its decrease of FW-2 revealed that the increasing influent EC (between 9 and 16 mS/ cm) might be a reason for the inhibition. Patescibacteria was dominated in Phase I and decreased in Transition Phase and Phase II. The reasons might be that the microbial community of the SMBR and its diversity was influenced by the higher influent EC and FW digestate and its controlling parameters. Fig. 5b shows the heatmap of microbial composition in the SMBR activated sludge at the genus level.

Furthermore, the microbial community diversity and richness were calculated using the Shannon-Wiener index, Simpson and Chao 1, and the diversity indices are shown in the supplementary material. The Shannon-Wiener index reflects species richness (diversity in ecology), and the Simpson index reflects the weight of the evenness in species. The Chao1 is an estimator based on abundance. According to the diversity indices, all samples showed similar diversity and richness at three phases.



Fig. 5a. The microbial communities at the phylum level at samples collected on day 31, 76, 118, 144, 161, 207 and 231 and labeled as (A) SW-1, SW-2, SW-3, (B) TS-1, TS-2, FW-1, FW-2, respectively.

3.4.2. Key functional groups related to nitrogen removal

In this study, the SMBR was successfully adapted to the SBNR process for the nitrogen removal, such that, the suppression of nitrite oxidation without delaying the ammonia oxidation is required to achieve successful nitritation on the stable SBNR process. Nitrosomonas and Nitrospira have been reported in the activated sludge as the most predominant AOB and NOB, respectively (Liu et al., 2017; Soliman and Eldyasti, 2016; Sui et al., 2018). A relatively high abundance of AOB and a low abundance of NOB were detected throughout this experiment. The Nitrosomonas was the only detected species among the AOB species, and Nitrospira showed low activity in all three phases. Nitrobacter (NOB Species) was not presented in all examined samples, which might be related to low DO concentration conditions, short SRT, and SMBR controlling parameters (refer to section 3.2.2) (Cao et al., 2017). Abundances of AOB and NOB in this study are illuminated in the supplementary material. However, the richness of Nitrosomonas was increased throughout the reactor operation (Phase I, Transition Phase and Phase II) except FW-2 sample, where the decline from 4.3% to 2.0% was observed. The results showed that the *Nitrosomonas* could tolerate for a varied range of EC.

The population of the denitrifiers was enriched in the activated sludge samples with a diversity of denitrifiers such as *Thauera*, *Diaphorobacter*, *Comamonadaceae* (*Ottowia*), *Flavobacterium*, *Paracoccus*, and *Rhodobacter*. The genus *Diaphorobacter* and *Thauera* were the most dominant heterotrophic denitrifiers observed in Phase I, Transition Phase and Phase II, respectively, and gradually increased in all phases. The dominance of *Thaurea* could have simplified the denitrifying process by utilizing sodium acetate as an external carbon source; *Thaurea* was also increased when the reactor operated under high ammonia concentration (Guo et al., 2009; Zou et al., 2020). *Flavobacterium* and *Paracoccus* were detected with low abundance in Phase I, and disappeared in Transition Phase and Phase II. The *Ottowia*, which is phylogenetically revealed to represents a distinct line of descent within the *Comamonadaceae* (Spring et al., 2004), was detected and identified as the 2nd



Fig. 5b. The heatmap of microbial communities at the genus level at samples collected on day 31,76,118,144,161,207 and 231 and labeled as (A)SW-1, SW-2, SW-3, TS-1, and (B) TS-2, FW-1, FW-2, respectively.

dominant genus in the Transition Phase as well Phase II. *Comamona-daceae* is facultative and has better denitrification efficiency than other denitrifiers. It signifies high denitrification performance during Phase II. As per the results that *Thaurea, Diaphorobacter and Ottowia* could tolerate

the higher EC while showing the inhibition of *Flavobacterium* and *Paracoccus*.

As shown in the supplementary material, the redundancy analysis (RDA) was performed to investigate the distributions of microbial communities with reactor environmental factors at the genus level. The sample of SW-2 and SW-3 results positively correlated with controlling parameters, e.g., MLSS, C/N, FA, FNA, nitrite, nitrate and EC, while SW-1 deviated. The sample of TS-1 and TS-2 showed a positive correlation with C/N, FA and nitrate, while FW-1 positively correlated to MLSS, NAR and nitrate. The overall RDA results showed a high correlation with dominant nitritaion and denitritaion bacterial population and environmental factors in all phases. In addition, to investigate the ecological correlation between the microbial community composition (in the phylum level) with environmental controlling factors and correlation on pollutants removal, the principal component analysis (PCA) was conducted and shown in Fig. 6. The maximum variation of PCA1 (92.21%, 75.38%) and PCA2 (7.79%, 18.09) for Phase I (Sample of SW-1, SW-2, SW-3) and Phase II (Sample of TS-1, TS-2, FW-1, FW-2), respectively. The Proteobacteria, Patescibacteria, Planctomycetes, Deinococcus, Verrucomicrobia were correlated to COD, TN and NH₄⁺-N removal owing to nitritation, while Bacteroidetes, Actinobacteria, Acidobacteria and Chloroflexi were assigned to TN and NH₄⁺-N removal owing to denitritation. The PCA analysis indicated that COD, TN and $NH_4^+ - N$ removal had a strong negative correlation in all three phases. Moreover, Proteobacteria and Bacteroidetes showed a strong positive correlation (p < 0.05) with EC throughout the experiment period, and it was further verified with the abundance of microbial community distribution (Fig. 5a, Fig. 5b and Fig. 6).

The microbial community shift of this study was consistent with a previously reported study, which was similar to swine wastewater treatment (Kim et al., 2004; Sui et al., 2018; Wu et al., 2015) and similar to digestate treatment (Wang et al., 2020; Zou et al., 2020). It suggests the applicability of the current approach as an alternative for swine wastewater and FW digestate treatment. Overall, the microbial community distribution showed (Fig. 5a, Fig. 5b, and Fig. 6) an adaptation of retained and survived microbes to the controlling parameters of SMBR. Furthermore, SMBR operation and controlling parameters have promoted the denitrifying population under limited oxygen conditions, under high ammonia load, and high influent EC to achieve successful carbon and nitrogen removal via the SBNR process.

4. Conclusions

In this study, an integrated short-cut process of MC and SBNR was introduced and compared for efficient swine wastewater and FW digestate treatment. Accordingly, the COD, TN, NH⁺₄ – N and TP removals for swine wastewater were 96.05 ± 0.2%, 97.30 ± 0.3%, 99.07 ± 0.2% and 96.25 ± 0.5% respectively, the respective FW digestate removals were 97.39 ± 0.2%, 97.44 ± 0.3%, 98.54 ± 0.2%, 95.59 ± 0.5% respectively. The NAR was well built up with the SBNR process and achieved 74.54% and 76.02% for swine wastewater and FW digestate, respectively. Total microbial community investigation showed that genus *Diaphorobacter* and *Thaurea were dominant* in denitritation, and *Nitrosomonas* was dominant in nitritation.

CRediT authorship contribution statement

Tharindu Ritigala: Investigation, Conceptualization, Methodology, Data curation, Formal analysis, Validation, Visualization, Writing original draft. Yanlin Chen: Formal analysis. Jiaxi Zheng: Resources. Hailu Demissie: Formal analysis, Writing - review & editing. Libing Zheng: Formal analysis. Dawei Yu: Writing - review & editing. Qianwen Sui: Writing - review & editing. Meixue Chen: Writing - review & editing. Jinxing Zhu: Resources. Hua Fan: Resources. Jiao Li: Resources. Qian Gao: Resources. Sujithra.K. Weragoda: Writing - review & editing. Rohan Weerasooriya: Writing - review & editing. K.B.S.N. Jinadasa: Writing - review & editing. Yuansong Wei: Conceptualization, Funding acquisition, Resources, Supervision, Writing - review & editing.



Fig. 6. Principal component analysis (PCA) correlation between dominant phyla bacterial and environment factors. (A) for sample SW-1, SW-2, SW-3 and (B) for TS-1, TS-2, FW-1 and FW-2.

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.

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Appendix A. Supplementary data

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References

- Antileo, C., Medina, H., Bornhardt, C., Muñoz, C., Jaramillo, F., Proal, J., 2013. Actuators monitoring system for real-time control of nitrification-denitrification via nitrite on long term operation. Chem. Eng. J. 223, 467–478. https://doi.org/10.1016/j. cej.2013.02.079.
- Cao, Y., Zhang, C., Rong, H., Zheng, G., Zhao, L., 2017. The effect of dissolved oxygen concentration (DO) on oxygen diffusion and bacterial community structure in moving bed sequencing batch reactor (MBSBR). Water Res. 108, 86–94. https://doi. org/10.1016/j.watres.2016.10.063.
- Chen, Y., Sui, Q., Yu, D., Zheng, L., Chen, M., Ritigala, T., Wei, Y., 2021. Development of a Short-Cut Combined Magnetic Coagulation-Sequence Batch Membrane Bioreactor for Swine Wastewater Treatment. Membranes (Basel). 11, 83. https://doi.org/ 10.3390/membranes11020083.
- Chini, A., Chiapetti, A., Ester, C., Goldschmidt, F., Fongaro, G., Treichel, H., Kunz, A., 2019. Evaluation of deammoni fi cation reactor performance and microrganisms community during treatment of digestate from swine sludge CSTR biodigester. J. Environ. Manage. 246, 19–26. https://doi.org/10.1016/j.jenvman.2019.05.113.
- Chiumenti, A., da Borso, F., Chiumenti, R., Teri, F., Segantin, P., 2013. Treatment of digestate from a co-digestion biogas plant by means of vacuum evaporation: Tests for process optimization and environmental sustainability. Waste Manag. 33 (6), 1339–1344. https://doi.org/10.1016/j.wasman.2013.02.023.
- Chon, K., Kyongshon, H., Cho, J., 2012. Bioresource Technology Membrane bioreactor and nanofiltration hybrid system for reclamation of municipal wastewater : Removal of nutrients, organic matter and micropollutants. Bioresour. Technol. 122, 181–188. https://doi.org/10.1016/j.biortech.2012.04.048.
- Ciudad, G., Rubilar, O., Muñoz, P., Ruiz, G., Chamy, R., Vergara, C., Jeison, D., 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. Process Biochem. 40 (5), 1715–1719. https://doi.org/10.1016/j.procbio.2004.06.058.
- Demirer, N., Ülgüdür, N., Ergüder, T.H., Uluda, S., 2019. High-rate anaerobic treatment of digestate using fi xed fi lm reactors *. Environ. Pollut. 252, 1622–1632. https:// doi.org/10.1016/j.envpol.2019.06.115.
- Demissie, H., An, G., Jiao, R., Ma, G., Liu, L., Sun, H., Wang, D., 2020. Removal of phenolic contaminants from water by in situ coated surfactant on Keggin-aluminum nanocluster and biodegradation. Chemosphere 269, 128692. https://doi.org/ 10.1016/j.chemosphere.2020.128692.
- Demissie, H., An, G., Jiao, R., Ritigala, T., Lu, S., Wang, D., 2021. Modification of high content nanocluster-based coagulation for rapid removal of dye from water and the mechanism. Sep. Purif. Technol. 259, 117845. https://doi.org/10.1016/j. seppur.2020.117845.
- Gao, D.-W., Peng, Y.-Z., Liang, H., Wang, P., 2003. Using Oxidation-Reduction Potential (ORP) and pH Value for Process Control of Shortcut Nitrification-Denitrification. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 38 (12), 2933–2942. https://doi.org/10.1081/ESE-120025842.
- Gonzalez-Tineo, P.A., Durán-Hinojosa, U., Delgadillo-Mirquez, L.R., Meza-Escalante, E. R., Gortáres-Moroyoqui, P., Ulloa-Mercado, R.G., Serrano-Palacios, D., 2020. Performance improvement of an integrated anaerobic-aerobic hybrid reactor for the treatment of swine wastewater. J. Water Process Eng. 34, 101164. https://doi.org/ 10.1016/j.jwpe.2020.101164.
- Guo, J., Peng, Y., Wang, S., Zheng, Y., Huang, H., Wang, Z., 2009. Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. Bioresour. Technol. 100 (11), 2796–2802. https://doi.org/10.1016/j. biortech.2008.12.036.
- Jaramillo, F., Orchard, M., Muñoz, C., Zamorano, M., Antileo, C., 2018. Advanced strategies to improve nitrification process in sequencing batch reactors - A review. J. Environ. Manage. 218, 154–164. https://doi.org/10.1016/j. jenvman.2018.04.019.
- Ji, J., Peng, Y., Mai, W., He, J., Wang, B., Li, X., Zhang, Q., 2018. Achieving advanced nitrogen removal from low C/N wastewater by combining endogenous partial denitrification with anammox in mainstream treatment. Bioresour. Technol. 270, 570–579. https://doi.org/10.1016/j.biortech.2018.08.124.
- Jubany, I., Lafuente, J., Baeza, J.A., Carrera, J., 2009. Total and stable washout of nitrite oxidizing bacteria from a nitrifying continuous activated sludge system using automatic control based on Oxygen Uptake Rate measurements. Water Res. 43 (11), 2761–2772. https://doi.org/10.1016/j.watres.2009.03.022.
- Kim, J.-H., Chen, M., Kishida, N., Sudo, R., 2004. Integrated real-time control strategy for nitrogen removal in swine wastewater treatment using sequencing batch reactors. Water Res. 38 (14-15), 3340–3348. https://doi.org/10.1016/j.watres.2004.05.006.
- Kornboonraksa, T., Lee, S.H., 2009. Factors affecting the performance of membrane bioreactor for piggery wastewater treatment. Bioresour. Technol. 100 (12), 2926–2932. https://doi.org/10.1016/j.biortech.2009.01.048.
- Liu, J., Zhang, H., Zhang, P., Wu, Y., Gou, X., Song, Y., Tian, Z., Zeng, G., 2017. Twostage anoxic/oxic combined membrane bioreactor system for landfill leachate treatment: Pollutant removal performances and microbial community. Bioresour. Technol. 243, 738–746. https://doi.org/10.1016/j.biortech.2017.07.002.
- Lotti, T., Burzi, O., Scaglione, D., Ramos, C.A., Ficara, E., Pérez, J., Carrera, J., 2019a. Two-stage granular sludge partial nitritation / anammox process for the treatment of

digestate from the anaerobic digestion of the organic fraction of municipal solid waste. Waste Manag. 100, 36-44. https://doi.org/10.1016/j.wasman.2019.08.044

- Lotti, T., Burzi, O., Scaglione, D., Ramos, C.A., Ficara, E., Pérez, J., Carrera, J., 2019b. Two-stage granular sludge partial nitritation/anammox process for the treatment of digestate from the anaerobic digestion of the organic fraction of municipal solid waste. Waste Manag. 100, 36–44. https://doi.org/10.1016/j.wasman.2019.08.044.
- Meng, J., Li, J., Li, J., Antwi, P., Deng, K., Wang, C., Buelna, G., 2015. Nitrogen removal from low COD/TN ratio manure-free piggery wastewater within an upflow microaerobic sludge reactor. Bioresour. Technol. 198, 884–890. https://doi.org/ 10.1016/j.biortech.2015.09.023.
- Ministry of Ecology and Environment of People's Republic of China, 2020. Second National Pollution Source Survey of China (2017-2020.06).
- National Bureau of Statistics People's Republic of China, 2019. National Statistical Report on Ecology and Environment.
- Peng, Y., Zhu, G., 2006. Biological nitrogen removal with nitrification and denitrification via nitrite pathway. Appl. Microbiol. Biotechnol. 73 (1), 15–26. https://doi.org/ 10.1007/s00253-006-0534-z.
- Peng, Y.Z., Ma, Y., Wang, S.Y., 2006. Improving nitrogen removal using on-line sensors in the A/O process. Biochem. Eng. J. 31 (1), 48–55. https://doi.org/10.1016/j. bei.2006.05.023.
- Ren, X., Xu, X., Xiao, Y., Chen, W., Song, K., 2019. Effective removal by coagulation of contaminants in concentrated leachate from municipal solid waste incineration power plants. Sci. Total Environ. 685, 392–400. https://doi.org/10.1016/j. scitotenv.2019.05.392.
- Ren, Y., Yu, M., Wu, C., Wang, Q., Gao, M., Huang, Q., 2018. A comprehensive review on food waste anaerobic digestion : Research updates and tendencies Bioresource Technology A comprehensive review on food waste anaerobic digestion : Research updates and tendencies. Bioresour. Technol. 0–1 https://doi.org/10.1016/j. biortech.2017.09.109.
- Ritigala, T., Demissie, H., Chen, Y., Zheng, J., Zheng, L., Zhu, J., Fan, H., Li, J., Wang, D., Weragoda, S.K., Weerasooriya, R., Wei, Y., 2021. Optimized pre-treatment of high strength food waste digestate by high content aluminum-nanocluster based magnetic, J. Environ. Sci. 104, 430–443. https://doi.org/10.1016/j.jes.2020.12.027.
- Scaglione, D., Lotti, T., Ficara, E., Malpei, F., 2017. Inhibition on anammox bacteria upon exposure to digestates from biogas plants treating the organic fraction of municipal solid waste and the role of conductivity. Waste Manag. 61, 213–219. https://doi. org/10.1016/j.wasman.2016.11.014.
- She, Z., Zhao, L., Zhang, X., Jin, C., Guo, L., Yang, S., Zhao, Y., Gao, M., 2016. Partial nitrification and denitrification in a sequencing batch reactor treating high-salinity wastewater. Chem. Eng. J. 288, 207–215. https://doi.org/10.1016/j. cei.2015.11.102.
- Soliman, M., Eldyasti, A., 2016. Development of partial nitrification as a first step of nitrite shunt process in a Sequential Batch Reactor (SBR) using Ammonium Oxidizing Bacteria (AOB) controlled by mixing regime. Bioresour. Technol. 221, 85–95. https://doi.org/10.1016/j.biortech.2016.09.023.
- Spring, S., Jäckel, U., Wagner, M., Kämpfer, P., 2004. Ottowia thiooxydans gen. nov., sp. nov., a novel facultatively anaerobic, N2O-producing bacterium isolated from activated sludge, and transfer of Aquaspirillum gracile to Hylemonella gracilis gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 54, 99–106. https://doi.org/10.1099/ ijs.0.02727-0.
- Sui, Q., Jiang, C., Yu, D., Chen, M., Zhang, J., Wang, Y., Wei, Y., 2018. Performance of a sequencing-batch membrane bioreactor (SMBR) with an automatic control strategy treating high-strength swine wastewater. J. Hazard. Mater. 342, 210–219. https:// doi.org/10.1016/j.jhazmat.2017.05.010.
- Sui, Q., Liu, C., Zhang, J., Dong, H., Zhu, Z., Wang, Y.i., 2016a. Response of nitrite accumulation and microbial community to free ammonia and dissolved oxygen treatment of high ammonium wastewater. Appl. Microbiol. Biotechnol. 100 (9), 4177–4187. https://doi.org/10.1007/s00253-015-7183-z.
- Sui, Q., Zhang, J., Chen, M., Tong, J., Wang, R., Wei, Y., 2016b. Distribution of antibiotic resistance genes (ARGs) in anaerobic digestion and land application of swine wastewater. Environ. Pollut. 213, 751–759. https://doi.org/10.1016/j. envpol.2016.03.038.
- Świątczak, P., Cydzik-Kwiatkowska, A., 2018. Treatment of Ammonium-Rich Digestate from Methane Fermentation Using Aerobic Granular Sludge. Air. Soil Pollut. 229 (8) https://doi.org/10.1007/s11270-018-3887-x.
- Świątczak, P., Cydzik-Kwiatkowska, A., Zielińska, M., 2019. Treatment of the liquid phase of digestate from a biogas plant for water reuse. Bioresour. Technol. 276, 226–235. https://doi.org/10.1016/j.biortech.2018.12.077.
- Wang, K., Wang, S., Zhu, R., Miao, L., Peng, Y., 2013. Advanced nitrogen removal from landfill leachate without addition of external carbon using a novel system coupling ASBR and modified SBR. Bioresour. Technol. 134, 212–218. https://doi.org/ 10.1016/j.biortech.2013.02.017.
- Wang, P., Qiao, Z., Li, X., Wu, D., Xie, B., 2020. Fate of integrons, antibiotic resistance genes and associated microbial community in food waste and its large-scale biotreatment systems. Environ. Int. 144, 106013. https://doi.org/10.1016/j. envint.2020.106013.
- Winkler, M.K., Straka, L., 2019. New directions in biological nitrogen removal and recovery from wastewater. Curr. Opin. Biotechnol. 57, 50–55. https://doi.org/ 10.1016/j.copbio.2018.12.007.
- Wu, X., Zhu, J., Cheng, J., Zhu, N., 2015. Optimization of Three Operating Parameters for a Two-Step Fed Sequencing Batch Reactor (SBR) System to Remove Nutrients from Swine Wastewater. Appl. Biochem. Biotechnol. 175 (6), 2857–2871. https://doi.org/ 10.1007/s12010-014-1467-0.
- Xia, A., Murphy, J.D., 2016. Microalgal Cultivation in Treating Liquid Digestate from Biogas Systems. Trends Biotechnol. 34, 264–275. https://doi.org/10.1016/j. tibtech.2015.12.010.

T. Ritigala et al.

- Xu, Z., Song, X., Li, Y., Li, G., Luo, W., 2019. Removal of antibiotics by sequencing-batch membrane bioreactor for swine wastewater treatment. Sci. Total Environ. 684, 23–30. https://doi.org/10.1016/j.scitotenv.2019.05.241.
- Yan, L., Liu, S., Liu, Q., Zhang, M., Liu, Y., Wen, Y., Chen, Z., Zhang, Y., Yang, Q., 2019. Improved performance of simultaneous nitrification and denitrification via nitrite in an oxygen-limited SBR by alternating the DO. Bioresour. Technol. 275, 153–162. https://doi.org/10.1016/j.biortech.2018.12.054.
- Yang, Q., Peng, Y., Liu, X., Zeng, W., Mino, T., Satoh, H., 2007. Nitrogen removal via nitrite from municipal wastewater at low temperatures using real-time control to optimize nitrifying communities. Environ. Sci. Technol. 41 (23), 8159–8164. https://doi.org/10.1021/es070850f.
- Yang, S., Xu, S., Mohammed, A., Guo, B., Vincent, S., Ashbolt, N.J., Liu, Y., 2019. International Biodeterioration & Biodegradation Anammox reactor optimization for

the treatment of ammonium rich digestate lagoon supernatant - Step feeding mitigates nitrite inhibition. Int. Biodeterior. Biodegrad. 143, 104733. https://doi.org/10.1016/j.ibiod.2019.104733.

- Zou, X., Zhou, Y., Guo, B., Shao, Y., Yang, S., Mohammed, A., Liu, Y., 2020. Single reactor nitritation-denitritation for high strength digested biosolid thickening lagoon supernatant treatment. Biochem. Eng. J. 160, 107630. https://doi.org/10.1016/j. bej.2020.107630.
- Zuriaga-Agustí, E., Mendoza-Roca, J.A., Bes-Piá, A., Alonso-Molina, J.L., Fernández-Giménez, E., Álvarez-Requena, C., Muñagorri-Mañueco, F., Ortiz-Villalobos, G., 2016. Comparison between mixed liquors of two side-stream membrane bioreactors treating wastewaters from waste management plants with high and low solids anaerobic digestion. Water Res. 100, 517–525. https://doi.org/10.1016/j. watres.2016.05.053.