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Assessment of organics and nitrogen removal of aerobic granular sludge with the alternating operation of oxic – anoxic – oxic phases and different feeding mode in sequential batch reactor

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Abstract

Organic matters and nitrogen removal efficiencies from industrial wastewater were evaluated by the aerobic granular sludge (AGS) process with the alternative oxic/anoxic conditions and different feeding regime in Sequencing Batch Reactor (SBR). The experiment was carried out in two lab-scale SBR (R1 and R2) with the same alternation of oxic-anoxic-oxic (O-A-O). Specifically, R1 was applied for one-step feeding at the beginning of each cycle and R2 for two-step feeding with one more filling in anoxic phase. Our study results showed that AGS remained stable and maintained excellent settling properties with low SVI of 42 – 45 mL gTSS⁻¹, and high sludge biomass of 7.46 – 7.53 g L⁻¹ was retained in both reactors. Specific Oxygen Uptake Rate (SOUR) was 80.70 and 87.90 mgO₂ gTSS⁻¹ h⁻¹ in R1 and R2 respectively that indicated the high biological activity of granular sludge. The high biomass retained and bioactivity of the organism in sludge leads to stable COD removal efficiency in both reactors about 88 – 91% during the experiment. In additional, total nitrogen (TN) removal efficiency of R2 (80.20 – 85.60%) was 13 – 15% higher than that of R1 (68.42 – 71.20%). Our study concluded that nitrogen removal via nitrification and denitrification was achieved in SBR operation with alternating of O-A-O, and two-step feeding was appropriated proposal for organic matters and nitrogen removal in single reactor. And step-feeding was an effective option in providing internal carbon source from wastewater as electron donor for denitrification in the anoxic phase instead of using external carbon source in SBR operation.

Keywords: aerobic granular sludge; sequencing batch reactor; nitrification and denitrification; step-feeding; oxic-anoxic-oxic phase

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1. Introduction

Aerobic granular sludge (AGS) has been studied for more than 15 years and showed several advantages compared to activated sludge in terms of a good settling property. Due to a large amount of active biomass accumulation in reactor, it could be able to withstand the fluctuation of pollution loading and the simultaneous removal of organic matter, nitrogen, and phosphorus [1, 2]. The Sequencing Batch Reactor (SBR) is considered a low-cost operation and higher flexible than continuous systems. It can be easily changed operation modes for combination of anaerobic, anoxic, or aerobic conditions in one cycle to achieve the removal of organic matter, nitrogen and phosphorus [3]. Especially, SBR is considered as suitable reactor for the cultivation, development, and maintenance of AGS because it could create favourable conditions for this process [1, 2]. Therefore, the combination of AGS and SBR with the flexible operation was a potential solution to remove organic matter and nitrogen

simultaneously in a single reactor. The biological removal of nitrogen involves two processes, nitrification occurred in an aerobic condition by autotrophic bacteria and denitrification performed by heterotrophic bacteria in an anoxic condition. By applying alternating aeration and non-aeration conditions, the oxic (O) and anoxic (A) phase could exist in a single reactor, and therefore, nitrogen could be removed by the nitrification and denitrification process in SBR [4 – 6]. In addition, it is well known the denitrification process requires the presence of carbon source as electron donor. It could be external carbon source by adding ethanol, acetate from the outside of the treatment flow or using internal carbon source was obtained from influent wastewater [6, 7]. In term of economical aspect, it is better to use internal carbon source from raw wastewater and step-feeding is an appropriate option in SBR operation.

Previous studies showed that there were certain combined oxic and anoxic phases in SBR operation to remove organic matter and nitrogen in a single reactor [5, 8 – 10]. It was reported that the application A-O combination with anoxic phase firstly appeared and followed by oxic phase [8, 9]. The advantage of A-O combination was that organic matters in the effluent filling could be used as carbon source for denitrifying nitrate of the previous phases or cycle without adding external carbon. However, A-O sequence leads to increasing nitrate at the end of the cycle due to the oxidation of ammonium in raw wastewater in the following aerobic conditions. Some studies indicated that the application of O-A with the oxic phase occurred at first and anoxic in following [5, 10]. The advantage of this combination was that organic matters could be removed and ammonium was oxidized to nitrate in the oxic phase, then nitrate was used in denitrification process in the following anoxic condition. In case of O-A, organic matter is consumed under aerobic condition resulted in reducing carbon source for denitrification while reactor turning into anoxic phase. In order to reduce this phenomenon, a step-feeding with the filling in the anoxic phases was recommended to provide internal carbon source to achieve the denitrification process without adding external carbon. In one hand, the step-feeding might save the external carbon source by using the internal carbon source within the influent wastewater for denitrification, but in another hand, it caused to increase ammonium concentration in the effluent.

Therefore, the proposal of oxic-anoxic-oxic (O-A-O) and step-feeding in anoxic phase would be a potential option in order to harmonize the advantages and disadvantages of both combinations to remove organic matters and nitrogen in a single reactor without adding external carbon source. The first oxic phase respond to organic matters removal and nitrification, then following the anoxic phase is for denitrification. Meanwhile, the last oxic phase after the O-A pair was used to remove residual organic matters and ammonium by step-feeding in anoxic phase. The purpose of this study is to evaluate organic matters and nitrogen removal efficiency of AGS by alternating of O-A-O operation with one-step and two-step feeding in SBR for treating industrial wastewater from Phu Bai Industrial Zone (Phu Bai IZ) in Thua Thien Hue Province, Viet Nam.

2. Materials and Methods

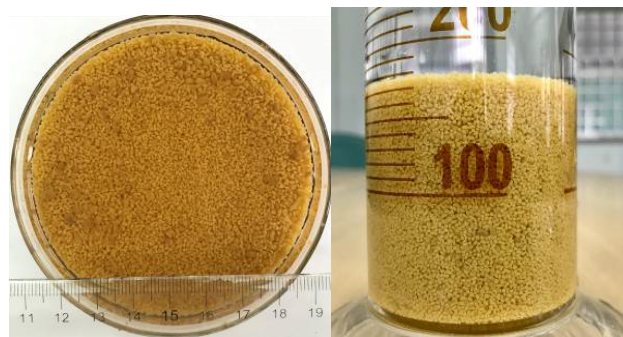
Materials

Aerobic granular sludge (AGS)

AGS was previously cultivated from activated sludge and stored at 2 – 3 °C in the refrigerator. Prior to the experimental design, the preserved AGS was reactivated and operated in SBR within one week using synthetic wastewater by Thanh *et al.* [11]. At beginning of the experiment, 1L of mixed liquor of AGS was put into each reactor. Characteristics of AGS used for experiment were shown in Table 1.

Table 1 Characteristics of Aerobic Granular Sludge used for experiment

No.	Characteristics	Unit	Value
1	Size of granules	mm	1 mm
2	Biomass (in Total Suspended Solids, TSS)	g L ⁻¹	6.13
3	Biomass (in Volatile Suspended Solid, VSS)	g L ⁻¹	5.61
4	Sludge Volumetric Index (SVI)	mL gTSS ⁻¹	40.00
5	Specific Oxygen Uptake Rate (SOUR)	mg O ₂ gTSS ⁻¹ h ⁻¹	86.20

**Fig. 1** Aerobic granular sludge used for experiment

Wastewater composition

Wastewater used for experiment was collected three times per week from the sewage system of Phu Bai Industrial Zone (Phu Bai IZ), Thua Thien Hue Province, Viet Nam. Samples were settled to reduce the suspended solids and preserved at 2 – 3°C to eliminate biodegradation. Carbon to nitrogen ratios (C:N) of the influent was about 15 – 22. In according to Metcalf and Eddy (2004), this C:N ratio was suitable for biological treatment process. Characteristics of Phu Bai IZ wastewater were shown in Table 2.

Table 2 Characteristics of Phu Bai IZ wastewater

No.	Parameters	Unit	Raw wastewater	Settled wastewater
1	pH	-	7.86 ± 0.63	7.82 ± 0.51
2	TSS	mg L ⁻¹	94.60 ± 10.55	42.60 ± 10.04
3	Biochemical Oxygen Demand, BOD ₅	mg L ⁻¹	356.80 ± 12.24	328.10 ± 6.36
4	Chemical Oxygen Demand, COD	mg L ⁻¹	463.40 ± 16.15	437.00 ± 14.54
5	Ammonium, NH ₄ ⁺ -N	mg L ⁻¹	29.60 ± 1.37	26.08 ± 2.54
6	Nitrate, NO ₃ ⁻ -N	mg L ⁻¹	0.87 ± 0.11	0.49 ± 0.12
7	Total Nitrogen, TN	mg L ⁻¹	43.60 ± 2.88	38.20 ± 1.48

Lab-scale Sequencing Batch Reactor (SBR)

Experiments were carried out in two lab-scale SBR (R1 and R2) with the same dimensions. The inner diameter was 8 cm and the total height was 100 cm. The working volume of the reactor was 4 L. Wastewater was filled and discharged by peristaltic pumps. They were automatically controlled by the timer to provide cyclic operation. After each cycle, wastewater was disported at level of 40 cm from the bottom of SBR, resulting in volumetric exchange rate was about 50%. Air was supplied into the reactor from bottom of SBR by air pumps with stone diffusers and airflow rate measured by flowmeter. The air pumps were set on/off by the timers in providing the alternating conditions of aerobic and anoxic. Reactors were operated at room temperature and pH between 7.40 – 8.10. Fig. 2 illustrates the layout of the SBR in the experimental design.

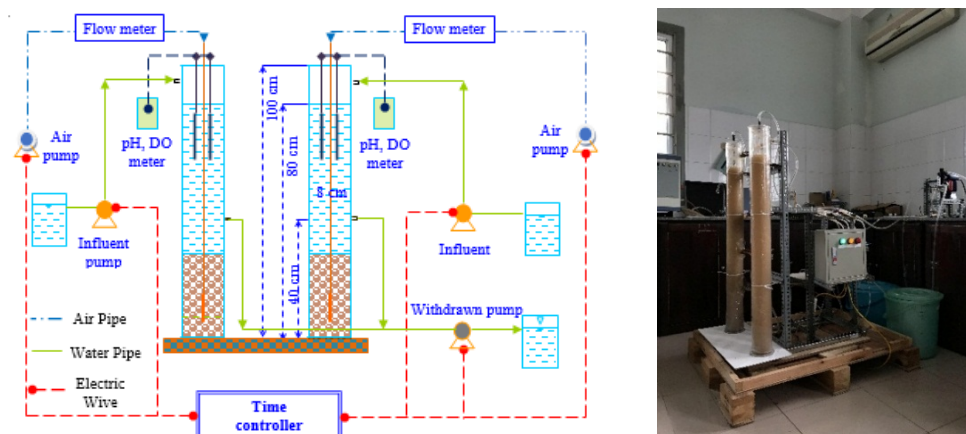


Fig. 2 Layout of lab-scale SBR system in the experimental design

Experimental design

Two reactors (symbolized as R1, R2) were operated sequentially and automatically in 240 minutes per cycle, consisting of influent feeding, reaction process, settling, and effluent withdrawal. Both reactors were designed to operate in the same alternating aeration and non-aeration conditions, 90 min of aeration with airflow 6 L min^{-1} ; 117 min of non-aeration and mixed with magnetic stirrer at the rate of 300 rpm and 30 min of aeration with airflow 2 L min^{-1} . This combination provided the alternating operation of oxic-anoxic-oxic phases (O-A-O) in both reactors. Oxic and anoxic alternation was considered to affect the distribution of microorganism responsible for nitrification and denitrification in a single reactor [5]. However, R1 applied one-step feeding and wastewater was fully filled at the beginning of each cycle, while R2 had 2-step feeding including the first filling (75% of influent volume) at beginning of the cycle, and the second (25% remaining) in the anoxic phase. The different feeding was adopted to evaluated the denitrification by using the internal organic matter from the wastewater instead of adding external carbon source like ethanol, acetate. Fig. 2 illustrated the detailed cycle operation in each reactor.

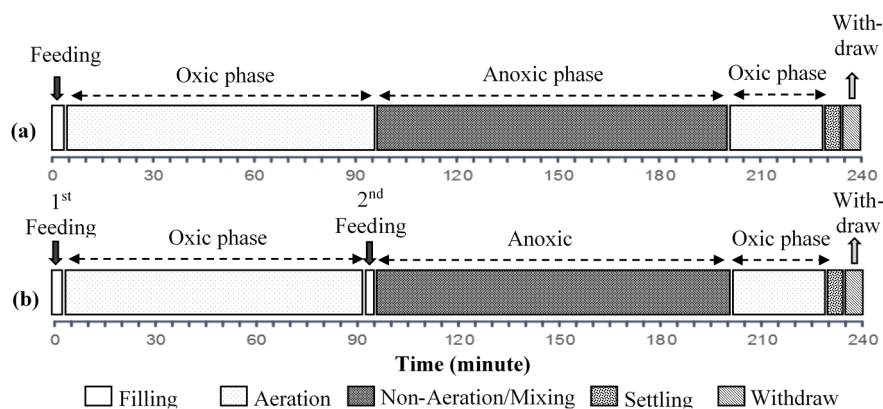


Fig. 3 Operational conditions of the typical cycle in R1 (a) and R2 (b)

Analysis methods

Sludge biomass, settling ability and activity

Sludge biomass concentration was measured by TSS and VSS. Sludge Volumetric Index (SVI, mL gTSS^{-1}) was used to assess the settling properties and calculated by the volume of the granules after 10 minutes settling with the biomass. Specific Oxygen Uptake Rate (SOUR, $\text{mgO}_2 \text{ gTSS}^{-1} \text{ min}^{-1}$) represents the bioactivity of heterotrophic in sludge. TSS, VSS, SVI and SOUR were determined according to Standard Methods for the Examination of Water and Wastewater [12].

Physical and chemical analysis

The pH and DO in a reactor were measured with an electrode (Orion Instruments) and portable DO meter (WTW-330i). BOD₅, COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N were analysed according to the APHA [12]. Samples were filtered by 0.45µm glass microfiber filter (Whatman, England) to remove suspended solids before analysis. For the determination of total nitrogen (TN), sample was digested by commercial reagents with digestion systems (TOA-DKK, Japan) and measured on TNP-10DKK equipment (Japan).

Calculation of SBR operational parameters

OLR, NLR and SRT were calculated in Equations 1 and 2 [6] [13].

$$\text{SRT} = \frac{V_{\text{SBR}} \times X_{\text{SBR}}}{Q_R \times X_R} = \frac{V_{\text{SBR}} \times X_{\text{SBR}}}{V_v \times n \times X_R} \quad \text{day} \quad (1)$$

$$\text{OLR and NLR} = \frac{Q_v \times C_v}{V_{\text{SBR}}} = \frac{V_v \times n \times C_v}{V_{\text{SBR}}} \times 10^{-6} \quad \begin{matrix} \text{kg COD m}^{-3} \text{ d}^{-1} \\ \text{kg NH}_4\text{-N m}^{-3} \text{ d}^{-1} \end{matrix} \quad (2)$$

n : cycle per day ($n=6$) V_{SBR} : Working volume of SBR (4 L) V_v : Filling volume (2 L)

C_v, C_r : Concentration of COD and NH₄⁺-N in influent and effluent (mg L⁻¹), respectively

X_{SBR}, X_R : Concentration of biomass in the reactor and in the effluent (mg L⁻¹), respectively

3. Results and discussion

The development and stability of aerobic granular sludge

Biomass concentration (measured as TSS) increased steadily over the first 3 weeks of the operation and reached high concentration at 7.20 and 7.40 g L⁻¹ in R1 and R2, respectively at day 20. From day 25 to day 28, sludge breakage resulted in decreasing biomass to 5.50 – 5.80 g L⁻¹ due to the washout of sludge in effluent withdrawal phase in both reactors. However, system was recovered quickly after 1 week of operation. At the end of the experiment period, high concentration of biomass remained stable at 7.46 and 7.53 g L⁻¹ in R1 and R2, respectively. Granular sludge breakage during the operation may a part of the development mechanism of AGS which was reported in many previous literature, consisting of the granular sludge formation, size development, granular break up and new sludge formation. [1, 2].

The AGS in both reactors had good settling properties with low SVI of 42 – 45 mL gTSS⁻¹. High rate of settling allowed greater biomass accumulation and therefore high SRT in both reactors was obtained in the range of 13 – 22 days (Fig. 4), this SRT value was suitable for nitrification [14]. In additional, SOUR value of sludge was 80.70 and 87.90 mgO₂ gTSS⁻¹ h⁻¹ in R1 and R2, respectively, it was indicated the high biological activity of sludge. The SOUR value was different from the study of Thanh, 118 mgO₂ gTSS⁻¹ h⁻¹ [11] and Song, 67.80 mgO₂ gTSS⁻¹ h⁻¹ [14] due to different operation and type of wastewater used in experiment.

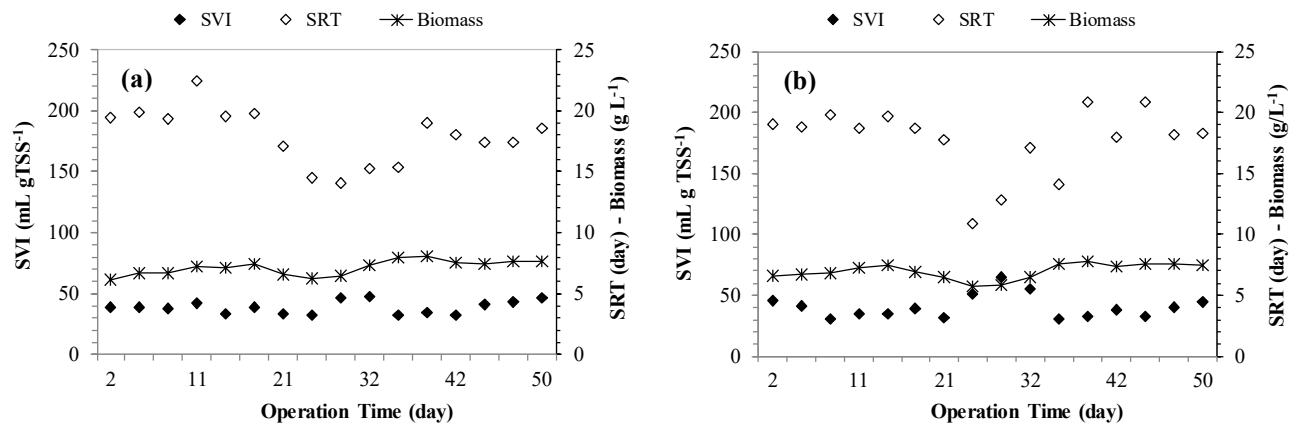


Fig. 4 The change in biomass sludge, SVI and SRT in R1 (a) and R2 (b) during operational day

Organic removal

During the experimental period, both reactors operated with the OLR of $1.22 - 1.34 \text{ kg COD m}^{-3} \text{ d}^{-1}$ and results showed that COD removal efficiency in R1 and R2 was stable in the range of 87 – 90% (Fig. 5). The high COD removal efficiency in the system was due to two reasons, high biomass retained in both reactors $7.46 - 7.53 \text{ g L}^{-1}$ (Fig. 4) and high bioactivity of the organism in sludge (SOUR was $80.70 - 87.90 \text{ mgO}_2 \text{ gTSS}^{-1} \text{ h}^{-1}$). These results agree with previous report by Nguyen [15], val del Río [16], and Arrojo [17] treating different kind of industrial: Tapioca Processing, dairy products and fish canning industry wastewater using aerobic granular sludge.

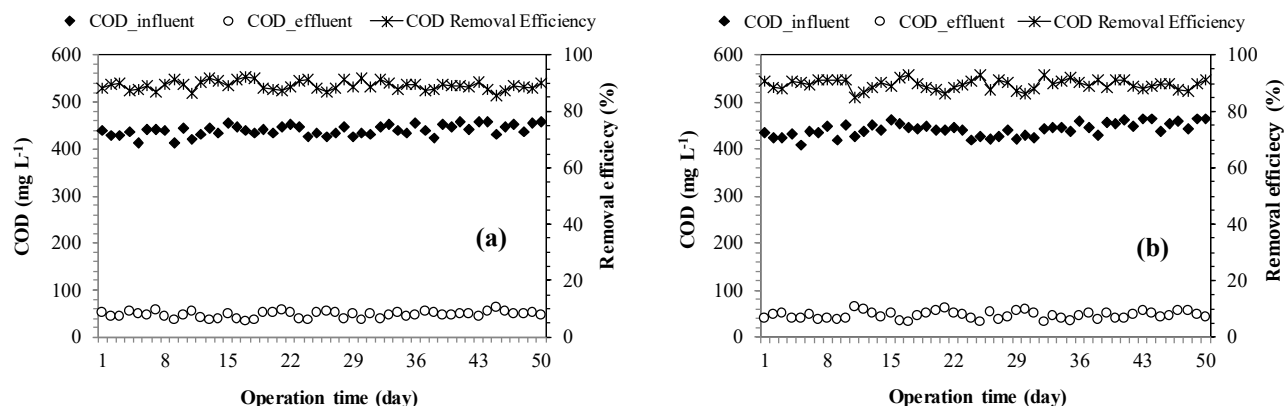


Fig. 5 The COD removal efficiency in R1 (a) and R2 (b) during operational day

Nitrogen removal

It can be seen that high and stable ammonium removal efficiency (about 97 – 99%) was achieved in both reactors (Fig. 6a, b). The study results showed that concentration of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ in the nt in both reactors was lower than 0.13 mg L^{-1} , but $\text{NO}_3^-\text{-N}$ concentration in effluent in the R1 and R2 was only $4.30 - 5.20$ and $2.80 - 3.50 \text{ mg L}^{-1}$, respectively. Additionally, both reactors had a relatively high total nitrogen (TN) removal efficiency, 68.40 – 71.20% and 80.20 – 85.60% in R1 and R2, respectively (Fig.7). It can be said that an amount of $\text{NO}_x\text{-N}$ might be lost via denitrification process in the anoxic phase in both reactors with the O-A-O operation. Recent studies confirmed that the autotrophic microorganism involved in organic matters removal and nitrification dominated on the surface of granular sludge, whereas, the heterotrophic microorganisms for denitrification was distributed in the deeper layer [1 – 3]. In Additional, the growth of these kinds of microorganisms could be maintained by alternating aeration and non-aeration strategy. Adav *et al.* [5] reported the coexist of the nitrifying and denitrifying bacteria in the surface layer of granules with the alternating O-A operation that were responsible for the nitrification and denitrification in the reactor. It can be explained for the nitrogen removal by nitrification and denitrification in both aerobic granular sludge reactors with the alternating O-A-O operation in SBR.

Moreover, the TN removal efficiency in R2 with two-step feeding was 13% higher than that in R1 with one-step feeding (Fig. 7). In case of the O-A-O operation with one-step feeding, organic matter was consumed in the first oxic phase may reduce the availability of organic matters and causing lack of carbon source as electron donor for denitrification in the following anoxic phase. Meanwhile, R2 applied two-step feeding with one more filling in anoxic phase was a better option in providing internal carbon source from raw wastewater for denitrification. It could be explained that the TN removal efficiency achieved in R2 was higher than that in R1. It was found that step-feeding was an efficient option to provide internal carbon source from wastewater as electron donor for denitrification in the anoxic phase instead of adding external carbon source. Our study results also confirmed that the alternating O-A-O operation with two-step feeding was efficient option to improve the nitrogen removal in SBR.

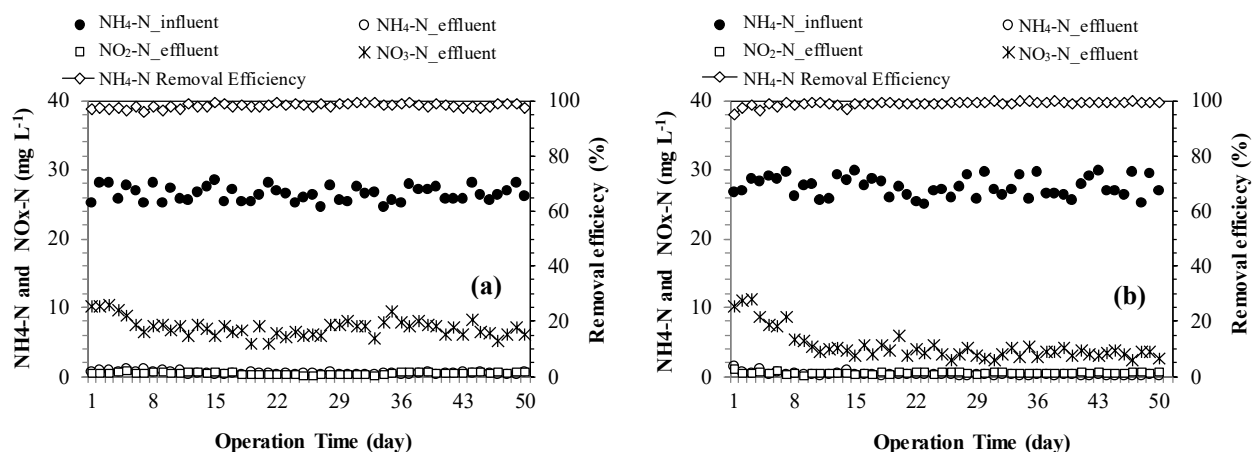


Fig. 6 Ammonium ($\text{NH}_4^+\text{-N}$) removal efficiency, concentration of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in R1 (a) and R2 (b) during operation period

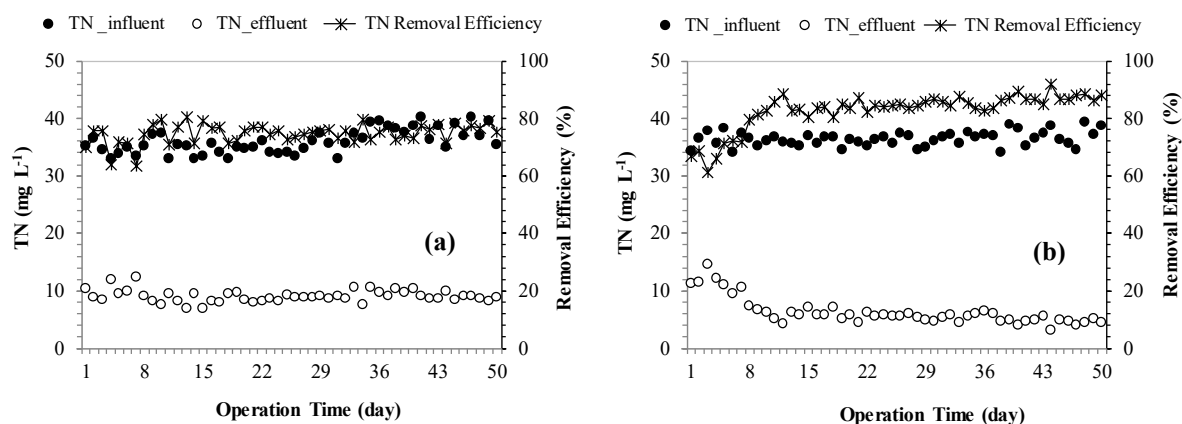


Fig. 7 Total nitrogen (TN) removal efficiency in R1 (a) and R2 (b) during operation time

Removal efficiency of aerobic granular sludge achieved in this study was agreed with recent studies [4, 15, 16, 18]. According to the data in Table 3, the removal efficiency of COD, $\text{NH}_4^+\text{-N}$ and TN obtained in this study were in accordance with other studies operating in the alternating oxic and anoxic for treating synthetic wastewater [4, 18]. Noticeably, to treat industrial wastewater by aerobic granular sludge in SBR, this study showed a better removal efficiency of COD and TN than others SBR operating without the alternating oxic and anoxic phase [15, 16].

Table 3. The removal efficiency of COD, NH₄⁺-N and TN in this study in comparison with literatures

No	Type of wastewater	Type of reactor and operation mode	COD _{inf} (mg L ⁻¹)/ OLR (kg COD m ⁻³ d ⁻¹)	NH ₄ ⁺ -N _{inf} (mg L ⁻¹)/ NLR (kg NH ₄ -N m ⁻³ d ⁻¹)	COD/ NH ₄ ⁺ -N/ TN removal (%)	References
1	Industrial wastewater (IW)	SBR: O-A-O + one-step feeding: 90 min (O)+107 min (A)+30 min (O)	444/1.22	26.64/0.11	87/98/75.40	This study
2	IW	SBR: O-A-O + two-step feeding: 90 min (O) + 107 min (A) + 30 min (O)	460/1.30	27.46/0.12	88/99/86.40	This study
3	IW (dairy products)	SBR: 171 min (O) + single step feeding	600/3.14	80/0.60	80/90/72	[16]
	IW (a fish canning industry)	SBR: 171 min (O) + single step feeding	430/1.27	65/0.15	93/85/15	[16]
4	IW (Tapioca Processing)	SBAR: 150 – 170 min (O) + single step feeding	800 – 1,200/5 – 10	95 – 182/-	96.30/82.60/68.80	[15]
5	Synthetic wastewater (SW)	SBR: A-O + step-feeding: 30 min (A) + 60 min (O) + 30 min (A) + 60 min (O) + 30 min (A) + 143 (O)	600/-	60/-	-/93 – 95	[4]
6	SW	SBR: A-O + step-feeding: 42 min (A) + 128 min (O)+42 min (A)+128 min (O)	800/-	300/-	98/70/-	[18]

“-”: not given (O): Oxidic phase (A): Anoxic phase SBAR: sequencing batch airlift reactor

4. Conclusion

Aerobic granular sludge maintained its stability with a low SVI of 42 – 45 mL gTSS⁻¹, high retained biomass of 7.46 – 7.53 g L⁻¹. High COD removal efficiency in the range of 89 – 90% was observed in both reactors. The removal of nitrogen via nitrification and denitrification was achieved in the alternating O-A-O operation, however, the two-step feeding with one filling in anoxic phase showed a better nitrogen removal than that of one-step feeding.

It was found that step-feeding was an efficient option to provide internal carbon source as electron donor from raw wastewater for denitrification in the anoxic phase instead of using external carbon source. In addition, adding the second oxidic phase of O-A-O operation is a suitable option to completely remove residual ammonium supplied by step-feeding. Although high organics and nitrogen removal efficiencies were achieved, this study was operated in a short-term in lab-scale SBR. Therefore, it is recommended to further investigate the granular sludge treatment ability in the long-term operation to assess the stability of the system.

5. Acknowledgement

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