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Influence of N/COD ratios and nitrogen forms on aerobic granulation in sequencing batch airlift reactor

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ABSTRACT

This study investigated the effects of different N/COD ratios (N as variable) and N forms on (i) the granulation and characteristics and (ii) pollutants removal of aerobic granular sludge. The 10/150 ratio resulted in the highest biomass concentration of 22,000 mg/L. The granules were measurable with the largest size (2.0 mm) in the culture of both forms of NH_4^+–N and NO_3^-–N (N/COD of 10/150) whilst the sole NH_4^+–N cultures (N/COD of 5/150 and 30/150) received smaller granules size of 0.2 and 1.1 mm, respectively. The NO_3^-–N source was found as the key factor determining the granule formation. With reference to pollutants treatment, the COD removal efficiencies were above 94% regardless of the N/COD ratios and nitrogen forms. Given this situation, the additional NO_3^-–N of 10/150 ratio (NH_4^+–N:NO_3^-–N = 1:1) could enhance the granule size and total nitrogen removal.

Keywords: Aerobic granular sludge; Simultaneous nitrification and denitrification; Nitrogen removal; N/COD ratio; Sequencing batch airlift reactor

1. Introduction

Aerobic granular sludge possesses prominent characteristics for wastewater treatment. It is more effective than activated sludge process [1]. It is of superior settling ability, high biomass and well-adapted to various pollution levels [2]. Its granulation occurs in a shorter period compared with 2–8 months of anaerobic granules [3]. It has a compact structure, diverse microbial community and notable simultaneous nitrification and denitrification (SND) capacity [2,4,5]. For these reasons, the aerobic granular sludge process has been implemented for treating various wastewater types [4,6,7]. For example, wastewaters of high pollutants load, such as livestock wastewater [8], brewery wastewater [9], rubber wastewater [10], petroleum wastewater [11], are handled efficiently by the aerobic granular sludge.

Granulation is a key success of this technology and it is determined by the operating conditions including hydraulic retention time, pH, dissolved oxygen and organic loading rate (OLR). Among those parameters, OLR is one of the important and decisive one because it would determine the nutrient levels for microbial consortium in the aerobic granular sludge [12] and would affect pollutants removal efficiency. Previously, numerous OLRs have been investigated, such as 2–15 kg COD/m^3 d [4], 2.7–22.5 kg COD/m^3 d [6],

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1.76–2.84 kg COD/m^3 d [13], 1.05–1.68 kg COD/m^3 d [14], 6.4–27 kg/m^3 d [15], 3–15 kg/m^3 d [16]. It can be seen that the studied OLR ranges are varied widely. It happens because other nutrients, especially N, are not considered. Therefore, the N/COD ratio has been used intensively to control the granulation and pollutants removal efficiency of aerobic granular sludge process and other biological treatment [12,17–20].

The N/COD ratio is a critical condition for aerobic granular process; however, the optimal range of N/COD ratio and type of involved N is still inconclusive. Previously, the N/COD ratio from 1/2 to 1 resulted in the unstable and disintegrated granules [19]. On the other hand, the N/COD ratio of 6/20 is found as enriching nitrifying bacteria granular, while N/COD ratio of 1/20 can enhance activities of heterotrophs [17,21]. Another important issue is that COD has been using as a variable for aerobic granular sludge's study. It would cause a knowledge gap because the effect of N loading rate and various N forms is not understood adequately. Also, the nitrogen form in the latest investigations relates to NH_4^+–N whereas NO_3^-–N form is missed [19,22,23]. As known, excessive NH_4^+–N would inhibit the nitrite oxidizing bacteria and worsen the balance of nitrification–denitrification system [24]. In turn, many microbial strains in aerobic granular sludge need NO_3^-–N for cell growth [25,26]. The role of N loading in N/COD ratio and N forms of both NH_4^+–N and NO_3^-–N needs to be resolved. It is necessary for practical applications. For those reasons, this study aims to explore the effect of N/COD ratios (N as a variable) and N forms on (i) the granulation and characteristic of aerobic granular sludge and (ii) its pollutants removal targeting at municipal wastewater in the SBAR.

2. Materials and methods

2.1. Synthetic wastewater and seed sludge

In this study, glucose was a main organic source in artificial wastewater for aerobic granules cultivation (Table 1). The components of the artificial wastewater were retrieved from the previous study of Thanh et al. [27]. Different N/COD ratios were prepared in three reactors by adjusting the amount of NH_4Cl and $NaNO_3$. Nitrogen forms in the influent wastewater were NH_4^+–N and NO_3^-–N and the N/COD ratios were designed as 5/150, 10/150 and 30/150. The pH was maintained at 7.5–9.5 by adjusting the dosage of $NaHCO_3$. The influent COD concentration of 1,200 mg/L was established for the entire experiment.

Table 1
Feed wastewater characteristics and N/COD ratios set up for reactors

Parameters	Reactor 1	Reactor 2	Reactor 3
N/COD ratio	5/150	10/150	30/150
COD (mg/L)	1,200	1,200	1,200
NH_4^+–N (mg/L)	40	40	240
NO_3^-–N (mg/L)	–	40	–
COD loading (kg COD/m^3 d)	3	3	3
N loading (kg N/m^3 d)	0.1	0.2	0.6
Acclimatization stage (d)	51	51	51
Stabilization stage (d)	90	90	90

The seed sludge was taken from a conventional activated sludge process in wastewater treatment plant. The experimented mixed liquor suspended solid (MLSS) concentration of sludge was 3,000 mg/L and the sludge volume index (SVI) was 124 mL/g. The sludge was acclimatized in 51 d (adaptation phase) prior to conducting experiments.

2.2. Sequencing batch airlift reactor

The experimental system consisted of three identical SBARs (Fig. 1), corresponded with three N/COD ratios designated. Those reactors were made of acrylic plastic. During the operation, wastewater was fed into three reactors using submersible pumps (Cole-Parmer, USA) located in an influent tank. A flow-direction baffle was installed at the center of the reactor to facilitate the flow condition as an elliptical orbit. This baffle would enhance shear force which generated stronger granules [28]. The working volume of one reactor was 8 L divided into two zones including raiser and downcomer (4.8 L and 3.2 L). The total volume of 4 L treated wastewater was withdrawn from each reactor after each cycle. The airflow velocity of 2.67 cm/s was supplied to each reactor via an air blower (Cole-Parmer, USA).

With reference to the operation, the system was run with 6 cycles/d. Each cycle was divided into four main stages including feeding (5 min), aeration (225 min), settling (3 min) and withdrawal (7 min). The total time of each operating cycle was 240 min. The volume exchange ratio was set up at 50%.

2.3. Experimental setup

The experiments were divided into two objectives and the applied N/COD ratios (e.g., 5/150, 10/150 and 30/150) were similar in all objectives. The experimental period was 140 d including the sludge acclimatization stage (day 1 to 50) and stabilization stage of 90 d (day 51 to 141). The experimental data of two objectives were collected in the stabilization stage. For the first objective, the granulation and characteristics of granular sludge including granules size, settling behavior and biomass growth, were examined. The COD and nitrogen removal efficiencies were studied in the second objective. The data of nitrogen and COD removal was started from day 72 to 113, and 21 to 141, respectively.

2.4. Analytical methods

The aerobic granular sludge was collected to record the granules size, settling behavior and biomass. The shape and size of granules were determined by an Olympus CX 21FS1 microscope with 40X and 100X magnification; and captured by 8 Megapixel camera. The SVI, MLSS and other parameters such as COD, NH_4^+–N, NO_3^-–N were measured according to the Standard Method of American Public Health Association [29].

The nitrogen balance was calculated as the formula below:

$$TN_{inf} = TN_{eff} + TN_{ass} + TN_{de} \quad (1)$$

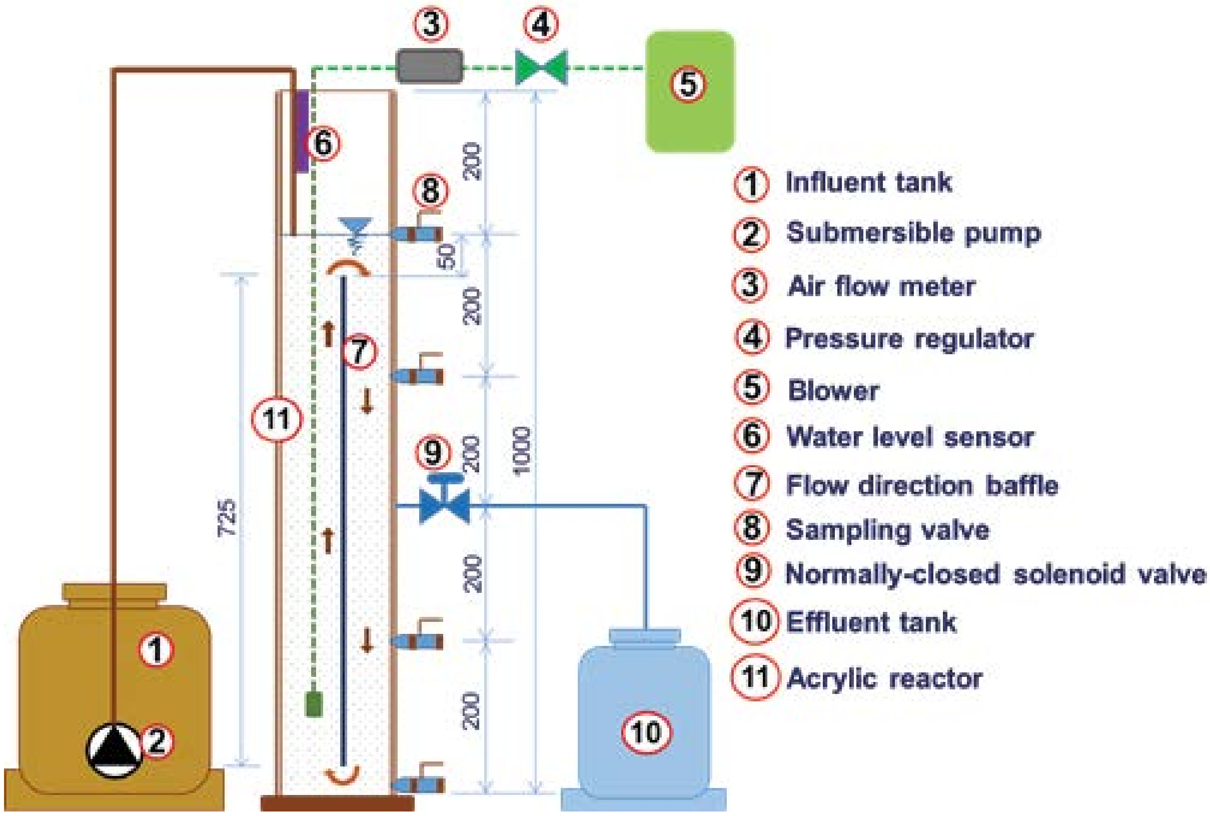

Fig. 1. Sketch of the SBAR.

where TN_{inf}: total nitrogen concentration in the influent (mg/L); TN_{eff}: total nitrogen concentration in the effluent (mg/L); TN_{ass}: total nitrogen assimilated in sludge biomass (mg/L); TN_{de}: total nitrogen denitrification (mg/L).

Nitrogen consumed in sludge biomass was estimated by the assimilated nitrogen in biomass (12% of sludge biomass) [30].

The nitrification rate and total nitrogen (TN) removal were calculated as below:

$$\text{Nitrification rate} = \frac{[NH_4-N]_0 - [NH_4-N]_t}{t} \quad (2)$$

$$\text{TN removal} = \frac{[TN]_0 - [TN]_t}{[TN]_0} 100\% \quad (3)$$

where $[NH_4–N]_0$: initial concentration of NH_4–N (mg/L); $[NH_4–N]_t$: concentration of NH_4–N at time t (mg/L); $[TN]_0$: initial total nitrogen (TN) concentration (mg/L); $[TN]_t$: concentration of total nitrogen at time t (mg/L).

2.5. Statistical analyses

The analysis of variance (ANOVA) was employed for the statistic in this study. The factorial ANOVA was implemented for COD and N removal efficiency. The data were presented as mean value ± standard deviation (mean ± SD) with replicated samples.

3. Results and discussion

3.1. Granules formation and characteristics

3.1.1. Granules size

After 51 d of the adaptation stage, granules in three reactors changed color from light black to brownish and light yellow (Fig. 2). In week 6th, initial granules were found in the three reactors. In the culture containing both ammonia and nitrate (N/COD = 10/150), granules formed faster than the reactors with only ammonia (N/COD = 5/150 & 30/150; Fig. 3a). Granules of 10/150 ratio were measurable from day 51st while it was on day 71st and 83rd for ratio 5/150 and 30/150, respectively. The addition of NO_3^-–N was a major reason given that it would be a substrate for microorganism and further supported the sludge development [12,31]. In this study, the time of granulation assisted by NO_3^-–N form was compatible to the average value of 30 to 60 d [32].

Of the granules size, three N/COD ratios differed from each other. For the 10/150 ratio with the presence of NO_3^-–N, its granules were the largest size (2.0 ± 0.3 mm). Other ratios of 30/150 and 5/150 created average granules size as 1.1 ± 0.1 mm and less than 0.2 mm (no deviation due to small size), respectively. The granules of ratio 5/150 failed to reach the matured size (1–3 mm) [32], due to the lack of nutrients. Elsewhere, the size of matured granules was known as 0.82 mm [33]. The granules size of 10/150 ratio was two-fold higher than that obtained from the study by He et al. [33]. Influent nitrogen concentration was found to impact strongly on the size of

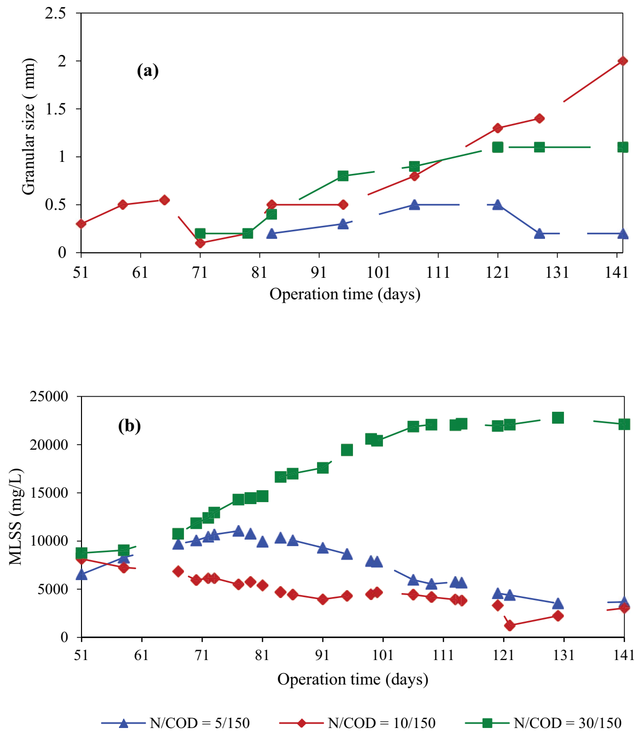

Fig. 2. (a) Size of granules, $p < 0.05$ indicates significant difference of granular size of three ratios. (b) Biomass concentration in steady state, $p < 0.05$ indicates significant difference of biomass concentration of three ratios. Both parameters were measured since day 51st.

aerobic granules. Insufficient nitrogen would reduce the specific growth rate of microorganism and affected the granules size accordingly [34]. Notably, the nitrogen source in those studies was from NH_4^+–N while this research experimented with a mixture of NH_4^+–N and NO_3^-–N. Thus, the NO_3^-–N was also beneficial for granular sludge cultivation.

3.1.2. Growth of biomass

The growth of biomass through NO_3^-–N consumption follows the below reaction:

$$16\ NO_3^- + 124\ CO_2 + 140\ H_2O + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138\ O_2 + 18\ HCO_3^- \quad (4)$$

Throughout the acclimatization stage, biomass increased steadily pursuant to all N/COD ratios (Fig. 2b). The ratio 30/150 outcompeted others. The biomass concentration of 30/150 ratio rose considerably from 3,000 to 22,000 mg/L during the time course of 141 d. The biomass concentrations of N/COD of 5/150 and 10/150 ratios were two-fold less than the value of 30/150 ratio that marked 11,000 (day 77th) and 8,150 mg/L (day 49th), respectively. After that time, the biomass of 5/150 and 10/150 ratios started decreasing. This happened because of the low nitrogen levels in the 5/150 and 10/150 ratios. Microbes have consumed the nitrogen compound extensively. The adequate nitrogen of 30/150 ratio promoted a strong increase of biomass concentration regardless

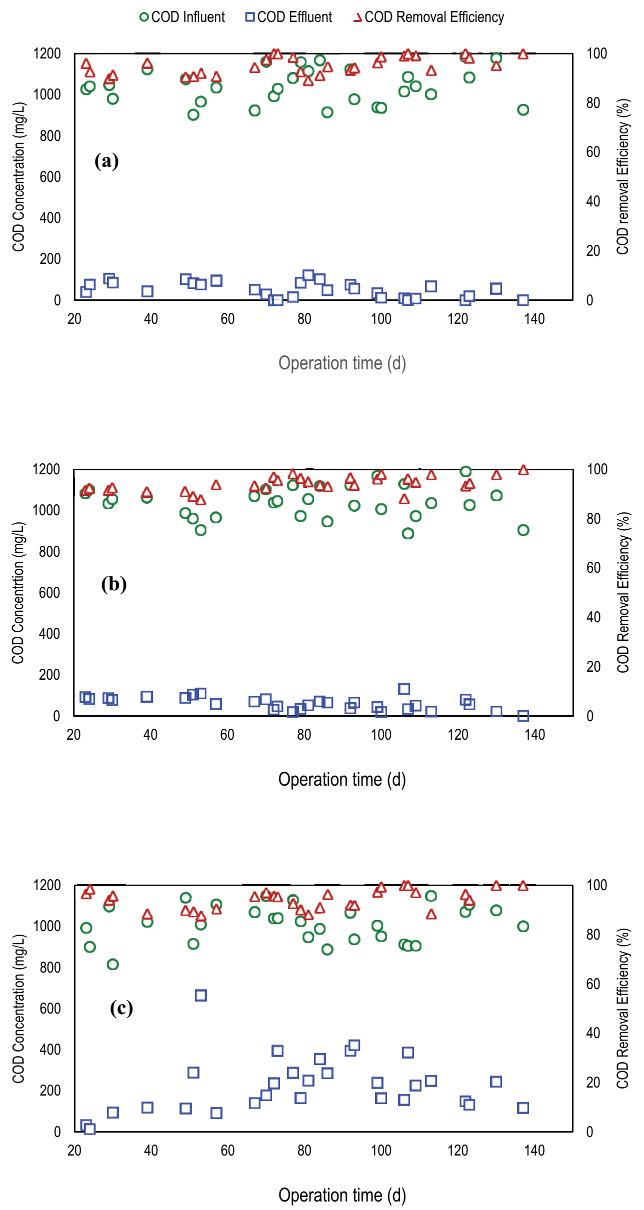

Fig. 3. COD removal in three ratios. (a) N/COD = 5/150, (b) N/COD = 10/150, and (c) N/COD = 30/150. Value and error bars are the average and standard deviation of 30 samples. $p = 0.54 > 0.05$ indicates insignificant difference of COD removal of three ratios.

of N forms. In the other studies, biomass concentration of aerobic granules was beyond 10,000 mg/L [27]. Specifically, the past study [35] reported the biomass could reach up to 15,000 mg/L; which was less than this study. Notably, the applied influent COD and NH_4^+–N concentration were 2,990 and 212 mg/L [35] in which 2.5 times higher than this work, given the N/COD of 10/150 was similar. Regarding the ratio N/CCOD of 10/150, although additional NO_3^-–N was supplied, the overall nitrogen concentration was still insufficient to feed aerobic microorganism. However, in the previous studies, extra NO_3^-–N would encourage the heterotroph denitrifiers and condition the denitrification process [36].

3.1.3. Settling behavior of granules

Settling behavior of the granular sludge was evaluated through SVI value. The SVI of initial seed sludge was 124 mL/g. After 30 d of the acclimatization period (day 30th to 40th), the SVI was from 20 to 40 mL/g for all ratios. From day 70th (steady stage), SVI of 30/150 ratio became stable which was around 14.6 mL/g. The SVI values of 5/150 and 10/150 ratio were higher slightly. The SVI of 10/150 ratio increased from 27 mL/g (day 43rd) to 94 mL/g (day 91st). Those values were still less than 100 mL/g indicating the well-settling performance of sludges in this study. As observed, the SVI was not affected by the increase of N/COD ratio. Similarly, it was suited at all N/COD [37].

3.2. Pollutants removal performance

3.3.1. COD removal

The average COD removal efficiencies in N/COD ratios of 5/150, 10/150 and 30/150 were 95%, 94% and 94%, respectively (Fig. 3). The COD removal efficiencies of three N/COD ratios were not statistically different. Thus, N/COD ratios and N forms did not have a significant influence on COD removal efficiency for COD below 1,200 mg/L. The COD removal efficiency in this study was quite stable, even at higher OLR, compared with 0.32–0.63 kg COD/m^3 d [38] and (0.3 kg COD/m^3 d) [28]. The nutrient level of 1,200 mg COD/L was sufficient for microorganism in this case. It is no doubt that this system could be operated with higher COD loading, rather than 3 kg/m^3 d. For example, Li et al. [39] pinpointed that an over 90% of COD removal was witnessed at COD loading rate from 4 to 13 kg COD/m^3 d; however, only 78% of COD could be eliminated at 20 kg COD/m^3 d. This study just experimented with a sole COD loading (3 kg COD/m^3 d).

3.3.2. Nitrogen removal

The N balance corresponded to three N/COD ratios is demonstrated in Fig. 4. For the ratio of 5/150, N removal efficiency was high throughout the experiment because of low N loading (0.1 kg N/m^3 d). The N removal efficiency kept rising and the average removal efficiency was 95% ± 0.7%. The N concentration in the effluent was lower than 6 mg/L. The N/COD ratio of 10/150 was operated with higher N loading (0.2 kg N/m^3 d) that facilitated cell formation and the denitrification process. Initially, N removal efficiency was higher than 60% ± 0.9% and reached the peak at 90% ± 1.5%. The results could be explained that the granules was forming at the time and excessive N was consumed consequently. However, the N removal efficiency turned to be unstable and started decreasing since day 67th due to the presence of filaments. The filamentous microorganisms were washed out subsequently resulting in the elevation of N removal efficiency since day 99th.

Compared with other ratios, the average N removal efficiency of 30/150 ratio was less of around 20.0%–49.6% due to highest N loading (0.6 kg N/m^3 d). The average N in the effluent accounted for 63.7% ± 2.3% while assimilated N and denitrification N only dominated 20% ± 0.3% and 16.3 ± 1.1%, respectively. At extremely high N loading of 54.5 kg NH_4^+–N/m^3 d, [23] achieved 84.6% of total N removal at N/COD of 30/150; yet, it was supported with a membrane bioreactor. Likewise, He et al. [40] successfully removed 96.56% ± 3.44% of NH_4^+–N and 93.88% ± 6.78% of total inorganic nitrogen with the similar SBR system, but lower nitrogen concentration (TN = 19.3 mg/L) compared with this study. Thus, the N/COD of 30/150 established herein was comparable with others and applicable in practical applications, aiming for high N loading wastewater.

The results exhibited that N removal efficiency decreased pursuant to the increment of N/COD ratio (Table 2). The balance of nitrification and denitrification is important to achieve high nitrogen efficiency [41,42]. In this study, the ratio of 5/150 possessed similar nitrification and denitrification rate; and thus, TN removal achieved high efficiency (99.5%). When N/COD ratio increased in the ratio 30/150, nitrification rate improved and denitrification decreased; resulting in the decline of TN efficiency. This was previously explained due to the high nitrogen loading rate. Feng et al. [43] came up with a similar conclusion applied to N/COD ratios of 1/20, 1/15 and 1/10. According to Feng et al. [43], the rise of N/COD ratio increased both ammonia and nitrite oxidizers population. In turn, the heterotrophic population decreased and denitrification rate slightly went downward. The same results were also documented elsewhere [44,45].

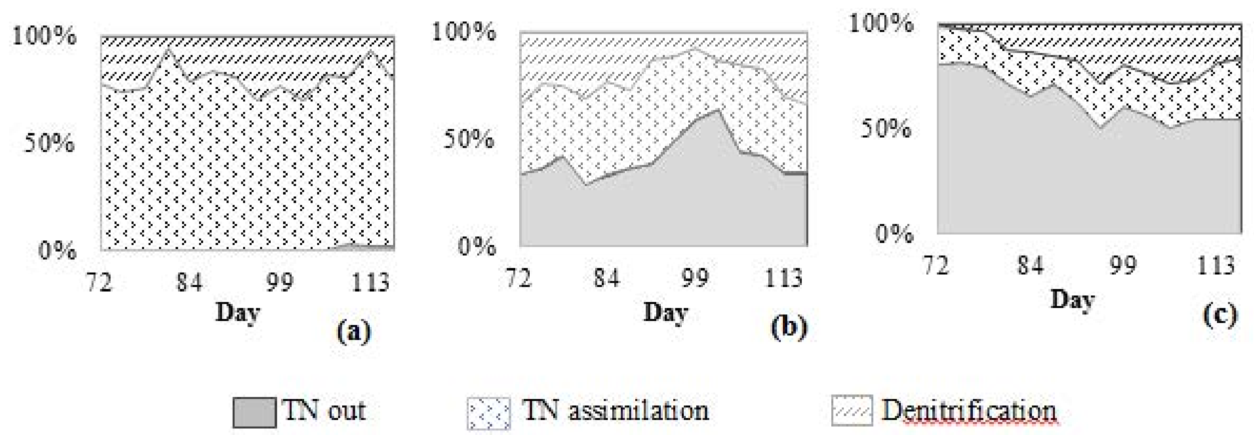

Fig. 4. Nitrogen balance of at different N/COD ratios: (a) N/COD = 5/150, (b) N/COD = 10/150, and (c) N/COD = 30/150.

Table 2
Nitrification and total nitrogen removal at different N/COD ratios

Reactor	Unit	1	2	3
N/COD ratio		5/150	10/150[a]	30/150
Nitrification rate	mg NH_4–N/L.h	32.4 ± 2.3	20.4 ± 1.4	52.1 ± 3.5
Specific nitrification rate	mg NH_4–N/h.mg MLSS	5.6 ± 0.3	3.6 ± 0.2	2.3 ± 0.1
TN removal efficiency**	(%)	99.5 ± 5.3	85.3 ± 3.2	45.7 ± 2.1

[a]50% NO_3–N, 50% NH_4^+–N.
**$p < 0.05$ indicates significant difference of TN removals of three ratios.

Table 3
Comparison with other related studies

Wastewater source	Reactor type	Nutrient level	N/COD ratio	Cycle time (h)	Characteristic of cultivated granule	COD removal efficiency (%)	N removal efficiency (%)	References
Synthetic wastewater	SBAR	1,200 mg COD/L, 3 kg COD/m³.d 40–240 mg N/L, 0.1–0.6 kg N/m³.d	5/150, 10/150, 30/150	4	Size: 1.1 ± 0.1–2 ± 0.3 mm SVI: 23–35 mL/g	94–95	49.6–95	This study
Traditional Chinese medicine waste1water	EGSB	4,000–5,500 mg/L, 4 to 13 kg COD/m³ d 50–57 mg NH_4^+–N/L	1/80–1/95	6–12	Size: 0–1,000 μm	78–94	–	[39]
Synthetic wastewater	SBR	200–6,000 mg COD/L 200 mg N/L	1/1, 1/2, 2/7, 1/5, 2/15	6	Size: 3.7 ± 0.6 μm SVI: 30–62 mL/g	63–94	17–54	[37]
Synthetic wastewater	SBAR	400 mg COD/L 100–400 mg N/L	1/1, 1/2, 1/4	2.4	Size: 250–889 μm Flocculent surface area: 1.82 to 3.14 m²/g	–	28–42	[19]
Synthetic wastewater	*SBR*	1,000 mg COD/L 50–300 mg N/L	5/100, 10/100, 15/100, 20/100, 30/100	6	Size: 0.25–0.51 mm	95[a]	85[a]	[46]
Synthetic wastewater	SBR	200–800 mg COD/L 200 mg N/L	200/0, 200/200, 200/400, 200/800	4	Size: 0.41–0.92 mm SVI: 32–40 mL/g	59–82	39–97	[48]
Pulp and paper industry WW	Pilot SBR	2,000–3,000 mg COD/L	–	24, 12, 8, 6	Size: 2–4 mm MLVSS: 7 -8 g/L SVI: 60–80 mL/g	88	–	[49]
Petroleum wastewater	SBR	600 mg COD/L	3–5/100	4.8	Size: 0.46–0.9 mm SVI: 30–80.6 mL/g	95	30–35 (NH_4^+–N) 35 TN	[11]

[a]Retrieved from graph.

Both Yang et al. [17] and Kocaturk and Erguder [37] indicated that the elevation of NH_4^+–N/COD ratio (≥30/150) enhanced the SND efficiency thanks to the augment of nitrification and nitrifying microbial populations. For the ratio 10/150, the TN removal was increased approximately two times higher than that of the ratio 30/150.

Most authors did not apply NO_3^-–N source in their studies [12,17,37]. Thus, through this study, it can be seen that not only N/COD ratio, the nitrate nitrogen concentration in the influent could elevate SND efficiency. The addition of NO_3^-–N could bring the two benefits as follows: (a) it served as a substrate in the nitrogen metabolic pathways of plentiful microbes such as *Proteobacteria*, *Azospira*, *Denitratisoma*, *Dechloromonas*, *Flavobacterium*, *Zoogloea*, *Pseudomonas* and *Thauera* [31]. As such, NO_3^-–N could be an electron acceptor for the denitrification process undertaken; and (b) saving oxygen consumption from the nitrification process [12].

In practice, the characteristics of wastewater varied type by type and this work initiated with synthetic wastewater. This observation needed a further study in which conducted with similar nitrogen loading, but different N forms, to accurately compare the effect of N forms, and explored with real wastewater for conclusive and solid outcomes.

3.3. Comparisons of SBAR system with other studies

The comparisons with other studies were documented with a focus on operating conditions, granules characteristics and pollutants removal efficiency (Table 3). This SBAR system performed competitively with other technologies such as SBR and expanded granular sludge bed (EGSB). With regard to the SBAR system of Luo et al. [19], the N removal efficiency and granules' size in this study were more significant. Three possible reasons were the longer cycle time, more appropriate N/COD ratio and the addition of NO_3^-–N source herein. For EGSB technology, the COD removal efficacy of this work was slightly higher given that the influent COD loading was apparently similar [39]. Notably, Li et al. [39] also processed with higher cycle time and nutrients loadings but received smaller granules size. It is likely that the N/COD obtained from the study of Li et al. [39] was lower than this work; thus, the granular size was smaller accordingly. Also, the involvement of NO_3^-–N made the performance of our SBAR better compared with the feed wastewater containing only NH_4^+–N form.

With reference to other SBR-based studies, the pollutants removal efficiencies fluctuated provided that COD removal efficiencies were approximately 90%. The N removal efficiencies were from 30% [11] to 85% [46]. The characteristics and type of applied wastewater might have a certain influence on the removal efficiency of pollutants. For example, petroleum wastewater contained toxic chemicals and they were harmful to microbes in the SBR system [11]. Herewith, the airlift condition of SBAR wielded the advantages from the efficient shear force, circulation and superficial gas flow [47]. One would be an alternative for wastewater treatment.

4. Practical applications and future perspectives

Looking at an opportunity for practical applications, the finding of this study is applicable to the N/COD ratio of 10/150 for wastewater treatment in real practice. From this achieved ratio, the blend wastewater can be used instead of a single wastewater source. For example, the wastewater of aquaculture sector is known with N:COD of 1:23 to 1:30 [50] and that is such an appropriate one for the granulation system. Or else, catfish farm wastewater with N/COD ratio of 1:7 can also be a proper target for SBAR application [51]. This study also contributes to the operation and maintenance of SBAR given that the system is likely stable since day 100th. Thus, this serves as manual for design and operation of the batch granulation system accordingly.

Another potential application relates to the amount of NO_3^-N in the influent wastewater (NH_4^+–N:NO_3^-–N = 1:1). As indicated beforehand, it offers numerous benefits. This approach should be refined to enhance the N removal efficiency of high nitrogen concentration wastewater.

5. Conclusion

In this study, different N/COD ratios and N forms were investigated for the aerobic granulation process in a SBAR. The N/COD ratio of 30/150 achieved the highest biomass concentration up to 22,000 mg/L. However, feeding the reactor with 30/150 ratio would not remove nitrogen efficiently due to overloading. The N/COD ratio of 10/150 (with NH_4^+–N:NO_3^-–N of 1:1) could enhance the granule size and facilitate the denitrification process. The COD removal (approximately 95%) was independent of N/COD ratios and nitrogen forms.

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