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Reactor configuration on the nitrifier community and kinetics of activated sludge process

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ABSTRACT

Both a nitrifying sequencing batch reactor (SBR) and a completely stirred tank reactor (CSTR) were operated under aerobic conditions. Microbial populations of both reactors were examined via fluorescent in situ hybridization (FISH) and kinetic properties such as ammonium/nitrite utilization rate and half-saturation constants (K_s) were investigated. In the SBR, dominant ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were rapid growth *r*-strategists (*Nitrosomonas europaea* and *Nitrobacter*). However, in the CSTR, the dominant AOB and NOB were slow growth *K*-strategists (*Nitrosospira* and *Nitrospira*). The half-saturation constants of AOB and NOB were 3.01 ± 0.97 and 4.22 ± 0.64 mg/L, respectively, for the SBR and 0.43 ± 0.19 and 2.15 ± 0.64 mg/L, respectively, for the CSTR. The nitrification rate of the SBR was 63% higher than that in the CSTR. The reactor configuration shows an evident selectivity for *K*/*r*-strategists. Thus, the difference in nitrification rates between the SBR and CSTR are likely caused by the combination of chemical reaction engineering kinetics and nitrifier communities.

Keywords: Reactor configuration; Microbial community; Nitrifier kinetics; *K*-Strategists; *r*-Strategists

1. Introduction

Biological treatment processes are "complex systems" where many different types of microbes grow and interact in a dynamic manner. Understanding the relationships between microbial diversity and bioreactor performance could facilitate the optimization of bioreactor design and thus enable the solution of bioreactor-related problems [1,2]. Biological nitrogen removal (BNR) is a key process for the removal of nitrogen from wastewater and has become more important due to increasingly stringent discharge regulations [3]. Nitrification is an important step in BNR from wastewater. The first step of nitrification (oxidation of ammonia to nitrite) is generally catalyzed by chemolithoautotrophic ammonia-oxidizing bacteria (AOB), which belong to two monophyletic groups within the β- and γ-*Proteobacteria*, and Archaea (AOA), which belong to *Thaumarchaeota* phylum [4]. In addition, van Kessel et al. [5] and Daims et al. [6,7] reported that nitrification can also be conducted by comammox bacteria. The second step (oxidation of nitrite to nitrate) is conducted by chemolithoautotrophic [8,9] and mixotrophic [10] nitrite-oxidizing bacteria (NOB).

The key nitrifiers involved in wastewater treatment can be distinguished via their specific growth rates and substrate affinity constants [11–13]. In an ecological context, *Nitrosomonas* and *Nitrobacter* genera are known as *r*-strategists, which show relatively high growth rates and high half-saturation constant K_s values for substrate ammonia/nitrite and prefer high concentration of substrates. *Nitrosospira* and *Nitrospira* on the other hand are *K*-strategists,

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which show relatively low growth rates and low K_S values and prefer low concentration of substrates [14,15]. Therefore, an improved understanding of the relationship between the nitrifying community and nitrification rate could potentially aid the improvement of both monitoring and control of biological nutrient removal systems.

Reactor configuration is a central factor in the design of water resource recovery facilities. So far, many studies have indicated that reactor configuration will influence the performance of nitrifiers. Chudoba et al. [16] compared the nitrification rate of plug flow to that of complete mix reactors, and a ~60% higher nitrification rate was found for the plug flow reactor. Still et al. [17] also reported a ~53% higher nitrifier growth rate when this was determined from sequencing batch reactor (SBR) compared with a continuous flow completely stirred tank reactor (CSTR). The SBR is normally accepted as an approximation of a plug flow reactor, while the CSTR is similar to a complete mix reactor. Given that even if nitrifiers' kinetic constants remained identical, a plug flow reactor would have a higher reaction rate than a complete mix reactor [18].

Reactor configuration may strongly influence the substrate distribution in time and space due to different influent supply regimes. Due to fluctuating and constant nitrogen (ammonia and nitrite) concentrations, SBR and CSTR wastewater supply regimes were hypothesized to support different predominant AOB and NOB, respectively, exhibiting distinct biokinetic properties [19,20]. Based on their different growth rate and substrate affinity, significant differences in nitrifying microbial community can be expected to be sparked in SBR (or Plug Flow) and CSTR during long-term operation. Therefore, the differences in nitrification rates could be caused by the development of different genera of AOB and NOB in both types of reactors, rather than by direct impact of different reactor configurations from the viewpoint of chemical reaction engineering kinetics.

An understanding of the relationship between microbial community, nitrification performance, and environment is important for the maintenance of conditions that promote the growth of faster nitrifiers, which in turn could provide useful feedback for plant design and operation. However, this area of work needs to be confirmed using modern tools such as gene probe analyses.

The main purpose of this study was to compare and contrast both AOB (*Nitrosospira* and *Nitrosomonas europaea*) and NOB (*Nitrospira* and *Nitrobacter*), which represented *K*- and *r*-strategists in the SBR and CSTR, respectively. These AOB and NOB were determined via fluorescence in situ hybridization (FISH) with strain-specific probes and the selected groups of bacteria were quantified via image software [21,22]. Parameter evaluation also included the performance of the reactors and nitrification kinetics for both types of treatment systems.

2. Materials and methods

2.1. Experimental set-up

Two reactors were used in this study. One was a SBR system and the other was a CSTR system. Both reactors consisted of toughened glass with identical cylindrical shape and effective volumes (5.0 L).

The SBR was operated with a hydraulic retention time of 8 h and a sludge retention time of 15 d at 20°C ± 1°C for 102 d. The nitrogen load was 0.15 kg/m^3 d, and the organic load was 1.06 kg/m^3 d. The dissolved oxygen (DO) concentration was controlled at 2–3 mg/L, and the pH value was controlled at 7.0–8.0 via addition of $NaHCO_3$ solution. The SBR was fed 2.5 L of synthetic wastewater six times a day and 4 h per cycle (feed, 2 min; oxic, 178 min; settle, 50 min; decant, 3 min; and idle, 7 min).

The CSTR was operated at the same conditions with the SBR, except that the feed was continuously at a speed of 15 L/d and the ratio of return activated sludge was 100%.

Each reactor intermittently or continuously received synthetic domestic wastewater (440 mg/L total chemical oxygen demand, 60 mg/L total nitrogen, and 9 mg/L total phosphorus). The feeding solution consisted of chemical compounds: 92 mg/L urea, 13 mg/L NH_4Cl, 80 mg/L Na-acetate, 132 mg/L Na-acetate·$3H_2O$, 17 mg/L peptone, 29 mg/L $MgHPO_4 \cdot 3H_2O$, and 23 mg/L K_2HPO_4. Food ingredients (calculated as chemical oxygen demand): 122 mg/L starch, 116 mg/L milk powder, 52 mg/L yeast, and 29 mg/L soy oil. Trace metals: 1.93×10^{-4} mg/L $Cr(NO_3)_3 \cdot 9H_2O$, 1.34×10^{-4} mg/L $CuCl_2 \cdot 2H_2O$, 2.70×10^{-5} mg/L $MnSO_4 \cdot H_2O$, 8.40×10^{-5} mg/L $NiSO_4 \cdot 6H_2O$, 2.50×10^{-5} mg/L $PbCl_2$, and 5.20×10^{-5} mg/L $ZnCl_2$.

The seed source was obtained from the oxic tank in the fourth wastewater treatment plant of Xi'an, and 5.0 L seed source was added to each reactor directly at the start of the experiment.

2.2. Physicochemical analyses

The analyses of NH_4^+-N, NO_2^--N, NO_3^--N, mixed liquor suspended solid (MLSS), and mixed liquor volatile suspended solid were conducted according to standard methods for the Examination of Water and Wastewater [23]. DO was measured with a DO meter (HI9143, Hanna Instruments, USA). Oxidation–reduction potential (ORP) and pH were measured with a ORP/pH meter (HI9025, Hanna Instruments, USA).

2.3. Biokinetics analysis

To evaluate the response of the communities to the increasing substrate load, kinetic studies of nitrification rates were conducted. Nitrifying biomass was harvested from both reactors at day 84 and were transferred into a 250 mL glass vessel containing a DO electrode (Seven2Go S9, Mettler Toledo, Switzerland) at 20°C.

To test the first step of nitrification, ammonia (ammonium chloride 1–30 mg NH_4^+-N/L) and alkalinity (sodium bicarbonate 0.5 g/L $NaHCO_3$ per 0.2 g/L NH_4Cl) were supplied in excess; to evaluate the second step of nitrification, ammonia was substituted with nitrite (sodium nitrite 1–30 mg NO_2^--N/L).

The medium was pre-aerated to achieve oxygen saturation prior to mixing with nitrifying biomass from the SBR and CSTR via magnetic stirring at 150 rpm. For AOB kinetics determination, sodium azide was added to ensure a working concentration of 24 μM for the suppression of NOB respiration, and for the NOB kinetics determination, allylthiourea was added to ensure a working concentration of 86 μM for the suppression of AOB respiration instead [24].

Endogenous decay of nitrifiers was assessed during the first 10 min of incubation. Next, the vessel was spiked with either ammonium chloride or nitrite sodium, and the decrease in nitrogen concentration (calculated according to the decrease in DO concentration) was monitored. The biokinetic parameters for nitrifier, that is, NH_4^+-N half-saturation constants for AOB ($K_{NH_4^+-N}$), maximum ammonium utilized rate of AOB (AUR_{max}), NO_2^--N half-saturation constants for NOB ($K_{NO_2^--N}$), and maximum nitrite utilized rate of NOB (NUR_{max}) were determined by fitting the nitrogen profile to the following equation:

$$r = \frac{r_{max} \times C}{(K_S + C)} \quad (1)$$

where r represents the nitrogen (ammonium or nitrite) utilization rate (mg N/L h) obtained via respirometry, C represents the nitrogen concentration (mg N/L), and K_S represents the nitrogen half-saturation constants for AOB (mg NH_4^+-N/L) or NOB (mg NO_2^--N/L).

2.4. Fluorescence in situ hybridization

Nitrifying biomass in each reactor was immediately fixed with paraformaldehyde for 3 h at 4°C and washed with 1 × phosphate-buffered saline thrice to remove residual paraformaldehyde, followed by a series of ethanol dehydration steps (each lasting 3 min) to successively reduce the moisture content by 50%, 80%, and 98% (V/V). FISH was conducted according to the protocol in Refs. [25,26]. Applied oligonucleotide probes and formamide percentages are shown in Table 1. Oligonucleotide probe details are available at probeBase (http://www.microbial-ecology.net/probebase/) [27]. The probes were labeled at the 5′ end with fluorescein isothiocyanate (FITC), indocarbocyanine dye Cy3 and Cy5. Samples were observed under a Leica TCSSP8X Confocal Microscope (Leica Microsystems, Mannheim, Germany) with an Ar laser (488 nm) and two HeNe lasers (543 and 633 nm). To quantify the probe-positive cells, image stacks were obtained via optical sectioning and were exported in TIFF format to the Leica Microsystems LASAF-TCSSP8 software. The percentage values of specific probe positive cells relative to EUB338mix-positive cells were averaged over at least 10 image stacks.

3. Results and discussion

3.1. Nitrification performance in the reactors

SBR and CSTR were operated for 100 d with either intermittent or continuously feeding of the same synthetic wastewater, respectively.

The profiles of NH_4^+-N in the effluent from the reactors are shown in Fig. 1(a). It could be seen that the NH_4^+-N concentration in the effluent of both reactors was relatively high; however, it gradually decreased below 1 mg/L at day 17, which may be because the nitrifiers in the seed source were trying to adapt to the new environmental conditions in the reactors during the start of reactors. Then, the effluent NH_4^+-N concentration in the SBR remained relatively stable, but gradually increased in the CSTR after day 17, reaching 9.48 mg/L at day 27, and then decreasing gradually. This was similar to the observations in the SBR; these phenomena will be explained by the shifts of AOB community further below.

The profiles of NO_2^--N in the effluent from the reactors are shown in Fig. 1(b). It could be seen that the NO_2^--N concentration of the effluent of the SBR accumulated more evidently compared with that in the CSTR. Nitrite accumulation was observed at day 9, and reached 17 mg/L at day 32, then quickly decreased and reached 0.32 mg/L at day 37. Nitrite accumulation was also observed in the CSTR during day 17 to 36; however, with 1–3 mg/L, the nitrite concentration was much lower than in the SBR; these phenomena will be explained by the shifts of NOB community further below.

The change of NH_4^+-N and NO_2^--N concentrations in the SBR and CSTR during a typical SBR cycle are shown in Fig. 2. In the typical SBR cycle, the NH_4^+-N concentration increased to almost 30 mg/L after feeding, then gradually decreased to below 1 mg/L at 120 min. The NO_2^--N concentration increased gradually and nitrite accumulation occurred accordingly; the value peaked at 13.60 mg/L and once ammonia oxidation was completed, the NO_2^--N concentration began to gradually decrease to less than 1 mg/L at 165 min. However, in the CSTR, the NH_4^+-N and NO_2^--N concentrations in the reactor were relatively stable in a range of 0.2–1.0 and 0.1–0.5 mg/L, respectively, and both were almost identical to the value in the effluent.

Table 1
Oligonucleotide probes applied for fluorescence in situ hybridization (FISH)

Probe	Sequence (5′–3′)	Specificity	FA (%)
NSO1225	CGCCATTGTATTACGTGTGA	Ammonia oxidizing beta-proteobacteria	35
Nsv443	CCGTGACCGTTTCGTTCCG	*Nitroso-spira, -lobus, -vibrio*	30
Nsm156	TATTAGCACATCTTTCGAT	*Nitrosomonas* spp., *Nitrosococcus mobilis*	35
Ntspa662	GGAATTCCGCGCTCCTCT	*Nitrospira*	35
Comp Ntspa662	GGA ATT CCG CTC TCC TCT	Competitor for Ntspa662	–
NIT3	CCTGTGCTCCATGCTCCG	*Nitrobacter*	40
Comp NIT3	CCT GTG CTC CAG GCT CCG	Competitor for NIT3	–
EUB338	GCT GCC TCC CGT AGG AGT	Most bacteria	0–40
EUB338 II	GCA GCC ACC CGT AGG TGT	Planctomycetales	0–40
EUB338 III	GCT GCC ACC CGT AGG TGT	Verrucomicrobiles	0–40

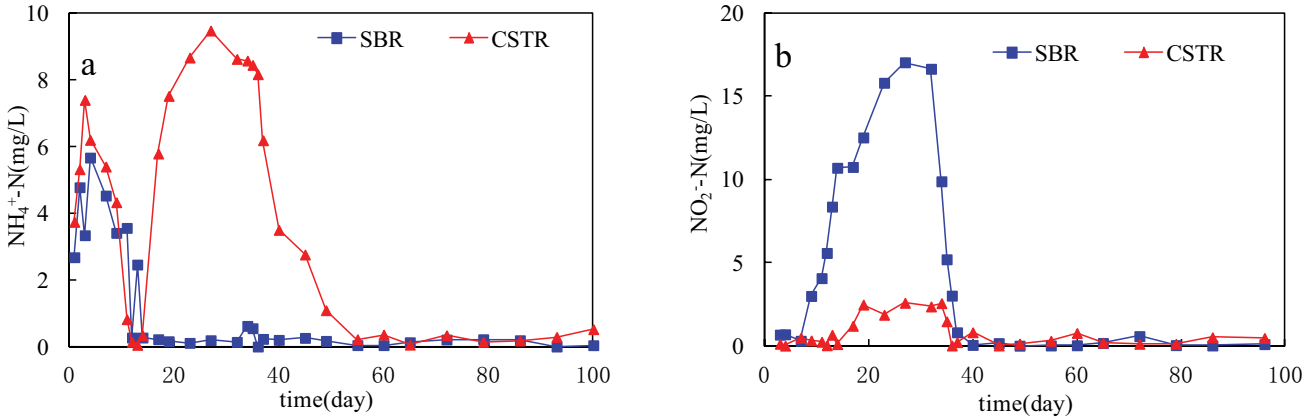

Fig. 1. NH_4^+-N (a) and NO_2^--N (b) profiles in the effluent from the SBR and CSTR.

After reactors were started-up, ammonia accumulation was evident in the CSTR, while nitrite accumulation was observed in the SBR before the reactors achieved stable states.

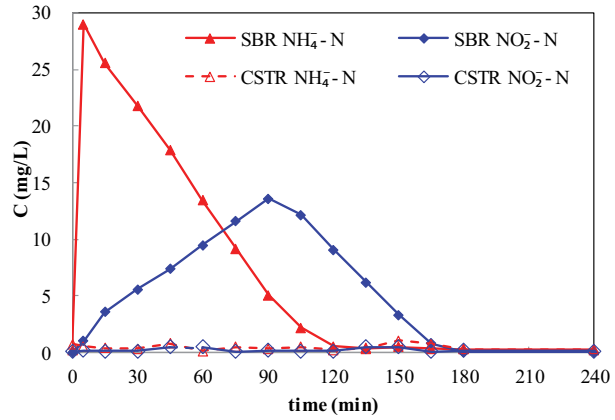

Fig. 2. NH_4^+-N and NO_2^--N profiles in SBR and CSTR during a typical SBR cycle.

In the SBR, the NH_4^+-N was almost 30 mg/L after feeding, then gradually decreased to almost depletion at 120 min; the NO_2^--N increased accordingly during this stage and reached a peak value of 13.6 mg/L when it began to decrease after NH_4^+-N was depleted. However, in the CSTR, NH_4^+-N and NO_2^--N remained at relatively stable and low values.

3.2. Nitrification activity

The seed source of both reactors was obtained from the oxic tank of the fourth wastewater treatment plant, which was operated with stable nitrification. The ammonium uptake rate (AUR) and nitrite uptake rate (NUR) of the seed source were 11.01 mg NH_4^+-N/L h and 12.42 mg NO_2^--N/L h, respectively.

After the reactors had achieved stable nitrification, the nitrification activity of the activated sludge was detected biweekly (day 56, day 70, day 84, and day 98). The average AUR of the SBR and CSTR were 16.55 ± 2.06 and 10.13 ± 0.73 mg NH_4^+-N/L h, respectively; the average NUR of the SBR and CSTR were 15.33 ± 2.02 and 9.34 ± 2.56 mg NO_2^--N/L h, respectively. These data are summarized in Table 2. The results show that the SBR achieves a much higher nitrification rate than the CSTR, the AUR and the NUR in the SBR were 63% and 64% higher than those of the CSTR, respectively. These values were similar to the results of Chudoba et al. [16] and Still et al. [17].

3.3. Population kinetics

The nitrification rate can depend on the initial substrate concentration if the nitrifying population is not the limiting factor. The "affinity constant" or "half-saturation constants" (K_S) concept is applied in wastewater treatment models to incorporate the effect substrate limitation exerts on process performance. The half-saturation constants K_S depend greatly on the system condition and should be evaluated on a case-to-case basis [11]. Repeated experiments with variable initial ammonium and nitrite concentrations were conducted in this study.

Figs. 3(a–d) show the initial AUR versus initial ammonia and initial NUR versus initial nitrite content for both reactors. It also shows the Monod equation (1) fitting curve.

Monod is the most commonly used kinetic approach to describe substrate dependent autotrophic growth and seemed appropriate to match the characteristics of both AOB and NOB performance.

According to the fitting results, the NH_4^+-N half-saturation constants $K_{NH_4^+\text{-}N}$ for AOB were 3.01 ± 0.97 and 0.43 ± 0.19 mg NH_4^+-N/L in the SBR and CSTR, respectively, and the NO_2^--N half-saturation constants $K_{NO_2^-\text{-}N}$ for NOB were 4.22 ± 0.18 and 2.15 ± 0.64 mg NO_2^--N/L in the SBR and CSTR, respectively.

The different responses to increasing substrate set point concentrations of both communities in batch tests confirmed

Table 2
Nitrification activity of seed source and activated sludge in both reactors at the stable stage

	Seed source	SBR	CSTR
AUR (mg N/L h)	11.01	16.55 ± 2.05	10.13 ± 0.73
NUR (mg N/L h)	12.42	15.33 ± 2.02	9.34 ± 2.56
VSS (g/L)	1.70	2.36 ± 0.21	2.12 ± 0.18

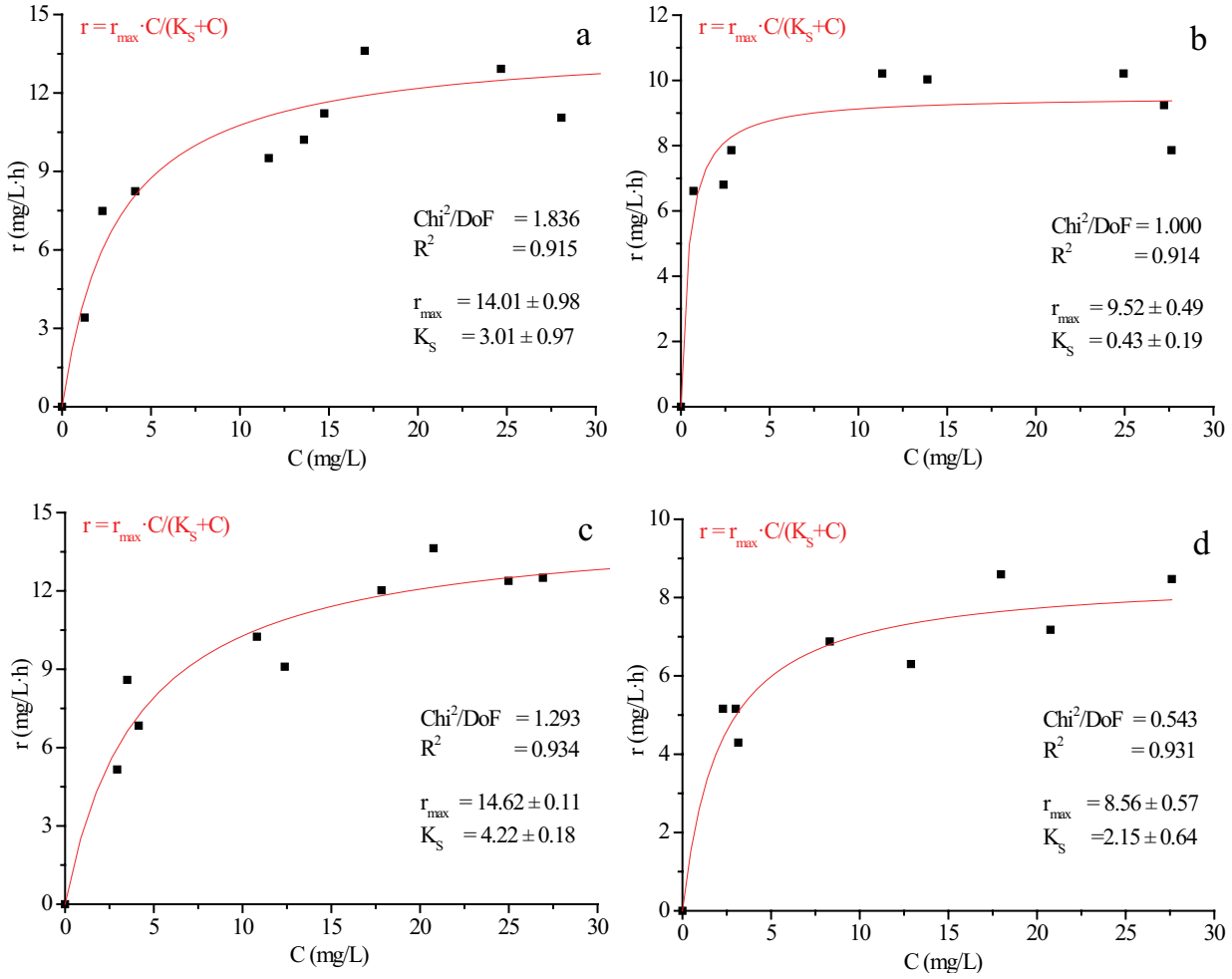

Fig. 3. Initial ammonia uptake rate (AUR) versus initial ammonia from SBR (a) and CSTR (b), initial nitrite uptake rate (NUR) versus initial nitrite content from SBR (c) and CSTR (d).

Half-saturation constants $K_{NH_4^+\text{-}N}$ for AOB were 3.01 ± 0.97 and 0.43 ± 0.19 mg NH_4^+-N/L in the SBR and CSTR, respectively; the half-saturation constants $K_{NO_2^-\text{-}N}$ for NOB were 4.22 ± 0.18 and 2.15 ± 0.64 mg NO_2^--N/L in the SBR and CSTR, respectively. The nitrifiers in the CSTR achieved higher affinity but lower activity than those in the SBR.

that their nitrification kinetics were fundamentally different. Both AUR and NUR increased much faster with increasing substrate in the SBR than in the CSTR. Furthermore, the half-saturation constants of AOB and NOB in the SBR were much higher than those of the CSTR. The kinetics indicates that the nitrifiers in the CSTR have much higher affinity and lower nitrification activity than in the SBR.

Different populations of nitrifiers could be responsible for different nitrification performances. This was further investigated by analyzing the most common populations of AOB and NOB via FISH.

3.4. Microbial community

FISH analyses were synchronized with batch tests of the nitrification activity biweekly after the reactors had achieved stable nitrification.

The percentage of nitrifiers/EUB (eubacterium) in the CSTR was slightly higher than in the SBR (Table 3 and Fig. 4). This may be due to the relatively lower settleability in the CSTR, caused by filamentous bulking and the MLSS in the CSTR (2.12 ± 0.18 g/L) was slightly lower than that in the SBR (2.36 ± 0.21 g/L). The amount of nitrifiers in both reactors was almost identical (2.12 g MLSS/L × 10.9% = 0.231 g nitrifiers/L for the CSTR, 2.36 g MLSS/L × 9.7% = 0.229 g nitrifiers/L for the SBR).

Fig. 5(a) shows the community structures of AOB in the seed source and both reactors during the stable stage. The analysis of the AOB community composition showed a clear

Table 3
Fraction of nitrifiers of the seed source and the activated sludge in both reactors at the stable stage

	Seed source	SBR	CSTR
AOB/EUB	4.3% ± 0.7%	4.3% ± 0.8%	4.1% ± 0.9%
NOB/EUB	5.6% ± 1.5%	5.4% ± 1.2%	6.8% ± 1.1%

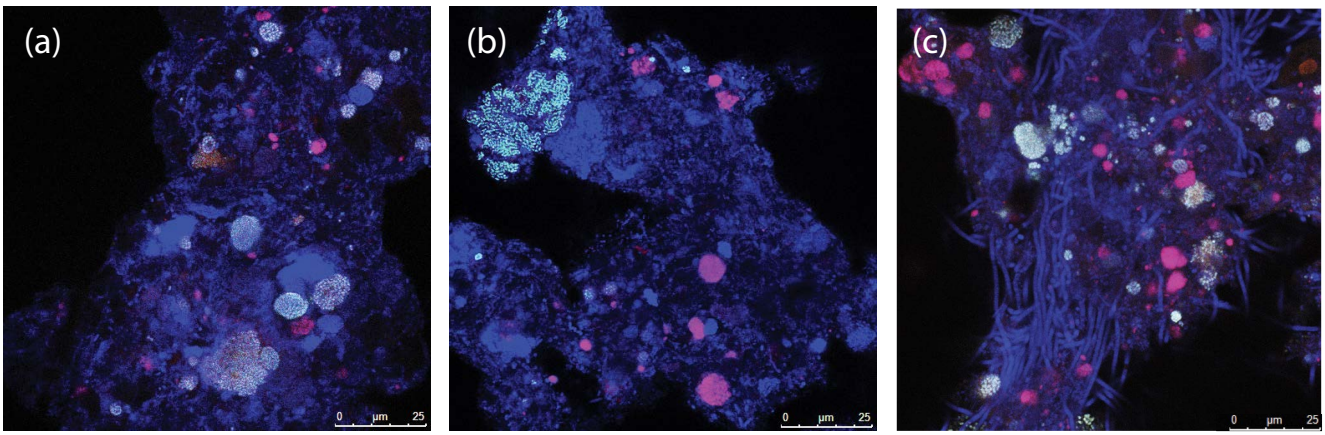

Fig. 4. Microbial community structure of nitrifying biomass in the (a) seed source, (b) SBR, and (c) CSTR. Samples from the seed source, SBR, and CSTR were hybridized with FITC-labeled AOB mix, Cy3-labeled NOB mix, and Cy5-labeled EUB to visualize the AOB (Green + Blue = Cyan), NOB (Red + Blue = Purple).

The amount of nitrifiers in the reactors was close to the seed source; however, the fraction of the nitrifiers/EUB in the CSTR was slightly higher than that in the SBR due to its relatively lower settleability which was caused by filamentous overgrowth.

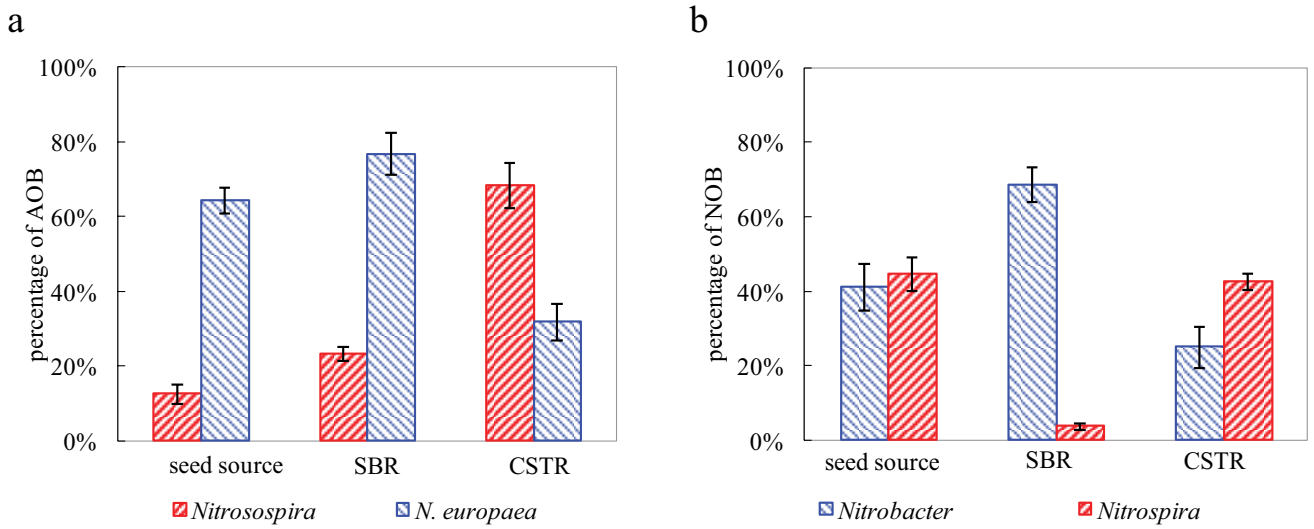

Fig. 5. Nitrifiers community structure in the seed source and reactors during the stable stage (n = 4): (a) AOB structure and (b) NOB structure.

The AOB community structure in the SBR resembles that in the seed source, the *r*-strategist (*N. europaea*) was the dominant AOB; however, in the CSTR, the dominant AOB was transferred to a *K*-strategist (*Nitrosospira*). The NOB community structure in the CSTR resembled that in the seed source: the *K*-strategist (*Nitrosospira* spp.) was the dominant NOB, while the dominant NOB was transferred to an *r*-strategist (*Nitrobacter*) in the SBR.

difference between both reactors. The dominating species was *N. europaea* accounting for 77% ± 6% of detected AOB in the SBR, resembling the seed source where the *N. europaea* was dominant AOB and accounted for 72% ± 4%. In contrast, the dominant AOB in the CSTR was shifted to the *Nitrosospira*, which accounted for 68% ± 6% of AOB, while *N. europaea* was decreased to 32% ± 5% of AOB.

These results can be explained with the *K*/*r* hypothesis, indicating *N. europaea* as an *r*-strategist, which has a relatively low substrate affinity and high adaptivity to environmental fluctuation; therefore, it should be dominant under high ammonia concentration and unstable environment [28–30], while *Nitrosospira* is a *K*-strategist, which has a relatively high substrate affinity and low adaptivity to environmental fluctuation; therefore, it should be dominant in a low ammonia concentration environment [12,31]. In the CSTR, the NH_4^+-N concentration was below 1 mg/L during the stable stage; therefore, the *K*-strategist (*Nitrosospira* spp.) was more competitive than the *r*-strategist (*N. europaea*). In contrast, in the SBR, the NH_4^+-N concentration was about 30 mg/L after the feed, and gradually decreased to below 1 mg/L in each cycle; therefore, the *r*-strategist (*N. europaea*) should be more competitive. These results are consistent with the findings of Dytczak et al. [14] and Terada et al. [20].

Figs. 1(a) and 5(a) also show that the shifts of dominated AOB in the CSTR may explain the ammonia accumulation in the CSTR during day 17 to 55 (Fig. 1(a)). During this period, most of the *r*-strategist (*N. europaea*) was washed out or had gradually faded, while the *K*-strategist (*Nitrosospira* spp.) gradually increased and ultimately dominated the CSTR, thus resulting in the accumulation of ammonia in the effluent of the CSTR.

In addition, combining Figs. 3(a) and 2(b) with Fig. 5(a), a clear distinction was observed between the *N. europaea*- and *Nitrosospira* spp.-dominated types of sludge in terms of ammonium half-saturation constants (K_S). *N. europaea*-dominated sludge (*r*-strategist) had higher "apparent" nitrite half-saturation constants and higher ammonia utilization rates compared with the *Nitrosospira* spp.-dominated types (*K*-strategist) of sludge. This correlated well with previous reports of *N. europaea* and *Nitrosospira* enrichment culture experiments as *r*- and *K*-strategists, respectively [12,30].

Fig. 5(b) shows the community structures of NOB in the seed source and both reactors during stable stage. In the seed source, *Nitrospira* showed a tendency to be dominant compared with *Nitrobacter*. However, the NOB community composition also exhibited a clear difference in both reactors. *Nitrobacter* was the predominant species and accounted for 90% ± 6% of the detected NOB (Probe NIT3 + Ntspa662) in the SBR, while *Nitrospira* was the dominant NOB in the CSTR, accounting for 63% ± 3% of detected NOB, which suggested the seed source that the *Nitrospira* was dominant AOB and accounted for 53% ± 4%.

These results can also be explained with the *K*/*r* hypothesis. In the CSTR, the NO_2^--N concentration always remained at a low level (0.1–0.5 mg/L) during the stable stage; therefore, the *K*-strategist (*Nitrospira*) was more competitive than the *r*-strategist (*Nitrobacter*); these results are consistent with Tangkitjawisut et al. [32] and Srithep et al. [33], in which only *Nitrospira* were detected in shrimp ponds where the nitrite concentrations were in a range of 0.02–0.17 mg NO_2^--N/L. However, in the SBR, the NO_2^--N concentration was about 0.2 mg/L after the feed, and increased to ~13.6 mg/L with the ammonia oxidation. When the ammonia oxidation process had finished, the nitrite concentration gradually decreased to 0.27 mg/L as shown in Fig. 2, therefore, the *r*-strategist (*Nitrobacter*) should be more competitive; these results are consistent with previous studies that reported the dominances of *Nitrobacter* at high nitrite concentrations [32].

In addition, as shown in Fig. 1(b), the nitrite accumulation in the SBR was much higher than that in the CSTR during day 9 to 37 (Fig. 1(b)), which may be caused by two reasons as follows: first, the ammonia in the SBR was almost transferred to nitrite (Fig. 1(a)), and then, the nitrite load should be higher in the SBR than that in the CSTR at the beginning of nitrite accumulation. Second, it can be inferred from Fig. 5(b) that most *K*-strategists (*Nitrospira*) were washed out or had gradually faded, while the *r*-strategist (*Nitrobacter* spp.) gradually increased and dominated the SBR finally during this period. Then, the shifts of NOB community resulted in sharp nitrite concentration fluctuations in the effluent of the SBR.

Combining Figs. 3(c) and 2(d) with Fig. 5(b) shows a clear distinction between *Nitrobacter*- and *Nitrospira*-dominated types of sludge in terms of nitrite half-saturation constants (K_S). *Nitrobacter*-dominated sludge (*r*-strategist) had higher "apparent" nitrite half-saturation constants and a higher nitrite utilized rate compared with *Nitrospira*-dominated types (*K*-strategist) of sludge. This correlated well with previous descriptions of *Nitrobacter* and *Nitrospira* enrichment culture experiments as *r*- and *K*-strategist, respectively [34].

Based on these results, it can be inferred that the difference in nitrification rates between the SBR and CSTR is caused by a combination of chemical reaction engineering kinetics and nitrifier community structure.

4. Conclusions

Reactor configuration poses an evident influence on nitrifier kinetics and the nitrifier community. Nitrifiers in the SBR have higher nitrification activity, but lower affinity than those of the CSTR. Furthermore, rapid growth *r*-strategists (*N. europaea* and *Nitrobacter*) were dominant in the SBR, while the slow-growing *K*-strategists (*Nitrosospira* and *Nitrospira* spp.) were dominant in the CSTR. The difference in the nitrification rate between the SBR and CSTR are likely due to the combination of reaction kinetics and nitrifier community.

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