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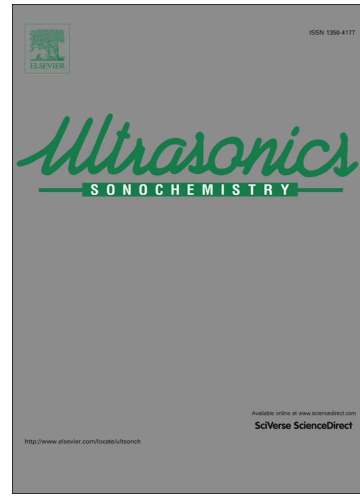

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Achieving mainstream nitrogen removal via the nitrite pathway from real municipal wastewater using intermittent ultrasonic treatment

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ABSTRACT

Achieving mainstream nitrogen removal via the nitrite pathway ($NH_4^+ \rightarrow NO_2^- \rightarrow N_2$) is highly beneficial for energy neutral/positive wastewater treatment. Our previous batch assays revealed that ultrasonic treatment can suppress nitrite-oxidizing bacteria (NOB) while enhancing the activity of ammonia-oxidizing bacteria (AOB). Based on this concept, this study investigated the feasibility of applying ultrasonication to achieve the nitrite pathway in mainstream wastewater treatment. Two lab-scale sequencing batch reactors were set-up in parallel and fed with real municipal wastewater. With 100% of the sludge treated every 12 h at a treatment energy input of 0.066 kJ per mg mixed liquor suspended solids, the nitrite pathway was rapidly (within two weeks) established in the experimental reactor with stable effluent nitrite accumulation ratio ($NO_2^-/(NO_2^- + NO_3^-)$) of above 80% and significantly decreased NOB population. In comparison, the control reactor always possessed the conventional nitrification and denitrification pathway. Economic analysis indicated that energy consumption is too high for practical applications. However, this technology may be used in conjunction with other technologies, whereby this ultrasonic treatment can be used infrequently (e.g. once every few months) when the nitrite pathway becomes unstable.

Keywords: Mainstream nitrogen removal; Nitrite pathway; Municipal wastewater; Nitrite-oxidizing bacteria (NOB) suppression; Intermittent ultrasonic treatment.

1. Introduction

Driven by requirement for energy neutral/positive wastewater treatment plants (WWTPs), mainstream nitrogen removal via the nitrite pathway ($NH_4^+ \rightarrow NO_2^- \rightarrow N_2$) has become extremely attractive in recent years because of its lower energy and carbon consumption in comparison with conventional nitrification/denitrification [1,2]. One key challenge for the nitrite pathway is to stop the oxidation of ammonia at nitrite rather than nitrate. To this end, nitrite-oxidizing bacteria (NOB) must be suppressed. Researchers have proposed many approaches, such as dissolved oxygen control [3], oxygen/ammonium flux ratio control [4], transient anoxia [5], free nitrous acid (FNA) [6] or free ammonia (FA) [7] –based sludge treatment, real-time aeration control [8] and maintaining required level of residual ammonium concentration (RAC) [9]. While all these strategies have been shown to suppress NOB to some extent, the stability of the suppression is still a critical issue to be fully addressed.

Ultrasound is cyclic sound pressure with a frequency greater than the upper limit of human hearing (> 20 kHz). Ultrasonic treatment has been considered a practical technology owing to its high efficiency, low instrumental requirements, significantly reduced process time compared with other conventional techniques and its economically viable performance [10,11]. The ultrasonication technology have been applied to many areas of environmental science and technology, i.e., in biocidal treatment of water. It has been previously reported that ultrasound can cause the destruction of micro-organisms, and thereby can be used effectively for water disinfection, especially in conjunction with chlorine [12].

In the WWTPs, ultrasonic pretreatment of sludge can improve performance of anaerobic digestion via destruction of cells [13,14]. It was also used in activated sludge systems to reduce excess sludge production through so-called 'ultrasonic lysis–cryptic growth' [15-16]. After ultrasonic treatment, mixed liquor properties were significantly changed with floc disintegration, organics release, temperature increase, microbial activity and pH variation [17].

Until recently, several recent studies further investigated the impacts of ultrasonic treatment on nitrogen removal performance during reactor operation. These studies showed that low-intensity ultrasound could increase the rates of nitrification [18,19], denitrification [20] or anammox [21,22] under optimized conditions. The selection of suitable parameter values for ultrasonic intensity or sludge treatment ratio (R_s, defined as percentage of treated sludge amount in the total reactor sludge amount) was important to avoid effluent quality deterioration [16]. In our recent study, ultrasound at a density of 0.09 kJ per mg volatile suspended solid enhanced start-up of nitritation process treating nitrogen-rich urine wastewater [23]. Moreover, using batch essays, our study revealed that ultrasonic treatment of sludge can suppress NOB and stimulate AOB. This finding suggests that ultrasonic treatment would provide a promising approach to enhance mainstream nitrogen removal via the nitrite pathway, but more tests are needed.

This study aims to evaluate the feasibility of applying ultrasonication to achieve the nitrite pathway in mainstream nitrogen removal. Fed with real municipal wastewater, two lab-scale sequencing batch reactors (SBR) with or without ultrasound treatment of sludge were operated in parallel. The parameters of ultrasonic energy density and R_s were tested at different levels to establish the nitrite pathway. The degree of nitrite accumulation was monitored, along with the overall nitrogen removal performance and the variation in the NOB and AOB populations. The impacts of the ultrasonic treatment on the sludge concentration and settleability were also assessed. The mechanisms responsible for achievement of the nitrite pathway and implications of the ultrasonication technology for mainstream deammonification are discussed.

2. Materials and methods

2.1 Reactor set-up, operation and monitoring

Experiments were performed in two laboratory-scale SBRs operated at ambient temperature. One was coupled with an ultrasound generator as experimental reactor and the other was set as control. The reactors were made from Plexiglas cylinder in duplicate. Each one had an effective volume of 9.0 L (height 40 cm and inner diameter 20 cm). Each cycle of the SBR operated with 12 hours, including influent (13 min), anoxic mixing (2.5 h), aeration (5.5 h), settling (50 min), effluent (10 min) and idling (167 min). The above feeding regime and cycle time give rise to a hydraulic retention time (HRT) of around 1.1 day. The sludge retention time (SRT) was maintained at 30–40 d with periodical discharge of excess activated sludge. Dissolved oxygen (DO) concentration was online monitored. The DO concentration was controlled at 0.6-0.8 mg/L during the aerobic period using the signal control system (Chemitec S423/C/OPT, Italy).

The two SBRs were operated for 250 days, during which the operating conditions of the control reactor remained the same, as described above. The operation of the experimental reactor consisted of five phases, as described below.

Phase I (Baseline phase, Day 0–120): the two SBRs were operated under the same conditions for 120 days to establish steady-state, as indicated by the almost identical ammonium, nitrite and nitrate concentrations over cycles, mixed liquor suspended solid (MLSS) concentration and sludge volume index (SVI).

Following that, Phases II–V (Day 120–250): in the experimental reactor, ultrasonic treatment was performed each 12 hour (equal to one SBR cycle) within the idling stage of each cycle. The activated sludge in the reactor was collected and concentrated, which resulted in a solids concentration in the range of 10–12 g MLSS/L. The concentrated sludge was then transferred into the ultrasonic treatment unit for treatment. After the treatment, the thickened sludge was mixed with the separated supernatant and returned to the experimental reactor (Fig. 1). Relatively low R_s (20%) was firstly used (Phase II), and then increasing the R_s up to 100% was introduced to investigate the impacts of different ultrasonic intensities on nitrogen removal performance (Phase III–V). Table 1 summarizes the operational conditions in different running phases in the experimental reactor. In this study, the used ultrasonic generator (ZJS–1000–500, Hangzhou Success Ultrasonic Equipment Co., Ltd) had a frequency of 40 kHz. The active power was 100 W. The inputting treatment irradiation energy was controlled by varying the treatment time. The applied ultrasonic energy density (E_S, kJ/mL) was calculated using

$$E_S = \frac{P \times t}{V} \quad (1)$$

where, P is the ultrasonic power (W), t is the irradiation time (s), V is the effective volume of the volumetric flask (L). So, the specific energy density (kJ/mg MLSS) was defined as the E_S per MLSS (g/L).

Figure 1

The ammonium in influent and ammonium, nitrite and nitrate concentrations in effluent were measured 2–4 times every week. Cycle studies in the two SBRs were performed 2 times every operational phases by analysing the nitrate, nitrite, and ammonium concentrations every 30−60 min in a cycle. MLSS concentration and SVI were detected 1–2 times for a week. The NOB and AOB populations at Steady States I and V were examined by real-time quantitative PCR.

2.2 Raw wastewater and seed sludge

Raw municipal wastewater was collected from a sewer pipe on the campus of Tsinghua University. It was firstly pumped into an intermediate tank (0.1 m^3) and stored at 4°C before being pumped into the SBRs. The collected wastewater contained average 235 ± 143 mg/L chemical oxygen demand (COD), 48.3 ± 12.7 mg/L total nitrogen (TN) and 45.9 ± 14.0 mg/L NH_4^+-N, as well as < 1.0 mg/L NO_2^--N and NO_3^--N. The influent COD/TN ratio was less than 5.0, which is deficient in organics for conventional biological nitrogen removal.

Inoculation was from a full-scale membrane bioreactor (MBR) treating municipal wastewater on the campus of Tsinghua University (Tsinghua Water Reuse, Beijing). The plant configured with an A/O process with operational SRT of 25–40 day produces

1200–1300 tons clean water per day for the university's water reuse. Good performance of organics and ammonium nitrogen removal with efficiencies above 90% were obtained during long-term operation of the MBR process. The seed sludge for the two SBRs was taken from the aerobic tank when the plant had stable nitrogen removal performance as well as relatively constant MLSS concentration.

2.3 Analytical methods

Measurements of COD, NH_4^+–N, NO_2^-–N, NO_3^-–N, total suspended solids (TSS) and MLSS concentrations were performed in the accordance with Standard Methods [24]. DO, pH and temperature were automatically recorded using a pH/DO meter (WTW, pH/Oxi340i). Nitrite accumulation ratio in the effluent was calculated by the percentage of the nitrite nitrogen concentration in the total nitrate and nitrite nitrogen concentrations.

2.4 DNA extraction and real-time quantitative PCR

The sludge samples were collected by concentrating at 5,000 rpm for 4 min. Fast DNA Spin Kit for Soil (MP Biomedicals, LLC, Solon, OH, USA) was applied following the manufacturer's instructions for genomic DNA extraction. Total DNA was eluted in 50 μL of sterile water and stored at -20°C until use. The concentration and purity of DNA were detected using a NanoDrop spectrophotometer (ND2000, Nanodrop, USA).

Quantitation of AOB, *Nitrospira*-like and *Nitrobactor*-like NOB gene in activated sludge were determined by real-time quantitative PCR. The specific primer pair CTO189f/CTO654r (5'－GGAGRAAAGCAGGGGATCG－3'/ 5'－CTAGCYTTGTAGTTTCAAACGC－3') was used for AOB [25]. NOB specific primers for *Nitrospira* and *Nitrobacter* were

NSR1113r/NSR1264f (5' － CCTGCTTTCAGTTGTTGCTACCG － 3'/ 5' － GTTTGCAGCGCTTTGTACCG － 3') [26] and P338f/Nb1000r (5' － ACTCCTACGGGAGGCAG－3'/ 5'－TGCGACCGGTCATGG－3') [27], respectively. A 20μL Reaction system was used, which contained 16.5 μL of 2×SybrGreen qPCR Master Mix, 0.8 μL of each primer (10 μM) and 2 μL of DNA template. Thermo-cycle conditions were set up on an ABI 7500 Real-Time PCR System (Applied Bio systems, USA) as follows: 10 min at 95°C for initial start, then 40 cycles of 10 s at 95°C, 34 s at 60°C and 15s at 95°C. Melting curves were obtained by 60 s at 60°C, 30 s at 95°C and 15 s at 60°C. The final quantitation of AOB, *Nitrospira* and *Nitrobacter* was calculated based on the standard curves generated during the amplification.

3. Results and discussion

3.1 Achievement of the nitrite pathway

Two SBRs were operated in parallel for 120 days to establish the steady state (Phase I), as indicated by the almost identical ammonium, nitrite and nitrate concentrations over cycles, biomass concentrations and sludge volume indices. Subsequently, the experimental reactor was performed with the intermittent ultrasonic treatment. As shown in Fig. 2a, with application of the low R_s the nitrification performance remained good (ammonia removal efficiency remained high at 99% with almost no effluent nitrite), whereas the effluent ammonium, nitrite and nitrate nitrogen concentrations were significantly affected by use of the high R_s even at three different levels of energy inputs. Specifically, the ultrasonic treatment of 1.2 kJ/mL made rapid build-up of effluent ammonium (Phase III), indicating that ultrasonic extensive energy input would cause loss of ammonia oxidation activity. With the energy shift to relative low-intensity of 0.6 kJ/mL, the ammonia oxidation process recovered (Phase IV). Then, with a middle energy value of 0.9 kJ/mL, the effluent nitrite-N concentration increased considerably, reached 10-20 mg/L and stabilized at this level over two months (Phase V). The effluent nitrate-N concentration decreased down to 1-3 mg/L. This gave rise to a stable effluent nitrite accumulation ratio of above 80% in the experimental reactor. By contrast, almost no nitrite accumulation was presented in the effluent of the control reactor during the overall operation (Fig. 2b). This indicates that the intermittent ultrasonic treatment of sludge, under the optimized conditions of 0.9 kJ/mL radiation energy, 100% sludge treatment ratio and 12 h interval time, was effective in establishing the mainstream nitrogen removal through the nitrite pathway.

Figure 2

NOB and AOB populations were quantitatively detected in the two SBRs. With operation as long as 120 days at relatively low DO ranging from 0.6−0.8 mg/L (Phase I), the numbers of *Nitrospira*-like NOB reached 10^9 gene copies per g-MLSS sludge both in the experimental and control reactors. In the sludge, the numbers of *Nitrospira*-like NOB detected were two orders of magnitude greater than those of *Nitrobacter*-like NOB, suggesting that *Nitrospira* was the dominant NOB in this study. On day 184, Phase V commenced, the number of *Nitrospira*-like NOB decreased approximately an order of magnitude in the sludge of the experimental SBR whereas the number was still at a similar level in the sludge of the counterpart (Fig. 3a). The low NOB population conclusively reveals that the majority of NOB were eliminated from the experimental SBR after implementing the intermittent ultrasonic treatment. In addition to that, the quantitative PCR results showed that the numbers of AOB population (copies per g-MLSS sludge) were not negatively affected following the commencement of ultrasonic treatment in Phase V (Fig. 3b). Consistently, the q-PCR analysis confirmed the expected accomplishment of nitrogen removal performance through the nitrite pathway.

Figure 3

3.2 Evaluating the biological nitrogen removal performance via the nitrite pathway

The nitrogenous compound concentrations in the periodic cycle studies were investigated to assess the impact of the nitrite pathway on the biological nitrogen removal performance. Fig. 4 shows the comparative results on typical variation of nitrogenous compound concentrations in Phase V between the control and experimental reactors. Anoxic denitrification and aerobic nitrification (from ammonium and nitrite to nitrate) were presented in a typical SBR operational cycle of the control reactor (Fig. 4a). The anoxic denitrification was not complete (nitrite was still presented at the end of anoxic period) because the influent biodegradable organic carbon source was inadequate. As such, the nitrite presented was converted to nitrate in the aerobic period again. In contrast, ammonium was almost completely oxidized to nitrite during the aerobic period in the experimental reactor, and consequently this gave rise to a short-cut denitrification with nitrite as electron acceptor during the anoxic period (Fig. 4b). The result indicated that the accomplishment of the nitrite pathway in anoxic/aerobic SBR could theoretically achieve more nitrogen removal with less consumption of organic carbon source and oxygen. Furthermore, with the intermittent ultrasonic treatment, total nitrogen removal was observed in the aerobic period (Fig. 4b). The rate of aerobic total nitrogen removal reached 2.07 mg N/(L·h), and was approximately half of the denitrification rate under anoxic condition (5.36 mg N/(L·h)). As the length of the aerobic period nearly doubled that of anoxic period, the amounts of total nitrogen loss were basically the same in the two periods. Therefore, the ultrasonication technology would be able to enhance both anoxic and aerobic nitrogen removal through the nitrite pathway, resulting in improved the overall biological nitrogen removal performance.

Figure 4

3.3 Impact of intermittent ultrasonic treatment on the sludge concentration and settleability

Fig. 5a shows that the sludge concentration in the experimental reactor over the experimental period was significantly lower under the intermittent ultrasonic treatment than the concentration in the control reactor. In Phase V, the average sludge concentration in the experimental reactor was reduced from 2431 ± 675 mg/L to 1366 ± 268 mg/L. The sludge reduction ratio is similar in Phase II when relative low-intensity and low R_S parameters were applied (Table 1). This indicates that the sludge reduction was not enhanced with the increment of R_S value.

Figure 5

As shown in Fig. 5b, SVI varied significantly over the experimental period and showed a clear correlation with the R_s. In Phase II, when the R_S is set as 20%, the SVI gradually decreased from 100 mL/g to 60 mL/g, and then maintained at a low level in the experimental reactor compared with the SVI value in the control reactor. The SVI rapidly increased to 120 mL/g in 10 days with application of the high R_S of 100% (Phase III), and reached a maximum value of 150 mL/g on day 202 during the start-up of partial nitrification (Phase V). Notwithstanding this variation of SVI, the decline in sludge settleability did not significantly deteriorate the effluent quality, i.e., the effluent TSS concentrations remained unchanged before and after the ultrasonic treatment (17.3 ± 10.1 mg/L vs 21.1 ± 19.7 mg/L).

3.4 The mechanism responsible for the nitrite pathway

The experimental results presented in Fig. 2-4 show that ultrasonic-assisted biological reaction should be responsible for the achievement of the nitrite pathway in the mainstream nitrogen removal. In the previous study [23], we reported ultrasonic bidirectional effect based on the results of AOB and NOB activities in batch assays, that is, AOB activity reached a peak level and then declined but NOB activity deteriorated continuously as the power intensity of ultrasound increased. This is consistent with the results in this work by using batch activity assays (Fig. S1). Hereby, we hypothesize that achieving the nitrite pathway in mainstream was stimulated by the ultrasonic bidirectional effect. The intermittent ultrasonic treatment suppressed NOB. So, observation of the effluent nitrite accumulation should be the resultant of different response of AOB and NOB activities towards the ultrasonic treatment.

The ultrasonic enhancement on the AOB activity has been reported previously. Zhang *et al.* [18] found that NH_4^+-N removal loading was improved by 16.5% in an ultrasonic enhanced reactor (35 kHz, 0.15 W/cm^2, and irradiation time of 10 min) compared with that in control reactor. Zheng *et al*. [19] also reported an increase in the NH_4^+-N removal loading up to 48.7-129.5% by use of low frequency and density ultrasound (40 kHz and 0.027 W/mL). According to recent investigation on the AOB growth kinetics, ultrasound likely reduced the activation energy of key enzymes involved in ammonium oxidation bacteria [28].

On the other hand, cell lysis by ultrasound had also been widely studied. Since 1960s, ultrasound had been generally recognized as a non-thermal sterilization technique. The studies on ultrasonic germicidal efficacy showed that the effects of irradiation time on four bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, were significant but different [29]. It had also been reported that *Pseudomonas fluorescens* was less resistant to ultrasound than *Streptococcus thermophilus* [30]. The mechanism of ultrasonic germicidal efficacy was believed to be cell disintegration and the germicidal efficacy varied among different bacterium because of the difference of complex intracytoplasmic membranes (i.e., Gram-positive bacteria have a cell wall made up of a rigid framework of a cross-linked peptidoglycan layer that is 10–15 times thicker than in Gram-negative bacteria) [31]. However, the commonly-known AOB and NOB in wastewater treatment are both Gram-negative bacteria. Another mechanism explanation was proposed by Gao *et al.* [32]. In their studies, generation of hydroxyl radicals and hydrogen peroxide were considered as main mechanism for bacterial inactivation by high-frequency ultrasound.

However, the inhibitory mechanism of ultrasound on the NOB activity is still rarely reported. It can be believed that the above sonochemical effects all have important impacts on the NOB behaviour, such as growth and decay rates, but these need to be further studied.

As known previously, ultrasonic treatment was effective in reducing waste activated sludge in sewage treatment [15,16]. In these studies, the ultrasonic conditions of specific energy densities of 13.3-26.7 kWh/kg TS (equal to 0.048-0.096 kJ/mg TS) and a treating frequency of 1-3 times/day were close to the operational parameters (0.032-0.073 kJ/mg MLSS and 2 time/day) in our study except that a high R_s up to 100% was only tested in this work (upper limit of 30% as previously reported in these studies). Consistently, excess sludge reduction could be achieved with a low parameter value of R_s (Fig. 5a). However, as observed in this study, the achievement of the nitrite pathway highly depends on the high R_s value. This finding is important because it demonstrated that sludge treatment ratio was a key factor to achieve the nitrite pathway in the mainstream bioreactor by implementing the ultrasonic-assisted biological reaction.

Previous studies also showed that low DO may play a role in achieving the nitrite pathway [3,33]. In this work, a control reactor was overall operated at low DO (0.6-0.8 mg/L), and *Nitrospira*-like NOB was well enriched in the activated sludge. This result is consistent with the results reported in a comparative study on long-term operation at high and low DO [34]. Therefore, low DO unlikely contributed for the NOB washout in our experimental reactor.

3.5 Implications of ultrasonication technology for mainstream deammonification

Currently, many researchers have put forward their efforts to the achievement of stable mainstream autotrophic nitrogen removal by partial nitritation and anammox. Our study demonstrated that mainstream nitrogen removal through nitrite pathway could be achieved by intermittently ultrasonic treatment of activated sludge. Ultrasonication is a well-established mechanical technology for enhancing sewage sludge disintegration and subsequent anaerobic digestion [13]. It had at least 20 full-scale and 17 pilot-scale installations, mostly in Germany [14]. These installed ultrasonic equipment can be modified for the treatment reported in this study, thereby resulting in benefits for the start-up or recovery of mainstream deammonification in these municipal WWTPs.

Moreover, the energy consumption needs to be properly considered in the practical application of the ultrasonication technology. Although the ultrasonication hold its advantage of enhancing AOB activity when the nitrite pathway is achieved compared with many other approaches, it is clear that continuous use of ultrasonication in the mainstream sludge treatment would be economically unfavourable (According to economic analysis and SI). However, the ultrasonication could be implemented and used to rapidly eliminate the NOB from activated sludge system when they get adapted to other treatment methods such as the FNA-based technology to achieving the nitrite pathway [35]. This suggests that a combination of multiple technologies such as ultrasonic and FNA-based sludge treatment would be useful for maintenance of long-term stable mainstream deammonification in the future. Based on the SBR operation and optimized ultrasonic conditions (0.9 kJ/mL, 100% sludge treatment ratio, interval time 12 h), we assumed that implementing the ultrasonication is needed to rapidly (within two weeks) exclude the adapted NOB once a year (365 days). The electric energy consumption can be calculated to be 0.7 kW·h/m^3 (in term of full-year sewage flow), which is relatively high compared with the corresponding cost in current municipal WWTPs (0.27-1.89 kW·h/m^3) [36]. The reason might be that most of the ultrasonic energy is applied to heterotrophic bacteria rather than nitrifiers. Considering that more efficient organic carbon may be recovered prior to the nitrogen removal process in next generation energy neutral/positive WWTP, the economic efficiency can be significantly improved with the perspective of implementing ultrasonication to establish the nitrite pathway in mainstream deammonifciation process.

4. Conclusions

Mainstream nitrogen removal through the nitrite pathway was successfully achieved in a conventional anoxic/aerobic SBR using intermittent ultrasonic treatment. It was demonstrated that the ultrasonic approach was effective to suppress the NOB and thus nitrite production with an effluent nitrite accumulation ratio of above 80% could be stably achieved. Following the ultrasonic establishment of the nitrite pathway, total nitrogen removal could occur under both anoxic and aerobic conditions. The occurrence mechanism of nitrite accumulation could be explained by the ultrasonic bidirectional effects on AOB and NOB activities. The increase in sludge treatment ratio from 20% to 100% was identified to be a key factor to achieve the nitrite pathway, and it slightly declined the sludge settleability yet did not significantly deteriorate the effluent quality. Further research is required to understand the sonochemical effects on the cell lysis of NOB, to explore the application strategy, and to decrease the energy consumption.

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Table 1. Ultrasonic conditions in different running phases in the experimental reactor.

Running phases	E_S (kJ/mL)	Irradiation time (min)	Specific energy density (kJ/mg MLSS)	R_s (%)
I (1–120 days)	0	0	0	0
II (121–150 days)	0.6	10	0.046 ± 0.007	20
III (151–167 days)	1.2	20	0.073 ± 0.003	100
IV (168–183days)	0.6	10	0.032 ± 0.000	100
V (184–268 days)	0.9	15	0.066 ± 0.013	100

Figure Captures

Fig. 1. Schematic diagram of the control and experimental systems.

Fig. 2. Profiles of influent and effluent ammonium, effluent nitrate and nitrite concentrations and nitrite accumulation ratio in the experimental reactor (a) and control reactor (b).

Fig. 3. Quantitatively detected NOB (a) and AOB (b) populations in the sludge of the experimental and control reactors.

Fig. 4. Typical variation of nitrogenous compound concentrations in Phase V at the condition of nitrate pathway along the control reactor (a) and nitrite pathway along the experimental reactor (a), respectively.

Fig. 5. Profiles of MLSS concentration (a) and SVI (b) in the experimental and control reactors.

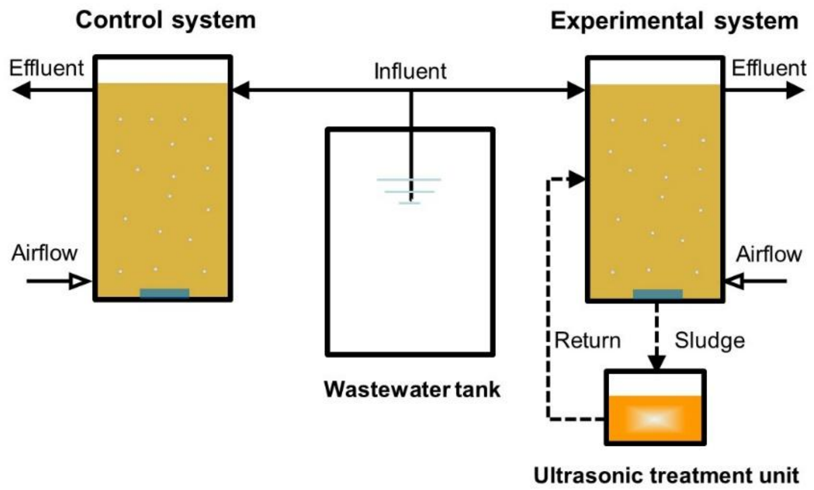

Fig. 1. Schematic diagram of the control and experimental systems.

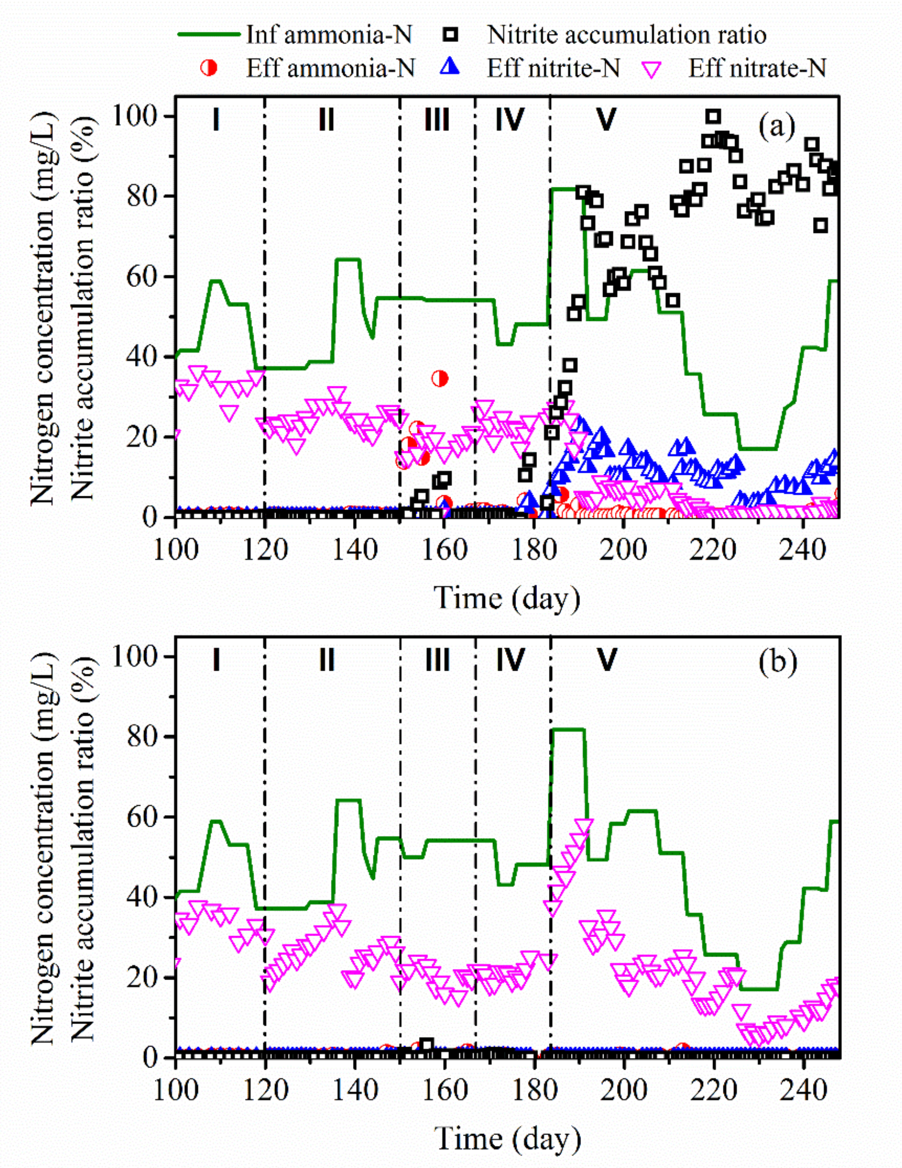

Fig. 2. Profiles of influent and effluent ammonium, effluent nitrate and nitrite concentrations and nitrite accumulation ratio in the experimental reactor (a) and control reactor (b).

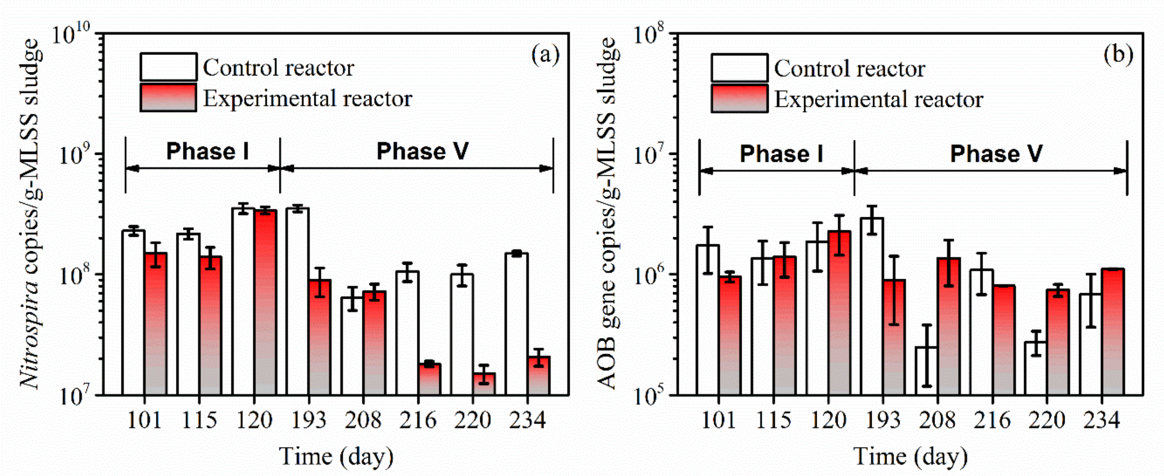

Fig. 3. Quantitatively detected NOB (a) and AOB (b) populations in the sludge of the experimental and control reactors.

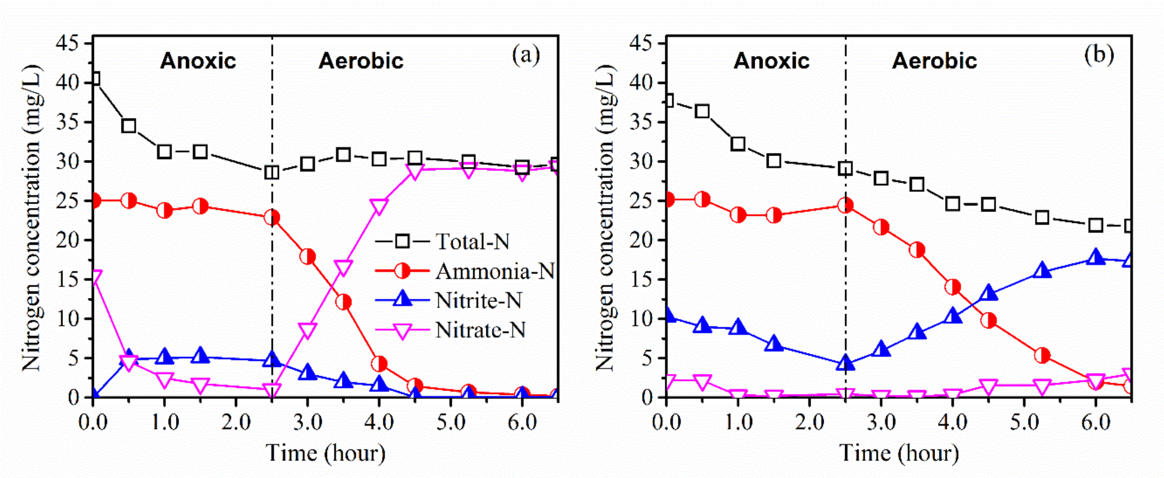

Fig. 4. Typical variation of nitrogenous compound concentrations in Phase V at the condition of nitrate pathway along the control reactor (a) and nitrite pathway along the experimental reactor (b), respectively.

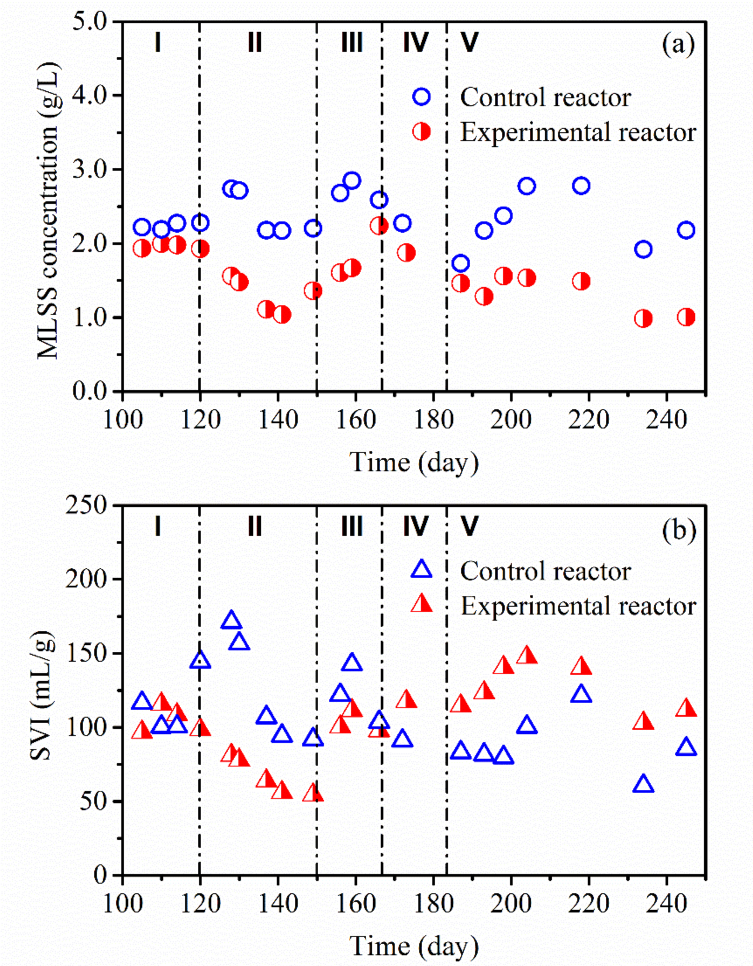

Fig. 5. Profiles of MLSS concentration (a) and SVI (b) in the experimental and control reactors.

Highlight

- Intermittent ultrasonic treatment is an effective way to establish nitrite pathway
- Population of nitrite-oxidizing bacteria is successfully suppressed
- Sludge treatment ratio (R_S) is identified to be an important operational parameter