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SHORT COMMUNICATION

Partial anammox achieved in full scale biofilm process for typical domestic wastewater treatment

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HIGHLIGHTS

- A full scale biofilm process was developed for typical domestic wastewater treatment.
- The HRT was 8 h and secondary sedimentation tank was omitted.
- *Candidatus Brocadia* were enriched in the HBR with an abundance of 2.89%.
- Anammox enabled a stable ammonium removal of ~15% in the anoxic zone.

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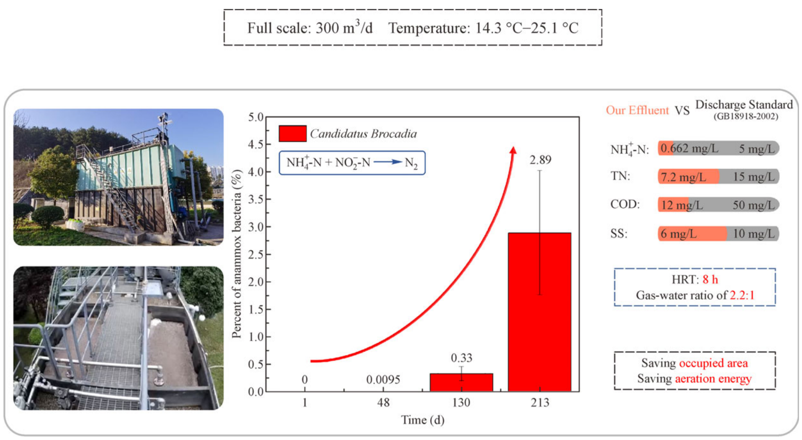
GRAPHIC ABSTRACT



ABSTRACT

The slow initiation of anammox for treating typical domestic wastewater and the relatively high footprint of wastewater treatment infrastructures are major concerns for practical wastewater treatment systems. Herein, a 300 m^3/d hybrid biofilm reactor (HBR) process was developed and operated with a short hydraulic retention time (HRT) of 8 h. The analysis of the bacterial community demonstrated that anammox were enriched in the anoxic zone of the HBR process. The percentage abundance of *Candidatus Brocadia* in the total bacterial community of the anoxic zone increased from 0 at Day 1 to 0.33% at Day 130 and then to 2.89% at Day 213. Based upon the activity of anammox bacteria, the removal of ammonia nitrogen (NH_4^+-N) in the anoxic zone was approximately 15%. This showed that the nitrogen transformation pathway was enhanced in the HBR system through partial anammox process in the anoxic zone. The final effluent contained 12 mg/L chemical oxygen demand (COD), 0.662 mg/L NH_4^+-N, 7.2 mg/L total nitrogen (TN), and 6 mg/L SS, indicating the effectiveness of the HBR process for treating real domestic wastewater.

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1 Introduction

Due to rapid urbanization, resource recovery through wastewater treatment has gradually become the next-generation theme for global wastewater sector. With the trend of turning wastewater treatment plants (WWTPs) from a site for removing certain pollutant into a site for water and energy reuse and generation, abundant cutting-edged technologies were developed to synergistically harness sustainable water supply, energy-efficient operation and multiple environmental benefits (Lu et al., 2018; Qu et al., 2019; Chen, 2020).

In traditional wastewater treatment processes, the conversion and removal of nitrogen determines the footprint and energy consumption. Nitrogen species in wastewater are finally converted into nitrogen gas mainly through autotrophic nitrification and heterotrophic denitrification, which results in the use of large land area and high energy consumption (Zhang et al., 2013; Jenni et al., 2014; Ji et al., 2014; Xiao et al., 2014). Anaerobic ammonium oxidation (anammox) is a promising nitrogen removal process developed in the late 1990s. Anammox bacteria can oxidize ammonium using nitrite as the electron acceptor, thus reducing the consumptions of oxygen and carbon by $>50\%$ each (Yang et al., 2017; Ding et al., 2020; Xu et al., 2021). Even though anammox bacteria has been demonstrated in more than 100 projects for the treatment of N-rich wastewater, stable anammox could be rarely achieved in main stream operations, especially in the weak domestic wastewater (Winkler et al., 2012; Ma et al., 2016; Cao et al., 2017; Li et al., 2019a). It is mainly because the sludge retention time (SRT) of a typical WWTP is generally not sufficient to retain the slow-growing anammox bacteria, whereas the anammox bacteria are also vulnerable to many environmental conditions including high dissolved oxygen and high organic carbon concentrations. To enhance the retention of biomass, cultivating the biofilm to enhance SRT is an effective strategy to enrich the slow-growing anammox biomass (Ali et al., 2015; Li et al., 2016; Gu et al., 2017; Zhang et al., 2019).

In this study, a hybrid biofilm reactor (HBR) was developed and operated in a 300 m^3/d full scale to achieve the goals of efficient nitrogen removal and low land occupation. The carrier's filling-ratio of the anoxic and aerobic zones were 60% and 75%, respectively, and the secondary sedimentation tank was omitted in the system. During the operation, nitrogen removal was enhanced by the addition of hydroxylamine and the application of intermittent aeration. The mechanism of efficient nitrogen removal was investigated, and the functional bacterial community was analyzed. To the best of our knowledge, this is the first investigations into the fast initiation of anammox in a full scale reactor for treating typical domestic wastewater.

2 Materials and methods

2.1 Full scale reactor design and operational conditions

A full scale HBR reactor was developed in this study, and consisted of an anoxic zone and two aerobic zones (Fig. 1(a)). The total working volumes of the anoxic and aerobic zones were 46.8 m^3, 32.0 m^3 and 21.3 m^3, respectively. Biological carriers (Fig. 1(b)) with a total volume of 68.1 m^3 were filled into the anoxic zone (60% filling; 28.1 m^3) and the two aerobic zones (75% filling; 24.0 m^3 and 16.0 m^3, respectively). Carriers in each reaction zone were fix-bedded for better holding the biofilm and filtrating SS, except when backwashing took place (Fig. S1). Detailed information about the carrier design and the operational conditions for the HBR process are presented in Table S1. The reactor was continuously operated and investigated for more than 200 days. The designed influent flow was 300 m^3/d, an HRT of 8 h was employed during the operation. There were two stages in the entire operational period: Stage 1 was the control group where no anammox occurred (Day 1 to Day 50). Stage 2 was the experimental stage where anammox bacteria were enriched (Day 51 to Day 213). Based upon the preliminary testing, an optimized hydroxylamine dosing of 5 mg/L was added to the first aerobic zone twice a day and an intermittent aeration strategy was applied to stimulate the short-cut nitrification in Stage 2.

All the tests were conducted at Guiyang, China during the time period of June–December, 2020. The influent wastewater temperature lied within the range of 14.3°C–25.1°C. The integrated water samples from each day were analyzed and used to better represent the overall performance of the whole day. The characteristics of the influent domestic wastewater in the HBR process listed in Table S2.

2.2 Analytical methods

The concentrations of NH_4^+-N, TN, nitrite (NO_2^--N), nitrate (NO_3^--N), COD, Total phosphorus (TP), and SS were measured according to the standard methods (APHA, 1998). Temperature and DO concentrations were monitored using a WTW340i probe (WTW Company, Germany). The anammox activity of the anoxic biofilms on the biological carrier was measured in a jar test that employed a medium containing ammonium and nitrite as the reactants, and minerals and trace elements to better support the microbial metabolism (van de Graaf et al., 1996). Bacterial community of the biofilm in the anoxic zone was analyzed at Day 1, Day 48, Day 130 and Day 213. On these specific time periods, five parallel samples were simultaneously taken from the anoxic zone to reduce the measurement bias. DNA extraction, PCR and high-throughput sequencing procedures were conducted according to a previous report (Du et al., 2017).

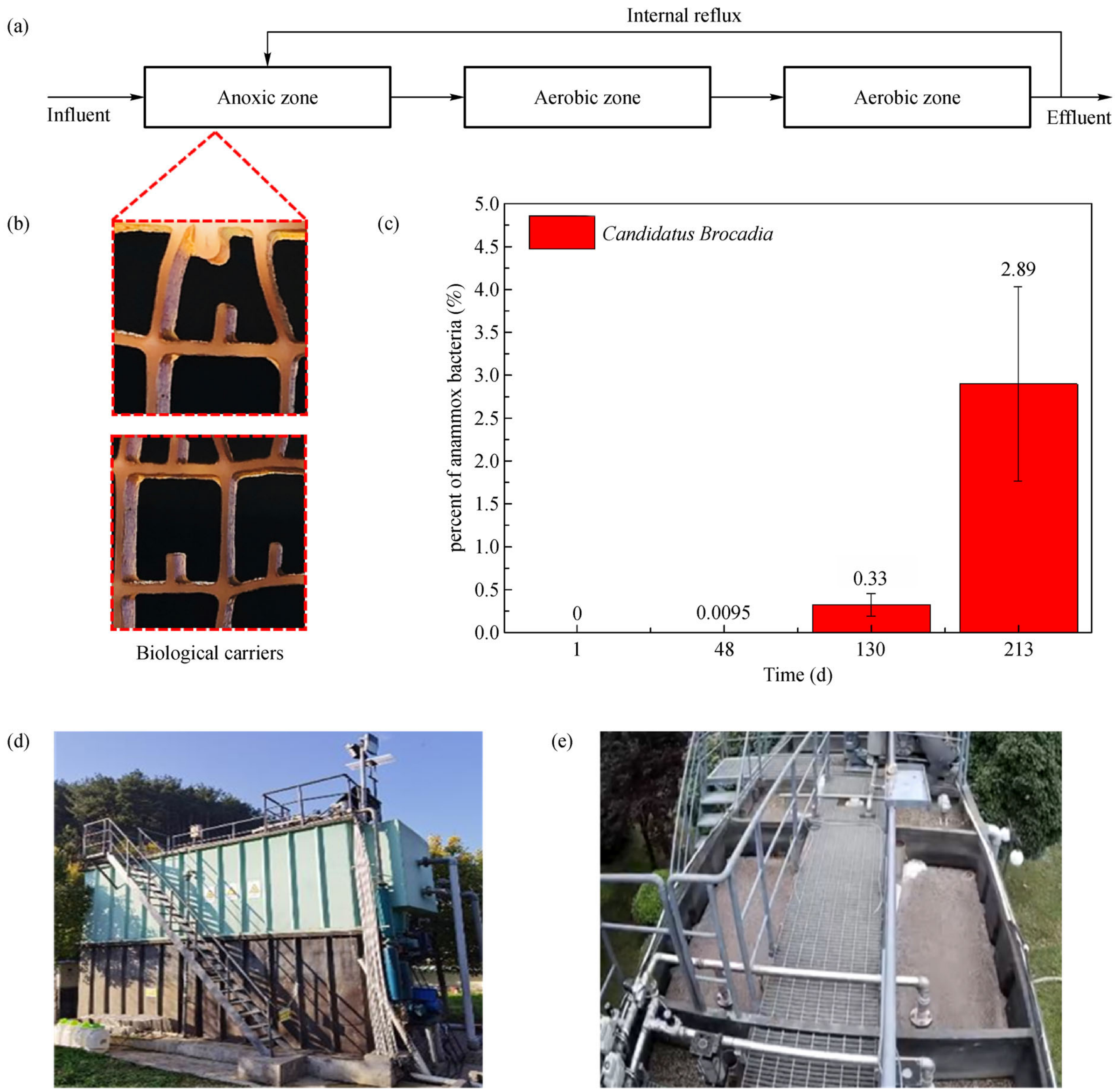

Fig. 1 The flow chart of the HBR system (a), the biological carriers in the anoxic zone showing biofilm attachment (b), biological community analysis showing the percentage abundance of anammox bacteria in the biofilm attached on the carriers sampled from the anoxic zone (c), and the side- and top-view of the full scale HBR system (d and e).

2.3 Calculations

Nitrite accumulation ratio was calculated using Eq. (1) (Zhang et al., 2017b):

$$\mathrm{NAR}(\%) = \left(\frac{\mathrm{NO_2^-}}{\mathrm{NO_2^-} + \mathrm{NO_3^-}}\right)*100\%, \quad (1)$$

where NO_2^- and NO_3^- (mg/L) were the concentrations of NO_2^--N and NO_3^--N in the effluent, respectively.

Anammox removal contribution ratio in the anoxic zone was calculated using Eq. (2).

$$R = \frac{\left(C_{inf} \cdot Q_{inf} + C_{eff} \cdot Q_{ref}\right) - C_{anx} \cdot \left(Q_{inf} + Q_{ref}\right)}{C_{inf} \cdot Q_{inf}}, \quad (2)$$

where C_{inf}, C_{eff}, and C_{anx} (g/m^3) represent the concentrations of NH_4^+-N in the influent, final effluent and anoxic zone effluent, respectively, and Q_{inf} and Q_{ref} (m^3/h) represent the flow rates of influent and internal reflux, respectively.

3 Results and discussion

3.1 Anammox enrichment in the anoxic zone

The enrichment of anammox in the HBR process was strongly evidenced by the increase in relative abundance of *Candidatus Brocadia* in the total bacterial community of the anoxic zone. The relative abundance of *Candidatus*

Brocadia increased from 0 at Day 1 to 0.33% at Day 130, and then to 2.89% at Day 213 (Fig. 1(c)). Considering the fact that the proportion of anammox bacteria is usually < 0.2% in full scale wastewater treatment systems (Wang et al., 2015a; Guo et al., 2017; Li et al., 2019a) (Table S3), the relative abundance of anammox bacteria of 2.89% in the HBR system (Figs. 1(d) and1(e)) was an order of magnitude higher than the previous studies. *Candidatus Brocadia* has a higher proliferation rate and can express more complete function than other anammox bacteria (Zhang et al., 2017a; Zhao et al., 2019). It is worth noticing that *Candidatus Brocadia* detected in this study was the same typical dominant genus detected in other anammox-based systems under mainstream conditions, especially for treating real domestic wastewater (Laureni et al., 2015; Li et al., 2019a).

The contribution of NH_4^+-N removal in the anoxic zone was shown in Fig. 2(a), whereas the accumulation of NO_2^--N in the aerobic zone was shown in the Fig. 2(b). In Stage 1, the contribution of NH_4^+-N removal in the anoxic zone was negative, indicating that the concentration of NH_4^+-N increased, which was likely caused by ammonification. Meanwhile, there was rarely any accumulation of the nitrite in the aerobic zone, indicating that no ammonium oxidation had occurred in Stage 1.

Starting from the beginning of Stage 2, hydroxylamine was added into the aerobic zone, and NO_2^--N could be detected in the final effluent after 7 days' operation (Fig. 2 (b)). Hydroxylamine has been shown to enable short-cut nitrification in some previous laboratory-scale studies (Xu et al., 2012; Wang et al., 2015b; Li et al., 2019b), while in the current work, the application of hydroxylamine in a full scale reactor was studied for the very first time to treat real domestic sewage. The ratio of the internal reflux from the aerobic zone to anoxic zone was 200%. After the short-cut nitrification had been achieved in the aerobic zone, NO_2^- could be recirculated back into the anoxic zone. Due to this reason, the influent NH_4^+-N and the refluxed NO_2^--N both existed in the anoxic zone, providing key substrates for stimulating the growth of the anammox bacteria. The contribution ratio of NH_4^+-N removal in the anoxic zone remained at *ca*. 15% since the 115th day (Fig. 2(a)). Moreover, 600 mL biological carriers were carefully taken out from the anoxic zone at Day 168 to measure the anammox activity in a jar test (Fig. S2). A specific anammox activity of 18.6 mg N/L/d was observed based on the decrease in the concentration of ammonium.

3.2 Comprehensive contaminant removal in the HBR system

The HBR system can stably remove NH_4^+-N, TN, COD, TP and SS (Fig. 3) within a short HRT of 8 h and a low gas-water ratio of *ca*. 2.2:1. The average removal efficiency for

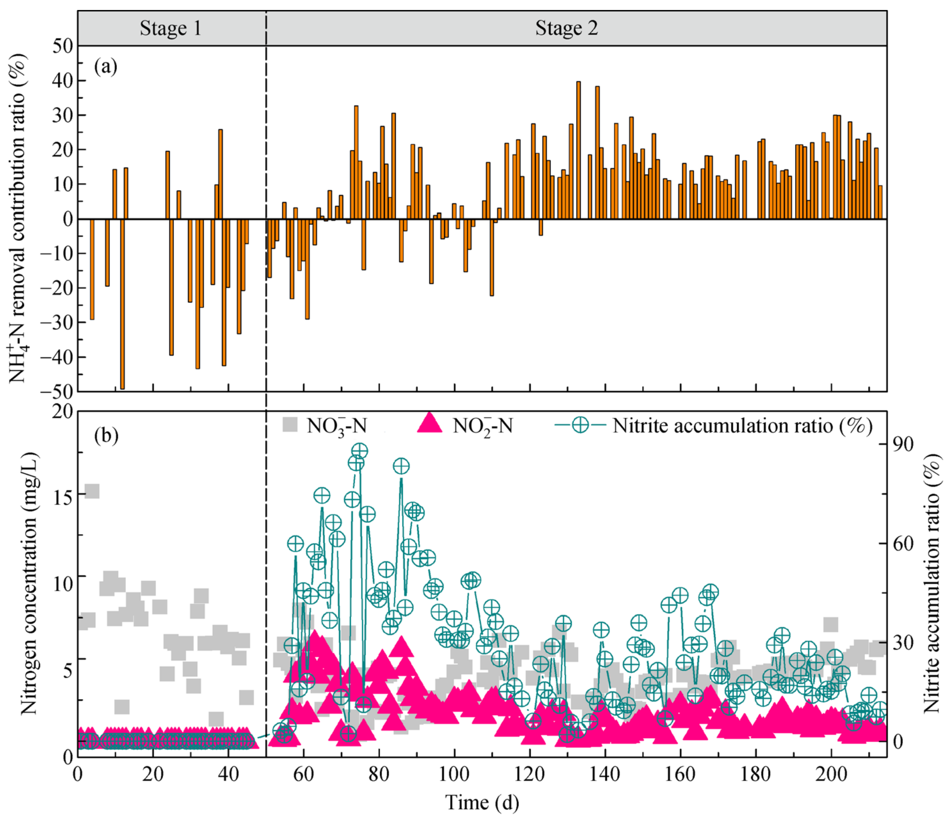

Fig. 2 Contribution ratio for NH_4^+-N removal in the anoxic zone (a) and NO_2^--N concentration in the final effluent (b).

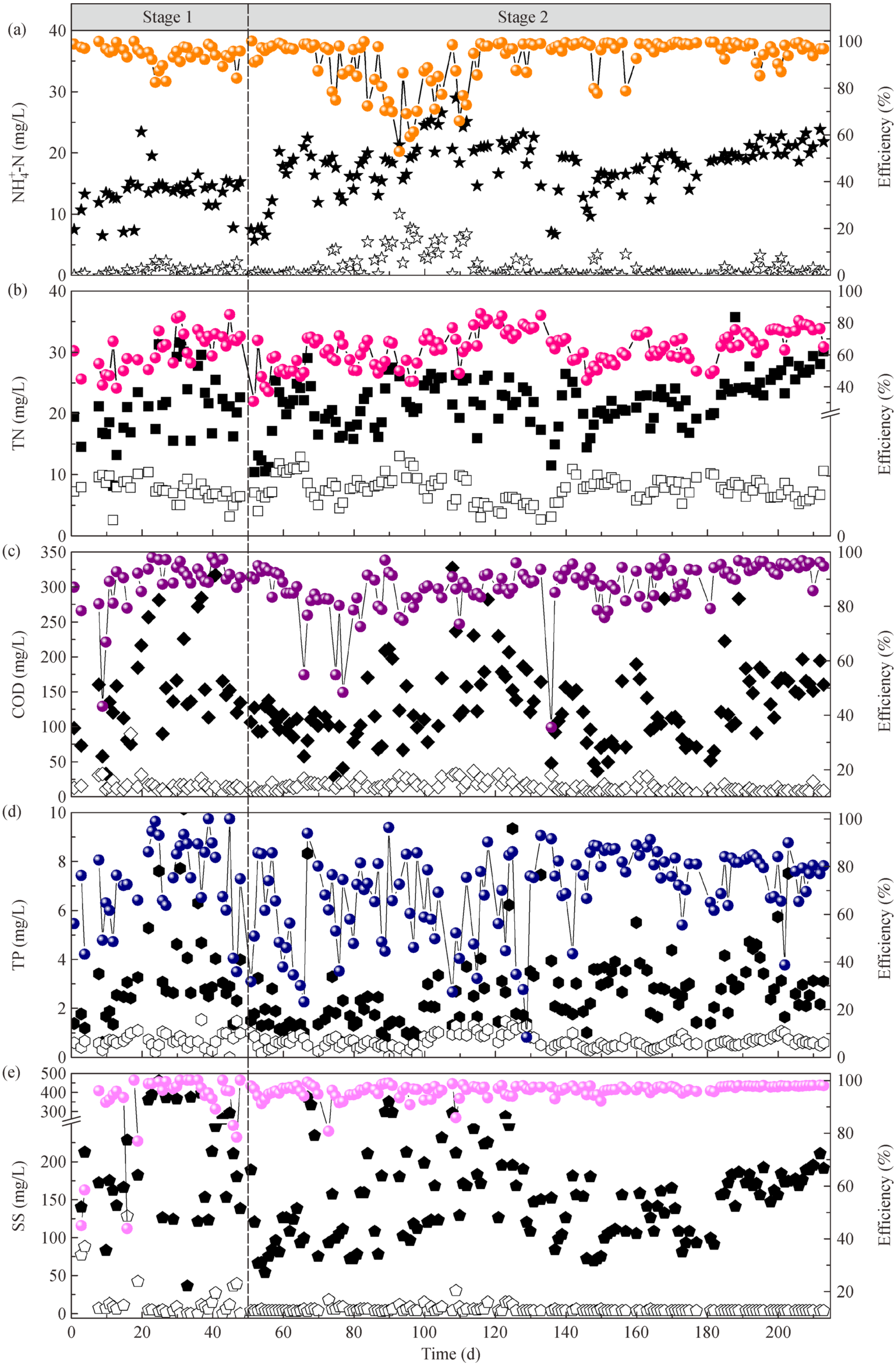

Fig. 3 Removal results for NH_4^+-N (a), TN (b), COD (c), TP (d), and SS (e) in the HBR process during the whole operation period. The solid symbols represent influent concentrations, the hollow symbols represent effluent concentrations, and the colored balls represent removal efficiencies.

NH_4^+-N was kept at a very high level of approximately 96.30% (Fig. 3(a)). An even lower gas-water ratio of 1.5:1 was also tested during the 82^{nd}–115^{th} day, however NH_4^+-N could not be completely removed (thus not meeting the strict Chinese standard) due to insufficient aeration. Therefore, the gas-water ratio was adjusted back to 2.2:1 for the rest of the operation. The average TN removal efficiency was 59.18% in Stage 1 with a influent C:N ratio of 8.8, while the TN removal efficiency in Stage 2 increased to 67.39% even with a lower influent C:N ratio of 6.0 (Fig. 3(b)). A better TN removal in Stage 2 was achieved under a disadvantageous influent condition, which was due to the effectiveness of the partial anammox in the anoxic zone, as discussed previously (Section 3.1).

Moreover, COD, TP, and SS could also be adequately removed in the HBR system (Figs. 3(c)–3(e)). For the influent average concentrations of COD, TP and SS of 135 mg/L, 2.80 mg/L and 147 mg/L in Stage 2, respectively, the corresponding average effluent concentrations of COD, TP and SS of 12 mg/L, 0.67 mg/L and 6 mg/L, respectively were obtained. During the whole testing period of over 7 months that spanned summer and winter, the effluent concentrations of COD and SS were stably up to the first Grade A standard of discharge standard of pollutants for domestic wastewater treatment plant (GB18918-2002, 50 mg/L for COD and 10 mg/L for SS). The values continuously met the standard despite huge fluctuations in the influent. It is noted that the SS removal was effective in the HBR system with an average removal efficiency of 95.99%. A photo of the influent and effluent water samples in Fig. S3 showed that the effluent looked very clear. This could be attributed to the excellent filtration effect of the fix-bedded carriers in the HBR operation.

3.3 Economic advantages of the HBR system

The fixed-bed of the biological carriers was formed by increasing the filling ration to 60%–75% and optimizing the operational parameters of aeration mode in the HBR system. Additionally, the flow pattern in each reaction zone was basically the plug flow, which improved the mass transfer rate and reduced the HRT. The resulting HRT of 8 h was shorter than that in a conventional process, which usually lies within the range of 12–18 h (Chang and Ouyang, 2000). Since the concentration of SS in the final effluent was reduced to a very low level of < 10 mg/L through physical filtration and biological adhesion effects, a secondary sedimentation tank was no longer needed in the HBR system. Therefore, land occupation for the proposed system could be greatly reduced compared to a conventional wastewater treatment plant. Meanwhile, the low gas-water ratio reduced the aeration in the HBR and further increased the energy efficiency.

4 Conclusions

In summary, anammox bacteria were effectively enriched in the anoxic biofilm in a full scale HBR system with a capacity of 300 m^3/d. The achieved partial anammox in the anoxic zone was shown to be very active and enhanced the total nitrogen removal. For the real domestic wastewater over a time span of 7 months, the average effluent concentrations of COD, nitrogen and SS stably met the Grade A standard of discharge standard of pollutants for domestic wastewater treatment plant (GB18918-2002) with just a short HRT of 8 h, representing a higher efficiency than a traditional treatment process. Considering the excellent treatment results and the economic benefits of this compact biofilm HBR system, we could expect it has a promising application potential, providing good energy efficiency and more sustainable land-use for wastewater treatment system.

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