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# Spontaneous initiation and maintenance of partial nitritation for household toilet wastewater treatment

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**ABSTRACT**

Partial nitritation (PN) can be achieved in the aerobic treatment of toilet wastewater (TW) independent of accurate control of working parameters due to its high concentration in free ammonia and a low ratio of alkalinity to total ammonia nitrogen. Although the mechanism of spontaneously realizing the ideal PN for TW has been clearly elaborated, few researches on the application of PN to household toilet wastewater (HTW) treatment are available. In this study, a biofilm reactor was established to study the PN of HTW, especially focusing on the effect of HTW drainage characteristics on the PN. After a 100-d operation, the results showed that the ideal PN can also be initiated and maintained spontaneously for HTW. Moreover, the drainage characteristics of HTW is beneficial for maintaining the stability of PN and achieving sludge reduction, which will support the establishment of subsequent partial nitritation anaerobic ammonia oxidation process for HTW.

*Keywords:* Single-family blackwater; Partial nitritation; Drainage characteristics; Sludge reduction; Microbial behavior

## 1. Introduction

The toilet revolution campaign of the Chinese government resulted in the installation of toilet facilities across China, despite the inadequate sewer network of rural regions. As such, wastewater treatment there is gradually becoming the research focusing as it signifies a promising strategy to fill the gap in rural sanitation. Compared to the centralized treatment mode, the decentralized treatment may be more suitable for the scattered rural areas due to the lower average construction cost per household [1]. As the smallest scale of decentralized treatment, single-family treatment fully plays its flexibility. The household Johkasou system is representative of single-family treatment, which is widely used in rural areas of Japan [2]. However, the construction cost of current household wastewater treatment technologies remains expensive. Moreover, the treatment effect is unstable because of the high fluctuation of both the amount and quality of household wastewater. The challenge of this unstable treatment effect mainly lies in the control of total nitrogen (TN). Toilet wastewater (TW) typically contains more than 90% total ammonia nitrogen (TAN, TAN = $NH_3$–N + $NH_4^+$–N) and TN, while only accounting for about 20% of the volume of household wastewater [3,4]. Therefore, the separate collection and treatment of TW is a cost-effective method to control N-pollution in household wastewater [5]. Furthermore, compared to centralized treatment, household treatment is relatively easier to achieve a separate collection of TW.

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TW is a typical high TAN and high pH wastewater. TAN and TN concentrations of the pretreated TW are in the range of 150–318 mg/L and 151–336 mg/L, respectively, with pH levels at about 8.5 [6,7]. Several technologies have been practically applied for nitrogen removal from TW, most of which are based on biological nitrification–denitrification where TW is diluted 10 to 20-fold; alternatively, advanced technologies and membrane processes can also be employed [6,8]. The advent of partial nitritation-anaerobic ammonia oxidation (PN/A) technology provides a new option for the nitrogen-removing process of TW [9].

The realization of PN is an essential prerequisite for the technology application. Interestingly, TW is a kind of wastewater with a high concentration in free ammonia (FA) and a low ratio of alkalinity to TAN, and the ideal PN can be achieved in the aerobic treatment without accurate control of working parameters [10], such as pH [11], dissolved oxygen (DO) [12], and sludge retention time [13]. The reason for that can be explained by the collaborative inhibition mechanism of FA and free nitrous acid (FNA). Specifically, FA concentration of TW is in the range of 20–80 mg/L [6,14], and FA begins to suppress the activity of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) from the range of 10–150 mg/L and 0–1 mg/L, respectively [15,16]. Therefore, the NOB can be readily washed out from the aerobic treatment process of TW because of the discriminative inhibition by FA. Additionally, the ratio of alkalinity to TAN in TW is ~3.80 g $CaCO_3$/gN [14], and it takes 7.14 g alkalinity to convert 1 g $NH_4^+$–N to $NO_2^-$–N. Therefore, the pH of TW will decrease to ~6.00 during the nitritation process due to the lack of alkalinity, and the FNA concentration in nitritation unit will be as high as 0.86 mg/L, which will suppress the activity of AOB [14,16]. To sum up, the synergistic inhibition of FA and FNA contributes to spontaneously achieving the ideal PN for TW.

So far, the mechanism of spontaneously realizing the ideal PN for TW in the aerobic treatment process has been clearly elaborated. In this study, the ideal PN is planned to be achieved in the aerobic treatment of household toilet wastewater (HTW), which is the foundation for the establishment of PN/A technology. The drainage period of HTW is evenly dispersed over the awake state of people, and the drainage frequency and volume are relatively stable [17]. Understandably, the study on the PN of HTW could involve the effect of household drainage characteristics on the PN, which is also the innovation of this paper. Therefore, the aim of this paper is to investigate the initiation and maintenance of PN for HTW, while researching the influence of HTW drainage characteristics on the PN.

## 2. Materials and methods

### 2.1. Experimental system

The experimental system consisted of two sequential vessels made of polyvinyl chloride (Fig. 1). The first vessel was used for TW pretreatment, mainly by physical separation and anaerobic reactions. It consisted of two sequential anaerobic units, the first of which had a working volume of 4.8 L and did not use packing; the second unit was packed with stereoscopic mesh filler (texture-polypropylene, porosity-97%, filling rate-70%) and had a working volume of 2.4 L.

The second vessel also consisted of two sequential units, the first of which was defined as the aerobic unit for aerobic reactions and the second was defined as the settling unit where the effluent settled and was discharged. Each of the units in the latter vessel had a working volume of 1.6 L. The aerobic unit was packed with spherical polypropylene fillers (diameter-25 mm, porosity-84%, filling rate-50%) with a filling rate of ~50%, at the bottom of which a sandy aerator (bubble stone, Chongguan, Shanghai, China) was placed for aeration. An outlet was arranged at the bottom of the settling unit for the drainage of possible excess sludge. The HTW flowed through all four units in sequence, driven by gravitational forces and eventually flowed out the experimental system after sedimentation.

### 2.2. Influent of anaerobic and aerobic unit

The influent of the anaerobic unit was taken from a TW collection tank, which collected wastewater from a flush toilet. A stirring device was set in the tank and will be turned on when feeding the experimental system. The hydraulic retention time (HRT) of TW in the tank was about 1 d. The influent of the aerobic unit was the effluent of the anaerobic unit. Table 1 gives the properties of the influent of the anaerobic and aerobic units (Fig. S1).

### 2.3. HRT and loading rate of the partial nitritation unit (aerobic unit)

Table 2 shows the HRT and loading rate of the PN unit (aerobic unit) for TW in this study and several references. As shown in Table 2, both the HRT and loading rate of the PN unit was within a reasonable range. The variations of these two parameters in studies may be due to the different aerobic technologies, feed strategies, and biomass of sludge.

### 2.4. Experiments

It is well understood that the frequency of excretion per person is about 6 times per day. The discharge volume

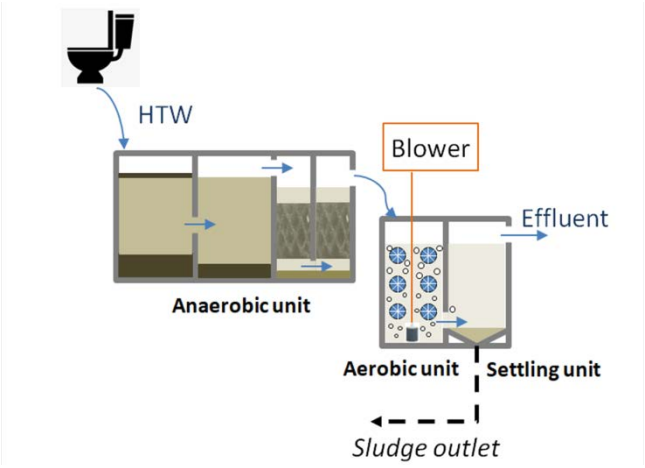


Fig. 1. Schematic diagram of the experimental system for the treatment of household toilet wastewater.

Table 1
Characteristics of the influent of anaerobic and aerobic unit

| Item | Influent of anaerobic unit | Influent of aerobic unit |
|---|---|---|
| pH | 8.75–8.95 | 8.51–8.79 |
| Chemical oxygen demand, (mg COD/L) | 510–1,232 (791) | 200–551 (394) |
| Total ammonia nitrogen, (mg N/L) | 162–357 (257) | 172–316 (238) |
| Total nitrogen, (mg N/L) | 227–404 (311) | 229–357 (281) |
| Alkalinity, (mg $CaCO_3$/L) | N.A. | 836–1,133 (992) |

N.A.: Not available;
Values in parentheses are the average of concentrations.

Table 2
HRT and loading rate of the partial nitritation unit for toilet wastewater in this study and references

| Item | This study | References [6,10,14] |
|---|---|---|
| Hydraulic retention time, (d) | 0.61 | 0.33–0.83 |
| Chemical oxygen demand loading rate, (g COD/d/$L_{Reactor}$) | 0.603 | 0.627–0.963 |
| Total ammonia nitrogen loading rate, (g N/d/$L_{Reactor}$) | 0.357 | 0.325–0.636 |

of TW per time is similar and the period of discharge is evenly distributed. Also, there is no TW discharge during bedtime. To simulate the daily drainage of family toilets as possible, the reactors were fed six times a day in batches, respectively at 8:30, 10:30, 14:30, 16:30, 19:30 and 21:30.

The reactor was filled with clean water before start-up. The operation of the reactor was divided into two phases, phase-1and phase-2, respectively. Phase-1 was from day 1 to day 34, and phase-2 was from day 35 to day 100. The volume load in phase-1 was 200 ml per time, and that in phase-2 was 400 mL per time.

Twice samplings are taken in one day for monitoring water qualities. The first sampling was at 8:30 before a feed, and the second sampling was at 21:30 before a feed. The water quality at a specific sampling day was expressed as the averaged values of the twice samples in the day.

During the experiment, no excess sludge was discharged from the settling unit, and the liquid temperature was 24°C–27°C.

### 2.5. Analytical methods

TAN, $NO_2^-$–N, $NO_3^-$–N, TN, suspended solids, and alkalinity were tested according to the standard methods [18]. Chemical oxygen demand (COD) was measured using a reagent bottle method (COD reagent 20–1,500 mg/L, Hach, USA). pH, DO, and the temperature was monitored using a pH/DO meter (HQ30D53000000-PHC10103/LDO10103, Hach, USA).

### 2.6. Analysis of the microbial community structure

On days 10, 30, and 90, the biofilm attached to the filler in the aerobic unit was extracted and stored in the dark at –20°C prior to the analysis of the microbial community structure. The bacterial community was analyzed by high-throughput sequencing, and primers 515F and 805R were selected to target the 16S rDNA V4 region (which is commonly used for bacterial community analysis). The biofilm samples were sent to Sangon Co., Ltd. (Shanghai, China) for pretreatment and pair-end sequencing using the Illumina MiSeq sequencing system (2xTaq Master Mix, Vazyme Biotech, Nanjing, China). Sample pretreatment consisted of the extraction of DNA, first and second PCR amplification, purification and recovery of DNA, and quantitative mixing. Based on the sequencing reads, data processing and statistical analyses were performed, which included distinguishing sample sequences and performing quality control. Then, operational taxonomic unit (OTU) cluster analysis, taxonomic classification analysis, phylogenetic analysis, and function prediction analysis were performed in sequence. The accession number of the high-throughput sequencing results is PRJNA670546.

### 2.7. Calculations

Eqs. (1) and (2) were used to calculate FA and FNA concentrations in solution, respectively [19]:

$$\mathrm{FA}\left(\mathrm{mg/L}\right)=\frac{17}{14}\times\frac{C_{\mathrm{TAN}}\times 10^{\mathrm{pH}}}{e^{\frac{6{,}334}{273+T}}+10^{\mathrm{pH}}} \tag{1}$$

$$\mathrm{FNA}\left(\mathrm{mg/L}\right)=\frac{47}{14}\times\frac{C_{\mathrm{NO_2^-N}}}{\left(e^{-\frac{2{,}300}{273+T}}\times 10^{\mathrm{pH}}\right)} \tag{2}$$

where $C_{\mathrm{TAN}}$ (mg/L) and $C_{\mathrm{NO_2^-N}}\left(\mathrm{mg/L}\right)$ represent the concentrations of TAN and $NO_2^-$–N, respectively, and $T$ (°C) represents the solution temperature.

## 3. Results and discussion

### 3.1. Initiation and maintenance of partial nitritation

#### 3.1.1. Variation of N-compounds concentration

The experimental period lasted for 100 d. Fig. 2a shows the variation of $NO_2^-$–N and TAN concentration in the aerobic unit effluent. Fig. 2b shows the variation of $NO_2^-$–N/$NH_4^+$–N and nitrite accumulation rate (NAR, $NO_2^-$–N/($NO_2^-$–N + $NO_3^-$–N) in the effluent of the aerobic unit.

The experimental system was filled with tap water before initiation and therefore, initially, the variation of water quality along the direction of flow was affected by both the dilution of pre-filled water and the reactions occurring in the system. Subsequently, the dilution effect lasted for several days and the biological reaction gradually became the dominant factor.

During the initial 10 d, the TAN concentration continuously increased, while the $NO_2^-$–N concentration was negligible (Fig. 2a). From day 15 to day 18, the TAN concentration decreased sharply from 157.5 to 99.2 mg/L, while the $NO_2^-$–N concentration simultaneously increased from 22.5 to 120.8 mg/L. Following this, N-compounds concentration in the effluent of the aerobic unit entered a stable period. The TAN and $NO_2^-$–N concentration combined, accounted for about 99% of TN, while the $NO_3^-$–N concentration was relatively negligible (Fig. 2b). These results showed that AOB was enriched in the reactor while NOB was not, and the nitritation was successfully initiated. From day 35 to day 100, although the influent volume load was doubled, the nitritation efficiency remained almost constant, and the $NO_3^-$–N concentration remained negligible.

In this study, the desired PN was readily achieved for HTW under the onsite treatment mode without the regulation of reaction conditions. The $NO_2^-$–N/$NH_4^+$–N in the effluent of the aerobic unit remained constant at about 1.3 after the startup of the experimental system (Fig. 2b), which is consistent with the results reported [10,14].

#### 3.1.2. Synergistic mechanism of inherent free ammonia and endogenous free nitrous acid

Fig. 3 gives the changes of FA, FNA concentration, and pH in the effluent of the aerobic unit. As shown in Fig. 3a, from day 1 to day 34, FA concentration initially increased and reached a maximum of 57.7 mg/L on day 11. Following this, the FA concentration sharply decreased and reached a negligible level (0.45 mg/L) on day 18. In the first 11 d of operation, FA concentration in the aerobic unit had a high level, resulting in the suppression of NOB, while AOB was enriched exclusively. This enabled the initiation of nitritation in the aerobic unit. From day 15 to day 18, the TAN

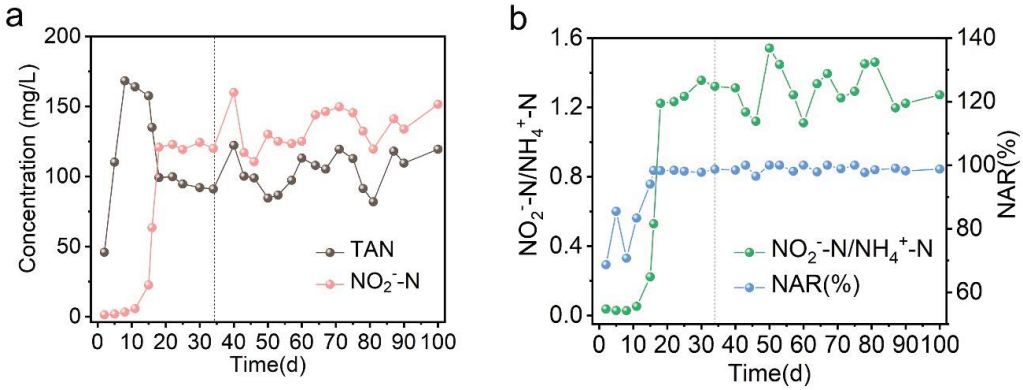


Fig. 2. (a) Variation of $NO_2^-$–N and total ammonia nitrogen concentration in the aerobic unit effluent and (b) variation of the ratio of $NO_2^-$–N to $NH_4^+$–N ($NO_2^-$–N/$NH_4^+$–N) and nitrite accumulation rate (NAR, $NO_2^-$–N/($NO_2^-$–N + $NO_3^-$–N) in the aerobic unit effluent.

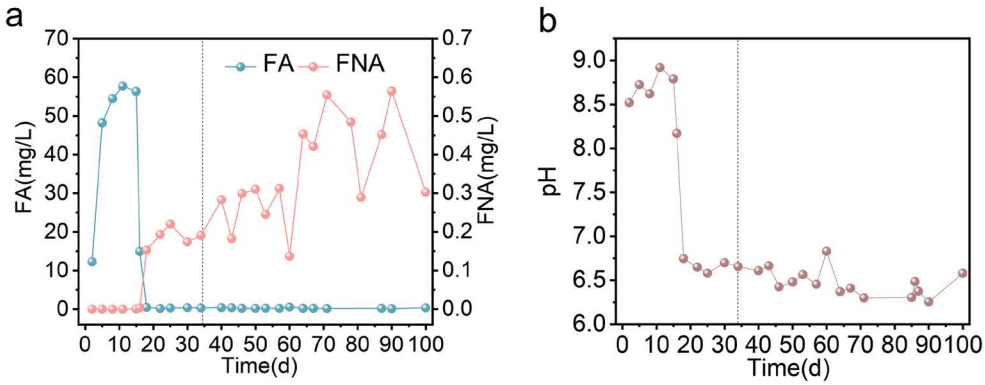


Fig. 3. Variation of (a) free ammonia, free nitrous acid concentration and (b) pH in the effluent of the aerobic unit (*T*, 24°C–27°C).

concentration sharply decreased, while the $NO_2^-$–N concentration correspondingly increased (Fig. 2a) and the pH decreased from 8.79 to 6.75 (Fig. 3b). These resulted in a decrease of FA concentration to a negligible level, while FNA simultaneously and rapidly increased from 2.56E-4 mg/L to 0.15 mg/L (Fig. 3a).

FNA exerts a broad range of antimicrobial effects on bacteria. It has previously been reported that NOB and AOB inhibition is initiated by FNA concentrations of 0.01–0.2 mg/L and 0.2–2.8 mg/L, respectively [15]. Therefore, NOB was selectively suppressed in the aerobic unit, which effectively explained why $NO_3^-$–N concentration in the aerobic unit remained negligible after FA concentration became very low from day 18 onward.

In summary, NOB showed a two-stage inhibition process. During the first stage (day 1-day 15), it was inhibited by FA, while during the second stage (after day 15) it was inhibited by FNA. Two stages switched simultaneously, resulting in NOB having almost no opportunity for growth and oxidizing $NO_2^-$–N to $NO_3^-$–N, which efficiently maintained the stability of nitritation. Following the successful startup of the experimental system, the calculated FNA concentration fluctuated in the range of 0.2 to 0.6 mg/L (Fig. 3a).

#### *3.1.3. Effect of the drainage characteristics of household toilet wastewater on the partial nitritation*

Fig. 4 shows the variation of wastewater quality in the aerobic unit before and after feed during a cycle. As shown in Fig. 4, the wastewater quality, both before and after a feed, varied regularly over a cycle. According to the variation before a feed, it can be found that the time-8:30 and -21:30 were two representative moments for the wastewater quality. The reactants (COD, TAN) of aerobic reaction reached the lowest and highest concentration at 8:30 and 21:30, respectively (Figs. 4a and c). Understandably, the difference in the wastewater quality before feed during a cycle is mainly related to a load of batch feed and the time interval of the adjacent batch. It has been established that the oxidation of organics and the nitritation of ammonia are two main reactions in the aerobic unit. Generally, longer interval time may result in more complete removal of reactants. Since the same volume of feed was applied each time (400 mL during the phase-2), COD and TAN concentration respectively reached the lowest values at 8:30 after the longest reaction time of 11 h ($21:30–8:30^{+1d}$) in a cycle. In addition, as shown in Figs. 4a and c, the COD and TAN concentration before feed showed an increasing trend overall from 8:30 to 21:30, which illustrates that a load of batch feed fails to be completely removed during the time interval of the adjacent batch, even the longest interval of 4 h (10:30–14:30). Therefore, in a feed cycle, with the batch increasing, the amounts of reactants failing to be completely removed would gradually accumulate and reach the peak before the last batch of the cycle (the time-21:30).

Similarly, the time-8:30 and -21:30 were also the representative moments for the variation of alkalinity, pH, FA, and FNA before feed (Figs. 4e–h). The reason is that the removal of reactant ammonia of aerobic reaction is accompanied by a decrease in alkalinity and pH, and FA and FNA are in turn functionally related to pH.

As shown in Fig. 4h, the FNA concentration at 8:30 before the feed was as high as ~0.8 mg/L. FNA is a broad biological inhibitor, which also inhibits the activity of AOB when the concentration increases to a certain level (0.2–2.8 mg/L). To verify whether the FNA concentration at 8:30 before a feed can completely inhibit the activity of AOB, the batch feed was only deliberately stopped on day 94, and the 0 h corresponded to 8:30 (Fig. 5). Interestingly, the $NH_4^+$–N concentration remained almost unchanged throughout the following 8 h, and the $NO_2^-$–N/$NH_4^+$–N in the aerobic unit remained stable at around 1.3.

It has been established that it takes 7.14 g alkalinity ($CaCO_3$) to convert 1 g $NH_4^+$–N to $NO_2^-$–N. In this study, the ratio of alkalinity to TAN concentration in the influent of PN unit was ~4.1 g $CaCO_3$/g N. Therefore, TAN in the influent is hard to be completely converted to $NO_2^-$–N. As the alkalinity is consumed, pH decreases and FNA is formed. When the concentration of FNA reached ~0.8 mg/L, the activity of AOB was completely inhibited, and the ratio of $NO_2^-$–N/$NH_4^+$–N in the aerobic unit was just ~1.3.

The FNA with high concentration can be used to treat aerobic sludge to reinforce the inhibition on NOB for initiating or restoring the nitritation [20]. As shown in Fig. 5, during the period from 21:30 to 8:30 of the next day, no TW flowed into the nitritation unit, which could keep the FNA in that unit at a high concentration of ~0.9 mg/L. This period contributes to a sustained inhibition on NOB, and this inhibition process was repeated daily, ensuring the stability of the nitritation reaction. This is also an inherent advantage of the onsite household treatment mode in the application of PN process.

### *3.2. Sludge reduction*

The technology of the aerobic unit in this study is a biofilm, which is different from the technology of activated sludge. There is no regular sludge reflux and discharge to maintain the biomass and activity of biofilm. Based on that, the strategy of sludge discharge was decided. If the excess sludge can be steadily stored at the bottom of the setting unit, no sludge will be discharged. When sludge uplift occurs in the setting unit, sludge discharge will be enforced.

Referring to this strategy, no sludge has been discharged from the experimental system for 100 d, that is, the excess sludge produced can be stably stored at the bottom of the setting unit. The reason for that contains three aspects.

Firstly, the sludge observed yield of the aerobic unit is low. The total biomass of the aerobic and setting unit was monitored on day 100. Specifically, the biomass of attached sludge (biofilm) and suspended sludge were 3.424 and 1.727 g SS, respectively (Fig. 6a). During this period, the total mass of COD removal in the aerobic unit was 63.596 g COD (Fig. 6b). Therefore, the sludge observed yield was 0.081 g SS/g COD, which is lower than the range in the researches on sludge reduction, 0.088–0.148 g SS/g COD [14,21].

Secondly, the settleability of excess sludge is good. The settleability was detected during a stable period, and the ratio of $SVI_5$ to $SVI_{30}$ was in the range of 0.833–1.000, which demonstrates good performance in the settleability. Therefore, the probability of sludge sloshed and flowing out

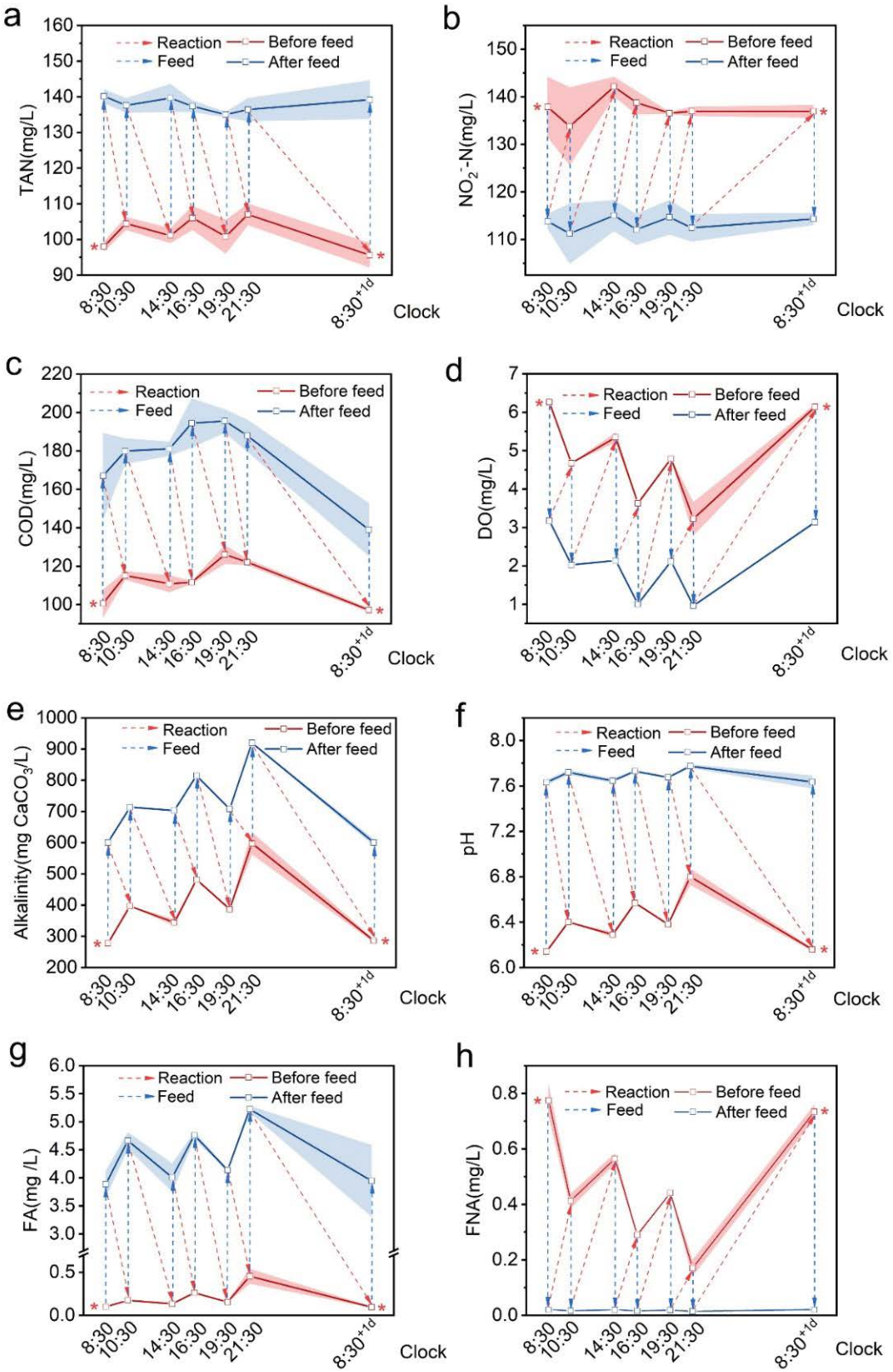


Fig. 4. Variation of (a) total ammonia nitrogen, (b) $NO_2^-$–N, (c) chemical oxygen demand, (d) dissolved oxygen, (e) alkalinity, (f) pH, (g) free ammonia, (h) free nitrous acid in the aerobic unit before and after feed during the phase-2 (on day 90 and day 98). '*' stands for the start of a feed cycle.

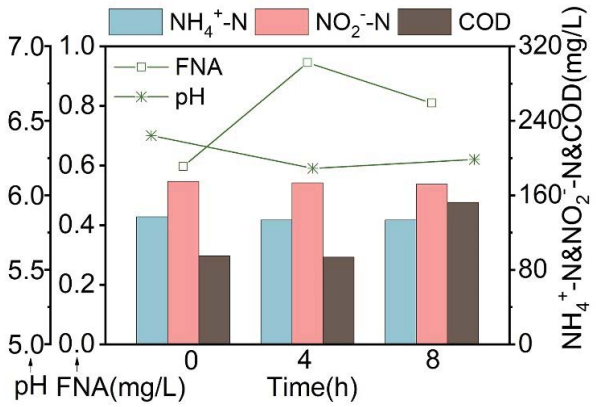


Fig. 5. Variation of $NH_4^+$–N, $NO_2^-$–N, chemical oxygen demand, pH, and free nitrous acid concentration in the aerobic unit without feed on day 94 (0 h corresponding to 8:30).

the setting unit due to the instantaneous feed was greatly reduced.

Thirdly, the stability of excess sludge is high. The HRT of the aerobic unit was as high as 14.7 h, and the aeration was also sufficient. Thus, the organic was fully removed, and the content of degradable organic flowing into the setting unit was relatively low, so the excess sludge can be stably stored at the bottom instead of floating out.

The reason for the three characteristics of excess sludge elaborated above originates from two aspects.

Firstly, the FNA concentration in the aerobic unit varied in the range of 0.15–0.80 mg/L. It has been established that FNA is a kind of biological inhibitor. When the concentration reaches 0.10 mg/L, FNA will generate an inhibitory effect on microorganisms. Therefore, in the range of 0.15–0.80 mg/L, microbial growth and proliferation in the aerobic unit were more or less inhibited by FNA, thus reducing the sludge observed yield. Besides, the long-term inhibition by FNA, not only led to the disruption of sludge but also contributed to the formation of granular sludge [22], which enhances the settleability of excess sludge [23].

Secondly, most of the time, the sludge in the aerobic unit was in a state of starvation. Based on the drainage characteristics of HTW, the reactor was not fed lasting for 11 h in the night. However, the aeration pump was still working, which makes the bacteria in the endogenous respiration phase. Thus, partial excess sludge was consumed, and the sludge observed yield was reduced [24].

### *3.3. Evolution of the microbial community structure of attached biofilm in the aerobic unit*

The Chao1 and Abundance-based Coverage Estimator (ACE) indexes are generally utilized to assess the community richness, while Shannon, Simpson, and coverage indexes enable the evaluation of community diversity. Specifically, these indexes are positively correlated to the community richness or diversity, except for the negatively correlated Simpson index. Hence, as shown in Table 3, both richness and diversity of the community (sampled on day 10) were significantly lower compared with the community sampled on day 30 or day 90.

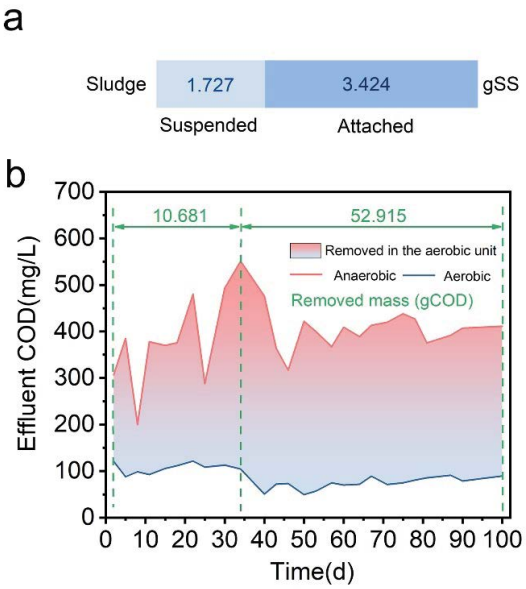


Fig. 6. (a) Sludge production and (b) chemical oxygen demand removal in the aerobic unit during the 100-d operation.

Fig. 7 presents the phylogenetic classification and relative abundance (RA) of bacteria at different levels. At the class level, the increases in RA of *Betaproteobacteria* primarily occurred because of the overall increase in *Comamonas*, *Thiobacillus*, and *Nitrosomonas* at the genus level. *Nitrosomonas* are AOB, and NOB was completely undetectable.

In this study, the RA of *Nitrosomonas* was 1.99% on day 100, which is indeed lower than the values reported, 3.00%–4.18% [14,25,26]. The reason for that may be related to the feed strategy. To simulate the drainage characteristics, the feed period of the experimental system was evenly dispersed from 8:30 to 21:30, and no HTW was fed into the system from 21:30 to $8:30^{+1d}$ but the aeration pump still working. During the period of no feed, FNA and DO concentrations were as high as 0.9 and 7.00 mg/L, respectively. The situation that the activity of *Nitrosomonas* is inhibited by the high FNA concentration and the endogenous respiration of bacteria triggered by the high DO will be developed. Therefore, the RA of *Nitrosomonas* was lower than the values reported.

The emergence and enrichment of the phylum *Planctomycetes* were synchronized with the enrichment of the genus *Nitrosomonas*. Moreover, all ANAMMOX bacteria that have been cataloged to date belong to *Planctomycetes*, and *Nitrosomonas* are indispensable bacteria in the ANAMMOX process. Additionally, it can be concluded that the microbial community structure of the biofilm sampled on day 10 was significantly distinct from those of samples collected on day 30 or day 90. The reason for this could be the emergence of *Nitrosomonas* on day 30. It has previously been established that the emergence of *Nitrosomonas* directly results in the generation and accumulation of FNA, which is a conclusive factor for the evolution of microbial systems. Likely, the end of evolution may be the most applicable state for the subsequent ANAMMOX process.

Table 3
Alpha diversity analysis of the microbial community of the attached biofilm in the aerobic unit

| Sample | Seq. number | OTU number | Shannon index | ACE index | Chao1 index | Coverage | Simpson |
|---|---|---|---|---|---|---|---|
| Day 10 | 35,550 | 213 | 1.59 | 402.60 | 314.64 | 1.00 | 0.44 |
| Day 30 | 43,226 | 790 | 3.85 | 1,035.70 | 999.52 | 0.99 | 0.08 |
| Day 90 | 39,416 | 750 | 3.88 | 1,033.56 | 1,042.46 | 0.99 | 0.06 |

| Phylum | Relative abundance(%) | | | Class | Relative abundance(%) | | | Genus | Relative abundance(%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | day10 | day30 | day90 | | day10 | day30 | day90 | | day10 | day30 | day90 |
| *Proteobacteria* | 88.01 | 79.47 | 70.25 | *Gammaproteobacteria* | 76.36 | 32.42 | 16.92 | *Acinetobacter* | 74.25 | 24.32 | 0 |
| | | | | | | | | *Psychrobacter* | 1.07 | 0 | 0 |
| | | | | | | | | *Dokdonella* | 0 | 1.14 | 0.3 |
| | | | | *Betaproteobacteria* | 8.46 | 29.74 | 44.43 | *Comamonas* | 0.49 | 8.52 | 8.75 |
| | | | | | | | | *Acidovorax* | 1.14 | 0.41 | 0 |
| | | | | | | | | *Thiobacillus* | 0 | 0 | 17.33 |
| | | | | | | | | *Nitrosomonas* | 0 | 1.01 | 1.99 |
| | | | | *Alphaproteobacteria* | 2.81 | 16.65 | 7.69 | *Novosphingobium* | 0 | 2.85 | 1.17 |
| | | | | | | | | *Altererythrobacter* | 0 | 3.35 | 1.36 |
| | | | | | | | | *Phenylobacterium* | 0 | 1.03 | 0.67 |
| *Bacteroidetes* | 10.21 | 12.45 | 20.83 | *Flavobacteriia* | 10.16 | 0.59 | 0.55 | *Chryseobacterium* | 9.84 | 0 | 0 |
| | | | | *Bacteroidia* | 0.01 | 1.24 | 10.45 | - | - | - | - |
| | | | | *Sphingobacteriia* | 0.04 | 2.04 | 5.61 | *Flavisolibacter* | 0 | 0 | 1.89 |
| | | | | | | | | *Terrimonas* | 0 | 0 | 1.32 |
| | | | | *Cytophagia* | 0 | 2.68 | 0 | *Leadbetterella* | 0 | 2.11 | 0 |
| *Planctomycetes* | 0 | 1.01 | 2.36 | *Planctomycetia* | 0 | 1.01 | 2.36 | *Zavarzinella* | 0 | 0 | 1.73 |
| *Gemmatimonadetes* | 0 | 0.35 | 1.03 | *Gemmatimonadetes* | 0 | 0.35 | 1.03 | *Gemmatimonas* | 0 | 0 | 1.03 |
| *unclassified* | 0 | 0.3 | 1.08 | *unclassified* | 0.03 | 7.32 | 6.41 | *unclassified* | 8.37 | 36.14 | 52.16 |

Fig. 7. Phylogenetic analysis and relative abundance of bacteria at different levels.

### 3.4. Discussion and implication

Similar to the aerobic treatment of TW [10], spontaneous initiation and maintenance of PN were also realized in the HTW treatment, which is the foundation for the establishment of PN/A technology. In addition, the drainage characteristics of HTW simultaneously provide two advantages for PN technology. Reducing the observed sludge yield is the first advantage [14,21], and continuously maintaining the stability of PN is the second [20]. The above two advantages are conducive to reducing the operation and maintenance cost of PN/A technology, which contributes to increasing the value of engineering applications.

The focus of the next job will be switched to the establishment and realization of subsequent ANAMMOX technology, as well as the effect of drainage fluctuation of HTW on the PN/A technology. The specifical challenge is how to achieve stable operation of the ANAMMOX process in the household treatment mode. To simulate the model, the feed strategy of the experimental system was decided according to the drainage characteristics of HTW, and DO concentration in the effluent of the PN unit was in the range of 3.00–6.00 mg/L at this situation. However, the effluent with such a concentration of DO is forbidden to feed the ANAMMOX reactor. Therefore, in the absence of manual regulation, how to ensure the oxygen demand for PN and simultaneously adjust the effluent DO of the PN unit for feeding the ANAMMOX reactor is the next job. At present, the work has been carried out, and it has been found that adjusting the structure of the experimental reactor is a practical method.

## 4. Conclusions

This study simulated the actual scenario of household toilet drainage, using the onsite mode to treat the HTW. The following conclusions were drawn.

- PN can be initiated and maintained spontaneously.
- Drainage characteristics of HTW are beneficial for maintaining the stability of PN and achieving the sludge reduction
- Emergence of *Nitrosomonas* is a determining factor in the structural changes of the microbial community of the aerobic unit.

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**Supplementary information**

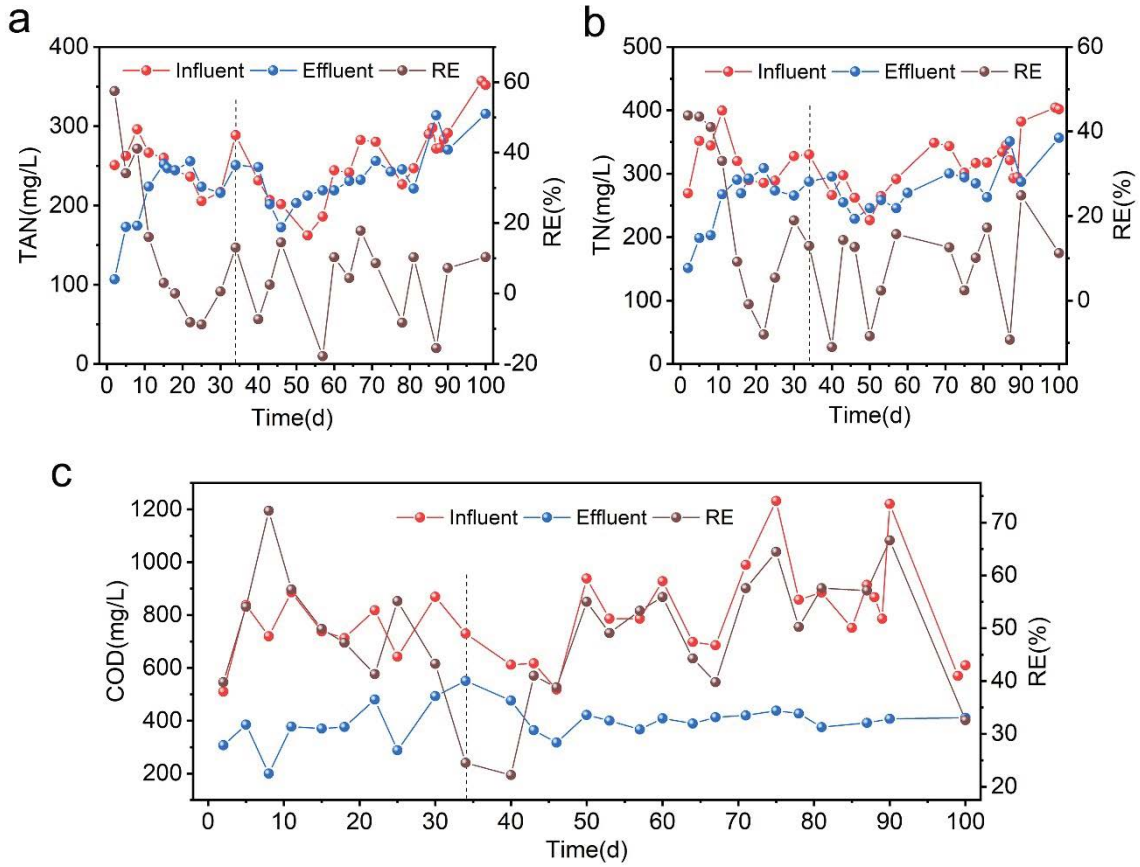


Fig. S1. Variation of (a) total ammonia nitrogen, (b) total nitrogen, and (c) chemical oxygen demand in the influent and effluent of the anaerobic unit and the corresponding removal efficiency.