Influence of seasonal temperature change on autotrophic nitrogen removal for mature landfill leachate treatment with high-ammonia by partial nitrification-Anammox process

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A B S T R A C T
In this study, a denitrification (DN)–partial nitritation (PN)–anaerobic ammonia oxidation (Anammox) system for the efficient nitrogen removal of mature landfill leachate was built with a zone-partitioning self-reflux biological reactor as the core device, and the effects of changes in seasonal temperature on the nitrogen removal in non-temperature-control environment were explored. The results showed that as the seasonal temperature decreased from 34°C to 11.3°C, the total nitrogen removal rate of the DN-PN-Anammox system gradually decreased from the peak value of 1.42 kg/(m²•day) to 0.49 kg/(m²•day). At low temperatures (~20°C), when the nitrogen load (NLR) of the system is not appropriate, the fluctuation of high NH₄⁺-N concentration in the landfill leachate greatly influenced the stability of the nitrogen removal. At temperatures of 11°C–15°C, the NLR of the system is controlled below 0.5 kg/(m²•day), which can achieve stable nitrogen removal and the nitrogen removal efficiency can reach above 96%. The abundance of Candidatus Brocadia gradually increased with the decrease of temperature. Nitrosomonas, Candidatus Brocadia and Candidatus Kuenenia as the main functional microorganisms in the low temperature.

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Introduction
Landfill leachate is a long-time byproduct that exists after landfills. Its composition is complex with the components of the waste, and changes continuously with landfill age, and season (Bhatt et al., 2017; Costa et al., 2019). The mature landfill leachate is waste liquid from a sealed landfill after several years of the landfill use. Due to the lack of a biodegradable organic carbon source (biochemical oxygen demand/total nitrogen (BOD/TN) is less than 0.3) and high NH₄⁺-N concentration (1000–5000 mg/L), mature landfill leachate is classified as difficult to treat wastewater (Qi et al., 2018). The use of conventional physicochemical methods (NH₃ stripping and gas-permeable membrane for NH₄⁺-N removal) to treat such wastewater with high NH₄⁺-N concentration can ensure that the effluent water quality is stable and meets the standards. However, when these methods are used, higher amounts of material and energy are consumed, it is difficult
to find the destination for the byproducts, and hardness and surfactants can interfere with the removal effect (Chang et al., 2018; Cingolani et al., 2018; Zhang et al., 2020). Compared with physicochemical technology, nitrification and denitrification have always been regarded as the most economical biological nitrogen-removal treatment techniques for wastewater and are widely used in the initial operation of landfills (Chen et al., 2019b; Miao et al., 2019). However, for the mature landfill leachate with high NH$_4^+$-N and low organic carbon contents, the traditional biological nitrogen removal process is faced with organic carbon deficiency, a lot of air is needed to control high dissolved oxygen (DO) in nitrification process, high ammonia toxicity, high reflux ratio of nitrifying liquid, and low total nitrogen load rate (TNLR). As a result, the cost of nitrogen removal for wastewater is increased, and sometimes even the stable operation of the process is affected (Luo et al., 2020; Plüg et al., 2015).

Partial nitrification (PN)—anaerobic ammonia oxidation (Anammox) as a new and highly efficient autotrophic biological nitrogen removal process, has received more attention in the treatment of industrial wastewater with high NH$_4^+$-N concentration (Li et al., 2017; Tan et al., 2020). In the PN-Anammox process, ammonium oxidizing bacteria (AOB) are used to convert 50% to 60% of NH$_4^+$ to NO$_2^-$ under aerobic conditions, and then anaerobic ammonium oxidizing bacteria (AnAOB) are used to convert NO$_2^-$-N and the remaining NH$_4^+$-N to 89% N$_2$ and 11% NO$_3^-$-N under anaerobic conditions (Chen et al., 2019a). This process has been applied in the small-scale study of nitrogen removal for landfill leachate and has yielded a good nitrogen removal effect (Miao et al., 2019). However, the functional microorganisms of PN-Anammox have a strong sensitivity to the environmental conditions (e.g., temperature, pH, substrate concentration) (Jin et al., 2012). There are few reports on the application of PN-Anammox to actual projects with complex and changing environments that showed good nitrogen removal effects (van der Star et al., 2007). In addition, many problems have not been paid attention to and solved when it is applied to practical wastewater or engineering (Nsenga Kumwimba et al., 2020; Tomaszewski et al., 2017). For example, the optimal temperature for AOB and AnAOB is 35°C. It is well known that the nitrogen removal efficiency of PN-Anammox decreases with the decrease of temperature (Tomaszewski et al., 2017). Many landfills do not have heating conditions, and seasonal temperature changes will inevitably affect the stability of the PN-Anammox system in nitrogen removal, but the related issues have not yet received attention. For actual wastewater, the decrease of temperature is not only the decrease of functional microbial activity, but also may bring other problems. The temperature drop easily leads to the rapid increase concentration of NH$_4^+$-N and BOD in the system (Mieczkowski et al.), and this will certainly result in multiple inhibitions on the activity of denitrification microorganisms of PN-Anammox systems, and the microbial community structure of the PN-Anammox process system also will become fragile. Moreover, there is a question whether the influence of toxic substances (heavy metals, organic substances) (Baderna et al., 2019; Ghosh et al., 2017) in landfill leachate on microbial community structure and functional microbial species will increase at low temperature.

These are the problems associated with the PN-Anammox process in landfill leachate engineering applications. Because of AOB needs oxygen and AnAOB does not need oxygen, it usually uses granular sludge and biofilm to create different DO gradients for microorganisms with different habits. This is done to achieve a symbiosis of two microorganisms in a single system (Li et al., 2018a) or to cultivate them in partitioned zones and remove nitrogen by coupling PN and Anammox in series (Xu et al., 2020b). When the temperature was controlled in the moderate range (31–34°C), a unique zone-partitioning self-reflux biological nitrogen removal device was successfully used in the early stage to efficiently couple PN-Anammox, and the total nitrogen removal rate (TNRR) reached 3.1 kg/(m$^3$·day) (Li et al., 2017). With this device as the core, the DN-PN-Anammox system was constructed and applied in mature landfill leachate treatment. The system showed a high nitrogen removal efficiency with TNRR reached 2.2 kg/(m$^3$·day) (Li et al., 2018b). However, the operating temperature of these devices is controlled at 30°C-35°C, and the actual temperature of landfill leachate is far lower than this value. Although the device was used in practical engineering and achieves good TNRR, it needs to keep moderate temperature, which will require a lot of heat energy consumption. It is worth noting that many researchers currently implement PN, Anammox or PN-Anammox processes running at low temperatures by simulating wastewater (Morales et al., 2016; Wang et al., 2018). However, the influence of seasonal temperature change on nitrogen removal in PN-Anammox process has not been paid attention, especially in the treatment of wastewater with high NH$_4^+$-N concentration and toxicity, such as landfill leachate.

Considering these, the current study investigated the effect of seasonal temperature difference on the nitrogen removal efficiency and functional microbial community structure of the DN-PN-Anammox system and explored the feasibility of achieving stable and efficient nitrogen removal of mature landfill leachate with high NH$_4^+$-N concentration at different temperatures. This study also intends to provide a technical reference for applying the DN-PN-Anammox process to different regions with temperature differences.

1. Materials and methods

1.1. Experimental device

The DN-PN-Anammox system was composed of an up-flow anaerobic sludge blanket (UASB) and a zone-partitioning self-reflux biological nitrogen removal device (Fig. 1). The pre-UASB reactor was mainly used as a denitrification unit. The zone-partitioning self-reflux integrated device was mainly used as the PN and Anammox coupling unit, and related dimensions were as reported by Li et al. (2018b). In the aerobic zone, the intake air volume was adjusted by a rotameter, and the DO was controlled at 0.1–0.4 mg/L. Throughout the experiment, only the temperature difference caused by local climate change (Suzhou, China) was used to study the effect of different temperatures on the nitrogen removal process.
1.2. Inoculated sludge and wastewater

The DN-PN-Anammox system was initially inoculated with sludge samples from granular sludge in sewage plants, mature nitritation biofilms cultured in laboratories, and Anammox granular sludge separately. Before this experiment was conducted, for over two years, the system had been used for removing nitrogen from mature landfill leachate at a temperature of 31°C–34°C, with a maximum TNRR of 2.2 kg/(m²•day). After other destructive experiments, such as reflux ratio, the reactor TNRR stabilized at 1.5 kg/(m²•day) (Li et al., 2018b). The influent was mature landfill leachate from Qizishan, Suzhou, not subjected to any pretreatment such as dilution, and the water quality characteristics were as described by Li et al. (2018b). The COD concentration was 2500 ± 250 mg/L and consisted primarily of non-biodegradable organic matter. The concentration of BOD₅ was 1100 ± 150 mg/L. The concentration of NH₄⁺-N at 2550 ± 200 mg/L.

1.3. Experimental method

When the influence of temperature on nitrogen removal was studied by the DN-PN-Anammox system, the heating device was removed, and the system temperature gradually reduced only by seasonal cooling. To avoid the influence of high NH₄⁺-N concentration on microbial activity during the system operation, when the effluent NH₄⁺-N concentration was higher than 100 mg/L, the hydraulic retention time (HRT) was extended to ensure that the microorganisms were not inhibited by free ammonia (FA). The microbial activity of each treatment unit was evaluated by the changes in the concentration of pollutants in the influent/effluent and different regions. The calculation method was based on the report by Li et al. (2018b). At the same time, sludge was sampled at different temperatures in different zones of the system during stable operation to analyze changes in the microbial community structure.

1.4. Measurements and methods

The concentrations of NH₄⁺-N, NO₃⁻-N, and NO₂⁻-N, chemical oxygen demand (COD) were measured based on the Standard Methods (APHA, 2005). The online monitor (WTW Company, Germany) was used to measure the DO, temperature, pH, and oxidation-reduction potential (ORP). The analysis method of microbial community structure was based on Li et al. (2018b).

2. Results and discussion

2.1. Effect of temperature on the nitrogen removal by DN-PN-Anammox system

Before the temperature control device was removed, the temperature of DN-PN-Anammox system was kept at 31°C–34°C. When the system was used to treat the mature landfill leachate with NH₄⁺-N concentration of 2250 mg/L - 3000 mg/L, the total nitrogen in the effluent was lower than 70 mg/L, nitrogen removal efficiency (TNRE) was higher than 96%, and TNRR was higher than 1.38 kg/(m²•day). All these indicate that the system had a good removal effect on high concentration NH₄⁺-N in landfill leachate through the stable control of appropriate temperature.

In the initial stage of the non-temperature control state (1–24 days), the HRT was still maintained at 1.53 day. The system temperature fluctuates between 32 and 34°C. Therefore, the nitrogen removal efficiency of the system did not change considerably. The influent NH₄⁺-N concentration was about 2250 mg/L; the effluent NH₄⁺-N and NO₂⁻-N concentrations were maintained at 15 mg/L and 6 mg/L, respectively; and the effluent NO₃⁻-N was almost 0 mg/L (Fig. 2a). The TNRR and TNRE of the whole system were maintained at 1.42 kg/(m²•day) and 97% or more, respectively (Fig. 2b). These results showed that the seasonal temperature difference of 30–35°C had no substantial effect on the nitrogen removal of the DN-PN-Anammox system. The temperature gradually decreased from the 25th day, and the concentration of NH₄⁺-N in the effluent showed a gradual upward trend. In order to avoid the accumulation of NH₄⁺-N to form a high concentration of FA affecting the system, the TNLR of the system was reduced by gradually extending the HRT. During day 30–50 of the system operation, the temperature was maintained at 25°C. At this time, the HRT was extended to 1.94 days. The NH₄⁺-N concentration of the influent was still maintained at about 2250 mg/L, and the NH₄⁺-N and NO₂⁻-N concentrations of the effluent were finally maintained at 20 mg/L and 12 mg/L, respectively. Meanwhile, a small amount of NO₃⁻-N in the effluent began to accumulate, reaching a maximum of 15 mg/L. Although the TNRE of the system could still reach over 95% due to a decrease in the TNLR, the TNRR dropped to 1.15 kg/(m²•day), with a decrease of 20%.

As the winter arrived, the temperature again fell rapidly. The temperature dropped and stabilized at 15°C–17°C during days 73–115 of the system operation. During this period, the TNLR of the system was continuously lowered by extending the HRT. However, due to the gradual increase in the NH₄⁺-N concentration of the landfill leachate and temperature fluctuations, the final effluent NH₄⁺-N concentration was still fluctuating, reaching a maximum of 106 mg/L. The TNRR also decreased and stabilized to 0.7 kg/(m²•day), a drop of 51%. Daverey et al. (2013) treated sludge digestion liquid in a single reactor with DN-PN-Anammox process and found that the
fluctuations of temperature and water quality (mainly COD and organic nitrogen) had a negative effect on the stability of synergistic nitrogen removal. Guo et al. (2015) found that when the temperature was less than 9.5°C, the nitrogen removal efficiency of the Anammox system became unstable. This indicates that with the decrease of temperature strip, the fluctuation of landfill leachate composition has a relatively great impact on the nitrogen removal efficiency of the system and the stability of the effluent quality.

With the temperature continued to fall to 11°C–12°C, the NH₄⁺-N concentration of the system effluent still increased rapidly, reaching a maximum of 211 mg/L. In order to treat the landfill leachate using the nitrogen removal system at a lower temperature, the HRT of system was extended to 5.09 days on day 139 of the system operation. Meanwhile, because of the effect of the rainy season, the NH₄⁺-N concentration of the influent at this stage gradually decreased and stabilized to about 2200 mg/L–2280 mg/L. Finally, the TNLR of the system decreased and stabilized to 0.5 kg/(m³•day); the corresponding NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N concentrations stabilized within 20 mg/L, 8 mg/L, and 45 mg/L, respectively (Fig. 2a), and the TNRE and TNRR were 97% and 0.49 kg/(m³•day), respectively (Fig. 2b), with a decrease of 65.7%.

In general, when the temperature of the DN-PN-Anammmox system was less than 30°C, the nitrogen removal efficiency showed a downward trend as the temperature decreased. If the TNLR of the system is not adjusted to a sufficiently low level in time, fluctuations in the nitrogen concentration during actual operation might easily lead to NH₄⁺-N accumulation in the effluent and unstable nitrogen removal efficiency. As long as the TNLR of the system was properly controlled, even when the system temperature dropped to 12°C, a good nitrogen removal efficiency and stable effluent water quality could be obtained.

2.2. Effect of seasonal temperature variation on different nitrogen conversion processes

2.2.1. Effect of seasonal temperature variation on the nitrogen removal efficiency of DN zone

Nitrite-oxidizing bacteria (NOB) has always existed in PN process (Arrigada et al., 2017), and Anammox process also produces about 11% NO₃⁻-N. Thus, the PN-Anammox process theoretically produces at least 242 mg/L NO₃⁻-N when processing NH₄⁺-N (2200–2300 mg/L) in mature landfill leachate, and this severely affects the effluent TN discharge. The landfill leachate used in this experiment contained about 1300 mg/L biodegradable COD (CODbio), accounting for 40% of the total COD. Directly introducing the landfill leachate into the PN-Anammox system will not only affect the nitrogen removal efficiency of the system but also increase the power consumption of gas supply in the aerobic zone (Li et al., 2020); therefore, the pre-denitification in the DN-PN-Anammmox system utilized denitification to simultaneously reduce NO₃⁻-N and CODbio. In the early stage of the UASB operation (temperature greater than 30°C), the effluent COD stabilized at 1420 mg/L–1500 mg/L (Fig. 3). However, with the gradual decrease in temperature, the COD concentration of the UASB effluent gradually increased to over 1600 mg/L, reaching a maximum of 1670 mg/L. Moreover, according to the effluent COD of the back-end zone-partitioning reflux device, this part of COD was not considerably reduced after the PN-Anammox process; thus, it can be inferred that easily degradable COD was removed in the UASB, and the COD of the effluent was the refractory organic matter (Li et al., 2020). This also reveals that the microorganisms in the DN system could degrade some refractory organic substances under moderate temperatures. As the temperature decreased, the activity of the functional microorganisms of the PN-Anammmox process decreased to varying de-
Fig. 3 – Effect of temperature change on the simultaneous removal of NO$_2^-$-N and organic matter in DN.

Fig. 4 – Effect of temperature change on nitrogen removal in PN-Anammox system.

degrees, resulting in the accumulation of NO$_2^-$-N and NO$_3^-$-N in the effluent (analyzed in Section 2.2.2). Therefore, the concentrations of NO$_2^-$-N and NO$_3^-$-N entering the UASB also showed a gradual increase (Fig. 4a). However, the NO$_3^-$-N concentration of the UASB effluent was almost zero during the entire operation period (Fig. 3). When the temperature was lower than 15°C, there was a distinct accumulation of NO$_2^-$-N (6–11 mg/L) in the UASB, and the highest concentration reached 14.17 mg/L. Ji et al. (2017) showed that when NO$_2^-$-N and NO$_3^-$-N were simultaneously present in the system, denitrifying bacteria preferentially used easily degradable COD and NO$_3^-$-N for shortcut denitrification, and then the remaining easily degradable COD reacted with NO$_2^-$-N. Theoretically, there is no reduction in NH$_4^+$-N concentration in the UASB, but based on the NH$_4^+$-N concentrations in the reflux water, the DN system had a loss of 20–30 mg/L in the NH$_4^+$-N concentration. Therefore, so it is speculated that there are some coupling processes of shortcut denitrification and Anammox
in the system, and this requires further in-depth exploration. In summary, the decrease in temperature could cause an increase in the COD in the system, but COD was not be degraded in the subsequent PN-Anammox process, which had no significant effect on the nitrogen removal system.

2.2.2. Effect of seasonal temperature variation on the PN-Anammox process

The PN-Anammox was coupled with the zone-partitioning self-reflux biological nitrogen removal device as the core device for the entire system to perform efficient nitrogen removal. Previous studies have shown that the device allows for the cultivation of functional microorganisms in partitioned zones suitable for different environmental needs (Li et al., 2017). In the initial operation period (1–24 day) when the temperature control device was removed, the nitrogen removal efficiency of the PN-Anammox process reached a maximum of 1.68 kg/(m\(^3\)•day), and TNRE reached over 98% due to the small temperature change. The nitrite production rate (NPR) in aerobic zone reached 1.5 kg/(m\(^3\)•day), and the nitrogen removal rate (NRR) of Anammox in the anaerobic zone reached 16.14 kg/(m\(^3\)•day) (Fig. 4). When the temperature was lowered to 25°C, NH\(_4\)\(^+\)-N in the aerobic zone and anaerobic zone began to accumulate gradually, and the highest concentrations reached 117 mg/L and 96.5 mg/L, respectively (Fig. 4a). As the TNLR of the system gradually decreased, the concentrations of NH\(_4\)\(^+\)-N in the aerobic and anaerobic zones decreased and finally stabilized at 55 mg/L and 40 mg/L, respectively. However, as the temperature continued to decrease, the NH\(_4\)\(^+\)-N concentration in each zone of the reactor fluctuated greatly, and the decrease in NH\(_4\)\(^+\)-N concentration could be achieved by only extending the HRT. When the reactor was operated to day 139, the NLR in the reactor decreased to 0.5 kg/(m\(^3\)•day), and NH\(_4\)\(^+\)-N decreased and stabilized at a relatively low level. At this time, NRR of the integrated device stabilized at above 0.49 kg/(m\(^3\)•day). Among them, the NPR of the aerobic zone decreased to 0.44 kg/(m\(^3\)•day), and the Anammox nitrogen removal rate of the anaerobic zone dropped to 4.7 kg/(m\(^3\)•day) (Fig. 4c); both values were decreased by 70.7% and 70.9%, respectively. A previous study showed that the growth rates of AnAOB at 30°C, 20°C, and 15°C were 0.14 day\(^{-1}\) (Sobotka et al., 2017), 0.02 day\(^{-1}\), and 0.009 day\(^{-1}\) (Lotti et al., 2014), respectively; the growth rates of AOB at 30°C, 20°C, and 15°C were 1.8 day\(^{-1}\), 0.8 day\(^{-1}\), and 0.523 day\(^{-1}\), respectively, indicating that AnAOB are more sensitive to temperature changes than AOB. Li et al. (2019) and De Clippeleir et al. (2013) used simulated wastewater to study the effect of temperature on PN-Anammox and found that AnAOB were more sensitive to temperature shocks. In the present study, when PN-Anammox was used to treat landfill leachate with high NH\(_4\)\(^+\)-N concentration, as the temperature decreased, PN and Anammox had the same sensitivity to temperature. It is speculated that other toxic substances in landfill leachate can produce an inhibitory effect on the PN system. Throughout the reactor operation, the NO\(_3\)\(^-\)-N concentration in the aerobic zone was maintained at 45 mg/L–55 mg/L, and the NO\(_2\)\(^-\)-N concentration in the anaerobic zone was 10 mg/L–15 mg/L. However, the NO\(_3\)\(^-\)-N concentrations in the aerobic zone and anaerobic zone showed an increasing trend simultaneously. Li et al. (2019) used simulated wastewater as influent and studied the effect of temperature on the PN-Anammox process. When the temperature was lower than 20°C, the dominant growth of AOB was interrupted; moreover, the NOB began to increase by large quantities, and the NO\(_3\)\(^-\)-N concentration in the effluent increased rapidly. A previous study showed that the effect of temperature on the NOB growth rate was significantly lower than that on the AOB growth rate. When the temperature dropped from 30°C to 20°C and then to 15°C, the NOB growth rate decreased from 1.182 day\(^{-1}\) to 0.788 day\(^{-1}\) and then to 0.642 day\(^{-1}\) (Zhu et al., 2008). Therefore, the NOB in the system is speculated to increase at low temperatures. However, due to the high concentration of NH\(_4\)\(^+\)-N in the landfill leachate, the FA concentration in aerobic and anaerobic zones fluctuated between 1 and 4 mg/L under low-temperature conditions (Fig. 4b), which had a certain inhibitory effect on the NOB.

2.3. Effect of temperature on functional microorganisms in various zones of the system

To further explore the effect of temperature on the nitrogen removal efficiency of the DN-PN-Anammox system, the structures of biological communities were analyzed for the sludge samples in the UASB and the aerobic zone and anaerobic of the zone-partitioning self-recycling device on day 0 (33°C), day 47 (25°C), day 115 (16°C), and day 139 (12°C) of the reactor operation.

The phylum level shows that during the process of gradual temperature decrease, Chloroflexi, Proteobacteria, Actinobacteria, and Bacteroidetes were the main phyla in the UASB (Fig. 5a). Among them, the abundance of Chloroflexi decreased from 43.8% to 37.1%; the abundances of Proteobacteria and Actinobacteria increased from 25.5% and 7.6% to 31.4% and 10.7%, respectively, and the rest did not change significantly. The main phyla in the aerobic zone of the integrated reactor were Proteobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, and Actinobacteria (Fig. 5c). In the anaerobic zone, the abundance of Chloroflexi first increased and then decreased (Fig. 5e). The abundance of Proteobacteria exhibited a distinct increase, while the abundances of other main phyla (Planctomycetes, Bacteroidetes, Actinobacteria, Acidobacteria, Gemmatimonadetes, and Deinococcus-Thermus) showed a substantial decrease. These results show that the temperature change had no significant effect on the phyla of the system but had a great impact on their abundances.

At the genetic level, no methanogens were found in the UASB, indicating that no methanization of organic matter occurred. The main bacterial genera included JG30-KF-CM66, SBR1031, SJA-28, Limnobacter, Nocardioides, and Aquamicrobium, among which Limnobacter and Anaerolineaceae can decompose refractory organics into small molecular organics available to microorganisms (Wang et al., 2018), and their abundances did not change with temperature (Fig. 5b). Truberapa plays an important role in the wastewater denitrification process and can tolerate harsh environments, and its abundance increases with decreasing temperature in various regions (Shan et al., 2017; Yan et al., 2014). Current researchers have found that truepera is an important functional microorganism in the shortcut denitrification process, which can convert NO\(_3\)\(^-\)-N into NO\(_2\)\(^-\)-N by using organic matter (Wang et al.,
tem, the activity of NOB will increase greatly, and the concentration of NO$_3^-$-N in the effluent will increase approximately (Wang et al., 2015). The reason why the abundance of NOB has not been improved in this study may be that NH$_4^+$-N continued to accumulate during the gradual decrease of temperature, which allowed the presence of the inhibitory effect of FA in the system. Candidatus Kuenenia was the main functional microorganism in the Anammox process (Fig. 5e), which is the same as that observed by other researchers when using Anammox to treat landfill leachate at medium temperature (Li et al., 2014; Phan et al., 2017). When the temperature was above 30°C, the abundance increases from 22.5% to 32.7% with the increase of nitrogen load at the initial stage of the operation. However, when the temperature decreased to 20°C–30°C, its abundance decreased and stabilized at about 21%, and no distinct change occurred in this temperature range. When the temperature was below 20°C, its abundance fell to 3.1%. The results indicate that temperature had a relatively great influence on the abundance of the functional microorganism Candidatus Kuenenia. The abundance of Candidatus Brocadiaceae, another bacterial species of Anammox, slightly in-
creased with decreasing temperature (from the initial 0.33% to 2.7%), but when the temperature was lower than 20°C, its abundance also dropped to 1.1%. In previous studies, the optimal growth temperatures for Candidatus Brocadia and Candidatus Kuenenia were 25°C–45°C (Oshiki et al., 2011) and 25°C–37°C, respectively (Egli et al., 2001). However, Lotti et al. (2015) found that Candidatus Brocadia and Candidatus Kuenenia are the main AnAOB in Anammox process for treatment landfill leachate at low temperature.

2.4. Exploring strategies for coping with temperature changes in different regions

Proper temperature control is the guarantee for the efficient and stable operation of biological systems. He et al. (2012) found that seasonal temperature changes greater than 6°C would affect the enhanced nitrogen removal efficiency of PN-Anammox in removing inorganic nitrogen in wetlands. The optimum temperature for AOB and AnAOB, the main functional microorganisms of PN-Anammox, to exert the best nitrogen removal efficiency was 30°C–35°C (He et al., 2012). Cui et al. (2015) indicated that in the application of PN-Anammox for the treatment of wastewater with high NH$_4^+$-N concentration at low temperatures, maintaining the optimal water temperature of functional microorganisms (30°C–35°C) would consume numerous heat sources; moreover, energy consumption would account for more than 50% of the entire process operating cost; therefore, heating the landfill leachate above 30°C will consume a large amount of heat energy, which will significantly increase the process operating cost.

To realize a stable operation, it is important to understand the influence of temperature changes on the PN-Anammox process and seek suitable control strategies. Guo et al. (2015) found that there was a significant linear relationship between the decrease of temperature and the decrease of nitrogen removal efficiency in Anammox reactor by using simulated wastewater. In the treatment of complex landfill leachate, the TNRR of the reactor did not show obvious linear relationship with the decrease of temperature. Comparing the correlations between temperature and the TNRR and AOB and AnAOB activities in this study reveals the following: Although the nitrogen removal efficiency of the DN-PN-Anammox process also gradually decreased with the temperature, when the temperature was higher than 20°C, the NPR of AOB, NRR of Anammox and the TNRR of the PN-Anammox system were stable under a certain load (Fig. 6). When the temperature was lower than 20°C, although the NPR of AOB and the NRR of Anammox could reach 0.44 kg/(m$^2$.day) and 4.7 kg/(m$^3$.day), respectively, the stability of the system worsened, and the effluent water quality was easily deteriorated. Therefore, when the DN-PN-Anammox treatment system is used for the nitrogen removal treatment of mature landfill leachate, it is best to control the temperature to above 20°C. In some cold areas, the system temperature cannot rise to 20°C, which poses a challenge in the engineering applications PN-Anammox. Therefore, the operation strategy and device type need to be improved continuously. For example, Gilbert et al. (2015) have shown that different reactor types have an important impact on nitrogen removal in the PN-anammox coupling system. The ability of mobile biofilm to enrich functional microorganisms was significantly better than that of suspended sludge under the low temperature environment (10°C–20°C). Wang et al. (2018) used a single reactor coupling DN and PN-Anammox to treat landfill leachate with NH$_4^+$-N concentration of 1900 mg/L and found that the NO$_3^-$-N concentration in the system effluent reached over 160 mg/L. However, in pre-denitrification under low-temperature environment using this system, organic matter could be effectively used for NO$_3^-$-N denitrification, and even when the temperature was reduced to 11°C–15°C, the effluent concentration of NO$_3^-$-N was less than 50 mg/L. Apparently, this system is very suitable for the nitrogen removal treatment of mature landfill leachate at low temperatures. This system can still greatly reduce the demand for organic carbon compared with the traditional nitrogen removal process, is a relatively economical nitrogen removal method under low temperature.

3. Conclusions

Seasonal temperature variation has great impact on the nitrogen removal efficiency and stability of DN-PN-Anammox system in the treatment of mature landfill leachate containing high NH$_4^+$-N. When the temperature fluctuates between 30 and 35°C, there was no effect on the NRE of the DN and PN-Anammox system. When the temperature was lower than 30°C, the decrease of microbial activity with the decrease of temperature and the fluctuation of influent NH$_4^+$-N concentration will easily lead to the accumulation of NH$_4^+$-N in aerobic and anaerobic zone, and the NRE of the PN-Anammox system will gradually decrease. When the temperature was 11°C–15°C, a reduction in the NRR of the system to 0.49 kg/(m$^2$.day) could ensure stable and efficient nitrogen removal. With the decrease of temperature, the pre-denitrification avoids the effect of accumulation of biodegradable organic matter on the
PN-Anammox process. The simultaneous removal of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$ is realized by shortcut denitrification and Anammox coupling in UASB.

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