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# APPLICATION OF COUPLING PARTIAL NITRIFICATION WITH ANAMMOX IN LANDFILL LEACHATE: A REVIEW

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**Abstract:** Leachate with high ammonia nitrogen and low carbon to nitrogen ratio (C/N) was produced by waste landfill. Furthermore, nitrogen removal in leachate has been always a hot research area all over the world. The traditional nitrogen removal technology in wastewater has always been occurred the consumption of resources, resulting in the increased cost. However, partial nitrification anammox (PN/A) has been used for removing the nitrogen due to the advantage of organic carbon source and reducing the cost, etc. Although numerous studies claimed nitrogen removal via PN/A process was able to achieve, few studies summarized the application and feasibility analysis of nitrogen removal in leachate by the process. Therefore, the latest research in landfill leachate via PN/A process was explained. Besides, influence factors in PN/A system and how to achieve stable nitrogen removal under inappropriate environment is described in the paper. The review provides a theoretical basis for the PN/A application.

## INTRODUCTION

The environmental issue has garnered attention with the high speed of urbanization and industrialization. And the nitrogen removal of wastewater has already become an important problem. The traditional methods of biological nitrogen removal involves aerobic nitrification and anaerobic denitrification (Zanetti et al., 2012). However, the conventional denitrification process has always been drawbacks (Gao et al., 2023). For example, large amounts of aeration and organic carbon sources (methanol etc.) need to be consumed resulting in increased operating costs, etc (Wang et al., 2017). Considering these drawbacks, many researchers have begun to focus on developing the new biological denitrification techniques. The anaerobic ammonium oxidation process (anammox) was first verified (Mulder et al., 1995). Anammox bacteria (AnAOB) converted ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) into nitrogen ( $\text{N}_2$ ) using

$\text{NO}_2^-\text{-N}$  as electron acceptor in an anaerobic or hypoxia environment (Ren et al., 2022). ANAMMOX has gradually attracted the attention of people due to the process with avoiding nearly all carbon source supplementation, less greenhouse gas emission ( $\text{N}_2\text{O}$ ) generated (Gonzalez-Martinez et al., 2017), 60% energy savings (Wanget al., 2022) and less sludge production (Z. Guo et al., 2022). It was regarded as an innovative and environmentally friendly process in biological nitrogen removal (Ni et al., 2010). As previously reported, at least over 300 anaerobic ammonia oxidation plants have been put into operation worldwide, and the amount will increase continuously in the future (Kumwimba et al., 2020; Wang et al., 2022).

$\text{NO}_2^-\text{-N}$  as electron acceptor is obtained by other means because it was hardly taken from the general natural water body (Singhet al., 2021). Thus, to obtain stable  $\text{NO}_2^-\text{-N}$  is the main goal to reach. In practical applications, partial nitrification (PN) was used in order to get sufficient  $\text{NO}_2^-\text{-N}$ , coupled with anammox process

(PN/A) have been applied to remove the biological nitrogen (Wang et al., 2017). PN/A has always been a hot spot in the areas of nitrogen removal of wastewater at the domestic and foreign research (Chen et al., 2021; Yang et al., 2022). PN/A includes two types of processes: the single-stage process of the same reactor and the two-stage process of multiple reactors. The influent  $\text{NH}_4^+\text{-N}$  about 50% under aerobic conditions was oxidized to  $\text{NO}_2^-\text{-N}$  by AOB, and AnAOB use  $\text{NO}_2^-\text{-N}$  from PN as the terminal electron acceptor to oxidize the remaining  $\text{NH}_4^+\text{-N}$  to  $\text{N}_2$  (Kartal et al., 2011). The single-stage process mainly includes aerobic deammonification (DEMON), and the two-stage process mainly include Single reactor High activity Ammonium Removal Over Nitrite (SHARON)-anammox process (Ren et al., 2022). Compared to the conventional nitrogen removal process, PN/A has plenty of advantage, which is no need for organic carbon by 100% due to ammonia-oxidizing bacteria (AOB) and AnAOB belonging to chemoautotrophic (Kosgey et al., 2022), reduce oxygen delivery (Cao et al., 2017) and better nitrogen removal effect (Zhang et al., 2020).

Currently, it is reported that the stable PN/A process have been successfully implemented in various wastewater. The research (Xiao et al., 2021) used a novel two-stage PN/A reactor for wastewater treatment, which was successfully achieved and the nitrogen removal efficiency (NRE) was 92.3%. Another (Wang et al., 2022) investigated the nitrogen removal efficiency (NRE) of the PNA process in SBR could be more than 90%. However, the application in landfill leachate and the encountered difficulty in operating conditions was rarely summarized. Therefore, varieties of factors in PN/A system and the application of landfill leachate are described in the paper. Meanwhile, applicability in others wastewater was also listed. The review provides a theoretical basis for the PN/A application.

## INFLUENCE FACTORS

$\text{NO}_2^-\text{-N}$  is an important intermediate for the successful linking of the PN and anammox process. In

the PN/A process,  $\text{NH}_4^+\text{-N}$  is oxidized to  $\text{NO}_2^-\text{-N}$  by AOB, then  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  are converted to diatomic nitrogen by AnAOB. The accumulation of  $\text{NO}_2^-\text{-N}$  is advantageous for the activity of AnAOB (Gao et al., 2021). However, NOB competes with AnAOB to oxidation  $\text{NO}_2^-\text{-N}$ , resulting in decreasing the removal of nitrogen and inhibition proliferation of AnAOB (Di et al., 2022). Therefore, AOB and AnAOB are enrichment and becomes the dominant species, and NOB is washed out in the system, which has been mainly goal and it is necessary to for implementing highly efficient nitrogen removal (Liu et al., 2021). According to previous reports, the activity of bacteria in PN/A process depend on various environmental factors. And dissolved oxygen (DO), pH, temperature, free ammonia (FA) and free nitrite acid (FNA) have been regarded as important parameters. Therefore, it is particularly important to identify the optimum range for microbial and the following is a detailed introduction.

## DO

AOB is aerobic bacteria and AnAOB is strictly anaerobic bacteria. The environments of aerobic and anaerobic is usually provided by aeration and is one of the most common strategies. However, compared to continuous aeration, researchers proposed that intermittent aeration was more beneficial to wash out NOB due to NOB had no sufficient  $\text{O}_2$  to oxidize  $\text{NO}_2^-\text{-N}$ , which contributed to improve the nitrite accumulation efficiency. Therefore, DO as one of the critical parameters have been widely applied for the successful initiation of the PNA process, and it was increasingly regarded as effective for  $\text{NO}_2^-\text{-N}$  accumulation. Wiesmann et al. confirmed that DO concentrations  $>1.8\text{mg/L}$  contributed to the survival of NOB (Wiesmann, 1994). However, previous study documented (Ma et al., 2017) that AOB had significantly sensitivity to low concentration of DO than that of NOB and NOB was easily suppressed, and it was due to the half-saturation constants of AOB less than that of NOB ( $K_{\text{AOB}} < K_{\text{NOB}}$ ). Tong also found that AOB represented a

significantly larger affinity to DO and had stronger ability of enrichment than that of NOB (Jia et al., 2022). However, in the one-stage PN/A process, the effect of AnAOB by DO was taken into consideration because the survival environment of AOB in the two-stage PN/A system was always under strict anaerobic conditions (Kumwimba et al., 2020). Strauss et al. found that the inhibition of AnAOB activity was reversibly by DO in 1997 (Strous et al., 1997). Therefore, by controlling the DO concentration in one-stage PN/A system have been used to inhibit NOB, it is not only less effect on the activity of AnAOB but assured high-performance nitrogen removal by the cooperation between AOB and AnAOB.

Researchers (Blackburne et al., 2008) controlled the concentration of DO at 0.4mg/L and successfully removed NOB to obtain enrichment of AOB in SHARON. The concentration of DO ranges from 0.2 to 0.4mg/L, the accumulation amount of AOB far greater than NOB, and it is consistent with the above results (Le et al., 2020). However, studies had reported that NOB is more sensitive to oxygen under long-term low DO condition (Liu et al., 2013). The researchers found that species of NOB like *Nitrospira* can adapt to low DO concentrations (the concentration is generally less than 0.5 mg/L) (Singh et al., 2022). The result was also confirmed in the Singh's experiment when DO concentrations is increased to 0.4-0.5mg/L. (Lei et al., 2021) found that the gene encoding hydroxylamine oxidation was affected because of DO concentrations in the range 0.30-0.43 mg/L, which reduced the activity of AOB. The PN run stably again and the accumulation of  $\text{NO}_2^-$ -N was restored when adjusted to 0.90-1.30mg/L. Although the situation did happen, the research shows that the activity of AnAOB was effected by DO concentrations >0.6mg/L and nitrogen removal is achieved in one-stage system at a DO concentrations 0.2-0.4mg/L (Liu et al., 2017). The presence of NOB did not limit AOB activity when the concentrations of DO was  $0.6 \pm 0.1$ mg/L and nitrogen removal was the best under the conditions (Zou et al., 2018). It was illustrated that both AOB and NOB were affected by low DO

concentrations but the activity of NOB was not easily recoverable (Li et al., 2011). (Chen et al., 2021) controlled DO concentration at 0.1-0.2 mg/L, and the PNA reaction was able to successfully remove nitrogen at about 70% effective. Based on above, although the data varied from researchers, the PN/A process was stable operation usually under DO concentration < 0.6 mg/L.

## FA and FNA

FA and FNA played an important parameter for the accumulation of  $\text{NO}_2^-$ -N, the calculation of FA and FNA concentrations was following by the formula (Qiao et al., 2010). The appropriate concentration of FA and FNA were considered as a inhibited for the activity of NOB to achieve the enrichment of AOB in PN (Gong et al., 2021). As reported in a previous study, FA concentration had an inhibitory impact on the activity of AOB and NOB in ranges of 10-150 mg/L and 0.1-1.0 mg/L, respectively, whereas FNA concentration ranged from 0.2 to 2.8 mg/l had an inhibitory impact on the activity of NOB (Anthonisen et al., 1976). (Vadivelu et al., 2007) proposed that the activity of NOB could be completely inhibited when the concentrations of FA and FNA were 6 mg/L and 0.02 mg/L respectively. The result was evidenced, along with the operation of A/O reactor, the final concentrations of FA and FNA were 1.23 mg/L and 0.095 mg/L respectively, which achieved the accumulation of  $\text{NO}_2^-$ -N and stable PN process (Wang et al., 2016).

However, some researchers proposed that the activity of NOB under above conditions could not be completely inhibited due to NOB in the long-term operation acclimated to the changes of FA and FNA, resulting in NOB still existed in the system (Villaverde, et al., 2000). The similar situation was found and demonstrated in Bin et al. research (Ma et al., 2017). Thus, the adaptive capacity of NOB in PN/A system become the crucial issue. Some approaches have been investigated to overcome the adaptive ability of NOB to provide sufficient  $\text{NO}_2^-$ -N for anammox process. An effective strategy was applied to suppress the activity of

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NOB, which was adopted by FNA shock sludge led to several communities of NOB was unable to acclimate and wash out in the system (D. Wang et al., 2016). Similar thought have been adopted in PN/A reactor, the sludge was respectively treated by FA and FNA (four hours in a week) which the stable PN/A process was obtained (Peng et al., 2020). Although several studies showed NOB suppression by return sludge was capable of occurring under FA and FNA, few studies have been conducted to reach a sufficient amount of  $\text{NO}_2^-$ -N. Therefore, it is still needed to find the optimum approach by experimental exploration.

## Temperature

Temperature had also a great influence on microbial activity. According to an Arrhenius-type equation (Hao et al., 2002), the risk of inactive for bacteria was increased due to high temperature but low temperature will inhibit activity. The adaptability and the maximum relative generation rate of AOB was stronger than that of NOB at elevated temperature, and the optimum temperature of AOB was approximately between 30 and 40 °C (Hellinga et al., 1998). Besides, the suitable temperature for the growth of AnAOB growth has been documented from 30 to 35 °C and it have a strong sensitivity to the temperature descending condition (Jetten et al., 2001). (Balmelle et al., 1992) found that the maximum accumulation of  $\text{NO}_2^-$ -N was obtained at temperature 35°C. Previous studies have shown that the conversion rate of  $\text{NH}_4^+$ -N to  $\text{NO}_2^-$ -N in PN process was successfully 81% at the temperature 37 °C (Cho et al., 2011). Wiśniewska et al. (Banachet et al., 2021) confirmed that the NRE reached 80% in anammox process when temperature was maintained at 30 °C. By controlling the temperature at 32-35°C in stage 1 and 2, the NRE could achieve 94.5%, in contrast, the NRE in stage 3 was declining at temperature decreasing from 35 to 24°C (Leet al., 2022). (Isanta et al., 2015) showed that the efficient of nitrogen removal had significantly decreased with increased temperature (from 35 to 46°C). Therefore, the data showed that the stable PN/A process and

increased the efficiency of nitrogen removal are achievement under the optimum temperature between 30 and 35 °C.

Nevertheless, the fluctuation in the seasonal temperature has an effect on the PN/A process and it is an obstacle hindering the efficiency of nitrogen removal, resulting in the microorganisms of PN/A system vulnerable. (Wanget al., 2014) also recorded the activity of microbial community structure and the NRE decreased as temperature drops. When temperature decreased from 22.2°C to 15°C, the decrease of NRR from the initial to the end was observed (Gong et al., 2020), which was consistent with the above finding. To address the mainly bottleneck at low temperature, controlling the nitrogen load (NLR) by adjusting the concentration of  $\text{NH}_4^+$ -N in the PN/A system was proposed to reach the increased efficiency of nitrogen removal, and the NRE achieved more than 96% in Li's study when the temperatures at  $13\pm 2^\circ\text{C}$  (Liet al., 2021). (Li et al., 2022) proposed carrier biofilms under the temperature changes from 29.1°C to 16.3°C was inoculated in the PN system to apply for treating low C/N sewage, and the result showed that the PN/A process was relatively stable and the NRE was maintain about 80%. (Ishimotoet al., 2021) proposed that the stable PN/A system was implemented in treating swine wastewater by controlling anammox granules to acclimate low temperature.

## RESEARCH APPLICATION

### Landfill leachate

Leachate with high ammonia nitrogen and low carbon to nitrogen ratio (C/N) was produced by waste landfill (Wanget al., 2022). Leachate has a complex composition and can have an impact on the environment (Qiu et al., 2022). The C/N of leachate will lead to increased treatment costs (Gabarró et al., 2012; Renouet al., 2008). And the problem of nitrogen removal from leachate in landfills has become the focus of work. The PN/A is attracting attention for its ability to solve the

problem of increased operating costs due to low C/N in the treatment of waste leachate, and the process is highly effective in nitrogen removal of leachate (Nhatet al., 2014). (Wu et al., 2020) studied the PNA treatment of leachate at low temperatures. Nitrogen removal was successfully achieved by a combination of four processes (UASB-A/O-ANAOR-ASBR). The removal rate of  $\text{NH}_4^+\text{-N}$  is more than 97%, and the removal rate of TN is about 94%. (Yan et al., 2022) used a fictional method to treat mature waste leachate. Ozone pretreatment is first applied to mature waste leachate, and granular sludge is used in a two-stage system of the PN/A process. The PN process can occur without controlling the exact amount of dissolved oxygen (DO) in the study. It also provides a new idea for future research work on PN/A. (Jiang et al., 2020) introduced aerobic biofilm combined with PNA. The reactor had the best effect on mature leachate treatment when the DO concentration was around 4.03mg/L by constantly adjusting. Compared with the traditional PNA process, the highest NRE was 96.7% in the reactor with biofilm. (Wang et al., 2022) designed a novel gas-rising reactor. An integrated PNA reaction is implemented in a new reactor to treat waste leachate. The reactor received a fast start-up and NRR was 1.54 kg N/m<sup>3</sup>·d.

### Other sewage

(Wanget al., 2017) used a medium-scale PN/A reactor to treat sludge digestate. The PN process was started up because of the effectively controlled concentration of FA and FNA by adjusting the environmental factors. The process provided a stable substrate for anammox. Eventually, the PN/A reactor was in stable operation and NRR was 1.23 kg N/m<sup>3</sup>/d at 148 days. (Silveiraet al., 2021) addressed the nitrogen removal in slaughterhouse wastewater by combining suspended biomass reactors and settling tanks for PN and Upflow Anaerobic Sludge Blanket (UASB) reactors for anammox. The COD/N was maintained at 0.4-0.6 mg COD/N. throughout the experiment. Studies have shown that PNA can successfully solve slaughterhouse

wastewater and the NRE ranged from 74.4% to 94%. (Lin et al., 2020) applied the PNA process to artificial wetlands to achieve the nitrogen removal of low ammonium wastewater. The optimal idle time and reaction time of the PN reactor were found to be 7.5 h and 16 h through trial. The ratio of nitrite to ammonia nitrogen in the effluent is close to the theoretical value of 1.32. (Daverey et al., 2013) added nitrite total auto-nitrification (CANON) to SBR to achieve an integrated PNA process for treating wastewater in the photovoltaic industry. By adjusting the NLR and HRT, the reactor was able to achieve 89% and 98% removal of TN and  $\text{NH}_4^+\text{-N}$ , respectively. (Y. Guo et al., 2022) studied the PNA process to treat anaerobic membrane bioreactor filtrate under low temperature conditions, and was eventually able to perform denitrification as well.

### CONCLUSION

Compared with the traditional technology, the advantage in PNA process is obvious, which has been used for full-scale application in the treatment of landfill leachate and other wastewater. At present, Combination of PN/A process and technology has attracted many researches to remove nitrogen in wastewater due to the high nitrogen removal efficiency. However, the accumulation of nitrite has been a problem. Furthermore, AnAOB was also a bottleneck because of hard to remain stable. Therefore, how to enhance the stability of PNA in nitrogen removal under the above difficulties is still priority and need more experimental exploration to find appropriate strategy.

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