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PERSPECTIVES

New perspectives in free nitrous acid (FNA) uses for sustainable wastewater management

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HIGHLIGHTS

- The historical development of free nitrous acid (FNA) technologies is reviewed.
- The roles of novel acid-tolerant ammonia oxidizers are highlighted.
- Acid-tolerant ammonia oxidizers can self-sustain high-level FNA production.
- The next-generation *in situ* FNA-based technologies are discussed.

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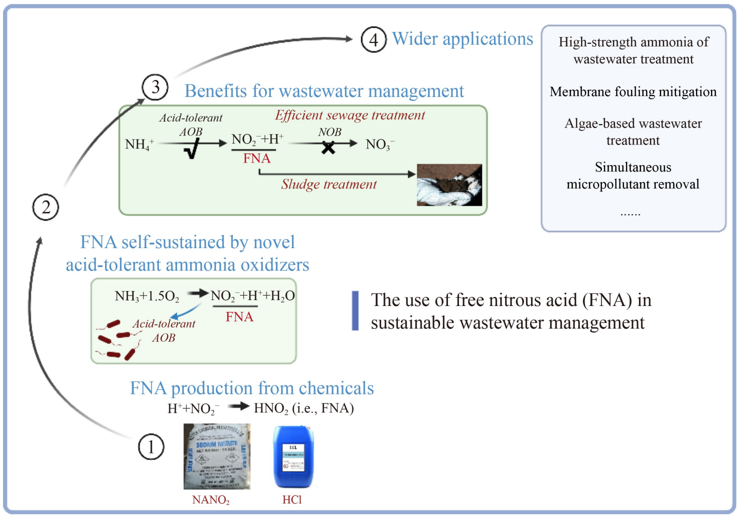
GRAPHIC ABSTRACT



ABSTRACT

The biocidal effects of free nitrous acid (FNA) have found applications in multiple units in an urban wastewater system, including sewer networks, wastewater treatment processes, and sludge treatment processes. However, these applications are associated with chemical costs as both nitrite and acid are needed to produce FNA at the required levels. The recent discovery of novel acid-tolerant ammonia oxidizers offers the possibility to produce FNA from domestic wastewater, enabling the development of next-generation FNA-based technologies capable of achieving self-sustaining FNA production. In this study, we focus on the concept of *in situ* FNA generation facilitated by acid-tolerant ammonia oxidizers and highlight the multiple benefits it creates, after a brief review of the historical development of FNA-based technologies. We will discuss how wastewater systems can be made more energy-efficient and sustainable by leveraging the potential of acid-tolerant ammonia oxidizers.

1 Background

Free nitrous acid (FNA), the protonated form of nitrite ($HNO_2 \leftrightarrow NO_2^- + H^+$), has garnered significant interest in

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wastewater management (Fig. 1). The early attention to FNA was due to its inhibitory effect on a broad range of microorganisms found in wastewater treatment systems, including nitrifiers, denitrifiers, polyphosphate-accumulating organisms, anammox bacteria, and methanogenic archaea (Anthonisen et al., 1976; Rake and Eagon, 1980; Strous et al., 1999; Saito et al., 2004). FNA at ppm-levels was later found to have potent bactericidal properties in both aerobic and anoxic conditions across wastewater management applications. While these effects were initially viewed as problematic due to their potential negative impact on wastewater treatment performance, recent research has leveraged the bacteriostatic and bactericidal properties of FNA to improve integrated wastewater management. In sewer systems, FNA is applied to mitigate pipe corrosion, odor, health hazards, and greenhouse gas emissions by inactivating sewer microbes (Jiang et al., 2011; Zuo et al., 2020). A field trial in Australia demonstrated that one single dose of FNA can achieve 80% sulfide reduction in a pressure sewer for up to 10 d, with a lower cost (only 0.01 $\$/m^3$ sewage) than other chemical methods (0.04–0.48 $\$/m^3$ sewage) (Jiang et al., 2013). In wastewater treatment, FNA is used to selectively eliminate nitrite-oxidizing bacteria (NOB) by side-stream sludge treatment, thereby achieving carbon and energy-efficient shortcut nitrogen removal (Wang et al., 2014). FNA treatment can be enhanced by coupling with other strategies (e.g., low dissolved oxygen and residual ammonium concentration control) in practical applications (Zheng et al., 2023). FNA is also employed in sludge management to improve sludge reduction, energy recovery, and pathogen removal (Pijuan et al., 2012; Wang et al., 2013a; 2013b). For instance, pre-treatment of full-scale wastewater activated sludge (WAS) for 24 h at an FNA concentration of 1.8 mg HNO_2–N/L enhanced volatile solids (VS) destruction by 17% ± 1% and increased dewaterability from 12.4% ± 0.4% to 14.1% ± 0.4% (Wei et al., 2018). Additionally, FNA is utilized in membrane systems to mitigate membrane fouling (Filloux et al., 2015), and in algae systems to facilitate algae harvesting (Bai et al., 2014). These progresses have been reviewed by Zhou et al. (2011) and Duan et al. (2020), which focused on the inhibitory and biocidal effects of FNA, respectively.

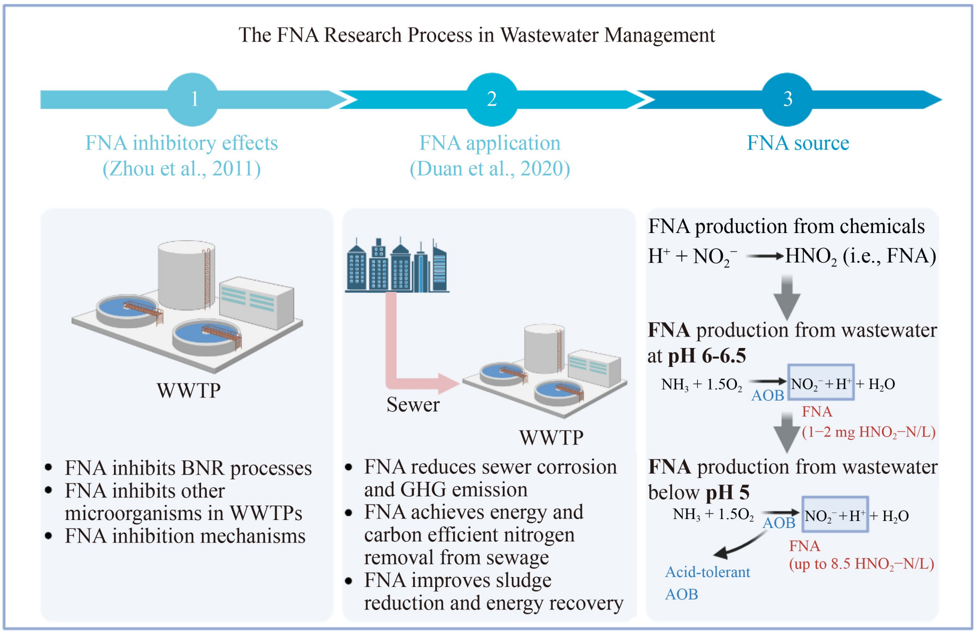

Fig. 1 Progress of the FNA research in the past 20 years.

The integration of FNA into urban wastewater systems requires sufficient FNA production (Fig. 1). The concentration of FNA, as a protonated form of nitrite, is jointly determined by the pH level and the nitrite concentration. The initial approach to produce FNA at ppm levels relied on chemical inputs (i.e., nitrite and acid), which generally results in high costs (Calderon et al., 2021). Subsequently, researchers have proposed an alternative method for FNA production from wastewater rich in ammonium, e.g., anaerobic digester liquor, urine wastewater, and landfill leachate (Law et al., 2015; Zheng et al., 2017). This method utilizes ammonia-oxidizing bacteria (AOB) to convert ammonia to nitrite through nitritation (Zuo et al., 2023). However, acids are required to overcome the buffer in the wastewater/sludge to be treated, to bring the pH down to a desirable level and achieve the required level of FNA, which incurs significant chemical costs. For instance, when the produced FNA is used to regularly treat a portion (20%–40%) of the activated sludge from the main line of wastewater treatment to achieve shortcut nitrogen removal, a pH level as low as 5.5 is required. To lower the pH, a large amount of acid is consumed, yet still not guaranteeing stable suppression of NOB due to their adaptation (Zahedi et al., 2016; Zhang et al., 2016; Su et al., 2023).

Due to the abovementioned challenges and the fact that many wastewater treatment facilities do not have such high-strength wastewater available, a new approach to producing FNA *in situ* from low-strength of wastewater (e.g., domestic wastewater) has been proposed. In this new concept, FNA at ppm-levels is formed at relatively low nitrite levels, with the wastewater pH lowered to acidic levels of pH 4–5. As canonical AOB in wastewater treatment systems cannot survive at such low pH levels, this approach relies on novel AOB strains that can tolerate acidic environments. In this context, this review will focus on the most recent advances in producing FNA *in situ* using novel acid-tolerant AOB and their applications to urban wastewater management.

2 Acid-tolerant ammonia oxidizers

Nitrosomonas, the predominant AOB in wastewater treatment, is known to be sensitive to acidic conditions, with its activity completely ceasing at pH values below 5.5 (Fumasoli et al., 2015). While acid-tolerant AOB like Nitrosospira, Nitrosococcus, and *Candidatus* (*Ca.*) Nitrosoglobus have been identified in naturally acidic environments such as acid lakes and soils at pH < 5.5 (Hayatsu et al., 1993; Hayatsu et al., 2017; Taylor and Bottomley, 2006), their establishment in wastewater treatment systems has not been achieved only until recent years.

The oxidation of one-mole ammonia generates two-mole protons, which causes the lowering of pH in wastewater, particularly when alkalinity is a limiting factor. In systems treating urine wastewater, for instance, β-proteobacterial Nitrosospira has been observed to outcompete *Nitrosomonas* at a pH of 4.6 (Fumasoli et al., 2016; Li et al., 2020; Meng et al., 2022). Some γ-proteobacterial AOB (e.g., *Ca.* Nitrosoglobus) have been found to be able to reduce pH to 2.2 in wastewater with a high ammonia content (Fumasoli et al., 2017; Wang et al., 2021a). Kinetic studies have revealed that *Ca.* Nitrosoglobus, among all known ammonia oxidizers, exhibits the highest resistance to FNA (Wang et al., 2021b). Specifically, the FNA concentration for a 50% activity inhibition of *Ca.* Nitrosoglobus was identified as high as 29.5 mg HNO_2–N/L, much higher than that (< 1 mg HNO_2–N/L) for Nitrosomonas (Wang et al., 2021b). Indeed, *Ca.* Nitrosoglobus, a newly identified γ-proteobacterial AOB, presents unique physiologic characteristics (Hayatsu et al., 2017). The *Ca.* Nitrosoglobus genome is generally smaller than other AOB genomes. In contrast to known γ-proteobacterial AOB isolated from marine environments, *Ca. Nitrosoglobus* is sensitive to salinity. Notably, this strain thrives in a pH range of 5–7.5 and endures highly acidic conditions down to pH 2 by forming cell aggregates. In strongly acidic soil, the ammonia monooxygenase subunit A gene and its transcript of *Ca.* Nitrosoglobus were found more abundant compared to ammonia-oxidizing archaea and β-proteobacterial AOB.

The discovery of acid-tolerant AOB offers the possibility of achieving *in situ* FNA accumulation through operating sewage and sludge treatment units in acidic operations. First, it allows for the achievement of FNA concentrations at ppm levels even in low-strength municipal wastewater. Taking 40 mg NO_2^-–N/L as an example, FNA can reach 0.95 mg HNO_2–N/L at pH 5.0 (22 °C). Such an *in situ* FNA concentration has been shown to effectively eliminate NOB (Zheng et al., 2018). Second, compared to the periodic treatment of biomass with FNA generated *ex situ*, the high *in situ* FNA concentration ensures the continuous exposure of the entire system to the harsh conditions, theoretically maximizing the benefits.

3 Benefits of *in situ* FNA self-sustained by acid-tolerant AOB for wastewater management

The strong FNA resistance of acid-tolerant AOB has multiple advantages for the treatment of domestic wastewater and sludge (Fig. 2).

3.1 Robust nitritation in mainstream treatment of domestic wastewater

The nitrite shunt and Partial Nitritation/Anammox (PN/A) processes in mainstream wastewater treatment have gained worldwide attention due to their advantage for energy-positive treatment of domestic wastewater (Liu et al., 2019). However, the key challenge in establishing nitrite shunt and PN/A is the stable suppression of NOB (Wang et al., 2021c; 2021d; Lu et al., 2023a; Zheng et al., 2023). Various approaches have been explored to eliminate NOB, including low dissolved oxygen, intermittent, short sludge retention time, starvation, side-stream sludge treatment using FNA, among other strategies (Wang et al., 2022a). However, it is increasingly experienced that NOB develop resistance to suppressions over an extended time, leading to the failure of these processes (Su et al., 2023). Moreover, these methods often result in partial inhibition of AOB, leading to a decrease in sewage treatment capacity.

In recent studies, acidic mainstream nitritation approaches have been developed using acid-tolerant AOB to overcome both AOB inhibition and NOB adaptation.

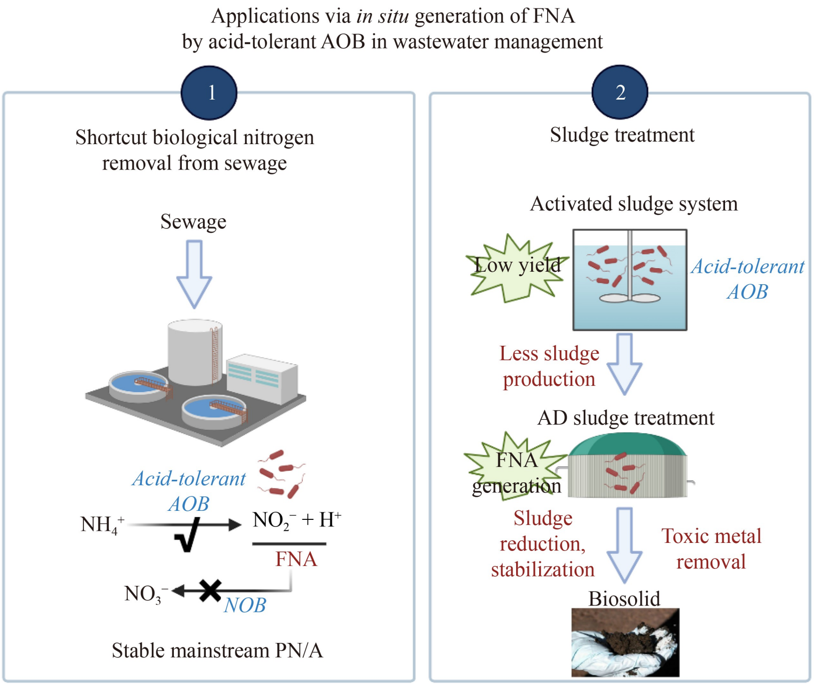

Fig. 2 Potential applications of FNA produced *in situ* by acid-tolerant AOB to sewage and sludge treatment.

Li et al. (2020) reported the successful establishment of stable nitrite accumulation for 200 d in a slightly acidic bioreactor (pH 5–6) dominated by *Nitrosospira*, with FNA levels reaching up to 2 mg HNO_2–N/L. Similarly, Wang et al. (2021c) demonstrated robust mainstream nitritation for 450 d in an acidic reactor (pH 4.5–5.0) with FNA levels of 3.0 ± 1.4 mg HNO_2–N/L, where *Ca. Nitrosoglobus* dominated the nitrifying community. Importantly, this level of FNA can completely suppress NOB, but still maintain the high activity of AOB, leading to a high nitritation rate of 200 mg N/(L·d), observed in the long term experiment of Wang et al. (2021c). Notably, the robustness of NOB suppression by this strategy is associated with the fact that the entire microbial community was exposed to FNA directly and continuously, leaving limited opportunity for NOB adaption as observed with other strategies (Meng et al., 2022).

3.2 Acidic sludge treatment for reduction, stabilization, and toxic metal removal

WWTPs generate large amounts of wasted activated sludge, which needs to be treated, transported and disposed of at a cost of up to 60% of the overall costs of WWTPs (Murthy et al., 2006). Anerobic digestion (AD) is the most used sludge treatment technology, which generates biogas and AD sludge. However, the subsequent treatment of AD sludge is still needed for further sludge reduction and stabilization. Recently, acidic aerobic digestion of AD sludge was proposed to improve the reduction and stabilization at ambient temperature (Wang et al., 2021e). The acidic condition in the experimental reactor was driven by acid-tolerant AOB, which drove the pH to 4.8 ± 0.2 as a result of ammonia oxidation. During this process, nitrite accumulated and reached around 200 mg N/L, while the FNA at 8.5 ± 1.8 mg HNO_2–N/L was therefore formed *in situ*. The high FNA and low pH led to a reduction of the total solids, volatile solids (VS) and non-volatile solids in the AD sludge by 25.2% ± 7.0%, 29.8% ± 4.3%, and 22.6% ± 5.5%, respectively. These reduction levels are significantly higher than those at near-neutral pH. Additionally, the acidic aerobic digestion significantly stabilized the AD sludge, decreasing the specific oxygen uptake rate (SOUR) by 72% to 0.5 ± 0.1 mg O_2/(g·VS h) (Wang et al., 2021e).

Moreover, the pH of the AD sludge has the potential to decrease to 2.0. Consequently, metals present in the AD sludge were extracted into the liquid phase. Notably, two of the most abundant metals, copper (Cu) and zinc (Zn), exhibited high solubilization efficiencies of 88% ± 4% and 96% ± 3%, respectively (Wang et al., 2021a). These findings showcase the viability of removing metals from biosolids without the need for external chemical addition. This not only ensures an economical disposal method but also promotes the safe reuse of excess sludge generated during biological sewage treatment. However, it was found that, at low pH, FNA rapidly decomposed to nitrate, involving several volatile intermediates, such as nitric oxide (NO), nitrogen dioxide (NO_2), and nitrogen trioxide (N_2O_3) (Lu et al., 2023b).

4 Perspectives and challenges

We have come to recognize that wastewater composition plays a critical role in applying acid-tolerant ammonia oxidizers for achieving more sustainable wastewater management. The alkalinity-to-ammonia ratio in wastewater is a pivotal factor that enables acid-tolerant AOB to effectively lower wastewater pH and function optimally in acidic conditions, without adding external acids. Building upon this fundamental principle, we propose broader possibilities through the utilization of acid-tolerant AOB to enhance wastewater management (Fig. 3).

Wastewater can be categorized into two groups based on its ammonia content. First, domestic wastewater is characterized by relatively low levels of ammonium and abundant alkalinity. Under these conditions, the utilization of acid-tolerant AOB can be challenging. Recent studies have introduced innovative approaches, such as CEPT or the mixing of domestic sewage with AD liquor, to reduce alkalinity (Hu et al., 2023; Wang et al., 2021c; 2022b). For instance, Hu et al. (2023) proposed an integrated system, consisting of a CEPT, an acidic PN, and an anammox to treat domestic wastewater. With the addition of $FeCl_3$ at 50 mg Fe/L during CEPT, the process effectively removed about 61% of COD and 90% of phosphate, along with a reduction in alkalinity. Following CEPT, stable nitrite accumulation was realized in an aerobic reactor operated at pH 4.35, facilitated by acid-tolerant AOB. The subsequent polishing in an anoxic reactor (Anammox) produced an effluent with satisfactory levels, containing COD at 41.9 ± 11.2 mg/L, total nitrogen at 5.1 ± 1.8 mg N/L, and phosphate at 0.3 ± 0.2 mg P/L. Importantly, this integrated system demonstrated the potential for energy self-sufficiency in domestic wastewater treatment. However, the stability and efficacy of these methods require systematic investigation via both pilot and full-scale applications. The second category of wastewater is high-strength ammonia wastewater, such as the source-separated urine, anaerobic digestion liquor, and landfill leachate. The low alkalinity and high ammonia concentration in these high-strength wastewaters render the feasibility of applying acid-tolerant ammonia oxidizers. Taking source-separated urine as an example, it represents only 1% of the total volume of municipal wastewater but contributes a substantial proportion of nitrogen (80%–90%) (Wald, 2022; Zuo et al., 2023a). Thus, separating human urine at its source holds great potential for reducing pollutant loads to wastewater, while biological nitrification is a

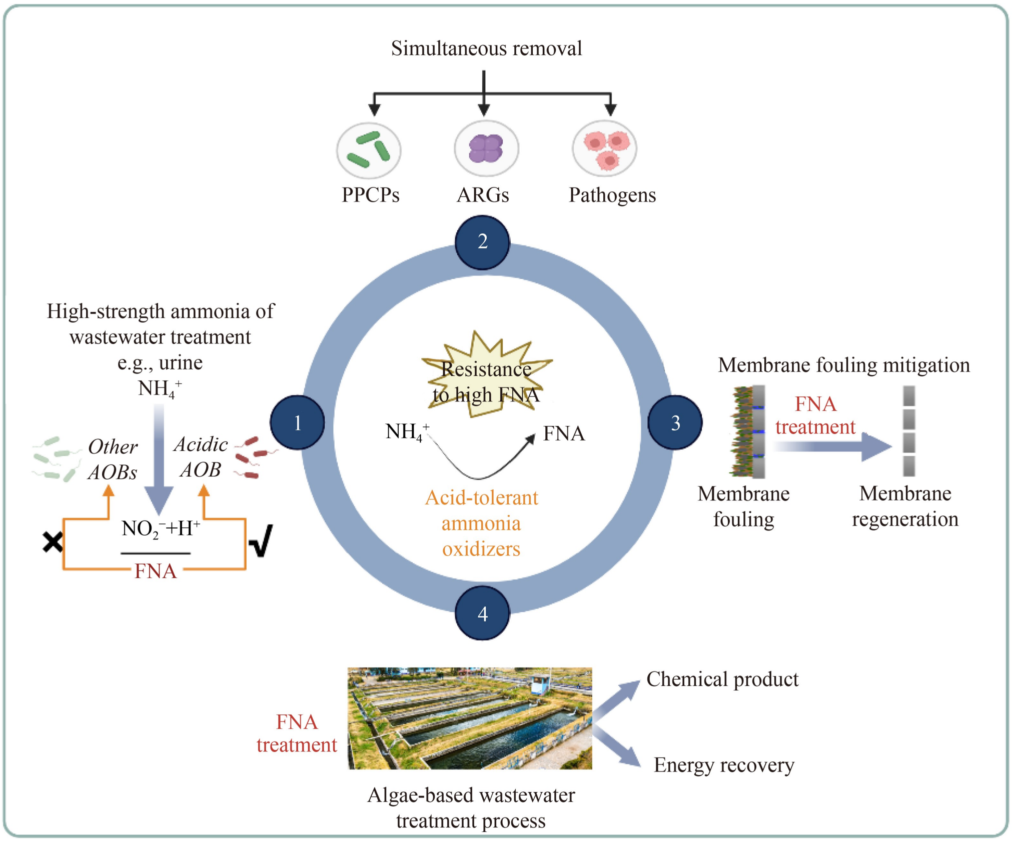

Fig. 3 Wider applications of FNA produced *in situ* for sustainable wastewater management.

cost-effective method for recovering nitrogen fertiliser from the source-separated urine (Li et al., 2021; Zuo et al., 2023b). From approximately 8000 mg N/L ammonia content of pure urine, about 4000 mg N/L nitrite can be formed due to alkalinity limitation, which can generate 3–10 mg HNO_2/L at an acidic pH of 6–6.5 (Zheng et al., 2017). While traditional AOB *Nitrosomonas* is inhibited by 50% by only 0.2 mg HNO_2/L (Hellinga et al., 1999), *Ca. Nitrosoglobus*, which can withstand much higher FNA concentrations (50% of activity loss at up to 29.5 mg HNO_2–N/L) (Wang et al., 2022b), can be a more promising candidate in these conditions.

Beyond leveraging acid-tolerant ammonia oxidizers to improve nitrogen removal and recovery from different types of wastewater, more applications may be developed by utilizing *in situ* generation of FNA. Some examples are provided:

1) Removal of Micropollutants: FNA has been shown to effectively reduce pharmaceuticals and personal care products, antibiotic-resistance genes and pathogens, fundamentally because of FNA-induced breakdown of macromolecular substances (Chislett et al., 2022a; 2022b; Liu et al., 2022). For example, sulfamethoxazole could be effectively removed by 95% ± 5% with the accumulation of FNA during urine nitritation (Cheng et al., 2021);

2) Pathogen Inactivation: In wastewater or sludge treatment, FNA-induced cell lysis may support pathogen inactivation. For example, it was observed that FNA pre-treatment of activated sludge improved pathogen reduction by 2.1 ± 0.2 logs in anaerobic digestion (Wei et al., 2018);

3) Mitigating Membrane Fouling: FNA was proven efficient in removing organic foulants and dissolving calcium (Czuba et al., 2022). However, the cleaning performance is often limited, likely due to the limited diffusion of FNA into the deep layer. For example, the FNA cleaning only recovered 74% of the water flux performance of a fouled forward osmosis membrane after ten cycles of cleaning (1 h cleaning for each cycle), while the water flux was recovered to > 90% by other cleaning reagents (e.g., NaOCl, EDTA) (Ab Hamid et al., 2018; Linares et al., 2012). Increasing FNA exposure concentration may be a solution. Using acid-tolerant AOB, a high FNA level of over 5 mg HNO_2–N/L can be easily generated from high-ammonium wastewater at a lower pH of 4.5. The high FNA and acidic pH are expected to recover membrane filtration performance more effectively.

4) Optimizing Algae-Based Wastewater Treatment: FNA was used as a novel algae pre-treatment method for boosting both product (crude lipids and triacyl glycerides via extraction) (Bai et al. 2014) and energy (biogas via anaerobic digestion) recovery. The integration of a Microalgal-bacterial consortium (MBC) presents a sustainable and efficient alternative to traditional activated sludge processes for wastewater treatment. Notably, a recent advancement involves combining the MBC process with nitritation (i.e., shortcut MBC), which offers additional advantages such as decreased organics and reduced aeration requirements (Abbew et al., 2022). The benefits may be more visible if FNA could be self-sustained by acid-tolerant ammonia oxidizers in the algae-based wastewater treatment system.

While the utilization of acid-tolerant AOB shows good promise for improving wastewater management, there may be some potential challenges associated with practical applications. The complexity of wastewater, including factors like salinity, heavy metals, and organic matter, could have unknown effects on acid-tolerant AOB, potentially limiting its effectiveness. Furthermore, the scalability of applying acid-tolerant AOB in urban mainstream sewage is currently underway. Additionally, it should be pointed out that acidic operations can trigger the disproportionation reaction of FNA, resulting in the chemical conversion of nitrite to nitrate with the production and emission of NO (Lu et al., 2023b; Udert et al., 2005; Wang et al., 2021c). Therefore, precise control of the acid pH range is crucial. The rate of this chemical reaction is dependent on FNA concentration (Zuo et al., 2022). Seeking strategies to minimize NO emissions while maintaining the effectiveness of FNA appears to be an important research question that warrants further exploration.

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Conflict of Interest The authors Yongzheng Peng and Zhiguo Yuan are Members of Advisory Board of *Frontiers of Environmental Science & Engineering*. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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