Nitrite-dependent microbial utilization for simultaneous removal of sulfide and methane in sewers

Zhiqiang Zuo [a,b,c], Yaxin Xing [a], Xi Lu [b], Tao Liu [b,d], Min Zheng [b,e], Miao Guo [f], Yanchen Liu [a,*], Xia Huang [a]

[a] *State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, PR China*
[b] *Australian Centre for Water and Environmental Biotechnology, The University of Queensland, St Lucia QLD 4072, Australia*
[c] *National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology, Engineering Research Center of Beijing, Beijing University of Technology, Beijing 100124, PR China*
[d] *Department of Civil and Environmental Engineering, The Hong Kong Polytechnic University, Hong Kong 999077, PR China*
[e] *Water Research Centre, School of Civil and Environmental Engineering, University of New South Wales, Sydney, New South Wales 2052, Australia*
[f] *Department of Engineering, King's College London, London WC2R 2LS, UK*

A B S T R A C T

Chemicals are commonly dosed in sewer systems to reduce the emission of hydrogen sulfide (H_2S) and methane (CH_4), incurring high costs and environmental concerns. Nitrite dosing is a promising approach as nitrite can be produced from urine wastewater, which is a feasible integrated water management strategy. However, nitrite dosing usually requires strict conditions, e.g., relatively high nitrite concentration (e.g., ~200 mg N/L) and acidic environment, to inhibit microorganisms. In contrast to "microbial inhibition", this study proposes "microbial utilization" concept, i.e., utilizing nitrite as a substrate for H_2S and CH_4 consumption in sewer. In a laboratory-scale sewer reactor, nitrite at a relatively low concentrations of 25–48 mg N/L was continuously dosed. Two nitrite-dependent microbial utilization processes, i.e., nitrite-dependent anaerobic methane oxidation (n-DAMO) and microbial sulfide oxidation, successfully occurred in conjunction with nitrite reduction. The occurrence of both processes achieved a 58 % reduction in dissolved methane and over 90 % sulfide removal in the sewer reactor, with microbial activities measured as 15.6 mg CH_4/(L·h) and 29.4 mg S/(L·h), respectively. High copy numbers of n-DAMO bacteria and sulfide-oxidizing bacteria (SOB) were detected in both sewer biofilms and sediments. Mechanism analysis confirmed that the dosed nitrite at a relatively low level did not cause the inhibition of sulfidogenic process due to the downward migration of activity zones in sewer sediments. Therefore, the proposed "microbial utilization" concept offers a new alternative for simultaneous removal of sulfide and methane in sewers.

1. Introduction

Hydrogen sulfide (H_2S) and methane (CH_4) are two significant contaminants in global sewer networks. The H_2S formation poses multiple threats including corrosion of concrete infrastructure, sewer odors, and health hazards for sewage treatment workers (Pikaar et al., 2014; Zuo et al., 2019). CH_4 is a potent greenhouse gas (GHG), with a global warming potential approximately 28 times that of CO_2 when measured over a 100-year period (Soued et al., 2022). Sewer systems are significant sources of CH_4 production, with a high rate of 1–2 g CH_4/(m^2·d), leading to a liquid-phase CH_4 concentration of up to 20–25 mg/L in real rising main sewers (Guisasola et al., 2008; Liu et al., 2015a). To address

both H_2S and CH_4 emissions in sewers, a well-known approach is to reduce the source production of sulfide and methane by suppressing the activities of SRB and MA in sewer biofilms and sediments (Zhang et al., 2023). This suppression can be primarily achieved by the dosing of various chemicals, such as free nitrous acid (FNA), free ammonia (FA) and ferrate (Jiang et al., 2011; Yan et al., 2023; Zuo et al., 2023b). However, microbial inhibition typically requires the exposure of sewer microbial communities to chemicals with high concentration for several hours, presenting a challenge in maintaining such harsh conditions in real sewers. Several factors, including chemical degradation and dilution along sewer pipes, tend to result in the relatively low efficiency of these approaches. For instance, previous reports have demonstrated that

0.2–0.3 mg HNO_2—N/L (e.g., ~200 mg NO_2^--N/L at pH=6) of FNA achieved 80 % inactivation of SRB in a lab-scale biofilm reactor (Jiang et al., 2011), but even 13.3 mg HNO_2—N/L of FNA was insufficient for sustained SRB inactivation in a real pressure sewer (Despot et al., 2021).

In-sewer dosing of nitrite has emerged as a practical approach because nitrite can be derived from partial nitridation ($NH_3+1.5O_2 \rightarrow NO_2^-+H_2O$) of high-strength wastewater (e.g., source-separated urine) (Mackey et al., 2016; Zuo et al., 2023a). Zheng et al. (2017) reported that using nitrite produced from urine would lower the operational costs by approximately 2/3 and GHG emissions by 1/3, compared with using commodity chemicals. Although nitrite is widely recognized as toxic to sewer microbes and causes microbial inhibition (Jiang et al., 2010; Mohanakrishnan et al., 2008), studies indicated that nitrite could also serve as an electron acceptor for sulfide removal (Eqs. (1) and 2), facilitated by sulfide oxidizing bacteria (SOB) like *Thiobacillus* (Mahmood et al., 2007; Yang et al., 2005). This microbial utilization mechanism inspired us to seek any potential microbial oxidation of methane by dosed nitrite, contradictory to the previous microbial inhibition mechanism. Searching in the literature, a novel microbial process of nitrite-dependent anaerobic methane oxidation (n-DAMO) was discovered (Raghoebarsing et al., 2006). This process was demonstrated to have an important role in mitigating methane in many natural environments like marine, wetland, and deep lakes (Deutzmann et al., 2014; Haroon et al., 2013; Hu et al., 2014). The bacteria responsible for n-DAMO belong to the genus "*Candidates*' Methylomirabilis" of the NC10 phylum, which can utilize methane as an electron donor and nitrite as an electron acceptor (Eq. (3)) (Ettwig et al., 2010). Consequently, we hypothesize that these bacteria, if present in sewers, are likely to act as a sink of methane, as do in natural environments. Over past years, various n-DAMO technologies have been developed to remove dissolved methane and nitrogen in wastewater treatment (An et al., 2024; Fan et al., 2023; Liu et al., 2020; van Kessel et al., 2018; Wang et al., 2023). Zuo et al. (2024) found that methane emission was reduced by half due to the growth of n-DAMO bacteria in sewers, pointing to the potential of utilizing microbes to enhance sewer management. However, a detailed approach to leveraging nitrite for both sulfide oxidation and DAMO processes in sewers is currently lacking, and the efficiencies of these processes remain unknown.

$$3HS^- + 8NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \tag{1}$$

$$3HS^- + 2NO_2^- + 5H^+ \rightarrow 3S^0 + N_2 + 4H_2O \tag{2}$$

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \tag{3}$$
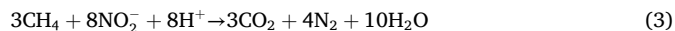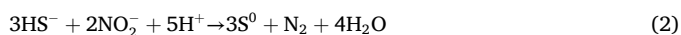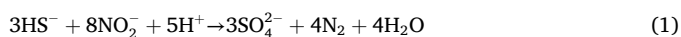
Therefore, this research aims to study in-sewer nitrite dosing for simultaneous sulfide and methane control via "microbial utilization" in addition to conventional "microbial inhibition". This microbial utilization process is expected to involve SOB and n-DAMO bacteria as key microbes which co-utilize nitrite as an electron acceptor to oxidize sulfide and methane simultaneously. To test this hypothesis, a lab-scale sewer reactor was operated for over 240 days with continuous nitrite dosing, during which sulfide and methane removal performance was systematically monitored. A comprehensive experimental approach involving cycle studies, activity batch tests and microbial analysis was employed to dissect the underlying mechanism behind the reactor's performance. Outcomes from this study suggest that nitrite-dependent microbial utilization concept hold promise as a useful method for sewer management.

2. Results and discussion

2.1. Simultaneous removal of methane and sulfide through nitrite dosing

A long-term experiment was conducted in the lab-scale sewer reactor receiving real wastewater that contained high concentrations of sulfide

and methane, produced from another identical lab-scale sewer reactor without any chemical dosing (control reactor). Fig. 1A shows the long-term performance of methane removal by nitrite dosing, as observed from the methane concentrations in the influent and effluent at varying nitrite dosages. In the initial 120 days at 33 mg N/L nitrite dosage (excluding days 35–45), the average methane concentration in the effluent was only 8.5 ± 2.9 mg CH_4/L. Compared with the influent level (18.5 ± 3.5 mg CH_4/L), the methane removal ratio reached around 53 %, demonstrating the stable occurrence of n-DAMO process through nitrite dosing. Mass balance analysis, based on the average removed amount of methane (i.e., approx.10 mg CH_4/L) and the stoichiometry of n-DAMO reaction (Eq. (3)), revealed that about 70 % of nitrite was consumed through the n-DAMO process, suggesting a significant contribution of n-DAMO in nitrite reduction. Simultaneously, the sulfide concentration in the effluent was also significantly reduced to approx. 8.1 ± 6.6 mg S/L during the first 120 days (excluding days 35–45) (Fig. 1B). An average sulfide removal ratio of ~71% was obtained, higher than the methane removal ratio (Fig. 1A). The simultaneous removal of methane and sulfide in the nitrite-dosed sewer reactor confirmed the reliability of the proposed microbial utilization approach.

During the period of day 35–45, reducing the nitrite concentration to 25 mg N/L resulted in a rapid recovery in effluent methane and sulfide concentrations (15 ± 2.3 mg CH_4/L and 16 ± 6 mg S/L). The relatively low ratio of 36 ± 9 % (methane) and 34 ± 24 % (sulfide) indicated that their removal relied on nitrite dosing concentration (Fig. 1C). As such, a subsequent increase in nitrite concentration to 44 mg N/L was conducted after day 120 to improve methane and sulfide removal performance. The results showed that the overall methane removal performance was slightly enhanced, with an average removal ratio of 58 % (Fig. 1C). However, it was observed that the influent dissolved methane concentration was unstable during day 165–182, which was only 7.4 ± 1.2 mg CH_4/L (Fig. 1A), due to the fluctuations of COD in raw sewage (Figure S1). Within this period, the effluent methane concentration only dropped to 3.6 ± 0.4 mg CH_4/L (Fig. 1A). The low methane removal amount suggests that methane removal also relies on methane concentration, in addition to nitrite dosing amount. After day 182, the influent dissolved methane concentration was stable in the range of 15–20 mg CH_4/L. The methane removal ratio at the steady state was 67 ± 4 %, which was significantly higher than that in the period with nitrite dosage of 33 mg N/L ($p < 0.05$).

In contrast, increasing the nitrite concentration to 48 mg N/L could effectively enhance sulfide removal (Fig. 1D). After day 150, the sulfide concentration in the effluent was only 1.2 ± 1.5 mg S/L, with a high removal ratio of over 90 % (Fig. 1D). Within this period, sulfide control was relatively stable, compared to the dynamic fluctuations in the effluent sulfide concentration before day 120 (Fig. 1B).

2.2. Evidence of nitrite-dependent microbial methane and sulfide oxidation

Copy numbers of n-DAMO bacteria and SOB. The abundance of n-DAMO bacteria and SOB was assessed using qPCR. n-DAMO bacteria were quantified by targeting both n-DAMO bacterial 16S rRNA genes and the functional gene *pomA* (Fig. 2A), while SOB bacteria were quantified using the functional gene *soxB* (Fig. 2B). The copy number of n-DAMO bacterial16S rRNA genes in sewer biofilm and sediment in the nitrite-dosed sewer reactor was found to be 1.4 (± 0.2) $\times 10^6$ copies/g sludge and 0.9 (± 0.2) $\times 10^6$ copies/g sludge, representing a substantial increase compared to those observed in the control reactor (0.1 (± 0.003) $\times 10^6$ copies/g sludge and 0.1 (± 0.02) $\times 10^6$ copies/g sludge) (Fig. 2A). They were in line with the increased abundance of the *pmoA* gene, collectively supporting the prevalence of n-DAMO bacteria in sewer biofilms and sediment in the nitrite-dosed sewer reactor (Fig. 2A). In addition, the abundance of the *soxB* gene in the nitrite-dosed sewer reactor was notably high, i.e. 329 (± 72) $\times 10^6$ copies/g sludge (biofilm) and 62 (± 13) $\times 10^6$ copies/g sludge (sediment), indicating the
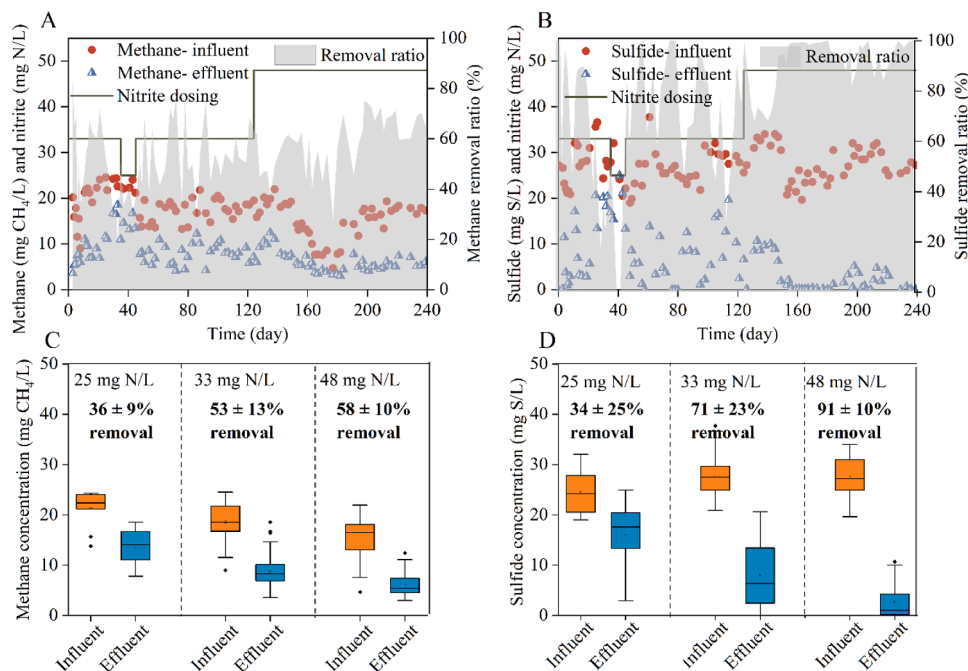
Fig. 1. Long-term removal performance of methane (A) and sulfide (B) in the sewer reactor receiving nitrite dosing and is summarized in box figures, respectively (C and D).
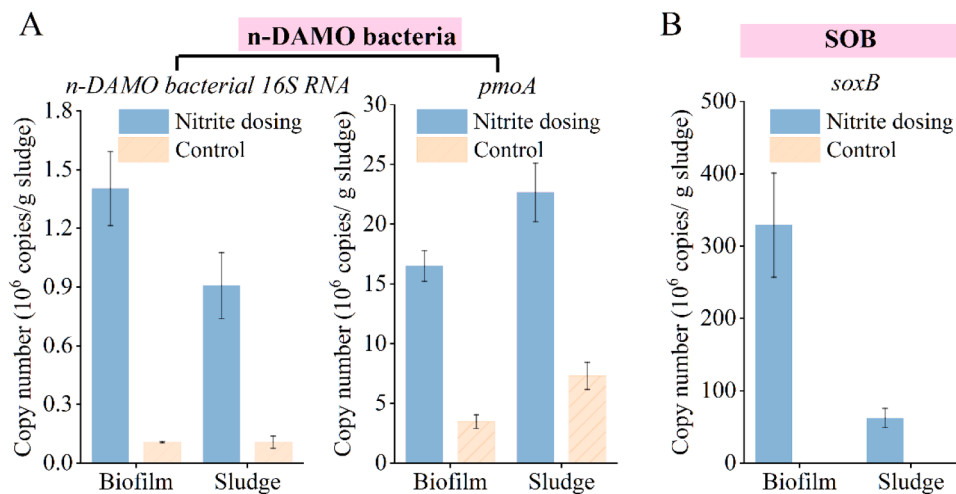

Fig. 2. The abundance (copy number) of n-DAMO bacteria related 16S RNA gene (A) and function gene *pmoA*, and SOB related function gene *soxB* (B) in the biofilm and sediment sludge in the nitrite-dosed sewer reactor and control reactor, respectively.

dominance of SOB under nitrite-dosed sewer conditions.

Nitrite-dependent methane and sulfide oxidation activity. Independent batch tests were carried out in triplicate to show the simultaneous oxidation of methane and sulfide coupled with nitrite reduction in the nitrite-dosed sewer reactor (Fig. 3). Following the dose of nitrite with an initial concentration of 22 mg N/L, sulfide and methane rapidly oxidized at an average rate of 29.4 mg S/(L·h) and 15.6 mg CH$_4$/(L·h), respectively, while nitrite also dropped at a comparable rate of 45.6 mg N/(L·h). The simultaneous decline in these three indicators suggests that n-DAMO and sulfide oxidation occur concurrently. According to Eq. (3), the nitrite reduction rate by n-DAMO bacteria was determined to be 36 mg N/(L·h), approx. 78 % of the observed total nitrite reduction rate. This ratio was close to the average contribution (~70 %) in the long-term result. In comparison, the same nitrite dosing test was conducted in the control reactor, but no methane and sulfide decline were observed (Figure S2), suggesting that both sulfide and methane oxidation are

driven microbially rather than chemically. To repeat the phenomenon of diminished methane removal observed between days 165–182 with a low influent methane concentration (Fig. 1A), an additional batch test was undertaken in the nitrite-dosed reactor with the initial methane concentration hovered around 6 mg CH$_4$/L. As expected, the fluctuations in methane levels were negligible (Figure S3), again suggesting that n-DAMO activity may be limited at low methane concentrations.

2.3. Cycling study results

To understand the dynamic fluctuation in sulfide and methane removal by nitrite dosing, a series of periodic cycle tests were conducted (days 41, 57, 139, and 184), as illustrated in Fig. 4. On day 41, with 10 mL nitrite (25 mg N/L) dosed into the reactor, the initial nitrite concentration reached around 4 mg N/L due to dilution (Fig. 4A). Following that, the nitrite concentration appeared to decrease, concomitant with
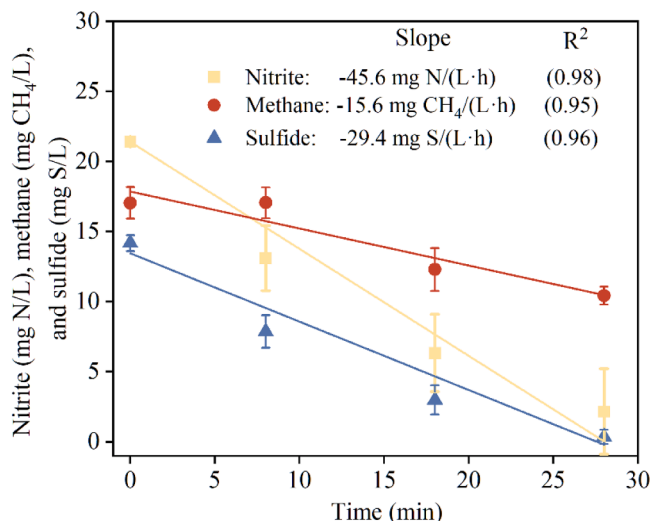
Fig. 3. Nitrite-dependent simultaneous methane and sulfide oxidation activities in the sewer reactor with nitrite dosing. The lines present the linear fitting, with the slope presenting the rates. The error bars are the results of triplicate tests.

the decrease in sulfide concentration. This concurrent decline indicates the occurrence of nitrite-dependent sulfide oxidation. However, after the depletion of nitrite after 20–30 min, the sulfide concentration showed a remarkable recovery and reached around 25 mg S/L at the end of the cycle — a high level observed before nitrite dosing. This suggests that the present nitrite dosage did not effectively inhibit SRB activity. Overall, the amount of removed sulfide via nitrite-dependent sulfide oxidation was offset by the production of sulfide once nitrite was depleted, leading to the worse sulfide removal performance during days 35–45 (Fig. 1B).

In comparison, at 33 mg N/L of nitrite dosage on day 57, a low sulfide concentration was evident in a cycle (Fig. 4B). After nitrite dosing, sulfide rapidly declined to zero, and was not re-produced after nitrite was depleted, which is probably due to the absence of available COD as the substrate. To further explore the key effect of COD on sulfide production rebound, Fig. 4C and 4D depict the cycle results with higher nitrite concentrations (48 mg N/L) on days 139 and 184, respectively. On day 139, a substantial recovery in sulfide production (Fig. 4C), like that in Fig. 4A, also occurred after the nitrite depletion, again suggesting the high tolerance of SRB activity to nitrite dosing. On day 184, there is no recovery in sulfide concentration (Fig. 4D), resembling the scenario of COD insufficiency in Fig. 4B. These results indicate that SRB were not inhibited even at the highest nitrite dosage of 48 mg N/L. Once nitrite was depleted, whether sulfide could be re-produced by SRB was dependent on the availability of COD.

Additionally, the variations in methane concentration were measured in all cycles. Methane concentrations exhibited a clear decline under three conditions (Figs. 4B-D), except for the neglected variation under 25 mg N/L nitrite dosing (Fig. 4A). This characteristic aligns with long-term methane removal performance (Fig. 1A and 1C). Notably, there is no recovery in methane production after the depletion of nitrite in all cycles, indicating a low methane production activity.

2.4. The inhibitory effect of nitrite on sulfidogenic and methanogenic processes

To ascertain the impact of nitrite dosing on methane and sulfide production activity, batch tests were conducted, as illustrated in Fig. 5A. The control lab-scale sewer reactor without any chemical dosing displayed a similar microbial activity between sulfide production and (4 mg S/(L·h)) and methane production (3.8 mg CH$_4$/(L·h)). In contrast, the experimental reactor, subjected to long-term nitrite dosing, exhibited significant changes.

Methane production activity dropped below 0.5 mg CH$_4$/(L·h), indicating effective inactivation of MA by nitrite dosing, consistent with
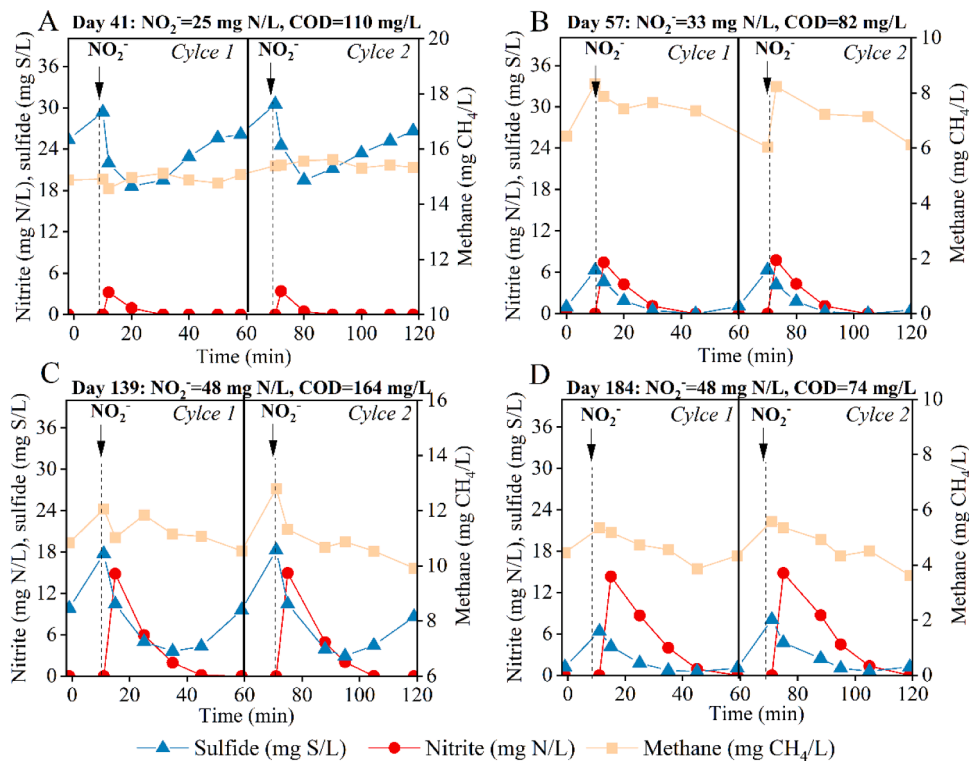

Fig. 4. Dynamic variations in the nitrite, sulfide, and methane concentrations during two typical cycles in the nitrite-dosed sewer reactor: day 41 (A), day 57 (B), day 139 (C) and day 184 (D).
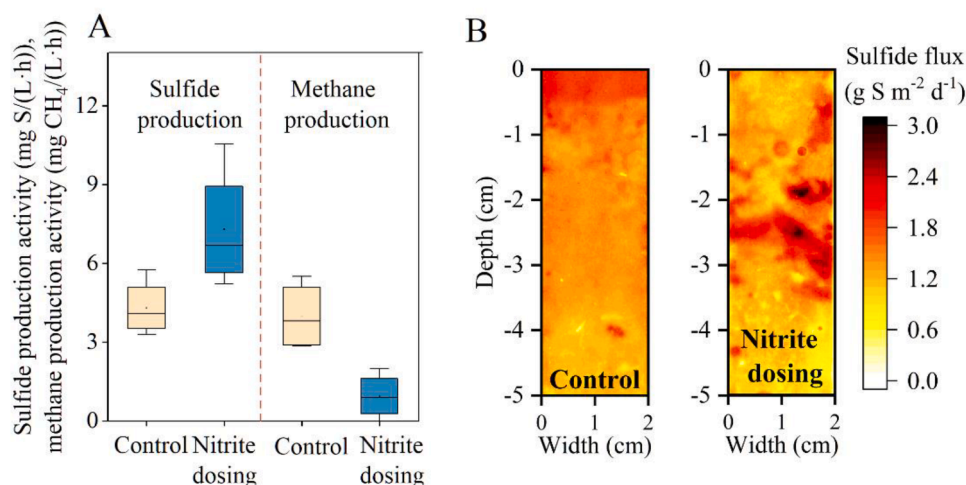
Fig. 5. Sulfide and methane production activities in the control reactor without chemical dosing and the experiment reactor with nitrite dosing (A), and the 2D profiles of sulfide flux in the sediments of both reactors (B). The depth of 0 cm represents the water-sediment interface.

previous studies reporting the sensitivity of MA to chemicals such as FA, FNA, and ferrate (Jiang et al., 2011; Yan et al., 2023; Zuo et al., 2023b). The low methane production activity reasonably explained no recovery of methane production after nitrite was depleted (Fig. 4).

In contrast, the long-term addition of nitrite enhanced rather than inhibited sulfide production activity (Fig. 5A). However, previous studies demonstrated that nitrite is toxic to most microbes including SRB (Mohanakrishnan et al., 2008). To delve into the enhanced mechanism of nitrite dosing on sulfidogenic activity, the novel diffusive gradient in thin films (DGT) technique was applied to measure microscale sulfidogenic activity within the sediment. Previous studies have shown that the DGT technique enables the direct identification of the hotspot layer of sulfide generation along the sediment depth (Zuo et al., 2020). Fig. 5B shows that the control reactor exhibited varying sulfide generation flux within the sediment, with the active zone located within the surface layer (0–0.5 cm) in the control reactor, with a sulfide production flux of 1.87 ± 0.04 g S m^{-2} d^{-1}, similar with previous reports. In contrast, under the long-term pressure of nitrite dosing, sulfide production flux with the surface layer dropped to 0.8 ± 0.01 g S m^{-2} d^{-1}. Instead, the sulfide generation hotspot shifted to the deeper layer (2–3 cm), providing the potential protection of sulfidogenic process against nitrite exposure (Fig. 5B). The downward migration of activity zones in sewer sediments has been reported in previous studies whose dosed various chemicals like nitrate and iron salts (Cao et al., 2019; Liu et al., 2015b).

2.5. Microbial utilization vs. microbial inhibition: implication for urban integrated water management

In sewer management, sulfide-induced pipe corrosion and odor are persistent challenges, prompting decades of focused attention and mitigation measures globally. As the imperative for achieving net-zero carbon emissions gains prominence, a corresponding commitment to reducing methane emissions in the sewer sector emerges. This study demonstrates a novel microbial utilization approach which is capable of removing sulfide and methane simultanously (*Path II*) (Fig. 6A). The long-term experimental result showed a 58 % removal of methane and a 91 % removal of sulfide at a practical nitrite dosing concentration (48 mg N/L), demonstrating the reliability of the proposed concept for sewer management.

Recent studies mainly focused on microbial inhibition strategies (*Path I*) to suppress source production of sulfide and methane within sewer biofilms or sediments (Fig. 6A). However, challenges remain. Microbial inhibition strategies require the identification of sulfide and methane production hotspots, which is difficult in actual sewer networks (Zuo et al., 2021). In addition, microbial inhibition usually relies on a several-hour exposure to chemicals with high concentrations, e.g., 200 mg N/L of nitrite at pH 6 for 8–24 h. This is difficult because the added chemicals can quickly degrade and dilute along the pipeline. SRB and MA are often protected within sewer biofilms and sediments, which may also result in the added chemicals having a weaker inactivation effect. As evidenced in Fig. 5B, nitrite dosing simulated a high sulfide production activity in the deep layer of sediments. A crucial reason for the limited application of microbial utilization (*path II*) was the lack of methane oxidation process in sewers. Until recently, we discovered the growth of n-DAMO bacteria in sewer systems, pointing the possibility of developing next-generation biotechnologies in sewer management (Zuo et al., 2024). Herein this study demonstrated the feasibility of leveraging the n-DAMO bacteria and SOB for methane and sulfide removal in sewer
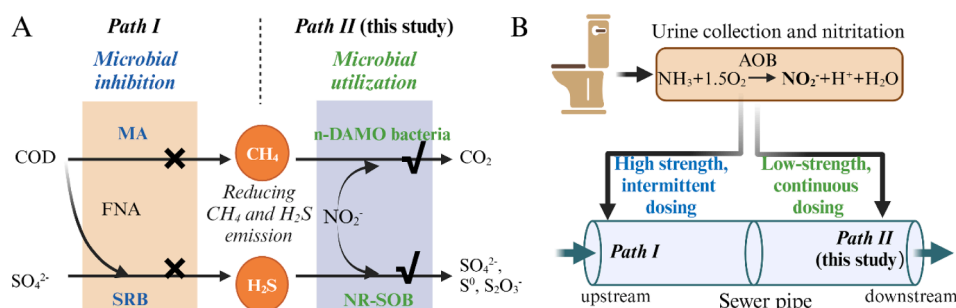

Fig. 6. A conceptual diagram involving two paths for simulaneous sulfide and methane removal from sewers (A), and a novel urban wastewater management concept integrating urine collection and nitritation based on two paths (B).

systems. Besides, the competition between sulfide oxidation and methane oxidation driven by nitrite reduction is comparable, but with methane consumption, n-DAMO activity will be significantly limited, whereas sulfide oxidation activity will remain high (Fig. 1). This difference warrants further studies.

While nitrite-dependent microbial utilization approach is attractive, the practical application may be limited to the associated high cost due to continuous nitrite inputs. The search for a sustainable nitrite source becomes imperative. Recent interest in the recycling of urine for sewer management presents a noteworthy advancement. Studies demonstrated that urine can serve as a stable and cost-effective source of nitrite through microbial nitritation, thereby delivering huge economic and environmental benefits (Mackey et al., 2016; Zheng et al., 2017). Based on the findings of this study, we can further propose a new urban integrated wastewater management concept, i.e., urine nitritation for sewer sulfide and methane oxidation (Fig. 6B). In earlier studies (Chen et al., 2013; Zheng et al., 2017; Zuo et al., 2023b), urine has been introduced into sewer systems for sulfide control, either by inhibition or oxidation mechnisam, but methane oxidation is missing. Here our proposed nitrite-dependent oxidation approach integrating n-DAMO and sulfide oxidation can simultaneously mtigiate sulfide and methane in sewers, thereby supporting sewer management from a renewed perspective. The added benefit of nitrogen pollutant removal underscores a paradigm shift of sewer system from the conventional "wastewater collection system" to a more advanced "wastewater treatment system", which encapsulates a forward-thinking vision for the future of urban wastewater management.

It is important to noted that this work remains a proof-of-concept study that requires further understanding and optimzation. For instance, it is estimated that ~70 % of nitrite was consumed through the n-DAMO process based on chemical analysis of long-term data (Fig. 1). A more accurate mass balance can be achieved using isotope tracing techniques. Besides, the proposed microbial utilization approach was demonstrated in a lab-scale preudo sewer system, highlighting the necessity for further scale-up testing and field trials. Real sewers operate under dynamic anaerobic/aerobic conditions with varying water quality. In our study, the approach demonstrated effectiveness only under anaerobic conditions, making it suitable for anaeobic scenarios in pressure main sewers. However, in gravity sewers, where oxygen is prevalent due to reaeration, the electron acceptor may switch to oxygen instead of nitrite. Additionally, the amount of available COD in wastewater influences the sulfide and methane oxidation effectiveness, as COD serves as the effective electron donor for nitrite. In addition to COD, ammonium may also used as an electron donor for nitrite through anaerobic ammonium oxidation. All these factors warrant systematic investigation. Finally, it is essential to emphasize that the feasibility of microbial utilization (*Path II*) does not render it a replacement for microbial inhibition (*Path I*). *Path II* is deemed suitable for extended sewer pipes with high concentrations of sulfide and methane originating from upstream pipes. Conversely, this control strategy may not be effective in sewer systems where methane and sulfide levels are low. This is a potential reason why previous studies did not discover n-DAMO process in sewer systems with upstream nitrite dosing. Therefore, the selection between the two paths necessitates a thoughtful consideration of real sewer structures and water quality. Moreover, coupling both paths is also possible under specific sewer conditions.

3. Conclusions

This study investigates in-sewer nitrite dosing for simultaneous sulfide and methane control via novel "microbial utilization" path in a simulated sewer reactor. The main findings are as follows:

1) Microbial utilization involves SOB and n-DAMO processes to co-utilize nitrite as an electron acceptor, achieving a 58 % reduction in dissolved methane and over 90 % sulfide removal from influent.

2) The n-DAMO and sulfide oxidation activities were comparable, measured as 15.6 mg CH$_4$/(L·h) and 29.4 mg S/(L·h), respectively, which was supported by high copy numbers of n-DAMO bacteria and SOB found in both sewer biofilms and sediments.

3) Nitrite-dependent microbial utilization strategy serves as a promising alternative for enhancing simultaneous sulfide and methane control in sewer management. Future research should focus on the feasibility of using nitrite produced from urine to drive the microbial utilization pathway.

4. Materials and methods

4.1. Sewer reactor

Nitrite dosing was implemented in a lab-scale sewer reactor to remove sulfide and methane from wastewater. The feeding of the reactor was the real domestic wastewater that contained high concentrations of sulfide and methane, daily produced from another identical lab-scale sewer reactor without any chemical dosing.

The sewer reactor had a volume of 1.8 L (12 cm height and 14 cm inner diameter) and was equipped with a mechanical stirrer (ZT 3IK15RGN—C) operating at 40 rpm. The sewer reactor was covered with a black cloth to simulate the dark environment of sewer systems. The reactor contained mature sewer sediment (about 5 cm deep) and sewer biofilm on the inner walls. The sewer reactor was inoculated with sludge containing n-DAMO bacteria *Candidatus Methylomirabilis*. The relative abundance of n-DAMO bacteria in the inoculum was about 2 %. The feeding pattern consisted of 20 cycles per day (once per hour, excluding 1:00–5:00am). Each feed pumping event was 10 min, delivering 0.3 L of wastewater into the reactor. The operating temperature was maintained at 20 °C ± 2 °C.

4.2. Long-term nitrite dosing experiments

Nitrite dosing was initiated on day 0. A nitrite stock solution (1 g N/L), prepared with sodium nitrite (NaNO$_2$), was introduced into the reactor after each sewage feeding event (i.e., 20 cycles per day), completing within 1 min via a peristaltic pump (Long, Model: BT100–2 J). The dosed nitrite volume varied, specifically with approximately 10 mL (0–35 days), 8 mL (35–45 days), 10 mL (45–120 days), and 13 mL (120–240 days). These volumes corresponded to 33, 25, 33, and 48 mg of NO$_2^-$-N/L in influent wastewater, respectively, which are in the practical range of the nitrite dosage in sewers (Mohanakrishnan et al., 2008).

Throughout the nitrite dosing operation, sulfide and methane concentrations in the influent and effluent of the reactor were measured 3–5 times per week. COD and nitrite concentrations in the influent and effluent were simultaneously monitored. Biofilm and sediment-surface (0–0.5 cm) samples in nitrite-dosed sewer reactor were collected at the end of reactor operation and stored at −80 °C for microbial analysis. In the meantime, the microbial samples from another lab sewer reactor without any chemical dosing were also collected as a control (i.e., control reactor).

Cycle studies were conducted at different periods (days 41, 57, 139 and 184) to gain insights into the dynamic characteristics of sulfide and methane removal by nitrite. In each cycle, 5 mL liquid was collected every 10–20 min to monitor the variations in sulfide, methane, and nitrite concentrations. Each cycle test was conducted in duplicate to prove the reliability of the data.

4.3. Bioactivity assessment

Nitrite-dependent methane and sulfide oxidation activities. To showcase the simultaneous oxidation of methane and sulfide coupling with nitrite reduction in the nitrite-dosed sewer reactor, independent batch tests were conducted in situ at the steady state. Long-term nitrite dosing was

halted before batch tests to obtain a high initial concentration of methane and sulfide. Subsequently, 18 mL of nitrite stock solution (1 g N/L) was added, resulting in an initial nitrite concentration of 20–25 mg N/L. Liquid samples were collected every 7 min, and the concentrations of the sulfide, methane, and nitrite in these samples were measured. Batch tests lasted 30 min and were performed in triplicate. Linear regression of concentrations versus time was applied to derive slopes representing nitrite-dependent methane and sulfide oxidation activities. For a control, the same nitrite dosing batch test was also performed in another identical lab-scale sewer reactor without any chemical dosing (control reactor).

Methane and sulfide production activities. To determine the impact of nitrite dosing on methane and sulfide production activities, bioactivity tests were conducted monthly for both the nitrite-dosed sewer reactor and the control reactor. The liquid in sewer reactors was replaced with re-injecting fresh raw wastewater at the beginning of the test, and 4–5 samples were collected over a 2-hour period for measuring dissolved sulfide and methane concentrations. Linear regression of concentrations versus time was applied to obtain slopes representing sulfidogenic and methanogenic activities.

DGT technique. At the end of the operation, a novel technique, namely diffusive gradient in thin films (DGT), was employed to measure two-dimensional, high-resolution sulfidogenic activity in sediments of the nitrite-dosed sewer reactor and the control reactor. The principle of DGT for sulfide measurement in sewer sediment was introduced in previous studies (Zuo et al., 2021, 2020). The DGT was prepared according to the reported study (Ding et al., 2012), with the probe provided by Easysensor Ltd. (www.easysensor.net). To initiate the DGT measurement, the liquid in sewer reactors was replaced with fresh raw wastewater at the beginning of the test. After 10 min, the DGT probe was vertically inserted into the sediment of the reactor. The reactors were hermetically sealed to prevent any H_2S loss during the measurement process. The probe was strategically positioned at the sediment-water interface using a ceramic knife. After 40 min, the DGT probes were carefully extracted for subsequent analysis (see more details in Text S1 of Supporting Information (SI)).

4.4. Analytical methods

The concentrations of COD, sulfide, and nitrite were determined using standard methods (Ministry of Environmental Protection, 2006). The sulfate and VFA concentrations were measured using ion chromatography (ICS-2000 Dionex, USA) and gas chromatography (GC-6890 N Agilent, USA), respectively. To measure the dissolved methane concentrations, 2 mL of wastewater was filtered through a 0.45-μm filter membrane using a syringe with a needle into a vacuum tube. After the gas-liquid equilibrium was attained overnight, a gas chromatography instrument (GC-7890A Agilent, USA) equipped with a flame ionization detector was used to determine the methane concentration in the gas phase. The methane concentration in the liquid phase was calculated using the mass balance equation and Henry's law (Guisasola et al., 2008). Additionally, the pH was monitored using a pH probe meter (WTW, pH/Oxi340i, Germany).

Real-time quantitative PCR (qPCR) was used to measure the abundance of the n-DAMO bacteria by quantifying the 16S rRNA genes and *pmoA* genes of n-DAMO bacteria in the collected samples. Specifically, the 16S rRNA gene of n-DAMO bacteria was determined using the primer sets of qp1F and qp1R, while the *pmoA* gene of n-DAMO bacteria was quantified using the primer sets of cmo182 and cmo568 (Hu et al., 2014). The detection of both genes made the quantification of n-DAMO bacteria more convincing. More details of qPCR were described in the previous study (Hu et al., 2014).

CRediT authorship contribution statement

Zhiqiang Zuo: Writing – review & editing, Writing – original draft,

Supervision, Methodology, Funding acquisition, Conceptualization. **Yaxin Xing:** Methodology, Investigation. **Xi Lu:** Validation. **Tao Liu:** Writing – review & editing. **Min Zheng:** Writing – review & editing, Supervision. **Miao Guo:** Writing – review & editing. **Yanchen Liu:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Xia Huang:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2024.100231.

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