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Review

# Path to zero emission of nitrous oxide in sewage treatment: is nitrification controllable or avoidable?

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Amid growing concerns over climate change, the need to reduce nitrous oxide ( $N_2O$ ) emissions from sewage treatment is more urgent than ever. Sewage treatment plants are significant sources of  $N_2O$  due to its production as an intermediate in nitrification and its release into the air during aeration. Effective management of the nitrification process is therefore vital for controlling or eliminating these emissions. Despite substantial efforts to quantify and understand  $N_2O$  emissions from sewage treatment, success in reducing them has been limited. This review discusses and proposes promising solutions for reducing  $N_2O$  emissions in sewage treatment, evaluates the potential of various strategies, and identifies ways to accelerate their development and implementation.

#### Addresses

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#### Introduction

Nitrous oxide ( $N_2O$ ) is a potent greenhouse gas (GHG) that has a global warming potential estimated to be approximately 273 times greater than carbon dioxide over a 100-year time frame [1].  $N_2O$  emissions significantly

contribute to climate change, accounting for approximately 6% of anthropogenic GHG emissions worldwide [1]. This underscores the importance and urgency of reducing N<sub>2</sub>O emissions to combat climate change effectively. As the sixth-largest emitter, the wastewater sector contributes 3% of total N<sub>2</sub>O emissions (Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2021). Setting path to zero emission of N<sub>2</sub>O is therefore critically needed for the wastewater industry.

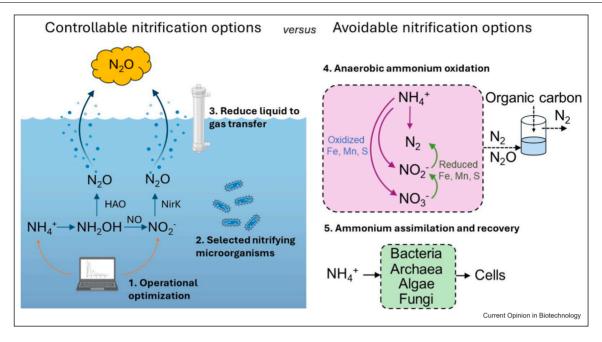
Nitrification, the biological oxidation of ammonium  $(NH_4^+)$  to nitrite  $(NO_2^-)$  and subsequently to nitrate  $(NO_3^-)$ , is a central process in global nitrogen cycling. For decades, sewage treatment plants (STPs) worldwide have employed the nitrification process to remove  $NH_4^+$  from sewage. When coupled with subsequent denitrification — the biological reduction of  $NO_3^-$  to dinitrogen gas  $(N_2)$  — this process effectively safeguards natural water bodies from eutrophication due to treated water discharge.

However, the nitrification unit presents a hotspot for N<sub>2</sub>O emissions, with an Intergovernmental Panel on Climate Change (IPCC)-estimated average emission level of 1.6% of total nitrogen removed in global STPs, although lower emission levels have been observed in cases with reduced influent nitrogen loads [1]. Microbial nitrification inherently produces N<sub>2</sub>O as an intermediate by incorporating two common pathways of hydroxylamine oxidation and nitrifier denitrification [2,3]. Furthermore, nitrification occurs with aeration, providing the oxygen necessary for nitrifying microorganisms to thrive. Diffused bubbling and surface aeration, which have been commonly installed in STPs, transfer liquid  $N_2O$  into the air, leading to  $N_2O$ emissions [4]. Controlling or avoiding N<sub>2</sub>O emissions through better management of the nitrification process is therefore vital.

Therefore, this paper aims to provide insights into solutions of controllable and avoidable nitrification for achieving the ambitious goal of nearly zero  $N_2O$  emissions in sewage management in the next decades, as illustrated in Figure 1. Notably, the focus is primarily on biological solutions, and thus, many promising physicochemical processes — such as membrane absorption or adsorption — are not covered.

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Figure 1



Potential solutions to address GHG N<sub>2</sub>O emissions from wastewater treatment.

## Controllable nitrification: operational optimization

During nitrification, intermediate compounds such as nitrite can enhance the formation of N<sub>2</sub>O, especially under suboptimal conditions like low oxygen levels or high ammonia concentrations [5]. Numerous laboratory, pilot, and full-scale studies have tested optimizing operational parameters for reducing N<sub>2</sub>O emissions [6,7]. These include aeration control, feed scheme optimization, and process optimization, with full-scale STPs showing N<sub>2</sub>O emission reductions of 30%-60% [7,8]. Understanding the mechanisms of N<sub>2</sub>O generation pathways is crucial, as operational parameters affect these pathways differently. This understanding can be achieved through close monitoring of N<sub>2</sub>O emissions and biological nitrogen removal performance. Analyzing and correlating the monitored results with plant operation control can help develop effective strategies. Additionally, the complexity of N<sub>2</sub>O generation mechanisms necessitates using mathematical models to evaluate the consequences of optimization on N2O emissions and treatment performance, yet it needs to be cautious with model predictions on absolute emissions. However, the extensive efforts required for monitoring, data analysis, and mathematical modeling can be prohibitive for implementing operational optimizations at STPs. Limited mitigation practices have been implemented due to the multiple challenges in process control optimization.

## Controllable nitrification: introducing selected nitrifying microorganisms

Nitrification is catalyzed by various nitrifying microorganisms, including the canonical ammonia-oxidizing bacteria (AOB) Nitrosomonas, commonly found in activated sludge of STPs [9]. Nitrosomonas in activated sludge produces N<sub>2</sub>O as a by-product, constituting several percentages of ammonia oxidized [10]. However, other ammonia oxidizers exhibit distinct nitrogen metabolism characteristics. For instance, complete ammonia-oxidizing (comammox) Nitrospira [11,12] and ammonia-oxidizing archaea (AOA) [13] lack the N<sub>2</sub>O-producing nitrifier denitrification pathway due to the absence of nitric oxide (NO) reductase in their genomes. Similarly, acid-tolerant AOB, such as Candidatus Nitrosacidococcus and Candidatus Nitrosoglobus, lack NO-producing nitrite reductase (NirK) [14,15]. Consequently, ammonia oxidation processes dominated by comammox [16–18], AOA, and acid-tolerant AOB [19] could exhibit lower N<sub>2</sub>O emissions compared to those led by canonical AOB Nitrosomonas [20,21].

An important research question of this solution is how to apply these low N<sub>2</sub>O producers for ammonia oxidation in sewage treatment. Comammox Nitrospira are ubiquitous in activated sludge and can become the dominant nitrifying microorganism in long sludge retention time or attached biofilm systems [22,23]. Additionally, comammox bacteria facilitate cost-effective nitrogen

removal by cooperating with anammox bacteria under oxygen-limited conditions [24,25], a potential that AOA may also share [26]. Acid-tolerant AOB are advantageous for performing robust, high-rate nitritation processes, providing nitrite for subsequent nitrogen removal by anammox bacteria [19,27]. Creating appropriate operating conditions can facilitate the replacement of canonical AOB with these alternative ammonia oxidizers, leading to substantially reduced N<sub>2</sub>O generation from nitrification.

#### Controllable nitrification: reducing liquid nitrous oxide transfer into air

As N<sub>2</sub>O is highly soluble in the aqueous phase (up to 1.5 g N/l at 15°C), liquid N<sub>2</sub>O generated during nitrification could theoretically be reduced to N<sub>2</sub> through subsequent denitrification, serving as a sink for N<sub>2</sub>O [28]. Significant reduction of N<sub>2</sub>O emissions can be achieved by mitigating the transfer of dissolved N2O from liquid to air. Managing liquid turbulence to lower the gas-liquid transfer coefficient (kLa) is crucial for controllable nitrification [29]. Reducing liquid turbulence must also ensure adequate oxygen supply to maintain effective nitrification.

A potential solution is the membrane-based aeration. Researchers have developed membrane-aerated biofilm reactors (MABR) as an emerging technology for air supply without forming bubbles, thereby reducing liquid turbulence compared with conventional bubbling methods. Membranes also promote biofilm formation, hosting diverse microbial communities, with nitrifiers growing inside the biofilm and denitrifiers on the outside. This arrangement theoretically allows most of the N<sub>2</sub>O produced within the biofilm to be consumed by denitrification before it reaches the bulk liquid. However, large-scale demonstrations remain limited, and further validation is needed for its potential as a low N<sub>2</sub>O emission process. A recent fullscale MABR study reported only 0.8% of the influent nitrogen load emitted as N2O but did not cover overall  $N_2O$  emissions from the integrated process [30]. Moreover, design of full-scale MABR usually requires coarse bubble aeration for biofilm scouring, which could lead to additional N<sub>2</sub>O emissions [31]. More research is still needed to evaluate the effectiveness of MABR in mitigating N<sub>2</sub>O emissions.

## Avoid nitrification through anaerobic ammonium conversion

Although nitrification has been an essential process in wastewater treatment for decades, eliminating it could be a potential solution for achieving zero  $N_2O$  emissions. This would require an alternative nitrogen removal process. Recent studies have reported the phenomenon of ammonium oxidation using electron acceptors such as sulfate [32,33], ferric iron [34,35], and manganese [36] instead of oxygen, For example,

$$2NH_4^+ + SO_4^{2-} \rightarrow S^0 + N_2 + 4H_2O$$
  
 $8NH_4^+ + 3SO_4^{2-} \rightarrow 4N_2 + 3HS^- + 12H_2O + 5H^+$   
 $NH_4^+ + 6Fe^{3+} + 4H_2O \rightarrow N_2 + 6Fe^{2+} + 10H^+$   
 $NH_4^+ + 3MnO_2 + 2H_2O \rightarrow N_2 + 3Mn^{2+} + 4OH^-$ 

These preliminary findings have laid a foundation for  $NH_4^+$  conversion to  $NO_2^-$ ,  $NO_3^-$ , or even  $N_2$  in the absence of oxygen. Consequently, aeration would no longer be necessary. This dramatic paradigm shift could significantly mitigate N2O emissions by minimizing liquid turbulence and reducing the likelihood of N<sub>2</sub>O being stripped into the air. Moreover, these anaerobic nitrogen-converting processes may not produce N<sub>2</sub>O as an intermediate, although the functional microorganisms responsible for them have not been fully identified or understood. Even if N<sub>2</sub>O is generated, it can be easily reduced to N2 through denitrification treatment of the off-gas due to the absence of oxygen, ensuring complete elimination of N<sub>2</sub>O emissions in sewage management. While recent studies have explored novel anaerobic ammonium conversion, most have been limited to observational phenomena and have rarely investigated N<sub>2</sub>O emissions in depth [36,37]. At this stage, this approach to mitigating N<sub>2</sub>O emissions remains in its infancy. However, if successful, it could revolutionize century-old sewage treatment practices. Further research is essential to identify the microorganisms responsible for these anaerobic processes, understand the involved nitrogen metabolisms, and evaluate the feasibility and effectiveness of using these reactions for sewage treatment.

## Avoid nitrification through ammonium assimilation and recovery

Another approach to avoid nitrification is to incorporate NH<sub>4</sub><sup>+</sup> into biomass, that is, assimilation, rather than oxidizing it to NO<sub>2</sub> or NO<sub>3</sub>, thereby preventing the formation of N2O altogether. Various microorganisms, including bacteria, archaea, algae, and fungi, assimilate NH<sub>4</sub><sup>+</sup> as a nitrogen source for growth. For instance, algae-based systems and heterotrophic bacteria can be employed to uptake NH<sub>4</sub><sup>+</sup> directly from sewage, with high yields of up to 0.45 g of biomass per gram of NH<sub>4</sub><sup>+</sup>-N [38]. Algae, in particular, can assimilate NH<sub>4</sub><sup>+</sup> rapidly and convert it into biomass, which can then be harvested and used as biofuel or fertilizer, creating a circular economy. Assimilation of NH<sub>4</sub><sup>+</sup> can be enhanced by optimizing the conditions for microbial growth, particularly in systems where microbial communities are tailored to efficiently assimilate NH<sub>4</sub><sup>+</sup>. Studies have identified high NH<sub>4</sub><sup>+</sup>-assimilating strains [39], indicating bioaugmentation may be a feasible strategy to enhance the efficiency of ammonium assimilation in existing facilities such as the high-rate activated sludge process for organic carbon and nitrogen removal. Additionally, purple phototrophic bacteria are capable of NH<sub>4</sub><sup>+</sup> assimilation under anaerobic and light conditions, with biomass yields often ranging from 0.3 to 0.6 g per g of NH<sub>4</sub><sup>+</sup>-N assimilated. Novel microbes, such as fungi, although less studied for NH<sub>4</sub><sup>+</sup> assimilation in wastewater contexts, may have the potential to meet the requirement for less organic carbon, necessitating further fundamental and applied research. Notably, a key limitation of these NH<sub>4</sub><sup>+</sup> assimilation processes is the requirement for substantial organic carbon, which is often insufficient in sewage.

Utilizing ion exchange to recover NH<sub>4</sub><sup>+</sup> can prevent nitrification and usually requires a semi-abiotic process to replace traditional biological nitrogen removal. This process could achieve high NH<sub>4</sub><sup>+</sup> removal efficiency [40] and avoid N<sub>2</sub>O emissions. However, despite these promising results, significant challenges remain in developing and scaling up this innovative treatment approach.

#### **End-of-pipe techniques**

End-of-the-pipe approaches offer a promising opportunity to achieve nearly zero N2O emissions by removing emitted N<sub>2</sub>O from off-gas. In some STPs in Australia, treatment facilities are fully covered to collect almost all off-gases emitted from the plant. Although off-gas treatment is primarily designed for odor control, it rarely addresses the issue of N<sub>2</sub>O. In industrial plants for nitric and adipic acid production, catalytic decomposition of NO<sub>x</sub>, including Selective Catalytic Reduction and Selective Non-Catalytic Reductions, has been widely implemented and proven effective in N<sub>2</sub>O removal [41]. However, these catalytic methods have not been reported for sewage off-gas treatment due to the significant oxygen and moisture content in sewage off-gas, which can significantly affect the effectiveness of the catalytic conversion of N<sub>2</sub>O [42]. Additionally, the hightemperature requirement (typically > 500 K) for catalytic decomposition implies high energy costs for preheating off-gas, challenging its economic feasibility.

Biological technologies usually offer a cost-effective option for sewage treatment. Studies have demonstrated the effectiveness of biological N<sub>2</sub>O removal in sewage off-gas, which relies on anoxic microbial reduction of N<sub>2</sub>O in the absence of oxygen [43–46]. However, removing oxygen from sewage off-gas is a costly step that requires substantial external organic carbons. This makes end-of-the-pipe biological technologies difficult to apply at current STPs with significant aeration and collection of significant oxygen in off-gas. In contrast,

these technologies are feasible in treatment systems of anaerobic ammonium conversion, which does not require aeration with oxygen supply.

Some studies have aimed to promote  $N_2O$  generation in sewage treatment, recovering  $N_2O$  as an energy resource, in contrast to reducing  $N_2O$  generation [47–49]. The heterotrophic coupled aerobic-anoxic nitrous decomposition operation process is the most investigated for  $N_2O$  recovery, while autotrophic processes, such as photoelectrotrophic denitrification, sulfur-driven denitrification, and hydrogenotrophic denitrification, are also presented as alternative [50]. Nonetheless, recovering  $N_2O$  from both heterotrophic and autotrophic denitrification processes still requires either  $NO_2^-$  or  $NO_3^-$ , leaving  $N_2O$  generated from the prior nitrification process unregulated.

# Technical maturity of solutions to reduce nitrous oxide

While some strategies for reducing N<sub>2</sub>O emissions in sewage treatment are commercially available and effective, many approaches aimed at avoiding nitrification are still in early stages of development. Controllable nitrification methods, which focus on optimizing the process of converting NH<sub>4</sub><sup>+</sup> into NO<sub>3</sub><sup>-</sup>, are already being used in many STPs. These methods include strategies like adjusting aeration to lower N<sub>2</sub>O emissions. However, these methods still require careful monitoring and complex management to be fully effective, which can be challenging and costly. On the other hand, methods that avoid nitrification altogether, such as using alternative microbial processes to treat NH<sub>4</sub><sup>+</sup> or using chemical treatments like ion exchange to capture NH<sub>4</sub><sup>+</sup>, are still in the experimental phase. These techniques hold great potential because they could prevent N<sub>2</sub>O from forming in the first place, but they require more testing to prove their feasibility in large-scale sewage plants. For example, using microbes that produce much less N<sub>2</sub>O during NH<sub>4</sub><sup>+</sup> conversion, like Comammox bacteria, could revolutionize treatment processes, but applying these organisms on a large scale has not yet been fully validated. Similarly, ion exchange systems could capture NH<sub>4</sub><sup>+</sup> efficiently, but their high cost and the need for further optimization have limited their widespread adoption. Overall, while controllable nitrification solutions are relatively mature, promising strategies for avoiding nitrification still need further research and refinement before they can be widely implemented.

#### **Conclusions**

Minimizing  $N_2O$  emissions from sewage treatment is crucial for mitigating climate impacts. This paper highlights the nitrification unit as a hotspot for  $N_2O$  emissions in current STPs and suggests either controllable or

avoidable nitrification as potential strategies for mitigation. Achieving controllable nitrification with low N<sub>2</sub>O emissions requires a comprehensive approach, including operational optimization, strategic management of nitrifving microorganisms, and membrane-based aeration. Avoiding nitrification offers substantial promise for reducing emissions by one to two orders of magnitude compared to current levels. This can be accomplished through anaerobic ammonium conversion for removal or ammonium assimilation for recovery, both of which show promise but necessitate further fundamental and applied research. End-of-pipe techniques can play a critical role when combined with other approaches to achieve the ultimate goal of nearly zero N<sub>2</sub>O emissions in future sewage management. Successfully integrating these diverse strategies is essential for the wastewater sector to significantly reduce its carbon footprint and contribute meaningfully to global climate mitigation efforts.

#### CRediT authorship contribution statement

Min Zheng: Conceptualization, Writing - original draft preparation, Writing - review & editing. James Lloyd: Writing - original draft preparation. Peter Wardrop: Writing – original draft preparation. Haoran Duan: Writing – original draft preparation. **Tao Liu**: Writing – original draft preparation. Liu Ye: Writing – review & editing. Bing-jie Ni: Writing – review & editing.

#### **Data Availability**

No data were used for the research described in the article.

#### **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.

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