

The Mechanism and Affecting Factors of Nitrous Oxide Emission in Wastewater Treatment

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Abstract

Nitrous oxide (N_2O) can be produced and directly emitted from wastewater treatment plants (WWTPs). N_2O not only is a greenhouse gas, with an approximately 300-fold warming effects compared to carbon dioxide, also reacts with ozone in the stratosphere leading to ozone layer depletion. The concentration of N_2O the atmosphere increasing at a rate of 0.2-0.3% per year. Wastewater treatment is a potential antropogenic source of N_2O to the atmosphere. In this review, the mechanisms of N_2O production and factors leading to N_2O emission in wastewater treatment are discussed.

Key words: nitrous oxide, greenhouse gas, nitrification, denitrification, wastewater treatment.

1. Introduction

Municipal wastewater treatment plants are emission sources of the greenhouse gases methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) [1]. The excess emission of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) causes climate changes that threaten the existence of numerous living things on Earth, including humans [2]. Among these greenhouse gases, nitrous oxide (N_2O) emissions draw attention since nitrous oxide (N_2O) is an extremely potent greenhouse gas that is approximately 300 times more powerful than carbon dioxide (CO_2) over its 120-year lifetime in the atmosphere [3]. N_2O is expected to be the dominant ozone-depleting substance emitted in the 21st century [4].

It is generally accepted that nitrogen removal processes in a wastewater treatment system are an antropogenic source of N_2O [5]. Conventionally, biological nitrogen removal is achieved by a combination of nitrification and denitrification processes. Nitrification is the stepwise autotrophic oxidation of ammonia (NH_4^+) to nitrite (NO_2) by ammonia oxidizing bacteria (AOB) and further to nitrate (NO_3) by nitrite-oxidizing bacteria (NOB). Denitrification is the reduction of nitrate (NO_3) to atmospheric nitrogen (N_2) by heterotrophic denitrifiers (HET), with nitrite (NO_2), nitric oxide (NO) and nitrous oxide (N_2O) as obligatory intermediates. N_2O can be an intermediate or end product in the metabolism of both nitrification and denitrification processes. Over the past decade, controlling the emission of N_2O has become an important part of the biological wastewater treatment process. A recent review by Kampschreur et al. [6] showed that there are large variations in the N_2O emissions from full-scale wastewater treatment plants (WWTPs) (0–14.6% of the nitrogen load) and lab-scale WWTPs (0–95% of the nitrogen load). Although some

researchers measured nitrous oxide emission from full-scale wastewater treatment plants, the impact of wastewater treatment plants on global N₂O budget is not yet clear. Moreover, knowledge about the condition where N₂O production is stimulated in wastewater treatment facilities is still lacking. Therefore, there is an increasing need to identify the exact mechanisms behind N₂O emission and how the emission is associated with the operation parameters in wastewater treatment plants. The objective of this review is to identify mechanisms that promote N₂O production and operating conditions that are responsible for N₂O emission.

2. Nitrous oxide production (N₂O) pathways

2.1. Nitrification

Nitrification is the oxidation of NH₄⁺ to NO₃⁻ via NO₂⁻. These reactions are produced by two groups of microorganisms, the first step to NO₂⁻ by ammonium oxidizing bacteria (AOB) and the second step by nitrite-oxidizing bacteria (NOB). The first step is catalyzed by ammonia monooxygenase where NH₄⁺ is oxidized to hydroxylamine (NH₂OH) with the reduction of one of the atoms of O₂. NH₂OH is an intermediate, which is oxidized into NO₂⁻, by the hydroxylamine oxidoreductase. The second step is catalyzed by nitrite oxidoreductase without detectable intermediate [7]. N₂O is also produced during nitrification through two pathways. N₂O can be formed during the incomplete oxidation of hydroxylamine [8,9]. N₂O can also be formed during ammonia oxidation through chemical decomposition of intermediates from the oxidation of NH₄⁺ to NO₂⁻ or of NO₂⁻ itself. This is usually regarded as a special form of chemodenitrification. However, this chemical production of N₂O is reported to occur only when the NO₂⁻ concentration is relatively high (>1 mM) and is scarcely found in activated sludge [10]. Finally, some ammonia oxidizing bacteria (AOB) reduce NO₂⁻ to N₂O or N₂ through a process called nitrifier denitrification [11,12].

2.2. Denitrification

During denitrification, NO₃⁻ is firstly reduced to NO₂⁻ by nitrate reductase (NaR), NO₂⁻ is then reduced to N₂O by nitrite reductase (NiR), and finally N₂O is reduced to molecular nitrogen (N₂) by nitrous oxide reductase (N₂OR). N₂O is an obligatory intermediate of the denitrification pathway [13]. However in low oxygen concentrations, N₂O can be the final product of denitrification instead of N₂ because N₂OR is the most sensitive enzyme to oxygen [14,15].

Nitrate ammonification is an anaerobic microbial pathway that transforms NO₃⁻ into NO₂⁻, and then to NH₄⁺. These reactions are catalyzed by nitrate and nitrite reductases. The conditions for nitrate ammonification (i.e., low redox potential, high concentrations of available NO₃⁻ and labile C) are similar to those for denitrification. Furthermore, the two reduction pathways can exist under anoxia or low oxygen concentrations [13].

3. Factors leading to N₂O emission

3.1. Dissolved oxygen

The dissolved oxygen concentration is considered a very important parameter for the removal of nitrogen by affecting the nitrification and denitrification, which have a great impact on N₂O emission. It is hypothesized that oxygen-limiting conditions during nitrification lead to an accumulation of nitrite, since NOB have a lower affinity to oxygen than AOB, possibly due to a different half-saturation constant [16] and therefore a lower activity. Given that AOB can use nitrite instead of oxygen as the electron acceptor, nitrifier denitrification by AOB is held responsible for the increased N₂O emissions [7,17]. A high N₂O production was observed under a DO level lower than 2 mg/L, especially in the range of 0.5 mg/L-1.0 mg/L [17]. Additionally, it has been reported that N₂O production can correspond to 10% of the nitrogen load at oxygen concentrations below 1 mg/L [18]. The large impact of the dissolved oxygen concentration on N₂O emission indicates that appropriate control is required in the nitrification tanks of a WWTP. Low dissolved oxygen concentrations in the nitrification tank will lead to local oxygen limitation and an increased N₂O production. At the same time, too high aeration rates in the nitrification tank may lead to an increased oxygen introduction to the denitrification tank which also may lead to enhanced N₂O emissions [6].

The impact of DO on N₂O emission in denitrification can be explained from two aspects: denitrifiers are facultative bacteria, and they can carry out both aerobic respiration and anaerobic respiration. The denitrifier will use O₂ first instead of NO₃⁻ and inhibit denitrification because energy from aerobic respiration is far greater than that from anaerobic respiration. On the other hand, the reduction of N₂O to N₂ will be limited by the activity of N₂OR inhibited by DO. The combined effect of these two aspects causes a large number of N₂O emissions [19].

3.2. Nitrite

NO₂⁻ is often considered to be one of the key parameters responsible for N₂O production [6,11]. Correlation between N₂O production and high NO₂⁻ concentration by AOB is reported in several full-scale studies [20]. In laboratory scale studies, artificial nitrite pulses of 10 mg/L led to a four- to eightfold increased N₂O emission during nitrification, depending on the oxygen concentration [7]. This was postulated to be due to the inhibition of the N₂O reductase by high concentrations of NO, NO₂⁻, or its equilibrium partner, The production of N₂O in the presence of NO₂⁻ could also be attributed to denitrification by AOB, under oxygen-limited conditions [21]. Some microorganisms, e.g. *Alcaligenes faecalis*, were reported to use NO₂⁻ rather than NO₃⁻ in N₂O production [22].

3.3. COD/N

Lack of biodegradable organic carbon is known to increase N₂O emission during denitrification [23,24]. Hanaki et al. [25] investigated the impact of various COD/N ratios (1.5, 2.5, 3.5 and 4.5) and observed that up to 10% of the nitrogen load was emitted as N₂O at the lowest COD/N ratio studied. In an intermittently aerated laboratory scale reactor, approximately 20–30% of influent N

was emitted as N₂O when the COD to N ratio was less than 3.5 [14]. A higher emission of N₂O was found when there was an influent COD/N lower than 6 in WWTP [26]. In a pure culture study of *A. faecalis*, even up to 32–64% was emitted as N₂O when organic carbon became limiting [27]. Addition of an external organic carbon source, methanol, led to an appreciable reduction of the N₂O emission from 4.5% to 0.2% of the nitrogen load [28].

3.4. pH

Hynes and Knowles [29] demonstrated that N₂O production by *Nitrosomonas europaea* in a fully aerated culture was dependent on both pH and the buffer that was used. N₂O emission was maximum at a pH of 8.5 and minimum at a pH of 6. Similar observations were made by Hanaki et al. [25], where N₂O emission during denitrification increased when the pH decreased from 8.5 to 6.5. Increased N₂O production rate of an enriched AOB culture at pH 8.0 when compared with pH 6.0 is also reported by Law et al. [30].

3.5. Consumption of internal storage compounds

Systems operated to achieve simultaneous nitrification, denitrification and phosphorus removal can encourage the growth of denitrifiers, such as polyphosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO) that are capable of storing organic carbon in the form of polyhydroxybutyrate (PHB). Laboratory scale studies on such systems show that denitrification by glycogen accumulating organisms leads to an increased N₂O emission [31,32,33]. GAO and PAO both employ a special mechanism to store organic carbon during anaerobic periods, involving storage compounds, which are finally degraded via their internal PHB pool. Biological phosphate removal specifically selects for PAO organisms, but at higher temperatures the presence of GAO cannot be prevented [34]. A possible mechanism for N₂O emission by organisms growing on storage compounds is the fact that PHB consumption is the rate-limiting step in these organisms [35,36]. This leads to competition for electrons between the denitrifying enzymes, apparently resulting in a higher NO reduction rate compared to the N₂O reduction rate. Schalk-Otte et al. [27] also observed that as soon as stored PHB became the growth substrate due to COD limitation, N₂O started to accumulate. Since PHB plays an essential ecological role in several wastewater treatment processes [37], it might be a general factor related to N₂O emission. Storage processes are important in WWTPs, as activated sludge processes intrinsically experience dynamic conditions of substrate availability. This effect is even more significant in biological phosphate removal systems and WWTPs with selectors for bulking sludge control. Conversely, a pure culture study of a PHB-degrading denitrifying organism did not show appreciable N₂O emission during denitrification, however, in this case PHB was not present as internal storage compound but as external substrate [38]. From a denitrifying biological phosphorus removing reactor no appreciable N₂O emission could be deduced (based on mass balance calculations, [39]). Further research should focus on finding whether there is an intrinsic relation between storage polymers and N₂O formation in denitrifying systems. It cannot be excluded that the N₂O emission from the studied phosphate removal systems by [31,32] and [33] were caused by nitrite accumulation either within the sludge flocs and granules or in the liquid phase. N₂O emission was reduced by the increase of phosphorus load, due to the decrease of N₂O yield by heterotrophic denitrification [40].

3.6. Rapidly changing process conditions

In several studies increased N₂O emission was observed in response to rapidly changing environmental conditions. Probably the metabolism of bacteria needs time to respond to the changes in environmental conditions, resulting in substantial peak emissions of N₂O. A strong correlation between the build-up of nitrite and N₂O emissions in combination with ammonia shock loads [41,42]. Transient changes in DO concentration are shown to cause immediate increase in N₂O production especially from AOB [17,43]. Yu et al. [43] reported a positive correlation between N₂O production and NH₄⁺ concentration in *N. europaea* cultures and concluded that N₂O production via NH₂OH oxidation contributed even more when ammonia oxidation is shifted from low towards its maximum specific activity. pH perturbation causes strong N₂O emissions. When pH decreases, nitrogen compounds in the bioreactors would be reduced to N₂O rather than N₂, because N₂O reductase is more inhibited than the other denitrifying reductase by low pH [44]. Bacterial populations subjected to continuously changing conditions can reduce their N₂O emission by adaptation. For example, *Alcaligenes faecalis*, was observed to reduce N₂O emission from 86% to 28% of nitrite converted after 10 cycles of pulse-wise substrate addition [27].

3.7. Other factors

Some factors that are connected in literature to N₂O emission might not be directly related to emission but cause changes of parameters that lead to N₂O production. An important parameter in this respect is nitrite. Short SRT, toxic compounds (like increased sulphide concentration), low temperatures, high salinity and increased ammonium concentrations have repeatedly been related to increased N₂O emissions. Alternatively, these process conditions may have resulted in nitrite accumulation that induces N₂O emission. The step-feed strategy was approved to be an effective approach to reduce N₂O production during nitrogen removal via nitrite [45].

An increase in salt concentration markedly influences N₂O emission both directly and indirectly via the inhibition of N₂O reductase activity. The indirect inhibition is due to the high concentration of dissolved oxygen which is transported from the oxic tank to the anoxic tank through the circulated liquid [46].

Temperature affects N₂O emission by affecting the overall nitrification and denitrification processes. Hu et al. [47] investigated the effect of temperature on N₂O emission from wastewater treatment process and higher N₂O fluxes was found at lower temperatures (temperature range: 15–35 °C),

4. Conclusion

Despite their relatively small contribution to the overall global greenhouse gas emissions, N₂O emissions from wastewater treatment plants is a significant factor in the greenhouse gas footprint of the total water chain. N₂O emissions from wastewater treatment processes vary substantially between plants depending on the design and operation of the plants, and the flow and

characteristics of wastewater. The main operational parameters leading to N₂O emission in WWTPs are: (i) low dissolved oxygen concentration in the nitrification and denitrification stages, (ii) increased nitrite concentrations in both the nitrification and denitrification stages, and (iii) a low COD/N ratio in the denitrification stage., but it remains unclear whether nitrifying or denitrifying microorganisms are the main source of N₂O emissions. The tendency of WWTPs to decrease their energy consumption by decreasing aeration could be adverse towards the greenhouse effect: even though it decreases CO₂ emission, this could be counteracted by the increased N₂O emission due to its 300-fold stronger greenhouse effect. Rapidly changing process conditions lead to increased N₂O emission, but adaptation can occur if systems repeatedly experience dynamic conditions. There are indications that growth on internal storage compounds can lead to N₂O emission, but the mechanism is unclear and scientific findings are contradictory. The relative contribution of nitrification and denitrification to the overall N₂O emission from WWTPs should be quantified. Because this will be dependent on environmental conditions, the dependency of the relative contributions on operational parameters is an important issue.

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