Factors Affecting N_2O Emissions from Wastewater Treatment Plants

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Wastewater treatment plants (WWTPs) generate significant amounts of greenhouse gases, including carbon dioxide, methane and nitrous oxide. Nitrous oxide (N_2O) is an important greenhouse gas with a global warming potential (GWP) 273 times that of carbon dioxide (CO_2), contributing to ozone layer depletion and climate change. Therefore, even small amounts of N_2O emissions can significantly contribute to total greenhouse gases (GHG) emissions. Thus, it can be concluded that the minimization of N_2O emissions and the identification of the factors controlling these emissions constitute a great challenge.

Keywords: N_2O production pathways ; hydroxylamine oxidation ; nitrifier denitrification ; heterotrophic denitrification ; influence of DO ; COD/N ; pH and temperature

1. Introduction

Wastewater treatment plants (WWTPs) generate significant amounts of greenhouse gases, including carbon dioxide, methane and nitrous oxide [1]. According to the IPCC 2023, the global anthropogenic emissions of greenhouse gases (GHGs) were 59 $GtCO_2$-eq, about 12% (6.5 $GtCO_2$-eq) higher than in 2010 and 54% (21 $GtCO_2$-eq) higher than in 1990 [2]. The contribution of each gas to the total GHG emissions varies: 79.4% for carbon dioxide, 11.5% for methane, and 6.2% for nitrous oxide, with the remainder consisting of fluorinated gases [3]. Nitrous oxide (N_2O) is an important greenhouse gas with a global warming potential (GWP) 273 times that of carbon dioxide (CO_2) [2], contributing to ozone layer depletion and climate change [4]. Global N_2O emissions were 2.7 GtCO2-eq, according to the IPCC 2023 [2]. Therefore, even small amounts of N_2O emissions can significantly contribute to total GHG emissions. Thus, it can be concluded that the minimization of N_2O emissions and the identification of the factors controlling these emissions constitute a great challenge.

2. History

Human activities such as agriculture and fossil fuel combustion, along with microbial processes occurring in biological wastewater treatment are the main sources of anthropogenic N_2O emissions [5][6][7][8]. Wastewater treatment as a sector contributes approximately 3% of the global anthropogenic N_2O emissions [1][9][10][11]. This percentage has increased in recent years [12][13][14]. Yao et al., 2022 reported that wastewater treatment was the fourth-largest source of N_2O emissions after agriculture, energy production, and other industrial production activities, accounting for 5.6% of total N_2O emissions. N_2O emissions originating from sludge disposal and treatment are not included in those from the wastewater treatment sector. The total amounts of N_2O produced from sludge incineration, reuse in cement, and composting are 645.0 kg N_2O/tonne, 294 kg N_2O/tonne and 0.37 kg N_2O/tonne, respectively [15]. N_2O emission from wastewater management contributes 26% of the total GHGs originating from the water sector, which includes drinking water production, water transport, wastewater and sludge treatment and discharge [10][16]. Numerous studies have confirmed that in biological wastewater treatment, the nitrification and denitrification processes occurring under aerobic and anaerobic/anoxic conditions are generally responsible for N_2O emissions [17][18][19][20][21][22][23][24][25][26]. Many researchers have investigated the mechanisms of N_2O production in WWTPs [5][25][27][28][29][30][31][32][33][34]. It is known that N_2O formation pathways include hydroxylamine oxidation, nitrifier denitrification and heterotrophic denitrification [35][36][37][38][39][40][41]. The main mechanism favoring N_2O production in WWTPs depends on the process configuration and operational parameters [49,50]. Goreau et al., 1980 concluded that the denitrification pathway of AOB was responsible for N_2O emissions. In order to reveal possible mechanisms of N_2O production, many factors affecting N_2O emissions have been reported: low dissolved oxygen concentration in aerobic conditions or high dissolved oxygen concentration in anoxic conditions, accumulation of nitrite, rapidly changing process conditions, pH, temperature or a low ratio of COD to nitrogen compounds during heterotrophic denitrification [27][42][43][44][45][46][47][48].

3. Influences

According to different N_2O production pathways, the main factors affecting N_2O emissions are the dissolved oxygen concentration (DO), the nitrite accumulation, the rapidly changing process conditions (e.g., high ammonia concentration and oxygen limitation), the substrate composition and COD/N ratio, the pH, and the temperature. The climatic zone, the location, the performance, and the influent characteristics of WWTPs also influence N_2O emissions. These factors are dependent on the various microorganism species (AOB, NOB, AOA), whereas the microorganisms present in each process are dependent on the substrate and process conditions.

3.1 Dissolved Oxygen Concentration (DO)

The dissolved oxygen concentration is considered a very important parameter controlling N_2O emissions during nitrification (nitration) or nitritation. Low DO concentrations during nitrification result in high N_2O emissions, which can be attributed to nitrifier denitrification [49][42][43][46][50][51][52]. Li et al., 2015 investigated the synergistic effect of DO and pH on N_2O emissions in a pilot-scale SBR process and reported that when DO was decreased from 3 to 0.5 mg/L, more NO_2^- was accumulated, resulting in N_2O production (nitrifier denitrification). Similar observations were made by Zheng et al., 1994 reporting that at DO < 1 mg/L N_2O production increased, due to nitrifier denitrification. In addition to nitrifier denitrification, the hydroxylamine oxidation pathway was found to contribute to N_2O emissions in the study of Peng et al., 2014. They demonstrated that the specific N_2O production rate increased from 0 to 1.9 mgN_2O-N/h/grVSS when DO concentration was increased from 0 to 3 mg/L [53]. With an increase in DO from 0.2 to 3 mg/L, the contribution of nitrifier denitrification by AOB decreased from 92% to 73%, accompanied by a corresponding increase in the contribution by the hydroxylamine oxidation pathway [53]. The transition from anoxic to aerobic conditions resulted in the accumulation of hydroxylamine and the formation of N_2O through the hydroxylamine oxidation pathway [54]. High N_2O production was observed under an increased aeration rate in a partial nitritation anammox reactor [55][34][56]. The stronger aeration, accompanied by an increased DO, stimulates stripping, leading to an increased proportion of the produced N_2O leaving via the gas phase [34]. Dissolved oxygen affects N_2O production during denitrification by inhibiting the synthesis and activity of nitrous oxide reductase, and its activity has been found to stop immediately when the denitrifying bacteria move from an anaerobic to an aerobic environment [57]. Nitrite reductase activity continues at a lower rate under the same transition, so that nitrous oxide emissions will occur [6].

3.2 Nitrite Accumulation

Nitrites are formed by AOB ammonium oxidation and by the reduction of heterotrophic bacteria nitrates. NO_2^- plays a key role in nitrous oxide production. NO_2^- accumulation increases nitrous oxide emissions during nitrification and denitrification. During nitrification, increased nitrite concentrations can lead to increased nitrifier denitrification by AOB and increased N_2O emissions [6][17][27][45][46][47][48][58][59]. High nitrite concentrations and low DO concentrations are known triggers for nitrite reductase and nitric oxide reductase expression in AOB, which favors N_2O production through the nitrifier denitrification pathway [60]. During nitritation–denitritation applying SBR process, increased nitrous oxide emissions were observed at high NO_2^- concentrations and DO < 1.5 mg/L [61]. This was related to a promoted expression of nitric oxide reductase gene or increased activity of NO_2^- reductase with increasing substrate concentration [62]. Thus, at lower DO (<1.5 mg/L), AOB denitrification was possibly responsible for N_2O production during nitritation. In heterotrophic denitrification, high NO_2^- concentrations inhibit complete denitrification, resulting in nitric oxide and nitrous oxide accumulation and increased nitrous oxide emissions [24][63][64][65][66][67]. Under elevated NO_2^- concentrations, NiR, NOR and N_2OR compete for electrons [68]. Limited generation of nitric oxide reductase under high concentrations of NO_2^- during denitrification has also been observed, resulting in NO accumulation [63]. This can further affect nitrous oxide emissions, as nitric oxide inhibits the activity of the enzymes involved in the denitrification process.

3.3 Rapidly Changing Process Conditions

In many studies, elevated nitrous oxide emissions were reported when the process conditions were changed rapidly (e.g., high ammonia concentration and oxygen limitation) [53][69]. Ammonia shock loads lead to incomplete nitrification, resulting in decreased nitrogen removal efficiency, NO_2^- accumulation and N_2O formation. Thus, the performance of the wastewater treatment plant also influences N_2O production. Oxygen limitation during nitrification could result in NO_2^- accumulation and N_2O formation (nitrifier denitrification pathway). Bacterial metabolism likely necessitates a period of adjustment to adapt to shifts in process conditions, leading to significant peaks in nitrous oxide emissions. Variations in bioreactors have also been observed, e.g., a decline in DO concentration owing to elevated influent loading or aeration rate limitation [69][70], resulting in increased N_2O production through the nitrifier denitrification pathway. Furthermore, the transition from anoxic conditions to aerobic conditions with the presence of accumulated NH_4^+ resulted in N_2O formation, suggesting that the hydroxylamine oxidation pathway is an important contributor in the formation of N_2O [55][54][71].

3.4 Substrate Composition and COD/N Ratio

The influent characteristics of a WWTP affect nitrous oxide emissions. The composition of different organic substrates is a major factor contributing to N_2O emissions. Limiting availability of biodegradable organic carbon hinders complete denitrification, resulting in N_2O accumulation [72][73], whereas excess carbon decreases N_2O production. Influent nitrogen plays an important role in N_2O emissions [5][6]. Thus, the COD/N ratio is an important factor controlling N_2O production. Several researchers have investigated the effect of organic substrates on N_2O emissions on the basis of experiments conducted at lab-scale using methanol, sodium acetate and mannitol as carbon sources [74][75][76]. In the study of Song et al., 2015, lower N_2O emissions were observed in the case of acetate compared to those emitted when using methanol as the carbon source. The N_2O emission factor was 2.3% of influent nitrogen for the methanol and 1.3% of influent nitrogen for the acetate, which was attributed to the fact that the biomass became more abundant in bacteria capable of reducing N_2O with acetate as carbon source [74]. Conversely, in the study of Adouani et al., 2010, the results showed that the highest N_2O and NO emissions were generated when using acetate as the carbon source, which was attributed to the diversity of denitrifying bacteria and their distinct metabolic pathways towards the added carbon substrates. The use of mannitol instead of sodium acetate as a carbon source resulted in lower N_2O conversion rates (21% for mannitol and 41% for sodium acetate) [76]. Microbial analysis showed that mannitol lowered the N_2OR enzyme inhibition caused by the high nitrite concentration in the partial nitrification system, thus enhancing heterotrophic denitrification. The presence of trace metals (e.g., Fe(II), Fe(III), Cu(II)) and other compounds in the substrate may affect N_2O emissions through abiotic reactions [77][78][54][79]. N_2O accumulation can be observed in the absence of sufficient Cu in natural waters or in the formation of nonbioavailable complexes with copper [80][81]. Increased N_2O emissions were observed during denitrification when the availability of biodegradable organic carbon was limited [72][73]. N_2OR is less competitive under limited COD, leading to N_2O production [68][82]. Schalk-Otte et al., 2000 conducted experiments in a pure culture, observing that when the availability of organic carbon became limited, 32–64% of the nitrogen load was emitted as nitrous oxide [83]. The various denitrification enzymes (NaR, NiR, NOR and N_2OR) compete for electrons when conditions of limited carbon sources are favored. NaR and NiR have relatively higher affinity for electrons than NOR and N_2OR [84], resulting in incomplete denitrification and N_2O formation. Another cause for increased N_2O emissions under conditions in which organic carbon is limited is the microbial consumption of internal storage compounds [6]. Nitrous oxide production is reduced when excess carbon is provided for the removal of electron competition [18]. Regarding the effect of different COD/N ratios (1.5, 2.5, 3.5 and 4.5) on N_2O emissions, it was shown that the highest N_2O production was obtained under the lowest COD/N ratio in laboratory-scale experiments [85]. Similar observations were made in the studies of Itokawa et al., 2001 and Andalib et al., 2018, where the highest N_2O production was reported when the COD/N ratio was below 3.5 [86][87]. Thus, it can be concluded that low COD/N ratios correspond to high nitrogen load in the influent, resulting in elevated N_2O production. Moreover, according to Law et al., 2012a, for complete denitrification, a COD/N ratio above 4 is required, with the optimal ratio ranging from 4 to 5 [88]. In agreement with the above statements, Gruber et al., 2021 demonstrated a weak positive correlation between C/N ratio and the N_2O emission factor [89]. However, Quan et al., 2012, employing three lab-scale aerobic granular SBRs, reported that lowering the nitrogen loading rate or, equivalently, raising the COD/N ratio did not hinder the heterotrophic denitrification process [90].

3.5 pH and Temperature

One of the major factors affecting nitrification in wastewater treatment is pH. Nitrification systems are sensitive to variations in pH [91]. Wastewater biological nitrification processes are accompanied by DO consumption and pH reduction. Although the optimal pH range for complete nitrification varies between 7.5 and 8 [28][30], the optimum pH for AOB and NOB growth ranges from 8.5 to 8.8 and from 8.3 to 9.3, respectively [92]. Thus, the activity of AOB and NOB can be affected by changes in pH [93], and pH can also cause changes in the concentrations of free ammonia (FA) and free nitrous acid (FNA). High pH shifts the equilibrium to FA, which is the substrate of AOB [94], and is inhibitory to nitrite-oxidizing bacteria (NOB) [95][96]. The ranges of FA concentrations that begin to inhibit nitrifying organisms are: 10 to 150 mg/L for AOB and 0.1 to 1.0 mg/L for NOB [95]. Low pH increases the FNA concentration, which inhibits both AOB and NOB [4][97]. The inhibition of both AOB and NOB was initiated at concentrations of FNA between 0.22 and 2.8 mg/L [95]. During nitrification, the highest N_2O production was observed at the lowest applied pH (pH = 6.0) [51]. NOB are strongly affected by low pH values (no activity was detected at pH =6.5) [98], thus resulting in the accumulation of nitrites. No inhibition was observed at high pH values (the activity was nearly the same for the pH range 7.5–9.95) [98]. In the partial nitrification (nitritation) process at DO = 0.7 mg/L, accumulation of nitrites was observed at high pH (pH = 7.85) [99], thus resulting in high production.

During denitrification, N_2O formation was observed at pH below 6.8 [100]. Similar observations were made by Hanaki et al., 1992, showing that the maximum N_2O emissions occurred when pH decreased from 8 to 6.5. This was attributed to N_2O reduction rate decreasing at low pH, resulting in N_2O accumulation [101]. Wastewater temperature plays a significant role

during nitrification. The mass transfer, chemical equilibrium and growth rate of both AOB and NOB are affected by temperature [93], and it could consequently be a major factor influencing nitrous oxide emissions. The temperatures at which the growth rates of AOB and NOB are maximized are 35 ^{o}C and 38 ^{o}C, respectively [102]. Van Hulle et al., 2007 suggested that the optimal temperatures for partial nitrification range from 35 ^{o}C to 45 ^{o}C [103]. However, only short-term effects on temperature were studied. Prolonged exposure to temperatures higher than 40 ^{o}C is likely to result in deactivation [104]. Hellinga et al., 1998 reported that at temperatures higher than 25 ^{o}C, the AOB specific growth rate increases and becomes higher than that of NOB. NOB can be washed out in activated sludge processes operating with high temperatures (30–35 ^{o}C), leading to the accumulation of nitrites and elevated N_2O emissions due to the nitrifier denitrification pathway [105]. It is known that denitrification rates increase with increasing temperature [106]. It is also known that increasing temperature decreases the solubility of N_2O.With a temperature increase from 25 ^{o}C to 35 ^{o}C, a reduction in the solubility of nitrous oxide in water of 23% was observed [107]. Thus, nitrous oxide solubility plays a key role in controlling nitrous oxide emissions [108]. A low nitrous oxide solubility at elevated temperature leads to more N_2O leaving the liquid phase before complete denitrification can be accomplished. Increasing the temperature from 10 ^{o}C to 20 ^{o}C leads to higher N_2O emissions (a 2.5-fold increase was measured in nitrous oxide emissions) [29]. Poh et al., 2015 investigated the impact of temperature on nitrous oxide emissions during denitrification. The specific reduction rates of nitrates, nitrites and nitrous oxide increased by 62%, 61% and 41%, respectively, when the temperature was increased from 25 ^{o}C to 35 ^{o}C. At 35 ^{o}C, although a higher N_2O reduction rate was observed, N_2O became less soluble in the mixed liquor, meaning that stripping was occurring more intensively. Thus, the dissolved N_2O was found to decrease continuously during the experiment because the stripping was occurring faster. As a result, although high temperatures are employed to increase the denitrification kinetics, they are expected to produce more emissions in the end.

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