Nitrification and denitrification processes for mitigation of nitrous oxide from waste water treatment plants for biovalorization: Challenges and opportunities

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Nitrification and denitrification processes for mitigation of nitrous oxide from waste water treatment plants for biovalorization: Challenges and opportunities

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ABSTRACT

Nitrous oxide (N_2O) is a potent greenhouse gas. Even though its emissions is much lesser than CO_2 but its global warming potential (GWP) is 298 times more than CO_2. N_2O emissions from wastewater treatment plants was caused due to incomplete nitrification or incomplete denitrification catalyzed by ammonia-oxidizing bacteria and heterotrophic denitrifiers. Low dissolved oxygen, high nitrite accumulation, change in optimal pH or temperature, fluctuation in C/N ratio, short solid retention time and non-availability of Cu ions were responsible for higher N_2O leakage. Regulation of enzyme metabolic pathways involved in N_2O production and reduction has also been reviewed. Sequential bioreactors, bioscrubbers, membrane biofilters usage have helped microbial nitrification-denitrification processes in succumbing N_2O production in wastewater treatment plants. Reduction of N_2O negativity has been studied through its valorization for the formation of value added products such as biopolymers has led to biorefinery approaches as an upcoming mitigation strategy.

1. Introduction

Nitrification and denitrification processes are microbial elimination of ammonium. During nitrification step, ammonium is oxidized to nitrate under aerobic conditions, while during denitrification step, nitrate is reduced to molecular nitrogen under anaerobic conditions. Nitrous oxide, third biggest contributor to manmade climate warming after carbon dioxide and methane is mainly produced into the environment by these processes. Since 1750, the atmospheric N_2O concentration has increased by about 16%, from around 270 ppb, to 319 ppb in 2005. Each year, the global N_2O emissions are increasing at an alarming rate that contributes approximately 6%–8% of the overall greenhouse effect (Guo et al., 2018). This concern is significant in the present century as N_2O has been considered as the most dominant ozone-depleting agent having a lifetime of 120 years due its global warming potential which is 298 that of CO_2 (Perez-Garcia et al., 2017; Guo et al., 2018; Zhao et al., 2018).

Recent studies have identified that the prime source of the anthropogenic N_2O emission is the wastewater treatment plants that contributes to both climate change and air pollution and thus concerns regarding the same is significantly growing day by day among urban water authorities and researchers (IPCC, 2015; Campos et al., 2016; Sweetapple et al., 2014). IPCC classified that out of seven sectors, wastewater treatment sector is assumed to be responsible for 4–5% (N_2O) of the global anthropogenic emission (Tumendelger et al., 2019). The microbial denitrification (both nitrifier or heterotrophic) that regularly takes place in a natural and engineered ecosystems is being accounted for 40%–85% of global N_2O emissions as N_2O is a known obligate intermediary product (Zhao et al., 2018; Braker and Conrad, 2011). 90% of the emitted N_2O is being released from the activated sludge process while 10% comes from the grit and sludge storage tanks. Other sources accounted for the global emission are such as human activities (40%–50%), fertilizers used in agricultural practices (80%), industries like adipic acid and nitric acid production, biomass and fossil combustion, landfill sites management (IPCC, 2015; Law et al., 2012; Ghosh and Thakur, 2017). Thus, the abatement of N_2O has become a prime concern for researchers and environmentalists so that they could contribute in the reduction of the N_2O magnitude to the atmosphere.

Parameters such as the dissolved oxygen concentration, nitrite accumulation, carbon source, the COD/N ratio, temperature, pH and most importantly the availability of Cu ions that play an integral part in the nitrous oxide reductase enzyme influences the N_2O generation during nitrification-denitrification processes and have always been the prime focus (Law et al., 2012; Frutos et al., 2017a–c; Zhao et al., 2018; Frutos et al., 2018). Wastewater treatment plants (WWTPs) are engineered designs to achieve high nitrogen conversion as well as removal rates but

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unfortunately it is not always technically feasible or cost-efficient to combat N_2O emissions thus biological end-of-the-pipe technologies such as parameter monitored bioreactors are being used for controlling N_2O abatement that could help in the improvement of the full-scale WWTPs designs to control the greenhouse gases (GHG) emissions. Most of the previous reviews have mainly focused on the reasons pertaining to N_2O emissions and its mitigation, however N_2O abatement could also be achieved through diverting this gas into value-added product formation that play an important role in extenuating the excess N_2O. Studies have reported N_2O application in synthetic chemistry, medical sector, biotechnology sector such as biovalorisation of N_2O to biopolymers as well as in biogas energy recovery (Severin, 2015; Ghosh et al., 2019; Frutos et al., 2017a–c).

This review critically addresses the nitrogen removal performance on environment in WWTPs and how microorganisms have played an immense role in the nitrification-denitrification mechanism underlying N_2O production and reduction (Guo et al., 2018; Medhi et al., 2017). It has focused on the key factors that particularly affects the N_2O release and have summarized the possibility to rectify them. This review not only focusses on the molecular insight of the enzyme mechanism valuable for monitoring the key biocatalytic processes for N_2O abatement to deep digger into genomics, transcriptomics and metabolomics analysis but will also help to realize the alternative future of N_2O treatment in production of value-added products to combat the climate change. Latest efforts with developed technologies for the possible mitigation purposes are also discussed along with their concerned challenges that still prevail and the opportunities that need to be identified and improved.

2. Persistence of nitrogen and organic pollutant in waste water

Nutrients are essential for the growth and survival of living organisms, and hence, are crucial for development and maintenance of healthy ecosystems. The health of freshwater bodies depends on the nutrients such as nitrogen, phosphorus and carbon that play a foremost role in maintaining the biological diversity of the aquatic ecosystem. The increasing anthropogenic activities to cope with the population explosion has led to the over-enrichment of these nutrients especially nitrogen and deterioration of the aquatic habitat. The excess input of the two main culprits, nitrogen (N) mainly in form of nitrate-N and phosphorus (P) leads to the occurrence of the ageing process eutrophication. Consequentially, the release of excess nutrients also pollutes the air and soil directing climate change and biodiversity misbalance. They are also the key pollutants in any wastewater treatment plants and their improper treatment ultimately pollutes the environment. Therefore, wastewater treatment should be done efficiently and water resources should be treated properly (Ghafari et al., 2008; Medhi and Thakur, 2018). The reactive nitrogen compounds in the form of ammonia, nitrite and nitrate exists in the wastewater treatment plants as well as the presence of nitrogen-containing compounds create serious problems to the environment when released (Medhi et al., 2017). There has been a 10-fold increase in the production rate of these reactive nitrogen compounds over a few decades into the biosphere and annually, 6.2% of the global reactive nitrogen escapes as nitrous oxide (N_2O) and contributes to the global warming (Medhi et al., 2017; Fagodiya et al., 2017). The most hazardous and widespread form of nitrogen is the nitrate-nitrogen, particularly recognized as contaminant in water bodies (Nancharaiah et al., 2016). Nitrogen pollution differs from country to country that greatly depends on the contributing sources such as extreme usage of fertilizers in crop production followed by the uncontrolled dumping of treated or raw sewage from domestic and industrials wastewater treatment plants are the main non-point and point sources respectively of nitrogen pollution in surface water bodies (Fig. 1). High concentrations of total nitrogen as N (approximately 20–70 mg/L) have been usually found to be present in domestic wastewater usually usually (Medhi and Thakur, 2018; Rout et al., 2017; Ma et al., 2015).

The urban wastewater along with nutrients, is constituted with different organic pollutants arising from households, industries, hospitals, agriculture, atmospheric deposition (dry or wet) etc. where, some are highly persistent while others are easily degraded and can be removed effectually. Recent studies in WWTPs have observed the occurrence of pharmaceuticals, pesticides, heavy metals, dioxins, hydrocarbons, plasticizers, bioactive and industrial compounds in effluent and sewage sludge that were required to undergo further treatment before their discharge into the environment (Gupta and Thakur, 2015, 2018). Polycyclic Aromatic Hydrocarbons (PAHs) are recognized as one of the most toxic organic contaminants from waste water and come under the priority pollutants list drafted by US-EPA and EU (Rathour et al., 2018; Gupta and Thakur, 2015). Polychlorinated Biphenyls (PCBs) and polychlorinated pesticides being highly toxic potentially accumulate in the sewage sludge and are mostly contributed by domestic and industrial sources. The improper disposal of pharmaceutical compounds that were most frequently detected in the effluent such as analgesics, anti-inflammatories, anti-hypertensives and lipid regulators have made them the current emerging pollutants in wastewater treatment plants (Cantwell et al., 2018; Comber et al., 2018; Guedes-Alonso et al., 2013). Therefore, isolation and application of pure cultures of potent bacterial strains or consortia as an advanced treatment method need to be added after the secondary clarification process in the existing sewage treatment facilities which could result in enhanced removal of certain persistent xenobiotics from effluent along with the recovery of the nitrogen to mitigate nitrous oxide from waste water.

3. Role of microorganisms in waste water treatment for nitrogen removal

N_2O is mainly released during the biological nutrient removal process in wastewater treatment plants and fundamentally follows the same nitrogen transformation processes of undergoing transition of aerobic or anoxic reactions carried out by the indigenous microbial cultures that usually exists in the other environments such as soil, freshwater and marine habitats. The two-step process of nitrogen bioelimination from wastewater consists of nitrification under strict aerobic conditions followed by denitrification under anoxic conditions. Ammonia primarily present in wastewaters are being oxidized to nitrite and eventually nitrate with the help of obligate aerobic autotrophs known as ammonia-oxidizing bacteria (AOB) such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosopira* involved in converting NH_3 to NO_2^- falls under the β-proteobacteria lineage while NO_2^- to NO_3^- conversion is fulfilled by nitrite-oxidizing bacteria (NOB) *Nitrobacter. Nitrospina*, *Nitrocystis* from the α-proteobacteria lineage deriving their energy from inorganic compounds. In the biogeochemical N_2 cycle, NO_2^- is a key intermediate and its fate determines whether the nitrogen remains fixed as nitrite, nitrate or is lost to the atmosphere as NO, N_2O or N_2. Thus recent studies have considered it important to focus on the NOB activity in WWTPs since its instability might cause tremendous ecological damage if nitrite from WWTPs leaks into natural waters (Daims et al., 2016). N_2O generation is caused under aerobic conditions in wastewaters by AOB and NOB through nitrifier denitrification and incomplete oxidation of hydroxylamine (NH_2OH) (Guo et al., 2018; Schreiber et al., 2012). Nitrifier denitrification which have been studied within the last two decades is generally believed to occur in oxygen-limiting conditions where the yielding of N_2O as final product instead of the atmospheric N_2.

Biological denitrification is the heterotrophic bioconversion process for the removal of nitrite and nitrate from the wastewaters under oxic/anoxic conditions, usually carried out by the facultative anaerobes such as gram-negative classes of α, β and γ Proteobacteria mainly *Paracoccus, Agrobacterium, Pseudomonas, Acinetobacter* and some gram-positive bacteria such as *Bacillus licheniformis* (Medhi et al., 2017; Li et al., 2015; Chen et al., 2019; Khanichaidecha et al., 2019). They

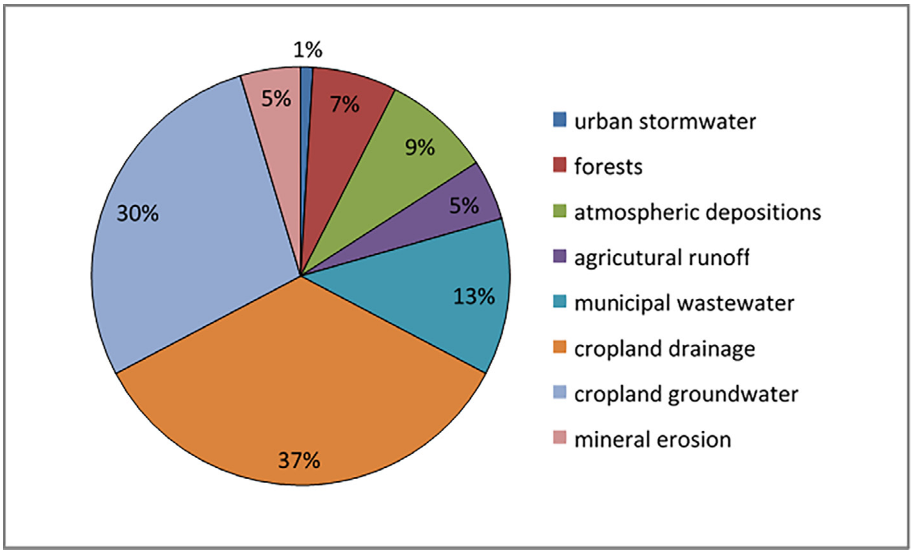

Fig. 1. Sources of nitrogen pollution in water bodies.

usually biotransform the oxidized nitrification products (NO_2^- and NO_3^-) and reduced them to dinitrogen where the intermediate product of is N_2O. Its production in wastewater treatment plants vary due to the partial denitrification or nitrogen imbalance of the enzyme-catalyzed processes. The promising potential application of heterotrophic-aerobic denitrifiers in simultaneous nitrification and denitrification has provided an advantage over conventional system as cost-effective, yielding minimum sludge as well as delivered complete nitrogen removal when used in single-aerobic reactors (Medhi et al., 2017; Li et al., 2015).

4. Conventional biochemical process of nitrification and denitrification in waste water

The two important biochemical reactions of nitrification and denitrification involving the conversion of ammonia to nitrate to molecular nitrogen (final end product) were recognized in the nineteenth century. The wide implementation of the traditional nitrification–denitrification processes at wastewater treatment plants help in decreasing reactive forms of nitrogen to below discharge limits with the use of aerobic autotrophic nitrifiers and anaerobic heterotrophic denitrifiers, which convert nitrogen containing compounds to molecular nitrogen gas (N_2) (Fig. 2).

4.1. Nitrification

In conventional BNR plants, NH_3 is converted to NO_2^- and NO_3^- under aerobic oxidation. Firstly, ammonia (NH_3) oxidized into nitrite (NO_2^-) involving enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) and secondly, oxidation of nitrite (NO_2^-) to nitrate (NO_3^-) in presence of enzyme nitrite oxidase. Incomplete oxidation of NH_2OH as well as autotrophic denitrification generates N_2O. NH_2OH is the intermediate product formed when AOB oxidizes NH_3 to NO_2^- under normal aerated conditions. But during unfavorable conditions HAO further undergoes two step catalyzation that involves the conversion of NH_2OH to nitrosyl radical (NOH) instead of NO_2^- and the subsequent polymerization and hydrolysis of NOH leads to the formation of N_2O as shown in the Eq. (1) (Guo et al., 2018; Sabba et al., 2015; Law et al., 2012). Recently a study conducted by Caranto et al. (2016) observed that under anaerobic conditions there was direct production of N_2O from NH_2OH by cyt P_{460} (a c-type heme of HAO) suggesting N_2O may be the main product of NH_2OH oxidation under both aerobic and anaerobic conditions. During nitrifier denitrification, NO_2^- is directly reduced to NO in presence of nitrite reductase (NiR) which was further reduced to N_2O catalyzed using nitric oxide reductase (NOR). Since AOB lack the presence of nitrous oxide reductase (NOS) no further conversion takes place making N_2O the end-product.

$$NH_2OH \rightarrow 2NOH \rightarrow N_2O_2H_2 \rightarrow N_2O + H_2O \tag{1}$$

4.2. Denitrification

Denitrification is the sequential process involving the dissimilatory reduction of one or both the ionic nitrogen oxides, nitrate (NO_3^-) and nitrite (NO_2^-) to gaseous nitrogen oxides, nitric oxide (NO), nitrous oxide (N_2O) and finally reduce to the ultimate product, dinitrogen (N_2) thus removing biologically available nitrogen and returning it to the atmosphere (Knowles, 1982). Traditionally, under typical conditions both nitrate and nitrite gets fully converted to atmospheric nitrogen but sometimes due to insufficient carbon sources, low dissolved oxygen (DO) and operational fluctuations or environmental conditions lead to improper denitrification and N_2O accumulation and emissions (Vázquez-Torres and Bäumler, 2016).

Even though denitrification occurs under anoxic/anaerobic phase, denitrifiers with their facultative anaerobic trait perform denitrifying activities under the presence of oxygen assisting the rise of N_2O as a denitrification intermediate. Many heterotrophic nitrifiers along with the oxidation of NH_3 can simultaneously perform aerobic denitrification (Medhi et al., 2017; Li et al., 2015). N_2O is generated via the reduction of nitrate to N_2 catalyzed by the nitric oxide reductase (NOR) which is present in the denitrifiers. Presently, the bioaugmentation of aerobic denitrifiers have promising potential for not only nitrogen removal but also equivalent amounts of nitrogen conversion to N_2 and N_2O through simultaneous nitrification and denitrification (SND) process in a single aerobic reactor that can be further applied in WWTPs (Chen et al., 2018).

5. Factors influencing N_2O emission

The parameters responsible for the N_2O emission has been described below (Fig. 3):

5.1. Dissolved oxygen (DO)

During nitrification, the DO concentration is a very crucial parameter that controls the emission of N_2O. Lower is the DO, higher will be

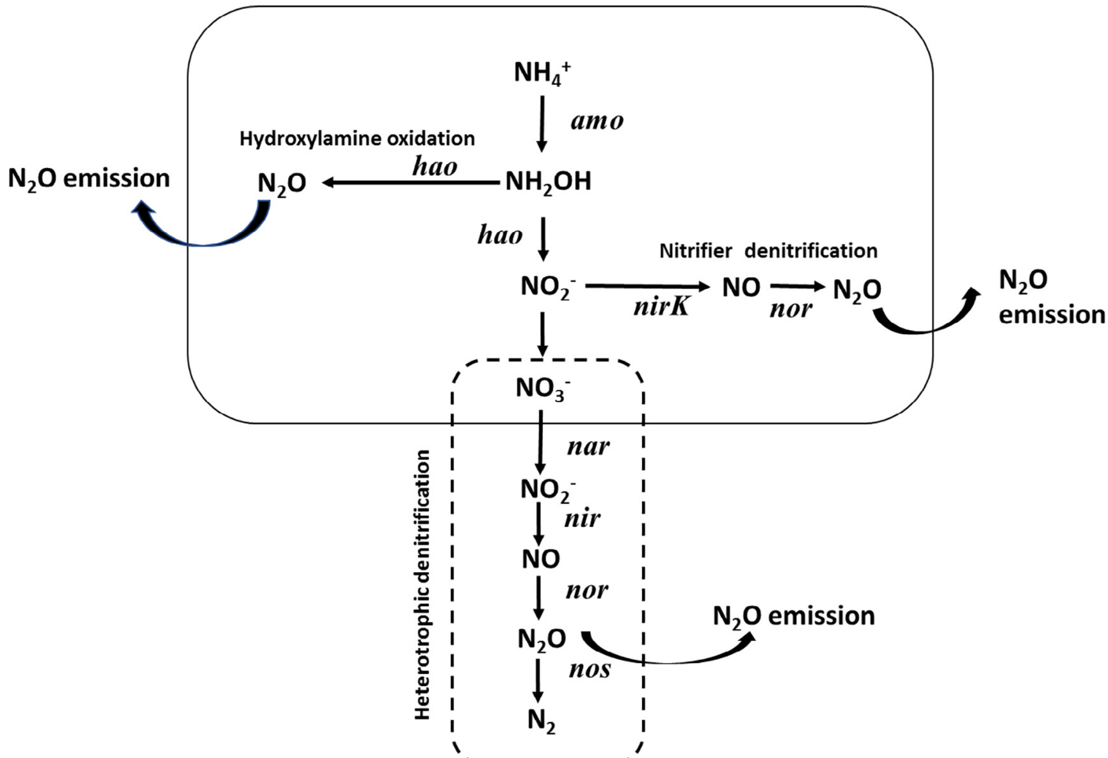

Fig. 2. Biological route entitled for N_2O production in nitrification-denitrification processes.

the N_2O emissions (Guo et al., 2018; Law et al., 2012; Kampschreur et al., 2009). Both nitrifier denitrification and incomplete NH_2OH oxidation occurs at low concentration of DO levels (ranging within 0.2–1.5 mg/L) and due to this oxygen limitation both are held responsible for the maximum emission of nitrous oxide (Frutos et al., 2018). AOB has stronger affinity toward oxygen as compared to NOB. During oxygen-limited situations AOB utilizes the nitrite as the electron acceptor that is being accumulated due to the inhibition of oxidation of nitrite to nitrate making way for N_2O emission as well as saving oxygen that will be required for the oxidation of ammonia to hydroxylamine.

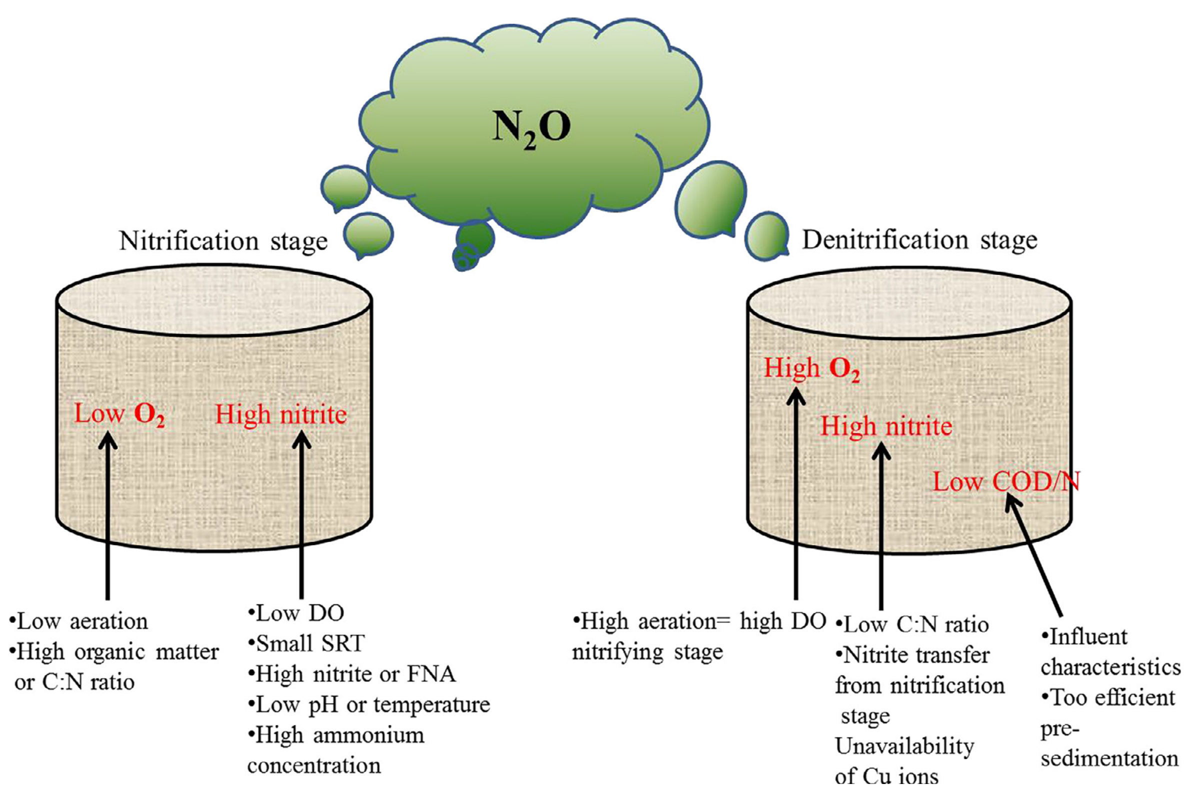

Fig. 3. Different key parameters responsible for N_2O emission.

N_2O emissions during denitrification process is also determined by the fluctuation of DO levels. Even though NR, NiR, NOR can tolerate the presence of oxygen but in case of NOS, it could get temporarily inactivated if exposed to even to low levels of oxygen since N_2O is very sensitive to the presence of oxygen, leading to N_2O emission even at very low concentration (Richardson et al., 2009). Tallec et al. (2006) performed lab-scale studies with actual urban wastewater to scrutinize the effect of oxygenation on nitrous oxide emission taking COD/N ratio of 2.8 and SRT more than 10 days demonstrated 0.1% of N_2O emission of the influent nitrogen at DO 1.0 mg/L and 0.04–0.06% at DO 2.1–6.2 mg/L implementing low DO as the main reason. Peng et al. (2018) investigated the effects of different DO concentrations (4, 2, 1 mg/L) on N_2O production via shortcut simultaneous nitrification and denitrification using a sequencing batch reactor that resulted highest N_2O production (127.6 mg/m^3) at a DO concentration of 2 mg/L which was 24.17 and 2.90 times the production at DO of 4 and 12 mg/L, respectively. The large impact of the dissolved oxygen concentration on N_2O emission indicates that proper control of the parameter is required in the wastewater treatment plants since low DO concentrations during nitrification and at the same time high aeration in the nitrification tank may lead to introduction of increased DO concentration during denitrification process, where both will ultimately lead to enhanced emission of N_2O.

5.2. Nitrite accumulation

The previous factor DO had stated that nitrite accumulation and its subsequent utilization will promote and lead to increase in N_2O emissions during nitrifier denitrification processes by AOB (Law et al., 2012; Guo et al., 2018). While in heterotrophic denitrification process, the increased nitrite concentration can hinder the denitrifying rate and lead to the accumulation of both NO/N_2O and moreover previous full-scale studies have established that the correlation between N_2O production and high nitrite concentration produced by AOB (Kampschreur et al., 2009; Kinh et al., 2017). Lab scale studies have also proved that utilization of 10 mg/L of NO_2^- concentration by a nitrifying mixed culture led to the escalation of N_2O production at higher DO concentrations (eightfold – 1 mg/L) (Tallec et al., 2006). 46% of total N_2O production was accounted during the coupling of $^{15}NH_2OH$ with $^{14}NO_2^-$ via N-nitrosation hybrid reaction indicating it as a predominant pathway while 51% of total N_2O production was observed when $^{15}NH_4^+$ was spiked into 400 mg/L of NO_2^- concentration using AOB-enriched biomass in SBR (Terada et al., 2017). High N_2O was observed due to the occurrence of incomplete nitrification at DO 0.5 mg/L and at SRT shorter than 5 days which led to the high accumulation of nitrite inhibiting further nitrification. It has been reported that the presence of free nitric acid (FNA) generated by nitrite also influences the N_2O production and is more potential in N_2O emission than nitrite (Guo et al., 2018). FNA passively diffuses across a cell membrane and directly reacts with the metabolic enzymes leading to toxicity in microorganisms and thus a high FNA concentration forces the AOB to employ denitrification to release increased N_2O thereby protecting themselves (Zhou et al., 2011).

5.3. C/N ratio

Availability and biodegrability of organic matter in the wastewater treatment plants are measured as chemical oxygen demand (COD) and is an important factor to govern the N_2O emissions during denitrification processes (Law et al., 2012). Denitrification rate largely depends on the source of carbon as well as concentration of available carbon which might vary according to different microorganisms and environmental conditions to achieve optimal removal. For complete denitrification, a C/N ratio of 4 is required. Low C/N ratio leads to improper denitrification while high C/N ratio leads to the nitrite accumulation resulting extra production of nitrous oxide (Ghafari et al., 2008). Decrease in carbon sources increases N_2O emissions during denitrification as the various enzymes in that process compete for the electrons where NOS is the weakest competitor which ultimately leads to incomplete denitrification (Guo et al., 2018). A study using *A. faecalis* reported that limiting carbon sources led to increased N_2O formation by 32–64%, while decreased N_2 production drastically (Schalk-Otte et al., 2000). N_2O emissions were raised up to 30% when the C/N ratio for treating high strength wastewater is below 3.5 while another study found that insufficient COD level leads to increased N_2O production (Itokawa et al., 2001; Kishida et al., 2004).

5.4. Availability of copper (Cu) ions

NOS encoded by the nosZ gene is a multi-copper enzyme containing two copper centers Cu_A and $Cu_{Z.}$ For the biosynthesis of N_2O reductase Cu is the most essential element and its availability determines the N_2O emissions. With sufficient supply of copper ions, they bind to the active site (Cu_Z) of nitrous oxide reductase enzyme that catalyzes the conversion of N_2O to N_2. Deficiency of the copper supply leads to incomplete biosynthesis of NOS making this enzyme inactive which results in the shifting of the end-product of heterotrophic denitrification from N_2 to N_2O (Paraskevopoulos et al., 2006). Zhu et al. (2013) observed that the addition of Cu to the denitrification tank increased the activity of the NOS enzyme together with a reduction of 50–73% in N_2O emissions. In denitrification, the presence of FNA competes with Cu ions for the active sites of NOS leading to a competitive inhibition resulting N_2O accumulation (Guo et al., 2018). Chen et al. (2012) evaluated the effect of the addition of Cu nanoparticles during anaerobic-microaerobic biological nitrogen removal for the generation of N_2O which resulted that the presence of 10 mg/L concentration of Cu NPs induced minimum N_2O generation as compared to the control tests with no Cu NPs improved the nitrogen removal. Although copper is demonstrated to increase the N_2O reductase activity and to reduce N_2O production in wastewater further investigation is need to be done.

5.5. Availability of emerging inorganic and organic contaminates

Emerging contaminants and organic hydrocarbons such as aliphatic, alicyclic, aromatic, polyaromatic present in waste water treatment plant inhibits growth of nitrifying and denitrifying microorganisms. In aerobic nitrifying processes, when O_2 was delivered as an electron acceptor, reduced contaminants were oxidized, e.g., benzene, toluene, surfactants and several hydrocarbons (Ontiveros-Valencia et al., 2018). Inhibition of the growth of microorganism can result in poor performance which can be enhanced by using a set of design and operation features as follows: gas pressure, membrane type, and surface loadings. The increase contaminants also act as substrates when primary energy source such as carbon gets depleted which leads to high COD/N ratio during nitrification resulting in incomplete nitrification and increased N_2O emissions. In pentachlorophenol (PCP) de-chlorination, other electron acceptors responsible for de-chlorination reported to be capable of denitrification (efficient nitrate removal) (Long et al., 2018; Shah and Thakur, 2002).

6. Molecular aspects of N_2O production

New molecular aspects in genomics, proteomics and metagenomics have opened up new pathways to elucidate and to understand the complexity of a single microorganism or a bacterial community and its relation to nitrogen removal through nitrification and denitrification. Several enzymes such as HAO and NOR found in AOB and denitrifiers respectively contribute to the N_2O production while NOS is the enzyme responsible for its consumption. N_2O is a response to the imbalance that occurs in between N_2O production and consumption. The proper functioning of these enzymes is very important as they need to be regulated and accumulation of nitric oxide or oxygen fluctuation need

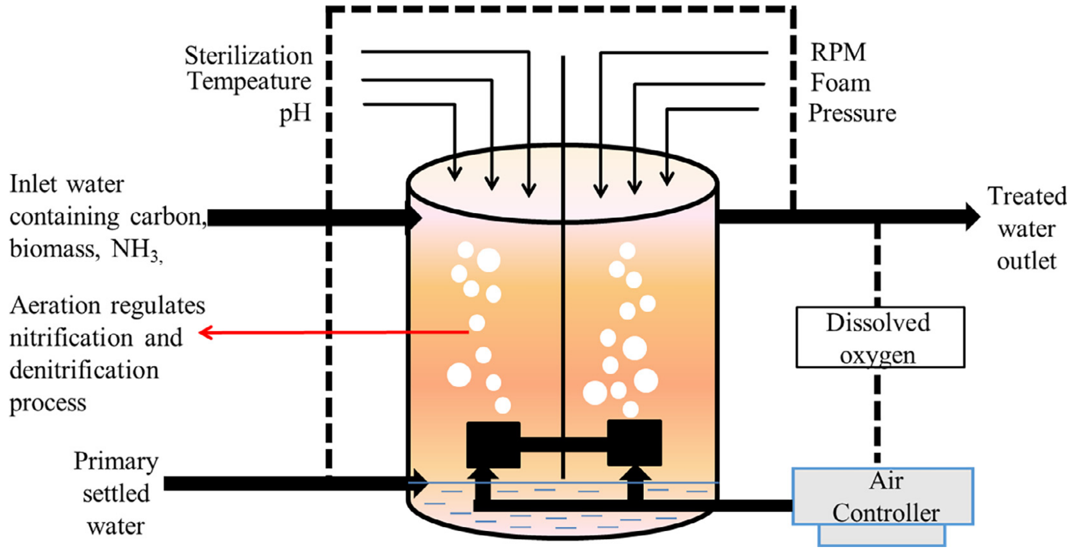

Fig. 4. Schematic diagram of a lab-scale bioreactor for monitoring operational conditions during nitrogen removal.

to be avoided (Olaya Abril et al., 2018; Sullivan et al., 2013). The reduction of NO_2^- to NO by the nitrite reductase (NiR) is the most vital point of the denitrification process as it signifies the conversion of a non-gaseous water-soluble nitrogen oxyanion to a N_2O gas. Globally, biological denitrification accounts for about 60% of total N_2O emissions to the atmosphere. Hu et al. (2015) demonstrated that nitrous oxide reduction is severely affected with acidic pH as it disturbs the nitrous oxide reductase activity at transcriptional or post-transcriptional level. NO_2^- accumulation at the gene expression and transcription level was investigated in AOB in response to low DO revealed that the mRNA concentrations for AMO and HAO in the growth phase of Nitrosomonas europaea were higher at lower DO along with elevated *nirK* and *norB* mRNA for nitrite reductase and NO reductase, respectively (Yu and Chandran, 2010). They suggested that *N. europaea* could efficiently metabolize NH_3 and NH_2OH for growth and also promote detoxification by the reduction of NO_2^-. The presence of *nir* and *nor* genes and absence of *nos* genes in the genome of AOB revealed that N_2O instead of N_2 is the end-product of nitrifier denitrification (Beaumont et al., 2005; Cantera and Stein, 2007). Terada et al. (2017) reported that N_2O production was boosted by $^{15}NH_2OH$ spiking, causing exponential increases in mRNA transcription levels of AOB functional genes encoding *haoA*, *nirK*, and *norB* genes. Spiro (2012) also explored the regulating environmental parameters ensuring optimal expression of the genes encoding the enzymes involved in N_2O production and consumption, whereas Schreiber et al. (2012) suggested the integration of molecular methods, isotope and microelectrode to understand and assess the relationship between environmental parameters, microbial community structure and gene regulation for N_2O emission rate. The whole genome of both *Paracoccus denitrificans* ISTOD1, *G. thermodenitrificans* KCTC 3902^T revealed complete set of denitrification genes suggesting complete conversion of reactive nitrogen compounds into N_2 gas but the absence of *nosZ* gene in *G. kaustophilus* HTA426 predicted the end-product of denitrification is N_2O (Medhi et al., 2018b; Lee et al., 2017). A proteomic study of *Paracoccus denitrificans* PD1222 has been conducted revealing that the gene expression levels, protein intensity and activity of the nitrous oxide reductase *nosZ* were found to be lower in presence of oxygen than in anoxic conditions (Olaya Abril et al., 2018). Oxygen and nitric oxide are regulated by *FnrP* and *Nnr* transcription regulators which controls the expressions of nitrous oxide reductase in *P. denitrificans* under nitrate-respiring denitrifying conditions (Bergaust et al., 2012). Thus, molecular insight also be taken into account along with biological processes to have a better understanding of the N_2O regulation.

7. Bioreactors for N_2O abatement

Controlled measures are being taken while treating nitrogen in nitrification and denitrification tanks of the traditional wastewater treatment plants but due to alteration of aerobic/anoxic design, unfavorable conditions, failure of the operating parameters or excessive nitrogen load disbalances the removal efficiency as well as removal rate of the persisting nitrogenous wastes. So, to address the proper N removal processes many controlled operating conditions, such as hydraulic retention time (HRT), sludge retention time (SRT), dissolved oxygen (DO) concentrations and organic loading rates have been studied using bioreactors where every parameter can be monitored properly. The production and reduction of N_2O wholly depends on the operating conditions (Ribeiro et al., 2018). Conventional alteration of aerobic-anaerobic treatment processes used for the reduction of both organic carbon and nitrogen concentration in an engineered wastewater treatment plants not only takes a good amount of time but these processes have also been found to operate at a high cost. Therefore, innovative bioreactors run at laboratory, pilot and full scale have been designed and operated in the last four decades consisting of a single reactor which will minimize the operational costs and reduce time wastage incorporating both aerobic and anaerobic processes as shown in Fig. 4. These technologies have consistently shown a high robustness, cost efficiency and low environmental impacts results from the operational processes consuming low energy (monitored temperature and pressure) (Lebrero et al., 2013; Estrada et al., 2012).

7.1. Sequencing batch reactors (SBR)

A single reactor where both aerobic-anaerobic processes take place in a sequence. Experiments conducted by Yang et al. (2009) investigated the mechanisms of N_2O production in domestic wastewater via nitrification nitrite using SBR resulted 1.5 times higher N_2O production than nitrogen removal via nitrate indicating that ammonia oxidation is the main source of N_2O production not nitrite oxidation or anoxic denitrification. SBR study using synthetic wastewater conducted under controlled aerobic and anaerobic phase consisting of influent ammonia concentration of 42 mg/L and DO 1.8–5.6 mg/L, reported 2.5–4.6% and 0–0.013% of N_2O emission from the influent nitrogen during the aerobic phase and the anaerobic phase respectively. Thus suggesting aerobic phase is essentially responsible for N_2O emission (Bhunia et al., 2010). Rodriguez-Caballero et al. (2015) established an SBR with aerated and non-aerated cycles to minimize N_2O emissions found that short oxic-anoxic cycles of 20–30 min minimized N_2O emission while longer cycles of 55 min generated higher N_2O of 140 ppm. A study reported the emission rate of N_2O using SBR with real wastewater and sodium acetate as external carbon source, COD of 425 mg/L and BOD of 380–400 mg/L, where it was observed that with the increase in aeration time, the emission rate of N_2O also increased approximately 0.13 mg/min/m^3g/MLVSS and subsequently getting reduced at low DO phase. This might be probably due to the incomplete denitrification ability by the microbial population and also the existence of excess nitrite that inhibits the N_2O reductase causing incomplete denitrification causing emission of N_2O (Li et al., 2010). Based on the above results, a step-feed SBR will serve as an effective method to reduce N_2O production from wastewaters during nitrogen removal mainly focusing on the nitrification phase.

7.2. Biofilters

Recently, the usage of off-gas treatment bioreactors such as biofilters have been assessed at lab-scale to study N_2O heterotrophic denitrification for the abatement of N_2O emissions from domestic wastewater. Hood (2011) evaluated a N_2O abatement from swine wastewater using biofilter packed with compost: woodchips (30:70) at a gas empty bed residence time (EBRT) of 7.6 s operating for 8 months and recorded that low concentration of N_2O led to low N_2O removal efficiencies (RE ~ 14–17%). Even a study conducted by Akdeniz et al. (2011) evaluated the performance of a biofilter packed with pine nuggets and lava rock at a gas EBRT of 5 s for the treatment of the exhaust gases from a swine manure and wastewater, recorded a low N_2O removal efficiency (RE ~ 0.7%) at the low inlet concentrations due to the presence of O_2 and low EBRT. The above factors hindered the N_2O biodegradation by the microbial community present in the biofilter. To achieve satisfactory N_2O abatement conventional biofilters were not able to support heterotrophic denitrification due to their inherent limitations.

7.3. Bioscrubbers

The bioscrubber in a biological system is represented by a combination of water adsorption column (gas scrubber) and a biological wastewater treatment plant (biological reactor) for treating wastewater. Frutos et al. (2015) evaluated N_2O emissions using an innovative bioscrubber consisting methanol as both carbon and electron donor source, where emitted N_2O-laden air was introduced at the bottom of a 2 L packed-bed absorption column operating con-currently with a trickling mineral salt medium (MSM) pumped out from a 3 L anoxic stirred tank reactor (STR) containing denitrifying microbial cultures immobilized in a polyurethane foam. The immobilized heterotrophic denitrifying population helped in absorbing the N_2O and then reduced to N_2 in the STR. Lab-scale studies were also evaluated using similar bioscrubber for the continuous abatement of N_2O where domestic wastewater was taken as the carbon and electron donor source as an operational strategy to reduce the overall operating costs procured from the external carbon supply, consisted of a packed bed absorption column (2 L) coupled to a fixed bed anoxic bioreactor (FBR) (two units of 3 and 7.5 L were evaluated) filled with polyurethane foam to support microbial immobilization achieved removal efficiencies of N_2O up to 94% showing a consistent performance (Frutos et al., 2016). The absorption of N_2O in the trickling wastewater followed by its reduction to N_2 by the anaerobic-denitrifying community in the FBR confirmed the operational feasibility of combining both bioremediation processes.

7.4. Nanomagnetic constituents

NOx released in the industrial flue gas is relatively challenging to handle but could also be entailed in the formation of harmless nitrogen gas. NO being poor water-soluble accounts for 90–95% of the NOx and cannot be removed through a simple alkali absorption process. Thus, the usage of metal complexes such as EDTA-Fe (II) can readily react with NO to facilitate its absorption and form dissolved metal-nitrosyl complexes such as EDTA-Fe(II)-NO (Sharif et al., 2018). This process is widely known as the wet scrubbing method for NO removal and using this method, a novel nanomagnetic adsorbent, Fe_3O_4-EDTA-Fe (II) (MEFe(II)) was developed for NO removal. The NO adsorbed by MEFe (II) was then converted to N_2O, a valuable compound in many industries using sulphite as the reductant. Under optimal pH conditions (7.5–8) required for NO adsorption and N_2O recovery, the produced N_2O was easily handled without its unwanted release to the atmosphere.

7.5. Airlift reactors

The reactors consist of a two-stage process composed by a first aerobic granular reactor followed by a second anoxic granular reactor for feasible technology to complex industrial wastewaters. The specialized required microorganisms are embedded on aerobic granular and feeding continuously. Frutos et al. (2017a–c) studied the continuous abatement of industrial N_2O emissions using methanol as a carbon and electron donor source using airlift reactors with low O_2 and high N_2O concentrations (1500–3500 ppm) of waste emissions from HNO_3 production plants might support the direct diffusion of N_2O emissions in denitrifying suspended cultures for abatement of N_2O. Another study used a continuous airlift reactor with aerobic granular biomass was established to treat synthetic wastewater mixed with aromatic compounds with varying DO of 0.5–4 mg/L, COD/N ratio of 0.65 mg/L resulted in 4.1% of nitrous oxide emission of the total influent nitrogen. At 0.5 and 1.0 mg/L of DO, higher N_2O emissions and between 1.0 and 4.0 mg/L of DO stable N_2O emissions were observed, produced as an intermediate product of nitrification in the aerobic reactor (Ramos et al., 2016). Pijuan et al. (2014) also observed similar N_2O trend using another granular airlift reactor performing nitrification of real reject wastewater.

7.6. Denitrifying bioreactors and membrane biofilm reactors

Natural process of denitrification can be enhanced by addition of a solid organic carbon source and maintenance of anoxic conditions for removal of nitrate from wastewaters. For efficient N removal along with water and air pollutants removal, denitrifying bioreactor popularly known as woodchip bioreactors has been gaining importance and has become an active research for treatment systems (Christianson and Schipper, 2016). Feyereisen et al. (2016) observed improved nitrate removal and reduced nitrous oxide production using woodchips amended with corn cobs. Bioreactor age, continuous emissions of H_2S, N_2O, CH_4 and sulfide formation can be detrimental to the environment.

Membrane biofilm reactor (MBfR) is a new environmental biotechnology tool that consists of microbial biofilm accumulating on a membrane having a significant mechanism of substrates delivery to the biofilm (Martin and Nerenberg, 2012). This reactor is primarily used for the delivery of gaseous substances such as oxygen, hydrogen, methane, CO, CO_2 to the biofilm harbouring a complex microbial community (Ontiveros-Valencia et al., 2018). However, maximum rates affects the microbial community's structure and its metabolic function. Different membrane composition and properties would affect the substrate delivery differently. H_2 being a universal electron donor for many microorganisms, the transfer of H_2 through MBfR has been used to reduce and detoxify water pollutants such as nitrate and improve denitrification ultimately lowering N_2O emissions.

8. Mitigation strategies of nitrous oxide

There is still a lack of understanding the N_2O production mechanism in nitrification-denitrification processes and the exact causes and pathways are still to be studied and revealed for elimination of these prominent and persistent green-house gas. In biological nutrient removal processes, the main focus for N_2O mitigation is the production of less N_2O subsequently its consumption. The leakage of N_2O during the wastewater treatment process is an indicator of a failed or less-efficient operating system specifically in the nitrification tank owing to toxic nutrient load or insufficient/excess aeration taking place (Law et al., 2012). The metabolic pathway of AOB causing N_2O production still require more elaboration but the rapid shifts in the parameters such as DO, pH, temperature, immediate nitrite spiking can be avoided for minimizing the N_2O emission into the environment to maintain a proper performance of wastewater treatment plants. The full-scale plants can be designed with modifications, configured and operated under more stable process conditions such as uniform DO concentration to achieve less N_2O and moreover to be equipped with designs that can sequester N_2O emissions from the effluent and relatively result in low emission (Yu et al., 2010). But for these, cost still remains a very big factor. As we have already discussed in this paper that low DO concentration at nitrification stage, nitrite accumulation at both nitrification-denitrification stage as well as carbon availability plays an important role in the N_2O production. Therefore, the first and foremost rule should be to target them as they can be avoided with controlled parameters to reduce the GHG emission. The oxygen input during the nitrification should be high enough so that balanced nitrifier denitrification take place also directing to nitrate oxidation for the reduction of nitrite accumulation to minimize N_2O emission. DO equal to or higher than 1.0 mg/L should be maintained to avoid high N_2O emissions. Higher aeration or increased DO during nitrification will result in the stripping of the highly water-soluble gas that remains in the liquid phase to emit as N_2O, thus, minimized stripping would give the indigenous microorganisms more time to consume it (Cui et al., 2015; Law et al., 2012). In case of simultaneous nitrification–denitrification processes, increased aeration will inhibit proper denitrification, thus the aeration parameter is the main focused area that need to be maintained properly (Kampschreur et al., 2009). However, increased aeration rate above 0.6 L/min, decreased the N_2O emission rate (Wu et al., 2014).

The traditional use of physical and chemical processes for catalytic reactions in treatment plants requires high maintenance and these costs prohibits the treatment of low to moderate N_2O concentrations (Campos et al., 2016). Thus, bioprocesses bioaugmented with heterotrophic nitrifying-denitrifying bacteria such as *Pseudomonas stutzeri*, *Paracoccus pantotrophus* or microalgae could be developed and studies have reported 75%–99% in concurrent reduction of nitrate and N_2O emissions and enhancing N_2 production (Read-Daily et al., 2016). The occurrence of high C/N ratio during denitrification processes can also lead to the minimization of the N_2O gas. Studies have reported that increased C/N ratio, nitrite act as the electron acceptor during denitrification leading to its increased removal rates which in turn decreased the N_2O emission factor (Wu et al., 2014; Medhi et al., 2018c). Some of the other factors that need to be monitored are high hydraulic retention times (HRT), engaging of a long solid retention time (SRT) during nitrification phase to maintain low ammonia concentration that would minimize nitrite accumulation (Campos et al., 2016). The anaerobic ammonium oxidation (Anammox) conversion process was first observed in a denitrifying fluidized bed reactor under lab-scale conditions, where bacterial growth depends on the utilization of ammonia in absence of oxygen. It can also be depicted as denitrifying process where nitrite reduction to nitrate takes place along with ammonia oxidation. The nitrate reduction does not follow the traditional process via N_2O and therefore emission of N_2O is not expected. This process yields gaseous N_2 as its end-product, thus this process need to be implemented more when nitrous oxide production need to be studied and eliminated (Kartal et al., 2007; Guo et al., 2018). *Nitrosomonas* sp. could successfully oxidize ammonium under both anaerobically as well as aerobically and can be used in N_2O reduction (Schmidt and Bock, 1997).

Another alternative for efficient biotechnological abatement of N_2O, is the production of value-added products utilizing nitrous oxide as substrate for establishing a biorefinery based process. Previous studies have reported as well as establish the biorefinery based N_2O abatement by producing commercial biopolymers, medicines or cosmetics to improve the economic sustainability of N_2O emission (explained in separate section) (Frutos et al., 2018). Lastly, the metabolic pathways involved in the cause and remedy of the N_2O emissions largely depend on the potential activity of the enzymes involved should be of central concern as they are the critical modulators for the same, therefore their analysis is a must for N_2O abatement. Molecular tools such as RT-qPCR has provided ways to assess the enzymes as well as the relationship between the gene expressions of nitrifiers or denitrifiers, required in N_2O emissions (Yu et al., 2010).

9. Biovalorization of nitrous oxide

The remarkable use of N_2O in different fields had started since it was first discovered (Severin, 2015). It has been popularly used in the medical field as a recreational drug, as a pain relief drug during childbirth, as a drug for treating patients with treatment-resistant depression and have been used as an anesthetic by dentists and doctors since the 19th century but presently its usage has been drastically reduced (Speth et al., 2013; Nagele et al., 2015). Its application has been also utilized as whipping agent for cream and as a fuel additive for rockets and motors (Zuck et al., 2012). As every coin has two sides, N_2O can be utilized for useful applications but its emission can also lead to harmful environmental impact like its participation as an ozone-depleting agent found in CFCs. However, its application in synthetic chemistry has turn heads to convert it into other less-toxic products with minimum environmental impact. N_2O is a well-known strong oxidant and its oxidation reactions result in the formation of the atmospheric N_2 (Severin, 2015; Sharif et al., 2018). Thus its application as O-atom donor, N-atom donor has been discussed operating under mild conditions (Fig. 5). Biovalorisation strategies for N_2O abatement is also a very important part for N_2O mitigation.

9.1. N_2O as O-atom donor

Oxidation reactions with N_2O via oxygen transfer results in the release of N_2. The utilization of N_2O allows selective oxidation reactions that are difficult to achieve with other oxidants such as O_2. Low valent silicon compounds are used as suitable substrates for performing selective O-atom transfer reactions with N_2O. N_2O has helped in the oxidation of disilanes, silylenes, carbine-stabilized Si (0) compounds as well as interestingly has react with a complex combining of metallosilylene with an osmium silylene (Severin, 2015). Recently, the oxidizing reactions of N_2O with low-valent germanium compounds have also been studied to form hydroxide compound (Yao et al., 2011). Similarly, germanones were formed when donor-stabilized germylenes were oxidized. Some other group compounds that react with N_2O under mild conditions were phosphines, sodium sulphite and boranes. N_2O also acts as O-atom donor during transition of metal complexes when metals such as Ti (III), V(III), Ni (0), zirconium come in contact with N_2O (Severin, 2015). The oxidation of Ni (0) carbonyl complex with N_2O was reported to produce a chelating carbonate ligand complex (Horn et al., 2012).

9.2. Metal-catalyzed reactions with N_2O

As discussed above that N_2O activation with metals suggested that metal-catalyzed oxidation reactions could be performed, carried out by heterogeneous catalysts or under the gas phase. One example that has been reported of the catalytic metal oxidation reactions with N_2O performed in homogeneous solution was the oxidation of phosphines to

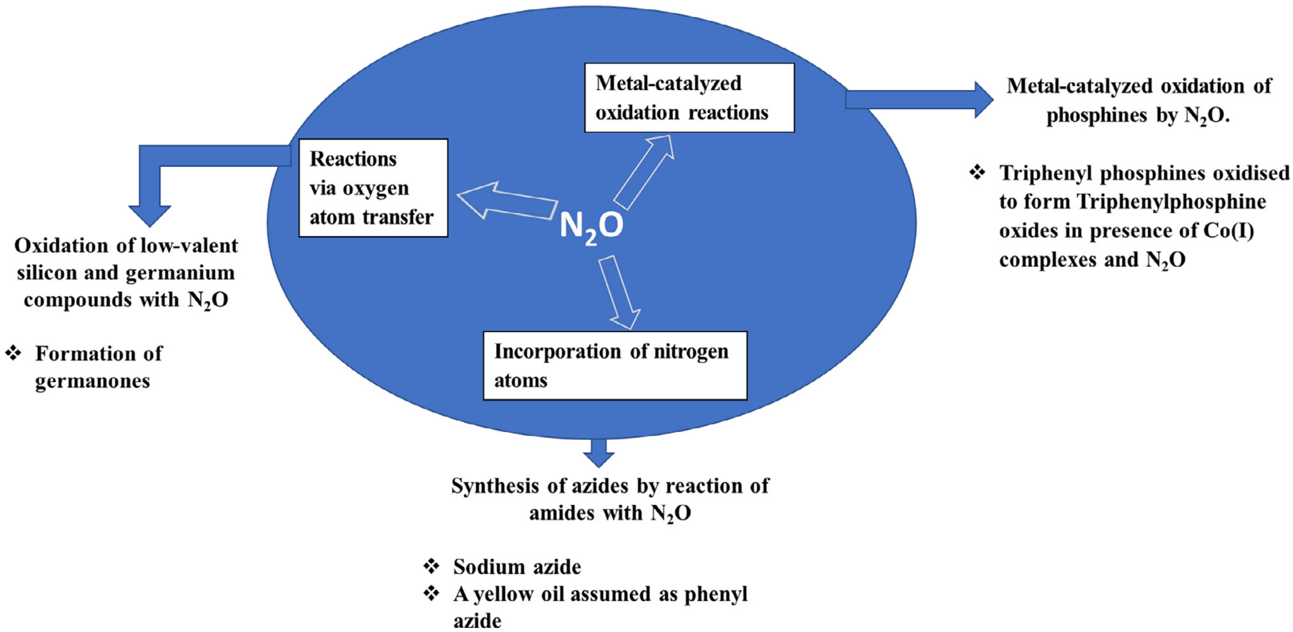

Fig. 5. N_2O applicability in synthetic chemistry.

phosphine oxides. It was believed that the hydride complex CoH (N_2) $(PPh_3)_3$ was able to catalyze the oxidation of PPh_3 resulting in $OPPh_3$. The presence of Co (I) complexes initiated the reduction of N_2O to N_2 was also studied (Severin, 2015). They also reported that competent catalyst system used for the conversion of phosphines to phosphine oxides by N_2O was the combination of $NiCl_2$ (DPPP) and n-BuLi. The oxidation of different organic substrates in presence of Ru-porphyrin as well as the O_2 atom transfer reactions mediated by organometallic Mo complexes were investigated by Yamada et al. (2001) and Yonke et al. (2011) respectively.

9.3. N_2O as N-atom donor

The reaction mechanisms in this section demonstrated the incorporation of nitrogen atoms of N_2O into the final product formation and their subsequent valorization. Severin (2015) reported the production of sodium azide upon exposure of $NaNH_2$ to N_2O at elevated temperatures and presently industries are using this reaction to produce sodium azide at a large scale. Even the reaction of KNH_2 with Zn $(NH_2)_2$ was found to be in the similar fashion. The same mechanism was investigated using ^{15}N-labelled nitrous oxide that demonstrated that $N^{15}NO$ and ^{15}NNO could be prepared by decomposition of either $NH_4{}^{15}NO_3$ or $^{15}NH_4NO_3$. The conversion of amides of aromatic compounds into azides was also studied where lithium anilide reacts with N_2O to form azobenzene, biphenyl and phenyl azide (Severin, 2015). The coupling of lithium amide and organomagnesium compounds with N_2O resulted in the formation of triazenes, which have been examined as potential anti-tumor drugs whereas triazene dacarbazine and temozolomide were being used for cancer treatment (Newell et al., 1990).

9.4. Polyhydroxyalkanote (PHA)

To combat the escalating climate change arise due to the emission of different GHG such as CO_2 and N_2O, formation of value-added products would help in the mitigation strategies. Biotechnologies exhibit eco-friendly route towards gaining this opportunity. One such product is the formation of biopolymers such as polyhydroxyalkanoates (PHA). The denitrifying bacteria such as *Paracoccus denitrificans*, *Pseudomonas stutzeri*, *Ralstonia eutropha* were capable of producing intracellular PHA as carbon storage source in presence of excess carbon in the media during nutrient starvation conditions (Barak and van Rijn, 2000; Saharan et al., 2014). Frutos et al. (2018) studied the simultaneous denitrification for N_2O abatement along with formation of value-added products such as poly (3-hydroxybutyrate) (PHB) or poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), types of bioplastics that shared properties with the conventional fossil-derived plastics. They used two bioreactors namely bubble column and airlift reactor for the co-production of biopolymers with the help of a pure *Paracoccus denitrificans* culture resulting 34–68% PHA according to the bacterial biomass weight along with N_2O removal efficiency of 87%. The influence of carbon source was also investigated where methanol, acetic acid and glycerol were used as feedstocks for biopolymer production revealed approximately 91% of N_2O removal efficiency along with the coproduction of PHBV (Frutos et al., 2017a–c). There have been many studies that evaluated the biological sequestration of GHG such as CO_2 with simultaneous accumulation of biopolymers as a cost-effective alternative for minimizing GHG emissions conceptualizing the biorefinery approach (Kumar et al., 2016; Maheshwari et al., 2018).

9.5. Production of extracellular polymeric substances

Usually it has been observed that nitrifying and denitrifying bacteria have the capability to produce extracellular polymeric substances (EPS), composed mainly of carbohydrate polymers and protein that holds the biofilm together as well as to the substratum (Flemming and Wingender, 2001). The EPS constituents also play an important role in helping the biofilms resist toxicants, predation, and desiccation. EPS found in the form of biofilms in MBfR could help in the maintenance of the biofilm integrity as well as mitigation of N_2O. The biofilm composed with 25% of heterotrophic bacteria was found to be associated with autotrophic and these H_2-oxidizing autotrophs provided substrates in the form of soluble microbial products (SMP) to the heterotrophs due to the hydrolysis of EPS (Tang et al., 2012). Recent work showed the correlation between the EPS accumulation and microbial metabolism associated with V (V) reduction and CH_4 oxidation (Lai et al., 2017). The presence of hydroxyl (–OH) and carboxyl (COO–) groups in the biofilm initiated the microbial binding along with the reduction of V (V). EPS are well known for their ability to adsorb or immobilize metals based on their hydroxyl, carboxyl, phosphate, and amine groups (Sheng et al., 2010).

9.6. Energy production through methanogenesis

Another alternative study was reported for the abatement of N_2O and rerouting its emission for energy production (Scherson et al., 2014). Energy recovery from wastewater is achieved through anaerobic digestion of organic matter and subsequent conversion to CH_4 and the recovered CH_4 is combusted in presence of oxygen to produce energy as electricity and heat. This research was focused on a new concept of maximizing the N_2O production, capturing its emission and ultimately using it for energy generation to distinguish N_2O could be treated as an upcoming renewable energy source. N_2O is a more powerful oxidant than O_2 and can increase energy recovery along with CH_4 combustion. The emitted N_2O from the nitrifying unit of Coupled Aerobic-anoxic Nitrous Decomposition Operation (CANDO) process, a new technology for removal of nitrogen from wastewater was injected into a biogas-fed engine at a full-scale SRT to burn along with methane that increased the combustion output by 5.7–7.3%. The bacterial community consist of *Comomonas*, *Clostridium* present in the CANDO was also able to produce PHB and helped in its oxidation for the reduction of nitrite to N_2O to be used in energy recovery.

10. Challenges and opportunities for nitrous oxide mitigation and biovalorization

N_2O emissions from wastewater treatment plants can be very significant in terms of their contribution to the overall global warming footprint as it leads to the depletion of the stratospheric ozone layer that results in the minimized protection from harmful UV rays as well as absorbs heat escaping to the space altering the greenhouse effect, contributing to climate warming. Despite their relatively small contribution to the overall global greenhouse gas emissions it has become important to understand actual mechanisms of its formation so that proper mitigation strategies can be deduced. The mechanisms of N_2O production is known but its emissions from wastewater treatment processes vary substantially as it entirely depends on the design and operational conditions, the flow rate and wastewater characteristics and thus N_2O emission could be avoided through proper process design and operation. Even though preliminary strategies have been developed but remain to be verified through full-scale applications. Since the past two decades, research opportunities in this field indicated the usage of bioreactor and their configurations such as the upcoming new technology of biofilm based membrane bioreactor and use of nanobiocomposite materials. In addition, evaluation and characterization of microbial ecology, the effects of hydraulic retention time to maintain low ammonia and nitrite concentrations in the media in bioreactor, proper optimization of pH, temperature and carbon on nitrate removal should be properly evaluated in laboratory-scale studies to answer the novel questions. There is an urgent need for large-scale research that could be used to evaluate field-scale issues such as longevity and management. The future must hold studies that look beyond the bioreactor "black box" through use of monitoring techniques as well as more advanced research in real-time continuous sensors to evaluate bioreactor performance under relatively rapid temperature, flow changes and its applications. The N_2O emission from activated sludge processes may be reduced by improvement of operational conditions viz. low dissolved oxygen concentration in the nitrification and the presence of oxygen in denitrification phases, high nitrite concentrations in both nitrification and denitrification stages, low COD/N ratio in the denitrification stage, sudden shifts of pH, dissolved oxygen, ammonia and nitrite concentrations, as well as transient anoxic and aerobic conditions.

Synthesis of materials by catalytic reactions with N_2O in recent years have been examined extensively since N_2O is found to be a cheap and eco-friendly oxidation agent. Nevertheless, it is possible to use N_2O as a donor of nitrogen atoms. Reactions of this kind are known for many decades, but applications in organic synthesis were sparse. Solution-based reactions with transition metal catalysts have shown only very limited success as to achieve those high temperatures and/or pressures were needed but resulted in low turnover numbers. For reactions conducted with N_2O, the transfer of oxygen is the most commonly reported mode of reactivity. The formation of side products, low yields, and the existence of more attractive alternative procedures have hampered the utilization of N_2O in N-atom transfer reactions which might improve in the near future. Last but not the least, production of biopolymers viz EPS, polyhydroxybutarate, high energy recovery of combusting nitrous oxide with methane and other GHG like CO_2 for biofuel and biorefineries which would open up significant cost-effective and eco-friendly opportunities in the 21st century.

11. Conclusion

This review has emphasized on emission of the ozone-depleting agent N_2O during nitrogen removal in wastewater treatment plants. The nitrification under aerobic conditions was observed to be the concerned phase as it led way for the N_2O leakage through hydroxylamine oxidation and incomplete nitrifier denitrification. DO has been the major factor affecting N_2O production and emission in both nitrification-denitrification processes. The new-age technologies of bioreactors with monitored parameters constituted popular treatments for the abatement of N_2O at commercial level. Valorization of N_2O in biopolymer and energy production has helped to identify it as an eco-friendly and sustainable renewable resource.

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Conflict of interest

The authors declare that they have no competing interests

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