Micropollutant Control in Wastewater Treatment: A Review of Harnessing Nitrification and Denitrification Biotransformation of Micropollutant

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Abstract – **Micropollutants, an array of organic compounds such as pharmaceuticals, personal care products, and agrochemicals, are pervasive in contemporary ecosystems, posing significant threats to environmental health even in trace concentrations. Therefore, exploring an efficient and effective technique to remediate these pollutants is essential. Nitrification–denitrification (ND) have emerged as one of the most sustainable treatment methods that effectively mitigate micropollutants while facilitating their biotransformation. This review provides a comprehensive analysis of the intricate interactions fundamentally and mechanically between the ND process and the influencing factors, such as dissolved oxygen (DO) concentration and pH optimization, which are vital to the success of micropollutant biotransformation. Insights gained from this examination contribute to a deeper understanding of microbial strategies, which offer potential avenues for sustainable environmental management and the protection of ecosystem integrity.**

Index Terms – **Biotransformation, Denitrification, Micropollutant, Nitrification, Wastewater treatment**

I. Introduction

Nitrification and denitrification (ND) are crucial processes in wastewater treatment and environmental sciences, playing a significant role in the fate and transformation of micropollutants such as pharmaceuticals, personal care products, and agrochemicals, are typically present in wastewater at concentrations ranging from a few nanograms per liter to several micrograms per liter (Suneethi et al., 2015). Despite their low concentrations, these compounds can be toxic, mutagenic, genotoxic, and disruptive to endocrine systems, raising concerns about their impact on environmental and human health (Alzate Marin, Caravelli and Zaritzky, 2016; Miao et al., 2019). Conventional wastewater treatment plants (WWTPs) primarily focus on removing pathogens, total suspended solids (TSS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) (James and Vijayanandan, 2023), whereas nitrogen and micropollutants are left behind in the discharged of the so-called treated wastewater (Phan et al., 2014). This partially treated wastewater discharge is a significant global concern, with an estimated 80% of wastewater worldwide being inadequately treated (WWAP, 2017). This underscores the urgent need for sustainable and cost-effective solutions for nitrogen and micropollutant removal.

Biological treatment methods, particularly those involving ND, are crucial in eliminating the amount of existing nitrogen and the majority of the micropollutants. Because ND processes utilize microbial activity to convert ammonia (NH□) into nitrogen gas (N□), simultaneously reducing nitrogen levels and transforming micropollutants. However, the efficiency of ND processes is influenced by various factors, including the types of pollutants, microbial community composition (the variety of microorganisms and their food [M\F]), and operational conditions such as DO concentration, pH, and hydraulic retention time (HRT), the retention time of the sludge (SRT), aeration time, temperature, salinity, the sludge characteristics, and reactor configuration (Smith, 1978; Wang et al., 2020). Because the sensitivity of the microorganisms increases exponentially with various sources of pollutants; hence, microbial sensors could be used to quantify nitrifiable compounds and detect the effects of nitrification inhibiting (Hammar, 2002). Reid (1907) explained that the efficiency of this system is indirectly related to the pore size of the used filter particles, so the finer the particles are the better the effluent will be to discharge. In addition, dissolved oxygen concentration (DO), the ratio of carbon to nitrogen (C:N), the variety of microorganisms and their food (M\F), the retention SRT, the retention time for the hydraulic (HRT), pH, aeration time, temperature, salinity, the sludge characteristics, and reactor configuration contribute in

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the efficiency determination (Smith, 1978; Wang et al., 2020). Each one of the mentioned factors has its contribution to the system efficiency, for example, existing DO is crucial in the ND process as it directly proportioned to removing efficiency of total nitrogen (91.17% total nitrogen removal at 1 mg/L DO concentration) (Huang et al., 2022).

NO_3^--N is not the only pollution that needs attention, micropollutants, such as pharmaceuticals, personal care products, industrial chemicals, and pesticides that are anthropogenic compounds (Luo et al., 2014) as well are vital in the cleaning process. The concentration of these micropollutants varies from a few ng/L to several µg/L (Wang and Wang, 2016). Even though these micropollutants exist in very low concentrations, they can be toxic, mutagenic, genotoxic, resistant to antibiotics, and disruptive to endocrine (Marti et al., 2014).

This review aims to synthesize current research on the effectiveness of ND in wastewater treatment, with a particular focus on the factors that enhance the process for both nitrogen and micropollutant removal. Therefore, by analyzing existing knowledge, the process's adaptability can be assessed in diverse treatment scenarios and explores the fate of micropollutants in these systems. The goal is to provide insights that will inform the development of more efficient and sustainable wastewater treatment strategies.

II. Mechanism and Pathway of Nd

According to Liu et al. (2010), depending on the existing microbial populations and the achieved redox conditions with the flocs' physical nature, the mechanisms in this process can be categorized into several pathways: direct conversion of ammonia into di-nitrogen gas, and autotrophic nitrification, heterotrophic denitrification, heterotrophic nitrification, and aerobic denitrification (Liu et al., 2010; James and Vijayanandan, 2023) (Fig. 1). Besides, the production of various microbial enzymes contributes to the biological degradation pathways. The floc size and density are essential contributors to the DO diffusion, Aeration rate and time, organic matter, and nitrogen concentration (He, Xue and Wang, 2009). The key factor in biological treatment is the microbial community, therefore enhancing the existence of the vital microorganisms based on the types of micropollutants through optimizing the environmental condition, such as carbon source, temperature, pH, aeration pattern, DO concentration, and free ammonia is crucial (Xiao and Tang, 2014). For example, autotrophic and heterotrophic bacteria grow in two different environments depending on the DO concentration, therefore, for these two different bacteria to coexist, enhancement should be the priority (Chang et al., 2019).

A. Conventional Autotrophic Nitrification and Heterotrophic Denitrification

All the sources of nitrogen (total nitrogen) when it reaches the sewer system immediately naturally undergo a series of transformations starting with the hydrolysis of organic nitrogen to ammonia (NH_3), then it automatically converts into ammonium (NH_4^+) depending on the pH of the water (American Water Works Association, 2013). The amount of NH_4 increases when the pH is low (acidic water) and vice versa (Bueno et al., 2018). The presence of novel bacteria in conventional nitrification is involved mostly in the establishment of the nitrification and denitrification of hydrolyzed sewage (Chai et al., 2019; Jia et al., 2020); thus, process will take place in mainly two stages; nitrification and then denitrification (Alzate Marin, Caravelli and Zaritzky, 2016).

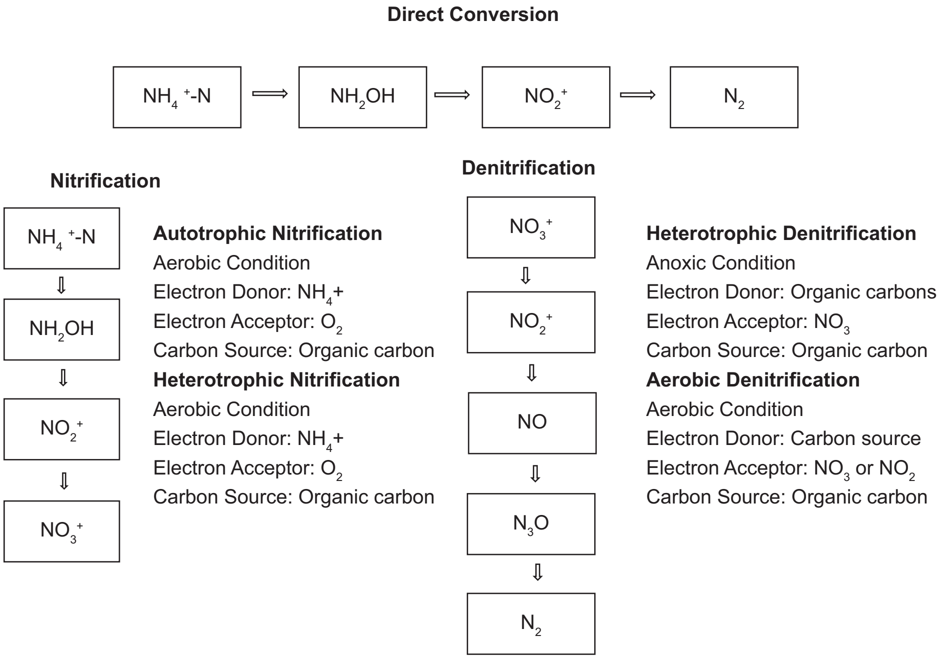

Fig. 1: Pathways of nitrogen transformation (James and Vijayanandan, 2023).

First: Autotrophic nitrification

The autotrophic bacteria (nitrifiers) using ammonia monooxygenase (AMO) and nitrite reductase enzymes convert the existing ammonium (NH_4^+-N) into nitrite (NO_2^--N) and then oxidize the latter into nitrate (NO_3^--N) in various biological processes using DO (Smith, 1978). In this stage, most total organic carbon is reduced compared to the anaerobic zone (50% of COD is removed) (Khin and Annachhatre, 2004; Alzate Marin et al., 2016). Furthermore, research indicates that nitrifying enzymes significantly contribute to the cometabolic biotransformation of organic micropollutants. This process involves the simultaneous oxidation of ammonia and the degradation of various pollutants, including pharmaceuticals, under nitrifying conditions (Kennes-Veiga, et al., 2022). In addition, micropollutant degradation can be enhanced by certain phosphorus-accumulating organisms (PAOs) during nitrification (Kolakovic et al., 2022).

The nitrification process is sensitive to environmental factors such as the depth of the wastewater, pH, temperature, and the presence of specific chemicals, for example, nitrification can be enhanced by adding CaO, which maintains a pH of 8–9, whereas inhibitory substances such as chlorine lime and aluminum sulfate can hinder the process (Smith, 1978; Thakur and Medhi, 2019). In addition, the stability of nitrification is often challenged by the accumulation of nitrite-oxidizing bacteria (NOB) in nitrite-rich conditions (Li et al., 2013), which can be mitigated by optimizing the growth environment for nitrifiers (Di Capua et al., 2022). However, this could be enhanced through optimum conditions provision for the microorganisms, which leads to a significant increase in the efficiency of the process hence overcoming the limitations (Abu Bakar et al., 2018; Ma et al., 2017) which also make the structure cost-effective (Yan et al., 2019; Yang and Yang, 2011).

Second: Heterotrophic denitrification

This is a key process in environmental engineering, that is performed by many different groups of microbes, such as *Bacillus cereus and Bacillus tequilensis* (Saïd et al., 2014). Following nitrification, when oxygen is depleted (under anoxic conditions), where heterotrophic bacteria use nitrate as an electron acceptor in the absence of oxygen for their respiration and the creation of nitrogen gas (N2) which bubbles out of the water (Zhang, Yang and Furukawa, 2010). This process not only reduces nitrogen levels but also decreases biochemical oxygen demand (BOD) by up to 80% as declared by Zhang, Yang and Furukawa, 2010. Interestingly, denitrification might occur even in well-oxygenated conditions within particulate matrices, where microcolonies of denitrifying bacteria metabolically shade each other (Smriga et al., 2021). Besides, Xu et al., (2015) explained that simultaneous nitrification and denitrification are more efficient and promising in removing nitrogen, chemical oxygen demand, sulfide, and micropollutants. Although stimulated nitrification–denitrification (ND) are cost-effective, consumes low energy, produces little sludge, and has a small footprint as elucidated by James and Vijayanandan in 2023, it cannot be applied to treat mainstream wastewater. During this process, nitrite and nitrate, nitrous oxide, and nitric oxide reductase are produced by denitrifiers to catalyze the reactions (Singh et al., 2022).

B. Heterotrophic Nitrification and Aerobic Denitrification

Denitrification can occur by different types of aerobic heterotrophic bacteria that produce N_2 gas using NO_3^--N as oxidizing agents; however, the vital enzyme that is essential in this process is periplasmic nitrate reductase, which is normally found in aerobic nitrifiers (Bucci et al., 2021; Ji et al., 2015; Qu et al., 2015); therefore, aerobic denitrifies (heterotrophic nitrification) utilize organic carbon to perform nitrification (Rout et al., 2017; Song et al., 2021). Removing nitrogen under saline conditions using isolated halophilic stains, and Halomonas campisalis ha3 was efficient (Guo et al., 2013). This process is particularly efficient in environments with low temperatures or high salinity, where traditional nitrification and denitrification processes might be less effective (Song et al., 2021).

C. Direct Conversion of Ammonia into Di-nitrogen Gas

In this process, some microorganisms, such as *Cupriavidus, and Thiosphaera pantotropha*, convert NH_4^+ to N_2 directly by first, producing hydroxylamine (NH_2OH) by AMO under aerobic conditions through hydroxylation of NH4 +-N, and next, oxidizing NH_2OH to NO_2 −-N by hydroxylamine oxidase, then the latter is directly transferred to N_2 (figure 1) (James and Vijayanandan, 2023). This pathway, while less common, highlights the diversity of microbial strategies available for nitrogen removal in wastewater treatment.

III. FACTORS AFFECTING ND

Physicochemical and operational parameters are the key factors that control the efficiency of this process; therefore, optimizing these factors helps in treating wastewater using the ND process. The essential factors that play a role in the procedure are as follows:

A. pH

In general, the performance of the ND system can be evaluated using pH as an indicator, because pH in the reactor controls the amount of the existing microorganisms and their types as well (Hayatsu, Katsuyama and Tago, 2021; Huang et al., 2023) 3.5 g alkalinity is produced due to the reduction of 1 g NO_3 –N in denitrification, whereas 7.14 g of alkalinity is consumed due to the oxidation of 1 g NH_4 +–N in nitrification; thus, pH can be maintained without any chemical additions (He, Xue and Wang, 2009). In addition, lead and copper are released from their bearing materials due to the reduction in pH and DO by nitrification (Zhang, Yang and Furukawa, 2010). Lead release increased from lead piping when pH was >7.5 (100 mg/L alkalinity as $CaCO_3$); however, soluble lead release increased 65 times more when pH was < 6.5 (American Water Works Association, 2013). Maintaining an optimal pH is critical for both processes, generally within the range of 6.5–7.5 for denitrification (Gan et al., 2019; Hayatsu, Katsuyama and Tago, 2021; Huang et al., 2023) and 8–8.4 for nitrification (He, Xue and Wang, 2009). For the highest specific rate of nitrate reduction, a pH of 10.5 may be required (Dhamole et al., 2008), whereas a range of 7–7.5 is optimal for overall ammonium and total nitrogen removal (Hossini et al., 2015). However, the acidophilic partial nitrification process recently has been developed for nitrification to occur effectively at a pH of lower than 6 even achieving stable nitrogen removal rates at 5.36 (Qian et al., 2019; F. Zhang et al., 2024). Therefore, the biotransformation of micropollutants' efficiency is determined significantly by the pH (Zhou et al., 2023)

B. Temperature

Temperature controls microbial growth, as it affects enzyme denaturation, metabolism rate, and the overall efficiency of the ND process (Zhang et al., 2009). This parameter is directly proportioned to the micropollutant biotransformation and ammonia oxidation rate; however, it is inversely proportional to DO concentration (Fernandez-Fontaina et al., 2012). Hence, inhabited denitrification occurs when the temperature gets lowered (around 15°C) (Kanda et al., 2016), while the removal efficiencies drop from 98.0% at 18°C to 78.1% at 13°C for nitrification (Zhang et al., 2019). The optimum temperature for nitrifiers is 22–27°C, whereas it is 20–40°C for denitrifiers (He, Xue and Wang, 2009). Nitrate nitrogen removal was nearly 99.26% at 40°C (Qu et al., 2022). The activity of certain microbial pathways increases at higher temperatures causing the N2O gas emission which leads to a potent greenhouse gas (Nair et al., 2021). Hence, enhancing the ND process requires maintaining a temperature range of 18–35°C (James and Vijayanandan, 2023).

C. Free Ammonia and Salinity

Free ammonia and salinity can significantly limit the existence of both the growth of ammonia-oxidizing bacteria (AOB) and NOB (Xiao and Tang, 2014; Zhu et al., 2015). The efficacy of AOB in degrading pharmaceutical compounds has been documented, as the broad substrate specificity of AOB allows them to metabolize a variety of micropollutants, thereby improving their removal from wastewater (Sharma et al., 2023).

It has been indicated that nitrification can be promoted when the concentration of free ammonia is nearly 10–15 mg/L, whereas *Nitrosomonas* which is essential for the effective nitrification processes becomes abundant the higher levels (Statiris et al., 2022; Sun et al., 2012).

Besides, salinity is inversely proportional to the ammonium oxidation rate; higher salinity levels decrease the ammonium oxidation rate, with a reduction by half observed when salinity increases from 2% to 1% (She et al., 2018). In addition, within high saline wastewater, halophilic or halotolerant species that are not that efficient at removing nitrogen will increase (Arumugham et al., 2024a; Zhou et al., 2023). At high salinity, ND can be enhanced through the NO_2 −-N pathway, because NOB are more sensitive to the salinity (Corsino et al., 2016).

D. DO Concentration

The existence of DO is a crucial factor that determines the type of bacteria that work on the nitrification (needs >2 mg/L) and denitrification (<0.2 mg/L) process (Pochana and Keller, 1999). High DO is necessary for the maximum removal of COD and $NH_4{}^+$N, as the availability of organic carbon is low in the flocs (James and Vijayanandan, 2023). However, nitrogen removal efficiency decreases when DO levels are higher than 3 mg/L, this also leads to increased nitrous oxide emissions (Li et al., 2020).

Sarioglu et al. (2009) manifest that around 1.8 mg O_2 per litter is sufficient to remove about 85–95% nitrogen for sustaining simultaneous ND in a membrane bioreactor. On the contrary, the persistence of certain micropollutants increases in the oxygen-activated sludge ND process (Levine, Meyer and Kish, 2006). Besides, the proliferation of heterotrophic bacteria is promoted due to the organic carbon utilization that leads to less organic carbon penetration into flocs (Liu et al., 2010), thus, with a high rate of DO, the electron acceptors shift from NO_3 −-N/NO_2 −-N to oxygen for denitrifies. Therefore, to improve the breakdown of micropollutants, recent advancements in wastewater treatments have focused on optimizing DO levels (Zhang et al., 2024).

E. Food/Microorganism (F/M)

The F/M ratio is essential for reducing competition between heterotrophic and autotrophic nitrifiers. Besides, the provision of a sufficient amount of carbon substrates for denitrification is important (James and Vijayanandan, 2023). A low C/N ratio typically enhances nitrification, whereas denitrification gets suppressed; thus, it is essential for micropollutants to be metabolized and transformed by microorganisms (Arumugham et al., 2024a). F/M can be increased due to the maintenance of a high concentration of mixed liquor volatile suspended solids in the membrane bioreactor, leading to an increase in NH4 +-N Removal (He, Xue and Wang, 2009).

F. HRT

The contact time between microorganisms and pollutants is important because the removal efficiency lowers once the contact time is insufficient (Wang et al., 2017a). HRT also impacts the diversity and richness of microbial communities, which are crucial for effective denitrification (Liu et al., 2010). Chang et al., (2019) explain that removing NH_4 +-N and total nitrogen (TN) decreases by 42.11%, and 49.5% when the retention time was lowered from 12 h to 4 h, respectively. Even with low HRT, the availability of carbon substrate can maintain high denitrification efficiency (Pous et al., 2017a; Wang et al., 2017a). Song et al., (2020) declared that, for maximizing nitrogen removal, it is necessary to have an optimal HRT of around 5–6 h, based on the influent nitrate concentration, whereas denitrification performance improves when HRT get increased, however, excessively long HRTs cause nitrite accumulation and decrease treatment efficiency (Wang et al., 2017b). A study highlighted that increasing HRT can improve denitrification performance by providing sufficient contact time between substrates and denitrifying bacteria. Optimized HRT ensures efficient hydraulic shear, which helps in forming denitrifying granular sludge. However, excessively long HRTs may lead to decreased treatment efficiency and nitrite accumulation, indicating a need for careful management of HRT to balance performance and efficiency (Pous et al., 2017b). In general, HRT through ND processes enhances the biotransformation of micropollutants (Ilies and Mavinic, 2001).

G. SRT

SRT is a critical loading parameter that influences the growth rate of microorganisms, nutrient transformations involved in ND, effluent concentrations, and treatment efficiency (Clara et al., 2005), especially in the secondary clarifier, where it can impact effluent quality (James et al., 2015). Longer retention times may provide microbes with more time to perform these processes, whereas shorter retention times could potentially limit their effectiveness; thus, optimizing SRT is essential in managing and enhancing the efficiency of ND systems. In general, SRT is longer than HRT to allow sufficient time for microbial reproduction (Clara et al., 2005). It has been indicated that for nitrification to be effective and efficient, 10–20 days is vital, whereas the optimal SRT is 10–30 days for efficient denitrification (Li and Wu, 2014).

H. Aeration Time

Ammonia-nitrogen oxidation is affected by the aeration rates and patterns, for instance, 99% nitrification efficiency was achieved with the aeration rates of 9 L-air/min (Mota et al., 2005). Besides, the removal and the composition of nitrifying bacterial communities greatly lie on lengths of aeration and non-aeration periods, for example, higher levels of certain AOB were achieved at short aeration times (e.g., 30 minutes), but for effective denitrification longer non-aeration periods (up to 4 h) was essential (Landi and Lu, 2022). Thus, oxidizing NH_4 +-N completely is based on the aeration time (Abbassi, et al., 2014); however, temperature plays an important role as well, which is inversely proportioned with aeration (Zhang et al., 2009). Over-aeration can lead to NO□□-N accumulation and the deterioration of nitrification efficiency (Peng et al., 2004). Recently, the importance of aeration has been interconnected with ND processes to achieve the most effective treatment of wastewater containing micropollutants (Ghasemi, Hasani Zonoozi and Hoseini Shamsabadi, 2024).

IV. Reactor Configuration

Two factors control the efficiency of the configuration: the gradient of DO concentration, and the creation of an anoxic microenvironment inside the flocs (Yan et al., 2019). Besides, the intermittent feeding and microbial community composition represent the reactor conditions that significantly influence the removal efficiency of micropollutants (Gonzalez-Gil, Carballa and Lema, 2017).

The reactor should be designed in a way that guarantees the coexistence of nitrifiers and denitrifiers at a gradient concentration of DO; furthermore, the formation of flocs that have optimum size and density is essential in the reactor (James and Vijayanandan, 2023). For instance, in the Closed Down-Flow Hanging Sponge Reactor, DO concentration should be 1.2 mg-O2/L to achieve significant nitrite production while maintaining high ammonium removal rates (Landi and Lu, 2022).

Zhang et al., in 2009, claimed that the thickness of biofilm in the attached growth system affects total nitrogen removal and organic carbon significantly. The thicker the biofilm is, first; the more diffusion of organic carbon occurs using less oxygen (Li and Irvin, 2007), second; a favorable anoxic environment denitrifying bacteria can be developed due to the penetration of oxygen (0.20–0.25 mm depth) into the thicker biofilm (Gieseke et al., 2002). Besides the thickness, aeration time is vital as well, for example, the penetration increases up to 1.5 mm when the time is increased up to 3hrs (James and Vijayanandan, 2023). To optimize and enhance, this process, prediction, and prevention of interferences of biotransforming micropollutants with a focus on the biodegradability of potential inhibitory compounds is essential (Pagga, Bachner and Strotmann, 2006). This can be simulated by a computer model (Sanz et al., 1996). For example, a model in continuous up-flow filters, which has been validated in a semi-scale filtration plant for nitrification was stimulated by Qi in 2009; while a kinetic model highlighted the role of carbon sources and the potential for nitrite accumulation in the denitrification process (Michioku et al., 2016).

V. Biotransformation

Biotransformation in the environment refers to how living organisms, particularly microorganisms, chemically modify or break down pollutants, toxins, or other organic compounds into less harmful or more easily degradable substances. This process plays a critical role in the natural detoxification of ecosystems and can involve various metabolic pathways, often leading to the complete mineralization of pollutants into basic inorganic compounds such as water, carbon dioxide, and minerals (Schwarzenbach, Gschwend and Imboden, 2017). Because all biological reactions are enzyme-catalyzed, biotransformation includes the vitro enzymatic reactions, metabolism of the compounds, and biosynthetic pathways in the plants (Doble, Kruthiventi and Gaikar, 2004). Recently, complete ammonia oxidizers (comammox), these bacteria are the complete ammonia oxidizers have been discovered that can oxidize ammonia to nitrate in a single step, hence enhancing micropollutant biotransformation (Han et al., 2019).

Biotransformation seems to be the key to developing eco-friendly methods, in which enzymes are mostly in control. They elucidate that there are six groups of enzymes: ligases catalyze, oxidoreductases catalyze oxidation-reduction, transferases mediate, hydrolases catalyze the hydrolysis, lyases catalyze, and isomerases (Radley et al., 2023). There are plenty of different micropollutants that have been treated using biotransformation, for instance, anti-cancer drugs (Gao et al., 2013). Every aspect of pesticide biotransformation in plants and microorganisms concludes that the persistent variation of pollutants in the process (Hall, Hoagland and Zablotowicz, 2000).

VI. Fate of Micropollutants

Micropollutants are emerging contaminants found in wastewater at low concentrations but with potentially harmful effects. Consuming water bodies that contain micropollutants is harmful to humans, therefore, removing them is vital (Phan et al., 2014). In general, the fate of micropollutants in WWTPs is governed by various processes, including biotransformation, photo-degradation, volatilization, and sorption which are commonly used in reducing micropollutants in treated effluent (Lakshminarasimman et al., 2018). However, the physicochemical properties of micropollutants and the treatment conditions determine the removal efficiency (Jonas et al., 2015).

Cometabolism is a primary degradable substrate used in this process, which produces biomass and acts as a source of electron donors (James and Vijayanandan, 2023). Besides the organic matter, micropollutants can act as an energy and carbon source for microbes; however, the ratio and concentration of both primary substrates and the micropollutants are essential (Dawas-Massalha et al., 2014; Tiwari et al., 2017).

In tandem with these, aeration, hydraulic, solid retention time, and redox condition are also critical operational parameters to determine the success of the process (Arumugham et al., 2024b). Nitrification helps the degradation of micropollutants through cometabolism (Dorival-García et al., 2013), for instance, ethinylestradiol, naproxen, and roxithromycin were transformed in a nitrification process (Suarez, Lema and Omil, 2010). This transformation is done through the production of the AMO enzymes by AOB (Dorival-García et al., 2013; Alvarino et al., 2018). That enzyme contributes to the degradation depending on the micropollutant's diffusion across the cell membrane, and their structure as well (Fernandez-Fontaina et al., 2012). Although not all micropollutants are degradable in nitrifying conditions, redox is the best condition for this purpose, due to mono- and di-oxygenase enzymes that are produced by both nitrifying and denitrifying bacteria (Dawas-Massalha et al., 2014; Hammer and Palmowski, 2021).

The redox conditions are vital in secreting various enzymes by microbial communities and the structure of the micropollutants is of great importance when it comes to biotransformation (Tiwari et al., 2017), for example, sulfamethoxazole, trimethoprim, and atenolol degrade perfectly in any condition (anaerobic, anoxic, and aerobic conditions); atenolol and trimethoprim were removed efficiently at anaerobic reactor (Alvarino et al., 2018; Lakshminarasimman et al., 2018); however, some others such as carbamazepine, diazepam, and diclofenac were not undergoing any biotransformation at all (Sipma et al., 2010). In general, biodegradation makes simpler, less toxic, or completely mineralized into CO_2 products (Tiwari et al., 2017). It is worth mentioning that during nitrification, microplastics affect negatively on ammonia oxidation rate, but positively on denitrification (Li et al., 2020).

VII. Conclusions

Nitrification and denitrification processes can help in biotransforming micropollutants and removing total nitrogen by harnessing the inherent capabilities of microorganisms to safeguard water quality. Recent research has highlighted the critical role of nitrifying enzymes in the cometabolic biotransformation of organic micropollutants. Besides, the discovery of comammox that are capable of oxidizing ammonia to nitrate in a single step, presents new opportunities for improving the efficiency of micropollutant biotransformation in wastewater treatment systems. Furthermore, enhanced biological phosphorus removal systems show a great contribution to micropollutant degradation by certain PAOs.

This process can be enhanced to make the process more efficient by controlling the gradient of DO in the same reactor within the flocs to co-exist with auto and heterotroph bacteria. Shifting from one mechanism to another depends on the microbial community, which can be influenced by operational parameters (e.g., DO, SRT, and HRT).

The efficiency of this system depends strongly on microbial diversity, environmental conditions (For example, the concentration of DO, the C:N ratio, microorganisms' food, the retention time for the hydraulic, pH, aeration time, temperature, salinity, the sludge retention ratio and sludge characteristics, and reactor configuration), and the specific nature of micropollutants. However, among the environmental factors, optimizing DO, and pH are the most critical parameters to the success of the process of micropollutant biotransformation. Besides, controlling sludge production caused by freeing N_2 into the atmosphere is challenging as well, thus innovating and adjusting a proper system is vital. ND process for micropollutant biotransformation was reviewed as a potential biological treatment process in removing carbon, nitrogen, and micropollutants from wastewater, which holds immense promise for sustainable and environmentally friendly solutions.

References

Abbassi, R., Kumar Yadav, A., Huang, S., and Jaffé, P.R., 2014. Laboratory study of nitrification, denitrification and anammox processes in membrane bioreactors considering periodic aeration. *Journal of Environmental Management*, 142, pp.53-59.

Abu Bakar, S.N.H., Abu Hasan, H., Mohammad, A.W., Sheikh Abdullah, S.R., Haan, T.Y., Ngteni, R., and Yusof, K.M.M., 2018. A review of moving-bed biofilm reactor technology for palm oil mill effluent treatment. *Journal of Cleaner Production*, 171, pp.1532-1545.

Alvarino, T., Suarez, S., Lema, J., and Omil, F., 2018. Understanding the sorption and biotransformation of organic micropollutants in innovative biological wastewater treatment technologies. *Science of the Total Environment*, 615, pp.297-306.

Alzate Marin, J.C., Caravelli, A.H., and Zaritzky, N.E., 2016. Nitrification and aerobic denitrification in anoxic-aerobic sequencing batch reactor. *Bioresource Technology*, 200, pp.380-387.

American Water Works Association, 2013. *Nitrification Prevention and Control in Drinking Water*, 2nd ed., AWWA Manual. American Water Works Association, Denver, CO.

Arumugham, T., Khudzari, J., Abdullah, N., Yuzir, A., Iwamoto, K., and Homma, K., 2024a. Research trends and future directions on nitrification and denitrification processes in biological nitrogen removal. *Journal of Environmental Chemical Engineering*, 12, p.111897.

Arumugham, T., Khudzari, J., Abdullah, N., Yuzir, A., Iwamoto, K., and Homma, K., 2024b. Research trends and future directions on nitrification and denitrification processes in biological nitrogen removal. *Journal of Environmental Chemical Engineering*, 12, p.111897.

Bucci, P., Coppotelli, B., Morelli, I., Zaritzky, N., and Caravelli, A., 2021. Heterotrophic nitrification-aerobic denitrification performance in a granular sequencing batch reactor supported by next generation sequencing. *International Biodeterioration and Biodegradation Society*, 160, p.105210.

Bueno, R.F., Piveli, R.P., Campos, F., and Sobrinho, P.A., 2018. Simultaneous nitrification and denitrification in the activated sludge systems of continuous flow. *Environmental Technology*, 39, pp.2641-2652.

Chai, H., Xiang, Y., Chen, R., Shao, Z., Gu, L., Li, L., and He, Q., 2019. Enhanced simultaneous nitrification and denitrification in treating low carbon-to-nitrogen ratio wastewater: Treatment performance and nitrogen removal pathway. *Bioresource Technology*, 280, pp.51-58.

Chang, M., Wang, Y., Pan, Y., Zhang, K., Lyu, L., Wang, M., and Zhu, T., 2019. Nitrogen removal from wastewater via simultaneous nitrification and denitrification using a biological folded non-aerated filter. *Bioresource Technology*, 289, p.121696.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., and Kroiss, H., 2005. The solids retention time-a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, 39, pp.97-106.

Corsino, S.F., Capodici, M., Morici, C., Torregrossa, M., and Viviani, G., 2016. Simultaneous nitritation-denitritation for the treatment of high-strength nitrogen in hypersaline wastewater by aerobic granular sludge. *Water Research*, 88, pp.329-336.

Dawas-Massalha, A., Gur-Reznik, S., Lerman, S., Sabbah, I., and Dosoretz, C.G., 2014. Co-metabolic oxidation of pharmaceutical compounds by a nitrifying bacterial enrichment. *Bioresource Technology*, 167, pp.336-342.

Di Capua, F., Iannacone, F., Sabba, F., and Esposito, G., 2022. Simultaneous nitrification-denitrification in biofilm systems for wastewater treatment: Key factors, potential routes, and engineered applications Author links open overlay panel. *Bioresource Technology*, 361, p.127702.

Doble, M., Kruthiventi, A.K., and Gaikar, V.G., 2004. *Biotransformations and Bioprocesses, Biotechnology and Bioprocessing Series.* Marcel Dekker, New York.

Dorival-García, N., Zafra-Gómez, A., Navalón, A., González-López, J., Hontoria, E., and Vílchez, J.L., 2013. Removal and degradation characteristics of quinolone antibiotics in laboratory-scale activated sludge reactors under aerobic, nitrifying and anoxic conditions. *The Journal of Environmental Management*, 120, pp.75-83.

Fernandez-Fontaina, E, Omil, F., Lema, J.M., and Carballa, M., 2012. Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. *Water Research*, 46, pp.5434-5444.

Fernandez-Fontaina, E., Omil, F., Lema, J.M., and Carballa, M., 2012. Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. *Water Research*, 46, pp.5434-5444.

Gao, F., Zhang, J.M., Wang, Z.G., Peng, W., Hu, H.L., and Fu, C.M., 2013. Biotransformation, a promising technology for anti-cancer drug development. *Asian Pacific Journal of Cancer Prevention*, 14, pp.5599-5608.

Ghasemi, M., Hasani Zonoozi, M., and Hoseini Shamsabadi, M.J., 2024. Simultaneous nitrification and denitrification pattern in aerated moving-bed sequencing batch reactor: Choosing appropriate SRT for different COD/N ratios. *Water Practice and Technology*, 19, pp.1920-1935.

Gieseke, A., Arnz, P., Amann, R., and Schramm, A., 2002. Simultaneous P and N removal in a sequencing batch biofilm reactor: Insights from reactor- and microscale investigations. *Water Research*, 36, pp.501-509.

Gonzalez-Gil, L., Carballa, M., and Lema, J.M., 2017. Cometabolic enzymatic transformation of organic micropollutants under methanogenic conditions. *Environmental Science and Technology*, 51, pp.2963-2971.

Guo, Y., Zhou, X., Li, Y., Li, K., Wang, C., Liu, J., Yan, D., Liu, Y., Yang, D., and Xing, J., 2013. Heterotrophic nitrification and aerobic denitrification by a novel *Halomonas campisalis*. *Biotechnology Letters*, 35, pp.2045-2049.

Hall, J.C., Hoagland, R.E., and Zablotowicz, R.M., 2000. *Pesticide Biotransformation in Plants and Microorganisms Similarities and Divergences*. American Chemical Society, UAS.

Hammar, F., 2002. History of modern genetics in Germany, In: Dutta, N.N., Hammar, F., Haralampidis, K., Karanth, N.G., König, A., Krishna, S.H., Kunze, G., Nagy, E., Orlich, B., Osbourn, A.E., Raghavarao, K.S.M.S., Riedel, K., Sahoo, G.C., Schomäcker, R., Srinivas, N.D., and Trojanowska, M. (Eds.), *History and Trends in Bioprocessing and Biotransformation, Advances in Biochemical Engineering/Biotechnology*. Springer, Berlin, Heidelberg, pp.1-29.

Hammer, L., and Palmowski, L., 2021. Fate of selected organic micropollutants during anaerobic sludge digestion. *Water Environment Research*, 93, pp.1910-1924.

Han, P., Yu, Y., Zhou, L., Tian, Z., Li, Z., Hou, L., Liu, M., Wu, Q., Wagner, M., and Men, Y., 2019. Specific micropollutant biotransformation pattern by the comammox bacterium *Nitrospira inopinata*. *Environmental Science and Technology*, 53, pp.8695-8705.

Hayatsu, M., Katsuyama, C., and Tago, K., 2021. Overview of recent researches on nitrifying microorganisms in soil. *Soil Science and Plant Nutrition*, 67, pp.619-632.

He, S., Xue, G., and Wang, B., 2009. Factors affecting simultaneous nitrification and de-nitrification (SND) and its kinetics model in membrane bioreactor. *Journal of Hazardous Materials*, 168, pp.704-710.

Huang, R., Meng, T., Liu, G., Gao, S., and Tian, J., 2022. Simultaneous nitrification and denitrification in membrane bioreactor: Effect of dissolved oxygen. *Journal of Environmental Management*, 323, p.116183.

Huang, S., Fu, Y., Zhang, H., Wang, C., Zou, C., and Lu, X., 2023. Research progress of novel bio-denitrification technology in deep wastewater treatment. *Frontiers in Microbiology*, 14, p.1284369.

Ilies, P., and Mavinic, D.S., 2001. Biological nitrification and denitrification of a simulated high ammonia landfill leachate using 4-stage Bardenpho systems: System startup and acclimation. *Canadian Journal of Civil Engineering*, 28, pp.85-97.

James, O.O., Cao, J.S., Kabo-Bah, A.T., and Wang, G., 2015. Assessing the impact of solids retention time (SRT) on the secondary clarifier capacity using the State Point Analysis. *KSCE Journal of Civil Engineering*. 19, pp.1265-1270.

James, S.N., and Vijayanandan, A., 2023. Recent advances in simultaneous nitrification and denitrification for nitrogen and micropollutant removal: A review. *Biodegradation* 34, pp.103-123.

Ji, B., Yang, K., Zhu, L., Jiang, Y., Wang, H., Zhou, J., and Zhang, H., 2015. Aerobic denitrification: A review of important advances of the last 30 years. *Biotechnology and Bioprocess Engineering*, 20, pp.643-651.

Jia, Y., Zhou, M., Chen, Y., Hu, Y., and Luo, J., 2020. Insight into short-cut of simultaneous nitrification and denitrification process in moving bed biofilm reactor: Effects of carbon to nitrogen ratio. *Chemical Engineering Journal*, 400, p.125905.

Jonas, M., Luca, R., Barry, D.A., and Christof, H., 2015. *A Review of the Fate of Micropollutants in Wastewater Treatment Plants. Wiley Period. Inc CORE Metadata Citation and Similar Papers at. Coreacuk Provided Infoscience École Polytechnique. Fédérale Lausanne*. Wiley, United States.

Kanda, R., Kishimoto, N., Hinobayashi, J., and Hashimoto, T., 2016. Effects of recirculation rate of nitrified liquor and temperature on biological nitrification-denitrification process using a trickling filter. *Water and Environment Journal*, 30, pp.190-196.

Kennes-Veiga, D.M., González-Gil, L., Carballa, M., and Lema, J.M., 2022. Enzymatic cometabolic biotransformation of organic micropollutants in wastewater treatment plants: A review. *Bioresource Technology*, 344, p.126291.

Khin, T., and Annachhatre, A.P., 2004. Novel microbial nitrogen removal processes. *Biotechnology Advances*, 22, pp.519-532.

Kolakovic, S., Salgado, R., Freitas, E.B., Bronze, M.R., Sekulic, M.T., Carvalho, G., Reis, M.A.M., and Oehmen, A., 2022. Diclofenac biotransformation in the enhanced biological phosphorus removal process. *Science of the Total Environment*, 806, p.151232.

Lakshminarasimman, N., Quiñones, O., Vanderford, B.J., Campo-Moreno, P., Dickenson, E.V., and McAvoy, D.C., 2018. Biotransformation and sorption of trace organic compounds in biological nutrient removal treatment systems. *Science of the Total Environment*, 640-641, pp.62-72.

Landi, A.I., and Lu, J., 2022. Effects of aeration rates and patterns on shortcut nitrification and denitrification Ali. *Journal of Environmental Protection*, 13, pp.640-656.

Levine, A.D., Meyer, M.T., and Kish, G., 2006. Evaluation of the persistence of micropollutants through pure□oxygen activated sludge nitrification and denitrification. *Water Environment Research*, 78, pp.2276-2285.

Li, A.J., Li, X.Y., Quan, X.C., and Yang, Z.F., 2013. Aerobic sludge granulation for partial nitrification of ammonia-rich inorganic wastewater. *Environmental Engineering and Management Journal*, 12, pp.1375-1380.

Li, B., and Irvin, S., 2007. The comparison of alkalinity and ORP as indicators for nitrification and denitrification in a sequencing batch reactor (SBR). *Biochemical Engineering Journal*, 34, pp.248-255.

Li, B., and Wu, G., 2014. Effects of sludge retention times on nutrient removal and nitrous oxide emission in biological nutrient removal processes. *International Journal of Environmental Research and Public Health*, 11, pp.3553-3569.

Li, L., Song, K., Yeerken, S., Geng, S., Liu, D., Dai, Z., Xie, F., Zhou, X., and Wang, Q., 2020. Effect evaluation of microplastics on activated sludge nitrification and denitrification. *Science of the Total Environment*, 707, p.135953.

Li, Y., Guo, J., Li, H., Song, Y., Chen, Z., Lu, C., Han, Y., and Hou, Y., 2020. Effect of dissolved oxygen on simultaneous removal of ammonia, nitrate and phosphorus via biological aerated filter with sulfur and pyrite as composite fillers. *Bioresource Technology*, 296, p.122340.

Liu, Y., Shi, H., Xia, L., Shi, H., Shen, T., Wang, Z., Wang, G., and Wang, Y., 2010. Study of operational conditions of simultaneous nitrification and denitrification in a Carrousel oxidation ditch for domestic wastewater treatment. *Bioresource Technology*, 101, pp.901-906.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., and Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, 473-474, pp.619-641.

Ma, W., Han, Y., Ma, W., Han, H., Zhu, H., Xu, C., Li, K., and Wang, D., 2017. Enhanced nitrogen removal from coal gasification wastewater by simultaneous nitrification and denitrification (SND) in an oxygen-limited aeration sequencing batch biofilm reactor. *Bioresource Technology*, 244, pp.84-91.

Marti, E., Huerta, B., Rodríguez-Mozaz, S., Barceló, D., Jofre, J., and Balcázar, J.L., 2014. Characterization of ciprofloxacin-resistant isolates from a wastewater treatment plant and its receiving river. *Water Research*, 61, pp.67-76.

Miao, L., Yang, G., Tao, T., and Peng, Y., 2019. Recent advances in nitrogen removal from landfill leachate using biological treatments - a review. *Journal of Environmental Management*, 235, pp.178-185.

Michioku, K., Taniura, H., and Inoue, K., 2016. Optimization of Nitrification/Denitrification Process in Landfill Leachate Treatment. In: *International Symposium on Ecohydraulics*. 11th ed Engineers Australia, Barton ACT, Melbourne, pp.342-349.

Mota, C., Head, M., Ridenoure, J., Cheng, J., and De Los Reyes, F., 2005. Effects of aeration cycles on nitrifying bacterial populations and nitrogen removal in intermittently aerated reactors. *Applied and Environmental Microbiology*, 71, pp.8565-8572.

Nair, D., Abalos, D., Philippot, L., Bru, D., Mateo-Marín, N., and Petersen, S.O., 2021. Soil and temperature effects on nitrification and denitrification modified N2O mitigation by 3,4-dimethylpyrazole phosphate. *Soil Biology and Biochemistry*, 157, p.108224.

Pagga, U., Bachner, J., and Strotmann, U., 2006. Inhibition of nitrification in laboratory tests and model wastewater treatment plants. *Chemosphere*, 65, pp.1-8.

Peng, Y.Z., Chen, Y., Peng, C.Y., Liu, M., Wang, S.Y., Song, X.Q., and Cui, Y.W., 2004. Nitrite accumulation by aeration controlled in sequencing batch reactors treating domestic wastewater. *Water Science and Technology*. 50, 35-43.

Phan, H.V., Hai, F.I., Kang, J., Dam, H.K., Zhang, R., Price, W.E., Broeckmann, A., and Nghiem, L.D., 2014. Simultaneous nitrification/denitrification and trace organic contaminant (TrOC) removal by an anoxic-aerobic membrane bioreactor (MBR). *Bioresource Technology*, 165, pp.96-104.

Pochana, K., and Keller, J., 1999. Study of factors affecting simultaneous nitrification and denitrification (SND). *Water Science Technologies*, 39, pp.61-68.

Pous, N., Puig, S., Balaguer, M.D., and Colprim, J., 2017a. Effect of hydraulic retention time and substrate availability in denitrifying bioelectrochemical systems. *Environmental Science: Water Research and Technology*, 3, pp.922-229.

Pous, N., Puig, S., Balaguer, M.D., and Colprim, J., 2017b. Effect of hydraulic retention time and substrate availability in denitrifying bioelectrochemical systems. *Environmental Science: Water Research and Technology*, 3, pp.922-229.

Qian, W., Ma, B., Li, X., Zhang, Q., and Peng, Y., 2019. Long-term effect of pH on denitrification: High pH benefits achieving partial-denitrification. *Bioresource Technology*, 278, pp.444-449.

Qu, D., Wang, C., Wang, Y., Zhou, R., and Ren, H., 2015. Heterotrophic nitrification and aerobic denitrification by a novel groundwater origin cold-adapted bacterium at low temperatures. *RSC Advances*, 5, pp.5149-5157.

Qu, W., Suo, L., Liu, R., Liu, M., Zhao, Y., Xia, L., Fan, Y., Zhang, Q., and Gao, Z., 2022. Influence of temperature on denitrification and microbial community structure and diversity: A laboratory study on nitrate removal from groundwater. *Water*, 14, p.436.

Radley, E., Davidson, J., Foster, J., Obexer, R., Bell, E.L., and Green, A.P., 2023. Engineering enzymes for environmental sustainability. *Angewandte Chemie International Edition*, 62, p.e202309305.

Reid, G., 1907. Nitrification of sewage. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 79, pp.58-74.

Rout, P.R., Bhunia, P., and Dash, R.R., 2017. Simultaneous removal of nitrogen and phosphorous from domestic wastewater using *Bacillus cereus* GS-5 strain exhibiting heterotrophic nitrification, aerobic denitrification and denitrifying phosphorous removal. *Bioresource Technology*, 244, pp.484-495.

Saïd, M., Ezzahra, A.F., Jamal, A., Mohammed, R., and Omar, A., 2014. Heterotrophic denitrification by Gram-positive bacteria: *Bacillus cereus* and *Bacillus tequilensis* 4. *International Journal of Scientific and Research Publications*, 4.

Sanz, J.P., Freund, M., and Hother, S., 1996. Nitrification and denitrification in continuous upflow filters - process modelling and optimization. *Water Science and Technology*, 34, pp.441-448.

Sarioglu, M., Insel, G., Artan, N., and Orhon, D., 2009. Model evaluation of simultaneous nitrification and denitrification in a membrane bioreactor operated without an anoxic reactor. *Journal of Membrane Science*, 337, pp.17-27.

Schwarzenbach, R.P., Gschwend, P.M., and Imboden, D.M., 2017. *Environmental Organic Chemistry*., 3rd ed. Wiley, Hoboken, NJ.

Sharma, P., Kanta Pandey, K., Lepcha, A., Sharma, S., Maurya, N., Kumar Sharma, S., Pradhan, R., and Kumar, R., 2023. Elucidating the potential of nitrifying bacteria in mitigating nitrogen pollution and its industrial application. *Microsphere*, 2, pp.246-259.

She, Z., Wu, L., Wang, Q., Gao, M., Jin, C., Zhao, Y., Zhao, L., and Guo, L., 2018. Salinity effect on simultaneous nitrification and denitrification, microbial characteristics in a hybrid sequencing batch biofilm reactor. *Bioprocess and Biosystems Engineering*, 41, pp.65-75.

Singh, V., Ormeci, B., Mishra, S., and Hussain, A., 2022. Simultaneous partial Nitrification, ANAMMOX and denitrification (SNAD) - A review of critical operating parameters and reactor configurations. *Chemical Engineering Journal*, 433, p.133677.

Sipma, J., Osuna, B., Collado, N., Monclús, H., Ferrero, G., Comas, J., and Rodriguez-Roda, I., 2010. Comparison of removal of pharmaceuticals in MBR and activated sludge systems. *Desalination*, 250, pp.653-659.

Smith, A.G., 1978. *Nitrification-Denitrification of Wastewater using a Single-Sludge System, Research Report - Research Program for the Abatement of Municipal Pollution "Project no. 71-1-20." Environment Canada : Obtained from Training and Technology Transfer Division (Water), Environmental Protection Service, Fisheries and Environment* Canada, Ottawa.

Smriga, S., Ciccarese, D., and Babbin, A.R., 2021. Denitrifying bacteria respond to and shape microscale gradients within particulate matrices. *Communications Biology*, 4, p.570.

Song, T., Zhang, X., Li, J., Wu, X., Feng, H., and Dong, W., 2021. A review of research progress of heterotrophic nitrification and aerobic denitrification microorganisms (HNADMs). *Science of the Total Environment*, 801, p.149319.

Song, X., Yang, X., Hallerman, E., Jiang, Y., and Huang, Z., 2020. Effects of hydraulic retention time and influent nitrate-N concentration on nitrogen removal and the microbial community of an aerobic denitrification reactor treating recirculating marine aquaculture system effluent. *Water*, 12, p.650.

Statiris, E., Dimopoulos, T., Petalas, N., Noutsopoulos, C., Mamais, D., and Mamais, S., 2022. Investigating the long and short-term effect of free ammonia and free nitrous acid levels on nitritation biomass of a sequencing batch reactor treating thermally pre-treated sludge reject water. *Bioresource Technology*, 362, p.127760.

Suarez, S., Lema, J.M., and Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. *Water Research*, 44, pp.3214-3224.

Sun, H., Jiang, T., Zhang, F., Zhang, P., Zhang, H., Yang, H., Lu, J., Ge, S., Ma, B., Ding, J., and Zhang, W., 2012. Understanding the effect of free ammonia on microbial nitrification mechanisms in suspended activated sludge bioreactors. *Environmental Research*, 200, 111737.

Suneethi, S., Keerthiga, G., Soundhar, R., Kanmani, M., Boobalan, T., Krithika, D., and Philip, L., 2015. Qualitative evaluation of small scale municipal Wastewater Treatment Plants (WWTPs) in South India. *Water Practice and Technology*, 10, pp.711-719.

Thakur, I.S., and Medhi, K., 2019. Nitrification and denitrification processes for mitigation of nitrous oxide from waste water treatment plants for biovalorization: Challenges and opportunities. *Bioresource Technology*, 282, pp.502-513.

Tiwari, B., Sellamuthu, B., Ouarda, Y., Drogui, P., Tyagi, R.D., and Buelna, G., 2017. Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. *Bioresource Technology*, 224, pp.1-12.

Wang, J., and Wang, S., 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: A review. *Journal of Environmental Management*, 182, pp.620-640.

Wang, J., Rong, H., Cao, Y., and Zhang, C., 2020. Factors affecting simultaneous nitrification and denitrification (SND) in a moving bed sequencing batch reactor (MBSBR) system as revealed by microbial community structures. *Bioprocess and Biosystems Engineering*, 43, pp.1833-1846.

Wang, Z., Fei, X., He, S., Huang, J., and Zhou, W., 2017a. Effects of hydraulic retention time and ratio on thiosulfate-driven autotrophic denitrification for nitrate removal from micro-polluted surface water. *Environmental Technology*, 38, pp.2835-2843.

Wang, Z., Fei, X., He, S., Huang, J., and Zhou, W., 2017b. Effects of hydraulic retention time and ratio on thiosulfate-driven autotrophic denitrification for nitrate removal from micro-polluted surface water. *Environmental Technology*, 38, pp.2835-2843.

Xiao, J., and Tang, J.H., 2014. Nitrogen removal with nitrification and denitrification via nitrite. *Advanced Materials Research*, 908, pp.175-178.

Xu, G., Feng, C., Fang, F., Chen, S., Xu, Y., and Wang, X., 2015. The heterotrophic-combined-with-autotrophic denitrification process: performance and interaction mechanisms. *Water Science and Technology*, 71, pp.1212-1218.

Yan, L., Liu, S., Liu, Q., Zhang, M., Liu, Y., Wen, Y., Chen, Z., Zhang, Y., and Yang, Q., 2019. Improved performance of simultaneous nitrification and denitrification via nitrite in an oxygen-limited SBR by alternating the DO. *Bioresource Technology*, 275, pp.153-162.

Yang, S., and Yang, F., 2011. Nitrogen removal via short-cut simultaneous nitrification and denitrification in an intermittently aerated moving bed membrane bioreactor. *Journal of Hazardous Materials*, 195, pp.318-323.

Zhang, F., Du, Z., Wang, J., Du, Y., and Peng, Y., 2024. Acidophilic partial nitrification (pH<6) facilitates ultra-efficient short-flow nitrogen transformation: Experimental validation and genomic insights. *Water Research*, 260, 121921.

Zhang, L., Wei, C., Zhang, K., Zhang, C., Fang, Q., and Li, S., 2009. Effects of temperature on simultaneous nitrification and denitrification via nitrite in a sequencing batch biofilm reactor. *Bioprocess and Biosystems Engineering*, 32, pp.175-182.

Zhang, L., Yang, J., and Furukawa, K., 2010. Stable and high-rate nitrogen removal from reject water by partial nitrification and subsequent anammox. *Journal of Bioscience and Bioengineering*, 110, pp.441-448.

Zhang, Q., Chen, X., Luo, W., Wu, H., Liu, X., Chen, W., Tang, J., and Zhang, L., 2019. Effects of Temperature on the characteristics of nitrogen removal and microbial community in post solid-phase denitrification biofilter process. *International Journal of Environmental Research and Public Health*, 16, p.4466.

Zhang, X.Y., Zeng, Y.W., Tao, R.D., Zhang, M., Zheng, M.M., Qu, M.J., and Mei, Y.J., 2024. Analysis of the microbial diveristy and the mechanism of simultaneous nitrification and denitrification in high nitrogen environments. *International Journal of Environmental Science and Technology*, 21, pp.1-14.

Zhou, Y., Zhu, Y., Zhu, J., Li, C., and Chen, G., 2023. A comprehensive review on wastewater nitrogen removal and its recovery processes. *International Journal of Environmental Research and Public Health*, 20, p.3429.

Zhu, S.M., Deng, Y.L., Ruan, Y.J., Guo, X.S., Shi, M.M., and Shen, J.Z., 2015. Biological denitrification using poly(butylene succinate) as carbon source and biofilm carrier for recirculating aquaculture system effluent treatment. *Bioresource Technology*, 192, pp.603-610.