

Review

# The Effect of Influent Characteristics and Operational Conditions over the Performance and Microbial Community Structure of Partial Nitrification Reactors

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**Abstract:** Nitrogen is a main contaminant of wastewater worldwide. Novel processes for nitrogen removal have been developed over the last several decades. One of these is the partial nitrification process. This process includes the oxidation of ammonium to nitrite without the generation of nitrate. The partial nitrification process has several advantages over traditional nitrification-denitrification processes for nitrogen removal from wastewaters. In addition, partial nitrification is required for anammox elimination of nitrogen from wastewater. Partial nitrification is affected by operational conditions and substances present in the influent, such as quinolone antibiotics. In this review, the impact that several operational conditions, such as temperature, pH, dissolved oxygen concentration, hydraulic retention time and solids retention time, have over the partial nitrification process is covered. The effect of quinolone antibiotics and other emerging contaminants are discussed. Finally, future perspectives for the partial nitrification process are commented upon.

**Keywords:** partial nitrification; operational conditions; antibiotic; wastewater

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## 1. Introduction

One of the most important environmental problems today is the discharge of wastewater from urban and industrial sources, since human activities have greatly accelerated and extended the natural cycles of nitrogen in the soil, water and atmosphere. In this way, different technologies have been used for the removal of different environmental pollutants, such as nitrogen, organic matter and phosphorus. In wastewater treatment plants, nitrogen is often removed by conventional biological processes, such as nitrification and heterotrophic denitrification. In particular, the conventional processes are not suitable for the treatment of effluents, such as a dewatering concentrate stream, due to their toxic effects to those microorganisms able to degrade the nitrogen. For this reason, alternative systems have been developed for the removal of this nutrient, such as partial nitrification/anaerobic ammonium oxidation (anammox) technology. Thanks to these novel technologies, high ammonium-low carbon effluents, such as reject water from anaerobic digesters, can be treated within the sludge pipeline, therefore avoiding its treatment within the activated sludge processes.

Antimicrobial agents are among the most commonly used pharmaceuticals. However, although antibiotics are of great importance for human health, they can also be a great problem for the environment. During wastewater treatment, antibiotics are drastically removed from the water stream, but their fate is associated with sewage sludge. In this sense, the biological processes of nitrification have been described as particularly sensitive to toxic substances, such as pesticides and antibiotics.

The partial nitrification process is a technique in which nitrification is achieved with nitrite as the intermediate under stable processing conditions, where only 50% of the ammonium in the influent is converted into nitrite. This system and its combination with the anammox process have led to the development of a new technology of great interest in the treatment of effluent with high nitrogen content.

The results obtained in partial nitrification bioreactors show that modifications to the operating conditions can dramatically affect the functioning of the partial nitrification technology. Specifically, a significant factor that produces changes in the microbial communities is hydraulic retention time (HRT) [1]. Thus, changes in the operating conditions, such as HRT, may produce changes in cellular physiology and the community level. Consequently, the performance of the biotransformation of ammonium into nitrite in a partial nitrification bioreactor can be directly affected by the HRT, which can be crucial for the optimization of nutrient removal rates and the implementation of control strategies.

Antibiotics, such as fluoroquinolones, have been reported [2,3] as able to impact the performance of the partial nitrification process, suggesting that there is a negative correlation between antibiotic concentration in the influent and performance of the partial nitrification process. Furthermore, the microbial community inside the bioreactors suffers a period of adaptation or a deep change with a significant reduction of the ammonium-oxidizing bacteria in response to the antibiotic concentration in the influent [3]. Additionally, the presence of antibiotics in the bioreactor increases the number of antibiotic-resistant bacteria. In this way, it could be suggested that the presence of selected antibiotics, such as quinolones, can seriously affect partial nitrification systems and select microorganisms with antibiotic resistance [3].

The objective of our article is to evaluate the influence of different environmental parameters, such as HRT, and the concentration of fluoroquinolone antibiotics on the performance of partial nitrification

systems. We also review the effects of these parameters on the microbial communities growing in bioreactors using molecular biology techniques.

## 2. Partial Nitrification Process: An Overview

Contamination caused by wastewater discharge has increased during the last decades, due to the increasing global population [1]. Nitrogen, one of the main contaminants of wastewater, has been identified to cause oxygen depletion and eutrophication in aquatic environments [4]. Wastewater engineering has traditionally removed nitrogen from wastewater through complete nitrification-denitrification processing to meet the water quality criteria for treated wastewater [1]. More restrictive standards for nitrogen in wastewater effluent are being imposed by new regulations, such as the European Union (EU) Water Frame Directive 91/271/EEC (Table 1). To achieve these standards in a cost-effective way, autotrophic nitrogen removal technologies, such as partial nitrification/anaerobic ammonium oxidation (anammox), DEMON (deammonification), OLAND (oxygen limited autotrophic nitrification/denitrification), and CANON (completely autotrophic nitrogen-removal over nitrate) [5,6], have been developed. Autotrophic nitrogen removal technologies are based on anammox bacteria, which can eliminate nitrogen by utilizing ammonium as a substrate and nitrite as the terminal electron acceptor in molar ratios of 1:1 [7]. These technologies have been successfully utilized at full-scale wastewater treatment plants for the treatment of reject water from anaerobic digesters (e.g., the partial nitrification/anammox process at Rotterdam wastewater treatment plant (WWTP) and the DEMON process at Apeldoorn WWTP and the CANON process at Olburgen WWTP, all located in The Netherlands). Anammox technologies can save up to 90% of costs with respect to traditional nitrification-denitrification processes for influents with a high ammonium concentration and low carbon content [8]. Among these technologies, the partial nitrification/anammox system has been developed as a two-step autotrophic nitrogen removal process. The first step involves the oxidation of ammonium to nitrite under aerobic conditions in such a way that roughly 50% of ammonium is oxidized [8]. This is achieved with partial nitrification technology. Today, there are six examples of full-scale partial nitrification plants in The Netherlands and one in the USA [9].

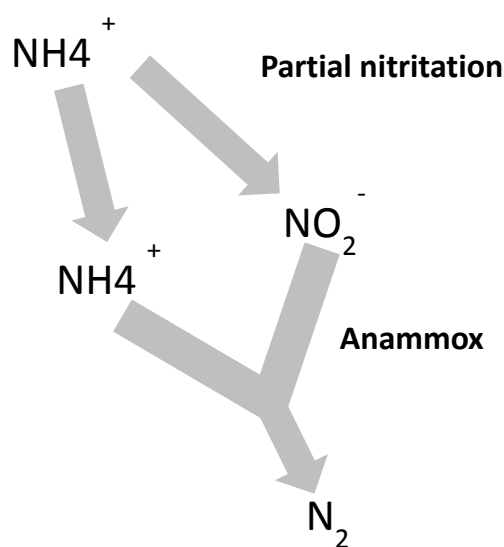
**Table 1.** European Directive 91/271/EEC Requirements in Matter of Nutrients (from [10]).

Parameter	Concentration	Minimum percentage of reduction <sup>1</sup>	Reference method of measurement
Total Phosphorus	2 mg/L (10,000–100,000 population equivalent) 1 mg/L (more than 100,000 population equivalent)	80	Molecular Absorption Spectrophotometry
Total Nitrogen <sup>2</sup>	15 mg/L (10,000–100,000 population equivalent) <sup>3</sup> 10 mg/L (more than 100,000 population equivalent) <sup>3</sup>	70–80	Molecular Absorption Spectrophotometry

Notes: <sup>1</sup> reduction in relation to the load of the influent; <sup>2</sup> total nitrogen means the sum of total Kjeldahl nitrogen (organic and ammoniacal nitrogen), nitrate-nitrogen, and nitrite-nitrogen; <sup>3</sup> these values for concentration are annual means, as referred to in Annex I, paragraph D.4[c]. However, the requirements for nitrogen may be checked using daily averages when it is proven, in accordance with Annex I, paragraph D.1, that the same level of protection is obtained. In this case, the daily average must not exceed 20 mg/L of total nitrogen for all of the samples when the temperature from the effluent in the biological reactor is higher than or equal to 12 °C. The conditions concerning temperature could be replaced by a limitation on the time of operation to account for regional climatic conditions.

In a partial nitrification system, ammonium is oxidized to nitrite (Figure 1). Partial nitrification systems were developed by the Delft University of Technology in 1996–1999 [11] and have several advantages over total nitrification-based technologies, such as 25% savings in aeration, 30% reduction of biomass generation, with a biomass yield of about  $0.15 \text{ g biomass (g NH}_4^+\text{-N)}^{-1}$  [12], and 20% less  $\text{CO}_2$  emission [11]. Partial nitrification bioreactors at the laboratory scale have been reported to successfully treat food processing and agriculture industry wastewater, reject wastewater and slaughterhouse wastewater or swine manure wastewater, among others [13–19].

**Figure 1.** Nitrogen cycle in the partial nitrification/anammox processes.



Within a partial nitrification reactor, the main performance is the oxidation of ammonium to nitrite, and therefore, there is an accumulation of nitrite in the system. Although nitrite has been thought not to accumulate in ecosystems, some reports show that it can accumulate in natural and engineered environments, such as soils, sediments and wastewater treatment plants [20]. This is achieved by the metabolism of ammonium-oxidizing bacteria (AOB).

AOB use ammonium mono-oxidase (AMO) enzyme to oxidize ammonium to hydroxylamine ( $\text{NH}_2\text{OH}$ ), using oxygen as an electron acceptor. Following this reaction, hydroxylamine is oxidized to nitrite with the mediation of hydroxylamine oxidoreductase (HAO), with hydrazine as an intermediate [11,21]. AOB are autotrophic microorganisms, so they utilize inorganic carbon as a carbon source. AOB communities belong to the  $\beta$ -Proteobacteria class, with species like *Nitrosomonas* sp., *Nitrosospira* sp., *Nitrosolobus* sp. and *Nitrosovibrio* sp., among others (Table 2). It has also been found that *Nitrosomonas* and *Nitrosospira* are the most popular genera among partial nitrification reactors, with *Nitrosospira* dominating under high-ammonium conditions [22]. Two different types of AOB bacteria have been differentiated so far: fast-growing AOB and slow-growing AOB. The difference between these two groups resides in the affinity for ammonium, which is higher in slow-growing AOB. Thus, slow-growing AOB, k-strategists, dominate in environments with an ammonium limitation. In partial nitrification reactors, the ammonium concentration is high, and therefore, fast-growing AOB, r-strategists, dominate [23]. Even though it is known that species of AOB can carry out denitrifying metabolism that reduces nitrite to nitric oxide, nitrous oxide and dinitrogen [9], it is thought that AOB only perform ammonium oxidation in partial nitrification reactors.

**Table 2.** References for the identification of ammonium-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anammox bacteria in engineered and natural ecosystems.

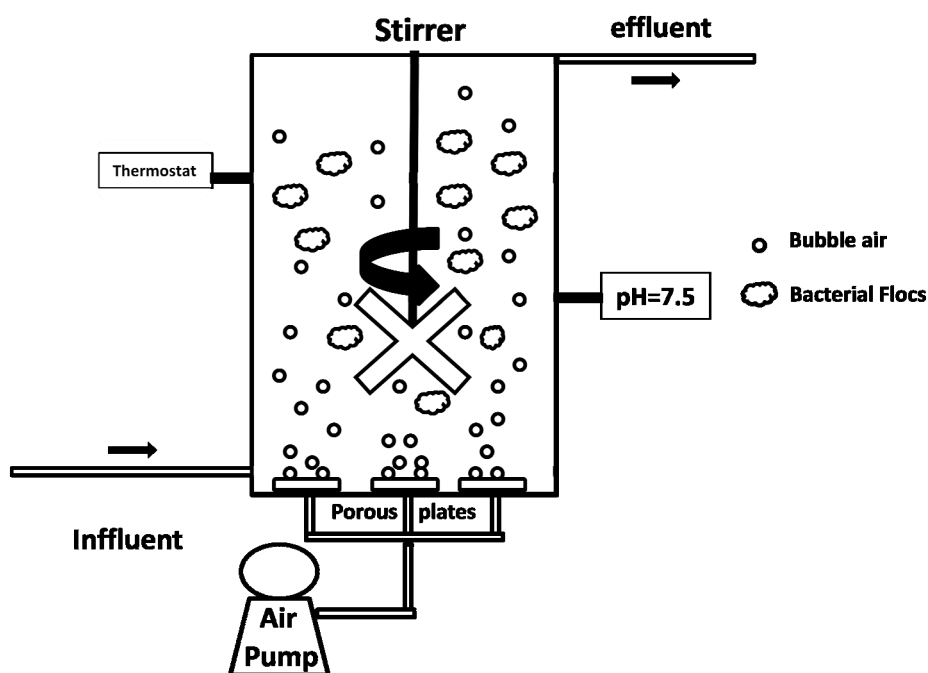
Species	Ecological Role	Class	References
<i>Nitrosomonas</i> sp.	NH <sub>4</sub> <sup>+</sup> oxidizer	β-Proteobacteria	[1,11,22,24–26]
<i>Nitrospira</i> sp.	NH <sub>4</sub> <sup>+</sup> oxidizer	β-Proteobacteria	[1,22,25]
<i>Nitrosolobus</i> sp.	NH <sub>4</sub> <sup>+</sup> oxidizer	β-Proteobacteria	-
<i>Nitrovibrio</i> sp.	NH <sub>4</sub> <sup>+</sup> oxidizer	β-Proteobacteria	[1]
<i>Nitrosococcus</i> sp.	NH <sub>4</sub> <sup>+</sup> oxidizer	γ-Proteobacteria	[24,26]
<i>Nitrobacter</i> sp.	NO <sub>2</sub> <sup>-</sup> oxidizer	α-Proteobacteria	[11,24]
<i>Nitrococcus</i> sp.	NO <sub>2</sub> <sup>-</sup> oxidizer	γ-Proteobacteria	-
<i>Nitrospina</i> sp.	NO <sub>2</sub> <sup>-</sup> oxidizer	Nitrospinae	-
<i>Nitrospira</i> sp.	NO <sub>2</sub> <sup>-</sup> oxidizer	Nitrospirae	[26]
<i>Brocadia</i> sp.	Anammox	Planctomycetia	[27]
<i>Kuenenia</i> sp.	Anammox	Planctomycetia	[28,29]
<i>Scalindua</i> sp.	Anammox	Planctomycetia	[30,31]

Nevertheless, AOB have to compete with other microbial communities inside a partial nitrification reactor. The main competitors of AOB are nitrite-oxidizing bacteria (NOB), which utilize oxygen for the oxidation of nitrite to nitrate (Table 2). As NOB metabolism utilizes nitrite as the metabolic substrate under aerobic conditions, partial nitrification systems represent viable environments for NOB to develop. The most common NOB species isolated from activated sludge systems belong to the genera, *Rubrivivax*, *Rhodobacter* and *Pseudomonas* [32,33]. NOB population development is the major problem related to partial nitrification operational performance. It has been stated that the achievement of the desired ammonium oxidation of a partial nitrification bioreactor depends on the understanding of the AOB and NOB community structure and the effect of operational conditions on AOB and NOB community dynamics [34]. If NOB communities are uncontrolled, nitrate will appear, due to complete nitrification of ammonium when ammonium loading declines from 0.5 kg N m<sup>-3</sup> day<sup>-1</sup>, with the consequent loss in the performance of the system. Therefore, controlling the NOB population is needed in order to achieve the desired nitrogen removal [11,22]. The different characteristics of AOB and NOB have been studied in order to develop strategies for NOB control in partial nitrification systems. These are based on temperature, dissolved oxygen, hydraulic retention time (HRT) and solid retention time (SRT), or free ammonia (FA) and free nitrous acid (FNA) concentrations, among others [14,24,35,36]. When 50% ammonium is oxidized under steady-state conditions, partial nitrification reactors have a relative abundance of 64% AOB and less than 5% NOB [37].

### 3. Effect of Operational Conditions and Quinolone Antibiotic over the Partial Nitrification Process

#### 3.1. Effect of Influent Characteristics

Partial nitrification reactors have been developed for the treatment of wastewater with low organic matter content and high concentrations of ammonium (Figure 2). The effects that different substances present in wastewater, such as ammonium and organic matter, have on the partial nitrification process have been extensively investigated.

**Figure 2.** Schematic of a fluidized bed partial nitrification reactor.

The ammonia loading rate (ALR) has been shown to affect the chemical composition of the effluent generated in a partial nitrification reactor. In a continuously-stirred tank reactor (CSTR) configuration, at an ALR from  $1.04 \text{ N-NH}_4^+ \text{ m}^{-3} \text{ day}^{-1}$  to  $1.81 \text{ N-NH}_4^+ \text{ m}^{-3} \text{ day}^{-1}$ , the composition of the effluent was stable and dominated by nitrite and ammonia, at a ratio of about 1.2:1, with a small fraction of nitrogen present as nitrate. A similar trend was observed in a biofilm reactor configuration at the ALR range of 2.15 to  $4.07 \text{ N-NH}_4^+ \text{ m}^{-3} \text{ day}^{-1}$ . This effluent composition was suitable for a further anammox treatment for nitrogen elimination. Lower ALRs lead to an excess of nitrate in the effluent for this purpose, and higher ALRs achieve an excess of ammonia in the effluent. In this way, ALR has been proposed as a practical way to control the performance of partial nitrification reactors and has been claimed to be more practical than other control strategies, such as dissolved oxygen (DO) control [38].

Ammonium concentration has been shown to affect the performance of partial nitrification reactors, due to the production of FA and FNA. Both AOB and NOB can be inhibited by their metabolic substrates and/or by-products. It has been found that FA and FNA can inhibit AOB and NOB [39–41]. In any case, NOB are much more sensitive than AOB. The activity of NOB becomes inhibited at FNA concentrations from  $0.26 \text{ mg HNO}_2\text{-N L}^{-1}$  [42], which is lower than the inhibition concentration for AOB,  $0.49 \text{ mg HNO}_2\text{-N L}^{-1}$  [41]. Inhibition by FNA is related to the donation of a proton to the electron transport chain, which impedes the transmembrane pH gradient for the synthesis of ATP [21]. NOB is inhibited by FA at concentrations ranging from 1 to  $7 \text{ mg NH}_3\text{-N L}^{-1}$ , while AOB starts to be inhibited at  $150 \text{ mg NH}_3\text{-N L}^{-1}$  [11]. Other authors have proposed different thresholds for the FA inhibition of NOB ( $1.75 \text{ mg NH}_3\text{-N L}^{-1}$ ) [42] and AOB ( $605 \text{ mg NH}_3\text{-N L}^{-1}$ ) [41]. Inhibition of NOB by FA is thought to result from the competition of FA with nitrite oxide reductase (NOR), an NOB enzyme involved in the oxidation of nitrite to nitrate [21]. It has been stated that AOB become inhibited by FA and FNA when nitrogen loading rates become higher than  $1.5 \text{ kg/m}^{-3} \text{ day}^{-1}$  [43]. Regardless of this, the adaptation of NOB and AOB communities to FA concentrations has been

widely covered by several authors [44–46]. Some *Nitrobacter* spp. strains, typical NOB in partial nitrification reactors, have been found to resist up to 40 mg NH<sub>3</sub>-N L<sup>-1</sup> FA [11], while AOB communities have been acclimated to FA concentrations of 122–224 mg L<sup>-1</sup> [24]. Therefore, control based on FA concentrations might not be an efficient, practical tool for the assessment of the performance of partial nitrification systems.

Hydroxylamine, an intermediate in the oxidation of ammonium to nitrite, was reported to have the capability of stabilizing a partial nitrification system operating at a high chemical oxygen demand to nitrogen (COD/N) ratio, low temperature and high DO concentration, due to its inhibitory effect on NOB populations [47]. Hydroxylamine has an inhibitory effect on NOB communities at 250 μM and on AOB populations at 2,000 μM; therefore, it selects for AOB to thrive in the system [11].

A certain amount of organic matter can enter a partial nitrification reactor with the influent. Organic matter entering a partial nitrification reactor affects its performance. One of the reasons is that organic matter favors the development of heterotrophs, which have a shorter duplication time than AOB and could therefore outcompete them for oxygen inside the bioreactor [48]. It has been reported that the stability of the partial nitrification process is disturbed by high COD/N ratios, due to the promotion of heterotrophic bacteria inside the system [49]. It has been shown that the C/N ratio does not affect the performance of the partial nitrification process at ammonium volumetric loading rates of 0.5 kg N-NH<sub>4</sub><sup>+</sup> m<sup>-3</sup> day<sup>-1</sup>. At higher ammonium volumetric loading rates, higher C/N ratios require higher DO concentrations if the same ammonium oxidation efficiency is desired [24]. In spite of the promotion of heterotrophic growth, the impact of total organic carbon (TOC) concentration on a partial nitrification system has been found to depend on carbon concentration. It has been reported that TOC concentrations are 0.2 g TOC L<sup>-1</sup>, as acetate stimulates ammonium oxidation in partial nitrification reactors, but also 0.3 g TOC L<sup>-1</sup>, as acetate decreases 10% ammonium conversion in these systems [6].

Another impact of organic matter on partial nitrification reactors is related to the carbonate-bicarbonate-carbon dioxide equilibrium. With the development of heterotrophs in the partial nitrification reactor, carbon dioxide is generated in the system. Given the pH range in which partial nitrification reactors operate (6.6–8.0), the equilibrium will select for HCO<sub>3</sub> as the dominant form of inorganic carbon. Carbonate will acidify the medium, thus reducing its capacity to balance the release of protons taking place during ammonium oxidation. Therefore, the addition of organic matter will lead to lower conversion rates of ammonium to nitrate [36].

In spite of this fact, it has been proven that the elimination of organic matter can be achieved in partial nitrification reactors at the same time as ammonium oxidation when carbon loading rates do not exceed 2 kg m<sup>-3</sup> day<sup>-1</sup> [48]. Accordingly, it has been proposed that, for the purpose of an anammox treatment train, a partial nitrification process following an anammox reactor is recommended when the influent contains a considerable amount of organic matter, which suggests the ability of partial nitrification systems to handle peaks of organic loading while maintaining a stable operational state [50]. In spite of this capacity, it has been found that the recovery of partial nitrification reactors after excessive loading of organic matter is a long process [42].

The addition of certain organic and inorganic compounds could play an important role in partial nitrification processes. It has been found that fulvic acid impacts nitrite accumulation in partial nitrification reactors, impeding ammonium oxidation when its concentration is below 0.002 mg L<sup>-1</sup> or over 0.07 mg L<sup>-1</sup>. It has also been reported that NOB are more sensitive than AOB to ortho-cresol, aniline

and phenol.  $\text{ClO}_2^-$  has been proven to inhibit NOB activity at 3 mM; thus, chlorine could be used as a control strategy for NOB population development [21].

### 3.2. Effect of Temperature

Temperature shows a clear relationship with ammonium oxidation in partial nitrification systems. It has been reported that, at a constant ammonium volumetric loading rate and DO concentration, higher temperatures lead to higher ammonium oxidation up to 35 °C. Beyond this point, higher temperatures lead to FA formation, and the activity of AOB becomes inhibited [24]. In similar studies, it was found that at 25 °C, ammonium oxidation reached values of up to 60% of those at 28–39 °C. At 41 °C, bacterial activity stops, and thus, ammonium oxidation does not take place. Furthermore, it has been observed that the ammonium uptake rate in partial nitrification reactors is maximum in the temperature range of 33–37 °C [51].

The bacterial community structure of partial nitrification reactors seems not to be influenced by temperature. Therefore, a difference in microbial activity due to temperature has been proposed as an explanation of differences in the performance of the system at different temperatures [52].

It has been found that NOB have slower growth rates than AOB when temperatures go up from 24 °C, while at temperatures as low as 15 °C, NOB attain domination over AOB in partial nitrification reactors [15,21]. It was reported that temperatures above 30 °C led to a prevalence of AOB over NOB, due to the faster growth rate of the former, so operating at temperatures higher than 30 °C will select for AOB [53].

Furthermore, the effect of temperature on the performance of a partial nitrification reactor has been explained by the formation of FA and FNA, due to shifts in the concentrations of FA and FNA produced in partial nitrification systems. In this way, while the FA concentration increases with temperature, from about 20 mg/L at 25 °C to 120 mg/L at 35 °C, FNA shows the opposite behavior, with 0.5 mg/L at 25 °C and 0.1 mg/L at 35 °C. Thus, at low temperatures, FNA is the main inhibitor of AOB, while at high temperatures, it is the combination of FA and FNA that inhibits ammonium oxidation [54].

In practice, partial nitrification reactors are commonly operated in the range of 30–35 °C to ensure that AOB outcompetes NOB [54]. However, even though partial nitrification reactors have been widely operated at 35 °C, there is not much difference in the practical operation between 25 °C and 35 °C in terms of growth of AOB and NOB; in practical operation, 25 °C is considered enough for the purpose of NOB control [21].

### 3.3. Effect of pH and DO

The pH in a partial nitrification reactor has an impact on its performance. In fact, pH has been reported as a key parameter affecting influent quality in models for laboratory-scale partial nitrification bioreactors [55]. It has been proposed that the influence of pH on ammonium oxidation in partial nitrification reactors is driven by three processes: activation and deactivation of nitrifying enzymes; changes in inorganic carbon concentrations; and changes in FA and FNA concentrations [20,56]. At higher pH, carbonate and bicarbonate are present at higher concentrations, and thus, the buffer capacity of the system increases. On the contrary, as the pH drops below 7.7, the equilibrium tends to carbon



dioxide, leading to a loss of buffer capacity [36]. High pH has been related to the formation of FA, which is the primary substrate for AOB, and has also been related to a decrease in FNA concentration, which is the primary substrate of NOB communities [38]. On the other hand, it has been demonstrated that nitrification does not occur below pH 6 [11,20]. Therefore, control of the pH in partial nitrification systems can select for AOB and inhibit NOB, due to the formation of FA and the limitation of FNA [45,57].

It has been reported that the optimal pH for *Nitrosomonas* spp., typical AOB, ranges between 7.9 and 8.2 and that for *Nitrobacter* spp., typical NOB, it ranges between 7.2 and 7.6, while the optimal pH for partial nitrification reactor operation ranges between 7.0 and 8.0 [11,38]. The higher tolerance of AOB to low pH is thought to result from their ability to develop thick extracellular polymeric substance (EPS) layers [58].

DO concentrations have an impact on the performance of a partial nitrification reactor. It has been found that ammonium oxidation increases with DO concentration, regardless of the C/N ratio, for the same ammonium volumetric loading rate [24].

Half saturation constant values for oxygen of AOB and NOB were reported to be 0.2–0.4 mg L<sup>-1</sup> and 1.2–1.5 mg L<sup>-1</sup>, respectively, which supports the hypothesis of the lower affinity for oxygen of NOB than AOB [11,21]. Following this, oxygen limitation inside partial nitrification reactors is an efficient way to control NOB development. Some studies state that the growth rate of AOB is higher than that of NOB when DO concentrations drop below 1 mg L<sup>-1</sup> [22]. Therefore, DO concentrations lower than 1 mg L<sup>-1</sup> are used to control NOB in partial nitrification reactors [59,60]. The accumulation of nitrite can be controlled in a short time by setting DO concentration to 0.4–0.8 mg L<sup>-1</sup>. Furthermore, at DO concentrations of 2 mg L<sup>-1</sup> or higher, a substantial accumulation of nitrate occurs in partial nitrification bioreactors [49]. Some authors used aeration of lower than 0.1 (m<sub>air</sub><sup>3</sup> day<sup>-1</sup>)/(kg N m<sup>-3</sup> day<sup>-1</sup>) in order to control nitrate accumulation [22]. In addition, low DO concentrations are related to increasing NO and N<sub>2</sub>O emissions [11].

Partial nitrification processes have been reported as responsible for the emissions of CH<sub>4</sub> and N<sub>2</sub>O to the atmosphere [61–64]. Even though CH<sub>4</sub> emissions have been related to soluble CH<sub>4</sub> stripped from reject wastewater treated in partial nitrification systems, N<sub>2</sub>O emissions have been correlated with DO concentration. In this way, higher DO concentrations inside the bioreactor will decrease the %N<sub>2</sub>O/N<sub>oxidized</sub> ratio. When normal operation parameters were used, emissions of 19.3% ± 7.5%N<sub>2</sub>O/N<sub>oxidized</sub> were found for a partial nitrification sequencing batch reactor (SBR) process [65].

### 3.4. Effect of HRT and SRT

It has been reported that *Nitrosomonas* spp., typical AOB [24], have a maximum growth rate of 0.54 ± 0.09 day<sup>-1</sup>, while *Nitrobacter* spp., typical NOB [24], have a maximum growth rate of 0.67 ± 0.03 day<sup>-1</sup> [66]. Other authors have reported similar values for a minimum doubling time of AOB (7–8 h) and NOB (10–13 h) [21]. Given the shorter doubling time of AOB compared with NOB, control of NOB populations can be achieved with the utilization of the HRT/SRT. If HRT is set shorter than the AOB doubling time, then no ammonium oxidation will occur, and if HRT is set longer than the NOB doubling time, then the system will undergo nitrate generation. For this reason, SRT should be set longer than the AOB doubling time, but shorter than the NOB doubling time. In addition to this,

partial nitrification bioreactors will avoid sludge retention, given that the recycling of biomass will make NOB persist in the system and, therefore, develop in it. For this reason, partial nitrification reactors are generally operated without sludge retention [8].

Conventionally, HRT and SRT are set up to be the same time in partial nitrification reactors. However, the development of non-coupled HRT and SRT partial nitrification bioreactors has been attempted by biofilter partial nitrification reactors. By setting up a biofilter process, SRT is increased with respect to HRT. It has been confirmed that biotransformation of ammonium to nitrite is three-times higher in biofilter partial nitrification reactors than in conventional partial nitrification reactors [1].

The impact of HRT on partial nitrification reactors has been evaluated by several authors. It was found that higher HRTs, at the same SRTs, led to higher oxidation of ammonium to nitrite [1,25,37]. Differences in ammonium oxidation at different HRTs are due to differences in microbial community structure inside the bioreactor at these HRTs. Differences in bacterial diversity have been reported in biofilter partial nitrification reactors for different HRTs. Within long HRTs of 12 h, the dominant phylotypes are *Nitrosomonas europaea* and *Nitrosomonas eutropha*, with the importance of *Diaphorobacter* sp. At moderately long HRTs of 9 h, the dominant species are *N. europaea*, *Nitrosospira* spp., and *Paracoccus* spp., with the presence of *Nitrosovibrio* spp., *Rhodobacter* spp. and *Catellibacterium* spp. [1,25]. A decrease in microbial diversity at high HRTs due to competition for ammonium has been observed, which links bacterial community structure to ammonium oxidation in partial nitrification reactors [1]. At high HRTs, the ammonium loading rate decreases in the system. Under these conditions, ammonium is scarce and bacteria with high affinity for ammonium will rapidly thrive inside the bioreactor, outcompeting other bacteria with a lower affinity for this substrate. The selection of bacteria with high ammonium affinity will increase the biotransformation rate of the system. Therefore, control of a biofilter partial nitrification reactor can be achieved by changing the HRT of the system [1].

The SRT also has an influence on bacterial communities in partial nitrification reactors. As a main control of NOB in the system, the SRT should be set lower than the duplication time of NOB, thus ensuring the washout of these bacteria from the reactor [8]. Nevertheless, a short SRT also leads to a loss of AOB biomass. In this way, partial nitrification reactors have been conventionally operated as suspended growth processes [30,67,68]. On the other hand, it has been confirmed that attached growth partial nitrification processes with attached/granular biomass have advantages over suspended growth partial nitrification processes, such as enhanced AOB biofilm formation [69]. Attached growth processes for partial nitrification systems have been successfully applied at the pilot-plant scale [1,5]. An efficient, stable, 50%–50% ammonium–nitrate ratio in the effluent has been achieved at optimum HRTs of 7 h [5]. In this sense, it can be said that HRTs lower than 7 h could not achieve the oxidation of half of the influent ammonium, with the consequent failure of the operation of the system. An analysis of bacterial community dynamics at shorter HRTs has not been attempted to date in biofilter partial nitrification reactors. It has been proven that attached growth processes offer up to 100-times higher biomass retention and 10-times lower loss of biomass and require a lower HRT than suspended growth processes [70].

### 3.5. Effect of Salinity, Starvation of Biomass and Emerging Contaminants

Salinity affects the performance of partial nitrification systems. It has been concluded that concentrations of 85 mM NaCl increases ammonium conversion by up to 30%. As the NaCl concentration rises, the system loses stimulation and tends to similar values of performance as those for the no-salinity scenario. Nevertheless, at NaCl concentrations of 256 mM and higher, the system loses the capacity for ammonium oxidation. At 342 mM NaCl, ammonium oxidation in the system is 70% less compared with no-salinity operation [4]. The sensitivity of AOB has been identified after short-term exposure to salinity, but the adaptation of *N. europaea* strains to high salinity conditions has also been observed [71,72].

The bacterial dynamics of AOB and NOB communities inside a partial nitrification bioreactor subjected to a long period of starvation were studied [73]. When the feed of a partial nitrification reactor ceases, ammonium availability starts to decrease due to AOB activity, until all of the ammonium has been oxidized. At this point, due to the lack of substrate, AOB communities exhibit a sharp decrease in relative abundance, from 77% to less than 1%. On the other hand, NOB communities gain relative abundance due to the availability of nitrite excreted by AOB. This trend stops when all of the nitrite is consumed within the system. When the starvation period ceases and the bioreactor is subjected to a regular feed again, AOB communities develop rapidly, achieving a steady state similar to that before the starvation period after 15 days.

Emerging contaminants (ECs) have been proposed as particular pollutants that, in fact, have never been studied before [74]. They came to the attention of the scientific community recently and are different from traditional pollutants. Contaminants of emerging concern today include pharmaceutical products, plasticizers, flame retardants, new-generation pesticides, cyanotoxins and more.

Pharmaceuticals have been reported as some of the most important ECs. They are present in urban and industrial wastewater treatment systems all around the world (an example from the analysis of pharmaceuticals from three wastewater treatment plants of Catalonia region, Spain, is given in Table 3) and have been targeted as contaminants of emerging concern [1]. As an example, different antibiotics have been detected at concentrations of up to 450 ng L<sup>-1</sup> in urban wastewater treatment plants [75]. In this context, it has been reported that the partial nitrification process can be drastically inhibited by quinolone antibiotics, such as ciprofloxacin [76]. Quinolone antibiotics have been found in influent urban wastewater in concentrations ranging up to 426 ng/L [3].

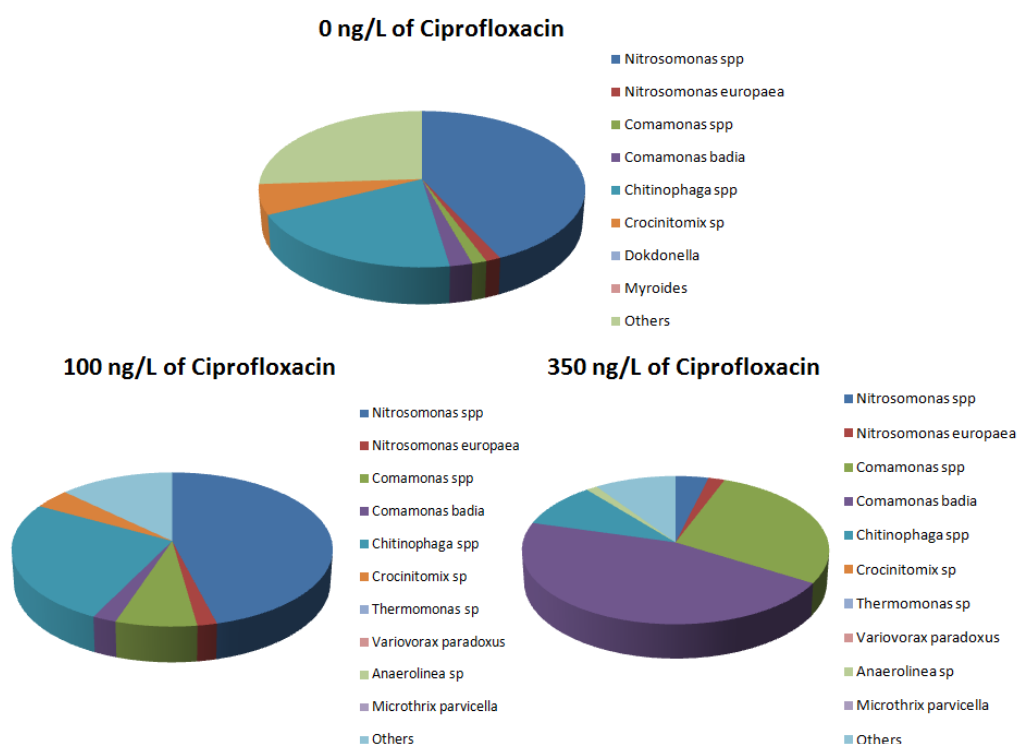
As indicated above, the effect of ciprofloxacin on the performance and the bacterial community dynamics of a partial nitrification reactor has been studied [3]. Ciprofloxacin at 100 ng L<sup>-1</sup> impacts the performance of the system, which loses 40% capacity of ammonium conversion during the first several days, but stabilizes within a month, showing a more efficient conversion of ammonium than before the antibiotic addition. On the other hand, concentrations up of 350 ng L<sup>-1</sup> ciprofloxacin drastically reduce ammonium oxidation, by 70%, during the first several days. When the system stabilizes, ammonium conversion recovers, but the performance reached is 30% lower compared with that at the beginning of the experiment. Biomass in the system decreases when ciprofloxacin is added, by about 40% for 100 ng L<sup>-1</sup> and 60% for 350 ng L<sup>-1</sup>, but at the end of the experiment, there is a net increase in the biomass (5% for the lower concentration and 80% for the higher concentration). In the case of 100 ng L<sup>-1</sup> ciprofloxacin, bacterial community dynamics start with a decrease of the relative abundance of AOB and end with a slight net increase in the importance of AOB with respect to the

starting structure. On the other hand, ciprofloxacin at concentrations of up to 350 ng L<sup>-1</sup> removes the AOB domination of the system, in favor of *Comamonas* strains. With time, AOB tend to grow in importance; however, the microbial community structure has still shifted completely, and the recovery of the system is impossible (Figure 3).

**Table 3.** Examples of pharmaceutical emerging contaminants (ECs) detected in influent and effluent wastewater of wastewater treatment plants (adapted from [77]).

Compound	Frequency of detection (%)	
	Influent wastewater	Effluent wastewater
Carbamazepine	100	100
Diazepam	54	54
Trimethoprim	100	96
Ketoprofen	100	54
Mevastatin	12	8
Clarithromycin	73	85
Salbutamol	69	58
Furosemide	100	96
Sulphamethazine	58	65
Diclofenac	92	100

**Figure 3.** Bacterial community structure of a biofilter partial nitritation bioreactor operating at stable nitrogen removal values subjected to the addition of ciprofloxacin antibiotic to its influent (adapted from [3]). It can be observed that the addition of 350 ng/L of ciprofloxacin dramatically changed the microbial community structure in the system. Furthermore, partial nitritation bioreactors can withstand ciprofloxacin concentrations of up to 100 ng/L with no significant impact on the microbiota.

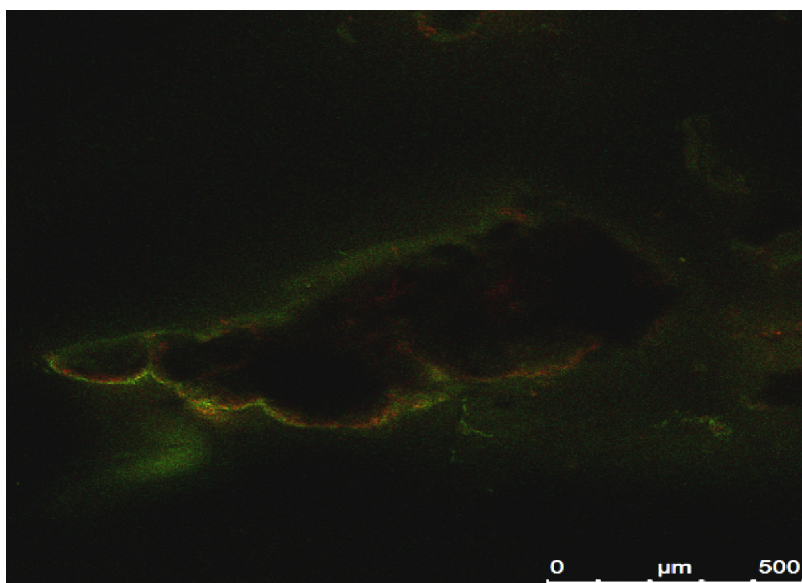


#### 4. Future Trends for the Partial Nitrification Process

The application of partial nitrification systems to nitrogen removal from wastewater is becoming more and more popular around the world. Today, full-scale plants that implement the partial nitrification/anammox system for nitrogen elimination from reject wastewater are a reality [3,76]. In addition, the oxidation of ammonium to nitrite is a necessary step for anammox bacteria (Table 2) to carry out autotrophic denitrification [3]. Therefore, the development of systems that rely on the anammox elimination of nitrogen will need to take into account partial nitrification processes.

A promising technology based on anammox microorganisms is the CANON process. In these systems, partial nitrification and anaerobic ammonium oxidation mediated by anammox bacteria take place in the same bioreactor. A coupled partial nitrification-anammox reaction is possible due to the syntrophic metabolism of AOB and anammox bacteria and its spatial organization in granular biomass (Figure 4) [78]. Therefore, the necessity for two different bioreactors for partial nitrification and the elimination of nitrogen via the anammox pathway, as in the partial nitrification/anammox process, is removed. In spite of their engineering advantages, CANON systems are more complicated in practical operation than partial nitrification bioreactors. The competition of NOB with AOB for oxygen and with anammox bacteria for nitrite is the major problem for the performance of these systems [79]. The growth of NOB inside CANON bioreactors is commonly avoided by limiting the DO concentration in the system. The selection of AOB and the inactivation of NOB coupled with the growth of anammox bacteria inside the same bioreactor arises as the immediate future application of partial nitrification.

**Figure 4.** Fluorescence *in situ* hybridization (FISH) image of a biomass granule from a CANON bioreactor. AOB (green) and anammox bacteria (red) are displaced next to each other; the spatial organization of AOB and anammox bacteria inside the granule is determined by the syntrophic relationships among the phylotypes.



On the other hand, the development of partial nitrification systems has yet to solve the main problem that these processes have: the development of NOB communities inside the bioreactor. Many steps can be taken to further avoid the growth of NOB species in partial nitrification reactors. One of these steps is

to turn partial nitrification processes into fully anaerobic processes. This can be done by taking into account the anaerobic nature of autotrophic ammonium oxidation [9].

Strains of *N. eutropha* were reported to perform anaerobic ammonium oxidation with the mediation of nitrogen dioxide as an electron acceptor [80]. Further investigation showed that dinitrogen tetroxide could also be utilized as the final electron acceptor for anaerobic autotrophic ammonium oxidation in an *N. eutropha* species [81]. Later, a new pathway for anaerobic autotrophic ammonium oxidation was proposed [81]. Here, dinitrogen tetroxide oxidizes ammonium to hydroxylamine and nitrous oxide with the mediation of the enzyme, AMO. In this way, for the purposes of anaerobic ammonium oxidation in *N. eutropha*, the presence of oxygen just causes nitrous oxide to become oxidized to nitrogen dioxide [82]. Furthermore, the study of the nitrogen cycle in oxygen-depleted marine areas suggests that anaerobic ammonium oxidation is linked to Mn, Fe and S reduction [83–86].

If AOB can perform anaerobic ammonium oxidation, then partial nitrification reactors could avoid the growth of NOB communities by setting up anaerobic conditions in the system. Further work on technologies relying on this pathway aims to change the future perspectives of autotrophic nitrogen removal processes.

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## Author Contributions

Alejandro Rodriguez-Sanchez has been primarily responsible for the compiled information in the review. He has been particularly concerned with the microbiology of AOB, NOB and anammox bacteria in autotrophic nitrogen removal systems; Alejandro Gonzalez-Martinez has been responsible for the technical supervision of the review, with knowledge in autotrophic nitrogen removal biotechnologies; Maria Victoria Martinez-Toledo has been responsible for the taxonomic analysis of the bacterial strains; Maria Jesus Garcia-Ruiz has been responsible for the operational parameters supervision; Francisco Osorio-Robles has been responsible for the micropollutant supervision; Jesus Gonzalez-Lopez is the general coordinator of the review and the main article writer.

## Conflicts of Interest

The authors declare no conflict of interest.

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