Review

Development of Strategies for AOB and NOB Competition Supported by Mathematical Modeling in Terms of Successful Deammonification Implementation for Energy-Efficient WWTPs

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Abstract: Novel technologies such as partial nitritation (PN) and partial denitritation (PDN) could be combined with the anammox-based process in order to alleviate energy input. The former combination, also noted as deammonification, has been intensively studied in a frame of lab and full-scale wastewater treatment in order to optimize operational costs and process efficiency. For the deammonification process, key functional microbes include ammonia-oxidizing bacteria (AOB) and anaerobic ammonia oxidation bacteria (AnAOB), which coexisting and interact with heterotrophs and nitrite oxidizing bacteria (NOB). The aim of the presented review was to summarize current knowledge about deammonification process principles, related to microbial interactions responsible for the process maintenance under varying operational conditions. Particular attention was paid to the factors influencing the targeted selection of AOB/AnAOB over the NOB and application of the mathematical modeling as a powerful tool enabling accelerated process optimization and characterization. Another reviewed aspect was the potential energetic and resources savings connected with deammonification application in relation to the technologies based on the conventional nitrification/denitrification processes.

Keywords: deammonification; NOB suppression; microbial interaction; influencing factors; energy consumption; model-based analysis; kinetics

1. Introduction

The excess amount of the nutrients (in particular nitrogen and phosphorus), released to the environment from the point sources, such as wastewater treatment plants (WWTPs), is associated with eutrophication process acceleration and threatens aquatic life. While effective phosphorus removal from municipal wastewater could be ensured by coagulation, in terms of nitrogen removal, reasonable costs are obtained exclusively for the biological treatment. Thus, the principles of biological nitrogen removal (BNR) methods have stimulated interests worldwide [1]. A combination of the nitrification and denitrification is a conventional method for removing nitrogen from wastewater [2]. In the frame of the first process—nitrification ammonium is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) (nitritation), and nitrite is subsequently converted to nitrate by nitrite-oxidizing-bacteria (NOB) (nitratation). After transition into anoxic conditions, under limited dissolved oxygen concentration, nitrite/nitrate is reduced to dinitrogen gas by heterotrophic denitrifies with organic compounds such as methanol, ethanol, acetate, or glucose, which serve as electron donors. On the one hand, systems based on conventional nitrification/denitrification (N/DN) enable to achieve high efficiencies of nitrogen

removal, on the other hand, such efficiency comes at high operational costs (basically due to high oxygen demand to ensure full nitrification) and sufficient amount of easy bioavailable carbon source for denitrification has to be assured [3,4]. For example, N/DN processes consumes approximately 50% to 90% of electricity under pilot-scale conditions, which is 15–49% of the total operational costs [5,6].

Currently, novel technologies of nitrogen removal have been intensively investigated in order to implement solutions for energy consumption savings and to lower carbon demand, which is based on partial nitration (PN) and partial denitrition (PDN). These approaches could be combined with the anammox-based process as shown in Figure 1.
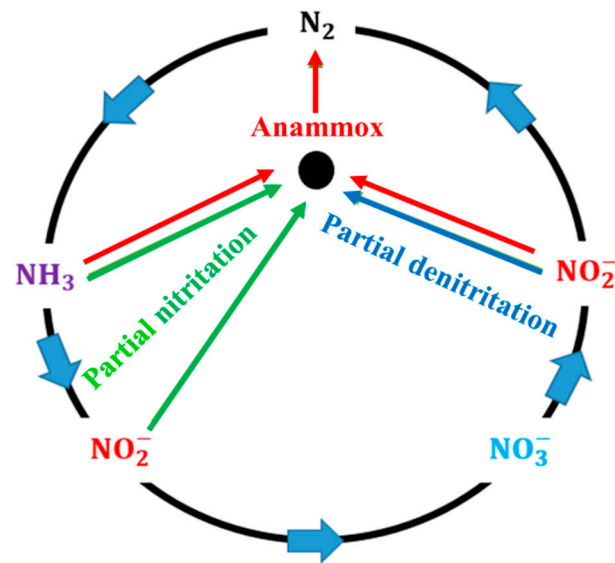

Figure 1. The schematic pathway of nitrogen transformation with implementation of technologies based on anammox process.

In particular, combination of the partial nitration (PN) and anaerobic ammonium oxidation (anammox, noted as A), so called deammonification, seems to be the most promising approach in that field [7,8]. During PN process, nearly 50% of ammonia is oxidized to nitrite and subsequently, the rest of ammonia with nitrite as an electron acceptor under anoxic conditions are converted together to nitrogen gas [1,4]. Several studies have proved that PN/A process can be successfully applied for lab and full-scale designs for side-stream lines in plants due to the elimination of carbon demand for denitrification and having cost-effective benefits [9,10]. This pathway has diverted attention due to its appropriate benefits such as cost-saving approaches, reducing the oxygen demand, inhibiting the activities of denitrifies, eliminating carbon affinity for denitrification, improving sludge handling process in comparison with conventional methods to remove nitrogen from WWTPs [11]. Deammonification-based technologies have been applied for more than 100 full-scale plants around the world [1,12]. Strass WWTP has been known as a forerunner plant that has used the deammonification method successfully since 2004 [2,13,14].

Up to date, different systems based on the deammonification process implementation have been validated, including single reactor for high activity ammonium removal over nitrite (SHARON), completely autotrophic nitrogen removal over nitrite (CANON) which is an example of SNAP (single-stage nitrogen removal using anammox and partial nitration), simultaneous partial nitrification, anammox, and denitrification (SNAD) (Figure 2) [15–18].
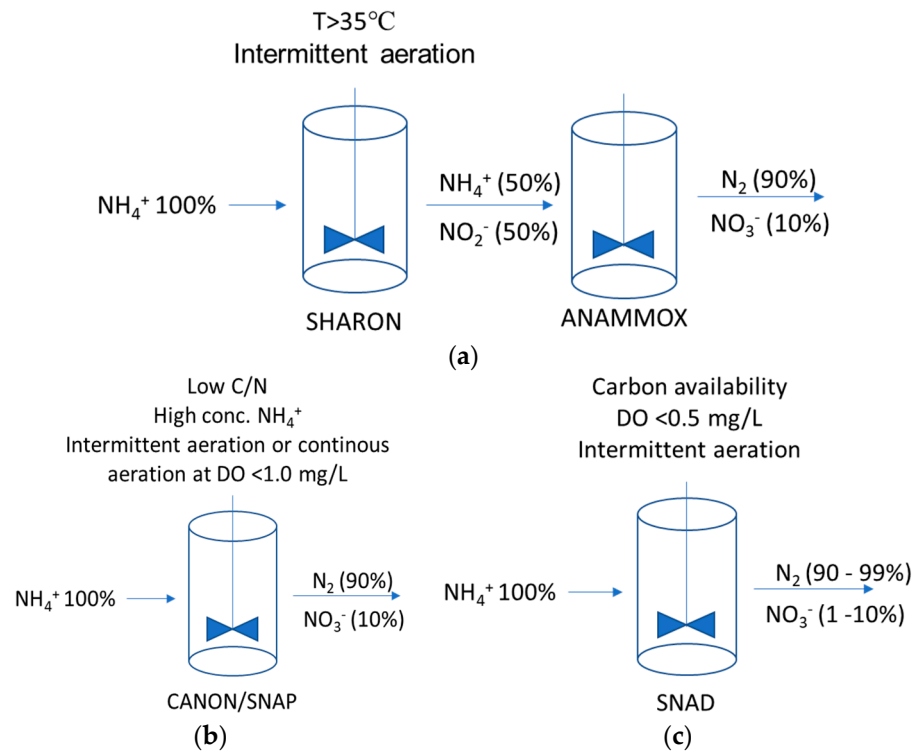
Figure 2. An overview of technologies based on the deammonification process: single reactor for high activity ammonium removal over nitrite (SHARON) (**a**) completely autotrophic nitrogen removal over nitrite CANON/ (single-stage nitrogen removal using anammox and partial nitration SNAP (**b**) simultaneous partial nitrification, anammox, and denitrification SNAD (**c**).

The aim of the presented review was to summarize current knowledge about deammonification process principles, related to microbial interactions responsible for the process maintenance under varying operational conditions. Particular attention was paid to the factors influencing the targeted selection of AOB/AnAOB over the NOB and application of the mathematical modeling as a powerful tool enabling accelerated process optimization and characterization. Another reviewed aspect was the potential energetic and resources savings connected with deammonification application in relation to the technologies based on the conventional nitrification/denitrification processes.

2. Microorganisms Interaction through Shortcut Nitrification Systems

Novel strategies (based on deammonification) are effectively developed in order to enrich biomass with AOB and AnAOB population or/and induce their activity, with parallel NOB suppression [16,19]. The interaction and competition between main internal bacteria including AOB, AnAOB, and NOB for common substrates are considered as a critical point for maintaining the stability of technologies based on the deammonification process (Table 1).

Table 1. Substrate and electron donors specified for the main microbial groups involved in nitrogen metabolism.

Functional Group	Electron Donor	Electron Acceptor
AOB	NH_4^+	O_2
NOB	NO_2^-	O_2
AnAOB	NH_4^+	NO_2^-

The main dependencies are: competition between AOB and AnAOB for NH_4^+–N as an electron donor; NOB and AnAOB contest for nitrite as both, electron acceptor and

donor; rivalry between AOB and NOB for oxygen substrate as an electron acceptor; AOB, NOB, and AnAOB competition for inorganic carbon (IC) [20,21].

Based on the processes kinetics performed by AOB and NOB, the minimal AOB/NOB ratio of their abundance in the biomass, in order to maintain the stability of the deammonification process, is considered around 2:1 [22]. In addition, activity of the nitrifying autotrophic bacteria and dynamics of their population can be affected by applied reactor type, for instance, application of the moving bed biofilm reactor (MBBR) enables to maintain the stable deammonification process under low-temperature conditions [23].

2.1. Ammonia Oxidizing Bacteria (AOB)

Currently, ammonia oxidizing bacteria responsible for the nitritation step are categorized into five genera: *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosorobrio*. Several reports have suggested that representatives of the *Nitrosomonas* genus, which belongs to the β subdivision of the *Proteobacteria*, constitutes commonly from 0.4 to 1.5% of the total biomass in the deammonification systems, at the same time, nitrifiers from the *Nitrosomonas* predominate AOB population at the share of 63–97.70% [21]. For instance, Huang et al., [23] who investigated the AOB community within one-stage PN/A process, have identified *Nitrosomonas* sp. HKU as the most abundant nitrifying bacterial group in the reactor [24].

The predominant role and the highest abundances of the *Nitrosomonas* genus in the frame of the AOB population in PN/A systems, is mainly attributed to their high substrate (as well under high ammonium concentrations) and oxygen affinities [4]. Yang et al. [24] observed that by decreasing ammonia concentration in the influent wastewater under mainstream conditions, the population of the AOB, validated by qPCR, decreased to 3.0×10^{10} copies/L in comprehension to 5.6×10^{11} copies/L, during the experimental stage where elevated ammonium nitrogen loads were applied [25].

Previous studies have revealed that maximum specific growth rate ($\mu_{AOB,max}$) of the AOB, which is around 0.81–0.85 day^{-1}, was obtained for pH and temperatures at the ranges of 7.5–8 and 30–35 °C, respectively [16].

While under strictly aerobic conditions, AOB growth is not limited by the electron acceptors availability, AOB are capable to maintain their growth under anoxic conditions relying on the remaining, limited DO concentration [25].

2.2. Nitrite Oxidizing Bacteria (NOB)

Nitrite oxidizing bacteria are catalyzers of the nitratation step during nitrification process. At the present, the NOB are classified within seven bacterial genera, *Nitrospira*, *Nitrobacter, Nitrotoga*, *Nitrococcus*, *Nitrospina*, and *Nitrolancea* [26,27]. NOB are known as Gram-negative bacteria with differential cell shapes such as rod, spiral, and spherical. Most of the NOBs genera are autotrophic and use CO_2 as a carbon source for the biomass synthesis. Based on the characterization of the biomass composition obtained from the WWTPs with the modern molecular tools (for instance, high-throughput sequencing of the 16S rRNA gene), representatives of the Nitrobacter and *Nitrospira* genera are recognized as the most abundant NOB in such ecosystems [28].

Yao and Peng. [21] have indicated that share of the NOB in the total bacterial communities of the biomass form the conventional WWTPs ranged from 1.0 to 5.0%, within which 60.0–92.0% were attributed to the representatives of the *Nitrospira* genus. NOB activity and growth rate depends on many operational factors, for instance, high level of SRT, low temperature, and concentration of ammonia, NOB growth in the system over AOB [29].

The predominance of the particular NOB genus, i.e., *Nitrospira* versus *Nitrobacter* is strongly dependent on the nitrite concentrations and availability [13]. Representatives of the *Nitrobacter* genus are adapted to higher nitrite concentrations in the contrary to the members of the *Nitrospira*, which prevail in the systems operated under moderate and relatively low nitrite loads/accumulation [7]. Moreover, limitation of the DO concentrations and extended SRTs are additional factors that promote *Nitrospira* growth over

Nitrobacter [30]. On the other hand, since *Nitrobacter* has a lower affinity for oxygen than *Nitrospira*, hence under low DO concentrations but with nitrite availability, *Nitrobacter* would inhibit the activity of *Nitrospira* [31,32].

Additional aspect that should be taken into account, is a selection strategy specified for the particular microbial group. In line with the r/K selection theory, organisms are classified into r-strategists or K-strategists based on their growth rate and affinity for the available substrates. In general, r-strategists are defined by a fast growth rate at the high substrate availability and under low competition level with other microorganism, whereas K-strategists grow faster when they are faced with limited substrate concentrations and are exposed to the strong competitiveness (Table 2) [2,33,34].

Particular genera of the AOB and NOB reflect variability in terms of r/K strategies. For instance, in terms of affinity to the dissolved oxygen, *Nitrosomonas europaea* and *Nitrobacter* sp. due to low affinity are known as r-strategists while *Nitrosospira* and *Nitrospira* with a higher affinity are recognized as K-strategists [7,35].

Table 2. Predominating species in different biological processes.

Reference	Main AOB	Main NOB	Characteristic Strategy	Ammonium Conversion Efficiency%	Biomass Type	Reactor
[36]	*Nitrosospira*	Nitrospira	k-strategists/k-strategists	Completely	Aerobic granulation	SBR
[37]	*Nitrosomonas*	*Nitrobacter*	k-strategists/r-strategists	90	Activated sludge + biofilm	lab-scale continuous-flow bioreactors
[16]	*Nitrosomonas europaea*	*Nitrospira*	r-strategists/k-strategists	99.4	Biofilm	SBR
[4]	*Nitrosomonas* sp.	-	r-strategists	91.9	Granular biomass	SBRs
[38]	*Nitrosospira* sp.	-	k-strategists	87.8	Cultivated activated sludge	MMBR
[39]	*Nitrosomonas*	*Nitrobacter*	r-strategists/r-strategists	>93	Granular biomass	SBR
[40]	*Nitrosomonas eutropha*	*Nitrobacter alkalicus*	k-strategists/r-strategists	From 21% to 99% *	Activated sludge	continuously stirred tank reactor (CSTR)

* Efficiencies range for 6 six independent reactors differing with operating conditions.

2.3. Anaerobic Ammonia Oxidizing Bacteria (AnAOB)

Anammox bacteria are responsible for the oxidation of the ammonium in the presence of the nitrite, which serve as an electron acceptor under limited dissolved oxygen concentration. In the consequence nitrogen is transformed to the gaseous nitrogen, while about 10% from the initial sum of the ammonium and nitrite is remaining in the solution as a nitrate. Anammox are chemoautotrophic bacteria, which are a member of the original order *Planctomycetales* in frame of the *Planctomycetes* phylum [2] divided into 6 genera, *Candidatus* Kuenenia, *Candidatus* Brocadia, *Candidatus* Jettenia, *Candidatus* Anammoxoglobus, *Candidatus* Scalindua, and *Candidatus* Anammoximicrobium. AnAOB are commonly found in the various natural and artificial ecosystems such as, full and laboratory-scale bioreactors dedicated for wastewater treatment based on granular sludge and biofilm, as well in the freshwater or marine environment. In accordance to the literature, AnAOB reflect low growth rate at the moderate temperatures, for instance, their doubling time has been estimated around 17 days at the 20 °C while, by increasing the temperature to 32–33 °C, the doubling time increased up to 17 days with the maximum growth rate of

0.0027 day^{-1} [13,41]. On the other hand, Gilbert et al. (2015) revealed that by gradual adaption, anammox activity was obtained at low temperatures (from 20 to 10 °C) [42].

Pereira et al. [43] revealed that the optimum ranges of pH and temperature for maintaining the stable growth rate for AnAOB are between 6.7–8.3 and 20–43 °C, respectively. Ye et al. [44] investigated the influence of starvation modes on AnAOB and subsequently nitrogen removal efficiency under SBR operation conditions, short-term starvations (1–4 days) lead to the increase in the tolerance and activity of AnAOB, as well as the enhanced nitrogen removal efficiency to around 82.5%.

3. Factors Affecting Microorganisms Activity in Shortcut Nitrification Process

Temperature, DO, and pH are key parameters in wastewater treatment and their impact on the bacterial metabolism has been the subject of intensive research in recent years (Table 3). Such major operating factors can directly affect AOB, NOB, and AnAOB activity, as well indirectly influence other parameters e.g., free ammonia (FA) and free nitrous acid (FNA), indicating the importance of interaction among these factors to inhibit NOB activity through the shortcut nitrification process (Figure 3). Therefore, the following discussions focus briefly on the effects of the main operating factors on shortcut nitrification process efficiency and activity of the particular bacterial groups involved in nitrogen metabolism.
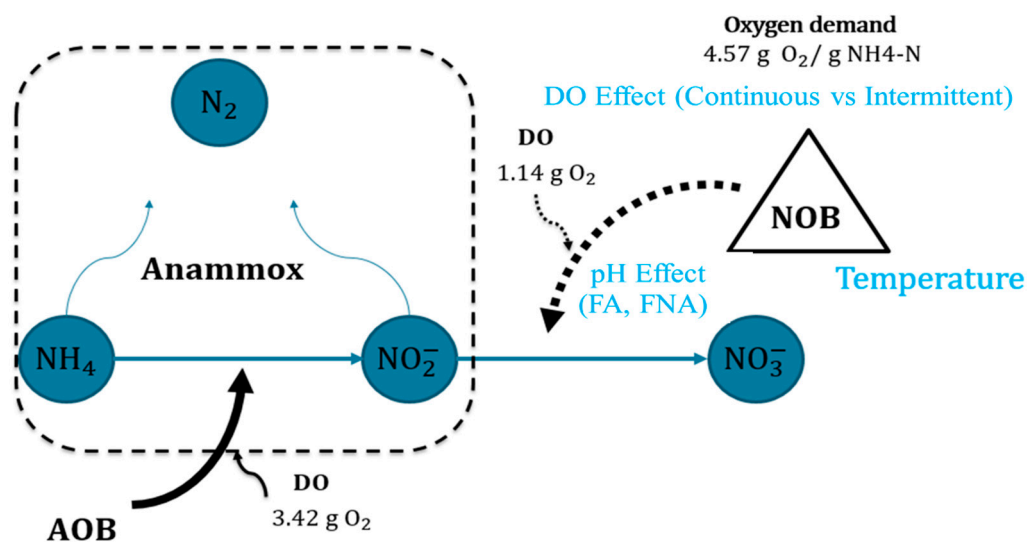

Figure 3. An overview of the role of influencing factor on nitrogen removal process. 3.42 g O$_2$—oxygen demand for ammonium oxidation to nitrite; 1.14 g O$_2$—oxygen demand for nitrite oxidation to nitrate; 4.57 g O$_2$—total oxygen demand for ammonium oxidation to nitrate [45].

3.1. Temperature

Based on the temperature dependency within nitrification process, obtaining optimal temperature can improve the shortcut nitrification process and maintain the beneficial ratio between AOB, NOB, and AnAOB. The specific growth rate of the AOB during partial nitritation increases more than NOB under temperatures higher than 25 °C [12]. By applying high temperatures and the low SRT, the inhibition of NOB activity, as well their washout from the system could be successfully achieved [46]. Furthermore, optimal temperatures for anammox-based technologies to achieve appropriate nitrogen removal efficiency have been proposed within the range 30–40 °C [47]. The highest activity of AnAOB was found at around 37 °C, whereas temperatures higher than 45 °C led to the full process inhibition [47,48]. Along with the temperature decrease, AOB outcompete AnAOB, lead to the accumulation of nitrite, ammonium, and subsequently nitrate within anammox-based systems. Rodriguez-Sanchez et al. [49] investigated the impact of the low temperatures down to 15 °C for a single-stage partial nitritation/anammox granular

sludge reactor, at the same time, 12.25% of the AOB and 9.50% of the AnAOB cooperated at an adequate level of mutual interaction for the stable nitrogen removal process [49].

Table 3. The interaction between major operational factors and alternative strategies.

Reference	Type of Reactor	DO (mg O$_2$/L)	pH	Temperature (°C)	Efficiency (%)	Strategy
[50]	Nitritation–anammox-one stage-MBBR-Biofilm	1.1–1.7	7.2–8.2	19–10	N removal 74–54	The process could recover under low temperature and stay stable by increasing DO concentration from 1.1–1.7 mg O$_2$/L.
[38]	MBR-one stage-activated sludge	Optimal 0.8–0.9	7.25–7.35	32–35	Nitrite accumulation in average 90.1	Low DO concentration together with elevated temperature and FA-FNA control were crucial factors for PN process.
[51]	IFAS	0.4–1.5	7.2–7.5	15 or 17	N removal 51	The attribution of intermittent aeration strategy with high DO concentration more than 1 mg O$_2$/L considered for controlling NOB activity.
[52]	One-stage deammonification systems	0.1–0.12	7.6–8.3	32	Ammonium removal in average, 72	The low range of DO was recovered by applying high temperature and FA (0.1–3 mg O$_2$/L) was effective for NOB inhibition.

Temperature reductions can influence the microbial community composition, thus affect shortcut nitrification efficiency. For instance, Lackner et al. [53] revealed that temperature decrease lower than 12 °C inhibited AOB and AnAOB activity, which resulted in drop of the ammonia conversion rate and nitrite accumulation for the both SBR and MBBR operated under lab scale.

The relation between temperature variations, the maximum specific growth rate of the microorganisms and activation energy could be described by (Arrhenius-type equation) (Equations (1) and (2)) in wastewater [54]. The theoretical range of temperature coefficients (θ) that shows the biomass activity has been reported between 1.02 to 1.10 for the high temperatures [55]. However, Guo et al. [56] showed a higher value for θ around 1.172 within lower temperatures 5–20 °C [56]. Furthermore, Sobotka et al., [20] found that θ within the batch test experiment was in agreement with the theoretical range, whereas by operating the long-term SBR test, temperature coefficients were 1.07 and 1.65 for (15–30 °C) and (11–15 °C) [21]. Activation energy factor (E_a) (Equation (2)) showed same responses with 111.5 kJ mol^{-1} under lower temperatures (5–20 °C) and 42.0 kJ mol^{-1} for warmer conditions (20–35 °C) [13,56]:

$$r_T = r_{293} \times \theta^{(T-293)}, \tag{1}$$

$$\mu_{\text{max},T} = \mu_{\text{max},20\,°C} \times \exp\left[-\frac{E_a}{RT}\right], \tag{2}$$

$$ln\,r_T = -\frac{E_a}{RT} + ln A$$

where:

$\mu_{max,T}$—Maximum specific growth rate (d^{-1});

$\mu_{max,20\,°C}$—Maximum specific growth rate (d^{-1}) under temperature at 20 °C;

E_a—The activation energy (KJ/mol);

R—8.314 (J/mol·K);

r_T—Reaction temperature at T;

θ—temperature coefficient;

A—the frequency factor for the reaction;

T—temperature in Kelvin.

Temperature fluctuations can influence the inhibitory impacts of free ammonia (FA) and free nitrous acid (FNA) on AOBs activities. Gabarró et al. [57] reported that FNA under both temperatures 25 and 35 °C affected AOBs activity averagely 35%, whereas FA only had an inhibitory impact nearly 22.1% at 35 °C [57].

On the contrary, the specific growth rate of AOB had a sharp drop around 1.5 times when the controlled temperature decrease from 25 to 15 °C was conducted [56]. Therefore, temperature changes influence the performance of the PN process due to the direct impact on the AOB and NOB population size. The growth rate of NOB within low temperatures between 10–20 °C, is usually higher than AOB, which leads to unfavorable AOB/NOB ration in the system and loss of the process stability [58].

However, partial nitration strategies were implemented successfully within temperatures lower than 20 °C. Isanta et al. [31] revealed that by simultaneous control of DO and ammonium concentrations ratio, partial nitration process could be successfully maintained under lowered temperatures. This strategy was also implemented by Bian et al. [59], where careful adjustment of low DO concentrations, in a continuous moving bed biofilm reactor, ensured efficient system operation, even under the lowest from the applied temperature of 6 °C. A stable autotrophic nitrogen removal process under temperature reduction from 20 to 10 °C in lab-scale conditions with granular sludge was successfully achieved with the nitrogen removal efficiency changing between 85–75%, when the DO concentration was increased up to 2.5 mg O_2/L [59].

Another solution for the successful PN/A systems operation under low temperatures is application of the technologies based on the high concentration of the biomass maintenance in the bioreactors such as MBBRs. For instance, Gilbert et al. [60] obtained stable anammox activity under temperature lower down 10 °C, with parallel NOB suppression. In another study by Zekker et al. [61], gradual biomass adaptation in MBBR to the lowered temperatures as low as 9 °C ensured comparable nitrogen removal rates as in case of the measurements conducted at the temperature 20 °C.

3.2. Dissolved Oxygen (DO) Set Point and Aeration Strategies

Dissolved oxygen concentration is considered as the main factor affecting activity of the bacterial communities and successful operation of the shortcut in the nitrogen removal processes. The stability of the partial nitrification/nitration relies on the inhibition of NOB activity, which depends on the dissolved oxygen concentration limitation during the reaction phase. Most researchers consider kinetic components that describe the oxygen dependency of bacteria, such as half-saturation constants specified for AOB and NOB in order to adjust the optimum DO set point in the system.

Cao et al. [62] observed a decreasing trend of the ammonium nitrogen concentration (conc. NH_4^+–N from 13.75 to 0.5 mg/L) in the effluent from the in moving bed sequencing batch reactor (MBSBR), when an increasing DO concentration was applied to the system. At the same time, the nitrate accumulation increased from 0 to 9.8 mg N/L. In another study, when DO concentration was set at 3.5 mg O_2/L conditions favoring for complete ammonia oxidation to nitrate were obtained [62].

Rodriguez-Sanchez et al. [38] revealed that value of the half-saturation constant for AOB and NOB were 0.2–0.4 and 1.2–1.5 mg/L, respectively, resulting that NOB activity is affected greater than AOB under low DO concentrations (0.8–0.9 mg O_2/L), leading to nitrite accumulation [38]. Li et al. [63] achieved total nitrogen removal efficiency nearly

81% under low DO concentration 0.6 ± 0.1 mg/L within a nitritation-anammox process in an up flow membrane-aerated biofilm reactor (UMABR).

The implementation of the low DO (0.5 mg O_2/L), SRT (4 days), and sludge return ratio (R = 2.5) in the MBR configuration has been proposed as a promising solution to suppress NOB activity along with a high rate of nitrite accumulation over 80% [11].

3.3. Intermittent Aeration Pattern

An intermittent aeration strategy is a cost-effective alternative for inhibiting NOB and enriching AnAOB due to the accumulation of nitrite and consuming by AnAOB under different shortcut nitrification systems [64,65].

Contradictory findings have been suggested in the literature regarding the application of aeration strategies including intermittent and continuous aeration. For example, a continuous pattern was considered more preferable than the intermittent aeration method, since higher removal rate of nitrogen and ammonium was attained along with shorter aeration period [1,66]. Furthermore, Sobotka et al. (2015) found that the accumulation of nitrite increased toward more than 100 mg N/L in the SBR under a continuous aeration pattern of which similar anammox activities were also observed based on the microbiological analysis [67].

On the other hand, the applicability and advantages of intermittent aeration strategy due to the inhibitory impact on NOB activities have gained strong interest worldwide. For instance, Y. Sun et al. (2017) reported that the intermittent strategy could promote the accumulation of nitrite, and after switching the continuous aeration method to the intermittent aeration system, NOB activities were dramatically limited, thus the stability of the reactor improved substantially [68]. Moreover, the nitrate build-up issue could be addressed under an intermittent aeration pattern (aeration 7 min/anoxic 21 min), resulting in the inhibition of NOB activities and higher nitrogen removal rate within the PN/A process [69]. The possible combination of intermittent aeration strategy under low temperature conditions was proposed for maintaining AOB growth rate and inhibiting NOB activities using the gradient DO level during a different aeration rate [70].

The sustainability of partial nitrification increased under an intermittent pattern due to the enrichment of AOB microorganisms and inhibition of NOB activities [71]. However, in mainstream conditions, intermittent aeration can influence the activities of AOB and then the system efficiency [72,73]. NOB activities would be inhibited by using an intermittent aeration cycle when NOB are not able to translate quickly from the anoxic to aerobic phase [74]. Miao et al. (2016) reported that NOB activity reduced from 14.6 to 2.82 mg N/(h·g_{VSS}) and nitrogen removal rate reached 73 mg N/(L·d) under intermittent conditions [69]. The nitritation process was achieved through intermittent-fed SBRs under 20–26 °C for 300 days operation, near 93% of ammonium oxidized to nitrite [75].

Blum et al. (2018) demonstrated that N_2O production decreased within partial nitritation in SBR when three major strategies were applied including, short-term cycles, low concentration of ammonium less than 100 mg NH_4^+–N/L, and high frequency in ON/OFF intermittent strategy [76].

The application of intermittent aeration with the ratio between the non-aerated and aerated period (1/3) showed to be particularly advantageous in a one-stage PN/A process including, the reduction in ammonium and nitrate concentration up to 17–20% within non-aerated phase, the maintenance of system stability even though the aeration time was decreased, the contribution of non-aerated phases in enriching AnAOB along with repressing NOB activity [77].

Bao et al. (2017) investigated the activity of AOB and NOB under high DO concentration, AOB/NOB ratio changed from 3/1 to 23/1, indicating direct relation by DO changes which proves the inhibitory impact of high DO concentration on NOB populations, especially *Nitrospira*-like bacteria [78,79].

3.4. pH Direct Effect

The pH dynamics through its effects on NH_3 and HNO_2 concentrations together with environmental variations can influence the community of AOB, NOB, and AnAOB, affecting nitrogen removal efficiency along with nitrous oxide emission. Sudden changes in FA and FNA concentrations changed by pH value can have an inhibitory effect on NOB activity. Therefore, the relationship between pH and FA-FNA, and their potential interaction with other operational factors such as DO and temperature, can affect NOB population under nitrite pathway methods.

The adjustment of pH between 7.5 to 8.5 could achieve a desirable effect on partial nitrification processes and the accumulation of nitrite [28]. Under decreasing pH values from 8.0 ± 0.1 to 7.5 ± 0.2, ammonia oxidation activities and following the amount of nitrite accumulation decreased, while the activity of atrophic bacteria was repressed when pH was adjusted at 6.5 in this test [80], whereas the increasing pH from 7.5 to 8.3 can enhance the rate of nitrite accumulation 1.68 ± 1.51 to $35.46 \pm 7.86\%$ [81].

Feng et al. (2017) revealed that optimum pH for AOB and NOB activities are estimated 7.4 and 6–7.5 under temperature 30 °C [13], whereas during another research study the fluctuation of pH between 7 and 8 would be a proper range to inhibit NOB under DO levels more than 0.5 mg O_2/L [82]. Likewise, a higher removal rate of ammonium was observed under high pH around 8 compared to other biofilters under lower pH at 7.6 [83].

Organic carbon and pH can influence the nitrification efficiency, hence, the bioaugmentation process could enhance the ammonia removal efficiency up to 95% [84]. Taylor et al. (2015) revealed pH as a major influencing factor on N_2O accumulation, where pH = 6 could inhibit microorganisms activities [85]. The contribution of pH variations indicated that the highest concentration of N_2O emissions occurred near to neutral pH within the partial nitrifying process [86,87].

With an increasing level of pH from 6.5 to 8 applied to an intermittently-fed SBR reactor, results showed that a seven-fold increase occurred in N_2O emissions and the $\Delta N_2O/\Delta NH_4^+$ ratio [88]. Furthermore, Massara et al. [89] confirmed that the maximum extent of N_2O production near 0.53 ± 0.04 (mg N_2O-N/h·g_{VSS}) could happen in pH = 8, and also a linear relation was observed between the AOR and the N_2O emissions within pH variation.

pH variations can affect the stability of the process due to the direct impact on FA and FNA concentrations. In previous research, it was reported that a reliable range of pH between 7–8 combined with low concentration of FA less than 2 mg NH_3/L can achieve a stable nitrogen removal rate and protect the AnAOB population and activity under the MBBR system [83,90].

Hultman equation [91]:

$$\mu_{\max}(\text{pH}) = \mu_{\max}(\text{pH})_{opt} \cdot \frac{1}{1 + k\left(10^{|\text{pH}_{opt} - \text{pH}|} - 1\right)}. \tag{3}$$

Michaelis equation (Antoniou et al. [92], Angelidaki et al. [93]):

$$\mu_{\max}(\text{pH}) = \mu_{\max}(\text{pH})_{0.5} \cdot \frac{1 + 2 \times 10^{0.5 \cdot (pK_{h1} - pK_{h2})}}{1 + 10^{(pK_{h1} - \text{pH})} + 10^{(\text{pH} - pK_{h2})}}. \tag{4}$$

3.5. FA and FNA Inhibition Effect

High concentrations of the ammonia in the influent or accumulation of the nitrite within the nitrification process, under proper conditions, lead to the occurrence of highly reactive and toxic free ammonia and free nitrous acid (FNA), respectively [94]. Especially, pH changes influence FA and FNA pool which interact to repress the growth rate of both AOB and NOB [95].

FA influences the activity of NOB more than that of AOB, while nitrite was accumulated due to the inhibitory effect of FA on *Nitrobacter* [96,97], for decreasing the activity

of AOB, FA concentrations in the range of 10–150 mg NH_3/L are needed whereas for inhibiting NOB, the range was 0.1–1 mg/L [98]. Moreover, increasing the amount of FA from 0 to 16.82 mg NH_3–N/L indicated the reduced effect of AOB and NOB activities down to 15.9% and 29.2%, respectively [99]. The nitrite was accumulated from 19.8–90% and nitritation successfully started up using the inhibitory effect of FA on NOB activities and real-time control strategy, under low temperatures (13.0–17.6 °C) [97]. Subsequently, the use of FA-based technologies could reduce the energy consumption rate from 0.27 to 0.14 kWh/m^3 sewage treated [100].

Furthermore, it has been reported that increases in FNA could reduce the activity of NOBs more significantly than that of AOBs, leading to the nitrite accumulation [7,101]. Moreover, it was demonstrated that the dominance of the AOB population and N_2O accumulation are quite sensitive to pH and FNA variations [86].

The AOB and NOB population could be affected by various concentrations of FNA, while the FNA concentration at the range of 0.42–1.72 mg/L, 50% of AOB was inhibited, NOB was completely inhibited, and the stabile PN/A process attained by applying an FNA concentration of 0.026–0.22 mg NH_3/L, indicating higher dependency of NOB to FNA changes than AOB [34,102,103].

An FNA-based strategy has also been considered as environmental friendly and economically beneficial methods due to producing methane and reducing the energy consumption for aeration, sludge handling, and sludge return line processes could also improve within applying FNA-based methods [104].

In the following equations [54], the relation of pH with FA and FNA concentrations (mg NH_3/L) is given:

$$FA = \frac{17}{14} \times \frac{TAN \times 10^{pH}}{e^{\frac{6344}{273+T}} + 10^{pH}},$$ (5)

$$FNA = \frac{47}{14} \times \frac{TNN}{e^{-\frac{2300}{273+T}} \times 10^{pH} + 1}.$$ (6)

Haldane equation:

$$\frac{\mu_{max}(pH)}{\mu_{max}(pH)_{opt}}(FA, FNA) = \frac{1}{\left(1 + \frac{K_{m,FA}}{FA} + \frac{FA}{K_{i,FA}}\right)} \cdot \frac{1}{\left(1 + \frac{K_{m,FNA}}{FNA} + \frac{FNA}{K_{i,FNA}}\right)},$$

where TAN is total ammonium nitrogen and TNN is total nitrite nitrogen. T in °C.

The pH changes based on (Equations (5) and (6)) can increase the concentration of FA within high pH and accumulate FNA during nitrification processes under low pH, therefore, the adjustment of pH in order to control the inhibitory effect of FA and FNA on autotrophic bacteria behaviors can lead to optimize the nitrogen removal efficiency.

The bioaugmentation strategy is proposed as an effective way to reduce the SRT under nitrification process, as well the possible reduction of aeration time occurred when excess sludge brought the from sludge treatment system added to the wastewater system [105].

4. Cost and Energy/Resources Reductions Comparing Conventional & Deammonification

It has been reported that the application of nitrite pathway methods coupled with anammox-based technologies can reduce operating and maintenance costs, including aeration, carbon demand, sludge disposal, and N_2O emissions up to 85% on average [106,107]. Moreover, nitrogen removal costs through the nitrite pathway lead to a great reduction in oxygen demand (more than 50% on average), as well reduces the energy consumption to remove 1 kg of the nitrogen from 2.4 kWh/N kg under conventional process to 1 kWh/N kg within PN/A technology [108,109]. Figure 4 compares the overall energy consumptions by the conventional and deammonification method, including oxygen, carbon, and N_2O emission. The level of overall energy consumption within the conventional method was

estimated at around 30 kWh/PE·year, which is almost twice higher in comprehension to the 17 kWh/PE·year estimated for the deammonification system [110].
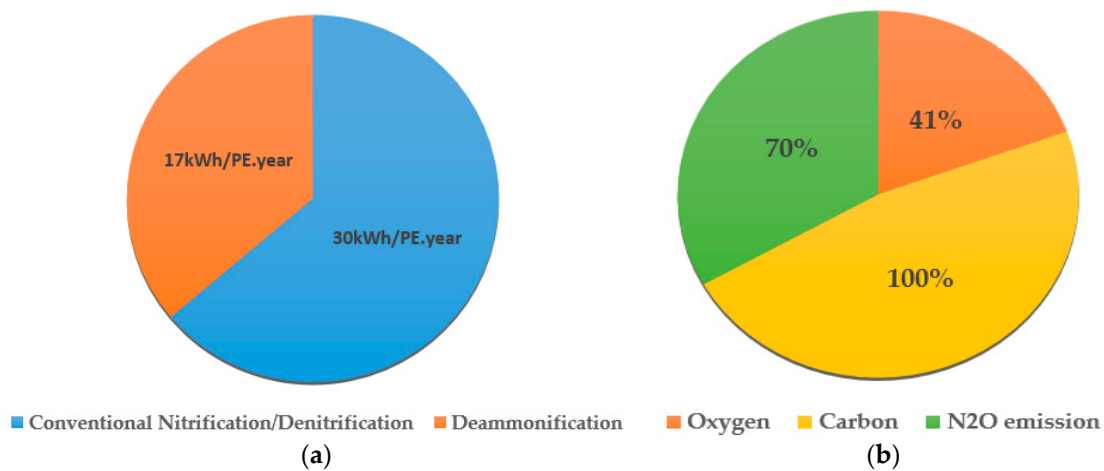

Figure 4. The role of deammonification vs. conventional nitrification/denitrification process on the decrease of overall amount of energy consumption (**a**) and oxygen consumption, nitrous oxide, and carbon from Wastewater Treatment Plant WWTP (**b**) based on [110,111].

4.1. Energy Consumption in One-Stage Deammonification Systems

In contrast, within one-stage reactors, partial nitrification and anammox processes occur simultaneously, hence the produced nitrite from the PN process is instantly converted to nitrogen gas by applying the anammox bacteria. Moreover, one-stage reactors have attracted more attention while nearly 88% of plants, due to cost-saving potentials and operational aspects, in comparison with two-stage configuration [1,112].

In addition, more than 50% of plants have made efforts to implement the deammonification process in WWTPs through the sequencing batch reactor (SBR) [66]. SBR would be operated with the use of granular anammox and suspended biomass to remove nutrients under intermittent or continues aeration within the lowest possible DO concentration [1,66].

The popularity of the deammonification method is highly developed for the side-stream with high temperature and highly concentrated ammonium around 1 g N/L in wastewater [12]. The implementation of this method in the side-stream line has beneficial results, since it decreases the competition between AOB and NOB bacteria under different affecting factors, including low DO concentrations, high temperatures, low SRTs, and free ammonia (FA) treatment [9,113]. Moreover, findings have demonstrated that the application of PN/A technology in the side-stream line is capable of removing total nitrogen from reject water, up to 88% with high ammonium removal efficiency near to 92% [4].

Therefore, the ideal configuration for the deammonification process in the side-stream would achieve advantages such as 60% and 85% reduction costs for aeration and sludge handling purposes, the use of accumulated nitrite as a substrate for AnAOB and the mini-mization of carbon demand approximately 100% for the denitrification process [106,114]. Moreover, within the deammonification process, a huge percentage of biodegradable organic matters convert to biogas due to the inhibition of denitrification bacteria activity, leading to expedite the procedure of nitrogen removal. [115].

To date, around 75% of PN/A reactors have been designed for side-stream line due to the energy-saving aspects while the feasibility of such methods has drawn special attention for the mainstream. The low concentration of ammonium (15–50 mg NH_4^+–N/L) and temperature (10–15 °C) in the mainstream as major factors can make challenges for process stability and the growth rate of microorganisms in comparison with side-stream [9,53].

Furthermore, different operating factors can affect the performance of the PN/A process in the mainstream, for instance, high ratio of COD/N in municipal wastewater

leads to the inhibition of AnAOB activity; recently, Al-Hazmi et al. (2019) successfully operated the PN/A process under a mild ratio of C/N (1–3), when the ratio increased from 1 to 3, total nitrogen removal efficiency increased from 78.96% to 82.92% and 36.7% to 63.3% in the 1st and 2nd series and, respectively [116]. Moreover, other components contributing to the PN/A process are: lower alkalinity conditions can increase the requirements for adjusting pH, the control of strong competition between AOB and NOB bacteria to use oxygen substrate, the accumulation of nitrite and the considerable start-up time >1 year due to the anammox-based technology to achieve desired nitrogen removal efficiency [15,114].

In addition, mathematical modeling prepares proper conditions to better understand the microbial competition under various operational and environmental changes in order to optimize the process of deammonification [117]. For instance, the application of a dynamic model was investigated to simulate the behavior of anammox within different DO concentrations and evaluate the changes in the microbial community during competition and interaction with other bacteria [118].

4.2. Energy Consumption in Two-Stage

Two-stage configuration, as an alternative strategy, is comprised of two separated processes. Within stage A considered as a pre-treatment process under high rate activated sludge (HRAS) and chemically enhanced primary treatment (CEPT), the COD concentration is reduced to achieve the lowest range of organic compounds in the effluent. Then, during stage B, the partial nitritation combined with the anammox process is implemented under low COD/N ratio (Figure 5). Due to the slow growth rate of the anammox bacteria, maintaining the balance between stage A-B and the controlling adequate retention time for the anammox process can influence the process stability [12]. One of the most important advantage of two-stage method is the reduction of competition between NOB and AnAOB over nitrite substrate compared with the one-stage method [31]. However, the implementation of the two-stage method based on the separated stages includes some drawbacks. For instance, higher maintenance costs, the challenge of mass balance between the output sludge for transferring to anaerobic digestion and returned sludge including high COD concentration in stage A, and as well as high temperature and SRT dependency of AnAOB for growing in the mainstream line which can cause challenges within the process [12].
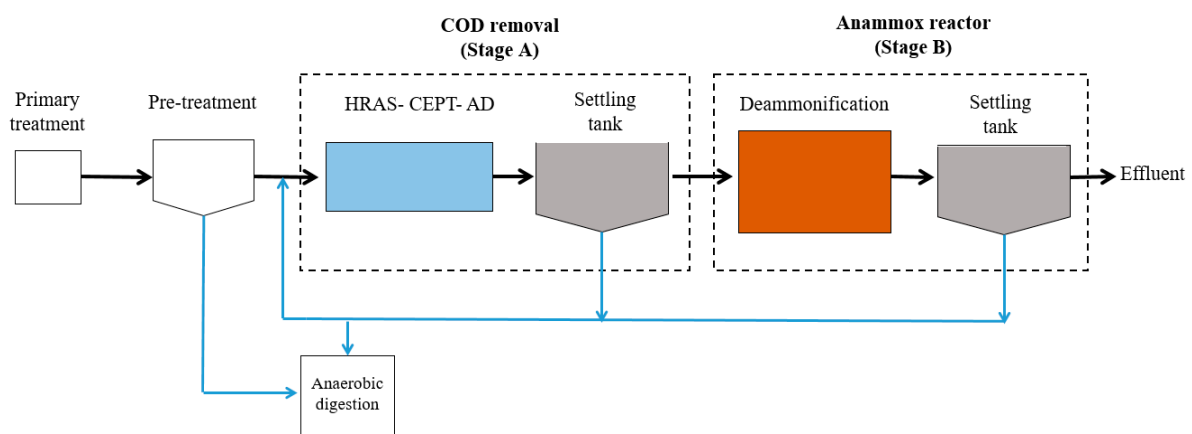

Figure 5. A two-stage configuration of the deammonification process based on [12].

For instance, a successful operation of two-stage reactor configuration was reported by Gu et al. [119], while within stage A, 58% of influent COD was recovered to methane gas and was followed by around 75% reduction of sludge production, as well as 87% of total inorganic nitrogen (TIN) was simultaneously removed from the system, afterward during stage B, the nitritation-denitritation process, together with anammox strategy, were applied to remove nitrogen effectively.

5. Mathematical Modeling for Shortcut Nitrification Processes

The mathematical models can be highly useful in order to simulate the behavior of kinetics within biological processes under different operating scenarios. These cost-effectiveness models can significantly save operation costs and time combined with applying modifications in both the lab and pilot scale. Despite the advantages in operation aspects, new models can make a bridge between past, present, and future to find the optimum range of influencing factors participating in nitrogen removal processes for enhancing the process efficiency (Figure 6).

5.1. Mathematical Kinetics Behavior of Microbial Community

The kinetic of parameters can attribute to better understanding of the impact of operational factors on the activities of AOB, AnAOB, and NOB and comparing the effect of various factors applied to the system in order to optimize the nitritation pathway and inhibit NOB activities. The most common kinetic approaches to understand the growth rate of nitrifying bacteria are based on the Monod equation [120].

The principle kinetic parameters within the Monod equation are the maximum specific growth rate of AOB ($\mu_{max,AOB}$) and NOB ($\mu_{max,NOB}$). Diverse parameters can influence the growth rate of AOB and NOB in model calibration, for instance: the substrate concentrations, endogenous decay rate, temperature, DO, and maximum specific growth rate [121]. Moreover, the growth rate of AOB is related to the concentration of ammonium in influent which could be followed by the NOB repression process. Oxygen is known as a common substrate for both AOB and NOB, hence the affinity of AOB and NOB for oxygen are represented by half-saturation constants ($K_{O_2,AOB}$, $K_{O_2,NOB}$) as sensitive parameters in the Monod equation [2].

In the following, an activated sludge model (ASM) based on Monod equations for the growth rate of AOB and NOB is presented:

$$\mu_{AOB} = \mu_{max,AOB} \times \left(\frac{S_{NH4}}{S_{NH4} + K_{NH4}} \right) \times \left(\frac{S_{O2}}{S_{O2} + K_{O2,AOB}} \right) - b_{AOB}, \tag{7}$$

$$\mu_{NOB} = \mu_{max,NOB} \times \left(\frac{S_{NO_2^-}}{S_{NO_2^-} + K_{NO_2^-}} \right) \times \left(\frac{S_{O2}}{S_{O2} + K_{O2,NOB}} \right) - b_{NOB}, \tag{8}$$

$$\mu_{AnAOB} = \mu_{max,AnAOB} \times \left(\frac{S_{NH4}}{S_{NH4} + K_{NH4}} \right) \times \left(\frac{S_{NO_2^-}}{S_{NO_2^-} + K_{NO_2^-}} \right) - b_{AnAOB}, \tag{9}$$

where:

μ_{max}—maximum growth rate (d^{-1});

S_{NH4}, S_{NO2}, $S_{O_2^-}$ concentration of ammonia (mg N/L), nitrite (mg N/L), DO (mg O$_2$/L);

K_{NH4}, $K_{NO_2^-}$, $K_{O_2^-}$ ammonia, nitrite half-saturation constant (mg N/L), oxygen half-saturation (mg/L) b is the decay rate (d^{-1}).

As well, as another application of the Monod equation for describing the inhibition approaches with different substrates have been shown [122]:

$$q = q_{max} \times \left[\exp\left(-\frac{S_{NH4}}{k_i} \right) - \exp\left(-\frac{S_{NH4}}{k_s} \right) \right], \tag{10}$$

$$q = q_{max} \times \left[\exp\left(-\frac{S_{NO_2^-}}{k_i} \right) - \exp\left(-\frac{S_{NO_2^-}}{k_s} \right) \right], \tag{11}$$

where:

q—Specific growth rate per day;

q_{max}—maximum specific growth rate per day for different bacteria;

S—substrate concentration (mg/L);

k_s and k_i are half saturation constant and inhibition constant (mg N/L), respectively.

5.2. The Application of Mathematical Models

An activated sludge model no.1 (ASM1) describing simultaneous nitrification and denitrification was developed under low DO concentration less than 0.6 mg O_2/L, the proposed model displayed the sensitivity of AOB and NOB rather than DO variations [123].

An optimization framework was applied by the use of ASM3 to adjust the optimal aeration mode within an intermittent strategy under a partial nitrification pathway, followed by reducing energy consumption, as well as the linear constraints of the model facilitated its application for a quicker optimization process [124].

The application of an integrated mathematical model was investigated to observe N_2O emissions by AOB and enhance nitrogen removal efficiency under controlled DO and nitrite concentrations [125]. In another paper, Guisasola et al. [126] utilized the ASM2d model to predict the behavior of N_2O emissions within the control of DO variations for a municipal wastewater under anaerobic/anoxic/oxic configuration, the maximum extent of N_2O emissions was estimated approximately 22% when DO and ammonium concentrations were controlled around 1.1 mg O_2/L and 40, respectively [126].
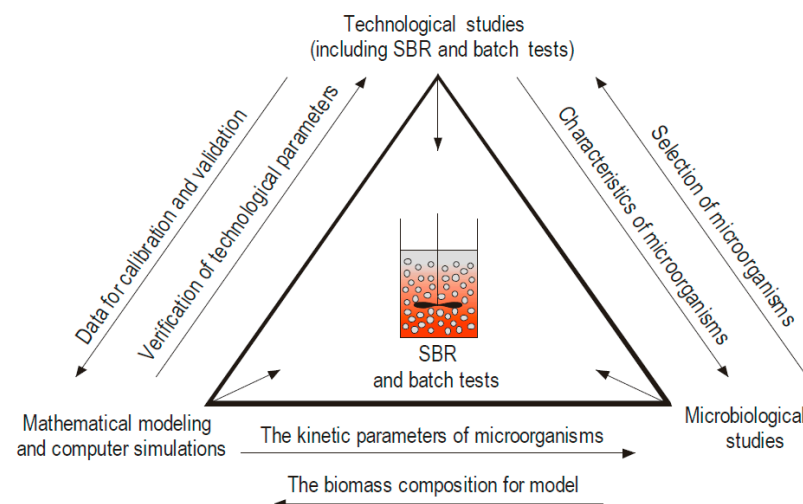

Figure 6. Interactions between technological, microbiological, mathematical modeling, and computer simulations approaches [127].

A recent modeling study predicted pH changes using modified nitritation and nitratation models, which developed to identify an optimal zone of influent ammonium, bicarbonate concentrations, and operating factors for achieving a high-efficient partial nitritation process [128].

A modified activated sludge model (ASM2d) was developed to simulate the behavior of major elements COD, ammonium, and phosphate variations which were monitored according to the same results as the original ASM2d model; interestingly, the oxygen uptake rate (OUR) computed by modified model showed higher value, around 5.7% compared with the basic model [129].

5.3. Model-Based Simulations of NOB Suppression

The one-dimensional multispecies biofilm model was used to simulate the performance of the granular sludge reactor, the proposed model was developed to determine the optimum range of operating parameters to attain a stable nitrogen removal process due to the inhibition of NOB activities constantly, based on the sensitivity analysis; K_{O_2}, NOB/K_{O_2}, AOB ratio was recognized as main factors on NOB repression [130].

The competition among r-AOB, K-AOB, and NOB was investigated by the development of a mathematical model which was solved by MATLAB 7.5 software. The model

outputs proved that autotrophic competitions are more dependent on SRT and NH_4^+ concentration within shortcut nitrification pathways [131].

In the same paper, Al-Omari et al. (2015) developed two strategies to repress NOB activity; the first one for a mainstream deammonification pilot was simulated based on the interaction among ammonium changes versus (nitrate + nitrite) (AVN), resulting in successful NOB out-selection and reduced additional carbon costs up to 60%. In following, the second one was the combined control of SRT and AOB seeding (Bioaugmentation) in order to maximize the AOB/NOB ratio for inhibiting NOB activity and recovering the AOB community [132].

An activated sludge No.1 (ASM1) was proposed to investigate the impact of different operating factors such as DO, SRT, and temperature on the nitrification process. The results generated by MATLAB/Simulink software showed that the DO was the most crucial factor, of which changes can extremely influence the process efficiency, as well as increasing DO was monitored as an effective strategy to improve the stability of the process within temperature variations [133].

Furthermore, the influence of DO limitation under intermittent strategy was evaluated by the development of an one-dimensional model. The results indicated that FA variations resulting by pH changes could primarily affect NOB activities, moreover, they revealed that in counter diffusion biofilm, pH adjustment is more crucial for suppressing NOB than DO changes [134].The growth rate of autotrophic bacteria within a partial nitrification process in the SBR system was described by the use of inhibition models and basic growth models based on Monod equation, the simulation outputs demonstrated that AOB retained and NOB washed out from the system under low DO concentrations in granular SBR as well as simultaneous inhibitory effect of FA and FNA varying sensitively by pH changes [135]. Examples of the mathematical models application have been summarized in Table 4.

Table 4. The application of different mathematical models within nitrogen removal processes.

Reference	Type of Reactor	Process	Ammonium Concentration (mg N/L)	Optimum DO (mg O_2/L)	Model Type	Strategy
[130]	Granular sludge	single-stage nitritation-anammox	-	1	One-dimensional multispecies biofilm model	Applying different DO and ammonium concentration
[136]	complete autotrophic nitrogen removal biofilm reactor	nitritation and anammox	<50	0.5	One-dimensional biofilm model	Biofilm characteristic, DO changes, ammonium concentrations effects on N_2O
[133]	membrane biological reactor	anaerobic-anoxic-aerobic	-	1–3	ASM1	Effect of DO, SRT and temperature
[134]	membrane-aerated biofilm reactors (MABRs)	nitritation	33		A pH-explicit 1-D multi-species nitrifying biofilm model (MSNBM)	Intermittent aeration, considering DO limitation, direct and indirect pH effects
[137]	UCT-MBR	nitrification and denitrification	-	<1.5	ASM2d	The prediction of N_2O based on uncertainty for optimizing plant processes
[128]	SBR	partial nitritation	19–84	2	ASM3	Predicting the behavior of operating and influent conditions for partial nitrification process

6. Perspectives

Several studies have shown that shortcut nitrification methods coupled with anammox processes have gained increasing attention due to being cost-effective. The implementation of such methods in the side-stream line for high-strength wastewater has beneficial results, since it decreases the competition between AOB and NOB under low DO concentration, high temperature, low solids retention time (SRT), and free ammonia (FA) treatment. However, the application of such technologies has become state of the art for mainstream conditions under lower ammonium concentrations and seasonal temperatures. To date, the deammonification process under both lab-scale and full-scale conditions have become popular due to an increase in reducing the oxygen demand followed by cost-saving achievements, the elimination of carbon demands for denitrification process, and the improvement of sludge handling aspects [13]. However, the performance of shortcut nitrification strategies including the deammonification process depends on the enrichment of AOB and AnAOB population and the inhibition of NOB activities. Recent studies have revealed a variety of perspectives to improve the performance of shortcut nitrification methods including:

1. The changes in reactor operation and configuration, for instance, the elimination of carbon within a partial nitrification process with the application of two-stage configuration [12].
2. The recognition of functional microbial communities and their features for considering the appropriate strategies to outcompete NOB species against the AOB community.
3. The identification of main operating factors affecting AOB and NOB competition within different phases in order to apply the optimum range of such parameters for maintaining the stability of the process. The main bottlenecks in this step are related to the differences of specific growth rates of bacteria under various operating conditions. For instance, the high DO concentrations can enhance the activity of both AOB and NOB, whereas due to the slower growth rate of AOB than NOB, the complete nitrification process could occur by AOB repression and nitrate accumulation. Moreover, AnAOB are sensitive to aerobic conditions while their activities will be inhibited under aerated processes. Therefore, the optimal operational conditions should be further considered for enriching AOB and AnAOB over NOB based on their dominating species (K and R-strategists).
4. Alternative and supplemental strategies have gained attention due to maintaining the stability of the nitritation process, but these strategies need to be further studied to suppress NOB activities based on the mutual interaction between different operating parameters. Such possible strategies might be: (1) intermittent aeration operation as an effective method to successfully inhibit NOB activities under optimal aeration frequency under aerobic/anoxic conditions; (2) gradual decrease of temperature coupled with applying biomass to the system as an alternative to perform shortcut nitrification methods even under lower temperatures [61]; (3) bioaugmentation strategy may be useful to increase the nitrogen removal efficiency, the enrichment of nitrifier activities, and quicker start-up even under lower temperatures and DO concentrations [105,138]; (4) FA and FNA can inhibit AOB and NOB activities, as well as this inhibitory impact of FA and FNA on NOB is much more than AOB, so this method should be further investigated to identify the optimum range for these variables based on pH changes [7]; (5) the use of supplemental process to make the nitritation process much more efficient and decrease the consequences of carbon sources on the growth rate of bacteria under mainstream conditions. For instance, the use of carbon substrate for developing novel technologies based on simultaneous partial nitrification, anammox, and denitrification (SNAD) [139]; (6) To date, extensive efforts have been done for evaluating the interesting possibility of applying different types of biomasses including suspended sludge, biofilm, and granules within the nitrogen removal processes. Furthermore, the combination of different biomasses such suspended sludge (the enrichment of AOB-NOB activities) and biofilm or granules (the improvement of anammox activity)

into reactors are gaining increasing interest for nitrite pathway methods which can enhance nitrogen removal efficiency (Figure 7, Table 5). Laureni et al. (2019) reported that the combination of biofilm with floc biomasses can have a significant effect on AnAOB and AOB-NOB activities, which was successfully followed by inhibiting NOB activity under decreasing DO concentration from 1.2 to 0.17 mg O_2/L (Figure 8) [140].
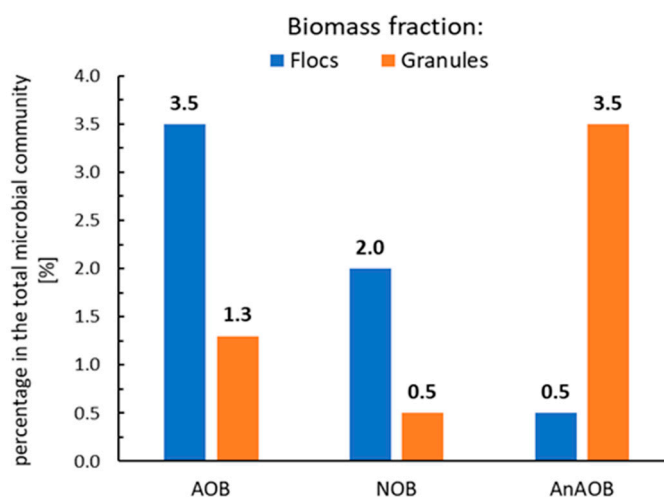

Figure 7. The abundance of different bacteria under the presence of flocs and granules based on [141].

Table 5. The introduction of different biomasses retention strategies in literature.

| Reference	Type of Reactor	Type of Biomass	AOB/NOB	DO (mg O_2/L)	Temperature (°C)	Removal Efficiency
[53]	PN-A	Biofilm	(AOB/NOB) > 1 Within >17 °C	0.44 ± 0.15	10–20	-
[31]	partial nitritation	Granules	72 ± 8 to 81/19 to 1	1–5	12.5	-
[139]	partial nitrification granules	Granules	0.4/-	2.5–7	25 ± 1	7.8–8.2%
[140]	partial nitritation	Granules	41 and 65/1.4 And 92 ± 4/1 ± 1	0.5–2.5	10	-
[141]	PN	Biofilm (R2) + Activated sludge(R1)	R1(62.5/6.5–1.8) R2(47.3/0.8–2.6)	0.3	14–16	ARE = 100%
[142]	SND	Biofilm	0.082 in DO = 4.5/0.766 in DO = 2.5	1.5–5.5	30 ± 2	83.73%
[143]	SBR	Activated sludge	3.93/1.09	0.1–0.6	26.6–16.6	Average of ammonium removal, 93%
[144]	PN/A	micro-granule	10.5/8.2	0.1–0.2	25	72 ± 10%
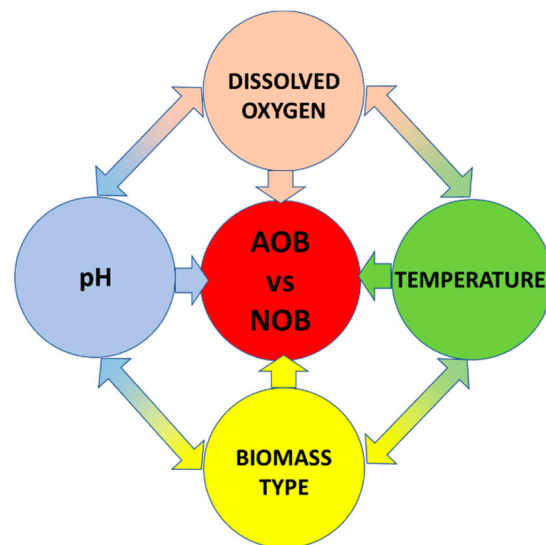
Figure 8. Interactions between different operational factors and ammonium oxidizing bacteria (AOB) - nitrite oxidizing bacteria (NOB) competition.

Model-based approaches could be useful for simulating the influence of operational parameters on nitrogen removal efficiency due to enhancement AOB and AnAOB activity, as well inhibiting the activity of NOBs for different configurations. Furthermore, the application of mathematical models would be further considered as a fast and fairly accurate optimization framework in order to calibrate models for prediction purposes.

7. Conclusions

The main challenge for implementing a shortcut in nitrogen removal processes from the wastewater is related to the strong competition between functional microbial groups responsible for nitrogen metabolism. In particular, activity of the NOB negatively affects the stability of the nitrogen removal processes which is the result of high operational and maintenance costs. Several studies have focused on the single impact of influencing factors on NOB activity (e.g., limited DO and low temperature conditions, intermittent aeration strategies, FA-FNA approaches); however, the simultaneous effect of various strategies is still represents a significant amount of missing knowledge under lab-scale and large-scale conditions. Therefore, the development of combined strategies for identifying the optimum operational conditions along with the use of a relevant type of biomass can kinetically inhibit NOB activity and enrich AOB activity, leading to simplify the nitrification process and decrease the additional aeration consumption and carbon source. Furthermore, model-based methods can be employed to better understand the kinetic behavior of different bacteria and investigate different inhibitory strategies for repressing NOB activity and subsequently, making the deammonification process easier to maintain, more cost-effective, and environmentally sustainable.

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