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Review

The Summary of Nitritation Process in Mainstream Wastewater Treatment

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Abstract: The application of the mainstream partial nitritation/anammox (PN/A) process is promising due to the huge cost reduction compared to traditional biological nitrogen removal. However, the nitrite production rate (NPR) of a biological nitritation pre-treatment process is relatively lower than the nitrite consumption rate in a pure anammox reactor with a high nitrogen loading rate (NLR). Thus, the NPR is the rate-limiting step for operating the PN/A process with a higher NLR. Various studies have attempted to improve mainstream NPR. A comprehensive review of these processes is needed for the actual application of the PN/A process. This study focuses on: (1) various nitrite production processes that have emerged in recent years; (2) the main microbial species and characteristics involved in biological nitritation; (3) the existing problems and the N_2O emission problem of these processes; and (4) a proposed novel and promising PN/A process facilitated with photocatalyst oxidation. This review is expected to provide references and a basis for the research on the nitritation step of the application of the mainstream PN/A process.

Keywords: nitrite production; nitritation; photocatalytic oxidation; mainstream wastewater; N_2O emission; anammox

Citation: Zhao, H.; Guo, Y.; Wang, Q.; Zhang, Z.; Wu, C.; Gao, M.; Liu, F. The Summary of Nitritation Process in Mainstream Wastewater Treatment. *Sustainability* **2022**, *14*, 16453. https://doi.org/10.3390/su142416453

Academic Editors: Yu-You Li, Yu Qin and Jiayuan Ji

Received: 7 November 2022
Accepted: 7 December 2022
Published: 8 December 2022

1. Introduction

Since its discovery in the 1990s, the anammox process has been widely developed as a promising technology due to its obvious advantages of energy reduction and high efficiency. The annamox process has shed light on the revolution of wastewater treatment plants (WWPTs) towards the energy-neutral even output installations [1,2]. In an anammox reaction, the reactants of ammonium (NH_4^+) and nitrite (NO_2^-) can be mainly converted to nitrogen gas to achieve nitrogen removal. The reaction ratio of NO_2^--N/NH_4^+-N is usually considered to be 1.146 for mainstream wastewater [3]. Given that the main nitrogen species in actual mainstream wastewater is NH_4^+-N, the required NO_2^--N/NH_4^+-N ratio for the anammox reaction can be achieved through the conversion of the part of influent NH_4^+-N to nitrite. However, the nitrite consumption rates of pure anammox reaction in many studies are noticeably higher than the nitrite production rate (NPR) in nitritation research for mainstream wastewater [4–6]. Thus, the nitritation process is the rate-limiting step of the partial nitritation/anammox (PN/A) process for chasing higher nitrogen removal rate (NRR) and strategies and solutions to improve NPR are urgently needed.

As is indicated in Figure 1, there are two main strategies for achieving nitrite production: the biological nitritation process and the physicochemical photocatalytic oxidation process. In the physicochemical photocatalytic oxidation process, the reaction involves oxidizing ammonia to nitrite with a combination of light and a photocatalyst. The main drawback of this method is that the remaining oxidant may oxidize nitrite to nitric acid. Current research shows that changing the type of catalyst used can effectively inhibit the oxidation of nitrite. TiO_2 modified with Ag_2O showed excellent purification performance in the study of photocatalytic oxidation treating 100 mgNH_4^+-N/L synthesis wastewater. Among them, the nitrite production efficiency (NPE) reached 40.8% with 0.12 kg/m^3/d NPR at a temperature of 21 °C, which is twice the catalytic effect of ordinary TiO_2 [7,8]. This method is promising because the temperature and ammonium concentration are close to mainstream wastewater treatment conditions. Additionally, photocatalytic oxidation will not cause N_2O emission like the biological nitritation process does and N_2O production can be ignored without aeration [9].

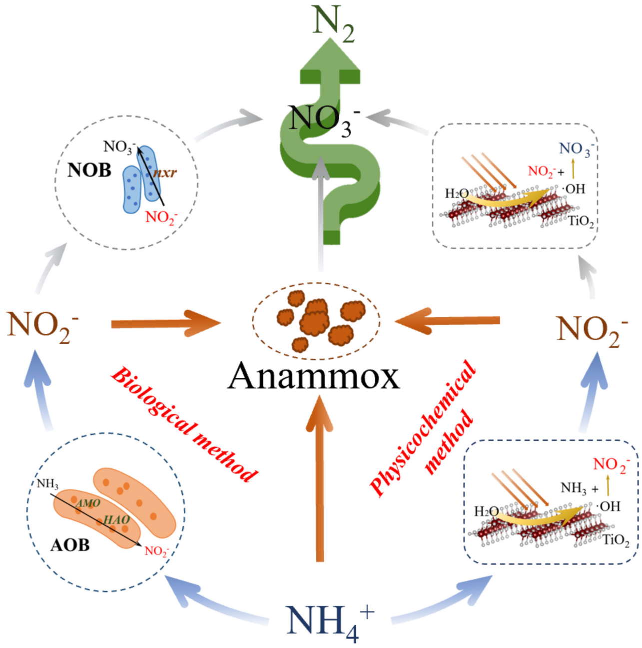

Figure 1. The nitrogen removal pathway of nitrite production and anammox process. Nitrite production process mainly includes a biological method and a physicochemical method.

The biological nitritation method is to oxidize ammonium to nitrite using ammonium-oxidizing bacteria (AOB). One of the major reasons for the difficulty in achieving a high NPR and NPE for biological nitritation is the presence of nitrite-oxidizing bacteria (NOB), which can further oxidize nitrite to nitrate. Generally, the control of operating conditions such as temperature, pH, free ammonium (FA), free nitrite acid (FNA), etc., can effectively suppress NOB [3,10]; however, the effect of these conditions cannot reach the requirements of anammox in mainstream wastewater [11,12]. Therefore, some studies have attempted to improve the NPR of nitritation through physical, chemical, and other assisted methods and have made promising progress. For example, in a previous study nitritation assisted by light for synthetic wastewater treatment was achieved with an NPR of 0.13 kg/m^3/d at 25 °C and influent 30 mg NH_4^+-N/L [13]. Another study obtained an NPR higher than 1.0 kg/m^3/d by adjusting salinity to 10 g NaCl/L at 30 °C and 100 mgNH_4^+-N/L [14]. However, NO_2^- may be reduced to Nitrous oxide (N_2O) by AOB at elevated nitrite concentrations or low oxygen conditions in the nitritation process, which is known as [15,16]. Higher NPR values increase the concentration of nitrite in the reactor, which may exacerbate nitrifier denitrification and higher NPR may therefore weaken the nitritation efficiency. However, the fluctuation of influent quality and quantity, low temperature, improper pH, and other harsh conditions will have unignorable effects in the application of biological nitritation and photocatalyst oxidation for actual mainstream treatment. Therefore, it is significant to conduct a comprehensive review of both these processes for selecting a properly effective method to achieve the efficient nitrite production and less N_2O in specific practical situations.

This paper summarizes the current methods of nitrite production, reviews the microbial main species and characteristics involved in biological nitritation process, outlines the existing problems and N_2O emissions in various nitrite production processes, and puts forward the promising process of photocatalytic oxidation for nitrite production and an anammox process for nitrogen removal.

2. Photocatalyst Oxidation

There are many physicochemical methods for wastewater ammonium removal including stripping [17], adsorption [18], photocatalytic oxidation [7], breakpoint chlorination [19] and more. These methods usually directly convert ammonium into harmless nitrogen or nitrate, except for photocatalytic oxidation, which can be used to generate nitrite and accumulate. Photocatalytic oxidation refers to a process in which light irradiates the surface of a photocatalyst, resulting in the electrons on its surface absorbing the photo energy and becoming photoexcited electrons. The photoexcited electrons convert to the empty conduction band from the filled valence band, leaving holes in the valence band. Subsequently, an electron–hole pair (e^--h^+) is generated [20]. Holes combine with H_2O to form a variety of oxidants, mainly hydroxyl radicals, and pollutants are degraded by reacting with hydroxyl radicals in the wastewater, as is shown in Equations (1) and (2) [21]. The main pollutant, ammonia, will be oxidized by hydroxyl radicals to various products such as $NO_3{}^-$, $NO_2{}^-$, and N_2.

$$H_2O + h^+ = OH^{\cdot} + H^+ \tag{1}$$

$$R - H + OH^{\cdot} = R'^{\cdot} + H_2O \tag{2}$$

Titanium dioxide (TiO_2)-based materials are promising photocatalysts for $NH_4{}^+$-N/NH_3 oxidation [7,9,22,23]. Nevertheless, the performance of the naked TiO_2 is unsatisfactory, due to the fact that recombination (in which the valence band holes and conduction band electrons simply recombine to liberate heat or light [24]) is easy to incur in most instances when light shines on TiO_2. However, TiO_2 modified with metals or their oxides can inhibit the recombination. Particles of metals or their oxides dispersed on the surface of TiO_2 can also produce electrons and holes. These electrons and holes change with electrons and holes produced by TiO_2 to inhibit the recombination [7,24]. Therefore, these photocatalysts have higher efficiency.

Modifying TiO_2 catalysts with different metals makes their surface adsorption energy intensity different. According to the difference of surface adsorption energy intensity, it has been suggested that the removal of ammonium can be divided into two ways [7,25], as is shown in Figure 2. In path I, the hydroxyl radical formed by Equation (1) reacts with ammonium to produce amino radicals and the two amino radicals combine to produce hydrazine. Hydrazine will be photochemically converted to diazene (N_2H_2). Finally, diazene can easily decompose into hydrogen and nitrogen under ambient temperature and pressure. The Pt and Pd surfaces have the best medium atomic nitrogen affinity for the production of dinitrogen, which makes the use of such catalysts more conducive to the production of nitrogen through path I [22]. In path II, ammonium molecules or amino radicals react with hydroxyl radicals to form hydroxylamine. Hydroxylamine is continuously oxidized by hydroxyl radicals to form nitrite ions and nitrate ions. The surface affinity of Ru and Rh is too strong to recombine and Au and Ag are too weak to produce active intermediates. Therefore, TiO_2 catalysts modified with Ru, Rh, Au and Ag are comprised to realize stable nitrite production. Some other catalysts can also be used to produce $NO_2{}^-$ and $NO_3{}^-$ in the photocatalytic oxidation reaction, such as atomic single layer graphic-C_3N_4 (SL g-C_3N_4) [26].

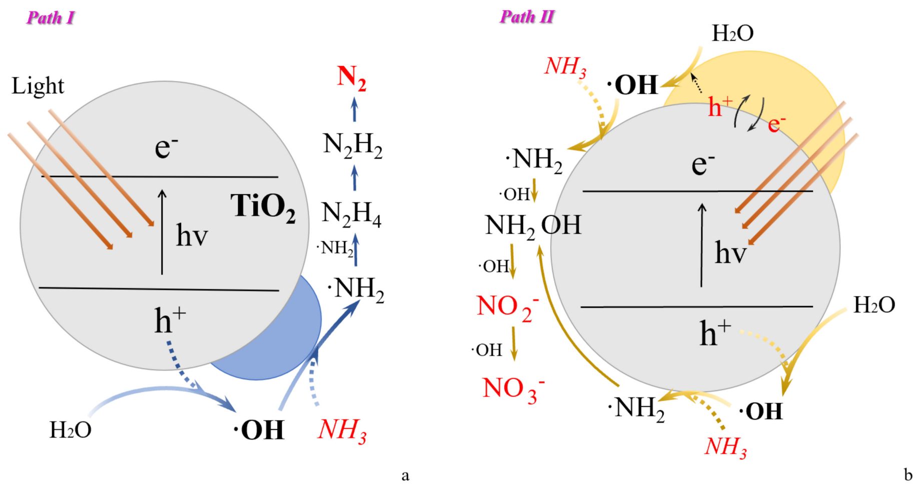

Figure 2. Possible reaction path of ammonium under photocatalytic oxidation: (**a**) the pathway to generate nitrogen; (**b**) the pathway to generate nitrogen.

Under ultraviolet light and extreme alkaline conditions (pH = 12.5), synthetic wastewater (130 mgNH_4^+-N/L) treated by TiO_2 showed results including a 26% ammonium-removal efficiency (ARE), a 25.5% NPE and a 0.13 kg/m^3/d NPR [8]. However, the reaction condition occurred at 30 °C and the high pH value is quite difficult to achieve under the actual treatment conditions of mainstream wastewater. Therefore, more suitable catalysts are needed. From above, using TiO_2 modified with Au, the synthetic mainstream wastewater (130 mgNH_4^+-N/L) treatment was achieved with a 27% ARE at 30 °C and a pH value of 10, and the NPE and NPR could reach 21.6%. and 0.11 kg/m^3/d, respectively [27]. It is obvious that this catalyst yielded a higher ARE with a lower pH condition, however, the NPE and NPR were lower than that achieved by naked TiO_2. Au and Ag are similar in surface adsorption energy intensity characteristic. Therefore, TiO_2 modified with Ag or Ag_2O may also be promising. When the pH value was 11 and the temperature was 21 °C, ARE could reach 47.3% through TiO_2 modified with Ag_2O for synthesis wastewater (100 mgNH_4^+-N/L) treatment, where the NPE and NPR were 40.8% and 0.12 kg/m^3/d, respectively [7]. Compared to the above catalysts, the temperature of the wastewater treated by this catalyst was lower, which is closer to the mainstream conditions and seems to be a more suitable choice. Additionally, the NPE of this catalyst was higher, so more nitrite could be produced to participate in anammox. Other types of catalysts were also investigated. The activated carbon (AC-$NiFe_2O_4$) catalyst bonded on the iron–nickel oxide could oxidize 87% of ammonium and almost all of it was converted to nitrite with 0.15 kg/m^3/d NPR at 25 °C and a pH of 10.5 in the presence of H_2O_2 [28]. This catalyst is also expected to be used in the nitrite production process of mainstream wastewater.

3. Biological Nitritation

The conversion of ammonium to nitrite achieved through biological metabolism is known as nitritation. A nitritation reaction is expressed in Equation (3) [29]. Nitritation-based nitrogen removal processes mainly include the nitrite shunt process and the PN/A process. The nitrite shunt process is composed of nitritation and denitritation, and the PN/A process consists of nitritation and anammox. The nitrite shunt process consists of two steps: nitritation and denitritation, which involve the oxidation of ammonium to nitrite by AOB followed by nitrite reduction to nitrogen using denitrifying bacteria [30]. Equations (4)–(7) express denitritation, the nitrite shunt process, anammox, and the PN/A reaction, respectively [29,31]. The main configuration of the nitrite shunt process is a one-stage mode [30,32,33], while the configuration of the PN/A process includes one-stage [34] and two-stage modes [35,36], as are shown in Figure 3. The two-stage mode can be further divided into two sub-modes. In the first sub-mode, the wastewater enters the anammox unit after meeting the requirement (NO_2^-/NH_4^+ ratio = 1.146) through the partial nitritation unit. In the second sub-mode, according to the theoretical calculation of the equations and under ideal conditions, part (53.4%) of the wastewater enters the nitritation unit where ammonium is completely oxidized into nitrite. Subsequently, the effluent of the nitritation unit is mixed with the remaining wastewater (46.6%) to meet the requirement (NO_2^-/NH_4^+ ratio = 1.146) and the mixed wastewater enters the anammox unit. However, in practice, AOB cannot convert all ammonia into nitrite. Therefore, the actual amount used to generate nitrous acid should be significantly higher than 53.4%. Some studies show that a proportion of 57.8–65.9% of mainstream wastewater is used to produce nitrous acid and, finally, 93% of the ammonia removal rate is achieved [36]. Compared to the nitrite shunt process, the PN/A process uses less oxygen based on the theoretical calculation (a 0.436 mol O_2/mol NH_4^+-N reduction in oxygen consumption) [37]. The nitrite shunt process has a higher NRE than PN/A because all ammonium is oxidized to nitrite and no nitrate is produced, but its obvious disadvantage is that a carbon source is required in the denitritation step [38]. This paper summarizes the biological nitritation process by dividing this section into two parts: pure nitritation process and assisted nitritation process for a more detailed introduction.

$$NH_4^+ + 1.238O_2 + 0.04HCO_3^- + 0.161CO_2 \rightarrow 0.04C_5H_7NO_2 + 0.96NO_2^- + 0.919H_2O + 1.919H^+ \quad (3)$$

$$0.12NH_4^+ + NO_2^- + 0.68CH_3COO^- + 1.57H^+ \rightarrow 0.5N_2 + 0.12C_5H_7NO_2 + 1.62H_2O + 0.75CO_2 \quad (4)$$

$$NH_4^+ + 1.11O_2 + 0.585CH_3COO^- + 0.036HCO_3^- \rightarrow 0.139C_5H_7NO_2 + 0.43N_2 + 0.369H^+ + 2.218H_2O + 0.502CO_2 \quad (5)$$

$$NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+ \rightarrow 0.986N_2 + 0.161NO_3^- \quad (6)$$

$$NH_4^+ + 0.674O_2 + 0.054HCO_3^- + 0.088CO_2 \rightarrow 0.022C_5H_7NO_2 + 0.449N_2 + 0.073NO_3^- + 1.018H^+ + 0.5H_2O \quad (7)$$

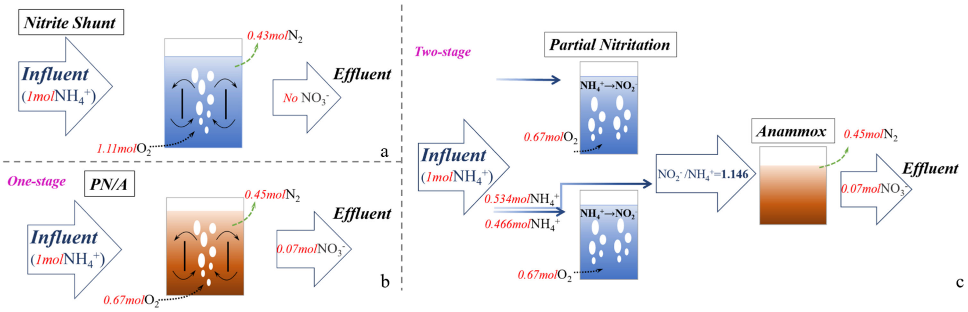

Figure 3. The process flow of nitritation−based nitrogen removal processes: (**a**) one-stage nitrite shunt process; (**b**) one-stage PN/A process; and (**c**) two-stage PN/A process.

3.1. Pure Nitritation

3.1.1. Research Progress

Pure nitritation refers to nitritation without an abiotic auxiliary method. NOB inhibition is an important issue in pure biological nitritation. Given that NOB can proliferate more rapidly than AOB in improper conditions, part of the nitrite oxidization will be in vain, which can finally decrease the NPE. Consequently, various measures in pure nitritation such as regulating and controlling temperature, DO, pH, sludge retention time (SRT) and hydraulic retention time (HRT) are adopted to inhibit NOB activity and growth. The main studies that achieved desirable nitritation through pure nitritation are shown in Table 1.

In Table 1, NPE, NPR and the nitrite accumulation ratio (NAR) are calculated as follows:

$$NPE = \frac{[NO_2^- \text{-} N]}{[NH_4^+ \text{-} N]_{inf}} \times 100\% \quad (8)$$

$$NPR = NLR \times NPE \quad (9)$$

$$NAR = \frac{[NO_2^- \text{-} N]}{\left[NH_4^+ \text{-} N\right]_{inf} - [NH_4^+ \text{-} N]_{eff}} \times 100\% \quad (10)$$

where $[NO_2^{-}\text{-N}]$ refers to nitrite generated, $[NH_4^{+}\text{-N}]_{inf}$, $[NH_4^{+}\text{-N}]_{eff}$ refer to the ammonium concentration of the influent and effluent, respectively, and NLR refers to the nitrogen load rate.

A previous study reported the highest NPR of 0.74 kg/m^3/d with a 57.63% NPE for treating synthetic wastewater (63 mgNH_4^{+}-N/L) at 25 °C [39]. A maximum NPE of 86.8% with 75 mg/L of the influent NH_4^{+}-N has also been reported, while the NPR merely reached a common level at 0.20 kg/m^3/d [40]. Thus, it can be observed that a relatively high NPR value may not be conducive to obtaining a high NPE and vice versa. The reason for this may be the effect of HRT on the operating efficiency of the process; a shorter HRT will increase the NLR and contribute to a higher NPR, but a shorter HRT will result in incomplete oxidation of the ammonia and a significant reduction in the NPE [5,6,41]. Table 1 indicates that temperature can significantly affect NPE. Generally, the NPE increases as the temperature increases. Moreover, the influent ammonium concentration exerts a great impact on NPR and NPE. NPE and NPR can reach relatively high values even at a low temperature with high influent ammonium concentration [39,40,42]. The NPR in most nitritation processes is lower than 0.1 kg/m^3/d due to the low ammonium concentration and low temperature of mainstream wastewater [43–45].

Table 1. Nitrite production ratio (NPR) and nitrite production efficiency (NPE) in recent pure nitritation process.

Wastewater	**Temp.**	**pH**	**NH_4^{+}-N_{inf}**	**DO**	**HRT**	**NAR**	**ARE**	**NPR**	**NPE**	**Refs.**
	°C		**mg/L**	**mg/L**	**h**	**%**	**%**	**kg/m^3/d**	**%**	
Syn-wastewater	10	8	70	1.2	5.7	88.58	50	0.13	44.29	[46]
Syn-wastewater	20	8	47	0.42	-	-	-	0.04	40.10	[43]
Real wastewater	20.1	7.29–7.55	75	-	8	96.1	90.3	0.20	86.78	[40]
Syn-wastewater	25	-	37	8	20	70.38	96	0.03	67.57	[44]
Syn-wastewater	25	7.8–8.5	63	-	1	-	-	0.74	57.63	[39]
Real wastewater	25.5	7.1–7.4	70	-	3	93	60	0.31	55.8	[42]
Real wastewater	25.5	7.25	33.4	0.21	4	65.66	81.44	0.10	48.02	[41]
Syn-wastewater	30	8	100	1.5	8	55.43	98.5	0.16	54.6	[47]
Syn-wastewater	32	7.8–8	50	0.5–1	24	59.36	96.35	0.03	51.37	[4]
Syn-wastewater	33	7.5–8.1	50	-	8	48.59	87.4	0.06	38.14	[5]
Municipal wastewater	34	7.5–8.0	45	-	-	43.09	82.8	-	32.05	
Syn-wastewater	35	8	50	-	2	31.98	93.8	0.18	30	[6]
Syn-wastewater	30–35	8–8.5	60	0.3	12	77.46	80	0.08	64.4	[48]

3.1.2. Microbial Information

Thus far, many kinds of AOB have been detected, as is shown in Table 2. Four genera, namely *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, and *Nitrosolobus*, are common [49]. The dominant AOB is generally the *Nitrosomonas* genus. However, under some specific conditions, the main AOB genera of the nitritation process are altered. A study has reported that, in a nitritation process realised by polyvinyl alcohol gel beads, the *Ignavibacterium* genus is the major AOB [5]. Different from common AOB, *Ignavibacterium* is a chemoheterotroph with a versatile metabolism in the phylum *Chlorobi*. Other research has demonstrated that the *Nitrosomonadaceae Ellin6067* genus could play a pivotal role in inappropriate growth environments such as low temperature or light [13]. These recently detected genera are few. Hence, their main characteristics are not comprehensive and need to be further studied.

Table 2. The reported AOB species and their main features in pure nitritation.

AOB		Limit of NaCl Tolerance	Limit of Ammonia Tolerance at pH 7.8	Optimum			Main Characteristics
Genus	**Species**	**%**	**mg/L**	**Temp. (°C)**	**pH**	**Salinity (%)**	
Nitrosomonas	*Nitrosomonas europaea*	2.9	6800	25–30	7.5–8.0	-	Common in WWTPs.
	Nitrosomonas aestuarii	4.1	5100	30	7.5–8.0	1.8	Salt requirement. Common in marine and estuarine waters.
	Nitrosomonas communis	1.8	3400	30	7.5–8.0	-	Common in soils.
	Nitrosomonas eutropha	2.9	8500	30	7.5–8.0	-	Tolerance of increasing ammonia concentrations. Common in municipal and industrial sewage disposal systems.
	Nitrosomonas halophilus	5.9	6800	30	7.5–8.0	1.5	Salt requirement. Common in brackish water.
	Nitrosomonas marine	4.7	3400	30	7.5–8.0	2.3	Salt requirement. Common in marine waters and salt lakes.
	Nitrosomonas mobilis	3.5	5100	30	7.5–8.0	0.6	Found from brackish water environments and sewage disposal plants.
	Nitrosomonas nitrosa	1.2	1700	30	7.5–8.0	-	Found in eutrophic environments.
	Nitrosomonas oligotropha	1.2	850	30	7.5–8.0	-	Tend to be found in low ammonium concentration or poor-ammonia environments.
	Nitrosomonas ureae	1.8	1700	30	7.5–8.0	-	Tend to be found in low ammonium concentration and soils.
	Nitrosomonas cryotolerans	3.5	6800	22–30	7.0–8.5	1.8	Can survive at a low ammonia concentration. Common in marine environments.
	Nitrosomonas sp. NP1	1.8	3400	30	7.5–8.0	-	A new AOB strain isolated from activated sludge.
	Nitrosomonas sp. Nm143	-	-	30	7.5–8.0	-	Common in intermediate brackish sites and estuarine sediments.
Ignavibacterium		-	-	33–35	7.5–8.6	-	Heterotrophic nitrifying bacteria. It can be observed as polyvinyl alcohol gel beads.
Nitrosococcus	*Nitrosococcus halophilus*	9.4	10,200	30	7.5–8.0	3.5–4.7	The habitat in salt lakes.
	Nitrosococcus oceani	-	17,000	30	7.5–8.0	2.3–2.9	Grows only in seawater.
Nitrosospira	*Nitrosospira briensis*	-	-	25–30	7.5	-	Low growth rate and low abundance. Common in grasslands, heath, forest soils, and mountainous areas.
Nitrosovibrio	*Nitrosovibrio tenuis*	-	-	25–30	7.7–7.8	-	Grows slowly. Common in oligotrophic soils or natural soils.
Nitrosolobus	*Nitrosolobus multiformis*	-	-	25–30	7.5	-	Common in agricultural amended soils and freshwater.

3.1.3. Influencing Factors

1. Dissolved oxygen:

Different concentrations of DO can cause different degrees of effects on AOB and NOB activities. Taking advantage of this difference, strict control of DO concentration in the reaction process can effectively inhibit NOB and produce a better nitrosation effect [43,50]. Studies have reported that, when DO concentration is below 0.51 mgO_2/L in the biofilm reactor, there is a complete and long-term NOB suppression resulting in a low relative nitrate production ratio (10%) [43]. Additionally, for traditional aeration, the oxygen mass transfer rate is limited by its solubility. Altering aeration can enhance oxygen transfer, thus promoting the growth of AOB and its dominance in the microbial community [51]. Oxygen transfer rate can be greatly improved (18 times) by using the micro–nano aeration method, allowing for a significant increase in DO concentration [44]. It can also considerably increase the abundance of hydroxylamine oxidoreductase (hao) and ammonium monooxygenase (amoA) to enhance the activity of AOB. Generally, high-DO concentrations have an adverse impact on nitritation. In a one-stage PN/A process, a high DO concentration inhibits the growth of anaerobic ammonium-oxidizing bacteria (AnAOB), weakens the inhibition of NOB, and leads to nitritation failure [44]. However, in the treatment of industrial wastewater, toxic pollutants contained in the wastewater selectively inhibit the NOB activity and growth—similar to inhibitors that assist in achieving the nitritation process—allowing nitritation at higher DO concentrations and reducing the problem of maintaining low-DO concentrations. However, in the low concentration of ammonia wastewater, selective inhibitors do not exist and efficient operation cannot be achieved by relying on DO concentration control alone.

2. Sludge retention time:

SRT directly affects nitritation efficiency. The minimum doubling time of AOB is 7–8 h shorter than that of NOB (i.e., 10–13 h) [52]. Therefore, SRT adjustment can change the structure of the microbial community. Researches have proven that with a short SRT (<2 d), NOB can be flushed successfully [53]. The production of nitrite is also promoted by a short SRT [54]. When selecting an SRT with a minimum reproduction time higher than that of NOB, conditions such as DO are required to suppress NOB [46]. However, when the SRT approaches infinity or sludge is not discharged, DO, temperature, and other factors may not effectively inhibit NOB and other methods may need to be introduced for assistance.

3. Temperature:

The temperature of the reactor is also an important influencing factor, and its influence is reflected in two main aspects. First, temperature affects microorganism activity. Studies have demonstrated that ARE is only 50% at 10 °C but can exceed 90% after warming to 27 °C [45,46]. The reason for this is mainly that NOB have lower growth rates than AOB when the temperature exceeds 24 °C, but the growth rates of NOB will be faster when the temperature is below 15 °C, making it dominant in the microbial community [55]. Secondly, temperature affects FA and FNA concentrations. The concentration of FA in the same solution at 25 °C is one-sixth of that at 35 °C, however, the concentration of FNA at 25 °C is five times higher than that at 35 °C [56]. Different FA and FNA concentrations will have different effects on AOB and NOB, so the effect of temperature changes on the nitritation process is multifaceted. In real wastewater treatment, nitritation operating conditions are generally below 25 °C and it is difficult to achieve stable operation only in terms of the effect of temperature on microbial activity. It is also necessary to combine some other conditions of control in addition to FA and FNA for efficient operation.

4. Potential of hydrogen:

The effect of pH is similar to that of temperature, and it involves the activity of AOB and NOB and the equilibrium of FA and FNA. The *Nitrosomonas* and *Nitrobacter* genera are the most common AOB and NOB, respectively, in the nitritation process. For the *Nitrosomonas* genus, the optimum pH varies from 7.9 to 8.2, whereas for the Nitrobacter genus it ranges from 7.2 to 7.6 [57]. Research has suggested that nitrite accumulation can be achieved at a pH greater than 7.5 [58]. Therefore, the general nitritation process will set the pH at about 7.8. The relationship amongst FA, FNA and pH is shown in Equations (11) and (12) [52,59].

$$FA(\text{mg/L}) = \frac{17}{14} \times \frac{NH_3\text{-}N \times 10^{pH}}{10^{pH} + \exp\left(\frac{6344}{273+T}\right)} \tag{11}$$

$$FNA(\text{mg/L}) = \frac{46}{14} \times \frac{NO_2\text{-}N}{10^{pH} \times \exp\left(-\frac{2300}{273+T}\right)} \tag{12}$$

5. Free nitrite acid:

Inhibition of NOB activity by FNA in wastewater is a common method. It was shown that the concentration of FNA in the range of 0.42–1.72 mgHNO$_2$-N/L reduced AOB activity by 50%, however, it could inhibit NOB in the range of 0.011–0.07 mgHNO$_2$-N/L and, in the range of 0.026–0.22 mg, HNO$_2$-N/L could completely inhibit NOB [60]. FNA during the nitritation process can cause NOB to be inhibited by the accumulation of nitrite production. The genera *Nitrospira* and *Nitrotoga* in NOB are not susceptible to the effect of FNA [33,61]; therefore the effect of FNA is not enough to completely inhibit NOB [43]. Additionally, the inhibitory effect of FNA on NOB is weakened for biofilm reactors. Even with a long treatment time of 24 h the biofilm will still protect NOB from inhibition [43]. Therefore, relying on FNA inhibition of NOB alone may not achieve efficient nitritation.

6. Free ammonium:

FA prevents the oxidation of ammonium and nitrite ions during the nitritation process. The inhibition of FA in nitrite oxidation by NOB begins at a concentration of 0.1–1.0 mgNH$_3$-N/L, while ammonium oxidation by AOB is inhibited when the concentration reaches the range of 10–150 mgNH$_3$-N/L FA [62]. Therefore, FA concentrations in the range of 1–10 mgNH$_3$-N/L were chosen to be suitable for nitritation. Research has reported that FA concentrations in the 5–10 mgNH$_3$-N/L range can effectively inhibit NOB and have no effect on the activity of AOB [63]. However, in general practical applications, FA concentrations higher than 10 mgNH$_3$-N/L are adopted to ensure complete NOB inhibition [64,65].

3.2. Assisted Nitritation Process

Several studies have attempted to improve the NPR to meet the requirements of the anammox reaction in recent years. Nitritation in these studies can be achieved through a number of methods including light, ultrasound, magnetic field, metal ions, salinity, and others. The main principles are shown in Figure 4 and the relevant data are in Table 3. The maximum NPR can reach 1.05 kg/m^3/d at 25 °C–26 °C and 200 mgNH$_4^+$-N/L, assisted by CuO NPs, with an NPE of 80% [66]. This value is significantly higher than that of other metals, and the study can largely satisfy the nitrite ion consumption rate of the anaerobic ammonia oxidation reaction. According to Table 3, HRT will significantly affect the NPR and a too-long HRT is not conducive to achieving a high NPR. Temperature is also an important parameter. The NPR and NPE will increase with temperature. This paper will present the factors influencing the assisted nitrification process through these 6 aspects.

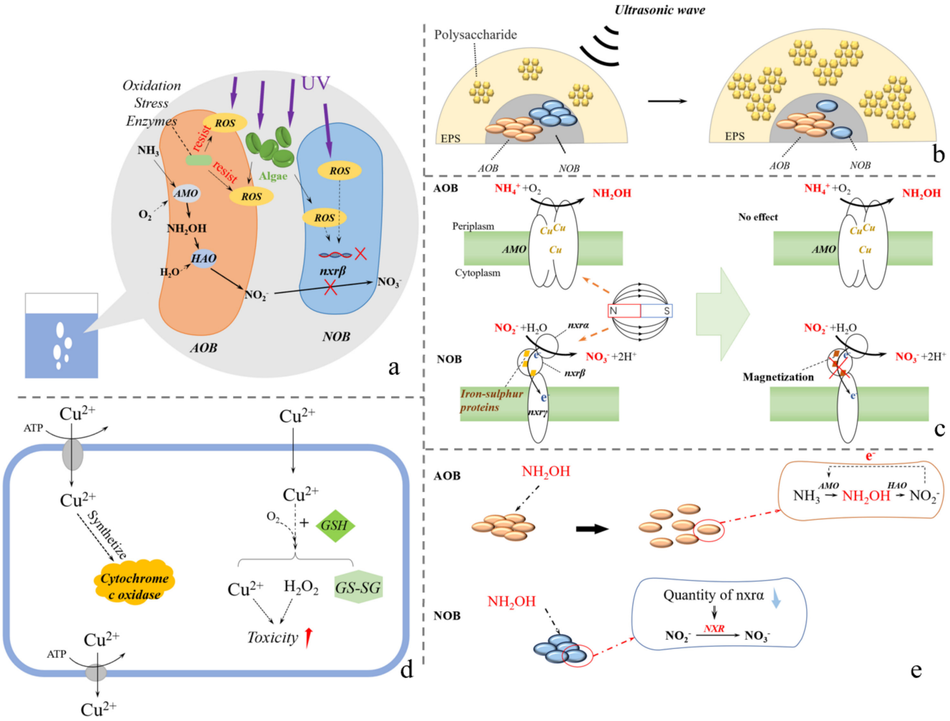

Figure 4. The principles of assisted nitritation process: (**a**) light-assisted nitritation process; (**b**) ultrasonic-assisted nitritation process; (**c**) magnetic field-assisted nitritation process; (**d**) copper ion-assisted nitritation process; and (**e**) hydroxylamine-assisted nitritation process.

Table 3. NPR and NPE in assisted nitritation processes.

Method	**Wastewater**	**Condition**	**Temp.**	**pH**	**NH_4^+-N$_{-inf}$**	**DO**	**HRT**	**NAR**	**ARE**	**NPR**	**NPE**	**Refs.**
			°C		**mg/L**	**mg/L**	**h**	**%**	**%**	**kg/m^3/d**	**%**	
Light	Syn-wastewater	1000 μmol/(m^{2}s)	25	7.4	30	1	3.85	70	100	0.13	70	[13]
	Syn-wastewater	0.87 μE/(Ls)	25–28	7.4–7.6	50	0.5	6	80	100	0.10	48	[67]
Ultrasound	Syn-wastewater	0.25 W/mL	18	7.5–8.5	60	-	16	90	100	0.05	50	[68]
	Syn-wastewater	0.25 W/mL	25	8.1	60	-	16	90	95	0.08	85.5	[69]
	Syn-wastewater	0.15 W/ml	-	8	60	-	16	85	95	0.07	80.75	[70]
Magnetic field	Syn-wastewater	15 mT	25	7.5–8.0	100	-	12	90.1	95	0.12	60	[71]
Metal ion	Syn-wastewater	Cu^{2+} 0.61 mg/L	18.1	8	125	8.7	5	-	90	0.23	38.4	[72]
	Syn-wastewater	Y^{3+} 5 mg/L	23	8.9	150	-	-	-	70	0.30	70	[73]
	Syn-wastewater	Zn^{2+} 10 mg/L	23–26	8.05	200	0.1–0.15	12	-	90	0.30	75	[74]
	Syn-wastewater	CuO NPs 5 mg/L	25–26	7.7–7.8	200	0.2	3.67	-	80	1.05	80	[66]
	Syn-wastewater	La^{3+} 5 mg/L	-	8.9	150	0.2	-	-	100	0.37	90	[75]
Salinity	Industrial wastewater	6.6 gNaCl/L	29	-	161	0.1–1.5	48.24	61.6	76.9	0.03	35.80	[76]
	Syn-wastewater	10 gNaCl/L	30	7.5–8.0	100	-	8	-	60	0.15	48.62	[14]
	Industrial wastewater	8.6 gNaCl/L	31	7.6	220	0.5–3.5	31.2	80	90	0.08	47	[77]
	Syn-wastewater	13.5 gNaCl/L	32	8	150	0.3	2.4	73.3	-	1.05	70	[78]
Others	Domestic sewage	NH_2OH 5 mg/L	19.5–28.2	7.9	70	3	3	-	100	0.28	50	[79]
	Syn-wastewater	PNCT 8 mg/L	20	7.50–7.80	70	-	6	94.94	-	0.23	81.1	[80]
	Syn-wastewater	formic acid 1380 mg/L	25	7.9	127.1	2	8.6	-	94.6	0.33	94.32	[81]
	Syn-wastewater	NH_2OH 10 mg/L	25	7.8–8.2	100	5	8	-	57	0.17	56.9	[82]
	Syn-wastewater	N_2H_4 2–5 mg/L	30	8	30	0.09–0.25	2	82	90	0.18	51.2	[83]
	Syn-wastewater	N_2H_4 2–5 mg/L	30	-	42	0.3	2.32	82.9	97.4	0.23	52.2	[84]
	Syn-wastewater	formic acid 1380 mg/L	-	7.9	120	-	4	-	95	0.62	86.45	[85]

3.2.1. Light

Light affects the growth and metabolism of some microorganisms in water. Previous studies have demonstrated that photosensitivity differences exist between AOB and NOB, with NOB being more sensitive to light than AOB [13,86]. This principle is shown in Figure 4a. Reactive oxygen species (ROS) can be generated in wastewater under light and can indirectly lead to a series of damages, mainly the oxidative damage of cell proteins, cell membranes, and nucleic acids [87,88]. However, AOB have a critical oxidation stress enzyme that can be used to protect themselves, while NOB do not [89,90]. Therefore, NOB will be affected by light, mainly in the form of NOB nxrβ gene un-expression and irreversible inhibition of NOB activity. Meanwhile, under light irradiation, a large amount of algae is produced in nitritation and forms a symbiotic system with AOB [67]. The algae also produce ROS, so this method can effectively inhibit NOB [91].

The effect of light intensity on NPE is very obvious. When the strength exceeds 300 μmol/(m^2·s), AOB were inhibited and nitritation fails [92]. Within a small range of light intensities, AOB activity initially increases and then decreases with an increase in light strength, whereas NOB activity directly decreases. Therefore, an optimum light intensity will be available to maximize the promotion of AOB and inhibit NOB, allowing nitritation to operate efficiently. Studies have observed a 1.31–1.43-fold increase in AOB activity at 25 °C under optimal conditions (p = 0.03–0.08 kJ/mgVSS) [13]. Moreover, the photoinhibition effect of AOB and NOB and the light time have a direct ratio [13]. Therefore, selecting light-assisted nitritation for long-term treatment can continuously inhibit the effect of NOB and make nitrite ions more likely to accumulate.

3.2.2. Ultrasonic

Research has found that low-frequency ultrasound can accelerate microbial growth by increasing the abundance of functional genes to promote signal transduction, cell movement, and membrane transport [68,93,94]. Differences in the effects of low-frequency ultrasound on AOB and NOB exist, and ultrasound at certain frequencies and intensities can promote the oxidation process of ammonia by AOB while inhibiting the oxidation of nitrite ions by NOB. During ultrasound-assisted nitritation process, an increase in extracellular polymer (EPS) content, especially a significant increase in polysaccharide (PS) content, was observed (shown in Figure 4b). EPSs have a positive effect on the efficiency of the mass transfer and the activity of AOB while inhibiting NOB activity [70]. Therefore, nitritation assisted by low-frequency ultrasound can be feasible.

The effect of low-frequency ultrasound intensity is similar to that of light. A suitable range also exists to increase the AOB activity and decrease the NOB activity [93]. Thus, this method is promising for use in mainstream wastewater treatment. Moreover, the temperature in the reactor can be considerably improved through ultrasound, weakening the inhibitory effect of low temperature on AOB. Generally, nitritation operations at 18 °C may fail according to the previous paper, but ultrasound-assisted nitritation can realise more than 90% of NAR [68]. Hence, the application of ultrasound can provide a good temperature environment for mainstream treatment [95].

3.2.3. Magnetic Field

Generally, microorganisms are magnetic to some extent. Thus, under an external magnetic field, enzyme activity and cell membrane permeability are affected to a certain extent [96,97]. Subsequently, the microbial metabolism also becomes affected. Nitrite oxidoreductase (nxr), which oxidizes nitrite to nitrate in NOB, can recruit iron–sulphur proteins to realise redox reaction [98], as is shown in Figure 4c. Iron is magnetised by the static magnetic field (SMF), but the active site of ammonium monooxygenase (AMO) in AOB is copper, which is not magnetised. Therefore, SMF only inhibits NOB [71]. The SMF makes the sludge gather closely and form an oxygen concentration gradient so that AOB are easily enriched while NOB are washed off [99]. The strength of the SMF also has an appropriate range to realise nitritation. Within this range, the DO utilisation rate is improved, nitritation is achieved faster than through pure biological nitritation, and the DO concentration is maintained at 0.5–0.7 mg/L at 35 °C [100]. Therefore, the magnetic field-assisted method is recommended for actual applications.

3.2.4. Metal Ions

Metal ions are indispensable elements for microbial growth and metabolism. As shown in Figure 4d, copper ions play a considerable role in cytochrome c oxidase and related enzymes that participate in cell respiration. Generally, copper ions enter cells through active and passive transport [101]. However, copper ions enter cells rapidly and not specifically through passive transport at a high periplasm concentration. The copper ions' concentration in the cytoplasm then increases and the copper ions promote the reaction with glutathione (GSH) and oxygen to produce oxidized disglutathione (GS-SG), copper ions, and H_2O_2 [102]. Copper ions are not removed, and H_2O_2 increases toxicity. This may cause gene mutation and reduce the growth rate. Other metal ions have different effects on microorganisms; for example, Mn^{2+} can promote the respiration of AnAOB [103], and Zn^{2+} can be used for the synthesis of some zinc-containing enzymes [103].

Generally, metal ions are important co-factors of metalloproteinases, and some enzymes have a low metal ion concentration [101]. However, at a high concentration metal ions chemically combine with some enzymes and affect the structures and activity of these enzymes, finally inhibiting microbial activity [104]. For example, at 1 mmol/L concentration, Cu^{2+} effectively inhibits the activity of AOB and NOB at 25 °C [105]. Therefore, the promotion or inhibition of AOB and NOB depends on the exposure dose [73]. Most studies have reported that an increase in dosage initially increases the activity of AOB to the peak then gradually decreases it [66,73–75]. However, the activity of NOB decreases directly. Therefore, a suitable dose range exists to achieve nitritation.

3.2.5. Salinity

For mainstream wastewater, high salinity greatly increases the difficulty of denitrification. The main reason for this is that high salinity may inhibit microbial enzymes and metabolism and decrease cell activity [106,107]. Furthermore, high salinity affects the osmotic pressure of cells and even leads to plasmolysis [78]. Studies have indicated that AOB activity is inhibited under high salinity (25 gNaCl/L) at 35 °C [108]. However, at a low concentration (13.5 gNaCl/L), salt can promote the growth of AOB at 32 °C, such as in *Unclassified Nitrosomonadaceae* [78]. By adding salt (5.2, 7.6 and 10.2 gNaCl/L), the NAR can increase to above 95% at 22 °C and 36.2 mgNH_4^+-N/L [109]. After high salinity acclimation, AOB and NOB show increased activity [110,111]. Thus, a low NPE may be obtained, but the NPR may increase [34,112]. Low NPE makes this method impossible to use alone.

3.2.6. Others

In addition to the categories provided above, other substances can inhibit NOB. These include hydroxylamine, hydrazine, formic acid, and some antibiotics. Hydroxylamine is the intermediate product of nitritation and a concentration of 10 mg/L can make nitritation highly stable at 25 °C and 100 mgNH_4^+-N/L [82]. As shown in Figure 4e, hydroxylamine disperses cells to improve the mass transfer efficiency for AOB. Abundant hydroxylamine promotes the production of nitrite, which produces many electrons to circulate to the AMO enzyme and increase the consumption of ammonia [113]. However, it may inhibit the growth of NOB by inhibiting the induction of nxr and decreasing the nxrα enzyme [82,114]. Therefore, hydroxylamine can be selected to assist in nitritation and this method can obtain high efficiency [115].

Hydrazine, being an intermediate product of anammox, can also promote nitritation. Exogenous hydrazine can be partly converted into hydroxylamine to inhibit NOB, and another part causes hydroxylamine to be produced disproportionately during nitritation to reduce the effect on AOB [83]. Hydrazine can also improve AnAOB activity and can be completely consumed without extra pollution, thereby making hydrazine a frequently selected inhibitor [84]. Studies have indicated that formic acid can effectively and selectively inhibit nxrβ gene transcription, leading to the inhibition of nitrite oxidation and promotion of nitrite accumulation [81,116]. Therefore, formic acid-assisted nitritation is also a suitable method. As a common antibiotic, PNCT can often be detected In wastewater [117]. PNCT can inhibit AOB and NOB through DNA and protein damage and destruction of the carbon fixation pathway [80]. However, the abundance of *Nitrosomonas* is always higher than that of *Nitrospira*, so AOB is always dominant in the reactor. Therefore, the PNCT-assisted method can also achieve stable nitritation.

4. N_2O Emission

As a major greenhouse gas in WWTPs, N_2O emissions weaken the advantage and efficiency of nitritation-based nitrogen removal processes due to nitrite consumption and environmental pollution. N_2O is produced in nitritation unit via two pathways: NH_2OH oxidation, and nitrifier denitrification by AOB [118,119]. The first case refers to the incomplete oxidation of NH_2OH produced in the nitritation N_2O. The other pathway describes how NO_2^- is reduced by AOB, as shown in Figure 5. Nitrifier denitrification is generally considered the main pathway in nitritation [15]. N_2O emission is affected by DO and nitrite concentration in the nitritation unit. The denitrification by AOB is stimulated and the N_2O emission increases when DO is less than 0.5 mg/L at 27 °C with 600 mgNH_4^+-N/L [120], whereas nitritation will be promoted and N_2O emission will be reduced with the increase of DO concentration [121]. The range (DO < 0.5 mg/L) is generally suitable to inhibit NOB and realise pure nitritation. Therefore, there is a great risk of N_2O emission at low-DO concentration in pure nitritation. The research demonstrated that nitrite concentration and N_2O emission are in a direct ratio [15]. In a one-stage process, nitrite is generated and consumed at the same time. In addition, the nitrogen removal rate of anammox in mainstream wastewater is greater than 1.2 kg/m^3/d; that is, nitrite consumption rate is greater than 0.70 kg/m^3/d [3]. The NPR is significantly lower than nitrite consumption rate according to Table 1. Therefore, nitrite cannot theoretically accumulate to produce N_2O in a one-stage process, and a one-stage process is more promising than a two-stage process. For photocatalytic oxidation, the NH_2OH produced by ammonia oxidation will be directly oxidized to nitrite under anaerobic conditions due to the strong oxidation of hydroxyl radicals [9]. The reduction of nitrite can be neglected under the condition of NH_2OH existence. Therefore, nitrite production through photocatalytic oxidation is also environmentally friendly.

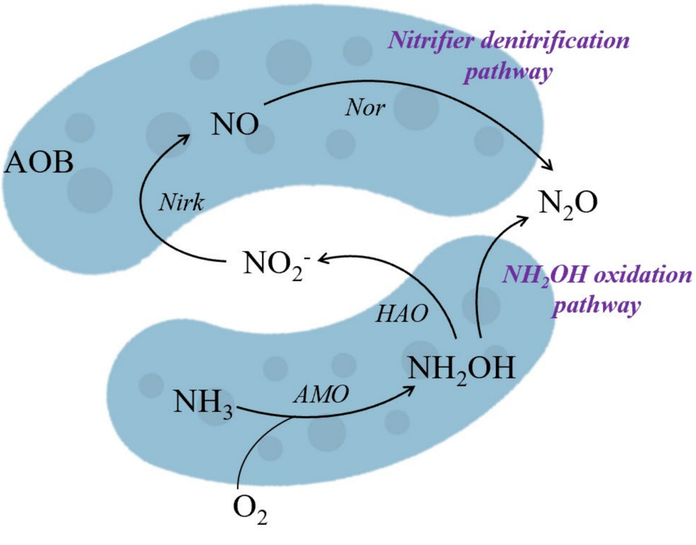

Figure 5. N_2O emission pathways in nitritation processes. N_2O can be emitted through nitrifier denitrification and the NH_2OH oxidation pathway.

5. Existing Problems

According to the above, photocatalytic oxidation is the only physicochemical method that can realize nitritation at present. However, this method is still in the lab-scale and has not been used in practice [25]. In its actual application, the chromaticity and turbidity of wastewater will significantly affect the efficiency. In addition, most of current studies tend to oxidize ammonium directly to nitrogen to reduce subsequent treatment [122]. This direction seems to be more environmentally friendly, but the treatment efficiency is poor. This case also results in less existing research and that the used catalysts are incomplete for nitrite production. Therefore, further research is needed in this direction.

There are some unresolved questions regarding these nitritation processes assisted by various methods. Light, ultrasonic, and magnetic field methods are more susceptible to fluctuations in water quality and quantity. For example, the density and morphology of sludge will have a significant impact on the shading for light-assisted nitritation [13] Additionally, use of light, ultrasonic, and magnetic field methods will increase energy consumption, so it makes sense to use less energy to achieve better results in the practical application of these methods. Metal ions and salinity are not suitable to assist in nitration alone given that long-term treatment of metal ions will increase the inhibition concentration and that there will still be a small amount of *Nitrospira* restoring activity when the salinity drops to a low concentration, even if the salt concentration is very high [77]. Cost of operation should also be considered. A higher concentration is required to more effectively inhibit NOB, but it will increase the cost. Organic compounds are frequently used for selective inhibitors. However, these inhibitors have some adverse other effects: hydroxylamine has unstable chemical properties, limited inhibition of Nitrobacter, and high chemical cost; hydrazine will inhibit AnAOB under mainstream conditions; formic acid is corrosive and difficult to store; and when antibiotics are incompletely removed they will aggravate the pollution of water quality [79,80,123]. Moreover, treatment of selective inhibitors will increase the operation cost and affect microorganisms other than NOB for the long term [124].

Given that photocatalytic oxidation has undeniable advantages, a two-stage PN/A process that combines photocatalytic oxidation for nitrite production and anammox for nitrogen removal can be designed for the follow-up treatment of the anaerobic digestion effluent of mainstream wastewater. The process flow is shown in Figure 6. First, wastewater will be treated in the COD removal unit before it enters the photocatalytic oxidation unit after passing through the setting tank and undergoing pH adjustment. The requirements of the anammox reaction will be met in this unit. Second, nitrogen will be removed in the anammox unit and water will be discharged after sedimentation. Unlike in the above-mentioned processes, the NPR of photocatalytic oxidation is higher and can meet the requirement of the anammox reaction at 21 °C and 100 mgNH_4^+-N/L. This process does not require aeration and does not produce sludge in photocatalytic oxidation, so operating costs will be significantly reduced. As this process does not consume chemicals, the effects on the subsequent anammox process, drug storage and environmental pollution need not be considered.

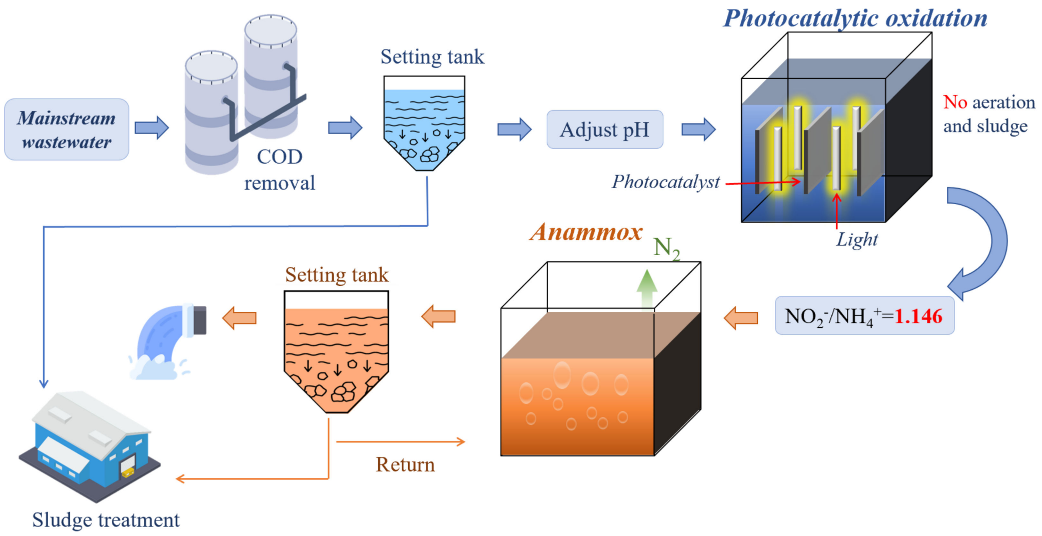

Figure 6. The novel two-stage nitrogen removal process.

6. Conclusions

This paper comprehensively summarizes nitrite production processes. The microbial main species and characteristics involved in biological nitritation processes are also reviewed. The principles of the various assisted nitritation processes and the influencing factors present are described in detail. A comparison of the studies reveals that the efficiency of nitritation is strongly influenced by HRT and temperature; the NPR increases as HRT shortens, while the NPE increases with increasing temperature. This provides a reference for the practical application of the PNA process. In addition, the N_2O emission in the nitrite production process is analysed, and a one-stage nitritation-based process and photocatalytic oxidation process are environmentally friendly. Finally, a two-stage process that combines photocatalytic oxidation and anammox is proposed. This process has a good prospect for development under mainstream conditions.

Author Contributions: H.Z. and Y.G. have reviewed the literature, summarized the information, prepared the tables and figures, and drafted the manuscript. F.L. and Z.Z. contributed to the designed figures in this work. C.W. and Q.W. contributed to the review and revision of the manuscript. M.G. planned and supervised the work. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key R&D Program of China (2019YFC1906304; 2019YFC1906302), and the National Environmental and Energy Base for International Science & Technology Cooperation.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated or analysed during the current study are available in the Web of Science repository [http://apps.webofknowledge.com/ accessed on 1 June 2022].

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

WWPTs: wastewater treatment plants; NPR: nitrite production rate; NPE: nitrite production efficiency; PN/A: partial nitritation/anammox; NRR: nitrogen removal rate; AOB: ammonium-oxidizing bacteria; NOB: nitrite-oxidizing bacteria; DO: dissolved oxygen; FA: free ammonium; FNA: free nitrite acid; N_2O: Nitrous oxide; SRT: sludge retention time; HRT: hydraulic retention time; NAR: nitrite accumulation ratio; hao: hydroxylamine oxidoreductase; AMO: ammonium monooxygenase; amoA: monooxygenase; AnAOB: anaerobic ammonium-oxidizing bacteria; ROS: reactive oxygen species;

EPS: extracellular polymer; PS: polysaccharide; nxr: nitrite oxidoreductase; SMF: static magnetic field; GSH: glutathione; GS-SG: disglutathione; ARE: ammonium removal efficiency.

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