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Review

Recent Advances in Autotrophic Biological Nitrogen Removal for Low Carbon Wastewater: A Review

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Abstract: Due to carbon source dependence, conventional biological nitrogen removal (BNR) processes based on heterotrophic denitrification are suffering from great bottlenecks. The autotrophic BNR process represented by sulfur-driven autotrophic denitrification (SDAD) and anaerobic ammonium oxidation (anammox) provides a viable alternative for addressing low carbon wastewater. Whether for low carbon municipal wastewater or industrial wastewater with high nitrogen, the SDAD and anammox process can be suitably positioned accordingly. Herein, the recent advances and challenges to autotrophic BNR process guided by SDAD and anammox are systematically reviewed. Specifically, the present applications and crucial operation factors were discussed in detail. Besides, the microscopic interpretation of the process was deepened in the viewpoint of functional microbial species and their physiological characteristics. Furthermore, the current limitations and some future research priorities over the applications were identified and discussed from multiple perspectives. The obtained knowledge would provide insights into the application and optimization of the autotrophic BNR process, which will contribute to the establishment of a new generation of efficient and energy-saving wastewater nitrogen removal systems.

Keywords: low carbon wastewater; autotrophic biological nitrogen removal; anammox; sulfur-driven autotrophic denitrification; functional microorganisms

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1. Introduction

Nitrogen, as an essential element, plays a crucial role in both natural environments and life activities [1]. Meanwhile, environmental issues related to nitrogen pollution have received increasing attention. It is reported that the more than 50% of the reactive-nitrogen input to the biosphere is due to human activities [2], which has led to increasing problems such as acid rain, groundwater pollution, and aquatic eutrophication [3]. Stricter emission standards and the pursuit of sustainable processes have greatly driven the development of nitrogen removal processes. Heterotrophic denitrification (HD), reducing nitrate (NO_3^--N) to N_2 with organics as electron donors, is currently the most widely applied biological nitrogen removal (BNR) process in municipal wastewater treatment plants (WWTP) [4]. However, the HD process is greatly limited by the dependence of the carbon source. Most municipal wastewater in China is characterized by low C/N [5]. Additionally, the availability of carbon in some nitrogen-laden wastewater, including groundwater and industrial wastewater, is relatively limited. In practice, external carbon sources are often supplied, which increases operating costs and the risk of secondary pollution. In contrast, the autotrophic BNR provides a promising alternative for the BNR process.

In autotrophic BNR, the conversion of nitrogen to N_2 is driven by the oxidation of inorganic substances [4]. Thus, the low carbon wastewater can be effectively treated by the autotrophic BNR process. Moreover, autotrophic BNR has the advantage of low sludge production due to the slow growth rate of autotrophic bacteria. According to the previous study, waste sludge treatment accounts for about 30% of the total operating cost of a WWTP [6]. As representatives of autotrophic BNR, anaerobic ammonium oxidation (anammox) and sulfur-driven autotrophic denitrification (SDAD) received the most extensive attention in the last decade. As shown in Figure 1, in the SDAD process, the electron donors required for the nitrate reduction is the reduced sulfur compounds. Herein, the SDAD process can also be applied to the removal of sulfur-containing pollutants along with the nitrogen removal of low carbon wastewater. The sulfate reduction autotrophic denitrification nitrification integrated (SANI) process is undoubtedly the most successful process in recent years, with SDAD as its technical core. It is reported that the SANI process can reduce residual sludge production in Hong Kong by up to 60–70%, while the facility land occupation will also be reduced by 30–40% [7]. On the other hand, optimization of key operation parameters and reduction of sulfate emissions are still challenges for the SDAD process. Anammox is a biological process in which ammonium is directly oxidized by nitrite to produce N_2 under anoxic conditions (Figure 1). This phenomenon was first reported in 1995 and has since attracted widespread attention in the field of BNR due to the low cost and high efficiency [8,9]. To date, more than 100 full-scale installations based on the anammox process have been put into practical operation worldwide [10]. However, the anammox process is still greatly limited by nitrite shortage, high environmental sensitivity, long start-up time, etc.

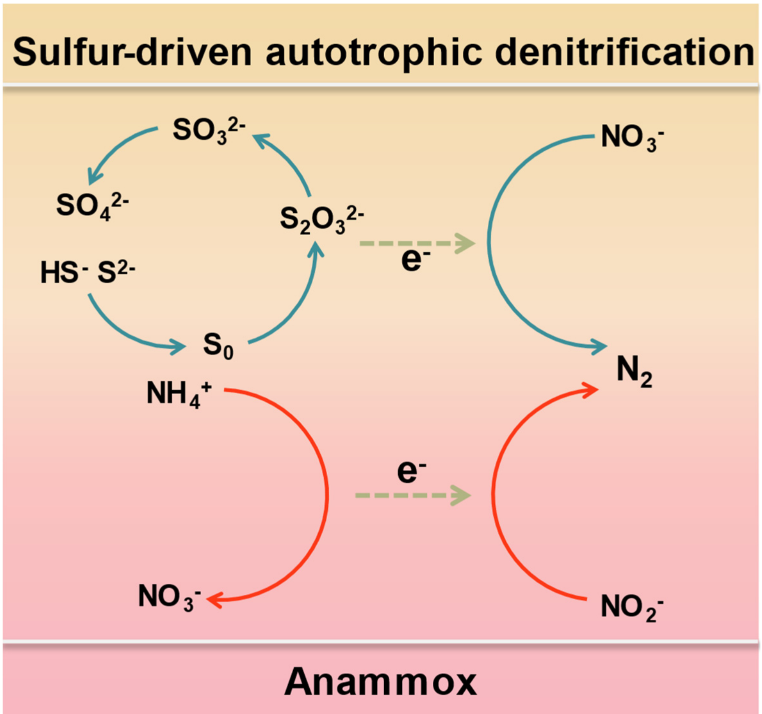

Figure 1. Conceptual diagrams for sulfur−driven autotrophic denitrification (SDAD) and anammox.

In addition to the above, H_2 can also drive autotrophic denitrification and is the cleanest electron donor. The H_2-based autotrophic denitrification process is favored for drinking water treatment due to the absence of undesirable products [11]. However, the application of the related process is limited by the low solubility, high cost and safety concerns of H_2 [11]. The construction of a membrane biofilm reactor (MBBR) provides a viable solution to the above issues. So far, the H_2-MBBR process has been successfully applied to actual groundwater nitrogen removal treatment at the pilot scale [12]. Correspondingly, the deterioration of nitrogen removal performance due to membrane fouling needs to be further optimized. Overall, the H_2-based autotrophic denitrification process still has limitations in terms of practical application, compared to SDAD and anammox. Thus, this study focuses on SDAD and anammox as the main lines to review the recent advances of autotrophic BNR technology.

Although autotrophic BNR processes represented by SDAD and anammox show great potential in treating low carbon wastewater, there are still bottlenecks. Thus, a systematic review and summary of the obtained knowledge would help to develop feasible strategies and provide guidance for the operation and optimization of the relevant processes. Herein, the recent advances and challenges of autotrophic BNR process guided by SDAD and anammox are comparatively summarized in this review, focusing on (1) the present applications and crucial operation factors, (2) functional bacteria, and (3) future perspectives and technical advantages in the context of carbon emission reduction. Such improved knowledge will lay the foundation for a more efficient and sustainable BNR process for low carbon wastewater.

2. Sulfur-Driven Autotrophic Denitrification Process

2.1. Sulfate Reduction Autotrophic Denitrification Nitrification Integrated Process

As a representative of SDAD, SANI has achieved great success in dealing with the mainstream nitrogen removal of urban wastewater in Hong Kong. As shown in Figure 2, SANI mainly contains the following three processes: (1) sulfate reduction generates reduced sulfide compounds accompanied by the oxidation of organic matter with the action of heterotrophic sulfate-reducing bacteria (hSRB); (2) reduction of nitrate to N_2 by the action of autotrophic sulfur oxidizing nitrate reducing bacteria (a-soNRB) using sulfide as an electron donor; (3) under aerobic conditions, ammonium is oxidized to nitrate and supplied to a-soNRB [7]. Due to the utilization of seawater, the sulfate content of urban wastewater in Hong Kong is generally 500 mg L^{-1}, which provides a sufficient reduction potential [13]. The growth yield of hSRB is 0.2 g VSS (volatile suspended solids) g^{-1} sulfate, which is much lower than that of heterotrophic denitrification bacteria (HB) (0.4 g VSS g^{-1} chemical oxygen demand (COD)) [13]. Thus, the low sludge yield is the most outstanding advantage of the SANI process. The full-scale SANI process (800–1000 m^3 day^{-1}) has been successfully operated, and it is estimated that 50% of operating costs could be reduced compared to the traditional activated sludge process [7]. However, when dealing with high nitrogen (low C/N) wastewater, SANI has the limitation of insufficient electron donation from sulfate reduction and severe acidification caused by nitrification. For high-strength ammonium wastewater, Wu et al. [14] introduced a microbial electrolysis cell (MEC) on the basis of SANI process, namely the e-SANI process, and achieved satisfactory nitrogen removal performance [14]. At a cathodic potential of -1.0 V, the e-SANI process achieved a nitrogen removal efficiency (NRE) of 56.9 $\pm$ 1.4%, which is a 22.0% improvement over the conventional SANI process. Besides, the residual sulfide was effectively oxidized to S^0, driven by the anodic potential, which reduced the risk of secondary contamination due to sulfur emissions. [14].

For inland areas, the sulfate content in urban wastewater is limited, so low-cost sulfur-rich resources should be a viable alternative. Based on the above, the wastewater produced by wet flue gas desulfurization (FGD) was introduced into SANI process (FGD-SANI process) as sulfur sources by a previous study [15]. The nitrogen was effectively removed in over 200 days of operation, and surprisingly the FGD-SANI process maintained stable performance at 5–10 °C, demonstrating the application potential in cold regions [15]. Furthermore, in the case of $S_2O_3{}^{2-}$ as the electron donor, the NRE of FGD-SANI reached 100%, and 35% of alkalinity could be recovered for the absorption of SO_2 in FGD process [16]. In addition, based on the autotrophic BNR process, Wang et al. (2005) achieved the treatment of high concentration sulfur and nitrogen containing wastewater by introducing the heterotrophic process [17]. Although the SANI process has shown great promise, more efforts are necessary on increasing nitrogen removal efficiency (reduce hydraulic retention time, HRT) and reducing sulfate emissions.

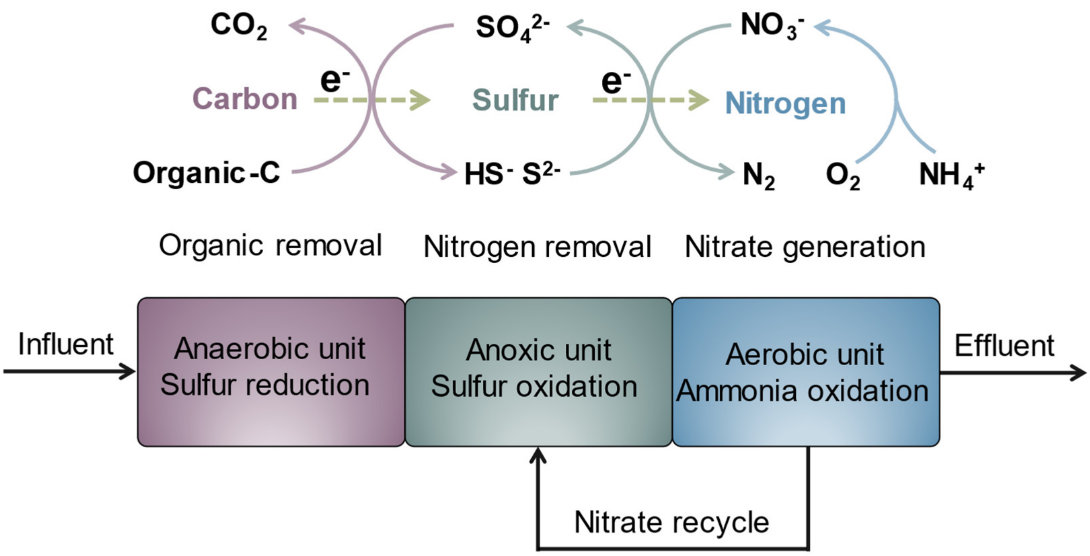

Figure 2. Schematic diagram for sulfate reduction autotrophic denitrification nitrification integrated (SANI) process.

2.2. S^0 as Electron Donor for SDAD Process

As the electron donor of the SDAD process, the denitrification efficiency of elemental sulfur follows the sequence of thiosulfate > sulfide > S^0 [18]. However, S^0 is more readily available and less costly (\$0.43 kg^{-1} nitrate) [19]. Thus, S^0 was considered as an ideal substrate for the SDAD process, and the S^0-packed bed was the most widely used. The SDAD process leads to acidification, and approximately 4.57 g alkalinity was required with the removal of one gram of nitrate [19]. A decrease in pH can potentially deteriorate the nitrogen removal performance. It has been reported that a pH less than 7.6 will result in a 25% decrease in denitrification activity of the SDAD process [20]. Therefore, the maintenance of relatively neutral conditions is critical for the construction of the SDAD system. In practical applications, limestone, calcite, crushed oyster shells, etc., were often applied as solid buffers [21]. In a pilot-scale S^0 packed bed reactor (S^0-PBR), a previous study optimized the mass ratio of S^0 to limestone ($CaCO_3$) to be 1:1 and achieved a high nitrogen removal rate of 500 g N m^{-3} day^{-1} [22]. The same results were also verified under full-scale application. Sahinkaya et al. [23] explored the process performance of full-scale S^0-PBR treating wastewater effluent from the secondary sedimentation tank under different S^0/limestone (1:1–3:1), and the results showed that a mass ratio of 1:1 drove better performance [23]. In addition to providing alkalinity, limestone can also provide inorganic carbon for the growth of microorganisms, and as a carrier contributes to the formation of biofilms [18].

Increasing the retention of biomass in the reactors has also attracted widespread interest, due to the slow growth rate of a-soNRB. In recent years, many studies have applied membrane bioreactor (MBR) technology to the S^0-based SDAD process and achieved satisfactory results. A previous study achieved complete removal of 25 mg N L^{-1} at an HRT of 2 h by equipping the ultrafiltration membrane [24]. Furthermore, a recent study had achieved efficient removal of 250 mg N L^{-1} by the S^0-based SDAD process in a MBR reactor [25]. However, membrane fouling due to colloidal sulfur particles can cause a negative impact on process performance and requires further investigation [26].

The solubility of S^0 is only 5 μg L^{-1} at 25 °C, which limits its bioavailability. Thus, improving the electron-providing efficiency and bioavailability of fillers is also a concern of current research. Zhu et al. (2018) achieved a significant improvement in process performance (720.35 g N m^{-3} d^{-1}) by using siderite ($FeCO_3$) as an additional electron donor and a solid buffer [27]. Besides, the production of sulfate in effluent was also reduced by about 15%, reducing the risk of secondary pollution. Another study determined an optimal volume ratio of 1:3 between S^0 and $FeCO_3$, and the related process was successfully applied at a pilot-scale for the treatment of secondary effluent [28]. In addition to adding exogenous strengthening substances, some studies have found that the particle size of S^0 will affect the mass transfer rate, which is related to the process performance [19]. The large surface area and small particle size have a positive effect on the performance of the S^0-based SDAD process, but can also lead to problems like clogging.

2.3. Sulfide as Electron Donor for SDAD Process

Some low carbon wastewaters also have high sulfide concentrations, such as acid mine wastewater, algal bloom water, etc. [29]. Sulfide emissions pose a series of environmental problems due to their odor, toxicity and corrosiveness. In addition to nitrogen removal, the SDAD process is also promising for desulfurization. As shown in Figure 3, it is generally accepted that the autotrophic denitrification process consists of the two following steps: r_1: S^{2-} oxidation to S^0 accompanied by the reduction of NO_3^-; r_2: S^0 is further oxidized to SO_4^{2-} with simultaneous production of N_2 [30]. It is reported that the reaction rate of r_1 is 3.31 times higher than that of r_2 [31], and the molar ratio of S/N is the key to determine the fate of sulfur in SDAD system. Huang et al. (2015) showed that the optimum molar ratio for the recovery of S^0 was 5:6 (S/N), and the optimal nitrogen removal performance was also achieved in this condition [32]. Chen et al. (2012) evaluated the recovery performance of S^0 at different S/N ratios (5:2, 5:5, and 5:8) and achieved the highest recovery rate of S^0 at 5:2 [33]. The differences in optimal molar ratio of S/N in different studies may be caused by the species of S and N [34], which will be discussed in next section. In addition to S/N, the flow pattern and operating loading rate of the reactor are also important considerations in determining S^0 recovery. Huang et al. (2021) found that the recovery of S^0 was maintained at 87% at high loading rate (1.87 S^{2-} kg m^{-3} d^{-1}; 1.19 kg NO_3^--N m^{-3} d^{-1}), while no S^0 production was observed at low loading (0.95 S^{2-} kg m^{-3} d^{-1}; 0.60 kg NO_3^--N m^{-3} d^{-1}) [35].

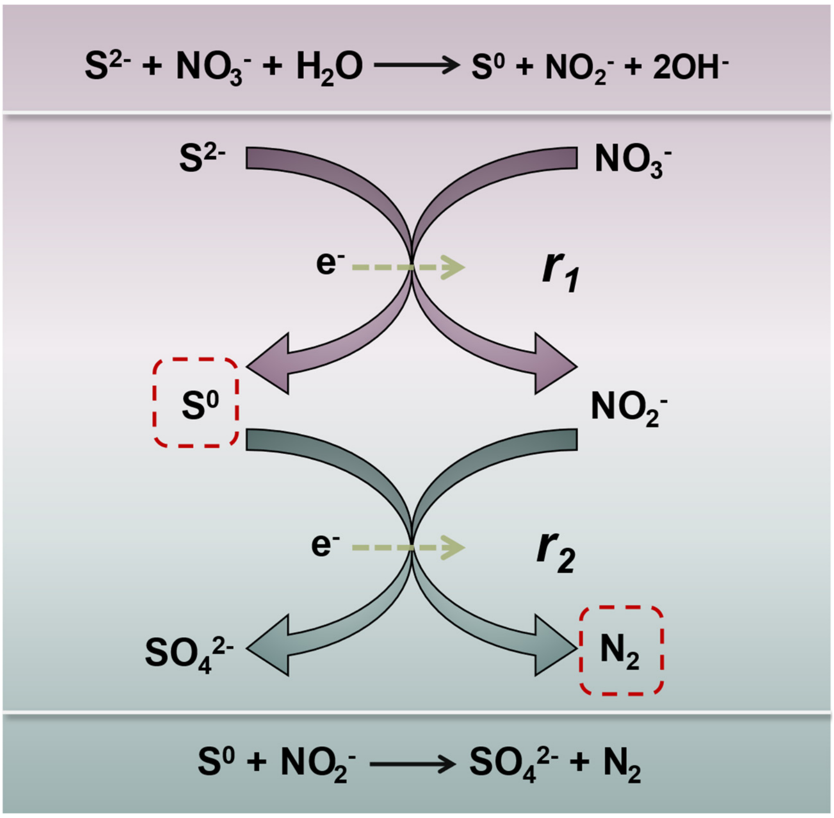

Figure 3. Reaction pathways of the SDAD process that leads to the formation of S^0 and N_2.

In situ generated S^0 in the SDAD system, if not discharged timely, will affect the mass transfer efficiency and thus reduce the process performance. When the pH > 9, the sulfur generated by the SDAD system is partially in the form of biogenic S^0 colloids, which leads to a technical bottleneck for the effective separation [36]. Flocculation techniques for sulfur in the effluent of SDAD process have been applied in some previous research. By dosing Zn^{2+} (500 mg L^{-1}), a previous study achieved 90.5% removal of effluent turbidity in the SDAD process, while 97.2% of the Zn^{2+} was also effectively removed [33]. Moreover, S^0 colloids exhibit a negative charge in waters and the electrostatic interaction with Zn^{2+} is the dominant cause of adsorption and precipitation. This study also provides a new path for the removal of zinc in wastewater. In another study, the use of 5 mg L^{-1} polymerized aluminum chloride (PAC) as flocculant resulted in a 98% recovery of S^0 in the SDAD process [37]. However, the adoption of additional flocculation units will increase the construction costs of the related processes. Therefore, the development of the in situ recovery technology of S^0 in the SDAD process should be investigated in future research.

2.4. Practical Applications of SDAD Process

The SDAD process has been widely implemented in the treatment of actual wastewater. In terms of municipal wastewater, the SANI process with SDAD as the core technology is undoubtedly the most successful case. With the application of the full-scale process [7], it is estimated that the SANI process can reduce the production of excess sludge by 90% and save 30–40% of the land occupation, which led to a 50% reduction of operating costs [34]. As to typical low carbon wastewaters, the denitrification process is also widely used to meet the demand for nitrogen removal from municipal tailwater. With S^0 as the electron donor, the SDAD process has been successfully applied to both pilot- and full-scale operations [23]. It is noteworthy that the BNR performance of the SDAD process can be maintained even at a low temperature of 6.4–9.8 °C [38], which provides a basis for the extension of the process. Besides, SDAD process has also been widely applied in the treatment of sulfide-containing and flue gas desulfurization wastewater [15,39]. Overall, in recent years, the SDAD process has made great progress in the application of treating low carbon wastewater. Nevertheless, the optimization of key parameters and the accumulation of engineering experience are still urgently needed.

3. Anammox Process

3.1. Mainstream Anammox Process

The discovery and application of anammox technology provides a viable solution to the problem of insufficient carbon sources for mainstream municipal wastewater. However, as far as the mainstream anammox process is concerned, the availability of nitrite is the primary bottleneck. Effective strategies for this problem mainly focus on partial nitrification (PN) and partial denitrification (PD) coupled with anammox. Herein, the PN-anammox (PN-A) and PD-anammox (PD-A) process are discussed respectively in detail as follows:

3.1.1. Partial Nitrification-Anammox (PN-A) Process

The PN-A process mainly consists of the two following steps, as shown in Figure 4a: (1) the accumulation of nitrite due to partial nitrification of ammonium under aerobic conditions; (2) the remaining ammonium is oxidized by nitrite to N_2 through anammox [40]. Compared with the traditional nitrogen removal process, in terms of the PN-A process, about 60% of the aeration consumption can be saved and does not rely on the carbon sources [8]. The achievement of PN is dependent on the control of dissolved oxygen (DO). In the PN system, ammonium oxidizing bacteria (AOB) have a lower oxygen half-saturation constant than nitrite oxidizing bacteria (NOB) [41]. Thus, AOB tends to dominate under low DO conditions. By controlling the DO at 0.15–0.18 mg L^{-1}, a previous study successfully achieved the inhibition of NOB activity under mainstream conditions, and the NRE of the PN-A system reached 70% during long-term operation [42]. However, the recent studies have shown that NOB can recover from the suppression of low DO, which is not conducive for the long-term stable operation of the PN-A system [43,44]. The research by Wang et al. [43] showed that NOB can adapt to the inhibition of low DO (0.3–0.4 mg L^{-1}) and gradually regain nitrite oxidation activity after 3 months [43]. The operation strategy of intermittent aeration provides a viable solution to the above problem. A previous study found that the AOB can recover its bioactivity from DO inhibition faster compared to NOB, which offers the possibility of nitrite accumulation under intermittent aeration [45]. Specifically, Xu et al. [46] showed that 0.5 mg L^{-1} of DO and 20 min of anoxic time effectively suppressed NOB and increased NRE of PN-A process by 40% [46]. By pre-anaerobic treatment, a recent study achieved more than 90% accumulation of nitrite (DO: 0.5–1.0 mg L^{-1}), and the NRE of PN-A process-treated low C/N domestic wastewater was stabilized at 92.06% [47]. Besides, the online control technology of DO also provides a guarantee for the optimization of the PN-A process, and it has been applied on a pilot-scale in China [44].

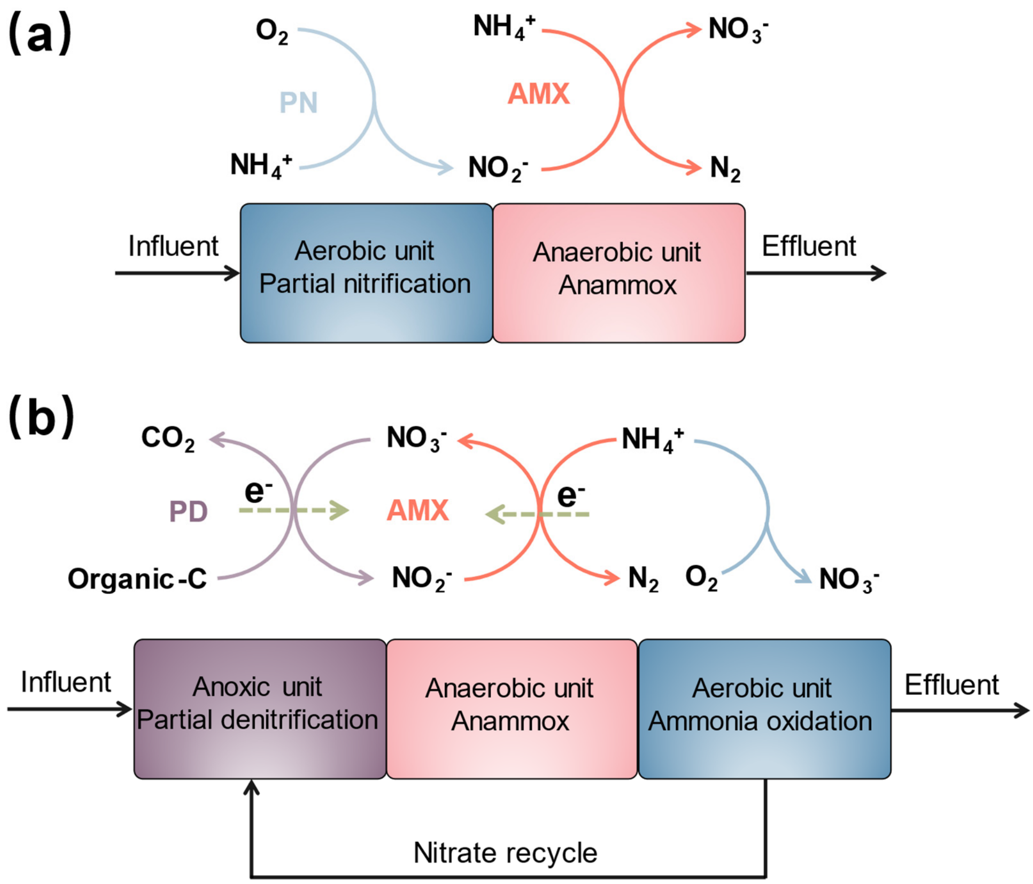

Figure 4. Schematic diagram for the partial nitrification−anammox (**a**) and the partial denitrification-anammox (**b**) processes.

Low temperature is another environmental factor that needs to be considered in mainstream PN-A processes. The theoretical temperature to maintain the activity of AOB and anammox bacteria (AnAOB), and eliminate NOB, is reported to be 25–35 °C [48]. However, the mainstream conditions (T < 15 °C) will lead to the inhibition of AnAOB. Therefore, the development of operating technologies related to the anammox process at low temperatures is also an important current consideration. It was found that AnAOB can gradually adapt to low temperatures through acclimation. After 10 days of acclimation, the study by Hu et al. (2013) achieved stable operation of PN-A system at 12 °C, and the NRE could be stabilized at 90% during 300 days of operation [49]. In addition, the maintenance of anammox biomass was also being proved as a strategy to increase the tolerance of the PN-A system to low temperature. The related research investigated the process performance of conventional PN-A vs. a biofilm-based system during the process from 20 to 10 °C [50]. The results illustrated that the biofilm-based PN-A system achieved a better nitrogen removal performance (0.007 g N L^{-1} d^{-1}). Furthermore, the maximum nitrite consumption efficiency was observed in the experimental group with 10 mm-carriers (compared with 2-mm), indicating a better anammox performance [50].

In addition to those mentioned above, some studies have attempted to optimize the PN-A process by exogenous administration of NOB inhibitors, such as hydroxylamine (NH_2OH, 2 mg L^{-1}) and hydrazine (N_2H_4, 2 mg L^{-1}) [48,51]. Despite the improved process performance, the practical scale-up of this initiative is still limited considering the operating costs. Overall, PN-A has been comprehensively confirmed as a feasible solution to applying anammox to mainstream wastewater nitrogen removal. However, engineering experience still needs to be accumulated for the full-scale application of PN-A process. Moreover, the construction of long-term stable NOB selection inhibition strategies is still a bottleneck and could be considered as the trend for future research.

3.1.2. Partial Denitrification-Anammox (PD-A) Process

The recently developed PD process offers another viable path for anammox application in mainstream wastewater nitrogen removal. PD refers to the control of the denitrification process in the nitrite phase (Figure 4b), which provides substrate for anammox while also consuming nitrate (11% production) produced by the anammox process [52]. Compared with traditional BNR process, 100% of aeration, 80% of external carbon source, and 64% of sludge production can be saved by the PD-A process [53]. Currently, a full-scale WWTP based on the PD-A process (25×10^4 m^{-3} d^{-1}) has been successfully constructed in Xi'an (107.40–109.49° E, 33.42–34.45° N), China, which demonstrates the attractive potential of PD-A in mainstream wastewater nitrogen removal [54]. The COD/N ratio is the decisive parameter to be considered first for the PD-A process. A high COD/N favors complete denitrification, which increases the possibility of AnAOB elimination in PD-A systems. Conversely, a low COD/N has been widely accepted as an effective strategy to achieve nitrite accumulation and anammox. Cao et al. (2019) systematically investigated the performance of the PD-A process at different COD/N ratios [53]. The results showed that the NRE of the process at low COD/N (0.5–1.7) can be maintained consistently above 80%, while C/N ratios above 4.0 will lead to rapid deterioration of the nitrogen removal performance [53]. Considering the characteristics of the actual municipal wastewater, the optimal range of the COD/N ratio for the PD-A process was recommended to be 2.0–3.5 in the last recent study [44].

As autotrophic bacteria, the growth rate of AnAOB is much lower than that of HB. Therefore, the maintenance of biomass concentration, especially anammox biomass, is equally important for the operation of the PD-A process. A recent study achieved effective enrichment of AnAOB (relative abundance: from 0.74–4.34%) by introducing a biofilm in an anoxic unit of PD-A system [55]. The same phenomenon has also been reported for a full-scale WWTP-based PD-A process, which highlights the crucial role of regulating biomass in practical applications. In addition, the bioavailability of the carbon source is also a key factor affecting the performance of PD-A. It is reported that readily biodegradable organics, compared to the slowly biodegradable, are more likely to lead to an excellent performance [56]. Although the PD-A process has shown great potential in the nitrogen removal of low carbon wastewater, there is still a knowledge gap in the regulation of the ecological niche balance between AnAOB and HB, which should be the focus of future research.

3.2. *Sidestream Anammox Process*

In addition to mainstream municipal wastewater, the anammox process also has great potential for treating high strength nitrogen-containing wastewater. In the upflow anaerobic sludge bed (UASB) reactor, Tang et al. [57] achieved a super-high nitrogen removal rate of 74.3–76.7 kg N m^{-3} d^{-1} for the anammox process [57], which is the highest level that has been reported so far to our best knowledge. In that study, the short HRT (0.16–0.11 h) resulted in high biomass concentrations (42.0–57.7 g VSS L^{-1}), which in turn led to a high performance [57]. A recent study has shown that aggregated anammox biomass achieves higher nitrogen removal performance compared to the planktonic flocs [58]. Thus, focusing on sludge aggregation and maintaining a high biomass is also an important regulatory measure for the sidestream anammox process. Despite acting as substrates, high concentrations of nitrite can lead to an inhibition of annamox, especially in the context of high nitrogen-containing wastewaters. The inhibition thresholds for nitrite have been reported in the range of 5–280 mg L^{-1} under different operating modes [59–61]. For a general continuous flow anammox process, Jin et al. (2012) suggested the concentration of 280 mg L^{-1} (influent) and 100 mg L^{-1} (effluent) as pre-warning values for nitrite inhibition [60].

It is worth noting that the effect of characteristic pollutants accompanying high nitrogen-containing wastewater on anammox should also be considered, such as antibiotics, heavy metals, etc., in livestock and poultry farming wastewater. Take norfloxacin (NOR), which had been widely detected in piggery wastewater [62], as an example. A previous study showed that a trace concentration of 1 μg L^{-1} of NOR can lead to a significant inhibition on the performance of anammox [63]. In another study, the anammox system exhibited tolerance to 50 mg L^{-1} of streptomycin, while 3 mg L^{-1} of spiramycin resulted in inhibition [64]. Thus, the inhibitory effect on anammox depends mainly on the type of pollutant. Table 1 summarizes the common pollutants and corresponding concentration thresholds that cause inhibition on anammox, including antibiotics [63–65], salinity [66–68], and heavy metals [69–71]. Detailed regulatory measures of anammox response to related pollutants have been discussed in previous studies [60,72]. The related strategies will help optimize the anammox process and expand its applications for different types of nitrogen-containing wastewaters.

Table 1. Summary of common inhibitors on anammox and the corresponding concentration thresholds.

Inhibition Conditions		Reactors	Concentration of Nitrogen (mg L^{-1})		Inhibition Threshold	References
			Ammonium	Nitrite		
Antibiotics	Norfloxacin	Biofilm reactor	50.1	51.4	1 ug L^{-1}	[63]
	Spiramycin	UASB	280	280	3 mg L^{-1}	[64]
	Erythromycin	UASB	280	280	0.1 mg L^{-1}	[65]
	Sulfamethoxazole	UASB	280	280	5 mg L^{-1}	[65]
	Tetracycline	UASB	280	280	0.1 mg L^{-1}	[65]
Salinity	NaCl	SBBR	400–472	520	12 g L^{-1}	[66]
	NaCl	UASB	191	325	20 g L^{-1}	[67]
	NaCl; KCl	Batch test	100	100	18.6 g L^{-1}	[68]
Heavy metals	Zn^{2+}	Batch test	-	-	6.9 mg L^{-1}	[70]
	Cu^{2+}	Batch test	100	100	12.9 mg L^{-1}	[71]
	Cd^{2+}	Batch test	100	120	11.16 mg L^{-1}	[69]
	Ag^{+}	Batch test	100	120	11.52 mg L^{-1}	[69]
	Hg^{2+}	Batch test	100	120	60.39 mg L^{-1}	[69]

UASB: upflow anaerobic sludge bed reactor; SBBR: sequencing batch biofilm reactor.

4. Sulfur-Driven Autotrophic Denitrification Coupled with Anammox Process

Regulating the SDAD process to achieve the accumulation of nitrite has also proven to be a viable alternative to provide substrates for anammox. Many sulfur (e.g., SO_3^{2-}, $S_2O_3^{2-}$, S^0, and S^{2-}) have been reported to be utilized by a-soNRB for partial denitrification [73], which provides theoretical support for the coupling of SDAD with anammox, namely sulfur-driven partial denitrification and anammox (SPDA) processes. The lower growth rate of a-soNRB (0.04–0.27 h^{-1}) will avoid competition with AnAOB [74], and therefore does not require relatively complex process control. In the SDAD system, the research by

Chen et al. (2018) showed that the affinity of S^0 for nitrate was significantly higher than that for nitrite, and on this basis, over 95% nitrite accumulation was successfully achieved [75]. The optimal pH and temperature for achieving partial denitrification in the SDAD process proved to be 8.5 and 35 °C, respectively, in that study [75]. Coupled with anammox on this basis, a recent study successfully realized the S^0-based SPDA process for the treatment of semiconductor wastewater (NH_4^+-N: 410 ± 20 mg L^{-1}; NO_3^--N: 640 ± 15 mg L^{-1}) and achieved a nitrogen removal rate of 4.11 kg N m^{-3} d^{-1} [76]. Thiosulfate ($S_2O_3^{2-}$) has likewise proven to be an ideal sulfur source for the establishment of the SPDA process. The research of Deng et al. [77] achieved 82.5% NRE for the thiosulfate-based SPDA process, and the contribution rate of anammox to nitrogen removal reached 90% [77]. Compared with the SDAD process, about 49% of electron consumption can be reduced by the thiosulfate-based SPDA process [77]. Sulfide often coexists with high concentrations of ammonium in anaerobic treatment tailwater, and is also an ideal electron donor for the SDAD process (as discussed in Section 2). Meanwhile, the toxicity of sulfide to anammox is commonly reported, which becomes a barrier to the related process. Sulfide-based SPDA process offers a new path for efficient treatment of related wastewaters. On the one hand, while providing nitrite, a-soNRB can also oxidize sulfide to S^0 or sulfate with low-toxicity, which provides AnAOB with a low-toxic environment. On the other hand, the establishment of the sulfide-based SPDA process, which is oriented to S^0 production, has the ability to desulfurize. Liu et al. [78] successfully realized the high accumulation of sulfide-based SPDA process in the EGSB reactor, and proposed that the optimal ratio of sulfide to nitrate is 1.31:1 [78]. Furthermore, a recent study has systematically evaluated the stability of the sulfide-based SPDA process in long-term operation (392 days), and 80% of stable NRE was achieved [79].

Although a-soNRB as autotrophic bacteria avoids competition with AnAOB, a long start-up time is required for the SPDA process due to the slow growth rate of functional bacteria. Therefore, it is of practical importance to establish a fast start-up technology for the SPDA process. A previous study found that anammox sludge could be an ideal inoculum for efficient start-up of the SDAD process [80]. By only changing the substrate composition of influent, a high-performance SDAD process was achieved in a UASB reactor with nitrate and sulfide removal rate of 28.45 kg N m^{-3} d^{-1} and 105.5 kg S m^{-3} d^{-1}, respectively [80]. The same result was also confirmed in another study, where interestingly the obtained SDAD process could achieve high anammox performance in a short period (nitrogen removal rate: 1.68 kg N m^{-3} day^{-1}) [81]. In other words, the SDAD process can be switched quickly between the anammox process without complicated regulation. Current studies on fast start-up of the SPDA process are still insufficient. The related findings mentioned above provide a new perspective for further studies.

5. Functional Microorganisms

The metabolic function of microorganisms is the key to the realization of the autotrophic biological nitrogen removal process. Thus, insight into the functional microbial species and their physiological characteristics is essential for process operation and optimization. Here, the functional microorganisms commonly reported in autotrophic nitrogen removal systems are systematically summarized and linked to process performance.

5.1. Autotrophic Sulfur Oxidizing Nitrate Reducing Bacteria (a-soNRB)

The a-soNRB involved in the ecosystem sulfur cycle are composed of two types of phototrophs and chemolithotrophs. In terms of water or wastewater treatment system, as shown in Table 2, the functional bacteria are mainly chemolithotrophs [82–90]. At the genus level, *Thiobacillus* and *Sulfurimonas* are the most commonly identified as the dominant bacteria in the SDAD process. Although both *Thiobacillus* and *Sulfurimonas* were reported to have the ability to reduce nitrate or nitrite (Table 2), the reduction potential is different. In the S^0-based SDAD system, the study by Chen et al. (2018) shown that *Thiobacillus* were significantly enriched when the electron acceptor was converted from nitrite to nitrate and resulted in a high-process performance [75]. In addition, organic carbon is also an important factor leading to the composition of functional bacteria. It is reported that *Thiobacillus* is sensitive to organic components and conversely *Sulfurovum* is more suitable for organics condition [85]. A recent related study found that during the change of the C/N ratios from 2.7 to 0, *Sulfurovum* was almost eliminated from the SDAD system (relative abundance: 15.4% to 0.9%), and instead *Thiobacillus* became the absolute dominant bacteria (relative abundance: 0.1% to 50.2%) [85]. In another study, the addition of acetate reduced the relative abundance of *Thiobacillus* from 51.4% to 39.4%; in contrast, *Thauera* was accumulated and accounted for 7.3% in the SDAD system [30]. Notably, as common heterotrophic bacteria [52], the sulfur oxidation capacity of *Thauera* has also been reported (Table 2). Therefore, when considering the application of the SDAD to low carbon wastewater treatment, the transformation of functional bacteria is actually a dynamic process. The interpretation of the dominant bacterial localization associated with function is essential for the regulation of related processes.

Table 2. Characteristics of main sulfur oxidizing bacteria in water and wastewater treatment processes.

Genera	Origins	Electron Donor	Electron Acceptor	Relative Abundance	References
Agrobacterium	S^0-packed bed	S^0; S^{2-}; $S_2O_3^{2-}$; SO_3^-; organic carbon	NO_3^-	-	[87]
Pseudomonas	EGSB	S^0; S^{2-}; organic carbon	NO_3^-; NO_2^-	27.60%	[30]
Paracoccus	MBBR	S^0; S^{2-}; $S_2O_3^{2-}$; organic carbon; H_2	NO_3^-	7.87%	[86]
Thiobacillus	SBR	S^0; S^{2-}; $S_2O_3^{2-}$; SO_3^-	NO_3^-; NO_2^-	50.20%	[18,85]
Thioalkalimicrobium	Sediments from lake	S^0; S^{2-}; $S_2O_3^{2-}$	NO_3^-	-	[18]
Thioalkalivibrio	Sediments from lake	S^0; S^{2-}; $S_2O_3^{2-}$	NO_3^-; O_2	-	[89]
Thioploca	Sediments from lake	S^0; S^{2-}; $S_2O_3^{2-}$; organic carbon	NO_3^-; O_2	-	[18,84]
Thauera	AnFB-MBR	S^0; S^{2-}; $S_2O_3^{2-}$; organic carbon	NO_3^-	11.90%	[34,83]
Sulfurimonas	SBR	S^0; S^{2-}; $S_2O_3^{2-}$; SO_3^-; organic carbon	NO_3^-; NO_2^-; O_2	11.80%	[34,75,90]
Sulfurovum	Deep-sea hydrothermal vent	S^0; $S_2O_3^{2-}$	NO_3^-; O_2	-	[82]
Sulfuricella	S^0-packed bed	S^0; $S_2O_3^{2-}$	NO_3^-	About 10%	[18]
Ferritrophicum	Sediments	S^0; S^{2-}; Fe^{2+}; H_2	NO_3^-	-	[88]

EGSB: expanded granular sludge bed; MBBR: moving bed biofilm reactor; SBR: sequencing batch reactor; AnFB-MBR: anoxic fluidized-bed membrane bioreactors.

As shown in Table 2, in addition to S^0 and sulfide, thiosulfate can be widely utilized by a-soNRB as an electron donor and is therefore considered an ideal substrate for autotrophic denitrification process. Thiosulfate is more likely to drive high denitrification performance due to its low toxicity with high bioavailability [18]. However, thiosulfate-driven autotrophic denitrification processes are predominantly laboratory-scale due to the relatively poor chemical stability and difficult engineering available for large quantities.

There are differences in the sulfur oxidation pathways among different genera, which directly determine the fate of sulfur in the SDAD system. In the case of *Thiobacillus* vs. *Sulfurimonas*, the sulfur oxidation pathway in the former is mainly regulated by the Dsr gene cluster, resulting in the formation of S^0. However, *Sulfurimonas* primarily uses the SoxCD gene cluster rather than Dsr, so S^0 formation has not been reported [40,91]. Besides, sulfur oxidation by *Pseudomonas* is mainly regulated by Sqr, leading to the accumulation of S^0 in the periplasm [4,18]. The S^0 transformation rate of up to 94% has been reported in the integrated autotrophic–heterotrophic denitrification (IAHD) system with *Pseudomonas* as the dominant genus [4]. Therefore, the selective enrichment of the corresponding functional bacteria is of great importance when constructing a desulfurization-oriented SDAD process.

5.2. Anammox Bacteria (AnAOB)

So far, all identified AnAOB belong to the phylum Planctomycetes under six candidatus genera as follows: *Kuenenia*, *Brocadia*, *Jettenia*, *Scalindua*, *Anammoximicrobium*, and *Anammoxoglobus* [92]. It is worth noting that different genera of AnAOB occupy different ecological niches in the natural environment and wastewater treatment systems, due to complex inter- and intraspecific relationships. In the wastewater treatment systems, *Candidatus* Brocadia, *Candidatus* Kuenenia, *Candidatus* Jettenia and *Candidatus* Scalindua are commonly identified as the dominant genera with the relative abundance of around 0.1–38.0% [93]. *Candidatus* Brocadia is widely reported as the typical dominant genus under the mainstream conditions, especially in full-scale structures. A recent study found that *Candidatus* Brocadia, which makes up only 0.4% relative abundance, can contribute 61–72% of total nitrogen removal in the mainstream anammox process treating domestic sewage [94]. Interestingly, the tolerance of *Candidatus* Brocadia to dissolved oxygen has been reported by some studies. The Brocadia-dominated biomass can recover biological activity after micro-oxygen (0.02 mg O_2 L^{-1}) inhibition [95]. Moreover, Ji et al. [96] elucidated the potential mechanism of oxygen detoxification in Ca. *Brocadia* sp. from a genetic and metabolic perspective, and grabbed the positive role of genes encoding cytochrome c peroxidase (Ccp) and superoxide dismutase (SOD) [96]. As shown in Figure 4, in the mainstream process, a certain amount of dissolved oxygen will flow into the anammox unit with nitrification liquid, either in the PN-A or PD-A system. Thus, the tolerance of AnAOB to dissolved oxygen should be further concerned in future research and application.

Unlike mainstream conditions, *Candidatus* Kuenenia are often enriched in lab-scale bioreactors with high nitrogen concentrations. So far, over 90% enrichment level of *Candidatus* Kuenenia has been achieved in laboratory bioreactor [97]. Besides, the tolerance of *Candidatus* Kuenenia to a wide range of contaminants has been widely reported (as discussed in Section 3.2), which highlights the application potential of *Candidatus* Kuenenia in sidestream conditions. A recent study found that *Candidatus* Kuenenia was more competitive than *Candidatus* Brocadia in the presence of fulvic acid at 15 °C [98]. In another study, the dominant genus shift from *Candidatus* Brocadia to *Candidatus* Kuenenia was observed in a biofilm reactor, as the nitrogen loading rate increased [99]. The ammonium and organic carbon co-metabolic capability of *Candidatus* Kuenenia may promote the survival of AnAOB under unfavorable conditions [100].

As the only reported marine anammox bacteria (genus level), *Candidatus* Scalindua was first discovered in the Black Sea and drew wide attention due to its high salinity tolerance [101]. It has been reported that the anammox activity of *Candidatus* Scalindua can be maintained at high salinity conditions of 30–50 g L^{-1} [102]. Besides, in a pilot-scale UASB reactor, Yokota et al. [101] successfully achieved the enrichment of *Candidatus* Scalindua (relative abundance: 20–30%) and the nitrogen removal rate of the reactor reached 10.7 kg N m^{-3} d^{-1} at a salinity of 27.0 g L^{-1} [101]. The high salinity tolerance of *Candidatus* Scalindua may benefit from its salt-in and compatible-solute strategies. On the one hand, under the salt-in strategy, cations (especially K^+) are pumped into the cytoplasm to match the osmotic pressure of the salinity environments. On the other hand, cells can also resist high osmotic pressure through the synthesis of small organic molecules, known as the compatible-solute strategy [102]. Although both of these strategies have been reported in *Candidatus* Scalindua, the choice of specific strategies varies considerably at the species level. In addition, the specific operating mechanism need to be further clarified. Interestingly, researchers have generally found that *Candidatus* Scalindua cannot be enriched in the laboratory with synthetic salt (e.g., NaCl or KCl) as substrates. However, two species of *Candidatus* Scalindua (*Ca*. S. brodae and Ca. S. wagneri) have been detected in WWTP (Pitsea, UK) treating landfill leachate [97]. Thus, further studies on the ecological distribution of AnAOB and its metabolic properties are apparently required.

6. Future Perspectives of the Application

Although the application of SDAD and the anammox-based autotrophic BNR process in low-carbon wastewater treatment has received increasing attention and progress in recent years, issues in terms of widespread dissemination and basic mechanisms remain unresolved. As shown in Figure 5, several current bottlenecks and possible solutions in the application are proposed as follows:

(1) Enrichment of functional bacteria. The slow growth rate of autotrophic bacteria on the one hand avoids the production of large amounts of residual sludge, but at the same time makes them vulnerable to be eliminated from the wastewater treatment system.

Thus, the enrichment of highly active and abundant functional bacteria should be considered. Previous studies have illustrated that the structure of biofilms and granular sludge lead to a high performance in autotrophic BNR processes [18,55,98]. Future research should focus on the aggregation behavior and enrichment of bacteria, decipher the formation process of biofilm and granular sludge, and provide microscopic interpretation and guidance for related processes.

(2) Community interactions. Whether in the SDAD or anammox process, functional bacteria always appear together with companion bacteria, but their functional and ecological significance is often overlooked. Therefore, exploring the intra- and interspecific interactions from the perspective of community, such as cross-feeding [103], quorum sensing [104], and functional redundancy [105], will aid further understanding of the autotrophic system. Moreover, it lays a foundation for the optimization of community structure and function via process performance.

(3) Metabolic pathways. Although the main metabolic pathways of a-soNRB and AnAOB have been clarified, there are still knowledge gaps regarding the metabolic diversity and coupling. With the development of molecular biological methods (e.g., metagenomic, metatranscriptomics, and proteomic analysis) in recent years, more unknown metabolic pathways have been discovered in the anammox or SDAD systems. The isolation of C, N and S co-metabolizing bacteria and the interpretation of their metabolic mechanisms [4,106], the discovery of new metabolism pathways such as ferric ammonium oxidation (Feammox) [107], nitrate/nitrite dependent anaerobic methane oxidation (n-DAMO) [8], and dissimilatory nitrate reduction to ammonium (DNRA) [108] have greatly expanded the knowledge of the natural nitrogen cycle. Besides, this information provides theoretical guidance for the broad application of wastewater nitrogen removal processes and the development of new technologies.

(4) Construction of nutritional conditions. The construction of an autotrophic condition is a prerequisite for the autotrophic BNR process. In terms of the SDAD process, improving the bioavailability of S^0 should be considered first. Although some strategies have been proven to be feasible, such as polysulfide mediation and surface modification of S^0 [18], the long-term stability still needs further optimization. It is recommended that future studies focus on the interfacial mass transfer between S^0 and biofilm via the immobilization of functional community groups, which has been somewhat neglected before. In terms of anammox, the stable supply of nitrite in wastewater treatment systems remains a concern. In addition to PN and PD, sulfur-based autotrophic PD strategy should be further investigated. Besides, coupling of multiple nitrite supply techniques may also be considered, as previous studies have shown that a relatively diverse microbial community structure is conducive to the maintenance of the macroscopic stability of the system [107].

(5) Key parameters. In addition to the key parameters such as pH, temperature, substrate ratio, HRT, etc., which have been discussed above, the combined effect of various parameters on the process performance should also be intensively investigated due to the complex situation in practice. On the other hand, more effective parameters for reflecting on the in-situ state of the biological treatment system should be further explored. Some recent studies have established real-time monitoring systems based on dissolved organic matter (DOM) spectral detection in wastewater treatment systems [109,110]. DOM contains a large number of soluble microbial products (SMP), and the changes of content and compositions have been shown to establish a good correlation with process performance. However, related studies in the autotrophic BNR process, especially for SDAD systems, have been rarely reported. Thus, the detection of parameters related to spectra (SMP) or signal molecules is of positive significance for the establishment of real-time monitoring and pre-warning systems.

(6) Environmental conditions. The bioactivity of functional bacteria is significantly related with the environmental conditions. In addition to the environmental parameters (e.g., pH and temperature, etc.) mentioned above, the changes of environmental conditions

caused by characteristic pollutants contained in low-carbon wastewater, such as antibiotics, heavy metals, and salinity, should be of concern. In addition to process performance, the fate of characteristic pollutants should also be focused to avoid secondary pollution as much as possible. The spatial distribution of environmental conditions in autotrophic BNR systems is often overlooked, but spatial differences in denitrification functions and community structure do exist [4,30]. Based on the focus of the spatial distribution and coupling of functional bacteria, the exploration of spatial differences in environmental conditions, especially in full-scale processes, is conducive to the design and optimization of related facilities.

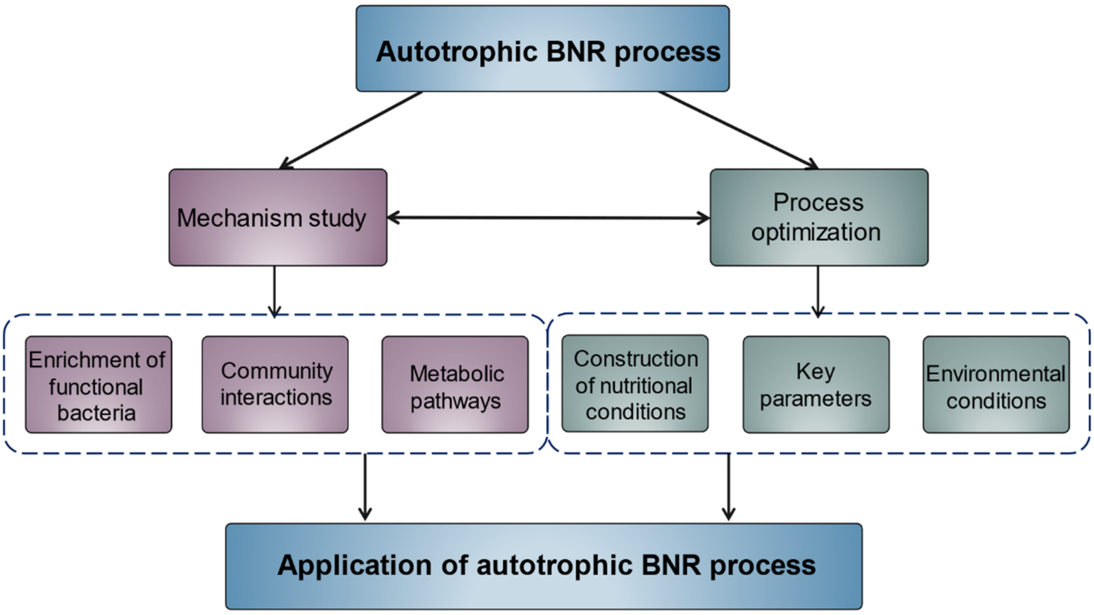

Figure 5. Proposed strategies for the optimization and application of the autotrophic biological nitrogen removal (BNR) process.

As a significant source of emissions, WWTPs emit large amounts of greenhouse gases represented by CO_2, which brings adverse effects to global climate change. According to the analysis, carbon emissions from WWTPs account for more than 3% of the total global carbon emissions [111]. From the energy point of view, the conventional HD-based BNR process is actually a carbon emitting and energy consuming process. Therefore, the construction of low carbon emission and energy autarky wastewater treatment system is a goal pursued globally. Autotrophic BNR technology allows for maximum retention of carbon in the wastewater, which can be recovered in the form of energy in subsequent anaerobic digestion units. Besides, autotrophic BNR technology also offers advantages in terms of reducing greenhouse gas (CO_2 and N_2O) emissions. Thus, the autotrophic BNR technology shows great potential for meeting the sustainable development of the global wastewater treatment industry.

7. Conclusions

In the present study, the advances and challenges of the autotrophic BNR process, mainly including SDAD and anammox, have been systematically reviewed. The related process offers a promising pathway for the treatment of low-carbon wastewater. The SANI, PN-A and PD-A processes can be considered in response to the insufficient carbon sources for mainstream municipal wastewater. Meanwhile, the production of residual sludge can be minimized due to the slow growth rate of autotrophic bacteria. The SPDA process, coupling SDAD with anammox, allows for complete autotrophic nitrogen removal so that the carbon source could be preserved to the maximum extent, which can be recovered in the anaerobic unit as energy. Improving the stability and efficiency of the process is still the current bottleneck. It is suggested to shed more light on microbial community succession and metabolism of functional bacteria, which can provide theoretical guidance of the optimization. For promotion and application, the accumulation of engineering experience and the acquisition of key parameters are also the focus of future research. Overall, in the context of carbon emission reduction and sustainable development, autotrophic BNR process shows great technical advantages and deserves to be one of the important main lines of future development in the field of wastewater treatment.

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