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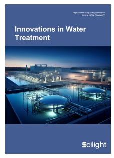

Mini Review

Enhancing the Stability of Partial Nitritation Anammox in Mainstream Municipal Wastewater Treatment: Biological Foundations, Control Strategies, and Future Perspectives

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Abstract: Mainstream anammox has attracted much attention as an energy-efficient nitrogen removal technology. However, its stable operation remains challenged by complex mainstream conditions, limiting engineering implementation. This review synthesized the biological foundations, operational control strategies, and engineering practices underlying stable mainstream anammox operation. Process stability depends on effective coupling between ammonia-oxidizing bacteria and anammox bacteria (AnAOB), sustained suppression of nitrite-oxidizing bacteria (NOB), and efficient enrichment and retention of AnAOB. Accordingly, key intensification strategies were highlighted, including fine-tuned dissolved oxygen control, decoupling of hydraulic and solids retention times, reinforcement of biofilm and granular sludge structures, and the use of physical selectors and membrane separation. Application cases demonstrate that, despite proven feasibility, autotrophic nitrogen removal in mainstream anammox remains limited and may decline during long-term operation. Finally, future research directions were proposed, including multi-omics-based elucidation of AnAOB metabolic regulation under low-temperature and low-substrate conditions, quantitative modeling of organic matter and anammox interactions, cross-scale linkages between microscale structure and macroscopic performance, and data-driven intelligent operation and control. This review provides a theoretical basis and practical guidance for the optimization and engineering application of mainstream anammox processes.

Keywords: mainstream anammox; partial nitritation anammox; NOB suppression; AnAOB retention; control strategy

1. Introduction

Against the backdrop of increasingly stringent water quality standards and the implementation of carbon neutrality strategies, municipal wastewater treatment systems are facing growing challenges in simultaneously achieving effective pollution control and carbon reduction [1,2]. Although conventional nitrification and denitrification processes are technologically mature and operationally reliable, their strong dependence on intensive aeration and external organic carbon addition results in high energy consumption and substantial carbon emissions [3,4]. These intrinsic limitations have increasingly emerged as a critical bottleneck constraining the transition of wastewater treatment plants (WWTPs) toward low-carbon operation. Consequently, the development and application of low-carbon wastewater treatment technologies have emerged as a major focus in the field of environmental engineering [5–7].

Anammox process, characterized by its independence from organic carbon addition, low aeration demand, and minimal sludge production, is widely regarded as a transformative pathway toward low-carbon wastewater treatment [8]. Over the past decades, the anammox process has been successfully implemented at full scale for the treatment of high-ammonium sidestream wastewaters, such as anaerobic digester effluents and landfill leachates [9,10], delivering substantial energy savings and carbon emission reductions. However, extending anammox from these high-strength, relatively controllable sidestream conditions to the hydraulically and chemically complex mainstream of municipal wastewater treatment remains highly challenging [11]. In contrast to sidestream systems, mainstream wastewater is typically characterized by low ammonium concentrations (20–60 mg/L), low operating temperatures (10–20 °C), the coexistence of organic carbon and inorganic nitrogen, and pronounced temporal fluctuations in both flow and composition. These environmental conditions are fundamentally misaligned with the physiological traits of anammox bacteria (AnAOB), which exhibit slow growth rates [12], limited tolerance to low temperatures [13], and high susceptibility to competition from heterotrophic bacteria (HB) [14]. As a result, mainstream anammox systems face significant challenges in start-up, operational stability, and long-term performance sustainability. In particular, under low-temperature and low-ammonium conditions, the effective suppression of nitrite-oxidizing bacteria (NOB) and the maintenance of stable partial nitritation (PN) remain major challenges [15]. Sustained enrichment and long-term retention of AnAOB further represent critical bottlenecks for the engineering application of mainstream anammox processes [16,17].

In recent years, research on mainstream anammox has intensified, leading to the development of diverse process configurations and enhancement strategies [18–20]. These include one-stage and two-stage partial nitritation anammox (PNA) systems [21], biofilm or granular sludge technologies [22,23], low dissolved oxygen (DO), and intermittent aeration control [24], as well as microbial regulation approaches driven by selective pressures [25,26]. Despite these advances at laboratory and pilot scales, the micro-ecological characteristics of mainstream anammox systems remain poorly understood. Current insights into the mechanisms underlying stable operation, as well as effective regulation and control strategies, are still limited. Moreover, several reported successful cases rely strongly on site-specific wastewater characteristics or highly complex control schemes [27,28], raising concerns regarding their reproducibility and broader applicability.

Therefore, this review systematically synthesized the microbiological foundations, operational parameter regulation, and engineering practices of mainstream anammox processes. The mechanisms underpinning stable operation and process control were examined in depth. The development potential and future directions of mainstream anammox within low-carbon wastewater treatment frameworks were further discussed. This work can provide a scientific basis and practical insights to support the optimization and engineering application of mainstream anammox processes.

2. Biological Mechanisms Underpinning the Stable Operation of Mainstream Anammox

The long-term stability of mainstream anammox processes fundamentally depends on the selective enrichment, spatial organization, and metabolic coordination of functional microbial communities. In contrast to sidestream systems, the selective pressures imposed on functional bacteria in mainstream systems are substantially weakened, leading to more complex microbial competition. Consequently, a systematic elucidation of the biological foundations underpinning stable operation is required, encompassing community structure, metabolic coupling, and the construction of micro-ecological niches.

2.1. Synergistic and Competitive Interactions among Functional Bacteria

The core functional microbial bacteria in mainstream anammox systems primarily comprise ammonia-oxidizing bacteria (AOB), NOB, AnAOB, and HB (Figure 1). Stable operation relies critically on efficient coupling between AOB and AnAOB, alongside the sustained suppression of NOB. The maximum specific growth rates (μ_{max}) of AOB and NOB are approximately 0.09 h^{-1} and 0.06 h^{-1}, respectively, whereas those of AnAOB are substantially lower (0.002–0.007 h^{-1}) [29]. In mainstream wastewater treatment systems, low ammonium concentrations and low temperatures further suppressed AOB metabolic activity, resulting in insufficient nitrite production for AnAOB. Moreover, under low-substrate conditions, NOB could exhibit higher substrate affinity and competitive growth potential [30,31], facilitating their resurgence in mainstream systems. Accordingly, stable operation fundamentally depends on whether the functional coupling between AOB and AnAOB is sufficiently robust to withstand the competitive advantage of NOB. This competition-driven balance defines the biological objectives underlying both operational parameter regulation and reactor configuration design.

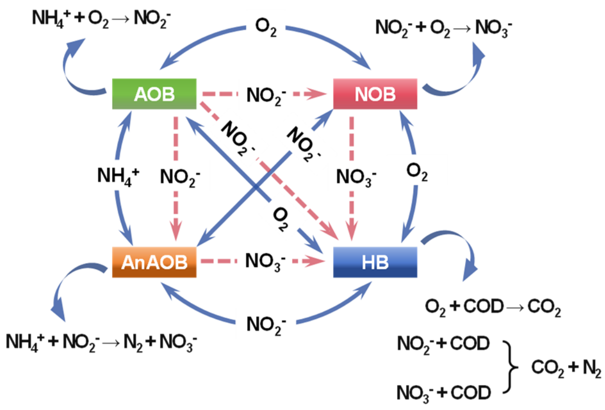

Figure 1. Key microbial bacteria and interactions in mainstream anammox systems.

2.2. Enrichment and Retention Mechanisms of AnAOB

AnAOB exhibit extremely low growth rates and are therefore highly susceptible to washout and competitive exclusion in mainstream systems. Achieving long-term stable operation, therefore, critically depends on the establishment of effective biomass retention mechanisms. These mechanisms extend the actual solids retention time of AnAOB and provide relatively stable ecological niches. Accumulating evidence indicates that biofilm and granular sludge structures represent the most important retention forms for AnAOB [32–34]. Furthermore, the aggregated structures revealed spatial stratification (Figure 2). AnAOB were preferentially observed in the inner layers of granules or biofilms, while AOB and HB dominated the outer layers [35]. This layered organization facilitates the formation of anaerobic microenvironments at the microscale and simultaneously buffers AnAOB against direct exposure to DO, organics, and inhibitory compounds [23,36]. Beyond biomass retention, granular and biofilm formation also substantially enhance the tolerance of AnAOB to low-temperature conditions [37]. The relatively stable mass-transfer environment within granules helps sustain basal AnAOB metabolic activity, allowing mainstream anammox systems to retain measurable nitrogen removal capacity during winter or under prolonged low-temperature operation.

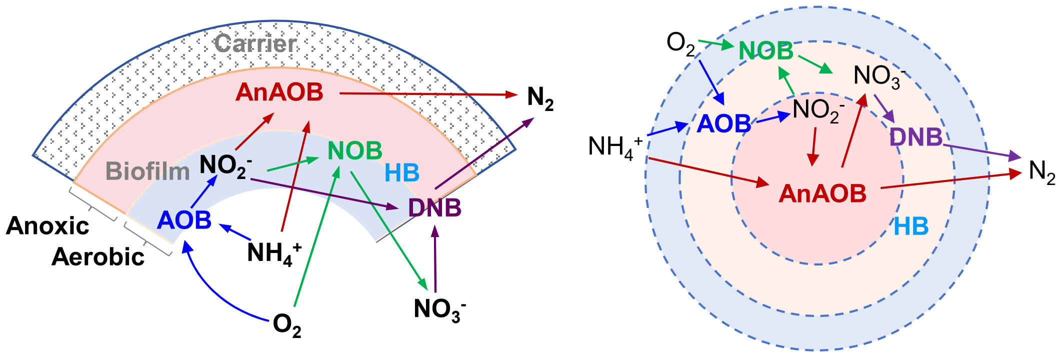

Figure 2. Stratified organization of functional microorganisms in biofilm and granular sludge anammox systems.

2.3. Biological Basis for the NOB Suppression

In mainstream anammox systems, the NOB suppression represents a fundamental biological prerequisite for sustaining PN and ensuring sufficient substrate supply for AnAOB (Figure 3). Many studies suggest that NOB suppression is not governed by a single factor, but rather emerges from the interplay of multiple biological mechanisms [38–40]. DO limitation is the most widely applied strategy for NOB suppression [41,42]. Compared with AOB, NOB generally exhibit a higher oxygen demand, and their metabolic rates decline more sharply under low-DO conditions [8]. However, under mainstream operation, particularly at low temperatures, the physiological differences in oxygen affinity between AOB and NOB tend to diminish, rendering DO-based control alone increasingly uncertain. Free ammonia (FA) and free nitrous acid (FNA) inhibition can also contribute to NOB suppression in mainstream systems [43,44], but their effectiveness is inherently constrained by the relatively low ammonium and nitrite concentrations characteristic of municipal wastewater. Consequently, FA/FNA inhibition typically functions as a complementary rather than a dominant control mechanism. Beyond chemical and operational factors, microbial spatial distribution and structure-induced selective pressure play a critical role in shaping NOB competitiveness [38]. In granular sludge and biofilm-based systems, NOB preferentially colonize outer layers, where they are more susceptible to shear stress, DO fluctuations, and substrate limitation [45,46]. This spatial disadvantage may place NOB at a competitive deficit relative to AOB and AnAOB, providing a mechanistic basis for achieving "ecological niche exclusion" through reactor architecture and biomass structuring.

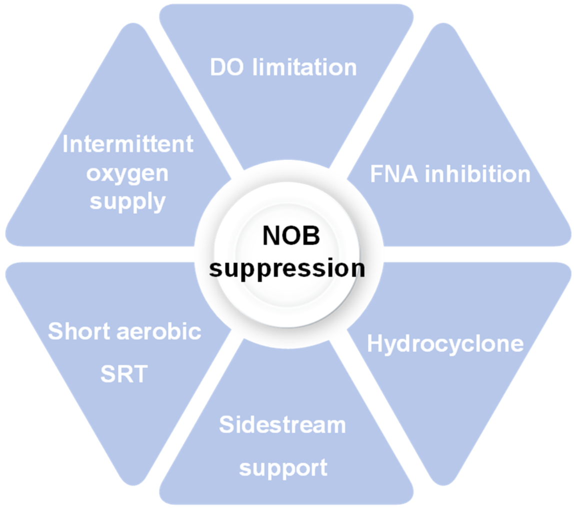

Figure 3. Microbiological mechanisms and control strategies for NOB suppression in mainstream anammox systems.

2.4. Dual Roles of Organics in Mainstream Anammox Systems

Unlike sidestream systems, mainstream municipal wastewater typically contains appreciable concentrations of biodegradable organics, which exerts a pronounced dual effect on the anammox process. On the one hand, the presence of organic substrates can stimulate HB that compete with AOB and AnAOB for key resources such as DO and nitrite, potentially suppressing AnAOB activity [47]. On the other hand, heterotrophic metabolism can indirectly benefit AnAOB by scavenging DO and thereby facilitating the formation of localized anaerobic microenvironments. In this context, partial denitrification and anammox (PDA) has recently been proposed as a potential synergistic nitrogen removal pathway under mainstream conditions [48,49]. By coupling organic carbon-driven nitrate reduction to nitrite with anammox, PDA can provide an additional nitrite source for AnAOB, partially alleviating nitrite limitation. However, the long-term stability and controllability of this pathway under fluctuating mainstream conditions remain insufficiently understood and warrant further investigation.

2.5. Micro-Niche Construction and Its Role in System Stability

From a biological perspective, stable operation of mainstream anammox does not arise from the dominance of a single microbial group, but rather from the orderly coexistence of multiple functional microorganisms across microscale spatial domains [50,51]. Functional stability emerges from a well-defined partitioning of micro-ecological niches. AOB dominate aerobic zones, AnAOB are confined to anoxic interiors, and heterotrophs regulate organic matter turnover and oxygen availability [22,52], collectively stabilizing system performance under dynamic mainstream conditions. Accordingly, both reactor configuration and operational parameter regulation should be designed to support the long-term maintenance of this micro-niche architecture. Stable mainstream anammox relies on efficient AOB-AnAOB coupling, sustained NOB suppression, effective AnAOB retention, and balanced regulation of HB activity [23]. Collectively, these processes reflect a competition and cooperation equilibrium among multiple functional bacteria under low-substrate and low-temperature conditions, accompanied by continuous micro-ecological niche restructuring. A deeper understanding of these mechanisms is essential for translating mainstream anammox from laboratory-scale studies to robust and stable full-scale implementation.

3. Control Strategies for Achieving Stable Mainstream Anammox Operation

Under mainstream conditions in municipal wastewater treatment, anammox systems are challenged by multiple adverse factors, including low ammonium concentrations, reduced temperatures, the coexistence of organic matter, and pronounced hydraulic fluctuations. Reliance on microbial self-adaptation alone is insufficient to ensure long-term stable operation. Instead, sustained stability requires systematic operational regulation and process intensification to impose effective and persistent selective pressures that maintain AOB and AnAOB cooperation while preventing the resurgence of NOB. This section, therefore provides a systematic overview of the key control strategies enabling stable operation of mainstream anammox systems (Table 1).

Table 1. Operational parameters and control strategies for achieving stable operation of mainstream anammox processes.

System	Operational Conditions	Results	Strategy	Reference
SBR with biofilm	DO = 0.7 mg/L, NH_4^+-N = 70–80 mg/L, COD = 200–300 mg/L, T = 30 °C	TNRE = 92.7%, AnAOB = 0.33%, DNB = 8.78%	Low DO & intermittent aeration	[53]
One-stage PNA with biocarriers	DO = 0.15 mg/L, NH_4^+-N = 50 mg/L, HRT = 2 h, T = 35 °C	TNRE = 70%, AnAOB = 29.5%, AOB = 6.8%	Low DO & Carrier	[23]
One-stage PNA with granules	DO = 0.2 mg/L, NH_4^+-N = 50 mg/L, HRT = 2 h, T = 35 °C	TNRE = 72.7%, AnAOB = 35.0%, SAA = 1.02 g-N/g-VSS/d SAOA = 0.93 g-N/g-VSS/d	Low DO & Granules	[46]
One-stage PNA MBBR	DO = 0.24 mg/L, NH_4^+-N = 50 mg/L, HRT = 2 h, T = 25 °C	TNRE = 71.7 ± 9.1%, AnAOB = 29.7%, AOB = 6.3%	Carriers & HRT	[54]
Two-stage PNA	pH = 7.8–8.3, NH_4^+-N = 45–55 mg/L, SRT = 15 d, HRT-PN = 1.16 h, T = 18.6 °C	TNRE = 92.8%, AOB = 1.1%, NOB = 0.12%	Low DO & SRT	[55]
PNA-CSTR with granules	DO = 0.4–0.6 mg/L, NH_4^+-N = 59.6 mg/L, HRT = 40 min, T = 23 °C	TNRE > 80%, SRT increased by 10 times, AnAOB increased by 2.6 times	HRT & SRT & Granules	[56]
One-stage PNA with granules	DO < 0.2 mg/L, NH_4^+-N = 500 mg/L, HRT = 10 min, T = 15 °C	TN removal rate = 0.97 kg/m^3/d, TNRE = 78%, AnAOB = 29.2%, AOB = 16%, NOB < 0.01%	Hydroxyapatite-based granular sludge	[37]
One-stage PNA-MBR	DO < 0.5 mg/L, NH_4^+-N = 50 mg/L, HRT = 1.7 h, T = 28 °C	TNRE = 89%, AnAOB = 11.4%, AOB = 1.7%	Membrane separation	[57]

3.1. Fine-Tuned DO Control Strategies

DO is the key regulatory parameter governing microbial competition in mainstream anammox processes. Owing to their higher oxygen affinity, AOB are selectively favored over NOB under oxygen-limited conditions. AnAOB can avoid direct oxygen exposure through spatial stratification within biofilms or granules. Consequently, maintaining a low DO level is critical for achieving effective partial nitritation. Many studies reported that stable PN typically required DO concentrations in the range of 0.2–0.5 mg/L [24,53], and in some cases even lower (<0.2 mg L^{-1}) [23]. A single-stage airlift PNA system achieved a total nitrogen removal efficiency (TNRE) of 71.8 ± 9.9% with influent NH_4^+-N of 50 mg/L and a DO level of 0.1 mg/L [46]. The formation of micro-granular sludge further mitigated the inhibitory effects of aeration on AnAOB. Although excessive aeration could suppress AnAOB activity and promote excessive NOB proliferation, both biofilm and floc systems have been shown to recover in situ performance under oxygen-limited conditions (DO = 0.05–0.20 mg/L) [23,58]. However, under low-temperature conditions, the differences in oxygen affinity between AOB and NOB become less pronounced, challenging the robustness of strategies based solely on constant low-DO control. In response, dynamic DO regulation and intermittent aeration have attracted increasing attention [59,60]. Dynamic modulation of aeration regimes introduces non-steady-state selective pressures that selectively suppress NOB, whereas AOB and AnAOB are maintained through spatial protection and metabolic coupling.

3.2. Decoupling HRT and SRT

Under mainstream conditions, the effective solids retention time (SRT) of AnAOB must substantially exceed their apparent growth rate to prevent washout with the effluent. Accordingly, decoupling hydraulic retention time (HRT) from SRT could ensure long-term AnAOB retention. Carrier-based biofilm technologies enable high SRT operation of AnAOB while maintaining relatively short HRTs to accommodate the hydraulic demands of municipal wastewater treatment [54]. In addition, staged configurations separating PN and anammox have been proposed as an effective means of coordinating HRT and SRT [21]. In such two-stage PNA systems, a short SRT in the PN reactor (15 days) facilitated the NOB washout, whereas a long SRT in the anammox reactor ensured sustained AnAOB retention [42]. Using coupled DO and SRT control, a two-stage PNA process achieved a high TNRE of 92.8% when treating mainstream municipal wastewater at 20 °C [55]. Additional studies employed hydrodynamic selection pressure by progressively reducing the HRT to enhance the activity of granular PNA sludge. A continuous-flow granular PNA system achieved a TNRE of approximately 80% at an extremely short HRT of 40 min [56]. Granule formation increased the effective SRT AnAOB by nearly an order of magnitude. These findings further confirm that coordinated manipulation of HRT and SRT during operation can substantially improve both the efficiency and stability of PNA systems [61]. It should be noted, however, that SRT optimization cannot be implemented in isolation and typically requires integrated consideration of DO, temperature, and HRT.

3.3. Enrichment Strategies of AnAOB

Current studies have demonstrated several effective strategies for ensuring the enrichment and long-term retention of AnAOB in mainstream systems. (1) Granular sludge formation. Granulation has been shown to sustain high functional activity and abundance of AnAOB, with *Candidatus Brocadia* reaching a relative abundance of 35.0% and an activity of 1.02 g N/g-VSS/d [58]. Moreover, the formation of hydroxyapatite-based autotrophic nitrogen-removal granules enabled a reactor to achieve a high nitrogen removal rate of 0.97 kg/m/d at 15 °C, markedly reducing the sensitivity of anammox communities to temperature decreases [37]. (2) Biofilm-based retention using carrier media. The addition of carrier materials promoted biofilm development in the PNA system, leading to the dominance of *Candidatus Brocadia* and *Nitrosomonas*, with relative abundances of 29.5% and 6.8%, respectively [23]. (3) Physical selection through hydraulic and density-based separation. Selective retention of dense and strongly aggregated biomass via settling selection or hydrocyclone separation can preferentially eliminate low-efficiency microbial populations and promote AnAOB enrichment [26]. Such "physical selectors" can effectively compensate for the limitations of chemical inhibition strategies under mainstream conditions. At the municipal wastewater treatment plant in Strass, Austria, hydrocyclone-based selection and retention of granular anammox sludge from the sidestream were used to reinforce the mainstream process [62]. This strategy achieved a TNRE of 84.1 ± 11.1% and resulted in a 46-fold increase in the absolute abundance of AnAOB. (4) Coupling mainstream anammox with membrane separation. Integration of mainstream anammox with membrane bioreactors (MBR) enables extremely high SRT operation through efficient solid-liquid separation. In a single-stage PNA-MBR system treating influent ammonium at 50 mg/L, a TNRE of 89% was achieved, with AnAOB and AOB relative abundances reaching 11.4% and 1.7%, respectively [57].

3.4. Strategies for Strengthening Low-Temperature Operation

Low temperature is widely recognized as a key constraint on the engineering application of mainstream anammox. To address the pronounced performance decline during winter operation, multiple enhancement strategies have been proposed. Long-term low-temperature acclimation could progressively select AnAOB communities with improved cold tolerance [63,64]. On the other hand, the establishment of biofilm- or granular-based architectures could create relatively stable microenvironments that partially buffer external temperature fluctuations [13,65].

Stable operation of mainstream anammox relies on the coordinated implementation of multi-level control strategies, including fine-tuned DO regulation, decoupling of HRT and SRT, reinforcement of biomass structure, and process integration. Collectively, these strategies aim to establish sustained selective pressures and stable micro-ecological niches, thereby preserving the functional advantage of AnAOB under the complex and dynamic conditions characteristic of mainstream wastewater. Future research should focus on the systematic integration of these control approaches, coupled with online monitoring and intelligent control, to enable long-term stable operation and facilitate the engineering-scale deployment of mainstream anammox.

4. Engineering Practices and Implications

Although substantial progress has been achieved for mainstream anammox at laboratory scale, its engineering application in full-scale municipal WWTPs remains at an exploratory stage. Existing application studies have demonstrated the considerable energy-saving potential of mainstream anammox, while also suggesting the challenges associated with maintaining long-term stable operation under complex real-world conditions. By synthesizing representative application cases, key operational characteristics, and practical insights were further summarized (Table 2), providing an engineering perspective to support the large-scale deployment of mainstream anammox processes.

Table 2. Pilot-scale and full-scale applications of mainstream anammox processes.

Scale	System	Operational Conditions	Performance	Reference
Pilot	Two-stage PNA, Q = 20 m^3/d	DO = 1.61–4.76 mg/L, NH_4^+-N = 34.01 ± 6.5 mg/L, COD = 102.91 ± 26.44 mg/L, T = 10.4–31.1 °C, HRT = 4.5–13.5 h	NAR = 75.04 ± 10.05%, Eff. TN = 10.91 ± 4.23 mg/L	[66]
Pilot	One-stage PNA-CSTR, Q = 1.2 m^3/d	NH_4^+-N = 50 mg/L, HRT = 4 h, pH = 8.0–8.3, T = 15 °C	TNRE = 37.6%, TN removal rate = 0.09 $kg/m^3/d$	[67]
Pilot	One-stage PNA-IFAS, Q = 10.02 m^3/d	NH_4^+-N = 35.6 ± 4.1 mg/L, C/N = 1.44, HRT = 4 h, pH = 8.0–8.3, T = 15 °C	NAR = 65.9%, Eff. TN = 12.0 mg/L	[68]
Pilot	Two-stage PNA, V = 600 L	NH_4^+-N = 43 ± 10 mg/L, COD = 66 ± 11 mg/L, T = 11–28 °C	NAR = 99%, TNRE = 80%	[69]
Full	Step-feed AO process, Q = 80 × 10^4 m^3/d	NH_4^+-N = 31 ± 3.7 mg/L, TN = 41 ± 4.2 mg/L, COD = 337 ± 41 mg/L, T = 28–32 °C	TNRE = 86%, Anammox contribution = 37.5%	[70]
Full	PN- fixed-film, Q = 4 × 10^4 m^3/d	NH_4^+-N = 53.4 mg/L, TN = 57.0 mg/L, COD = 350 mg/L, T = 11.6–28.9 °C	TNRE = 91.8 ± 4.6%, Eff. TN = 4.5 ± 2.3 mg/L	[71]
Full	Adsorption-Biodegradation process, Q = 2.65 × 10^4 m^3/d	NH_4^+-N = 26 mg/L, TN = 44 mg/L, COD = 605 mg/L,	TNRE = 70%, Eff. TN < 13.2 mg/L	[62]
Full	AAO-MBBR, Q = 25 × 10^4 m^3/d	NH_4^+-N = 20.3–40.8 mg/L, HRT = 10 h, SRT = 14–18 d, T = 10.7–25.2 °C	Eff. TN = 8.0 ± 1.5 mg/L, Anammox contribution = 15.9%	[72]

4.1. Advances in the Application of Mainstream Anammox Processes

Based on strategies for enhancing the enrichment and retention of AnAOB, numerous studies have investigated the feasibility of mainstream anammox at the pilot scale. Chen et al. utilized zeolite-based carriers to exploit their ammonium adsorption capacity in a two-stage PNA pilot system, achieving stable partial nitritation with a nitrite accumulation rate (NAR) of 75.04 ± 10.05% and an effluent TN concentration of 10.91 ± 4.23 mg/L [66]. In another pilot-scale IFAS system, effective NOB suppression was realized through the coupled control of low DO (0.4 ± 0.2 mg/L), FNA, and residual ammonium concentration, resulting in a *Candidatus Brocadia* abundance of 0.74% ± 0.21% and effluent TN below 10 mg/L [73]. Chao et al. further demonstrated that a single-stage PNA-IFAS system employing biofilm carriers could sustain stable nitrogen removal performance of 65.9% even under low-temperature conditions (15 °C) [68].

Despite these encouraging pilot-scale results, full-scale implementation of mainstream anammox in municipal WWTPs remains limited. At the Changi water reclamation plant in Singapore, a step-feed anoxic-oxic (AO) configuration enabled the establishment of a PNA pathway, with autotrophic nitrogen removal and heterotrophic denitrification contributing 37.5% and 27.1%, respectively [70]. This case, however, benefited from favorable climatic conditions, as wastewater temperatures in Singapore typically range from 28 to 32 °C. Zhang et al. retrofitted a conventional wastewater treatment plant with a PNA fixed-film system and achieved stable effluent TN concentrations of 4.5 ± 2.3 mg L^{-1} through combined chemical inhibition and ecological niche selection [71]. In this system, *Candidatus Brocadia* reached a high relative abundance of 17.87%, and its nitrogen removal activity was approximately eight times higher than that of heterotrophic denitrification. At the Strass WWTP in Austria, periodic transfer of sidestream anammox biomass to the mainstream, coupled with hydrocyclone-based selection, enabled effective NOB suppression during cold winter conditions and periods of high organic loading [62]. At the Fourth WWTP in Xi'an, unexpected enrichment of AnAOB was observed following carrier addition in the anoxic zone originally intended to enhance denitrification, with the anammox pathway contributing up to 15.9% of total nitrogen removal [72]. These engineering cases demonstrate the technical feasibility of activating the anammox pathway in mainstream municipal wastewater. However, the contribution of autotrophic nitrogen removal remains relatively limited in most systems and, in some cases, exhibits a declining trend over prolonged operation [74]. To date, robust, reproducible, and widely transferable full-scale applications of mainstream anammox are still lacking.

4.2. Insights from Engineering Practice

Engineering case studies consistently indicate that the presence of effective AnAOB retention mechanisms is a decisive factor for successful system performance [75]. Biofilms, granular sludge, or high-efficiency solid-liquid separation exhibits markedly greater operational stability than conventional suspended-growth systems [76]. This highlights the importance of incorporating structural reinforcement strategies at the design stage. In addition, dynamic adjustment of aeration intensity, internal recirculation, and operational cycles has proven effective in mitigating the impacts of influent and load fluctuations, thereby reducing the risk of process instability [73,77]. Online monitoring of nitrite, nitrate, and DO plays a critical role in assessing system status and preventing NOB resurgence [78,79]. Several studies have implemented early-warning frameworks based on trends in key operational indicators, enabling proactive intervention prior to system failure [41,42].

Successful operation typically depends on the coordinated matching of influent characteristics, process configuration, and operational management. Compared with sidestream anammox, mainstream systems require more refined operational control and the integration of multiple reinforcement measures. Therefore, mainstream anammox is better positioned as a low-carbon nitrogen removal enhancement unit or a stage-specific process, rather than as a direct and universal replacement for conventional nitrification-denitrification systems.

5. Future Prospects

Mainstream anammox shows substantial potential for reducing energy consumption and carbon emissions in municipal wastewater treatment. Nonetheless, achieving stable and reproducible full-scale implementation remains challenged by unresolved scientific and technical bottlenecks. Future research should advance from a deeper mechanistic understanding toward the systematic integration of process design and control strategies, enabling long-term stable operation and large-scale deployment of the mainstream anammox process.

5.1. Metabolic Mechanisms of AnAOB under Low-Temperature and Low-Substrate Conditions

Low temperature and low ammonium availability are defining features of mainstream conditions that distinguish them from sidestream systems [15]. These factors are also the primary constraints on anammox activity and operational stability. Although some AnAOB communities have been reported to exhibit a degree of cold tolerance [80,81], systematic understanding of their metabolic regulation mechanisms, energy allocation strategies, and temperature-dependent variations in key enzymatic activities remains limited. Future studies should integrate multi-omics approaches, including metagenomics, transcriptomics, proteomics, and metabolomics. These analyses can elucidate the molecular-level physiological regulation of anammox bacteria under low-temperature and low-substrate conditions. Such efforts are essential to identify rate-limiting steps in anammox metabolism and to pinpoint functionally cold-tolerant AnAOB lineages, thereby providing a theoretical basis for targeted acclimation and biological enhancement strategies.

5.2. Quantitative Mechanistic Modeling of Organics-Anammox Interactions

Organic matter is inevitably present in mainstream municipal wastewater and exerts a pronounced dual effect on anammox systems [52,82]. However, quantitative relationships between organic matter concentration, composition, and AnAOB activity remain poorly defined, which limits the rational design of fine-tuned control strategies. The direct and indirect impacts of different organic fractions on AnAOB require further investigation. In addition, the boundaries between competition and cooperation among HB, AOB, and AnAOB under multi-substrate conditions remain unclear. Developing predictive kinetic and ecological models that describe organic matter and anammox interactions will be essential for advancing precise control of mainstream anammox processes.

5.3. Cross-Scale Linkages between Microscale Structure and Macroscopic Process Performance

Stable operation of mainstream anammox strongly depends on microscale structures such as granular sludge and biofilms [83,84]. However, current understanding of the mechanisms by which microscale spatial structures influence macroscopic nitrogen removal performance and system resilience remains largely qualitative. The response of functional microbial spatial distribution within granules or biofilms to microscale gradients of oxygen, substrates, and inhibitory compounds also requires further investigation. Integrating advanced microscopic imaging and microelectrode techniques with reactor-scale modeling will enable the development of cross-scale structure-function-performance frameworks, thereby providing mechanistic guidance for reactor design and operational optimization.

5.4. Intelligent Monitoring and Data-Driven Operational Control

AnAOB are highly sensitive to operational conditions, rendering conventional experience-based control strategies inadequate for coping with the complexity and variability of real-world operation. With advances in online sensing technologies and data science, intelligent control is emerging as a promising tool for enhancing system stability [85,86]. Integrated online monitoring platforms can be established based on multiple parameters, including NH_4^+, NO_2^-, NO_3^-, and DO. Machine learning approaches can be applied to support operational state identification and load prediction. Such data-driven strategies enable dynamic optimization of operational parameters and are expected to improve system resilience and operational reliability.

Author Contributions

H.W.: writing—original draft preparation, conceptualization, writing—reviewing and editing; Y.L.: conceptualization, visualization; S.L.: validation, visualization; X.D.: writing—reviewing and editing, supervision. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

No data was used for the research described in the article.

Conflicts of Interest

Given the role as Young Editorial Board Member, Hong Wang had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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