Performance of Anammox Processes for Wastewater Treatment: A Critical Review on Effects of Operational Conditions and Environmental Stresses

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Abstract: The anaerobic ammonium oxidation (anammox) process is well-known as a low-energy consuming and eco-friendly technology for treating nitrogen-rich wastewater. Although the anammox reaction was widely investigated in terms of its application in many wastewater treatment processes, practical anammox application at the pilot and industrial scales is limited because nitrogen removal efficiency and anammox activity are dependent on many operational factors such as temperature, pH, dissolved oxygen concentration, nitrogen loading, and organic matter content. In practical application, anammox bacteria are possibly vulnerable to non-essential compounds such as sulfides, toxic metal elements, alcohols, phenols, and antibiotics that are potential inhibitors owing to the complexity of the wastewater stream. This review systematically summarizes up-to-date studies on the effect of various operational factors on nitrogen removal performance along with reactor type, mode of operation (batch or continuous), and cultured anammox bacterial species. The effect of potential anammox inhibition factors such as high nitrite concentration, high salinity, sulfides, toxic metal elements, and toxic organic compounds is listed with a thorough interpretation of the synergistic and antagonistic toxicity of these inhibitors. Finally, the strategy for optimization of anammox processes for wastewater treatment is suggested, and the importance of future studies on anammox applications is indicated.

Keywords: anammox; optimization; operational factors; toxic compounds; inhibition

1. Introduction

Implementation of anaerobic ammonium oxidation (anammox) in many water technologies has gained increasing attention since it was first discovered in the 1980s [1]. The application of the anammox process in wastewater systems has resulted in higher rates of nitrogen removal and lower energy requirements than those for conventional nitrogen removal [2–5]. Because of these advantages, anammox bacteria were selected for use in wastewater management owing to their high salt tolerance, wide temperature tolerance, and high capability for the removal of organic waste. Moreover, the anammox process is known to be responsible for 50% of nitrogen turnover in marine environments at various temperature and salinity conditions [6,7].

Anammox bacteria belonging to Planctomycetes contain a membrane-bound organelle that can convert ammonium and nitrite to dinitrogen gas using a process involving the production of the toxic and extremely energy-rich hydrazine intermediate [8]. During wastewater treatment, high organic matter in the wastewater is degraded to carbon dioxide by microorganisms through an aeration system known as the activated sludge process. This process is energy intensive, particularly for

the aeration systems. Conventional denitrification often requires additional organic matter for the reduction of nitrate to dinitrogen gas [8]. In combination with the anammox process in wastewater treatment, the remaining waste containing high ammonium will continue to be degraded by anammox; the problem of sludge in the treatment process can thus be mitigated [9–11]. The water treatment process has been recognized to be a two-step process, comprising a sidestream and a mainstream process, which are divided based on influent sources and bacterial involvement. The introduction of the mainstream process enables the decoupling of carbon and nitrogen removal and maximizes energy recovery through a carbon-concentrating pre-treatment process that channels more carbon to an anaerobic digester (or an up-flow anaerobic sludge blanket (UASB) reactor) for biogas generation [12]. There are two consecutive steps in the mainstream process. First, the primary conversion of ammonium to nitrite facilitated by ammonium-oxidizing bacteria (AOB), which is known as partial nitritation (PN). In the second step, the remaining ammonium reacts with nitrite to produce dinitrogen gas supported by anaerobic oxidizing bacteria named anammox (A). The partial nitritation and anammox (PN/A) process has been studied and applied at numerous scales of wastewater treatment to improve energy utilization efficiency [12–14]. The PN/A process provides environmental advantages over conventional nitrogen removal such as 60% reduction in oxygen demand, elimination of 95% of the biodegradable carbon, and 80% reduction in sludge, which are the main purposes of modern wastewater treatment [2,15–18].

Unlike that of other bacteria, the growth rate of anammox bacteria is quite slow, with the exponential phase being 10–22 days [19] or within 10–12 days when cultured at 35 °C; therefore, its application is limited to the operation of continuous wastewater treatment processes containing high concentrations of ammonium or other organic matter. Environmental factors play an important role in triggering high bacterial growth rates. High nitrogen removal performance can be enhanced by optimizing the operating conditions and limiting environmental stresses. However, it is difficult to create such conditions in an operating bioreactor system. An investigation of growth characteristics of anammox bacteria and their response to various environmental perturbations can help reveal their capacity to adapt in these limiting conditions.

There are many challenges in maintaining the operational condition of anammox systems, either in the mainstream or the sidestream, to achieve a stable high nitrogen removal rate. In the mainstream, a longer start-up period, lower nitrogen concentrations, and inconsistency in loading rate become the most important challenges that may affect the inflexibility and instability of effluent nitrogen concentrations [2,20]. The difficulties in controlling organic solids in the influent and microspatial aeration have been documented as principal problems [14]. In addition, inhibition by exogenous compounds, such sulfides, phenols, alcohols, antibiotics, or toxic metals in substrate media, is an important factor to be considered [21–24]. Although nitrite is a substrate of the anammox reaction, at a concentration higher than 100 mg/L, it becomes toxic to anammox growth [25–28]. The competition for nutrient and oxygen among bacteria can inhibit the capacity for growth and anammox activity, which should be taken into account [12,13,29,30]. Nevertheless, environmental parameters such as temperature, oxygen concentration, organic carbon content, nitrogen concentration, and pH are important factors influencing the mainstream process, while the sidestream process is mainly affected by high organic carbon content in the system. The influence of environmental factors in each bioreactor, whether in the operational mainstream or in sidestream processes, was previously investigated at different scales of operation including in industrial and municipal wastewater treatment units or in batch reactors at laboratory scales [2,4]. However, these investigations were unable to provide clear evidence to understand the challenges posed by environmental factors. Furthermore, the capacity of anammox bacteria to adapt to unwanted toxic compounds in the anammox processes has not been systematically summarized and discussed.

Based on these current limitations in the literature, here, we systematically summarized and interpreted the performance of the anammox process for wastewater treatment focusing on operational conditions and environmental stress factors that may directly interfere with proper anammox performance. Up-to-date information regarding anammox studies on wastewater treatment has

been summarized here to identify optimal strategies for the efficient operation of the anammox process, a critical aspect that is currently a focus of research.

2. Effects of Operational Conditions on Anammox Performance

2.1. Temperature

Temperature is one of the most important factors that strongly influences the anammox process. The fluctuation in temperature in the bioreactor can change the physical response of annamox and affects nitrogen removal efficiency [29,31–33] (Table 1). Temperature has a considerable effect on anammox growth and adaptation by controlling nitrogen removal, triggering many inhibitory effects, affecting microbial community structure, and influencing the stability of low nitrogen effluent when it is used in various bioreactors [3,8,25,34]. In the natural environment, anammox bacteria can be found in low (−5 to 4 °C) and high (60 to 80 °C) temperature ranges [35–37]. The acclimation of anammox bacteria to a wide range of temperatures enables their applicability to various wastewater treatment systems.

The mainstream process is known to operate at a lower temperature, mostly around 15 °C, to achieve a high nitrogen removal efficiency (70%–90%) at low oxygen concentrations [38]. However, a previous study revealed that the application of the mainstream process with the PN/A process at low temperatures of 10–15 °C resulted in low nitrogen removal rates and often in increased nitrogen concentrations in the effluent [12], mainly in the form of nitrate. Decreasing the temperature can be beneficial to enable highly competitive nitrite-oxidizing bacteria (NOB), which are more active than AOB, to produce more nitrate, despite the concentration ratio of AOB to NOB in the sludge being relatively constant [31]. In general, autotrophic nitrogen removal technologies in the mainstream process should be operated within temperatures ranges of 25 °C to a maximum of 40 °C as the optimal operating temperature.

At 30–40 °C, anammox bacteria can grow and perform better, preventing future adverse effects owing to inhibition and generating high organic carbon consumption to help reduce greenhouse gas emissions, especially of CO_2 and N_2O [22,25,27,39–41]. When the temperature drops from 30 to 15 °C, or from 30 to 10 °C, the specific anammox activity declines by approximately ten-fold [31,42]. However, a study by Gilbert et al. [43] revealed that the specific activity of anammox was irregular and inconsistent while acclimating in temperatures of 10, 20, and 30 °C. A few studies also reported substrate inhibition by the availability of free ammonia, high concentration of nitrite, and toxic metals when operated at a high temperature of over 35 °C [44,45]. Some previous studies confirmed that a temperature range between 40–45 °C can be detrimental to anammox bacteria, causing lysis in bacterial cells because of the release of cytochrome c after temperature shock and consequently reducing the anammox activity [40,42,46].

The optimal operating temperature for the sidestream anammox process has also been reported to be around 35–40 °C, higher than the temperature applied in the mainstream process [25,47]. The PN/A process in the sidestream wastewater treatment was also used for the treatment of dewatering liquor from anaerobic digesters fed with municipal and industrial wastewater [12]. Currently, PN/A is mainly used to remove ammonium in the effluent from anaerobic tanks in the mainstream process. Several studies have investigated full-scale sidestream anammox installations for the treatment of digested sewage, landfill leachate, and rejected water [14,34,48]. They found that the sidestream anammox process required high temperatures, usually higher than 30 °C, to generate high anammox activity and nitrogen removal efficiency [8,30,43,49,50].

Recently, there is increasing interest in anammox performance at low temperatures to enable the use of the technology at high latitude regions at a temperature range of 10–20 °C [31,41]. The cold anammox bacteria usually involved in temperatures around 5−6 °C belong to *Brocadia,* particularly species "*Candidatus* Brocadia sinica" and "*C.* Brocadia fulgida" [31]. Anammox species "*C.* Kuenenia stuttgartiensis" was also found in a continuous bioreactor system at a temperature lower than 20 °C [47].

Even though these anammox bacteria can acclimate to the temperature range of 10–12 °C [43], optimal activity is usually observed at warm temperature, between 18 and 23 °C, with high growth rate, high specific anammox activity, and less free ammonia and free nitrous acid [41]. Furthermore, at high latitudes, anammox bacteria decrease their growth rate owing to the decrease in enzyme activity, density of cytoplasm, and low mass transfer rate [12,49,51]. Moreover, operating anammox systems at low temperatures can stimulate an increase in the amount of ladderane fatty acids, which reduce membrane permeability and passive diffusion of protons out of the anammoxosome, and this prevents ATP synthesis, thereby reducing metabolism [41,52–54]. The instability of temperature in the mainstream and sidestream anammox processes makes extrapolation in operational design and maintenance challenging [55,56].

Table 1. Influence of temperature and pH level on anammox performance.

Temp.	pH	Anammox Species	Reactor Capacity	Bioreactor Type	Removal Rate	Growth Rate	Ref.
35–46 °C	7.5–8.5	*Brocadia anammoxidans, Brocadia fulgida, Brocadia* sp., and *"Candidatus Kuenenia"*	10.0 L	SBR (Sequencing bioreactor)	0.25 g N L^{-1} d^{-1}	-	[40]
45–55 °C	7–8	*Kuenenia stuttgartiensis*	1.0 L	SBR	-	-	[42]
15 °C	7–8	*Kuenenia stuttgartiensis*	1.0 L	SBR	0.05 g N L^{-1} d^{-1}	Slow growth	[42]
37 °C	7.86	*"Candidatus* Brocadia anammoxidans"	1.25 L	Single PN/A up flow reactor	0.22–0.35 Kg N m^{-3} d^{-1}	High growth but no value mentioned	[9]
20 °C	7	*"Candidatus* Brocadia"	10.0 L	MBR (Membrane bioreactor)	-	0.0011 d^{-1}	[57]
30 °C	7	*"Candidatus* Brocadia"	10.0 L	MBR	-	0.33 d^{-1}	[57]
29 °C	7.5	*"Candidatus* Brocadia fulgida"	12.0 L	SBR	100–400 mg N L^{-1} d^{-1}	0.015–0.095 d^{-1}	[58]
15 °C	7.4	*"Candidatus* Brocadia"	12.0 L	SBR-PN/A	20–40 mg N L^{-1} d^{-1}	-	[2]
13–18 °C	7.5–7.9	*"Candidatus* Kuenenia"	2.5 L	UASB	5.16–5.40 kg N m^{-3} d^{-1}	Lower growth rate	[41]
23–28 °C	7.5–7.9	*"Candidatus* Kuenenia"	2.5 L	UASB	5.72 kg N m^{-3} d^{-1}	-	[41]
10–20 °C	7.3	*"Candidatus* Brocadia fulgida"	2.7 L	SBAR	0.4 g N L^{-1} d^{-1}	High growth rate at 15 °C	[29]
24 °C	7.8	*"Candidatus* Brocadia", "C. Scalindua"*	1.5 L	SBR	0.048–0.156 g N L^{-1} d^{-1}	-	[59]
30 °C	-	-	8.0 L	UASB	5.72 Kg N m^{-3} d^{-1}	High growth rate but not specific number	[60]
16 °C	-	-	8.0 L	UASB	2.28 Kg N m^{-3} d^{-1}	Slightly low in growth rate	[60]
29–30 °C	8.0–8.1	*Brocadia fulgida*	200–1200 L	SBR	0.6 g N L^{-1} d^{-1}	-	[61]

The capacity for growth in anammox bacteria is strongly influenced by temperature. Previous studies investigating the growth rate of both anammox bacteria and AOB at the temperature range between 20–30 °C observed that AOB had a higher growth rate than anammox (0.7–0.9 d^{-1} [62] vs. 0.05–0.09 d^{-1} [25,63], respectively). The slow growth of anammox bacteria (10–12 days at 35 °C) limits their application at such temperature, particularly for influent with high concentrations of ammonium [8,54,63]. Unlike other microorganisms, anammox bacteria possess an extremely long reproduction time. Bacterial cells typically divide only once a week for a single cell or twice a week for aggregated cells [52,64]. However, the advantage of anammox bacteria is that the bacteria can be dormant if exposed to unfavorable conditions and become re-active under favorable conditions along with doubling time reached within 2 to 5 days [65]. When anammox bacteria have adapted to 15 °C, their biomass can easily increase by about two-fold and result in a higher nitrogen removal rate than the non-adapted anammox [31,42,66]. An ambient temperature of 21−25 °C was recommended to obtain

a low nitrogen effluent in a single-stage anaerobic bioreactor [47], an up-flow column reactor [67], a municipal wastewater treatment reactor [12,56], and a sequencing batch reactor [33]. The presence of high diversity of anammox species resulted in various strategies for survival and adaptation being incorporated [3,33]. *Brocadia* and *Kuenenia* could adapt well to a low temperature range from 10−20 °C, but they also performed better at the temperature range of 20−30 °C [41] although their activity was lower than that at the optimal temperature of 30 °C [8,33,42,67]. A study by Lotti et al. [31] reported that the highest growth rate can be found at 30 °C, and the biomass was mostly occupied by "*Candidatus* Brocadia sp.", which is similar to findings in the mainstream PN/A step-feed activated sludge system operated in the Changi Water Reclamation Plant, Singapore [12] and in anammox membrane bioreactors operated at full scale in Rotterdam [57].

The solid retention time in the operational anammox process also plays an important role because it can affect specific anammox activity when the non-active and non-anammox cells in the reactor increased therein. Therefore, temperature control in the system should be considered with respect to solid retention time [57]. The operation of anammox membrane bioreactors at 37 °C, fed with low-ammonium influent (100 mg-N/L), achieved high biomass and a high nitrogen removal rate compared to those at the same temperature but operated with an influent with higher ammonium content [8,68]. Activating the anammox population under such low nitrogen concentrations has become a strategy for the application of anammox for domestic wastewater treatment systems [69]. Thus far, the activation of anammox bacteria at such low or high temperatures is likely to depend on the adaptability of specific anammox species.

2.2. pH

The change in culture pH can influence the anammox process greatly owing to the accumulation of toxic compounds which inhibits anammox activity. Common pH values applied in anammox systems are listed in Table 1. Maintaining pH is an important aspect of sustaining high nitrogen removal over a long period [3,70]. Previous studies found that pH ranging from 6.5 to 8.3 can support growth and activity of anammox bacteria [25,67]. A review by Tomaszewsky et al. [71] suggested that a pH range of 7–8 is suitable to anammox and seems to be the ideal range for avoiding the inhibition of anammox by high free ammonia and free nitrous acid. For example, free ammonia concentration increased when pH was increased but a decrease facilitated accumulation of free nitrous acid. The low permeability of the anammox bacterial membrane and the limited diffusion ability of protons can protect the anammox bacteria from alkaline or acid conditions [37]. However, the anammox cell was observed to contain two compartments when cultured at different pH (6.3 and 7.3), revealing the presence of a proton motive force over the intracytoplasmic membrane [3]. A study by Jetten et al. [39] indicated that the optimum anammox activity and growth were obtained at the pH range of 6.7–8.3 and the maximum activity was found at pH up to 8.0.

The pH of the anammox enrichment culture also determined the dominant anammox bacteria in the anammox process. *Brocadia anammoxidans* and "*Candidatus* Anammoxoglobus propionicus" were observed to be dominant in the enrichment from aerobic granules and leachate sludge, respectively, when the pH of the enrichment culture was controlled from 6.8–7.0 [72]. However, anammox species "*Candidatus* Brocadia anammoxidans" [9,63,73–75] and *Kuenenia stuttgartiensis* [45,63] were found predominantly in anammox systems with pH of 7.8–8 [76]. Therefore, controlling pH closely coordinates the efficiency of nitrogen removal of anammox reactors. The implementation of anammox in a single bioreactor is a novel approach that can remove the nitrogen from both municipal and industrial wastewaters by controlling pH and dissolved oxygen through aeration [33,77]. The single-stage anammox process can achieve an energy-cost saving of 25% compared to the conventional process provided that stability of pH and aeration is controlled [59,77].

2.3. Dissolved Oxygen (DO)

Anammox bacteria conventionally grow and are active in critical DO regions, which has been proven by several studies in oxygen minimal zones [78–82]. Studies have investigated the anammox activity at a very low oxygen concentration, even below the detection limit (0.01 mg L^{-1}), addressing the sensitivity of anammox bacteria to oxygen levels [34,81] (Table 2). Anoxic conditions or low oxygen concentrations support anammox metabolism particularly in terms of enzyme production [78,81,82] and activate the induction of the anammox reaction [27,34]. The application of oxygen microelectrode profiling in the sequencing batch reactor (SBR) anammox system highlighted the influence of low oxygen levels on the size and type of anammox biomass [83]. Granular biomass anammox can produce a similar nitrogen removal rate of 600 mg N/(L·d) at both DO concentrations of 1 and 8 mg/L, even though at the latter DO concentration, oxygen can fully penetrate the bacterial cells in the granular biomass [83]. In contrast, neither growth nor nitrogen removal was observed with an anammox biofilm exposed to a DO level of 8 mg/L in another study [84]. A study by Liu et al. [84] indicated that the anammox activity at a DO level lower than 0.04 mg/L can generate a high rate of nitrogen removal (2.1 kg N/(m^3·d).

Table 2. Effect of dissolved oxygen (DO) on the performance of anammox systems.

DO Level	Specific Bioreactor	Reactor Capacity	Problems	Removal Rate	Ref.
2–8 mg O$_2$ L^{-1}	SBR	2.6 L	Increasing O$_2$ from 2 to 8 mg L^{-1} decreased the removal rate	46–380 g N L^{-1} d^{-1}	[61]
<0.04 mg O$_2$ L^{-1}	NRBC (non-woven rotating biological contactor)	7 L	Increasing O$_2$ in the system reduced the AOB activity, nitrogen removal rate, and caused high nitrite concentrations	2.1 kg N L^{-1} d^{-1}	[84]
<0.3 mg O$_2$ L^{-1}	SBR	10 L	Suspended anammox biomass activity inhibited by low temperature	6–8 mg N L^{-1} d^{-1}	[43]
0.4 mg O$_2$ L^{-1}	SBR	1200 L	Thin layers of granules can easily be penetrated by oxygen	0.6 g N L^{-1} d^{-1}	[83]
3.0 mg O$_2$ L^{-1}	SBR	200 L	More difficult for oxygen to penetrate thick layers and it protected anammox from non-suitable liquid media conditions (benefit to anammox)	0.6 g N L^{-1} d^{-1}	[83]
0.4–4 mg O$_2$ L^{-1}	SBR	10 L	Appearance of AOB in anoxic conditions	0.25 g N L^{-1} d^{-1}	[40]
0.18 mg O$_2$ L^{-1}	SBR-MBBR (moving bed biofilm reactor)	12 L	High nitrite and ammonia concentrations during low temperature, <11 °C, anammox activity decreases	0.047 g N L^{-1} d^{-1}	[2]
0.15 mg O$_2$ L^{-1}	SBR-HMBBR	12 L	High nitrite and ammonia concentrations during low temperature, <11 °C, anammox activity decreases	0.026 g N L^{-1} d^{-1}	[2]
0.4 mg O$_2$ L^{-1}	CFR (continuous flow reactor)	12 L	Slow response of anammox because of lower loading rate and low anammox biomass	0.42 g NH$_4$ -N L^{-1}	[85]

Increasing the DO level gradually could be a better operational strategy to increase the adaptability of anammox and other symbiont bacteria within the system. Liu et al. [84] provided a strategy based on the anammox symbiont and other oxygen-consuming bacteria such as *Nitrosomonas eutropha* to create anoxic conditions for anammox bacteria activity. Wang et al. [86] and Liu et al. [84], using fluorescent in situ hybridization analysis, observed that the anammox bacterial population reached 70% of total bacteria in biofilms after the oxygen adaptation stage. This suggested that the application of anammox in an environment with moderate DO level in the presence of symbiont bacteria could be considered. Controlling the DO levels in the reactor would support the oxidation of ammonium to nitrite and prevent the oxidation of nitrite to nitrate, which is challenging for the anammox reaction [77,87].

When a stable DO level is maintained in the reactor, the conversion rate of ammonium to nitrite and nitrite to nitrate was found to be different owing to the diversified population of AOB and NOB in various systems [56]. The common AOB found in the anammox system were *Nitrosomonas eutropha*,

N. europaea, and *Nitrosospira* sp. [77]. *Nitrosomonas* was reported to be the dominant genus of AOB. It can uptake oxygen faster and exist in the anammox system for a long period to enhance the nitritation process. In addition, *N. eutropha* is beneficial to the anammox system in that it protects anammox bacteria from oxygen inhibition [84,88].

2.4. Nitrogen Loading

Besides temperature, pH, and DO, nitrogen concentration is another important factor directly affecting the anammox process. Some previous studies revealed that various loading rates of nitrogen in the form of ammonium can considerably influence nitrogen removal rate, growth capability, dominant bacterial species, and optimization potential of the long-term anammox process [20,59] (Table 3). Ammonium concentration was responsible for the successful growth of anammox bacteria and their nitrogen removal capability; however, the ammonium concentration should be correlated to the density of anammox bacteria. Low ammonium concentration coupled with high cell density would result in low anammox activity [25]. In contrast, the supply of improper ammonium and nitrite levels would stimulate the growth of unwanted bacteria, thereafter interfering with anammox activity [25].

Table 3. Effect of nitrogen loading on the performance of anammox systems.

Nitrogen Loading Rate	Reactor Type	Culture Period	Nitrogen Removal Rate	Removal Efficiency	Nitrogen Effluent	Problems	Ref.
0.1–0.31 kg N L^{-1} d^{-1}	MBFR	730-d	0.2 kg N L^{-1} d^{-1}	91.7%–94.7%	<5 mg N L^{-1}	High effluent nitrogen in the system causes unsatisfactory nutrient ratio 1.32:1 (nitrite to ammonium) at 20 °C	[89]
40–61 mg N L^{-1} d^{-1}	MBBR	240-d	30–47 mg N L^{-1} d^{-1}	73%–91%	5.7 mg N L^{-1}	No interruption because nitrogen load was less	[2]
38–42 mg N L^{-1}	Hybrid MBBR	240-d	26 mg N L^{-1} d^{-1}	63%	8 mg N L^{-1}	Less stable in removing nitrogen owing to increase in nitrate produced	[2]
206–291 mg N L^{-1}	SBR	155-d	150–200 mg N L^{-1}	80%	100 mg N L^{-1}	Reduced removal rate because of high salt concentrations >3.5–15 g NaCl L^{-1}	[59]
220–262 mg N L^{-1}	SBR	445-d	50–100 mg N L^{-1} d^{-1}	<80%	200–300 mg N L^{-1}	High salt and nitrite levels >75 mg L^{-1}, decline in anammox activity	[59]
1.2–1.34 kg N L^{-1} d^{-1}	SBR	101-d	0.71–0.98 kg N L^{-1} d^{-1}	66%–75%	20 mg N L^{-1}	Reduced anammox activity by reducing enzyme activity at low temperature	[90]
1800 mg N L^{-1} d^{-1}	UASB	580-d	1800–2000 mg N L^{-1} d^{-1}	nd	18 mg N L^{-1}	Source of the influent came from municipal WWTP (wastewater treatment plant) comprising heavy metals that inhibit anammox activity	[4]
0.32 kg N L^{-1} d^{-1}	SBR	240-d	<800 mg N L^{-1} d^{-1}	46%–75%	<50 mg N L^{-1}	High inorganic carbon content in the sludge promoted high accumulation of other bacteria and then reduced NRR (Nitrogen revomal rate)	[50]
0.78–0.90 g N L^{-1} d^{-1}	SBR (a)	955-d	400–500 mg N L^{-1} d^{-1}	47%–55%	-	Reducing temperature to 15 °C can directly reduce the nitrogen removal up to 16%	[83]
0.76–0.79 g N L^{-1} d^{-1}	SBR (b)	1120-d	190–440 mg N L^{-1} d^{-1}	16%–57%	-	Reduced temperature reduced the removal ability of anammox	[83]
0.11–0.31 g N L^{-1} d^{-1}	SBR (c)	186-d, 1676-d	40–80 mg N L^{-1} d^{-1}	10%–70%	<20 mg N L^{-1}	Lower temperature reduced ability of NRR, while adapted anammox can perform better NRR	[83]

(a) Operated at 20 °C with nitrogen concentration 200 mg NH_4^+ L^{-1}, (b) at 15 °C with nitrogen concentration 200 mg NH_4^+ L^{-1}, (c) at 15 °C with nitrogen concentration 50–75 mg NH_4^+ L^{-1}.

Anammox bacteria preferred a low nitrogen concentration for faster activation [71]. The abundant availability of nitrite and nitrate (higher than 0.07 g/L) at an early stage can interrupt anammox growth, whereas a lower nitrite concentration (up to 0.04 g/L) can enhance anammox growth and specific

anammox activity [71]. The maximum specific anammox activity (0.035 g N/(g VSS·h) was obtained in an SBR when the nitrogen concentration was controlled close to the stoichiometric nitrite/ammonium molar ratio (1.32) [91]. Free ammonia level of 35–40 mg N/L [43,92] or 20–30 mg N/L [93] could decrease anammox activity by up to 50%. Both ammonium and nitrite concentrations in the range of several hundred milligrams per liter [26] to several grams per liter [34] can influence anammox activity regardless of loading rates.

As with temperature, anammox bacteria need to be acclimatized to adapt to high or low nitrogen concentrations in order to support anammox metabolism and removal capability. Adapted anammox bacteria in an SBR bioreactor could remove up to 70% of nitrogen at a concentration load of 1 g NH$_4$-N/L over 60 days [61], whereas 80% nitrogen removal efficiency was achieved within 30 days in the anammox reactor fed with fish-canning wastewater containing 0.105 and 0.203 g N/L [59]. In addition, 87% nitrogen removal was successfully achieved after 150 d of operation with influent of 0.2 g N/L and 9.2 g/L of NaCl [59]; however, when a high nitrogen loading rate of 1.7 kg N/(m^3·d) coupled with high NaCl concentration of 30 g/L was introduced to the reactor, the nitrogen removal rate decreased significantly owing to the deterioration of anammox bacteria under severe hypersaline inhibition. High salinity can inhibit the synthesis of many enzymes and decrease cell metabolism via plasmolysis [22]. However, the appropriate addition of salt to the influent can mitigate the effect of high nitrogen loading into anammox reactors.

With the ultimate aim to mitigate the limitation of the anammox process for treating wastewater containing high nitrogen concentration, anammox bacteria were introduced in reactors of various types and capacities to observe treatment efficiency and energy consumption [43]. Operation of the anammox process with different sources of biomass should be applied using different adaptation strategies. The anammox process operated with a granular sludge will adapt to a nitrogen level different from that operated with suspended anammox biofilms. Anammox bacteria in the form of large granules will be less affected by nitrogen concentration than a smaller anammox biofilm [43]. Previous studies reported that a granular anammox sludge can remove 80% ammonium, corresponding to a maximum removal rate of 14 kg N/(m^3·d) [94], whereas a suspended anammox biofilm can achieve an average removal rate of 12 kg N/(m^3·d) [95]. Such a high rate of nitrogen removal by the suspended anammox biofilm could be because of the high cell density [96]. Furthermore, there are many other micro-environmental factors simultaneously affecting the performance of anammox systems including organic matter and DO concentration in the influent, mass transfer capability of biofilms, and micro-environment in the bioreactor as well as aeration strategy [43,95]. To avoid any interruption to the anammox process for a long period, a progressive adaptation of anammox biomass to particular bioreactors with various nitrogen concentrations and nitrogen loadings is required for process optimization [61,83,96].

2.5. Carbon Sources

Municipal wastewater usually contains a certain amount of organic matter. However, the availability of high levels of organic carbon in the sludge waste somehow reduces the efficiency of anammox activity [70]. The anammox process is well recognized as a cost-efficient and sustainable alternative for nitrogen removal from wastewater sources with low C/N ratios [56]. The investigation on the effect of the form of nitrogen such as ammonium, nitrate, and nitrite in anammox systems has been widely conducted; however, there is little information on the effect of organic carbon to nitrogen (C/N) ratio (Table 4). Jin et al. [22] systematically summarized previous studies on the inhibition of anammox activity by toxic and non-toxic organic compounds. Anammox activity could not be sustained at COD/N ratios higher than 2 in a membrane-aerated biofilm reactor [13]. At the same COD/N ratio, rate of nitrogen removal decreased by 50% in a co-diffusion conventional biofilm reactor [13]. The availability of high organic matter in the wastewater treatment system has a negative effect on removal of ammonium by the anammox process owing to the competition between anammox bacteria and heterotrophic denitrifying bacteria. Anammox symbionts can typically oxidize a multitude of organic compounds with simultaneous reduction in nitrate and/or nitrite [97–101]. During the

anammox operation, cell lysis or solubilization of unwanted material in the system resulted in the increase of organic matter from 6 ± 2 mg/L to 327 ± 21 mg/L, and this directly reduced the performance of anammox bacteria [77]. The study by García-Ruiz et al. [102] revealed that the nitrogen removal efficiency was not affected by the organic carbon level of 100 mg COD (chemical oxygen demand) L^{-1} during the operation of a CANON biofilter system in which coexisting AOB, anammox, and denitrifiers were fed with an influent with an ammonium concentration of 320 mg/L. However, the operation of the same bioreactor without organic carbon in the presence of anammox and AOB showed a significant decrease in nitrogen removal efficiency. The study by Kartal et al. [103] showed the possibility of a metabolic pathway for anammox using carbon as a substrate in the presence of heterotrophic denitrifiers. Anammox bacteria can benefit under this condition because these denitrifiers consume oxygen to metabolize the organic carbon [104]. Anammox bacteria "*Candidatus* Anammoxoglobus propionicus" was found to out-compete other denitrifiers for propionate as the electron donor [103]. "*Candidatus* Brocadia fulgida" can consume some organic carbon forms such as propionate, formate, and dimethylamine [105].

Table 4. Effect of nitrogen loading on the performance of anammox systems.

Influent Carbon (COD/IC)	Reactor Type	Culture Period	Carbon Concentration Effluent	Nitrogen Removal Efficiency/ Effluent Nitrogen	Problems	Ref.
70.6–284.1 COD mg L^{-1}	ABR	91 d	50 mg L^{-1}	89%–96%	High COD concentration damages the anammox activity	[106]
100 mg COD·L^{-1}	CANON	60 d	nd	85%	Lower carbon resulted in high removal rate	[102]
400 mg COD L^{-1}	CANON	60 d	nd	68.1%	Partial inhibition of nitrogen removal rate and increase in the heterotrophic bacteria	[102]
533 mg COD L^{-1}	MBBR	280 d	40 mg COD L^{-1}	73%–91%	No interruption because nitrogen load was less	[2]
533 mg COD L^{-1}	Hybrid MBBR	210 d	33 mg COD L^{-1}	63%	Less stable in removing nitrogen owing to increase in nitrate produced	[2]
60 mg IC L^{-1}	Fixed bed Reactor	39 d	nd	4–4.5 kg N L^{-1} d^{-1}	High IC reduced the NRR	[107]
<10 mg IC L^{-1}	Fixed bed Reactor	39 d	4 mg C L^{-1}	3.5 kg N L^{-1} d^{-1}	Low IC reduced the anammox removal of nitrogen, the NRR, and the saturation found at 1.2 mg L^{-1}	[107]
90 mg IC L^{-1}	Lab Scale PN/A	40 d	nd	78%	Low nitrite was produced because of imbalance in bacterial population in the process	[108]
9.6 mg IC L^{-1}	Lab Scale PN/A	86 d	nd	46%	Anammox was outcompeted by NOB for nitrite; then, nitrate becomes abundant	[108]

In contrast to organic carbon, studies on the role of inorganic carbon in the anammox process are still limited. The limitation of inorganic carbon hindered the reproduction of anammox bacteria and thereafter delayed the performance of anammox [18,107]. The limitation of inorganic carbon and the competition for organic carbon with other symbiont bacteria is a challenge in the maintenance of the mainstream anammox process.

3. Effect of Environmental Stresses on Anammox Performance

3.1. Inhibition by Nitrite

According to the anammox equation, the theoretical ratio of nitrite to ammonium is 1.32:1. Although nitrite is an essential substrate for the anammox process, nitrite becomes toxic to some anammox bacteria [109,110] when it is accumulated in the anammox systems owing to the shortage

of ammonium for a theoretical anammox reaction. The nitrite inhibition level varied in different anammox systems and with different anammox inoculation sources (Table 5). The highest nitrite concentration tested for anammox was 1000 mg/L [111]. Anammox sludge dominated by "*C. Brocadia*" in a serum bottle was found to be totally inhibited when introduced to 1000 mg/L of NO_2^--N and in the batch test, and its activity decreased by 50% at a nitrite concentration of 400 mg/L [111]. The nitrite concentration where activity of anammox is decreased by 50% is commonly referred to as IC_{50}. The IC_{50} was also different for different anammox inoculum sources. In the same investigation with different anammox inoculation sources, UASB granular sludge was found to have the highest IC_{50} (240 mg/L), whereas the IC_{50} with MBBR (moving bed biofilm reactor) biofilms and SBR sludge was 85 and 98 mg/L, respectively [112]. The IC_{50} for the anammox biomass dominated by "*Candidatus* Kuenenia stuttgartiensis" in a batch test using serum vial was found to be 350 mg/L [26]. The IC_{50} value was not significantly different among physical types (suspended and granular) of anammox bacteria in a serum batch-test [113]. In contrast, the presence/absence of ammonium in the culture medium significantly affected the IC_{50} value [109]. In the presence of ammonium, 384 mg/L of nitrite could decrease the activity of anammox bacteria by 50%. However, in the absence of ammonium, only 53 mg/L of nitrite could inhibit 50% anammox bacterial activity.

Table 5. Effect of nitrite on anammox performance.

Seeding Sources	Reactor	Operation Mode	NO_2^--N Concentration (mg/L)	Effect	Ref.
Anammox sludge dominated by *Brocadia*	Serum bottle	Batch test	400 1000	Inhibition by 50% Total inhibition	[111]
Anammox sludge from pilot scale SBR (40 L)	1.1-L reactor	Batch test	30 60	Losses in activity Maximum nitrite removal rate decreased by 25%	[114]
Anammox biomass dominated by "*Candidatus* Kuenenia stuttgartiensis"	Serum vial	Batch test	350	Inhibition by 50%	[26]
Anammox biofilm	SBR reactor 5 L	Continuous	<240	No inhibition	[55]
Anaerobic granular sludge from UASB (upflow anaerobic sludge blanket reactor) reactor	UBF reactor	Continuous	380	Nitrogen removal sharply decreased	[70]
Anammox sludge entrapped in a polyethylene glycol (PEG) gel carrier	Cylindrical reactor (500 mL)	Continuous	750	Activity decreased by 10%	[115]
Granular anammox sludge	Serum bottle	Batch test	561	Inhibition	[92]
MBBR biofilm SBR sludge UASB granular sludge	Air-tight 800 mL bottle	Batch test	85 98 240	Inhibition by 50% Inhibition by 50% Inhibition by 50%	[112]
Biofilm carriers taken from the MBBR	1.2 L vessel	Batch test	100 160	Activity decreased by 26% Total inhibition	[116]
Suspended anammox culture Granular anammox enrichment	Serum flask (160 mL)	Batch test	151 185	Inhibition by 50% Inhibition by 50%	[113]
Anammox granular sludge	Serum flasks (160 mL)	Batch test	53 384	Inhibition by 50% in the absence of ammonium Inhibition by 50% in the presence of ammonium	[109]
Loose biomass taken from the MBBR	Serum flask (800 mL)	Batch test	52	Activity decreased by >35%	[117]

As summarized in Table 5, the inhibition concentration of nitrite seems to be higher in the continuous test than in the batch test. No inhibition was observed for anammox bacteria at nitrite concentrations lower than 240 mg/L in a 5 L SBR reactor [55]. Anammox bacterial activity decreased by only 10% at a nitrite concentration of 750 mg/L in a cylindrical reactor operated with a continuous mode [115], at which nitrite concentration and anammox bacteria in most of the batch operations decreased by 50%. In another study with a continuous UBF (upflow blanket filter) reactor, nitrogen removal by the anammox granular sludge decreased sharply at 380 mg/L of nitrite [114].

The most nitrite-sensitive anammox bacteria were found in a batch test in a 1.1 L reactor with inoculum taken from a pilot-scale SBR (40 L), where the anammox activity was lost from the nitrite concentration of 30 mg/L onward [114]. When nitrite concentration increased to 60 mg/L, nitrite removal rate decreased by 25%. Total anammox activity inhibition was reported in a batch test with anammox biofilm carriers taken from an MBBR with nitrate concentration of 160 mg/L [116] which is quite a low concentration compared to that in other studies. However, in the same experiment with a nitrite concentration of 100 mg/L, anammox activity decreased by only 26%. Generally, the effect of nitrite concentration on anammox bacteria activity cannot be predicted and can only be determined through experimentation. In order to maintain effective performance of the anammox system, nitrite concentration should be controlled at an appropriate threshold which is determined previously.

3.2. Inhibition by Sulfide

Sulfide is commonly found in an anaerobic wastewater system owing to the degradation of organic matter and reduction in sulfate under anaerobic conditions. Understanding the effect of sulfide on activities of anammox bacteria is necessary for the operation of an anammox system. However, the number of studies on sulfide inhibition is more limited than those on nitrite inhibition. In a batch test with anammox granular sludge in serum bottles, anammox bacterial activity was halved when influent with 264 mg/L of sulfide-S was added [51] (Table 6). The same anammox seeding source in an UASB (upflow anaerobic sludge blanket reactor) reactor with continuous influent containing 40 mg/L of sulfide-S showed a decrease of 17.2% in nitrogen removal activity. This indicated a significant difference in the effect of sulfide on the same anammox bacterial community when the operation mode and reactor were changed. Total anammox activity inhibition was reported at 160 mg/L of sulfide-S [26] in a batch test using serum vials and anammox biomass dominated by "*Candidatus* Kuenenia stuttgartiensis". However, in another study, anammox mixed sludge exposed to 192 mg/L of sulfide-S only showed a decrease of 35.6% in nitrogen removal activity [118]. The percentage decrease in anammox activity is in proportion to the increase in sulfide-S concentration in the anammox system. In most cases, anammox activity can recover when no more sulfide exists in the influent. However, exposure of anammox to sulfide-S of 320 mg/L resulted in irreversible inhibition [113].

In fact, the toxic effect of sulfide was found to be attributed to unionized sulfides (soluble H_2S) and not ionized forms such as HS^- and S^{2-}. A small concentration of soluble H_2S could significantly inhibit anammox activity. The IC_{50} of soluble H_2S was found to be 1.023 mg/L for suspended anammox enrichment culture in serum flasks for a batch test [113]. This number was slightly higher for granular anammox enrichment in the same investigation. The concentration of soluble H_2S is typically dependent on the pH of the medium, total sulfide added, and the headspace to liquid volume ratio, and can be calculated using these values [113]. Therefore, the control of the amount of unionized sulfide in the anammox system is more important than that of the total sulfide in the influent. In the anammox system, there could be a joint toxic effect from various inhibitors [118]. This joint toxicity could be antagonistic or synergistic. The inhibitory effect of sulfide to anammox granular sludge in a serum bottle was observed to be generally synergistic with oxytetracycline and phenol, both of which can be potentially found in wastewater [118].

Table 6. Effect of sulfide on anammox performance.

Seeding Sources	Reactor	Operation Mode	Sulfide-S Concentration (mg/L)	Effect	Ref.
Suspended enrichment culture Granular anammox enrichment	Serum flasks	Batch test	1.023 (H_2S) 3.751 (H_2S)	Inhibition by 50% Inhibition by 50%	[113]
Anammox granular sludge	Serum bottles Up-flow anaerobic sludge blanket reactor	Batch test Continuous	264 40	Inhibition by 50% Activity decreased by 17.2%	[51]
Anammox mixed sludge	Serum bottles	Batch test	48 96 192	Activity decreased by 14.0% Activity decreased by 21.2% Activity decreased by 35.6%	[118]
Anammox biomass dominated by "*Candidatus* Kuenenia stuttgartiensis"	Serum vial	Batch test	160	Total inhibition	[26]
Suspended and granular anammox culture	Serum flasks	Batch test	320	Irreversible inhibition	[113]

3.3. Inhibition by Toxic Metals

Toxic metals are usually present in some types of wastewater such as landfill leachate and industrial discharge. It is well-known that a high concentration of metals is normally toxic to microorganisms because metals cannot be degraded and thus accumulate in cells, disrupting disrupt cell metabolism [119]. An in-depth understanding of the effect of toxic metals on anammox activities would facilitate the application of anammox for the removal of nitrogen from these kinds of wastewater. The commonly investigated toxic metals in anammox systems are Cu, Zn, Hg, Cd, Ag, and Pb.

3.3.1. Copper

Cu is one of the essential elements to living organisms at micro concentrations, but it becomes very toxic at high concentrations. The toxic effect of Cu on microorganisms has been attributed to the soluble form of Cu, which is typically found as Cu(II). The inhibitory effect of Cu(II) on anammox has been investigated in many studies [28,119–124] (Table 7). The toxic concentration of Cu(II) on anammox bacteria was determined to be quite low, and varied from 1.9 to 19.3 mg/L. Anammox bacterial activity was found to decrease by 50% upon exposure to Cu(II) concentrations of 1.9 mg/L [28] and 4.2 mg/L [124] in a batch test using serum bottles. At a slightly higher concentration of Cu (II) (5 mg/L), anammox activity was observed to decrease by more than 10% in the continuous feeding mode of a 500 mL reactor dominated by Planctomycetes [123]. However, in the same continuous mode in a 2.7 L reactor inoculated with anammox sludge from a 1 year operating reactor, anammox activity was not significantly inhibited when the influent wastewater contained 5.95 mg/L of Cu(II) [121]. In the same study, when the influent Cu(II) level increased to 12.6 mg/L, a clear inhibition of anammox activity was recorded. The inhibition level of Cu(II) in the continuous mode seemed to be higher than that in the batch mode, which was similar to the toxic effect of nitrite. However, this was not true for the inhibition of Cu(II) on anammox activity because for a Cu(II) level of up to 16.3 mg/L in a batch test using serum bottles, anammox activity was only 20.1% [120]. The IC_{50} of Cu(II) in two other studies was 12.6 mg/L [122] and 19.3 mg/L [119]. Therefore, it can be said that the inhibition level of Cu(II) on anammox activity might generally depend on the seeding source.

Table 7. Effect of toxic metals on anammox performance.

Seeding Sources	Reactor	Operation Mode	Toxic Metals and Concentration (mg/L)	Effect	Ref.
Anammox sludge dominated by KSU-1 strain	120 mL serum vials	Batch test	Cd: 11.16 Ag: 11.52 Hg: 60.35 Pb: 40	Inhibition by 50% Inhibition by 50% Inhibition by 50% Activity decreased by 7.19%	[128]
Anammox sludge dominated by "*Candidatus* Kuenenia stuttgartiensis"	160 mL serum flask	Batch test	Cu: 16.3 Zn: 20.0	Activity decreased by 20.1%	[120]
Non-acclimated microbial sludge	Serum bottles	Batch test	Zn: 6.9	Inhibition by 50%	[125]
Acclimated microbial sludge	Stirring SBBR (2.5 L)	Continuous	Zn: <10	Stimulated anammox performance	[125]
Anammox sludge from 1-year operating reactor	2.7 L reactor	Continuous	Cu: 5.95 Cu: 12.6	No significant inhibition Clear inhibition	[121]
Anammox granular sludge	1 L UASB reactors	Batch test	Cu: 12.6	Inhibition by 50%	[122]
granular anammox biomass	340 mL serum bottles	Batch test	Cu: 1.9 Zn: 3.9	Inhibition by 50% Inhibition by 50%	[28]
Anammox sludge dominated by "*Candidatus*" and Planctomycetes	500 mL reactor	Continuous feeding	Ni: 5 Cu: 5 Co: 5 Zn: 10 Mo: 0.2	Anammox activity decreased by more than 10%	[123]
Anammox sludge dominated by "*Candidatus* Brocadia"	120 mL serum bottle	Batch test	Cd: 7.00 Hg: 2.33 Pb: 10.40 Cr: 9.84 As: 60	Inhibition by 50% Inhibition by 50% Inhibition by 50% Inhibition by 50% Activity decreased by 29.67%	[129]
Granular anammox biomass	160 mL serum bottles	Batch test	Cu: 4.2 Zn: 7.6 Cd: 11.2 Ni: 48.6 Mo: 22.7 Pb: 6.0	Inhibition by 50% Inhibition by 50% Inhibition by 50% Inhibition by 50% Moderately inhibitory Moderately inhibitory	[124]
Anammox granular sludge	1 L UASB reactor	Continuous	Zn: 25	Inhibition by 50%	[126]
Anammox sludge and nitrification sludge	Anammox biofilter reactor	Continuous	Zn: 20	Irreversible inhibition	[127]
Granular sludge dominated by *Brocadia fulgida*	25 mL vials	Batch test	Cu: 19.3 Cr: 26.9 Pb: 45.6 Zn: 59.1 Ni: 69.2 Cd: 174.6 Mn: 175.8	Inhibition by 50%	[119]

3.3.2. Zinc

In addition to Cu(II), the other metal commonly investigated in anammox studies is Zn(II). The toxicity of Zn(II) towards anammox bacteria appears to be less than that of Cu(II) because the inhibition concentration of Zn(II) has been reported to be higher (3.9–59.1 mg/L) than that of Cu(II) (Table 7). The IC_{50} value was determined from several batch tests using serum bottles as 3.9 mg/L [28], 6.9 mg/L [125], and 7.6 mg/L [124]. In the continuous mode operation with a bigger reactor, this value was recorded at 25 mg/L [126], which is much higher than those of the batch tests. In addition, the inhibition of Zn(II) on an anammox sludge and nitrification sludge in an anammox biofilter reactor was found to be irreversible when Zn(II) concentration was increased to 20 mg/L [127]. The activity of anammox sludge dominated by Planctomycetes was decreased by more than 10% in a 500 mL reactor

fed with continuous influent containing 10 mg/L of Zn(II) [123]. A decrease of 20.1% in the activity of anammox sludge dominated by "*Candidatus* Kuenenia stuttgartiensis" was observed in a batch test with 160 mL serum flasks [120]. In contrast to these observations, anammox performance was found to be stimulated in the continuous operation of a stirring 2.5 L SBBR with acclimated microbial sludge [125]. In particular, the IC_{50} value of Zn(II) for granular sludge dominated by "*C. Brocadia fulgida*" was extremely high (59.1 mg/L) [119].

3.3.3. Cadmium

Cd is a biologically toxic metal with well-recognized adverse effects on most human organs. Cd(II) occurs in wastewater owing to the increasing use of cadmium in modern industry. Currently, the inhibitory effect of Cd(II) on anammox bacteria has only been investigated at a batch scale. In a batch test using 120-mL serum bottles, the performance of anammox sludge dominated by "*Candidatus Brocadia*" was found to be decreased by 50% at a Cd(II) concentration of 7 mg/L [129]. Similarly, the IC_{50} values of Cd(II) for anammox bacteria in other batch tests were 11.16 mg/L [128] and 11.2 mg/L [124]. The highest IC_{50} of Cd(II) (174.6 mg/L) was found with granular sludge dominated by "*C. Brocadia fulgida*" [119].

3.3.4. Lead

Similar to Cd(II), the toxic effect of Pb(II) has only been investigated in batch tests using serum bottles. The IC_{50} of Pb(II) was determined differently in studies using different anammox sludges. The Pb(II) concentration of 10.4 mg/L decreased the activity of anammox sludge dominated by "*Candidatus* Brocadia" up to 50% [129], whereas this level of reduction in inhibition was found at Pb(II) concentration of 45.6 mg/L in a 25 mL vial test using granular sludge dominated by "*Candidatus Brocadia fulgida*" [119]. However, the activity of anammox sludge dominated by the KSU-1 strain decreased by only 7.19% upon exposure to Pb(II) concentration of 40 mg/L [128]. In addition, the toxic effect of Pb(II) was said to be moderately inhibitory in a batch test with granular anammox biomass at a Pb(II) concentration of 6 mg/L [124].

3.3.5. Nickel

Ni is one of the microelements that is recognized to be essential to organisms at very low levels. However, it becomes highly toxic with chronic exposure at high levels [130]. The toxic effect of Ni(II) on anammox activity has not been as extensively investigated as that of the other toxic metals listed thus far. However, the current literature provides data regarding Ni(II) inhibition in both continuous mode and batch operations [119,123,124]. In the continuous feeding of a 500 mL reactor, anammox activity decreased by 10% upon exposure to an Ni(II) concentration of 5 mg/L [123]. The IC_{50} values of Ni(II) in the batch test of 160 mL serum bottles and 25 mL vials were 48.6 mg/L [124] and 69.2 mg/L [119], respectively.

3.3.6. Other Toxic Metals

Other toxic metals such as Hg, Mo, Cr, Ag, Co, and As have been investigated in several studies operating at batch or continuous feeding modes. The IC_{50} of Hg(II) for anammox sludge dominated by the KSU-1 strain was 60.35 mg/L [128], which is significantly different from that for anammox sludge dominated by "*Candidatus* Brocadia" (2.33 mg/L) [129] with the same reactor volume and operation mode. Mo was found to be moderately inhibitory to anammox activity at a level of 22.7 mg/L in a batch test [124], whereas in another study with continuous feeding, 0.2 mg/L of Mo could decrease anammox activity by more than 10% [123]. The IC_{50} values of Cr(VI) were 9.84 mg/L for a batch test using 120 mL serum bottles [129] and 26.9 mg/L for a batch test using 25 mL vials [119]. As a well-known antibacterial agent, Ag(I) could decrease anammox activity by up to 50% at an exposure level of 11.52 mg/L [128]. A Co(II) concentration of 5 mg/L could decrease anammox activity by more than 10% in the continuous feeding mode of a 500 mL reactor. As(III), which is more toxic than As(V),

was investigated for its inhibitory effect on anammox sludge dominated by "*Candidatus* Brocadia" [129]. The activity of anammox bacteria was decreased by 29.67% upon exposure to an As(III) concentration of 60 mg/L [129]. Mn is known to be a less toxic metal; IC$_{50}$ of Mn(II) was 175 mg/L in a batch test with 25 mL vials [119].

3.4. Inhibition by Toxic Organic Compounds

Organic compounds occurring in wastewater are usually divided into two groups, non-toxic organic compounds and toxic organic compounds. Non-toxic organic compounds can serve as a carbon source for microorganisms. However, most anammox bacteria are chemoautotrophic microorganisms that utilize inorganic carbon such as CO_2, $CO_3{}^{2-}$, and $HCO_3{}^-$ as the only carbon source [131]. Therefore, the presence of non-toxic organic compounds at high levels can cause an adverse effect on the anammox system because heterotrophic bacteria grow on organic carbon and compete with the anammox bacteria [99]. In cases where anammox bacteria might consume organic carbon, they would use these as a substrate, rather than ammonium and nitrite, in the presence of high concentrations of organic carbon. These reasons contribute to the negative effect of high organic content on anammox systems. This review focuses more on the toxic organic compounds which commonly occur in wastewater.

3.4.1. Alcohols

Alcohol, especially ethanol, is commonly used as a disinfectant and sanitizing agent. The inhibitory effect of alcohol on anammox activity has been investigated previously [47,70,132–134]. During the initial stage of anammox enrichment from anaerobic sludge, the alcohol fermentation from organic matter can occur simultaneously because anammox is an anaerobic system. Methanol is the most toxic compound among alcohols because it can be converted to formaldehyde inside the cell and destroy cell metabolism [135]. It has been reported that in marine sediment, methanol can completely inhibit the anammox process at a concentration of 96–128 mg/L, because at this concentration, the denitrification process was stimulated [132]. In another study [133], methanol was considered to be the most toxic inhibitor, causing a complete and irreversible inhibition of anammox activity at a methanol concentration as low as 16 mg/L. However, the anammox activity in a batch test was found to be decreased by 71% in the presence of 160 mg/L methanol [47], whereas the addition of 1 mM methanol decreased anammox activity by up to 86% [134]. The difference in methanol resistance in the anammox process could be attributed to the difference in anammox species of microbial communities. Methanol-resistant anammox was successfully enriched from methanogenesis sludge [136]. This methanol-resistant anammox sludge was dominated by "*Candidatus* Brocadia".

3.4.2. Phenol

Phenol and phenolic compounds are not commonly found in domestic wastewater but are present in industrial wastewater from chemical industries, petroleum refineries, coal conversion, and fiberboard manufacturing [137–139]. A highly phenol-resistant anammox consortium was successfully enriched through a long adaptation process for treating wastewater from coke-ovens [140]. Anammox activity decreased when phenol concentration increased from 50 to 550 mg/L, but gradually recovered after a period of acclimation and even improved after being adapted. In the presence of other inhibitory agents such as sulfide and Cu(II), the toxic effect of phenol was generally synergistic [118]. In fact, the cooperative inhibition of phenol and Cu(III) was synergistic at a low phenol concentration of 75 mg/L, whereas it was antagonistic at a high phenol concentration of 300 mg/L [118]. The inhibitory effect of phenol was also found to be synergistic with that of thiocyanate [141]. Anammox activity decreased by more than 90% upon short-term exposure to a 100 mg/L phenol and thiocyanate mixture. Recently, more studies have focused on the inhibitory effect on anammox by phenol and have reported that the inhibition of anammox activity by phenol was restorable. Anammox performance was significantly suppressed upon exposure to phenol concentrations of 12.5–50 mg/L for nearly 200 days [142]. However, when phenol was depleted from the influent, the anammox performance recovered after 81 d. It was

interesting that the recovery model with phenol as an inhibitor was very different from the test for sulfide as the inhibitor. In another study [143], an influent containing 50 mg/L of phenol could significantly depress the anammox performance of 1 L UASB reactors seeded with anammox granular sludge. The IC_{50} value of phenol was also defined at 678.2 mg/L in a batch test under the same conditions. The granule characteristics and stoichiometric ratios of anammox were observed to be changed under the stress of phenol. In depth, the presence of phenol changed the microbial community considerably in an SBR anammox system [144]. The anammox bacterial population was reduced from 14.7% to 10.1% and phenol-degrading bacteria were selectively enriched in the presence of phenol. The anammox bacterial community even shifted from "*Candidatus* Kuenenia stuttgartiensis" to "*Candidatus* Brocadia sinica" upon exposure to a mixture of phenol and thiocyanate at 16–32 mg/L over the long term of 262 d [141]. Phenol also exhibited an inhibitory effect on partial nitration activity in a batch test with an IC_{50} value of 5.6 mg/L [145]. Until now, phenol has been observed to block the synthesis of hydrazine as the key enzyme in anammoxosomes, which is the main mechanism for immediate inhibition of anammox activity [146].

Other phenolic compounds have also been revealed to have an inhibitory effect on anammox activity similar to that of phenol. It was reported that 2,4-dinitrophenol concentrations of 37 and 15 mg/L partially inhibited the anammox activity of biomass-enriched municipal sludge [147]. In another study [148], the addition of 37 mg/L 2,4-dinitrophenol decreased anammox activity by up to 53%, and anammox activity was completely inhibited when 2,4-dinitrophenol concentration was increased to 368 mg/L. The effect of phenolic compounds on anammox activity was dependent on the types of phenolic compounds [149]. Previous investigation with anammox granules dominated by *Brocadia* sp. indicated that *o*-cresol and *o*-chlorophenol resulted in a toxic effect to anammox bacteria. Anammox performance did not recover after bacterial granules were washed from *o*-cresol and *o*-chlorophenol, whereas the presence of *p*-nitrophenol and quinoline exhibited an inhibitory effect on anammox activity. When anammox granules were washed from *p*-nitrophenol and quinoline, specific anammox activity was almost recovered to the initial stage without inhibitors. Exposure of the anammox granules to a higher concentration of phenolic compounds would result in a higher reduction in anammox activity [149]. The joint effect of *o*-cresol, *o*-chlorophenol, *p*-nitrophenol, and quinoline was determined to be synergistic.

3.4.3. Antibiotics

Antibiotics are widely applied in humans, animals, and fisheries to treat infection and especially used in aquaculture and breeding farms as prophylaxis. Therefore, antibiotics are typically detected in various livestock wastewaters [150]. Owing to their ubiquitous migration, antibiotics are now found in all aquatic environments at various levels [151]. A thorough understanding on the effect of antibiotics on anammox performance is required. Currently, a wide range of antibiotics such as penicillin, chloramphenicol, ampicillin, tetracycline, sulfathiazole, oxytetracycline, amoxicillin, florfenicol, sulfamethazine, and oxytetracycline were the most investigated antibiotics for the anammox system (Table 8).

Antibiotics have a strong negative effect on anammox activity. Higher antibiotic concentrations resulted in lower nitrogen removal in an anammox system [148]. Anammox activity decreased by 17% in the presence of 1 mg/L penicillin and decreased to 36% when penicillin concentration increased to 100 mg/L. More than 90% of anammox activity was inhibited by 200 mg/L chloramphenicol or 800 mg/L ampicillin. Chloramphenicol of 200 g/L could decrease anammox activity by 98% for the first 3 d of exposure; however, anammox activity was slightly improved after 3 d of incubation owing to acclimation [148]. Similarly, anammox activity was decreased by 20% to 80% in a batch test using 25 mL serum vials with tetracycline hydrochloride at 100–1000 mg/L or chloramphenicol at 250–1000 mg/L [152]. However, low concentration of antibiotics such as allylthiourea and chloramphenicol did not show a meaningful inhibition on anammox activity [26]. Allylthiourea of 1 mg/L decreased anammox activity by more than 20%, whereas chloramphenicol of 1 mg/L did not result in any inhibition.

The level of toxicity of antibiotics on anammox bacteria also depended on the type of antibiotics. The IC_{50} value of sulfathiazole in a batch test using 200 mL vials was found to be 650 mg/L, whereas this value for oxytetracycline was determined to be 1100 mg/L [28]. In a continuous operation of anammox dominated by "*Candidatus* Kuenenia stuttgartiensis", florfenicol at 20 mg/L could decrease anammox activity by up to 50% [153]. Amoxicillin at 150 mg/L showed a severe inhibition, whereas sulfamethazine at 200 mg/L only exhibited a slight inhibition on anammox activity of the same anammox granules.

Table 8. Effect of antibiotics on anammox performance.

Seeding Sources	Reactor	Operation Mode	Antibiotics and Concentration (mg/L)	Effect	Ref.
Sludge from the denitrifying fluidized bed reactor	500 mL serum bottle	Batch test	Penicillin: 1 Penicillin: 100 Chloramphenicol: 20 Chloramphenicol: 200 Ampicillin: 400 Ampicillin: 800	Activity decreased by 17% Activity decreased by 36% Activity decreased by 36% Activity decreased by 98% for first 3 d and 68% after 3 d Activity decreased by 71% Activity decreased by 94%	[148]
Granular Anammox sludge	25 mL serum vials	Batch test	Tetracycline hydrochloride: 100–1000 Chloramphenicol: 250–1000	Activity decreased from 20% to 80% Activity decreased from 20% to 80%	[152]
Granular Anammox sludge	1 L SBR reactor	Continuous	Tetracycline hydrochloride: 10 Chloramphenicol: 20	Activity decreased by 60% Activity decreased by 80% after	[152]
Anammox biomass dominated by "*Candidatus* Kuenenia stuttgartiensis"	25 mL serum vials	Batch test	Allylthiourea: 1 Chloramphenicol: 1	Activity decreased by more than 20% - No inhibition	[26]
Granular anammox biomass	200 mL vials	Batch test	Sulfathiazole: 650 Oxytetracycline: 1100	Inhibition by 50% Inhibition by 50%	[28]
Anammox seed granules dominated by "*Candidatus* Kuenenia stuttgartiensis"	1 L UASB reactor	Continuous	Amoxicillin: 150 Florfenicol: 20 Sulfamethazine: 200	Severely inhibited Inhibition by 50% Slight inhibition	[153]
Anammox seed granules dominated by "*Candidatus* Kuenenia stuttgartiensis"	1 L UASB reactor	Continuous	Oxytetracycline: 2	Specific anammox activity decreased by 81.3%	[154]
Anammox bacteria from an SBR	1 L serum bottle	Batch test	Oxytetracycline: 10–100	Complete inhibition	[155]
Anammox mixed culture from UASB reactor	160 mL serum bottle	Batch test	Oxytetracycline: 517.5	Inhibition by 50%	[156]
Anammox mixed culture from UASB reactor	1 L UASB	Continuous	Oxytetracycline: 50	Activity loss of 90.4%	[156]

The inhibitory effect of the same antibiotic compound can differ with different anammox seeding sources and operation conditions. The IC_{50} of oxytetracycline in a batch test with an anammox mixed culture from an UASB reactor was found to be 517.5 mg/L [156]. However, complete inhibition of anammox activity was observed with oxytetracycline concentrations from 10 to 100 mg/L in a 1 L serum bottle test [155]. The toxic concentration of oxytetracycline seems to be lower in the continuous operation. A continuous influent with 2 mg/L oxytetracycline in a 1 L UASB reactor decreased specific anammox activity by 81.3% [154]. In another continuous-mode study [156], an anammox activity loss of 90.4% was observed with 50 mg/L oxytetracycline. In a 1 L SBR reactor inoculated with granular

anammox sludge operated in continuous mode, 10 mg/L tetracycline hydrochloride and 20 mg/L chloramphenicol decreased anammox activities by 60% and 80%, respectively [152].

The toxic effect of antibiotics was also investigated in the presence of other inhibitors. The cooperative toxicity of oxytetracycline and Cu(II) on the anammox granular sludge from an UASB reactor was observed to be antagonistic, whereas the combination effect of oxytetracycline and sulfide was generally synergistic [118]. The toxic effect of an oxytetracycline and Cu(II) mixture was always smaller than that of the individual compounds. At a low Cu(II) concentration, the toxic effects of the mixture decreased with the increase in oxytetracycline concentration, indicating that the addition of oxytetracycline weakened the toxic effect of Cu(II) on anammox bacteria. In a study seeking to acclimatize anammox bacteria with oxytetracycline, it was found that anammox bacteria can resist the toxicity of oxytetracycline via the efflux pumping mechanism [154]. In addition, anammox performance was totally recovered after 2 mg/L of oxytetracycline was withdrawn completely from the reactor. The feasibility of anammox application for treating antibiotic-containing wastewater has also been indicated previously [153]. After long-term acclimatization, anammox sludge dominated by "*Candidatus* Kuenenia stuttgartiensis" can achieve a resistance to 60 mg/L amoxicillin, 10 mg/L florfenicol, and 100 mg/L sulfamethazine.

3.5. Inhibition by Salinity

Saline solution creates high osmotic pressure that can kill bacteria owing to water loss from cells [157]. Therefore, high-salinity wastewater might not be treatable by any biological system. High-salinity wastewater is usually generated from food waste leachate, seafood processing industries, textile dyeing, and tanneries [158]. However, the anammox process could be a potential candidate for these high-salinity wastewaters because the anammox reaction has also been detected in marine environments [158]. Recently, it was reported that fresh anammox sludge can also be resistant to high salinity and perform stably under extreme high salt concentration after a long-term acclimation [159–161] (Table 9).

Table 9. Effect of salinity on anammox performance.

Seeding Sources	Reactor	Operation Mode	Salts and Concentration (g/L)	Effect	Ref.
Anammox sludge from an SBR	2 L SBR	Batch	NaCl: 5–10	Improved biomass retention Anammox activity slightly reduced initially but gradually increases	[161]
Mature anammox sludge	3 L SBR	Continuous	NaCl: 0–30	Enhanced the aggregation of anammox biomass Stimulated the activity at concentrations of 6–15 g/L Activity decreased at concentrations higher than 15 g/L	[162]
Anammox sludge	50 L lab-scale RBC	Batch test	NaCl: 6 NaCl: 30 (shock load)	No significant effect 96% loss in anammox activity for non-adapted biomass 58% loss in anammox activity for adapted biomass	[163]
Anammox sludge dominated by "*Candidatus* Kuenenia stuttgartiensis"	25 mL vials	Batch test	NaCl: 13.45 Na$_2$SO$_4$: 11.36 KCl: 14.9	Inhibition by 50% Inhibition by 50% Inhibition by 50%	[26]
Anaerobic activated sludge	5 L UASB bioreactor	Batch test	NaCl: 30 (shock load)	Activity decreased by 67.5% for non-adapted biomass Activity decreased by 45.1% for adapted biomass	[164]
Freshwater anammox sludge	2.8 L up-flow fixed-bed column reactor	Continuous	NaCl: 30 NaCl: >30	Stable nitrogen removal rate Nitrogen removal sharply declined	[165]

Table 9. *Cont.*

Seeding Sources	Reactor	Operation Mode	Salts and Concentration (g/L)	Effect	Ref.
Anammox bacterium KU2	7 L up-flow column reactor that	Continuous	NaCl: 30	Stable nitrogen removal after a period of adaptation	[67]
Anammox sludge	2.5 L UASB reactor	Continuous	NaCl: 5–60	Sludge retention time decreased with the increase in NaCl load Nitrogen removal rate decreased significantly with NaCl > 10 g/L	[166]
Marine anammox bacteria dominated by Planctomycete UKU-1	0.2 L up-flow column reactor containing	Continuous	NaCl: 0–75	Stable nitrogen removal with NaCl < 50 g/L Activity significantly declined with NaCl of 75 g/L	[167]
Anammox granule dominated by "*Candidatus* Brocadia fulgida"	10 L MBBR	Continuous	NaCl: 0–15	Complete inhibition at 15 g/L	[168]
Anammox sludge	1 L UASB reactor	Continuous	NaCl: 5–30	Performance degraded at NaCl higher than 15 g/L	[169]
Anammox sludge	1 L SBR	Continuous	NaCl: 5, 15 CaCl$_2$: 5	5 g/L of NaCl and CaCl$_2$ favored the formation of anammox biofilm Inhibitory effect observed at NaCl 15 g/L	[170]
Anammox bacteria from estuarine and coastal wetlands	12 mL vials	Batch test	NaCl: 0–40	Maximal anammox activity at NaCl 5 g/L Inhibition at NaCl higher than 30 g/L	[171]
Anammox sludge	6 L UASB reactor	Continuous	NaCl: 8–38	Activity inhibition from shock load of 8–38 g/L NaCl Nitrogen removal decreased slightly and stable at NaCl range of 8–18 g/L	[172]
Anammox sludge	5 L non-woven biofilm reactors	Continuous	NaCl: 0–20	Nitrogen removal deteriorated at NaCl higher than 10 g/L	[173]
Fresh water anammox sludge	350 mL UASB reactors	Continuous	NaCl: 3–30	Dominant anammox bacteria shifted from "*Candidatus* Brocadia fulgida" to "*C.* Kuenenia stuttgartiensis"	[159]
Anammox sludge	2.2 L UASB reactor	Continuous	NaCl: 35.1	Anammox performance collapsed	[160]

The effect of salinity on anammox performance was investigated in a wide range, up to 75 g/L, of NaCl. A marine anammox bacterial consortium dominated by Planctomycete UKU-1 can resist a salt concentration of 75 g/L, but the nitrogen removal rate significantly declined [167]. Stable nitrogen removal was observed in this system with NaCl concentrations lower than 50 g/L. Most fresh water anammox bacteria were adversely affected at a salt shock load of 30 g/L NaCl and higher [160,163–165,171]. The response of anammox activity to salinity exposure typically depends on the adaptation process. Without adaptation, anammox activity in a 5 L UASB bioreactor decreased by 67.5% in the presence of 30 g/L NaCl [164], whereas the activity of adapted anammox biomass decreased by only 45.1% under the same salinity condition. Similarly, these values in a 50 L lab scale RBC were 96% and 58% for non-adapted and adapted anammox biomass, respectively [163]. No inhibition was detected when this reactor was fed with influent having salinity of 6 g/L. At a smaller scale batch test with 25 mL vials using anammox sludge dominated by "*Candidatus* Kuenenia stuttgartiensis", the IC$_{50}$ value of NaCl was determined at 13.45 g/L [26]. The inhibition of salinity shock on nitrogen removal performance of anammox was generally found in the range from 10–15 g/L [162,166,168–170,172,173]. A complete inhibition of anammox activity was observed in a 10 L MBBR reactor continuously fed with NaCl of 15 g/L [168]. Nitrogen removal rate decreased significantly with NaCl levels higher than 10 g/L loaded to a 2.5 L UASB reactor [166].

Except for the inhibitory effect on anammox activity at high salinity, the presence of salt in the anammox system exhibited various benefits to the operation of the anammox process. In a 2 L SBR reactor, the presence of 5–10 g/L NaCl improved the retention of biomass, which also improved nitrogen-removal performance [161]. Under this condition, anammox activity was slightly reduced at the initial stages but gradually increased thereafter. Similarly, the presence of NaCl was observed to enhance the aggregation of anammox biomass and stimulate anammox activity at NaCl concentrations of 6–15 g/L [162]. In another study [170], the addition of 5 g/L NaCl and $CaCl_2$ favored the formation of anammox biofilms in an 1 L SBR reactor operated in continuous mode. In addition, the maximal anammox activity was observed only at an NaCl concentration of 5 g/L [171]. The stable activity of anammox under the transition from low to high salinity was explained through the change in dominant anammox bacteria. The 454 pyrosequencing of a freshwater anammox community indicated that dominant anammox bacteria shifted from "*Candidatus* Brocadia fulgida" to "*C.* Kuenenia stuttgartiensis" when the salinity of the influent changed from 3 to 30 g/L during a 40 day adaptation [159]. There was also strong evidence that the increase in salinity triggered significant changes in the functional proteins of anammox bacteria in a 5 L non-woven biofilm reactor [173], which could be a main mechanism for salinity resistance in anammox.

4. Optimizing Strategies for the Efficient Performance of the Anammox Process

Several factors directly affect nitrogen-removal performance during the operation of an anammox system for wastewater treatment. Among them, temperature, pH, DO, nitrogen loading, and carbon source content primarily need to be precisely controlled to produce a steady effluent meeting the discharge requirements. The anammox process was observed to perform efficiently at high ambient temperatures and the practical low temperatures during winters represents the bottleneck in commercializing this technology in wastewater treatment. Therefore, an intensive investigation on the metabolism of key functional anammox bacteria at low temperatures through metagenomic analysis would enhance the feasibility of the wide application of anammox because a good performance of the anammox process at low temperatures has been recorded previously [41]. Monitoring pH of the influent to maintain it within the favorable range of anammox bacteria (pH 6.7–8.3) is imperative because extremely low or high pH is detrimental to the nitrogen-removal efficiency of the anammox system. In the one-stage anammox process, the real-time control of DO through intermittent aeration is the most effective technique for balancing nitrite and ammonium levels for the anammox reaction and suppressing NOB activity [33]. Real wastewaters commonly contain high nitrogen concentration. Therefore, controlling influent nitrogen below the inhibition threshold by a reasonable loading rate and effluent recirculation would improve and stabilize the nitrogen removal rate in anammox systems. The presence of excess organic carbon sources in the influent of anammox systems can be overcome by controlling the activity of heterotrophic bacteria in a synergetic relationship with anammox bacteria. The heterotrophic bacteria might be heterotrophic denitrifiers that could partially remove the nitrate present in the systems or simple organic oxidizers that can maintain a suitable anaerobic niche around anammox granules. In another strategy, the excess organic carbon in the wastewater influent can be recycled and reused through further studies on the carbon footprint and capture technologies.

The sudden occurrence of some uncommon toxic organic and inorganic compounds owing to environmental disasters or accidents and the complexity of industrial wastewater can deteriorate or inhibit the normal activity of anammox systems. The recovery of anammox performance after deterioration can be achieved through adjustment of operating parameters together with the careful monitoring of performance, activity, and microbial dynamic changes in the anammox system. However, the thorough prior understanding of the relationship between operating parameters and the toxic effects of inhibitors on anammox bacteria, the adaptability of the current anammox sludge to the inhibitors, and the capability for recovery after deterioration of anammox systems is required. In this scheme, the pre-acclimation of the anammox sludge to these inhibitors before official operation might be an efficient precaution. When anammox sludge density was significantly reduced owing to the

inhibitor, the addition of fresh anammox sludge through a stable influent loading might balance the anammox growth factor and relieve the activity loss through inhibition.

5. Conclusions

A stable and high nitrogen removal rate can only be achieved if operational parameters such as temperature, pH, DO, nitrogen concentration, and carbon concentration are controlled at appropriate levels. Under the same operational conditions, the nitrogen removal efficiency of various anammox systems could differ owing to the difference in dominant anammox species because the metabolism of each anammox community is different. Therefore, the operational conditions for each anammox type should be investigated individually to determine precise optimal conditions. Anammox inhibition by hyper-saturation of substrates such as nitrite, hypersalinity, or toxic compounds such as sulfides, toxic metals, phenols, alcohols, and antibiotics can be controlled and prevented using proper strategies. Some specific anammox species can perform efficiently under these severe environments in the presence of toxic compounds. Therefore, a careful understanding of both the characteristics of the wastewater influent and the existing anammox community of the bioreactor plays an important role in its operation. To date, major research on the anammox process has been limited to lab-scale research reactors and synthetic wastewaters. The investigation of real wastewaters such as landfill leachate, pharmaceutical wastewater, and swine wastewater with the effect of multiple environmental factors at pilot and industrial scales is required for the future study of anammox application. A complete online-monitoring system coupled with automatic control of operational factors developed through the assistance of artificial intelligent technology would be another interesting topic in future research on anammox.

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