

Effects of Inhibition Conditions on Anammox process

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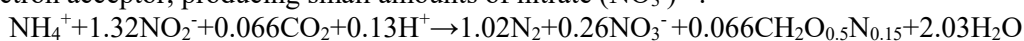
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Abstract. Anaerobic ammonium oxidation (Anammox) is a very suitable process for the treatment of nitrogen-rich wastewater, which is a promising new biological nitrogen removal process, and has a good application prospects. However, the Anammox process is inhibited by many factors, which hinders the process improvement and the application of the Anammox process. Such as organic, temperature, salts, heavy metals, phosphates, sulfides, pH and other inhibitors are usually present in practical applications. We have reviewed the previous researches on the inhibition of Anammox processes. The effect of the substrate on the anaerobic oxide is mainly caused by free ammonia or nitrite nitrogen. Most heavy metals inhibit Anammox growth and activity. The inhibition of organic matter depends on the content of organic matter and species. High salinity inhibits Anammox activity. Dissolved oxygen allows the flora to be in a balanced state. The optimum pH and temperature, as well as other factors, can provide a good growth environment for Anammox. The knowledge of inhibition on Anammox will help prevent the application and improvement of the Anammox process.

1. Introduction

Nitrogen removal has attracted increasing attention due to the potential role of nitrogen in eutrophication. Biological nitrogen removal has many advantages over physical or chemical methods. Conventionally, sequential nitrification and heterotrophic denitrification are typically used for nitrogen removal and the removal of organic matter from wastewater ^[1]. These procedures require organic, chemical reagents and aeration. Anammox (anaerobic ammonium oxidation) is a novel, environmentally friendly, cost-effective technology with a high nitrogen removal potential ^[2,3,4]. During the Anammox reaction, ammonium (NH₄⁺) is oxidized to gaseous N₂ using nitrite (NO₂⁻) as an electron acceptor, producing small amounts of nitrate (NO₃⁻) ^[5]:



However, the application and industrialization of the Anammox process are limited by the growth characteristics of anaerobic bacteria and the presence of a wide range of inhibitory factors in nitrogen-enriched wastewater. Anammox bacteria have a slow growth rate (their doubling time at 30–40°C is approximately 10–14 d ^[5,6,7,8], a low cell yield ^[5,9], and a high sensitivity to changing environmental conditions ^[10,11], which make them extremely difficult to cultivate. Although the growth rate can be increased by optimizing the operating conditions, the doubling time can be reduced to 4.8 d ^[14], or even as low as 1.8 d ^[12], but the culture is still very difficult. The complexity of industrial wastewater and wastewater composition tends to make the Anammox process harder to start ^[6], making process performance more susceptible to suppression and slowing Anammox activity from inhibition ^[11,13].



2. Substrate inhibition

Any bacteria on the substrate concentration have a certain range of adaptation, low concentration of bacteria is difficult to protect the growth rate, the concentration is too high will inhibit the bacteria and interfere with the metabolism of bacteria^[14]. Anammox of the main substrate is ammonia and nitrite nitrogen, generally to ensure the reactivity, we must also consider the matrix concentration and the ratio between the two substrates. It is reported that the Anammox process is free from ammonium concentrations up to 1 g N L^{-1} of the inhibition^[10]. However, other studies have reported that high levels of ammonia inhibit the Anammox process^[10,15,16]. Dapena-Mora et al.^[16] observed that hemi-inhibitory concentration of ammonium of 770 mg L^{-1} , free ammonia rather than ammonium itself, is a real inhibitor. Strous found that ammonia nitrogen on the Anammox inhibition limit of 1000 mg L^{-1} , and nitrite nitrogen on the Anammox inhibitory effect is much stronger, the suppression limit of only 100 mg L^{-1} ^[10].

3. Organic matter inhibition

The Anammox bacteria community is a self-sustaining microorganism with carbon dioxide(CO_2) as the main carbon source^[17]. Sufficient enough inorganic carbon can promote the growth of Anammox bacteria, but also can enhance its ability to move^[18]. However, organic matter is generally considered to be an inhibitor of Anammox^[19]. In fact, organic carbon, inorganic carbon, and ammonia salts are coexisting in real water, and high concentrations of organic carbon often limit the use of Anammox. Therefore, it is necessary to deploy a mixed microbial population capable of performing Anammox in the current organic compounds^[20]. In this mixed system based on Anammox, the denitrification performance is due to the coexistence of Anammox and denitrification. the toleration of organic carbon by Anammox was due to denitrification and cell lyses^[19]. It has been shown that if you keep enough of the high concentration of sludge to stay while increasing the rate of influent COD/N, Anammox can coexist with some heterotrophic bacteria. The local denitrification/Anammox process shows and promotes this mixed Anammox-based system in which denitrification consumes COD, Anammox produces nitrates, but meanwhile supplied nitrite for anammox^[21,22,23]. However, Anammox is easy to lose their ability to move, even from the system disappeared, because the rapid growth of denitrifying bacteria and cell growth is increasing^[24]. Therefore, it is necessary to find out how to get a solid reaction system, which could affect the denitrification performance as the main factors.

4. Dissolved oxygen (DO) concentration inhibition

Conventional views have shown that the Anammox process is temporarily inhibited in the case of a low dissolved oxygen concentration of 0.032 mg L^{-1} , and then it is recovered under anoxic conditions^[10]. However, oxygen will consume a portion of the respiration of the microbial system members before reaching the Anammox cells, just like a one-step reactor. In a limited oxygen reactor, or in an increasing dissolved oxygen reactor, Anammox can co-exist with aerobic bacteria (AOA and AOB)^[25]. This will consume oxygen for the anaerobic ammonium oxide, which consumes toxic nitrite for AOA and AOB, which will help denitrify. Through further cultivation, Anammox bacteria will gradually adapt to the high dissolved oxygen concentration is about 8 mg L^{-1} , in the long-term aeration conditions, most of the activities of Anammox bacteria will be slightly reduced^[25]. Recent researches have shown that Anammox can survive in the aerobic tank of urban wastewater treatment, even in the case of dissolved oxygen over 2 mg L^{-1} . This proves a more powerful ability than originally thought^[26]. This result indicates that the conditions for limiting oxygen in the sewage treatment process can be mitigated or avoided.

5. Temperature inhibition

Temperature affects cell growth and metabolic activity. In general, cell growth is faster at high temperature, so Anammox technology can only be limited to the treatment of high temperature wastewater. The activation energy of Anammox bacteria is very similar to that of ammonia-oxidizing bacteria^[27]. Anammox operating temperature is about $6\text{--}43^\circ\text{C}$; at less than 15°C or higher than 40°C ,

its reaction rate will decline rapidly^[28]. Despite these limitations, however, the removal rate of nitrogen was $2.28 \text{ kg.m}^{-3}.\text{d}^{-1}$ by Anammox in wastewater with a low intensity of only 16°C ^[29]. More recently, more researches have been conducted in a reactor to treat pretreatment of municipal wastewater at a temperature of 29°C , a nitrogen removal rate of $0.465 \text{ kg.m}^{-3}.\text{d}^{-1}$, and a decrease of $0.046 \text{ kg.m}^{-3}.\text{d}^{-1}$ at 12.5°C ^[30,31]. It was also observed that as the temperature decreased ($31.2\text{--}2.5^\circ\text{C}$) and increased in the Anammox reactor, the denitrification performance drastically decreased and slowly recovered.

6. Salinity inhibition

Salinity is known to have toxic effects on bacteria and is also capable of altering the microbial community. Moreover, salinity also significantly affects the physical and biochemical properties of activated sludge^[32,33]. Several researchers have studied the effect of salinity on the Anammox process^[34, 35]. A previous study has shown that Anammox bacteria acclimated to salinity can tolerate levels up to 75 g L^{-1} NaCl^[35,36]. However, it is reported that, even with extended acclimatization, freshwater Anammox sludge can not be applied in Anammox treatment at salt concentrations higher than 30 g L^{-1} . Currently, the adaption of Anammox to salt is considered as one of the best ways to overcome saline inhibitions, and thus, some researchers have focused on acclimation strategies^[35,37]. Salinity is often highly variable in industrial wastewaters, especially with regard to concentration and discharging time. These variable conditions can lead to fluctuant salt concentrations that create a transient shock^[38]. However, little is known about the impact of transient salinity shock loads on Anammox process performance. The relevant research results and the corresponding Anammox under different operating conditions are shown in Table 1. Further researches need to be undertaken.

Table 1. Research on the Salinity inhibition of Anammox.

Reactor	Salts	Concentration g L^{-1}	Operation mode	Effect	References
Up-flow column reactor	NaCl	20	Long-term test	Significant inhibition	[39]
Up-flow column reactor	NaCl	30	Long-term test	A stable NRR of $4.5 \pm 0.1 \text{ kg N m}^{-3}.\text{d}^{-1}$	[39]
up-flow fixed bed column reactor	NaCl	30	Long-term test	Stable NRR of $1.7 \text{ kg N m}^{-3}.\text{d}^{-1}$	[40]
	NaCl	>30	Long-term test	NRR sharply declined	[40]
UASB	NaCl	>10	Long-term test	losing much biomass	[41]
SBR	NaCl	<7	Long-term test	Not affect the anammox activity	[42]

7. Heavy metals inhibition

Heavy metals are not biodegradable and can accumulate in organisms, causing bioaccumulative toxicity. Some nitrogen-rich wastewater, such as landfill leachate, usually contains high levels of heavy metal ions. Understanding of heavy metals for Anammox inhibition will help remove nitrogen from these wastewater. Typically, 1 mmol L^{-1} HgCl_2 completely inhibits Anammox activity^[43]. Although the study of heavy metal inhibition of Anammox is rare^[44]. The relevant research results and the corresponding Anammox are summarized in Table 2, showing heavy metals are not easily biodegradable and can accumulate in organisms, causing biological accumulation toxicity. The further studies should be undertaken.

Table 2. The Heavy metals inhibition of Anammox.

inhibitor	Operation mode	inoculation	Concentration (mg L^{-1})	Effect	reference
Cu^{2+}	Batch	Anammox	12.9	IC_{50}	[45]
Cu^{2+}	Long-term	Anammox	5.0	94% loss in activity	[45]
Cd^{2+}	Batch	Anammox	11.16 ± 0.42	IC_{50}	[46]
Ag^+	Batch	Anammox	11.52 ± 0.49	IC_{50}	[46]
Hg^{2+}	Batch	Anammox	60.35 ± 2.47	IC_{50}	[46]
Zn^{2+}	Batch	Anammox	7.6	IC_{50}	[47]
Ni^{2+}	Batch	Anammox	48.6	IC_{50}	[47]

8. Other condition inhibition on anammox

8.1. Phosphate and sulfide inhibition

Phosphates and sulfides are two common inorganic inhibitors of the Anammox process. Phosphate inhibits Anammox activity^[44,48]. SO_4^{2-} is usually reduced to H_2S ^[48] in anaerobic digestion, leading to sulfide inhibition of Anammox^[48,49]. The results are differences in dominant species (or sowing sludge) and operating conditions. Different studies have shown that sulfide and phosphate have different effects on Anammox in Table 3. However, the most commonly used Anammox species in the study of phosphate inhibition or sulfide inhibition is *Candidatus Kuenenia stuttgartiensis*, which should further study the inhibitory effects on phosphates or sulfides from other Anammox species.

Table 3. The phosphate and sulfide inhibition of Anammox.

Inhibitor	Operation mod	Inoculation sludge (dominant species)	Concentration (mmol L ⁻¹)	Effect	References
Phosphate	Batch	Denitrifying FBR sludge	5 or 50	-100%	[49]
	Batch	Nitrifying RBC biofilm (<i>Candidatus Kuenenia stuttgartiensis</i>)	20	No inhibition	[50]
	Batch	Anammox bacteria (<i>Candidatus Kuenenia stuttgartiensis</i>)	20	-50%	[48]
	Batch	RBC biofilm (<i>Candidatus Kuenenia stuttgartiensis</i>)	1.8	-37%	[51]
			3.6	-80%	
			9.2	-80%	
	Batch	Anammox bacteria	<1 >2	No inhibition inhibition	[44]
Sulfide	Batch	Anammox Biofilm (<i>Candidatus Brocadia sinica</i>)	20 (phosphorus)	-20%	[52]
	Batch	Denitrifying FBR sludge	1or5	Advance	[49]
	Continuous flow	Denitrifying FBR sludge	2 (A pulse feed)	20%	[49]
	Continuous flow	Denitrifying FBR sludge	2(Intermittent feeding)	60%	[49]
	Batch	Anammox bacteria (<i>Candidatus Kuenenia stuttgartiensis</i>)	2-January	-60%	[48]
	Batch	Anammox bacteria(<i>Candidatus Kuenenia stuttgartiensis</i>)	>5	-100%	[48]

8.2. pH inhibition

In the Anammox process, pH is a very important environmental conditions. The effect on the Anammox process is mainly due to its effect on bacteria and the matrix. Ammonia nitrogen and nitrite are two major reaction matrices in Anammox. Schalk et al. suggested that hydroxylamine is an important step in Anammox. When pH is too low, the rate of nitrite conversion to hydroxylamine is significantly slower; when pH is too high, ammonia is excessively converted to nitrite nitrogen. That is, too high or too low pH may make the rate of Anammox reaction decreased^[53]. According to Jettn et al., the optimum pH range for Anammox is between 6.7-8.3 and the maximum reaction rate is at pH 8.0. It also have focused on the effect of pH on the concentrations of ammonia and nitrite nitrogen , the effect on the Anammox was described by the double substrate dual inhibitor model, and it was suggested that the Anammox activity was higher at pH 7.5-8.3^[54].

9. Conclusions

The inhibition of Anammox is a major obstacle to the popularization and application of the Anammox biological nitrogen removal process. At concentrations above the inhibition threshold, the substrate (ammonia and nitrite), organic matter, salinity, heavy metals, phosphates, sulfides and pH, can inhibit anaerobic reactivity. The inhibitory effects of these Anammox inhibitors are related to the exposure dose and operating conditions of the Anammox species. In the moderate dose range, some so-called inhibitors (such as NaCl and some organic matter) contradictically promote the nitrogen removal of Anammox. Since the culture of pure Anammox bacteria has not been studied, some consensus has

been achieved. We believe that this differential experiment may be caused by different Anammox species or Anammox substrate or/and metabolic diversity and operating conditions. Further studies of the following aspects of Anammox inhibition are suggested:

(1) Studies of nitrogen removal by Anammox in specific wastewater are required. Researches on the specific inhibition challenges presented by various specific kinds of wastewater would allow a more targeted strategy for Anammox inhibition relief.

(2) The inhibition effects of inhibitors on various Anammox species need to be studied. The Anammox inhibition based on Anammox species caused by ammonium to nitrite ratio, organic matter, salts, heavy metals, phosphate, sulfide and pH are still insufficient, further and broader studies are necessary.

(3) The need for composite inhibition studies. The inhibitory effect on the Anammox process is often dependent on multiple inhibitors. The study of multiple inhibitory factors in the researches of Anammox composite inhibition is relatively less; therefore, further researches into composite inhibition are suggested.

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