Role and application of quorum sensing in anaerobic ammonium oxidation (anammox) process: A review

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Role and application of quorum sensing in anaerobic ammonium oxidation (anammox) process: A review

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ABSTRACT

Anaerobic ammonium oxidation (anammox) is a promising biological nitrogen removal process, due to its advantages of high efficiency and low cost. However, problems remain in the application, such as long startup period and susceptibility to environmental variations. Quorum sensing (QS), as a means of bacterial communication, attracts more attentions in regulating aggregation behavior and microbial density. Many physiological characteristics of anammox bacteria have been confirmed to be associated with QS, including specific anammox activity, growth rate and the production of extracellular polymeric substances (EPS), which directly affect the performance of anammox process. Therefore, a comprehensive understanding of the QS in anammox process is prerequisite. This work systematically reviewed the role and application of QS in the anammox process with the focus on mechanism. Additionally, current challenges and research needs were proposed.

KEYWORDS Anammox; application; role; signaling mechanism; quorum sensing

1. Introduction

Anaerobic ammonium oxidation (anammox) is a chemoautotrophic biological process in which ammonium is transformed into dinitrogen (N₂) gas with nitrite as the electron acceptor (van de Graaf et al., 1995). This phenomenon was first reported in a denitrifying fluidized bed reactor in 1995 (Mulder, Graaf, Robertson, & Kuenen, 1995). Due to its unique metabolic pathway, anammox, with its high efficiency and low cost, has received extensive attention in the field of wastewater treatment (Arrigo, 2005; Strous & Jetten, 2004). The implementation of anammox technique depends on the activity of anammox bacteria, which belong to the phylum...
Planctomycetes (Zhu et al., 2011). The anammox bacteria have a strong aggregation ability. When the cell density is higher than \(10^{10}–10^{11}\) per L, anammox bacteria’s bioactivity presents (Ali et al., 2018; Kartal, van Niftrik, Keltjens, Op den Camp, & Jetten, 2012) implying the potential existence of quorum sensing (QS) (Shrout & Nerenberg, 2012).

QS, as a communication approach among bacteria, was first discovered in *Vibrio fischeri* (Nealson & Hastings, 1979). Briefly, bacteria with a QS mechanism can synthesize and release chemical signal molecules, whose external concentration is positively correlated with cell density. When the concentration of signal molecules in the microenvironment reaches a threshold, QS bacteria will regulate the expression of related genes and trigger corresponding behaviors, including biofilm formation and bioluminescence, etc, in response (Davies et al., 1998; Engebrecht, Nealson, & Silverman, 1983; Waters & Bassler, 2005).

In terms of intracellular, the secondary messenger bis-(3’–50’)-cyclic dimeric guanosine monophosphate (c-di-GMP) is also critical for regulating the microbial metabolism. Signal molecules are essentially the “language” of the communication, and different kinds of QS bacteria use correspondingly different “languages” (Table 1). The signal molecules present in bacterial systems mainly include acyl-homoserine lactones (AHLs), autoinducing peptides (AIP), autoinducer 2 (AI-2) and diffusible signal factor (DSF), while the intracellular signal is mainly secondary messenger c-di-GMP (Deng, Wu, Tao, & Zhang, 2011; Hengge, 2009; Miller & Bassler, 2001; Shrout & Nerenberg, 2012; Waters & Bassler, 2005).

Although more than 100 full-scale anammox installations have been built around the world, the anammox process is still suffering from several major drawbacks (Lackner et al., 2014). Due to the slow growth rate (doubling time of 10–14 days) and growth yield (0.11 g VSS g\(^{-1}\)NH\(_4^+\)-N) of anammox bacteria (van Dongen, Jetten, & Van Loosdrecht, 2001),

<table>
<thead>
<tr>
<th>Signal</th>
<th>Characteristics</th>
<th>Source</th>
<th>Regulatory genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyl-homoserine lactones (AHLs)</td>
<td>Intraspecific signals</td>
<td>Gram-negative bacteria</td>
<td>luxI luxM hdtS agrC agrB agrD</td>
</tr>
<tr>
<td>Autoinducing peptides (AIP)</td>
<td>Intraspecific signals</td>
<td>Gram-positive bacteria</td>
<td>luxS luxQ</td>
</tr>
<tr>
<td>Autoinducer 2 (AI-2)</td>
<td>Interspecific signals</td>
<td>Gram-negative and gram-positive bacteria</td>
<td>rpfF rpfC</td>
</tr>
<tr>
<td>Diffusible signal factor (DSF)</td>
<td>Intracellular signal</td>
<td>Gram-negative and gram-positive bacteria</td>
<td>DGCG genes unknown</td>
</tr>
</tbody>
</table>

Table 1. Common signals and characteristics found in bacteria.
anammox processes often require long startup periods. The first industrial-scale anammox reactor was constructed in Rotterdam, the Netherlands, with a startup time of 3.5 years (van der Star et al., 2007). In addition, anammox bacteria are susceptible to many environmental conditions, such as pH, substrate concentration, temperature, and the presence of heavy metals (Jin, Yang, Yu, & Zheng, 2012), which make the stable operation of the anammox process difficult. Traditional methods like bio-augmentation and optimization of bioreactor configuration have been adopted (Tang, Guo, Wu, et al., 2018; Zhang, Yang, Sun, Tian, & Jin, 2018), but the results and stability obtained by these methods were not always satisfactory. The emergence of QS-based technology provides new perspectives and ideas for the optimization and regulation of the anammox process.

The first application of QS-based technology to the field of wastewater treatment was to solve the problem of membrane biofouling. In the membrane bioreactors (MBRs) system, membrane biofouling was successfully alleviated by adding orcin kidney acylase that can inactivate the AHLs molecule by amide bond cleavage (Yeon et al., 2009). In addition, QS-based strategies have also been applied to the granulation of sludge and the regulation of microbial community in wastewater treatment systems (Maddela et al., 2019; Xiao, Yaohari, Zhou, Sze, & Stuckey, 2019). By inoculating AI-2-regulating Escherichia coli culture, Xiao et al. (2019) successfully regulated the activities of Firmicutes and Synergistetes in the anaerobic digestion system and improved the biogas production during the toxic shock. The QS in anammox system was first mentioned in 1999 due to density-dependent activity of anammox bacteria (Strous et al., 1999). Many studies have focused on the role of QS in anammox bacteria and attempted to improve performance robustness and controllability in the anammox process through QS-based technology (Guo, Liu, Tang, & Yang, 2017; Tang, Guo, Wu, et al., 2018; Zhang, Li, et al., 2019; Zhao, Zhang, Zhang, & Yang, 2018).

The potential for QS-based technology has been reviewed previously in the field of wastewater treatment (Huang et al., 2016; Shrout & Nerenberg, 2012). This review aims to present a detailed comparative summary of previous and current research on QS in the anammox process, focusing on (1) application of QS in anammox process, (2) QS signaling mechanisms of anammox bacteria, and (3) ecological significance. In addition, the direction and main bottlenecks of the research on QS in the anammox process have also been discussed.

2. Application of QS in anammox process

QS, as a density-dependent mechanism of regulating microbial collective behavior, has received increasing attentions in the field of biological
wastewater treatment in recent years (Maddela et al., 2019). Studies have demonstrated the widespread presence of QS bacteria in biological wastewater treatment systems (Huang et al., 2016; Shrout & Nerenberg, 2012), which also provides ideas for the optimization of anammox processes based on QS technology. Therefore, the applications of QS in anammox process was further discussed in this section based on the previous researches.

2.1. Accelerating the startup of the anammox process

The major bottleneck in the practical application of the anammox process is the long startup times (Ali et al., 2015). Therefore, increasing the growth metabolic rate and the activity of anammox bacteria is expected to fundamentally solve this problem. On the one hand, QS regulation of the growth metabolism of anammox bacteria has been demonstrated in several studies (Liu et al., 2018; Tang, Guo, Wu, et al., 2018; Tang, Liu, Zhang, & Zhuang, 2015). Tang, Guo, Wu, et al. (2018) confirmed that AHLs-mediated QS could affect the growth of anammox bacteria by regulating LysoPC (20:0) metabolism. On the other hand, QS regulation also shows great potential in increasing the activity of anammox bacteria. By adding 150 µM C₆-HSL to a UASB reactor, anammox granular sludge increased its activity by 16%, and its special anammox activity (SAA) reached 1.08 kg N kg⁻¹ VSS d⁻¹ (Zhang et al., 2019). Meanwhile, in a study of Tang, Guo, Wu, et al. (2018) the addition of 2 µM 3OC₆-HSL, C₆-HSL and C₈-HSL significantly increased SAA by approximately 10%. It is noteworthy that due to the limited solubility of the signaling molecules, the higher concentrations were adopted to guarantee that the concentration of AHLs in the supernatant reached to the threshold of QS system (Tang et al., 2015). In fact, Zhao et al. (2018) successfully realized the fast startup of the anammox process within 66 days using AHL-containing supernatant. The nitrogen loading rate (NLR) was finally stabilized at 0.8 mg N m⁻³ d⁻¹, the nitrogen removal efficiency (NRE) of the reactor reached 98%.

In addition to improving the activity and growth rate of anammox bacteria, the QS-based technology also increases the secretion of EPS (Tang et al., 2015; Zhang et al., 2019), thus accelerating the aggregation of anammox bacteria in the initial startup stage. Furthermore, the regulation of the content of EPS by QS is realized by mediating the synthesis of uridine diphosphate-N-acetylgalactosamine (UDP-GlcNAc), suggesting that the regulation is indeed carried out at the metabolic level (Tang, Guo, Wu, et al., 2018). In brief, QS-based technology can be considered as an effective means for the fast startup of anammox process. More importantly, this regulation is at the metabolic level, which also provides a new perspective for the regulation of the anammox process.
2.2. Increasing the stability and resistance of the anammox process

Due to the high sensitivity of anammox bacteria to the changes in conditions, the anammox process has poor operational flexibility and limited application range (Jin et al., 2012). Therefore, there is increasing interest in improving the performance robustness of anammox and its tolerance to adverse environments.

QS has shown great potential in regulating the stability and balance of microorganisms in wastewater treatment systems (Shrout & Nerenberg, 2012). Valle, Bailey, Whiteley, and Manefield (2004) found that the community composition and function of activated sludge were changed by the addition of 2 μM exogenous AHLs. Similarly, the stability of community composition and function is essential for the stable operation of the anammox process. Furthermore, the positive regulation of the activity and aggregation ability of anammox bacteria by QS has been confirmed (Table 2).

Tang, Guo, Wu, et al. (2018) found that the addition of 2 μM C₈-HSL

Table 2. Signal substances and their potential functions present in anammox systems.a

<table>
<thead>
<tr>
<th>Type of process</th>
<th>Source</th>
<th>Functions</th>
<th>Impact on anammox</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₄-HSL</td>
<td>Batch test</td>
<td>Induce the expression of anammox active genes 1. Control the electron transport carriers 2. Promote the synthesis of exopolysaccharides</td>
<td>Increase anammox activity 1. Increase anammox activity 2. Increase the aggregation ability of anammox consortia</td>
<td>Liu et al. (2018)</td>
</tr>
<tr>
<td>C₈-HSL</td>
<td>Anammox bio-fermentor</td>
<td>Unknown</td>
<td>Increase anammox activity 1. Increase anammox activity 2. Increase the aggregation ability of anammox consortia</td>
<td>Tang et al. (2015)</td>
</tr>
<tr>
<td>C₁₂-HSL</td>
<td>The biofilm reactor</td>
<td>Unknown</td>
<td>Increase anammox activity 1. Increase anammox activity 2. Increase the aggregation ability of anammox consortia</td>
<td>De Clippeleir et al. (2011)</td>
</tr>
<tr>
<td>C-di-GMP</td>
<td>Anammox bio-fermentor</td>
<td>Promoted the growth of heterotrophic bacteria Adjusting the synthesis of EPS</td>
<td>Increase anammox activity 1. Increase anammox activity 2. Increase the aggregation ability of anammox consortia</td>
<td>Guo et al. (2017)</td>
</tr>
</tbody>
</table>

The AHLs with no substituent at three-carbon positions are expressed as Cₙ-HSL according to the length (n) of acyl side-chain, and 3OCₙ-HSL by having a substituent at three-carbon positions.
significantly increased the anammox floc sizes by 24% compared to the control. Additionally, Zhang et al. (2019) also detected that the addition of C₈-HSL increased the relative hydrophobicity of anammox granules by 28%, thereby increasing the settleability of the sludge. On the one hand, the acquisition of highly active anammox bacteria will improve the performance of the anammox process. On the other hand, the granulation of anammox biomass is beneficial for the retention of biomass in the anammox process (Dapena-Mora, Campos, Mosquera-Corral, Jetten, & Méndez, 2004; Lu et al., 2012). Achieving the above two aspects are crucial for improving the stability of the anammox process. To date, the highest SAA value reported in the literature is 5.6 ± 0.9 kg N kg⁻¹ VSS d⁻¹, which is derived from the anammox granules system (Tang et al., 2011). Maintaining the granular structure of sludge has also become the key to the achievement of a high SAA. Additionally, biogranulation is one of the best approaches to increase bacterial quantity and retention (Zhu et al., 2018). QS-based technology provides the possibility to simultaneously and rapidly achieve these goals.

Many studies have shown that some features of actual wastewater can inhibit the anammox process, including low temperature (He et al., 2018; Jin, Ma, & Yu, 2013); the presence of metal nanoparticles (Xu, Cheng, et al., 2019; Zhang, Cheng, et al., 2018), antibiotics (Fernandez, Mosquera-Corral, Campos, & Mendez, 2009; Meng, Sheng, & Meng, 2019; Zhang et al., 2015), or heavy metal (Yang et al., 2013; Yu et al., 2019; Zhang, Chen, et al., 2018); and salinity (Chen, Ma, Ji, Ni, & Jin, 2014; Liu, Yamamoto, Nishiyama, Fujii, & Furukawa, 2009). Thus, improving the tolerance to adverse conditions is critical to increasing the availability and range of anammox processes. Although it is possible to achieve the above purposes by means of acclimation, some such methods have severe faults such as long acclimation times and unstable results (Table 3). The relationship between the QS of bacteria and changes in environmental conditions has always been the focus of attention, and many studies have reported that bacteria can adapt to diverse environments through QS regulation (Montgomery, Charlesworth, LeBard, Visscher, & Burns, 2013; Zhao et al., 2019). As a barrier to the contact of bacteria with the external environment, EPS is considered to be beneficial for the survival of microorganisms in stressful environments (Liu, Liu, & Tay, 2004). Under the impact of 30 g⁻¹ NaCl, the EPS content of anammox granules was increased by 58.31% in a short term to alleviate salinity stress (Ma et al., 2012). While, dosing 2 µM AHLs can significantly increase the content of EPS by 25.5% (Tang, Guo, Wu, et al., 2018). Therefore, it is possible to make anammox bacteria respond in advance through QS technology, such as improving the production of EPS and promoting floc aggregation before experiencing
environmental stress. This may be an effective way to improve the resistance of anammox process.

A recent study has shown that the abundance of signal molecules (C6-HSL and C8-HSL) released by anammox granules increases significantly when the anammox process is subjected to substrate shock (2.1 g TN L\(^{-1}\)) (Zhang, Zhang, et al., 2019). Sun, Guan, Wang, and Wu (2019) found that biological nitrogen removal systems based on nitritation and anammox, the addition of organics reduces AHL-QS activity. These studies provide an improved understanding of the QS response to environmental changes and the opportunity for the optimization of anammox process based QS regulation.

3. Does the QS mechanism exist in anammox bacteria?

The ability to aggregate is a general feature of anammox bacteria. In wastewater treatment systems, the anammox sludge generally exists in the form of granular sludge and biofilm (Chen, Zheng, & Shen, 2013), which has been verified to be closely related to QS. AHL-based QS could regulate the aggregation of *Rhodobacter sphaeroides* (Parsek & Greenberg, 2005). *Vibrio cholerae* was able to regulated the secretion of exopolysaccharide through QS, thus affecting the aggregation of bacteria and adhesion to surfaces (Hammer & Bassler, 2003; Parsek & Greenberg, 2005; Zhu & Mekalanos, 2003). A similar phenomenon was also observed in *Aeromonas* and *Xanthomonas* in water and wastewater treatment systems (Maddela et al., 2019; Shrout & Nerenberg, 2012). Therefore, the aggregation ability of anammox bacteria may also

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### Table 3. The acclimation of the anammox process for treating special wastewater.

<table>
<thead>
<tr>
<th>Acclimation target</th>
<th>Result</th>
<th>Seeding sludge</th>
<th>Reactor</th>
<th>Days</th>
<th>NRR (kg N m(^{-3}) d(^{-1}))</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low temperature</td>
<td>13 °C</td>
<td>Anammox sludge</td>
<td>UASBa</td>
<td>199</td>
<td>5.24</td>
<td>He et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>9.1 ± 0.5 °C</td>
<td>Anammox ground sludge</td>
<td>UASBa</td>
<td>150</td>
<td>5.5 ± 0.5</td>
<td>Jin et al. (2013)</td>
</tr>
<tr>
<td>Salinity</td>
<td>30 g NaCl L(^{-1})</td>
<td>Anammox ground sludge</td>
<td>UASBa</td>
<td>163</td>
<td>2.14</td>
<td>Chen et al. (2014)</td>
</tr>
<tr>
<td>Heavy metal</td>
<td>33 g NaCl L(^{-1})</td>
<td>Anammox sludge</td>
<td>UASBa</td>
<td>180</td>
<td>1.7</td>
<td>Liu et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>10 mg Zn(II) L(^{-1})</td>
<td>Anammox sludge</td>
<td>UASBa</td>
<td>80</td>
<td>0.60</td>
<td>Zhang, Chen, et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>5 mg Cu(II) L(^{-1})</td>
<td>Anammox biofilm</td>
<td>FBBRb</td>
<td>194</td>
<td>7.6-14.0</td>
<td>Yang et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>0.5 mg Cr(VI) L(^{-1})</td>
<td>Anammox ground sludge</td>
<td>UASBa</td>
<td>160</td>
<td>about 4.51 ± 0.41</td>
<td>Yu et al. (2019)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>200 mg sulfamethazine L(^{-1})</td>
<td>Anammox ground sludge</td>
<td>UASBa</td>
<td>92</td>
<td>about 6</td>
<td>Zhang et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>1 mg tetracycline L(^{-1})</td>
<td>Anammox biofilm</td>
<td>Biofilm reactor</td>
<td>500</td>
<td>about 0.4</td>
<td>Meng et al. (2019)</td>
</tr>
<tr>
<td></td>
<td>1 mg Chlorotetracycline L(^{-1})</td>
<td>Anammox ground sludge</td>
<td>SBRc</td>
<td>100</td>
<td>–</td>
<td>Fernandez et al. (2009)</td>
</tr>
</tbody>
</table>

aNASB-upflow anaerobic sludge blanket.

bFBBR-fixed bed biofilm reactor.

cSBR-sequencing batch reactor.
associate with QS. Previous studies have confirmed that the aggregation of anammox bacteria was regulated by QS (Guo et al., 2017; Tang, Guo, Wu, et al., 2018), and this regulation was mainly achieved by affecting the content of EPS (As discussed in Section 2.1).

The presence of signal molecules is the most direct evidence for the existence of QS. Till now, all researches on signal substances in the anammox system have focused on two types: AHLs and c-di-GMP (Table 2). AHLs, as unique signal molecules for gram-negative bacteria, were commonly detected in anammox systems (De Clippeleir et al., 2011). Except for C12-HSL, most identified AHL-like signaling molecules have a positive effect on the anammox process (Table 2). This may be due to the fact that C12-HSL could facilitate heterotrophic metabolism and the growth of heterotrophic bacteria in the microbial community (Verhagen & Laanbroek, 1991). It is worth noting that many signal molecules, such as C6-HSL, 3OC6-HSL, and 3OC8-HSL are critical for the production of extracellular polymeric substances (EPS), and an increase in EPS will promote the formation of anammox aggregates (granules and biofilm) (Guo et al., 2017; Tang, Guo, Wu, et al., 2018). Furthermore, Zhao, Zhang, Zou, and Yang (2016) observed the disintegration of anammox granular sludge with the addition of vanillin and porcine kidney acylase (an AHLs inhibitor), reconfirming that the aggregation behavior of anammox bacteria is regulated by QS.

The development of molecular biology methods has also provided a new perspective for better understanding of QS in anammox systems. Using metagenomics-based technology, Strous et al. (2006) found the synthesis genes of S-adenosyl methionine (SAM) and acyl-acyl carrier protein (acyl-ACP), two necessary biomolecules for the synthesis of AHL, in Candidatus Kuenenia stuttgartiensis (Ding et al., 2013; Strous et al., 2006). This was the first study to provide evidence for the existence of QS in anammox bacteria at the metabolic level. In recent years, several bacterial communication genes (BCGs) that regulate the synthesis of signaling molecules and the intracellular signals have been found in anammox systems. For intracellular signals, the diguanylate cyclases (DGC5) genes, responsible for the synthesis of c-di-GMP, have been found in the anammox system (Sun et al., 2019). Furthermore, using multiple protein sequence alignment techniques, thirteen genes encoding putative c-di-GMP metabolic enzymes exist in anammox organism Candidatus Jettenia caeni (Guo et al., 2017). For extracellular signal molecules, BCGs within gram-negative bacteria have all been reported in the anammox system. A recent study reported that hdtS-type AHL synthase genes (jqsI-1 and jqsI-2) in Candidatus Jettenia caeni could successfully expressed in vitro (Tang, Guo, Zhu, Tao, & Liu, 2019). Sun et al. (2019) observed AI-2 regulators genes (lsrF) had a high abundance in the anammox system and demonstrated that Candidatus
Kuenenia could regulate the expression of related genes through the AI-2-mediated QS system. In addition, the DSF synthetic gene *rpfF* was also found in an anammox system and may be the primary communication engine in interspecific communication due to its high abundance (Tang, Guo, Jiang, & Liu, 2018). The discovery of multiple BCGs not only confirms the existence of QS in anammox bacteria, but also highlights the complexity of their QS signaling mechanism.

4. Possible QS signaling mechanisms in anammox bacteria

Studies have shown that many physiological behaviors of anammox bacteria are regulated by QS and different kinds of signaling molecules play different roles in anammox systems (Table 2). In order to deeply understand the above phenomenon, two possible QS signaling mechanisms in anammox bacteria were proposed based on the current researches.

4.1. Hierarchical QS signaling mechanism

In the hierarchical QS signaling mechanism system, the operation of a downstream QS channel is regulated by a superior QS channel (Lee & Zhang, 2015). The expression of a particular gene often requires the concentrations of multiple signal molecules to reach a threshold at the same time, which makes some behaviors that transform bacteria only occur under certain conditions (Lee & Zhang, 2015; Williams & Cámara, 2009). In fact, the activity of anammox bacteria, EPS content, and the relative abundance of BCGs change during the enrichment of anammox bacteria (Tang, Guo, Jiang, et al., 2018; Tang, Zheng, Chai, & Min, 2013). Furthermore, some molecules are involved in mediating multiple metabolic pathways of anammox bacteria mentioned above (Tang, Guo, Wu, et al., 2018; Zhang, Li, et al., 2019), which can be explained by the hierarchical QS signaling. For example, in the study by Tang, Guo, Wu, et al. (2018), the addition of 3OC6-HSL promoted the activity of anammox bacteria, the growth rate, and the synthesis of EPS. If the 3OC6-HSL controls the superior QS channel, the operation of the downstream QS channel can also be affected. Different QS channels control the different multiple metabolic pathways of anammox bacteria, thus the multiple physiological characteristics of anammox bacteria change with the increase of 3OC6-HSL concentration. Such characteristics provide evidence for the existence of a hierarchical QS signaling mechanism in anammox bacteria. *Pseudomonas aeruginosa* has a typical hierarchical QS signaling mechanism (Whiteley, Diggle, & Greenberg, 2017). Based on this, the hierarchical QS signaling mechanism in anammox bacteria was proposed (Figure 1A).
This system includes the superior AMXI-AMXR channel and the downstream GRMI-GRMR and EPSI-EPSR QS channels. Under the regulation of the amxl gene, the AMXI protein synthesizes the signaling molecule 3OC₆-HSL and releases it to the outside of the cell. Then, 3OC₆-HSL binds to the receptor protein AMXR, regulated by the amxr gene, to form the AMXR-3OC₆-HSL protein complex. On the one hand, the AMXR-3OC₆-HSL complex will trigger the expression of related genes in the anammox process to realize the anammox function of anammox bacteria (Liu, Hu, & Guo, 2019); on the other hand, the AMXR-3OC₆-HSL complex can also positively regulate the expression of related genes in the GRMI-GRMR and EPSI-EPSR QS channels. In the GRMI-GRMR QS channel, GRMI protein is responsible for the synthesis of AHL1 signal molecule, and GRMR protein is the cognate cytoplasmic receptor of AHL1; these proteins are

Figure 1. Schematic of possible anammox bacteria QS signaling mechanisms. (A) The hierarchical QS signaling mechanism of anammox bacteria at high cell density; (B) the parallel QS signaling mechanism of anammox bacteria at low cell density. The solid line represents what is happening, and the dotted line indicates that it has not occurred. The operation process of the two QS signaling mechanisms is described in the text.
regulated by grmI and grmR genes, respectively. The complex of GRMR protein and AHL1, GRMR-AHL1, can positively regulate gene expression related to the growth and metabolism of anammox bacteria (Tang, Guo, Wu, et al., 2018). In the EPSI-EPSR QS channel, the signaling molecule 3OC₆-HSL produced by EPSI protein will bind to the cognate cytoplasmic receptor (EPSR protein) to form the EPSI-3OC₆-HSL protein complex, which can modulate the expression of genes related to the formation and hydrolysis of EPS (Sun, Guan, Zeng, He, & Wu, 2018). The synthesis of EPSI and EPSR proteins is regulated by epsI and epsR, respectively.

When the cell density of anammox bacteria in the environment is relatively low, the extracellular concentration of 3OC₆-HSL will not reach the threshold to initiate the QS system. Thus, the anammox bacteria will not perform anammox due to the absence of the AMXR-3OC₆-HSL protein complex. Meanwhile, the slow growth of anammox bacteria and EPS synthesis are regulated by other metabolic pathways, such as the c-di-GMP pathway (Guo et al., 2017). This phase is the lag phase in the whole anammox bacteria enrichment process (Tang et al., 2013). As anammox bacteria proliferation and population increase, the extracellular concentration of 3OC₆-HSL increases because there are more signal producers present. When the extracellular concentration of 3OC₆-HSL is above the threshold, the AMXR-3OC₆-HSL protein complex is formed, which triggers the anammox reactions of anammox bacteria. Simultaneously, the GRMI-GRMR and EPSI-EPSR QS channels are also activated by the AMXR-3OC₆-HSL protein complex and accelerate the growth of anammox bacteria and the synthesis of EPS. Macroscopically, anammox granular sludge and biofilm were observed to form gradually, which is called the activity elevation phase (Tang et al., 2013; Wang, Wang, Yuan, Luo, & Kwame Indira, 2019). With further enrichment of anammox bacteria, the abundance of the AMXR-3OC₆-HSL protein complex in cells increases significantly, and the expression of genes related to the hydrolysis of EPS is triggered to hydrolyze the excess EPS. The excessive production of EPS will increase the mass transfer resistance of anammox granular sludge to substrate uptake (Shi et al., 2017; Xu, Kang, et al., 2019). Thus, anammox bacteria can dynamically regulate the synthesis and hydrolysis of EPS through the metabolic pathway mediated by the QS system, further maintaining the stability of the anammox bacteria population morphology.

4.2. Parallel QS signaling mechanism

The parallel QS signaling mechanism is a QS operation mode to arrange multiple QS channels in parallel and trigger downstream gene expression through simultaneous input of multiple signals (Miller, Skorupski, Lenz, Taylor, & Bassler, 2002). Many bacterial density-dependent physiological
characteristics of anammox bacteria, such as the realization of anammox function and the secretion of EPS, can also be well explained by the parallel QS signaling mechanism. *Vibrio cholera* has a typical parallel QS system (Miller et al., 2002). The QS operating mechanism of the anammox bacteria was predicted to be that shown in Figure 1B. It should be noted that the parallel QS system in *V. cholera* closely resembled a “redundant detector”, meaning that the effects of each individual QS channel can regulate the expression of related genes (Hurley & Bassler, 2017).

Anammox bacteria possess three parallel acyl-HSL QS channels, AMXI-AMXqsR, AMXS-AMXPQ and AMXA-AMXqsS, which are mediated by the signal molecules C₆-HSL, C₈-HSL, and 3OC₆-HSL, respectively. AMXI, AMXS, and AMX are responsible for the synthesis of signal molecules under the regulation of *amxI*, *amxS*, and *amxA*, respectively. Similarly, the receptor proteins AMXqsR, AMXPQ, and AMXqsS are expressed from the genes *amxqsR*, *amxPQ*, and *amxqsS*, respectively. Without binding to the signal molecules, the three receptor proteins mentioned above activate protein AMXO via phosphorylation through the protein AMXU. Activated AMXO promotes transcription of small RNAs (sRNAs), which in turn activate the expression of the EPSA protein and repress the production of the AMXR protein (Jung, Chapman, & Ng, 2015). As transcriptional regulators, protein EPSA and AMXR can upregulate the expression of genes involved in the synthesis of EPS and the anammox process, respectively.

The genes related to the synthesis of EPS will be triggered when the concentrations of these three signal molecules are below their detection threshold, but the anammox process will be blocked. At this phase, bacteria synthesize of EPS without expressing anammox activity. The secretion of EPS increases the aggregation ability of anammox bacteria (Jia et al., 2017). With the continuous accumulation of anammox bacteria, the density of anammox bacteria cells in the local environment gradually increases, and the anammox granular sludge and biofilm form correspondingly (Tang et al., 2013; Wang et al., 2019). AMXqsR, AMXPQ, and AMXqsS bind to their cognate signals when the signal molecules accumulate to high levels. Simultaneously, the resulting protein complex will reverse the flow of phosphate in the downstream QS channels, thereby terminating the transcription of small RNAₜ (sRNAs) (Jung et al., 2015). At this time, anammox bacteria realize the anammox function and slow the synthesis of EPS (Zhang, Li, et al., 2019).

The function of the different signal molecules in Table 2 can be well explained by these two QS signaling mechanisms. However the actual situation may be more complicated, because there may also be a competitive and synergistic relationship between different QS channels, such as that in the QS systems in *P. aeruginosa* and *Bacillus subtilis* (Waters & Bassler,
In addition, different species of bacteria use different QS systems (Eberl, 2006; Laue et al., 2000), which provides the possibility for a diversity of QS signaling mechanisms in anammox bacteria. Therefore, these two QS signaling mechanisms of anammox bacteria provide only a guide and reference for future research. To understand the QS mechanism in anammox bacteria, more research, especially at the metabolic and genetic levels, is urgently needed.

5. Ecological significance

Since the first discovery of anammox bacteria in manmade ecosystems, many studies have reported the widespread distribution of anammox bacteria in various natural habitats, including terrestrial ecosystems, anoxic marine sediments, and some special ecosystems (Hu, Shen, Xu, & Zheng, 2011). In a variety of ecosystems, anammox bacteria always appear together with other bacteria, including nitrifying bacteria, denitrifying bacteria, and anaerobic methane oxidation bacteria (Dalsgaard, Canfield, Petersen, Thamdrup, & Acuña-González, 2003; Lam et al., 2007; Yang et al., 2012). There is a competition for space and substrates between the anammox bacteria and the symbiotic bacteria (Ding et al., 2013). The QS system in microorganisms often plays a vital role in the rational use of resource and space (An, Goo, Kim, Seo, & Hwang, 2014; Zhao et al., 2019). The QS system could also regulate the gathering of anammox bacteria, and then occupy more resources and space to make the anammox bacteria become the dominant species in some environmental conditions. To some extent, this also determines that anammox bacteria function as a key part in the global nitrogen cycle (Jetten, 2008). According to statistics, the nitrogen production by the anaerobic ammonia oxidation process in marine environments is approximately 50% of the global nitrogen production (Humbert et al., 2010). In addition, anammox bacteria could also connect with other bacteria through the QS system. A current study proposed that Candidatus Kuenenia could interact with Chloroflexi and Proteobacteria by using diverse QS systems in nitrogen metabolism and biofilm formation (Sun et al., 2019).

QS systems can also play a crucial part in the intraspecific equilibrium of microorganisms. An et al. (2014) verified that Burkholderia glumae could slow the primary metabolism of an individual under crowded conditions through an AHL-mediated QS system, thus ensuring a stable population. Similarly, this is also most likely the case in the population of anammox bacteria. Because anammox bacteria generally appear as aggregates in nature, the cell density is high in the local space (Fuchsman, Staley, Oakley, Kirkpatrick, & Murray, 2012). To maintain the stability of the population,
it is particularly important to ensure the effective utilization of energy and resources (An et al., 2014). As a means of regulating gene expression according to cell density, the QS system in anammox bacteria could be an effective way to accomplish the above functions.

Many studies have confirmed that the QS system in bacteria can coordinate the production and utilization of extracellular products to stabilize social cooperation in microbial populations (Dandekar, Chugani, & Greenberg, 2012; Köhler, Perron, Buckling, & van Delden, 2010; Schuster, Sexton, & Hense, 2017). The QS system can reasonably allocate tasks of synthesizing and secreting of extracellular products like EPS. In most cases, EPS is, to some extent, a public good of anammox bacteria. A recent study showed that the regulation of public goods mediated by the QS system is essential to maintain the stability of the microbial population during the evolutionary process (Zhao et al., 2019). In the process of evolution, anammox bacteria will inevitably face the dilemma of public goods, and the QS system provides a powerful means to solve the above problems.

6. Perspectives

In the present study, the QS of anammox systems, including the QS signaling mechanism of anammox bacteria and its potential utilization in the anammox process, have been reviewed. As a high-efficiency and energy-saving method for nitrogen removal, the anammox process has promising prospects (Lackner et al., 2014; Strous, Van Gerven, Zheng, Kuenen, & Jetten, 1997). While QS regulation might not be the “master key” to questions pertaining to the anammox process, there is great potential to improve current practices by considering QS-related functions. Thus, using an approach combining QS-related regulation with other measures may be the best way to improve use of the anammox process. Several research needs and possible solutions were proposed here:

1. Multiple signals in anammox systems. Prior studies that have noted the importance of AHLs in the QS of anammox systems (Guo et al., 2017; Tang et al., 2015). However, knowledge of the existence of other kinds of signal molecules, such as DSF and AI-2, and their potential roles in anammox systems is still insufficient, and further and broader studies are necessary.

2. Pure cultures of anammox bacteria. Pure cultures of anammox bacteria are of great significance to explore their QS signaling mechanism. Whole-genome sequencing of anammox bacteria can help us to fully understand their QS signaling mechanism. However, most of the traditional isolation methods failed to obtain a pure culture of anammox
bacteria in enrichment (Jetten et al., 2001). Therefore, the development of pure-culture or bacterial enrichment technology is necessary.

3. Interspecific QS in anammox systems. From an application perspective, microorganisms in the mainstream anammox process must be part of a mixed-culture system due to the complex composition of actual wastewater (Reino, Suárez-Ojeda, Pérez, & Carrera, 2018). Thus, studying QS in the anammox community will improve our understanding of the relationship between anammox bacteria and other symbiotic bacteria, further contributing to better application of the anammox process.

4. The acquisition of QS-engineering bacteria. The regulation of the anammox process by adding exogenous signal molecules is the most effective means of QS-based regulation known at present. However, this approach is difficult to apply at large scales due to the high cost. Therefore, obtaining more strains with a signal molecule synthesis function and maintaining them active in anammox systems could be a possible solution. Some QS-regulated bacteria have been isolated and proven to be valuable in wastewater treatment (Cheong et al., 2013; Ergön-Can, Köse-Mutlu, Koyuncu, & Lee, 2019).

5. The response of QS to variable conditions in anammox system. As mentioned above, exploring the transformations of QS (signal molecules and BCGs) in anammox systems in different environments has great significance for engineering and ecological applications. Connecting QS with environmental changes in anammox systems may facilitate the establishment of anammox fingerprint systems and prewarning systems. However, related studies are relatively rare; thus, carrying out further studies into the QS of anammox systems in different environments is suggested.

6. Possible drawback of the QS-based technology. First, there is a risk that QS regulation may have negative effects the function of the anammox system. Because QS may influence the bacterial physiological behavior and disrupt the balance of microbial structure in the anammox system. For example, Tang et al. (2015) found that C_{12}-HSL had an inhibitory effect on the anammox activity. Thus, a deep understanding of the QS system in anammox bacteria is essential for mitigating its potential negative effects. Second, it is worth pondering whether the QS-based technology is cost-effective. Both dosing signal molecule chemicals and inoculating QS engineering bacteria suffer from economic obstacles (Maddela et al., 2019). Therefore, future applications of the QS-based technology need to consider the economy benefit.

Focusing on the above questions suggests considering the following possible methods or technologies. For pure cultures of anammox bacteria,
both microplate cultivation and density-gradient centrifugation have shown great potential (Mohammadi, Mowla, Esmaeilzadeh, & Ghasemi, 2018; Vartoukian, Palmer, & Wade, 2010). Anammox cells with a purity of more than 99.5% were obtained by density-gradient centrifugation (Strous et al., 2006). The rapid development of multiomics analysis has also provided a culture-independent means to explore microbial metabolic pathways and interspecific correlations (Lawson et al., 2017; Tang, Guo, Jiang, et al., 2018). In terms of qualitative and quantitative analysis of signal molecules, bacterial biosensors and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) are two commonly analytical methods (Huang et al., 2016). In recent years, the emergence of several novel techniques, such as nano-liquid chromatography-tandem mass spectrometry (Nano LC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy, has improved the accurate and rapid analysis of signal molecules (Maddela et al., 2019).

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**References**


