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Roles of autoinducer-2 mediated quorum sensing in wastewater treatment

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ABSTRACT

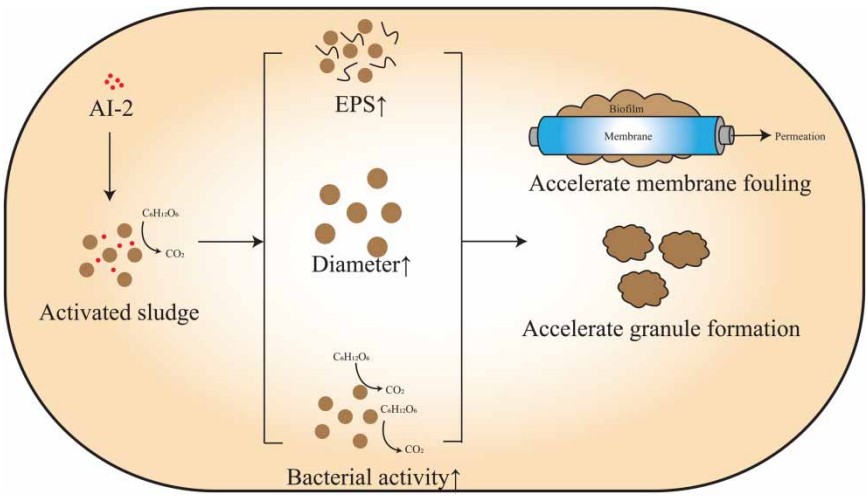
Quorum sensing (QS) is considered to be a promising regulation method for biological wastewater treatment (WWT) due to its regulation in extracellular substances (EPS) production, biofilm formation, granulation, colonization and bacterial activity and stability. Recently, autoinducer-2 (AI-2), a kind of interspecies communication QS signal molecule, is being increasingly reported for its roles in regulating bacterial gene expression and aggregation. Consequently, AI-2 mediated QS system is considered as a promising regulatory approach in WWT processes. This article systematically reviews the effects of AI-2-mediated QS system on bacterial behavior and its high potential for real-world applications in different WWT systems. Given the extensive presence of AI-2, AI-2 mediated QS could cooperate with other signal molecules in WWT processes, which suggests that the interactions among multiple signal molecules might be underestimated in the previous studies. The differences between AI-2 and AHL signaling molecules are also compared. Furthermore, the attempts at AI-2 regulated QS in pollution control of different WWT systems are summarized, while some challenges and defects still require targeted research in the future.

Key words: autoinducer-2, bacteria behavior, quorum sensing, wastewater treatment

HIGHLIGHTS

- The AI-2 mediated QS is a potential regulatory approach in WWT processes.
- The differences between AI-2 and AHL signaling molecules are compared.
- The interaction among multiple signal molecules might be underestimated in the previous studies.
- Possible applications of QQ mechanism based on AI-2 need to be further studied.

GRAPHICAL ABSTRACT



ABBREVIATIONS LIST

Aerobic granular sludge	AGS
Anaerobic ammonium oxidation	Anammox
Anaerobic granular sludge	AnGS
Anammox bacteria	AnAOB
Autoinducer-2	AI-2
Autoinducer peptide	AIP
Cyclic diguanylate	c-di-GMP
Diffusible signaling factor	DSF
Extracellular substances	EPS
Fluorescence resonance energy transfer	FRET
Hydrophobicity	RH
Loosely bound EPS	LB-EPS
Membrane bioreactors	MBR
Moving bed biofilm reactors	MBBRs
N-acyl homoserine lactones	AHLs
Pentachlorophenol	PCP
Polysaccharides	PS
Proteins	PN
Pseudomonas quinolone signal	PQS
Quorum quenching	QQ
Quorum sensing	QS
S-adenosylhomocysteine nucleosidase	Pfs
S-adenosyl-L-homocysteine	SAH
S-adenosyl-L-methionine	SAM
S-ribosylhomocysteine	SRH
Slightly soluble EPS	SMP
Tightly bound EPS	TB-EPS
Ultraviolet	UV
Wastewater treatment	WWT
Ydroxyl-palmitic acid methyl ester	PAME
4,5-Dihydroxy-2,3-pentanedione	DPD
50-methylthioadenosine	MTA

1. INTRODUCTION

In recent years, the discovery of quorum sensing (QS) has changed the understanding of bacteria as single cells that do not communicate with each other (Nealson & Hastings 1979). QS, as a cell-to-cell communication process, can induce the expression of specific genes and coordinate the behavior of bacteria in response to the changes of population density (Papenfort & Bassler 2016). Bacteria monitor population density by secreting and sensing the concentration of QS signal molecules. When the concentration of QS signal molecules reaches a certain threshold, the expression of specific genes would be triggered to regulate many physiological functions of bacteria; for instance, symbiosis, toxicity, binding, antibiotic production, sporulation and biofilm formation (Antunes *et al.* 2010; Rutherford & Bassler 2012). According to the different QS mechanisms, signal molecules can be divided into three types (Xiao *et al.* 2020): (1) *N*-acyl homoserine lactones (AHLs) produced by Gram-negative bacteria; (2) Autoinducer peptide (AIP) based on Gram-positive bacteria QS systems; (3) Autoinducer-2 (AI-2) utilized in interspecific communication. However, recent studies have shown that QS signals are not limited to the above three types, but also include: pseudomonas quinolone signal (PQS), diffusible signaling factor (DSF), ydroxyl-palmitic acid methyl ester (PAME) and cyclic diguanylate (c-di-GMP) (Whiteley *et al.* 2017).

At present, many researches have confirmed the important role of QS in biological wastewater treatment (WWT) processes (Chen *et al.* 2017; Whiteley *et al.* 2017; Maddela *et al.* 2019). QS-regulated behavior can improve the efficiency of biological treatment by regulating community structure, promoting extracellular substances (EPS) formation, and improving bacterial activity. The first article about QS in WWT was published in 1997 with *N. europaea* as the research target. In this research, the lag phase of starved *N. europaea* cell suspensions was shortened due to the addition of AHLs when ammonium was resupplied into its system (Batchelor *et al.* 1997). To date, QS has been applied to activated sludge, biofilm, granular sludge,

biological denitrification and other water treatment systems, and some researches have achieved positive results (Chen *et al.* 2017; Li *et al.* 2018). The influence and function of AI-2-based QS in several typical WWT systems are shown in Table 1.

Current achievements and development trends are mainly focused on the application of AHLs in the field of WWT, while studies on AI-2 with respect to biological pollutant removal and bioaugmentation are rarely reviewed. As a universal language of interspecific communication, AI-2 can also promote EPS production, regulate community structure and bacterial activity by adjusting the expression of related genes, thus affecting the physiological functions of bacteria. Therefore, it is critically important to explore the technology of combining AI-2 with biological WWT. Focusing on AI-2 signals, the specific aim of this review is to describe the knowledge background of AI-2 based QS system, analyze their potential roles in biofilm processes, granular sludge formation, anaerobic ammonium oxidation (anammox) process and bioaugmentation, and then discuss the effect of external control strategy to improve WWT performance, evaluate its feasibility and application prospect comprehensively.

2. FUNDAMENTALS OF AI-2 BASED QS

2.1. AI-2 signal molecule

In 1993, Bassler *et al.* (1993) found that the mutant *Vibrio harveyi* had defects in AHL synthesis, but it still remained capable of QS-dependent gene activation. Therefore, the possibility of establishing a new type of QS circuit was proposed, and its signal molecule was called AI-2. AI-2 is produced by LuxS, which is an enzyme that exists in many bacterial species, so the production of AI-2 is not limited to one bacterial species but is thought to be capable of interspecies communication (Bassler *et al.* 1997; Surette & Bassler 1998; Pereira *et al.* 2013).

To date, two AI-2 synthetic pathways have been proposed. The typical synthesis pathway suggests that AI-2 is produced by S-adenosyl-L-methionine (SAM) through three enzymatic steps (Winzer *et al.* 2003). (1) As a methyl donor, SAM lead to the formation of S-adenosyl-L-homocysteine (SAH) through SAM-dependent methyltransferase reactions. (2) The second step is catalyzed by S-adenosylhomocysteine nucleosidase (Pfs), which is responsible for the hydrolysis of the glycosidic bond in both 50-methylthioadenosine (MTA) and (SAH). SAH serves as a substrate to guide the formation of adenine and S-ribosylhomocysteine (SRH). (3) Finally, LuxS converts SRH into homocysteine and 4,5-Dihydroxy-2,3-pentanedione (DPD). In addition, another semi-biosynthetic pathway has been found in hyperthermophiles (with Pfs, without LuxS) (Nichols *et al.* 2009): (1) hyperthermophiles use SAH hydrolase to cleave SAH into adenosine and homocysteine. (2) Adenosine is converted by nucleoside phosphorylase to ribose-1-phosphate, and then isomerized from phospho-sugar mutase to ribose-5-phosphate. (3) Finally, ribose-5-phosphate is converted into DPD and AI-2 by thermal induction. DPD is unstable and can form different furan compounds spontaneously as AI-2 or precursors of AI-2 (Figure 1).

Three types of AI-2 receptors have been identified, including LuxP protein in *Vibrios*, LsrB protein in *Escherichia coli* and *Salmonella Typhimurium*, and RbsB protein in *Aggregatibacter actinomycetemcomitans* (Zhao *et al.* 2018). LuxP and LsrB receptors have different functions: After binding to AI-2, the LuxP receptor triggers a cascade of signal transduction and regulates downstream gene expression. Instead, LsrB internalizes AI-2, and the internalized AI-2 is phosphorylated and binds to LsrR protein to initiate the expression of the Lsr system, thus accelerating the transformation of AI-2. In addition, the absence of these three receptors in some microorganisms capable of utilizing AI-2 suggests that AI-2 receptors may not widespread, or that other types of AI-2 receptors have not been discovered (Han *et al.* 2015).

Table 1 | Influence and functions of AI-2 QS in typical WWT systems

System	Influence	Function	Usage	References
Membrane biofouling	Negative	Promotion of EPS production for biofilm formation	Controlling membrane biofouling by reducing AI-2's concentration	Shrout & Nerenberg (2012)
Aerobic granular sludge	Positive	Promotion of EPS production	Accelerating sludge granulation	Liu *et al.* (2016)
Anaerobic granular sludge	Positive	Promotion of EPS production	Accelerating sludge granulation	Ding *et al.* (2015a)
Anammox bacteria	Positive	Improving the growth rate and microbial activity of bacteria	Shortening the start-up time	Wu (2018)

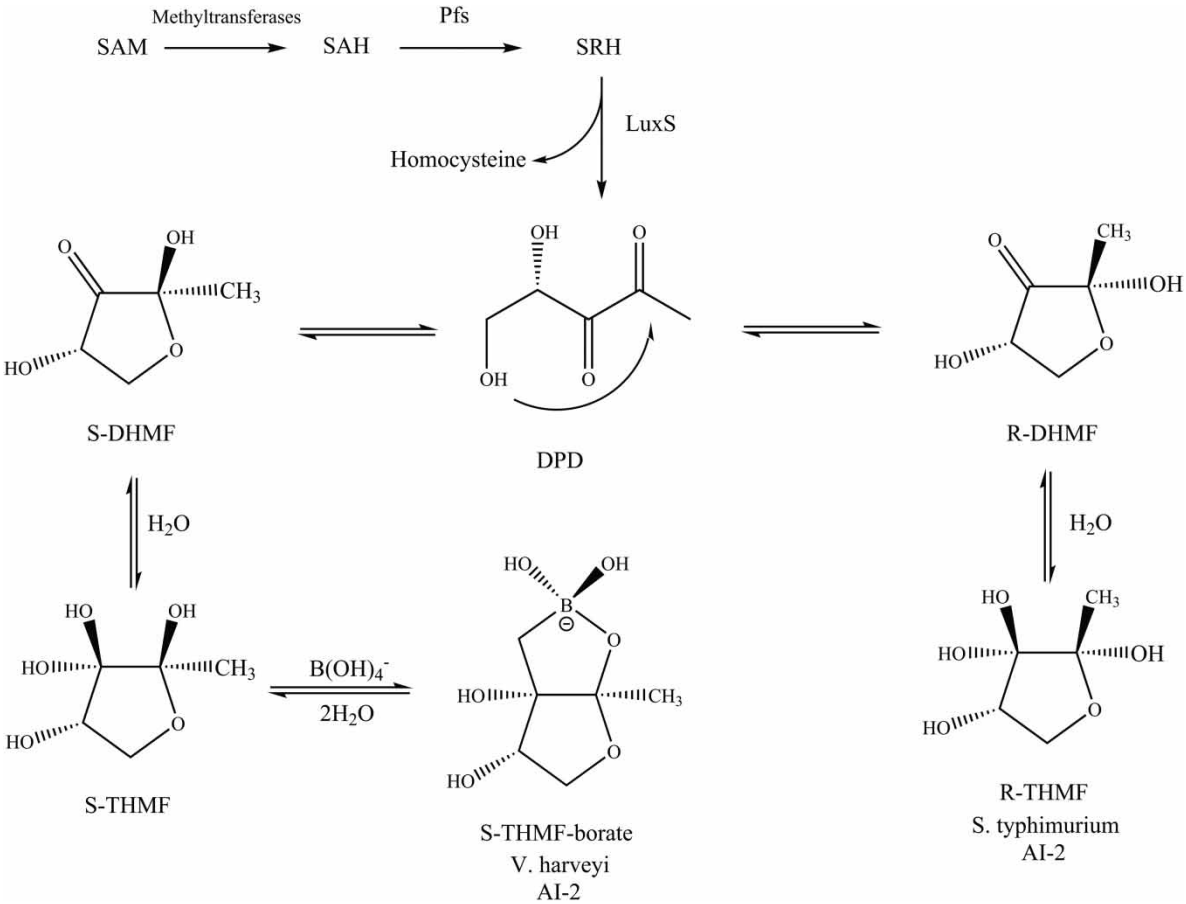


Figure 1 | Proposed pathway for formation of AI-2 (modified from Taga 2005): DPD spontaneously cycles into two stereoisomers, S-DHMF and R-DHMF. S-DHMF and R-DHMF are added with water to form S-THMF and *S. typhimurium* AI-2, respectively. Boric acid is added to S-THMF to form *V.harveyi* AI-2.

As shown in Figures 2 and 3, the regulation mechanism of AI-2 with LuxP and LsrB as receptors have been well studied (Pereira *et al.* 2013). (1) *Vibrio harveyi* QS (Figure 2): AI-2 is detected by LuxP/LuxQ (AI-2 receptor), causing a phosphorylation cascade to regulate the transcription of many genes. When the cell density is low and the AI-2 content is low, LuxQ behaves as a kinase, which phosphorylates LuxO through LuxU phosphorylation transport reaction, thereby inhibiting LuxR transcription and related gene expression. At high cell density, AI-2 is recognized and bound by LuxP, which converts LuxQ from kinase to phosphatase, thereby reversing the cascade reaction of phosphorylation. Unphosphorylated LuxO is inactive, resulting in LuxR production. (2) *Escherichia coli* QS (Figure 3): At the early stage of bacterial growth, little AI-2 is extracellular, LsrR was inhibited and the expression of Lsr system was inhibited. With cell growth and extracellular AI-2 accumulation, AI-2 is phosphorylated by LsrK and combined with LsrR. The Lsr-dependent transport system starts, and AI-2 binds to LsrB and is internalized by the Lsr transporter. More P-AI-2 is produced and the Lsr transporter is further induced. As a result, AI-2 input increased and extracellular AI-2 level decreased rapidly.

AI-2 activities fluctuate during the growth cycle of bacteria, and its maximum value usually appears from early exponential phase to stationary phase under suitable growth conditions. Beyond intrinsic variation of AI-2 concentration in bacteria, AI-2 production is also influenced by different environmental factors, such as pH, temperature, salinity, carbon sources, etc (Zhao *et al.* 2018). Acidic shock in *Lactobacillus* spp. could increase AI-2 activity and enhance luxS gene transcription (Moslehi-Jenabian *et al.* 2009). Incubation temperature of microorganisms has a complex effect on AI-2 production: The highest AI-2 activity of *Streptococcus pneumoniae* is 37 °C, but the optimal growth temperature of *Streptococcus intermedius* is not the optimal temperature for AI-2 production (Ahmed *et al.* 2009). The influence of temperature on AI-2 of *Lactobacillus* is species specific; that is, the AI-2 activity of *Lactobacillus rhamnosus* enhances with the increase of temperature, while the

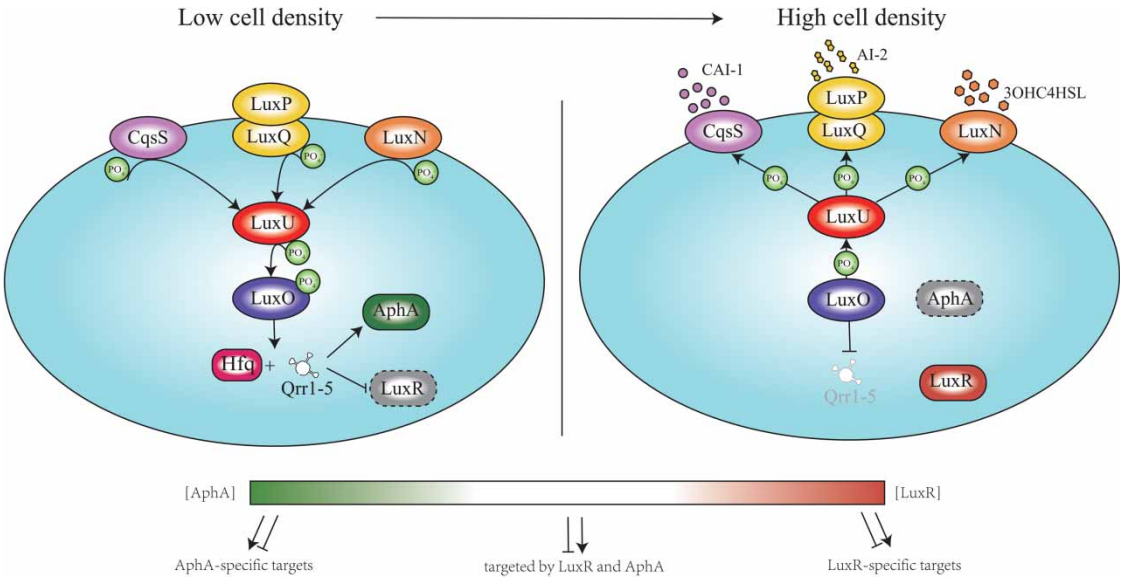


Figure 2 | QS in *Vibrio harveyi* (modified from Pereira *et al.* 2013): AI-2 is detected by LuxP/LuxQ (AI-2 receptor), causing a phosphorylation cascade to regulate the transcription of multiple genes. When the cell density is low and the AI-2 content is low, LuxR transcription and related gene expression are inhibited. At high cell density, AI-2 is recognized and bound by LuxP, and then the phosphorylation cascade is reversed, leading to the production of LuxR.

activity of AI-2 produced by *Lactobacillus plantaceus* presents variability within a narrow temperature range (Yeo *et al.* 2015). Salinity inhibits the production of AI-2 by *Aeromonas hydrophila* (Jahid *et al.* 2015), while the activity of AI-2 in *Lactobacillus* shows an opposite trend, increasing with the increase of salinity (Yeo *et al.* 2015). Carbon sources promote the production of AI-2, among which glucose has the most significant promotion. Adding glucose to the medium is helpful to enhance the activity of AI-2 (Zhang *et al.* 2008). The effects of carbon sources on AI-2 activity are also species specific. Three quarters of *lactobacilli* spp. need an appropriate amount of galactose to achieve the maximum yield of AI-2, while only one *L. plantarum* strain has a negative correlation between AI-2 production and the availability of carbon sources (Yeo *et al.* 2015).

2.2. Analytical methods for AI-2 detection

Quantitative detection of AI-2 is very important for studying its related physiological and biochemical processes. At present, AI-2 detection methods are mainly as follows (Table 2):

(1) Bacterial biosensor assay: *Vibrio harveyi* BB170 is often used as a biosensor strain for rapid and sensitive detection of AI-2 (Vilchez *et al.* 2007). The culture supernatant containing AI-2 can induce the luminescence of *Vibrio harveyi*, and its luminescence value is used to reflect the activity of AI-2. This method has a short detection time and low detection limit. The optimized *V. harveyi* BB170 bioassay has a detection limit of 25 nM. but it cannot directly quantify AI-2. In order to ensure the accuracy and repeatability of the test results, several points should be taken seriously. First, it is necessary to add iron ions to the autoinducer bioassay medium because iron ions can increase the reproducibility of luminescence and bioassays, while other trace elements can inhibit luminescence or cause false positive results (Vilchez *et al.* 2007). Then, the medium composition of the tested bacteria affects the luminous intensity. For example, glucose concentration can cause the bioassay results to deviate from standard values (Turovskiy & Chikindas 2006).

(2) Fluorescence resonance energy transfer (FRET): When AI-2 binds to a protein fusion named CLPY (Cyan Fluorescent Protein-LuxP-Yellow Fluorescent Protein fusion), the conformational change of LuxP will trigger FRET (Rajamani *et al.* 2007). This method is more sensitive than the bacterial biosensor, but it is not suitable for AI-2 quantification and is easily disturbed by other species in complex biological samples.

(3) Fluorescence biosensors: AI-2 receptor proteins modified with fluorescent dyes show fluorescence changes after binding to AI-2 (Zhu & Pei 2018). This method can be used for real-time monitoring of AI-2 and accurately reveal the precise changes in AI-2 production. Nevertheless, due to the limitation of ligand binding affinity, this method can only react

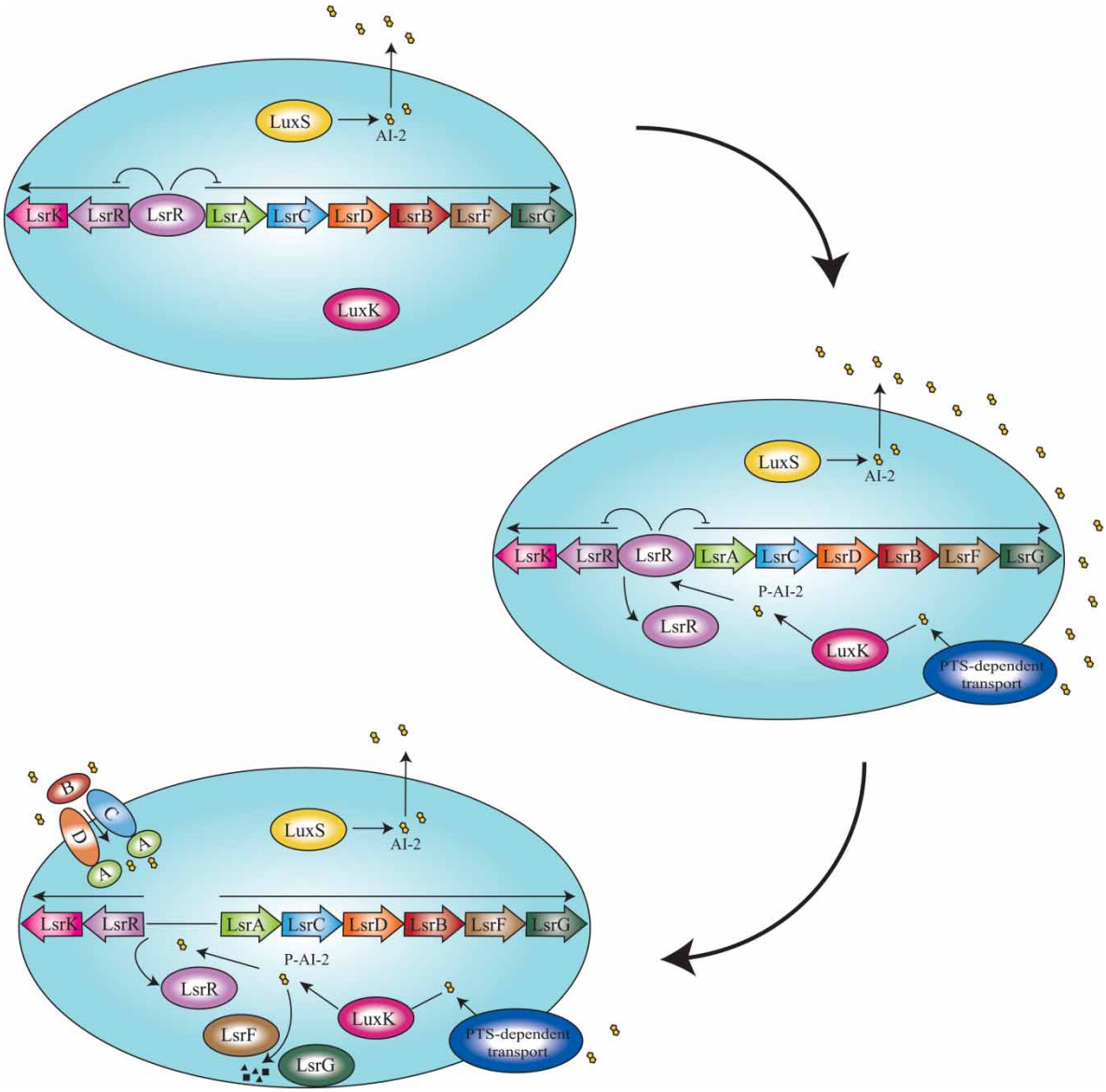


Figure 3 | QS in *Escherichia coli* (Modified from Pereira *et al.* 2013): In the early stages of bacterial growth, the Lsr system is inhibited. With cell growth and extracellular AI-2 accumulation, the Lsr-dependent transport system is activated and AI-2 is internalized by the Lsr transporter. As a result, AI-2 input increases and extracellular AI-2 levels rapidly decline.

on the part of DPD converted to borate, making the determination sensitive to the concentration of borate. And the linear range of this method is very narrow (1–20 μM).

(4) Chromatography: Several novel chromatography techniques are emerging to analyze and provide more accurate details of AI-2 after derivatization, mainly including gas chromatography-mass spectrometry (GC-MS) (Thiel *et al.* 2009), liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Xu *et al.* 2017) and precolumn derivatization-solid phase extraction-high performance liquid chromatography with fluorescence detector (HPLC-FLD) (Song *et al.* 2014). Chromatography has a lower detection limit and a wider linear range, which can generally meet the requirements of AI-2 detection. However, the pre-treatment process is complicated, such as two-step derivatization, solid phase extraction and concentration. In addition, the derived structure is different from DPD, and whether it can accurately detect various forms of AI-2 after the derivation is worth further determination.

Accurate detection of AI-2 is helpful to reveal the QS regulation mechanism under different conditions. At present, the combination of multiple detection and quantification methods may increase the confidence of future research. However,

Table 2 | Comparison of AI-2 detection methods

Detection methods	Limit of detection	Advantages	Disadvantages
Bacterial biosensor assay	25 nM	Short detection time Low detection limit	Not quantifiable Long time (3–6 h) Easy to get false positive results
Fluorescence resonance energy transfer (FRET)	1×10^{-10} M	Rapid (3 min) Respond to nonborate forms of AI-2. More sensitive than bacterial biosensor assay	Susceptible to interference from other species in complex biological samples
Fluorescence biosensors	1.0 μM	Fast detection speed Wide detection range No interference from sugars and DPD analogues Real-time monitor	Sensitive to the concentration of borate Narrow linear range
Chromatography	10 ng/mL	Wide linear range (10–14,000 ng/mL) Low detection limit	Complicated pre-treatment process

due to the complexity of the WWT environment, it is still necessary to develop a more traceable, stable, fast and accurate AI-2 detection method.

3. REGULATION OF AI-2-BASED QS IN BIOLOGICAL WWT PROCESSES

3.1. The effect of AI-2 signal molecules in biofilm formation

Bacterial biofilms are ubiquitous in almost every water environmental system. For WWT processes, biofilms play both positive and negative roles (Chen *et al.* 2017). Stable biofilms are crucial for some biological treatments, such as trickling filters, moving bed biofilm reactors (MBBRs) and granular sludge. In contrast, biofilms and associated EPS can cause biofouling of nanofiltration (NF) systems and membrane bioreactors (MBRs). AI-2 signal molecules are closely related to biofilm formation, and are mainly controlled by affecting bacterial attachment and EPS synthesis. As bacteria metabolites, EPS is thought to be the main substrate for bacterial aggregation and bridges between bacterial cells and other particles, thus fixing biofilm cells together.

The role of AI-2 in pure culture is undisputable. AI-2 has gained increasing attention for its positive role in improving the quality and thickness of biofilms. Adding AI-2 increases the quality of biofilm in *E. coli* by 30 times, while the LsR-deficient strain (lack of the ability to transport AI-2) fails to stimulate biofilm formation under the same conditions (Barrios *et al.* 2006). Biofilm growth without AI-2 is delayed or even stalled, but can be recovered with AI-2. Wang *et al.* (2017b) constructed a *Streptococcus gordonii* luxS deficient strain to investigate the effects of luxS on the dual-species biofilm formed by *Streptococcus gordonii* and *Streptococcus mutans*. The results showed that the luxS disruption in *Streptococcus gordonii* destroyed dual-species biofilm formation, while the exogenous addition of AI-2 could restore biofilm formation and virulence genes expression. Similarly, the reactive oxygen species photogenerated by TiO_2 can inhibit the secretion of AI-2 by *E. coli* K12 to delay the development of biofilm. The development of biofilm is restored with the addition of a certain dose of DPD (Xiao *et al.* 2016). Furthermore, AI-2 signaling molecules can also regulate biofilm formation of several bacteria, including *Escherichia coli*, *Shewanella onedensis*, *Porphyromonas gingivalis*, *Salmonella typhi* and *Streptococcus mutans* (Shrout & Nerenberg 2012). Similar to AHLs, the regulation of AI-2 presents a dose-dependent pattern of low concentration promotion and high concentration inhibition. Adding 10 nM exogenous AI-2 to *Pseudomonas aeruginosa* PAO1 promotes biofilm formation and the production of virulence factors, while 100 nM exogenous AI-2 has the opposite effects (Li *et al.* 2015). AI-2 also plays an important role in biofilm formation in WWT. The amount of biofilms on nylon membrane is positively correlated with AI-2 content, indicating that AI-2 mediated QS participate in biofilm formation (Xu & Liu 2010). AHL and AI-2 are also detected on contaminated reverse osmosis membranes in WWT plant, and 60% of bacteria on the membrane surface promote biofilm formation through effective intraspecific and interspecific communication (Kim *et al.* 2009).

3.2. The effect of AI-2 signal molecules in granulation

3.2.1. Aerobic granular sludge

Aerobic granular sludge (AGS), a spherical biofilm composed of self-immobilized cells without carrier, breaks through the limitations of aerobic biological treatment, such as treatment of high strength organic wastewater, bioremediation of toxic aromatic pollutants including phenol, toluene, pyridine and textile dyes, removal of nitrogen, phosphate, sulphate and nuclear waste and adsorption of heavy metals (Adav *et al.* 2008). The formation of AGS and QS was first reported by Jiang *et al.* (2006), who described the presence of AI-2 in two co-aggregating bacterial strains *Propioniferax*-like PG-02 and *Comamonas* sp PG-08. Many characteristics of QS regulated by QS are responsible for the formation and stability of granular sludge, such as aggregation, denitrification, biofilm formation, EPS synthesis and organic matter degradation (Huang *et al.* 2019). The roles of QS signal molecules (AHLs, AI-2 and c-di-GMP) in AGS are shown in Table 3.

AI-2 is crucial to AGS maturation since it promotes the aggregation of small particles into large particles, but it does not participate in the initial aggregation of particles (Xiong & Liu 2010). The AI-2-mediated QS system is activated to continuously regulate the growth and maturation of AGS only when the biological density reaches a threshold (around 1.025 g/mL) (Xiong & Liu 2012). Moreover, the activity of AI-2 in the particles is significantly higher than that in aqueous solution, indicating that the AI-2 in the particles may have a more direct impact on the stability and function of AGS (Chen *et al.* 2014). With the further study, researchers have found that there is a close relationship between AI-2 activity, EPS content and aerobic granulation degree during AGS formation. AGS has a high biomass shell and a relatively low biomass core, which supplement the high accumulation of hydrophobic EPS in the outermost layer to protect the structural stability and integrity of the aerobic granule. And the structure of hydrophobic EPS is significantly affected by endogenous or exogenous signal molecules (AHL and AI-2). A higher level of AI-2 is conducive to EPS production and the increase of cell adhesion, which ultimately accelerates the formation of AGS (Sun *et al.* 2016). Meanwhile, the maintenance of AGS is largely dependent on the skeleton formed by the interaction of polysaccharides (PS) and proteins (PN) in EPS, since the decrease in PS and PN in the decomposed AGS occurs simultaneously with the decrease in AI-2 concentration (Xiong & Liu 2013). Furthermore, the environmental pressure of starvation can also promote the production of large molecular weight EPS by stimulating the secretion of AI-2, leading to cell adhesiveness enhancement and granule formation (Liu *et al.* 2016). Based on the above, the relevant QS mechanism in AGS formation may be that AI-2 stimulates cells to secrete EPS, forms the PS-PN framework and promotes bacterial attachment, and then leads to changes in particle size, density, quantity and other physicochemical properties of AGS, as well as the improvement of settling performance.

In summary, bacteria form granules with PS-PN as the basic framework, and there are communities composed of a large number of species in granules, which have a variety of metabolic functions. The whole composition and various performance of the community depend on the interaction between different microorganisms, and signal molecules are the 'bridge' for communication between microorganisms. Higher AI-2 concentrations may promote the reactor to undergo extensive microbial evolution (Sun *et al.* 2016). In addition, AI-2 also promotes the growth of bacteria related to cell adhesion and EPS secretion, such as *Rhizobium* sp., *Arthrobacter* sp. (Liu *et al.* 2016). Therefore, it is of great significance to study the influence of AI-2 on bacterial interaction and the cooperative behavior at community level.

3.2.2. Anaerobic granular sludge

Anaerobic granular sludge (AnGS) is commonly used to treat wastewater with a large range of organic loads. Typical AnGS treatment processes, such as up-flow anaerobic sludge bed, expanded granular sludge bed and static granular bed reactor, have shown excellent performance in laboratory and pilot-scale experiments (Lim & Kim 2014). Compared with AGS, QS in AnGS is more complex and involves a variety of signal molecules, which makes it more difficult to study. A relatively complete regulatory mechanism has not been formed according to the current researches.

Table 3 | Roles of QS signal molecules in AGS

Signal molecules	Roles in AGS	References
AHLs	Enhance bacterial growth, PN production, and microbial activity	Wu *et al.* (2017)
AI-2	Enhance cell adhesion by regulating EPS, and accelerate AGS formation	Sun *et al.* (2016)
c-di-GMP	Causes bacteria to adhere to each other and form a biofilm	Wang *et al.* (2017a)

In order to intuitively demonstrate the performance of AI-2 signal molecules in the AnGS system, it is necessary to investigate the distribution of different signal molecules in the granulation process. The distribution of QS signal molecules in AnGS is regular, with 80% of AI-2 distributed in the water phase, while 70% of DSF was distributed in the sludge phase. Previous studies have investigated the regulation of three signal molecules (AHLs, AI-2, DSF) on AnGS granulation (Figure 4), and found that AHLs and AI-2 are favorable for granular growth while DSF inhibits this process (Ding *et al.* 2015b). Meanwhile, there is a positive correlation between DSF and AI-2 although their effects were opposite in granulation. (Feng *et al.* 2014). Correlation analysis also show that the relative AI-2 content is negatively correlated with the average diameter of AnGS, which may be caused by the smaller size granular secreting more AI-2 to facilitate the compaction between large particles. When the particle size reaches its peak, the secretion tends to be stable.

Current studies have shown that the molecular structure and synthesis of AI-2 in AnGS may be influenced by environmental factors. In an alkaline medium, the degradation of AHL leads to a low AHL content, which is difficult to reach the threshold to initiate the regulation of related genes. Therefore, the synthesis of EPS is mainly promoted by AI-2. Under neutral and weak alkaline conditions, the concentration of AI-2 increase while DSF decline, which is conducive to the enhancement of particle strength and relative hydrophobicity (RH) in AnGS, resulting in smoother surface and larger particle size. However, the trend in acidic medium conditions is opposite. With the increase of DSF content, the content of AI-2 decreases, leading to a decrease of RH and particle strength, and a corresponding decline of AnGS (Ding *et al.* 2015a). In addition, unbalanced nitrogen supply and organic shock loads also have a certain effect on QS of AnGS. In the process of unbalanced nitrogen supply, the regulation of AI-2 and DSF reduce the granule strength and diameter, leading to the degradation of granular sludge (Ding *et al.* 2016). With excessive accumulation of volatile fatty acids and sharp decline of pH, the content of AI-2 decreases while the content of AHLs and DSF increases under organic shock loading conditions. Changes in signal molecules cause the disintegration of AnGS, which has an irreversible effect on the sludge community (Ding *et al.* 2017). These previous studies suggest that QS mechanism is different under different environmental conditions, which provides a new way to improve the efficiency of the reactor under adverse environment.

In summary, the QS mechanism of AI-2 regulation in AnGS is similar to AGS: AI-2 promotes AnGS formation by enhancing interspecific bacterial communication, increasing EPS production and accelerating the increase of particle size. Different signal molecules may have opposite effects during granulation, AHL and AI-2 promote granulation while DSF inhibits granulation. Under different environment conditions, these signal modules show different potentials in mitigating adverse environmental effects. It is clear that there are interactions between multiple signal molecules in AnGS. Therefore, more attention is still needed to understand the combined regulation mechanism of multiple signal molecules to improve the efficiency of the AnGS reactor.

3.2.3. Anammox

Anaerobic ammonium oxidation (anammox) is a process in which the anammox bacteria (AnAOB) convert nitrite into nitrogen gas under anoxic conditions using ammonium as the electron donor (Ma *et al.* 2016). Compared with the traditional nitrogen removal process, anammox does not need aeration and an additional organic carbon source, which greatly reduces the treatment cost. However, there are problems in practical application such as long start-up period and susceptibility to environmental variations. Many physiological characteristics of AnAOB have been proved to be related to QS, including the

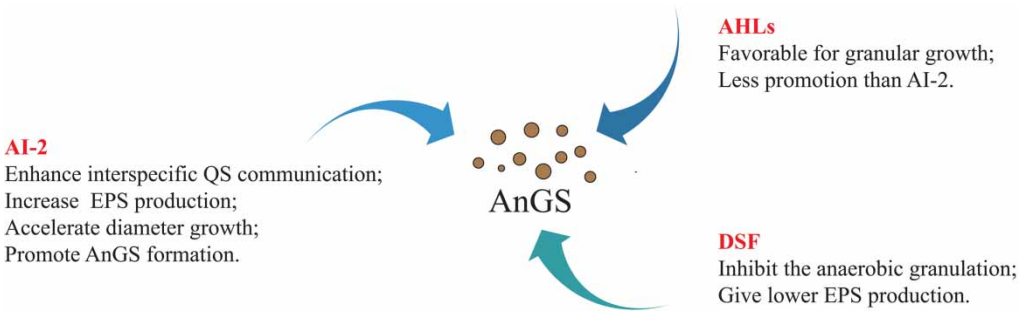


Figure 4 | The efficiency of AHLs, AI-2 and DSF on regulating AnGS granulation: AHLs and AI-2 are favorable for granular growth while DSF inhibits this process.

specific anammox activity, growth rate and EPS production, which directly affect the performance of anammox process (Zhang *et al.* 2021). At present, a purebred strain of AnAOB has not been obtained, which makes it difficult for the related research.

The study of signal molecules in anammox systems mainly focuses on two types: AHLs and c-di-GMP. Although few studies on AI-2 mediated QS have been conducted, there is still evidence that AI-2 can regulate the related functions of AnAOB. On the one hand, the AI-2 regulatory gene (lsrF) has a high abundance in the anammox system, and *Candidatus* Kuenenia has been proved to regulate the expression of related genes through AI-2-mediated QS (Sun *et al.* 2018). On the other hand, as the activator of AI-2, Boron can promote the rapid start of anammox and the enrichment of AnAOB (Wu 2018; Su *et al.* 2019). Boron shows great potential in improving the operation efficiency of the anammox reactor: Boron can promote the secretion of EPS, thus forming granular sludge with larger diameter and tighter structure (Wu 2018). Moreover, Su *et al.* (2019) found that Boron not only shortened the start-up time of anammox reactor, but also increased its total nitrogen load. After reactor initiation, some AnAOB, such as *Candidatus* jettenia, became the dominant flora.

At present, the AI-2 regulatory genes found in anammox and the promoting effect of Boron on AnAOB enrichment and EPS secretion indicate the importance of AI-2 in anammox. However, there is a lack of evidence to directly detect AI-2 from anammox, so more studies are needed to support the existence and role of AI-2 mediated QS in anammox process. Furthermore, pure cultures of AnAOB are of great significance for the study of their QS mechanism.

3.3. The effect of AI-2 signal molecules in bioaugmentation

Bioaugmentation is a technology which can improve the catabolism of specific compounds (such as refractory organics) by adding bacteria or compounds to WWT reactors (Herrero & Stuckey 2015). Biodegradation performance mainly depends on microbial activity and environmental conditions. AI-2 signal molecules enhance bacterial colonization by promoting EPS production and biofilm formation, so as to improving the efficiency of bioaugmentation.

Studies have shown that AI-2 exchange between different bacteria and improves biological treatment efficiency by promoting EPS production. Jiang *et al.* (2006) found that in co-culture process, highly efficient phenol degrading bacteria PG-02 and strong aggregation bacteria PG-08 communicate through AI-2. The two strains aggregated through the interaction of lectin-saccharide with the adhesin protein on PG-02 strain and the complementary sugar receptor on PG-08 strain, and the degradation efficiency of phenol was increased by 2–5 times. Similarly, AI-2 may play a key role in bacterial colonization. Wang *et al.* (2014) studied the QS system of tobacco wastewater bioaugmented treatment system inoculated with *Pseudomardia* HF-1: AI-2 was significantly increased in the successfully inoculated system, and the short strand AHL did not change. While in the failed inoculated system, AI-2 did not change, and the short-chain AHL increased threefold.

AI-2 has also shown great potential in promoting the recovery of anaerobic digestion system from the toxic shock response (Xiao *et al.* 2019): AI-2 levels in anaerobic sludge are mainly controlled by *Firmicutes*. And AI-2 partially regulates the toxic shock response of anaerobic sludge by affecting the activity of *Firmicutes* and *Synergistetes*. A mutant culture of *Escherichia coli* is added to the system to reduce the AI-2 levels in anaerobic digestion, thus producing the highest biogas production during pentachlorophenol (PCP) shock. The reason may be that reducing the AI-2 level can reduce acetylation and promote the production of hydrogen and methane, thereby reducing the accumulation of volatile fatty acids and increasing the production of methane during PCP shock.

4. EXTERNAL STIMULUS STRATEGY BASED ON AI-2-QS REGULATION MECHANISM

4.1. Regulation of QQ-QS interaction on WWT process optimization

Quorum quenching (QQ), as an antagonistic process of QS, coexists with QS in various biological WWT systems. To date, most of the comments on QQ-based regulation technology have been limited to AHL, with only a few focusing on AI-2 strategies (Roy *et al.* 2011; Pereira *et al.* 2013).

Optimizing the WWT process by adjusting the interaction between QS and QQ has become a promising control method (Table 4). Biofilm attaches to the membrane surface in the MBR reactor will form membrane biofouling, resulting in the decrease of membrane flux and the blockage of membrane pores. In addition, it also leads to the increase of operation investment, which is one of the main factors restricting the widespread use of MBRs. Given the importance of QS systems to biofilm formation, it is not surprising that some host organisms or competing bacteria may benefit from interfering with or disrupting biofilm formation. QQ inhibits QS mainly by blocking the synthesis of signal molecules, degrading signal molecules or interfering receptors, so as to achieve control of membrane biofouling (Hong 2019). Many studies have reported bacteria in MBR benefit from these colony QQ activities. The first report on the application of bacterial QQ to control MBR biofouling was

Table 4 | External stimulus strategy based on AI-2 QS regulation mechanism

External stimulus strategy	Control method	Example	References
Regulation of QQ-QS interaction	Interfering QS system by controlling some physical or chemical conditions	DNP interfere with AI-2 synthesis	Xu & Liu (2010)
	Promoting QQ system by adding QQ bacteria	QQ bacteria reduce the concentration of DPD	Lee *et al.* (2018)
Accelerate reactor start-up	Boron	Boron enhances the activity of AI-2	Zhang *et al.* (2011)
	Bacterial cultures containing AI-2	AI-2 contained in intracellular extract from mature granules accelerate sludge granulation	Ren *et al.* (2010)

published in 2009, which delayed membrane biofouling by inhibiting AHL production (Yeon *et al.* 2009). Considering the widespread of AI-2 in biofilm formation, some researchers proposed to inhibit biofilm formation by regulating the concentration of AI-2, so as to achieve membrane biofouling control. At present, there are two main ways for AI-2 mediated QS controlled membrane biofouling: interfering with the QS system by controlling some physical or chemical conditions and promoting the QQ system by adding QQ bacteria (Figure 5). Certain physical or chemical conditions can interfere with QS by inhibiting the production of AI-2. It has been found that 2,4-dinitrophenol (DNP) can interfere with AI-2 synthesis to reduce AI-2 production, thus reducing the number of microorganisms attached to the solid surface and effectively controlling membrane biofouling (Xu & Liu 2010). Besides, D-amino can reduce the synthesis of AI-2, e-DNA and EPS, and subsequently reduced microbial attachment on the surface of hydrophilic glass and hydrophobic polypropylene (Xu & Liu 2011). Cai & Liu (2016) analyzed the effect of on-line chemical cleaning on membrane biofouling in MBR reactors. The result demonstrated that the MBR membrane treated with a high concentration of NaClO had a higher rate of membrane biofouling, because NaClO as a membrane cleaning agent may cause bacterial dissolution, and the released EPS and AI-2 promoted the colonization of microorganisms on the membrane surface. In addition, ultraviolet (UV) can interfere with

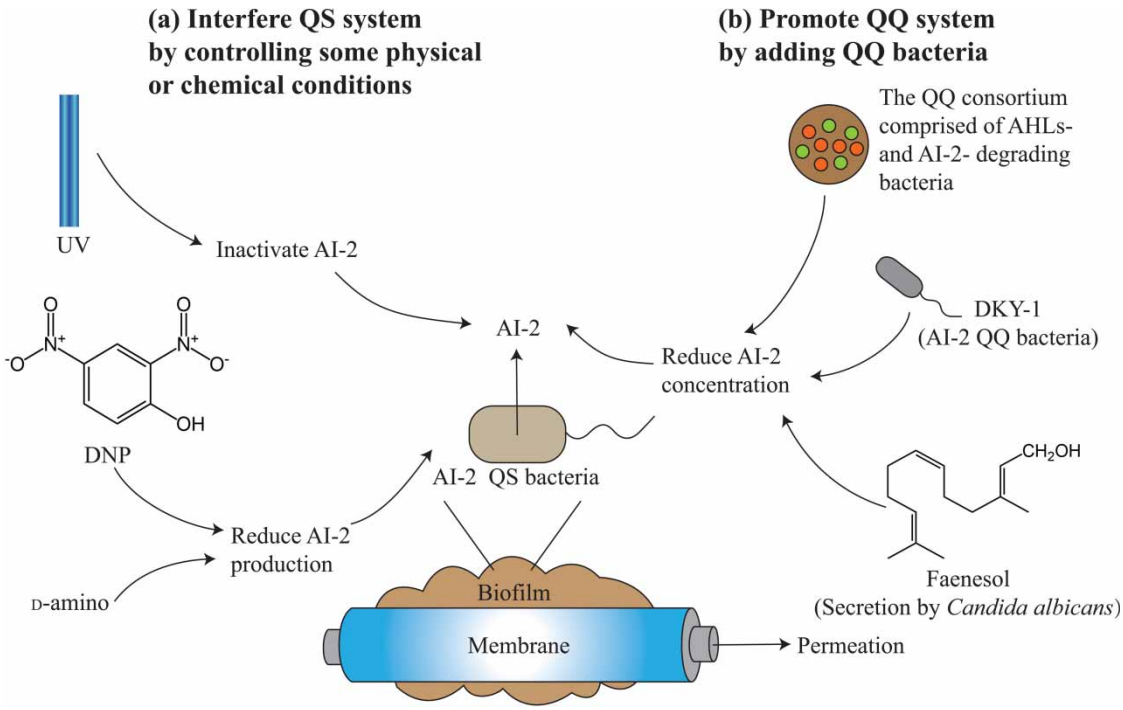


Figure 5 | The means of membrane biofouling controlled by AI-2-mediated QS. (a) Interfere with the QS system by controlling some physical or chemical conditions: DNP and D-amino can reduce the synthesis of AI-2, and UV can inactivate AI-2. (b) Promote the QQ system by adding QQ bacteria: QQ bacteria or QQ agents can delay membrane biofouling by reducing AI-2 concentration.

biofilm growth on membrane surfaces and inactivate AHL and AI-2 signal molecules, mainly because nitrate-regulated UV photodegradation inhibits bacterial QS (Zhang *et al.* 2019).

QQ bacteria also have high biological control potential in the process of MBR membrane fouling. Under stable organic load, the addition of the QQ consortium composed of AHLs and AI-2 degrading bacteria delay the biological contamination of MBR, which is 3 times higher than that of the control (Waheed *et al.* 2017). However, the continuous increase of organic loading will greatly reduce the function of QQ bacteria. Introducing native QQ bacteria (*Acinetobacter* sp. DKY-1) into the MBR can significantly reduce DPD concentration, thereby delaying membrane biofouling (Lee *et al.* 2018). Similarly, secretions from certain bacteria (e.g., *Candida albicans*) can also be used as QQ agents to substantially mitigate membrane biofouling via the suppression of AI-2 QS (Lee *et al.* 2016). Considering the competition between the added QQ bacteria and the native bacteria in MBR operation, researchers tried to fix QQ bacteria on certain carriers to ensure long-time survival in the practical application process, thus achieving stable membrane biofouling control (Ni & Wang 2019). Although QQ technology can effectively reduce membrane biofouling, more attention yet to be paid to investigate QQ-microorganisms, including isolation of QQ-microorganisms, the adaptability of microorganisms, and the effect of introducing QQ-microorganisms on the whole system.

Based on the important role of biofilm in WWT, it is of great practical significance to promote or inhibit the growth of biofilm by controlling QS signal molecules. As mentioned earlier, we can note that QQ-based technologies show significant impact on membrane biofouling as an attractive and economical method. These methods have been extensively studied and applied in MBRs. But further studies are still needed, including the effective combination of QS technology with traditional membrane biofouling control technology, the side effects of QS technology on biological treatment system, the combination of multiple QS technologies, and the reduction of the cost of QS technology.

4.2. Accelerate the start-up of the WWT process

The long startup time of biofilm, anammox process and granulation is the main bottleneck in application. The effectiveness of AI-2-based QS in promoting bacterial growth and activity will allow researchers to focus on using QS to accelerate the start-up of WWT process.

In current research, Boron and bacterial cultures containing AI-2 are often used to accelerate the granulation process of granular sludge (Table 4). AI-2 contained in intracellular extract from mature granules can accelerate sludge granulation, which may be the gene expression of particle adhesion growth induced by QS, leading to granule formation and its stable structure (Ren *et al.* 2010). The addition of *Vibrio harveyi* BB170 culture supernatant can increase EPS production and produce AnGS with larger particle size, due to the secretion of AI-2 by *Vibrio harveyi* in the culture supernatant (Ding *et al.* 2015b). Besides, as an important component of AI-2, Boron can trigger AI-2 mediated QS and accelerate intercellular communication, thereby improving the aggregation of bacteria in the initial startup stage. In the process of sludge granulation, the addition of Boron significantly enhances the activity of AI-2, improves the sedimentation performance of sludge, and finally accelerates the growth of AGS (Zhang *et al.* 2011). Boron can also realize the rapid start-up of the anammox process. It significantly promotes the enrichment of AnAOB, stimulates the secretion of EPS, and increases the total nitrogen load of the reactor, thus forming granular sludge with larger diameter and tighter structure (Wu 2018). Moreover, some AnAOB, such as *Candidatus* jettenia, become the dominant flora (Su *et al.* 2019).

AI-2 regulation shows great potential in improving the efficiency of the reactor and accelerating the start-up of certain WWT processes. This may be because AI-2 mediated interspecific communication allows more bacteria to participate in granulation, thus secreting more EPS to accelerate sludge accumulation. What's more, this promotion is at metabolic level, which deserves further study.

5. COMPARISON BETWEEN AI-2 MEDIA QS AND AHL MEDIA QS

Both AHLs and AI-2 have shown great potential for QS-based regulation, such as mediating formation biofilm and GS growth. However, one question may be overlooked: what is the difference between the two signaling molecules in QS-based strategy? It is necessary to study the relationship and difference between the two signal molecules to clarify the QS coordination mechanism and improve WWT efficiency. Due to different physical and chemical properties, they are distributed in different environmental conditions and then play different roles.

The distribution of signal molecules may affect the QS mechanism, mainly depends on the molecular weight and solubility of signal molecules. Long-chain AHLs are difficult to diffuse due to their low solubility in water, which may lead to cell-to-cell communication only within a short distance (Decho *et al.* 2011). In granular sludge, AI-2 and short-chain and medium-chain

AHLs are mainly distributed in the aqueous phase, while long-chain AHLs are mainly distributed in the sludge phase (Feng *et al.* 2014). Similarly, different parts of EPS are regulated by different signaling molecules (Wang *et al.* 2018). The content of AI-2 is the highest in slightly soluble EPS (SMP), followed by tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS). On the contrary, AHLs reach the highest content in TB-EPS, followed by SMB and LB-EPS. During the granulation process, the expression of AI-2 gene enhances the interspecific communication of bacteria, thereby inducing the transformation of bacteria from suspension growth to attachment growth. When the particle size reaches the limit, AI-2 drops to a lower level. It is worth noting that although AI-2 can promote EPS production during the start-up phase of the reactor, there is no evidence showing that AI-2 is related to EPS production in mature reactor. On the contrary, most AHLs are significantly positively correlated with EPS production in mature reactor, indicating that AHLs may be beneficial to EPS production process.

Environmental conditions are another key factor affecting the secretion of signal molecules. Ding *et al.* (2015a) found that when the pH increased from 5 to 9, the contents of C4-HSL, C6-HSL, 3-oxo-C6-HSL, C8-HSL and 3-oxo-C8-HSL decreased by 33.55%, 96.42%, 87.83%, 97.69% and 95.08%, respectively. The change of AI-2 content is opposite to that of AHLs: the synthesis of AI-2 is inhibited under acidic conditions (pH = 5), and its relative content is only 63.4% of that under alkaline conditions (pH = 9). These results indicate that different types of bacteria have different adaptations to pH, leading to changes in the secretion of signal molecules. Under the condition of unbalanced carbon and nitrogen supply in AnGS system, the content of AHLs first increases and then decreases, while the content of AI-2 decreases. In this process, AHLs increase the stability of AnGS by increasing the amount of LB-EPS, but this is not enough to balance the particle degradation caused by the low concentration of AI-2 (Ding *et al.* 2016). The effects of different signal molecules will be superimposed on the system under environmental pressure. Under the condition of organic shock loads, the content of AHLs increases after shock, which promotes the synthesis of PS and weakens the influence of shock loads, indicating that AHLs promote the recovery of AnGS. Conversely, the rapid decrease in AI-2 content results in a decrease in EPS content and AnGS diameter. During the OLR restoration stage, the contents of AHLs and AI-2 recover, but the granular sludge can't prevent the disintegration of AnGS. The results show that the effect of organic shock loads on sludge community is irreversible, indicating that interspecies communication plays an important role in particle stability (Ding *et al.* 2017).

Both exogenous AHLs and AI-2 can promote the growth of microorganisms, but they show different potentials. Ding *et al.* (2015b) studied the effects of adding AHLs (C4-HSL) and AI-2 on AnGS, and found that compared with AHLs-mediated QS, AI-2 mediated QS showed a faster granulation rate, higher EPS production, and more filamentous bacteria aggregation. The main reason may be that interspecific communication allows more species of bacteria (including Gram-positive and Gram-negative bacteria) to participate in granulation. AHLs-mediated QS maintains a higher COD removal rate, which may be due to the specific promotion of AHL(C4-HSL) on the COD degradation bacteria. However, the effects of other members of the AHLs family on AnGS has yet to be determined.

In summary, the distribution of signal molecules and environmental conditions, such as pH value, environmental pressure or different parts of EPS, have a great influence on the concentrations of AHLs and AI-2 (Feng *et al.* 2014; Wang *et al.* 2018). The relatively high concentrations of signal molecules dominate the current QS regulatory mechanism (Ding *et al.* 2015a, 2016, 2017). Bacteria regulated by AHLs have a relatively high participation in WWT process, leading to the improvement of WWT efficiency, while AI-2 may need to mobilize more types of bacteria to work together. Therefore, it is of great practical significance to select the appropriate signal molecules according to the regulatory purpose, and more connections and differences between AHLs and AI-2 deserve further study in the future.

6. CONCLUSIONS AND PROSPECTS

This article has summarized the role and application of AI-2 from two aspects: the regulation function and external stimulus conditions in WWT. Moreover, the regulation strategies of AHLs and AI-2 are also compared. The most significant feature of AI-2 mediated QS is that interspecific communication can promote more species of microorganisms to participate in community succession compared with AHLs-mediated intraspecific communication, thus enabling the WWT system to achieve stability faster. However, there is no direct evidence that the AI-2 pathway can be directly regulated, and it is usually activated under some specific conditions, such as the GS particle size growth stage and the initial stage of reactor startup. Several aspects still urgently await further investigation:

(1) **The difference between AHL-mediated QS and AI-2 mediated QS**. Understanding the distribution and roles of these two signaling molecules under different environmental conditions, their respective contribution ratio and their impact on community structure is expected to better develop QS based biological regulation strategies. To clarify the roles of these two signals in WWT processes is expected to further play the advantages of different signal molecules.

(2) **The role of AI-2 mediated QS in anammox**. Little is known about the roles of AI-2 mediated QS in anammox. The effects of AI-2 on the sedimentation and particle size of anammox sludge, the characteristics and functions of EPS, and the microbial community are urgent research topics in the future.

(3) **Interaction of multiple signaling molecules**. Determining the conditions and interaction mechanisms of multiple signaling molecules is worthwhile. Microorganisms in sewage treatment systems exist in the form of mixed bacteria. Previous studies mainly focused on the QS mechanism of single signal molecules, the regulation of AI-2 on a certain type of pure-cultured bacteria or a certain function. Hence, relevant mechanisms implied in AI-2 interactions with other signal molecules, as well as the regulation mechanism of AI-2 in the mixed bacteria system in wastewater systems require to be studied more deeply.

(4) **Possible application of QQ mechanism based on AI-2**. AI-2 based QQ technology provides a new approach to control membrane biofouling. Further research on QQ may focus on the exploitation of more appropriate QQ bacteria and immobilized microorganism techniques. Immobilization technology can protect target bacteria from environmental pressure, so it can be more widely used in MBR membrane pollution control. In addition, the existence of QQ might interfere with QS research. Therefore, it is necessary to evaluate the role of QQ to eliminate interference.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Adav, S. S., Lee, D., Show, K. & Tay, J. 2008 Aerobic granular sludge: recent advances. *Biotechnology Advances* **26** (5), 411–423.

Ahmed, N. A. A. M., Petersen, F. C. & Scheie, A. A. 2009 Biofilm formation and autoinducer-2 signaling in *Streptococcus intermedius*: role of thermal and pH factors. *Oral Microbiology and Immunology* **23** (6), 492–497.

Antunes, L. C. M., Ferreira, R. B. R., Buckner, M. M. C. & Finlay, B. B. 2010 Quorum sensing in bacterial virulence. *Microbiology* **156** (8), 2271–2282.

Barrios, A. F. G., Zuo, R., Hashimoto, Y., Li, Y., Bentley, W. E. & Wood, T. K. 2006 Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, B3022). *Journal of Bacteriology* **188** (1), 305–316.

Bassler, B. L., Wright, M., Showalter, R. E. & Silverman, M. R. 1993 Intercellular signaling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Molecular Microbiology* **9** (4), 773–786.

Bassler, B. L., Greenberg, E. P. & Stevens, A. M. 1997 Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *Journal of Bacteriology* **179** (12), 4043–4045.

Batchelor, S. E., Cooper, M., Chhabra, S. R., Glover, L. A., Stewart, G. S. A. B., Williams, P. & Prosser, J. I. 1997 Cell density-regulated recovery of starved biofilm populations of ammonia-oxidizing bacteria. *Applied and Environmental Microbiology* **63** (6), 2281–2286.

Cai, W. & Liu, Y. 2016 Enhanced membrane biofouling potential by on-line chemical cleaning in membrane bioreactor. *Journal of Membrane Science* **511**, 84–91.

Chen, G., Jun, H., Bi, J. & Guan, M. 2014 Quorum sensing of aerobic granular sludge tolerating high carbon and nitrogen loads. *Chinese Journal of Applied and Environmental Biology* **20** (01), 73–79 (in Chinese).

Chen, H., Li, A., Cui, D., Wang, Q., Wu, D., Cui, C. & Ma, F. 2017 N-Acyl-homoserine lactones and autoinducer-2-mediated quorum sensing during wastewater treatment. *Applied Microbiology and Biotechnology* **102**, 1119–1130.

Decho, A., Frey, R. & Ferry, J. 2011 Chemical challenges to bacterial AHL signaling in the environment. *Chemical Reviews* **111** (1), 86–99.

Ding, Y., Feng, H., Huang, W., Li, N., Zhou, Y., Wang, M., Xueqin, Z. & Shen, D. 2015a The effect of quorum sensing on anaerobic granular sludge in different pH conditions. *Biochemical Engineering Journal* **103**, 270–276.

Ding, Y., Feng, H., Huang, W., Shen, D. & Wang, M. 2015b A sustainable method for effective regulation of anaerobic granular sludge artificially increasing the concentration of signal molecules by cultivating a secreting strain. *Bioresource Technology* **196**, 273–278.

Ding, Y., Feng, H., Zhao, Z., Shen, D., Wang, M. & Zhou, Y. 2016 The effect of quorum sensing on mature anaerobic granular sludge in unbalanced nitrogen supply. *Water, Air, and Soil Pollution* **334** (227), 1–11.

Ding, Y., Feng, H., Shen, D., N, L., Wan, M. & Zhou, Y. 2017 The effect of organic shock loads on the stability of anaerobic granular sludge. *Environmental Technology* **38** (23), 3026–3033.

Feng, H., Ding, Y., Wang, M., Zhou, G., Zheng, X., He, H., Zhang, X., Shen, D. & Shentu, J. 2014 Where are signal molecules likely to be located in anaerobic granular sludge? *Water Research* **50**, 1–9.

Han, X., Liu, L., Fan, G., Zhang, Y., Xu, D., Zuo, J., Wang, S., Wang, X., Tian, M., Ding, C. & Yu, S. 2015 *Riemerella anatipestifer* lacks luxS, but can uptake exogenous autoinducer-2 to regulate biofilm formation. *Research in Microbiology* **166** (6), 486–493.

Herrero, M. & Stuckey, D. C. 2015 Bioaugmentation and its application in wastewater treatment: a review. *Chemosphere* **140**, 119–128.

Hong, Q. 2019 Research progress of quorum sensing and quorum quenching technology in application of MBR Membrane Fouling Control. *Water Purification Technology* **38** (6), 63–68 (in Chinese).

Huang, J., Yi, K., Zeng, G., Shi, Y. & Yu, H. 2019 The role of quorum sensing in granular sludge: impact and future application: a review. *Chemosphere* **236**, 124310.

Jahid, I. K., Mizan, F. R., Ha, A. J. & Ha, S. 2015 Effect of salinity and incubation time of planktonic cells on biofilm formation, motility, exoprotease production, and quorum sensing of *Aeromonas hydrophila*. *Food Microbiology* **49**, 142–151.

Jiang, H. L., Tay, J. H., Maszenan, A. M. & Tay, T. L. 2006 Enhanced phenol biodegradation and aerobic granulation by two coaggregating bacterial strains. *Environmental Science Technology* **40** (19), 6137–6142.

Kim, S., Lee, S., Hong, S., Oh, Y., Seoul, M., Kweon, J. & Kim, T. 2009 Biofouling of reverse osmosis membranes: microbial quorum sensing and fouling propensity. *Desalination* **247** (1–3), 303–315.

Lee, K., Lee, S., Sang, H. L., Kim, S. R., Oh, H. S., Park, P. K., Choo, K. H., Kim, Y. W., Lee, J. K. & Lee, C. H. 2016 Fungal quorum quenching: a paradigm shift for energy savings in membrane bioreactor (MBR) for wastewater treatment. *Environmental Science and Technology* **50** (20), 10914–10922.

Lee, K., Kim, Y., Lee, S., Lee, S. H., Nahm, C. H., Kwon, H., Park, P., Choo, K., Koyuncu, I., Drews, A., Lee, C. & Lee, J. 2018 Stopping autoinducer-2 chatter by means of an indigenous bacterium (*Acinetobacter* sp. DKY-1): a new antibiofouling strategy in a membrane bioreactor for wastewater treatment. *Environmental Science and Technology* **52** (11), 6237–6245.

Li, H., Li, X., Wang, Z., Fu, Y., Ai, Q., Dong, Y. & Yu, J. 2015 Autoinducer-2 regulates *Pseudomonas aeruginosa* PAO1 biofilm formation and virulence production in a dose-dependent manner. *BMC Microbiology* **15**, 192.

Li, Y., Fei, X., Jiao, X., Wang, L. & Xing, Y. 2018 Progress on the regulation of quorum sensing in wastewater treatment. *Chinese Journal of Applied Ecology* **29** (03), 1015–1022 (in Chinese).

Lim, S. J. & Kim, T. 2014 Applicability and trends of anaerobic granular sludge treatment processes. *Biomass and Bioenergy* **60**, 189–202.

Liu, X., Sun, S., Ma, B., Zhang, C., Wan, C. & Lee, D. 2016 Understanding of aerobic granulation enhanced by starvation in the perspective of quorum sensing. *Applied Microbiology and Biotechnology* **100**, 3747–3755.

Ma, B., Wang, S., Cao, S., Miao, Y., Jia, F., Du, R. & Peng, Y. 2016 Biological nitrogen removal from sewage via anammox: recent advances. *Bioresource Technology* **200**, 981–990.

Maddela, N. R., Sheng, B., Yuan, S., Zhou, Z., Villamar-Torres, R. & Meng, F. 2019 Roles of quorum sensing in biological wastewater treatment: a critical review. *Chemosphere* **221**, 619–629.

Moslehi-Jenabian, S., Gori, K. & Jespersen, L. 2009 AI-2 signalling is induced by acidic shock in probiotic strains of *Lactobacillus* spp. *International Journal of Food Microbiology* **135** (3), 295–302.

Nealson, K. H. & Hastings, J. W. 1979 Bacterial bioluminescence: its control and ecological significance. *Microbiological Reviews* **43** (4), 496–518.

Ni, L. & Wang, Y. 2019 Advances in MBR biofouling control based on quorum sensing and quenching. *Journal of Harbin Institute of Technology* **51** (08), 191–200. (in Chinese).

Nichols, J. D., Johnson, M. R., Chung-Jung, C. & Kelly, R. M. 2009 Temperature, not LuxS, mediates AI-2 formation in hydrothermal habitats. *FEMS Microbiology Ecology* **68** (2), 173–181.

Papenfort, K. & Bassler, B. L. 2016 Quorum sensing signal–response systems in Gram-negative bacteria. *Nature Reviews Microbiology* **14**, 576–588.

Pereira, C. S., Thompson, J. A. & Xavier, K. B. 2013 AI-2-mediated signalling in bacteria. *FEMS Microbiology Reviews* **37** (2), 1–26.

Rajamani, S., Zhu, J., Pei, D. & Sayre, R. 2007 A LuxP-FRET-based reporter for the detection and quantification of AI-2 bacterial quorum-sensing signal compounds. *Biochemistry* **46**, 3990–3997.

Ren, T. T., Yu, H. Q. & Li, X. Y. 2010 The quorum-sensing effect of aerobic granules on bacterial adhesion, biofilm formation, and sludge granulation. *Applied Microbiology and Biotechnology* **88** (3), 789–797.

Roy, V., Adams, B. L. & Bentley, W. E. 2011 Developing next generation antimicrobials by intercepting AI-2 mediated quorum sensing. *Enzyme & Microbial Technology* **49** (2), 113–123.

Rutherford, S. T. & Bassler, B. L. B. 2012 Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harbor Perspectives in Medicine* **2** (11), a12427.

Shrout, J. D. & Nerenberg, R. 2012 Monitoring bacterial twitter: does quorum sensing determine the behavior of water and wastewater treatment biofilms? *Environmental Science & Technology* **46** (4), 1995–2005.

Song, X. N., Qiu, H. B., Xiao, X. & Yu, H. Q. 2014 Determination of autoinducer-2 in biological samples by high-performance liquid chromatography with fluorescence detection using pre-column derivatization. *Journal of Chromatography A* **1361**, 162–168.

Su, Y., Wu, G., Rong, H., Huang, X., Tan, B. & Chu, Z. 2019 Effect of boron on start-up process and microbial community in anaerobic ammonium oxidation reactor. *China Water and Wastewater* **35** (13), 6–10. (in Chinese).

Sun, S., Liu, X., Ma, B., Wan, C. & Lee, D. 2016 The role of autoinducer-2 in aerobic granulation using alternating feed loadings strategy. *Bioresource Technology* **201**, 58–64.

Sun, Y., Guan, Y., Wang, H. & Wu, G. 2018 Autotrophic nitrogen removal in combined nitritation and Anammox systems through intermittent aeration and possible microbial interactions by quorum sensing analysis. *Bioresource Technology* **272**, 146–155.

Surette, M. G. & Bassler, B. L. 1998 Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proceedings of the National Academy of Sciences USA* **95** (12), 7046–7050.

Taga, M. E. 2005 Methods for analysis of bacterial autoinducer-2 production. *Current Protocols in Microbiology* Chapter 1, Unit 1C.1.

Thiel, V., Vilchez, R., Sztajer, H., Wagner-Döbler, I. & Schulz, S. 2009 Identification, quantification, and determination of the absolute configuration of the bacterial quorum-sensing signal autoinducer-2 by gas chromatography-mass spectrometry. *ChemBioChem* **10**, 479–485.

Turovskiy, Y. & Chikindas, M. L. 2006 Autoinducer-2 bioassay is a qualitative, not quantitative method influenced by glucose. *Journal of Microbiological Methods* **66** (3), 497–503.

Vilchez, R., Lemme, A., Thiel, V., Schulz, S., Sztajer, H. & Wagner-Döbler, I. 2007 Analyzing traces of autoinducer-2 requires standardization of the *Vibrio harveyi* bioassay. *Analytical and Bioanalytical Chemistry* **387**, 489–496.

Waheed, H., Xiao, Y., Hashmi, I., Stuckey, D. & Zhou, Y. 2017 Insights into quorum quenching mechanisms to control membrane biofouling under changing organic loading rates. *Chemosphere* **182**, 40–47.

Wang, M. Z., He, H. Z., Zheng, X., Feng, H. J., Lv, Z. M. & Shen, D. S. 2014 Effect of *Pseudomonas* sp. HF-1 inoculum on construction of a bioaugmented system for tobacco wastewater treatment: analysis from quorum sensing. *Environmental Science and Pollution Research* **21**, 7945–7955.

Wang, S., Shi, W., Tang, T., Wang, Y., Zhi, L., Lv, J. & Li, J. 2017a Function of quorum sensing and cell signaling in the formation of aerobic granular sludge. *Reviews in Environmental Science and Bio/Technology* **16** (1), 1–13.

Wang, X., Li, X. & Ling, J. 2017b *Streptococcus gordonii* LuxS/autoinducer-2 quorum-sensing system modulates the dual-species biofilm formation with *Streptococcus mutans*. *Journal of Basic Microbiology* **57** (7), 605–616.

Wang, J., Ding, L., Li, K., Huang, H., Hu, H., Geng, J., Xu, K. & Ren, H. 2018 Estimation of spatial distribution of quorum sensing signaling in sequencing batch biofilm reactor (SBBR) biofilms. *Science of the Total Environment* **612**, 405–414.

Whiteley, M., Diggle, S. P. & Greenberg, E. P. 2017 Progress in and promise of bacterial quorum sensing research. *Nature* **551** (7680), 313–320.

Winzer, K., Hardie, K. R. & Williams, P. 2003 Luxs and autoinducer-2: their contribution to quorum sensing and metabolism in bacteria. *Advances in Applied Microbiology* **53**, 291–396.

Wu, L. J., Li, A. J., Hou, B. L. & Li, M. X. 2017 Exogenous addition of cellular extract N-acyl-homoserine-lactones accelerated the granulation of autotrophic nitrifying sludge. *International Biodeterioration & Biodegradation* **118**, 119–125.

Wu, G. 2018 *Effect of AI-2 Activating Factor (Boron) on Sludge Granulation and Microbial Community of Anammox Reactor*. Guangzhou University, Guangzhou. (in Chinese).

Xiao, X., Zhu, W., Liu, Q., Yuan, H., Li, W., Wu, L., Li, Q. & Yu, H. 2016 Impairment of biofilm formation by TiO_2 photocatalysis through quorum quenching. *Environmental Science & Technology* **50** (21), 11895–11902.

Xiao, Y., Yaohari, H., Zhou, Z., Sze, C. C. & Stuckey, D. C. 2019 Autoinducer-2-mediated quorum sensing partially regulates the toxic shock response of anaerobic digestion. *Water Research* **158**, 94–105.

Xiao, M., Wu, R., Tan, C. & Li, P. 2020 Recent advances of quorum sensing system and quorum sensing inhibitors regulating bacterial biofilm formation. *Food Science* **41** (13), 227–234. (in Chinese).

Xiong, Y. & Liu, Y. 2010 Involvement of ATP and autoinducer-2 in aerobic granulation. *Biotechnology and Bioengineering* **105** (1), 51–58.

Xiong, Y. & Liu, Y. 2012 Essential roles of eDNA and AI-2 in aerobic granulation in sequencing batch reactors operated at different settling times. *Applied Microbiology and Biotechnology* **93** (6), 2645–2651.

Xiong, Y. & Liu, Y. 2013 Importance of extracellular proteins in maintaining structural integrity of aerobic granules. *Colloids and Surfaces B Biointerfaces* **112**, 435–440.

Xu, H. & Liu, Y. 2010 Control of microbial attachment by inhibition of ATP and ATP-mediated autoinducer-2. *Biotechnology and Bioengineering* **107** (1), 31–36.

Xu, H. & Liu, Y. 2011 Reduced microbial attachment by D-amino acid-inhibited AI-2 and EPS production. *Water Research* **45** (17), 5796–5804.

Xu, F., Song, X., Cai, P., Sheng, G. & Yu, H. 2017 Quantitative determination of AI-2 quorum-sensing signal of bacteria using high performance liquid chromatography-tandem mass spectrometry. *Journal of Environmental Sciences* **52**, 204–209.

Yeo, S., Park, H., Yosep, J., Park, S., Jaesik, Y., Lee, J., Mathara, M. J., Shin, H. & Holzapfel, W. 2015 Influence of gastrointestinal stress on autoinducer-2 activity of two *Lactobacillus* species. *FEMS Microbiology Ecology* **91** (7), fiv065.

Yeon, K., Cheong, W., Oh, H., Lee, W., Hwang, B., Lee, C., Beyenal, H. & Lewandowski, Z. 2009 Quorum sensing: a new biofouling control paradigm in a membrane bioreactor for advanced wastewater treatment. *Environmental Science & Technology* **43** (2), 380–385.

Zhang, M., Sun, K. & Sun, L. 2008 Regulation of autoinducer 2 production and luxS expression in a pathogenic *Edwardsiella tarda* strain. *Microbiology* **154** (7), 2060–2069.

Zhang, S., Yu, X., Guo, F. & Wu, Z. 2011 Effect of interspecies quorum sensing on the formation of aerobic granular sludge. *Water Science and Technology* **64** (6), 1284–1290.

Zhang, X., Lee, K., Yu, H., Mameda, N. & Choo, K. 2019 Photolytic quorum quenching: a new anti-biofouling strategy for membrane bioreactors. *Chemical Engineering Journal* **378**, 122235.

Zhang, Q., Fan, N., Fu, J., Huang, B. & Jin, R. 2021 Role and application of quorum sensing in anaerobic ammonium oxidation (anammox) process: a review. *Critical Reviews in Environmental Science and Technology* **51** (6), 626–648.

Zhao, J., Quan, C., Jin, L. & Chen, M. 2018 Production, detection and application perspectives of quorum sensing autoinducer-2 in bacteria. *Journal of Biotechnology* **268**, 53–60.

Zhu, J. & Pei, D. 2008 A LuxP-based fluorescent sensor for bacterial autoinducer II. *ACS Chemical Biology* **3**, 110–119.

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