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Review

Research Progress on the Influence Factors of the Quorum Sensing System Regulating the Growth of Wastewater Treatment Biofilm

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Abstract

Biofilms represent a prevalent and highly adaptable microbial lifestyle across diverse environments. With increasing understanding of quorum sensing (QS), its crucial role in regulating biofilm development in wastewater treatment systems has gained widespread recognition. This review systematically summarizes the role of QS in biofilm formation, encompassing the stages of initial attachment, irreversible adhesion, maturation, and dispersal. Additionally, the impacts of conventional environmental factors and specific stressors on QS-mediated biofilm regulation are discussed. Finally, the review highlights the positive regulatory effects of QS on biofilm performance. This review aims to offer practical insights into enhancing biofilm stability and optimizing wastewater treatment efficiency through QS-based regulatory strategies.

Keywords: biofilm; wastewater treatment system; quorum sensing; strengthening measures

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1. Introduction

Biofilms are structured communities of microbial cells, embedded within a self-produced matrix of extracellular polymers (EPS) and other biomolecules. These formations are complex and dynamic, often exhibiting three-dimensional structures and aggregation patterns [1]. Biofilms represent the predominant mode of microbial growth in both terrestrial and aquatic environments, offering protection against various threats, such as predation and exposure to biological or chemical toxins. They are also resilient to stressful conditions like dehydration and nutrient deficiencies [2].

Biofilms play a significant role in wastewater treatment systems, acting as ecosystems composed of various microorganisms. These ecosystems are essential in processes such as wastewater treatment and drinking water purification. Rapid biofilm formation is critical for the efficient start-up of biological wastewater treatment systems [3]. The development and establishment of biofilms are pivotal steps in these systems, offering numerous advantages, including long biomass retention, strong environmental adaptability, high biomass concentration, and operational flexibility [4]. In addition, granular sludge is regarded as a specialized form of biofilm, characterized by a dense granular structure where microorganisms were tightly clustered and encased within EPS [5].

Quorum sensing (QS) is a sophisticated communication system employed by microbial communities to coordinate collective behaviors. By synthesizing, releasing, and detecting small diffusible signaling molecules known as autoinducers, microbes could monitor their population density and regulate gene expression accordingly [6]. In biofilms, QS enable microorganisms to respond to environmental changes in a highly coordinated manner, playing a critical role in the transition from a planktonic (free-swimming) state to a sessile (surface-attached) lifestyle, thereby promoting biofilm formation [7].

QS is crucial in orchestrating the sequential stages of biofilm development, including initial surface attachment, maturation, and eventual dispersal of cells, by regulating specific gene expression in response to cell density [8]. Particularly during the dispersal phase, QS facilitates the detachment of cells from mature biofilms, allowing for colonization of new niches, biofilm turnover, and microbial dissemination [4]. Moreover, QS modulates key biofilm characteristics such as thickness, density, and robustness, which are vital for maintaining biofilm functionality in wastewater treatment systems [3,7,9]. Through precise regulation of these traits, QS enable microbial communities to balance biofilm stability with environmental adaptability.

QS enhances microbial adhesion and resistance to mechanical stress, subsequently facilitating the formation of biofilms [10,11]. With growing research interest in microbial communication, the pivotal role of QS in biofilm growth and regulation within wastewater treatment contexts has become increasingly evident [7]. Recent studies have reviewed the impact of quorum sensing (QS) on biofilm formation and extracellular polymeric substances (EPS) synthesis [12], as well as the regulatory mechanisms of QS on biofilms in biological wastewater treatment systems [13,14]. However, a comprehensive review of the effects of QS on wastewater treatment biofilms at different growth stages and under different environmental conditions, especially in the presence of specific pressure sources such as heavy metals, antibiotic resistance genes, and microplastics and nanoplastics, is still necessary.

This review aims to systematically summarize (1) the effects of QS on different stages of biofilm formation, (2) the influence of conventional and specific environmental factors on biofilm-related QS systems, and (3) the role of QS in enhancing the performance and stability of biofilm-based systems. This review offers a theoretical foundation for the application of QS in the active regulation of biofilm behavior, providing insights to support the enhancement of stability and treatment performance in wastewater biofilm systems.

2. The Effect of the QS System on Biofilm Formation and Regulation

QS plays a pivotal role in all stages of biofilm development, encompassing the initial attachment, irreversible attachment, and maturation phases. Furthermore, QS also governs the dispersal phase that occurred after biofilm maturation, as shown in Figure 1.

2.1. Effect of QS on the Initial Attachment of Biofilm

The initial stage of biofilm formation was characterized by the reversible attachment of bacteria to the surface of the carrier. During this phase, microorganisms adhered loosely and reversibly to surfaces, primarily through weak physical interactions including Lifshitz-van der Waals forces and electrostatic forces [15]. The sparse population of attached microorganisms and the weak nature of these interactions made the microorganisms particularly susceptible to detachment by external forces, such as fluid flow and shear stress [16].

QS system plays a crucial role in promoting initial bacterial attachment by regulating surface structures and the expression of adhesion-related genes. For instance, in *Pseudomonas aeruginosa*, the addition of C6-HSL and C8-HSL signal molecules led to the

overexpression of functional genes (*lasI*, *lasR*, *rhlI*, *rhlR*), which in turn promoted bacterial motility [17]. Specifically, increased swimming motility facilitated the transfer of *Pseudomonas aeruginosa* from the culture medium to the carrier surface. Subsequently, enhanced swarming motility further strengthened bacterial adhesion and proliferation on the carrier surface [17,18]. Moreover, in *Bacillus subtilis*, the ComQXPA (ComQ-ComX-ComP-ComA) QS system regulated biofilm formation by controlling the production of surface-active substances, such as surfactin. Surfactin reduced repulsive forces between bacteria and surfaces, facilitating contact and altering surface hydrophobicity to enhance wetting and diffusion on hydrophobic surfaces. Additionally, it enabled bacteria to remain on the surface long enough to initiate biofilm formation without permanent adhesion, thus maintaining motility [19].

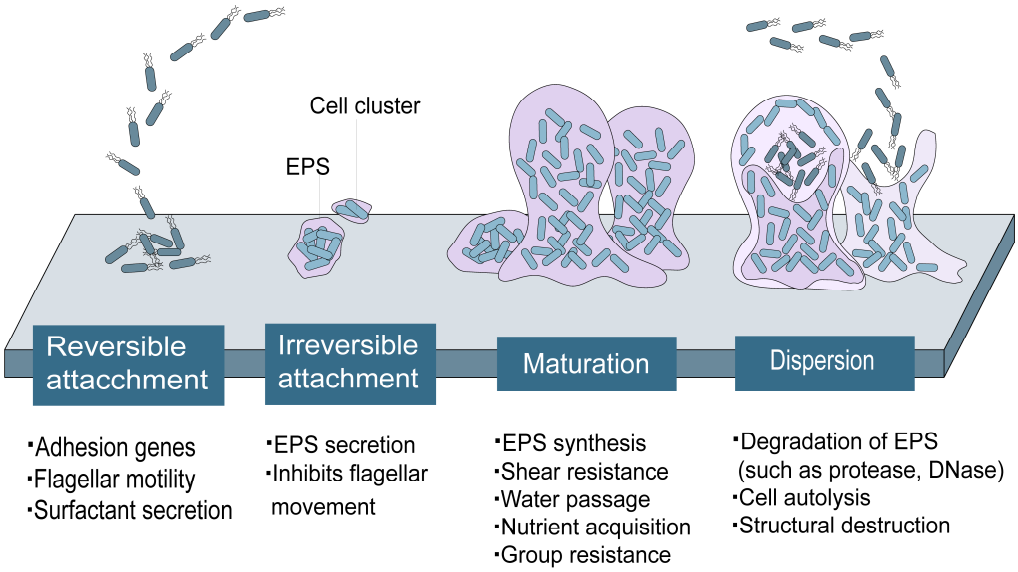

Figure 1. Schematic diagram of the biofilm formation process and the regulation of QS on different growth stages of biofilm.

The initial attachment phase played a crucial role in biofilm formation during wastewater treatment processes. To explore the regulatory effects of acyl-homoserine lactones (AHLs) on biofilm adhesion in wastewater systems, researchers introduced the QS strain *Sphingomonas rubra* sp. nov., capable of secreting C12-HSL and C10-HSL, into a biofilm reactor system [20]. The system was inoculated with aerobic activated sludge collected from both municipal and industrial wastewater treatment system. Exogenous AHLs significantly reduced the duration of the reversible adhesion phase in wastewater systems. Furthermore, with the addition of exogenous AHLs, the biofilm thickness in municipal and industrial wastewater increased significantly. Conversely, the addition of acylase extended the reversible adhesion phase while simultaneously reducing biofilm thickness and density, thereby delaying initial biofilm formation. These findings provided compelling evidence for the regulatory role of QS in biofilm development [21].

Collectively, QS signaling was found to modulate bacterial-surface interactions by altering surface wettability and enhancing adhesion capabilities during the reversible attachment phase. These modifications ultimately facilitated the initial bacterial adhesion and subsequent biofilm formation.

2.2. The Effect of the QS System on the Irreversible Adhesion Stage

During the irreversible attachment phase of biofilm, QS-mediated regulation primarily involved the secretion of EPS. The enhanced EPS production formed protective substrates

that strengthened bacterial-surface adhesion and reinforced the stability of the irreversible attachment phase. Subsequently, the attached cells initiated division and aggregation processes, promoting the formation of a microcolony [22,23]. Notably, AHLs and circulating di-GMP(c-di-GMP), as two key bacterial signaling molecules, played an important role in regulating the transition from biofilm formation to irreversible adhesion [23].

AHLs were the main signaling molecules in QS systems. Produced by LuxI family synthetases, they enabled cell-to-cell communication through diffusion, controlling biofilm formation, virulence, and antibiotic production [24]. For example, in *Pseudomonas aeruginosa*, EPS excretion was significantly stimulated by exogenous AHLs. The total content of EPS gradually increased from 3.15 mg/g VSS of the control to 6.22 and 5.40 mg/g VSS of 25 μM C6-HSL and C8-HSL, respectively. The increase in EPS content stimulated the transformation of reversible adhesion to the surface and transformed into an irreversible condensed three-dimensional polymer network, which was connected to each other and instantaneously fixed biofilm cells, promoting the irreversible binding of microorganisms to the surface [17]. In addition, AHLs produced by *Acinetobacter baumannii* could interact with the AHLs receptor *abaR*, which was presented on the surface of adjacent bacterial cells, through membrane diffusion in the environment. Because signal molecules activated the transcription of biofilm-related genes, the interaction between bacterial cell surface factors and biological or non-biological surfaces promoted the irreversible adhesion of bacteria [25].

c-di-GMP functioned as a second messenger molecule, belonging to the class of cyclic nucleotide signaling molecules. Through binding to diverse effector proteins (PN), including transcription factors and enzymes, c-di-GMP modulated EPS synthesis, flagellar motility, and cell cycle progression, facilitating the transition of bacteria from a planktonic to a sessile state [26,27]. For example, in *Salmonella* motility, c-di-GMP bound to the flagellar brake PN YcgR, directly inhibiting flagellar motor rotation and promoting bacterial-surface attachment to the irreversible stage [28]. In *Pseudomonas aeruginosa*, c-di-GMP facilitated the transition from reversible to irreversible attachment by modulating flagellar reversal rates and colony movement. Moreover, *Pseudomonas fluorescens* regulated the cell surface localization of the large adhesion PN LapA through c-di-GMP. This regulation facilitated the transition in the growth process of *Pseudomonas fluorescens* biofilm from reversible adsorption to irreversible adsorption [29].

2.3. The Effect of QS System on the Mature Stage of Biofilm

During the maturation phase of biofilm development, complex three-dimensional structures emerged, characterized by the formation of microcolonies, water channels, and intricate matrix networks [27]. At this critical stage, QS primarily functioned to regulate the stability of mature biofilms.

During this phase, QS signaling maintained biofilm stability through the regulation of EPS and other adhesion molecule formation, while simultaneously enhancing resistance to external stressors [30]. In *Pseudomonas aeruginosa*, the *LasI/R* system was confirmed to maintain biofilm structural stability through the regulation toxins and extracellular enzymes production [10]. During this phase, the QS-regulated EPS matrix provided structural support and protection against host immune responses and antimicrobial agents, and it also facilitated the dissemination of beneficial mutations within biofilm communities and improved nutrient acquisition capabilities [31]. Research findings revealed that the microbial hierarchical architecture of mature biofilms, including colony spatial distribution and EPS composition and thickness, remained relatively stable. This stability primarily stemmed from the physical barrier effect of EPS and complex microorganism interactions [16].

Overall, QS could continuously regulated EPS-related genes, ensuring the stability of biofilm, improving resistance to shear forces, antimicrobial agents, and environmental stressors.

2.4. The Effect of QS System on the Shedding Stage

During the dispersal phase of biofilm development, bacterial cells detached from the biofilm matrix and transitioned back to a planktonic state, a critical process for biofilm expansion and colonization of new habitats.

QS signaling molecules played a crucial regulatory role in coordinating bacterial dispersal and reattachment behaviors during biofilm shedding. As the biofilm aged, it accumulated harmful compounds and depleted resources, prompting cells to disperse to new regions to access nutrients, grow, and eliminate waste. During this phase, QS served as an effective mechanism to facilitate the release of bacteria from biofilms, enabling them to aggregate on other surfaces [32]. This mechanism was crucial in multispecies biofilm communities, as QS signaling synchronized dispersal and reattachment of diverse bacterial populations, facilitating microbial population expansion and niche colonization. QS was found to initiate autolysis processes within specific bacterial populations, resulting in the programmed self-decomposition of certain cells. This controlled cellular disintegration compromised biofilm structural integrity, thereby facilitating the detachment and dispersal of remaining viable cells [31].

In addition, QS was found to induce the secretion of extracellular enzymes in bacteria, including proteases and deoxyribonuclease I, which degraded EPS components and destabilized biofilm structures, ultimately promoting cellular detachment. The previous study showed that deoxyribonuclease I, secreted by QS-induced bacteria, effectively decomposed extracellular DNA (eDNA), leading to biofilm dispersal across various bacterial species. This phenomenon was particularly evident in *Staphylococcus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* strains [33]. In *Xanthomonas campestris*, DSF signaling molecules regulated biofilm dispersion by modulating the synthesis of enzymes responsible for degrading EPS [31].

In general, the QS system primarily achieved the diffusion stage of biofilm by controlling enzyme release, cell autolysis, and stress response.

3. The Influence of Conventional Factors on the Regulation of Biofilm by the QS System

3.1. Substrate

The concentration of substrate was identified as a critical factor for reactor performance, and it also influenced the secretion and composition of signaling molecules, further affecting the characteristic of biofilm [34].

At low substrate concentrations, microbial metabolic activity typically exhibited a decreasing trend. Conversely, excessive substrate concentrations could also lead to process inhibition due to the accumulation of byproducts resulting from overloading [35]. In the anammox biofilm system, more QS organisms were enriched at high concentration of NH_4^+-N (110 mg/L), and more AHLs synthesis were induced, which may help to improve anammox activity. In addition, the content of PN and hydrophobic amino acids in EPS also increased, thereby further regulating the development of anammox biofilms and making the biofilm structure denser [36]. In the anammox granular sludge system, high nitrogen loading disrupted sludge settling by triggering excess bound extracellular polymeric substances (B-EPS) production through bacterial signaling, making granules less dense [37]. Zhang, et al. [38] found that the levels of C6-HSL and C8-HSL in the ANAMMOX granules rose sharply as the substrate concentration increased from 200 mg/L to 1200 mg/L, triggering excessive EPS secretion and impairing granule sedimentation.

Furthermore, it was noteworthy that the type of substrate also played a significant role in modulating the QS system within biofilms. In the denitrifying phosphorus removal (DPR) system, the self-inducer, regulator, and transporter increased by 4.5%, 8.2%, and 10.0%, respectively, as the electron acceptor shifted from nitrate to nitrite. This phenomenon showed that the increase in biofilm synthetic PN genes corresponded to a rise in PN content within EPS after the electron acceptor changing from nitrate to nitrite, leading to irreversible biofilm adhesion [39].

3.2. Operation Parameters

Changes of operating conditions could influence QS of microorganisms, which subsequently affected the characteristics of biofilms.

Yan, et al. [40] investigated the induction mechanism of AHLs in Expanded Granular Sludge Bed (EGSB) under different reflux ratios. The concentration of C4-HSL was observed to fluctuate between 1.33 ng/g MLVSS and 5.33 ng/g MLVSS as the reflux ratio increased from 50% to 500%. The increasing trend of C4-HSL concentration led to an increase in the secretion of EPS, which in turn promoted cell adhesion within the wastewater treatment system, further facilitating the formation and maintenance of anaerobic granular sludge (AnGS).

Sludge retention time (SRT) played a significant role in system performance, microbial structure, and QS in activated sludge bioreactors. Zhang, et al. [41] investigated the role of SRTs on the microbial enrichment and granulation of the aerobic granular sludge (AGS) system. The results demonstrated that a 6-day SRT reactor achieved higher total nitrogen (TN) removal efficiency and produced more compact granules, and microbial families such as *Xanthomonadaceae*, *Rhodobacteraceae*, and *Hyphomonadaceae*, which are associated with AHL production and EPS secretion, were enriched under the 6-day SRT condition.

3.3. Temperature

As an environmental factor, temperature was found to influence the release of self-inducers and the role of QS in biofilm systems. It was observed that when the temperature decreased from 36 °C to 15 °C, the concentrations of AHLs in both the aqueous and biomass phases decreased, leading to a weakening of QS activity in the anammox system, particularly for C6-HSL and C8-HSL [42]. Tang and Zhao [43] used a pilot-scale Anaerobic-Anoxic-Oxic (AAO) reactor to explore the internal mechanism of biofilm denitrification at low temperatures, and they found that the QS system regulated c-di-GMP levels by controlling the synthesis of diguanylate cyclase (DGC) and phosphodiesterase (PDE). As temperature decreased, the abundance of these enzymes declined, reducing c-di-GMP levels, which were crucial for EPS secretion. This led to lower EPS production, especially TB-EPS, weakening microbial adhesion and aggregation.

3.4. pH

It was well known that pH, as one of the most common environmental factors, played a significant role in bacterial biofilm growth and QS. The study of Ding, et al. [44] found that pH significantly affected signaling-molecule synthesis and stabilization. In neutral and mildly alkaline conditions, higher Autoinducer-2 (AI-2) and lower Diffusible Factor Signal (DSF) improved sludge hydrophobicity and integrity, forming larger and more uniform particles. In acidic conditions, lower AI-2 and higher DSF reduced hydrophobicity and particle strength, leading to sludge destabilization and degradation.

Zhang, et al. [45] investigated the variations in AHL concentrations within the anammox granule system. They found that AHL signaling molecules sharply declined under extremely low pH conditions, leading to the deterioration of granule stability and metabolic activity. Under weakly acidic conditions (pH 6.0–7.0), the release of C8-HSL and C6-HSL

was enhanced, which promoted EPS production and increased the PN/PS ratio, thereby helping the granules maintain their structure and activity under unfavorable pH environments. In contrast, under alkaline conditions (pH 7.5–9.0), AHL release progressively declined with increasing pH. At strongly alkaline conditions (pH = 9.0), the AHL-based QS system lost its regulatory function over EPS synthesis. Consequently, anammox granules demonstrated poorer recovery under alkaline conditions than under acidic conditions. These findings highlighted the superior stability and activity of anammox granules under neutral to weakly acidic conditions compared to alkaline environments.

3.5. Other Conventional Parameters

In some sewage treatment plants, some biochemical tanks were easily influenced by prolonged light exposure. In *Pseudomonas aeruginosa* biofilm, the synthesis of C4-HSL and 3O-C12-HSL was inhibited by LED lighting, further influencing the maturation of biofilm. In addition, the concentration of EPS in *Pseudomonas aeruginosa* biofilm decreased after LED lighting treatment [46]. Light intensity was confirmed as the key factor influencing algae absorption capacity, nutrient uptake, and biomass growth in algae-bacterial systems, thereby affecting microbial metabolic processes and the synthesis of signal molecules. Zhang, et al. [47] found that the production of C6-HSL and 3OC8-HSL was higher under low light intensity conditions in the algal-bacterial granular sludge (ABGS) system.

Salinity affected the QS system in the bacterial biofilms. When the salt concentration reached to 20 mg/L and 30 mg/L, the secretion of C6-HSL and C8-HSL were stimulated, further promoting the EPS release, extracellular electron transfer, and oxidative stress [48]. In electroactive biofilm (EAB) systems, microorganisms secreted higher levels of AHLs with increasing salinity. This enhanced AHL production stimulated EPS synthesis, thereby improving microbial resistance to high-salinity environments [49].

4. The Influence of Emerging Factors on the Regulation of Biofilm by the QS System

4.1. Metal Nanoparticles

Metal-based nanoparticles were widely used in nearly every aspect of daily life, including semiconductors, microelectronics, personal care, textiles, and food products [50]. Various studies reported that metal nanoparticles were widely detected in the natural environment and sewage treatment plants [51], and exerted significant effects on the QS systems.

Metal-based nanoparticles could promote the QS system of wastewater treatment biofilm. In the study of Wang, et al. [52], 50 mg/L nZVI increased the abundance of c-di-GMP synthesis PN from 148 rpmr to 252 rpmr in the microbial community of the anammox granular sludge system, and decreased the abundance of c-di-GMP degradation PN from 238 rpmr to 204 rpmr, which led to the enrichment of c-di-GMP in the microbial community. The enrichment of c-di-GMP promoted the secretion of EPS by bacteria, which was conducive to the formation of sludge particles in the anammox reactor.

Moreover, metal-based nanoparticles had an inhibitory effect on the QS system of wastewater treatment biofilms. In the biofilm system enrichment with *Pseudomonas aeruginosa*, de Celis, et al. [53] found that the gene expression level of acyl-homoserine lactone synthase (*lasI*) decreased in the presence of Ag and ZnO NP, leading to the inhibition of the QS process and, consequently, impairing biofilm formation by disrupting the biofilm structure.

4.2. Antibiotics and Antibiotic Resistance Genes

The abuse of antibiotics led to the transfer of antibiotic resistance genes (ARGs) and the emergence of antibiotic-resistant bacteria (ARBs) in the environment, posing risks to human health. Antibiotics were primarily discharged into water bodies through medical, animal husbandry, aquaculture, and industrial wastewater. Various types of antibiotics and ARGs were frequently detected in aquatic environments [54]. Numerous studies reported that antibiotics and ARGs could affect the QS system.

Previous studies reported that tetracycline (TET), a commonly used antibiotic, interfered with the QS pathway of *Pseudomonas aeruginosa* strains biofilm and upregulated rhamnolipid synthesis genes, further promoting biofilm formation and enhancing bacterial motility [55]. Similarly, sulfamethoxazole (SMX) disrupted the QS system by inhibiting the expression of key genes, including the *Pseudomonas* quinolone signal (PQS) biosynthetic genes (*pqsA* and *pqsC*), the N-acyl-homoserine lactone receptor genes (*lasR* for 3OC12-HSL and *rhlR* for C4-HSL), and the rhamnolipid synthesis gene (*rhlA*). These molecular-level disruptions ultimately led to significant alterations in microbial community composition and biofilm development [56].

4.3. Microplastics and Nanoplastics

Microplastics (MPs), defined as particles smaller than 5 mm in diameter, and nanoplastics (NPs), which result from further degradation into particles smaller than 100 nm via weathering and ultraviolet irradiation, have attracted growing global concern due to their widespread presence in both aquatic and terrestrial ecosystems [57]. Recently studies demonstrated that MPs and NPs could interfere with the QS system within biofilm matrices.

Ma, et al. [58] investigated the effects of different MPs (PA and PE) on biofilm formation in microbial communities within constructed wetland systems. The study revealed that microplastics, particularly PA, significantly downregulated QS pathway genes by inhibiting the secretion of QS signaling molecules (e.g., AI). Additionally, the c-di-GMP regulatory pathway was disrupted, leading to alterations in key biofilm characteristics. Furthermore, EPS synthesis was significantly reduced (with PN and PS contents decreased by 26.35% and 28.99%, respectively), weakening biofilm structure and formation. This led to reduced pollutant resistance and nitrogen conversion efficiency, ultimately promoting biofilm dispersal. Also, in aerobic granular denitrification system, the signaling-molecule-mediated QS system was significantly attenuated following prolonged exposure to 20 mg/L NPs [59].

5. Strengthening Effect of QS on the Biofilm System

5.1. Enhancement of EPS Production by QS

Various studies reported that exogenous of signal molecules could enhance the EPS production of the biofilm system, further strengthening the stability of the biofilm (Figure 2).

Gao, et al. [60] studied the effect of QS signaling molecules on EPS production during biofilm formation. They added 0, 20, 40, and 60 μg/L of C4-HSL to strain LC-1 in early biofilm development. Compared to the control group, C4-HSL significantly increased EPS PS and PN contents, with higher doses showing greater effects, especially on PS. The results indicated that C4-HSL promoted PS secretion to accelerate biofilm formation. Additionally, C6-HSL enhanced extracellular PN and PS production, with a stronger impact on PN secretion. Fang, et al. [61] also showed that exogenous C6-HSL and 3OC12-HSL significantly improved key features of *Geobacter soli* cathodic electroactive biofilms (EABs), including biomass accumulation, cell viability, EPS production, and redox activity of outermost EPS PNs. These synergistic effects ultimately enhanced biofilm activation efficiency.

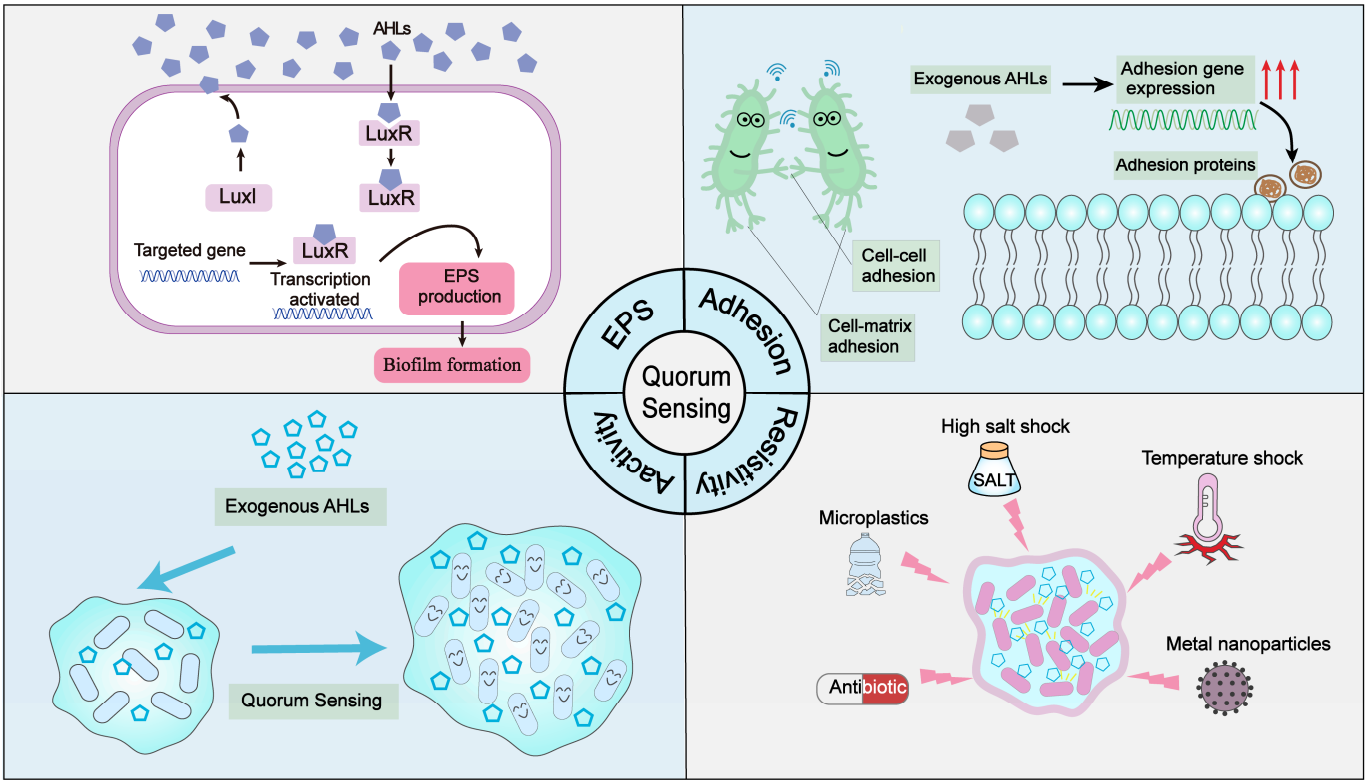

Figure 2. Strengthening of biofilm by QS system mediated mainly by exogenous AHLs. (The red arrow in the upper right corner represents enhancement).

5.2. The Enhancement of Biofilm Adhesion Ability by QS

The formation of biofilms was closely linked to the growth and adhesion efficiency of functional microorganisms.

Qian, et al. [17] demonstrated that the adhesion capacity of *Pseudomonas aeruginosa* was significantly influenced by AHLs. Specifically, as the concentration of C6-HSL increased from 0 to 25 μM, the adhesion rate of *Pseudomonas aeruginosa* rose from 9.9% to 33.5%. Similarly, Wang, et al. [11] supplemented pure-cultured *Sphingomonas rubra* biofilms with different AHLs and found that C4-HSL, C7-HSL, 3OC10-HSL, and C14-HSL significantly enhanced bacterial adhesion. The optimal concentrations for these AHLs were 100 ng/L, 100 μg/L, 1000 μg/L, and 10 μg/L, respectively, with C14-HSL (10 μg/L) exhibiting the most pronounced effect, yielding a cell index (CI) value 2.26 times higher than the control.

5.3. Enhancement of Biofilm Activity by QS

Recent studies demonstrated that AHLs, as central QS signal molecules, could significantly enhance bacterial metabolic activity in biofilm communities, thereby improving pollutant degradation efficiency and overall performance in wastewater treatment systems.

Shi, et al. [62] introduced the C4-HSL into the microbial electrolysis cell (MEC) biocathode for sulfate-containing wastewater treatment and found that addition of C4-HSL enhanced both sulfate reduction efficiency and biocathode stability, with the proportion of living cells in the biofilm increasing by 22%. Similarly, Hu, et al. [63] reported that exogenous of 5 nM AHLs significantly enhanced the EPS concentration, biofilm biomass and dehydrogenase activity. These improvements were attributed to the positive regulation of microbial metabolism and extracellular polymer production via QS mechanisms. Wang, et al. [64] showed that exogenous of C6-HSL and C8-HSL significantly enhanced biofilm formation and EPS secretion in *Acinetobacter* sp., further improving cell surface hydrophobicity and microbial aggregation. Moreover, the result of fluorescence spec-

troscopy showed a 50% rise in tryptophan-like fluorescence intensity, reflecting elevated metabolic activity.

5.4. The Enhancement of Biofilm Shock Resistance by QS

Numerous studies demonstrated that the activation of QS-mediated protective mechanisms significantly enhanced biofilm resistance to adverse conditions.

Lv, et al. [65] added AHLs to AnGS following temperature shock and found that exogenous AHLs promoted EPS production, enhanced PN secretion, and improved sludge granule integrity and size. Additionally, AHLs activated protective mechanisms by increasing the PN-to-PS ratio and hydrophobic groups in EPS.

Zhou, et al. [66] showed that exogenous signaling molecules (3OC12-HSL) significantly improved the salt shock resistance of electroactive biofilms (EABs). Under 10% salinity shock, EABs supplemented with QS signals achieved a current density recovery rate of 50.7%, compared to a 70% and 93.3% decrease in the blank and QS-quenched groups, respectively. The protective effect was attributed to three mechanisms: (1) QS promoted denser biofilm structures and enhanced cell viability (as revealed by CLSM); (2) QS increased PS secretion in EPS, forming a hydrated barrier that reduced Na+ penetration; and (3) QS reshaped the microbial community by enriching electroactive genera like *Pseudomonas* (20.0%), which secreted more protective EPS. In addition, QS also boosted biofilm stability by enhancing metabolic pathways such as glycan biosynthesis (0.82% vs. 0.78% in the control). These results highlight the potential of QS signaling to enhance EAB resilience in saline wastewater treatment.

Jiang, et al. [67] demonstrated that exogenous AHLs, i.e., C4-HSL, C6-HSL, and C8-HSL, improved the resistance of AnGS to microplastic toxicity. Exogenous of signal molecules activated QS responses, upregulated antioxidant gene expression to alleviate oxidative stress, enhanced energy metabolism, and promoted the synthesis of PNs, PSs, and phospholipids in EPS to rebuild microbial protective barriers. In addition, AHLs enriched methanogenic pathways and activated key enzymes, accelerating the functional recovery of AnGS.

6. Conclusions

In summary, QS played a key role in regulating biofilm development at all stages. Environmental factors such as temperature, pH, nanoparticles, and antibiotics could influence QS pathways, thereby affecting microbial activity, EPS composition, and gene expression. Targeted regulation of QS, including the addition of AHLs or similar signaling molecules, could enhance biofilm resistance by strengthening structure, improving metabolic function, and activating stress responses. These strategies offer valuable potential for improving biofilm performance in wastewater treatment and other environmental applications.

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