Quorum sensing in water and wastewater treatment biofilms

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Abstract

Fixed film processes and activated sludge processes are two main families of wastewater treatment systems which all refer to the heterogeneous microbial communities. Meanwhile, biofilms in drinking water distribution systems (DWDS) and biofouling in membrane systems are significant problems in the water and wastewater treatment which reduce the microbial quality of drinking water and limit the development of membrane system respectively. Since biofilms and quorum sensing (QS) as two microbial social behaviors have been inextricably linked, a number of studies have focused on the role of QS signaling and QS inhibition in the processes of water and wastewater treatment, which will help us engineer these biological treatment processes successfully and develop promising approaches for control of microbial adhesion, colonization and biofilm formation. This review gives a summary of recent known QS mechanisms and their role in biofilm formation for different species. Particular attentions are dedicated to the signaling molecules involved in some microbial granulation processes and the potential applications by some of their natural and synthetic analogues in the treatment of membrane biofouling.

Key words

Quorum sensing, Biofilm formation, Granular sludge, Membrane system, Drinking water

Introduction

The biofilm habitat represents a primary mode of growth and living for the bacteria. Wherever there is water, a surface support and available nutrients, a biofilm will form. In water and wastewater treatment systems, biofilms are found in many bio-treatment processes. While robust biofilms in fixed film process and granular sludge process are needed for effective sewage treatment, biofouling in membrane bioreactors and drinking water distribution systems (DWDS) is primal problem for effective and safe operation of the systems (Le-Clech et al., 2006; Aday et al., 2008; Zhu et al., 2010; Wingender and Flemming, 2011). Membrane biofouling reduces the membrane flux and increases the filtration pressure, whereas biofilms in DWDS are prone to release pathogens and corrode pipes (Percival and Walker, 1999; Le-Clech Chen and Fane, 2006; Xiong and Liu, 2010; Wingender and Flemming, 2011). Thus, biofilms in water and wastewater industry should be manipulated on the basis of the function.

Essential external environmental conditions affecting biofilm formation in water and wastewater treatment systems has been thoroughly described which included nutrient level, material and temperature (Liu and Tay, 2002; Ndiongue et al., 2005; Meng et al., 2009). However, much less attention has been paid to the effects of cell–cell communication and signaling on biofilms in the systems. Quorum sensing (QS) is a mechanism that involves the synthesis, release and detection of signal molecules called autoinducers (AIs) (Fuqua et al., 1994). Bacteria can utilize such systems to monitor their population density and to activate in specific gene expression which enables them to behave in a coordinated fashion (Waters and Bassler, 2005; Jayaraman and Wood, 2008). So far, numerous QS signal molecules and circuits have been identified in a diverse group of bacteria genera including those commonly associated with water and wastewater systems (Shrout and...
Nerenberg, 2012). And there is evidence that QS systems contribute to biofilm development in some bacteria, which not only participate in the construction of growing biofilms, but also involve in the dissolution of developed biofilms (Parsek and Greenberg, 2005; Moons et al., 2009). Since biofilms in water and wastewater systems are more likely to grow in polymicrobial communities, the occurrence of QS systems in biofilms in such systems seems to be universal. However, the effects of QS systems in the formation, composition and behavior of biofilms in the environmental engineering processes need to be understood. Recently, because QS is central to controlling biofilm formation and virulence expression in many pathogenic bacteria, interference with this regulation offers an important target for controlling diseases (Antunes et al., 2010; Francofolini and Donelli, 2010). In water and wastewater treatment industry, manipulating the QS systems may be a viable strategy to govern biofilms in such systems, no matter for biofilm promotion or prevention. The advantages of QS method are low or even nontoxic, high antibiofouling efficiency and low risk of bacterial resistance development.

This article describes the different quorum sensing signals and pathways in bacteria. In the context of biofilms are evaluate the role of QS signals in biofilms of water and wastewater treatment systems and the possibility of QS systems be manipulated to govern biofilms in such systems.

**QS in bacteria**: Quorum sensing (QS), a form of bacterial cell-cell communication, is a regulation of gene expression in response to fluctuations in cell-population density (Miller and Bassler, 2001). In this process, bacteria produce AIs intracellularly and then release them outside. The concentration of AIs in the environment will increase as the bacterial population expands. And when it reaches a threshold level, the cognate receptors will bind to the AIs and trigger the downstream gene expression that controls a broad range of bacterial activities, such as biofilm formation, competence, bioluminescence, virulence factor secretion, antibiotic production, sporulation and so on (Bassler, 2002; Waters and Bassler, 2005). In bacteria, QS is a widely spread phenomenon, and enables bacteria to adapt and survive in a continuously changing environments.

Bacteria can respond to a wide range of AIs. So far, there are three well-defined types of AIs associated with QS in bacteria, acyl homoserine lactones (AHLs), autoinducer oligopeptides (AIP), and autoinducer-2 (AI-2). While AHLs and AIP are predominately used as signaling molecules by Gram-negative and Gram-positive bacteria, respectively, AI-2 is universally used for interspecies communication of both Gram-positive and Gram-negative bacteria (Miller and Bassler, 2001; Waters and Bassler, 2005; von Bodman et al., 2008). Other bacterial QS molecules include *Streptomyces* butyrolactones, diketopiperazines (DKPs) and indole (Gamard et al., 1997; Holden et al., 1999; Lee and Lee, 2010; Deng et al., 2011). These structurally diverse signals provide a platform for bacterial intraspecies and interspecies crosstalk.

AHL-mediated communication is the most widely studied and the best understood model for QS. In the circuit, AHL synthase encoded by LuxI homologue synthesizes AHL molecules. Short side-chain AHLs diffuse freely across cell membranes, whereas long side-chain AHLs have to use active efflux to partition to the membrane (Pearson et al., 1999). Upon reaching a threshold concentration in the extracellular medium, AHL molecules are perceived by cytoplasmic LuxR family proteins to regulate the downstream processes (Parsek and Greenberg, 2000). However, the mechanism of signal recognition and sensing in Gram-positive bacteria differ from that in Gram-negative bacteria. Sensing and recognition of the AIP occurs through a two-component signal transduction system, in which the AIP binds to a membrane-bound histidine kinase sensor and the binding information is relayed to the cell through phosphorylation of response regulator proteins that ultimately bind to the promoter of target genes to regulate gene expression (Kleerebezem et al., 1997). Referring to the AI-2-mediated QS, AI-2 synthesis is catalyzed by a highly conserved enzyme LuxS, while signals are perceived by the cell in different manners depending on the system (De Keersmaecker et al., 2006). Understanding the mechanisms of QS circuitry at the molecular level provides the basis for incorporating QS circuitry in the practical applications.

**QS and biofilm formation**: QS systems have been believed to play a prominent role in biofilm development. Biofilm formation is a step-wise process involving adhesion of cells to surface, microcolonies growth and maturation into expanding structures, and detachment of some aged microorganisms or debris (Hall-Stoodley et al., 2004). QS systems appear to be involved in all stages of biofilm formation (Parsek and Greenberg, 2005). Several microbial factors have been shown to influence biofilm formation, including motility, surface appendage expression, and extracellular polymeric substance matrix (EPS) production. QS-regulated cell surface properties alteration seems to translate to a biofilm phenotype variation (Parsek and Greenberg, 2005; Irie and Parsek, 2008).

The influence of QS on biofilm formation was first described in *Pseudomonas aeruginosa* by the Davies et al. (1998). However, subsequent studies showed that the effect of QS in *P. aeruginosa* on biofilms structure was dependent upon experimental conditions (Shroud et al., 2006). And *P. aeruginosa* employed several QS circuits to affect biofilm development. Both las and rhl acyl-HSL QS systems of *P.
*aeruginosa* were involved in rhamnolipid production which was required for maintenance of architecture in structured biofilms (Schuster and Peter Greenberg, 2006). So understanding the link between QS and biofilm formation is a complicated matter.

QS systems have been shown to be involved in biofilm formation for a variety of species. In Gram-positive bacterium *Staphylococcus aureus*, the cyclic-peptide-dependent QS system represses several surface adhesions including fibrinogen- and fibronectin-binding proteins which inhibit the contact with the host matrix and subsequent biofilm initiation (Yarwood and Schleifer, 2003). In Gram-negative bacterium *Serratia liquefaciens*, Acyl-HSL-based quorum sensing has been implicated in biofilm maturation. QS system regulates the swarming motility of *S. liquefaciens* resulting in heterogenous biofilms (Labbate *et al.*, 2004). AI-2 and LuxS QS has also been linked to the growth of biofilms. For example, AI-2 dependent QS in *Escherichia coli* alter the bacterial motility property and increase the biofilm formation (Barrios *et al.*, 2006). Moreover, the DSF-based QS system in *Xanthomonas campestris* has been proved to influence biofilm dispersal. *X. campestris* uses QS to produce an extracellular mannosidase in order to cleave xanthan which promote cell aggregation during biofilm formation (Dow *et al.*, 2003).

So far, several bacterial species are identified in wastewater systems and are known to possess QS mechanisms. Some have been proved to link QS to biofilm formation. *Bacillus subtilis* and *Vibrio cholerae* have been observed to use QS systems to control EPS synthesis and biofilm formation (Hammer and Bassler, 2003; Auger *et al.*, 2006). *Burkholderia* sp. is common bacteria in the membrane system. The cepI/R quorum-sensing system of *Burkholderia cepacia* has been shown to control biofilm maturation (Huber *et al.*, 2001). However, other bacteria species have also been detected in biofilms in many water and wastewater treatment systems, yet their precise roles of QS system in biofilm formation are still not clear. Such bacteria include *Acinetobacter* sp., *Arcobacter* sp., *Legionella* sp., *Sphingomonas* sp. and so on. Further studies need to be carried out to understand whether their QS systems are involved in biofilm development.

**Biofilms in water and wastewater treatment systems:** Biological treatment technology has long been the mainstay of the wastewater treatment. The mechanism of the method is the use of bacteria and other microorganisms to take advantage of the environmental constituents to provide the energy for microbial metabolism and the building blocks for cell synthesis, and thereby remove contaminants from the wastewater. With respect to the state of biomass involved in the reactors, the biological treatment processes can be classified into two main groups: fixed-film system and activated sludge system.

Fixed-film processes include trickling filters, rotating biological contactors and several types of reactors with fixed and moving beds. They are all dependent upon the adhesion of microbial cells to form a biofilm on the inert support medium, which usually have large specific surface area for maximum biofilm development. However, the thickness of the biofilms is critical for the wastewater purification, as oxygen can only diffuse for a certain distance through the biofilms before being utilized leaving the deeper layers of the biofilms anoxic or anaerobic. In the outer aerobic layers, biofilms are exceptionally complex communities dominated by filamentous bacteria and also containing protozoa, small metazoan, and sometimes some vertebrates (Lazarova and Manen, 1995). These heterotrophic microorganisms play an important role for the degradation of organic matter from the wastewater. It is considered that only the surface layer of the biofilms is efficient in the terms of oxidation, and so that only a thin layer of film on the reactors is required for efficient purification (Capdeville and Rols, 1992). Meanwhile, anaerobic bacteria are the most common microbes in the inner layer of biofilms because of oxygen limitation. It has been reported that the inner anaerobic biofilms comprise a greatly reduced density of microorganisms, and a larger percentage of non-viable bacteria (Lazarova and Manen, 1995). During the filter operation, excessive biofilms on the substratum are not recommended. This is because thick growth of biofilms does induce its instability and detachment from the supports, resulting in the blocking of void spaces between medium allowing the oxygen transfer and wastewater movement but not the reduction of treatment efficiency.

In the activated sludge plants, biomass is kept in suspension by stirring and aeration to contact with sewage. This biomass is also a mixture of bacteria and protozoa, together with some nematodes and rotifers. However, it is considered that activated sludge has a fairly less complex mixture of species as compared with that of biofilms on the packing of fixed-film reactors (Lazarova and Manen, 1995). The overall cell biomass in activated sludge is usually thought of as aggregated communities call flocs rather than as freely dispersed cells. Aerobic granular sludge is a gradual process developed from conventional activated sludge. The distinctions between these two processes are that during microbial granulation, regular, dense and strong granular structure forms and excellent settling property occurs, whereas activated sludge only has a very loose, fluffy and irregular structure and relies on settlement tank to accomplish the separation of sludge and treated effluent. However, the aerobic granular sludge shares many features
of biofilm systems, even though granules form through self-
imobilization of microorganisms by packing with different
bacteria species, which not fit the strict definition of
microorganisms growing on a solid support surface. The
fact is that, because oxygen and nutrient limitations in the
deeper layers of granule, there is a layered structure of
granule, which resulting in gradients of microbial populations
from aerobic bacteria, anaerobic bacteria to dead microbial
cells with the depth form the granule surface (Liu et al.,
2002). Consequently, since larger granules are easy to fall
apart due to lysis in the inner part and smaller granules
have more live cells within a given volume, smaller granules
are more effective for aerobic wastewater treatment.
Furthermore, the morphology, density and size of granular
sludge are, as in the biofilm systems, also influenced by the
hydraulic shear forces and corresponding detachment in the
reactors.

Membrane systems have been widely used in
drinking water production, wastewater reclamation and sea
and brackish water desalination due to its high water quality
and compact process design (Viswanathan et al., 2000).
However, membrane biofouling is the bottleneck that hinders
the wide application of membrane systems, because it
results in the reduced membrane flux and increased filtration
pressure, poor separation performance and contaminated
water product, frequent membrane clean and replacement,
and subsequently high operation costs (Xiong and Liu,
2010). Membrane biofouling is initiated from the attachment
of living microorganisms in the feedwater to the membrane
surface. Then, the newly immobilized microorganisms may
grow and multiply with the nutrients in the feed, forming a
biofilm layer. In contrast to other abiotic fouling, such as
organic and inorganic substances which can mostly be
removed by the efficient pretreatment, biofouling is rarely
preventable as most systems are not sterile so microorganisms can reseed and regrow at the expense of biodegradable substances in the water, turning them into metabolic products and biomass. Thus effective strategies are needed for the biofouling control in the membrane process.

In DWDS, the biofilms adhered on the surface of
pipes can be found anywhere, ranging from the starting
point of the pipes to the end of the water tap. Though
multiple approaches have been used to prevent biofilm
formation in such systems, such as keeping low nutrient
and employing residual disinfectant in the treated water
entering the distribution system, biofilms can grow eventually. Biofilm growth in DWDS can lead to
microbiological deterioration, aesthetic problem and pipe
corrosion (Wingender and Flemming, 2011). Thus
approaches to preventing or removing biofilms in DWDS
also should be developed.

**QS in water and wastewater treatment systems:** In recent
years, there has been an increasing interest in the influence
of quorum sensing signaling molecules related to treatment
performance in water and wastewater treatment systems.
Signaling compounds have been detected in different water
and wastewater environments such as activated sludge,
granule sludge, and biofouling in the membrane bioreactor.
The underlying mechanisms involved in the relationship
between quorum sensing and microbial function in these
systems are needed to be better understood, which may be
applied further for enhancement or prevention of microbial
aggregation, such as rapid granulation for wastewater
treatment or inhibition of biofouling in the membrane
bioreactor.

**Promotion of aerobic granulation by QS signals:** Activated
sludge is usually used as seed to cultivate aerobic granular
sludge. The nature of activated sludge is bioaggregates or
flocs in water treatment systems, and the bacterial community
residing in the activated seed sludge is important for the
aerobic granulation process as it may also constitute the
majority of bacteria in the aerobic granules. It appears that
QS signals can be found in the activated sludge. In a study
by Valle et al. (2004), it was reported that seven
proteobacterial strains producing compounds with AHL-
like activity were isolated from phenol-degrading activated
sludge. Moreover, addition of AHLs to the sludge samples
sustained the phenol degradation property, whereas the
sludge community function faltered and even lost without
such addition. However, a dominant functional member of
the *Thauera* genus was transiently supplanted by a member of
the *Comomonas* genus in response to AHL addition. It
suggested that AHLs played a role in mediating microbial
community dynamics and growth behavior in an industrial
activated sludge process. Similarity, AHLs were detected
from municipal, hospital, or pharmaceutical activated
sludges, and six different *Aeromonas* strains and one
*Pseudomonas* strain producing AHL-like autoinducers
were isolated from one such municipal activated sludge sample,
indicating that AHL-producers were ubiquitous in activated
sludge biomass treating different effluents (Morgan-
Sagastume et al., 2005).

Aerobic granule is a special formation of biofilm
growth of bacteria in the attached-growth mode. QS signals
also can be detected in the aerobic granular sludge. Liu et al.
(2010) used bacterial biosensors to detect AHL signals
in the sequencing batch reactors, which treated newsprint
effluent under low phosphate conditions, and reported that
more AHL formation was found in granular sludge than in
suspended floc sludge. In another study, based on the
UPLC-MS detection, possible AHL fragment is found only
for the GS extracts but not for the AS extracts, and it is much more significant for the GS intracellular extract (Ren et al., 2010). While AHL signals are merely related to the Gram-negative bacteria communication, AI-2 as an interspecies signaling molecule may be more universal in the complex water treatment systems. Some studies have reported that AI-2 signals can be detected in the biomass during the overall period of aerobic granulation (Xiong and Liu, 2010; Zhang et al., 2011). These evidences demonstrated the likelihood of production of many kinds of QS signal chemicals by granular sludge and indicated that quorum sensing could play an important role in the aerobic granulation.

So far, there are only a few publications in which authors have attempted to elucidate the possible mechanisms of QS mediated aerobic granulation. Xiong and Liu (2010) investigated the production of AI-2 signals by biomass over granule formation process. They found that AI-2 content of biomass was closely associated with the population or biomass density, in which AI-2 content of biomass started to increase markedly until the biomass density reached a critical value and once the threshold density of biomass was achieved, the AI-2 regulated change of bacterial communities occurred. These results suggested that AI-2 was essentially involved in maturation of aerobic granule, whereas it might not necessarily be required for initiating aerobic granulation. In another study, taken into account the presence of QS signals in aerobic granules, the effect of granular sludge cellular substances on the bacterial granulation process was examined (Ren et al., 2010). As a result, dosing with the mature granular sludge intracellular substances was effective in accelerating the sludge granulation process. It was considered that the QS signal substances produced by the granular sludge induced attached-growth mode bacteria in the reactor, which in turn facilitated the formation of aerobic granules and the maintenance of granular structures. In a recent study, boron as an essential element for the formation of boron complexed to (R)-4, 5-dihydroxy-2,3-pentanedione (DPD), an AI-2 signal precursor, was added to a sequencing batch reactor to stimulate aerobic granular sludge growth (Zhang et al., 2011). The result showed that boron significantly accelerated the aerobic granulation. Moreover, it was speculated that AI-2 mediated EPS production was responsible for the rapid formation of aerobic granules. Thus, increasing the intensity of QS signal molecules and its precursors in the reactor at early startup stage can initiate the bacterial granulation rapidly and make the granular structures stable, which greatly enhance the aerobic granule formation process.

**Control of membrane biofouling by QS inhibitors**: The involvement of QS in the regulation of initial microbial attachment and biofilm formation has paved the way for an intense search of strategies that can control membrane biofouling by disruption or inhibition of microbial QS systems. Therefore, studies of QS related membrane biofouling and corresponding membrane controls are discussed herein.

In view of membrane biofouling in nature is microbial attachment to membrane surface, QS signaling molecules appears to be related to membrane biofouling. Kim et al. (2009) found that both AHL and AI-2 signals could be detected on fouled RO membranes from a real water treatment plant, and that 60% of bacterial species found on the fouled membrane surface could produce at least one type of signals. In a study of biofouling in a laboratory-scale continuous membrane reactor (MBR), Yeon et al. (2009) found that AHL autoinducers were produced in the MBR, and that at least three different AHLs were in the biocake, of which N-octanoyl-homoserine lactone was the most abundant. In addition, strong AHL activity in biocake was observed simultaneously with abrupt increase in the transmembrane pressure (TMP), which implies that there was a close correlation between AHL mediated-QS and membrane biofouling. Recently, Xu and Liu (2010) reported that microbial biofilm on a nylon membrane was positively related to the AI-2 content, indicating that AI-2 mediated-QS might also be involved in the membrane biofouling. Thus, it is clear that QS systems, not only intraspecies communication but also interspecies communication, are extensively associated with membrane biofouling.

Since the signal molecule AHL is the key factor in the bacterial communication process that leads to biofilms, AHL-degrading enzymes, which inactivate the synthesized AHL signals, are expected to be a promising group of QS inhibitors to control membrane biofouling. The AHL-degrading enzymes are often classified into two groups: AHL lactonases which hydrolyse the lactone ring in AHLs, and AHL acylases which liberate a free homoserine lactone and a fatty acid (Dong and Zhang, 2005). Paul et al. (2009) reported that the biofilm formation on RO membrane surface with two selected biofouling bacteria Aeromonas hydrophila and Pseudomonas putida, which had been isolated from a biofouled RO membrane system, was suppressed in the presence of Acylase I at a concentration of 60 ug ml⁻¹. The biofilm mass on the RO membrane surface was found to gradually reduce with increasing concentration of Acylase I, however, once reached a critical concentration, further increase in the concentration of Acylase I did not result in further significant biofilm reduction. It was worth noting that Acylase I only reduced the biofilm development significantly, but did not prevent the biofilm formation completely, even at higher concentration of the enzyme. Recently, Yeon et al. (2009) have applied the Acylase I in a continuous MBR to alleviate the membrane biofouling. AS a result, the addition of Acylase I at a concentration of 10
mg l⁻¹ retarded the TMP rise compared with that of the control reactor, indicating that acylase I alleviated the membrane biofouling by quenching AHL autoinducers. Meanwhile, the organics biodegradation efficiency of the MBR was not affected by the addition of Acylase I. Furthermore, in order to promote the use of the quorum quenching technique in the MBR under a long-term continuous operation, Yeon et al. (2009) have developed a magnetic enzyme carrier (MEC), which immobilizes the quorum quenching enzyme (Acylase I) on magnetic particles. Batch type MBR experiment showed that TMP in the MEC reactor maintained during the subsequence operation, whereas that TMP in the free enzyme increased rapidly to that in the control reactor after first operation, which means that the MEC was much more stable than free enzyme in MBR systems. With the application of MEC in the continuous MBR, TMP maintained almost its initial value for the entire operations of 200 hr, whereas the membrane module in control MBR have to be replaced after 48 hr of operation due to rapid increase of TMP, which revealed that MEC reduced membrane biofouling to a large extent in the continuous MBR operation. In summary, AHL-degrading enzymes, such as Acylase I, seem to have great potential for the efficient biofouling control in the actual MBR systems. Oh et al. (2012) also encapsulated quorum quenching bacteria Escherichia coli and Rhodococcus sp. into microporous membranes and obtained a successful control of biofouling in a laboratory scale MBR.

Besides signal-degrading enzymes, most of the efforts in search of QS inhibitors have focused on the structural similarity of signal molecules, which block the signal receptor proteins and therefore prevent activation of the target gene expression (González and Keshavan, 2006). Halogenated furanone and their synthetic analogs have been proved to possess AHL-antagonistic activity, which can be attributed to a structural similarity to AHLs (Givskov et al., 1996; Maneifeld et al., 2000). It has been reported that these furanone-type QS inhibitors have ability to inhibit some AHL-regulated biofilm-related phenotypes, such as swarming motility, flagella-driven movement, extracellular biosurfactant production, and eventually control microbial attachment and biofilm formation by several bacterial species (Hentzer et al., 2002). Recently, a non-halogenated, commercially available 2(5H)-furanone was found to inhibit the AHL molecules with varying chain lengths and significantly reduced the biofilm mass of Aeromonas hydrophila isolated from a biologically fouled RO membrane on polyurethane surface, which suggested that 2(5H)-furanone could be used as potential QS inhibitor compounds that reduced the biofouling on RO membranes (Ponnusamy et al., 2010). However, furanone compounds are considered to be toxicity and chemically unstable, and not suitable for the water purification system. So far, some natural compounds extracted from plants also can inhibit bacteria QS systems. In a study by Ponnusamy et al. (2009), vanillin (4-hydroxy-3-methoxybenzaldehyde) from vanilla beans, a well-known food flavoring agent, showed significant inhibition in short-chain and long-chain AHL molecules, and repressed the biofilm formation by a reverse osmosis (RO) membrane biofilm bacteria A. hydrophila on polyurethane surface. Furthermore, in a CDC reactor under continuous flow conditions, the A. hydrophila biofilm development on RO membrane was considerably suppressed in the case of biofilm grown in the presence of vanillin (Kappachery et al., 2010). However, its 1-day old pre-formed biofilm on a membrane could not be removed and prevented from further development. Thus, it is speculated that vanillin may be used for sustainable and eco-friendly control of membrane biofouling in real MBR systems.

Blockage of signals production also can inhibit the QS systems. Since the extent of microbial attachment is associated with the AI-2 content in the suspended microorganisms, disrupting the production of interspecies AI-2 signals seems to be an alternative way to control multispecies biofilms. Several researchers have (Xu and Liu, 2010; Xu and Liu, 2011) have shown that 2,4-dinitrophenol (DNP) could inhibit ATP synthesis, resulting in lower production of AI-2, which thereby not only inhibit membrane biofouling but also enhance biofilm detachment from nylon membrane.

**Future direction**: Consideration of existence of QS system in the water treatment systems, and its potential to promote or control the biofilms in these engineered systems, which thereby impacting the system operation and efficiency, comprehensive understanding of QS processes in multispecies biofilms under complex environmental conditions is needed to be explored. However, the QS mechanisms are well studied in some model bacterial species, and little is known about QS in water technology system related species. Additionally, in most cases, the effects of QS molecules and QS inhibitors on bacterial biofilms have been investigated with pure culture under laboratory conditions. Since water treatment systems are complex, there is a need to perform such experiments within mixed cultures in pilot-scale systems under natural conditions. Furthermore, more naturally produced QS inhibitors, as well as synthesized QS derivates, should be explored. For water technology systems, some nontoxic inhibitors targeted for a wide range of bacterial species are more preferable. Overall, the induction and inhibition of QS in water and wastewater treatment systems are important future directions.

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Quorum sensing in water and wastewater treatment biofilms

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