Chemosphere 180 (2017) 396–411

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Review

Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: An overview

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HIGHLIGHTS

- EPS are of importance for microbial aggregates in biological wastewater treatments.
- EPS control can cause changes in microbial aggregates and system performance.
- EPS elevation has great potential in promoting microbial aggregates performance.
- EPS limitation has great potential in alleviating membrane fouling in MBRs.

ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form
2 April 2017
Accepted 10 April 2017
Available online 12 April 2017

Handling Editor: A. Adalberto Noyola

Keywords:
Extracellular polymeric substances (EPS)
Biological wastewater treatment systems
Membrane bioreactors (MBRs)
Microbial aggregates
Quorum sensing (QS)
Membrane fouling

ABSTRACT

Extracellular polymeric substances (EPS) are present both outside of the cells and in the interior of microbial aggregates, and account for a main component in microbial aggregates. EPS can influence the properties and functions of microbial aggregates in biological wastewater treatment systems, and specifically EPS are involved in biofilm formation and stability, sludge behaviors as well as sequencing batch reactors (SBRs) granulation whereas they are also responsible for membrane fouling in membrane bioreactors (MBRs). EPS exhibit dual roles in biological wastewater treatments, and hence the control of available EPS can be expected to lead to changes in microbial aggregate properties, thereby improving system performance. In this review, current updated knowledge with regard to EPS basics including their formation mechanisms, important properties, key component functions as well as sub-fraction differentiation is given. EPS roles in biological wastewater treatments are also briefly summarized. Special emphasis is laid on EPS controlling strategies which would have the great potential in promoting microbial aggregates performance and in alleviating membrane fouling, including limitation strategies (inhibition of quorum sensing (QS) systems, regulation of environmental conditions, enzymatic degradation of key components, energy uncoupling etc.) and elevation strategies (enhancement of QS systems, addition of exogenous agents etc.). Those strategies have been confirmed to be feasible and promising to enhance system performance, and they would be a research niche that deserves further study.

Contents

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http://dx.doi.org/10.1016/j.chemosphere.2017.04.042
0045-6535/

1. Introduction

The production of extracellular polymeric substances (EPS) is a general attribute of microorganisms in natural environments and occurs in prokaryotic and in eukaryotic microorganisms (Wingender et al., 1999a). EPS are a complex high-molecular-weight mixture of polymers, consisting of polysaccharides, proteins, humic acids, uronic acids, nucleic acids, lipids etc. (Flemming and Wingender, 2001b). The accumulation of EPS happens by a number of different mechanisms including excretion, secretion, cell lysis and sorption (Flemming and Wingender, 2001a). In biological wastewater treatments, microorganisms are in the form of microbial aggregates such as biofilms, sludge flocs and granules (Ni et al., 2009; Leng et al., 2015b; Jiang et al., 2016a), and the EPS are found to form as a layer around microbial aggregates to provide a three-dimensional protective matrix against external stress, described as “house of cells” (Flemming and Wingender, 2001a, b). It was reported that EPS were a main component in biofilms, and proportion of EPS varied from 50% to 80% (w/w) of total biofilms weight (Flemming and Wingender, 2010; Leng et al., 2015a; Meng et al., 2016). Many attempts have been made to investigate the chemical compositions and physicochemical properties of EPS, especially that of biofilms, sludge flocs and granules (Flemming and Wingender, 2001a, b; Sheng and Yu, 2006; Huang et al., 2008; Ni et al., 2009; Cao et al., 2015; Zhu et al., 2015). In recent years, the effects of EPS on properties and functions of microbial aggregates in biological wastewater treatments are paid much attention, and the EPS are proved to exhibit important roles in mass transfer (Characklis et al., 2013; Jiang et al., 2015), surface charge (Wang et al., 2006; Cao et al., 2016), flocculation, settleability, dewatering ability (Yang and Li, 2009; Peng et al., 2012; Jiang et al., 2016b), stability (Adav et al., 2008; Xiong and Liu, 2013), adhesion (Omoike and Chorover, 2006; Li et al., 2013), and formation (Zhang et al., 2014; Guo et al., 2016a; Huang et al., 2017) of microbial aggregates. Meanwhile, the occurrence or production of EPS was also reported to be responsible for membrane fouling in membrane bioreactors (MBRs), and some researchers have attributed the scaling of MBR modules to loosely bound EPS, and polysaccharides of EPS are key factor for membrane fouling (Drews et al., 2006b; Wang et al., 2009a). It should be noted that interaction of EPS with membrane surface is not well established till date, and there have been numerous theories or considerations reported in the literature which are often contradictory. Put simply, there is no doubt that EPS in microbial aggregates exhibit beneficial or detrimental role in biological wastewater treatment systems and it is expected that control of available EPS can cause changes in microbial aggregate properties and reactor performance.

Currently, several excellent reviews have highlighted roles of EPS, particularly that of microbial aggregates (Liu and Fang, 2003; Liu et al., 2004; Flemming and Wingender, 2010), in biological wastewater treatments (Sheng et al., 2010; More et al., 2014; Ding et al., 2015c; Li et al., 2015; Salama et al., 2016), in metal biosorption and bioremediation (Pal and Paul, 2008; Li and Yu, 2014), and in membrane fouling (Drews et al., 2006a; Malaeb et al., 2013; Lin et al., 2014c). However, considering the dual roles of EPS in biological wastewater treatments, there is still no consolidated report so far, which highlights EPS controlling strategies for enhancement of system performance. This review will present current state-of-the-art knowledge with regard to EPS basics and their brief roles for microbial aggregates in biological wastewater treatment systems, and special emphasis has been laid on EPS controlling strategies (limitation or elevation), which will provide useful information to scientists and engineers who work in this field.

2. Basics of extracellular polymeric substances (EPS)

2.1. Formation mechanisms of EPS

The formation of EPS by microbial aggregates has various origins including active secretion from microorganisms, cell surface material shedding, cell lysis, and sorption from environment (Wingender et al., 1999a; Liu and Fang, 2003). It is clear that the formation of EPS involves complicated mechanisms. EPS may be actively secreted by the living cells, and various specific pathways of biosynthesis and discrete export machineries involving the translocation of EPS across bacterial membranes to cell surface or into the surroundings have been described for bacterial proteins (for review see, e.g., Filloux et al., 1998) and polysaccharides (for review see, e.g., Jonas and Farah, 1998). Another mechanism of EPS release is spontaneous liberation of integral cellular components from outer membrane of Gram-negative bacteria which occurs by formation of the outer membrane-derived vesicles (Beveridge et al., 1997; Li et al., 1998). Release of cellular material by surface blebbing during normal growth may be the result of metabolic turnover

processes (Wingender et al., 1999b). Death and lysis of cells contribute to the release of cellular compounds into the surroundings as part of the EPS. The EPS shed from microbial aggregates can be adsorbed in other places (Harris and Mitchell, 1973; Wingender et al., 1999a). It is known that EPS production is strongly associated with microbial growth and substrate consumption, and thus is influenced by many factors that govern bacterial metabolism (Laspidou and Rittmann, 2002; Ye et al., 2011a; Ni and Yu, 2012). Due to biodegradation of EPS, production of EPS can also be regarded as the result of excretion and consumption of microbial cells in a certain condition (Laspidou and Rittmann, 2002). Currently, it was reported that one of important regulatory mechanisms controlling EPS production was quorum sensing (QS) regulation (Waters and Bassler, 2005). QS is a signaling system between cells that functions via chemical signals such as N-acyl homoserine lactone (AHL) that allows the expression of specific genes in a manner that depends on population density (Shrout and Nerenberg, 2012; Huang et al., 2016b). There are two types of QS signaling systems used by bacteria including the autoinducer-1 (AI-1) type mainly involved in intra-species communication and the AI-2 type provided for inter-species interaction (And and Bassler, 2001). It is noted that QS regulatory mechanisms on EPS production are diverse in different bacteria species (Kalia, 2013). It has also already been clear that second messenger cyclic diguanylate (c-di-GMP) positively modulated the production of EPS matrix components at transcriptional and allosteric level for several Gram-negative species (Simm et al., 2004; Hickman et al., 2005; Thormann et al., 2006; Gjermansen et al., 2007; Borlee et al., 2010). The emerging paradigm was that high intracellular levels of c-di-GMP promoted biofilm lifestyle through EPS matrix production whereas lower levels of c-di-GMP promoted motility and the planktonic lifestyle. A clear manifestation of this principle was seen in *Pseudomonas aeruginosa* where CdrA expressed in response to the high c-di-GMP was secreted outside the cell and bond to the Psl polysaccharide contributing to the EPS matrix production (Starkey et al., 2009; Borlee et al., 2010). Certainly, formation of EPS has been believed to serve many functions, and EPS formation mechanisms from physiological determinants and molecular aspects are worthy of the continuous exploration in future research.

2.2. Important properties of EPS

EPS exhibit great effect on properties of microbial aggregates based on their special characteristics including adsorption, adhesion, hydrophobicity/hydrophilicity, surface charge and biodegradation. EPS typically contain abundant charged functional groups and non-polar groups (e.g., aromatics, aliphatics in proteins and hydrophobic region in polysaccharides), which indicates that EPS are amphoteric to some extent (Nguyen et al., 2012; Kim et al., 2015). Hydrophobicity/hydrophilicity of EPS is related with EPS composition and characteristics, and directly influence adhesion, flocculation, settling and dewatering properties of microbial aggregates (Liu and Fang, 2003). Based on the presence of abundant charged groups, EPS have a high binding capacity with heavy metals (Liu and Fang, 2002; Feng et al., 2010; Ha et al., 2010), and the adsorption followed the Langmuir or Freundlich equations (Bhaskar and Bhosle, 2006; Moon et al., 2006). EPS, by means of presence of multivalent cations (e.g., Mg^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}) were found to exhibit great roles on structure of microbial aggregates and their flocculation (Li et al., 2012; Konczak et al., 2014). The hydrophobic regions in EPS also contribute to organic pollutants adsorption (Esparza-Soto and Westerhoff, 2003; Sheng et al., 2008). In addition, EPS exhibited high adhesion to substratum surfaces, altered substrata physico-chemistry, and affected initial microbial adhesion processes (Feng et al., 2009) whereas adhesion of microbial cells to solid surface resulting from EPS deposition also resulted in biofouling or biocorrosion (Sand and Gehrke, 2006; Lin et al., 2014c). EPS had part of high molecular weight (MW) molecules and a broad MW distribution (Tansel et al., 2006), and MW distribution of EPS can affect stability of sludge flocs, and exhibit a role in membrane fouling (Duan et al., 2013). Parts of EPS can be reused by their own producers and by other microorganisms for cell proliferation in case of substrate shortage (Zhang and Bishop, 2003). Importantly, it should be noted that EPS degradation with reduction of EPS contents also would cause deflocculation of sludge flocs, exhibiting the complicated effects on membrane fouling.

2.3. Elucidation of the key components of EPS

The EPS key components have been believed to include proteins, polysaccharides, humic-like substances, uronic acids, nucleic acids and lipids (Wingender et al., 1999a; D'Abzac et al., 2010a; D'Abzac et al., 2010b), and certain studies have suggested that compositions and properties of EPS rather than quantity, had greater influences on some functions of microbial aggregates (Basuvaraj et al., 2015). Different components exhibit their own specific roles, largely determining properties and functions of EPS and microbial aggregates. Specifically, the main roles of extracellular proteins in microbial aggregates were multivalent cations and organic molecules binding, as well as in catalysis and degradation (Zhang et al., 2015). Furthermore, amino acid compositions and secondary structures of proteins in EPS significantly contributed to hydrophobic interactions and further to high aggregation activity of microbial aggregates (Hou et al., 2015; Yin et al., 2015). Polysaccharides had high molecular weight (>100 KDa), and the structure of the long carbon backbone with active side chains was responsible for high flocculation of EPS (Yuan et al., 2011; Yin et al., 2015; Huang et al., 2016e). The gel-forming property of EPS resulting from high-MW and cross-linked structure of polysaccharides was expected to exhibit some influences on cell aggregation (Wang et al., 2009b; Seviour et al., 2010). Specifically, due to the high abundance of some functional groups, such as carboxyl ($-COO^-$), roles of polysaccharides in cell aggregation could be greatly improved with the presence of multivalent ions (Yuan et al., 2011; Yin et al., 2015; Huang et al., 2016d). Besides the pure form of polysaccharides and proteins, those molecules also present in composite form through covalent bonds, and their composition and structure have been confirmed to exhibit effects on formation and stability of microbial aggregates (Garnier et al., 2005; Park and Novak, 2009; Bourven et al., 2015; Ding et al., 2015b). The important properties of humic substances were reported to be adhesion, as well as electron donor or acceptor, and exhibited minor roles in flocculation and biosorption of EPS, impact of which largely depended on their concentration and the nature (Wingender et al., 1999a; Flemming and Wingender, 2010; More et al., 2014). Extracellular DNA (eDNA) was reported to function as a scaffold offering structural integrity to the EPS matrix, and subsequently determine the three-dimensional architecture of biofilms (Wingender et al., 1999a; Flemming and Wingender, 2010; Das et al., 2013). eDNA influenced zeta potential and hydrodynamic diameter of bacterial cell, and cell surface hydrophobicity, and the thermodynamic analyses revealed that eDNA could introduce the favorable acid-base interaction responsible for bacterial aggregation and adhesion to surface, as well as biofilm formation (Das et al., 2010, 2011a, 2011b). Extracellular lipids with surface-active properties have been proved to protect bacteria in surface environments from strong surface tension of surrounding water, thereby facilitating bacterial growth on solid surfaces (Wingender et al., 1999a; Flemming and Wingender, 2010).

Up to now, detailed roles of EPS components are still continuing

to explore, and it remains arguable that which component of EPS shows major part in function studies (e.g., flocculation, adsorption, stability) of microbial aggregates. However, according to Seviour et al. (2009), proteins and polysaccharides both were able to form the gels whereas polysaccharides had a lower critical gelling concentration than proteins, and given that proteins and polysaccharides were cross-linking as composite, an attempt to differentiate their own roles appeared to be misleading (Huang et al., 2002; Seviour et al., 2009; Ding et al., 2015c). Hence, a different view, that more attention should be focused on mechanisms of such cross-links rather than finding which EPS component is major role, could be of great importance in further study of microbial aggregates. Meanwhile, it is also worth noting that composition and content of EPS in microbial aggregates are highly variable, which is due to factors such as substrate type, growth phase, sludge type, operation condition, extraction technique, and analytical method (Nielsen and Jahn, 1999; Raszka et al., 2006; Tang et al., 2008; Sheng et al., 2010), and thus the further study of EPS composition pattern at different factors should be needed.

2.4. Differentiation among the sub-fractions of EPS

EPS can be subdivided into two major fractions: bound EPS (capsular polymers, loosely bound polymers, condensed gels, sheaths, and attached organic materials) and soluble EPS (soluble macromolecules, slimes, and colloids) (Nielsen and Jahn, 1999; Laspidou and Rittmann, 2002; Comte et al., 2006; Yu et al., 2008). Soluble EPS, also called soluble microbial products SMP, are weakly bound with cells or absolutely dissolved into bulk solution whereas bound EPS form a discrete covering layer with distinct margin outside all cell walls (Laspidou and Rittmann, 2002; Yu et al., 2009). For the sake of convenience, the accepted EPS definition in literature and this review without being specified are bound EPS. Moreover, bound EPS exhibit a dynamic double-layer-like structure (Fig. 1) (Nielsen and Jahn, 1999; Lin et al., 2014c), and can be classified as tightly bound EPS (TB-EPS) forming inner layer and loosely bound EPS (LB-EPS) diffusing in outer layer by extraction methodology (Li and Yang, 2007).

There exist the differences in physical and chemical nature of these different EPS fractions, and functions of microbial aggregates through different fractions of EPS are distinct. Soluble and bound EPS were reported to have different component contents, which leads to differences in metal biosorption activity and organic pollutant binding capacity (Comte and Baudu, 2006; Pan et al., 2010). Moreover, it was also found that soluble and bound EPS in the treated sludge by different bacterial strains exhibited the distinct kaolin flocculation and dewatering potential (Subramanian et al., 2010; More et al., 2012). In term of further fractions (TB-EPS and LB-EPS) of bound EPS, Tsai et al. (2008) found that large molecules (MW > 20000Da) in TB-EPS accounted for 22% of the total dissolved organic carbon (DOC), and were protein-like substances of high aromaticity, whereas LB-EPS were organic-acid-like substances of low-intermediate aromaticity. In a study by Guo et al. (2016b), the findings revealed that hydrophobic groups, especially protein-related N-H, were present in greater proportion in TB-EPS extracted from activated sludge, and TB-EPS fraction contained a greater proportion of proteins and a smaller percentage of polysaccharides than did LB-EPS (Basuvaraj et al., 2015; Guo et al., 2016b). Different bound EPS fractions possess different characteristics of organic matters and cations, and Yu et al. (2009) showed that the TB-EPS extracted from sludge exhibited the characteristics of the high contents of macromolecules (330-1200 KDa) and trivalent cations (Fe^{3+} and Al^{3+}) as compared to that of other fractions, contributing to a greater flocculating rate to kaolin suspensions. On the other hand, Li and Yang (2007) suggested that polysaccharide rich LB-EPS exhibited negative effect on bioflocculation, sludge settleability and dewatering. The performance parameters of sludge-water separation, such as sludge volume index SVI, sludge retention time SRT and effluent suspended solids ESS, were strongly relevant to LB-EPS content in sludge whereas no

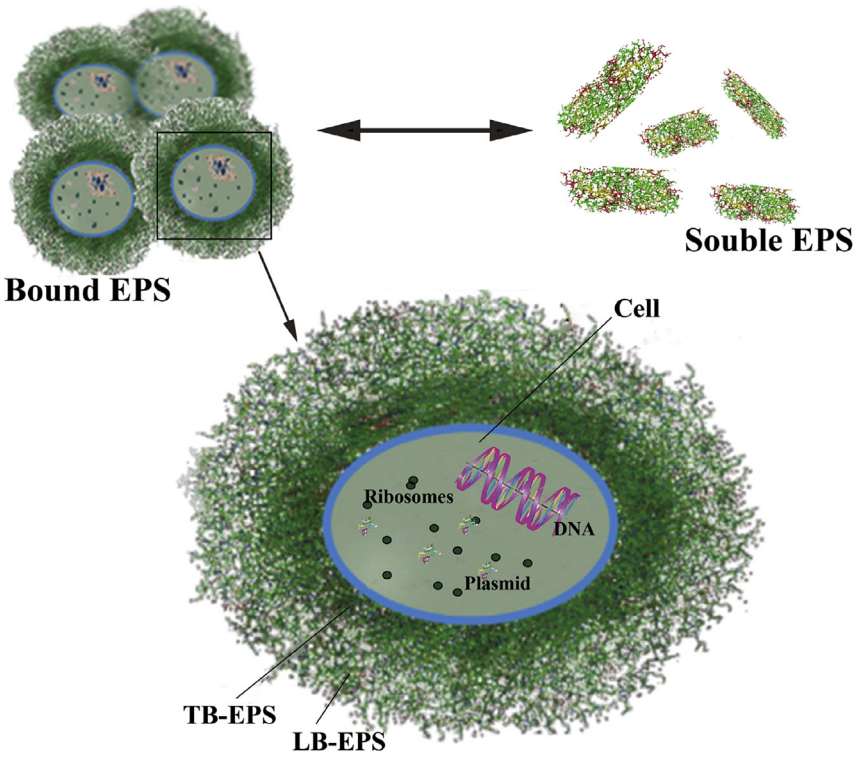

Fig. 1. Schematic of extracellular polymeric substances (EPS) structure.

correlation was found with TB-EPS amount. Although LB-EPS content was less than 1/5 of the total EPS in this study, excessive EPS in the form of LB-EPS would deteriorate cell attachment and weaken microbial aggregate structure, which resulted in poor bioflocculation, greater cell erosion and retarded sludge-water separation. In addition, the fouling potentials of membrane by different EPS fractions have been proved to be distinct, and LB-EPS was more significantly correlated with membrane fouling as compared with TB-EPS fraction (Ramesh et al., 2007; Wang et al., 2009a). Certainly, the characteristics of different EPS fractions have different effects on microbial aggregates, and further identification and elucidation on roles of TB-EPS, LB-EPS and SMP in microbial aggregates could be of great importance in manipulating functions of microbial aggregates.

3. EPS implications in biological wastewater treatments

In biological wastewater treatments, the presence of EPS has been confirmed and observed through various electron microscopy techniques. Currently, several excellent reviews have present the potential roles of EPS of microbial aggregates in biological wastewater treatment systems (Sheng et al., 2010; Lin et al., 2014c; More et al., 2014; Ding et al., 2015c; Salama et al., 2016), and the EPS are proved to involve in both beneficial and detrimental characteristics of microbial aggregates, and then influence performance of biological wastewater treatments. Hence, in the following, the overall roles of EPS in three common biological wastewater treatments including fixed-film processes, activated sludge processes and MBRs, will be briefly summarized and discussed.

3.1. EPS overall roles in fixed-film processes

Fixed-film processes are all dependent on the adhesion of microbial cells to form a biofilm on the inert support medium with a large specific surface area for maximum biofilm development to remove dissolved and colloidal organic pollutants (Shrout and Nerenberg, 2012; Huang et al., 2016b). Specifically, biofilm represents the core component in biofilm reactors, and its property is crucial to overall performance and efficiency of treatment as well as effluent quality. In most biofilms, microorganisms are less than 10% of the dry mass whereas the EPS matrix is over 90% (Flemming and Wingender, 2010). The EPS matrix has been confirmed to provide mechanical stability of biofilms, mediate their adhesion to surfaces and then develop cohesive, three-dimensional architecture that interconnects and transiently immobilizes biofilm cells (Flemming and Wingender, 2010; Flemming et al., 2016; Huang et al., 2016a). In addition, the EPS matrix in biofilms not only acted as a versatile external digestive system by retention of extracellular enzymes, enabling them to metabolize dissolved, colloidal and solid biopolymers from water phase, but also acted as a recycling centre by keeping all of the components of available lysed cells (Flemming and Wingender, 2010; Tielen et al., 2013; Flemming et al., 2016). Put simply, there is no biofilm without an EPS matrix; EPS and biofilm properties are strongly associated, and it expects that EPS could largely determine properties of biofilms, thereby affecting performance of fixed-film processes.

3.2. EPS overall roles in activated sludge processes

Activated sludge processes are based on biological coagulation, adsorption and oxidation of microorganisms in the sludge to remove the bulk of organic compounds. Specifically, sludge properties are crucial to overall performance and efficiency of the treatment as well as effluent quality. EPS are predominant constituents of sludge, and the mass of EPS represents up to 80% of the mass of activated sludge (Yu et al., 2006). In conventional activated sludge processes, EPS were involved in the sludge structure and the interactions between cells. EPS were expected to govern sludge stability, and a higher EPS content in the sludge would result in greater sludge stability (Mikkelsen and Nielsen, 2001; Adav et al., 2008). Sludge hydrophobicity increased with protein content of EPS, and an increased hydrophobicity generally leads to better flocculation (Liu and Fang, 2003). Most studies showed that sludge settled less well, as measured by SVI, with increased amount of EPS, and thus EPS exhibited the negative effect on sludge settleability (Jin et al., 2003). The EPS were also considered as key factors in dewatering ability of activated sludge, and an increase in EPS generally resulted in a poorer sludge dewatering whereas the inconsistent results had also been found that sludge dewatering improved as EPS content increased (Jin et al., 2004; Sheng et al., 2010). In sequencing batch reactor activated sludge processes (SBRs), EPS are of importance for formation as well as structural and functional integrity of aerobic granule sludge. Specifically, EPS exhibit essential roles in sludge granulation, and can bind cells through ion bridging interaction, hydrophobic interaction and polymer entanglement, serving to enhance and promote the formation of granules (Liu et al., 2004; Wang et al., 2005; Sheng et al., 2010; Ding et al., 2015c). Put simply, EPS and sludge properties are highly linked, and it expects that the EPS could largely determine the properties of sludge, thereby affecting performance of activated sludge systems.

3.3. EPS overall roles in membrane bioreactors (MBRs)

Membrane bioreactors (MBRs) have been considered as a high quality wastewater treatment technology of choice for various wastewater treatments, which consist of common bioreactors with membrane filtration units for biomass retention (Huang et al., 2010, 2016b). Not surprisingly, membrane fouling has been, and continuous to be, one of the main obstacles for their wider application (Huang et al., 2010). In the MBRs, EPS have been identified as important membrane foulants, and generally concentrations and characteristics of EPS are two important factors that determine the extent and severity of fouling condition (Gao et al., 2011; Silva et al., 2016). Specifically, EPS exhibit profound impacts on membrane fouling in MBRs through different mechanisms including pore clogging, formation of gel layer and cake layer, changes of foulants layer and osmotic pressure effect (Zhang et al., 2006; Lin et al., 2010). Specific resistance of extracted EPS alone was reported to be of the order of 10^{16}-10^{17} $m^{-1}kg^{-1}$ (Nagaoka et al., 1996), which was at least 1000 times higher than the specific filtration resistance of flocs (Hong et al., 2007). In addition, EPS can be considered as material base or medium of membrane fouling in MBRs, through which, other foulants such as SMP (Laspidou and Rittmann, 2002), dissolved organic matters (DOM) (Meng et al., 2009), biopolymer clusters (BPC) (Wang and Li, 2008), and colloids (Lin et al., 2014b) could exhibit roles in membrane fouling in a direct or indirect manner. Certainly, EPS exert considerable influences on the characteristics of sludge mixture liquor such as surface properties and morphology, which is also regarded as key fouling factor in MBRs (Lin et al., 2014a). Put simply, EPS and membrane fouling are closely related, and it suggests that EPS could largely affect membrane performance, thereby influencing the performance of MBRs.

Based on the two-sided roles of available EPS in biological wastewater treatments, combining with EPS basics in microbial aggregates described in Sections 2.2–2.4 (i.e. correlations between properties, compositions and sub-fractions of EPS and microbial aggregates), it appears to be a promising strategy to manipulate and improve system performance by control of EPS. Therefore, the controlling strategies of available EPS (i.e. limitation and elevation

of EPS), which would have great potential in promoting microbial aggregates performance and in alleviating membrane fouling, should be worthy of attention.

4. Limitation and elevation strategies of available EPS

4.1. Limitation strategies of available EPS

4.1.1. Inhibition of quorum sensing (QS) systems

Quorum sensing (QS) is a cell-to-cell communication process where bacteria can secrete and sense chemical signals, and then regulate gene expression in response to population density (Shrout and Nerenberg, 2012; Huang et al., 2016b). Numerous types of signaling molecules used by bacteria and a variety of different genes regulated by QS for different bacteria have been investigated in depth. One of the important signals, N-acyl homoserine (AHLs) produced by Gram-negative bacteria, has been found to influence EPS production, biofilm formation, surface motility, interspecies competition, and growth regulation (Shrout and Nerenberg, 2012). Specifically in AHL dependent QS regulatory system for Gram-negative bacteria, AHLs are produced constitutively by AHL synthase gene (such as *luxI*), and are distributed out cells at a low cell density, and get accumulated in surroundings (Rasmussen and Givskov, 2006; Kalia, 2013). At higher density, AHL signals bind to and activate their receptor protein (LuxR), and then activated AHL-LuxR protein complex most often homodimerizes and binds adjacent to the QS promoters, which activates expression of target genes (e.g., EPS formation) controlled by QS (Fig. 2) (Geske et al., 2008; Huang et al., 2016b). Biological wastewater treatments contain large quantities of dense microbial consortia in the form of biofilm, flocs or granules, in which Gram negative bacteria hold the dominant position and use AHLs as their major language to communicate with each other (Dobretsov et al., 2009). It has been found that *Vibrio cholerae*, transcriptional regulator hapR-AHL, was responsible for EPS synthesis and biofilm formation (Hammer and Bassler, 2003). *Pseudomonas* and *Xanthomonas* were also confirmed to produce AHLs and diffusible signal factor (DSF) respectively, to trigger EPS synthesis (Sakuragi and Kolter, 2007; He and Zhang, 2008). Moreover, AHLs been detected in both activated sludge and aerobic granular sludge, and high contents of AHLs in granular sludge were positive related with higher EPS content (Ren et al., 2010; Li et al., 2014b; Lv et al., 2014). Further, Li and Yu (2014) showed that AHL-based QS could regulate EPS contents and its component proportion in aerobic granulation. In addition, it was also reported that EPS production in mixed liquor and on membrane surface in MBRs was strongly dependent on AHL-based QS systems (Yeon et al., 2009; Kim et al., 2011, 2013a; Maqbool et al., 2015).

Based on QS-regulated control of EPS production, it is possible to influence EPS production by disrupting QS in a directed manner (Waters and Bassler, 2005). Currently, interference with QS systems can be achieved mainly by inhibiting the synthesis of signals, degrading signals, interfering with signal receptors, or blocking the formation of signal/receptor complex (Fig. 3) (Lade et al., 2014b, a; Zhang and Li, 2016). The molecules for inhibition of signal-based QS systems or signal-controlled phenotypes are called as quorum sensing inhibitors (QSIs). Many natural compounds were considered as QSIs, which have similar structure to those of QS signals and thus antagonize them and also have ability to degrade LuxR/LasR signal receptors (Teplitski et al., 2011; Lade et al., 2014b), and the presence of those has been found to significantly influence EPS production. For example, the *piper betle* extract (PBE) could inhibit the production of AHLs, as well as reduce biofilm formation and EPS production caused by *Pseudomonas aeruginosa* and bacterial consortium without raising selective pressure for microorganism growth (Siddiqui et al., 2012a, 2012b). Inactivation of AHLs by vanillin was reported to cause the depression and proportion changes in EPS components, and weaken the stability and strength of granular sludge (Lv et al., 2014; Zhao et al., 2016). In addition to QSIs, enzymatic inactivation of AHLs, including AHL-lactonases,

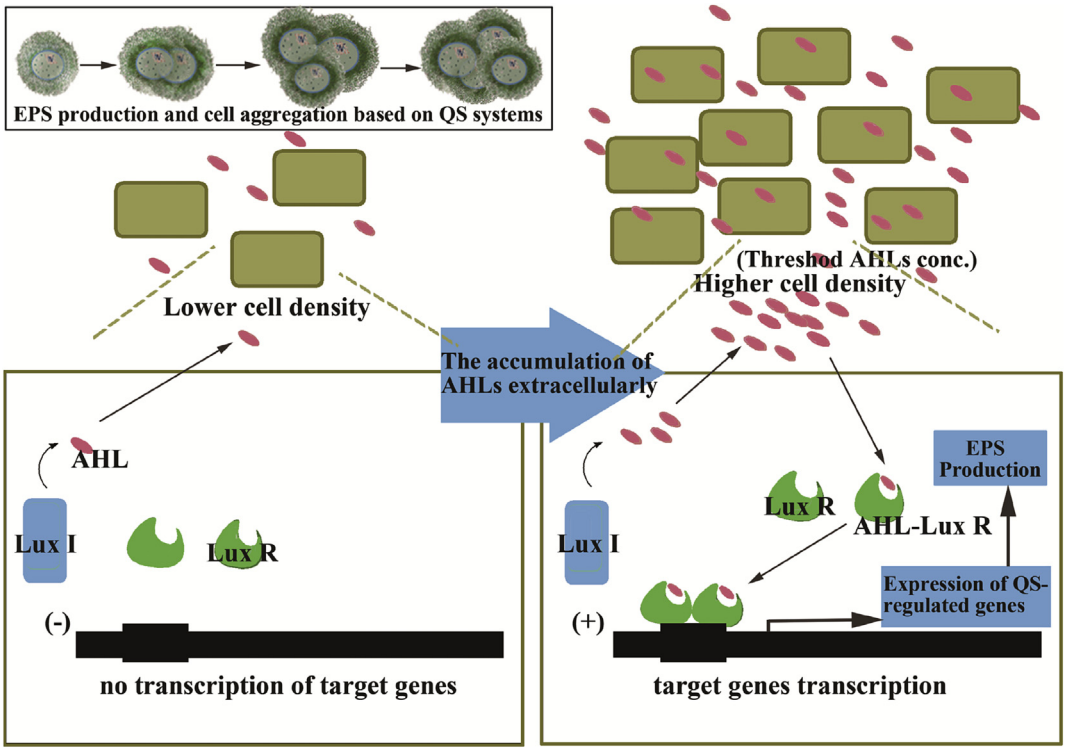

Fig. 2. Schematic representation of AHL-dependent LuxI/LuxR regulatory system.

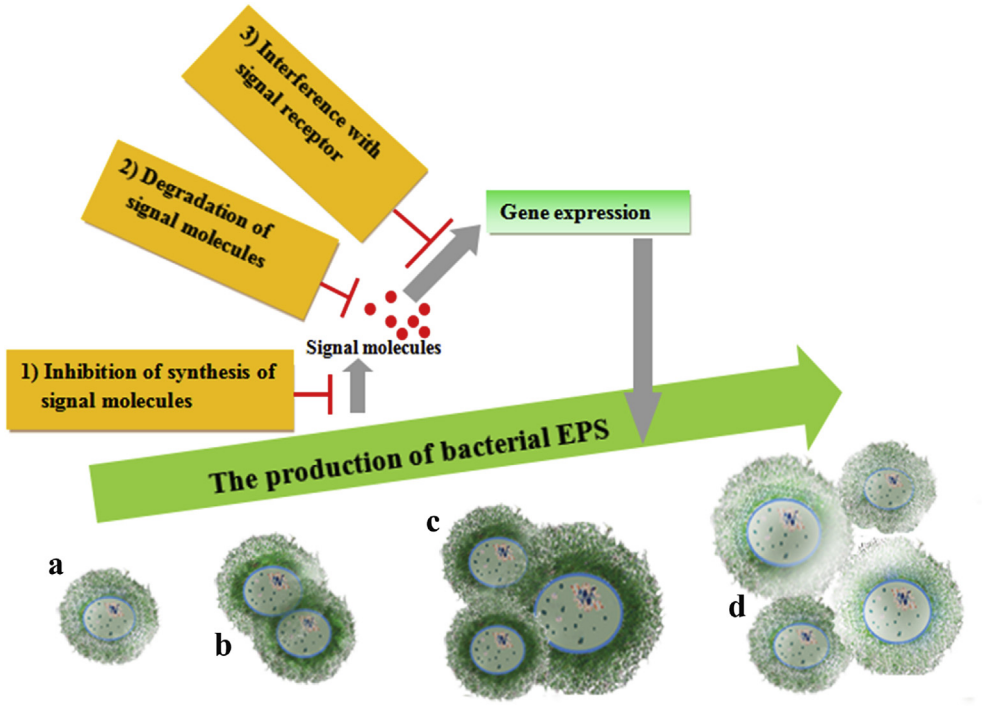

Fig. 3. Schematic diagrams of the interruption of QS systems and EPS inhibition. Fig. 3a–c represents EPS production process (e.g., the increase of EPS contents and cell aggregation); Fig. 3d represents EPS inhibition by QS interruption (e.g., the decrease of EPS contents and cell aggregation).

AHL-acylases and oxidoreductases, is a feasible alternative to inhibit bacterial QS systems for limitation of EPS production. Specifically, acylases can degrade AHLs by hydrolyzing amide moieties, and have been noted in various bacteria such as *P. aeruginosa* (Sio et al., 2006) and *Bacillus cereus* (Sunder et al., 2012). Lactonases degraded AHLs through hydrolysis of core lactone ring, and are of bacterial origin such as *Agrobacterium tumefaciens* (Liu et al., 2007) and *Rhodococcus* sp. (Uroz et al., 2008). Oxidoreductases from *Rhodococcus erythropolis* and *Bacillus megaterium* are found to catalyze AHL acyl side chain in an oxidative or reductive manner, and make AHLs structure modified without degradation (Uroz et al., 2005; Chowdhary et al., 2007). Certainly, the disruption of QS by enzymatic inactivation of AHLs has been proved to inhibit EPS production, induce EPS matrix damaged and disrupt stability of granular sludge (Li and Zhu, 2014; Li et al., 2014a). Currently, in order to overcome limitation of short half-life and low efficiency utilizing free enzymes, immobilized enzymes on solid materials including nanofitration (NF) (Kim et al., 2011) and magnetic enzyme carrier (MECs) (Yeon et al., 2009; Lee et al., 2014), as well as encapsulation of bacteria producing these enzymes (i.e. quorum quenching bacteria) (Oh et al., 2012; Kim et al., 2013b; Cheong et al., 2014), have also been applied to inactivate AHLs and reduce EPS production, contributing to mitigation of membrane fouling. It is well-known that the QS system is not a must for microbial growth (Lade et al., 2014b; Huang et al., 2016b; Zhang and Li, 2016), and it appears from above discussions that gaining better understanding for the QS-disruption strategies would be of great importance for limitation of available EPS without influencing microbial growth.

4.1.2. Optimization of external environmental conditions

The EPS secretion from the cells in their growth environment can be influenced by environmental conditions governing bacterial metabolism, and those conditions can be classified into three categories: feedwater characteristics, operational parameters and exogenous substances (Fig. 4). Specifically in term of feedwater characteristics, a profound cognition is that substrate type has great effect on microbial communities in sludge and microbial metabolism, and hence influences EPS production. Li and Yang (2007) found that activated sludge fed with acetate had lower EPS production than that fed on glucose, but the acetate-sludge had higher LB-EPS whereas TB-EPS and protein contents were independent of carbon source (Ye et al., 2011a). Changing the wastewater from chemical, leather and dye to wine and municipal treatments was reported to cause an increase in protein and a decrease in DNA of EPS (Sponza, 2002, 2003). Meanwhile, substrate concentration also has greater influence on EPS production and composition (Ye et al., 2011a). A profound emphasis is given to C/N (carbon/nitrogen) ratio in relation to EPS, and Ye et al. (2011b) reported that the C/N ratio of 20 was favorable for EPS production, and in LB-EPS polysaccharide content decreased whereas protein content increased at decreased C/N radio (from 100 to 20). It was found that EPS production was improved in case of phosphorus deficiency (Liu et al., 2006; Fang et al., 2009), and nitrogen deficiency was relation with increased protein contents in EPS (Sponza, 2002). In addition, presence of salinity in feedwater has been associated with production of EPS, and an increase in salinity resulted in an increase in EPS content as well as the change of functional groups and conformations in EPS components (Reid et al., 2006; Kokabian et al., 2013). Specifically in term of operational parameters, pH, temperature, and dissolved oxygen (DO) are important factors affecting production of EPS. The effect of pH on EPS production varied with different microorganisms, medium composition and operational conditions (Shu and Lung, 2004). The pH determined the morphological changes of the cells; generally the optimal medium pH for EPS production of the same microorganism varied between 5.0 and 7.0 (More et al., 2014). Some of the microorganisms produced more EPS in acidic pH 5.5–6.5 (Lee JWYeomans et al., 1999). The extreme pH profiles (pH 2.0–3.0 or pH $\geq$ 10) inhibited not only the process of microbial growth but also the EPS biosynthesis due to cell morphological change (Lindsay et al., 2000; Czaczyk and Myszka, 2007; Guo et al.,

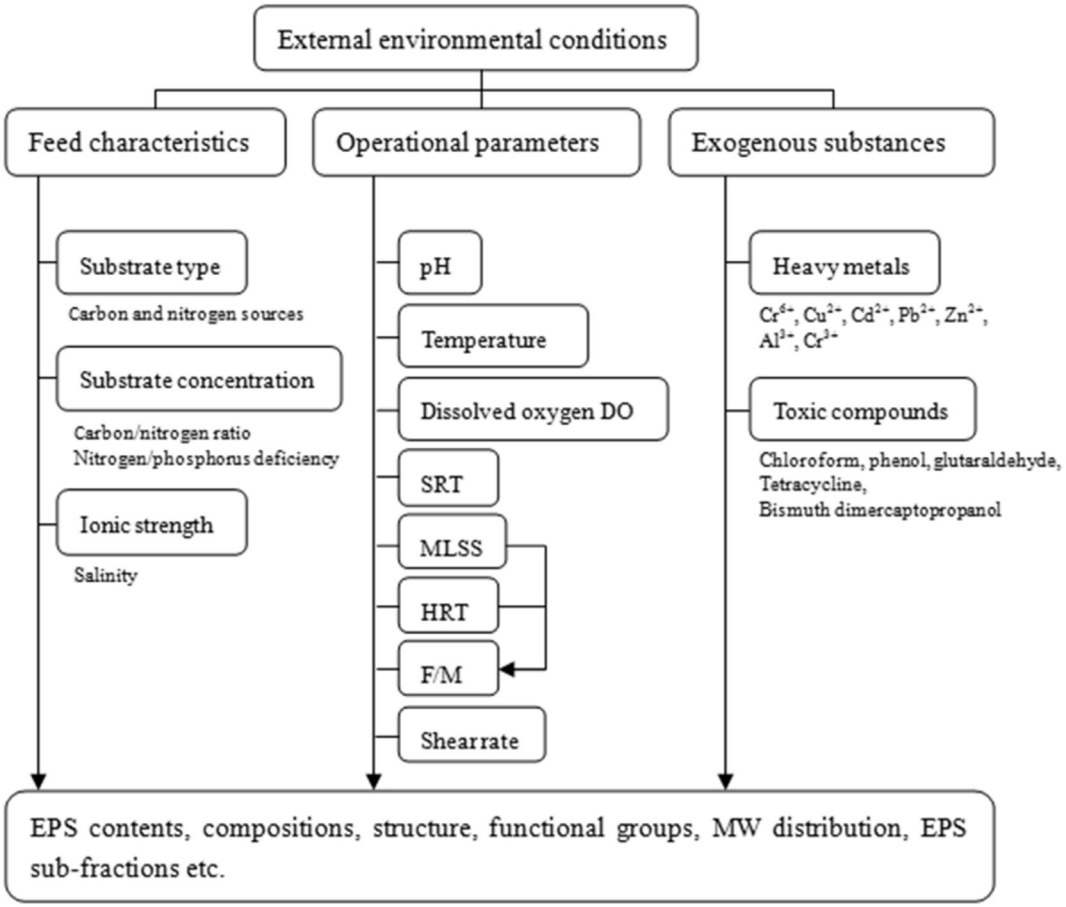

Fig. 4. Schematic of external environmental conditions affecting EPS characteristics.

2010). The pH also was found to influence MW distribution of EPS, and the relatively high MW EPS with a lower yield was obtained at low pH while the relatively low MW EPS with a high yield was obtained at higher pH (Shu and Lung, 2004). Moreover, both extreme high and low temperature have been also reported to increase the production of EPS and cause the change of EPS composition (Wang et al., 2010; Gao et al., 2012), which might be as a response of bacteria to protect themselves against environment stress. It can be seen that low DO caused EPS production, and generally the increases in polysaccharide, protein, and DNA levels of EPS were observed at DO level as low as 0.5–2 mg/L (Kim et al., 2006; Gao et al., 2011). In addition, other operational parameters, including sludge retention time SRT, mixed liquor suspended solids MLSS and hydraulic retention time HRT, also had complex relationship with the production of EPS, and the results in literature were somewhat contradictory. For instance, some researchers found that an increase in SRT was positively related to the total EPS quantity as well as the contents of proteins and polysaccharides in EPS (Sesay et al., 2006; Patsios and Karabelas, 2011) while others had suggested that EPS was negatively related to SRT (Ahmed et al., 2007; Duan et al., 2015), even or independent of SRT (Liao et al., 2001; Li and Yang, 2007). The inconsistent or even controversial conclusions might be partly attributed to different system types and evaluation methods used in those studies, and unknown mechanisms controlling EPS production in sludge flocs. It should be noted that MLSS and HRT are parameters relating food to microorganism (F/M) ratio exerted on bacteria. An increase in F/M ratio in a certain range would induce more EPS production, which is due to that EPS production is growth-related and in proportion to substrate utilization (Dvorak et al., 2011). Another operational factor, shear rate, can also affect EPS contents and compositions. It was found that sludge EPS content increased with an increase in shear rate or aeration intensity (Adav et al., 2007), and specifically polysaccharide contents in EPS increased with increasing air flow rate whereas protein contents remained almost unchanged at various air flow rates, which indicated that shear may stimulate bacteria to produce more polysaccharides (Shin et al., 2001). Specifically in term of presence of exogenous toxic substances, notably, in case of the condition of heavy metals (e.g., Cr^{6+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+} and Cr^{3+}) and some toxic compounds (e.g., chloroform, phenol, glutaraldehyde and tetracycline), more EPS were produced in activated sludge and biofilms, which might be as a vital self-protection mechanism of microbial cells against the harsh environment (Priester et al., 2006; Shi et al., 2013; Li and Yu, 2014), and specifically the high production of protein far exceeded than that of other components in EPS (Shi et al., 2013). However, toxic substances, such as bismuth dimercaptopropanol, can also directly inhibit production of EPS (Badireddy et al., 2008). Badireddy et al. (2008) reported that EPS expression by *Brevundimonas diminuta* was greatly reduced at levels below the minimum inhibitory concentration, and specifically total polysaccharide and protein contents decreased by approximately 95% during a 5-day period at a level near the minimum inhibitory concentration.

There is no doubt that external environmental conditions exhibit significant effect on EPS production and composition, although the inconsistent or even controversial results are always obtained from different studies. Hence, it suggests that limitation of available EPS could be achieved by optimizing adjustment of

external environmental conditions, along with meeting favorable conditions for microbial growth. Meanwhile, in the future, more detailed investigations on the relationship between environmental conditions and EPS in a certain system would be essential and conductive to achieve the better operable strategies to limit available EPS.

4.1.3. Enzymatic degradation of the key components

The key components of EPS possess their own roles and contribute to maintain the EPS matrix structure and functions, and thus the disruption of key components in EPS could be a feasible and effective approach for limitation of available EPS. Currently, proteins and polysaccharides are two main constituents of the most EPS secreted by bacteria, and hence two main EPS-degrading enzymes including proteolytic enzymes for protein hydrolysis (e.g., proteinase K, trypsin, subtilisin etc.) and polysaccharases for polysaccharide hydrolysis (e.g., Dispersin B, Mutanase, dextranase etc.) have been reported and applied for disruption of EPS matrix structure and further disintegration of microbial aggregates such as biofilms and aerobic sludge (Loiselle and Anderson, 2003; Petersen et al., 2005; Chaignon et al., 2007; Leroy et al., 2008a; Flemming and Wingender, 2010; Guezennec et al., 2012; Xiong and Liu, 2013). More specifically, proteinase K, as a broad-spectrum protease, is known to have specificity to cleave the peptide bonds at carboxylic side of aliphatic, aromatic, or hydrophobic amino acids (Kim et al., 2009; Lv et al., 2014). It was found that the content of extracellular proteins (PN) of EPS in aerobic granules was reduced by 40% after a 2-day exposure to 1 mg mL^{-1} proteinase K whereas only 11% of reduction in the control free of proteinase K, contributing to collapse of the EPS matrix and subsequent disintegration of aerobic granule (Xiong and Liu, 2013). Fredheim et al. (2009) reported that the 0.1 mg mL^{-1} proteinase K by hydrolyzing the PN in EPS matrix resulted in 98% detachment of 52 *Staphylococcus haemolyticus* isolate biofilms. Trypsin, as a serine protease, is specific for peptide bonds of lysine and arginine (Chaignon et al., 2007). Gilan and Sivan (2013) found that obvious reduction of *Rhodococcus ruber* (C208) biofilms in presence of trypsin was the result of hydrolysis of PN of EPS in biofilms. Subtilisin is a serine endopeptidase with a broad substrate range (Leroy et al., 2008b), and since proteins were present in a great amount in *Pseudoalteromonas* sp. D41 EPS components, and addition of subtilisin resulted in 99.5% inhibition of bacterial adhesion and 87% detachment in detachment assay (Leroy et al., 2008a, b). The above results indicated that such proteolytic enzymes by PN degradation in EPS could remove established biofilms effectively, which provides the great potential towards the effective control of EPS-mediated microbial attachment on membrane surface, contributing to mitigation of membrane fouling. Some polysaccharases are also available for disruption of the EPS matrix structure, significantly contributing to the inhibition of microbial attachment and membrane fouling. For example, it was found that Dispersin B, which could degrade N-acetylglucosamine-containing extracellular polysaccharide, effectively released and detached *Staphylococcus epidermidis* biofilms on plastic surfaces (Kaplan et al., 2004). Mutanase and dextranase were two specific sugar-degrading enzymes, and had ability to inhibit *Streptococcus* strain biofilms development (Hayacibara et al., 2004; Wiater et al., 2004). It expects that a joint action of protein- and polysaccharide-degrading enzymes could be a more effective strategy to limit the available EPS, providing an improved method against biofilms on membrane surface. Additionally, EPS of various origins possess extracellular DNA (eDNA), and eDNA, being the longest molecule in EPS and shown to be associated with proteins and polysaccharides, could well be the most important component of EPS keeping all its constituents together (Flemming and Wingender, 2010; Swartjes et al., 2013). Whitchurch et al. (2002) reported that the presence of DNaseI(DeoxyribonucleaseI), an enzyme which can non-specifically cleave DNA by breaking phosphodiester bonds of phosphate backbone via hydrolysis, would damage EPS matrix and prevent biofilm formation. Swartjes et al. (2013) reported that DNaseIcoating by disrupting eDNA in EPS, strongly reduced adhesion of *Staphylococcus aureus* (95%) and *Pseudomonas aeruginosa* (99%) and prevented biofilm formation up to 14 h, without influencing cell adhesion and proliferation. Thus, DNase can be a feasible and effective alternative for disruption of available EPS, thereby contributing to inhibition of microbial attachment and membrane fouling.

The enzymatic approach for disruption of EPS is considered to be nontoxic and environmentally friendly, however, it also has some inherent drawbacks concerning the nature of enzyme (e.g., instability, high pH-, temperature, and salt concentration- sensitivity), which may limit its large-scale application to some extent (Malaeb et al., 2013). In general, immobilization strategy can improve enzyme stability, activity and selectivity, and immobilized enzymes are usually active and highly stable against a broader range of environmental conditions than free enzymes (Cordeiro and Werner, 2011). Enzymatic disruption of EPS would be more sustainable and effective through enzymatic immobilization. EPS produced by microorganisms are a complex mixture composed of macromolecules, and hence its degradation efficiency by enzymatic disruption can be greatly dependent upon presence or availability of multiple enzymes, and some successful applications for EPS degradation by combined use of various enzymes have been reported (Allie et al., 2003; Joung-Hyun et al., 2008).

4.1.4. Energy uncoupling of the ATP synthesis

According to the chemiosmotic theory, adenosine triphosphate (ATP), which serves as a primary bioenergy currency of life, is generated by consuming the proton motive force (PMF) produced by coupling electron transport and oxidative phosphorylation (Jiang and Liu, 2012; Feng et al., 2014). Uncoupling of electron transport or oxidative phosphorylation is an effective approach to inhibit energy production, which can be achieved via adding various chemical uncouplers (Jiang and Liu, 2010; Malaeb et al., 2013). Meanwhile, the EPS have been found to facilitate cell-to-cell interaction, and further enhance microbial structure by formation of a polymeric matrix, whereas the synthesis of macromolecules in EPS (e.g., proteins, polysaccharides, DNA and lipid) is highly ATP dependent (Table 1) (Russell, 2007; Jiang and Liu, 2010; Xiong and Liu, 2010). As can be showed in Table 1, the calculation results were provided for energy demanding of various macromolecules, where the protein was the most costly polymer to synthesize, which accounted for 36.4 mmol ATP/(gram protein) whereas polysaccharide was relatively inexpensive to synthesize with 12.6 mmol ATP/(gram polysaccharide) that represented only one third of energy required for protein synthesis. It suggests from above contents that energy uncoupling for inhibition of ATP synthesis would have a profound impact on EPS synthesis. For example, 3, 3′, 4′, 5-tetrachlorosalicylanilide TCS as a typical uncoupler of oxidative phosphorylation is weak acid with substantial lipid solubility, and can carry protons across the cellular membrane disrupting PMF and hence inhibiting ATP synthesis. Jiang and Liu

Table 1
ATP needed to synthesize protein, polysaccharide, nucleic acid, and lipid.

Macromolecule	ATP requirement (mmol/g)
Protein	36.4
Polysaccharide	12.6
DNA	18.8
Lipid	2.0

(2010) reported that the net synthesis of cell ATP in the presence of TCS (4 mg/L) was reduced by 75% as compared to the control free of TCS, and at a result a greatly large reduction in PN content of EPS was clearly observed whereas reduction in PS content was relative with a small amount, resulting in the failure of aerobic granular sludge biofilms formation. It was known that Nitrophenols (NP) also functioned as chemical uncouplers in oxidative phosphorylation (Liang and Hu, 2012). Liang and Hu (2012) found that ATP level (0.06 ± 0.01 mg ATP/g VSS) of biomass after NP loading in MBRs was much lower than that (0.33 ± 0.09 mg ATP/g VSS) of biomass before NP exposure, resulting in reduced release of EPS from 26.98 mg/g VSS to 20.52 mg/g VSS with less biomass on membrane surface. Besides, it has also been revealed that AI-2, a universal signal, regulated EPS synthesis (Gao et al., 2009; De Araujo et al., 2010), and AI-2-mediated EPS production is highly ATP dependent (Xu et al., 2012; Jiang and Liu, 2013), for that the synthesis of AI-2 signals uses S-adenosylmethionine as the substrate, and specifically S-adenosylmethionine is made from ATP and methionine by methionine adenosyltransferase (Stevenson and Babb, 2002). Xu and Liu (2010) reported that dissipation of ATP synthesis by 2, 4-dinitrophenol DNP (a typical chemical uncoupler) could inhibit microbial AI-2 production and substantially reduce microbial attachment. Xu et al. (2012) reported that ATP content was reduced by 10-fold after contacting with 8 mg/L TCS for 2 h, which resulted in AI-2 contents reduction by 57% and inhibition of AI-2-regulated EPS production where PS content decreased by 36% and PN content reduced by 25%. AHL-based QS systems and EPS production are highly related (described in Section 4.1.1), and similar mechanism (ATP-dependent AHL synthesis) and phenomenon (reduced AHL contents and EPS production through inhibition of ATP) were also revealed in aerobic granulation (Jiang and Liu, 2012, 2013).

It suggests from above discussions that the inhibition of ATP synthesis by addition of chemical uncouplers could be a feasible alternative for limitation of available EPS. However, it has disadvantage that most ATP uncouplers, such as 2, 4-dinitrophenol DNP, 3, 3′, 4′, 5-tetrachlorosalicylanilide TCS, 2, 4-dichlorophenol DCP and pentachlorophenol PCP, can be classified as aromatic compounds and those chemicals are potentially recalcitrant/toxic, thereby in turn limiting their practical application (Jiang and Liu, 2010; Xiong and Liu, 2010; Xu and Liu, 2010; Malaeb et al., 2013).

4.1.5. Other potential strategies

Addition of proper adsorbent agents such as powdered activated carbon PAC (Basu et al., 2016; Kaya et al., 2016), zeolite (Park et al., 2004; Zhichao et al., 2012), diatomite (Liu et al., 2011), and clay (Yi et al., 2013) has been found to reduce EPS concentration in sludge mixture and mitigate membrane fouling, which might be due to that those agents not only possess an great adsorption performance but also act as excellent carrier to provide favorable place for microbial population to grow and further increase enzymatic activity for biodegradation of EPS. Currently, EPS degradation by low-concentration ozonation was studied where protein contents was significantly reduced while polysaccharide contents only had a slightly decrease, and molecular weight of proteins and polysaccharides dropped significantly (Meng et al., 2016). The measure by low dosage of ozone for effective removal of part EPS and sludge modification has been developed and applied into MBRs for the mitigation of membrane fouling (Huang and Wu, 2008; Wu and Huang, 2010). In addition, it has become increasingly clear in term of molecular mechanisms affecting bacterial EPS production that the intracellular secondary messenger cyclic diguanylate (c-di-GMP) is positively involved in the production of EPS matrix components such as certain polysaccharides and proteins, DNA, and rhamnolipids in Gram-negative species (Tischler and Camilli, 2004; Thormann et al., 2006; Borlee et al., 2010; Harmsen et al., 2010), and thus additional researches into the cyclic di-GMP signaling disruption would be a new and important target for limitation of available EPS. Certainly, in addition to above strategies for limitation of available EPS, continuous exploration and development of more effective strategies for EPS limitation are still highly desirable in the future.

4.2. Elevation strategies of available EPS

4.2.1. Enhancement of QS systems

The positive correlation has been characterized between bacterial QS systems and EPS production. However, compared with the well-studied QS inhibition strategy for limitation of available EPS (described in Section 4.1.1), research on the enhancement strategy of QS systems for elevation of available EPS is relatively limited. Currently, the idea is that by adding signal molecules, gene expression for EPS production would be reinforced to produce more EPS. This strategy has been studied in single species populations. In a study by González et al. (2013), a significant enhancement of EPS and capsular polysaccharide production in *Acidithiobacillus ferrooxidans* was got by the addition of C_{14}-AHLs mixture (0.5 μM), improving cell adhesion to substrates. Presumably, the addition of AHL signals reduced the time needed to reach a threshold concentration that would be required for autoinduction and EPS production (González et al., 2013). It was also reported that the addition of three kinds of signals including C_6-HSL, 3-oxo-C_6-HSL and 3-oxo-C_8-HSL resulted in a significant increase in the induction of EPS formation of *Pseudomonas* sp. HF-1 in culture media, contributing formation and development of HF-1 biofilms (Wang et al., 2012). Moreover, this strategy also exhibits the roles in mixed-culture bacterial systems. In AHLs add-back studies of SBRs system by Tan et al. (2014), exogenous addition of 40 μL AHLs significantly increased production of both extracellular polysaccharides (14–36%) and proteins (7–16%), as well as microbial aggregation by floccular sludge community. Moreover, production of extracellular polysaccharides was induced in a signal concentration-dependent manner, and addition of 3-oxo-C_6-HSL at 100 and 5000 nM increased polysaccharides by 18% and 36%, respectively (Tan et al., 2014). In another SBBR exogenous adding study by Hu et al. (2016a), the production of EPS changed after dosing with the nanomolar level (100, 1000 and 5000 nM) of exogenous AHLs in stable and mature biofilm whereas this change was not a signal concentration-dependent manner, which presented a little discrepant with Tan et al. (2014), and it can be due to differences in term of bacteria community composition and biomass concentration in different systems. Moreover, production of EPS sub-fractions through AHLs addition in biofilm reactors was specifically revealed by Hu et al. (2016b). The production of SMP and TB-EPS significantly increased after adding AHLs at 5 nM for 15 days at 10.31% for SMP and 4.21% for TB-EPS whereas the quantity of LB-EPS clearly increased with 50 and 500 nM AHLs addition (Hu et al., 2016b). It refers that response of different EPS sub-fractions to AHL signals might be different.

It appears form above discussions that enhancement of QS systems by exogenous addition of AHLs should be a feasible alternative to elevate available EPS. However, there may be some drawbacks for AHL exogenous addition. The AHL high cost limits its wide use for both laboratory and practical engineering researches, and adding the identical synthetic signals may not maintain a continuous positive effect (Ding et al., 2015a; Huang et al., 2016c). To date, to overcome those drawbacks, Ding et al. (2015a, b, c) have developed a designated microbial strain which secrets a specific type of signals to increase the concentration of “beneficial” signals to combine the native signals to produce the higher EPS for anaerobic granulation, which might provide an exogenous

supplementation of AHL-producing bacteria for elevation of available EPS instead of adding AHLs directly. The strategy presented here can achieve an in-situ, sustainable, and economic QS regulation system which will benefit future applications in available EPS elevation. Certainly, in future, more sustainable and economic QS enhancement strategies should be continuously explored for elevation of available EPS.

4.2.2. Addition of the exogenous additives

Addition of the exogenous additives, including adsorbent agents, inorganic/organic coagulants, carriers and other chemical agents, can influence EPS content in flocs and modify sludge properties. Adsorbent agents such as powdered activated carbon (PAC) and zeolite (Khan et al., 2012; Yuniarto et al., 2013) are reported to have the ability of absorbing SMP and colloids, and increasing EPS content. Specifically, Yuniarto et al. (2013) found that an increase of EPS (in which carbohydrates increased by 53% and proteins increased by 45.9%) in MBR with addition of PAC (4 g/L) as compared with that without PAC addition, contributing to form the bigger and better flocs structure. Variation of EPS content with PAC dosage was also reported, with a decrease in EPS content at lower PAC concentration of 0.75 g/L, followed by a rise at an increase PAC dosage of 1.5 g/L (Ying and Ping, 2006). However, it should be worth noting that the contradictory findings, that PAC addition caused a reduction in EPS content inside microbial flocs based on its adsorption and biodegradable effects (Kim et al., 1998; Lin et al., 2011; Ng et al., 2013; Basu et al., 2016; Kaya et al., 2016), even or showed no effect on sludge EPS contents but altered the SMP composition (Satyawali and Balakrishnan, 2009), have been reported. Similarly, addition of 2 g/L zeolite into MBRs was found to increase concentration of EPS, in which polysaccharide contents in EPS increased by 29% and protein contents increased by 19.1% (Yuniarto et al., 2013). On the contrary, Zhichao et al. (2012) revealed that adding zeolite into the conventional activated sludge processes could lower the contents of LB-EPS, TB-EPS and EPS, contributing to sludge better settling ability. Obviously, there is still no clear consensus concerning effect of PAC/zeolite on EPS production, which may be due to the complex nature of different biological systems and the differences in experimental methods of EPS. Currently, adding inorganic coagulants including aluminium sulfate, polyaluminium chloride (PACl), ferric chloride and polymeric ferric sulfate as well as organic coagulants including polyacrylamide (PAM) and chitosan into MBRs, have been found to decrease SMP and colloids, concurrently increase EPS content in flocs, enlarge flocs size, and mitigate membrane fouling (Ji et al., 2010; Pendashteh et al., 2011). In term of inorganic flocculants, Fe^{3+} flocculants presented more significant influence on EPS contents than Al^{3+} flocculants, and it could be attributed to the fact that Fe^{3+} hydrolysis produced more H^{+} and the presence of free H^{+} could simulate microbe to excrete more EPS (Ji et al., 2010). It had also been reported that addition of calcium, magnesium and other metal ions with coagulation properties could produce a great increase in EPS amount, which accelerated the process of microbial aggregating and formation of aerobic granules (Ni et al., 2013; Liu et al., 2014; Deng et al., 2016). Besides, the addition of a novel cationic polymer (MPE50) into MBR was reported to increase the bound EPS concentration as compared with control MBR (Zhang et al., 2010; Khan et al., 2012), which could be explained by notion of the SMP enmeshment in flocculated bioflocs resulting in the higher concentration of the bound EPS.

It suggests from above discussions that exogenous supplement of proper additives could provide possibility for elevation of available EPS although inconsistent results are always obtained from different studies. Meanwhile, it should be worth noting that addition of chemical agents may cause side effects by producing by-products and/or increasing sludge volume (Lin et al., 2013), concurrently demanding high costs of the added chemicals (Guo et al., 2010). Therefore, it is clear that continuous exploration of highly certain, economic and effective additives as well as their detailed mechanisms affecting EPS production is necessary and important for better elevation of available EPS in future.

4.2.3. Other potential strategies

External environmental conditions influencing production and composition of EPS have been summarized and discussed in Section 4.1.2. Specifically, it is clear that the changes of specific external conditions, including substrate types and concentration, salinity, temperature, pH, DO, MLSS, SRT, HRT, shear rate and metal ions, would exhibit positive or negative effect on EPS production and composition, and therefore favorable conditions for elevation of available EPS can be achieved by the appropriate adjustment of those conditions. Currently, the starvation period has been investigated as a vital influential factor for EPS production in SBRs sludge granulation, and it was found that sludge cultivated using a prolonged starvation period was favorable to the increase of total EPS amount and large MW EPS secretion by microorganisms, which might be considered as an effective survival strategy resisting external threats, while excessive starvation would result in degradation of EPS because EPS can be as both carbon source and energy source of cells during starvation (Liu and Tay, 2007; Pijuan et al., 2009; Li et al., 2014b; Liu et al., 2016). Hence, starvation could be controlled in reasonable range to maintain higher EPS production, and different starvation periods have been investigated to increase EPS production and achieve aerobic granulation effectively in SBRs. Certainly, in addition to above mentioned strategies for elevation of available EPS, continuous exploration of more feasible and effective strategies would be a research niche in the future.

5. Remarks

EPS are of importance for functions and characteristics of microbial aggregates in biological wastewater treatments, among which EPS are involved in both beneficial and detrimental characteristics of microbial aggregates. Specifically, EPS involve in biofilm formation and stability, sludge behaviors as well as SBR granulation whereas they are also responsible for membrane fouling in MBRs. This review emphasizes that controlling strategies of available EPS, specifically limitation strategies targeting inhibition of QS systems, control of external environmental conditions, degradation of the key components, uncoupling of ATP synthesis and application of other strategies as well as elevation strategies targeting enhancement of QS systems, addition of the exogenous agents and application of other strategies, would have great potential in promoting performance of microbial aggregates and in alleviating membrane fouling. It appears that control of available EPS would be a feasible approach for performance enhancement of biological wastewater treatments, and more intensive research effort is definitely needed in the future. Specifically, the following areas should be studied:

1) *Development of EPS controlling strategies.* As current mentioned strategies have drawbacks to some extent, more new EPS controlling strategies with eco-friendliness, high efficiency, low cost and long-term durability should be continuously pursued for performance enhancement of biological wastewater treatments.
2) *Identifying characteristic changes in EPS sub-fractions under the condition of different EPS controlling strategies.* EPS sub-fractions have been confirmed to exhibit different roles for microbial aggregates in biological wastewater treatments; however, in previous studies much attention has been paid on changes in

total amount and key component contents of EPS rather than in EPS sub-fraction characteristics under the condition of EPS controlling strategies. Identification and elucidation of characteristic changes of TB-EPS, LB-EPS and soluble EPS after those strategies should be deeply explored.

3) *Elucidation of precise mechanisms of QS-regulated EPS strategies.* QS system is an emerging and important regulatory mechanism controlling EPS production. It is worth noting that QS regulatory systems are diverse in different bacteria species, and thus detailed studies of elevation or limitation of QS in a certain system would be of importance to understand how these influence EPS production and characteristics, especially the limitation of EPS in MBRs. Exploration concerning QS inhibition and membrane fouling mitigation is of great interest in future research.
4) *Establishment of immobilization techniques in EPS limitation strategies.* In term of enzymatic or bacterial disruption of QS regulated EPS production and enzymatic degradation of the key components, stability, activity and durability of enzyme and bacteria are the key factors, and immobilization strategies would provide possibility to achieve these factors. Therefore, establishment of more stable and relatively simple immobilization methods is still highly desirable in the future.
5) *Exploration of uncertainty of EPS controlling strategies including the control of external environmental conditions and addition of exogenous agents.* Currently, there exist inconsistent or even controversial conclusions for some EPS control strategies; those might be partly of complex nature of different biological systems, non-uniform EPS extraction and evaluation methods, as well as different preponderant mechanisms affecting EPS production by different conditions. Therefore, in the future, continuous exploration for comparison on the relationship between EPS and control strategies in certain system and unified experiment methods will be essential and conductive to achieve the better strategies to control available EPS.

Acknowledgment

This study was financially supported by the National Natural Science Foundation of China (51578222, 51178172, 51521006, 51308076 and 51378190), the Project of Chinese Ministry of Education (113049A), the Research Found for the Program for Changjiang Scholals and Innovative Research Team in University (IRT-13R17).

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