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Minireview

Physiological response of electroactive bacteria via secretion of extracellular polymeric substances in microbial electrochemical processes: a review

Liping Huang[a,*], Gianluca Li Puma[b,*]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering, Ministry of Education (MOE), School of Environmental Science and Technology, Dalian University of Technology, Dalian, 116024, China

[b] Environmental Nanocatalysis & Photoreaction Engineering, Department of Chemical Engineering, Loughborough University, Loughborough, LE11 3TU, United Kingdom

E-mail address: lipinghuang@dlut.edu.cn (L. Huang).
g.lipuma@lboro.ac.uk (G. Li Puma)

The authors declare no competing financial interest.

Abstract

Microbial electrochemical processes involving microbial catalyzed electrochemical reactions are directly/indirectly related to the electroactive bacteria (EAB) physiological secretion of extracellular polymeric substances (EPS) with favorable biofilm dispersion. EPS harbor cytochrome-like substances, and thus accelerate extracellular electron transfer (EET) processes resulting in the simultaneous removal of environmental contaminants or in the conversion of dissolved CO_2 to key-block chemicals. This review holistically documents case-studies in the last five years focusing on EAB and their physiological release of EPS in response to external stimuli. The important role played by EPS in the performance of microbial electrochemical systems and the relationship of the EPS compositional diversity with the process parameters is reviewed. Targeting the physiological response of electroactive bacteria towards the release of EPS with precise compositional diversity provides tremendous opportunities for optimizing microbial electrochemical processes. This review provides kernels, quantitative approaches and promising advanced techniques guiding further research directions in this exciting field.

Keywords electroactive bacteria; microbial electrochemical technology; microbial physiological metabolism; extracellular polymeric substances; extracellular electron transfer

1. Introduction

Microbial electrochemical processes such as microbial electrosynthesis systems (MESs), microbial fuel cells (MFCs), and microbial electrolysis cells (MECs), also collectively called as bioelectrochemical systems (BESs) have emerged as new methods for wastewaters treatment, carbon sequestration and CO_2 conversion to key-block chemicals [1-3]. In such systems, the interphase region joining the electrode material and the supported electroactive bacteria (EAB), either exoelectrogens or electrotrophs, often controls the efficiency of many microbial electrochemical processes. Frequently, the fundamental structural and thermodynamic properties mismatch at the biotic/abiotic interface, results in lower charge transfer rate at the hetero-junctions and this aspect is central to the design and development of efficient electromicrobiological devices [4].

Microbiologically, the cellular response of EAB to electrical stimuli is extremely complex. While model dissimilatory metal-reducing bacteria *Geobacter* and *Shewanella* have been extensively studied [5], the nature of their microbial interaction with the electrode, especially at the single-bacterium level, remains unclear [6]. The EAB-electrode electronic contact can be augmented through artificial direct conjugation with redox cofactors that decrease the electron tunneling/hopping distance, or through the utilization of external molecular electron mediators that facilitate charge transfer via intermediate redox reactions [2,5].

Extracellular polymeric substances (EPS) physiologically secreted by microorganisms in variable amount and with a compositional diversity as a protection against unfavorable external conditions or external stimuli, play a central role in electron transfer and redox activities [7-8]. The EPS matrix made of various classes of macromolecules and hydrolysates including polysaccharides (40 – 95%), proteins (1 –

60%), lipids (1 – 60%), nucleic acids (1 –10%) and humic substances makes over 90% of the cell dry mass [7-8]. The composition and physicochemical properties of EPS are normally bacterial dependent and the highest EPS quantities are produced towards the end of the growth log-phase and during the stationary phase. Non-conductive polysaccharides are normally responsible for cell aggregation, adhesion and for the maintenance of cell envelope integrity and form scaffolds attached to biocarrier assisting the structural stability of EAB, affecting bacterial adaptability and biofilm electroactivity [8-10]. Conversely, many EPS proteins are redox-active and engage in extracellular electron transfer (EET), contributing to EPS conductivity [8-10]. Zhao's group summarized the conductivity and the EPS redox properties from an electrochemical perspective [11] and confirmed the harboring of cytochromes *c* and flavins in EPS from three bacteria [9]. Dominguez-Benetton et al. predicted the likely significant roles of electrochemically-tunable EPS not yet been addressed as a strategy to achieve efficient metal recovery in microbial electro-metallurgical processes [12].

The present review discusses recent efforts that describe the tunable physiological secretion of EPS by mixed or pure EAB cultures (Fig. 1, Tables 1-2) in response to external stimuli in MESs, emphasizing the impact of electrode potential, circuital current, electrode hybridization, light irradiation, electrolyte composition, nutrient limitation, chemical signaling molecules and toxic contaminants. Quantitative approaches and promising advanced techniques guiding further research directions in this exciting field will be discussed. In an effort to minimize overlap, preliminary aspects of the functions, properties and constituents of EPS by conventional bacteria or EAB has been reviewed elsewhere [7,11-12] and will not be addressed here.

2. Mixed EAB cultures

2.1 Impact of electrode potential/applied voltage

Electrode potential has been frequently used to determine the activity of mixed EAB cultures via regulating EPS composition and redox properties (Table 1). Low anode potentials (e.g., 0.2 V vs. SHE) have been shown to yield the highest current and EPS redox capacity favoring the secretion of proteins or humic substances in response to Ag^+ shock [13] or under ammonia inhibition [14-15]. However, the electrode potential was generally optimized under the respective experimental conditions [13-15] and was not correlated with the secretion of proteins or humic substances. In another study, an intermittent anode potential (–0.13 V) increased the amount of soluble EPS and the proteins fraction achieving 27–43% of the total charge, which compared to 15% and lower proteins under a continuous current [16]. Such behavior corroborated with the charge storage in the form of polymeric substances in the soluble EPS and the predominant model EAB of *Geobacter* sp. in mixed biofilms (Table 1). Collectively, higher EPS mass and proteins fraction appeared to be achieved by applying an appropriate low voltage and intermitted anode potentials in mixed biofilms.

While lipopolysaccharide has been frequently related with bacterial adhesion [7-8], the homologous protein structure has evolved into conductive substances (especially increasing the contents of redox enzymes and humic-like substances), and thus contributed to successful establishment of EET conditions, which confers a selective advantage in many pure or mixed cultures [9,11]. However, the presence of EPS producing species may lead to the integration of other species that do not synthesize matrix polymers in conventional mixed-species biofilms [7]. The EPS components in these mixed biofilms do not always originate from all cells present and neither always from the dominant species, nor do the different EPS add equally to the structure and properties of the resulting biofilms [7,16]. Thus, screening the EPS release by pure EAB as a function of electrode potential/applied voltage is tremendously attractive as a mean to control the performance of BESs. Moreover, considering the variations obtained in the measurement of EPS with different analytical and extraction methods [7-8], as well as the use of non-standard experimental conditions (e.g., electrolyte composition, liquid volume, reactor architecture) [13-16] (Table 1), the precise and quantitative tuning of the electrode potential towards the physiological release of EPS become a crucial factor to optimize the performance of BESs.

2.2 Hybrid electrode/conductive material

Carbon-based electrodes hybridized with metals have been reported to regulate the secretion of EPS by mixed EAB cultures via the changes in the expression of the associated functional genes (Table 1) [10,17]. Both the EPS mass secreted by syntrophic microbial communities and the carbohydrate-to-protein ratio significantly increased using a Ag-carbon electrode [17]. The EPS mass was correlated to 30% increase in the expression of the *Lux*R gene, which led to the increase of quorum signaling subsequently inducing EPS secretion, whereas the carbohydrate-to-protein ratio increase resulted from the decrease in expressions of various EET associated functional genes (*omc*B, *omc*C, *omc*E, *omc*Z, *omc*S and *pil*A), and smaller abundance of *Geobacter* species [17]. Fe_1Co, Fe_4Co and $Fe_{10}Co$ hybridized electrodes [10] have shown to create selective pressure on the adhesion of electroactive bacteria to the electrode surface achieving the enrichment of *Geobacter*-dominate electroactive biofilms. Such processes promoted the homogeneous distribution of a biofilm with structural integrity components (e.g. β-D-glucopyranose, α-D-glucopyranose polysaccharides) across its vertical depth, which stimulated the secretion of extracellular proteins in the inner biofilm affecting the EPS redox activity. These studies open new avenues to design highly electroactive bacteria via regulating the secretion of EPS and the expression of the associated genes.

2.3 Electrolyte

Since the EPS matrix dominates the architecture of conventional biofilm, the external factors influencing the architecture of biofilms, including the hydrodynamic conditions, the concentration of nutrients, the bacterial motility and even the intercellular communication mechanism, reasonably also affect the total amount and the compositional diversity of the EPS [7-8]. Currently, the electrolyte composition, the carbon source, and the presence of endogenous chemical signals, have been proven to induce various extents of physiological EPS secretion by mixed EAB cultures and different electron storages (Table 1).

Limited acetate (0.1 g/L) imposed a selective pressure which developed a thinner anodic biofilm (54% decrease) with significant decrease of insulating polysaccharides and appreciable abundance of *Geobacter* up to 88%, resulting in 90% increase in circuital current [18]. Conversely, with 1.0 g/L acetate the production of polysaccharides increased three-fold. Thus, the carbon source exerts a significant regulating effect, and lowering its amount decreases the physiological secretion of polysaccharides, increasing the EAB activity. In consequence, the treatment of diluted wastewaters providing limited carbon sources should favor the efficient performance of BESs and high EAB activity [18-19]. Moreover, through periodic glucose dosing (0 – 100 mg/L), the autotrophic oxygen-reducing biocathode experienced a prolonged startup period with lower currents and EPS mass, although the EPS mass and current increased after the formation of a mature biofilm [20]. Thus, dosing the carbon source achieves a desired EAB response via the self-alteration of the physiological secretion of tunable EPS.

Quorum sensing (QS) molecules (N-acyl-homoserine lactones) endogenously secreted by microorganisms to initiate cell-cell communication, cell movement, chemical biosynthesis or biofilm formation, have been correlated to increased EPS concentrations, compositional diversity and redox activity, although they did not alter the proportion of *Geoboacter* sp. [21]. Their presence increased the EPS conductivity and improved the degradation of chloramphenicol in MFCs [22]. Considering the secretion of different QS molecules and the response by different cognate regulator proteins in co-culture of *Pseudomonas* sp. and *Acinetobacter* sp., endogenous QS molecules enable mixed EAB to accomplish sophisticated electrogenesis functions [21-22]. Further investigations should prove the common and specific aspects of these correlative endogenous QS molecules with EPS secretion by mixed cultures, and possibly their role in EET and cell-cell communication.

The presence of contaminants in the electrolyte such as formaldehyde led to the increase in number of dead/broken anodic EAB cells and the decrease in EPS, which were 40% and 28% at 1 mM, and 91% and 75% at 10 mM, respectively [23]. Formaldehyde also disturbed the bacterial community, decreased the proportion of *Geobacter* species and up-regulated intracellular reactive oxygen species, superoxide dismutase activity, lactate dehydrogenase activity and adenosine triphosphate activity.

2.4 Light irradiation

Inorganic light absorbers produce electrons or reducing equivalents, which are synergistically utilized by EAB to reduce inorganic carbon to key-block chemicals such as acetate [1]. However, the impact and role of EPS secreted by EAB in these BESs under light irradiation conditions remain unclear.

The hybrid electrode incorporating CdS improved EPS production by mixed cultures, increasing the proportion of proteins and humic substances, the activity of ATPase and catalase, and the abundance of *Xanthomonadaceae*, *Hyphomonadaceae* and *Sphingobacteriales* achieving efficient nitrate removal [24] (Table 1). The EPS facilitated the separation of electron-hole of CdS nanoparticles under light irradiation and mediated electron transfer between CdS and periphytic biological processes for nitrate removal.

Heavy metals (Cd, Ni and Cs) in light absorbers are generally toxic toward EAB and thus deteriorate BESs performance, particularly under facultative or aerobic conditions [1,27]. These metals dissolved in the electrolyte after prolonged operation become environmentally undesirable. The stability of light absorbers and their impact on the physiological secretion of EPS should therefore be assessed over prolonged system operation. Moreover, a balance needs to be struck between electrode bacterial coverage and exposure of light absorbers to achieve high process efficiency, particularly in large cell sizes.

Conversely, in the absence of semiconductive catalysts and under darkness, anodic EAB biofilms densely formed on ITO glass working electrode achieved 196 – 5556 times higher current with a dominance of *Geobacter* species (85%), while under illumination (600–1200 lux) the predominance of *Rhodopseudomonas* species (66–75%) and storage of polysaccharides over proteins decreased the EET process [25]. Similarly, under illumination a graphite felt bioanode augmented the production of polysaccharides rather than proteins, exhibiting synergetic current and illumination for degrading EPS in activated sludge with higher polysaccharides content in soluble

EPS [26]. The dense and thick biofilms covering the working electrodes, and the absence of light-generated electrons or reducing equivalents, further explained the alteration of the bacterial community solely due to illumination exposure conditions [25-26].

Since each strain of a mixed microbial community secretes EPS components with varying properties, multiple EPS secretion mechanisms often interact in a synergistic manner. Hence, it is important to distinguish the relative impact of each independent mechanism, and synergistic/antagonistic feedbacks in complex mechanisms. The interpretation of the metabolic and biochemical functionality of pure cultures becomes necessary to interpret the behavior of mixed EAB cultures. This might require a multidisciplinary approach incorporating spectroscopy, nano-particle engineering, ecological and molecule techniques to tease apart the various regulating mechanisms.

3. Pure EAB cultures

A few model Gram-negative (*Geobacter*, *Shewanella* and *Desulfovibrio*) and Gram-positive (*Paenibacillus dendritiformis*) EAB along with some electrotrophs (*Desulfuromonas*, *Geomonas* and *Geothrix*) have been extensively investigated [5]. However, only a few studies have correlated the physiologically release of EPS to the operating conditions of microbial electrochemical processes (Tables 1-2) as summarized below.

3.1 Electrode potential/current

The production of tunable EPS under different redox potentials might be regulated by cells maintaining a balancing function between cell protection and EET process [8]. The electrochemical anodic respiration of *Geobacter sulfurreducens* strongly promoted EPS production, with increases 10–30 folds in proteins, 3.5–7.0 folds in polysaccharides, and 10 folds in uronic acid and eDNA, as compared to fumarate-respiration [28] (Table 1). While the substances transport from the cytoplasm or periplasm beyond the outer membrane improved during anodic respiration, thus accumulating soluble EPS in the electrolyte [28], the genes up-regulation responsible for EPS expression was observed in *Geobacter soli* through the endogenous QS molecule (N-acylhomoserine lactones) [29]. Similarly, electrochemical and spectroscopic analysis of EPS secreted by deficient EAB, detected a flavoprotein secreted by the *Saccharomyces cerevisiae* [30]. These species allow multistep electron hopping processes between the EPS spatially confined redox-flavoproteins/*c*-type cytochromes, or EET by diffusion of these mobile substances across the EPS matrix (Fig. 2). The flavoproteins and *c*-type cytochromes matched the charge storage of reduced cell components, like cytochromes and flavins bound to proteins on the cell surface [5,16].

The model EAB *Geobacter soli* regulated the EPS secretion to balance EET efficiency and cell-protection. The biofilm closer to the electrode produced an excess of extracellular redox-active proteins rather than polysaccharides at low potentials (0.0–0.2 V), regulating the EET efficiency of the biofilm, while the electrically nonconductive extracellular polysaccharide dominated interior layers at 0.6–0.8 V [31] (Table 1) providing a physical barriers for cells protection [8,10,28]. In constrast, *Shewanella* species suppressed the production of extracellular polysaccharide and stimulated the secretion of proteins at higher redox potentials [32]. This observation along with direct electron transfer in Gram-positive *Paenibacillus dendritiformis* [33] via conductive EPS matrix rich in pili/nanowires and multiple cytochromes along with high EPS secretion with abundance of *G. anodireducens* than *G. sulfurreducens* under a strong electrical field [34] (Table 1), stressed the relationship among potential/current and EAB strain on physiological secretion of tunable EPS with variable composition, significantly affecting system performance.

Specifically, the electrode-EAB adhesion force closely relates to polysaccharides [9-10]. Using *in situ* AFM-based electrochemical single-cell force spectroscopy, the unfolding behaviors of outer membrane appendages was shown to be dominantly impacted by the dynamic bacterial EET processes [6]. Thus, the correlation among the bacterial adhesion force and the EPS components at single cell level (nano Newton scale) should be further investigated to progress our knowledge on the critical role of EPS in EET.

3.2 Hybrid electrode/conductive material

The deposition of metals on the cathode by the microbial reduction of ions may impart higher electronic conductivity than the microbial EPS pili (ca. 0.1 S/cm). Thus, the migration of Ag(I) from a hybridized r-GO/Ag into the *Shewanella oneidensis* cellular membrane, reduced Ag(I) to Ag nanoparticles by proteins linked to lactate oxidative metabolism, facilitating electron transfer from internal electron carriers to the anode [35].

The lipopolysaccharide role in EPS during metals reduction is peculiar. Two lipopolysaccharide isoforms (with/without a terminal methyl-quinovosamine sugar) produced by *Geobacter sulfurreducens* PCA growing on fumarate, were only expressed as the shorter and more hydrophilic variant when reducing iron oxides. The lipopolysaccharides changes in the outer membrane further determined the cells hydrophilicity, increasing outer membrane vesiculation, disrupting the biofilm structure [36].

Using surface-enhanced Raman spectroscopy, the electron transfer mechanism of the EPS by common EAB (*Shewanella oneidensis* and *Geobacter sulfurreducens*) or non-EAB (*Escherichia coli* and *Bacillus subtilis*) was elucidated [37] (Table 1). The redox properties of EPS adsorbed on Ni and Ag nanoparticles and the dominant role of redox porphyrin in cytochrome *c* in the EPS of the EAB *S. oneidensis* and *G. sulfurreducens* were confirmed. Conversely, the EPS secreted by non-EAB such as *E. coli* and *B. subtilis* after adding Ni and Ag nanoparticles showed lower redox properties. Among closely related EAB species, *Geobacter sulfurreducens* dominated over *Geobacter anodireducens* in early biofilms formation producing higher EPS mass [38]. These results demonstrate the importance of EAB species-dependence for both EPS secretion and redox property. Further work should systematically quantify these case-by-case results, to further understand the role of EPS in EET.

3.3 Electrolyte

The QS compound N-acylhomoserine lactones endogenously secreted by *G. soli* GSS01 enhanced the relative abundance of external membrane proteins and EPS production by up-regulating the expression of key enzymes, facilitating biofilm formation and its electrochemical activity [29] (Table 1). Differently, exogenous N-acylhomoserine lactones shortened the start-up periods of both cathodic and anodic *G. soli* GSS01 biofilms and enhanced cell viability and EPS production achieving higher current and efficient denitrification through the formation of amide II and stronger H-bond between the carbonyl and amide of the surface proteins [29,39]. The diverse N-acylhomoserine lactones endogenous and exogenous secretion mechanisms in the EPS of *G. soli* GSS01 and its impact on EET, suggests further investigations on chemical signals by pure EAB cultures.

Besides QS compounds, carbon sources and toxic contaminants (e.g., heavy metals) in the electrolyte impact the EPS secretion by pure cultures. The indigenous electrotrophs (*Serratia marcescens, Citrobacter* sp., *Pseudomonas* sp., *Pseudomonas delhiensis* and *Ochrobactrum anthropi*) opportunistically metabolized organic (acetate) and inorganic carbon (HCO_3^-) while EPS were secreted with a compositional diversity under Cd(II) or Cu(II) stress conditions [40-44] (Table 2). This mixotrophic electrotrophic regulative metabolism of these electrotrophs released tunable EPS that protected the cells from heavy metal stress and under limited inorganic/organic carbon sources circumstances [43]. The relationship among EPS mass and composition in the presence of different heavy metals concentrations, cathode potential/circuital current, biofilms/planktonic cells, and magnetic field (Table 2), demonstrated the comprehensive tunability of the EPS of *S. marcescens* [40-41].

Specifically and in single-chamber reactors, pure EAB such as *Citrobacter* sp., *Pseudomonas* sp., *Pseudomonas delhiensis*, or *Ochrobactrum anthropic* on both electrodes secreted similar EPS quantities under Cd(II) stress [44]. The EPS composition was further influenced by the concentration of acetate, the circuital current, and the location of the cells (biofilms or planktonic cells) [40,44-45].

Currently, the qualitative comparison of EPS is limited by the lack of methods for precise discrimination of EPS composition and functions [7,12], even under the similar EPS extraction conditions [40-45,46]. The quantitative characteristics of EPS still need to be clarified to regulate the optimal performance of electrotrophic bacteria.

3.4 Light irradiation

Despite extensive investigations of non-photosynthetic model EAB (*Sporomusa ovata*, *Moorella thermoacetica* and *Methanosarcina barkeri*) in BESs incorporating light irradiated semiconductive materials immobilized on electrodes [1], the physiological secretion of EPS and their roles in system performance remains largely underexplored. Moreover, the non-model, anaerobic, non-photosynthetic *S. marcescens* Q1 and *Stenotrophomonas* sp. JY6 immobilized on semiconducting $WO_3/MoO_3/g\text{-}C_3N_4$ biocathodes physiologically secreted EPS under light and circuital current stimuli, affecting their EET [47]. The EPS compositional diversity was correlated to light irradiation and to the electrotrophic species, whereas the EPS mass was related to the cellular electron transfer rates. Varying the ratios of indirect (via H_2) and direct EET processes decreased EPS release and inorganic carbon conversion under a transitional period, allowing control over the cellular electron transfer process for efficient conversion of inorganic carbon to acetate in MESs. Oxygen exposure and the non-photosynthetic electrotrophs nature (aerobic/anoxygenic/anaerobic) might substantially impact the compositional diversity of EPS in complex BESs under light irradiation conditions. While only a very few electrotrophic species have been explored in these BESs [1-3,5,49-50], extensive studies about EPS released by other model/non-model electrotrophs and their tunable roles along with oxygen exposure conditions would be warranted.

Conversely, in the absence of semiconductive materials and in the presence of illumination, some photosynthetic bacteria e.g., the purple bacterium *Rhodopseudomonas palustris* immobilized on graphite anodes secreted EPS under anoxygenic conditions, and formed $^3EPS^*$ reactive species and $^{\cdot}OH$, which contributed to efficient sulfadiazine oxidation in single-chamber MECs [48]. The much higher EPS amount observed under illumination conditions, in comparison to open circuit conditions with the same illumination, suggested the stimulation of EPS secretion by photosynthetic electron extraction. The higher fraction of extracellular proteins at electrode potentials of 0.2 V or 0.4 V, and the higher amount of humic acid at 0.0 V or 0.4 V, along with more polysaccharides at 0.6 V, demonstrated the physiological regulation of this bacterium through the secretion of EPS components in response to electrode potential. In addition, oxygen favored the formation of active species $^3EPS^*$ by photosynthetic electrotrophs [48], whereas anoxic conditions preferred the formation of detrimental active species (e.g., $^{\cdot}O_3$ and $^{\cdot}OH$) to [26]. Thus, the tunable roles of EPS in these photosynthetic electrotrophs-assembled systems are also condition-dependent and should be cautiously evaluated in further investigations.

4. Perspectives and conclusions

The EPS role in microbial electrochemical processes, reviewed here, shows tremendous opportunities for tuning the compositional diversity of EPS to optimize contaminant removal and/or electrosynthesis of key block chemicals. Studies with either mixed or pure EAB cultures, on the physiological secretion of tunable EPS in response to process parameters (electrode potential/current, hybrid of electrode, light irradiation, and electrolyte) have primarily focused on proof-of-concepts, and have at most optimized a limited set of operational parameters (e.g., electrode potential). Further investigations on tuning EPS for engaging in EET and regulating EPS via the changes in expression of associated functional genes, are still needed.

Many investigations have focused on determining the EPS release by EAB during electrons exchange with the electrode but have not focused on manipulating the EPS secretion to optimize contaminants removal and/or CO_2 conversion to key-block chemicals. Further insights in tunable EPS-directed EAB metabolism could provide new routes towards contaminants removal and detoxification. Since extremophiles strive under environmental stress conditions (e.g., carbon limitation, high salinity, low/high temperatures, and the presence of heavy metals/contaminants), clarifying the diversity of their EPS secretion and quantifying their roles in the electromicrobiological processes is particularly promising to develop efficient BESs able to treat multiple contaminants in complex environments. Moreover, identifying these EPS with novel electronic properties in these extremophiles is likely to contribute to new applications in the biomedical field.

Prospecting through the EAB physiological metabolism and functional EPS is laborious using currents methods. The extrapolation of previous results on the role played by EPS components in response to external stress could strive further progress. The broad phylogenetic EAB diversity capable of variable physiological EPS release might inspire the next generation of electromicrobiological technologies, particularly for environmental detoxification.

Future research endeavors on EPS quantification, should focus on developing new molecular level measurement techniques that determine the compositional diversity of EPS produced by EAB. Current EPS extraction methods use non-standardized methods which hinders the comparison of the EPS structural complexity from different studies [7-8,37,51]. Additionally, the bioelectrochemical technologies diversity (e.g., anode/cathode, mixed/pure cultures, anodic/cathodic electrolyte) has differentiated the working principles and operating conditions [1-3,5,52]. The differences in the EAB physiological response to external stimuli in terms of amount and compositional diversity of EPS further complicates their interpretation. For this reason, non-destructive *in situ* spectroscopic and high-resolution molecular characterization techniques should increasingly replace conventional EPS extraction and *in-vitro* characterization, to identify the compositional diversity and molecular structure of EPS probing into dynamic EPS-involved microprocesses [8]. A global EPS molecules database should be established, each with unique spectral fingerprint validated by advanced molecular characterization techniques, through which the true compositional and structural properties of EPS molecules, and their dynamic behaviors are accurately discriminated and interpreted. Understanding the EPS production mechanisms at a molecular level should provide guidance for developing tunable BESs.

Besides EPS quantification under dynamic conditions, the quantitative evaluation of the EPS redox capacity is also needed. Electrochemical methods such as cyclic voltammetry and chronoamperometry typically yield peculiar results due to the complexity of the EPS functional groups [8,11]. Instead, the mediated electrochemical oxidation method recently developed might provide a more accurate quantification of the EPS electron exchange capacity [8,53]. This method coupled with *in situ* spectroscopy and high-resolution molecular characterization techniques might provide deeper mechanistic and quantitative knowledge of the EPS redox capacity.

Declaration of interest

None

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Captions:

Figure 1 Illustration of secretion of EPS by EAB and process parameters regulating EPS secretion.

Figure 2 Illustration of electron transfer of *c*-type cytochromes, flavoproteins, or both *c*-type cytochromes and flavoproteins in the EPS secreted by the corresponding pure cultures of EAB.

Table 1 EPS secreted by mixed or pure cultures of exoelectrogens or electrotrophs.

Table 2 Components of EPS released by pure cultures of electrotrophs under stressful heavy metal conditions

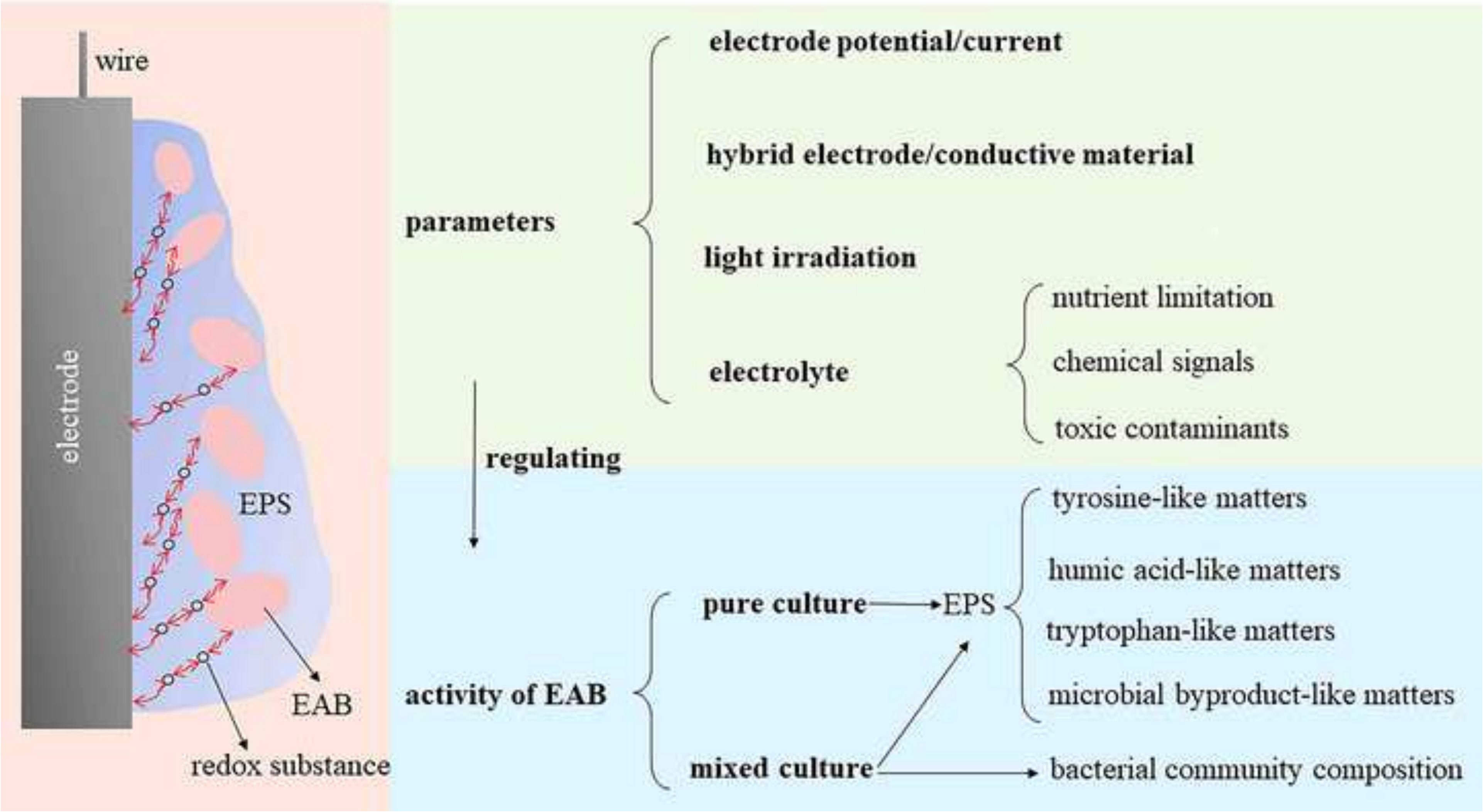
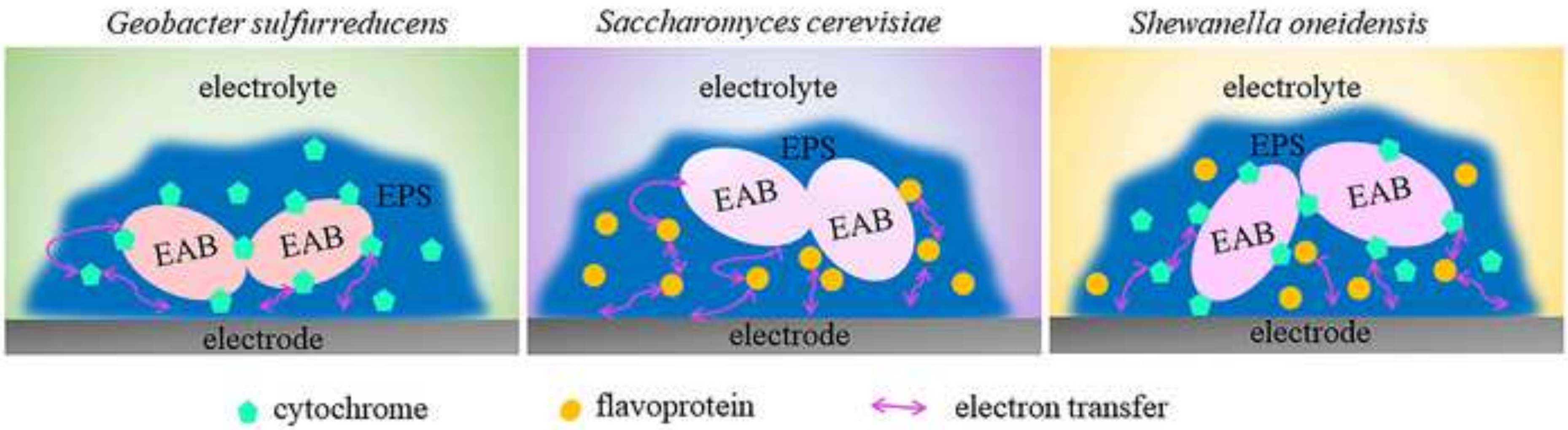

wire
electrode
EPS
EAB
redox substance
parameters
electrode potential/current
hybrid electrode/conductive material
light irradiation
electrolyte
nutrient limitation
chemical signals
toxic contaminants
regulating
activity of EAB
pure culture
EPS
tyrosine-like matters
humic acid-like matters
tryptophan-like matters
microbial byproduct-like matters
mixed culture
bacterial community composition

Figure 2



Geobacter sulfurreducens

Saccharomyces cerevisiae

Shewanella oneidensis

Table 1 EPS secreted by mixed or pure cultures of exoelectrogens or electrotrophs.

Condition	Reactor	Poised potential (V, vs. SHE)[a], or power production (W/m^2)[b] or (A/m^2)[c]	Working electrode and EAB	Performance	EPS secretion	Reference
Electrolyte (chemical signal: N-acylhomoserine lactones)	Dual-chamber MFCs	1.3[c]	Mixed culture; anode	electron transfer mechanism of EPS	Exogenous and endogenous quorum signaling molecules increased concentration and redox activities of EPS, in addition to enhanced diversity of EPS components by endogeous signaling molecules; Abundance of *Geoboacter* sp. increased with exogenous quorum signaling molecules	[21]
hybrid electrode (Ag, 50 mg/L)	Dual-chamber MECs	0.2[a]	Mixed culture; anode	Role of EPS in EET	More EPS were produced while carbohydrate to protein ratio in EPS increased (0.7 vs. 0.4); Quorum signaling gene increased by 30%; EET associated genes (*omc*B, *omc*C, *omc*E, *omc*Z, pilA) decreased; *Geobacter* species decreased	[17]
Electrolyte (acetate limitation)	Three-electrode reactor	–0.6 ~ 0.2[a]	Mixed culture; anode	Current, biofilm structure, viability and thickness	Low acetate concentration imposed a selective pressure to enrich *Geobacter*, and the thin biofilm had a high electroactivity due to decrease of insulative polysaccharide in EPS.	[18]
Electrolyte (glucose shock)	Dual-chamber MFCs	0.009 A (100 mg/L) 0.005 A (50 mg/L) 0.004 A (20 mg/L) 0.001 A (0 mg/L)	Mixed culture; oxygen-reducing biocathode	Current, biofilm structure	Adding glucose as an organic shock initially prolonged the startup time of biocathodes with a decrease in current. The currents surpassed the control without glucose addition for the mature biofilm, ascribed to production of more EPS	[20]
Electrolyte (formaldehyde: 1 – 10 mg/L)	Three-electrode reactor	–0.4 ~ 0.4[a]	Mixed culture; anode	Current, biofilm structure	Total protein and EPS decreased; the proportion of *Stenotrophomonas* increased and the *Geobacter* was inhibited	[23]

Potential/current	Single-chamber MECs	0.0 ~ 0.6^{a}	Mixed culture; anode	Potential-dependent EPS secretion for Ag$^+$ shock	A 0.2 V achieved the highest anti-shock capability by Ag$^+$ exposure, consistent with the highest loosely bound EPS and tightly bound EPS, dominant with proteins and humic acids	[13]
		0.1 ~ 2.5^{a}	Mixed culture; both electrodes	Potential-dependent EPS for nitrogen removal	More EPS with more ratios of proteins to polysaccharides at low applied voltage (≤ 1.5 V), matching with trend of nitrogen removal. Applied voltage reduced microbial diversity, but increased the abundance of *Candidatus* Scalindua at 1.5 V	[15]
Intermittent anode potential/current	Dual-chamber MECs	–0.13^{a}	Mixed culture; anode	Influential intermittent potential on EPS	An intermittent anode potential (–0.13 V) achieved 27 – 43% of total charge as soluble EPS with more proteins, compared to 15% in the controls	[16]
Light irradiation (semiconductive CdS)	Three-electrode reactors	0.4^{a}	Mixed culture; anode	Nitrate removal	More EPS with more proportion of proteins and humic substances, increased activities of ATPase and catalase, and promoted abundance of *Xanthomonadaceae*, *Hyphomonadaceae* and *Sphingobacteriales*	[24]
Illumination (ITO/glass with photosynthetic bacteria)	Three-electrode reactors	0.0^{a}	Mixed culture; anode	Effect of illumination exposure on EET	Illumination increased polysaccharides with decrease of proteins in EPS, and thus increased *Rhodopseudomonas* with decrease in *Geobacter* sp.	[25]
Illumination (graphite felt with photosynthetic bacteria)	Dual-chamber MFCs	0.039^{b} (light and current)	Mixed culture; anode	Effect of light exposure on degradation of EPS in activated sludge	Light and current synergetically improved performance with dominant *Acinetobacter*, *Escherichia/shigella*, *Petrimonas* and *Syntrophomonas*	[26]
		0.078^{b} (only current)			Single current promoted degradation of proteins in EPS with enrichment of *Proteiniphilum* and *Cloacibacillus*	
		--- (only light)			Single light improved total EPS especially polysaccharides in soluble EPS with dominant *Pseudomonas* and *Acinetobacter*	
		OCC				

Illumination (graphite plate with photosynthetic bacterium)	Single-chamber MECs	0.0 ~ 0.6^{a}	*Rhodopseudomonas palustris*; anode	Role of EPS in sulfadiazine degradation MECs	3EPS* was formed under illumination conditions, dominantly (accounting for 90%) contributing to sulfadiazine removal, in addition to $^{\cdot}$OH. More total EPS stimulated by photosynthetic electron extraction (0.4 V); More extracellular proteins at 0.2 V and 0.4 V, more humic acid at 0.0 V and 0.4 V, and more polysaccharides at 0.6 V	[48]
		OCC				
Hybrid electrode (Ni and Ag nanoparticles)	Three-electrode reactors	---	*Shewanella oneidensis*; anode	Role of EPS in EET	Higher contents of protein in EPS of *S. oneidensis* and *G. sulfurreducens* than those of nonelectroactive bacteria; Higher content of humic substances in EPS of *E. coli* than that of *B.subtilis*	[37]
			G. sulfurreducens			
			Escherichia coli			
			Bacillus subtilis			
Electrolyte (N-acylhomoserine lactones)	Dual-chamber MECs	–0.4^{a}	*Geobacter soli* GSS01; cathode	Role of chemical signals in denitrification	External added chemical signals shortened start-up of cathodic biofilm, improved cell viability and EPS production, and thus enhanced denitrification	[39]
	Dual-chamber MECs	0.5^{a}	*G. soli* GSS01; anode	Role of chemical signals	Chemical signal molecules shortened start-up, improved external membrane proteins and EPS by up-regulating expression of enzymes participating in EPS production	[29]
Potential/current	Dual-chamber MECs	0.6 – 0.8^{a}	*G. soli* GSS01; anode	Role of EPS in EET	Extracellular polysaccharide-dominated interior layers served as physical barriers for protecting cells from poised electrodes	[32]
		0.0 – 0.2^{a}			More extracellular redox-active proteins and less extracellular polysaccharides (closer to electrode)	
	Dual-chamber MFCs	0.002^{c}	*G. sulfurreducens*; anode	Role of EPS in EET	Anodic respiration promoted EPS production: proteins 10-30 folds, polysaccharides 3.5-7.0 times, and uronic acid and eDNA 10-fold more than the controls of anodic fumarate-respiring	[28]
	Three-electrode	---	*Saccharomyces*	Role of yeast	Flavoprotein was confined in EPS, and responsible for hopping	[30]

	reactors		*cerevisiae*	secreted EPS in EET	processes between spatial confined redox-flavoproteins in the EPS, or diffusion of mobile flavoprotein across EPS matrix between cell and electrode surface	
	Dual-chamber MECs	0.0[a]	*G. sulfurreducens* PCA and *G. anodireducens* SD-1	Response of different pure cultures to current	More *G. anodireducens* than *G. sulfurreducens* at strong electrical fields, and connected to chemotaxis, c-di-GMP, fatty acid metabolism, pilus, oxidative phosphorylation and transcription, resulting in more EPS and rapid cell proliferation	[34]
	Dual-chamber MFCs	0.005[c]	*Paenibacillus dendritiformis* MA-72; anode	EET in Gram-positive bacteria	This Gram-positive bacterium exhibited a direct electron transfer through the conductive extracellular polymer matrix via pili/nanowires and multiple cytochromes	[33]

Table 2 Components of EPS released by pure cultures of electrotrophs under stressful heavy metal conditions and analyzed by Three-dimensional fluorescence excitation-emission matrix spectroscopy

Strain	Origin	Carbon source	Stressful condition		EPS components					Refs.
					Tyrosine-like matters	Tryptophan-like materials	humic acid-like matters		soluble microbial byproduct-like matter	
							fulvic acid (ultraviolet region)	fulvic acid (visible region)		
Stenotrophomonas maltophilia JY1 (GenBank MZ701893)	Cu(II)	HCO_3^-	Cu(II)	–600 mV	0	+	0	+ +	+	[45]
				–900 mV	0	+	0	+ +	+	
Citrobacter sp. JY3 (GenBank MZ701895)	Cu(II)		Cu(II)	–600 mV	0	+	+ +	+	+	
				–900 mV	0	+	+ +	+ +	+	
Pseudomonas aeruginosa JY5 (GenBank MZ701899)	Cu(II)		Cu(II)	–600 mV	0	+	0	+	+	
				–900 mV	0	+	0	+ +	+ +	
Citrobacter sp. JY3 (GenBank MZ701895)	Cd(II)	acetate	Cd(II) (40 mg/L)	CCC	0	+ +	+	+	+ +	[44]
				OCC	0	+ +	+ +	+ +	+	
Pseudomonas sp. X3 (GenBank MZ703193)				CCC	0	+	+ +	+ +	+ +	
				OCC	0	+ +	+	+	+	
Pseudomonas delhiensis X5 (GenBank MZ703197)				CCC	0	+ +	0	+	+ +	
				OCC	0	+ +	+	+	+	
Ochrobactrum anthropi X7 (GenBank MZ703195)				CCC	0	+ +	+	+	+ +	
				OCC	0	+	+ +	+ +	+	
Stenotrophomonas sp. JY6 (GenBank MT982677.1)	Cu(II)	HCO_3^-	light irradiation		0	+	0	0	+	[47]
			no irradiation control		0	+	0	0	+	
Serratia marcescens Q1 (GenBank MT982676.1)	Cu(II)	HCO_3^-	Cu(II)	biofilms	0	+ +	+ +	+	+	[40]
				plankton cells	0	+	+ +	+	+ +	

			magnetic field		0	+ +	+ +	+ +	+ +	[41]
			no magnetic field control		0	+	0	0	+	
			OCC		0	+	0	0	+	
			magnetic field and Cr(VI)		0	+ +	+ +	+ +	+	[42]
			no magnetic field control		0	+	+	+	+	
			no Cr(VI) control		0	+	+	+	+ +	
			light irradiation		+ +	+ +	+ +	+ +	+ +	[47]
			no irradiation control		+	+	0	+	+	
Pseudomonas sp. X3 (GenBank MZ703193)	Cd(II)	HCO_3^-	Cd(II)	CCC	0	+	+	+	+ +	[46]
				OCC	+	+	+	+	+	
Pseudomonas delhiensis X5 (GenBank MZ703197)				CCC	0	+	+	+	+ +	
				OCC	+	+	+	+	+	
Ochrobactrum anthropi X7 (GenBank MZ703195)				CCC	+	+ +	+	+ +	+ +	
				OCC	+	+	+	+	+	

Note: + + means more components of EPS; + indicates less components of EPS; CCC: closed circuital conditions; OCC: open circuital conditions

Highlights:

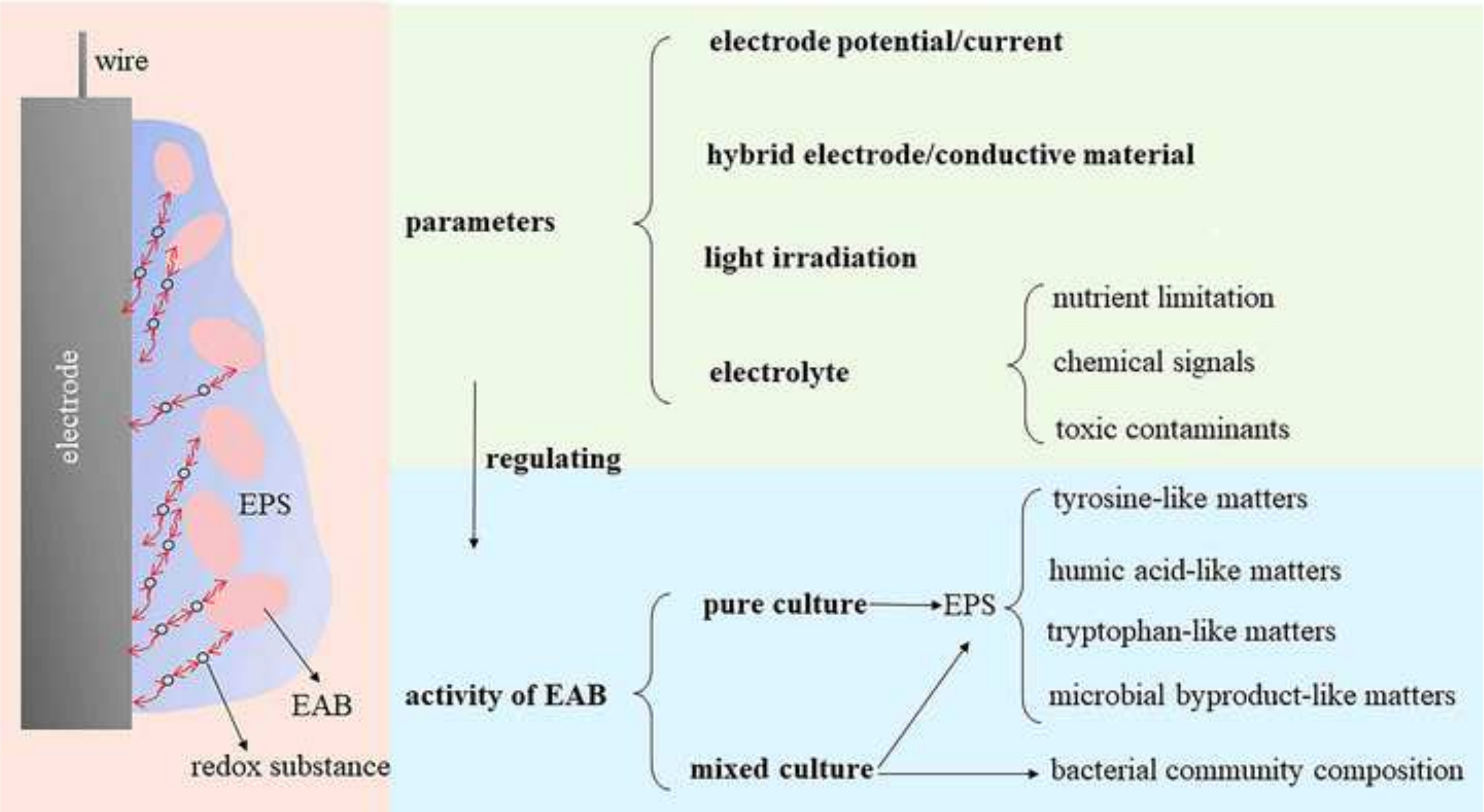
→ EAB secrete tunable EPS in response to changes of process parameters;

→ Pure and mixed cultures EAB regulate EPS composition and redox properties;

→ EPS harbor flavoproteins and *c*-type cytochromes for multistep hopping processes;

→ Quantifying composites and structures of EPS, and their dynamics should be exploited.

Graphical Abstract



Declaration of Interest Statement

The authors Liping Huang (lipinghuang@dlut.edu.cn) and Gianluca Li Puma (g.lipuma@lboro.ac.uk) declare no interest conflict.