Review

Extracellular Polymeric Substances and Biocorrosion/Biofouling: Recent Advances and Future Perspectives

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Abstract: Microbial cells secrete extracellular polymeric substances (EPS) to adhere to material surfaces, if they get in contact with solid materials such as metals. After phase equilibrium, microorganisms can adhere firmly to the metal surfaces causing metal dissolution and corrosion. Attachment and adhesion of microorganisms via EPS increase the possibility and the rate of metal corrosion. Many components of EPS are electrochemical and redox active, making them closely related to metal corrosion. Functional groups in EPS have specific adsorption ability, causing them to play a key role in biocorrosion. This review emphasizes EPS properties related to metal corrosion and protection and the underlying microbially influenced corrosion (MIC) mechanisms. Future perspectives regarding a comprehensive study of MIC mechanisms and green methodologies for corrosion protection are provided.

Keywords: extracellular polymeric substances; microbially influenced corrosion; corrosion protection

1. Introduction

Hydrated biopolymers including polysaccharides, proteins, nucleic acids, and lipids are secreted to encase and immobilize cells. These biopolymers are responsible for the macroscopic appearance of biofilms (Figure 1), which is frequently referred to as "slime" [1]. It is complicated to fully characterize and differentiate EPS composition from cellular components or transiently produced macromolecules. Biochemical and structural identification is hindered by the fact that a diverse array of saccharides with complex linkages are produced, which is defined as "the dark matter of biofilms" [2,3]. EPS were originally abbreviated for extracellular polysaccharides. Later, it was discovered that these so-called extracellular slimes also contain proteins, lipids, humic acids, and other substances. Therefore, EPS were then officially renamed as extracellular polymeric substances [4].
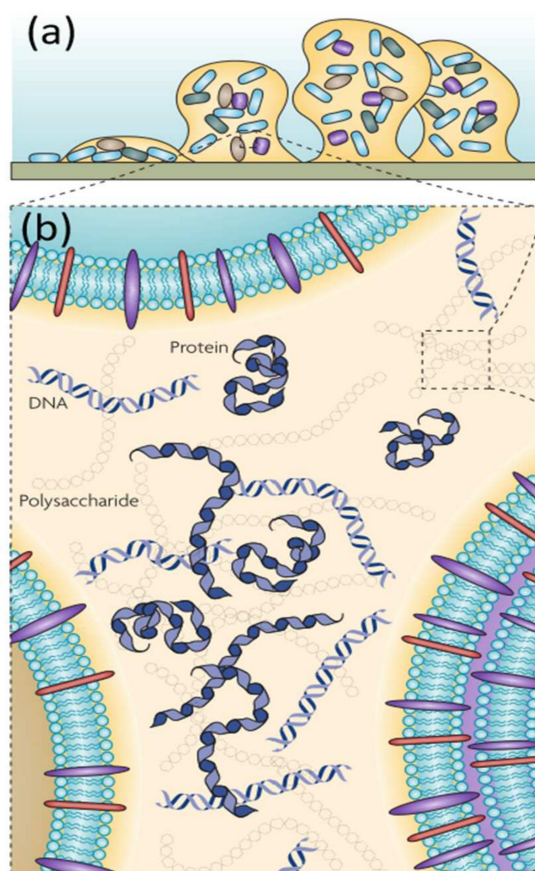
Figure 1. (**a**) The model of a bacterial biofilm attached to a solid surface; biofilm formation begins if bacteria attach to a solid surface and then divide to form microcolonies and produce EPS. Due to bacterial division and EPS formation, different kinds of microorganisms are attracted to enter the consortia/film, and a mature biofilm is gradually formed; (**b**) The major matrix components (polysaccharides, proteins, lipids, and nucleic acid) are mainly distributed between cells, and there are differences in different regions of EPS. Images are from [3]. Reproduced with permission from Springer Nature.

Metal/material corrosion caused by microorganisms attached to the surface of metallic materials is termed as MIC. The problem of MIC causes hundreds of millions of dollars in loss to the global economy every year [5]. Microorganisms secrete EPS to attach and adhere on metal surfaces [3,6]. The interactions of some EPS and biofilms with metals result in metal dissolution. There are many types of microorganisms causing MIC, such as sulfate-reducing prokaryotes (SRP) [7–12], manganese/iron-oxidizing bacteria (MOB/IOB) [13,14], iron-reducing bacteria (IRB) [15,16], methanogenic and halophilic archaea [17,18], nitrate-reducing bacteria (NRB) [19,20], and others [21,22]. Table 1 summarizes some representatives of SRP, iron oxidizers/manganese oxidizers, iron reducers, sulfur compound oxidizers, acid producing bacteria/fungi, and microbes that secrete organic acids and produce EPS. Detailed summary is shown in Table 1.

These MIC causing microorganisms are embedded sometimes in an EPS matrix and appear in the form of biofilms [23]. EPS play an important role in the formation of biofilms. They are mainly responsible for the structural and functional integrity of biofilms (Figure 1). The EPS components determine the cohesion, integrity and stress resistance of biofilms and are considered to be the key components determining the physical, chemical, and biological properties of biofilms [3,24]. These properties will determine the corrosion processes of metals.

Table 1. MIC causing microorganisms and their characteristics, summarized and modified on the basis of [25,26].

Type	Aerobic/Anaerobic	Corrosion Agents	Mechanism of Corrosion	References
Sulfate reducers *Desulfovibrio* sp. *Desulfomonas* sp. *Desulfotomaculum kuznetsovii* *Archaeoglobus fulgidus*	Anaerobic	H_2S and FeS	Cathodic depolarization by hydrogen uptake, anodic depolarization by corrosive iron sulfides, electrons extracted from Fe^0	[6,8,11,27–29]
Iron oxidizers/ manganese oxidizers *Gallionella* sp. *Mariprofundus ferrooxydans* *Leptothrix* sp. *Mariprofundus* sp. *Bacillus* sp.	Aerobic	Fe^{2+} to Fe^{3+} and Mn^{2+} to Mn^{4+}: Iron oxide and manganese dioxide formation	Deposition of cathodically reactive ferric and manganic oxides	[14,15,30–33]
Iron reducers *Pseudomonas* sp. *Shewanella* sp. *Geobacter sulfurreducens*	Aerobic	Reduce Fe^{3+} to Fe^{2+}, Mn^{4+} to Mn^{2+} manganese or iron oxide reduction	Reduction of iron and manganese oxides	[15,31,34]
Sulfur compound oxidizers *Thiobacillus* sp. *Acidithiobacillus ferrooxidans* *Acidithiobacillus caldus*	Aerobic	H_2SO_4	Acids corrode metal	[35–37]
Acid producing bacteria and fungi *Clostridium* sp. *Fusarium* sp. *Penicillium* sp. *Hormoconis* sp. *Bacillus subtilis* *Marinobacter* sp.	Aerobic and anaerobic	Acids	Dissolve iron, chelate copper, zinc, and iron	[38–42]
Slime (EPS) forming bacteria/almost all microorganism *Clostridium* sp. *Bacillus* sp. *Desulfovibrio* sp. *Pseudomonas* sp.	Aerobic and anaerobic	extracellular polymeric substances (biofilm) or surface compounds/ions	Exopolymers capable of binding metal ions	[43–46]
Methanogens *Methanobacterium* sp. *Methanococcus* sp.	Anaerobic	Extracellar hydrogenases, acids, and CO_2	Methane production with direct iron oxidation; syntrophic interaction with fermentative microbes or SRP; deposition of cathodically reactive ferric oxides; consumption of hydrogen generated by CO_2 corrosion	[17,47–49]

Current studies on EPS involved in MIC mainly include the analysis of the corrosion behavior of metals in EPS solutions extracted from corrosion-causing microorganisms and exploration of the relationship between EPS and metal corrosion by analyzing surface morphology and composition of metallic materials, environmental parameters, and electrochemical behavior, or by in-vitro simulating the composition of EPS by artificially adding

sodium alginate, bovine serum albumin (BSA), and cytochrome *c* to mimic extracellular polysaccharides, proteins, and electrochemically active components [50]. The corrosion effects of EPS produced by various bacteria/archaea on metal corrosion are quite different [51,52]. Existing studies have found that some functional groups in EPS will complex metal ions (iron ions, copper ions, etc.) and, thus, accelerate the dissolution of the anode causing corrosion [53,54]. However, there are also studies showing that the attachment of EPS has an inhibitory effect on the corrosion of a metal, mainly because EPS adsorb on the surface of a material and form a protective film. The negatively charged groups in EPS chelate the metal cations (such as Ca^{2+} and Mg^{2+}) in the solution, which results in a dense protective film on the surface of a material, reducing cathodic polarization and, thus, inhibiting corrosion [44,55–57]. When copper was immersed in 3.5 wt.% NaCl solution containing EPS produced by an aerotolerant and unidentified *Desulfovibrio*, copper corrosion was promoted in EPS solution for long-term immersion of 11 days, although corrosion was inhibited for a short time of 2 h. Functional residues in the EPS like hydroxyl, carbonyl, carboxyl, and phosphate groups are the main ones to react with Cu, influencing the corrosion behavior of copper in 3.5 wt.% NaCl solution [51]. When 70Cu-30Ni alloy was immersed in EPS produced by *Pseudomonas* NCIMB 2021, a protein-adsorbed metal oxide layer was formed on the surface of 70Cu-30Ni alloy. It inhibited the corrosion of 70Cu-30Ni alloy [58].

To a large extent, microbial corrosion is due to the formation of a biofilm by microorganisms attached to the metal surface. Biofilms are the environment allowing for a survival of microbial cells [3]. Therefore, understanding EPS properties and the role of EPS in the corrosion process is of great significance for knowing the mechanisms of microbial corrosion and for microbial corrosion protection. This review describes EPS analysis, characterization methods, EPS characteristics, and the relationship between EPS and corrosion. In this way, it helps to understand the role of EPS in the corrosion process and provides basic information for corrosion protection.

2. EPS Properties

The EPS components determine its structure, function, and reactivity. The diversity of the chemical composition and the properties of EPS play a decisive role in biofilm development and metal corrosion.

2.1. Components

Usually, polysaccharides and proteins make up the major part of microbial EPS, in addition to nucleic acids, humic substances, and lipids as shown in Figure 1b. EPS biochemical composition can be influenced by many factors such as microbial species, substrates, and the downstream extraction methods [59]. The composition of EPS can change during the corrosion process. EPS derived from SRP of *Desulfovibrionaceae* and *Desulfobacteriaceae* species were mainly composed of 60% protein, 37% polysaccharides, and 3% hydrocarbons. Adsorbed EPS on mild steel immersed in the 10-fold diluted EPS solution changed significantly after 60 days; molecular fractions of proteins, polysaccharides and hydrocarbons were changed from 0.64, 0.31, and 0.05 to 0.52, 0.08, and 0.40 [60]. Interestingly, hydrocarbons were detected in EPS materials at the beginning of the experiment. This may be attributed to the used medium based on seawater from Hong Kong Harbor. This harbor was contaminated with hydrocarbons as most harbors are. Most likely, residual hydrocarbons from contaminated seawater were absorbed in EPS. Nevertheless, we believe that conclusion was not justified completely and need a comprehensive investigation (e.g., purification of EPS), or the authors had made a mistake of using hydrocarbon instead of lipids. Functional EPS-groups such as carboxyl and hydroxyl play an important role in bacterial aggregation and biofilm formation [61]. The composition of EPS components changes with the microbial community and the environment, in which microorganisms are located [62,63]. Atalah et al. conducted a characterization of EPS from a thermophilic consortium isolated from a corroded airplane engine and found that the polysaccharides

are composed mainly of mannose and glucose residues. The predominant protein was surface (S)-layer protein [64]. The composition of EPS from an unidentified thermophilic SRB strain isolated from the Bohai oilfield in China was dependent on the culture stage. The highest EPS concentrations were extracted from a 14-day old culture [65]. Pressure affects the secretion of EPS. Under high pressure of 35 MPa, *Halanaerobium* spp. secrete more proteins than that of 0.1 MPa probably to increase adhesion [66].

2.2. Adhesion

EPS are inherently adhesive. The adhesion not only plays an important role in the formation of biofilms, but also increases the possibility of metal corrosion [67]. Studies described that the adhesion of EPS is related to a S-layer protein, which can act as an adhesive to adjacent cells, thereby further enhancing the aggregation of microorganisms during the formation of biofilms [64,68]. EPS can also increase cell adhesion through hydrophobic forces (primary effect) and/or electrostatic forces (secondary effect) [69]. Acetyl groups are a common substituent of extracellular polysaccharides and increase the adhesion and cohesion of EPS, which may alter the structure of a biofilm [70]. Mayer et al. measured the viscosity of EPS of *Pseudomonas (P.) aeruginosa* using nuclear magnetic resonance (NMR) and viscometry and found that electrostatic interactions and hydrogen bonding were the main reasons for the strong biofilm binding of EPS [71]. Thus, the adhesive properties of EPS increase the accumulation of microorganisms on metal surfaces and the formation of biofilms.

2.3. Redox Active EPS and Role in Electron Transfer

Many EPS components have been proven to have redox properties and electrochemical activity. Redox active EPS compounds play an important role in the process of electron transfer [72,73]. Extracellular electron transfer is an important component of microbial respiration [74,75]. Metallic materials are used commonly by microorganisms as electron donors [72,76]. Extracellular electron transfer is achieved mainly through direct or indirect electron transfer. The direct electron transfer occurs mainly through *c*-type cytochromes or conductive nanowires, while the indirect electron transfer happens mainly through electron shuttles such as phenazine, humic acids, or flavin (Figure 2). The specific path of bacterial extracellular electron transfer through cytochromes and nanowires is shown in Figure 3 [72,77–79]. Membrane-bound cytochrome *c* is a redox protein involved in electron transfer, which contributes often to MIC processes. Through proteomic analysis of the EPS of a *Shewanella* sp. HRCR-1 biofilm, 58 extracellular and outer membrane proteins were identified, including the *c*-type cytochromes Mtr C and Omc A [80]. The electroactive bacteria *Shewanella (S.) oneidensis* contain heme-binding proteins, which are redox components in the EPS [72]. Humic acids are an important component of EPS and can be used as electron shuttle to support microbial growth [81]. In addition, microorganisms can use humic acids as exocellular electron mediator for anaerobic respiration of organic compounds, which is effected by an indirect electron transfer [82]. EPS components can function as electron shuttle in the corrosion process. EPS can combine with iron ions to cause anodic dissolution and promote iron corrosion [54]. EPS can increase the electronic potential and reduce the electronic resistance, thereby increasing the corrosion current by promoting electron transfer and metal dissolution [69]. The metal ions bound in the EPS potentially can act as electron shuttle and transfer electrons to distant microorganisms [83]. Extracellular DNA (eDNA) is also an important component of EPS. Studies have found that eDNA can combine with the electron shuttle compound phenazine to promote the process of extracellular electron transport. Pyocyanin (PYO) and phenazine carboxamide can combine with eDNA to be retained in EPS, thus promoting an effective electron transport (Figure 4) [84].

At present, although it has been proven that some substances in EPS have redox properties, the role of EPS for the corrosion caused by microorganisms using extracellular electron transport remains under debate. The pathway of electron transfer in EPS remains

unclear, as shown in Figure 2. Some studies indicate that electron hopping is the most likely hypothesis for electrons to pass through EPS [85]. Studies have also found that EPS polysaccharides can hinder electron transport. These substances remain inactive substances and do not promote the microbial reduction process [50,86].
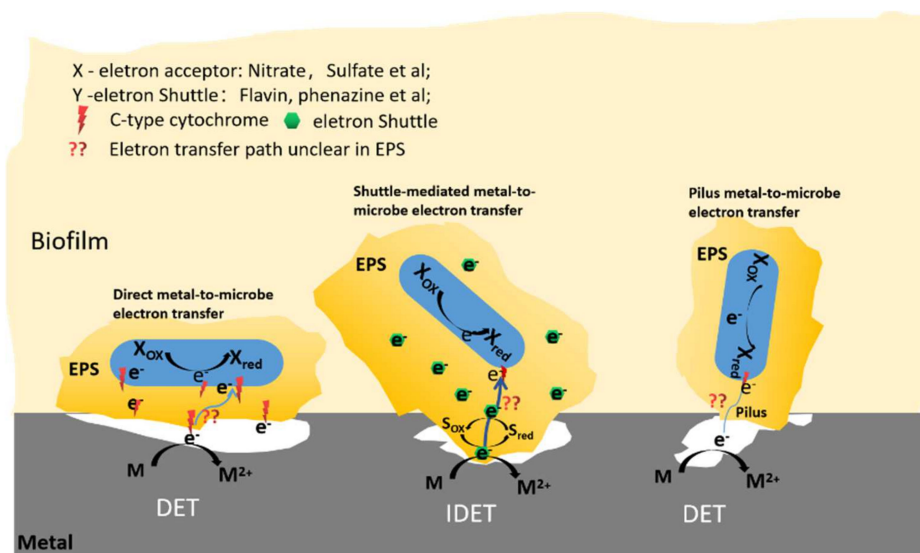

Figure 2. A hypothetical electron transfer pathway in presence and absence of EPS. DET, direct electron transfer; IDET, indirect electron transfer. Adapted from [78].
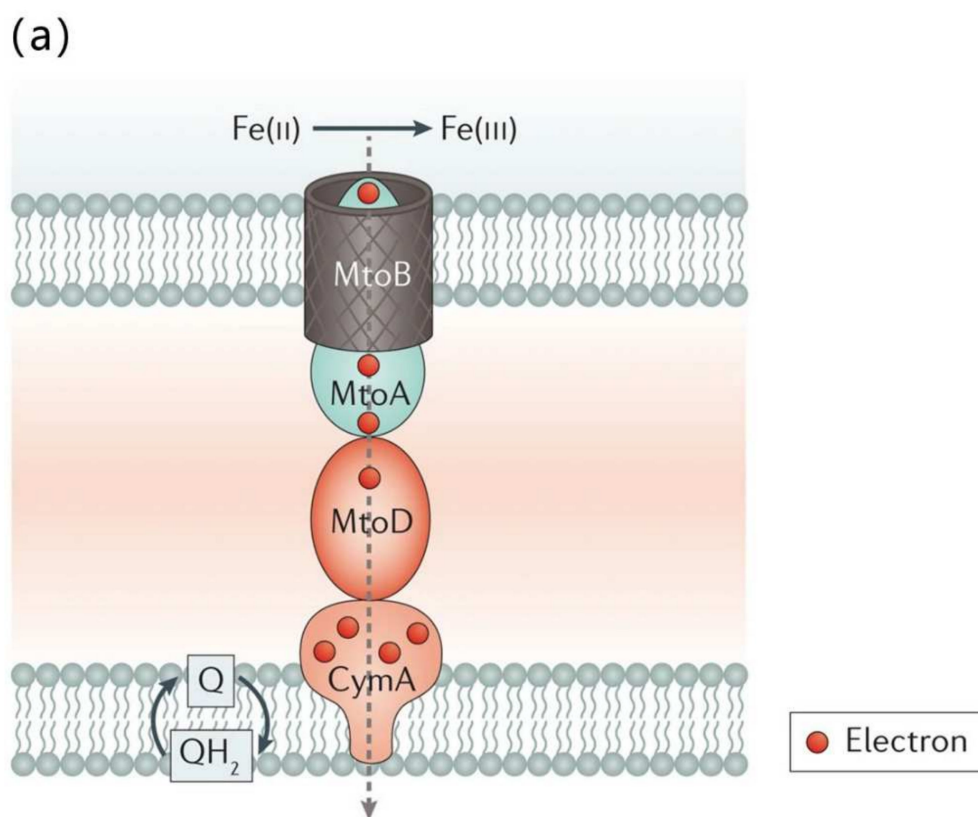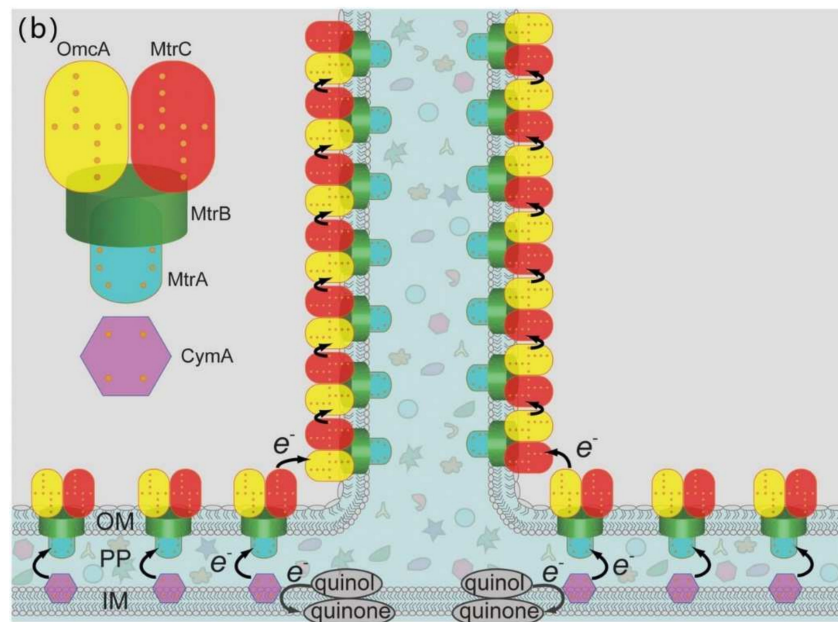

Figure 3. *Cont.*

Figure 3. (**a**) *Sideroxydans lithotrophicus* ES-1 uses *c*-type cytochromes (*c*-Cyts) for direct electron transport. The metal oxidation pathway (Mto) of *Sideroxydans lithotrophicus* ES-1 is MtoA (a multihaem *c*-Cyts (mtrA) homolog), MtoB (a porin-like outer membrane protein MtrB homolog), and MtoD (a mono-haem *c*-Cyt) and CymA (a multihaem *c*-Cyt), electrons transferred from extracellular Fe(II) to quinone (Q) in the inner cytoplasmic membrane and cytoplasmic membrane, respectively [72]. Reproduced with permission from Springer Nature. (**b**) Proposed structural model for nanowires of *S. oneidensis* MR-1. *S. oneidensis* MR-1 nanowires are outer membrane (OM) and periplasmic (PP) extension including various *c*-type cytochromes such as OmcA, MtrC, MtrB, MtrA, for electron transfer between these cytochromes, thus enabling extracellular electron transfer in bacteria [79]. Reproduced with permission from PANS.
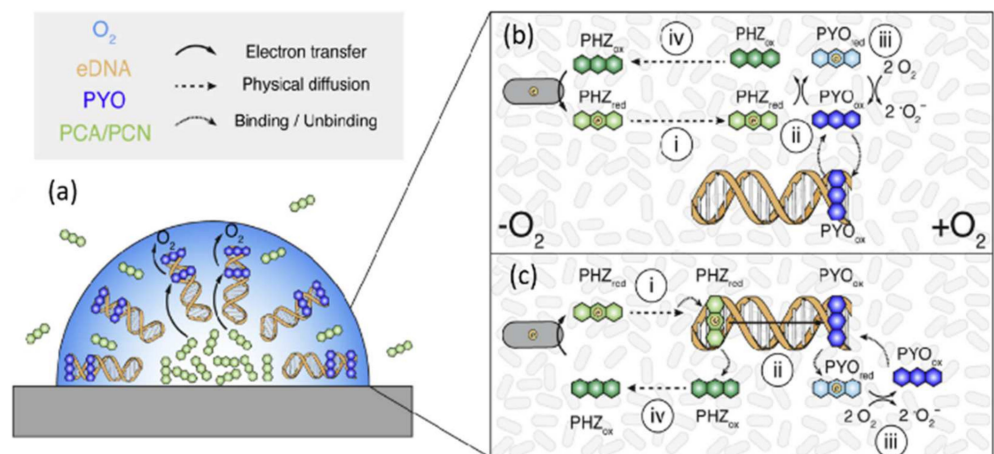
Figure 4. Schematic diagram of eDNA and the electron shuttle compound phenazine bind in biofilm. (**a**) Phenazine binds eDNA to transfer electrons to oxygen in biofilms; (**b**,**c**) two mechanisms of eDNA-mediated phenoazine electron transport [84]. Reproduced with permission from Elsevier.

3. EPS Extraction

The choice of EPS extraction greatly affects, qualitatively and quantitatively, the recovery of EPS and its constituents and whether they are obtained in a native state. Therefore, it is very important to choose an appropriate extraction method for studying EPS involved in biocorrosion. The extraction method must avoid the damage of microbial

cells. Meanwhile, it is necessary to ensure the extraction efficiency. Up to now, several methods for EPS extraction have been published, as listed below. The specific extraction method is shown in the Supplementary Figure S1.

(1) Physical methods: these methods mainly use an external force to separate EPS from microbial cells and transfer the EPS components to the solution. Commonly used methods include high-speed centrifugation [87], heat extraction [85] and ultrasonic extraction [88]. Among them, the heat extraction may destroy the cell structure of microorganisms due to high temperature application, so that intracellular substances may be released into the EPS solution. This method compromises the EPS purity. Additionally, high temperature may deactivate some redox active substances and enzymes in EPS. Thus, it is necessary to control the temperature and extraction conditions. Dai et al. studied five methods for EPS extraction from *S. oneidensis* MR-1: centrifugation (control), heating (40, 45, 50, and 60 °C), and treatments with H_2SO_4, ethylenediaminetetraacetic acid (EDTA), and NaOH. They found for this bacterium that heat (40 °C) and EDTA treatments were the most suitable methods for EPS extraction considering both the low cell lysis and high EPS content [89].

(2) Chemical methods: these methods add appropriate amounts of chemical reagents and/or solvents to the cell suspension to extract EPS. Water-soluble EPS macromolecules are solubilized and removed into the extraction medium. Common chemical methods are the use of alkali [90], formaldehyde/NaOH [91], NaCl [92], Ethanol [93], Crown ether [94], EDTA, and cationic resins [91]. The concentration of the extractants should be controlled properly, otherwise cell damage or even disruption may be caused. The cation exchange resin method is the most commonly used one. Sodium ions in the cation exchange resin are removed by divalent cations bound in the EPS of the bacteria. This weakens the forces between the extracellular polymers and the bacterial cell. In the next step, centrifugation as physical force can separate the EPS constituents from the cells [95,96].

EPS from *Acidiphilium* 3.2Sup(5) were investigated using five methods: EDTA, NaOH, ion exchange resin, heating, and centrifugation. A high EPS extraction was obtained using EDTA. This method also produced a lesser degree of cellular lysis. Nevertheless, both the amount and the chemical composition of EPS strongly depended on the extraction method used [97]. So far, none of these methods can extract all EPS constituents [98]. Some constituents remain attached to the microbial cells. Consequently, there is no universal EPS extraction method for all microbial EPS type. The extraction process must be adapted to the specific type of biofilm under study and the purpose of the research (Supplementary Table S1).

4. EPS Characterization

EPS are a gel, thus their composition and structure cannot be observed directly. Special characterization techniques are required to analyze structure and properties of EPS. These include in-situ observation of the composition of EPS without damaging it.

One non-destructive method is the use of fluorescently labeled lectins combined with confocal laser scanning microscopy (CLSM) to observe and analyze EPS in biofilms in situ [99]. Lectins are proteins or glycoproteins of plants, animals, or microbial origin. They bind to carbohydrates with a characteristic specificity [100,101]. Fluorescently labelled lectins can be used as probes to investigate an EPS composition enabling the microscopic in situ detection of EPS and its constituents in their distribution in biofilms [102–104]. Different types of lectins are used to observe various glycoconjugates in EPS (Figure 5). Different lectins can specifically recognize a specific sugar monomer; for example, the lectin of *Canavalia ensiformis* (Con A) can recognize glucose and mannose residues, the one of *Vicia graminea* (VGA) can recognize n-acetylglucosamine, etc. For details of the lectins see [103]. Strathmann et al. used fluorescently labelled lectins in combination with epifluorescence microscopy and CLSM to visualize and characterize carbohydrate-containing EPS in biofilms of *P. aeruginosa* [101]. Zhang et al. used fluorescently labeled lectins to study the glycoconjugates of EPS secreted by three meso- and thermoacidophilic metal-oxidizing archaea, namely *Ferroplasma acidiphilum*, *Sulfolobus metallicus*, and an unindenfied

Acidianus sp. on pyrite [103]. They found that EPS of the three archaea are divided into compact and loose EPS. These archaea produced different EPS glycoconjugates on the surface of pyrite [103]. Zippel and Neu et al. characterized the *in situ* distribution of EPS glycoconjugates in tufa-associated biofilms of two German hard-water creeks by employing fluorescence lectin-binding analysis [105].

In addition to visual analysis of EPS in biofilms, ^{1}H and ^{13}C NMR, Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy [98], and X-ray photoelectron spectroscopy (XPS) are used to analyze and identify EPS compounds. The three-dimensional excitation emission matrix (3D-EEM) can also be used to analyze the composition of the EPS. In addition to the above methods used to characterize EPS, there have been many new types of spectroscopic methods for *in situ* characterization of EPS components and functional groups in recent years. Maqbool et al. combined the use of stable isotope analysis and 13C-cross-polarization magic-angle spinning (CPMAS) NMR to examine the extent of the formation of new EPS and the turnover cycles of different carbon structures in EPS [106]. Lin et al. proved carboxyl-rich acidic polysaccharides in EPS by sulfur K-edge X-ray absorption near edge structure (XANES) analysis [107]. Fang et al. used X-ray absorption fine structure (XAFS) spectroscopy to study the adsorption of EPS on goethite extracted from *P. putida* [108]. XAFS results demonstrated that phosphate groups in EPS can form monodentate inner-sphere complexes at pH 3.0, while they form bidentate inner-sphere complexes at pH 9.0 [108]. Scanning transmission X-ray microscopy (STXM) and XAFS are used to characterize the interactions between *Arbuscular mycorrhizal* EPS and Cr ions [109]. STXM allows to obtain EPS structure correlated spectra [110]. The distribution of metal ions with different valence states (such as iron ions) on the surface of EPS can be observed by STXM [86]. EPS at the cell-pyrite interface can be observed by STXM- based C-NEXAFS analysis [111].
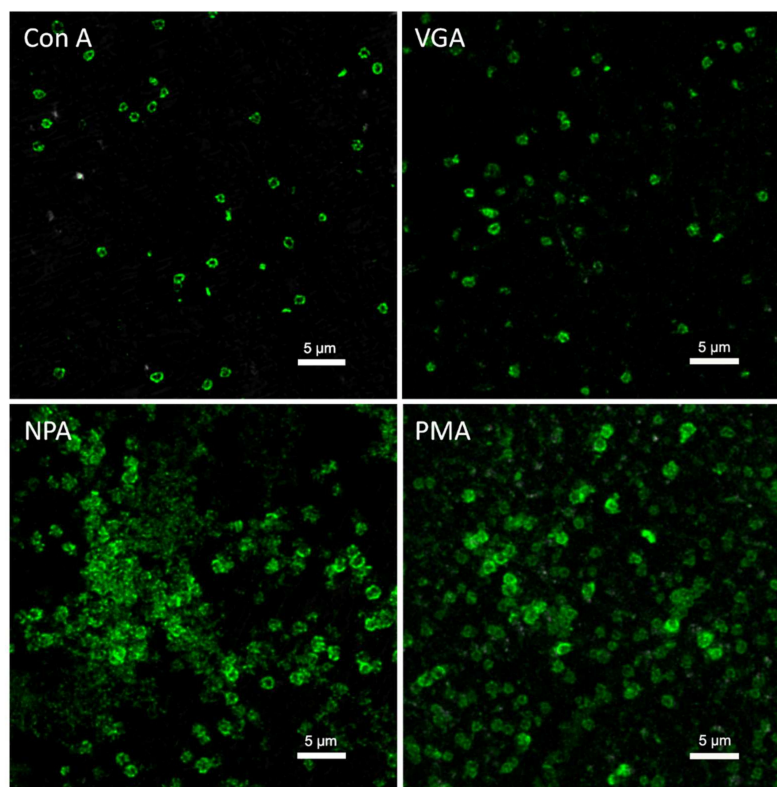
Figure 5. Images of *Sulfolobus metallicus*[T] biofilms on elemental sulfur stained by Fluorescein isothiocyanate (FITC)-labeled lectins Con A, VGA, NPA (*Narcissus pseudonarcissus*), and PMA (*Polygonatum multiflorum*) are shown. Images were reproduced from [104] with permission from Springer Nature.

5. Detection of Cell Lysis

No matter which method is used for EPS extraction, the extent of cell lysis caused by EPS extraction must be determined and taken into consideration for the validity of the results and conclusions. A harsh extraction of EPS can disrupt the integrity of the cells and lead to a release of intracellular compounds such as polysaccharides and proteins. This is affecting the complexity of the analysis of EPS components and often makes conclusions on the role of EPS constituents impossible. Alginate extracted from *P. aeruginosa* contains uronic acids and alginate does not exist in cells. Therefore, alginate can be used as a sign of contamination with cellular components [112]. N-acetylglucosamine (NAG) is one of the monomers of peptidoglycan in the cell wall. Thus, the degree of cell damage caused by EPS extraction can be judged by detecting the concentration of NAG [113]. The higher the concentration of NAG in the EPS, the more cells have been damaged. Glucose-6-phosphate dehydrogenase (G6PDH) is an enzyme in the cytoplasm of the cell and can be used as a sign of cell lysis [114]. The cell lysis indicated by G6PDH seems to be dependent on the extraction procedure, e.g., stirring intensity, extraction time, dose of the extraction agent [95]. Wang et al. used ATP for quantifying the EPS contamination by intracellular compounds due to cell lysis [115]. The viability of the cells can also be detected by using the LIVE and DEAD staining method to confirm that cells are still active after stripping off the EPS [116]. However, it must be noted that LIVE and DEAD staining regents are only calibrated against the standard cultures and the calculated live/dead ratio may not reveal the true proportion of living/dead cells for these target cultures in each experimental condition (H.-C. Flemming, personal communication). Scanning electron microscopy (SEM) can be used to observe the changes in the morphology of the cells before and after EPS extraction. Atomic force microscopy (AFM) can also be used to observe the morphology of microorganisms before and after EPS extraction [85,86]. The determination of cell damage aims mainly to detect intracellular substances outside of cells to judge about possible contamination of the EPS extraction. According to the needs of an actual test, the detection of cell lysis needs to be selected. In addition to the detection of landmark substances, AFM and SEM can be combined to detect and observe the activity and morphology of bacteria after extracting EPS.

6. EPS and Biocorrosion

At present, it can be ascertained that EPS can accelerate or inhibit corrosion depending on the microorgansims and environments. The following chapter is divided into two parts to discuss the relationships between EPS and biocorrosion.

6.1. EPS Accelerated Corrosion

The acceleration of metal corrosion caused by EPS mainly includes the following points: (1) EPS contain a large number of anionic groups such as carboxyl, hydroxyl and/or amino groups, etc. These groups can chelate metal ions, and thus promote the occurrence of corrosion [117]; Copper metal was corroded after 11 days of immersion in EPS extracted from the sulfate reducing bacterium *Desulfovibrio* sp. Copper corrosion is promoted due to the destruction of the protective Cu_2O film by EPS and its constituents. The main reason causing corrosion was that the hydroxyl, carbonyl, carboxyl, and phosphate groups in EPS reacted with Cu^+ in the Cu_2O protective film, which destroyed it finally [51]. The functional groups in the EPS play an important role for the shuttling electrons in the corrosion process of iron metals. EPS combined with iron ions lead to anodic dissolution promoting Fe corrosion [54]. EPS can increase the electric potential and reduce the electric resistance of materials causing an increase of the corrosion current. Thereby, they are promoting electron transfer and metal dissolution [52]. If microorganisms come into contact with metal surfaces, EPS have been shown to chelate iron ions and stimulate the interaction between microorganisms and metal ions [83]; (2) EPS can change the chemical properties of corrosion products and their morphology, and promote the corrosion of metals [68]; When a metal is immersed in EPS extracted from an iron-oxidizing bacterium, accelerated corro-

sion was detected [52]. Arkan et al. found that *Desulfovibrio* sp. led to the pitting corrosion of 316L SS, mainly due to the production of extracellular proteins [118]. Li et al. performed electrochemical and surface analyses for mild steel and pure copper and found that EPS accumulated on the metal surface. This resulted in an heterogenous biofilm and the formation of oxygen concentration cell causing corrosion, leading to severe pitting [119]. Zhang et al. analysed the in situ microbial diversity in artificial surface-associated marine biofilms and found that EPS-related genes were more abundant in copper-associated biofilms than that in aluminium-associated biofilms. They thus proposed that copper corrosion in natural marine environments was attributed to the heterogeneous microenvironments within the biofilms caused by the EPS [120]. Fang et al. found that EPS increased the corrosion of mild steel due to its acidity and its binding with iron [121]. Interestingly, it was reported that EPS production of *Halanaerobium congolense* WG8 increased, if the hydraulic pressure increased. Consequently, MIC was accelerated [66]. This study points out the necessity of studying microbial physiology and MIC under high pressure conditions such as hydraulically fractured shales or deep-sea environments; (3) When corrosion of metals is accelerated with biofilm formation and growth, anodic dissolution can occur. The metallic dissolution would be accelerated to form complexes with EPS. Consequently, the over-potential of metallic dissolution would decrease, and mixed potential should be shifted in the less noble direction, although Little et al. repeatedly described the potential shift in nobilization in the noble direction as an important characteristic for MIC [122,123]. When a metal is immersed in EPS extracted from unidentified iron-oxidizing bacteria isolated from the sludge of a Sinopec oilfield in China, accelerated corrosion was detected. It was found by potentiodynamic polarization curve that the corrosion current was higher and the corrosion potential was lower in the solution containing EPS in comparison with that containing no EPS, and EPS promoted the cathodic reaction processes [52].

6.2. EPS Inhibit Corrosion

EPS can adsorb on the metal surface and form a biofilm, which acts as a protective layer, hinders the transmission of oxygen, and separates a metal surface from a corrosive environment, thus, metal corrosion is inhibited [53,54,124–127]. The negatively charged functional groups in EPS, such as $-NH_2$, -COOH, -C-O-C, and -OH, first adsorb positively charged metal ions to form complexes. Then, EPS interact with metal ions by oxidation and precipitation to form biomineralized layers on the metal surface, which can protect metals from corrosive environments such as seawater [56,128,129]. The EPS control the kinetic pathway of $CaCO_3$, biomineralizing nucleation and $CaCO_3$ crystal growth. The formation mechanism of a biomineralized film is illustrated in Figure 6 [56,130]. Microorganisms with an over-production of EPS form a biomineralization film on the surface of a metal and protect it from corrosion. However, if microorganisms secrete only a small amount of EPS, a complete biological mineralization cannot be achieved. Consequently, accelerated corrosion due to the formation of concentrated elements on the surface will occur [130]. The researchers extracted polysaccharides from EPS and showed that the hydroxyl groups in the extracellular polysaccharides can adsorb/chelate Fe^{2+}/Fe^{3+} ions and attach to the surface of carbon steel. In this way, a dense protective film is formed, which reduces the diffusion rate of oxygen and Cl^- ions and, thus, inhibits the corrosion of carbon steel [131,132]. Li et al. found that EPS can protect X65 steel by preventing bacterial cells of SRP from adhering to the surface of X65 steel and blocking SRP from gaining electrons from the metal [133]. Recently, it was shown that a marine isolate of *P. stutzeri* can promote the formation of a protective biomineralization film, which had a good inhibition effect against steel corrosion [134]. *S. putrefaciens* could use cell walls as the nucleation sites to induce the formation of a protective biomineralized layer, which contained calcite and EPS on the steel surface [129].

In summary, the role of EPS in corrosion depends mainly on: (1) whether the functional groups in the EPS can interact with metal ions; (2) whether the film formed by EPS adsorption on the metal surface is closed or patchy to isolate the metal from the corrosive

environment; (3) whether EPS components such as proteins and/or humic acids can carry out electron transfer and are related to the redox state of metals. In any case, the influence of EPS and its component for the corrosion of metals need to be explored further.
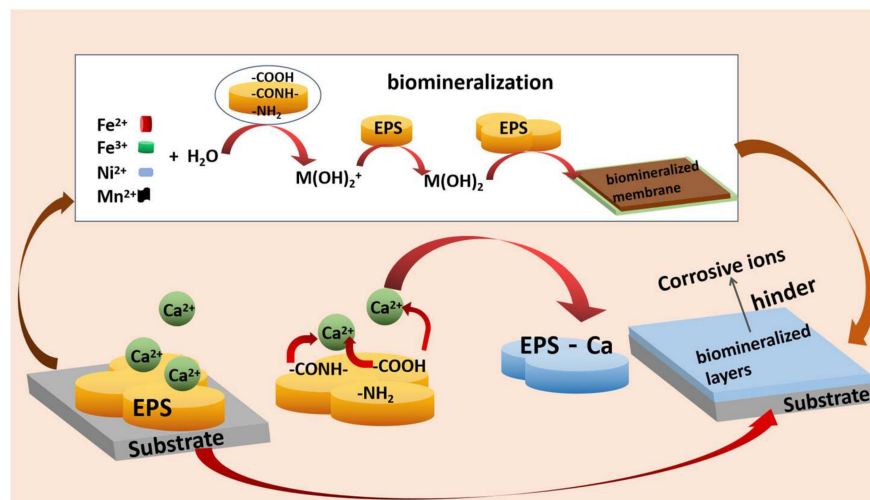
Figure 6. Diagram of formation of a biomineralized film. Adapted from [56]. Reproduced with permission from Elsevier.

6.3. Application of EPS as a Corrosion Inhibitor

EPS can be used as a new type of "green" corrosion inhibitor for metals [44]. They can be adsorbed on the metal surface and complexed with metal ions to form a protective film. This film acts as a barrier isolating a metal surface from a corrosive environment. Thereby the reaction between cathode and the anode is prevented. Moradi et al. found that EPS produced by *Vibrio neocaledonicus* can inhibit the corrosion of carbon steel in seawater up to ±95% [57]. The EPS extracted from waste-activated sludge also has a strong inhibitory effect on carbon steel, which is comparable to commercial corrosion inhibitors [124]. EPS secreted by a new isolate of *Marinobacter aquaeolei* has a corrosion inhibition efficiency of more than 91% for X80 steel, that proves that these EPS are an effective corrosion inhibitor [125]. As a corrosion inhibitor, an admixture based on EPS 180 exopolysaccharides was used in coatings. It has a good inhibitory effect on the corrosion of reinforced concrete. As a corrosion inhibitor, EPS will not affect the water absorption of concrete [135]. Differently functionalised cyclodextrins as EPS-analogue substances were tested to evaluate their inhibition effect on MIC by *Desulfovibrio vulgaris* under anaerobic conditions [136]. Out of the 25 tested compounds, (polymerised) carboxy(m)ethylated cyclodextrins showed the highest protective effect against MIC with 77% lower corrosion rates after 21 d incubation.

7. Perspective and Future Directions

New technical methods have been applied to explore the relationship between microorganisms and EPS and metal corrosion. The gene knockout method can control and adjust EPS secretion and verify the functional roles of EPS components [130]. Bioinformatics, mutational studies, and transcriptional profiling of biofilms have been used for identification of additional EPS components. Wu et al. identified multiple genes of *Pandoraea* sp. XY-2 involved in exopolysaccharide synthesis and EPS formation by bioinformatic analysis [137]. Cao et al. used a global proteomic approach and identified a total of 58 extracellular and outer membrane proteins in the EPS [80]. Twenty redox proteins were found in extracted EPS, such as *c*-type cytochromes, Mtr C, and Omc A, which have been implicated to participate in extracellular electron transfer [80,138]. The use of fluorescent markers such as lectins and CLSM can realize the visual and in situ analysis of EPS [103,139]. Lectins can

quickly identify carbohydrates in EPS and can be used to determine the role of EPS in microbial corrosion.

The study of EPS relevant to MIC is mainly limited in laboratory studies by the use of pure cultures. The data may not reflect the complexity of MIC processes. The mechanism of metal corrosion in natural biofilm environments is unclear still. Due to the complexity of EPS components and the diversity of EPS functions, the role EPS play in the process of corrosion requires further research. Improved knowledge regarding the relationship between biofilms/EPS and metal corrosion will provide further insights into the mechanism and the prevention of MIC.

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