

# Special Issue: Biofilm Composition and Applications

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Biofilms can be formed on both biotic and abiotic surfaces, including on living tissues, indwelling medical devices, industrial or portable water system piping, and natural aquatic systems [1]. It has been estimated that biofilms account for over 80% of the chronic and recurrent microbial infections in humans [2]. Biofilms are composed of microorganisms embedded in a self-produced extracellular matrix (ECM) composed of extracellular polymeric substances (EPS) such as polysaccharides, proteins, nucleic acids (e-DNA and e-RNA), lipids, and other biomolecules [3]. EPS accounts for 50%–90% of the total organic carbon of biofilms [4]. In addition to the above components, water (up to 97%) is a major part of biofilm and facilitates the flow of nutrients inside the biofilm matrix [5]. The composition and structure of biofilms can vary depending on the type of microorganism, the host environment, the availability of nutrients, shear stress, etc. [6]. Noncellular materials such as corrosion particles, mineral crystals, clay or slit particles, or blood components may be found in the biofilm matrix depending on the type of environment in which the biofilm has formed [7]. These medical devices commonly contain pure culture biofilms [7]. However, water system biofilms are highly complex and contain filamentous bacteria, freshwater diatoms, clay material, corrosion products, etc. [7].

EPS components are vital to providing structural and functional attributes to the biofilm, which can be generally classified into physical and chemical properties [8]. The biofilms of Gram-negative bacteria contain polysaccharides that are neutral or polyanionic due to the presence of uronic acids (such as D-glucuronic, D-galacturonic, and mannuronic acids) or ketal-linked pyruvates, which provides a greater binding force in a developed biofilm [4,9]. In the case of Gram-positive bacteria, the chemical composition of EPS is primarily cationic [9]. The primary conformation of the biofilm is determined by the composition and structure of the polysaccharides [9]. Many of the bacterial EPS that contain 1,3- or 1,4- $\beta$ -linked hexose residues tend to be more rigid, less deformable, and poorly soluble or insoluble. Generally, EPS may be hydrophobic, although it can be both hydrophilic and hydrophobic [9]. Since EPS is highly hydrated, it helps to prevent desiccation in some natural biofilms [9]. Additionally, EPS may contribute to antimicrobial resistance by facilitating the mass transport of antibiotics through the biofilm, mostly by directly binding to these agents [10]. EPS is not generally uniform, and different organisms produce varying amounts of EPS [11]. The minerals serve as an essential component of the EPS and support the morphogenesis of bacterial colonies [12]. In some cases, it also provides structural integrity to the biofilm matrix and acts as a scaffold that protects the bacterial cells from shear forces and antimicrobial agents [13]. EPS also promotes cell adhesion to solid substrates and cohesion among bacterial cells that are important for biofilm formation [14]. In addition to providing protection against antimicrobials, EPS also offers physical stability and resistance to mechanical removal. The viscoelasticity of mature biofilms makes them difficult to remove, even under high mechanical pressure and sustained fluid shear stress [15].

Though biofilms are often considered to be destructive in the clinical and industrial fields, many biofilms are potentially beneficial. Recently, biofilms have been intentionally engineered for various applications (antibacterial, food fermentation, biofertilizer, filtration,



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biofouling, prevention of corrosion, antimicrobial agents, wastewater treatment, bioremediation, and microbial fuel cells) in food, agriculture, medicine, the environment, and other fields [16–22].

Bacterial biofilms on the surfaces of leaves, roots, and stems act as biocontrol agents that protect the plants from soil-borne pathogens [16,18,23]. In addition, beneficial biofilm-forming bacteria have the potential to be employed as biofertilizers, as they can promote plant growth through nitrogen fixation, phytohormone production, disease suppression, etc. [24,25]. For example, *Bacillus subtilis* is a prominent rhizobacterium and is an efficient biocontrol agent and growth-promotion agent due to its ability to form robust biofilms and to synthesize several antagonistic metabolites, including lipopeptides, bacteriocins, and siderophores [18,24,26,27]. The lipopeptide known as surfactin protects plants from *Pseudomonas syringae* infection [24,28].

Currently, there is increasing interest in the use of biofilm-forming microorganisms as bioremediation agents, as they convert hazardous environmental substances (pollutants such as oil spills; organic pollutants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls; and polychlorinated ethenes, heavy metals, dyes, explosives, pesticides, and pharmaceutical products) into less toxic or harmless compounds [20,29]. The microorganisms living in biofilms display the highest tolerance to contaminants as well as increased survival and adaptation to toxic environments compared to their planktonic counterparts. The bacteria that can remediate environmental pollutants include *Pseudomonas*, *Rhodococcus*, *Burkholderia*, *Dehalococcoides*, *Arthrobacter*, *Bacillus*, *Alcanivorax*, and *Cycloclasticus* [30,31].

Nowadays, biofilm-forming organisms are considered to be potential agents for the neutralization and degradation of the organic and inorganic contaminants present in wastewater [32]. In addition, excess nutrients (nitrogen and phosphorus) in the wastewater also need to be removed to avoid anoxia [32]. Thus, the microorganisms used to treat wastewater often include denitrifying ones or those capable of neutralizing phosphorus [33]. Currently, biofilm reactors for the treatment of wastewater have been developed and include membrane reactors, rotating contactors, fluidized beds, moving beds, etc. [34]. Biofilms can also be applied in bioelectrochemical systems (BESs; [35]). BESs are bioreactors in which microorganisms act as biocatalysts and convert the chemical energy present in the organic waste to electrical energy using oxidation–reduction reactions [36]. Thus, BESs are referred to as systems that facilitate wastewater treatment, bioremediation, and the production of power, fuels, and chemicals [37].

Recently, biofilms have been employed to efficiently prevent corrosion in drinking water distribution systems as well as in the food processing, medical, and marine industries [38–40]. Various strategies have been deployed to prevent corrosion by utilizing biofilm-forming bacteria. Strategies include the removal of corrosive substances such as oxygen by aerobic bacteria; preventing the growth of bacteria that induce corrosion via the antimicrobial compounds produced within the biofilm; the production of protective coats such as  $\gamma$ -polyglutamate; and blocking the metal dissolution caused by biofilms [38–40]. The successful application of biofilm-forming bacteria as anticorrosive agents has been reported for steel, copper, and aluminum [40]. Anticorrosive approaches achieved using beneficial biofilms represent new and promising strategies that require more attention.

There is an increasing demand for bacterial biofilms to be engineered for the various applications described in the previous paragraphs. It is of importance to perform in-depth studies to understand the unique properties of bacterial biofilms (both natural and artificial) and to exploit them for improved performance. I hope this Special Issue provides a platform for discussing recent advancements in beneficial bacterial biofilms for various applications.

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