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Perspective

Detection and Characterization of Bacterial Biofilms and Biofilm-Based Sensors

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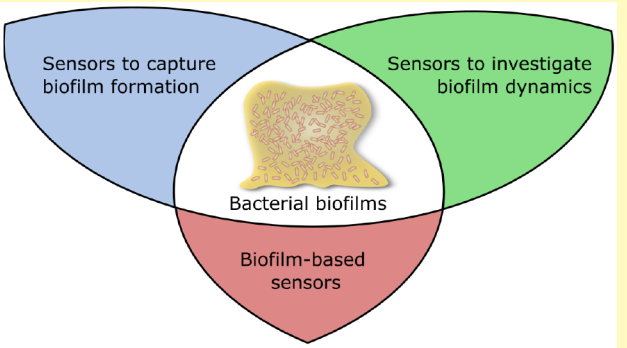
ABSTRACT: Microbial biofilms have caused serious concerns in healthcare, medical, and food industries because of their intrinsic resistance against conventional antibiotics and cleaning procedures and their capability to firmly adhere on surfaces for persistent contamination. These global issues strongly motivate researchers to develop novel methodologies to investigate the kinetics underlying biofilm formation, to understand the response of the biofilm with different chemical and physical treatments, and to identify biofilm-specific drugs with high-throughput screenings. Meanwhile microbial biofilms can also be utilized positively as sensing elements in cell-based sensors due to their strong adhesion on surfaces. In this perspective, we provide an overview on the connections between sensing and microbial biofilms, focusing on tools used to investigate biofilm properties, kinetics, and their response to chemicals or physical agents, and biofilm-based sensors, a type of biosensor using the bacterial biofilm as a biorecognition element to capture the presence of the target of interest by measuring the metabolic activity of the immobilized microbial cells. Finally we discuss possible new research directions for the development of robust and rapid biofilm related sensors with high temporal and spatial resolutions, pertinent to a wide range of applications.



KEYWORDS: *biofilms, sensors, real-time monitoring, cell-based biosensors, microbial fuel cells, microfluidic sensors, toxicity sensors*

Bacterial biofilms are aggregates of microorganisms in which cells are embedded in a self-produced matrix of extracellular polymeric substances (EPSs).[1−6] This condition is significantly different from planktonic bacteria, where bacterial cells freely move in a bulk solution. Cells in a multilayered biofilm experience cell-to-cell interactions, either within the biofilms in direct contact with the solid surface or in flocs where mobile biofilms are formed without adhering on a surface. Biofilm formation is a complex microbial process involving different development phases, some of which are specific to the type of bacteria involved.[7−9] A general description of bacterial biofilm formation usually entails three major stages, highlighted in Figure 1. First, planktonic bacteria adhere onto a surface (i.e., early adhesion), which is promoted by a conditioning layer consisting of organic and inorganic molecules that are either secreted by the bacteria approaching the surface or settled from the bulk solution. The interactions between the bacteria and the components of the conditioning layer and the bacterial appendages (i.e., cilia, flagelli, and curli) allow the planktonic cells to adhere onto the surface (i.e., early adhesion of bacteria). Once the adhesion becomes irreversible, the bacteria start to proliferate, aggregate into clusters, and secrete EPS, eventually losing their mobility to form the initial biofilm (stage 1 in Figure 1). Bacteria further multiply to develop more biofilm until it reaches the maximum volume, which corresponds to the maturation phase (stage 2 in Figure 1). When the biofilm approaches a critical thickness, it starts to release planktonic bacteria into the bulk environment to colonize new surfaces (stage 3 in Figure 1).

The EPS matrix, also labeled as the "house of the biofilm cells", mostly consists of polysaccharides, proteins, lipids, and extracellular DNA (eDNA).[10] The production and secretion of EPS require massive investment from cell resources and energy. Unlike free-living bacterial cells, the EPS can create a unique local microenvironment for various functionalities. First, the EPS matrix facilitates intercellular interactions and horizontal gene transfer. It also improves the resource capture and the surface adhesion and offers digestive capacity, protection against external agents, and inhibition of bacterial dehydration.[10,11] Utilizing these activated features, the EPS matrix can hamper antibiotic penetration into the biofilm and accumulate cell products which then degrade the drugs and induce phenotypic

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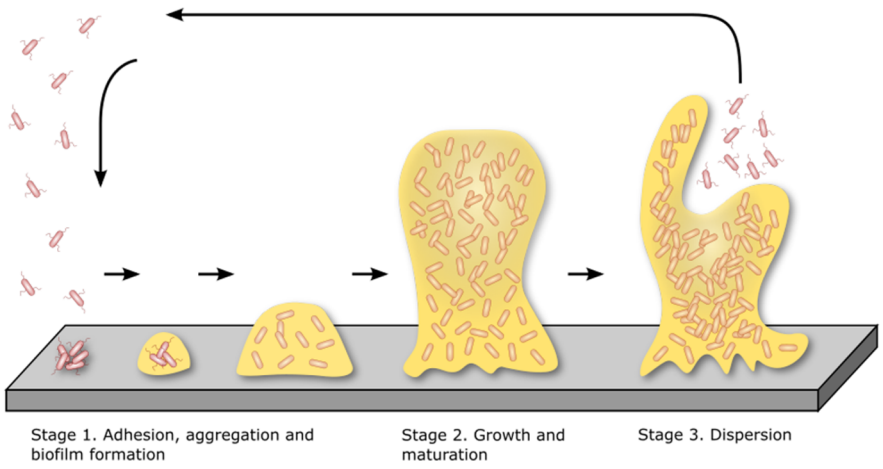

Figure 1. Key stages of biofilm development and life cycle. (1) Adhesion, aggregation, and biofilm formation. Bacterial appendages, such as curli, cilia, and flagella, promote cell adhesion. (2) Biofilm growth and maturation. The interaction between bacteria and the substrate is stabilized by the extracellular polymeric substances (EPSs), which improve surface adhesion and provide both mechanical and chemical protection for the microorganisms. (3) Dispersion. Planktonic bacteria are released for further colonization.

differentiation.[12−14] In addition, the gradient of nutrients and bacterial metabolites in the biofilm results in areas where cells are in a "dormant" state (i.e., nongrowing cells with extremely reduced metabolic activity[15,16]). These cells are highly immune to conventional antibiotics that typically target growing and metabolically active bacteria.[17] As a result, bacterial biofilm treatments require antibiotic doses hundreds or even thousands times higher than those required to eradicate planktonic bacteria.[18−20]

Because of their peculiar properties, bacterial biofilms have attracted tremendous attention in various industrial and medical fields. For instance, the use of cells in bioconversion and biotransformation process is limited by the poor stability and adhesion properties of planktonic cells. The intrinsic resistance, stability, and improved surface adhesion provided by the EPS matrix to the microbial cells have been exploited for industrial applications such as bioremediation, waste treatment, and production of fine chemicals and biofuels.[21] While it is possible to take advantage of biofilms for certain industrial applications, bacterial biofilms have also become a serious healthcare issue[22] as they display resistance against eradication procedures and antimicrobial agents[20,23,24] and are responsible for persistent infections and contaminations. Therefore, there is an urgent need to develop novel drugs and therapeutic strategies to eliminate bacterial biofilms[25,26] and establish surface treatment protocols to prevent bacterial adhesion and biofilm formation.[27,28] Another important issue related to biofilm contamination is biofouling. Specifically, biofilms in drinking water distribution networks can evolve into a temporary or transient source of pathogenic microorganisms.[29−31]

Concerns about bacterial biofilms in water pollution[32,33] and antibiotic resistance[34,35] have been recently highlighted by the World Health Organization (WHO), stressing the importance of developing novel and sensitive biofilm characterization strategies which can also be adopted to screen biofilm-specific drugs. Within this context, new sensing approaches offer promising opportunities to tackle the biofilm challenge with different prospects.[36] To limit the scope of this perspective, we focus on the sensing aspects of bacterial biofilms. For simplicity, we divide the ongoing research effort under two groups. The first group focuses on the sensing tools and devices that characterize and detect the bacterial biofilm formation and biofilm response to different treatments (e.g., drugs, eradication procedures, chemicals, etc.). The second group discusses biofilm-based sensors,[37,38] i.e., biosensors exploiting selective metabolism to detect the presence of desired targets. Specifically, the improved cell vitality and surface adhesion provided by the EPS matrix of microbial biofilms provide unique opportunities to solve two crucial issues of cell-based sensors: (1) the need to keep the microorganisms alive and healthy to perform the sensing task; (2) the need to couple the microbial cells with the transducer of the sensor. These two intertwined research efforts will be discussed in detail in the next sections.

SENSORS TO DETECT AND CHARACTERIZE BIOFILMS

The issues associated with bacterial biofilms underpin the quest for innovative methodologies involving both direct imaging[39,40] and sensing[41] to capture the formation of complex microbiological structures of biofilms with detailed characterizations.

While imaging allows researchers to obtain visual information on the biofilm (e.g., using dyes to highlight specific features of the biofilm),[42] sensing methodologies focus on the quantification of analytes or the characterization of the biofilm properties.[43] In particular, new sensing devices aim to overcome some of the limitations of the current interrogation methods for biofilm studies, which usually involve invasive and destructive end-point analysis. Sensors have been employed to study biofilms with two major aspects: (1) biofilm growth[41] and (2) dynamics of specific indicators in the biofilm.[44] The biofilm growth research usually focuses on the dynamics of biofilm formation to identify various stages of biofilm assembly, with the goal being to understand how the biofilm development process is affected by various chemical or physical cues. A wide range of sensing devices for biofilm evaluation rely on the optical, electrochemical, and mechanical transducers. On the other hand, phenomena taking place inside the biofilm matrix need to be probed by examining the spatial and temporal dynamics of physicochemical indicators such as the oxygen level, pH, temperature, ions, and certain biomolecules. Due to the heterogeneity nature of the biofilm structure, specific sensing techniques need to be determined accordingly. An overview of

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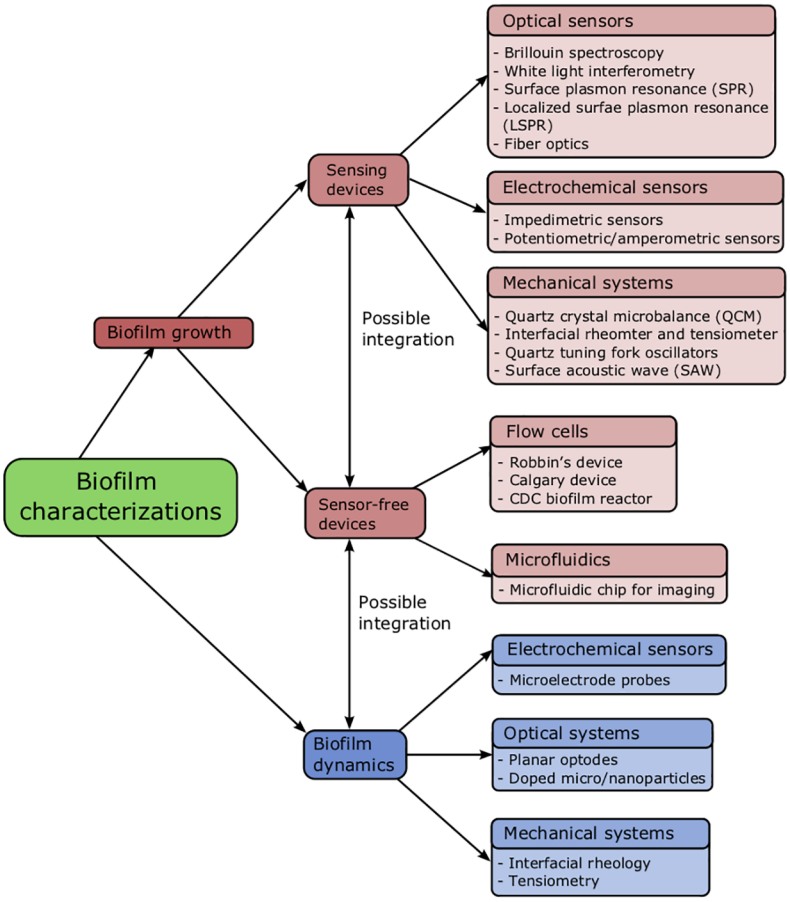

Figure 2. Different detection approaches to study bacterial biofilm formation and dynamics.

the major detection techniques used to characterize biofilms is shown in Figure 2.

DEVICES TO MONITOR BIOFILM FORMATION

Sensor-Free Devices. Conventional platforms to measure the growth and formation of biofilms employ both micro- and macroscale reactors with either static devices or flow cells. Static devices, such as multiwell plates, are commonly used in microbiology laboratories.[45] However, the difficulties in replenishing the culture medium over time can lead to the accumulation of waste and planktonic cells during the active measurement. On the other hand, flow cells, such as Robbin's device,[46] Calgary device,[47] and the Centers for Disease Control (CDC) biofilm reactor,[48] provide more reproducible and better control of the biofilm formation, but offer only end-point disruptive analysis. Miniaturized versions of these conventional systems mitigate some of the limitations and can be integrated with new sensing devices. These technologies offer portability, high-throughput analysis, reduced sample volume, and, most importantly, the ability to perform real-time and nondestructive biofilm characterizations. Utilizing these features sheds new insights on the biofilm growth, cell-to-cell interactions in the biofilm, and potential mechanisms of antibiotic resistance against biofilms.[49−52]

Microfluidic platforms have also been employed to study the effect of variables such as pH, flow rate, and temperature on biofilm formation with high reproducibility and throughput.[53,54] They offer the possibility to study biofilms in a highly controlled microenvironment that can be used to mimic real environments or in vivo conditions.[55] These systems can sometimes operate as stand-alone devices but often need to be integrated with standard biofilm characterization platforms involving confocal microscopy, colony forming units (CFUs), or crystal violet staining assays. Since these methodologies require sample treatment, labeling agents, optical corrections, and invasive procedures, only a few microfluidic platforms have been adopted for real-time nondisruptive analysis.[41] For example, microscopy-based techniques need continuous correction of the lens focus to capture the variations in biofilm thickness.

Sensing Devices. Non-invasive characterization of microbial biofilms has been achieved by using sensing technologies based on the optical, electrochemical, and mechanical transducers. For instance, optical detection systems offer high detection sensitivity but require extensive data collection and analysis.[56] Some examples include confocal reflection microscopy (CRM),[57] synchrotron radiation-based Fourier transform infrared (SR-FTIR),[58,59]spectromicroscopy, Brillouin spectroscopy,[60] white light interferometry,[61] surface plasmon resonance (SPR),[62,63]localized surface plasmon resonance (LSPR),[64] and fiber optics.[65,66]

Electrochemical (EC) sensors have also been applied to characterize biofilms,[67] grouped under impedimetric and potentiometric/amperometric sensors. The impedimetric sensing principle serves the foundation of the so-called impedance microbiology (IM),[68] where the change in the impedance between two electrodes that are exposed to the bacteria is used to identify the presence of microorganisms. This approach has allowed researchers to detect bacterial growth in real time by

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Table 1. Overview of the Existing Techniques to Study the Biofilm Growth

Approaches to study biofilm formation					
Detection devices	**Examples**	**Target**	**Pros**	**Cons**	**References**
Flow cells	Robbin's device, Calgary device, and CDC biofilm reactor	Biofilm growth, antibiotic resistance and eradication procedures	High reproducibility and control of biofilm formation	Only end-point disruptive analysis	46–48
Microfluidics	Microfluidic platforms		High-throughput analysis, reduced sample volume, and possible integration with sensing devices	When operating as stand-alone devices, they need to be coupled with standard complex and disruptive characterization techniques	53,54
Optical sensors	CRM, SR-FTIR, Brillouin spectroscopy, white light interferometry, SPR, LSPR, and fiber optics		High sensitivity	Extensive data collection and processing	56–66
Electrochemical sensors	Impedimetric and potentiometric/amperometric sensors		Sensitive, suitable for miniaturization and well-established methodologies	Cross sensitivity to mass and charge transport of analytes which affects the sensor stability	50,69–78,78–81,83–85
Mechanical systems	QCM, SAW devices, quartz tuning fork oscillators, interfacial rheometer and tensiometer		Cost-effective, real-time analysis	Cross sensitivity against density and viscoelastic properties in the bulk media	88–95

measuring the variations in the conductance, impedance, or capacitance of the bacterial solution.[50,69−78,78−82] Briefly, the bacteria and the extracellular components of the biofilm are dielectric species which affect the overall impedance of the system. Therefore, by monitoring the impedance in the solution over time, the biofilm formation and the subsequent change in the composition and metabolism of the biofilm can be inferred.[50]

Other EC sensors for biofilm characterizations include potentiometric and amperometric devices, which are based on the measurement of the faradaic current generated by the redox species produced by the biofilm in contact with the electrode surface. The main advantage of this approach lies in its ability to capture both the early adhesion stage of the cells[83,84] and the production of specific electroactive metabolites,[85] enabling real-time monitoring of specific biochemical activities of the microbes (this aspect will be discussed further in the next section).[44]

The last group of sensing devices used to study biofilm growth and formation is guided by mechanical systems such as quartz crystal microbalance (QCM),[86−88] quartz tuning fork oscillators,[89] surface acoustic wave (SAW) sensors,[90] and interfacial rheometry and tensiometry.[91−94] Some of these devices incorporate a piezoelectric component which gives an electrical response once inorganic or organic species (including cells) are deposited, adsorbed, or bonded to the device surface. These systems are used to capture biofilm formation in real time with high temporal resolution with relatively low costs. In addition, some QCM systems allow dissipation monitoring (i.e., QCM-D),[95] providing additional information on the mechanical properties of the adhering biofilm, which are crucial while optimizing eradication procedures or designing bacteria repellent surfaces. By measuring the mechanical response (i.e., interfacial elasticity and interfacial tension) of adsorbed bacteria or biofilm on a surface using interfacial rheometry and tensiometry, bacteria adsorption kinetics and their adhesion property to a given surface can be deduced.[91−94]

The methodologies used to study biofilm growth described in this section are summarized in Table 1 and Figure 2.

SENSORS TO INVESTIGATE BIOFILM PROCESSES AND THEIR METABOLISM

Alternative sensing techniques have also been developed to understand the temporal spatial dynamics of specific metabolites in the biofilm structure.[44] Inherent difficulties arise due to the high heterogeneity of microbial biofilms, non-uniformity in the structural and biochemical composition within the EPS matrix, and high degree of phenotypic variability of the bacteria inside the biofilm. In addition, invasive techniques to measure the properties of bacterial cells, cell density, and EPS characteristics can easily affect the biofilm and its activities.[96] For instance, microelectrode probes have been applied in some cases to perforate the biofilm, thus compromising the physical structure of the biofilm and altering the cell permeability.[97,98] Another complication is associated with the chemical treatment of biofilms during characterizations: when the biofilm is stained with specific dyes, the biochemical and biophysical properties of the bacterial cells can be potentially altered. The issues described above motivate researchers to develop less invasive approaches to monitor relevant indicators such as pH or oxygen level inside the biofilm.

Microelectrode and electrical sensors have been used to measure the level of oxygen,[99−101] pH,[102−104] concentration of ions (e.g., ammonium, nitrite, nitrate,[105] Ca^{2+},[106,107] etc.), and glucose and temperature variations[108] in the biofilm. These sensors are robust and flexible but can be affected by cross-sensitivity or interference (e.g., pH and temperature).[109] In addition, electrical sensors usually need to touch or pierce the biofilm, which in turn can affect the measurement accuracy and reduce its reproducibility.

Optical imaging systems based on confocal and fluorescence microscopy involving planar optodes (i.e., polymeric films embedded with oxygen-sensitive luminescent probes[113]) and labeled micro- and nanoparticles have been used to detect changes of oxygen and pH in the biofilm. Since planar optodes are 2D polymer films on which the cells adhere to form the biofilm, they cannot reveal information inside the biofilm,[114−116,126] whereas micro- and nanoparticles doped with fluorescent or luminescent dyes (that are sensitive to pH or oxygen changes in the biofilm) can be dispersed inside the EPS matrix, thereby providing 3D mapping of the oxygen concentration and pH inside the biofilm. Their nondestructive nature also offers reduced stress for in situ studies of the biofilm.[117−119] Fluorescent probes have also been used to detect heavy metal (Fe^{3+}, Cu^{2+}, Zn^{2+}, and Hg^{2+}) adsorption by bacterial biofilms that are relevant to bioremediation applications.[120]

Among the biofilm metabolites, glucose, *N*-acyl homoserine lactone (HSL), and cyclic di-GMP (c-di-GMP) have received

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Table 2. Overview of the Existing Sensors to Investigate Biofilm Dynamics

devices to study the dynamics of specific indicators in the biofilm					
detection principle	examples	target	pros	cons	ref
electrochemical sensors	microelectrodes probes	oxygen, pH, ions, temperature, metabolites	sensitive and easy-to-use	invasive, analysis can easily compromise the structure of the biofilm, with potential cross-sensitivity	97−108, 110−112
optical systems	planar optodes, labeled micro/nanoparticles	oxygen, pH, heavy metals, metabolites	low-invasive analysis and high sensitivity	laborious with additional instrumentation, organic dyes sometimes toxic, low solubility, and poor diffusion	113−124
mechanical systems	interfacial rheometry and tensiometry	interfacial material parameters for adhesion properties	real-time, in situ, and low-invasive analysis	validation and calibration with control samples	91−94, 125

particular attention. Glucose is the primary energy and carbon source for bacteria; therefore, its consumption is a clear indication of the biofilm metabolic activity. Thus, glucose has been routinely detected by using standard electrochemical sensors.[110,111] HLS and c-di-GMP are both important signaling molecules. HLS has been quantified by using fluorescence[121] and colorimetric[122] sensors, while c-di-GMPn in the biofilm has been detected by fluorescence-,[123] EC-,[112] and luminescence-based[124] sensors.

In a nutshell, various sensing approaches have been used to monitor the dynamics of specific indicators (e.g., oxygen level, pH, ions and metabolites concentrations, etc.) inside bacterial biofilms. The most appropriate technique for a specific application depends on various factors such as the type of the target and the tolerance level of the biofilm under invasive procedures. For instance, electrochemical sensors based on microelectrodes are extremely sensitive and reliable. However, they need to touch and penetrate the biofilm to perform the measurement, thus potentially compromising the structure of the biofilm. On the other hand, optical approaches relying on planar optodes and micro- and nanoparticles are less invasive. However, caution is needed considering the toxicity impact of the dyes and chemicals on the bacteria.

In terms of the mechanical systems, interfacial elasticity and interfacial tension are reliable indicators of bacterial adsorption kinetics and biofilm formation.[92] Specifically, bacterial adsorption at a hydrophobic interface results in the increase of interfacial elasticity and decrease of the interfacial tension. These material parameters can be measured using rheological and tensiometric methods and further correlated with the bacterial adhesion characteristics.[91,93,94]

An overview of the methodologies described in this section is summarized in Table 2 and Figure 2.

BACTERIAL BIOFILMS AS SENSING ELEMENTS IN BIOSENSORS DEVICES

Even though microbial biofilms have detrimental impacts in the natural environment and in medical and industrial processes, biofilms can also work to our advantages. The improved surface adhesion and cell vitality can overcome two of the major limitations of cell biosensors, i.e., the need to immobilize the cells and keeping them healthy and active in a prolonged period. Therefore, it is possible to exploit biofilms as sensing elements in a detection device. A schematic showing different transduction devices being coupled with biofilms is illustrated in Figure 3. Specifically, biofilm-based biosensors have been used for real-time and in situ environmental and process monitoring.[127] Detection systems based on microorganisms usually rely on luminescent and electroactive bacteria, as they can spontaneously generate fluorescence and electric outputs in response

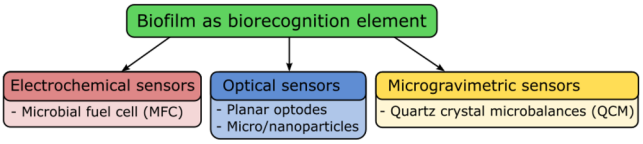

Figure 3. Sensing approaches involving bacterial biofilm as recognition element.

to the stress factor without the need of any additional genetic modification.

Bioassays involving naturally bioluminescent bacteria such as *Vibrio fischeri* (*V. fischeri*) are widely used for toxicity testing[128] since they offer short assay time and easy operation with low cost. However, most of these testing approaches rely on bacterial suspensions, which are not stable long term and require significant maintenance. Therefore, it is challenging to adopt bacterial suspensions for real-time monitoring. On the other hand, electroactive biofilms (EABs)[127,129] (i.e., biofilms consisting of electroactive bacteria) have been employed in electrochemical sensors for real-time analysis.[130] While conventional immobilization of bacteria on electrodes might require harmful or toxic chemicals, biofilm formation around the surface of an electrode is a "natural" immobilization process which preserves the functionality of the microorganism. Moreover, the biofilm matrix is extremely robust and is capable of self-maintenance and recovery, thus enabling long-term operations (e.g., some EAB-based sensors have been used continuously for years[131]). Finally, and most importantly, the consumption of organic compounds in the biofilm results in the production of electrical energy which can be used to sustain the device itself. For example, microbial fuel cell (MFC)[132] is widely used by utilizing EABs to investigate water quality.[133−135]EAB-based sensors are usually deployed as early warning devices for generic water indicators to track biochemical oxygen levels,[136] or contaminants (i.e., heavy metals and organic pollutants).[134,137,138]

One of the most important applications of MFCs is the detection of the BOD (biochemical oxygen demand) level,[139−141] an indicator to evaluate organic water pollution. BOD is usually measured by comparing the dissolved oxygen (DO) in a water sample before and after the incubation with microorganisms. This measurement is typically performed over 5 days at 20 °C. The use of EABs reduces the detection time significantly (1 day or less is needed[142]) and enables the MFCs to capture the temporal dynamics of BOD on site.[143] In addition, the capability of EAB-based MFC to biodegrade a wide range of substrates (e.g., glucose, acetate, and volatile fatty acids) also enhances the accuracy of the BOD quantification.[137] However, the lack of selectivity strongly limits the use of MFCs for the quantification of specific organic pollutants.

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Table 3. Overview of the Characteristics of the Biofilm-Based Sensors

biofilms as sensing elements					
detection principle	examples	target	pros	cons	ref
electrochemical	MFC	BOD, heavy metals, and organic pollutants	Cheap, self-sustained, suitable for early warning sensing	poor selectivity, cross-sensitivity	127, 129, 132−135
optical	fluorescence, bioluminescence	heavy metals, BOD	fast and sensitive response	complexity and cost of the optical components, only a few strains available	158, 159
microgravimetric	QCM	heavy metals	cheap and selective	limited to heavy metals	160

MFCs have also been used as toxicity sensors, since the metabolism of the bacteria in the EAB is typically inhibited by the presence of organic pollutants[144] or heavy metals.[145] This "toxic shock" on the bacteria results in a rapid drop of the current output of the device, which can be used as an early warning signal indicating the presence of the pollutant, which can be followed by traditional analysis to identify the specific contaminant. Even for this application, the lack of selectivity and the poor sensitivity are problematic drawbacks.[146] However, since MFC devices are relatively cheap, self-sustaining, and easy to handle, they have been used for early monitoring of in-field water quality in groundwater, rivers, and industrial effluents.[147] Examples of in-field EABs-biosensors include monitoring sewage treatment plants,[148−151] industrial wastewater leakages,[141,152,153] groundwater,[154] oil leakages,[155] and acid rain that can affect crop production.[156]

MFC devices involving bacterial biofilms have also been implemented for selective detection using different types of transduction principles. For instance, Carafa et al.[157] have recently used pulse amplitude modulated (PAM) fluorometry to capture changes in the photosynthetic yield, non-photochemical quenching, and basal fluorescence in river biofilms upon exposure to heavy metals (i.e., zinc, copper, nickel, arsenic, and lead). Specifically, non-photochemical quenching is effective to track acute effects, while the photosynthetic yield and basal fluorescence are robust indicators for chronic exposures. An alternative optical/electrochemical method was proposed by Qi et al.[158] who used a *V. fisheri* biofilm to develop a dual-mode sensor to capture the presence of Cu^{2+} ions. The comparison of these two transduction principles showed that the bioluminescence-based detection provided quicker response and was thus more sensitive than the electrical signal based detection for capturing the toxic shock. Fluorescence-based biosensors incorporating a biofilm of *Pseudomonas putida* (*P. putida*) with fiber optics have also been used for the fast quantification of the BOD.[159] Recently, our research group used the biofilm of *Delftia acidovorans* (*D. acidovorans*) coupled with a microgravimetric transducer such as QCM, to quantify gold ions in an aqueous environment.[160] The micoroganism *D. acidovorans* is capable of neutralizing ionic gold (toxic for the bacteria), in the form of "nanonuggets", that are effectively detected by the QCM directly on the *D. acidovorans* biofilm in real time for over 24 h. The gold neutralization process is a defense mechanism (biomineralization), which is relatively selective and common to many microorganisms that are able to neutralize different types of heavy metal ions in the form of dense nanoparticles.

The various types of biofilm-based biosensors, applications, and targets are summarized in Table 3.

ONGOING RESEARCH AND FUTURE DEVELOPMENTS

Bioflim Growth and Characterization. The importance of bacterial biofilms due to the rise of antibiotic resistance[34,35] and water pollution[32,33] motivates the quest for reliable detection tools to characterize and measure these microbial structures. Conventional techniques to study biofilm formation and assembly rely on macroscale devices, which provide only end-point invasive characterization. On the other hand, many sensing techniques are non-invasive and offer real-time investigations while controlling the environmental conditions in which the biofilm is formed, thus greatly supporting the screening of novel antibiofilm drugs and the development of biofilm treatments. While these sensing devices have the potential to support biofilm research, much work is still needed to improve their portability and simplicity to achieve point of care applications.

Concerning the study of biofilm dynamics, the main issues are the heterogeneous structure and composition of the biofilm which evolves over time. Some sensing and imaging techniques have tackled this challenge by providing information on the changes occurring in the biofilm during maturation and in response to different stimuli, such as antibiotic treatments. However, several key issues need to be addressed: i.e., how to minimize the perturbation and disruption of the biofilm during the sensing procedure, and how to improve the spatial and temporal resolution of the sensing techniques. In addition, there is a need to extend the current sensing techniques for other relevant biofilm targets, such as metabolites, signaling molecules, antibiotics, etc. Specifically, considering the detection systems described in this perspective, microleectrodes are well-established and offer cheap and sensitive measurements to quantify several biofilm indicators. However, their major drawback is the need to pierce the biofilm to perform sensing, thus damaging the biofilm during the process, which significantly affects the reproducibility of the measurement. On the other hand, micro- and nanoparticle-based detection is less invasive but requires expensive and complex equipment, such as confocal microscope, to perform the analysis. Therefore, the major aim for biofilm dynamics research is to develop sensing methodologies that can provide 3D mapping of metabolites inside the biofilm without perturbing the biofilm structure while keeping the analysis easy and cheap.

Bioflim-Based Sensors. The use of biofilms as sensing elements offers unique advantages in comparison with traditional laboratory and sensing techniques. However, to take full advantage of the possibility to perform early warning in situ monitoring of water environments, several aspects of biofilm-based sensing should be further explored.

First, the biofilm response is a complex process which depends on several environmental parameters such as temperature, pH, ionic strength, etc.,[161,162] and might be affected by the complexity of the sample matrix. Therefore, sample pretreat-

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ment and a more controlled sensing environment might potentially enhance the accuracy of the detection device. However, sample pretreatments require the implementation of additional moduli, so a cost−benefit analysis is needed during the design process. In addition, a better control of biofilm assembly and formation is fundamental to improving the reproducibility of these devices and strictly depends on the environment in which the biofilm is formed. The physicochemical characteristics of the immobilization surface also affect the biofilm formation and performances.[163] Ideally, these surfaces should be cheap, biocompatible, and robust for long-term monitoring applications.

Another important aspect concerns the response of the microorganism to specific stimuli such as the presence of the target of interest. A better understanding of how the bacterial microorganism interacts with specific stimuli is crucial to improving both selectivity and sensitivity against specific targets. For instance, we showed that biomineralizing bacteria[160] can be used for the selective detection of gold ions, and this approach can be extended to other heavy metals since many microorganisms are known for similar metal detoxification processes.[164,165]Another example is the optical biosensors based on the intrinsic fluorescence of some bacterial strains,[157,158]which might improve the sensitivity in comparison to electrochemical techniques. Nevertheless, the main challenge of biofilm-based biosensors lies in the lack of selectivity, which can be potentially tackled by introducing a genetically modified microorganism to improve the sensing performance[166] and open promising opportunities to achieve direct control of the detection mechanism, thus improving the detection selectivity.

Finally, the temporal stability and the storage conditions of the biofilms should be carefully studied.[167,168] Refrigeration, desiccation, and freezing are standard techniques to store biological materials. However, their effect on microbial viability and activity should be carefully investigated.

All issues described above have generated new research directions in material science, surface chemistry, microfluidics, and biotechnology. One important aspect of biofilm-based sensor development is the miniaturization for shortened response time and portability of the sensing device.[169−171] This can be achieved with the help of microfluidic technology.[82] Microfluidics allows researchers to control the microenvironment in which the biofilm forms and pave the way for parallel assays for multiplex selective measurements, this being of paramount importance for drug screening studies. Another emerging area of research is the integration of sensors in medical devices and implants to monitor potential biofilm infection in situ and in real time.

CONCLUSIONS

Bacterial biofilms have drawn critical concerns and received increasing attention in different disciplines, ranging from healthcare and medicine to microbiology, chemistry, engineering, and environmental science. To probe the biofilm properties, an ideal sensing device should provide real-time analysis and high temporal and spatial resolution, with the flexibility to couple with electrochemistry, optical, and mechanical transducers, in an integrated microfluidic platform. On the other hand, it is possible to take advantage of the unusual properties of biofilms to develop biofilm-based sensors where biofilm itself serves as the detection element responsible for the detection. These types of cell-based biosensors are cheap and robust, require low maintenance, and can operate continuously for a long time, thus making them favorable sensing devices for remote areas and online monitoring. They have been used to measure indicators of water pollution and have deployed as early warning devices to capture the release of toxic compounds in the aqueous environment, which can alert authorities to take prompt actions. However, the main drawbacks of these systems are the low reproducibility, poor selectivity, and limited understanding of the cellular metabolism involved in the sensing process. We are hopeful that the information entailed in this perspective will build further synergy among microbiologists, biotechnologists, chemists, material scientists, and engineers to develop novel biofilm related sensors for many cross-cutting applications.

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Notes

The authors declare no competing financial interest.

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