Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Electrochemistry

Review Article

Advances in electrochemical detection of bacterial biofilm metabolites

Niloofar Haghighian and Ritu Kataky

Abstract

Bacterial biofilms are structured communities of microorganisms that play a critical role in various industries and healthcare settings, contributing to chronic infections and biofouling issues. Understanding the metabolites produced by bacterial biofilms is of paramount importance to detect their growth patterns, virulence, and responses to treatment strategies. Electrochemical detection has emerged as a powerful and versatile tool for real-time, label-free, and sensitive analysis of bacterial biofilm metabolites. This review paper investigates recent breakthroughs in the field of electrochemical detection, focusing on the principles, methodologies, and applications of this cutting-edge technology. It includes a comprehensive examination of electrochemical sensors and their various modifications, designed to enhance sensitivity and specificity. Finally, the paper emphasisesing the potential for novel electrochemical techniques and their integration into clinical and industrial settings.

Addresses

Department of Chemistry, University of Durham, Lower Mountjoy, Durham DH1 3LE, UK

Corresponding author: Kataky, Ritu (Ritu.Kataky@durham.ac.uk)

Current Opinion in Electrochemistry 2024, **46**:101486

This review comes from a themed issue on **Sensors and Biosensors (2024)**

Edited by **Rasa Pauliukaitė**

For a complete overview see the Issue and the Editorial

Available online 14 March 2024

https://doi.org/10.1016/j.coelec.2024.101486

Keywords

Biofilms, Bacteria, Quorum sensing, Electrochemical, Detection.

Introduction

Bacterial biofilms represent complex, multicellular microbial communities that are enclosed within a self-produced extracellular matrix [1,2]. These biofilms hold significant implications across various domains, including healthcare, water treatment, food processing, and industrial settings [3–6]. Biofilm growth, virulence, and response to treatments are dependent on metabolites [7]. Real-time monitoring of these compounds is essential for understanding biofilm behaviour, enabling customised interventions and more effective treatment strategies [8]. Traditional methods for studying biofilm metabolites, such as mass spectrometry and chromatography, while valuable, often involve time-consuming and complicated sample preparation [9].

This review highlights electrochemical detection as a powerful and innovative technology. Electrochemical sensors offer real-time [10], label-free, and sensitive analysis of bacterial biofilm metabolites. The advantages include rapid detection [11,12], reduced sample preparation, and the potential for on-site monitoring.

Bacterial biofilm metabolites

Bacterial biofilms generate a diverse array of metabolites crucial to their formation, communication, and survival. Critical to the establishment of biofilm formation are the Extracellular Polymeric Substances (EPS) which provide structural support, comprising polysaccharides, protein, and nucleic acids [13–15]. Biofilm formation is induced by Quorum Sensing (QS), a phenomenon that relies on the production of self-generated signalling molecules of small molecular mass. These molecules accumulate extracellularly in cultures and at sufficient concentrations, initiate the transduction of signals that leads to cell communication and biofilm formation. Once formed, the EPS matrix is usually about 0.2–1.0 μm thick and enables the biofilm to become established by protecting the bacteria from environmental change. As the biofilm grows, the morphology and topology are characterised by unique pillar shapes that protrude from the surface to increase the accessible surface area and enable maximum nutrient adsorption. Typically, a biofilm is established after 48 h and any planktonic bacteria are liberated to form new colonies elsewhere. Quorum sensing molecules such as acyl-homoserine lactones (AHLs) and autoinducing peptides (AIPs), facilitate communication among bacteria, coordinating biofilm development [15]. Organic acids including acetic acid and formic acid, emerge as metabolic byproducts influencing the local microenvironment in bacterial biofilms, simultaneously, extracellular enzymes, such as

proteases and lipases, actively modify the biofilm matrix and aid in nutrient acquisition. Secondary metabolites often exhibit antimicrobial properties [16–18]. Specific molecules such as cyclic dimeric guanosine monophosphate (c-di-GMP) control the transition between planktonic and biofilm states [19,20]. Many components of EPS, such as DNA, humic acids, and some proteins, are redox-active or conductive/semiconductive. Xiao and co-workers proposed that the EPS store electroactive substances (such as flavins and c-type cytochromes), and act as electron transport media [21]. This enables EPS-enveloped cells to transport extracellularly, electrons to acceptors or from donors where electron hopping is the most likely molecular mechanism for electrochemical ET through EPS. This electron transfer process is complex depending on the spatial and temporal distribution of the electron transfer moieties. A reversible electrochemical system can be created when bacterial cells are permanently adhered to an electrode surface that enables electron transfer to take place between the electrode surface and the biofilm.

The transfer of electrons can occur via direct electron transfer (DET) where there are no diffusional redox species involved in the transfer of electrons that rely solely on the bacteria exhibiting membrane-bound electron transport relay proteins that enable electron transfer to an exogenous anode [22]. Criteria of the proteins are that they must make physical contact with the electrode surface; consequently, only bacteria located at the periphery of the biofilm in the first monolayer will be electrochemically active. However, microbes can also transfer electrons directly by producing highly conducting nanowires (NW) or 'pillis' [23]. Electron transfer can also proceed via mediated electron transfer (MET) which involves extracellular redox shuttles to transfer electrons between cells. Mediated electron transfer is accessible due to the secretion of redox metabolites via secondary metabolic pathways; a terminal electron acceptor molecule that behaves as the mediator is able to transfer electrons from the bacterial cell either to the electron-accepting anode or into the aerobic layers of the biofilm where it is re-oxidised and can be used in subsequent redox cycles [15].

Understanding these biofilm metabolites and their redox properties is crucial for developing strategies to control biofilm formation. This knowledge has broad applications, addressing challenges in medicine, industry, and environmental science, such as medical device infections and industrial biofouling. Targeting these metabolites can disrupt communication, compromise structure, or inhibit protective substance production, offering potential solutions in healthcare [24], industrial processes [25], and environmental management [26].

Principles of electrochemical detection

Electrochemical sensors offer real-time, label-free detection with high specificity [27]. Various types of electrochemical sensors have been developed, including amperometric [28], potentiometric [29], and impedance-based [30]. The following section of the manuscript provides an overview of various electrochemical methods used to detect biofilm metabolites.

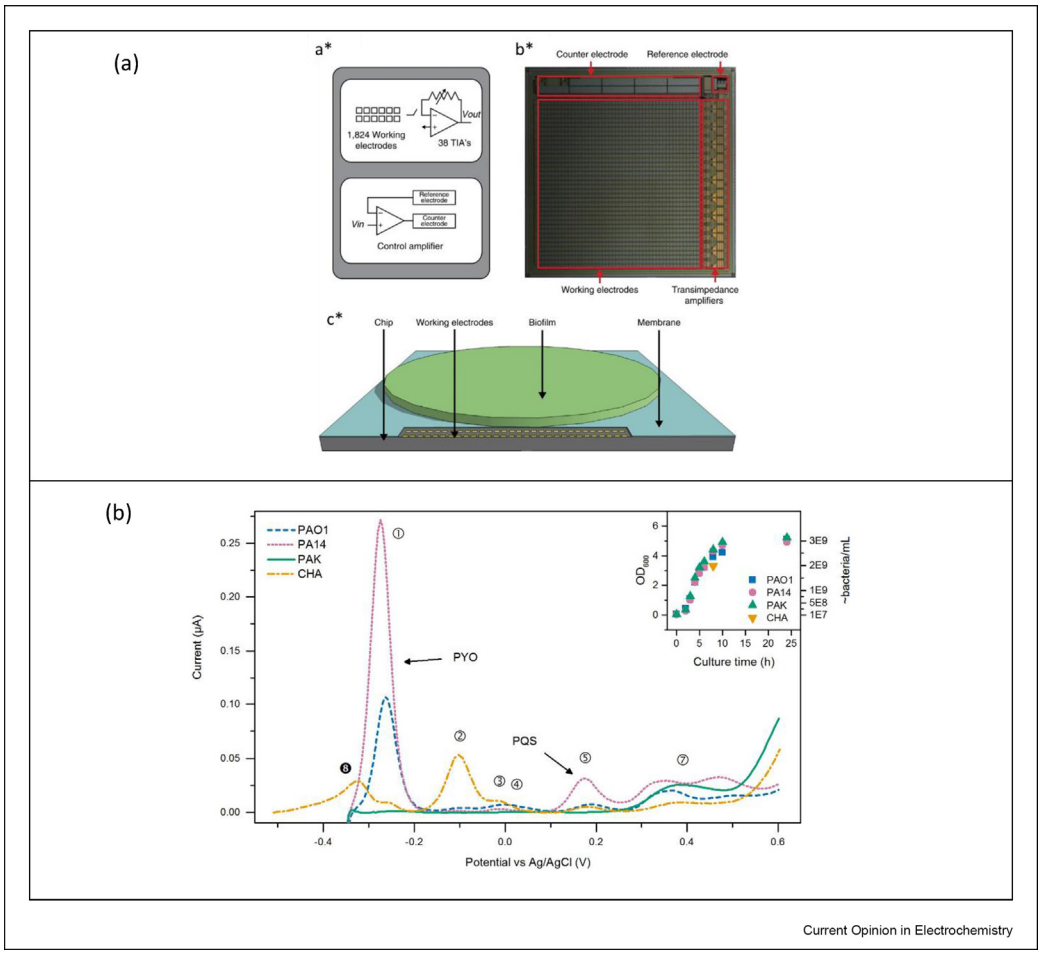
Identification and quantification of bacterial species

One of the key points of electrochemical detection of bacterial biofilm metabolites is the quantification of small molecule metabolites. Bellin et al. [7] described an integrated circuit-based electrochemical sensor which employs square wave voltammetry with a gold microelectrode for detecting redox-active metabolites in biofilms. They quantified four distinct redox-active phenazines metabolites, with concentrations as low as 2.6 μM. Phenazine production in both wild-type and mutant *Pseudomonas aeruginosa* PA14 colony biofilms was characterised. Then they [31] designed an "Electrochemical camera chip" for simultaneously imaging multiple redox-active phenazine metabolites produced (Figure 1a). The chip was used to analyse mutants with various capacities for phenazine production, revealing the distribution of phenazine-1-carboxylic acid (PCA) throughout the colony, with 5-methyl-phenazine-1-carboxylic acid (5-MCA) and pyocyanin (PYO) localised to the colony edge.

Oziat et al. [32] focused on the electrochemical detection of redox molecules secreted by different strains of *P. aeruginosa* (Figure 1b). In the study, the authors compared square wave voltammetry (SWV) responses of supernatants from different *P. aeruginosa* strains (PAO1, PA14, PAK, and CHA) after 8 h of growth using a Glassy Carbon-working electrode. The comparison of the SWV responses at a specific time point (8 h of growth) revealed distinct patterns for each strain, indicating differences in the production of redox-active molecules such as pyocyanin (PYO) and Pseudomonas quinolone signal (PQS). The study highlighted the specificity and sensitivity of electrochemical sensors in distinguishing between bacterial biofilm metabolites, suggesting their potential as fingerprint or 'ID-card' for rapid bacteria strain identification.

Signalling molecules play a key role in the communication and coordination within bacterial biofilms. Buzid et al. [33] presented the use of boron-doped diamond (BDD) electrode for the simultaneous detection of 2-heptyl-3-hydroxy-4-quinolone (PQS), 2-heptyl-4-hydroxyquinoline (HHQ), and pyocyanin (PYO) in *P. aeruginosa*. They optimised differential pulse voltammetry (DPV) for the analysis of these molecules in bacterial culture supernatants. The limit of detections (LODs) for each molecule was reported as PYO: 50 nM,

Figure 1



(**a**) Illustrates the electrochemical camera imaging platform. (**a***) The block diagram outlines the components of the electrochemical camera chip. (**b***) An optical micrograph showcases the electrochemical camera chip, emphasising the integrated electrodes and amplifiers. The chip measures 1 cm × 1 cm. (**c***) A diagram of the imaging platform highlights the electrochemical camera chip, working electrodes, biofilm, and membrane [31]. (**b**) Presents a comparison of the SWV responses (step 5 mV, amplitude 10 mV, and frequency 2 Hz) on the Glassy Carbon-working electrode of PAO1, PA14, PAK, and CHA supernatants after 8 h of growth. The insert shows the temporal evolution of OD600 and corresponding bacterial concentrations [32].

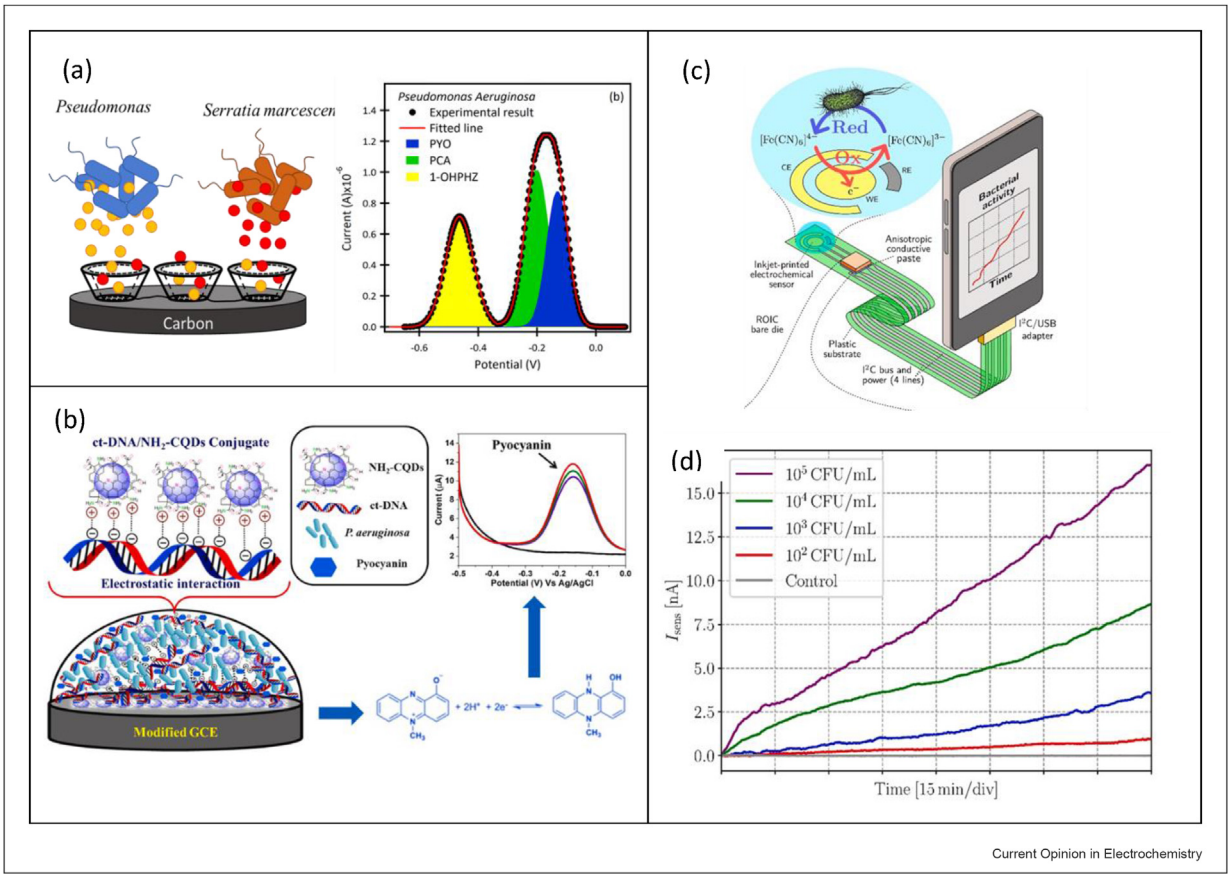
HHQ: 250 nM, and PQS: 250 nM, demonstrating the sensitivity of DPV in detecting these quorum-sensing signalling molecules at the nanomolar range.

Electrochemical sensor modifications

To enhance the sensitivity and specificity of electrochemical sensors in detecting bacterial biofilm metabolites, researchers have developed a range of sensor modifications. Haghighian & Kataky [29] modified a screen-printed electrode with β-cyclodextrin to create nanocavities for inclusion of the analytes (Figure 2a). The study used Square Wave Adsorption Stripping Voltammetry (SWAdSV) to detect metabolites from *Pseudomonas fluorescens*, *P. aeruginosa*, and *Serratia marcescens* during biofilm formation. The resulting SWAdSVs exhibited distinct peaks corresponding to redox behaviour, showcasing the modified electrode's superior performance over the bare electrode. The modified electrode shows a low detection limit of $4 \times 10^{-8} M$, indicating high sensitivity in detecting trace amounts of bacterial metabolites at specific stripping potentials offering a promising tool for fingerprinting bacterial species.

Thulasinathan et al. [34] designed an electrochemical sensor for the detection of pyocyanin (PYO), a key quorum-sensing molecule associated with *P. aeruginosa* infections. The approach involved mimicking the extracellular DNA interaction of PYO, utilising calf thymus DNA (ct-DNA) functionalised amine-containing carbon quantum dots (CQDs) as a biomimetic receptor (Figure 2b). The ct-DNA-based biosensor exhibited remarkable sensitivity, detecting PYO concentrations as low as 37 nM in real urine

Figure 2



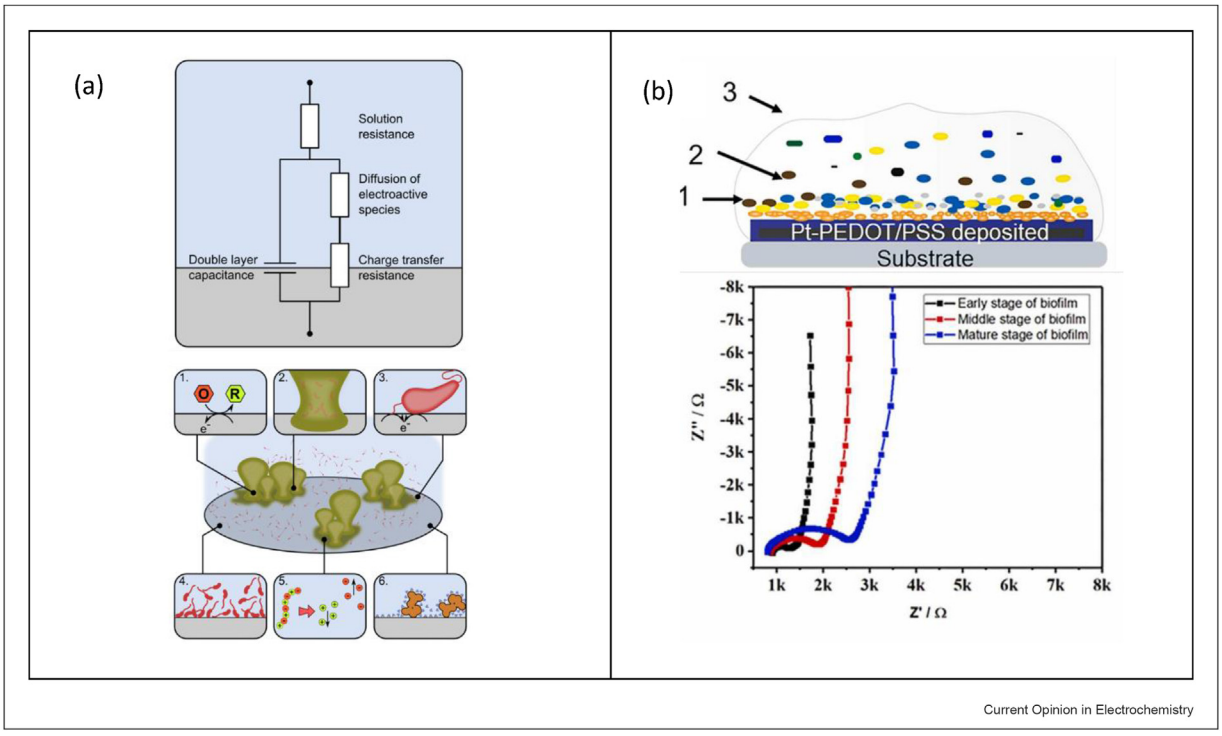
(**a**) Depicts SWAdSV curves of the β-CD modified electrode after 72 h in *P. aeruginosa* cultures, revealing multiple peaks corresponding to 1-OHPHZ (yellow), PCA (green), and PYO (blue) at –0.46 V, –0.20 V, and –0.13 V, respectively [29]. (**b**) Illustrates electrode modification with ct-DNA/NH2-CQDs assembly and the electrochemical measurement of PYO in various samples [34]. (**c**) Showcases the disposable platform and self-noise cancellation concept, presenting an all-in-one amperometric platform for ultrasensitive bacterial detection. (**d**) Highlights the measured chronoamperometry at different bacterial concentrations [35]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

samples, and demonstrated notable selectivity in the presence of interfering species. Calibration and validation were conducted across various sample types, including buffer solutions, microbial culture media, artificial urine, and real urine samples. Aymerich and coworkers [35] developed a disposable all-in-one amperometric platform with self-noise cancellation for ultrasensitive bacterial sensing (Figure 2c). The sensor used in the platform is fabricated using mask-less inject printing technology on flexible polyethylene naphthalate (PEN) substrate. The sensor includes gold-working electrode and counter electrode and a silver pseudo-reference electrode. The platform incorporates a flicker noise cancellation mechanism, to improve the signal-to-noise ratio and enhanced the accuracy of measurement Live bacterial concentration as low as $10^2 CFU/mL$ from a 50 μL droplet sample, (equivalent to 5 microorganisms) was achieved in less than 1 h (Figure 2d). Overall, the paper presents a promising approach to ultrasensitive bacterial sensing using a disposable, integrated platform.

Electrochemical impedance spectroscopy (EIS) is widely employed for bacterial detection and biofilm quantification during biofilm development [30]. Ward et al. [36] have developed a cost-effective biosensor for detecting *P. aeruginosa* using electrochemical impedance spectroscopy (Figure 3a). The biosensor was tested in a polymicrobial competition model with *Staphylococcus aureus*, and the results showed that it was able to detect the presence of *P. aeruginosa* by comparing the impedance signature of the culture media with and without *P. aeruginosa*.

Kumar and colleagues [37] have designed a non-invasive impedance-based technique to monitor real-time biofilm growth on various substrates. The impedance sensor was optimised for sensitivity and reproducibility by

Figure 3



(**a**) Illustrates the impedance signature of an electrochemical system, determined by factors such as solution resistance, redox compounds, diffusion gradients, and electrolyte composition near the electrode surface. Microorganisms can influence this signature by (1) producing electroactive secondary metabolites for charge transfer, (2) biofilm matrix attachment affecting capacitance and/or charge transfer, (3) direct microbial attachment through pili, flagella, and outer membrane proteins facilitating charge transfer, (4) outer cell membrane contact at high cell densities affecting capacitance, (5) breakdown of nutrients reducing solution resistance, and (6) protein/macromolecule adsorption on the electrode surface influencing double layer capacitance [36]. (b) Representative data of Nyquist plot of different stages of biofilm growth on glass substrate and schematic diagram of the complex biological process on top of the impedance sensor, (1) regarding the protein adsorption, (2) is the initial bacterial adhesion, and (3) in biofilm matured [37].

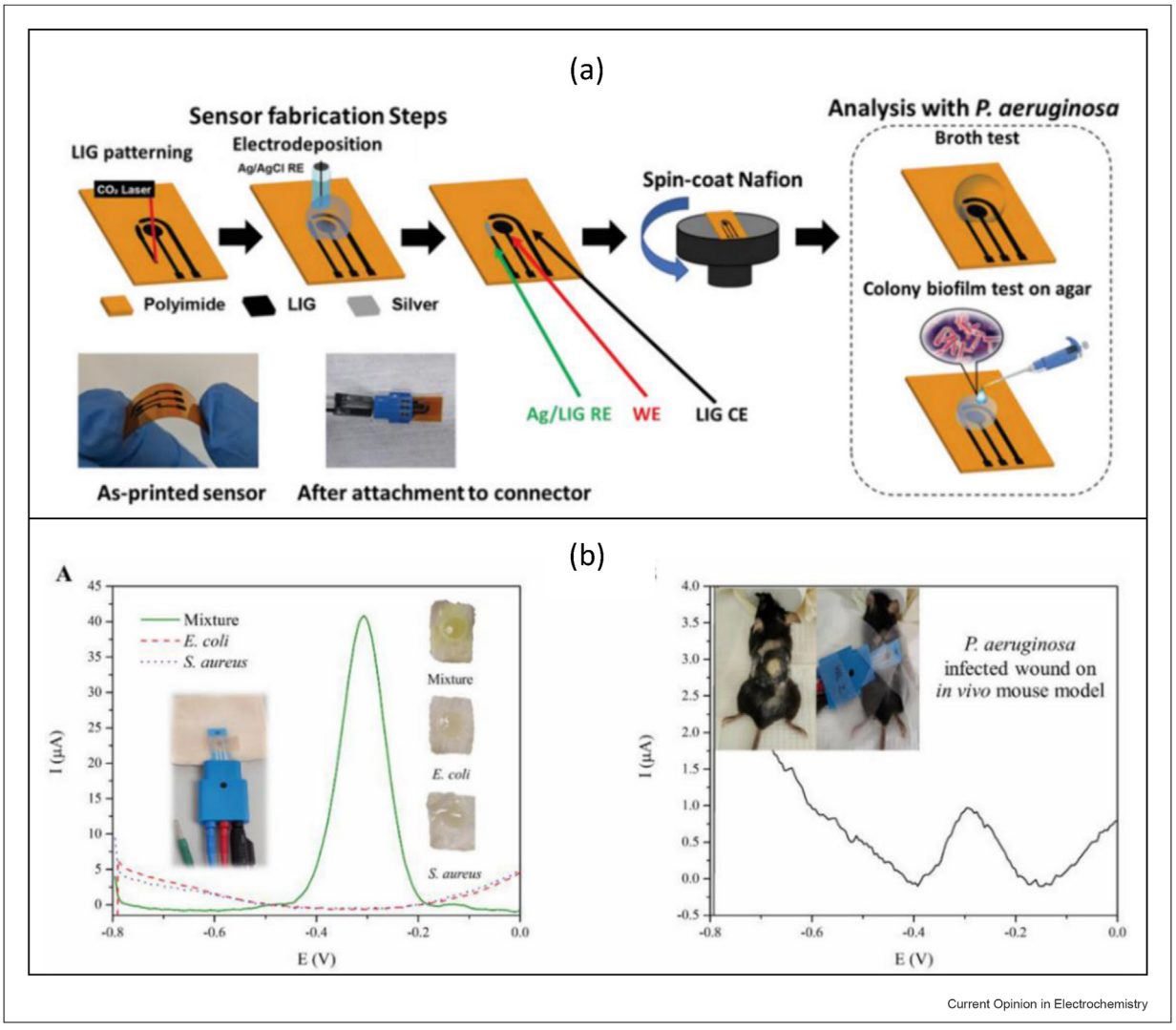
modifying the electrode surface (Pt wire) with poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) PEDOT: PSS. The PEDOT: PSS-coated electrode enhances protein adsorption and bacterial attachment through its porous structure, increased surface area and improved electrical conductivity (Figure 3b). The large electrochemical surface area (ECSA) of the impedance sensor can enhance the detection of biofilm growth and improve the performance of the sensor in protein-rich and complex bacterial growth media. In assessing EIS sensors for monitoring biofilm growth in industrial settings, McGlennen et al. [38] explored microfabricated sensors with polypyrrole (PPy) and poly (4-styrenesulfonic acid) (PSS) coatings for stable time-resolved EIS measurements under abiotic and biofilm growth conditions. These modified sensors were integrated into a 3D-printed flow cell for live-cell confocal laser scanning microscopy imaging and impedance measurements, demonstrating the potential for real-time biofilm detection in various environments.

Applications in medical diagnostics and environmental monitoring

Clinical and healthcare settings

The application of electrochemical sensors is not limited to the laboratory environment. Zhou et al. [39] designed flexible electrochemical sensors for *P. aeruginosa* detection in wound care management (Figure 4a). The sensors, based on SWV methods, targeted phenazines detection on laser-induced graphene with molybdenum polysulfide (MoSx) on a polyimide substrate. Evaluation in different mediums, including wound simulations, confirmed specificity for *P. aeruginosa* using control experiment with *Escherichia coli*, a bacterium that does not produce phenazine. The sensor showed reliability across the clinical range, by the ability to detect phenazine produced by *P. aeruginosa* in a wound-simulating medium with limit of detection (LOD) of approximately $1.3 \times 10^{-6} M$. This LOD was considered sufficient for detecting and monitoring phenazine in *P. aeruginosa*, even in complex clinical

Figure 4



(**a**) The sensor fabrication and functionalisation processes involve several key steps outlined in a schematic. Initially, a laser engraving machine creates the 3-electrode pattern. Subsequently, the working electrode (WE) undergoes functionalisation with Pt nanoparticles, while the reference electrode (RE) undergoes silver (Ag) electrodeposition. To complete the sensor, a Nafion membrane is spin-coated and attached to the connector for electrochemical testing. Two sets of electrochemical tests are conducted using the sensors: an in vitro analysis with broth and wound-simulating medium (WSM) and a long-term monitoring of colony biofilms on agar [39]. (**b**) Square Wave Voltammetry (SWV) current responses for detecting pyocyanin in wounds are illustrated in (A) an ex vivo porcine skin model and (B) an in vivo animal model [40].

sample such as sputum. Overall, Real-time monitoring of *P. aeruginosa* biofilms over several days demonstrated the sensors' potential for early infection diagnosis, especially in wound dressings.

Thirabowonkitphithan et al. [40] developed an electrochemical detection approach based on a multi-walled carbon nanotube/polyvinyl alcohol (MWCNTs/PVA) hydrogel-modified sensor for pyocyanin in wound (Figure 4b). Driven by the imperative to combine wound exudate absorbency with electrical conductivity, hydrogels were chosen for their biocompatibility and 3D network structures. The integration of MWCNTs enhanced mechanical robustness and surface area, enabling pyocyanin detection without sample preparation. The resulting sensor, applied on a flexible polycarbonate substrate, demonstrated reliability across the clinical range.

Industrial and environmental monitoring

Electrochemical detection holds significant potential for industrial and environmental monitoring. Bimakr et al. [41] showed this potential by employing graphite and stainless-steel electrodes for biofilm sensing in chlorinated drinking water system. The study utilised electrochemical impedance spectroscopy (EIS) and open circuit potential (OCP) to detect the biofilm growth, highlighting the effectiveness of these electrochemical sensing techniques in detecting biofilm growth within drinking water distribution systems (DWDSs). The research showed that stainless steel is 10-fold more sensitive than graphite for detecting changes in biofilm formation.

Further, within the food industry research, Liustrovaite et al. [42] presented a novel electrochemical sensor for highly specific detection of pathogenic *Listeria monocytogenes* - an undesirable pathogen in the food industry. The key innovation in this study was using molecularly imprinted polypyrrole (MI-Ppy) layer on electrodes. The tailored binding sites within the MI-Ppy layer on Pt and SPCE electrodes allowed for the entrapment of *L. monocytogenes* bacteria templates within the polymer matrix. The presence of the Ppy-based under-layer further enhanced the formation of the MIP-Ppy sensing layer by reducing direct interaction with the electrode surface. This strategy facilitated the removal of the bacterial templated post-synthesis, ensuring the formation of a clean, specific sensing layer for accurate detection. Pulsed amperometric detection assesses sensor performance, providing a limit of detection (LOD) at 70 CFU/mL and a detection range spanning from 300 to 6700 CFU/mL in this study.

Conclusion

Electrochemical detection offers a promising approach for non-labelled, relatively cheap technology for unravelling the complex world of biofilm communities. This review paper has highlighted some developments in electrochemical techniques addressing the detection and monitoring of bacterial biofilms in real environments. Amperometric, impedance methods and electrode arrays for identifying and monitoring spatial distributions of bacterial metabolites in real time are exciting and innovative. This is just the tip of the iceberg. The real challenges lie in the ability to detect with remote sensing capabilities, in harsh media such as brackish water, whole blood, food and drinks applications amongst others. In this era of anti-microbial resistance (AMR) threatening health, food security, and livelihoods amongst others, understanding and targeting bacterial activity is one of the greatest global challenges as identified by UN and WHO.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Sauer Karin, Paul Stoodley, Darla M, Goeres, Hall-Stoodley Luanne, Burmølle Mette, Stewart Philip S, Bjarnsholt Thomas: **The biofilm life cycle: expanding the conceptual model of biofilm formation**. *Nat Rev Microbiol* 2022, **20**.
2. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S: **Biofilms: an emergent form of bacterial life**. *Nat Rev Microbiol* 2016, **14**:563–575.
3. Shineh Ghazal, Mobaraki Mohammadmahdi, Perves Bappy Mohammad Jabed, Mills David K: **Biofilm Formation, and related impacts on healthcare, food processing and packaging, industrial manufacturing, marine industries, and sanitation–A review**. *Appl Microbiol* 2023, **3**:629–665.
4. Nunez Charles, Kostoulias Xenia, Anton Y: **Peleg, Francesca Short, Yue Qu, "A comprehensive comparison of biofilm formation and capsule production for bacterial survival on hospital surfaces,"**. *Biofilms* 2023, **5**, 100105.
5. Srey Sokunrotanak, Jahid Iqbal Kabir, Ha Sang-Do: **Biofilm formation in food industries: a food safety concern**. *Food Control* 2013, **31**:572–585.
6. Ganesh Kumar C, Anand SK: **Significance of microbial biofilms in food industry: a review**. *Int J Food Microbiol* 1998, **42**:9–27.
7. ** Bellin Daniel L, Hassan Sakhtah, Rosenstein Jacob K, Levine Peter M, Jordan Thimot, Emmett Kevin, Lars E, Dietrich P, Shepard Kenneth L: **Integrated circuit-based electrochemical sensor for spatially resolved detection of redox-active metabolites in biofilms**. *Nat Commun* 2014, **5**:3256.

This paper appears to be a significant contribution to biofilm research, offering a novel and powerful technique that has the potential to impact various scientific disciplines. The method's innovation lies in its ability to detect and quantify metabolites, offering comprehensive insights into biofilm metabolic activities, with potential environmental applications.

8. Funari Riccardo, Shen Amy Q: **Detection and characterization of bacterial biofilms and biofilm-based sensors**. *ACS Sens* 2022, **7**:347–357.
9. Fang Jinshu, Dorrestein Pieter C: **Emerging mass spectrometry techniques for the direct analysis of microbial colonies**. *Curr Opin Microbiol* 2014, **19**:120–129.
10. Simoska Olja, Gaffney Erin M, Minteer Shelley D, Franzetti Andrea, Cristiani Pierangela, Grattieri Matteo, Santoro Carlo: **Recent trends and advances in microbial electrochemical sensing technologies: an overview**. *Curr Opin Electrochem* 2021, **30**, 100762.
11. Simoska Olja, Stevenson Keith J: **Electrochemical sensors for rapid diagnosis of pathogens in real time**. *Analyst* 2019, **144**:6461–6478.
12. Kim Seonghwan, Yu Guiduk, Kim Taeyoung, Shin Kyusoon, Yoon Jeyong: **Rapid bacterial detection with an interdigitated**

array electrode by electrochemical impedance spectroscopy. *Electrochim Acta* 2012, **82**:126–131.

13. Flemming HC, Wingender J: **The biofilm matrix**. *Nat Rev Microbiol* 2010, **8**:623–633.

14. Wingender J, Neu TR, Flemming H-C: **What are bacterial extracellular polymeric substances?**. In *Microbial extracellular polymeric substances*; 1999:1–19.

15. Kataky Ritu, Knowles Emma: **Biofilm formation on abiotic surfaces and their redox activity**. *Curr Opin Electrochem* 2018, **12**:121–128.

16. Dürig A, Kouskoumvekaki I, Vejborg RM, Klemm P: **Chemo-informatics-assisted development of new anti-biofilm compounds**. *Appl Microbiol Biotechnol* 2010, **87**:309–317.

17. Herget K, Frerichs H, Pfitzner F, Tahir MN, Tremel W: **Functional enzyme mimics for oxidative halogenation reactions that combat biofilm formation**. In *Nanozymology: connecting biology and nanotechnology*; 2020:978–981.

18. Goel Nikky, Ghosh Moumita, Jain Deepti, Sinha Rajeshwari, Khare Sunil Kumar: **Inhibition and eradication of Pseudomonas aeruginosa biofilms by secondary metabolites of Nocardiopsis lucentensis EMB25**. *RSC Med Chem* 2023, **14**: 745–756.

19. Manner C, Dias Teixeira R, Saha D, *et al.*: **A genetic switch controls Pseudomonas aeruginosa surface colonization**. *Nat Microbiol* 2023, **8**:1520–1533.

20. Gomez JB, Waters CM, Gomez JB, Waters CM: **Switching on cyclic di-GMP heterogeneity in Pseudomonas aeruginosa biofilms**. *Nat Microbiol* 2023, **8**:1380–1381.

21. Xiao Yong, Zhang Enhua, Zhang Jingdong, Youfen Dai, Zhaohui Yang, Hans E, Christensen Https M, Jens Ulstrup, Feng Zhao: **Extracellular polymeric substances are transient media for microbial extracellular electron transfer**. *Sci Adv* 2017, **3**, 1700623.

22. Borole Abhijeet P, Reguera Gemma, Bradley Ringeisen, Wang Zhi-Wu, Feng Yujie, Hong Kim Byung: **Electroactive biofilms: current status and future research needs**. *Energy Environ Sci* 2011, **4**:4813–4834.

23. Reguera Gemma, McCarthy Kevin D, Mehta Teena, Nicoll Julie S, Tuominen Mark T, Lovley Derek R: **Extracellular electron transfer via microbial**. *Nature* 2005, **435**:1098–1101.

24. Fontana-Escartín Adrián, El Hauadi Karima, Lanzalaco Sonia, Maria M, Pérez-madrigal, Armelin Elaine, Turon Pau, Alemán Carlos: **PreparationandCharacterizationofFunctionalizedSurgicalMeshesforEarlyDetectionofBacterialInfections**. *ACS Biomater Sci Eng* 2023, **9**:1104–1115.

25. Alonso VPP, Gonçalves MPMBB, de Brito FAE, Barboza GR, Rocha LDO, Silva NCC: **Dry surface biofilms in the food processing industry: an overview on surface characteristics, adhesion and biofilm formation, detection of biofilms, and dry sanitization methods**. *Compr Rev Food Sci Food Saf* 2023, **22**:688–713.

26. Ahmed Barhoum, Selma Hamimed, Slimi Hamda, Othmani Amina, Fatehy M, Abdel-Haleem, Bechelany Mikhael: **Modern designs of electrochemical sensor platforms for environmental analyses: principles, nanofabrication opportunities, and challenges**. *Trends Environ Anal Chem* 2023, **38**, e00199.

27. Babauta Jerome, Ryan Renslow, Haluk Beyenal Zbigniew Lewandowskiand: **Electrochemically active biofilms: facts and fiction. A review**. *Biofouling* 2012, **28**:789–812.

28. Pires Leonardo, Sachsenheimer Kai, Kleintschek Tanja, Waldbaur Ansgar, Schwartz Thomas, Bastian E: **Rapp, Online monitoring of biofilm growth and activity using a combined multi-channel impedimetric and amperometric sensor**. *Biosens Bioelectron* 2013, **47**:157–163.

29. * Haghighian Niloofar, Kataky Ritu: **Rapid fingerprinting of bacterial species using nanocavities created on screen-printed electrodes modified by β-cyclodextrin**. *Sens. Diagn.* 2023, **2**: 1228–1235.

n this interesting study polymeric β-cyclodextrin (β-CD) modified electrode provides a rapid and sensitive method for identifying bacterial species, with a low detection limit of 4 × 10–8 M, and is disposable, making it a cost-effective and practical solution for identifying bacterial species in a range of applications.

30. Ameer Sikander, Ibrahim Hussam, Usama Yaseen Muhammad, Kulsoom Fnu, Cinti Stefano, Sher Mazhar: **Electrochemical impedance spectroscopy-based sensing of biofilms: a comprehensive review**. *Biosensors* 2023, **13**:777.

31. ** Bellin Daniel L, Hassan Sakhtah, Zhang Yihan, Price-Whelan Alexa, Dietrich Lars EP, Shepard Kenneth L: **Electrochemical camera chip for simultaneous imaging of multiple metabolites in biofilms**. *Nat Commun* 2016, **7**, 10535.

This paper is important because it introduces an innovative "Electrochemical camera chip" for real-time imaging of redox-active phenazine metabolites in microbial biofilms, providing high-resolution spatial monitoring and valuable insights into biochemical processes.

32. * Julie Oziat, Thibaut Cohu, Elsen Sylvie, Gougis Maxime, Malliaras George G, Pascal Mailley: **Electrochemical detection of redox molecules secreted by Pseudomonas aeruginosa – Part 1: electrochemical signatures of different strains**. *Bioelectrochemistry* 2021, **140**, 107747.

This paper is interesting because it introduces a simple and cost-effective electrochemical method for identifying bacterial strains based on their unique redox signatures, enabling rapid in situ detection and potential improvements in antibiotic management

33. Buzid A, Shang F, Reen FJ, Muimhneacháin EÓ, Clarke SL, Zhou L, Luong JH, O'Gara F, McGlacken GP, Glennon JD: **Molecular signature of Pseudomonas aeruginosa with simultaneous nanomolar detection of quorum sensing signaling molecules at a boron-doped diamond electrode**. *Sci Rep* 2016, **6**, 30001.

34. Thulasinathan Boobalan, Sujatha D, Murugan Sethupathi, Panda Subhendu K, Veerapandian Murugan, Manickam Pandiaraj: **DNA-functionalized carbon quantum dots for electrochemical detection of pyocyanin: a quorum sensing molecule in Pseudomonas aeruginosa**. *Biosens Bioelectron* 2023, **227**, 115156.

35. * Aymerich Joan, Ferrer-Vilanova Amparo, Cisneros-Fernández Jose, Escudé-Pujol Roger, Guirado Gonzalo, Terés Lluís, Michele Dei, Muñoz-Berbel Xavier, Serra-Graells Francisco: **Ultrasensitive bacterial sensing using a disposable all-in-one amperometric platform with self-noise cancellation**. *Biosens Bioelectron* 2023, **234**, 115342.

The paper is interesting because it presents a novel approach to ultrasensitive bacterial sensing using a disposable, integrated platform with self-noise cancellation. The platform has high sensitivity and accuracy in detecting low bacterial concentrations and has potential applications in various fields.

36. * Ward AC, Connolly P, Tucker NP: **Pseudomonas aeruginosa can be detected in a polymicrobial competition model using impedance spectroscopy with a novel biosensor**. *PLoS One* 2014, **9**, e91732.

The paper is important because it presents a novel biosensor using impedance spectroscopy to detect *Pseudomonas aeruginosa* in a polymicrobial model, offering potential for low-cost clinical diagnostics.

37. Kumar S, Nguyen AT, Goswami S, Ferracane J, Koley D: **Real-time monitoring of biofilm formation using a noninvasive impedance-based method**. *Sensor Actuator B Chem* 2023, **376**, 133034.

38. McGlennen Matthew, Dieser Markus, Foreman Christine M, Warnat Stephan: **Using electrochemical impedance spectroscopy to study biofilm growth in a 3D-printed flow cell system**. *Biosens Bioelectron X* 2023, **14**, 100326.

39. * Zhou Keren, Kammarchedu Vinay, Butler Derrick, Khamsi Pouya Soltan: **Electrochemical sensors based on MoSx-functionalized**. *Adv Healthcare Mater* 2022, **11**, 2200773.

This paper is interesting because it introduces a novel flexible electrochemical sensor based on MoSx-functionalized laser-induced graphene, offering high specificity and sensitivity for detecting phenazines produced by *P. aeruginosa*. The real-time monitoring capability of *P. aeruginosa* biofilms over several days adds significant value for potential clinical applications.

40. Thirabowonkitphithan Pannawich, Phuengmaung Pornpimol, Leelahavanichkul Asada, Laiwattanapaisal Wanida: **MWCNTs/PVA hydrogel-modified electrochemical sensors for ex vivo and in vivo detection of pyocyanin biomarker for Pseudomonas aeruginosa wound infection**. *ACS Appl Electron Mater* 2023, **5**:821–831.

41. Bimakr Fahimeh, Ginige Maneesha P, Kaksonen Anna H, Sutton David C, Puzon Geoffrey J, Cheng Ka Yu: **Assessing graphite and stainless-steel for electrochemical sensing of biofilm growth in chlorinated drinking water systems**. *Sensor Actuator B Chem* 2018, **277**:526–534.

42. Liustrovaite V, Pogorielov M, Boguzaite R, Ratautaite V, Ramanaviciene A, Pilvenyte G, Holubnycha V, Korniienko V, Diedkova K, Viter R, *et al.*: **Towards electrochemical sensor based on molecularly imprinted polypyrrole for the detection of bacteria—Listeria monocytogenes**. *Polymers* 2023, **15**:1597.