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Online biofilm monitoring is missing in technical systems: how to build stronger case-studies?

Ana Pereira[1,2]✉ and Luis F. Melo[1,2]

Using physical tools to assess online, real-time, continuous information about biofilms in technical water systems is a key component of an early-warning antifouling strategy. However, online biofilm monitoring is not yet relevant in real-field practice, nor in lab studies. In this review we analyse online biofilm monitoring from an academic perspective to provide insights on what science can improve to bring it to the spotlight of biofouling management and prevention. We argue the need to involve a broader community of biofilm researchers on the use of online monitoring to deepen biofilm studies (e.g. linking biofilm features, dynamics and operational impact) as well as the need for more, and better detailed studies. This will, consequently, reinforce the added value of biofilm monitoring as part of an early-warning antifouling strategy while bridging the techniques' potential to the real-field needs. Finally, we propose a framework to improve laboratorial and field studies.

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INTRODUCTION

The question of how to tackle unwanted biofilms in technical water systems, like heat exchangers, ultrafiltration or reverse osmosis systems, has been addressed by Flemming and Melo (1995)[1], following the discussion held at the 'unwanted biofilms workshop'. The authors highlighted the need for 'an exhaustive literature review about biofilm detection and monitoring methods, discussing their merits and limits, and the development of techniques which allow the monitoring of biofilm growth in-situ, online, automatically and in real time' and the need 'to develop concepts for advanced antifouling strategies'. It was also recognized that biofilm detection should be taken as a step forward to overcome limitations and costs associated with biofilm assessment through conventional indirect indicators.

Two decades after this challenge, interesting reviews have been written[2–4] but there is still a huge gap between the potential of physical sensors, their added-value to antifouling approaches and their effective implementation in real-field systems[5,6]. The number of papers found for 'online biofilm monitoring' shows that this specific area of biofilm research has not gathered much attention from the biofilm scientific community. As discussed in section 'Online Biofilm Monitoring Overview', most techniques were just reported twice or three times, without follow up applications. Most papers fail to provide enough level of details (e.g., probe surface area or the effect of interferents) regarding the monitoring techniques and the experimental approaches, to allow proper research advances or to provide historical accumulated data that contribute to effective progress of the state-of-the-art.

As scientists, we need to demonstrate and convince our peers and industry of the added value of online biofilm monitoring. Probably we must step back and start reinforcing the potential of these tools in biofilm research (lab focus), to deepen biofilms studies, and to better understand the relation between biofilm features and their operational impact. For that, more and better detailed lab and field case-studies are needed. By analysing the successful pathway accomplished by the Membrane Fouling Simulator[7]—a monitoring system that assesses biofouling across spiral wound membranes—we propose a framework to improve online biofilm monitoring related studies. Although detailed technical aspects of the monitoring systems are not discussed here, the reader can obtain this information from the extensive list of relevant publications suggested in the present paper. The review overview is schematically shown in Fig. 1.

SETTING-UP EXPECTATIONS—CHALLENGES OF ONLINE BIOFILM MONITORING

The function-driven role of online biofilm monitoring

Biofilms in technical water systems are complex ecosystems that entrap a miscellany of components beyond microorganisms and EPS (Extracellular Polymeric Substances)[8,9]. These ecosystems are the result of local conditions (e.g. hydrodynamics, surface materials, temperature, local chemistry) making biofilms' structure and dynamics heterogeneous[10] in time and space.

Biofilm monitoring has a function-driven role[11], related to: (a) the meaning of the change in biofilm attribute(s)—what is happening with the biofilm? and (b) the interpretation of what does that variation mean under an operational perspective. According to Lewandowsky[12], if biofilms are considered agents of certain activities, biofilm function is the (operational) result of the biofilm action. For example, the accumulation of biofilm on reverse osmosis or ultrafiltration systems might have different functions: increase energy consumption, reduction of permeate quantity, decrease of water quality, shorter membranes' lifetime.

Biofouling (the operational negative impact of biofilms in technical systems[13]) is thus a consequence of the place where biofilms occur and of the biofilms' properties. It is only above a certain level[13]—the so-called 'threshold of interference' - that biofilms become an operational problem.

It is interesting to note that across literature 'biofilm monitoring' and 'biofouling monitoring' are used interchangeably. This is probably because biofouling definition is also function-driven.

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[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal. [2]ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.
✉email: aalex@fe.up.pt

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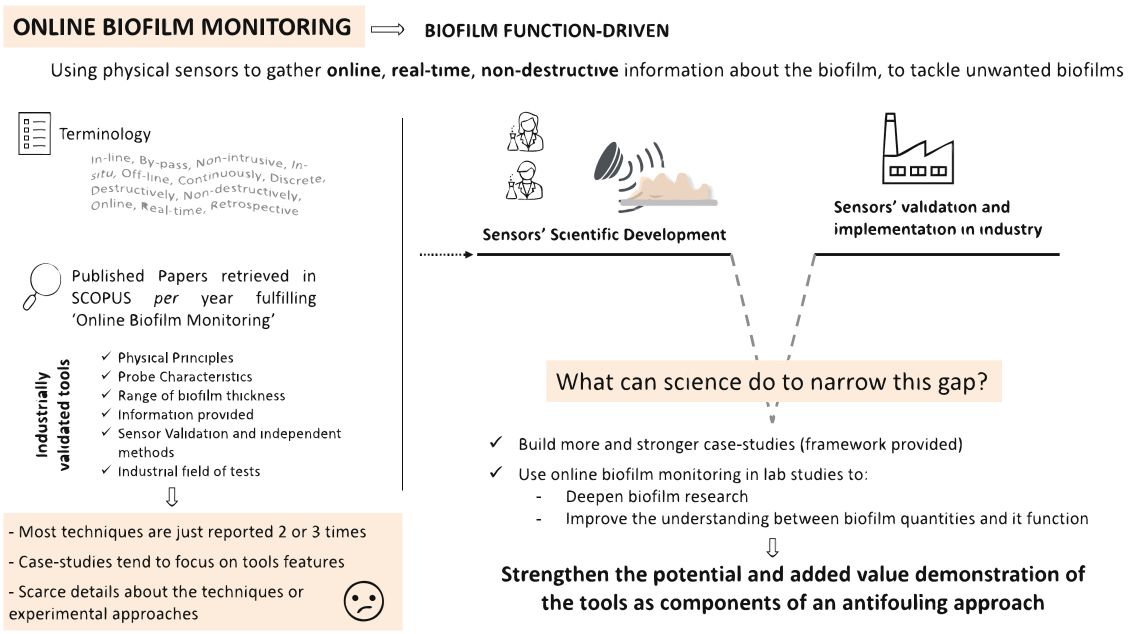


Fig. 1 Approach undertaken to accomplish this review work. (1) Setting-up expectations for online biofilm monitoring towards its function-driven role and terminology definition. (2) Overview of the 'online biofilm monitoring' tools industrially validated and identification of main drawbacks. (3) Proposal of a framework to build stronger case studies to narrow the gap between sensors' scientific development and its industrial validation.

Physical Sensors—analysing the interference caused by the biofilm in the input signal

Biofilm monitoring can be accomplished in different ways[14,15], such as: (a) directly assessing parts of the system and sampling/removing biofilms (via for example swabbing or scratching); (b) implementation of coupons on representative sites that are periodically removed for laboratorial analysis; (c) installation of in-situ physical sensors that provide online, real-time, and non-destructively information about the deposits attached to a given surface. Gathering results in options (a) and (b) is time-consuming, information is retrospective regarding the sample collection, and it refers to a discrete monitoring process as biofilm evaluation just occurs from time-to-time. Only option (c) fits into an antifouling integrated monitoring perspective, that relies on early-warning information about the biofilm formation potential and its removal.

Monitoring is based on the identification of the interference caused by the biofilm on an input signal[2]—schematically represented in Fig. 2. It usually encompasses the following steps: (1) emission of an input signal in the direction of the surface under investigation (where the biofilm builds-up); (2) the presence of biofilm changes the characteristic(s) of the input signal into an output signal; (3) detection and measurement of the signal shifts via signal processing and analysis; (4) translation of the measured physical quantities [e.g. voltage shifts] into biofilm properties. The approaches to address point (4) are further discussed in section' Strategies to validate the output provided by the sensors'.

Putting a monitoring system together is, in a larger extent, about choosing the right set of conditions (excitation signal, sensor, physical configuration) that maximizes the signal property(ies) to better 'describe' the interference caused by the biofilm, minimizing the interference from external factors. It is also about finding out how changes in the signal property(ies) are related with the biofilm characteristics[16].

Terminology

Associated with biofilm monitoring, often comes a panoply of designations like: online, in-situ, non-destructive, real-time, etc that aim to contextualize how the measurement/biofilm analysis is performed. Literature seems to point-out towards a common understanding of these terminologies, yet a clear definition is not easily found. For clarification purposes the following definitions are considered:

1. Where is the monitoring system installed? In-line—the monitoring tools are directly placed at the core of the process to be monitored (e.g. at a water circuit pipeline); By-pass/side-stream—monitoring probes are placed in a by-pass piping constantly fed with bulk water from the main water system; Non-intrusive/ non-invasive—the deposit does not build-up directly on the monitoring probe; the probe is placed externally to the monitored surface (can be in-line or in by-pass).
2. Where is the biofilm sample analysed? In-situ—the measurement is performed at the place of interest (in-situ is the Latin word for 'in place'); Off-line—samples are taken from the place of interest and biofilm is analysed externally.
3. Which is the time-rate of the measurements? Continuously—measurement is accomplished over time in a way that it can detect changes within a small timespan; Discrete—measurement occurs from time-to-time (discretely in time).
4. What happens to the biofilm sample? Non-destructively—measurement is performed without destroying the biofilm sample; Destructively—biofilm samples are destroyed during the analysis.
5. How is the measurement information accessed? Online—immediate access to measured information via computer-based protocols (intrinsically related to an in-situ, continuous, real-time measurement); Real-time—output information is immediately generated as measurement happens; Retrospective—the results are accessed with a delay regarding the measurement.

The definition proposed herein regarding 'online' does not fit the ones that are proposed in other industrial practices. For example, the pharmaceutical industry classifications for the Process Analytical Technology—an approach to operationalize product quality management practices by monitoring critical process parameters[17]—defines online methods equivalently to

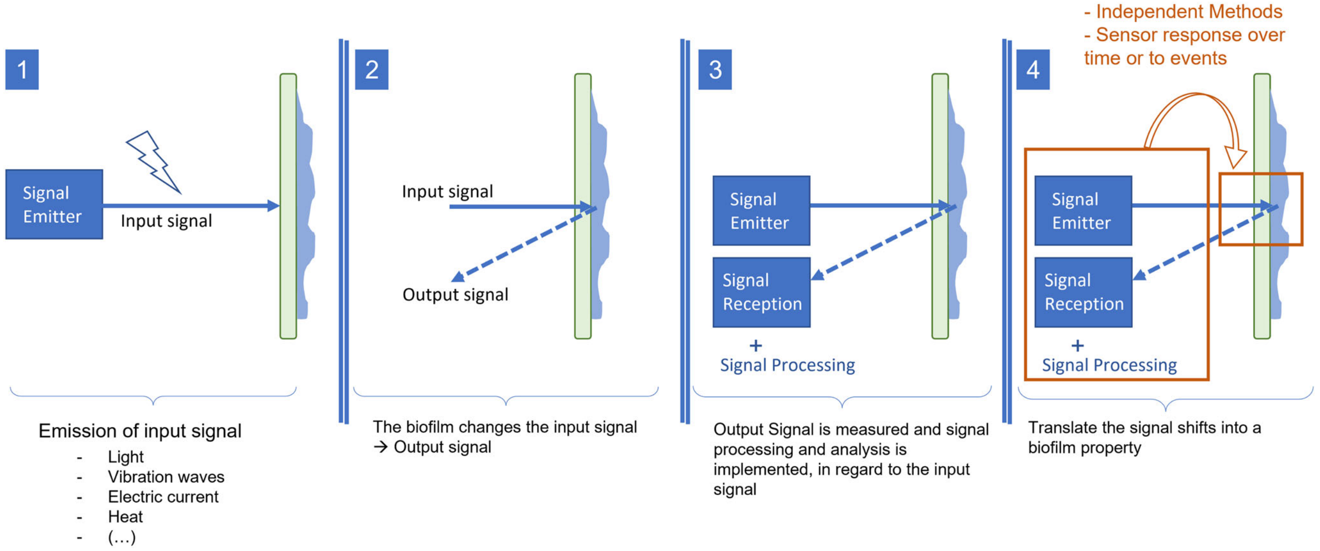


Fig. 2 The monitoring principle schematic representation. The process included the: (1) emission of an input signal; (2) the interference of the biofilm on the input signal (into the outputs signal); (3) output signal measurement; (4) translation of the signal shifts into a biofilm property.

our former definition of by-pass. In pharmaceutical processes or even the oil industry[17], the monitoring focus is on the bulk fluid. However, in biofilm monitoring the concern is with the surface where the biofilm builds-up. Additionally, the biofilm characteristics are the direct result of the bulk water taken from the main process, but also depend on the by-pass local conditions where the online measurement occurs[5]. As so, looking into biofilm monitoring related literature, it seems that 'online' is used to make a point that a physical sensor is used, and that information is accessed simultaneously with measurement (suggesting a computer-based acquisition process). For example, the work describing the fibre optical sensor (FOS)[18] states that the sensor head was installed directly at the piping system (according to our definition: in-line) providing an 'in-situ, online, in real-time and non-destructively' information about the deposit.

Each time these terms are used it is important to clearly mention what they are referring to. For example, according to Donlan, R.[19], real-time monitors are the ones that allow the 'installation and removal of test substrata from the device'. Our understanding, on the contrary, is that real-time monitors provide output information immediately as the measurement happens, without the need to remove the test substrata.

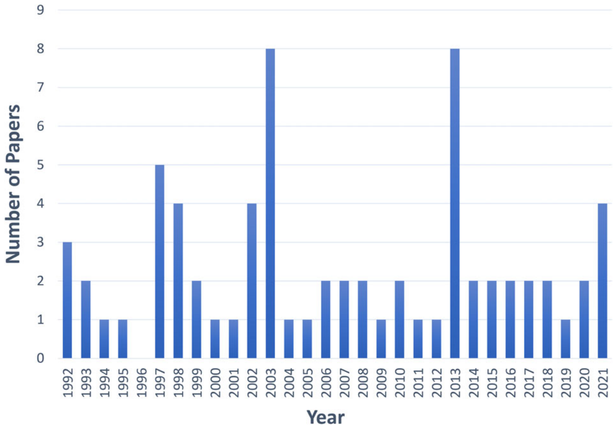


Fig. 3 Number of published papers per year retrieved from Scopus search engine when using the following keywords restrictions: (*Online* OR *On-line*) AND (*Biofouling* OR *Biofilm*) AND (*Monitoring* OR *Monitor*). The list of references considered (after eliminating the publications that were out of the scope of the present paper, through abstract and/or title analysis) is available in Supplementary Table 1.

ONLINE BIOFILM MONITORING OVERVIEW

To briefly overview the 'online biofilm monitoring' field of study over time, the following keywords were used at the SCOPUS search engine: 'biofouling OR biofilm' AND 'monitoring OR monitor' AND 'online OR on-line'. Figure 3 shows the refined retrieved number of documents per year that fulfilled the search criteria. This refined number of documents was obtained after eliminating the references that were out of the scope of the present papers, based on the title and/ or abstract text.

Data shown in Fig. 3 (list of publications provided in Supplementary Table 1) suggest that 'online biofouling monitoring' is not a field that gathers much attention from the scientific community. In the last decade less than 40 publications have been retrieved in this search (corresponding to an average of 2–3 papers per year), which does not follow the increasing importance of biofilm research area[20,21]. The techniques discussed in the following sections are not restricted to the ones listed in Supplementary Table 1 as they include other monitoring tools that were found through other search databases, in reference literature[2] or on the internet.

It is important to highlight that a comparison and technical details about the different methods for monitoring biofilm formation are out of the scope of the present review, which is more focused on the programmatic (strategic planning) issues. For that, the following literature should be addressed: Janknecht and Melo[2], Flemming[4], Nivens et al.[3], or Polman et al.[22].

Scope of biofilm monitoring testing

Monitoring techniques fall in one of two scopes of testing: (a) tools that were only evaluated in lab studies (not tested/ feasible for industrial use); (b) tools industrially validated. Tools included in (b), in principle, can also be used for laboratorial studies purposes.

Tools only validated in laboratory applications. Many tools considered in Fig. 3 are suitable for biofilm lab studies, but due to their sophisticated instrumentation, complex technical

Table 1. Overview of online biofilm monitoring tools industrially tested.

Online, real-time, non-destructive biofilm monitoring tools validated in industrial applications

Method	Technique |Physical Principle|	Probe Characteristics	Range of Biofilm Thicknesses	Operational information provided	Sensor Validation and Independent Methods	Industrial field tested
Optical	OPTIQUAD*[15] |Light fluorescence, refraction, transmission, and scattering|	Probe surface area[#]: 300 mm^2	Thin biofilms (1–50 μm, >10^6 cells/cm^2)[#]	Distinguishes biological and non-biological deposits; Biomass viability	Cell density (CFU/cm^2) –[DAPI]; Viability [NADH + ATP]; Inorganic [calculated]; Assess cleaning effect	Drinking water pilot test system (in-situ)
Optical	BioDART*[44] |Light Absorption/ Transmittance|	Probe surface area: information not found	Medium-thick biofilms (20–150 μm)	Biofilm dynamics; Microbial Activity	Thickness [CLSM]; Assess impact of biocide suppression and filter failure	Cooling system (side-stream)
Optical	Fiber Optical Sensor[14,18,43] (FOS) | Light Scattering|	Sensor tip diameter: 0.2–0.5 mm	Thin biofilms (>10^5–10^{10} CFU/cm^2), <7 μm	Biofilm dynamics; Biocide optimization	Cell density (CFU/cm^2) – [DAPI]; Assess cleaning effect (sensor head and piping system)	Brewery water system (in-situ)
Optical	Differential Turbidity Measurement[45] (DTM) | Turbidity|	Sensor window diameter: 18 mm	No information found about biofilm thickness	Biofilm dynamics; Distinguish organic and inorganic	Pressure drop [differential pressure meters]; Slime measuring board; Thickness [estimated via volume]; Assess cleaning effect	Paper machine whitewater circuit (side-stream)
Optical	Optical Fiber Sensor[46] | Evanescent Field Attenuation|	Sensor tip diameter: 0.98 mm	Thick biofilms (<1000 μm)	Biofilm dynamics	Thermal flux sensor; Thickness [not specified]	Cooling System (by-pass)
Vibration	OnGuard* 3B analyzer™[46,47] | Heat Transfer + Ultrasound propagation|	Probe surface area: 4645 mm^2	Medium-thick biofilms (25–100 μm)	Biofilm dynamics; Discrimination between biotic/abiotic deposits	Thickness *vs* heat exchanger fouling factor; Thickness [OCT]; Optimization of biocide program	Cooling system (by-pass)
Vibration	Mechatronic Surface Sensor[49,50,81,82] (MSS) |Vibration Propagation|	Probe surface area: 2200 mm^2	Medium-thick biofilms (10–1000 μm)[#]	Biofilm dynamics; Biocide optimization	Wet/dry density (mg/cm^3) – Weight, thickness [Micrometer]; Assess impact of unexpected events	Cooling system (by-pass)
Electrochemical	ALVIM* Sensor[58,59] |Cathodic Depolarization|	Probe surface area[#]: 500 mm^2	Initial bacterial Layer (1–2 μm)[#]	MIC prevention; Biofilm growth; Optimization of oxidant biocide dosages	Cells density [DAPI]	Cooling water system + Pilot reverse-osmosis (in-situ)
Electrochemical	BIOX[56,57] |Cathodic Depolarization|	Probe surface area: no information found	Early-stage biofilm (<10^7 CFU/cm^2)	MIC prevention; Optimization of oxidant biocide treatments	Surface coverage (CFU/cm^2) – direct counts	Cooling System (by-pass)
Electrochemical	BioGeorge*[53–55] |Cathodic Depolarization|	Probe Surface area: no information found	Initial bacterial Layer	Microbial activity; Biocide optimization (2001)	Microbial Activity [ATP]	Cooling System (by-pass)

*Commercially available sensor.

[#] specific information gathered from the documentation of the 'Biofilm Monitoring Workshop', organized by Hans-Curt Flemming in 2010, Germany (the authors have attended the Workshop).

All the considered techniques provide online, real-time, non-destructive information about the attached biofilm. The independent methods used are referenced in '[]' and the acronyms stand for: *DAPI* 4′,6-diamidino-2-phenylindole, *NADH* Nicotinamide Adenine Dinucleotide, *ATP* Adenosine Triphosphate, *CLSM* Confocal Laser Scanning Microscopy), *OCT* Optical Coherence Tomography, *SEM* scanning electron microscope, *MIC* Microbial Induced Corrosion.

operation and data interpretation are not friendly for industrial usage. This is not surprising as laboratorial biofilm research also needs online and real-time tools to deepen biofilm studies[23] and it is very likely that the initial validation of techniques starts under controlled laboratorial conditions. Some examples are the Quartz Crystal Microbalance[3,24,25] (QCM), that measures the changes in the resonant frequency of a piezoelectric quartz probe or the Photoacoustic Spectroscopy[26–28] (PAS) that takes advantage of the absorbed electromagnetic radiation inside the sample. A detailed overview of online monitoring tools validated in laboratory might also consider techniques like the Isothermal microcalorimetry[29], the Surface Acoustic Wave[30] or time-invariant heat transfer[31], whose advantages and disadvantages have been discussed by Wieland et al.[32].

There are several sensors that showed potential for online biofilm monitoring under laboratorial conditions and that claim suitability for future implementation in field systems, although there are no reports yet on their application to industrial situations. The Microwave Sensor[33] (based on electromagnetic waves) detects early-stage biofilms, with focus on biofouling and corrosion prevention and biocides dosage optimization. Ultrasound techniques were found to provide online and non-intrusively information about the biofilm quantification[34] and the use of wave pulse echo seems to be suitable for biofouling control in industrial applications[35]. It is important to highlight the works using UTDR (Ultrasonic Time Domain Reflectometry) in reverse osmosis modules[36,37]. Although most UTDR works address inorganic fouling, the technique adaptation[37] to ‘canary cell’ showed promising results for non-invasive real-time detection of biofouling in high pressure membrane processes, and for biofilm monitoring in wastewater applications[38].

Boukazia et al.[39] applied differential thermal measurements (hot wire method) to assess online and in-situ, the thickness and nature of the attached deposits. The spectrophotometric system (BioSpec)[40] was found to detect, in-situ, in real-time, continuously, and non-destructively changes in biomass and metabolic activity. The technique is intended for biofilm fundamental research studies (lab application) and to address mass-function relations. The use of electrochemical impedance spectroscopy[41] (EIS) to monitor biofilm dynamics showed to be a promising approach for industrial applications to assess the biofilm build-up potential and efficacy in disinfection programs.

Tools validated in industrial applications. Table 1 provides the list of tools that match the following criteria: (a) provide online, real-time, non-destructive information about the attached deposit; and (b) industrially tested devices. For each monitoring tool, the table includes the information provided by the sensor, the independent method(s) used to assess the biofilm characteristics and the industrial field where the tool has been validated. The references encompass the field tests and lab studies for each technique under consideration.

The criteria used in ‘Biofilm Thickness’ was based on Flemming et al.[14] who proposed a rough classification of biofilms based on their thickness: thin (<10 µm), medium-thick (10–200 µm) or thick (>200 µm).

Tools, physical principles, and stages of biofilm development: Online biofilm monitoring sensors tested in industrial studies rely upon three main underlying physical methods: optical, vibration or electrochemical measurements.

Optical-based techniques take advantage of the interference caused by the deposit formation in the light/optical signals. The effect of the biofilm on the optical signals can be examined under distinct approaches[42], including: scattering (FOS), attenuation (Optical Fiber Sensor), absorption/transmission (BioDart), reflection, photoacoustic, or the combination of several of approaches (OPTIQUAD, ROHNE OptosensGmbH, Neuss, Germany).

Optical sensors cover a wider biofilm development range, from thin to thicker biofilms. For example, the FOS[18] has been tested in the water system of a brewery to assess the impact that the deposits build-up/removal have on the backscattered light intensity. It can measure between 10^5 and 10^7 CFU/cm^2 but it is not suitable for biofilms thicker than 7 µm[43]. The OPTIQUAD (commercialized by KROHNE Optosens GmbH) was evaluated in a drinking water pilot testing unit[15]. The probe can simultaneously measure light fluorescence, refraction, transmission and scattering. It is able to distinguish biotic from abiotic deposits and the accumulation of biofilm regarding the proteins and biological activity, within a thickness range of 1–50 µm[15]. Another commercially available sensor is the BioDART (Chemaqua, United States) which assesses continuously the reduction in light transmittance as deposits form within a narrow lumen detector tube. This configuration amplifies the biofilm growth, and the overall BFI (Biofouling Index) is correlated with the biofilm quantity[44]. BioDART is suitable to detect biofilm thicknesses between 20 and 120 µm[44].

The Differential Turbidity Measurement[45] (DTM) takes advantage of the difference between the comparative turbidity measured in continuously cleaned and non-cleaned optical windows of the sensor. Although a clear indication about the thickness measurement range was not found, the DTM should be able to measure thicker biofilms[14]. The Optical Fiber Sensor[46] was also found to be suitable for thick biofilms (<1000 µm) by exploiting evanescent field attenuation through refractive index, absorption, and scattering modulation.

Vibration techniques take advantage of the echos generated in response to mechanical stimulations[2]. Such response changes as deposits starts to build-up/detach from the monitored surface. Vibration tools can assess deposits dynamics from larger surface areas and thus minimizing biasing problems associated to biofilm heterogeneity. The vibration techniques described in Table 1, depending on the frequency range they use, can be classified into: ultrasound (e.g., OnGuard) or low-frequency acoustic technique (e.g., MSS/DSS). The OnGuard$^{\text{TM}}$ 3B Analyzer[47] (commercialized by Solenis) performs the ultrasonic measurement on a heated surface, enabling a detection based on two parameters: heat transfer reduction and the travel time decrease of the ultrasonic wave. Such combination follows the build-up/removal of the attached deposits (thicknesses: 20–120 µm) and provides information about the predominant nature of the attached deposits[48] (soft—organic/biological or hard—scaling). The MSS[49,50] takes advantage of the effect that the mass/density of the attached layer has on the vibration properties of the wave that is propagated along the monitored surface. This technique is suitable for biofilm thicknesses between 10 and 1000 µm. It was successfully evaluated in by-pass of a cooling water system as an early-warning indicator of biofilm (or other deposits) build-up/removal. It can distinguish soft from hard layers.

Electrochemical techniques are reported in several references in Supplementary Table 1, even though most publications have been presented in Conference Proceedings—not easily available to the research community. Electrochemical tools take advantage of the effect that biofilm components (organic and inorganic substances and microorganisms’ metabolic activity) have on the electrochemical characteristics[51]. Given the specificity and nature of the interactions, these techniques are suitable to monitor the initial stage of biofilm development[51,52]. The measurement is usually accomplished with two or more electrodes of different materials immersed in the bulk fluid. There are several approaches to assess information of early-stages biofilm formation, including cathodic depolarisation—the underlying measuring principle behind the probes BioGeorge[53–55], BIOX[56,57], and Alvim Sensor[58,59]. The three tools are products of the same research activity[52], and all have been tested in by-pass of cooling systems to optimize oxidant biocides dosage and MIC prevention. The BIOX measures the

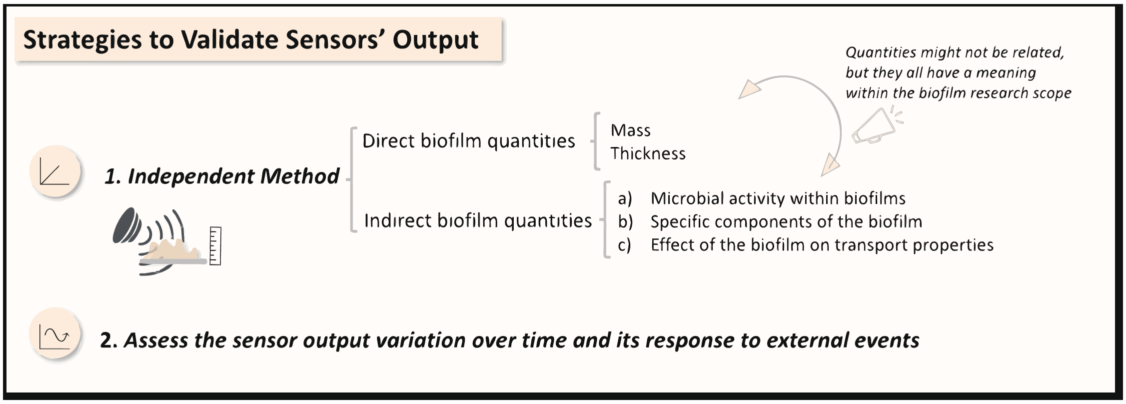


Fig. 4 Strategies to validate online biofilm monitoring sensors' output information. Validation methods can rely upon: (1) the use of an independent method, through direct or indirect biofilm quantification and/or (2) the assessment of the variation of the sensor's output over time in response to external events.

impact of cathodic discharge of oxygen caused by bacteria in the biofilm (at the working electrode) and the effect of the oxidant biocides species in the water[56]. The BIOX is limited to surface coverages below 10^7 CFU/cm^2. Alvim sensor is a refined version of the other two techniques with higher sensitivity and increased upper detection limit[58]. Sensor's output information is related with the bacteria surface area coverage[58].

Strategies to validate the output provided by the sensors: Regardless the physical principles behind the techniques, sensor's evaluation is usually accomplished in two ways, as illustrated in Fig. 4.

1st Strategy—independent method

The first strategy uses an external reference (independent) method to validate the biofilm interference on the input physical signal[16], and to translate such signal into a biofilm property. The choice of independent methods depends on the sensors' physical principle and on the biofilm information the sensor is measuring. It can target different biofilm properties[60], such as direct biofilm quantities, in the case of mass or thickness or indirect biofilm quantities when considering: (a) microbial activity within biofilms (measured as ATP, or viable cell counts, via different staining methods like DAPI or Live/Dead staining) which is important to assess the physiological state of the microorganisms[61]; (b) specific constituents of the biofilm (e.g. polysaccharide content, diversity of microbial species) that inform about the biofilm composition; or, (c) the effect of biofilm on transport properties (e.g. pressure loss, heat transfer resistance). Methods for measuring microbial activity and specific components of the biofilm are usually very sensitive, but direct quantities are essential for the determination of biofilm build-up/removal rates and stoichiometry. Not all biofilm properties have a direct relation with each other, however they all have a meaning to the biofilm scientific community[60], depending on the biofilm function that is being studied in each case.

Table 1 shows that techniques concerned with biofilm early-stages tend to use biofilm indirect quantities like cells viability—NADPH, ATP or viable cell counts. However, thickness is clearly the most consensual parameter addressed. This is not surprising as thickness is an important structural characteristic[62] (such as porosity, morphology, and density) of biofilm, and also shapes the bacterial communities in the biofilm. These structural features 'translate' the effect of the environmental factors affecting the biofilm dynamics (growth/ detachment) and its function[62].

2nd Strategy—assess the sensor output variation over time and its response to external events

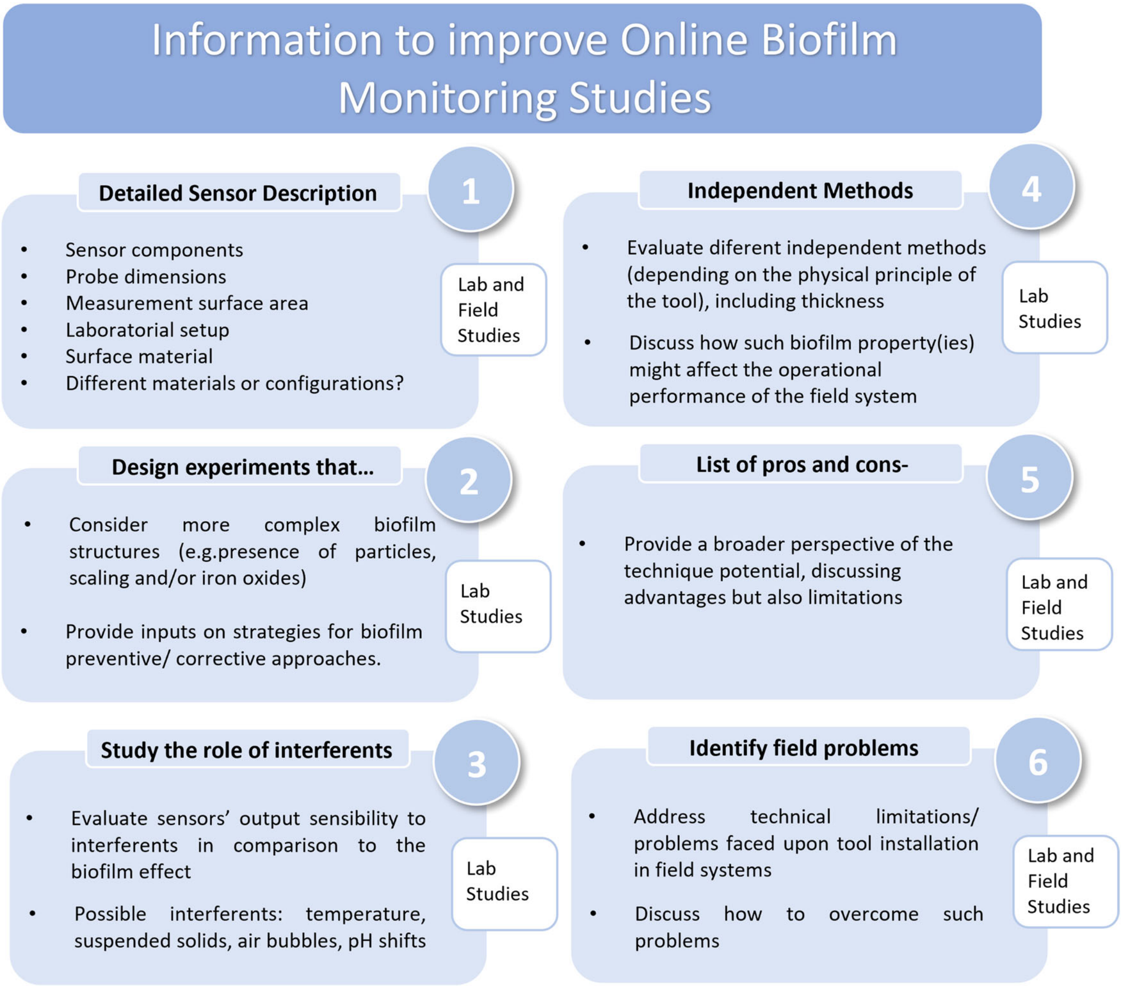
The second strategy focus on analysing the sensor output over time and its response to the variation of external events/ processual parameters. The expected impact of such variation in the output response is, in principle, known. For example, in most of the studies listed in Table 1, the effect of biocide/ disinfectant dosage on the sensors' response is studied. Given that sensors' outputs are related to biofilm properties, one might expect that an increase in the biocide dosage will for example decrease the viability of the microorganisms or detach biomass. In both cases, the sensor output information is expected to decrease. Usually, this analysis is focused on the trend of the sensor response over time[5]—is it increasing? is it decreasing? How fast and how far is it increasing/ decreasing?

As a generalization, independent methods (1st Strategy) are used in a laboratorial stage to establish a correlation, under specific conditions, between the sensor output and the biofilm quantity. Field trials focus on following the sensors response over time (2nd Strategy). It is important to note that in most cases the correlation between biofilm properties and sensor response is not directly implementable in field conditions as it is usually affected by local external interferents. From an operational perspective, in most of the cases, it is the information given by the 2nd strategy that will be used.

STRENGTHENING CASE-STUDIES (IN LAB AND FIELD) APPLICATIONS

It is difficult to convince a broad community of scientists and practioners about the relevancy of online biofilm monitoring without providing clear insights on the topic. In this specific area of biofilm study, it seems that the cumulative knowledge that makes science and development evolve is restricted to two or three papers per technique. Most of the overviewed techniques are just reported twice or three times and do not seem to be consistently used/ tested/ validated or improved over time. Similarly, it is difficult to follow up what happened with some of the tools listed in Table 1: are they commercially available? Was their name changed?

Even commercially available techniques mentioned in scientific literature have scarce information available. For example, Neicsh et al.[63] reported the use of Deposens (Lagotec GmbH - Magdeburg, Germany)—an online biofilm sensor based on heat transfer measurement—with the aim to study the sensor ability to quantify biofilm on microbial fuel cells electrodes. But, in the company webpage (www.lagotec.de, accessed in 14/10/2022), the information about the sensor is limited and the authors could not find significant information about case-studies or sensor's



Information to improve Online Biofilm Monitoring Studies

Detailed Sensor Description 1

- Sensor components
- Probe dimensions
- Measurement surface area
- Laboratorial setup
- Surface material
- Different materials or configurations?

Lab and Field Studies

Independent Methods 4

- Evaluate diferent independent methods (depending on the physical principle of the tool), including thickness
- Discuss how such biofilm property(ies) might affect the operational performance of the field system

Lab Studies

Design experiments that… 2

- Consider more complex biofilm structures (e.g.presence of particles, scaling and/or iron oxides)
- Provide inputs on strategies for biofilm preventive/ corrective approaches.

Lab Studies

List of pros and cons- 5

- Provide a broader perspective of the technique potential, discussing advantages but also limitations

Lab and Field Studies

Study the role of interferents 3

- Evaluate sensors' output sensibility to interferents in comparison to the biofilm effect
- Possible interferents: temperature, suspended solids, air bubbles, pH shifts

Lab Studies

Identify field problems 6

- Address technical limitations/ problems faced upon tool installation in field systems
- Discuss how to overcome such problems

Lab and Field Studies

Fig. 5 Framework to accomplish more detailed studies on online biofilm monitoring. Information to improve such studies should consider the: sensor description, proper design experiments, role of interferents, independent methods used, list of advantages and disadvantages, field problems encountered when testing the monitoring tools.

validation in the lab nor in field applications. Another example is the NeoSense[64] which, according to Crattele et al.[65] is now part of Aqualabo (en.aqualabo.fr, accessed in 18/07/2022) under the name of SkidSens[66]. The sensor integrates heating and temperature measuring elements into a MEMS technology and the measurement is based on the resistance caused by the attached deposit to heat transfer[66]. In this case, apart from the scarce information about the sensor(s), it is difficult to follow up the technique developments as they have different commercial tradenames not clearly communicated.

There is comprehensively a conflict of interests between the scientific development and the commercial exploitation of techniques. But, to deepen the concepts behind the definition of the biofilm threshold of interference and the relation between the biofilm dynamics or extent[5] and the operational impact of biofilm in field systems, case-studies should be completed and disclosed to a wider audience beyond the commercial domain. An open development practice will ultimately strengthen the arguments for the need of 'online biofilm monitoring' benefiting all the stakeholders.

Framework to build stronger case-studies

While overviewing literature, the authors came across specific items that are just slightly (or not at all) considered in several manuscripts. These points are schematically presented in Fig. 5 and can provide a framework to improve future studies on this topic while enabling a better comparison among techniques.

Strengthening the added value of online biofilm monitoring

To change the *status quo*, it is key to strengthen the relevance of online monitoring tools within the scope of biofilm study. As Mauricio et al.[35] proposed, it is essential to 'bridge the gap between current understanding of biofilm fundamentals and monitoring control systems' and, as Cloete[67] pointed-out, 'the advantage of biofouling monitoring needs to be demonstrated'. One might take as an example the MFS—Membrane Fouling Simulator. Since Vrouwenvelder et al.[7] described the technique, it became increasingly important on the study of biofouling related aspects in spiral wound reverse osmosis (RO) membrane. Based on the fact that biofouling is the key problem in membrane operations and that it occurs in a greater extent in the first membrane modules, the MFS is a small system that provides a representative assessment of what happens in spiral wound membranes, by measuring the pressure drop across the feed spacer channel. Kim et al.[68], provides an overview of several steps and works accomplished with the MFS. It is interesting to note that the focus of the studies is not restricted to demonstrating the potential of the tool as an early-warning for biofouling[69], but rather on establishing the rationale behind the design, implementation, independent methods used, among others. Studies also provide information that have an operational component (a direct bridge from research to practice), targeting for example the design of RO systems (e.g. feed-spacer geometry[70]) or strategies to biofouling prevention/ control (e.g. biocide dosage[71] or the role of phosphate dosage[72]).

A similar strategy is being accomplished with the Optical Coherence Tomography (OCT), that although more suitable for lab application, is being used in a diversified range of biofilm studies[73] while reinforcing the technique added value. OCT is a potential tool for 'non-invasive, label-free, real-time, in-situ' imaging of biofilms' and to assess the fluid-biofilm interactions at the mesoscale[74,75]. It has been used in several domains of biofilms' studies, such as biofilm structure and volumetric characteristics[76], understand the role of biofilm structure in membrane systems[77,78], microbial growth dynamics[79], and gathered important contributions from image processing (BISCAP[80]).

The optimization and validation of online biofilm monitoring tools within the scope of antifouling programs, requires interdisciplinarity and is time-consuming, inhibiting industry involvement[4,5] in this process. Strengthening the added value of online monitoring must rely on several, well designed scientific studies, that use the tools to improve the understanding of different aspects of the biofilm's behaviour[23] dynamics, their interactions with the ecosystems, and that address the biofilm function. By the end of the day, this approach will reinforce the monitoring tools potential, will provide advances of the biofilm research state-of-the-art and provide valuable insights for field practice.

Rethinking the strategy to address online biofilm monitoring from a research level can bring us closer to the idea of Flemming (2003)[4] that 'it is only a matter of time until biofilm monitoring will be a state-of-the-art technique, using many different approaches'.

In conclusion, online biofilm monitoring in technical systems is clearly a complex process. Biofilms change (in function and structure) in time and space as a response to local environmental conditions. Biofilm monitoring has an intrinsic function-driven role, as the final goal is to address the operational impact that biofilms have/ might have in each technical system. Furthermore, biofilm monitoring depends on interdisciplinary knowledge far beyond the strict biofilms' expertise *(electronics, robotics, etc)*. Public perception of the environmental, energy consumption and health issues related to biofilm formation already imposes much more effective biofilm assessment approaches that provide fast digitalized and accurate information as well as defined decision-support tools.

This review paper aims to stimulate and guide the broader community of researchers to use online monitoring techniques on biofilm studies. This will be decisive to establish more and better envisioned scientific work, that goes beyond the demonstration of technological abilities, as well as to deepen the biofilm understanding and to reinforce the added value of online biofilm monitoring as part of antifouling monitoring approaches in technical systems.

We provide here a framework to improve future laboratorial and field studies.

DATA AVAILABILITY

The data sources analyzed and discussed are provided in this paper. Data marked with # in Table 1 can be requested to the corresponding author (A.P.).

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Ana Pereira.