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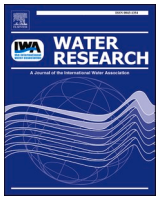

Automation of on-site microbial water quality monitoring from source to tap: Challenges and perspectives☆

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ABSTRACT

Ensuring the provision of safe drinking water necessitates thorough monitoring of microbial water quality. While traditional culture-based enumeration of bacterial indicators has served as the gold standard in compliance monitoring since the late 19th century, recent advancements in microbial sensor technology, driven by automation and digitalization, are revolutionizing on-site monitoring capabilities. These innovations offer unparalleled potential for automated, high temporal frequency monitoring with remote, real-time data transmission.

With regulatory frameworks increasingly favouring risk-based approaches to microbial risk management throughout the drinking water supply chain, we are witnessing a paradigm shift towards the adoption of microbial sensors. This review offers a comprehensive examination of the latest developments and accomplishments in automated on-site monitoring of microbial water quality.

Beginning with an elucidation of key terminology and an overview of available sensor technologies, we explore how these cutting-edge tools can enhance our understanding of microbial dynamics in the sourcing, treatment, and distribution of drinking water, and how this knowledge can be translated into operational management. Despite the promise of microbial sensors, significant challenges remain. Drawing from insights gathered from an international online survey targeting drinking water utilities, we discuss the analytical, economic, and legal barriers that must be overcome for the implementation of automated on-site monitoring of microbial water quality.

This review serves as a vital resource for researchers, utilities, and policymakers operating in water microbiology and sensor technology. While it is addressing drinking water more specifically, the presented concepts and tools can be extrapolated to recreational waters or wastewater management, with the shared goal to ensure sustainable management of water resources and protection of public health.

Abbreviations: AOM, Automated on-site monitoring; ATP, adenosine triphosphate; ALP, alkaline phosphatase; CCP, critical control point; DWTP, drinking water treatment plant; FCM, flow cytometry; FIB, faecal indicator bacteria; GAC, granulated activated carbon; GUS, ß-D-glucuronidase; GLU, ß-D-glucosidase; GAL, ß-D-galactosidase; HABs, harmful algae blooms; HPC, heterotrophic plate count; HNA, high nucleic acid; HLF, humic like fluorescence; ICC, intact cell count; LNA, low nucleic acid; PC, phycocyanin; SAC254, specific ultraviolet absorbance at 254nm; TCC, total cell count; TLF, tryptophane-like fluorescence.

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1. Introduction

Safeguarding microbial quality of drinking water is at the core of human wellbeing. Across the world, drinking water is sourced either from groundwater or surface water resources, or from desalination of seawater in some regions with limited freshwater resources. In addition, (in)direct water reuse is increasingly becoming a supplementary source of drinking water due to the escalating challenges to the integrity and sustainability of natural water supplies. For any of these water resources, the microbial quality and safety of treated and distributed drinking water must always be assured to avoid consumer exposure to waterborne or opportunistic pathogens, or to algae toxins (WHO, 2023). Waterborne outbreaks associated with drinking water have continued to occur despite economic mandates and knowledge resources to prevent them (Hrudey and Hrudey, 2019). While the implementation of water management policies has globally led to reduced disease burden, outbreaks have notably been attributed to catchment and distribution-associated deficiencies (Ligon and Bartram, 2016). In catchments, sewage contamination or rainfall-induced runoff impair microbial water quality by introducing bacterial, viral, and protozoan pathogens of faecal origin (Burnet et al., 2014; Gibson, 2014; Kirschner et al., 2017; Kistemann et al., 2002). Microbial quality can also deteriorate within the drinking water supply system due to excess nutrients and subsequent shifts in the native aquatic microbial community. This can lead to taste and odour issues, and excess growth of microorganisms including opportunistic pathogens such as *Legionella pneumophila* and *Pseudomonas aeruginosa* (Bédard et al., 2016; Favere et al., 2021a; Park et al., 2021). Drinking water quality impairments are expected to be promoted by climate change due to increased temperatures, more frequent droughts, floods and other extreme events threatening drinking water supplies and infrastructures (Landsman et al., 2019; Leveque et al., 2021; Tang et al., 2022).

According to the World Health Organization (WHO), the most reliable means of ensuring safe drinking water is achieved through the implementation of Water Safety Plans (WSPs) (WHO, 2023). This risk-based approach aims to identify, manage, and control hazards across the drinking water supply chain to meet health-based targets by identifying and characterizing critical control points (CCPs). From the point of view of microbial safety, these objectives are achieved through (1) system assessment, including the identification of contamination sources and measures to control them, (2) operational monitoring to control these measures during operation, and (3) a final verification of the quality of the produced drinking water to ensure regulatory compliance. System assessment comprises the characterisation of the raw water resource using microbial and non-microbial information, depending on system specificities. It also comprises the definition of control measures, such as source protection and water treatment (pathogen log-reduction targets). Operational monitoring controls the performance of individual components of the drinking water system at CCPs. It is currently performed using sensors for high frequency measurements of physicochemical water quality parameters (such as pH, conductivity, turbidity, flow rate, chlorine concentration, UV transmission and UV radiation). Final verification is undertaken at all three above-mentioned levels including the final product, using a panel of microbial and non-microbial parameters, as required and/or recommended by the local legislation.

Microbial measurements are therefore an integral part of all three WSP levels. Current investigative and monitoring approaches rely on discrete sampling at fixed frequencies using cultivation-based parameters that involve long sample-to-result times, typically 1 – 3 days (*E. coli*, enterococci, heterotrophic plate count at 22 °C) up to 7 days (*Legionella*). More frequent microbial measurements can greatly enhance system and process understanding, especially because of inherent fluctuations in microbial water quality that occur over short time scales (days to hours or even minutes) (Kistemann et al., 2002; Lautenschlager et al., 2010; Nescerecka et al., 2014; Stadler et al., 2008; Zamyadi et al., 2012). High frequency measurements of physicochemical proxy parameters only indirectly identify potential microbial hazardous events (Jung et al., 2014).

The need for rapid, automated microbial water quality monitoring has therefore been repeatedly emphasised over the years as illustrated by previous literature reviews, which identified promising technologies for microbial water quality sensing, but considered them not yet ready for deployment within existing operations (Lopez-Roldan et al., 2013; Rompré et al., 2002; Storey et al., 2011). Tatari et al. (2016) provided an updated overview of commercially available, under development, and research-level sensors for drinking water and highlighted the difficult and slow process of new sensor development and manufacturing. The authors concluded that automated near-real time monitoring of total bacteria in drinking water was feasible, but that the 'ideal sensor' as defined by drinking water utilities (focusing on total coliforms, and *E. coli*) was not yet available. The International Water Association explored various interdisciplinary aspects of sensing technology, including business, legislation, and educational needs (IWA, 2018).

With the rapid market growth of automated on-site technologies, end-users can be overwhelmed with commercial information regarding the anticipated performance of new emerging technologies. Critical analysis of the requirements of each end-user is thus essential before investing in additional monitoring capability. The present critical review is based on an analysis of the concerns and challenges expressed by water utilities regarding the implementation of automated on-site monitoring (AOM) of microbial water quality. Its primary objective is to provide critical insights into capabilities and constraints of these technologies to enable informed decision-making and their effective adoption towards enhanced water safety management. This review commences by proposing a standardised terminology to facilitate comparisons between various concepts, technologies, and microbial targets. It then examines recent implementations of AOM of microbial water quality across the drinking water supply chain, addressing their advantages and limitations in filling critical knowledge gaps. Lastly, the review discusses the challenges associated with AOM of microbial water quality considering perspectives from both the research community and water utilities. Although the primary focus is on drinking water, the insights presented in this review extend to other contexts (e.g., recreational water) where timely microbial quality data is essential.

2. Terminology

Consistent, clear terminology allows avoiding misconceptions and ambiguity between disciplines. Table 1 defines recurrent terms found in scientific peer-reviewed literature to harmonise usage and support consistency. Within this context, a microbial sensor is a device that measures, converts, and transmits microbial-related signals automatically and it conducts on-site measurements that can be either at-line (sub-samples taken from the water stream) or in-line (in the water stream). Most microbial sensors to date (presented in Table 2) conduct at-line monitoring *per se* as they automatically withdraw a sub-sample from the water stream (water resource or drinking water pipe). Some technologies enable real-time monitoring of microbial water quality (results within <1 min) (Besmer et al., 2017b; Fujioka et al., 2019), but the majority of investigations to date report near real-time measurements (results within 10 and 15 min) at sampling frequencies that vary depending on the studied system and microbial target (Besmer and Hammes, 2016; Burnet et al., 2019b; Buysschaert et al., 2018; Ender et al., 2017; Højris et al., 2016; Prest et al., 2021; Ryzinska-Paier et al., 2014; Stadler et al., 2016). In addition to the *in-situ*, automated and high frequency nature of this monitoring approach, the term "online" refers to the automated transmission of generated data to a server for remote visualization (and possible control of the instrument). Not all automated on-site instruments are necessarily online. Also, some on-site technologies are only partially automated. Nevertheless, they complement existing fully automated ones with a shared goal of delivering more

Table 1
Definition of main terms commonly used in the field of automated on-site monitoring of microbial water quality.

Term	Definition
Microbial sensor	A sensor is an automatic measurement device which continuously (or at a given frequency) gives an output signal proportional to the value of one or more determinants in a solution which it measures (ISO, 2003). A microbial sensor is an analytical device that interacts at varying degrees of specificity with a compound (e.g., ATP, DNA, enzyme) of a microorganism, converts that interaction into an (electrical) signal and amplifies it. It can further process, display, (and transmit) the signal.
Real-time	Shown/communicated at the same time as events happen (Cambridge Dictionary, 2025).
Near real-time	Shown/communicated within seconds-to-minutes as events happen.
On-site	On-site measurement of microbial water quality involves samples that are not removed from the premises prior to measurement. The measurement is carried out within seconds (e.g., phycocyanin or TLF sensing), multiple minutes (e.g., FCM or enzymatics) or hours (e.g., automated cultivation-based assays). On-site measurement can be conducted either in-line or at-line (see definitions below).
Online	Online measurements are on-site measurements that involve immediate transmission of generated results. The device is connected to the internet for results visualization and can sometimes be controlled remotely too.
In-line	In-line measurements involve sensors that are placed directly in the path of the water (Ramsay, 2018). (e.g., phycocyanin or TLF sensor)
At-line	At-line measurements involve sub-samples that are removed from the mainstream but measured automatically on the premises (Ramsay, 2018). (e.g., FCM, enzymatics)
Automated	Carried out by machines or computers without needing human control (Cambridge Dictionary, 2025).
Proxy parameter	A parameter that is used as an indicator of the presence of another parameter in the absence of a direct measure (Demeter et al., 2020).

rapid microbial water quality results. In this review, we are adopting the term "automated on-site monitoring", abbreviated "AOM", which encompasses both fully- and semi-automated in situ technologies that measure microbial parameters at high temporal frequency (see Table 1 for additional definitions). Other authors have used *automated high frequency monitoring (AHFM)* (Marcé et al., 2016) or *continuous water quality monitoring (CWQM)* (Carmi, 2019) to describe similar concepts.

3. Technologies for automated on-site monitoring of microbial water quality

Diverse technologies have been developed, tested, and deployed for AOM of microbial water quality in the last 15–20 years involving interdisciplinary expertise in microbiology, chemistry, engineering, and electronics. Signals report on microbial communities (essentially bacteria and cyanobacteria) either directly (e.g., flow cytometry) or indirectly via a specific biochemical parameter (e.g., ATP-metry, enzymatics, phycocyanin sensing) (Table 2). Physicochemical variables, such as turbidity, are also suggested as proxies for microbial parameters based on habitat-specific associations.

3.1. Which technologies are available?

Microbial water quality monitoring and control is currently undergoing a paradigm shift in data acquisition enabled by on-site measurement through automation of some or all the steps (sampling, cultivation/direct detection, and results reporting). Innovations in "sensing" technologies are ever evolving, and the dynamic sensor market spans a continuum of technologies with new ones continually being introduced while others are discontinued. Whereas most of the existing technologies rely on the high sensitivity of fluorescence-based measurements, luminescence and optical sensors have also been developed (Table 2). A summary of sensor technologies is presented in Table S1,

Table 2
Characteristics of existing technologies for fully automated on-site microbial water quality monitoring. Semi-automated methods are also mentioned, although they need further development until full automation. Given their usefulness in microbial water quality monitoring, they are mentioned alongside fully automated ones to provide a more global picture on current methodologies.

Method	Sampling		Reagents		Target					Fully automated		Signal transduction				Example technology
	Continuous	Discrete	No	Yes	Total cells	Viable cells**	Faecal indicator***	Pathogen	Other	Yes	No	Optic	Fluorescence	Luminescence	Chromogenic	
Physicochemical proxy (TLF, HLF)*	■		■						■	■			■			UviLux
Cultivation-dependent detection		■		■		■	■			■		■	■		■	ColiFast ALARM, Fluidion ALERT
		■		■			■				■		■		■	TECTA
Direct cell detection		■		■	■	■				■			■			BactoSense, onCyt (flow cytometers),
	■		■		■					■		■				BACMON****, IMD-W (direct imaging)
Biomolecule detection		■		■		■				■				■		EZ-ATP (ATP-metry)
		■		■			■	■		■			■			Kraken Sense (qPCR)
	■		■						■	■			■			EXO2, Wimo (phycocyanin probes)
Microbial (enzymatic) activity measurement		■		■		■	■			■			■			ColiMinder, BACTcontrol

* Tryptophan-like fluorescence, humic-like fluorescence. Specific fluorescent dissolved organic matter peaks that have been proposed as a proxy for detecting faecal contamination** Viable cells and/or metabolically-active cells in case of ATP measurement technologies

*** Faecal indicator bacteria can be culturable and/or viable bot not culturable (metabolically active) when detected by enzymatic measurements. Free enzyme released from dead cells can also be detected unless the technology includes a filtration step for sample concentration. In case of qPCR, besides culturable/viable and VBNC cells, dead cells and free DNA can also be detected.

**** BACMON has been discontinued in February 2020

including references to scientific studies that demonstrate their benefit for microbial water quality monitoring. For several years, AOM technologies based on FCM, enzymatics, and optics demonstrated their added value over traditional methods by providing microbial water quality data at unprecedented temporal resolution and with results being delivered remotely and in (near) real-time (Besmer et al., 2016; Burnet et al., 2019a; Demeter et al., 2020; Højris et al., 2016; Sorensen et al., 2018b; Zamyadi et al., 2014).

From a regulatory point of view, microbial methods may be categorised as standardised, alternative, or complimentary (Zibuschka et al., 2017). Currently, all technologies for AOM of microbial water quality are complimentary methods. While standardised parameters (e.g., cultivation-based detection of *E. coli*) form the basis of national/international regulations, alternative methods are methodological equivalents to standardised methods. Complimentary methods can provide supplementary insights and a more comprehensive understanding of the water resource and supply system (Zibuschka et al., 2017). However, certain AOM technologies have likely the potential to become new standards in the future (and maybe legally binding) based on convincing scientific facts, practical and economic experience, and conscientious standardisation processes.

This section and the information summarized in Table S1 provide a detailed overview of AOM technologies commercialised for the (drinking) water industry and their measured parameters, microbial features, and detection methods. Given the usefulness of some semi-automated methods that accelerate in situ enumeration of culturable faecal indicator bacteria (FIB), the section also features such assays.

3.2. What are the detection targets?

Automated on-site technologies primarily target prokaryotes (bacteria and cyanobacteria). Recent recommendations from WHO and new EU guidelines increasingly emphasize the need for virological indicators, such as somatic coliphages, to assess water quality because pathogenic viruses do not necessarily correlate with standard bacteriological indicators (EU, 2020). Consequently, new rapid on-site methods for coliphage detection in water have been developed (Muniesa et al., 2018; Rames and Macdonald, 2019). These methods show promise but are not yet as mature as current sensing technologies commercialized for bacteriological water quality monitoring. Current automated on-site instruments predominantly target bacteria through various detection methods based on cultivation, cell, or biomolecule detection to measure the presence, abundance and/or population activity (Fig. 1). Some technologies provide insights into the entire microbial community using parameters such as total cell count (TCC) or adenosine triphosphate (ATP). Others focus on specific subpopulations of the microbial community using parameters such as intact cell count (ICC) or enzymatic activity measurements (e.g., beta-d-glucuronidase (GUS) activity associated with faecal contamination) (Table S1). Lastly, operational monitoring practices commonly involve physicochemical parameters for

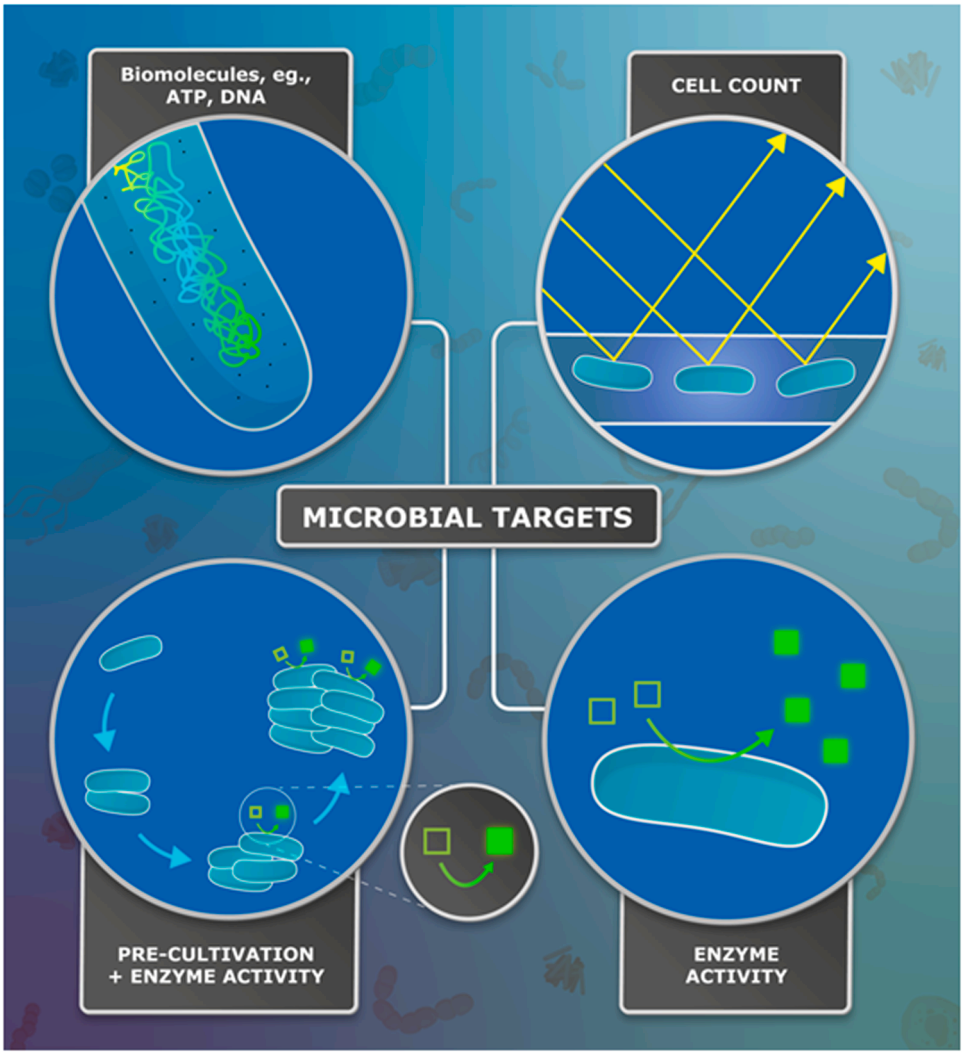

Fig. 1. Microbial targets for automated on-site monitoring of microbial water quality.

tracking water quality changes that may or may not be related to microbial contamination during drinking water treatment and supply. The section therefore starts with an overview on these proxy parameters.

3.2.1. Physicochemical proxy parameters for water quality changes

Microorganisms commonly share similar sources and transport pathways with particulate and dissolved matter, making certain physicochemical parameters suitable proxies of microbial contamination. For example, turbidity is easily measured with automated optical methods, and it is frequently used by utilities for online water quality monitoring. Deviations from baseline values reflect changes in raw water quality, variations in treatment performance, or ingressions into the distribution network (WHO, 2017). The parameter Specific Ultraviolet Absorbance at 254 nm (SUVA, SAC254, or UV254) (Weishaar et al., 2003) enables the automatic, online monitoring of a specific fraction of dissolved organic matter concentration. Given its correlation with faecal pollution under certain field conditions at alpine karst springs, it is used in the management of such springs for drinking water supply (Page et al., 2017; Stadler et al., 2010). More recently, fluorescence parameters at specific excitation/emission wavelength pairs, such as for tryptophan and humic-like fluorescence (TLF and HLF, respectively), have been suggested as proxies of wastewater-derived contamination (Corsi et al., 2021; Sorensen et al., 2018a, 2015). Physicochemical proxies for microbial contamination always need prior knowledge on the pollution sources and their dynamics within the catchment for correct online signal interpretation (Stadler et al., 2010).

3.2.2. Automated detection of microbial targets

Cultivation-dependent detection

The ever-growing interest in rapid results for faecal indicator bacteria (FIB) led to the development of semi to fully automated technologies (Table 2) based on enzymatic activity measurements combined with a prior cultivation step using selective media (Angelescu et al., 2019; Bramburger et al., 2015; Tryland et al., 2015). Given the similarity of both the selective cultivation step and the specific enzymatic activity assay to the one used in standardised FIB enumeration methods (e.g., Colilert), these rapid cultivation-dependent methods represent the closest alignment with regulated FIB parameters. Nevertheless, sample-to-result times still exceed several hours (Table S1). In these instances, only 1–2 measurements per day can be performed thereby missing possible peaks of fecal contamination.

Direct cell counting

Flow cytometry (FCM) is a direct detection method able to discriminate between particles (bacteria, protozoa, viruses, cell fragments, inorganic debris, etc.) by both light scattering and fluorescence. Fluorescence-based detection is typically facilitated by direct staining of nucleic acids with fluorescent dyes (Safford and Bischel, 2019; Van Nevel et al., 2017a). FCM offers insights beyond TCC, including information on cell size, nucleic acid content (high nucleic acid, HNA, and low nucleic acid, LNA content cells) and cell membrane integrity (intact cell count, ICC) using specific fluorescent stains or gating. The usage of multiple stains requires colorimetric compensation to prevent cells dyed with different stains from being counted similarly. Flow cytometry can also reveal microbial community patterns through phenotypic fingerprinting that consist in statistical analyses of multivariate FCM data (e.g., size, fluorescent colour, fluorescence intensity) which represents the distribution of raw data in the whole flow cytometric signal space (De Roy et al., 2012; Favere et al., 2020; Sadler et al., 2020). Evaluation studies have demonstrated the high reproducibility of FCM compared to epifluorescence microscopy, the standard cell counting method as reviewed in (Safford and Bischel, 2019) and fully automated on-site FCM enables near real-time enumeration of bacteria in water (Besmer et al., 2014). However, differences in the settings (gating, staining, etc.) may yield different results and thereby prevent direct comparison of results from different methods and applications. Also, automated wide-spread application of fingerprinting is not yet possible. Besides FCM, bacteria may also be enumerated using reagentless 3D imaging coupled to image-processing algorithms to distinguish bacterial cells from abiotic particles (Højris et al., 2016).

Biomolecule detection

Of the many potential biomolecular targets like nucleic acids or lipids, the measurement of ATP by rapid manual assays was a pioneering development for drinking water. ATP is the energy carrier of the cell and has long been considered an indicator of viable microbial biomass (Hammes et al., 2010). Methods can differentiate between total ATP and intracellular ATP, the latter often being correlated with ICC and intact biovolume (cell size) parameters measured by FCM in various types of water matrices (Hammes et al., 2010; Van Nevel et al., 2017a; Zhang et al., 2019). In recent years, ATP measurement has been automated for on-site monitoring purposes but only a few studies have demonstrated its feasibility in real-world conditions (de Vera and Wert, 2019; Favere et al., 2021b; Hansen et al., 2019) (Table S1).

Nucleic acids can be detected on-site using automated PCR. These systems have been explored particularly in coastal waters, but they are still in research and development phases (Fernández-Baca et al., 2021; Sepulveda et al., 2020; Yamahara et al., 2015). Recent developments demonstrate the feasibility of an automated on-site qPCR biosensor for high frequency monitoring of *Legionella pneumophila* in cooling towers (Trigui et al., 2024), however, there is currently no scientific literature available to substantiate its capability in drinking water contexts.

Real-time detection of specific algal pigments such as phycocyanin (the phycobilisome pigment of blue-green cyanobacteria) or chlorophyll *a* can be achieved by submersible in situ fluorometers to indirectly monitor cyanobacterial cells in water without sample pre-treatment like incubation or labelling (Brient et al., 2008; Henderson et al., 2015; McQuaid et al., 2011). While fluorescence measurements can be affected by various sources of interferences, simultaneously generated physical-chemical parameters can be applied by users to improve those measurements (Rousso et al., 2021; Zamyadi et al., 2016).

Enzymatic activity measurement

Methods for enzymatic activity detection have been automated for over a decade (Demeter et al., 2020). Using the same fluorogenic or analogous substrates as the ones used in selective media for culture-based detection of FIB, they rely on fluorometric measurements of enzymatic activity without the need for prior amplification of the signal (Table S1). The most common automated enzymatic method targets β-d-glucuronidase (GUS), present in many human gut bacteria (Pollet et al., 2017), including *E. coli*. It can be measured directly as ecto- and extracellular enzyme activity rate (Farnleitner et al., 2002). Other enzymatic targets include β-d-galactosidase (GAL), β-d-glucosidase (GLU) and alkaline phosphatase (ALP) expressed by total coliforms, intestinal enterococci, and all metabolically active bacteria, respectively (Favere et al., 2021b; Fiksdal and Tryland, 2008). Although possible interferences with non-target bacteria or algae have been reported (Baudart et al., 2009; Davies et al., 1994), and the relationship with cultivation-based fecal indicator bacteria varies widely among different water resources, GUS was suggested as a conservative biochemical surrogate to bacterial faecal pollution (Demeter et al., 2020). ALP activity, just like ATP concentration, is considered a viable biomass-associated parameter.

3.3. Where and why are they implemented?

The introduction of new automated on-site technologies for rapid microbial water quality assessment and monitoring has revolutionised data acquisition, greatly enhancing our ability to comprehend and manage microbial hazards and potential risks, particularly within the framework of water safety planning. It is important to note that reporting for legislative compliance currently remains unattainable without corresponding regulatory frameworks for sensors, but that AOM does effectively complement conventional culture-based assays.

In general, two main microbial hazards define at least two primary

application areas for AOM technologies. The first area is concerned with detecting external microbial ingress that could introduce intestinal pathogens or toxigenic cyanobacteria into the drinking water supply chain. These technologies are primarily deployed in monitoring raw, source water quality (e.g., surface water) and target FIB (*E. coli*, intestinal enterococci, faecal coliforms) or cyanobacteria (phycocyanin) (Burnet et al., 2019b; Ender et al., 2017; McQuaid et al., 2011; Ryzinska-Paier et al., 2014; Sylvestre et al., 2020). In some water sources such as karstic groundwater supplies, they can also target the entire microbial community using for instance automated on-site FCM (Besmer et al., 2016; Page et al., 2017). For DWTPs abstracting water from sources prone to cyanobacterial blooms, there is a risk for accumulation and breakthrough of cells and toxins through the water treatment processes, which prompts the use of phycocyanin sensors to identify CCPs and adopt appropriate control strategies (Ma et al., 2023; Zamyadi et al., 2014, 2012).

The second application area involves technologies that monitor changes in microbial water quality resulting from internal processes such as regrowth or release of biofilm-associated microorganisms during drinking water treatment and distribution (Prest et al., 2021).

Within these two application areas, technologies offer information on microbial risk to varying degrees, depending on their target (specific bacterial communities), and whether they measure metabolic activity, viability, or total cells. Some technologies deployed for the former application area currently do not enable reliable detection of external (e.g., faecal) microbial inputs in treated and drinking water because of too low sensitivities. Decisions regarding microbial targets and the desired endpoints (total, active, viable cells) thus play a pivotal role in choosing the appropriate technology.

4. Automated on-site microbial water quality monitoring from source to tap - recent applications

The following sections follow the source-to-tap continuum to illustrate how automated on-site technologies can enhance water safety planning by advancing our understanding on microbiological water quality dynamics (Fig. 2). We also highlight specific use cases where existing monitoring strategies already support effective drinking water supply management, thereby making the additional benefits of microbial sensors less obvious.

4.1. Source water quality and water abstraction

Source water protection and selective water abstraction are critical first steps in ensuring a high-quality water supply (WHO, 2019). Drinking water is primarily produced from groundwater and surface water. These resources vary widely in terms of microbiological quality and dynamics, and they range from pristine, oligotrophic groundwater resources (including protected, deep aquifers) to shallow or fractured aquifers vulnerable to infiltration, as well as surface waters impacted by wastewater discharges, surface runoff, and/or harmful algal blooms. The unique characteristics of each water resource determine which microbial parameters, physicochemical proxies, or a combination thereof is ideally suited to describe system dynamics and identify microbiological hazards.

4.1.1. Faecal pollution

Given the health risks associated with microbiological faecal contamination (i.e. waterborne pathogens) and water supply, monitoring is strictly regulated and traditionally accomplished using standardised cultivation-based enumeration of FIB such as *E. coli* and more recently, the viral faecal indicator somatic coliphages (EU, 2020).

Cultivation-dependent AOM technologies follow similar principles, relying on the selective cultivation and subsequent sensitive detection of specific enzymatic activity such as GUS or GLU for enumeration of *E. coli* and intestinal enterococci, respectively (Tables 2 and S1). Fully automated on-site systems such as ColiFast ALARM (Tryland et al., 2015), ALERT Fluidion (Angelescu et al., 2019) as well as semi-automated ones (e.g. TECTA) (Bramburger et al., 2015) have been employed for in situ enumeration of *E. coli* in drinking water supplies and in recreational water bodies (Table S1). Despite streamlining and accelerating the enumeration of FIB, sample-to-result times still exceed several hours (up to 15 h), limiting same-day decision-making.

The first account of cultivation-independent AOM based on specific enzymatic activity determination in water raised questions about the performance of GUS activity. At an alpine karst spring impacted by

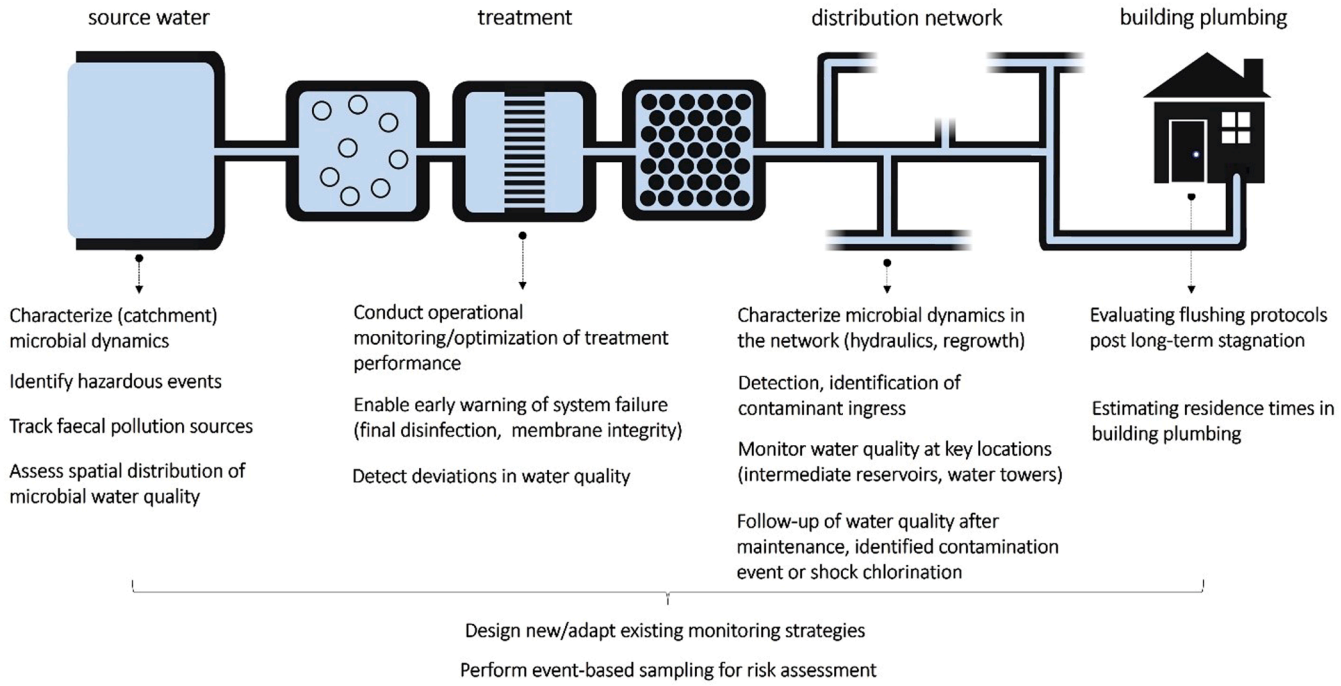

Fig. 2. Application areas for automated on-site monitoring of microbial water quality along the source-to-tap continuum.

ruminant faecal input, long-term GUS activity correlated stronger with UV254, turbidity and discharge rate than with *E. coli* by standard methods (Ryzinska-Paier et al., 2014). In contrast, Frank et al. (2022) found that GUS activity showed equally high correlations with *E. coli,* total organic carbon, small particle size fractions, and turbidity at two alpine karst springs. At a drinking water intake from a river in the Greater Montreal Area, Canada, Burnet et al. (2019b) reported a direct association between faecal pollution and GUS activity over 2.5-years of monitoring and identified a wastewater treatment plant among many upstream sewage releases as the primary source of faecal pollution (Fig. 3A). These and other studies highlight the faecal indication value of GUS activity at higher contamination levels (especially from point sources) but also emphasise potential location-specific differences or limitations, especially for diffuse or remote faecal sources (Demeter 2020).

Physicochemical parameters may also offer insights into external microbial inputs. At an alpine karstic spring, Stadler et al. (2010) found UV254 to be an early warning proxy of *E. coli*, with its signal increasing three to six hours earlier. Fluorescent dissolved organic matter peaks such as TLF and HLF have further been proposed as proxies for detecting faecal contamination (Baker et al., 2015; Fox et al., 2022; Sorensen et al., 2015). In a study of groundwater supplies in the UK, both TLF and HLF correlated more strongly to *E. coli* and total bacterial cell count than to turbidity (Sorensen et al., 2018b). More recently, Bedell et al. (2022) developed and validated an on-site TLF sensor for continuous monitoring of faecal contamination in river water. Using an integrated machine learning model that accounted for fouling and improved noise reduction, TLF levels of four sensors installed at one location predicted faecal contamination levels (based on WHO *E. coli*-based risk categories) with an overall accuracy of 64 %. UV254, TLF and/or HLF are reagentless sensor technologies (no complex liquid handling) and thus are cost-effective and require low maintenance. Nonetheless, a widespread use requires addressing optical interferences (light absorption and scattering), biofouling, inference with other molecules, and standardisation (Bedell et al., 2022; Offenbaume et al., 2020; Ward et al., 2021).

Although AOM offers unprecedented data resolution thanks to the technical maturity of such systems, further research is needed to better understand the indicator capacities of above discussed parameters and to establish catchment-type specific relationships with i) faecal pollution, ii) waterborne pathogen occurrence and iii) infection- or health risks in drinking water supplies (Demeter et al., 2020). Nevertheless, AOM has great potential to provide key information on microbiological pollution dynamics within natural systems that complement or surpass traditional approaches (e.g., Fig. 3A), bringing water safety management to a new level of continuous system monitoring and understanding.

4.1.2. *Surface runoff, aquifer vulnerability and system characteristics*

Water resources harbour their own natural water microbiomes, and shifts or fluctuations can serve as sensitive indicators of potential microbiological water quality changes (Farnleitner et al. 2005; Savio et al. 2018). However, the type of information is of indirect nature, i.e. microbiome changes are not necessarily linked with a general water quality deterioration but must be set into the context of the water resource characteristics and specific situation. Automated cell counting via on-site FCM has been applied in rivers as well as alluvial and karstic/fractured groundwater systems for several weeks, revealing both periodic and aperiodic fluctuations in TCC at most sites (Besmer et al., 2016, 2014; Besmer and Hammes, 2016; Page et al., 2017) (e.g., Fig. 3B). Periodic, diurnal patterns observed in a river were likely driven by autochthonous aquatic processes (Besmer et al., 2014), while diurnal patterns in an alluvial aquifer were attributed to changes in hydrological conditions due to intermittent water abstraction (Besmer et al., 2016). Sporadic fluctuations were observed at all sites in response to rainfall events, signifying allochthonous inputs of microbial cells. Page et al. (2017) combined TCC data with high-resolution time series of physicochemical parameters (turbidity, electric conductivity, temperature, discharge and UV254), providing a more nuanced picture of system dynamics. AOM of the entire bacterial community (i.e., total cell counts, intact cell counts, etc.) holds great promises for source water monitoring. However, further scientific investigations are needed to better understand the specific indicator value(s) of microbiome shifts (quantitatively and qualitatively) in relation to catchment type, pollution characteristics, and background conditions of the water resource.

4.1.3. *Harmful algal blooms*

Climate change is fuelling the development of harmful algal blooms (HABs) compelling water authorities worldwide to adopt strategies for managing and controlling toxin exposure, including establishing public

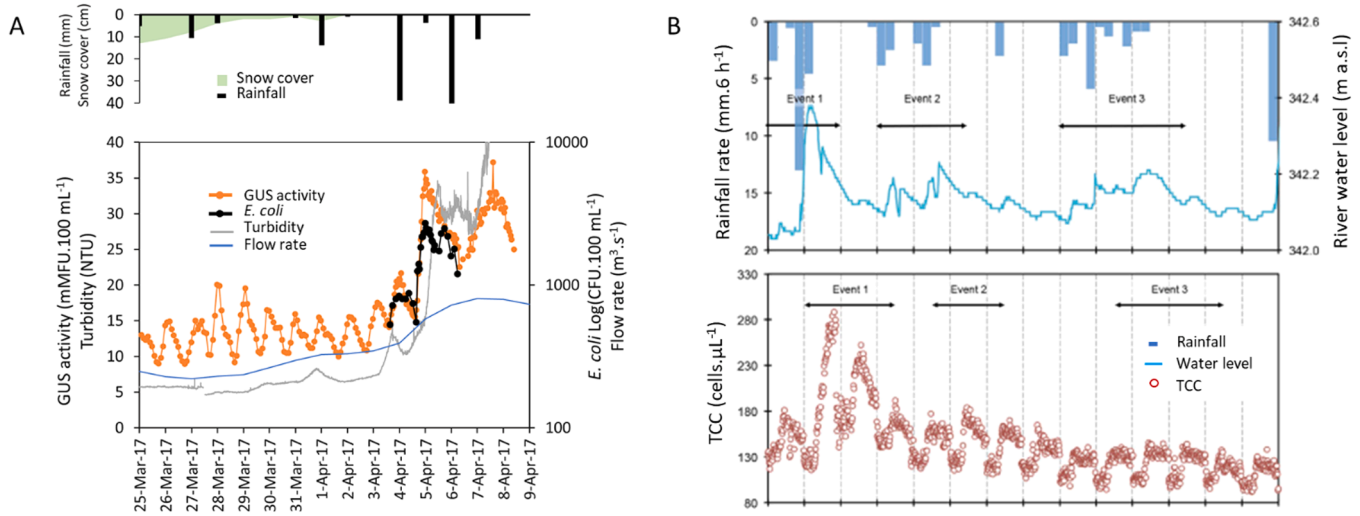

Fig. 3. Automated on-site monitoring of microbial water quality in the source. (A) Recurrent daily GUS activity fluctuations (hourly measurements) in an urban river illustrate continuous discharge of UV-treated effluents at an upstream sewage plant (adapted from Burnet et al. (2019b)). The contamination peaks observed after the rainfall in early April matched those observed for culturable *E. coli* (hourly samples) whereas turbidity peaks (or river discharge rate) did not. (B) Similar daily fluctuations of total cell count (TCC) were measured by online FCM in groundwater in an extraction well, but they were caused by the daily regional groundwater extraction. A TCC peak of higher amplitude was observed following a rainfall episode (event 1), indicating the vulnerability of the groundwater to riverbank filtrate (adapted from (Besmer et al., 2016)).

safety alert levels (Chorus and Welker, 2021). Given that cyanobacteria biomass and community composition exhibit highly variable spatio-temporal patterns, effective monitoring of threshold exceedances require analytical tools that confidently capture fluctuations at (ideally) sub-daily to hourly resolution. Traditional methods like microscopy or molecular assays cannot fulfil these requirements as they rely on periodic grab samples, are resource-intensive, and require significant technical expertise. In contrast, fluorescence spectroscopy offers high measurement frequencies (< 1 min), low cost and operational simplicity. It has therefore been increasingly adopted by the water industry to monitor HABs in drinking water supplies (Bertone et al., 2018).

Many studies have demonstrated the value of commercial fluorescence-based sensors in measuring phycocyanin concentrations to estimate cyanobacterial biomass (Brient et al., 2008; McQuaid et al., 2011; Zamyadi et al., 2014). Fluorescence probes can be installed on submersible (multi-parameter) sondes for near real-time AOM of cyanobacteria dynamics over long periods (Rome et al., 2021), for depth profiling (Brient et al., 2008; Wilkinson et al., 2020) or HAB monitoring in large water bodies (Chaffin et al., 2018; Qin et al., 2015). Typically, the sensors measure chlorophyll *a* alongside phycocyanin, providing insights into the relative dynamics of cyanobacteria and other phytoplankton communities (green algae, diatoms, etc.). Coupled with physicochemical sensors (e.g., temperature, dissolved oxygen, pH, turbidity, conductivity), fluorescence sensors offer high-resolution data revealing hourly and daily variations of key environmental variables, which would remain unseen using traditional grab-sampling approaches (Khac et al., 2018; Ma et al., 2024; Qin et al., 2015; Rome et al., 2021). However, significant uncertainties and variations in the correlations between taxonomic cell counts and fluorescence probe readings can be associated with the type of fluorescence probe, taxonomic composition, light exposure history, and water matrix-related interferences (Choo et al., 2018; Ranjbar et al., 2024; Zamyadi et al., 2016). This strongly suggests that site and instrument-specific calibrations must be established for phycocyanin probes to be used within a response framework for cyanobacteria risk management (Ma et al., 2024).

4.2. Drinking water treatment

Water treatment plants play a major frontline role in protecting public health. Each treatment step contributes to the removal or inactivation of microorganisms and to shaping the microbial community entering the distribution system (Li et al., 2017; Pinto et al., 2014). Treatment processes are validated using cultivation-based microbial indicators (e.g., bacterial spores, phages) or reference pathogens, while their technical performance is ensured using online physicochemical parameter monitoring (e.g., disinfectant concentration, discharge, UV transmission, UV-radiation). However, offline FCM and ATP have demonstrated their efficacy and value in the operational monitoring of drinking water treatment plants (DWTPs) (Helmi et al., 2014; Van Nevel et al., 2017a; Vital et al., 2012). AOM offers potential further benefits for several applications and can serve various purposes for plant operators (Ramsay, 2018).

4.2.1. Selection of operational parameters and monitoring frequency

For DWTPs treating deep groundwater or spring water with stable water quality, AOM using microbial sensors can be applied temporarily to identify operational (proxy) parameters for use as indicators of microbial contamination risks (Sinreich and Pochon, 2023). The appropriate frequency for laboratory-based microbial water quality monitoring (hourly/daily/weekly) in these stable environments can also be defined using such a temporary deployment of a microbial sensor (Besmer et al., 2016). For water treatment processes characterised by predictable operations, such as regular backwash cycles of rapid sand filters or granular activated carbon (GAC) filters, short-term studies employing AOM offer insights into normal operation variability in microbial parameters. Fig. 4 provides two application cases of AOM during short-term studies to establish the effect of switching between two rapid sand filter treatment trains (Fig. 4a) or the effect of backwash cycles of ceramic microfiltration membranes (Fig. 4b), and highlights cases where a microbial sensor did (or did not) provide additional value compared to physicochemical sensors. Similarly, Favere et al. (2021b) showed that during a GAC filter backwash, microbial signals (TCC, ATP concentration, ALP activity) increased in comparison to filtration cycle concentrations and temporarily exceeded a defined baseline level typically for less than an hour (based on 30-minute measurements). This finding confirms common knowledge by operators about post backwash breakthrough of turbidity and/or pathogens. In practice, the filters are put back into production based on turbidity limits, others after a given time limit. In some cases, turbidity is thus already used as an operational (proxy) parameter, but AOM can help identify the time required for the microbiological parameter to return to a normal level after a backwash event and/or the corresponding turbidity level that can serve as a reference value to put the filter back in production.

4.2.2. Operational control monitoring

In challenging environments characterised by diverse sources of sanitary risks that cannot be directly linked with specific plant operational parameters, AOM can function as a strategic approach for detecting microbial events and compromised water quality and safety. For instance, while ALP activity or intracellular ATP concentrations are extremely low directly after chlorination (Appels et al., 2018; Prest et al., 2021), an increase in the microbial signal can be an instant indication of disinfection failure. In such cases, appropriate measures could be taken immediately to manage the risk upon notification on a given threshold being exceeded. At a facility treating surface water by pre-chlorination and rapid sand filtration, followed by either ozonation and GAC filtration or ultrafiltration and reverse osmosis, Appels et al. (2018) observed shifts in ALP activity following rapid sand filtration in response to the raw water source. These changes were linked to a transition from river water to groundwater prompted by the degradation of river water quality due to contamination by sewage overflows from a nearby wastewater treatment plant. In such cases, when risks are associated with the raw water used in drinking water production, prioritizing monitoring efforts at the source is advisable. If source monitoring is impractical, surveillance should be conducted at the earliest treatment stage possible. This allows for the assessment of potential attenuation, contributing to more effective risk assessment considerations along the treatment train. Using automated on-site FCM, Besmer and Hammes (2016) provided strong evidence of diverse microbial dynamics within drinking water treatment systems. The study concurrently analysed microbial dynamics in raw (spring) water and after multi-barrier treatment involving flocculation, ultrafiltration, ozonation and GAC filtration. Treatment could buffer and hence mitigate the short-term microbial peaks in raw water following rainfall. Unexpected periodic fluctuations were identified after GAC filters and attributed to fluctuating water abstraction rates impacting detachment and dilution processes. High-frequency monitoring revealed these dynamics and thereby highlighted mitigation opportunities through improved management if appropriate. Buysschaert et al. (2018) and Fujioka et al. (2019) also demonstrated the capability of on-site FCM in monitoring ultrafiltration and reverse osmosis membranes, highlighting that such AOM technologies can provide more comprehensive information for system integrity than traditional monitoring methods such as turbidity. In some cases though, a microbial sensor may not be the most suitable choice, as other physicochemical sensing devices such as particle counters, may provide (at least) similar information (Fig. 4b).

Cyanobacteria risk management relies on alert levels that are based on total cyanobacteria cell biovolumes considered to be the best indicator of the potential health risks associated with cyanobacteria and their toxins (Chorus and Welker, 2021). Semi-continuous monitoring of cyanobacteria biomass using phycocyanin probes has enabled to observe trends in raw, clarified, filtered, chlorinated and sludge supernatant in

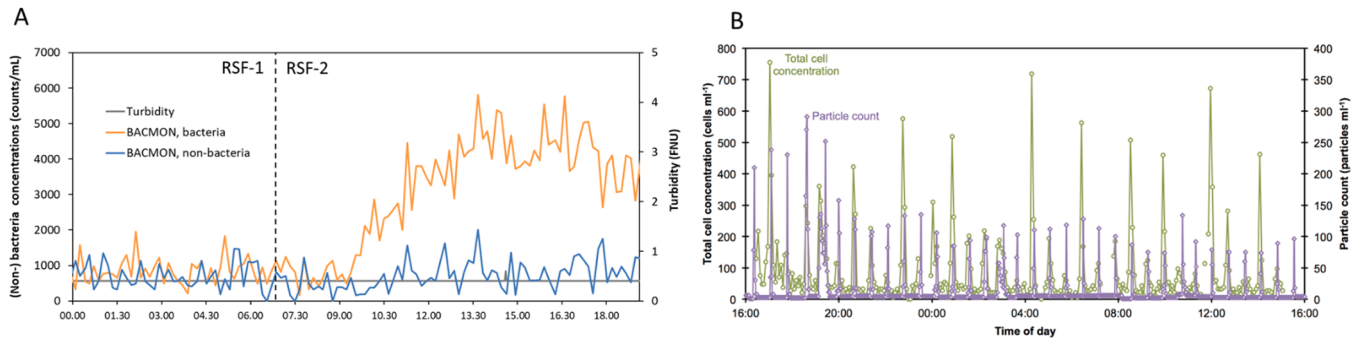

Fig. 4. Automated on-site monitoring of microbial water quality during water treatment. (A) Effect of changing treatment train (RSF, rapid sand filtration) on treated drinking water at Pipda utility, Belgium, where operation change (RSF 1 to RSF 2) caused an increase in bacterial cell counts measured by 3D-scanning of bacteria coupled to image-processing algorithms (BACMON), while no change in non-bacterial counts was monitored. Turbidity (black line) did not fluctuate during the operational change (van Bel et al., 2020). (B) Automated on-site monitoring of total cell concentrations after ceramic membranes throughout several backwash cycles. Particle counts provide the same information as total cell counts, questioning the added value of the microbial parameter in this case (Prest, Besmer et al., unpublished data).

DWTPs and provided near real-time log removal efficacy of the treatment barriers (Almuhtaram et al., 2018; Henderson et al., 2015; Ma et al., 2024; Zamyadi et al., 2014). Strong correlations between cyanobacteria biovolume and phycocyanin log removal efficacies have been established for the clarification process and they are unrelated to turbidity removal (Ma et al., 2024). Using multi-flow systems and a single fluorescence probe for high frequency monitoring, raw water risk as well as short-term treatment breakthrough and daily mean efficacies of processes can easily be assessed at low cost, allowing for the identification of periods of vulnerability and disfunction, rapid treatment adjustment and the prevention of cyanobacteria and toxin breakthrough into treated water (Ma et al., 2023; Zamyadi et al., 2012). In view of microbial safety, AOM should thus be considered as a complimentary tool for operation monitoring of treatment steps, where failure can cause a direct sanitary threat and where an early warning system is essential.

4.3. *Drinking water distribution*

The hygienic quality of drinking water in a drinking water distribution system (DWDS) can be influenced by various sources of pressure transients in the pipes, potential accidental intrusions but also by the growth of bacterial communities (biological instability) within the system (Courtois et al., 2018). Drinking water leaving the treatment plant should remain stable in terms of microbial community characteristics (abundance, viability, and composition) throughout the entire DWDS (Favere et al., 2021a). In practice, this is difficult to achieve because of the complex dynamics within large-scale DWDSs (El-Chakhtoura et al., 2015). Instability can lead to esthetical (taste and odour), technical (corrosion, biofouling) and sanitary (growth of opportunistic pathogens) issues. Although no specific guidelines exist to define acceptable changes in the biological stability of drinking water (Prest et al., 2016), guideline values have been established in the Netherlands for biologically stable (unchlorinated) water to prevent regrowth in the DWDS (van der Wielen et al., 2023). In the European Drinking Water Directive stability was recently defined as 'no abnormal change' (EU, 2020).

Changes in heterotrophic plate counts, bacterial cell concentrations, ATP concentrations or bacterial community composition have been observed with increasing residence times in both chlorinated (with decreasing disinfectant residual) and non-chlorinated distribution systems (Schleich et al., 2019; van der Wielen and van der Kooij, 2010). Generally, changes in biostability can be examined and monitored through conventional monitoring programs involving grab samples collected at different distribution locations over time (Lautenschlager et al., 2010; Nescerecka et al., 2014; Vital et al., 2012). More recently, studies have demonstrated the usefulness of AOM systems to shed new light on the fine-scale variations in bacterial concentrations and community composition within DWDSs (Farhat et al., 2020; Gabrielli et al., 2021; Prest et al., 2021).

4.3.1. *Monitoring microbial water quality at strategic locations: treatment outlet and intermediate reservoirs*

Strategic places of the distribution system such as treatment plant or intermediate reservoir outlets are the primary places for the deployment of microbial sensors for microbial water quality monitoring. Water quality at the treatment outlet is critical and AOM can be considered both to safeguard microbial safety and to provide a reference microbial water quality baseline over time (seasons, years) of the water entering the DWDS. At the outlet of a storage reservoir at a Dutch drinking water utility, Prest et al. (2018) used FCM to monitor TCC and ICC. Before entering the reservoir, treated water underwent secondary disinfection with chlorine dioxide. The results showed how ICC responded to the residence time in the reservoir: during low water consumption at night, elevated water levels in the storage reservoir and extended residence times correlated with a reduction in ICC. In contrast, TCC did not show any daily pattern (Fig. 5a).

Intermediate reservoirs and water towers are also critical points in DWDSs, where settling of particles and growth of micro- and macroorganisms may occur in addition to prolonged residence times. For instance, using online 3D scanning of bacteria in a Danish intermediate reservoir where water enters and exits through the same pipe, Højris et al. (2016) observed a noticeable shift in bacterial cell counts before and after water passage through the reservoir. Intermediate reservoirs are occasionally used to blend waters from different sources, to ensure sufficient capacity and a reliable drinking water supply in case of source shortage or contamination. At a water tower where drinking water produced from two different sources (surface water and groundwater) is mixed, Favere et al. (2020) revealed discernible changes in cell counts with the transition from a mixed to a single source water stream (Fig. 5b). The study further demonstrated the value of FCM fingerprints for early detection of shifts in microbial populations. Similar approaches could also prove useful in situations where contamination occurs in reservoirs due to poor infrastructure maintenance (Říhová Ambrožová, 2020). It should be kept in mind though that such community shifts do not necessarily imply sanitary risks for consumers.

4.3.2. *Ensuring distribution of safe drinking water*

Operational issues in drinking water distribution systems, such as pipe burst, leakages, low pressure leading to intrusion of surrounding groundwater, backflows, unintentional cross-connection with lower quality water, or poor practices during maintenance work on distribution pipes, can lead to contamination of drinking water with pathogenic microorganisms (Hrudey and Hrudey, 2019). Water quality checks after

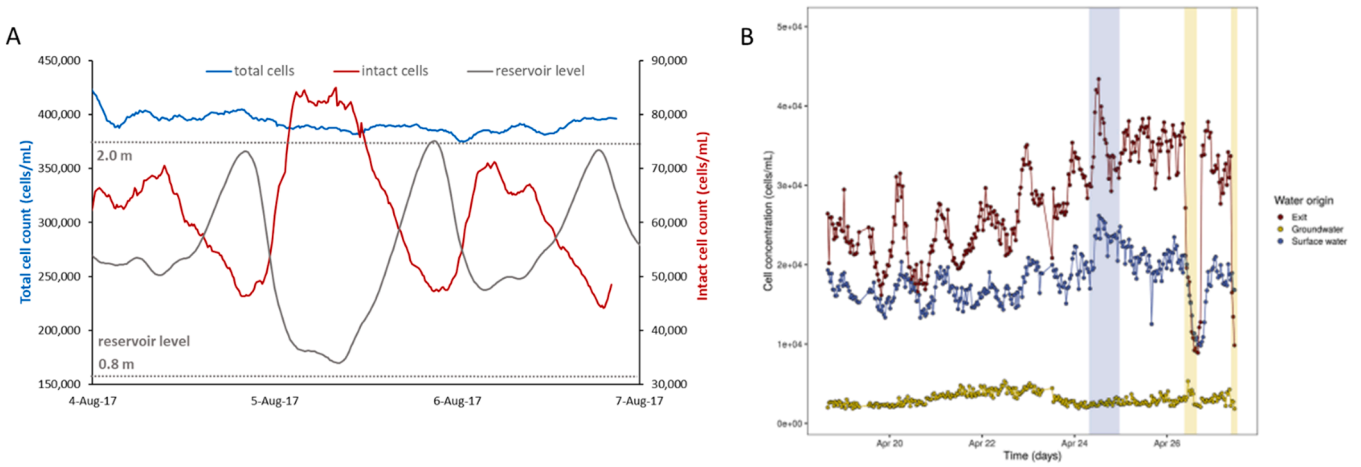

Fig. 5. Automated on-site monitoring of microbial water quality during drinking water distribution. (A) Effect of residence time and chlorination on the signal of the intact cell count (ICC). Total cell count (TCC) did not show any daily pattern associated with residence time (adapted from (Prest et al., 2018). (B) Continuous monitoring of TCC in the effluent of an intermediate reservoir shows clear signal shifts when the proportions of either surface water or groundwater increase (blue-shaded or yellow-shaded area, respectively) (Favere et al., 2020).

interventions on distribution pipes (or when a contamination event is suspected) are done with sample-to-result times between one (e.g., using qPCR) to several days (plate counting) (Brown and Hussain, 2003). This approach provides limited means to water utilities to detect the cause of the contamination event, while in the meantime the consumer is given a boil water advisory or urged to drink bottled water. There is thus a need for more rapid and on-site methods as to improve the detection of contamination events.

Considering the relatively high costs and maintenance requirements of microbial sensors, implementation of AOM of microbial water quality or early warning of system failure (e.g., pipe burst) is not realistic in DWDSs that typically represent hundreds to thousands of pipe kilometres. However, one could envision the use of such sensors locally and for short time periods e.g., after a pipe burst, shock chlorination or maintenance work to test for potential contamination and evaluate when the water is safe to be delivered to the consumers. Props et al. (2018) proposed an advanced FCM fingerprinting approach using multiple community FCM metrics and various statistical tools, which enabled to detect various contamination events at laboratory scale. Van Nevel et al. (2017b) also showed that FCM cell count, and fingerprinting can be used to evaluate when the water quality is back to its reference level upstream of the maintenance work. The latter study was performed by taking one sample per hour before and after the maintenance action and analyses were performed in the laboratory within 2 to 9 h after sampling. This approach provided faster results than the traditional culture-based one, but it could be even more efficient in case of automated on-site FCM. A recent paper also pointed to ATP as a potential alternative method to heterotrophic plate count testing for release-to-service after scheduled or emergency repairs, yet additional research needs to identify ATP concentrations or thresholds matching heterotrophic plate count outcomes (van der Waals et al., 2024). On laboratory scale, Besmer et al. (2017b) demonstrated the feasibility of using automated FCM to follow the efficiency of shock chlorination after a contamination event and the subsequent wash-in by regular drinking water. Microbial sensors can further be used for short periods of time for identification of the origin of non-compliance events. For example, the use of automated 3D scanning of bacteria helped identify a daily release of bacteria in the morning that led to routine plate count analysis exceeding the drinking water guideline values at a Danish cattle slaughterhouse (Højris et al., 2016). Once the origin is identified, the cause of the non-compliance can be targeted and solved more efficiently. Additional (semi)-automated on-site technologies providing information on the presence/absence or the activity of indicator organisms (e.g., total coliforms, faecal coliforms, *E. coli*, intestinal enterococci) (Table S1) offer the opportunity for added assurance that water can be safely put back into operation. Before this can be implemented though, semi-automated culture-based technologies need to reduce sample-to-result times while fully automated technologies still require higher sensitivities to detect FIB in drinking water.

5. Challenges ahead

In view of the conservative evolution of legislations in the water sector, the implementation of relatively recently available commercial AOM technologies reflects a proactive initiative by utilities or water management authorities to improve microbial hazard assessment, system performance understanding and operational insights. This was shown in an online survey conducted by the authors in 2019 across 75 utilities in 18 countries (see supplementary tables S2-S4 and figures S1-S3), which revealed a growing interest in the drinking water industry for deploying AOM technologies. At the same time though, it emphasized the multiple challenges to widespread adoption. Most (>75 %) of utilities had already tested/implemented AOM of microbial water quality (Fig. S1), either for specific microbial communities (e.g., GUS activity) or for total microbial community (e.g., FCM, ATP-metry). A high proportion of respondents represented large utilities in industrialized countries, which are typically more inclined to engage in testing new technologies. However, a substantial number of smaller utilities were also surveyed, revealing that most had not yet been involved in AOM of microbial water quality. Among those that had not tested AOM, half indicated no plans to do so in near future (Fig. S2).

For survey respondents, the three main barriers to implementation of AOM of microbial water quality were financial constraints, followed by analytical issues and absence of a regulatory framework. Other concerns challenging the overall acceptance of these new tools within the industry were raised within the remaining 26 % of responses (Table 3).

5.1. Capital and operating costs

The capital and operational costs of implementing AOM systems were identified as the primary barrier particularly for smaller water utilities. Survey respondents revealed that substantial upfront (CAPEX) and recurring costs (OPEX, i.e., reagents, software subscription and updates, instrument maintenance) deter implementation despite the potential for long term savings and improved efficiency. A common issue is a lack of understanding as to whether AOM systems and higher

Table 3
Major barriers to implementation and possible solutions identified by drinking water utilities. The latter were asked to list three main barriers and three main solutions to the identified barriers (see suppl. mat. for methods).

Barriers to implementation Categories	Number of responses	Frequency
Cost (Capital expenditures, operating expenses)	42	28 %
Analytical performance	36	24 %
Absence of regulation	33	22 %
Acceptance	13	9 %
Lack of information, understanding	7	5 %
Reporting, unnecessary warnings	6	4 %
New responsibilities	3	2 %
Expertise (technical and data-related)	7	5 %
Logistics (monitoring spots, integration in existing schemes)	3	2 %
Technical support	1	1 %
TOTAL	151	100 %
Solutions Categories	Number of responses	Frequency
Further technology validation and/or developments	42	25 %
Cost reduction & funding	24	14 %
Accreditation/acceptance	18	11 %
Demonstrate clear needs/benefits	15	9 %
Capacity building & education	13	8 %
Data interpretation & management and communication	11	6 %
Adaptation of legislation	10	6 %
User-friendliness	8	5 %
Involvement of government/regulator	6	4 %
Pilot studies	6	4 %
Outreach	5	3 %
Improve fundamental knowledge	5	3 %
Change in mentalities	4	2 %
Upskill suppliers	3	2 %
TOTAL	170	100 %

temporal resolution data provide enough value to offset these costs. A quantitative cost-benefit analysis by Seifert-Dähnn et al. (2021) for raw sourced drinking water suggests that the benefits of automated high-frequency monitoring often outweigh the costs under site-specific conditions, particularly for systems frequently and severely affected by environmental changes with detrimental consequences for human health.

Unfortunately, the advantages of AOM are often only evident after installation, making pre-investment justification challenging. Budget constraints and increasing scrutiny over expenditures typically necessitate cost-benefit evaluations before funds are allocated. This creates a paradox where utilities are hesitant to commit to technologies that could mitigate risks and reduce the (long-term) costs of managing microbial hazards. By facilitating the detection of short-lived, extreme and unpredictable events that conventional low-frequency monitoring often misses, AOM systems can provide cost savings compared to manual sampling programmes (McBride and Rose, 2018) and reduce investigation costs for non-compliance of drinking water supplies which are often dominated by staffing expenses (Ellis et al., 2018). Whalen et al. (2018) estimated the potential cost savings associated with anticipating the location and extent of a microbiological contamination in a distribution system. Such early information could lead to savings in chemical disinfectant or flushing (or both), limit massive expenses from boil water advisories, and improve infrastructure life by preventing corrosion and delay costly infrastructure investments. Altogether, an average utility serving 10,000 customers could reduce annual costs by >500,000 USD (Whalen et al., 2018).

Understanding the niche application of AOM based on system scale and risk level can provide valuable guidance for strategic installation. Scalability is a concern for utilities managing numerous drinking water service deliveries. Early warning systems may indeed require greater spatial coverage, especially in densely populated or vulnerable areas. While the benefits of AOM may scale linearly with the number of people served, costs may increase non-linearly due to economies of scale. Overcoming financial constraints therefore requires a comprehensive evaluation of lifetime costs against accrued benefits tailored to specific contexts, demonstrating the tangible value of AOM for risk management, regulatory compliance and operational improvements. Clearer cost-benefit insights are needed to support strategic investment in scalable, site-appropriate AOM technologies and a shift toward proactive data driven approaches that safeguard public health and ensure sustainable water management.

5.2. Analytical performance

The analytical performance of AOM systems also emerged as a critical concern reflecting scepticism about the reliability and accuracy of AOM systems to effectively monitor microbial water quality compared to existing culture-based methods. The survey highlighted that respondents are wary of discrepancies noting that correlations between AOM and culture-based methods are inconsistent particularly for technologies like flow cytometry (FCM) and enzymatic assays, making the transition to new technologies challenging. However, while it is tempting to think of a microbial sensor as the rapid equivalent of a standard culture-based assay, the scientific basis for microbial detection and the output signal often differs (Section 3.2.2), thereby challenging the validation process. For instance, while HPC is a useful operational tool for monitoring general bacteriological water quality throughout the treatment process and within the distribution system, it only detects a very small fraction (0.001–8.3 %) of the total bacterial population in drinking water. In contrast, newer methods like FCM which enumerate all bacterial cells, consistently report higher bacterial counts, making direct comparisons with culture-based methods problematic (Hammes et al., 2008; Van Nevel et al., 2017a). Similar challenges are observed with other automated monitoring technologies. In a validation study demonstrating the high precision and robustness of automated GUS activity measurements, Burnet et al. (2019a) reported significant correlations with culturable *E. coli* at concentrations above 1,000 CFU/100 mL. However, correlations were weaker at lower concentrations, which the authors hypothesized was attributable to a larger proportion of viable but non-culturable *E. coli* cells undetected by culture-based assays.

The direct application of validation procedures for traditional cultivation-based techniques to microbial sensors is thus inadequate due to fundamental differences between the two approaches. The use of AOM is also not without its own issues. Anomalies identified by AOM can result in unnecessary interventions, reduced operational efficiency, and diminished trust in the technology. This may undermine risk management strategies, divert resources from genuine hazards, and potentially disrupt service delivery. Sensitivity and specificity are thus critical to detecting target microbial populations without interference from background communities or compounds. For instance, fluorescence-based sensors are subject to various sources of matrix-related interferences that can affect measurement results (see Section 3.2.2). Also, detecting abnormal changes in microbial water quality using FCM can be challenging in source or non-disinfected drinking water with high background levels of naturally occurring bacteria.

Few studies have compared the performance of different microbial sensors for water quality monitoring (Adomat et al., 2020; Prest et al., 2021; Stadler et al., 2016). A recent investigation by Favere et al. (2021b) evaluated six commercially available devices in a DWTP after activated carbon filtration. Under normal operating conditions, all devices demonstrated high sensitivity and responsive detection to abrupt changes such as filter backwash events. For simulated rain- and groundwater contamination scenarios, enzymatic analysis, ATP-metry and flow cytometric fingerprinting outperformed standard plate

counting methods in sensitivity and speed of detection. These findings emphasize the importance of aligning technology selection with specific applications, and should be balanced between sensitivity, cost, and maintenance.

Sensitivity, specificity, accuracy, precision and representativeness are essential features of the ideal microbial sensor. Whereas such analytical features have been demonstrated for offline technologies such as FCM (e.g., BAG, 2012), their automated on-site versions need further validation under various relevant deployment conditions. Establishing these standards is critical for their integration into routine water quality monitoring and enhancing the ability of utilities to manage risks effectively. Moreover, when the primary goal of AOM is to detect elevated microbial health risks, pathogen testing or epidemiological investigations should ideally be involved to provide a clearer understanding of the sensor's indicator value for microbial hazard detection and its practical relevance to compliance monitoring for risk management.

5.3. Absence of regulatory frameworks

The absence of regulatory guidelines or thresholds for new microbial water quality parameters constitutes a further significant barrier to practical implementation of robust sensors as these frameworks dictate industry standards and monitoring practices. The survey revealed that current monitoring methods were often (too) deeply rooted in established patterns of action (Fig. S3). Without regulatory acceptance, utilities may face uncertainty in deploying AOM systems and hesitate to adopt these technologies due to apprehension of future non-compliance or the need to retrofit systems to meet eventual standards. Integrating automated technologies with legacy systems can be complex and may require significant modifications to existing infrastructure. Furthermore, in situations where an AOM system may report abnormal changes in microbial water quality while legally binding parameters remain below thresholds values, it remains unclear how water suppliers should deal with such discrepancies. Nevertheless, surveyed respondents believed that benefits can be gained from implementing the new monitoring tools and that there was room for innovation (Fig. S3). Given that most FIB in regulatory standards were developed and used over several decades, it will be challenging to overcome the regulatory barrier for the use of AOM data to demonstrate compliance with microbiological standards. The adoption of AOM will therefore rather be driven by their integration into early warning systems that enable utilities to proactively control the risks to decrease the likelihood of having to manage failures and communicate it to the consumers (Imran, 2018).

5.4. Data handling, interpretation and communication

Other challenges identified in the survey included technical issues such as data management, where utilities reported difficulties in processing and interpreting substantial amounts of new data generated by AOM systems. Managing large volumes of data demands a robust processing infrastructure and increasingly sophisticated analytics, including AI and machine learning, to derive meaningful insights, forecast trends, and support decision-making (Pérez-Beltrán et al., 2024). Also, there is a lack of expertise to fully capitalise on the value of these information data streams preventing utilities from integrating AOM results effectively into decision-making and operational strategies. Optimal monitoring schemes should thus primarily detect meaningful changes in microbial water quality, rather than reporting absolute values such as maximum cell counts, ATP concentrations, or enzymatic activities, as these measures may not indicate a specific health risk. Importantly, there are no universal upper thresholds for these parameters (Prest et al., 2016; Van Nevel et al., 2017a). As discussed above, the implementation of AOM into early warning systems will likely accelerate the adoption of this monitoring approach by establishing site-specific thresholds (Sorensen et al., 2018a,b). To interpret AOM results for potential microbial risk, nuanced signal analysis will be needed requiring a thorough understanding of signal dynamics. The range of signal concentrations can be site-specific, and the method of interpreting signals should also account for context. For example, transient short-term signal peaks in the raw water might pose less risk compared to sustained elevated signals within the treatment train where the likelihood of pathogen (or toxin) breakthrough increases.

Finally, as the world has become hyperconnected and consumers are getting more informed about the products they consume, any perceived failure in water quality can be disseminated widely almost instantaneously via social media. Elevated consumer awareness will therefore inevitably impact the amount of data, their measurement frequency and the subsequent reporting to consumers, which will influence regulatory frameworks but also add to utilities operating costs (Whalen et al., 2018).

6. Future perspectives

6.1. Optimising monitoring strategies

The recent development of AOM systems offers new opportunities for the acquisition of extensive datasets on microbial water quality that can elucidate the multiple scales of spatiotemporal variation (Fig. 2) (Burnet et al., 2019b; Page et al., 2017; Rome et al., 2021; Stadler et al., 2019; Zamyadi et al., 2014). High resolution monitoring can help designing useful site-specific thresholds to observe changes in microbial water quality over a range of magnitudes and time intervals (Chaffin et al., 2018; Izydorczyk et al., 2009; Sorensen et al., 2018a). Yet, as discussed in Section 5 above, high resolution monitoring over extended periods is presently impractical, notably because of the associated costs. System-specific fluctuations in microbial water quality therefore need to be captured using short intensive monitoring campaigns to e.g. increase the probability of detecting microbial changes induced by precipitation events in the source (Besmer et al., 2017a; Sylvestre et al., 2021) or to optimize routine monitoring of the DWDS by increasing sample representativity (Gabrielli et al., 2021). Using optimized monitoring strategies, meaningful data on FIB (Offenbaume et al., 2020), pathogens (Sylvestre et al., 2021) and microbial/chemical source tracking markers (Hachad et al., 2024) can be collected to support risk assessment at strategic points and times along the supply chain. Sample collection can even be automated by triggering an autosampler using a microbial sensor as suggested by Ryzinska-Paier et al. (2014) and demonstrated by Burnet et al. (2021) who proposed automated collection of samples for pathogens and microbial source tracking markers during an intermittent contamination event in an urban river.

There is also significant promise in leveraging data from conventionally deployed sensors to enable a tiered approach to water quality monitoring, providing more in-depth information and offering a more comprehensive understanding and characterisation of the supply chain. This approach allows complementary sensors (e.g., turbidity, temperature, chlorine, pH or dissolved oxygen) to provide additional context and insights into the environmental conditions and response of the DW system to infer changes in microbial communities (Reynaert et al., 2023). Integrating AOM systems with these technologies can enhance the reliability of monitoring to detect or predict microbial contamination events to support an adaptive management strategy. A combination of microbial, physical and chemical sensor signals that could be optimized by specific AI-driven algorithms (e.g., machine learning) would offer tailored solutions to utilities, taking into account their objectives as well as site-specific considerations. This could apply to i) system assessment and quantitative microbial risk assessment (QMRA) by establishing robust log-reduction targets through pathogen measurement, to ii) system verification to assess for instance whether routine physicochemical proxies are reliably indicating microbial water quality challenges and to iii) linking AOM of microbial water quality to laboratory-based standard parameters (culture-based enumeration of

indicators) at critical points in time and space to assess the complementarity of both systems.

6.2. Developing new AOM technologies

Whereas AOM technologies have been designed for monitoring bacteria and archaea (or proxies thereof), recent advancements in flow virometry pipelines for the quantification of viruses by FCM (Safford et al., 2023) could complement the AOM toolbox in the future. Also, new environmental DNA (eDNA) for micro- and macroorganism detection could help unravelling the complex dynamics and interplay of highly heterogeneous communities of the DWDS biome (protists, invertebrates, bacteria, fungi and archaea) and ultimately revolutionize microbial water quality monitoring (Pluym et al., 2024). AOM using amplification of nucleic acids (i.e., DNA or RNA) has not yet been implemented in the drinking water sector though due to upstream sample processing requirements, as well as the need for specific instrumentation and supporting infrastructure. Nonetheless, recent developments in nucleic acid detection technologies and sample processing workflows that have advanced their portability will likely make such monitoring feasible in the future. This includes miniaturization of laboratory instrumentation for DNA extraction and preparation for sequencing (Edwards et al., 2022; Pomerantz et al., 2022), gene-targeted or metagenomic sequencing on the nanopore MinION platform (Deamer et al., 2016), as well as specific PCR based assays (e.g., qPCR, loop-mediated isothermal amplification) for other microorganisms including pathogenic ones (Karthe et al., 2016). Here it is important to note that nucleic acid-based methods could be utilized to infer viability for the whole community or specific microbial targets by using membrane integrity as a metric of viability (Fittipaldi et al., 2012), as is used in FCM. Nonetheless, such procedures require extensive sample treatment prior to DNA extraction and PCR, which themselves may compromise the membrane integrity of stressed cells. As a result, the implementation of such approach will require strategies to include internal controls to ensure results are reliable.

Besides developments in molecular tools, the recent deployment of imaging-based approaches for microbial monitoring in the drinking water sector has been enabled by several advances in the development of low-cost open-source imaging platforms (Pollina et al., 2022). The latter may allow for high-resolution monitoring of source waters and when combined with the appropriate data analyses approaches (Fung et al., 2023; Xu et al., 2022), they may allow for quantitative monitoring of cyanobacteria in source waters for instance. The application of such platforms for microbial monitoring in other parts of the source-to-tap continuum is less likely though due to lower cell concentrations and smaller cell sizes.

Overall, the feasibility of AOM may still be sample-specific and dictated by overall microbial concentrations as well as concentrations of target microbes. As those do decrease along the source-to-tap continuum, large sample volumes will be required to harvest microbial cells (and nucleic acids) above background contamination levels. A complementary approach could thus be biofilm monitoring (e.g., Im et al., 2024), which could provide critical insights into microbial activity and help assess the biological stability of DW supply of drinking water supplies. This however still needs to be demonstrated in various field settings.

Aside from the development and deployment of novel platforms or assays for AOM, there is a need for systematic studies that test the utility of machine learning approaches to infer microbial contamination events based on conventional sensor data (Housh and Ostfeld, 2015; Zhong et al., 2021). Such efforts will require a significant upfront investment of resources for the collection and curation of existing datasets for the training and validation of machine learning models as well as the creation of new experimental datasets.

7. Concluding remarks

The future of AOM using microbial sensors in the water industry is set to transform water quality management through technological advancements and integration with digital systems for online (real-time) information. As customers increasingly demand transparency about the quality of their water, utilities will need to adapt by implementing sensors and managing the information they generate to maintain their social license to operate. Next-generation sensors are expected to offer improved sensitivity and specificity enabling the detection of microbial contaminants at lower thresholds and in more challenging environments. Advances in machine learning and AI will enhance data interpretation providing predictive analytics and real-time decision support for water utilities allowing them to respond proactively to potential risks. As regulatory frameworks evolve to incorporate real-time monitoring, microbial sensors for AOM will play a pivotal role in meeting stringent safety standards and addressing emerging challenges such as climate change-induced variability in water quality to ensure delivery of safe drinking water and protection of public health.

CRediT authorship contribution statement

J.B. Burnet: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **K. Demeter:** Writing – review & editing, Conceptualization. **S. Dorner:** Writing – review & editing, Funding acquisition. **A.H. Farnleitner:** Writing – review & editing, Conceptualization. **F. Hammes:** Writing – review & editing, Conceptualization. **A.J. Pinto:** Writing – review & editing. **E.I. Prest:** Writing – review & editing. **M. Prévost:** Writing – review & editing. **R. Stott:** Writing – review & editing. **N van Bel:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ameet Pinto reports a relationship with Water Research Journal that includes: board membership. Co-author Ameet Pinto serves as an editor for Water Research Journal. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2025.123121.

Data availability

Data will be made available on request.

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