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Executive Danish summary

HOFOR A/S, Aarhus Vand A/S, VandCenter Syd A/S og Kalundborg Forsyning A/S har taget initiativ til at styrke udviklingen af sensorer/sensorteknologier, der tillader fuldskala implementering af online overvågning af mikrobiel drikkevandskvalitet. På vegne af forsyningerne har DTU Miljø udarbejdet et state of the art overblik over sensorer i relation til mikrobiel vandkvalitet, der kan guide forsyningerne i det videre arbejde.

Opgaven blev udført ved:

- *Opsummering af erfaringer med sensorer til monitering af mikrobiel vandkvalitet fra danske vandforsyninger*

  Spørgeskemaer om driftserfaringer med sensorer til monitering af mikrobiel vandkvalitet blev udsendt til udvalgte forsyninger, der betragtes som banebrydende inden for sensor området og/eller som har haft større forureningssager inden for de seneste årtier.
- *Fokuseret internet søgning*

  Søgeord relateret til online sensorer til monitering af mikrobiel vandkvalitet blev anvendt til at identificere teknologier på markedet og under udvikling, herunder også nuværende manuelle metoder med potentiale for automatisering.
- *Opgørelse af relevante forskningsprojekter*

  Relevante forskningsprojekter, hovedsageligt nationale og europæiske, blev identificeret i relevante databaser og deres primære formål gennemgået.
- *Dialog med sensorproducenter*

  Tekniske informationer om sensorer, deres anvendelse og drift samt (hvor muligt) valideringsdokumentation blev indhentet igennem e-mail korrespondance eller korte telefoninterviews med sensorproducenter.
- *Telefoninterviews med fagfolk*

  Rikke Hansen (3V), Pernille Ingildsen (Kalundborg Forsyning) og Jeppe Resen Amossen (Harper & Vedel) blev interviewet på anbefaling af forsyningerne til at give indblik i sensorudviklingen i Danmark og for at opnå yderligere information om udfordringer og gennembrud inden for området.
- *Gennemgang af den videnskabelige litteratur*

  Hovedfokus for litteraturgennemgangen var review-artikler om sensorer samt analysemetoder, der potentielt vil kunne automatiseres i en sensor. Gennemgangen sigtede på at identificere styrker og svagheder ved de enkelte metoder.

Resultaterne blev evalueret med udgangspunkt i forsyningernes definition af 'den ideelle sensor', der skal kunne:

- Installeres online eller at-line
- Detekter indikatororganismer (*E. coli* og total coliforme)
- Have høj følsomhed til detektion af lave koncentrationer af mikroorganismer i drikkevand
- Give hurtigt svar
- Kræve lav vedligeholdelse
- Give lave forekomst af falske-positive

Resultaterne blev grupperet i tre kategorier:

- Teknologier på markedet
- Teknologier under udvikling eller i dokumentationsfase
- Teknologier på forskningsniveau

Resultater

Markedet for og udviklingen af sensorer til monitering af mikrobiel vandkvalitet er et ofte ugennemskueligt område. Udviklingen styres bl.a. af tilgængelige bevillinger, hvorved producenter og udviklingsforløb til stadighed starter op og lukker ned. Producenterne ønsker at sælge – enten deres produkt eller næste udviklingstrin - så det er nødvendigt kritisk at evaluere præsenterede data, da producenterne kan være for ambitiøse på deres produkters vegne, både med hensyn til udviklingshorisont og formåen. Dette gælder især, hvis driftserfaringer overføres fra et miljø til et andet. Drikkevand er karakteriseret ved et næringsfattigt miljø, hvor bakterierne har lavt energiniveau. Specifikke bakteriegrupper vil udgøre en meget lille andel af baggrundsbakterieniveauet, der vil have stor artsdiversitet. Det er derfor ikke altid muligt at anvende koncepter eller erfaringer fra andre miljøer, som fx fødevareproduktion eller medicinalindustri, da disse ofte er karakteriseret ved høje næringsniveauer og kraftig vækst af få kulturer.

Udvikling af sensorer til monitering af mikrobiel vandkvalitet kræver tid og ressourcer. Udviklingsforløb vil ofte strække sig over år eller årtier, og vil ofte involvere mange forskellige projektforløb, da én bevilling sjældent vil være tilstrækkeligt til at dække alle udviklingstrin.

Der blev identificeret 12 sensorer på markedet (jævnfør rapportens Tabel 1). Sensorerne kan opdeles i tre kategorier:

- Sensorer, der detekterer indikatororganismerne *E. coli* og totale coliforme ved enzymatisk reaktion. Disse sensorer kræver alle inkubationstid, hvilket giver forsinkelse på svaret.
- Sensorer, der optisk detekterer totalt bakterieantal ved mikroskopering eller billedgenkendelse. Disse sensorer har en kort svartid, men en forurening vil blive detekteret som en stigning i totalt bakterieantal. Det er derfor nødvendigt at evaluere resultatet imod en referenceperiode for at kunne afgøre, om en afvigelse skyldes en potential forurening eller driftsforhold (fx returskyl, boringsskift, hydrauliske ændringer). En forurening skal være tilstrækkelig stor til at give et udslag over 'støj'-niveauet for at blive detekteret. Dermed er der en risiko for, at forureninger ikke opdages, hvis de kun giver anledning til mindre ændringer i totalt bakterieantal.
- Sensorer, der detekterer totale bakterieniveauer målt som total bakterieaktivitet ved ATP. Sensorerne giver hurtigt svar, men som for de optisk baserede sensorer kræves en evaluering af resultatet imod en referenceperiode.

I tillæg til sensorer, der allerede er på markedet, blev der identificeret fem sensorer under udvikling (jævnfør rapportens Tabel 2).

En række analysemetoder vil potentielt på sigt kunne integreres i et sensorformat (jævnfør rapportens Tabel 3), men det vil kræve et betydeligt forsknings- og udviklingsarbejde:

- Immunoassays
- PCR (polymerase kædereaktion)
- FISH (fluorescens in situ hybridisering)
- Elektrisk detektion
- Flowcytometri
- Raman spektroskopi
- 'Microfluidic' systemer som platform

En række igangværende projekter blev identificeret inden for området (jævnfør rapportens Tabel 4), som kan påvirke den fremtidige sensorudvikling (bevillingsgiver i parentes):

- Future Water (VTUF/MUDP)
- Detektion af *E. coli* DNA i lednings-vand (VTUF)
- Real-time vandkvalitetsmåling i vandsektoren ved elektrisk detektering (VTUF/MUDP)
- AQUAWARN (EU, FP7-SME-2013)
- SMARTWATER4EUROPE (EU, FP7-CP)
- Aquavalens (EU, FP7)

- AquaSHIELD (EU, H2020)
- AQUAVIR (EU, FP7-CP)

Der findes i dag ikke en sensor, der opfylder alle kriterierne for 'den ideelle sensor'. Den optimale løsning er muligvis heller ikke én sensor til alle formål. Det bør i de enkelte situationer fastlægges, hvad man ønsker at monitere for, og herefter definere hvordan dette bedst opnås. En kombination af sensorer (herunder også sensorer til monitering af fysisk-kemiske parametre) kan vise sig at være den bedste løsning. Det kan også overvejes at fastlægge moniteringsstrategi og sensorvalg individuelt for separate dele af forsyningssystemet eller ud fra konkrete scenarier, da det kan være forskellige analyseparametre (fx specifikke organismer eller totalt bakterietal), der passer bedst til forskellige moniteringsformål. Dette sammenkæder sensorvalg og moniteringsstrategi med forsyningernes identificerede risici i egne systemer, hvilket er en opgave tæt knyttet til forsyningernes DDS-arbejde.

Preface

The utilities HOFOR A/S, Aarhus Vand A/S, VandCenter Syd A/S and Kalundborg Forsyning A/S have taken the initiative to boost the development of sensors for monitoring of microbial drinking water quality. This report is the result of a collaboration between the utilities and DTU Environment, and aims to provide a state of the art overview within the field of microbial sensors to guide utilities in decisions on future monitoring investments. The utilities were represented by Anne Esbjørn (VandCenter Syd A/S), Ann-Katrin Pedersen (HOFOR A/S), Jørn-Ole Andreasen (Aarhus Vand A/S) and Pernille Ingildsen (Kalundborg Forsyning A/S).

1 Introduction

Monitoring of drinking water quality is essential to document that the distributed water fulfils the required quality standards and is safe for human consumption. Contamination risks can be identified throughout the water supply system, from well to the waterworks, but most risks are associated with the distribution system[1]. Traditionally, water quality is monitored by grab sampling and laboratory analysis which holds the disadvantage that the information is delayed and temporal changes are not detected. To timely react on quality changes and thereby prevent risks to public health during a contamination case, continuous online monitoring with (close to) real-time results is needed.

Online sensors for monitoring physical and chemical parameters such as pressure, flow, temperature, pH, conductivity, dissolved oxygen and turbidity have been implemented by Danish utilities during the last decades to improve understanding of the distribution system dynamics[2]. Although these parameters can sometimes indirectly identify severe microbial contamination events, the demand for sensors that can directly detect microbial parameters is increasing.

The main challenge of developing microbial water quality sensors is to shorten the days-long incubation time required by the traditional culture based methods to provide results. These methods identify the presence of indicator microorganisms such as *E. coli* and total coliforms that indicates contamination. It is important to note that the typical concentration of naturally occurring bacteria in drinking water ranges between 10^4 and 10^6 cell/mL, meaning that sensors need to detect either the presence of indicators within this background or an increase in the total bacteria concentration caused by contamination. Several microbial sensor technologies have emerged on the market and others are in the validation or research stage, but further development and documentation are still needed before their use as routine monitoring methods is established.

The aim of this report was to provide a state of the art overview within the field of microbial sensors, presented in three categories: 1) Technologies currently available on the market 2) Technologies in the development and documentation phase 3) Technologies at the research level.

2 Methodology

The state of the art within the field of sensors for microbial drinking water quality monitoring was established by:

- *Summarizing the experiences of Danish utilities with microbial sensors*
  Utilities considered as first movers and/or having experienced larger contamination cases within the last decades were invited to complete a questionnaire regarding their experiences with microbial water quality sensors.
- *Focused internet search*
  Search terms associated with online microbial sensors were used to identify existing technologies and technologies under development, including methods that currently require manual handling but have the potential to become automated.
- *Compiling a list of relevant research projects*
  Research projects, primarily at the national and European level were identified in relevant databases and the main goals were reviewed.
- *Dialog with sensor producers*
  E-mail correspondence or short phone interviews were used to compile technical information about the reviewed technologies, their implementation and, where possible, validation documentation was obtained.
- *Phone interviews with professionals recommended by the utilities*
  Rikke Hansen (3V), Pernille Ingildsen (Kalundborg Forsyning) and Jeppe Resen Amossen (Harper & Vedel) were interviewed to provide insight on the current status of sensor development in Denmark, and to obtain additional information on the challenges or the breakthroughs in the field.
- *Scientific literature study*
  The main focus was on review articles on sensors and research methods that have the potential to be implemented in future sensor technologies. The literature search aimed to identify strengths and weaknesses of each of the methods.

The utilities described the 'ideal sensor' as fulfilling the following points:

- Online and at-line installation
- Detection of indicator microorganisms (*E. coli* and total coliforms)
- High sensitivity to detect very low concentrations of indicator microorganisms in drinking water
- Rapid response
- Low maintenance requirements

- Low false alarm occurrence

Results are presented and discussed in three categories:

- *Technologies currently available on the market*
  The currently available technologies are presented and their strengths and weakness are discussed including experiences from utilities responding to questionnaires.
- *Technologies in the development and documentation phase*
  Technologies in the development and documentation phase are discussed, including how close they are to be launched on the market and the challenges that are yet to be resolved.
- *Technologies at the research level*
  The aims and expected outcomes of ongoing research projects in the field are presented to signal the future direction in sensor development.

3 Results

3.1 Technologies currently available on the market

The sensor market is extremely fast-changing, with new technologies being released and manufacturing companies closing down or being taken over by larger ones. This means that market overviews tend to outdate after a very short time as e.g. the reviews of Storey et al 2011[3] and Lopez-Roldan et al 2013[4].

By creating an overview of the 2015 market, 12 rapid microbial water quality monitoring technologies were identified (Table 1). The technologies were grouped according to target and principle: 1) specific indicator organisms by enzymatic activity 2) total bacteria concentrations 3) total bacteria activity by ATP (Adenosine TriPhosphate). The main challenge of performing this screening has been the lack of transparency in the information provided by the manufacturing companies, as their main interest is to promote their products. Often, detailed validation information or technical details are not disclosed due to commercialization interests. Therefore the information provided by the manufacturing companies should be interpreted with care, especially when conclusions are drawn from arbitrary validation tests and when satisfactory performance in other fields is assumed to apply also for drinking water. Some of the reviewed technologies were developed and are mostly used in other fields e.g. the 'Colimínder' for wastewater monitoring, the 'Desktop microscope' for medical research and the 'Biocounter' for the beverage industry. These technologies are however included in this section because they have the potential or are currently being validated for use in drinking water.

3.1.1 Detection of specific indicator microorganisms by enzymatic activity

Sensors to monitor microbial water quality target either specific indicator microorganisms, such as *E. coli* and total coliforms, or measure total bacterial activity or concentration e.g. by ATP or direct cell counts. A large share of the available technologies are automated versions of the widely used 'Colilert' (Idexx) test kit, and measure *E. coli* and total coliforms by fluorescence/colour detection of enzymatic activity[28]. This includes the 'ALARM', 'CALM', 'Coliguard', 'aquaBio', 'TECTA' and 'Colimínder' technologies (Table 1). In brief, coliform bacteria use the enzymes β-glucuronidase to metabolise the substrate (colour reaction) and *E. coli* uses β-galactosidase (fluorescence)[30]. However, some studies have shown that other bacteria may cause false positives if they are present at high concentrations[31-33], but this is typically not the case for drinking water systems. The challenge of implementing this method into an automated at-line system is to detect indicator organisms at the low concentration range relevant for drinking water. These low concentration levels require incubation time that allows multiplication of cells in order to be detected. Thus, sensors using this measuring principle can only provide results with hours delay (Table 1). The result is usually expressed as enzymatic activity and although several correlations have been proposed[35-36], conversion to cell numbers is not straightforward[12].

Table 1. Overview of the currently available market technologies to monitor microbial water quality.

Technology name	ALARM[18]	CALM[15]	Coliguard BACTcontrol)[11]	aquaBio[9]	TECTA[7]	ColiMinder[5]
Manufacturing company	Colifast A/S	Colifast A/S	MicroLAN (mbOnline)	ADASA	Veolia	VMW (Vienna Monitoring Solutions)
Country	Norway	Norway	The Netherlands	Spain	Canada/ Switzerland	Austria
Analysed parameter	Total / thermo-tolerant coliforms or *E. coli*	Total coliforms, *E. coli*, *P. Aeruginosa* (two simultaneously)	Total coliforms or *E. coli*	Total coliforms and *E. coli* simultaneously	Total coliforms and *E. coli* simultaneously	Total coliforms, *E. coli*, or total bacteria
Measuring principle	β-galactosidase activity (yellow) and β-glucuroni-dase activity (fluorescence)	β-galactosidase activity (yellow) , β-glucuroni-dase activity (fluorescence) and substrate hydrolysation	β-galactosidase activity (yellow) and β-glucuroni-dase activity (fluorescence)	β-galactosidase activity (yellow) and β-glucuroni-dase activity (fluorescence)	β-galactosidase activity (yellow) and β-glucuroni-dase activity (fluorescence)	β-galactosidase activity (yellow), β-glucuroni-dase activity (fluores-cence) and alkaline phosphatase activity
Current field of application	Drinking water	Raw water, process water, wastewater	Drinking water, bathing water, process water	Drinking water, wastewater, bathing water	Drinking water, wastewater, reclaim water	Sewage, surface water and soon tested in drinking water
Validation documentation	ETV[19],[20]	EU project DEMOWATERCOLI [16]	Research paper[12]	Currently not	ETV[8]	N/A
Measuring unit	Presence/absence	Presence / absence, MPN, CFU	pmol MUF/min/100 mL	MPN/100 mL	Presence/absence and estimation of bacteria level	MFU/100 mL
Nominal detection limit	1 viable target organism/100 mL	1 CFU/mL depending on measuring mode	0.1 pmol MUF/100 mL	1 viable target organism/100 mL	1 viable target organism/100 mL	0.8 mMFU/100 mL
Current installation mode	at-line	at-line	at-line	at-line	Manual sampling loading/autosampling	at-line
Response time*	6-15 h (max 1 sample/day)	4-12 h	2-4 h	3-18 h	2-18 h	10-30 min (only tested for surface water) [6]
Sampling volume	100 mL	25-100 mL	20-3000 mL	N/A	N/A	6.5 mL
Maintenance	Reagent refill every 20 tests, yearly service	Reagent refill weekly to monthly. Service twice a year	Reagents refill every 1-3 months for 4 tests/day[13]	Every 15 d reagent replacement & monthly cleaning	N/A	N/A for drinking water – currently being tested
Market availability & cost	For sale. Approximate price : 190,000 DKK[17]	More expensive than ALARM[17]	Sale or rental agreement options. For sale. Price: 300,000 DKK[14]	For sale. Price is 174,000 DKK plus 15,000 DKK yearly maintenance[10]	135,000 DKK	For rent
Comments	Experience from HOFOR A/S, Trondheim and Stavanger utilities	Experience from Stavanger utility	New version targeting enterococci under development (expected release in 2016)[13] Experience by HOFOR A/S and Nordvand A/S		Did not reply to inquiry. Experience from Svendborg Vand A/S, Nordvand A/S and HOFOR A/S with the manual version	Currently working on new version that incorporates pc[6]

N/A: Information not available, CFU: Colony forming unit, MUF: Methylumbelliferyl, MPN: Most probable number, MFU: Modified Fishman Units, * Low response times only applies to high

Table 1. Overview of the currently available market technologies to monitor microbial water quality (cont.)

Technology name	Aquascope[27]	Desktop microscope[25]	7000 RMS[24]	BACMON[23]	EZ-ATP[22]	Biocounter[21]
Manufacturing company	Biotrack	4-deep	Mettler Toledo A/S	Grundfos A/S	Applitek	Biosensores
Country	The Netherlands	Canada	International	Denmark	Israel, Belgium	Spain
Analysed parameter	Total bacteria, *E. coli,* Enterococci or *Legionella*	Particles, bacteria or other organisms	Bacteria and particles (simultaneously)	Particles and total bacteria	ATP	N/A
Measuring principle	Filter cytometry & FISH	Optical	Optical	Optical	Chemiluminescence	N/A
Current field of application	N/A	Medical field (cancer research, urine and blood tests)	Pharmaceutical waters	Drinking water	Drinking water and wastewater	Wastewater, beverage industry
Validation documentation	N/A	N/A	N/A	Manuscript in preparation	N/A	N/A
Measuring unit	N/A	Particles or cells/mL	Bacteria and particles/mL	Cells and particles/mL	N/A	N/A
Nominal detection limit	1 cell/mL	N/A for drinking water	1 Biocount (correlation not given)	160 cells/mL	N/A	10^3 cells/ mL
Current installation mode	N/A	Can be installed in with flow through cell[26]	at-line	at-line	at-line	at-line
Response time	20-45 min	Immediate[26]	N/A	10 min	N/A	15 min
Sampling volume	1-500 mL	N/A	N/A	6 µL fixed in the flow cell	N/A	N/A
Maintenance	N/A	Every day wiping glass and flushing flow through cell[26]	N/A	Regular change of flow through cell	N/A	N/A
Market availability & cost	N/A	Initial rental eventually followed by purchase. Sale price: 200,000 DKK hardware & software + min. 40,000 DKK for training[26]	N/A	For rent	N/A	N/A
Comments	Did not reply to inquiry	Microscope needs operator at least remotely[26]	American technology formerly manufactured by Instant Bioscan just acquired from an International company. Information not available	Experience from TREFOR Vand A/S, Aarhus Vand A/S and HOFOR A/S	Did not reply to inquiry	Did not reply to inquiry

N/A : Information not available, FISH: Fluorescence in situ hybridization

Among the sensors based on enzymatic activities, the 'ALARM', 'CALM' 'Coliguard' and 'aquaBio' need reagent refill at specific time intervals of weeks to months, depending on sampling frequency[10, 13, 37-38]. These technologies need also yearly or twice-a-year maintenance service. The 'TECTA' is not a fully automated technology, but requires manual sample loading[7]. The technology is included in this section because it can be combined with an automated sampler from the same manufacturer[7]. No information was available about the maintenance requirement of the 'Coliminder'.

Well documented validation is essential to ensure that the technologies are reliable and suitable for drinking water monitoring. The 'ALARM' and 'TECTA' technologies have received an ETV (Environmental Technology Verification) from the US EPA[8, 19], which is a recognised validation process. The 'Coliguard' technology was tested by continuous monitoring of two groundwater sites for two years[12], while the 'CALM' technology has been validated as part of a the EU project DEMOWATERCOLI[16]. No information was available for validation of the 'aquaBio' technology in drinking water. In all cases, it is important that these technologies are validated in a systematic and unbiased way in drinking water systems. End-users should be critical when evaluating the validation material and the results presented by the manufacturing companies, especially if the technology is new and has no references of *in situ* use.

All the enzyme activity based sensors, except from the 'Coliminder' and the 'aquaBio', have been applied at the participating utilities. The 'ALARM' sensor has been used at HOFOR A/S to measure total coliforms or *E. coli* [39]. The sensor samples and provides data every 24 hours (response time 6 - 15 hours) and is only used for water quality monitoring outside normal working hours[39]. The utility considers the monitoring of either total coliforms or *E. coli* (not for both at the same time) and the presence/absence output a disadvantage[39]. On the positive side, the utility believes that the operation is quite stable and without excessive maintenance needs[39]. Trondheim utility, supplying about 180,000-200,000 people, has used the 'ALARM' sensor continuously for 3-4 years[38]. The facility treats infiltrated surface water and the sensor is installed at the raw water intake to monitor for contamination from the surrounding agricultural area[38]. Sampling and analysis is performed every 24 hours, while manual sampling and traditional culture based analysis are done once a week[38]. Several occurrences of manure contamination or sewage leakage in the area has been detected by the 'ALARM' and correlated roughly with the results of grab sampling and culture based analysis, even though samples were not collected at the same locations and time[38]. Overall, Trondheim utility believes that the sensor is a good contamination indicator implemented in a user friendly system that only requires a short training course for the operators[38]. They are currently planning to install one more 'ALARM' at the lake water intake point[38]. Rogaland (Stavanger) utility serves about 300,000 people and is currently using two 'ALARM' sensors and two 'CALM' sensors[37]. The 'CALM' sensors are installed at the two surface water intake points and monitor for *E. coli* every 4 hours. Contamination cases have been detected and correlated roughly with

weekly grab samples and traditional culture based analyses, although also in these cases samples were taken at different points and times[37]. Their overall experience is positive and the only expressed concern is the high initial and analysis cost, especially of the 'CALM' sensor[37]. They are however planning to continue using them[37].

The 'Coliguard' sensor has been used at Nordvand A/S and HOFOR A/S to measure *E. coli* and total coliforms[10, 39]. The sensor samples four times a day and has a low detection limit[39]. However, the utilities report high maintenance needs and consider it an expensive technology[10, 39].

The 'TECTA' sensor has been used by Nordvand A/S, HOFOR A/S and Svendborg Vand A/S in its manual version, i.e. without the incorporated auto-sampler[10, 39, 40]. The utilities' experience with the sensor is rather positive, as they find it to be user-friendly and the response time of 2-18 hours is shorter than the traditional culture based methods, yet giving accurate results. However, the measurements are only partly quantitative. The sensor is mainly used during holidays, when the laboratory personnel is not at work[39].

Costs range from 135,000 to 300,000 DKK for the technologies that provided exact pricing information (Table 1). This only includes the initial capital cost, and the operational cost related e.g. to reagent refill etc. needs to be added. Service is also needed once or twice a year, adding accordingly to the overall costs of the sensors.

3.1.2 Measurement of total bacteria concentrations by optical methods

The 'BACMON', 'Desktop microscope' and '7000 RMS' technologies use optical methods to detect microbial cells in water and thus do not require any incubation time. These technologies aim to recognise bacteria from particles or even specific indicator bacteria by image analysis[23, 25-26]. The 'BACMON' sensor provides a measure of total particles and bacterial cells present in the water[23], aiming to detect sudden changes from an established background level. The 'Desktop microscope' states to recognise specific indicator bacteria based on multiple morphological parameters integrated in algorithms that classify the detected objects into taxons[25]. The sensor however, may be seriously challenged by the high diversity of bacteria and low concentration of indicator microorganisms in drinking water. Distinction between the different bacteria is particularly challenging, and will normally require several additional tests besides microscopic observation. The '7000 RMS' sensor was originally developed in the US under the name 'RMS-ON90'. The initial manufacturing company Instant Bioscan Inc. was recently acquired from Mettler Toledo A/S, and due to the ongoing training of the responsible personnel very limited information was available at the time of the interview[41].

The 'BACMON' sensor is fully automated, but requires change of the flow cell at regular time intervals that may vary depending on the type of water[23]. The 'Desktop microscope' needs external handling via a computer that operates the microscope communication[26]. This can be done remotely, but daily wiping of the glass surface and flushing of the flow cell is required[26]. No information about maintenance of the '7000 RMS' sensor was available.

The 'BACMON' sensor has been validated during long term installation at Danish waterworks, although the detailed data has not been published yet[23]. The sensor has been installed at HOFOR A/S, Aarhus Vand A/S[42] and TREFOR Vand A/S[43]. Among the positive features of the sensor, TREFOR Vand A/S reports stable operation, good user interface and little maintenance required[43]. The disadvantage mentioned by TREFOR A/S is the difficult result interpretation, but the utility plans to continue the use of it[43]. HOFOR A/S uses 'BACMON' at points in the distribution system where the retention time is long and may affect the microbial water quality[39]. No validation or user experience information was available for the 'Desktop microscope' and '7000 RMS' technologies.

Exact cost information was only available for the 'Desktop microscope' and is approximately 200,000 DKK plus 40,000 DKK for training[26].

3.1.3 Measurement of total bacterial activity by ATP

ATP (Adenosine TriPhosphate) is the main energy carrier molecule in all living cells and hence measuring the ATP concentration gives an indication of total bacterial activity. ATP measuring kits have been widely used in the food and beverage industry[45-47]. The 'EZ-ATP' sensor is an automated ATP analyser developed by Applitek, which can also be applied for drinking water monitoring[22]. No information on maintenance, cost or user experience was available for the 'EZ-ATP' sensor.

3.2 Technologies under development or validation

An overview of five technologies in the development or validation stage is presented in Table 2. This overview includes technologies developed at least at the prototype level, which currently are under testing. Some of these technologies aim to become the automated version of already developed manual methods, e.g. 'Bactiline' and 'Minilab'. Technologies partly developed by closed-down companies are not included in this section, but presented separately in Appendix I.

Compiling technical details about technologies under development is even more challenging than the methods currently on the market, because companies are particularly reluctant to disclose any information before they launch their product. Often, it is also difficult to realise how far these technologies are in the development process, as manufacturing companies tend to be too optimistic about their progress and the expected time of market release.

Mycometer A/S is currently working on automation of their assay kit 'Bactiquant', which has been used by several Danish utilities including Frederikshavn Forsyning A/S[50], Svendborg Vand A/S[40], DIN Forsyning A/S[51], Nordvand A/S[10] and Energiforsyningen A/S[52]. The technology is based on enzymatic activity fluorescence and states to detect several gram negative and positive bacteria, thus providing a measure of total bacteria levels in the water[53]. Currently two prototypes of the automated version have been developed and are tested under laboratory conditions[54]. The next step is field testing of the prototype that is scheduled for 2016 within the EU founded project SMARTWATER4EUROPE[54]. Among the positive features of 'Bactiquant', utilities mention the rapid and quantitative response of the method[10, 40, 52]. The method requires establishment of a site-specific background level depending on bacteria composition, meaning a higher reading on site A than on site B is not necessarily an indication of higher bacteria numbers on site A. Direct comparison between readings for e.g. Aarhus and Copenhagen is thus not possible, which the utilities consider a drawback[40]. However, a utility has managed to formulate system specific upper and lower boundary levels based on statistical processing of data from a reference period[10]. Cases of increase in bacteria numbers detected by traditional culture based methods but not detected by 'Bactiquant' readings have been experienced[51]. Such deviations need explanations to maintain the utilities' trust in the method.

SBT Aqua ApS is developing a microfluidic device intended to measure total bacterial levels in drinking water based on impedance flow cytometry[55]. Impedance flow cytometry is a technique used mainly in medical research, that essentially detects cells through their dielectric properties[56]. The main advantage of this method is that no specific markers or reagents are needed[56]. Implementation of this method in the microfluidic scale has to deal with several challenges, such as clogging of the channels and high sensitivity needed to detect small changes of impedance from the established background level. Also, the method aims to detect total bacteria levels and not specific indicator microorganisms. At the moment, a prototype installed at Vilstrup Waterworks (Verdo Vand A/S) was operational for two months, although only its functionality was tested and no measurements were done[57]. Measurements have so far been done only under laboratory conditions, and next tests include measurements with wastewater dilutions and in situ measurements at the waterworks[57].

The MiniLab technology is already fully developed by Optiqua, even though the current version requires manual sampling loading[58]. The technology was initially developed to determine the concentration of low molecular weight contaminants and for affinity or binding studies of biomolecules[58]. Detection is based on an optical method that measures the refractive index by Mach-Zehnder interferometry[58]. In brief, the interferometer is integrated in a chip and measures changes in refractive index (bending of light in different media) between a sensing branch, where specific antibodies are immobilised, and a reference branch[59-60]. The sensing cartridge can be reused and needs regeneration every 100-500 tests,

depending on the concentration of the targeted compound or bacteria strain[61]. The company is currently developing a version targeting specific bacteria in drinking water and aims to automate the method within the scope of the aquaSHIELD EU project[61]. Again, the main expected challenge this method will face, is the much higher sensitivity needed to detect bacteria in drinking water compared to other compounds and biomolecules in contaminated samples.

Nwater is currently developing the MicroLab technology, also aiming to detect bacteria in drinking water based on microscopic sensing[65]. Because of their current commercialization negotiations with a project partner, no further information was disclosed at this point[66].

Blusense Diagnostics is a newly founded company that has developed a microfluidic sensor to measure protein and bacteria concentrations in urine and blood. The aim is to apply the same principle for detection of specific bacteria in drinking water[63]. The method combines DNA amplification and binding of targeted nucleotide sequences on detection probes fixed on magnetic nanoparticles in a microfluidic system[70-72]. The project is currently funded by Vandsektorens Teknologiudviklingsfond (VTUF) with Kalundborg Forsyning A/S as one of the project partners, and the expected completion date is by the end of 2016[63-64]. The expected advantage of this method is the rapid detection of specific microorganisms without the need for a growth incubation time. However, the high bacteria diversity and low bacteria concentrations in drinking water may challenge the contact chances between the extracted DNA from these microorganisms and the matching probes.

Development of the above technologies indicates that the field of microbial sensors is rapidly advancing. Although these technologies appear promising, the challenges current market methods experience such as the high sensitivity required, rapid response and low maintenance requirements need to be overcome. According to the manufacturers, release of the above technologies is expected within the next couple of years, if the final development is not subject to major delays.

Table 2. Overview of technologies at the development or validation stage.

Technology name	Bactiline[54]	N/A[55]	MiniLab[58]	MicroLab[65]	N/A
Manufacturing company	**Mycometer A/S**	**SBT Aqua ApS**	**Optiqua (Optisense)**	**Nwater**	**Blusense Diagnostics ApS[62]**
Manufacturing company background	Sells Bactiquant test kit for Detection of bacteria in water[53]	Spin-off from DTU Nanotech	Sells the EventLab technology for chemical contaminant monitoring	N/A	Spin-off from DTU Nanotech
Country of development	Denmark	Denmark	The Netherlands/ Singapore	Finland	Denmark
Analysed parameter	Activity of several gram positive and negative bacteria	Total bacteria	Total bacteria	N/A	Specific bacteria strains[63]
Measuring principle	Enzymatic activity and fluorescence	Impedance flow cytometry	Optical chip inferometry with specific antibody binding	Optical	Molecular extraction and detection of specific genes[63]
Intended application	Drinking water	Drinking water	Drinking water	Drinking water	Drinking water[63]
Validation/ development stage	Developed prototype(s) are currently validated in the laboratory. To follow validation at Vitens water-works (The Netherlands) within EU project SMARTWATER4EUROP[54]	Prototype tested for two months at continuous operation at Vilstrup Vandværk. No measurements done, only robustness of microfluidic system tested[55, 57]	Current version requires manual handling and lack testing in drinking water. Further development and automation during 2016/2017[61]	Prototype developed[66]	Currently the prototype has been developed for detection of proteins in blood and bacteria in urine and not tested in drinking water[63]
Expected release	2016[54]	End 2016[57]	2017	2017[66]	End 2016[63]
Intended installation mode	at-line	at-line	at-line	at-line	N/A
Comments	An automated version of the Bactiquant test[54]	-	Automated combined version of the EventLab and MiniLab (already on the market) is being developed within the aquaSHIELD EU project[61]	-	-
Funding	EU (FP 7) via SMARTWATER4EUROPE project[54]	MUDP[68] and VTUF[69]	EU (H2020) via aquaSHIELD project[67]	N/A	VTUF[64]

N/A : Information not available

3.3 Technologies at the research level

3.3.1 Methods with potential for sensor application

In addition to the methods discussed in section 3.1 and 3.2 which are already implemented in prototype systems, other methods are currently being investigated for their potential to be integrated in a sensor. Table 3 presents an overview of the six most promising methods, along with their main advantages and disadvantages. None of methods provides a practical and simple operational unit in their current version or they are limited to research use.

Immunoassays are commonly used to capture target microorganisms, as they selectively bind to the corresponding anti-bodies[4]. Specific antibodies are commercially available for most indicators, such as *E. coli*, enterococci and *Salmonella*[73]. Immunoassays are therefore often used as a separation or pre-concentration step to be combined with other detection methods[74]. Immunoassays can be automated and integrated in lab-on-a-chip systems[4, 74-75]. Non-targeted microorganisms may be captured as well, giving false positive results[4]. Another significant issue is the very small fraction of the target microorganisms in drinking water, so pre-concentration of large water samples may be required to provide contact opportunity between the antibody and the antigen, necessary to capture the targeted cells. Immunoassays have up to now been used in combination with ATP analyses in water and food[76-81], and in combination with other electrical[75, 82-83] and optical[75, 84] detection methods. Sensitivity of these combined methods may vary a lot, depending on both the efficiency of the immunoassay step and the sensitivity of the detection step, but has in some studies reached as low as of 20 CFU/100 mL in in-situ freshwater samples[80-81].

Polymerase chain reaction (PCR) is a commonly used laboratory molecular method to amplify targeted DNA sequences in a sample to enable subsequent detection and quantification[4]. The amplified DNA sequences are specified by the chosen primers, which essentially are short nucleotide sequences that match the end of the interest region[4]. Primers for a wide variety of microorganisms are currently available, making PCR a powerful detection and quantification tool[4]. Recent research has identified specific primers for *E. coli* and for a broad range of coliforms, making the method suitable for detection of traditionally used indicator organism[44]. The main disadvantage of the method is that is quite complex, although significant research effort is currently focusing on microfluidic method implementation[74, 97]. Microfluidic PCR has the advantage of being faster and potentially less expensive than the traditional laboratory protocol due to the small volumes of expensive reagents used[97]. A commercial implementation of on-chip PCR is already available by Rheonix[96] and has been reported to detect *Cryptosporidium* in water samples[74], while more systems are expected to emerge at the market in the near future.

Fluorescence in situ hybridization (FISH) is another molecular method using fluorescent RNA probes binding to complementary sequences[4]. The typical protocol includes cell treatment with appropriate chemical fixatives, followed by hybridization under stringent conditions with specific probes[4]. Stained cells are detected by epifluorescence microscopy[4]. The advantage of targeting RNA instead of DNA is that detection is more sensitive due to the higher number of copies available and that only viable cells are included[4]. FISH has been integrated in a microfluidic device followed by flow cytometry to detect *E. coli* in pure cultures[95]. The main disadvantage is that due to the low concentrations in drinking water, pre-concentration may be needed[4].

Electrical detection of microbial cells may be based on electrochemical methods[4, 75, 90], on measurement of electrical impedance[90] or on piezoelectric biosensing[90]. The common principle of these methods is measurement of electrical conductance or charge by an electrode. Specifically, electrochemical methods measure the light emitted by labels when they are stimulated electrochemically at the electrode[4]. These labels are typically attached to biological binding reagents and are used for solid-phase binding assays e.g. nucleic-acid hybridization assays of sandwich immunoassays[4]. In principle these assays involve two nucleic acid fragments, one immobilised on a surface and another suitably labelled for use as a hybridization probe[98]. When both fragments are mixed with a sample containing the target sequence, they hybridize and form a complex that becomes attached to the solid support[98]. The advantage of this method is that the sample nucleic acid does not need to be immobilised[98]. Electrical impedance biosensors measure microbial metabolism via an increase in both conductance and capacitance causing a decrease in impedance[90]. Lastly, piezoelectric biosensors use typically immobilised antibodies to coat the sensor surface by the bound bacteria so that the mass of the crystal changes and the resonance frequency of oscillation decreases proportionally[90]. The main advantage of these methods is the high potential for online and microfluidic implementation[74-75, 90]. However, only few studies have demonstrated the application of electrical methods to detect microorganisms in drinking water systems[74].

Flow cytometry is a method to quantify cells in suspension by letting a flow stream of single cells pass through a laser beam and record the forward-scattered light and side-scattered light, as well as fluorescence signals resulting from the beam disturbance[89]. The method has been established for more than 30 years in medical and cancer research, and has been extensively applied in laboratories to quantify bacteria, yeast cells, algae and protozoa during the last two decades[89]. The main advantages of the method is that it is rapid, sensitive and compatible with various staining and labelling methods[89]. Flow cytometry has been implemented in an automated laboratory system measuring bacteria in drinking water within a concentration range of 10^3 to 10^6 cell/mL[91]. An additional advantage of the method is that it can be implemented in a microfluidic system as demonstrated in previous studies[92-93].

A drawback is that result interpretation, especially at the low concentration levels relevant for drinking water systems, can be very subjective and proper standardization of the counting process is essential[89].

Raman spectroscopy is a method that measures inelastic scattering of monochromatic light following excitation[4, 86]. Biological molecules including nucleic acids, proteins, lipids and carbohydrates generate specific Raman spectra that provide biochemical information on the composition and structure of the cells, so that single microorganisms can be identified from the whole cell spectra[4, 86]. High sensitivity for identification of single bacterial cells and the possibility to obtain molecular information without using expensive reagents are the main advantages of the method[4, 86, 88]. Also the method has potential for microfluidic implementation[84]. Raman spectroscopy is however a detection method that needs to be combined with physical or chemical immobilization of the cells[86], which is possible either with optical tweezers or on an antibody coated surface[4, 86].

Microfluidic systems are an potential platform for developing laboratory methods into new sensors. Besides the compact design, microfluidic systems are preferred for their low production cost and small reagent volume requirements, which decrease operational cost. Microfluidic systems are not yet established for use in drinking water systems as a number of challenges still need to be resolved. These include clogging of the microfluidic channels by particles, lime scale or even biofilms after a short time of continuous operation. These risks can be partly reduced by a preceding filtration step, even though it increases system complexity. These filters also need to be cleaned or replaced at regular times. Clogging may actually not be a major issue if the microfluidic cartridge is easily replaceable and cheap, but can still become a considerable maintenance requirement. The main concern with microfluidic systems is the reliability of results, since the very small volume of water sampled may not include organisms only present in low concentrations. Integrated concentration steps are a possible solution to increase the sampled water volume, but they add complexity and maintenance requirements to the system.

Concentration of bacteria from the water can be a way to increase sensitivity in relation to many of the described methods, both for sensors currently available on the market as well as for sensors under development. Several versions of concentration equipment are available, e.g. the 'Døgnprøvetager' (volume sampler) developed by HOFOR A/S[49], and 'Pansi1000' and 'Alonda 1000' from Amphi-bac[48], which are all based on different filtration techniques.

Table 3. Overview of microbial monitoring methods used in research.

Method	Immunoassays	Polymerase chain reaction (PCR)	Fluorescence in situ hybridization (FISH)	Electrical	Flow cytometry	Raman spectroscopy
Principle	Antigen-antibody specific binding[4] Used to capture the target microorganisms and are typically combined with a detection method[74]	DNA amplification and detection by dyes or probes[4]	RNA hybridization and microscopic detection of fluorescent probes bound in cells[4]	Different methods where signal is detected by an electrode. Are typically combined with immunomagnetic separation[75, 82-83]	Labelled cells are suspended in a flow stream that passes through a laser beam[89-90]	Measurement of inelastically scattered light after excitation[4]. Provides quantitative and qualitative information[85]
Sub-categories	Fluorescent immunoassays[4, 90] Immunomagnetic assays[76-81] Immunosorbent assays[4, 90]	-	-	Electrochemical[4, 75, 90] Electrical impedance[90] Piezoelectric[90]	-	Surface enhanced Raman spectroscopy (SERS)[3-4] Lazer tweezer Raman spectroscopy (LTRS)[3-4]
Current application	Various types of water and food to detect *E. coli* [4, 76, 79-81, 90] *Salmonella* [77] *Legionella* [78], Enterococci[80]	Various types of water[4]	Groundwater to detect *E. coli* and bacteria involved in chromium dioxide reduction in a contaminated site[95]	*E. coli* [90] *Staphylococcus* [90] enterotoxin B[82] *Salmonella* [83, 90] *Cryptosporidium* [94]	Bacteria[89] Yeast cells[89] Algae[89] Protozoa[89] Viruses[89]	*E. coli*[86-87] *Streptococcus*[86] *Enterococcus*[86] Bacteria spores[88]
Advantages	High automation potential[4] Can be combined with several detection methods and can target a wide range of microorganisms Potential for lab-on-a-chip implementation[74-75]	Highly sensitive[4] Specific[4] Rapid[4] Potential to detect several micro-organisms in a single reaction[4, 44] Potential for lab-on-a-chip implementation[74-75, 96]	Allows the detection of viable but non-culturable cells[4] Can be combined with flow cytometry in a microfluidic system[95]	Potential for online and lab-on-a-chip implementation[74-75, 90]	Rapid and accurate[89] Compatible with various staining and labelling methods[89] Implemented in an automated and on-line system [91] and in lab-on-a-chip systems[92-93]	Very sensitive[4] Identification of single bacteria cells[86] Molecular information with no reagents use[86, 88] Potential for microfluidic implementation[84]
Disadvantages	Capturing of non-targeted microorganisms[4] Rapid but not quantitative[4] Sample concentration needed[4]	Complex method	Enrichment steps may be required[4]	Piezoelectric sensors require incubation time[90]	Subjective data interpretation[89] Bacteria detection requires high sensitivity of detection instruments[90] High cost[90]	Cells must be immobilised by physical or chemical contact[86]

3.3.2 Ongoing research

Sensor development and implementation has been the topic of numerous research projects (see examples of concluded projects in appendix II). Currently three large projects are funded at the national level and five large projects are funded at the European level (Table 4). Overall these projects aim either to develop new sensors, or to further validate and optimise already prototyped sensors. Information about these projects and their specific aims is available online, but as the projects are ongoing, publications or outcomes are not yet publically available. Some large European projects e.g. the SMARTWATER4EUROPE and the Aquavalens have midterm deliverables that are kept confidential, and only short summaries are publically available, not disclosing any progress details. Therefore it is not possible to establish the current status and achievements of these projects.

At the national level, the Vandsektorens Teknologiudviklingsfond and the Danish Ministry of the Environment have (partly) funded 3 projects in the sensor field (Table 4). Among them, the 'Future water' project is by far the largest, with a total budget of 20,904,000 DKK[99]. The project aims to resolve several challenges within the drinking water sector and is organised in eight work packages[99-100]. One of the work packages aims to provide a critical analysis of commercial and near-commercial technologies and to develop a system composed of individual alarm, auto-sampling and characterization units[100]. Partners involved in this work package, which is completed by the end of 2015, are Ringkøbing-Skjern Forsyning A/S, VIA University College, Amphi-Bac ApS, Alectia A/S, Minus 10dB ApS [99-100]. Data from the project was not available at the time of finalising the present report. Another approach to water quality monitoring was taken in work package 2[29] by Krüger A/S, Aarhus Vand A/S and VandCenter Syd, who aimed at developing algorithms to process large amount of data generated by online sensors. The aim was to apply the software in day-to-day operations, system optimization and fault finding.

Blusense diagnostics ApS is also currently involved in a project partly funded by VTUF and with a total budget of 4,124,000 DKK[64]. The aim is to test an already developed prototype that detects proteins and bacteria in urine and blood (described in section 3.2) for detection of *E. coli* in drinking water[64]. The project ends March 1st 2016[64].

Lastly, SBT Aqua ApS was also granted a fund from VTUF in the project 'Real-time water quality monitoring by electrical detection', with a total budget of 2,642,000 DKK[69]. The project aims to validate the technology described in section 3.2 by long term installation and monitoring at the waterworks[69]. The project ends September 1st 2016[69].

Table 4. Overview of larger ongoing national or European research projects.

Research project or method name	Future water WP 8- Biosensors[100]	Detection of *E. coli* DNA in distribution systems[64]	Real-time water quality monitoring by electrical detection[69]	AQUAWARN[103]	SMARTWATER4-EUROPE[107-108]	Aquavalens[104-105]	AquaSHIELD[103]	AQUAVIR[101-102]
Involved institutions and companies	Ringkøbing-Skjern Forsyning A/S VIA University College Amphi-Bac ApS Alectia A/S Minus 10dB ApS	Blusense Diagnostics	SBT Aqua Verdo Vand A/S Svendborg Vand A/S Sønderborg Vandforsyning A/S Guldborgsund Forsyning/Nykø-bing Waterworks	6 EU partners	Mycometer A/S and 19 EU partners	DTU Environment National Food Institute Højmarklaborato-riet A/S IPU A/S Nordvand A/S and 34 EU partners	Optiqua (Optisens)	DTU Nanotech Unisensor A/S Delta A/S DHI A/S and 10 EU partners
Country	Denmark	Denmark	Denmark	UK and other EU countries	Several EU countries	Several EU countries	The Netherlands	Several EU countries
Duration	Sept 2013 - Dec 2015	Jul 2015- Mar 2016	Jan 2015- Sept 2016	Dec 2013- Nov 2015	Jan 2014- Dec 2017	Feb 2013- Jan 2018	Jan 2015- Jan 2017	Nov 2013- Oct 2016
Relevant aim	Review of existing systems and potential development of single components for alarm, automated sampling and characterization of a contamination	Development of a cheap sensor to monitor *E. coli* in the distribution system within 30 min	Further development of the SBT Aqua sensor	Development of an integrated deployable device for the detection of pollution in water using state-of-the-art microfluidic technology	Further development and integration of sensor technologies	Development of automated detection methods for microbial water quality monitoring	Integration of existing technologies EventLab and MiniLab into a single sensor to detection chemical and microbial contamination	Development of a microfluidic automated virus analyser
Funding	VTUF, MUDP	VTUF	VTUF, MUDP	EU (FP7-SME-2013)	EU (FP7-CP)	EU (FP7)	EU (H2020)	EU (FP7-CP)
Contact	VandCenter Syd A/S (H. Juul)	Blusense Diagnostics ApS	SBT Aqua ApS (G.E. Skands)	T.E. LABORATORIES LIMITED	Vitens utility, The Netherlands	University of East Anglia	Optiqua (Optisens)	DTU Nanotech

N/A: Information not available

At the European level, the project 'AQUAWARN' has been granted a 1,294,659 EUR total budget and ends November 30th 2015. This project aim was to develop an integrated deployable device for the detection of contamination in water using microfluidic technology[103]. The 'AQUAWARN' device aims to be used for monitoring of selected water quality parameters in wastewater and environmental waters[103], although no specifications are given about the targeted parameters or the measurement principles. The envisioned device will be low-cost and transportable, and will be linked to a process control device and an auto-sampler. The data or an eventual alarm will be sent to a mobile phone or a laptop[103]. The project is coordinated by T. E. LABORATORIES LIMITED, an Irish environmental laboratory and chemical manufacturing company, and includes three other private companies, the Dublin City University and the UK Natural Environment Research Council[103].

'SMARTWATER4EUROPE' is a larger EU project, granted 10,043,233 EUR for a three-year period ending December 2017[107]. The project overall aims to demonstrate integrated solutions for water supply and is organised in 12 work packages, of which one specifically aims to further develop sensor technologies and to validate their use at selected demonstration sites[108]. The 'Bactiline' technology developed by Mycometer A/S will be tested at Vitens utility, which is the largest water supply company in The Netherlands[54]. The project consortium consists of 12 small-medium enterprises (SMEs), three water utilities, three research institutes, one company and two platform organisations[107].

'Aquavalens' is another large EU project relevant for water quality sensors, with a total budget of 11,909,166 EUR[105]. The project runs for five years and ends January 31st 2018[105]. The overall aim is to develop methods and practices to detect pathogens in drinking water and in water used for food preparation[104-105]. The project is organised in 15 work packages grouped in four clusters or main development phases[104]. Within these, DTU Environment is involved in a work package that aims to develop an automated platform for detection, based on ATP concentration measurements. DTU Environment is also involved in another work package that aims to test the developed method in large scale water supplies[104]. The project overall involves 18 academic and non-profit organizations and 21 SMEs across Europe[104].

The 'aquaSHIELD' project has been granted 1,123,136 EUR to further develop an integrated sensor solution developed by the Dutch company Optiqua[67]. Specifically, the aim of the project is to combine two already developed sensor components for online monitoring, which monitor chlorine residual, and performs rapid screening of a set of high priority threat substances[67]. The project involves only Optiqua and ends January 1st 2017[67].

Lastly, the 'AQUAVIR' project has been granted 5,246,429 EUR for three years, and ends October 31st 2016[102]. The project aims to develop a portable, on-site microfluidic system to detect viruses in different

freshwater water bodies[101-102]. The virus particles will be concentrated and detected by electrical read-out in the microfluidic cartridge[102]. Viruses in focus are norovirus, Hepatitis A and rotavirus and the target detection limit is 0.01-1 virus/L[102]. The project is coordinated by DTU Nanotech and involves 13 more partners[101].

Overall, research projects have set ambitious goals towards the development of optimised sensor systems. Apart from the 'aquaSHIELD' project, the above projects are collaborations between academic partners and private companies. Such collaborations ensure that the work is scientific founded and at the same time focuses on the development of market technologies for full-scale application.

4 Discussion

4.1 Where are we today?

Sensors for monitoring of microbial drinking water quality have seen great development in the recent years, but still the 'ideal sensor' as defined by the utilities (with total coliforms and *E. coli* in focus) is not yet available. Development of new sensors is a time consuming and complicated process, and it is important to realise that there is a long way from an initial conceptual idea to successful development of a new microbial sensor. The process generally stretches over years or decades and requires substantial funding as demonstrated by the list of current projects (Table 4). It is fairly common that a new technology is developed and validated within several research projects, as one funding source typically is not sufficient to cover all development stages. Insufficient funding can be a contributing factor to why concepts of the past have not managed to become fully developed into new technologies. Another contributing factor can be that the principle of the concept is not suited for application in drinking water. Drinking water is characterised by a large number of bacteria with high diversity living in an oligotrophic environment, thus being constantly starved with a low energy turnover. It is therefore not always possible to transfer a technology developed for other fields e.g. food industry or medical diagnostics, as these environments are often characterised by high nutrients levels and growth of single or few bacterial strains.

With the current state of the art, microbial sensors can be divided into two main categories, sensors targeting specific microorganisms and sensors targeting total bacteria levels.

4.2 Sensors targeting specific microorganisms

The primary reason to monitor microbial drinking water quality is to prevent pathogenic organisms from reaching the consumers through the water. It is problematic to detect specific pathogenic organisms, due to low numbers of pathogens, high background levels of bacteria and complicated and/or time consuming analysis methods. Therefore monitoring is instead based on indicator organisms i.e. organisms present in large numbers together with the pathogens, which are more simpel to detect. Historically, total coliforms and *E. coli* have been used as indicators for contamination (*E. coli* specifically for faecal contamination). The indicators are not necessary pathogenic themselves, in fact only few *E. coli* strains are pathogenic[110].

The utilities have long time experience with monitoring of total coliforms and *E. coli*, and there thus exist extensive historical reference material. Monitoring of total coliforms and *E. coli* is part of the Danish regulation of microbial water quality, with a guideline value of less than one coliform or *E. coli* per 100 mL of water[34] (the same as the detection limit of the current guideline method). The indicator

monitoring is comparable to looking for a needle in a haystack, meaning that a method with high sensitivity is required.

Today, most sensors targeting indicator microorganisms are based on enzymatic activity measurements and are essentially automated versions of the Colilert test kit (Table 1). These methods need an incubation time to reach sufficiently high cell numbers for detection of a colour reaction, and thus real-time detection is not possible.

Close to real-time detection of indicator organisms might be possible in the future by sensors based on molecular methods e. g. hybridization and PCR. However, these methods need yet to overcome significant challenges before becoming implemented in a sensor context, e.g. successfully bringing the target microorganisms in contact with the coated surface so the specific binding can take place. Additionally, due to the low concentrations of the indicators in drinking water, a pre-concentration step is likely to be needed, which is an additional challenge for integration in an automated system.

If molecular methods are to be integrated in a sensor, it expands the possibilities of targeting specific organisms and it should then be considered whether to monitor for specific pathogenic bacteria, protozoa or viruses and not only for indicators. Enhanced detection of specific organisms may also open up the discussion on whether total coliforms and *E. coli* are the optimal indicator organisms or if others may be used.

4.3 Sensors targeting total bacteria levels

The alternative to sensors for monitoring specific microorganisms are sensors for monitoring total bacteria levels, either by cell numbers or by ATP or enzymatic activity measurement. These sensor technologies are rapid and give close to real-time response.

Sensors for total bacteria levels are used to identify changes from a background level. Therefore establishment of background levels and variations under normal operating conditions for the specific system and location is necessary. Variation can be identified at two levels:

1. Variations due to normal operating conditions e.g. hydraulic conditions, well combination, filter backwashing etc.
2. Variation due to contaminations entering the system

Identifying a contamination as a deviation in the total bacteria level demands a good understanding of and experience with the system. Optimisation of system operation can give a more stable and distinct variation pattern, making it easier to identify variations. The use of algorithms referring to a defined

reference period can be a necessary tool to correlate variations and operation conditions, and thereby making it possible to identify variation, which might indicate contamination. An unexpected variation can be caused by other factors than a contamination, but a variation which cannot immediately be explained by normal operation conditions should lead to further investigations.

Since bacteria concentrations in drinking water are in the range of 10^4-10^6 cells/ml it will be difficult to detect a small contamination over the noise on the background level.

4.4 Combination of sensors

The 'ideal sensor' is ultimately a single sensor that combines several of the above advantages and overcomes the shortcomings discussed previously. This might be an unrealistic and too ambitious goal, and instead a combination of sensors may be the way ahead. Sensors for monitoring microbial quality combined with sensors for monitoring physicochemical parameters (e.g. turbidity, oxygen, conductivity, pH, temperature) can provide more information about a potential contamination. Sensors for physicochemical parameters are fully developed and available from many technology providers in different designs and set-ups. Multiple sensor set-ups are also available as e.g. the 'Intellisonde' technology developed by Intellitect Water Limited that combines monitoring of 11 physicochemical parameters in a single sensor[111].

Monitoring of several parameters simultaneously has the disadvantage of increasing the total cost, since more sensors need to be purchased and maintained, potentially becoming unaffordable for smaller utilities. An additional consideration is the large amount of data that needs to be evaluated. In depth knowledge of the system is crucial when navigating through this increased data log to ensure meaningful interpretation of variations. Algorithms specifically fitted for each system can be a necessary tool to identify an unexpected variation that requires further action. This is currently approached in the research project Future Water[29].

4.5 Monitoring approach

Microbial monitoring has traditionally focused on identifying contaminations. An alternative monitoring approach is to prevent the contaminations from occurring by monitoring of barrier efficiency and of high risk points, such as valves. These monitoring schemes may differ significantly from utility to utility depending on the system set-up and on the specific high risk points in each individual system.

Instead of planning for a universal monitoring strategy, it can be meaningful to design monitoring strategies for specific sections of the system or for specific scenarios. This demands that each utility performs a system analysis to identify potential risks for different sections of the system, and identifies what kind of information would be most beneficial to acquire in each case. This is a process closely

linked to the utilities' HACCP work (in Danish DDS) and scenario-based monitoring planning can be a tool to optimise monitoring strategy and prepare for acute situations.

Appendix

I. Technologies developed by closed down companies

Table I presents an overview of partly developed technologies from closed down companies. Limited information about these technologies is available online, and it is not known how far they actually got in the development phase and why these companies closed down. Both Early Warning inc. and Heed Diagnostics ApS worked on a molecular method that aimed to detect specific microorganisms by RNA hybridization on surface. The 'Biosentry' method was based on optical recognition of specific bacteria and protozoa, although no details on this method were available.

Table I. Overview of technologies (partly) developed by closed down companies.

Technology name	Early warning[115]	Biosentry[114]	N/A
Manufacturing company	**Early warning Inc.**	**Jmar**	**Heed Diagnostics ApS**[112-113]
Country	USA	USA	Denmark
Analysed parameter	Bacteria, protozoa, viruses	Specific bacteria and protozoa	*E.coli* and planned to extended to total coliforms and *Bacteroides*
Measuring principle	Magnetic bead separation and RNA hybridization on surface	Optical	Concentration and RNA hybridization
Field of application	Drinking water	Drinking water, food and beverage industry	Drinking water
Measuring unit	Cells/L	N/A	N/A
Intended installation mode	at-line	at-line	at-line
Response time	2-3 h	N/A	N/A
Sampling volume	10 L	N/A	N/A
Comments	Spin-off company from NASA. System was for rental	-	Technology was tested in the laboratory

N/A: Information not available

II. Concluded research projects

Table II presents an overview of concluded national and international projects relevant for microbial sensor development. The 'DEMOWATERCOLI' EU project was granted 1,165,988 EUR to test and validate the 'CALM' technology developed by Colifast[16]. No publication or final report is available from this project. Heed Diagnostics ApS was granted 2,492,000 DKK to test the 2nd generation prototype for measuring bacteria in drinking water based on RNA hybridization[116]. Within this project, the method was further developed[113], although following, the project the company closed down. The project 'AQUA fingerprint' aimed to demonstrate an online method based on fluorescence to identify faecal contamination in drinking, surface, overflow and swimming pool water[117]. Project partners were DTU Environment, DTU Aqua, TREFOR A/S and Krüger A/S[118]. The project concluded that the method was robust with potential to be implemented in an online system that can be modified to target other microorganisms of interest[117]. Lastly, Amphi-Bac ApS was granted 2,060,000 DKK for a project aiming to develop a DNA kit to identify sources of microbial contamination[119]. According to the final report of the project, the kit was partly developed, although not yet ready for commercializsation[120].

Table II. Overview of concluded sensor projects.

Research project or method name	DEMOWATERCOLI[16]	Compatibility assessment and field testing for a bacteria sensor[112, 116]	AQUA fingerprint[118]	DNA kit to identify sources of microbial contamination in drinking water[119]
Involved institutions and companies	4 EU partners	Heed Diagnostics Aps (closed down company)	DTU Environment DTU Aqua TREFOR A/S and Krüger A/S	Amphi-Bac ApS
Country(ies)	Norway, UK, Italy, France	Denmark	Denmark	Denmark
Duration	Jan 2001- Nov 2003	Jan 2012- Jun 2013	Mar 2009- Nov 2010	Jan 2012- Jan 2015
Relevant aim	Validation of the Colifast 'CALM' technology	Testing of a 2nd generation chip prototype for measuring bacteria	Use of florescence measurements to identify faecal contamination in different types of water	Development of a kit that reveals the source of DNA present in water e.g. from bacteria, snails etc.
Publication(s)	N/A	Final report for VTUF[113]	Final report for Naturstyrelsen[117]	Final report for VTUF[120]
Funding	DEM - Demonstration contracts (FP 5)	VTUF	Miljøstyrelsen	VTUF
Contact	Colifast A/S	Harper & Vedel (J.R. Amossen)	DTU Environment (Emeritus E. Arvin)	Amphi-Bac (S. Bastholm)

N/A : Information not available

III. Manual methods

Table III presents an overview of manual methods for monitoring microbial drinking water quality, which has a potential for automation. Within these methods, the 'Microsnap', 'Colilert' and 'ScanVIT' target specific indicator microorganisms, while 'Bactiquant' measures bacterial activity level and 'Cyflowcube' measures total bacteria levels (Table III). 'Colilert' (the most commonly used test kit for total coliforms and *E. coli*) and 'Microsnap' are based on measurement of enzymatic activity[28], as described in section 3.1.1. The same principle, also used by 'ScanVIT', is a method based on fluorescence microscopy that detects *E. coli* and total coliforms within 3 hours[121]. 'Bactiquant' measures activity of several gram positive and negative bacteria based on enzymatic activity and is currently being automated into the 'Bactiline' technology[54]. 'Cyflowcube' measures total bacteria concentration by flow cytometry[122].

Table III. Overview of most common manual methods to detect specific indicator microorganisms or total bacteria levels.

Method name	Bactiquant[53]	Microsnap[124]	Colilert [28]	Cyflowcube[122]	ScanVIT[121]
Manufacturing company	Mycometer A/S	Hygiena	Idexx	Sysmex Partec	Vermicon
Country	Denmark	USA	Worldwide	Germany (and worldwide)	Germany
Analysed parameter	Several gram positive and negative bacteria	*E.coli* Enterobacteriaceae Total coliforms	*E. coli* Total coliforms	Total bacteria	*E. coli* Total coliforms
Principle	Enzymatic activity and fluorescence	Enzymatic activity/ Biomarkers and fluorescence	β-galactosidase activity (yellow) and β-glucuroni-dase activity (fluorescence)	Flow cytometry	Fluorescence microscopy
Current field of application	Drinking water	Food, water, surfaces	Drinking water	Drinking water	Drinking and bathing water
Validation documentation	ETV[125]	N/A	USA EPA approved	N/A	N/A
Nominal detection limit	N/A	10 CFU/mL	N/A	1000 cells/mL[123]	N/A
Response time	< 1h	7- 8 h depending on concentration	18 h	N/A	3-12 h

N/A : Information not available, CFU: Colony forming units

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