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The use of the inline bacterial sensors BACMON and BACTcontrol to measure the bacterial water quality

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Preface

This TKI project on the performance of the microbial sensors BACTcontrol and BACMON started in January 2017 with laboratory analyses and validation experiments. In the summer of 2018 the two sensors were tested in an online setting at different full-scale locations of the several partners.

In June 2019 the project group was informed that Grundfos will stop all services of the BACMON per 1 February 2020. Although this date lies outside of the time span of the TKI project, it was discussed whether, and if so, how we should continue the project as was originally planned at the start of the project. The main objection was that when one of the partners becomes interested in the BACMON sensor after testing, it will not be available to purchase. For several reasons it was decided that the project will be continued as if the BACMON was not withdrawn from the market and that all results of the BACMON (laboratory studies and full-scale locations) should be reported. There are several reasons for this:

- A large part of the project is to compare both sensors to laboratory analyses but also to compare them to each other. Especially in the full-scale locations, in which in an online setting the sensors were tested and were not frequently compared with laboratory analyses. For example peaks detected with the BACTcontrol can be verified with the measurements of the BACMON sensor.
- The BACMON measures the total number of cells (bacteria) but there are more online sensors available that are based on the principle of measuring total number of cells, although the technique might be different (flow cytometry, enzyme based, etc). By analyzing and reporting the bacterial data of the BACMON, information is obtained including insight in how this parameter can be useful in the future for monitoring the water quality at the drinking water production, drinking water distribution, surface water or cooling tower water. As it is always unknown what future brings, maybe the BACMON or a comparable device will be on the market in the future and the current results may be necessary.
- The TKI grant of this project was granted based on the project plan in which was described that both sensors would be tested. Although it is possible, it is not easy to deviate from the project plan in such a major way as leaving the BACMON out of the report.

Summary

The online microbiological sensors BACTcontrol and BACMON are new, rapid, online methods to determine total bacteria numbers or activity. These methods might be suitable for the online monitoring of any microbial disturbances in different water types and/or of the effect of control measures on water quality. However, before they can be reliably implemented, supplementary experimental research is needed. The aim of this TKI project was to validate both sensors under laboratory conditions, test their performance at several full-scale locations and compare the result with each other as well as with other parameters that were determined at these full-scale locations.

The validation of the BACMON and the BACTcontrol under laboratory conditions were done with dilution series of different water types. The results from this validation showed that in general the BACMON data correlated well with ATP and the bacterial cell numbers determined with flow cytometry or microscopy. Despite the good correlation between the bacterial numbers measured with the BACMON sensor, flow cytometry and microscopy, the bacterial numbers obtained with the BACMON were 1 to 2 log units lower than bacterial numbers obtained with the other two methods. The same result was observed with the water types tested under full-scale conditions. The BACTcontrol data from the dilution series of a surface water sample showed good correlations with the other biomass or cell number data, but such correlations were not observed for the dilution series of other water types.

Subsequently, both sensors were tested at full-scale drinking water locations of Pidpa and Vitens, full-scale surface and cooling tower water of Uniper and full-scale raw water and raw water treated with rapid sand filters at the WRK in Nieuwegein. In general, the BACMON sensor performed well with most of the different water types tested at the full-scale locations. The only exception to this was one of the surface water types, where the performance was less due to high concentrations of non-bacterial particles. These high concentrations caused that only a small volume (on average 15% of the normal volume) could be analyzed with the BACMON sensor. Furthermore, the detection limit of the sensor is relatively low (300 – 500 bacterial cells/ml) for the water types tested. Although the BACTcontrol sensor could measure enzymatic activities in the water types tested, technical errors were regularly observed. These included too high pressure, detection fault, injection volumes of reagents incorrect, air bubbles in the system, influence on activity after reagents are changed, unknown malfunctions and higher enzymatic activity values directly after sensor maintenance. Furthermore, the detection limit corresponds to around 1×10^4 bacterial cells/ml, which is around the total number of cells that can be present in some drinking water types in the Netherlands.

It was also studied whether event detections by the BACMON or BACTcontrol sensor could be related to operational parameters at these full-scale sites. The peaks observed in the BACMON data were in general related to operational parameters at the full-scale location, such as change in decanter used or back-flushing of sand filters. Furthermore, a forced peak event at one of these locations also resulted in higher bacterial numbers detected by the BACMON sensor. The BACMON data from surface water and surface water treated with rapid sand filtration showed regular repetitive day/night patterns, which was also observed with the turbidity sensor. However, there were also peaks

observed in bacterial or non-bacterial numbers with the BACMON sensor on these water types that did not coincidence with operational or other parameters. The data from the BACTcontrol sensor on the drinking water types showed no or poor relations with changes in operational parameters (e.g. change in decanters, backwash flushing of sand filters). In addition, a forced peak event in one of these drinking water systems was not detected with the BACTcontrol sensor.

During a short period of the monitoring time at the full-scale locations, samples were taken to determine different microbiological parameters at the laboratory and those results were compared with the sensor data. The BACMON or the BACTcontrol data were in general not correlated to ATP or cell numbers determined with flow cytometry or microscopy. Moreover, also ATP, cell numbers determined with flow cytometry and cell numbers determined with microscopy were not correlated for most water types. Finally, the BACMON and BACTcontrol data from these full-scale locations were also not correlated with each other.

For the BACMON sensor it is concluded (i) that the sensor performs technically well on drinking water, cooling tower water and surface water after rapid sand filtration, (ii) that the bacterial numbers, quantified with the BACMON sensor, flow cytometry and microscopy, in dilutions of the same water sample correlate well with each other, but that such correlations were not observed at the full-scale locations, (iii) that the users were generally positive about using the sensor and the results that were obtained, and (iv) that in general the BACMON sensor is a reliable sensor to detect events that influence bacterial water quality.

For the BACTcontrol sensor it is concluded (i) that currently the BACTcontrol sensor cannot be reliably used to continuously measure the total bacterial activity in water, (ii) that in general the alkaline phosphatase enzymatic activity determined with the BACTcontrol in water does not correlate with other biomass or cell number parameters, (iii) that the users were less positive than for the BACMON sensor, mainly because of the malfunctions of the BACTcontrol sensor and (iv) that for now the BACTcontrol sensor is not suitable to reliably detect changes in water quality that relate to operational parameters or other water quality parameters.

Because the BACMON sensor has been taken out of the market, no further recommendations are made for this sensor. For the BACTcontrol sensor it is recommended to improve and optimize the BACTcontrol sensor in order to significantly reduce the technical errors, malfunction and reduce the detection limit of the sensor.

Contents

1 Introduction

1.1 Online sensoring

Development of methods for real-time monitoring of microbiological drinking water quality opens new opportunities for the waterworks. At the same time, the rapid development of data communication opens many new opportunities in monitoring, intelligent control and security that can be used by the waterworks to secure safe water to their customers (Skovhus and Højris 2018).

Routine sampling, transport of samples and the subsequent manual microbiological water quality analyses in the laboratory are time-consuming activities, which means that the test results of classical culturing methods are only known one to a few days after the water has left the waterworks. In addition, many types of microorganisms cannot be grown in the laboratory, despite being viable in their natural environment (Byrd et al 1991; Allen et al. 2004; Chowdhury 2012; Li et al., 2014), so these organisms are not measured in routine assays that use cultivating techniques. In the event of an outbreak of potentially pathogenic microorganisms, waterworks are rarely aware that something is wrong before it is too late: when analyses are ready for interpretation, drinking water has long been distributed to the consumers who first discover and experience the consequence of a critical pollution.

Online monitoring allows utilities to provide greater assurance of good drinking water quality and to respond in a timely manner to events affecting drinking water quality. However, there are also challenges related to the new sensor technologies: the interpretation of many data and the subsequent decision-making process requires increased system knowledge among the persons responsible for this task. Sensors located throughout the distribution system have the potential to follow water quality changes through a distribution network, but this will only be profitable in a situation where the sensors are relatively inexpensive and maintenance free. There is also a challenge in comparing results of online sensors with laboratory methods, making it difficult for sensor technology to be accepted as a substitute for traditional methods; not the least because the cultivation-based methods often produce results that cannot be directly compared to other parameters such as cell numbers or ATP, because often only a small part of e.g. the bacteria in a water sample appear in plate count analysis.

Ultimately, the best solution is probably not one sensor that meets all requirements, but rather a division of tasks into event alarms, automatic sampling and laboratory characterization. In such a system, sensors based on different principles would provide the alarm while advanced automatic samplers combined with laboratory analyses could provide additional characterization. With this diverse system approach, many contamination events can be detected and diagnosed, regardless of whether they have occurred abruptly or over time.

1.2 Current knowledge/validation studies/full-scale locations already published

The water quality within a drinking water distribution network, particularly regarding microbiological parameters, may be highly dynamic, due to alternating demands, changing water supplies, maintenance work, etc. The probability of catching a periodic

pollution event, by grab sampling, is thus very small (Besmer & Hammes 2016; Pinto et al. 2014) and the chance of acting on it even smaller.

Attempting to close the gap between time-consuming grab-sampling followed by laboratory analyses and highly dynamic water distribution, many water works have turned to fast, but indirect, measurements like conductivity, oxygen level, pH, and turbidity, which continuously are becoming more robust and maintenance free (Banna et al. 2014; Raich 2013; Storey et al. 2011; Lee, A. et al. 2012). However, they do not measure changes in microbiological load, but merely provide indicators that may or may not correlate with the number and activity of microorganisms. Also, since these parameters respond to more than just bacterial content, they are likely to show false positives and false negatives in terms of microbiological pollution detection.

Methods that target microorganisms more directly do exist, though they are mostly automated laboratory methods (Wang et al. 2013) or require handling of samples prior to analysis (Lopez-Roldan et al. 2013). Some of the most recent methods that have been tested in the field include flow cytometry (Priest et al. 2013), ATP (Vang et al. 2014), and optical bacteria detection (Højris et al. 2016). Both flow cytometry and ATP monitoring requires sensitive chemicals, creates waste, and requires regular maintenance. The latter optical technology is, on the other hand, chemical free and requires little maintenance. It was recently demonstrated in laboratory and field tests to be able to monitor the concentration of bacteria and abiotic particles with a ten-minute resolution (Højris et al. 2016; Højris et al. 2018).

Other monitoring solutions directly targets indicator organisms like *Escherichia* coli (Appels et al. 2018) utilizing detection of specific enzyme activities. Online versions of such methods, like the BACTcontrol and ColiMinder, are available and have been demonstrated to work in a range of different water qualities (Albrechtsen et al. 2018).

Using online sensors for monitoring total and/or specific bacteria supports far more advanced optimization than what is usually applied. By closely following trends and dynamics knowledge of the interconnections between operational parameters and microbiological water quality is increased. An example hereof was published by Bertelli and coworkers, investigating the effect of reducing chlorine levels in drinking water on the total bacterial number (Bertelli et al. 2018).

Traditional microbiological monitoring of water samples relies on collecting grab water samples and quantifying the pathogenic microorganisms after culturing either by counting of colony forming units (CFU) on plates or by presence/absence tests including Most Probable Number (MPN) tests. Modern methods for microbiological detection, like enzymatic fluorescence techniques, aim at mitigating several drawbacks of existing standard microbiological tests (Whalen et al., 2018; Appels et al., 2018):

- Analysis time: Time between sampling and the results of standard analysis is > 24h to days. Online methods like BACTcontrol improve and speed-up the detectability of incidents (‘early-warning system’). The short reaction time (< 2 hours) is an advantage of the enzymatic monitoring technique.
- Analysis frequency: The cost of sampling and analysis limits the number of sampling sites and the sampling frequencies resulting in very low probabilities of detecting contamination incidents. Online monitoring systems embedded in the treatment plant and distribution system can detect and characterize bacteriological targets in real- time, all time.

Assessing the metabolic activity of microorganisms, the BACTcontrol also overcomes two other limitations of traditional methods:

- Clumping/particle attachment: Bacteria clumping to particles lead to an underestimation of actual numbers present in the sample.
- Culturability: Most water microorganisms cannot be cultured, leading to an underestimation of the total bacteria population. Enzyme activity is also shown in viable but non-culturable bacteria.

Compared to alternative promising microbiological monitoring techniques (i.e. Optical, ATP and flow cytometry methods), fluorescence techniques measuring enzymatic activity are the most reliable and ready alternatives to culture methods capable of detecting specific indicator organisms.

The key value proposition of BACTcontrol against other enzyme activity online monitor systems is its capability of measuring multiple specific bacteria (*E.coli,* coliforms, *Enterococci,* total bacteria activity).

1.3 General and more specific information BACTcontrol and BACMON, including measurement principle

1.3.1 BACMON

The GRUNDFOS BACMON sensor is in essence an automatic, intelligent microscope. Based on patented 3D scanning optics it classifies all particles as bacteria or non-bacteria, by automatically moving a digital microscope over a flow cell. The robust algorithm classifies particles by evaluating 59 quantified image parameters (Højris et al. 2016; Olesen et al. 2018).

Always online and fully integrated into available platforms, data is available within minutes for access on a mobile device or PC.

The technology is capable of distinguishing and quantifying bacteria and non-bacteria in pure and mixed suspensions and the quantification correlates with total bacterial counts (Højris et al., 2016; 2018).

The sensor consists of:

1. An optical flow cell holding the water sample during analysis
2. An imaging setup, with an LED light source, a magnification lens and a CMOS based camera arrangement, angled with respect to the flow cell to scan a volume.
3. An image analysis system identifying and classifying individual particles based on the recorded stacks

Figure 1 shows a schematic of the flow cell and imaging setup, consisting of a light source, lens and a camera. The imaging setup is angled with respect to the flow cell, to be able to scan a volume. During a measurement, the imaging setup tracks along the flow cell, acquiring up to thousand images in the process.

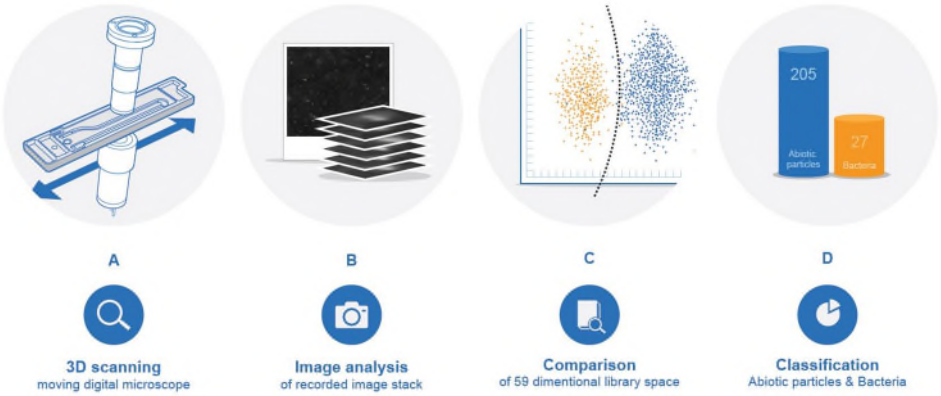


Figure 1. Schematic of sampling and image processing in BACMON (Højris et al. 2016).

Water is led through the flow cell by source pressure or by a peristaltic inlet pump in case of a non-pressurised source (Figure 1A). Initiating each measurement, a water sample is sealed inside the flow cell by two valves. The flow cell is designed to handle pressurised sources to minimise degassing and subsequent bubble formation. The design of the flow cell minimises internal circulation causing the particles to be spatially fixed. The tilted imaging setup is then scanned along the length of the flow cell causing the acquired images to span a fixed volume. After sampling and image acquisition, the source water is used to thoroughly flush the cell, preparing it for the next sample.

Figure 1B shows an image stack of a particle coming into focus and out again as the tilted image plane moves across it. Not only does this allow BACMON to evaluate the particle in focus, but it also gives information about how the particle behaves over time. All this is condensed into information about whether it is a bacterium or a non-bacteria in the subsequent image processing.

The step size of the imaging setup is less than the depth of field for the imaging setup to ensure all particles are imaged in the focus. At the same time, this has the consequence that each particle will be found on many images acquired during a scan, in and out of the focus plane. Due to the tilting of the acquisition setup, a particular particle may show up as out of focus on the first images, in focus on the middle ones, and out of focus again on the later ones.

Each identified particle is evaluated and characterised according to 59 different parameters. These parameters are used to compare the particle to a preinstalled library, and through a neural network classifier determine whether the identified particle is “bacteria” or “non-bacteria” (Figure 1C). As not only information about the particle in focus is revealed, but also information about the diffraction of light by the particular particle, this technique enables classification of specific particle classes, such as bacteria and non-bacteria.

Classification of particles in “bacteria” and “non-bacteria”. The concentration per sample (total count) is shown in count/ml (Figure 1D).

1.3.2 BACTcontrol

The BACTcontrol detects microbiological activity in water samples using enzymatic reactions that make specific bacteria visible for fluorescence detection.

The BACTcontrol monitors the different enzyme activities as indicators for the presence of bacterial contamination. The enzyme activity is detected by adding a substrate-specific reagents containing fluorescent indicators (see Table 1). The reagents hydrolyse with the enzymes to 4-methylumbelliferone (MUF) which fluoresces after excitation via UV irradiation (λex 360 nm; λem 450 nm).

Table 1. Pathogen specific enzymes detected and reagents used in BACTcontrol online monitoring device

Target organism	Enzyme	Reagent
E. coli	ß-glucuronidase (GLUC)	4-methylumbelliferyl-ß-D-glucuronide (MUG)
Coliforms	ß-galactosidase (GAL)	4-methylumbelliferyl-ß-D-galactopyranoside (MUGal)
Enterococci	ß-glucosidase (GLUCAN)	4-methylumbelliferyl-ß-D-glucopyranoside (MUGlu)
Total activity	alkaline phosphatase (ALP)	4-methylumbelliferyl-ß-D-phosphate (MUP)

The BACTcontrol analyser consists of a reactor with two chambers that are separated by a ceramic reusable filter with a pore size of 0.45µm. In the reactor, the water sample is concentrated by the filter, the temperature is stabilized and the enzymatic reaction is started, while the water sample is constantly stirred by a magnetic stirrer. Further the device includes a fluorescence detector to measure the enzymatic activities (Figure 2).

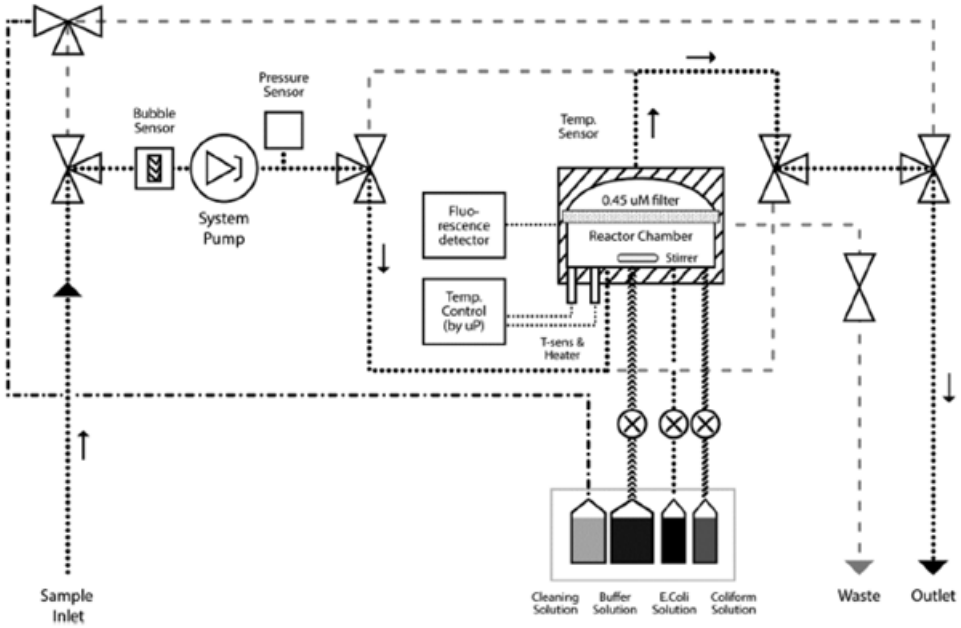


Figure 2. Schematic overview of the BACTcontrol system (Appels et al., 2018)

Prior to each measurement, the water sample is pumped from the water source through the reactor chamber at flow rates from 1 to 24 ml per minute, the time needed for the filtering depends on the volume that has to be filtered and the condition of the filter. The sampled water volume is also measured by the pump during this process.

After setting the temperature inside the reaction chamber to the optimum temperature (44 ± 0.1 ˚C for GLUC, 36 ± 0.1 ˚C for GAL, 37 ± 0.1 ˚C for GLUCAN, 45 ± 0.1 ˚C for ALP), the stabilization followed by the actual measurement of the fluorescence intensity can take place during a 20-minute incubation period. The fluorescence intensity of the fluorometer has been calibrated using a standard with a concentration of 1,000 nM MUF. This calibration allows the fluorometer to measure the production rate of MUF, which directly corresponds to the hydrolysis rate of the substrate, the fluorescence intensity is converted into MUF production per time and volume (pmol MUF * min * 100 ml^{-1}).

The increase in fluorescence is automatically saved to the BACTcontrol computer and the slope of the signal in the steady state phase is used to calculate the enzymatic activity by ordinary least square linear regression analysis. Furthermore, the software calculates a limit of detection (DL) for each measurement performed. For this statistical approach, the measurement is regarded as significant if the average signal during the measurement exceeds threefold the standard deviation in relation to the theoretical zero line of the reaction. The DL calculation is determined after the stabilization period (only during 20 minutes of incubation), from where the slope of the regression curve is determined until the end of this phase.

After each measurement, a cleaning / disinfection procedure is performed by the device, which comprises the injection of a chlorine solution and a heating procedure within the reactor to eliminate residues of the measuring process within the system.

1.4 Study aim

The described online microbiological sensors BACTcontrol and BACMON are new, rapid, online methods to determine total bacteria numbers. These methods are possibly suitable for the online monitoring of any microbial disturbances in different water types and/or of the effect of control measures on water quality. However, before they can be reliably implemented, supplementary experimental research is needed.

In this report the use of these sensors on different water types was tested and the performance of the sensors was compared with other, proven laboratory methods (e.g., cell counts, ATP, culture). This includes determining the detection range and detection limit of the online monitoring systems. Following laboratory studies (chapter 2) the sensors are tested in four full-scale locations where they monitor the microbiological quality of several water types continuously.

2 Laboratory validation studies

The BACTcontrol and BACMON are new, rapid, online methods to determine total bacteria numbers. These methods are possibly suitable for the online monitoring of microbial disturbances and/or of the effect of control measures on water quality of different water types (drinking water, cooling water, surface water and/or ground water). However, before they can be reliably implemented, supplementary experimental research is needed. In this chapter the performance of the sensors is compared to other, proven laboratory methods to quantify cell numbers (e.g., cell counts, culture) or biomass (ATP). The validation, including determining the detection range and detection limit, is performed on different water types.

Table 2. The water type, sample location and specifics of each water type used for validation studies.

Water samples	Sample location	Specifics	Chapters
Drinking water	KWR (Tull en 't Waal)	Produced from ground water. Comparable to Danish drinking water (e.g. Ca, Fe, NH_4^+)	2.2.1 2.2.2.1 2.2.2.2
	Andijk	Produced from surface water (IJsselmeer), without dune infiltration	2.2.2.3 2.2.2.4
	Weesperkarspel	Produced from surface water, without dune infiltration	2.2.1 2.2.2.1
Cooling tower water	Uniper	Surface water is concentrated and disinfected with ozone, an antiscalant is added	2.2.1 2.2.2.1 2.2.2.3 2.2.2.4
Surface water	Lekkanaal	Intake point of surface water for drinking water production	2.2.2.3 2.2.2.4
	Hollandse IJssel	Intake point for cooling towers of Uniper	2.2.2.1
Untreated ground water	Amersfoortseweg	Oxic, with relatively low concentration of organic carbon and iron.	2.2.1 2.2.2.3 2.2.2.4
	Havelterberg	Anoxic, with a relatively low concentration of organic carbon and iron	2.2.2.3 2.2.2.4
	Eindhoven	Anoxic, with a relatively medium concentration of organic carbon and high iron concentration	2.2.2.3 2.2.2.4
	Nieuwegein	Anoxic, with a relatively medium concentration of organic carbon and high iron concentration	2.2.2.3 2.2.2.4
	Linschoten	Anoxic, with a relatively medium concentration of organic carbon and iron	2.2.2.2 2.2.2.3 2.2.2.4
	Spannenburg	Anoxic, with relatively high concentrations of organic carbon and iron.	2.2.1

2.1 Methods

2.1.1 Water sources

For the validation studies water samples from several water sources were tested. In Table 2 some general characteristics of the sampled water sources are given and in Figure 3 the geographical locations are shown.

Figure 3. Location of all drinking water production locations from Table 2.

2.1.2 Initial comparison of laboratory analyses with microbial sensors

As an initial test drinking water, untreated ground water and cooling tower water (Table 2, chapter 2.1.1) were analysed with several methods (BACMON, BACTcontrol, ATP, total cell and membrane-intact cell counts with flow cytometry [TCC-FCM-total and TCC-FCM-intact], plate counts with HPC and total cell count using fluorescence microscopy [TCC-MS]; Table 3). The average values of each parameter were used for the graphs and statistical analyses to compare the different analyses to each other. The water was sampled in clean jerry cans or large glass bottles, stored at 4˚C for a maximum of 24 hours and tested at the KWR laboratory for the described parameters.

Table 3. Microbiological analyses performed in the different experiments. Given is in which paragraph the methods and results of the specific experiment are discussed. For each analysis the number of replicates is given.

Experiment	Paragraph	BACMON	BACTcontrol	ATP, total	ATP, cellular	HPC	TCC-FCM	TCC-MS
Initial	2.1.2 2.2.1	7	1	3	-	2	5	2
Cultured bacteria	2.1.3.1 2.2.2.1 2.2.2.2	5-11	1	3	3	-	2	2
Natural	2.1.3.2 2.2.2.3 2.2.2.4	4-12	1	3	3	-	2	2

2.1.3 Validation of BACMON and BACTcontrol to laboratory analyses

2.1.3.1 Cultured bacteria

A comprehensive validation was subsequently performed using several water types (ground water, surface water, drinking water, cooling tower water, Table 2). All water samples were filtrated (0.22 um) to remove all bacteria while at the same time keeping the water matrix unchanged as much as possible. However, particles present in the water matrix will be removed as well by the filtration, which affects the matrix composition and, therefore, probably the measurements. After filtration the filtrated water was stored at 4˚C for a maximum of 24 hours before the start of the experiment.

Two bacterial strains (*Pseudomonas fluorescens* P17 and *Spirillum spp*. NOX) were dosed in a dilution series to this filtrated water. These bacteria were originally isolated from drinking water (van der Kooij, 1992) and are commonly used in experiments to determine the easy assimilable organic carbon (AOC) content of a water sample. Before dosing, these two bacteria were precultured by two methods: in medium containing low concentrations of nutrients (mineral medium) and in medium with a high nutrient concentration (LLB, lab lemco broth). At the day the maximum number of bacteria was reached, the bacteria were 100x concentrated by centrifugation. This day with maximum yield was predicted based on previous experience and confirmed by culture analyses of which the results were available 2-3 days later. A dilution series was made in the supernatant after centrifugation and these dilutions were added to the filtrated water matrix. The dilution series were made in the supernatant to try to prevent stress or an osmotic shock what may happen when the dilutions would have been performed in sterile water. After addition of the bacteria to the filtrated water matrix the water sample was left at room temperature for one hour to allow bacteria to recover from the stress of centrifugation and being mixed with a different water type. It is assumed to be unlikely that the dosed bacteria will grow within this hour. To prevent possible growth (or die-off) during the experiment, all analyses of one dilution were performed at the same time.

Each spiked water sample was analysed with several methods (Table 3): BACMON, BACTcontrol, ATP, HPC and total cell count using microscopy (TCC-MS). The average of these measurements was used for the graphs and statistical analyses.

Drinking water of the KWR laboratory (Vitens, Tull en ‘t Waal) filtered through a 0.22 um filter served as a negative control.

2.1.3.2 Natural bacteria

In addition to using cultured bacteria as described above, bacteria naturally present in the several water types (ground water, surface water, drinking water, cooling tower water; Table 2) were used as well to make a dilution series.

To this end, the water source was sampled and divided in two parts. One part remained untreated and was stored at room temperature until the start of the experiment within 24 hours. This was done because cooling the water sample is assumed to slow down the metabolism of the bacteria and therefore may affect the BACTcontrol measurements which are based on enzymatic activity. The other part of the water sample was filtrated using a cross-flow filter (hemoflow, pore size: 30 kDa, approximately 3 – 10 nm). The hemoflow permeate (containing the filtered water without bacteria and larger particles) was harvested in sterile bottles (autoclaved, followed by flushing with ultrapure water and filtrated 70% ethanol to remove any particles that may still be present). The permeate was stored at 4˚C until the experiments were performed within 24 hours after sampling.

A dilution series of typically 8-10 dilutions was made of unfiltrated source water in the respective permeate using sterile and flushed bottles (as described above) and sterile equipment. All dilutions were stored at room temperature in the dark until the analyses were performed. On each dilution the following analyses were performed (Table 3): BACTcontrol, BACMON, number of total and membrane-intact cells with flow cytometry (TCC-FCM, total and living), ATP (total and cellular), total cell count with fluorescence microscopy (TCC-MS). In all graphs, tables and statistical analyses the average value for each parameter is shown.

All analyses of one dilution were performed within one hour of each other so as to prevent effects of possible growth, die-off or changes in the water matrix during the waiting time that may influence the analyses. Due to the longer analysis time of the BACTcontrol (one analysis every ~2.5 hour) not all dilutions were measured with the BACTcontrol.

The BACTcontrol has an internal, integrated, cleaning cycle (heating to 65˚C and dosing of hypochlorite) that runs after every analysis. The BACMON was not cleaned between measurements of the different dilutions of one water source, but the system was flushed for a few minutes with the new sample water before start of the analyses. Directly before the start of each experiment of one water source, and in between experiments on different water sources, the BACMON was manually cleaned according to the protocol of Grundfos. All analyses of the dilution series were performed in such an order that first the most diluted sample was measured (containing the least amount of bacteria and particles), followed by a less diluted sample and the last sample to be analysed contained the highest number of bacteria and particles.

2.1.4 Microbiological analyses

The BACMON and BACTcontrol analyses were compared to microbiological laboratory analyses commonly used in water: ATP, LLA culture plates, heterotrophic plate count (HPC), number of total and membrane-intact cells with flow cytometry (TCC-FCM) and total cell count with fluorescence microscopy (TCC-MS). Although not all of these parameters are good indicators for biomass, for convenient reason these parameters are called biomass analyses or parameters in this report. These parameters were chosen because they are often used (ATP), compulsory monitoring methods in the drinking water guidelines (HPC), easy to count the number of cultured cells that are

dosed to a water sample (LLA culture plates) or are methods in which the number of cells present in a water sample is measured (TCC-FCM and TCC-MS). The methods and working principle of the sensors and analyses are described below.

2.1.4.1 ATP

ATP was measured in accordance with NEN-EN 16421:2014. The measurements are based on an enzymatic reaction of luciferin with luciferase in the presence of ATP. The amount of light produced (described in Relative Light Units, RLU) is converted to the ATP concentration using a standard curve. In all experiments the total ATP concentration is given. When indicated also the cellular ATP concentration is given, this is only the ATP that is present in cells. Total ATP also includes ATP that is present as a free molecule in the water sample. Due to a methodological artifact, the calculated cellular ATP levels might be negative. To solve this, when the cellular ATP levels of the dilution with lowest number of cells were negative, the ATP levels were artificially set to '0' ng/l. The other cellular ATP levels within that dilution series were adapted accordingly. In the report this is called 'ATP (cellular, corrected)'. For example, the cellular ATP concentration of the dilution with the lowest number of cells is -6 ng/l and 38 ng/l for the dilution with the highest number of cells. To both 6 ng/l ATP is added yielding 'ATP (cellular, corrected)' levels of respectively 0 ng/l and 44 ng/l.

2.1.4.2 LLA culture plates

In experiments where *Pseudomonas fluorescens* P17 and *Spirillum spp*. NOX were dosed, the number of bacteria was determined by spread-plating the water sample in decimal solutions on a general and rich agar medium (lab lemco agar [LLA] plates). Subsequently, the number of colonies was counted after 48 – 72 hours incubation at 25˚C.

2.1.4.3 Heterotrophic Plate Count (HPC)

The number of HPC bacteria was determined in accordance with NEN-EN-ISO 6222. In short, 1 ml of water sample (or a decimal dilution) was incubated on Place Count Agar (PCA) at 22˚C for 68 hours, after which the number of colonies was counted.

2.1.4.4 Total or membrane-intact cell counts with flow cytometry (TCC-FCM)

The water sample (1 ml) was incubated with SYBR Green (stains DNA of bacteria with a permeable and impermeable bacterial membrane) and propidium iodide (stains DNA of bacteria with a permeable bacterial membrane only) and measured in a BD FACS Calibur. The detection limit is 1000 cells/ml. The total number of cells is equal to the number of SYBR green stained cells (TCC-FCM-total). The number of membrane-intact cells (as indication for living cells) were determined by calculating the difference between SYBR green and propidium iodide stained cells (TCC-FCM-intact).

2.1.4.5 Total cell count with fluorescence microscopy (TCC-MS)

The water sample (10 – 100 ml) was filtrated on a black 0.22 µm polycarbonate filter. The bacteria on the filter were dyed with acridineorange which stains bacterial DNA and RNA of all cells (living and death). After a washing and drying step the filter was visually analyzed using a fluorescence microscope and the number of bacteria was counted. The microscopic preparations were stored at -20˚C and analyzed within 1 month after they were frozen.

2.1.5 Statistical analyses

The average of multiple measurements of one water sample, or dilution, was calculated (e.g. triplicate measurements of ATP on one dilution are averaged) and used in the

statistical analyses. To test whether the results from the BACMON, BACTcontrol and/or one of the biomass parameters are significantly correlated to each other, a linear regression analysis (Pearson correlation) was performed. All measurements were log-transformed before the linear regression analysis was performed. Measurements below the detection limit ('<'-value) were not taken into account but removed from the dataset used for the linear regression analysis. Furthermore, when the number of data points (N) was two or lower, a linear regression analysis cannot be performed.

Only if the two tested parameters are significantly correlated ($p < 0.05$) in the linear regression analysis, the r^2 of the fitted linear trend line is given. The value of the r^2 indicates if the strength of the statistically significant correlation between the two parameters is excellent ($r^2 > 0.9$), good ($0.9 < r^2 < 0.7$), moderate ($0.5 < r^2 < 0.7$) or bad ($r^2 < 0.5$). These values were arbitrarily chosen.

2.1.6 Sampling at full-scale locations

At four full-scale locations a sampling campaign was performed using auto samplers. A 800-ml water sample was taken from a continuously refreshed overflow tank over the time course of 8 minutes. The samples were stored in sterile bottles at 4°C for 24-36 hours until the TCC-MS, TCC-FCM and ATP analyses were performed. To be able to compare the results of these laboratory analyses with the BACMON and BACTcontrol, two measurements of the BACMON (measurements performed immediately before, within or immediately after sampling) were averaged.

All sensors, including the auto sampler, were cleaned thoroughly after moving from one full-scale location to another.

2.2 Results

2.2.1 Initial comparison of laboratory analyses with microbial sensors

As a first test all biomass and cell count analyses were performed on drinking water, untreated ground water and cooling tower water (Figure 4, Figure 6, Figure 8). The two types of drinking water, although produced from different source waters, show similar results for all parameters. The ATP levels (2.4 – 5.2 ng/l), HPC levels (1 – 6 cfu/ml), number of membrane-intact cells (TCC-FCM-intact: 6.8 – 9.9x10^4 cells/ml) and the total number of cells (TCC-FCM-total: 7.1 – 10.6x10^4 cells/ml; TCC-MS: 1.6 – 3.3x10^4 cells/ml) are normal for drinking water. The BACMON measurements of both bacteria and non-bacteria are consistent and show little variation, although numbers are lower (1.3 – 3.5x10^3 cells/ml) than measured with flow cytometry and fluorescence microscopy. The two devices of the BACTcontrol do not behave entirely comparable: the measurements on the second device (two right red and blue bars in Figure 4) yield higher results compared to the other device. However, within one device a 10 times higher filtration volume consistently yields a 6-8x higher enzymatic activity.

The HPC number is about 3 – 5 log lower compared to the results of the BACMON, TCC-FCM-total, TCC-FCM-intact and TCC-MS. This phenomenon is known as the great plate count anomaly, i.e. the majority of the bacterial cells in drinking water cannot be cultured on HPC medium. The total number of bacterial cells that are counted with TCC-FCM and TCC-MS, the two 'cell count methods' in this experiment, are 0.65 – 1.9 log higher compared to the BACMON. The TCC-MS yields slightly lower counts compared to TCC-FCM (about 0.5 - 0.7 log). Whether the TCC-FCM and TCC-MS overestimate the number of cells in the water, or the BACMON underestimates the number of cells cannot be concluded from these results.

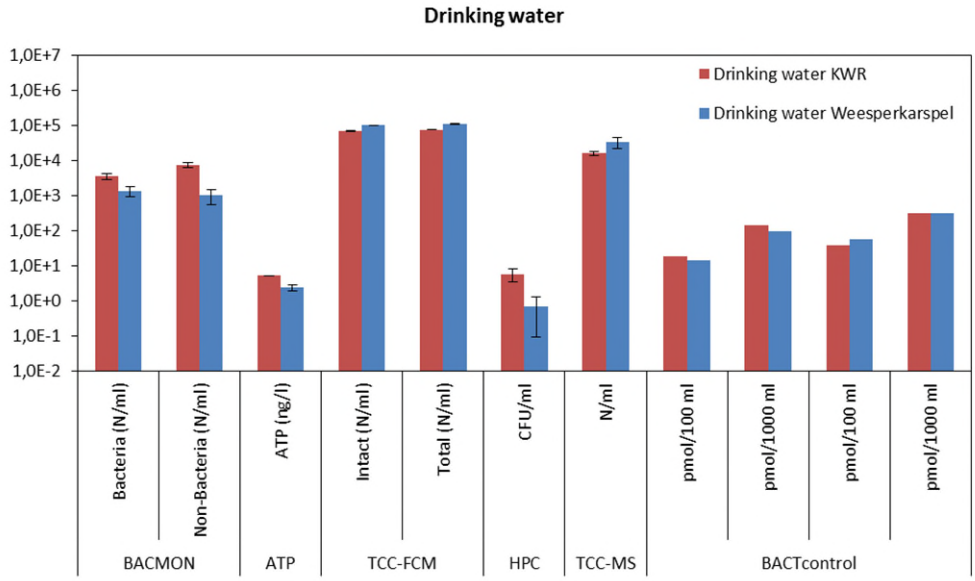


Figure 4. Comparison of biomass parameters on drinking water (sampled at KWR and from the distribution system of Weesperkarspel; Table 2). Given is the average with SD. In chapter 2.1.2 the number of repetitions for each parameter is given. The measurements on the BACTcontrol were performed on two devices and for two filtration volumes (100 and 1000 ml).

The BACMON was not able to measure cells in the untreated ground water from Spannenburg, also one of the measurements with BACTcontrol produced a negative result (Figure 6). After opening the reaction chamber of the BACTcontrol the filter showed to be clogged with iron and sediment-like particles from the Spannenburg ground water. Untreated ground water from Spannenburg contains high levels of iron which oxidize to yellow rust particles upon contact with oxygen during sampling (Figure 5). The yellow color most likely interferes with the microscopy analysis that takes place in the BACMON. However, if the sensors are applied in an online setting, this is unlikely to happen, assuming that the untreated ground water does not come in contact with oxygen during online monitoring at location. Both sensors had no problem with measuring microbial numbers or activity on ground water from Amersfoortseweg. None of the laboratory analyses (ATP, TCC-FCM, HPC, TCC-MS) showed any problems in measuring ground water from Spannenburg and Amersfoortseweg.

Figure 5. Untreated ground water from Spannenburg (left) and Amersfoortseweg (right).

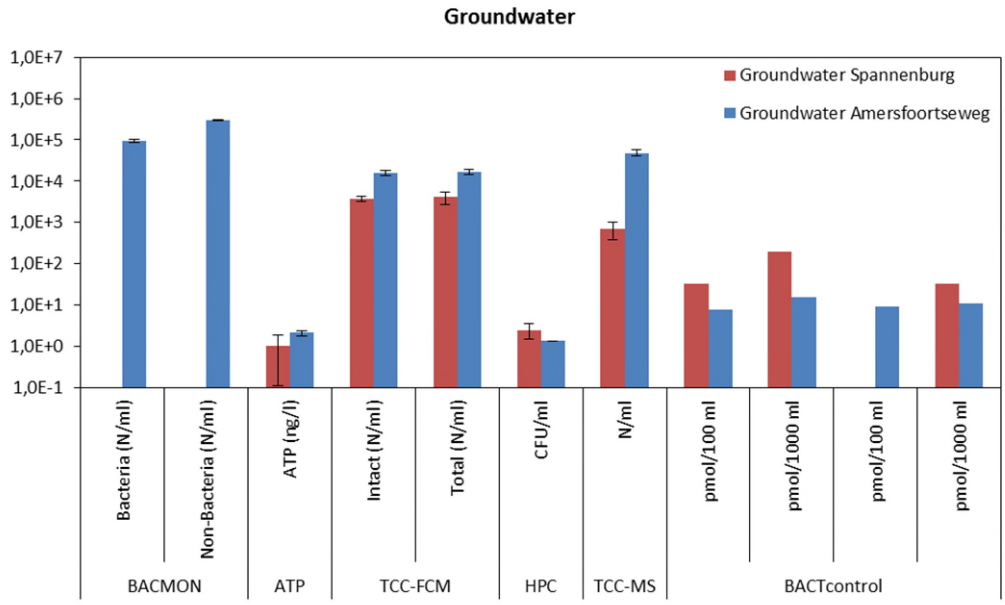


Figure 6. Comparison of biomass parameters on untreated ground water (sampled at Spannenburg and Amersfoortseweg). Given is the average with SD. In chapter 2.1.2 the number of repetitions for each paramter is given. The measurements on the BACTcontrol were performed on two devices and for two filtration volumes.

The BACTcontrol results of ground water from Amersfoortseweg show that when the filtration volume is 10x higher (1000 vs 100 ml), the enzymatic activity only increases 1.2 – 6x. Possible reasons for this can be that the cell number is too low for the BACTcontrol and thus the enzymatic activity is around the detection limit, despite the 10x larger volume tested the enzymatic activity remains around the detection limit. The measured variation is then caused by background noise. Other options are that the enzymatic activity in bacteria in ground water is very low and thus below the detection limit and the measurements results are again caused by background noise. A technical explanation could be that filtration of difficult water types causes rapid clogging of the filter and/or the pressure in the reaction chamber becomes too high due to which the measurement of the filtrated volume is inaccurate. Such technical problems are unlikely to be the cause for this sample as ground water from Amersfoortseweg is considered a clean water type and undergoes only very limited treatment during drinking water production (only marble filtration).

For both the BACMON and BACTcontrol it proved difficult to measure microbial cells or bacterial activity in cooling tower water. After filtration of a few samples the filter of the BACTcontrol was clogged and a white slimy layer was present on the filter (Figure 7). Only 6 out of 8 measurements were successful. The BACMON also had some difficulties and only 10 out of 14 measurements yielded a result.

Figure 7. Filter of the BACTcontrol after measuring cooling tower water. A white, slimy layer is present on the lower side of the filter.

The two cooling tower water samples yielded nearly identical results for most analyses (Figure 8). ATP (21 – 23 ng/l) and HPC levels (45 – 75 cfu/ml) are about 1 -1.5 log higher compared to ground water and drinking water. Also the number of cells as measured with TCC-FCM (total: 1.8 – 1.9x10^6 cells/ml), TCC-MS (1.6 – 2.4x10^6 cells/ml) and BACMON (4.2 – 4.8x10^4 cells/ml) are higher compared to the other water types. The BACMON results are 1 – 2 log lower compared to TCC-MS and TCC-FCM-total. The difference between total number of cells and cells with an intact membrane is largest compared to the other two water types (TCC-FCM-intact: 4.0 – 4.6 x10^5 cells/ml), indicating that a large fraction of the cells contain damaged cell membranes and these cells are considered to be dead.

The BACTcontrol shows more variation between the two water samples on the two devices (36.1 – 91.6 pmol/100 ml). Due to the problem with filter clogging, the results of the 1000 ml filtration are probably not representative and not discussed here.

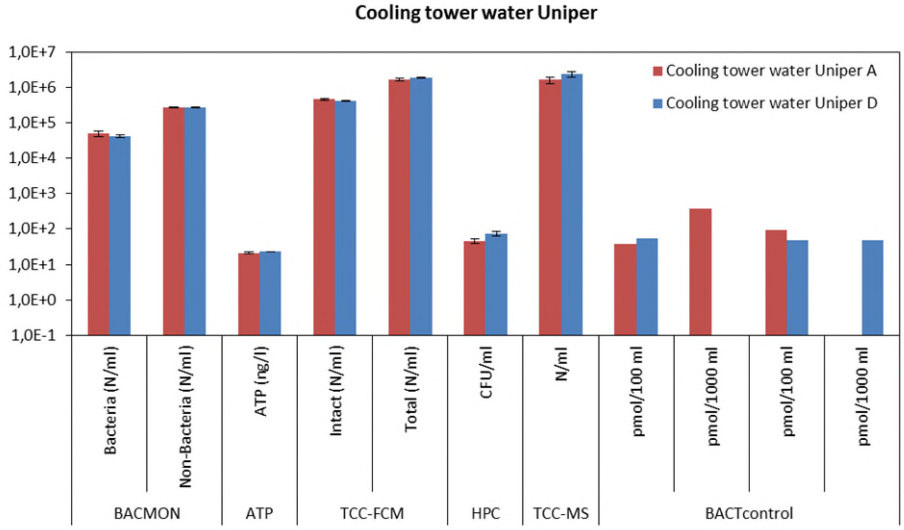


Figure 8. Comparison of biomass parameters on cooling tower water (sampled on one day at two location in the same cooling tower plant). Given is the average with SD. In chapter 2.1.2 the number of repetitions for each paramter is given. The measurements on the BACTcontrol were performed on two devices and for two filtration volumes.

Due to the low number of measurements (6 in total), no correlation studies or statistics have been performed. Such an analysis has been done in the other experiments from which the results are described below (paragraph 2.2.2).

This initial comparison shows that most water types can be analyzed with the BACMON and BACTcontrol, although iron-rich ground water and cooling tower water cause some problems with measuring cell number or microbial activity with these two sensors in the tested set-up.

2.2.2 Comparison of biomass parameters with sensors in dilution experiments

Several approaches were applied to make dilution series of bacteria in different water matrices and analyze these with the several biomass and cell count parameters. The results of the three approaches are described below.

2.2.2.1 Bacteria cultured in mineral medium

P. fluorescens P17 and *Spirillum spp*. NOX bacteria were individually cultured in mineral medium, containing limited nutrients, to mimic the situation in most natural water sources (mainly drinking water and surface water). The graphs of the dilution series are given in 0 and Figure 9. These graphs shows that the dilution series were not completely successful, especially the dilutions that were supposed to have cell numbers below ~$5x10^3$ cells/ml, remain at a cell concentration that is too high. This problem was caused by the dilution series being made in the supernatant after centrifugation. It was assumed that all cells would be concentrated by centrifugation and hardly any would be left in the supernatant. However, the number of cells in the supernatant remained relatively high ($1x10^3$ – $1x10^4$ cells/ml). As a consequence, the dilutions that were supposed to contain lower cell numbers were affected by the cell concentration in the supernatant. In the last experiment (cooling tower water, D) the supernatant was filtrated before it was used in the dilution series. This yielded better results and the dilutions with low cell numbers, especially with LLA culture, were successfully prepared with this filtrated supernatant.

Experiments show that bacteria grown in mineral medium are detected with the BACMON, but their activity is very low and cannot reliably be detected with the BACTcontrol sensor (0, Figure 9). Within one water type the enzymatic activity hardly varies between the different dilutions, regardless of the number of cells or the ATP-levels that are present. In most dilution series the data points are grouped together at around $1x10^3$ – $1x10^4$ cells/ml (counted with the BACMON) except for one outlying data point with a high number of cells (or ATP). This was caused by the described problems with making the dilution series. Consequently, a correlation analyses was not performed, because such a correlation analysis would only provide information on the background ATP and cell numbers in the used dilution solution.

As bacteria cultured in mineral medium are not or hardly detected with the BACTcontrol, this method is not suitable for validation purposes. Although the reason is not known why these bacteria cannot be detected in the BACTcontrol, laboratory studies have shown that the presence of phosphate in the mineral medium negatively regulates the alkaline phosphatase enzyme (Friedberg and Avigad, 1967; Fernley and Walker, 1967). Since this enzyme is measured in the BACTcontrol, we hypothesize that the disturbing influence of phosphate on the enzyme is the reason for non-detecting any signal. Another option is that due to the low level of nutrients the bacteria are

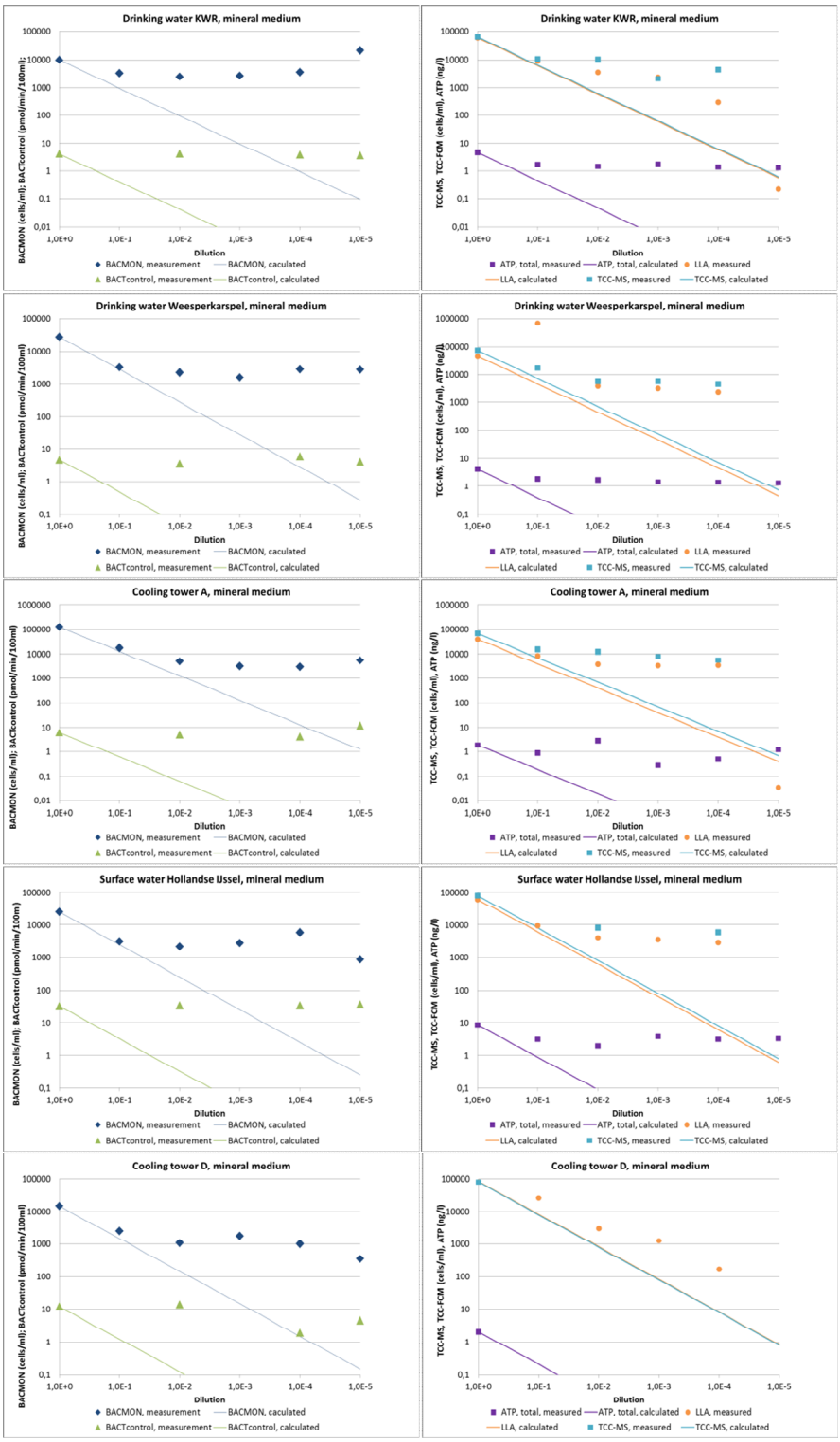


Figure 9. Measured (marker points) and predicted/calculated (line) values for the microbiological analyses of each dilution series. The calculated values are based on the first data point (highest value) that was measured, the following datapoints were calculated based on the applied dilution series. E.g. a 10-fold dilution series with 6 steps and $1x10^6$ cells/ml as highest measured value, will yield $1x10^5$ - $1x10^4$ - $1x10^3$ - $1x10^2$ - $1x10^1$ cells/ml as calculated value.

metabolically not active enough (i.e. the alkaline phosphate enzyme concentration is too low) and thus not detected.

Therefore, other strategies for the BACTcontrol were tested. Experiments for which bacteria were grown in mineral medium with 100x or 10000x less phosphate yielded no positive results in the BACTcontrol (data not shown), demonstrating that the low activity of the bacteria is the cause of not detecting a signal with the BACTcontrol sensor.

An alternative is, therefore, to use bacteria that are cultured with high nutrient concentrations and thus become metabolically very active which would make them visible for the BACTcontrol assay. Another option is to use bacteria derived from natural sources (drinking water, surface water, etc). Both options were tested and the results are described below.

2.2.2.2 Bacteria cultured in rich medium

The *P. fluorescens* P17 and *Spirillum spp*. NOX bacteria were individually cultured in rich medium (LLB) and diluted in filtrated drinking water (10 and 12 April 2018) or in filtrated untreated ground water from production location Linschoten (12 June 2018).

This approach yielded dilution series that could be measured with both the BACMON and BACTcontrol (0, Figure 10). Especially the measurements with LLA show proper dilution series as was expected. The other analyses often show a good dilution range with higher cell numbers, but at lower cell numbers the dilution flattens off, because the cell numbers and biomass reach the detection limit of the ATP and flow cytometer method. At which cell numbers the analysis results flatten off, varies sometimes between the analyses and the flattened-off results will probably negatively impact the correlation between the cell number methods and the other methods.

Due to a processing error, the BACMON did not measure two dilutions with the highest number of *Spirillum spp*. NOX bacteria (experiment: 12 April 2018, Figure 10). As a consequence only three dilutions were measured and no statistically significant correlations between the BACMON results and all other tested parameters was found for experiments done on 12 April 2018 (Table 4). However, good correlations were obtained for the experiments done on 12 June with the BACMON and the other parameters. The BACTcontrol did show good correlation with all tested biomass parameters for all experiments.

The graphs (0, Figure 10) show that when cell numbers (counted with TCC-MS, LLA or BACMON) are below ~$1x10^4$ cells/ml, the enzymatic activity measured with the BACTcontrol no longer decreases with decreasing cell numbers but remains at around 10 – 40 pmol/volume. This suggests that the BACTcontrol is not sensitive enough to distinguish between water samples with cell numbers below $1x10^4$ cells/ml and that the background baseline of the method is around 10-40 pmol/volume. To some extent the BACMON seems not able to distinguish between water samples when cell numbers are below ~ $5x10^2$ cells/ml. This is more or less related to the technical detection limit of 167 cells/ml. Detection of one, two or three cells mainly depends on the homogeneity of the water sample and on coincidence. Samples that by coincidence are just above the BACMON detection limit will negatively affect the correlations that are calculated.

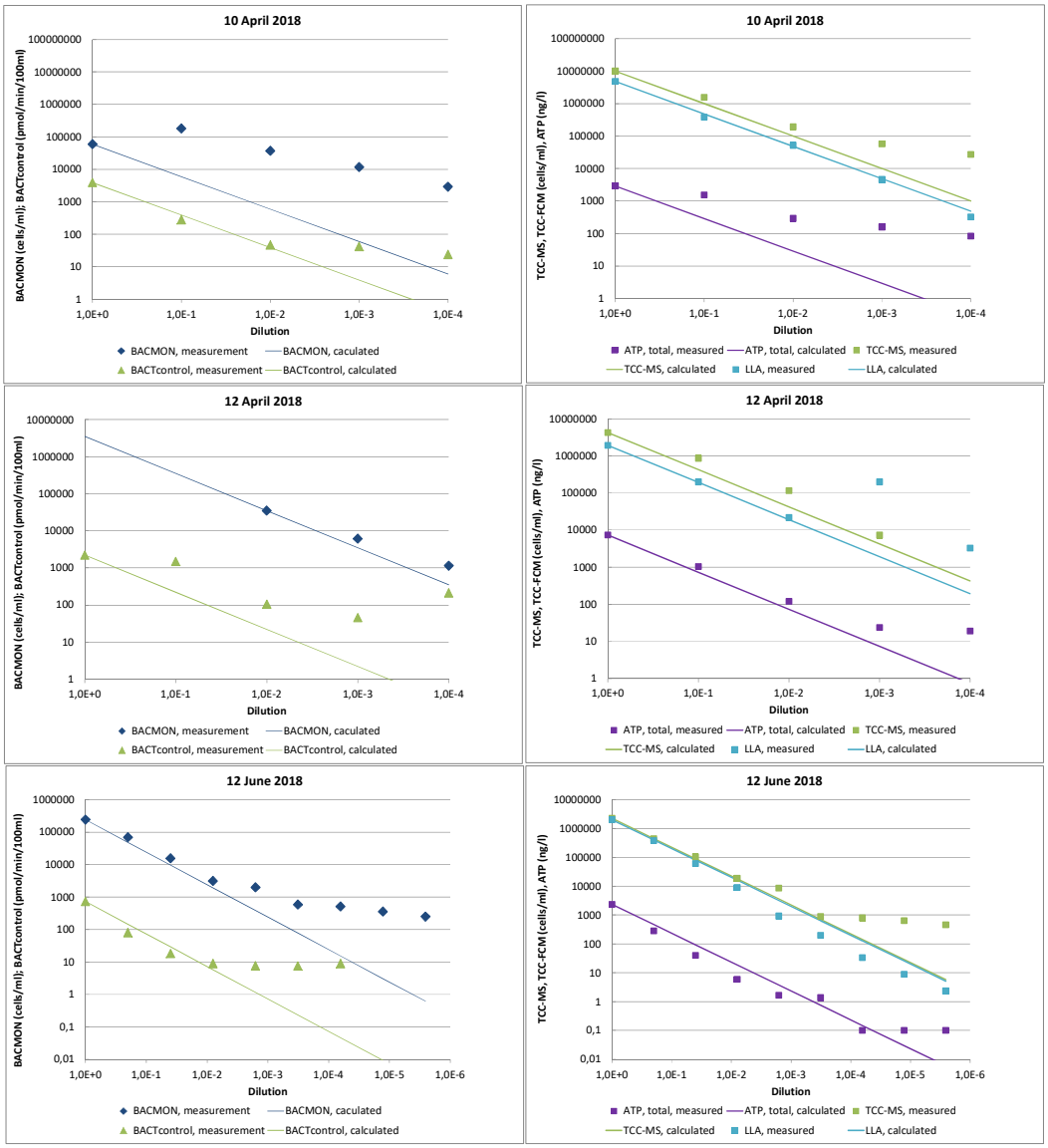


Figure 10. Measured (marker points) and predicted/calculated (line) values for the microbiological analyses of each dilution series. The calculated values are based on the first data point (highest value) that was measured, the following datapoints were calculated based on the applied dilution series. E.g. a 10-fold dilution series with 6 steps and $1x10^6$ cells/ml as highest measured value, will yield $1x10^5$ - $1x10^4$ - $1x10^3$ - $1x10^2$ - $1x10^1$ cells/ml as calculated value.

The BACTcontrol is thus capable of measuring cultured *Pseudomonas fluorescens* P17 and *Spirillum spp*. NOX bacteria grown under rich nutrient conditions and in general yields good correlations with the other biomass parameters (Table 4. Linear regression analysis of dilutions series of P17 en NOX bacteria cultured in rich medium in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given with a qualification of the r^2-value (good, moderate, bad). Graphs are shown in 0. The linear regression analyses were performed on log transformed data.. In contrast, the BACMON results did not significantly correlate with the biomass parameters with the experiments done in April 2018. However, good correlations were observed with the experiment performed in June. One explanation could be that the cultured cells are difficult to detect, although

this seems unlikely as this was done before (Højris, *et al.*, 2016). Probably the low number of dilutions measured partly causes the high p-values, especially since the experiments with a high number of dilutions analyzed (June 2018) showed significant correlations with excellent r^2 values.

Table 4. Linear regression analysis of dilutions series of P17 en NOX bacteria cultured in rich medium in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p < 0.05$) and if so the r^2 is given with a qualification of the r^2-value (good, moderate, bad). Graphs are shown in 0. The linear regression analyses were performed on log transformed data.

		BACMON vs				BACTcontrol vs		
		ATP	LLA	TCC-MS	BACTcontrol	ATP	LLA	TCC-MS
all data	**N**	13	16	15	14	16	17	16
	p<0.05	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	r^2	0.79	0.84	0.90	0.46	0.76	0.63	0.80
		Good	Good	Excellent	Bad	Good	Moderate	Good
10-4-2018 (P17)	**N**	4	4	4	4	5	5	5
	p<0.05	No	No	No	No	Yes	Yes	Yes
	r^2					0.88	0.83	0.93
						Good	Good	Excellent
12-04-2018 (NOX)	**N**	3*	3*	2*	3*	5	5	4
	p<0.05	No	No	ND#	No	Yes	No	Yes
	r^2					0.78		0.91
						Good		Excellent
12-06-2018 (P17+NOX)	**N**	6	9	9	7	6	7	7
	p<0.05	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	r^2	0.97	0.94	0.99	0.83	0.92	0.70	0.76
		Excellent	Excellent	Excellent	Good	Excellent	Good	Good

* Due to a processing error not all dilutions were measured in the BACMON, (partly) causing the p-value to be larger > 0.05.

ND = not determined, because only one or two data points were obtained.

Highly active bacteria (pregrown in rich media) thus seems to be a suitable, but not the most ideal method for comparing both sensors to laboratory biomass and cell count analyses, because it is assumed not to mimic the real-life situation with natural bacteria in (drinking) water.

2.2.2.3 Natural bacteria

The third alternative, dilutions series of natural bacteria derived from different water sources, proved successful. Dilution series have been performed with surface water (Lekkanaal, Table 5, Figure 11), cooling tower water (Uniper, Table 6, Figure 12), drinking water (Andijk, Table 7, Figure 13) and ground water (Amersfoortseweg, Eindhoven, Linschoten and Nieuwegein, Table 8, Figure 14). In the studies at full-scale locations (chapters 3, 4, 5, 6) a large number of water samples from the first three water types will be analyzed by the BACMON and BACTcontrol sensors and compared to laboratory analyses. This will yield a large, additional dataset (described in chapter 7.2) in which the sensors can be compared to laboratory analyses. Therefore, from drinking

water, surface water and cooling tower water only one or two water sources were tested in a dilution series. However, untreated ground water is not part of the full-scale locations and therefore water from multiple locations was tested.

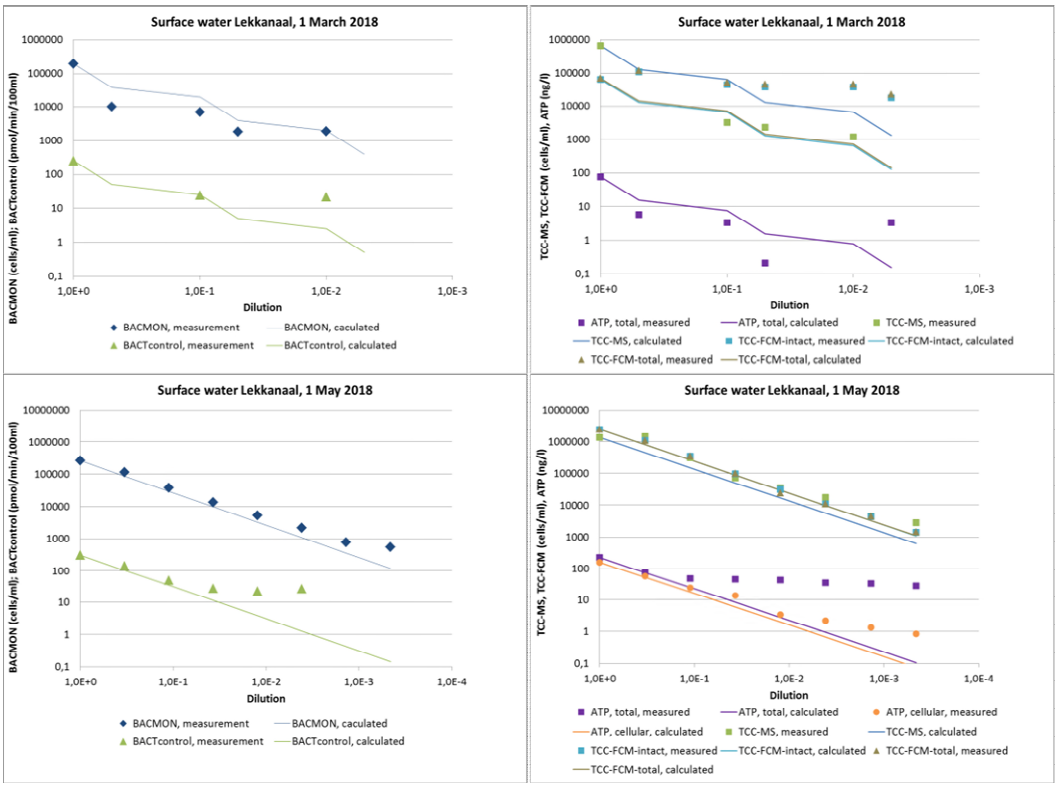


Figure 11. Measured (marker points) and predicted/calculated (line) values for the microbiological analyses of each dilution series. The calculated values are based on the first data point (highest value) that was measured, the following datapoints were calculated based on the applied dilution series. E.g. a 4-fold dilution series with 6 steps and $1x10^6$ cells/ml as highest measured value, will yield $2.5x10^5$ – $6.25x10^4$ – $1.56x10^4$ – $3.91x10^3$ – $9.77x10^2$ cells/ml as calculated values.

In the first dilution experiment (1 March 2018) only the results of both sensors are correlated to each other and the BACMON is correlated to the cell counts measured by TCC-MS. The relatively high cell count observed with TCC-FCM at lower BACMON or BACTcontrol data are probably the cause of the lack of a correlation between these methods (0, Figure 11).This suggests that some unknown component of the permeate is detected with the TCC-FCM. Another option could be that the number of measurements of the first experiments is too low (N = 3 – 6) to yield significant correlations. In contrast, the dilution series of natural bacteria in permeate of surface water show strong correlations between the BACMON and BACTcontrol with ATP, TCC-FCM, TCC-MS and to each other for the experiment of 1 May 2018 (p-value < 0.05; Table 5),, which might have been cause by the higher number of measurements that was performed (N = 6 – 8) in the second experiment compared to the first.

In the second experiment (1 May 2018), the permeate after hemoflow filtration contains large amounts of free ATP but very low concentrations of cellular ATP (39.7 ng/l vs 1.2 ng/l), which is in contrast to the untreated surface water that mainly contains cellular

ATP (160.9 ng/l vs 66.2 ng/l). The same phenomenon was seen in ATP levels of cooling tower water: untreated water contained 11.5 ng/l free ATP and 165.7 ng/l cellular ATP whereas the permeate contained 85.9 ng/l free ATP and <1 ng/l cellular ATP. These results show that during hemoflow filtration many cells are stressed or broken and release ATP in the surrounding water. Therefore, two kinds of ATP were measured in the second experiment (1 May 2018): total ATP and cellular ATP corrected for negative measurements (see chapter 2.1.4.1 for explanation). However, the differences between total ATP and cellular ATP (corrected) are minimal and both are significantly correlated to the BACMON and BACTcontrol.

Table 5. Linear regression analysis of dilutions series of surface water in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given. Graphs are shown in 0. The linear regression analyses were performed on log transformed data.

Water source and sampling date	Parameter 1	Parameter 2	N	p<0.05	r^2	
Lekkanaal Surface water 1 March 2018	BACMON	ATP	5	No		
		TCC-MS	4	Yes	0.94	Excellent
		TCC-FCM-intact	5	No		
		TCC-FCM-total	5	No		
		BACTcontrol	4	Yes	0.92	Excellent
	BACTcontrol	ATP	4	No		
		TCC-MS	3	No		
		TCC-FCM-intact	4	No		
		TCC-FCM-total	4	No		
Lekkanaal Surface water 1 May 2018	BACMON	ATP (total)	8	Yes	0.75	Good
		ATP (cellular, corrected)	8	Yes	0.99	Excellent
		TCC-MS	8	Yes	0.98	Excellent
		TCC-FCM-intact	8	Yes	0.99	Excellent
		TCC-FCM-total	8	Yes	0.99	Excellent
		BACTcontrol	6	Yes	0.86	Good
	BACTcontrol	ATP (total)	6	Yes	0.86	Good
		ATP (cellular)	6	Yes	0.87	Good
		ATP (cellular, corrected)	6	Yes	0.85	Good
		TCC-MS	6	Yes	0.84	Good
		TCC-FCM-intact	6	Yes	0.84	Good
		TCC-FCM-total	8	Yes	0.75	Good

As was seen in earlier experiments, at cell numbers below about 1-3x10^4 cells/ml the enzymatic activity of the BACTcontrol remains relatively stable. The BACMON levels off when cell numbers are below about 300 – 500 cells/ml.

Analysis of a dilution series in cooling tower water yielded good correlations between the BACMON and all tested biomass and cell count parameters, except for total-ATP (Table 6, 0, Figure 12). The BACTcontrol is only significantly correlated to the total- and cellular-ATP concentrations. ATP concentrations only noticeably increase when cell numbers are above $1x10^5$ cells/ml (measured with TCC-MS). At lower cell numbers the total and cellular ATP concentration remains stable.

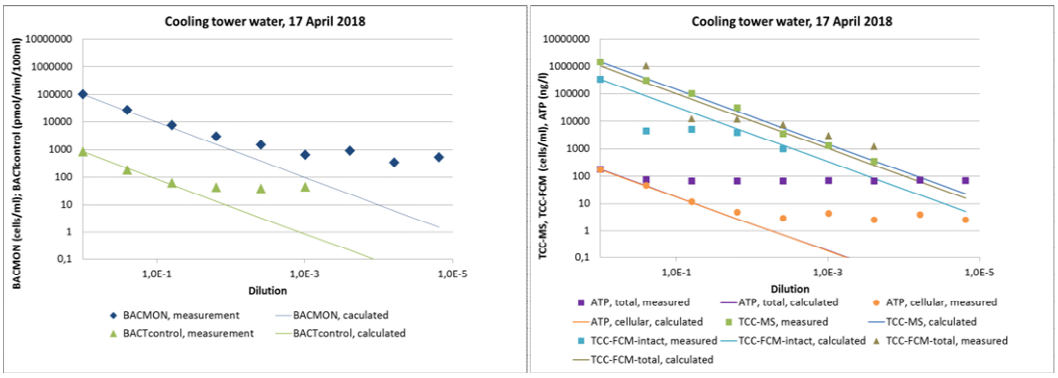


Figure 12. Measured (marker points) and predicted/calculated (line) values for the microbiological analyses of each dilution series. The calculated values are based on the first data point (highest value) that was measured, the following datapoints were calculated based on the applied dilution series. E.g. a 4-fold dilution series with 6 steps and $1x10^6$ cells/ml as highest measured value, will yield $2.5x10^5$ – $6.25x10^4$ – $1.56x10^4$ – $3.91x10^3$ – $9.77x10^2$ cells/ml as calculated values.

In contrast to the experiments that were performed for the initial comparison (chapter 2.2.1) the BACTcontrol did not have any problems with measuring the water samples. This suggests that the hemoflow filtration removes the compound that causes the filter of the BACTcontrol to clog.

Table 6. Linear regression analysis of dilutions series of cooling tower water in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given. Graphs are shown in 0. The linear regression analyses were performed on log transformed data.

Water source and sampling date	Parameter 1	Parameter 2	N	p<0.05	r^2	
Uniper Cooling tower 17 April 2018	BACMON	ATP (total)	8	No		
		ATP (cellular, corrected)	8	Yes	0.81	Good
		TCC-MS	6	Yes	0.87	Good
		TCC-FCM-intact	4	Yes	0.60	Moderate
		TCC-FCM-total	4	Yes	0.71	Good
		BACTcontrol	4	Yes	0.92	Excellent
	BACTcontrol	ATP (total)	5	Yes	0.77	Good
		ATP (cellular, corrected)	5	Yes	0.96	Excellent
		TCC-MS	5	No		
		TCC-FCM-intact	5	No		
		TCC-FCM-total	5	No		

Also in this experiment the enzymatic activity of the BACTcontrol levels off at cell numbers below about 1x10^4 cells/ml. The BACMON levels off when cell numbers are below about 300 – 500 cells/ml.

The dilution series in drinking water from Andijk show a moderate or good correlation between the BACMON and all biomass and cell count parameters (Table 7), but the BACTcontrol is not correlated to any of the parameters and also the two sensors are not correlated to each other. This is caused by high enzymatic activities that do not decrease while the cell number do decrease (Figure 13).

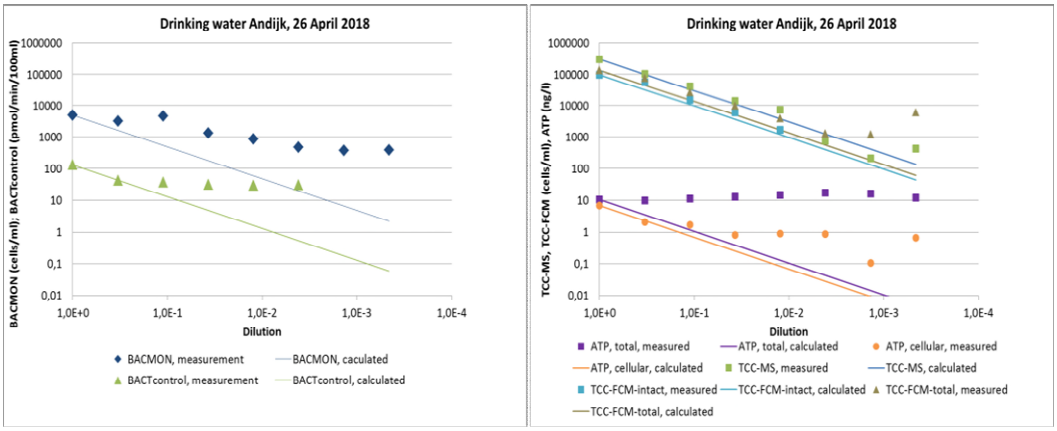


Figure 13. Measured (marker points) and predicted/calculated (line) values for the microbiological analyses of each dilution series. The calculated values are based on the first data point (highest value) that was measured, the following datapoints were calculated based on the applied dilution series. E.g. a 4-fold dilution series with 6 steps and 1x10^6 cells/ml as highest measured value, will yield 2.5x10^5 – 6.25x10^4 – 1.56x10^4 – 3.91x10^3 – 9.77x10^2 cells/ml as calculated values.

The total and cellular ATP concentrations differ, but for none an apparent dilution series is visible: whereas the total ATP concentration is stable, or slightly decreases, with increasing cell numbers, the cellular ATP concentrations remains stable until about 1x10^4 cells/ml and then increases (0, Figure 13). This is probably caused by high free ATP levels in the permeate, which is used to make the dilutions, that disturb the ATP measurements. However, the different between total and cellular ATP does not seem to affect the statistical correlations.

Table 7. Linear regression analysis of dilutions series of drinking water in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given. Graphs are shown in 0. The linear regression analyses were performed on log transformed data.

Water source and sampling date	Parameter 1	Parameter 2	N	p<0.05	r^2	
Andijk	BACMON	ATP (total)	8	Yes	0.66	Moderate
Drinking water		ATP (cellular, corrected)	8	Yes	0.67	Moderate
25 April 2018		TCC-MS	8	Yes	0.77	Good
		TCC-FCM-intact	5	Yes	0.76	Good
		TCC-FCM-total	8	Yes	0.83	Good
		BACTcontrol	6	No		
	BACTcontrol	ATP (total)	6	No		
		ATP (cellular, corrected)	6	No		
		TCC-MS	6	No		
		TCC-FCM-intact	5	No		
		TCC-FCM-total	6	No		

Four types of untreated ground water were tested, but only in water from Amersfoortseweg and Eindhoven sometimes statistically significant correlations were found between the BACMON, BACTcontrol and TCC-MS (Table 8). The BACTcontrol shows no correlation at all with the biomass and cell count parameters.

The lack of correlations observed for ground water measurements can be partly explained:

- ATP is below the detection limit in most ground waters and thus also in almost all dilutions that are prepared from this ground water. As a consequence the number of measurements (N in Table 8) is low and a regression analysis cannot be performed.
- Ground water from Linschoten and especially Nieuwegein yielded negative enzymatic activities in the BACTcontrol. These results were not included in the correlation analysis and thus the number of measurements (N in Table 8) is often too low to perform a regression analysis.
- The BACMON hardly detected any cells in the ground water of Nieuwegein and returned results of <167 cells/ml. These results were not included in the correlation analysis and thus the number of measurements (N in Table 8) is often too low to perform a regression analysis.
- The BACMON results of Eindhoven are strangely opposite to all other parameters (0). The number of cells measured with the BACMON decreases upon increase of cell number (measured with TCC-MS and TCC-FCM). This suggests that the results of the dilutions are exchanged, however we could not find any evidence for this. This supposed error does of course affect the regression analysis.

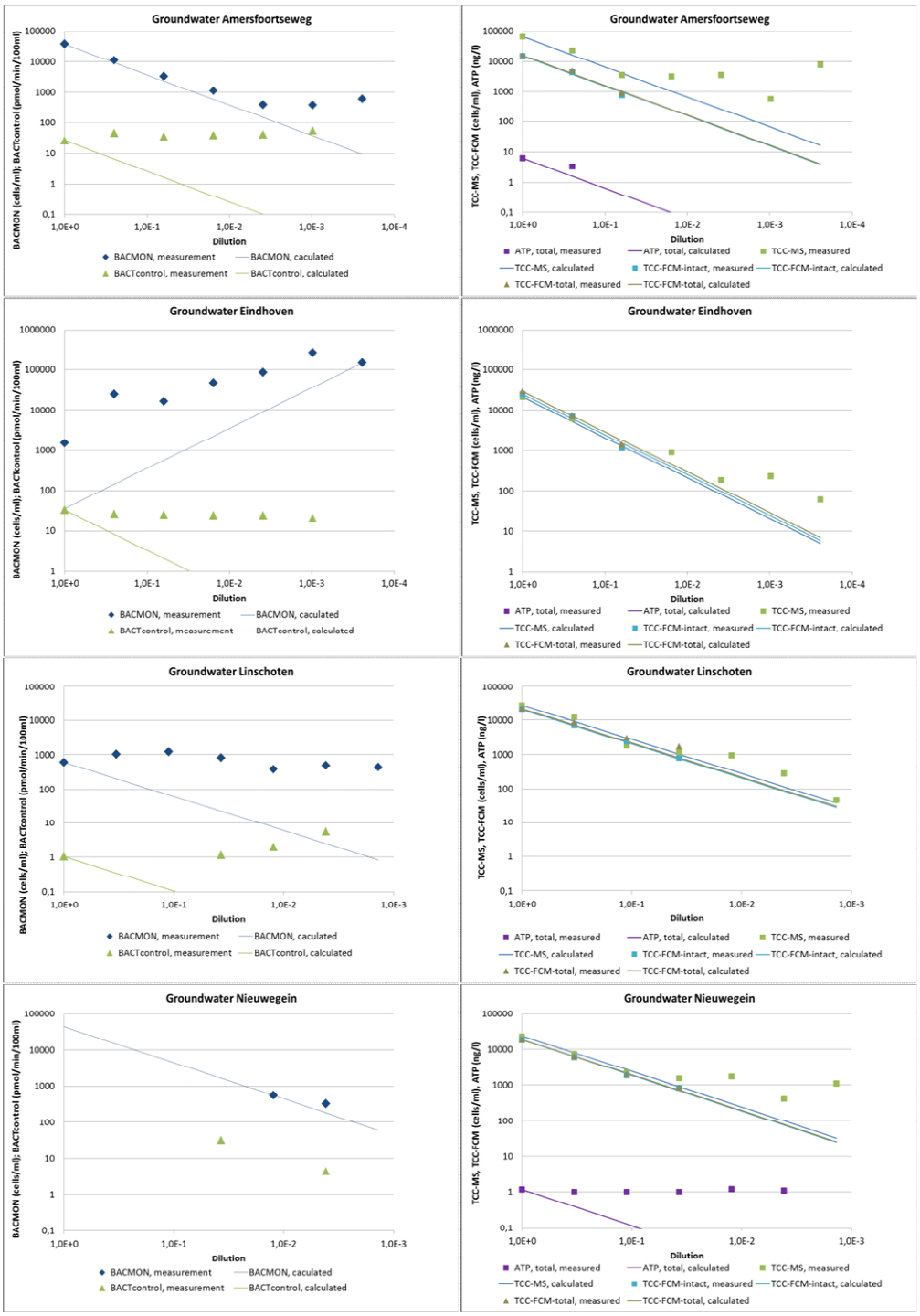


Figure 14. Measured (marker points) and predicted/calculated (line) values for the microbiological analyses of each dilution series. The calculated values are based on the first data point (highest value) that was measured, the following datapoints were calculated based on the applied dilution series. E.g. a 4-fold dilution series with 6 steps and $1x10^6$ cells/ml as highest measured value, will yield $2.5x10^5$ – $6.25x10^4$ – $1.56x10^4$ – $3.91x10^3$ – $9.77x10^2$ cells/ml as calculated values.

Table 8. Linear regression analysis of dilutions series of untreated ground water in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given. Graphs are shown in 0. The linear regression analyses were performed on log transformed data.

Water source/date	Parameter 1	Parameter 2	N	p<0.05	r^2	
Amersfoortseweg Ground water 19 June 2018	BACMON	ATP	2	ND#		
		TCC-MS	7	Yes	0.70	Good
		TCC-FCM-intact	3	No^		
		TCC-FCM-total	3	No^		
		BACTcontrol	6	No		
	BACTcontrol	ATP	2	ND#		
		TCC-MS	6	No		
		TCC-FCM-intact	3	No^		
		TCC-FCM-total	3	No^		
Eindhoven Ground water 26 June 2018	BACMON	ATP	0	ND#*		
		TCC-MS	7	Yes*	0.81	Good
		TCC-FCM-intact	3	No^		
		TCC-FCM-total	3	No^		
		BACTcontrol	6	Yes*	0.94	Excellent
	BACTcontrol	ATP	0	ND#*		
		TCC-MS	6	Yes*	0.76	Good
		TCC-FCM-intact	3	No^		
		TCC-FCM-total	3	No^		
Linschoten Ground water 10 July 2018	BACMON	ATP	1	ND#		
		TCC-MS	7	No		
		TCC-FCM-intact	4	No^		
		TCC-FCM-total	6	No^		
		BACTcontrol	4	No		
	BACTcontrol	ATP	0	ND#		
		TCC-MS	4	No		
		TCC-FCM-intact	2	ND#		
		TCC-FCM-total	3	No^		
Nieuwegein Ground water 18 July 2018	BACMON	ATP	2	ND#		
		TCC-MS	2	ND#		
		TCC-FCM-intact	0	ND#		
		TCC-FCM-total	0	ND#		
		BACTcontrol	1	ND#		
	BACTcontrol	ATP	2	ND#		
		TCC-MS	2	ND#		
		TCC-FCM-intact	0	ND#		
		TCC-FCM-total	0	ND#		

ND# = not determined, because none, one or two data points were obtained.

* Correlation considered to be not relevant due to strange measurement (see text or 0).

^ Low N because cell numbers are below detection limit of TCC-FCM

In a subsequent analysis, all data points of the dilution series (natural bacteria of all water types) were merged into one dataset and used for correlation analysis (Table 9). For all comparisons the correlation was significant ($p<0.05$) but the strength of the trend line (r^2) varied. The BACMON was mainly correlated to the cell count methods TCC-MS and TCC-FCM. The BACTcontrol was badly correlated to all biomass parameters. The results of both sensors correlated significantly to each other, but the strength of the correlation was classified as bad.

Table 9. Linear regression analysis of dilutions series of all water types in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given. Datapoints from Eindhoven water samples are not included in this analysis. The linear regression analyses were performed on log transformed data.

Water source	Parameter 1	Parameter 2	N	p<0.05	r^2	
All data points, all water sources	BACMON	ATP	37	Yes	0.11	Bad
		TCC-MS	47	Yes	0.67	Moderate
		TCC-FCM-intact	49	Yes	0.59	Moderate
		TCC-FCM-total	49	Yes	0.64	Moderate
		BACTcontrol	36	Yes	0.43	Bad
	BACTcontrol	ATP	30	Yes	0.44	Bad
		TCC-MS	38	Yes	0.26	Bad
		TCC-FCM-intact	38	Yes	0.10	Bad
		TCC-FCM-total	38	Yes	0.24	Bad

Inspection of the graphs (0) shows that the BACTcontrol results hardly decrease further when cell numbers (measured with TCC-MS and TCC-FCM) are below about $1x10^4$ cell/ml. This confirms what was found in the dilution series performed with bacteria cultured in rich medium (chapter 2.2.2.2). The background noise is thus a problem for measurement of lower cell numbers. Only in untreated ground water of Nieuwegein and Linschoten lower (or even negative) enzymatic activity was measured.

For the BACMON the detection limit seems to be around 300 – 600 cells/ml, again comparable with the results of the dilution series with bacteria cultured in mineral medium (chapter 2.2.2.1) or rich medium (chapter 2.2.2.2).

2.2.2.4 Untreated water samples

The water samples that were used for the validation experiments with natural bacteria (chapters 2.2.1 and 2.2.2.3) were also measured undiluted, without any prior treatment. In total 14 water samples of different water types were analyzed with BACMON, BACTcontrol, ATP, TCC-MS and TCC-FCM. Of four water samples also HPC was measured (Table 10, 0).

The regression analyses show only a moderate correlation between the BACMON and ATP and the total number of cells (TCC-FCM), and between the BACTcontrol and ATP. This suggests that although the BACMON and BACTcontrol correlate well with the biomass and/or cell count parameters in some of the validation studies of one water type, hardly any correlation seems to be present when results of different water types are combined.

Table 10. Linear regression analysis of measurements on untreated water samples (surface, drinking, ground and cooling tower water) in laboratory studies. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p < 0.05$) and if so the r^2 is given. Graphs are shown in 0. The linear regression analyses were performed on log transformed data.

Parameter 1	Parameter 2	N	p<0.05	r^2	
BACMON	ATP	11	No		
	TCC-MS	12	Yes	0.52	Moderate
	TCC-FCM-intact	12	No		
	TCC-FCM-total	12	No		
	HPC	3	No		
	BACTcontrol	12	Yes	0.42	Bad
BACTcontrol	ATP	12	Yes	0.74	Moderate
	TCC-MS	13	Yes	0.33	Bad
	TCC-FCM-intact	13	Yes	0.31	Bad
	TCC-FCM-total	13	Yes	0.31	Bad
	HPC	4	No		

2.3 Discussion and conclusions

2.3.1 Correlation with biomass parameters

Bacteria cultured in mineral medium were not properly detected with the BACTcontrol, but were detected with the BACMON. However, due to problems with preparing the dilution series, the variation in cell numbers was limited which yielded no correlation or no realistic correlations for the BACMON (Table 11 - Table 13). Culturing bacteria in rich medium showed better correlations between the BACMON, BACTcontrol and biomass parameters (Table 11 - Table 13). Especially the BACTcontrol correlates well with the biomass parameters, whereas the BACMON only showed good correlations when larger sample numbers were tested. It proved difficult to find a method to acquire added bacteria that can be detected with both sensors and that mimics the real-life situation. Although culturing bacteria in rich medium seems to work for both sensors, as long as the number of samples is sufficient, these bacteria are expected to be very different from bacteria that are naturally present in different water sources.

It is, therefore, better to validate these sensors with bacteria that are naturally present in (drinking) water. These tests with natural bacteria showed moderate to excellent correlations between the BACMON and all biomass parameters for surface water, cooling water and drinking water, but not for ground water. This absence of correlation with ground water was caused by low sample numbers and low cell numbers (below the detection limit of TCC-FCM; Table 8). The BACTcontrol only correlated well with the biomass and cell count parameters in one surface water sample and with the cellular ATP concentration for cooling water, but not in the other water samples with natural bacteria.

Table 11. Overview of all correlation analyses performed with the BACMON. Only the qualification of the correlation is given: - (analysis not performed), ND (not determined, due to low number of samples), no (no significant correlation), bad (significant correlation, $r^2 < 0.5$), moderate (significant correlation, $0.5 < r^2 < 0.7$), good (significant correlation, $0.7 > r^2 > 0.9$), excellent (significant correlation, $r^2 > 0.9$). Original data can be found in Table 4 - Table 10.

			BACMON, bacteria					
			ATP (total)	ATP (cellular)	LLA	TCC-MS	TCC-FCM, intact	TCC-FCM, total
Mineral medium	Drinking water	KWR	No	-	No	No	-	-
		Weeperkarspel	Good	-	No	Good	-	-
	Cooling water	Uniper, Unit A	No	-	No	Excellent	-	-
		Uniper, Unit D	ND	-	No	ND	-	-
	Surface water	Hollandse IJssel	No	-	No	No	-	-
Rich medium	Drinking water	KWR	No	-	No	No	-	-
		KWR	No	-	No	ND	-	-
	Ground water	Linschoten	Excellent	-	Excellent	Excellent	-	-
Natural bacteria	Surface water	Lekkanaal	No	-	-	Excellent	No	No
		Lekkanaal	Good	Excellent	-	Excellent	Excellent	Excellent
	Cooling water	Uniper	No	Good	-	Good	Excellent	Good
	Drinking water	Andijk	Moderate	Moderate	-	Good	Good	Good
	Ground water	Amersfoortseweg	ND	-	-	Good	No	No
		Nieuwegein	ND	-	-	ND	ND	ND
		Linschoten	ND	-	-	No	No	No
		Eindhoven	ND	-	-	Good	No	No

The number of bacteria (cultured in mineral or rich medium) counted with the BACMON are comparable to the LLA but are about 0 – 1 log lower compared to TCC-MS results. For natural bacteria the same difference is observed between the BACMON and TCC-MS and TCC-FCM (0 - 0). This is comparable to earlier studies with pure *E. coli* cultures where the BACMON underestimated the number of cells with 0.25 – 0.5 log compared to culture (Højris *et al.*; 2016).

These results show that the BACMON can reliably measure natural bacteria in drinking water, surface water and cooling water and that the trends are in general comparable to the parameters TCC-MS, TCC-FCM and (cellular) ATP (Table 11). In addition, the results with the BACTcontrol seems to imply that the BACTcontrol is unable to reliably measure natural bacteria and the result are often not related to the biomass or cell count parameters (Table 12).

Table 12. Overview of all correlation analyses performed with the BACTcontrol. Only the qualification of the correlation is given: - (analysis not performed), ND (not determined, due to low number of samples), no (no significant correlation), bad (significant correlation, $r^2 < 0.5$), moderate (significant correlation, $0.5 < r^2 < 0.7$), good (significant correlation, $0.7 > r^2 > 0.9$), excellent (significant correlation, $r^2 > 0.9$). Original data can be found in **Error! Reference source not found.** - Table 9.

			BACTcontrol					
			ATP (total)	ATP (cellular)	LLA	TCC-MS	TCC-FCM, intact	TCC-FCM, total
Mineral medium	Drinking water	KWR	No	-	No	No	-	-
		Weeperkarspel	No	-	No	No	-	-
	Cooling water	Uniper, Unit A	No	-	No	No	-	-
		Uniper, Unit D	ND	-	No	ND	-	-
	Surface water	Hollandse IJssel	No	-	Excellent*	Excellent*	-	-
Rich medium	Drinking water	KWR	Good	-	Good	Excellent	-	-
		KWR	Good	-	No	Excellent	-	-
	Ground water	Linschoten	Excellent	-	Good	Good	-	-
Natural bacteria	Surface water	Lekkanaal	No	-	-	No	No	No
		Lekkanaal	Good	Good	-	Good	Good	Good
	Cooling water	Uniper	Good	Excellent	-	No	No	No
	Drinking water	Andijk	No	No	-	No	No	No
	Ground water	Amersfoortseweg	ND	-	-	No	No	No
		Nieuwegein	ND	-	-	ND	ND	ND
		Linschoten	ND	-	-	No	ND	No
		Eindhoven	ND	-	-	Good	No	No

Table 13. Overview of all correlation analyses of the BACMON compared to the BACTcontrol. Only the qualification of the correlation is given: - (analysis not performed), ND (not determined, due to low number of samples), no (no significant correlation), bad (significant correlation, $r^2 < 0.5$), moderate (significant correlation, $0.5 < r^2 < 0.7$), good (significant correlation, $0.7 > r^2 > 0.9$), excellent (significant correlation, $r^2 > 0.9$). Original data can be found in **Error! Reference source not found.** - Table 9.

	BACMON versus BACTcontrol						
Mineral medium	Drinking water	KWR	No	Natural bacateria	Surface water	Lekkanaal	Excellent
		Weeperkarspel	No			Lekkanaal	Good
	Cooling water	Uniper, Unit A	No		Cooling water	Uniper	Good
		Uniper, Unit D	No		Drinking water	Andijk	No
	Surface water	Hollandse IJssel	No		Ground water	Amersfoortseweg	No
Rich medium	Drinking water	KWR	No			Nieuwegein	ND
		KWR	No			Linschoten	No
	Ground water	Linschoten	Good			Eindhoven	Excellent

2.3.2 Detection limit

The detection limit of the BACMON and BACTcontrol is not strongly influenced by the origin of the bacteria (cultured or natural bacteria). The BACTcontrol has a detection limit of about $1x10^4$ cells/ml (counted with TCC-MS, TCC-FCM or LLA). When cell numbers are below this level, the enzymatic activity measured with the BACTcontrol no longer decreases but levels off at around 10 – 40 pmol/volume. As a consequence, water sources with cell numbers below this limit cannot be reliably measured with the BACTcontrol. As a consequence, events where cell numbers in water rise or decrease will not be detected by the BACTcontrol when the cell numbers remains below $1x10^4$ cells/ml.

The theoretical detection limit of the BACMON is 167 cells/ml. The validation experiments show that at around 300 – 500 cells/ml the cell numbers no longer decrease which is close to the theoretical detection limit. However, the BACMON hardly ever yielded a result of 0 cells/ml (<167 cells/ml), even if other biomass parameters did yield bacterial counts below 167 cells/ml. This is caused by the difficult process of cleaning all bottles, flow cells, tubes, etc well enough to remove all particles and bacterial cells. The detection of 1, 2 or 3 cells in 6 µl (the measurement volume of the BACMON) leads to a concentration of respectively 167, 334 or 501 cells/ml but due to the low cell numbers this partly relies on coincidence.

Of both methods the upper detection limit was not reached. The theoretical detection limit of the BACMON lies at around $1\text{-}5x10^6$ cells/ml, above this level the results become less reliable (Højris et al., 2016). Of the BACTcontrol no theoretical upper detection limit is known.

2.3.3 Suitability for water types

Both sensors encountered some problems in measuring untreated ground water. The BACTcontrol measurements on ground water with natural bacteria yielded very low enzymatic activities. A possible explanation could be the low cell numbers in ground water and the metabolic state of the cells. If the cells are metabolically active to a limited extent, it might be possible that the alkaline phosphatase enzyme is not active enough to detect with the BACTcontrol. The BACMON measurements yielded constant bacterial numbers, despite the fact that the TCC-MS and TCC-FCM methods showed decreasing cell numbers due to the dilution series. However, the BACMON measurements on Amersfoortseweg (paragraph 2.2.2.3) were an exception and showed better results.

Drinking water, surface water and cooling tower water all have high enough cell numbers for both sensors to be able to measure these cell number or activity. Untreated ground water, however, may be more problematic. Still, measuring ground water in an online setting, thereby avoiding the sometimes anoxic water coming into contact with oxygen for example, might yield better results but this has to be tested in the field.

Overall we conclude from our results that the BACMON can reliably measure (part of the) cell numbers in drinking water, surface water and cooling water. The BACTcontrol can perhaps reliably measure microbial activity in surface water, but not in the other water types tested. Furthermore, the performance of the sensors in an online setting is not taken into account. This will be done in the tests at the full-scale locations (chapters 3 - 6).

3 Full-scale location at Oud-Turnhout, Pidpa

3.1 Description full-scale location

3.1.1 General introduction

Pidpa is a water company located in the province of Antwerp (Belgium). Pidpa provides drinking water to more than 500.000 customers with an infrastructure of 11 water production centers (WPC), 59 water towers, 27 boost pump station and more than 13.000 km of distribution network. Pidpa is also active in sewerage management and process water.

3.1.2 Water type and production process

The water type for both test locations used in this project, is drinking water made from anaerobic ground water at water production center (WPC) Oud-Turnhout. This WPC has three different raw water sources (Arendonk, Ravels and Oud-Turnhout; Figure 15). These ground waters are transported to the production location and treated in different mixtures in different treatment trains. The first step of every treatment train is aeration (addition of oxygen and partial removal of gases like methane, hydrogen sulfide, carbon dioxide). Iron removal is the next step and is done by sand filtration or decantation (with lime dosing). Ammonia and manganese are removed in a subsequent rapid sand filtration step. The treated water is stored in reservoirs and after UV disinfection pumped into the distribution network. The distribution network delivers to the water tower or takes water from the water tower according to the available pressure (demand) in the network.

The treatment train with double sand filtration receives raw water from the three different water sources but mainly (80 – 90%) from the sources Ravels and Arendonk. This treatment line is in continuous operation at 300-450 m³/h. The two other treatment lines have both a separate decantation step followed by a common sand filtration. Both decanters receive raw water from Oud-Turnhout but from different wells. This causes a difference in raw water quality between both decanters. The largest difference lies in the higher methane (1.6 versus 0.3 mg/l methane) and higher organic matter (4.2 versus 2.6 mg/l TNPOC) for decanter 1 (DC1). Both decanters work in an alternating regime at 400 m³/h for about 24 h. This means that most of the time one decanter is not in service during 24 h.

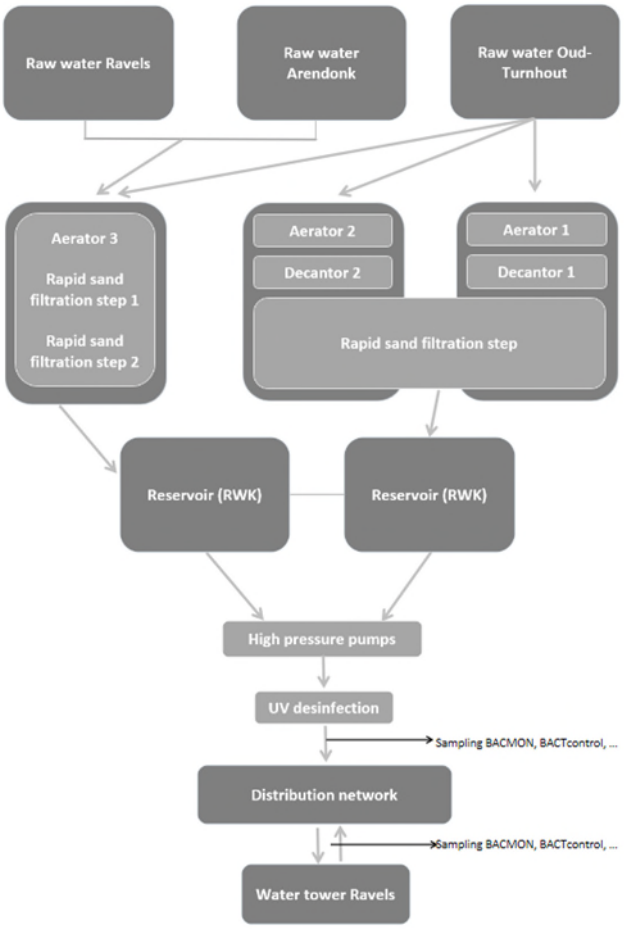


Figure 15. Schematic drawing of the water treatment plant at Oud-Turnhout (WPC OT). The sampling location of the sensors is indicated.

3.1.3 Location

WPC Oud-Turnhout, located in the province of Antwerp (Belgium; Figure 16), is one of the 11 drinking water production centers from Pidpa. It has a maximum production capacity of 36000 m³/day. The water tower in Ravels was built in 1975 and has a reservoir of 1000 m³.

For Pidpa the tests at the full-scale location were performed at WPC Oud-Turnhout (WPC OT; Figure 17) and the water tower in Ravels (WT RV; Figure 18). At both locations the BACMON sensor has been operational since February 2017 and the sensors showed already some correlations with operational parameters (shortly discussed in 3.1.4).

Figure 16. Location of WPC Oud-Turnhout in province of Antwerp, Belgium.

Figure 17. Overview of WPC Oud-Turnhout were the microbiological sensors were tested.

Figure 18. Water tower in Ravels were the microbiological sensors were tested.

3.1.4 Study aim

Historic data of Pidpa show a correlation between the number of bacteria at the high pressure pumps (clear water leaving the reservoirs) measured with the BACMON and which decanter was in service. Most of the time when decanter number 1 (Figure 15) was in service the bacterial number was higher at the high pressure pumps. This increase was also monitored after transport trough the distribution network at the water tower in Ravels (WT RV) with an average residence time in the distribution network around 16 h (depending on water flow, pressure, etc.).

The aim for this study was:

- To compare data from the BACMON and BACTcontrol and test whether differences caused by which decantor is in service can be measured. Do both sensors give the same or additional information with respect to this effect?
- To create an event with an increase of bacteria at the high pressure pumps and test whether this can be monitored with both sensors and with laboratory measured biomass parameters. At the same time test the effect of the distribution network on the bacterial number of this peak event.
- To look if bacterial regrowth can be measured. Due to a relatively high organic matter content (TNPOC: 3 mg/l) the area for water delivery around Oud-Turnhout has higher numbers of *Aeromonas* bacteria during warm periods. Can this effect also be seen with an online measurement technique for total bacteria?

The first hypothesis was that both sensors can measure the variation in bacterial numbers, both at WPC Oud-Turnhout and after transport to the water tower in Ravels, and that are caused by changes by which decanter is in service.

The second hypothesis was that both sensors can measure regrowth in the distribution network that is in concordance with regrowth of *Aeromonas*

Therefore, the data of both sensors are compared to each other and related (amongst others) to the flows of both decantors. A peak event in bacterial numbers will be created by delaying the switch between decantors for a period of 48 hours (instead of 24 hours).

3.1.5 Installation of microbiological sensors

The sensors were placed after the high pressure pumps of WPC Oud-Turnhout (after UV disinfection) and at the water tower in Ravels in the pipeline going to the elevated reservoir. At both locations already a BACMON sensor (owned by Pidpa) was in service. In Figure 19 a schematic drawing of the installed sensors is given.

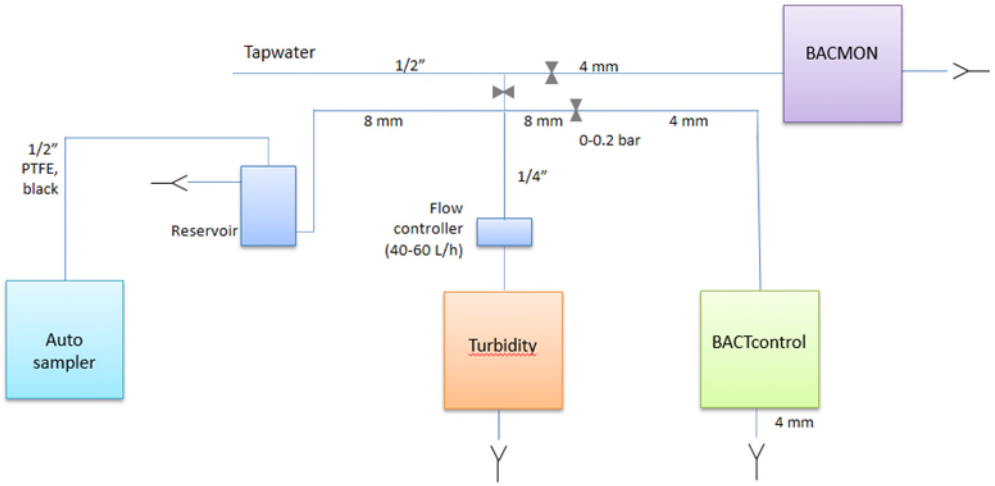


Figure 19. Schematic drawing of the installation of the BACMON, BACTcontrol and turbidity sensors at the water treatment plant at Oud-Turnhout (WPC OT) and water tower of Ravels. The installation site of the sensors is indicated in Figure 15. The type of tubes that was used is indicated.

3.2 Results

3.2.1 BACMON, BACTcontrol and turbidity results

At WPC Oud-Turnhout the results of the BACMON sensor showed one small 24 h period with a data interruption (5-11-2018) due to a problem with the Pidpa SCADA server (Figure 20, top graph). The number of bacteria varies between ~500 – 3000 cells/ml, the number of particles is lower (0 – 2000 cells/ml). Overall there is a larger variation (factor 2-4) in bacteria than in non-bacteria (factor 2). There was a clear relationship between which decanter was in service and the amount of bacteria at the high pressure pumps (discussed later in Figure 29, paragraph 3.2.3).

At the water tower in Ravels the same interruption occurred in the data as at the WPC Oud-Turnhout (Figure 20, lower graph). The variation in bacterial numbers is larger than the number of non-bacterial particles. The average values in the water tower are a little lower for both bacteria and non-bacteria compared to the WPC (Figure 21, top graph). The other difference with the WPC data lies in a larger amount of smaller peaks (for bacteria and non-bacteria). Possible causes for this are the impact of flow variations

and changing pressure in the distribution system and the alternating water flow in the water tower that causes a rising and descending water level.

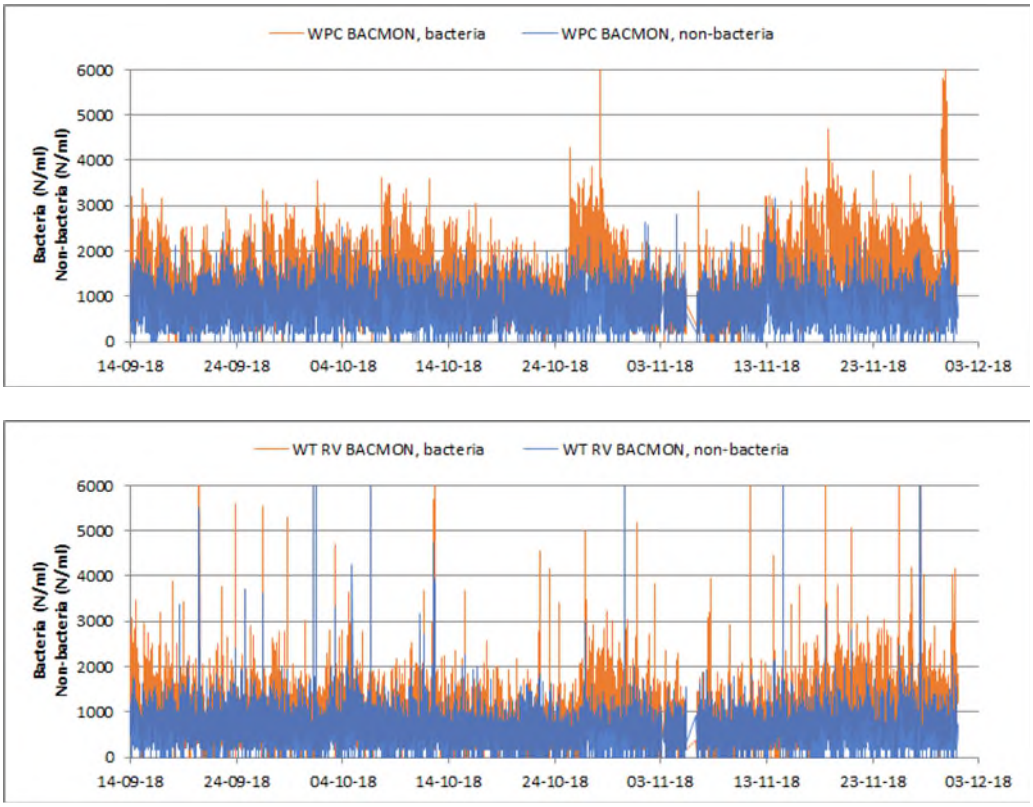
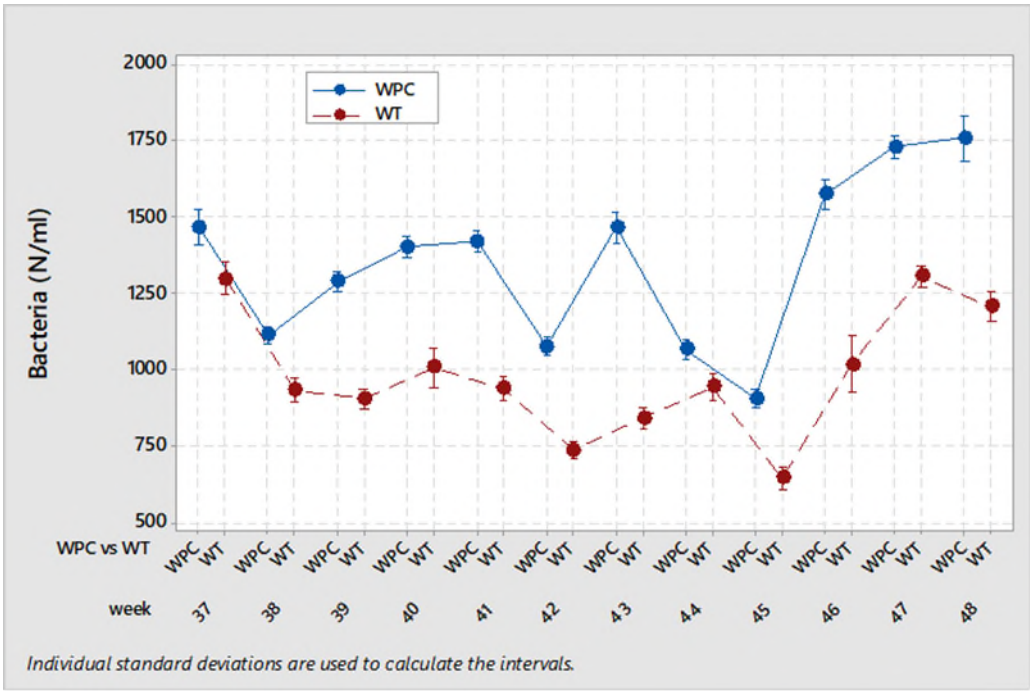


Figure 20. BACMON measurements (bacteria: orange; non-bacteria: blue) at WPC Oud-Turnhout (top) and the water tower in Ravels (bottom).



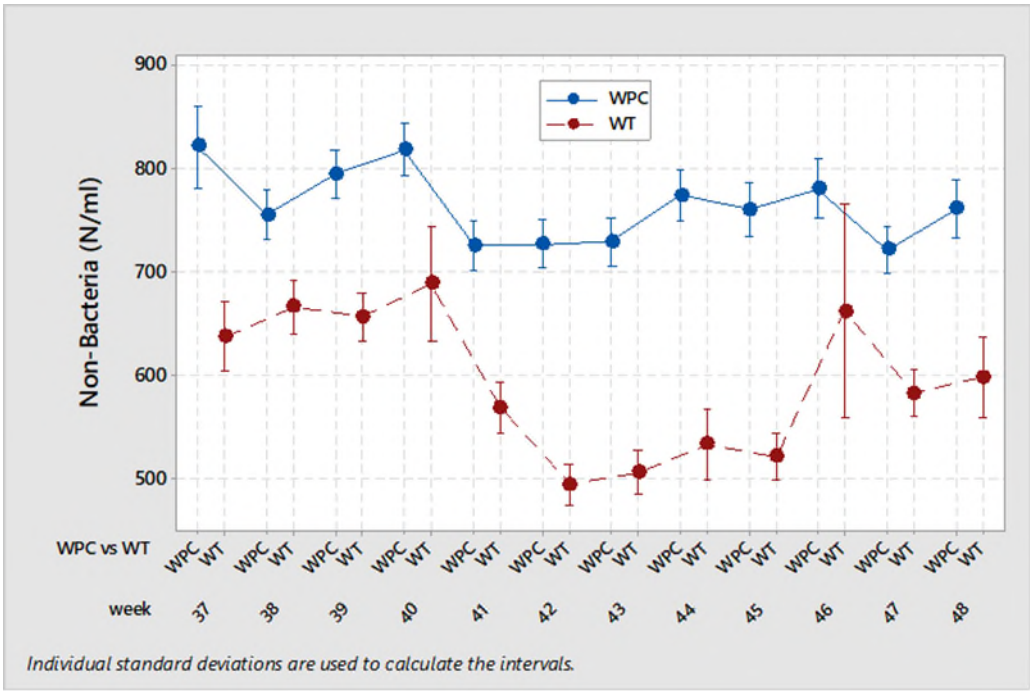


Figure 21. Average number of bacteria (top) and non-bacteria (bottom) per week at WPC Oud-Turnhout (blue) and the water tower at Ravels (red). Given is the average of about 1000 measurements (per week: 7 days x 24 hours x 6 measurements per hour = 1008 measurements), with the standard deviation.

The data of the BACTcontrol at the WPC (Figure 22, top) showed a few interruptions due to different causes: pressure too high, detection limit too high, detector fault, faulty reagent volumes, air in system, period with no cleaning agent and changing reagent. The rise in value from 24-9-2018 until begin October is possibly a drift in the signal because the values become high for drinking water (normal values for drinking water

are 40 ± 30 pmol/min/100 ml) and the ceramic filter had to be replaced on 8-10-2018. The sudden drop on the 25-10-2018 is most likely to be explained by changing to a reagent for enzymatic activity from another supplier. After 25-10-2018 the data shows a factor 2 variation in data but no correlation with operational data could be found (further details in paragraph 3.2.3).

The BACTcontrol data at the water tower (Figure 22, bottom) had a large period with doubtful data because of the absence of cleaning agent in the system. During the first week enzymatic activities at the WPC were higher in comparison with the water tower and during the last two weeks WPC and water tower showed similar amounts. Data of the last two weeks seem to be the most reliable because of the different interruptions and maintenance actions during the first period. This suggests that the number of bacteria does not vary between WPC and the water tower and thus that regrowth does not occur, is limited and can, therefore, not be detected or results in a change in microbial community composition, but not in cell numbers.

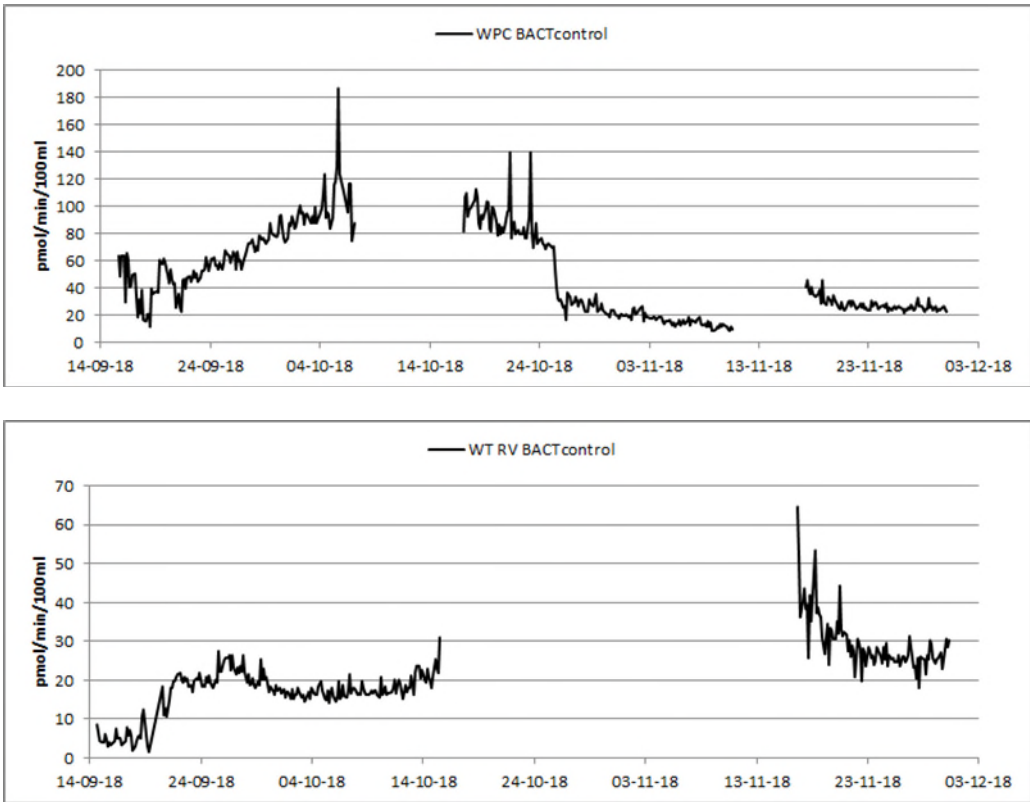


Figure 22 BACTcontrol measurements for the WPC (top) and the water tower (bottom)

Turbidity at the WPC showed only small overall variation (between 0.4 and 0.6 FNU) with a few peaks (Figure 23, Figure 24). Turbidity at the water tower showed a little more overall variation (between 0.2 and 0.6 FNU) in comparison to the WPC and also a few peaks occurred. The difference in turbidity (on average weekly basis) is shown in Figure 24 and confirms the small difference in average value and the higher standard deviation on the data of the water tower.

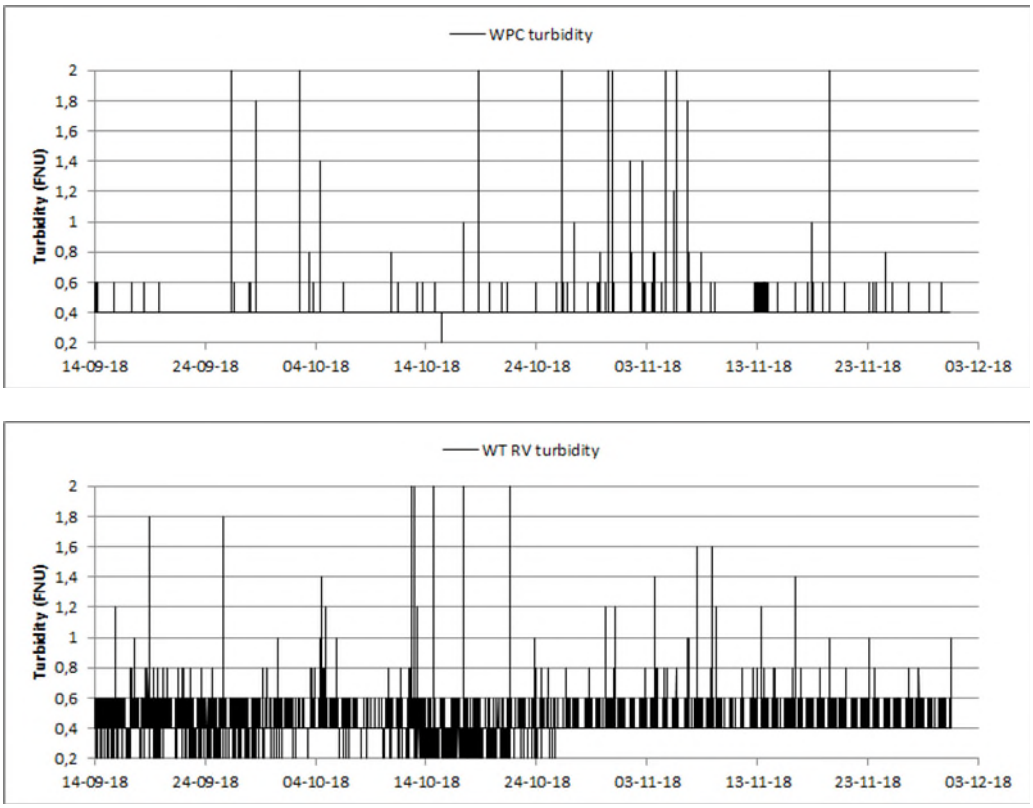


Figure 23. Turbidity measurements for the WPC (top) and the water tower (bottom)

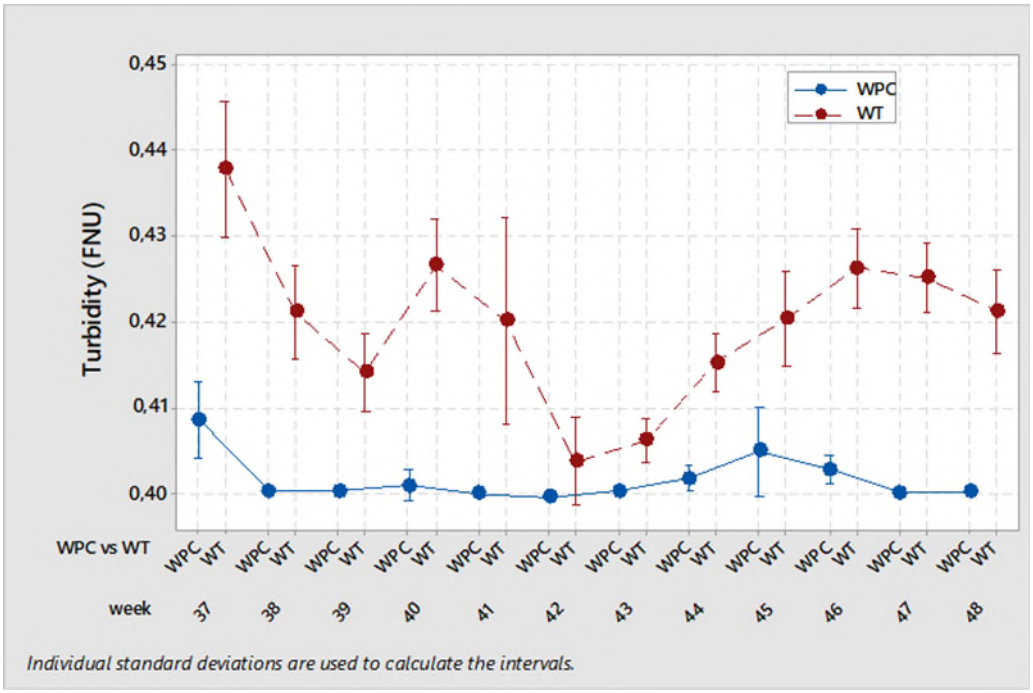


Figure 24. Average number of turbidity (FNU) per week at WPC (red) and at the water tower (blue)

3.2.2 Comparison of data from the three sensors to each other

The overall data from BACMON and BACTcontrol in the WPC or water tower do not show a comparable trend in bacterial numbers or enzymatic activity (Figure 25). The 24 hours variation that is visible with the BACMON is not observed with the BACTcontrol results. Trends with operational parameters that are seen with BACMON are not visible with the BACTcontrol (examples in paragraph 3.2.3).

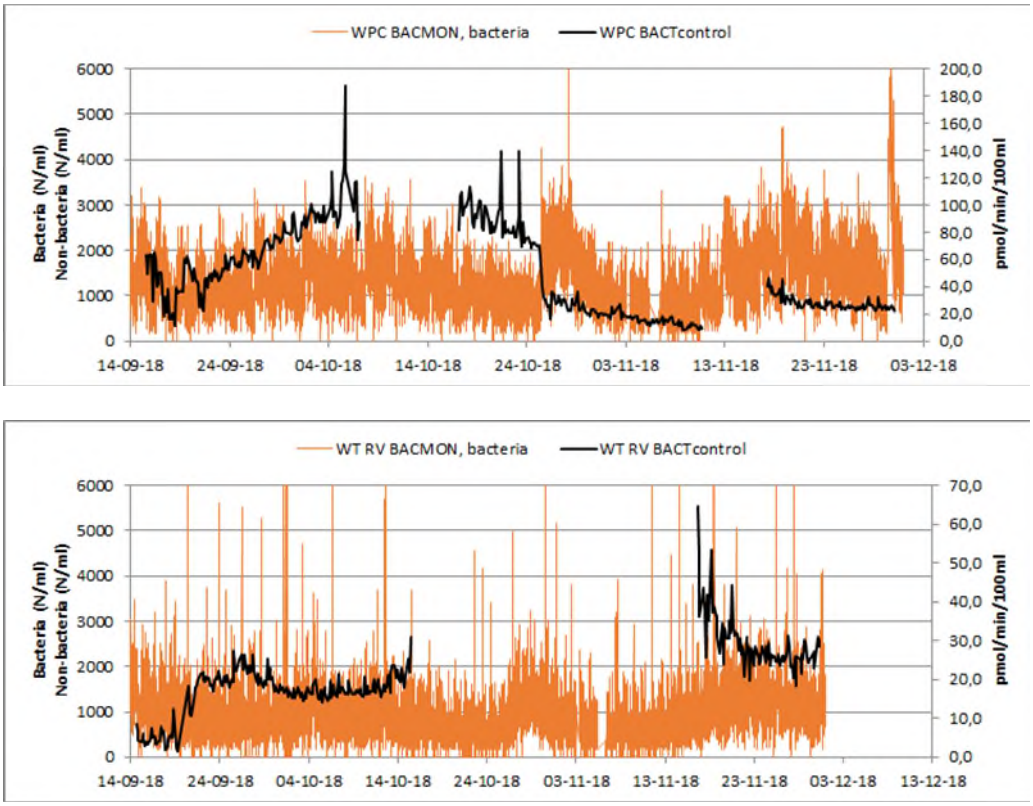


Figure 25. Comparison of BACMON (orange) and BACTcontrol results (black) at WPC Oud-Turnhout (top) and the water tower in Ravels (bottom).

For making a more statistical comparison between BACMON and BACTcontrol each value of enzymatic activity (BACTcontrol measurement every 3 hours) is compared to the average of the 3 nearest (in time) BACMON measurements (sample every 10 minutes). The scatterplot of the whole dataset of the WPC shows no relation between BACMON and BACTcontrol (Figure 26, top graph). When only data points after 25-10-2018 are used (which is considered to be the most reliable data for BACTcontrol) there is a significant linear correlation between BACMON (bacteria) and BACTcontrol, although the R^2 remains below 0.5 ($r^2 = 0.48$; Figure 26, bottom graph).

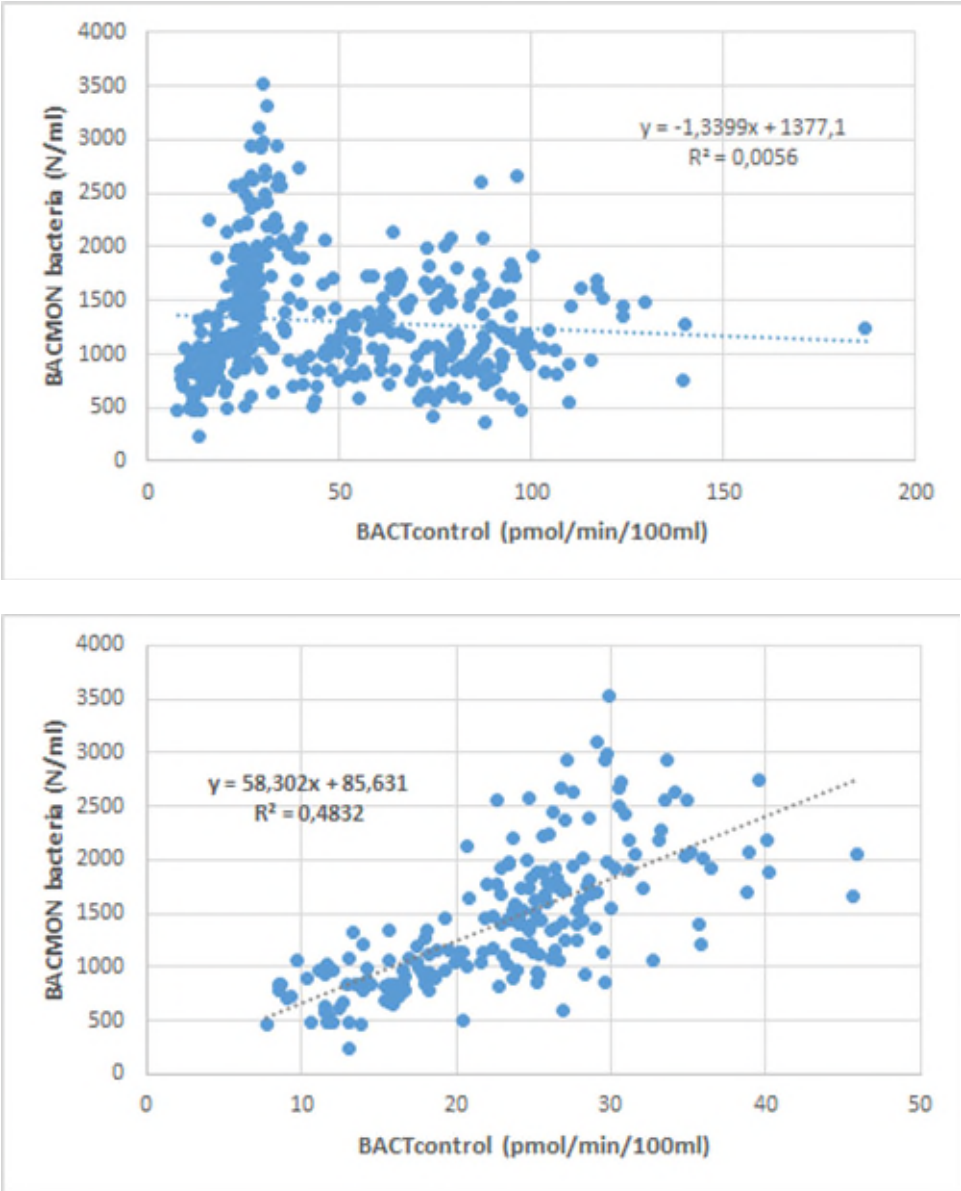


Figure 26. Scatterplot of bacteria (BACMON) and enzymatic activity (BACTcontrol) at WPC. Each value of enzymatic activity is compared to the average of the 3 nearest (in time) BACMON measurements. A linear correlation is shown. Top graph: all datapoints of the duration of the full-scale experiment. Bottom graph: only datapoints after 25-10-2018.

A comparison of the BACMON and BACTcontrol sensors at the water tower in Ravels shows no correlation between both sensors (Figure 27). Data starting from 16-11-2018 (which is considered the most reliable data for BACTcontrol) are shown.

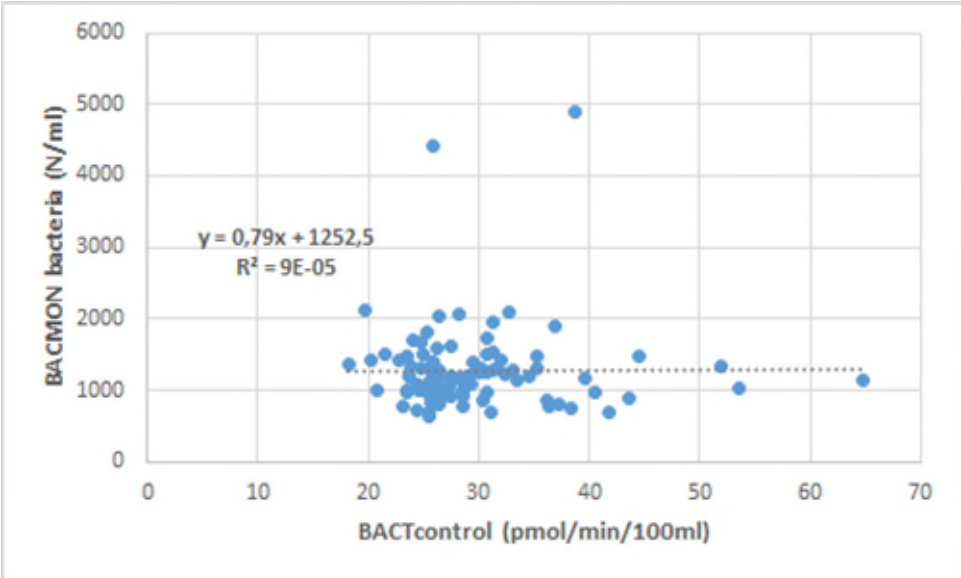


Figure 27. Scatterplot of bacteria (BACMON) and enzymatic activity (BACTcontrol) at the water tower. Each value of enzymatic activity is compared to the average of the 3 nearest (in time) BACMON measurements. A linear correlation is shown. All datapoints from 16-11-2018 are included.

At some events with higher number of non-bacteria (BACMON) there is a clear relation between turbidity and non-bacteria of the BACMON (Figure 28). But not every peak of the BACMON is detected by the turbidity sensor and vice versa. For example, the non-bacteria peak in around 12-10-19 13:00 has no peak in turbidity but the non-bacteria peak around 12-10-19 17:00 has a corresponding peak in turbidity. By using a scatterplot of BACMON (non-bacteria) and turbidity at the WPC or water tower, no correlation between both can be found**Error! Reference source not found.**.

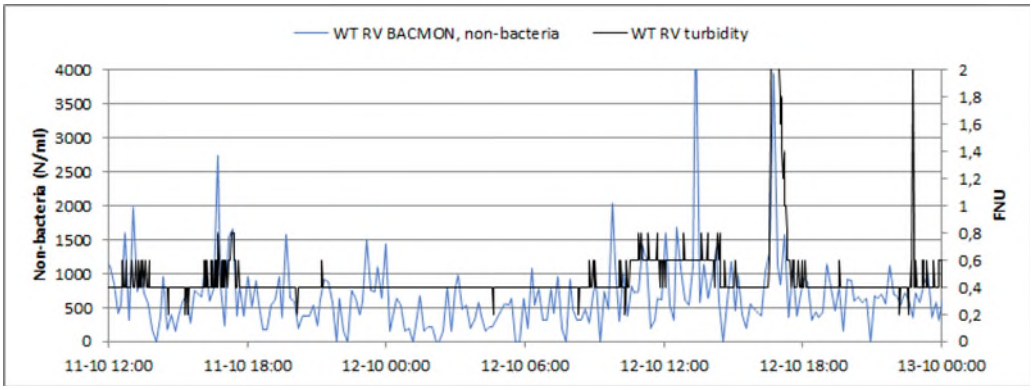


Figure 28. Turbudity measurements (black) and non-bacteria numbers of the BACMON (blue) at the water tower in Ravels.

3.2.3 Comparison of process data with microbial sensors

The BACMON results at the WPC show that alternating (every 24 h) between the two treatment lines with decanter (DC1 or DC2) results in a different water quality at the high pressure pumps (Figure 29, Figure 30). The bacterial count at the high pressure pumps is higher when decanter 1 (DC1) is in service compared to when decanter 2 (DC2) is in service (Figure 29). The same conclusion can be made based on the average values of bacterial numbers depending on whether DC1 (blue) or DC 2 (red) is in service (Figure 30), the average number of bacteria is higher when DC1 is in use. One of the possible explanations of this event is the difference in water quality of both decanters. Decanter 1 has a higher concentration of methane and organic carbon (TNPOC) in the raw water than decanter 2 which is caused by differences in water quality of the different raw water sources (paragraph 3.1.2). This probably causes a higher bacterial yield in the treatment due to more biological oxidation of methane and/or more bacterial growth on biodegradable organic carbon.

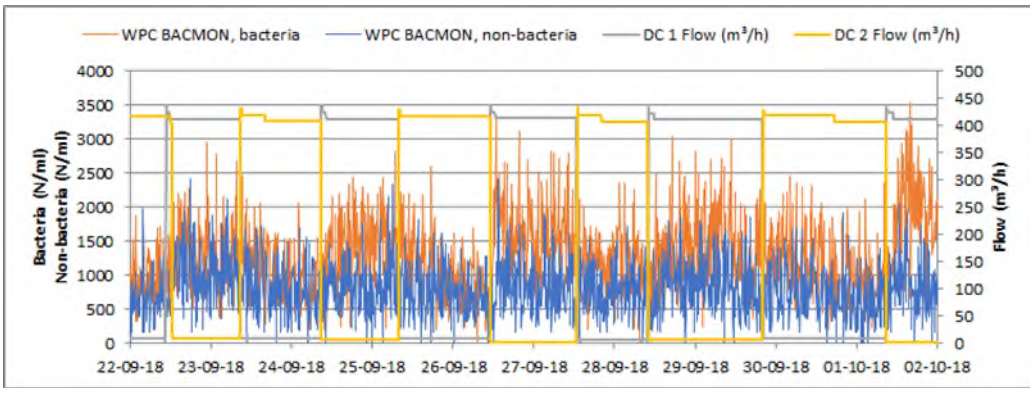


Figure 29. BACMON measurements (bacteria: orange; non-bacteria: blue) at WPC Oud-Turnhout compared with the flow of decantor 1 (grey) and 2 (yellow).

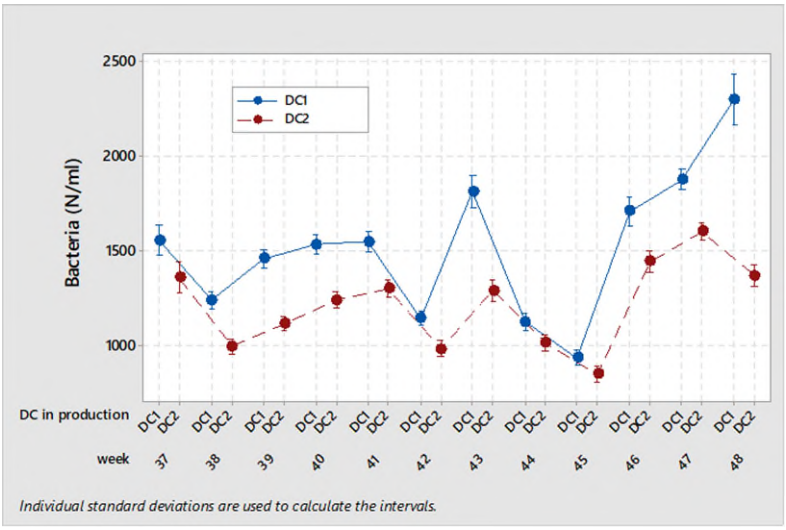


Figure 30. Average number of bacteria per week at WPC Oud-Turnhout when decantor 1 (DC1, blue) or decantor 2 (DC2, red) is in use. Given is the average of about 500 measurements per decantor per week (total per week: 7 days x 24 hours x 6 measurements per hour = 1008 measurements), with the standard deviation.

For WPC Oud-Turnhout the phenomenon of bacterial differences between which decanter is in service accounts for variation of a factor 2-4 in the bacterial data (DC1 in service creates 2-4 times higher bacterial values at the high pressure pumps). During the test period there were no other peaks that could clearly be linked to operational data. On other Pidpa production locations where also BACMON sensors are installed directly after the high pressure pumps relations were found between (as these production locations and sensors are not part of this study, no underlying data are shown):

- Turbidity after filtration and BACMON
- Turbidity after decantation and BACMON
- Changing high pressure pumps and BACMON
- Large changes in flow rate of treatment plant and BACMON
- Water standing still in water tower and BACMON

The pattern of alternating between the treatment lines (difference between DC1 and DC2 in service) is not visible with the BACTcontrol (Figure 31). No other visual correlations with operational parameters at the treatment plant were found.

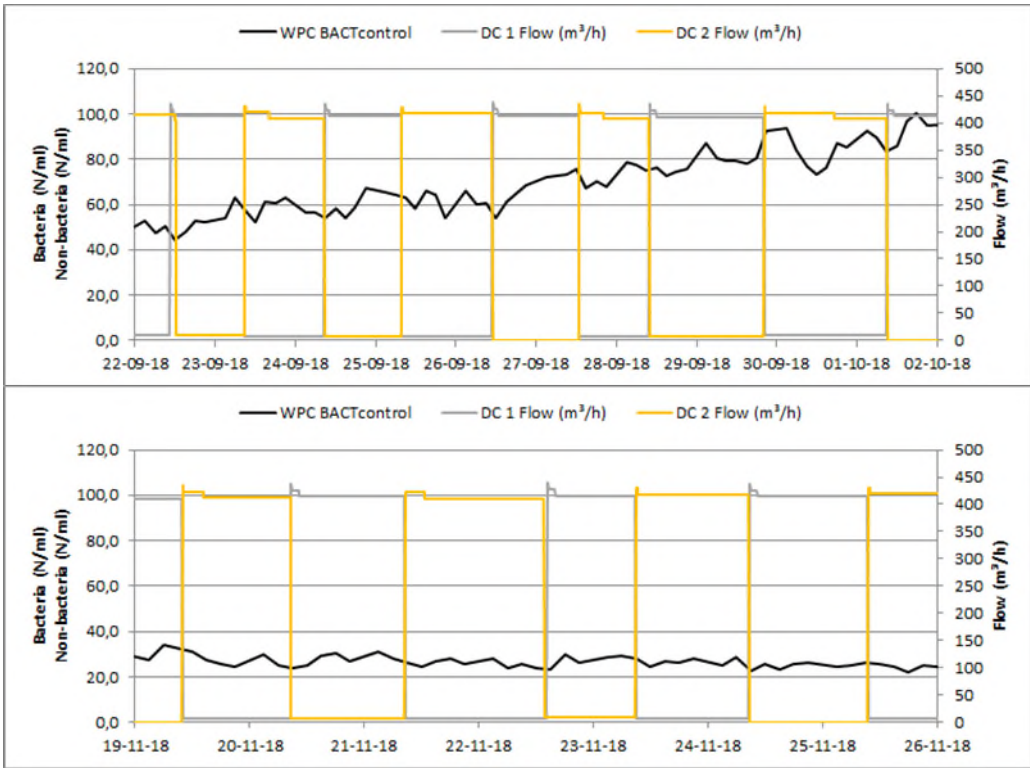


Figure 31. BACTcontrol measurements (black) at WPC Oud-Turnhout compared with the flow of decantor 1 (grey) and 2 (yellow).

During the day the water level in a water tower changes due to differences in water demand. When the water level in the tower is rising, water from the distribution network enters the water tower. When the water level descends, water flows out of the tower into the distribution network. In some cases water coming in has a different bacterial count than the water that is going out of the water tower. This can for example be seen in Figure 32, where around 9:00 the water level starts rising (water out of the distribution network flows into the water tower, so the BACMON measures this 'distribution system water') and bacterial count varies between 1000 and 3000 bacteria per ml. From around 15:00 the water level is descending and water coming out of the water tower is analyzed. The bacteria concentration of this water is < 1000 bacteria/ml so the water in the water tower has at this moment a lower bacterial content than the water of the distribution network. These data show that the number of bacteria in the water tower is lower compared to the pipes in the distribution system.

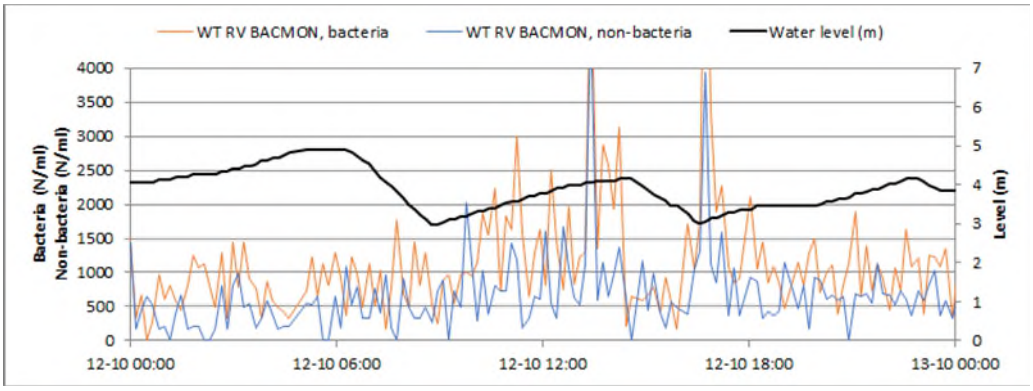


Figure 32. BACMON measurements (bacteria: orange; non-bacteria: blue) at the water tower in Ravels compared with the water level of the water tower (black).

3.2.4 Forced peak event

A peak event was forced by delaying the switch between decanters for a period of 48 hours (instead of 24 h). This action causes a rise in bacterial count (at the high pressure pumps) from 1000 to 5000 bacteria per ml, but no significant increase in non-bacteria (Figure 33, top graph). The rise in bacterial numbers is possible due to water standing still in the decantor for 48 h before it flows out of the decanters, and the different raw water quality for decanter 1 and 2 as was described earlier. This change at the WPC was not detected by the BACTcontol (Figure 33, bottom graph). The bacterial peak from BACMON WPC was also detected (with a lower maximum value) at the water tower in Ravels (WT RV) around 24 h later. At this moment no BACTcontrol data were available because the full-scale period was stopped 1 h before. In chapter 7.2 the results are discussed further, and compared with the laboratory biomass parameters.

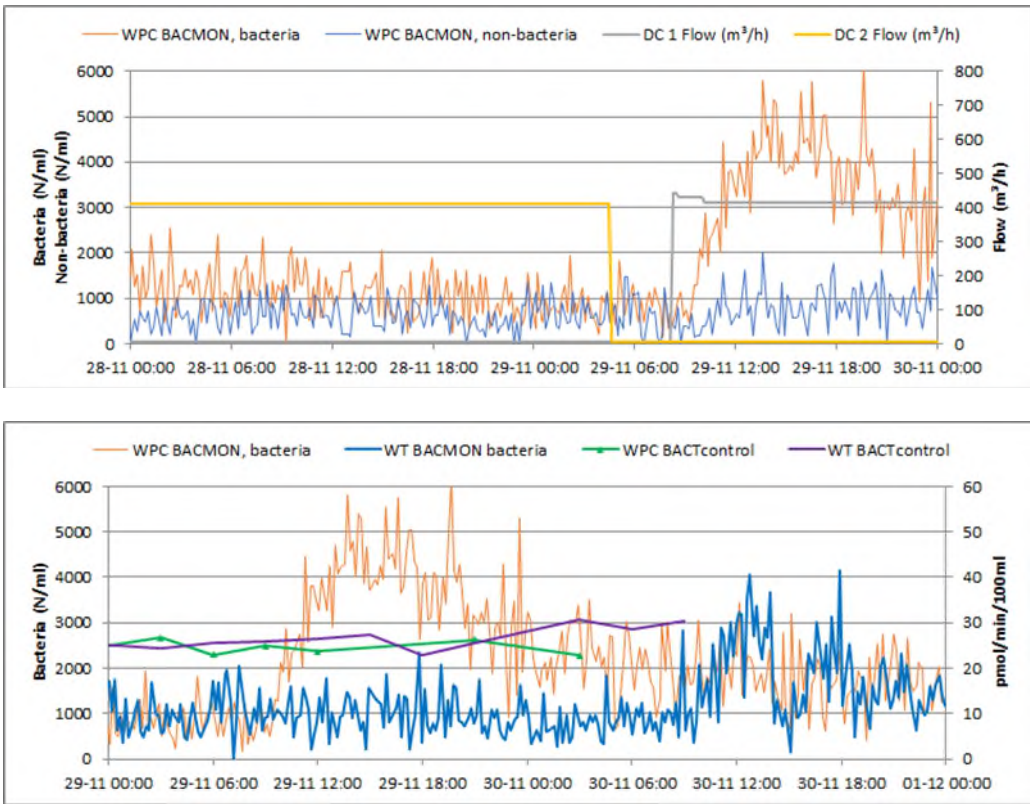


Figure 33. BACMON measurements at the WPC and at the water tower in Ravels compared with BACTcontrol.

3.3 Discussion and conclusions

3.3.1 Suitability of microbiological sensors for online measurements at the full-scale location

Pidpa is convinced that the BACMON sensor is useful to investigate the process stability of the ground water treatment processes on a continuous basis. The use of the BACMON sensor at WPC Oud-Turnhout led to the discovery of difference in water quality produced from different treatment lines. This was not found with the routine laboratory analyses done in the normal Pidpa monitoring program at the high pressure pumps (e.g. heterotrophic plate count, *Aeromonas*, flowcytometry). This might have been caused by the limited numbers of analyzed samples in the routine program and/or due to the (too) low sensitivity of the analysis to detect the differences observed with the BACMON sensor. The use of the BACMON at different WPC creates extra value and insights in the bacterial and particle stability after the treatment processes. Considering all these positive characteristics of the BACMON sensor, it is very disappointing that Grundfos decided to take such a reliable, sustainable and low maintenance sensor out of the market.

The BACTcontrol did not show such reliable results or correlations with operational parameters as the BACMON sensor for studying process stability of the overall ground water treatment process. This is probably due to the lower values of enzymatic activity of treated ground water or the sample interval of 3 hours which is rather big. In addition, many measurements were not successful due to different interruptions (e.g.

no cleaning agent in system) and maintenance actions (e.g. change in detector settings).

3.3.2 Conclusions

The microbial differences between which decantor is in service were detected with the BACMON sensor, but not with the BACTcontrol.

The BACMON sensor but not the BACTcontrol sensor detected transport of bacterial peaks from WPC trough the distribution network and up to the water tower. Regrowth in the distribution system was not observed by the BACMON, since the average bacterial number (on weekly basis) was always a little lower in the water tower compared to the WPC. The BACTcontrol also did not yield evidence for bacterial regrowth which would result in a higher enzymatic activity.

3.3.3 Lessons learned from the full-scale location

- The reagent free BACMON sensor has a big advantage compared to the reagent BACTcontrol sensor, because it will not have possible problems with dosing amounts, empty reagents, etc;
- Continuous measurements in (drinking) water create more insights compared to grab samples in a routine monitoring program.
- Not every peak can be explained, but a factor 3-4 change in bacterial count in the water measured with the BACMON can (in this case) always be coupled to an operational technical parameter.

4 Full-scale location at Uniper

4.1 Description full-scale location

4.1.1 General introduction

Uniper produces and supplies electricity, heat, and gas to customers in the Netherlands and plays an important role in ensuring the energy supply in the southwestern part of the Netherlands. Uniper also aims to supply a range of innovative energy products to their partners and customers throughout the southwestern region of the Netherlands (Figure 34). This includes providing steam to industrial facilities located near the power plants of Uniper and using biomass and industrial by-products (such as biopropane) to generate power and heat.

In addition, Uniper provides district heating to residents of Leiden, Rotterdam and The Hague for many years with technologically advanced gas-fired power plants.

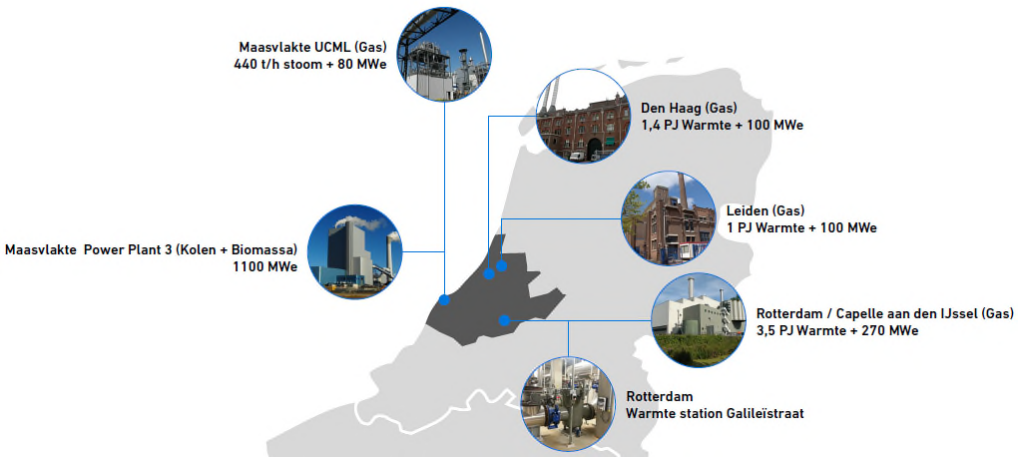


Figure 34. production locations of Uniper in the southwestern part of the Netherlands.

4.1.2 Location, water type and production process

In this study the microbiological sensors were installed at the power plant in Rotterdam/Capelle aan den IJssel (RoCa, Figure 34). This power plant, commissioned in 1982 and expanded in 1996, uses natural gas to produce heat and electricity (net output of 264 MW). The power plant includes a cooling tower with four different compartments that is required to discharge the excess heat which is produced by the power plant. The heat is transferred to the cooling water in the cooling towers using heat exchangers.

The RoCa power plant uses surface water derived from the river Hollandse Ijssel as cooling water. After intake the water is transported underground for approximately five km to the cooling towers of the power plant (Figure 35). Upon arrival the water is filtered by sand filtration, the pH is corrected and ozone and an anti-scaling component

(Kurita) are added after which the water enters the cooling system. Replacing the cooling water with new water is based on the conductivity.

Legionella growth is controlled by implementation of a management and monitoring plan. Regularly ATP and monthly *Legionella* measurements are performed and the cooling tower system is operated via ISSO 55.3 and AI32 regulations.

Figure 35. Location of Uniper, the approximate intake location of surface water (located on approximately 5 km distance from Uniper) is given. A short distance upstream of the intake point a waste water treatment plant (WWTP) is located.

4.1.3 Study aim

Growth of *Legionella pneumophila* can occur in cooling towers due to the appropriate temperature and the availability of high nutrient concentrations. Currently companies have to monitor cooling towers for the presence of *Legionella* and act if the number exceeds the legal limit. This monitoring is based on culture methods and takes 7 days before a result is available. The aim of this study was to test online microbiological sensors on cooling tower water and surface water used as source for cooling tower water. Such sensors have hardly been tested on such water types and, therefore, it was tested if the sensors can be applied to these water types and if these sensors can be used to predict an increase in bacterial growth, which might be related to enhanced *Legionella* numbers in the water.

4.1.4 Installation of microbiological sensors

The microbiological sensors were installed on 5 December 2018 at two points in the cooling system (**Error! Reference source not found.**). The first location is the incoming surface water, before filtration and ozone dosage (sampling point 'Hollandse Ijssel' in **Error! Reference source not found.**), the second location is the cooling water from the

cooling towers (sampling point 'cooling tower water' in **Error! Reference source not found.**).

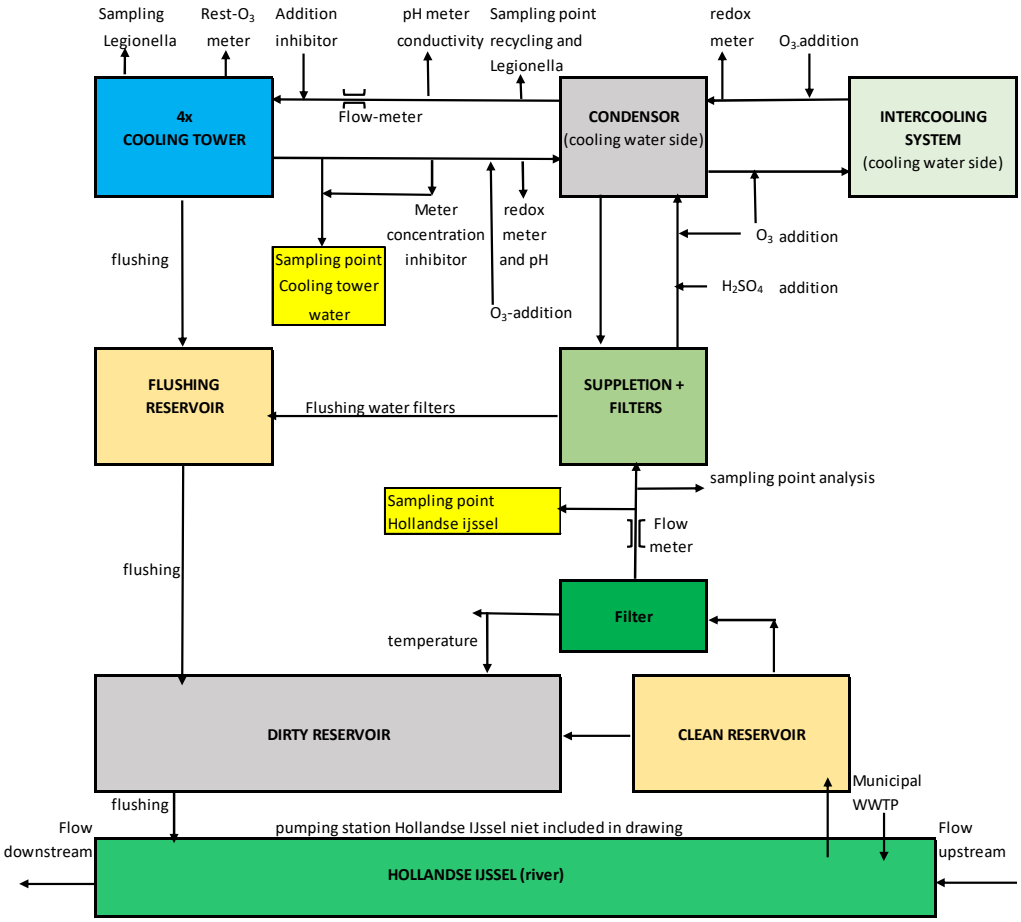


Figure 36. Schematic drawing of the installation of the sensors on cooling tower and surface water at Uniper.

In **Error! Reference source not found.** and **Error! Reference source not found.** a schematic drawing of the installed sensors is given. On 4 January 2019 a pressure reducer was installed to lower the variation in water flow and pressure. The large pressure variation caused problems with the measurements of the sensors as the sensors have different requirements for the water pressure.

Due to the high turbidity of both water types, the flow cell of the BACMON fouled quickly and was therefore replaced weekly. The turbidity sensor was cleaned regularly. However, the surface water was too dirty to reliably measure the turbidity. The BACTcontrol was not able to measure the cooling tower water, as the ceramic filter clogged after only a few measurements. Several attempts to solve this problem were unsuccessful and the BACTcontrol sensor was turned off. The filter of the BACTcontrol measuring surface water was also regularly replaced.

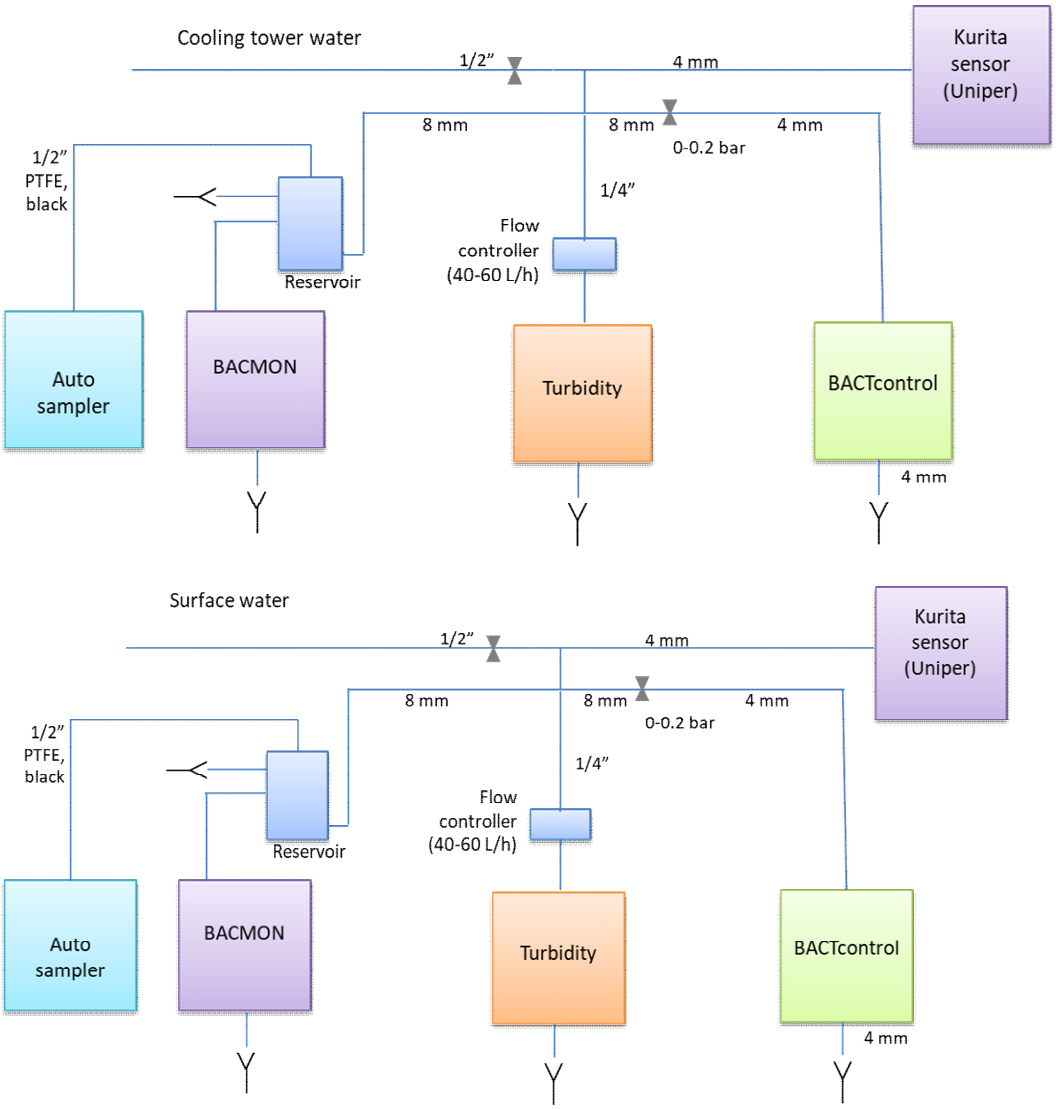


Figure 37. Schematic drawing of the installation of the BACMON, BACTcontrol and turbidity sensors on cooling tower water (top) and surface water (bottom) at Uniper up until 4 January 2019. The installation site of the sensors is indicated in Figure 47. The type of tubes that was used is indicated.

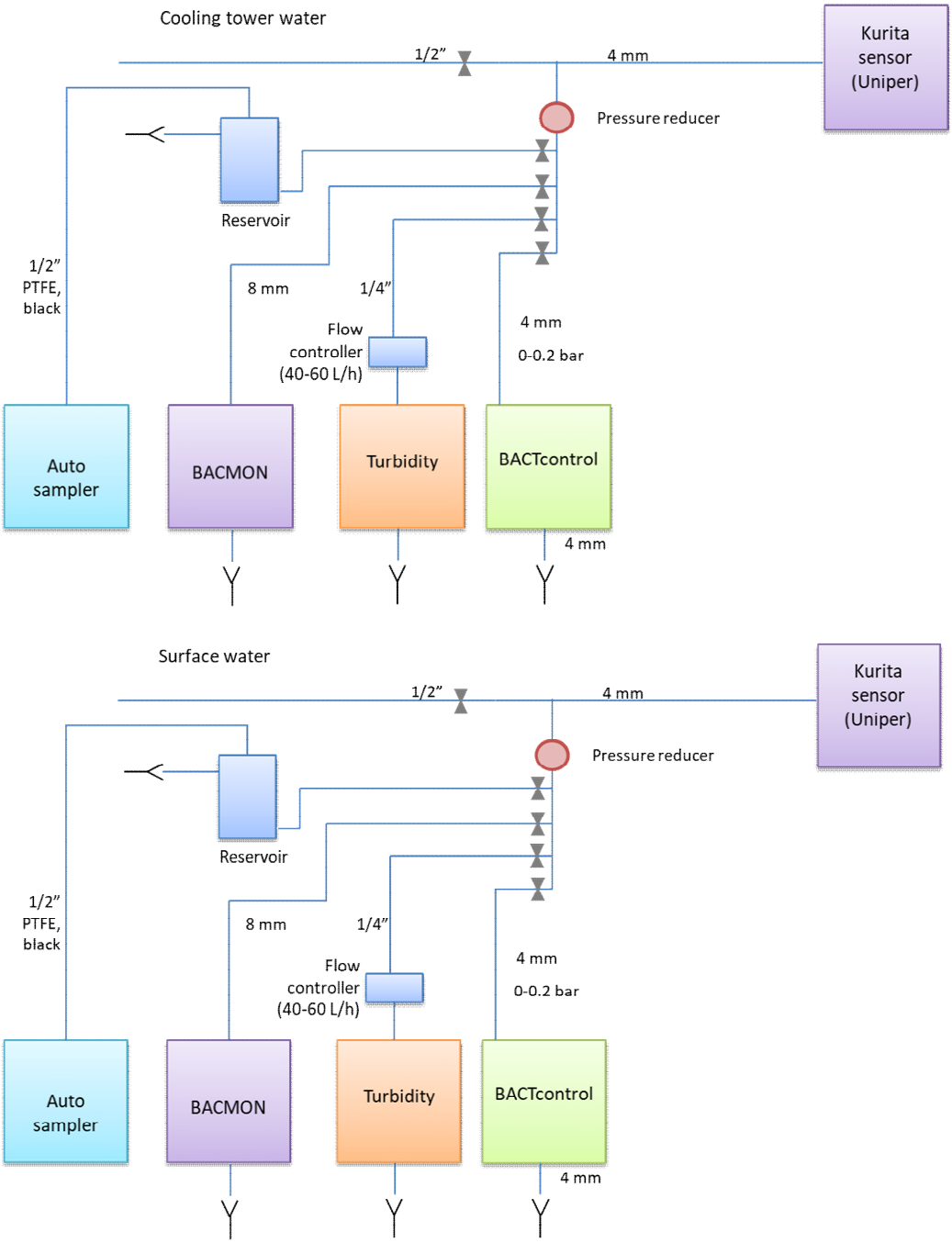


Figure 38. Schematic drawing of the installation of the BACMON, BACTcontrol and turbidity sensors on cooling tower water (top) and surface water (bottom) at Uniper after 4 January 2019. On 4 January 2019 a pressure reducer was installed. The installation site of the sensors is indicated in Figure 47. The type of tubes that was used is indicated.

4.2 Results

4.2.1 Results of sensors (BACMON, BACTcontrol, turbidity)

Turbidity data
The turbidity of the incoming surface water could not be reliable measured, due to quick fouling on the sensor. Therefore, only the turbidity data of the cooling tower water is provided (Figure 39). A slight increase in turbidity was seen at the end of December, begin of January.

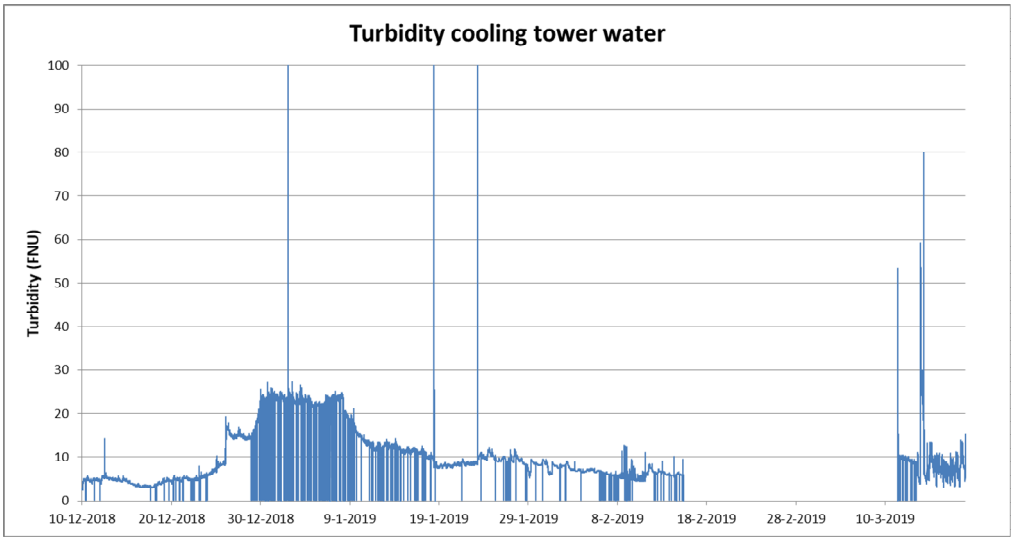


Figure 39. Turbidity (FNU) data of the cooling tower water.

BACMON data
The results of the BACMON sensors on the incoming surface water and cooling tower water at Uniper are shown in Figure 40. The bacterial and non-bacterial numbers in these waters are much higher than measured in drinking water (chapters **Error! Reference source not found.** and **Error! Reference source not found.**), which is normal when surface water is compared to drinking water. There are no clear events in the surface water. The bacteria show very short peak moments (in the order of minutes) in the surface water, but disturbances that lasted longer were not observed for bacteria during the whole monitoring period. A similar pattern was observed for the bacterial numbers that the BACMON monitored in the cooling tower water, although the bacterial numbers were lower in cooling tower water (average bacterial number in surface water was 251.190 N/ml, whereas for cooling tower water the average bacterial number was 135.773 N/ml). In addition, there was a malfunction of the BACMON sensor for several days in December 2018 and February 2019. This was caused by a too high fouling of the flow cell resulting in an error and no results were obtained. After replacement of the flow cell, solving the error and flushing the BACMON sensor, the system was functioning again.

The non-bacteria measured by the BACMON show a different pattern than the bacteria. At several moments the non-bacterial numbers increase for a longer period of time. In the surface water there is for instance an increase from January 18 till 22, after which non-bacterial numbers remain high until January 28. In the cooling tower water, non-bacterial numbers increase from December 30 till January 12, which coincidence with

an increase for turbidity (Figure 39). In contrast to the turbidity data, the non-bacterial numbers remained high till the beginning of February. It was observed that the non-bacterial numbers were higher in cooling tower water than in surface water, which is the opposite as was observed for bacterial numbers. It could be that during ozone disinfection bacteria are killed and maybe disintegrate, rendering them from being counted as a bacterium to being counted as being as one or multiple particles (non-bacteria).

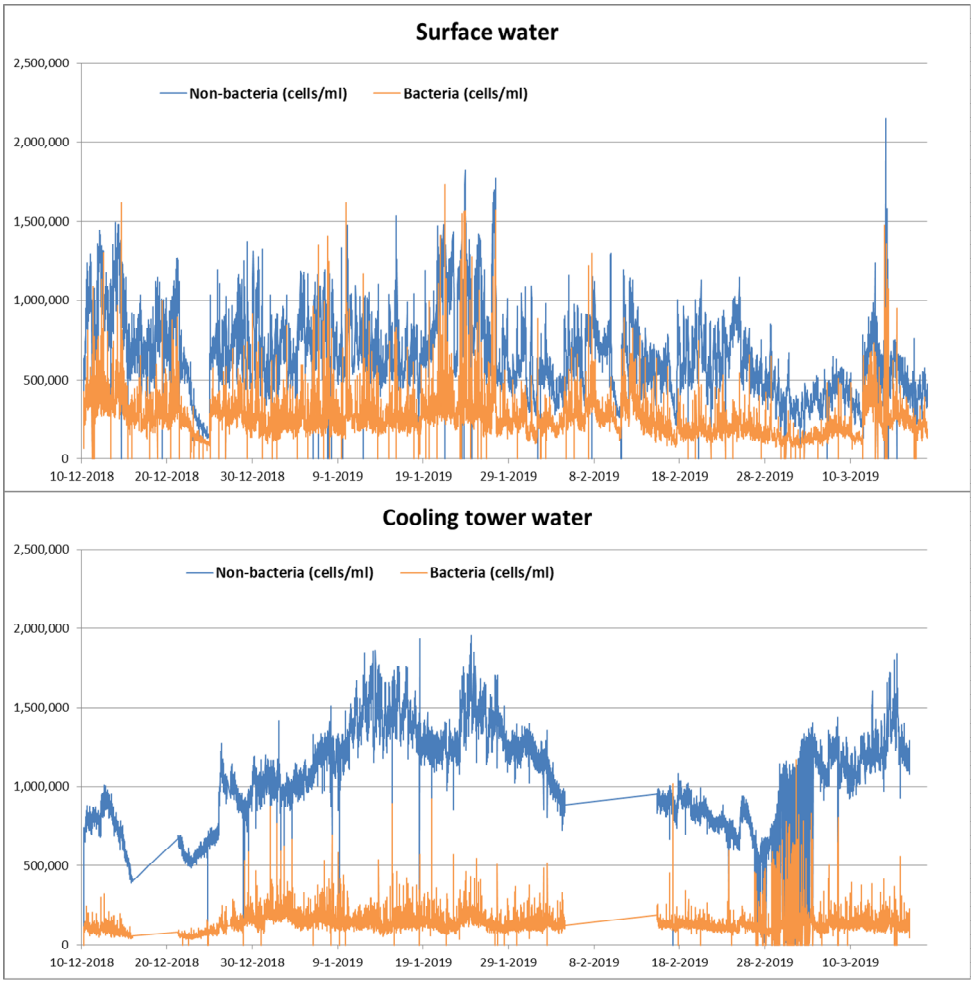


Figure 40. Results of the BACMON sensor measuring surface water (top) and cooling tower water (bottom). Given is the number of bacteria and non-bacteria. During two periods the flow cell fouling of the BACMON measuring cooling tower water was too high resulting in an error and no measurements were performed during that time.

BACTcontrol data

The BACTcontrol was also tested on the incoming surface water, but for a shorter period of time (19-01-2019 till 18-03-2019; Figure 41). The enzymatic activity data from the BACTcontrol showed some small and a few high peaks. The higher peaks were observed on 11/12 February (maximum activity: 1749 pmol/100 ml/min) and on 5 March (maximum activity: 1381 pmol/100 ml/min). Multiple data points showed increased enzymatic activity at the first peak, whereas the second high peak was related

to a single data point. These peaks could not be related to any maintenance activities of the sensor.
The cooling tower water was only measured for a short amount of time (20-02-2019 till 04-03-2019) with the BACTcontrol sensor (Figure 41). During this period a very high peak was observed on 1 March (maximum activity: 2571 pmol/100 ml/min), but this was caused by air bubbles trapped in the system. A smaller peak was observed on 26 February (maximum activity: 1305 pmol/100 ml/min) but that peak was related to replacement of the filter in the system. Finally a negative activity was measured on 4 March, which was due to a clogged filter. Because of these technical errors, problems with clogging of the filter and the short monitoring period, the BACTcontrol data for the cooling tower water cannot be used to reliable identify the microbial enzymatic activity in the cooling tower water.

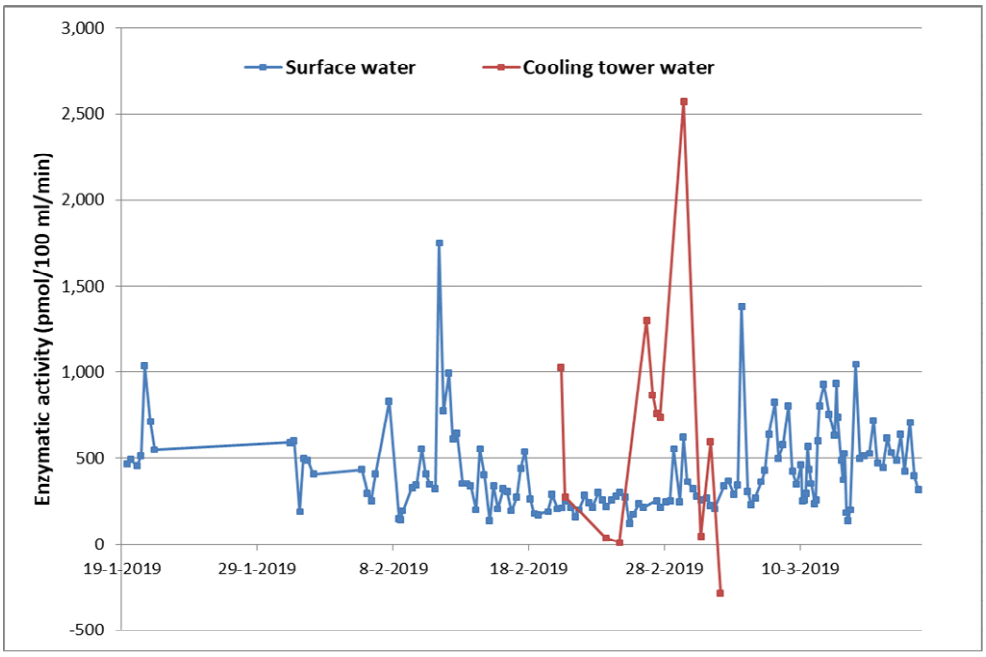


Figure 41. Results of the BACTcontrol measuring surface and cooling tower water.

4.2.2 Comparison of data from the three sensors to each other

The BACMON and the BACTcontrol data from the surface water were also plotted in one graph (Figure 42). The data in this figure shows that there seems to be overlap in some of the peaks that were observed with the BACMON and BACTcontrol. To study that in more detail, these peak moments were plotted in separate graphs (

Figure 43). From 9 till 14 February, the BACMON and the BACTcontrol data were similar with both showing a peak around 11/12 February. However, a clear difference between the BACMON and BACTcontrol data was observed between 4 and 7 March, where the BACTcontrol showed a peak, whereas the BACMON data remained stable at a low level. Finally, between 11 and 16 March an intermediate situation was observed with a small BACTcontrol peak at 11/12 March that was not observed for the BACMON data, while at 14 March both sensors showed a peak.

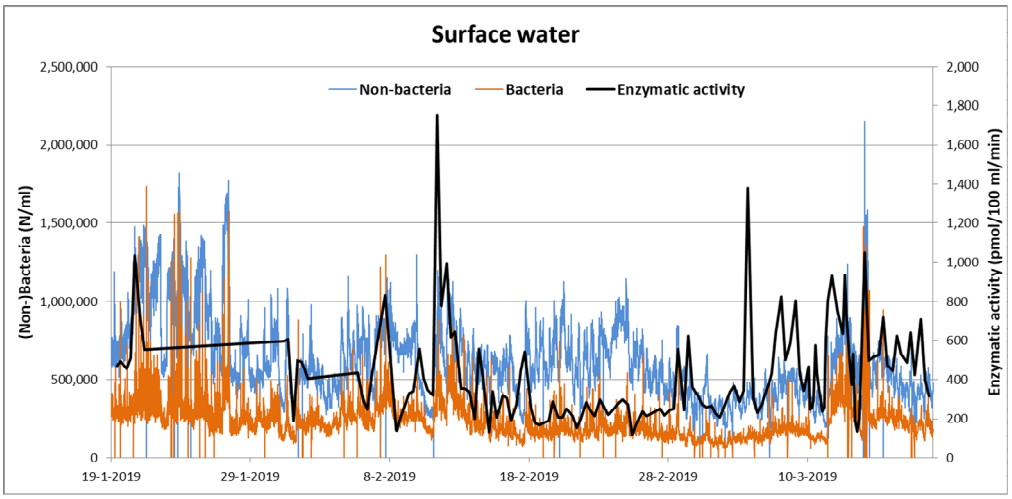


Figure 42. Comparison of BACTcontrol (enzymatic activity) and BACMON measuring surface water.

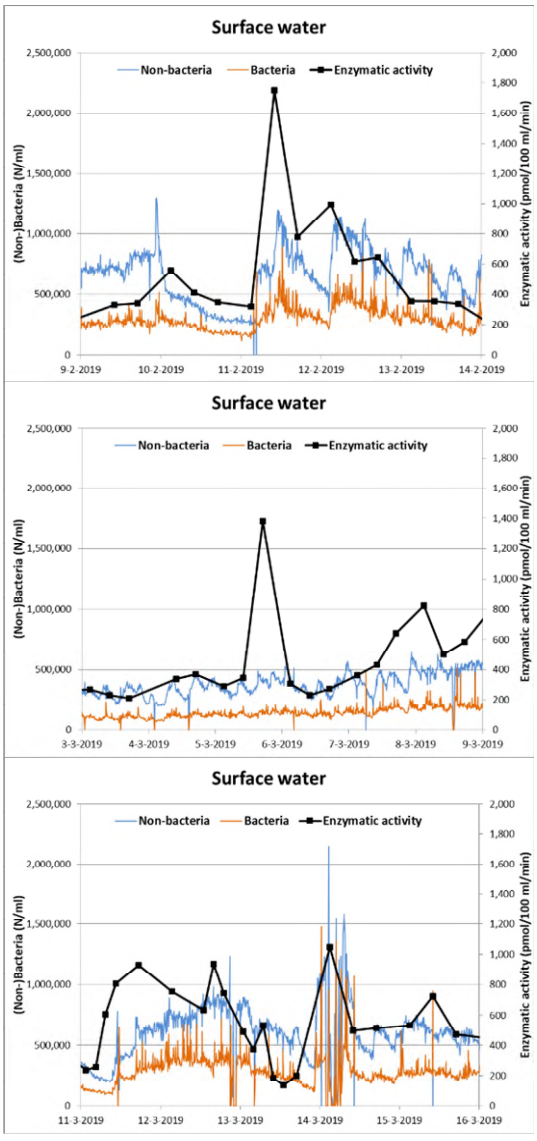


Figure 43. Detailed comparison of BACTcontrol (enzymatic activity) and BACMON measuring surface water.

The BACMON data was only compared to the turbidity data for the cooling tower water, because the turbidity data for the incoming surface water was unreliable. The data shows that there does not seem to be a relation between the turbidity data and the bacterial or non-bacterial data measured with the BACMON sensor (Figure 44).

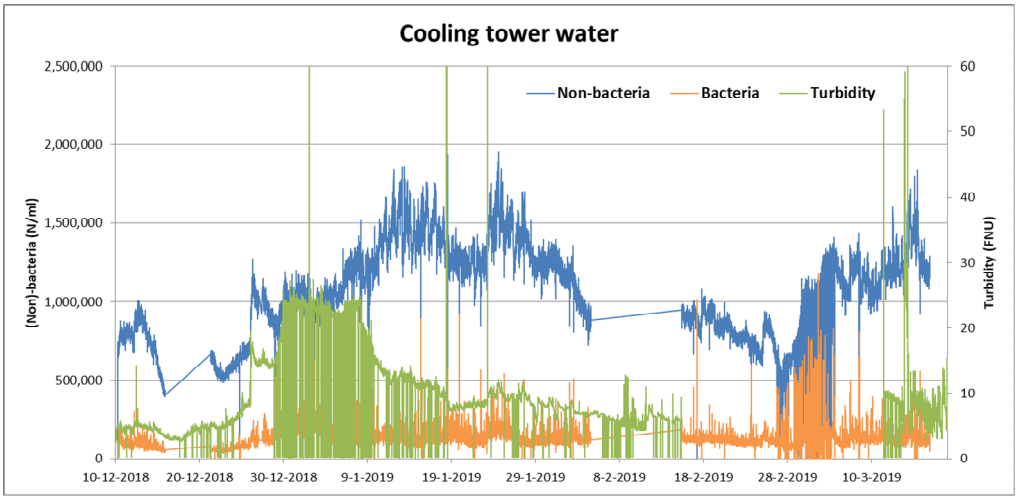


Figure 44. Comparison of BACMON and the turbidity sensor measuring cooling tower water.

4.2.3 Comparison of microbial sensor data with physical/chemical parameters

Uniper measures a number of additional physical/chemical parameters for the incoming surface water (flow and temperature) and cooling tower water (Kurita, flow, pH, redox, thickening, ozone residual, temperature and flushing flow). This data was also compared to the BACMON and BACTcontrol data. The bacterial numbers measured with the BACMON sensor were relatively constant in the cooling tower water, which was in accordance with the other physical/chemical parameters measured for this water type. This might indicate a relationship between the bacterial numbers counted with the BACMON and the other physical/chemical parameters, but this remains difficult to conclude, since the variation in each of these parameters was very low.

The non-bacterial numbers in the cooling tower water, measured with the BACMON sensor, was compared to the physical/chemical parameters that are measured by Uniper for the cooling tower water (e.g. flow, EGV, pH, redox, ozone residue, temperature). The non-bacteria increased from the end of December and beginning of January, but the physical/ chemical parameters did not increase during that time.

4.2.4 Comparison of microbial sensor data with ATP

Uniper also measures irregularly bacterial ATP concentrations in the surface water and cooling tower water. These ATP concentrations were plotted together with the bacterial numbers that the BACMON sensor measured in these water types (Figure 45). During the BACMON monitoring period two ATP peaks were observed in the surface water (Hollandse IJssel). The bacterial numbers measured with the BACMON sensor showed a more modest peak at the same time of the first bacterial ATP peak. The second ATP peak was measured at March 12, and at that time the bacterial numbers where twice as

high compared to the three previous time points where ATP was measured, which indicates that both the bacterial ATP and the bacterial numbers were considerable higher at this second ATP peak. Two large ATP peaks were also observed in the cooling tower water, but both ATP peaks did not coincide with higher bacterial numbers measured with the BACMON sensor. Overall, the data thus shows that in general the ATP data is non-congruent with the bacterial numbers determined with the BACMON sensor. However, it remains unclear from these data which parameter (ATP or bacterial numbers) is the most reliable parameter for the bacterial biomass in both water types.

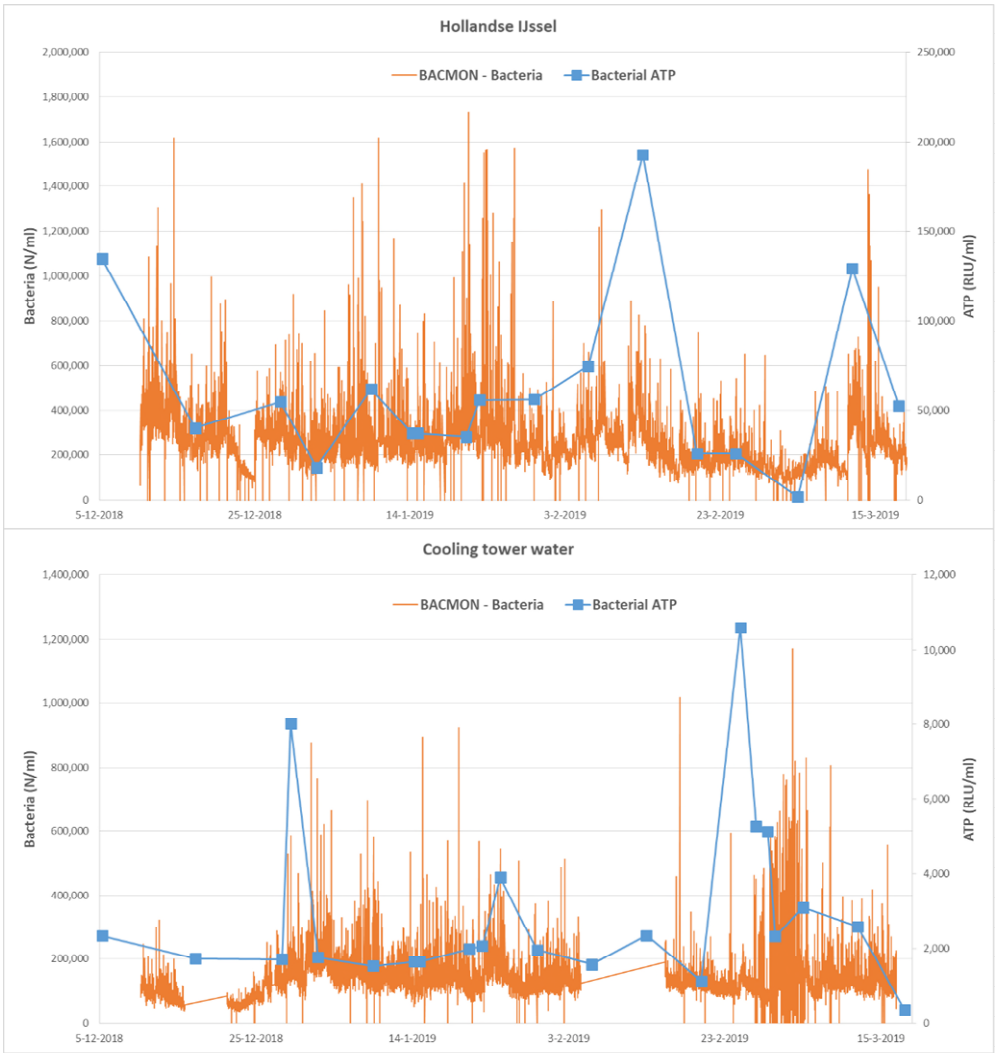


Figure 45. Comparison of bacterial number, measured with the BACMON, with ATP measurements performed by Uniper.

4.3 Discussion

4.3.1 Are the microbiological sensors suitable for studying the 'microbiological problem' at the full-scale location

The BACMON sensor was capable to measure bacterial and non-bacterial numbers in both the surface water and cooling tower water and the results from the BACMON sensor showed that these numbers were relatively stable during the monitoring period in the winter.

The BACTcontrol sensor was able to measure the enzymatic activity in the surface water and the data from the sensor showed that there were numerous peaks in enzymatic activity during the monitoring period. It remains uncertain whether all these peaks relate to changes in microbiological water quality, since other parameters did not always show an aberrant water quality at these enzymatic activity peaks. The BACTcontrol sensor did not work well with the cooling tower water, because the filters used in the BACTcontrol got clogged quickly with cooling tower water.

4.3.2 Application of the sensors for surface water and cooling tower water

The aim of applying the sensor to these locations was to test whether the sensors can be applied to these water types and if these sensors can be used to predict an increase in bacterial growth, which might be related to enhanced *Legionella* numbers in the water. As indicated above, the BACMON sensor can be applied to both water types (incoming surface water and cooling tower water). The BACTcontrol sensor could be applied to the incoming surface water, but seems to be less suitable for the cooling tower water that contained an ozone residue.

It is difficult from the data to conclude whether the sensors predict an increase in bacterial growth. During most of the monitoring period the data from both sensors on surface water and the BACMON data on cooling tower water were stable, indicating that bacterial growth in the water types was stable. This was supported by the physical/chemical parameters, most of the ATP measurements and the Legionella data (six measurements in cooling tower water during the monitoring period, which were always below 100 cfu/l). Still, some small peaks with the BACMON sensor and larger peaks with the BACTcontrol sensor or ATP measurements were observed in both water types. These peaks did not always match with each other, which makes it difficult to conclude whether the bacteria sensors reliable detect an increase in bacterial growth or missed an increase in bacterial growth. When it is assumed that the ATP measurements reliable detect an increase in bacterial growth, than most of the increase in bacterial growth (i.e. increase in ATP) were not detected with the BACMON or BACTcontrol sensors.

5 Full-scale location at Havelterberg, Vitens

5.1 Description full-scale location

5.1.1 General introduction

Vitens is the largest drinking water company in The Netherlands. Vitens delivers top quality drinking water to 5.6 million people and companies in the provinces Flevoland, Friesland, Gelderland, Utrecht and Overijssel and some municipalities in Drenthe and Noord-Holland. Annually Vitens delivers 350 million m^3 water with 1,400 employees, 100 water treatment works and 49.000 kilometers of water mains.

Vitens selected production site Havelterberg for the placement of the microbiological sensors. Havelterberg is located in the North of Meppel, close to Steenwijk. Havelterberg extracts yearly 6 million m^3 drinking water which get mixed with water from Ruinerwold. Two locations at the production site Havelterberg were selected: the incoming water from Ruinerwold from 'Water Maatschappij Drenthe' (WMD) and the produced water to be delivered to Steenwijkerwold. For this project the turbidity sensor, the auto sampler, BACMON and BACTControl were placed at both spots.

5.1.2 Water type and production

Production site Havelterberg has 22 ground water wells. First step in the production process is aeration, followed by NaOH dosing (increase of pH to increase iron removal) and marble filtration (Figure 46). In the clean water reservoir the produced water from Havelterberg is mixed with water taken in from Ruinerwold. After the reservoir part of the water is disinfected by UV before it is delivered to Steenwijkerwold. Both drinking waters are soft (Ruinerwold: 1.70 mmol/l or 9.5°D and Havelterberg: 1.36 mmol/l or 7.7°D).

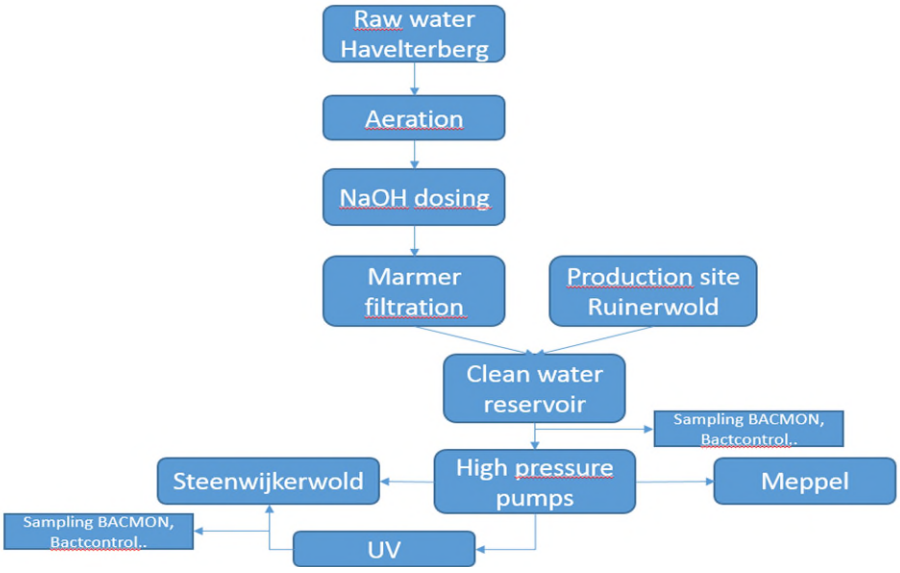


Figure 46. Overview of production site Havelterberg. The sampling site for the sensors is given.

5.1.3 Location

The sensors were tested at a drinking water treatment plant that is located in Havelterberg, the Netherlands (Figure 47).

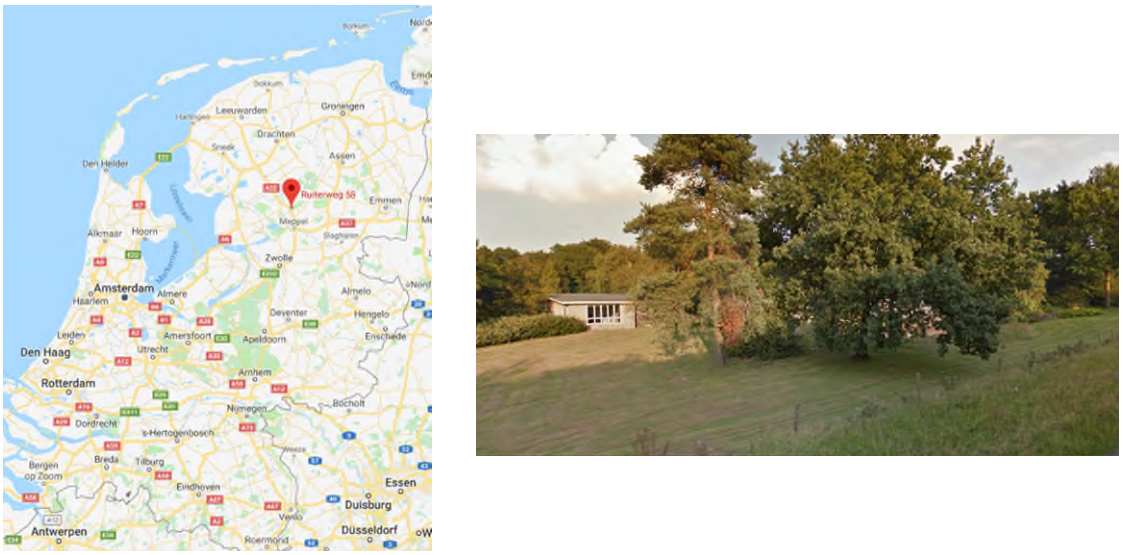


Figure 47. Production site Havelterberg, the Netherlands

5.1.4 Study aim

The main reason Vitens selected Havelterberg for the placement of the sensors is to find out what causes the presence of coliform bacteria that were found a few times in the outgoing water to Steenwijkerwold.

The underlying questions are:

- Does the water coming from Ruinerwold cause the sporadic detection of coliform bacteria?
- Are the coliform bacteria coming from the water produced at production site Havelterberg or Ruinerwold?
- Is there a process that causes the development of coliforms?
- Get a better insight into the influence of the water from Ruinerwold on the outgoing water.
- Get to know the sensors and the added value of these sensors for Vitens.

It will be tested whether the sensors will show some events in the water coming from Ruinerwold and/or also in the outgoing water for Steenwijkerwold caused by (unknown) production processes or whether the sensors do not detect events.

Therefore, two points were selected for installation of the sensors.

5.1.5 Installation of microbiological sensors

The sensors were placed at:

- Incoming water Ruinerwold (‘Inkoop Ruinerwold ‘): water that Vitens buys from WMD
- Produced drinking water for Steenwijkerwold (‘Uitgaand rein Steenwijkerwold’): Clear drinking water that is delivered to customers in Steenwijkerwold (a mixture of 1/3 Ruinerwold water, 2/3 Havelterberg water)

In Figure 48 and Figure 49 a schematic drawing of the installed sensors is given.

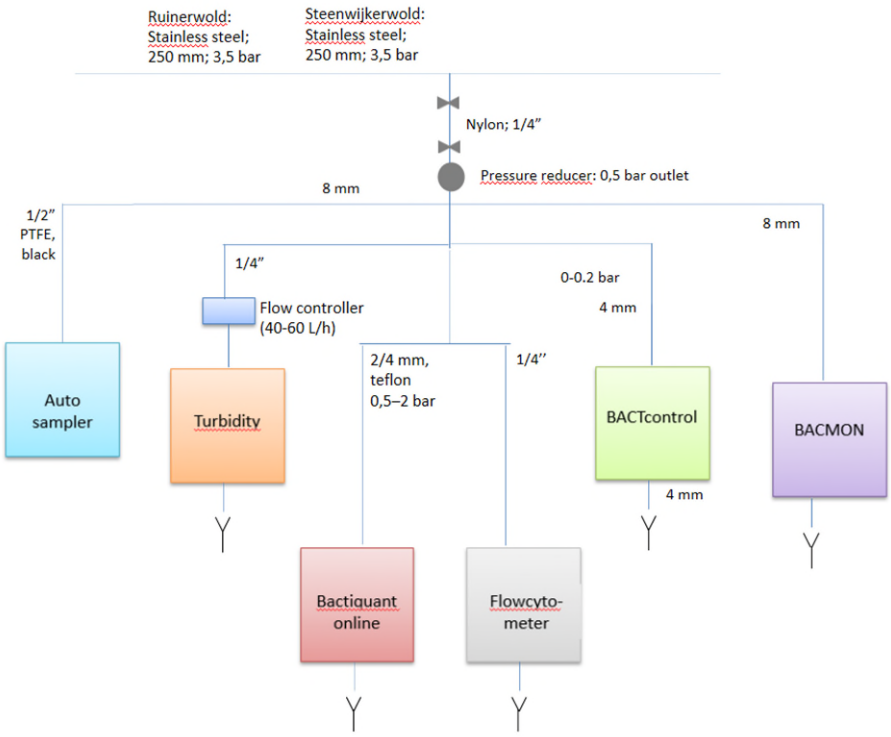


Figure 48. Schematic overview of the sensors measuring the outgoing water to Steenwijkerwold

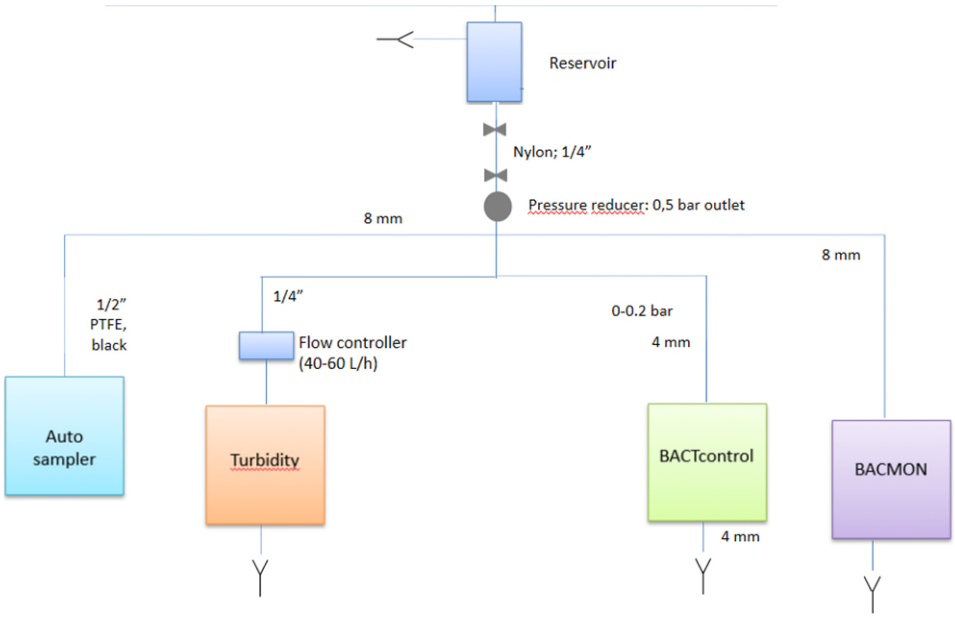


Figure 49. Schematic overview of the sensors measuring the incoming water from Ruinerwold

5.2 Results

5.2.1 BACMON, BACTcontrol, turbidity

5.2.1.1 BACMON

The results of the BACMON from 18-4-2019 till 4-6-2019 are shown in Figure 50 and Figure 51. Some of the peaks of the outgoing water to Steenwijkerwold (Figure 50) were evaluated and compared to the results of the other sensors and with the operational parameters at Havelterberg (Figure 53 - Figure 56). Because the operational conditions at the production site of Ruinerwold and the transport from Ruinerwold to Havelterberg are unknown, no explanation can be given for the variation in the (non-)bacterial numbers in the incoming water from Ruinerwold (Figure 51). The large peak of particles between 11 and 15 May cannot be compared to the outgoing water of Steenwijkerwold due to an error in the BACMON.

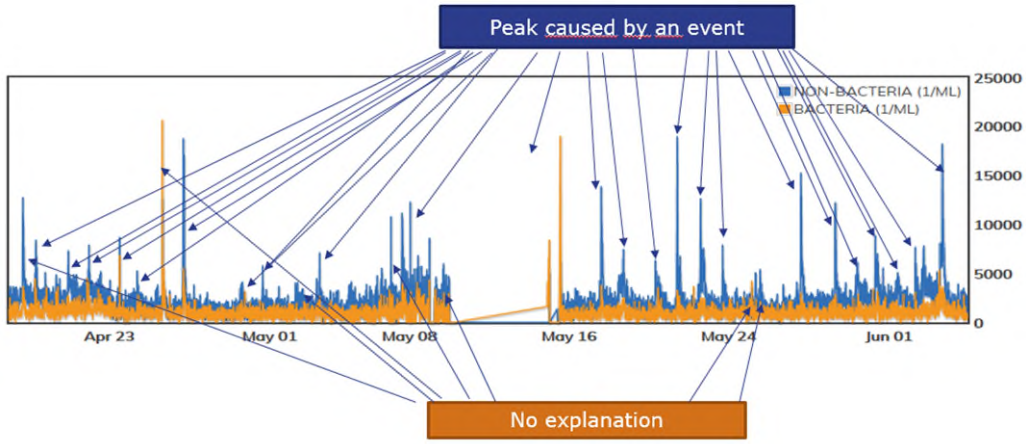


Figure 50. BACMON results of the outgoing water to Steenwijkerwold. For each peak in bacterial or non-bacterial number it is indicated if an explanation could be found.

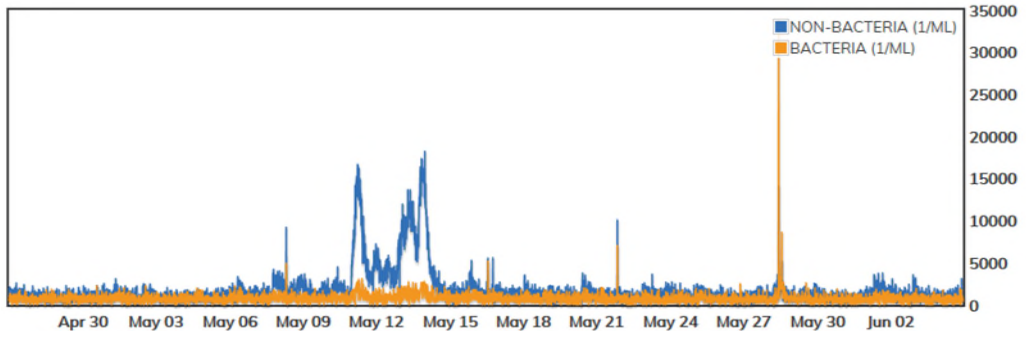


Figure 51. BACMON results of the incoming water from Ruinerwold. As the operational conditions at the production site of Ruinerwold and the transport from Ruinerwold to Havelterberg are unknown, no explanation can be given for the peaks.

Figure 52. BACMON at Havelterberg

Examples of peaks in the number of (non-)bacteria are given together with the possible causes for these peaks (Figures 53 – figure 56). However, in seven cases no explanation could be found for peaks with the BACMON and an example of such a peak is given in Figure 53.

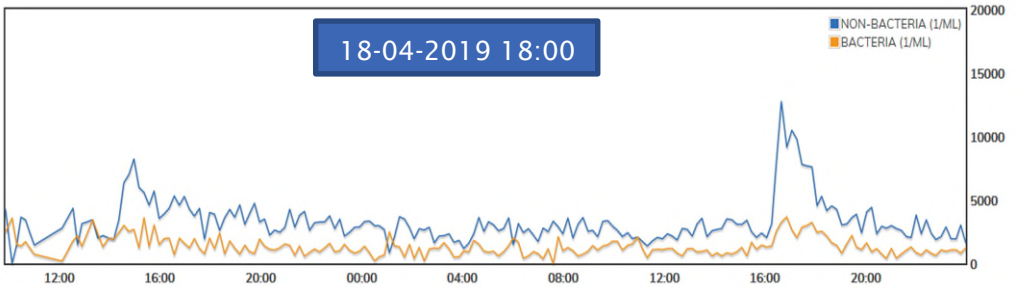


Figure 53. peak event BACMON with no explanation

Many peaks were caused by filter flushes, in total 13 times a peak was caused by a filter flush. Two examples are given in Figure 54. At these moments the S:can (turbidity) of Vitens showed no or a small increase. The BACTcontrol showed a small increase (first example) or was unreliable (second example).

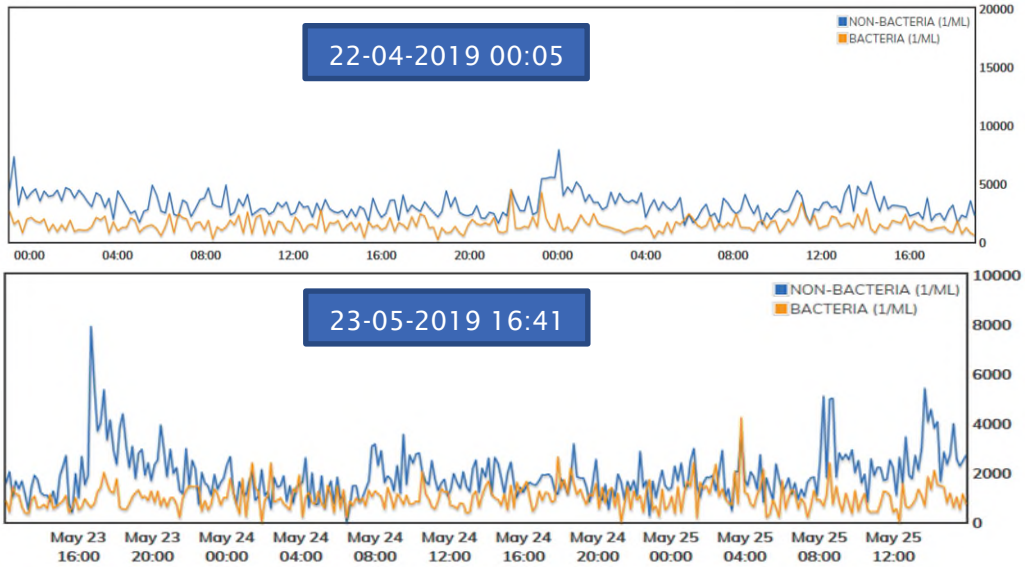


Figure 54. Peak event BACMON caused by filter flush

Furthermore, topping up of the marble filters caused a BACMON peak five times. Two examples are given in Figure 55. In the first example the turbidity (S:can) and enzymatic activity (BACTcontrol) increased (Figure 55, top). The second example showed no variation in turbidity and a small increase with the BACTcontrol (Figure 55, bottom).

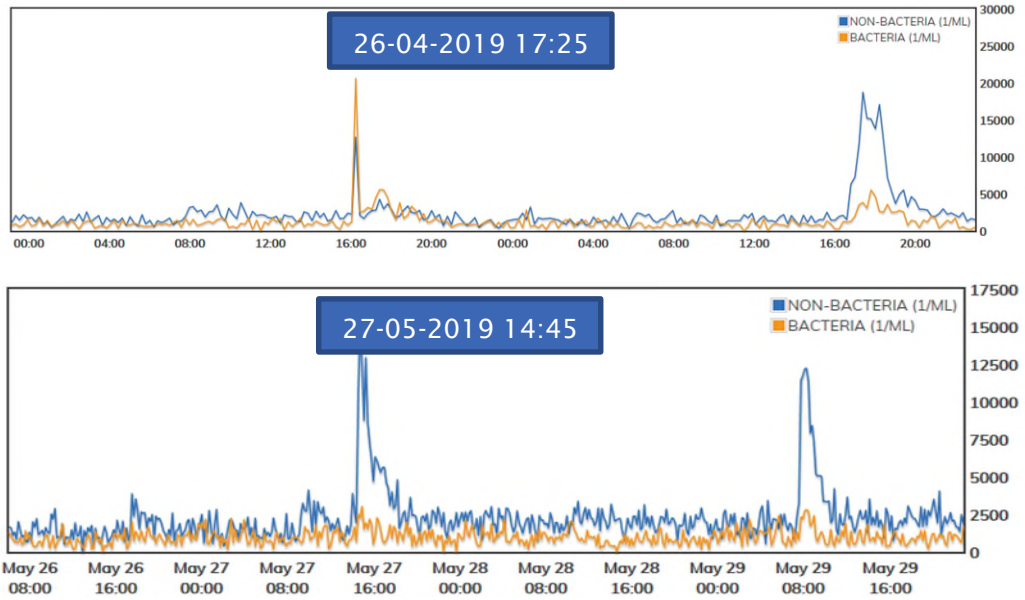


Figure 55. Peak events BACMON caused by topping up marble

Once a peak was caused by leakage in the pump well (Figure 56). Both turbidity and the BACTcontrol did not detect this peak in non-bacteria particles.

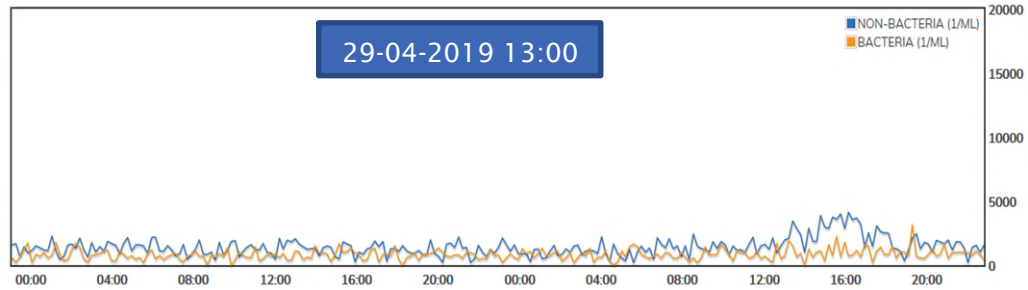


Figure 56. Peak event BACMON caused by pump well leakage

On 15 April 2019 the UV was turned on to treat the outgoing water to Steenwijkerwold. The Bactiquant and BactoSense sensors detected this event, but the BACTcontrol did not. Unfortunately, the BACMON had a malfunction from 14 – 17 April 2019.

5.2.1.2 BACTcontrol

The results of the BACTcontrol from 26-3-2019 until 3-6-2019 are shown in Figure 58 and Figure 59. The enzymatic activity was constantly low in the incoming water from Ruinerwold and varied between 6 and 16 pmol/min/100 ml (Figure 59).

The enzymatic activity of the outgoing water to Steenwijkerwold was relatively constant, but higher compared to the Ruinerwold water, until around 22 April 2019 (10 – 30 pmol/min/100 ml; Figure 58). After this date the enzymatic activity steadily increased, which could have been caused by a bacterial contamination in one of the reagentia. Therefore, these results are unreliable.

Figure 57. BactControl at production site Havelterberg

Figure 58. Enzymatic activities (measured with the BACTcontrol) in the outgoing water to Steenwijkerwold

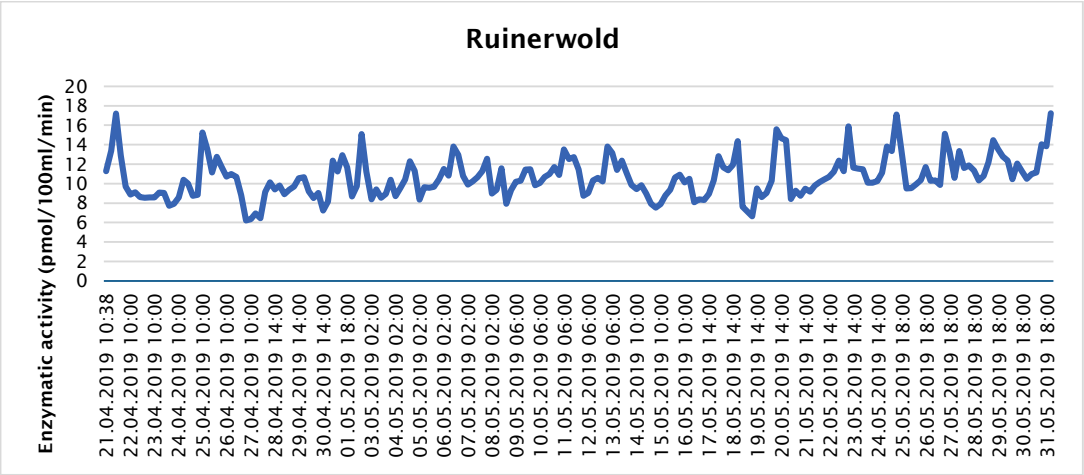


Figure 59. Enzymatic activities (measured with the BACTcontrol) of the incoming water from Ruinerwold

5.2.2 Comparison of BACMON with BACTcontrol

The results of the BACMON and BACTcontrol are different from each other (Figure 60 and Figure 61). When the BACTcontrol measured peak events, no significant increase is found with the BACMON and vice versa. In addition, the BACTcontrol results from the outgoing water to Steenwijkerwold increase after 20 April 2019, probably because of a malfunction in the system. As a consequence, these results from the BACTcontrol cannot be compared with the BACMON. The BACMON yields stable results for almost the entire testing period.

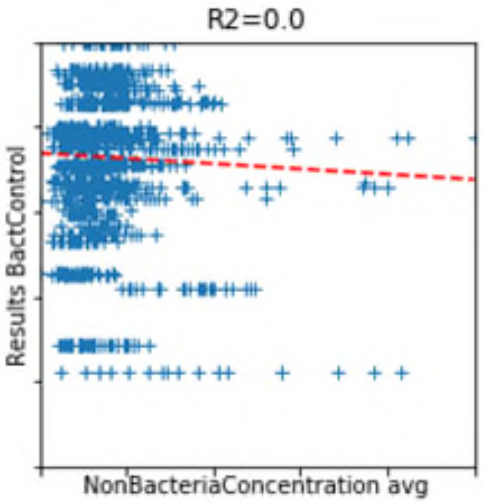


Figure 60. Scatterplot BactControl (enzymatic activity) and BACMON (Non-bacterial numbers). A lineair correlation is shown.

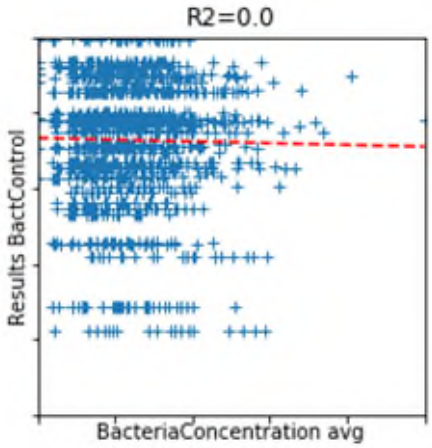


Figure 61. Scatterplot BactControl (enzymatic activity) and BACMON (BacteriaConcentration). A lineair correlation is shown.

5.2.3 Comparison of BACMON and BACTcontrol with other sensors

Besides the BACMON and BACTcontrol, Vitens has also tested other microbiological sensors at the same time on the outgoing water of Steenwijkerwold. These sensors measure turbidity, enzyme activity (Bactiquant) and total cell numbers, intact membrane cell numbers, compromised membrane cell numbers, high nucleic acid cell numbers and low nucleic acid cell numbers (Bactosense). The BACMON and BACTcontrol results were compared with these other parameters to determine whether some parameters were correlated (Figure 62 and Figure 63). No clear correlations between the bacterial and non-bacterial numbers measured with the BACMON and the other parameters were observed. The same result was obtained when the BACTcontrol data was compared to the data from the other sensors. These results were surprising as we would expect that the bacterial numbers measured with the BACMON correlated to the bacterial cell numbers that were measured with the Bactosense flow cytometer. In addition, it would have also been logical when the results from the enzymatic assay performed in the BACTcontrol correlated with the results from the enzymatic assay performed in the Bactiquant. The lack of correlations in these cases demonstrate that the tested sensors do not measure the same parameters. This could be advantageous, because in that case a suite of sensors might better describe the microbiological quality than when every sensor shows the same result.

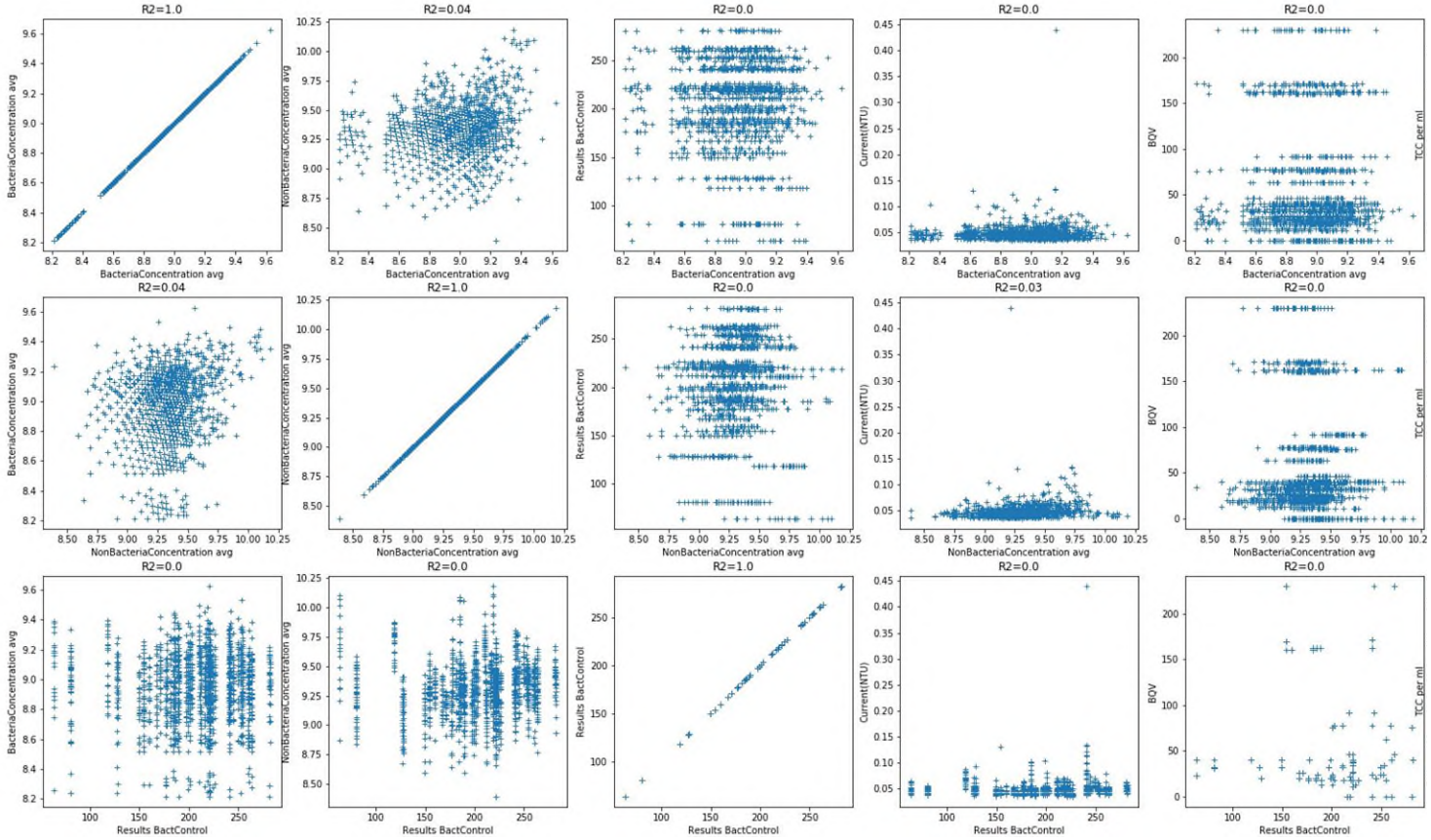


Figure 62. Correlation between BACMON, BACTcontrol, turbidity and enzymatic (BQV from BACTIQUANT) data

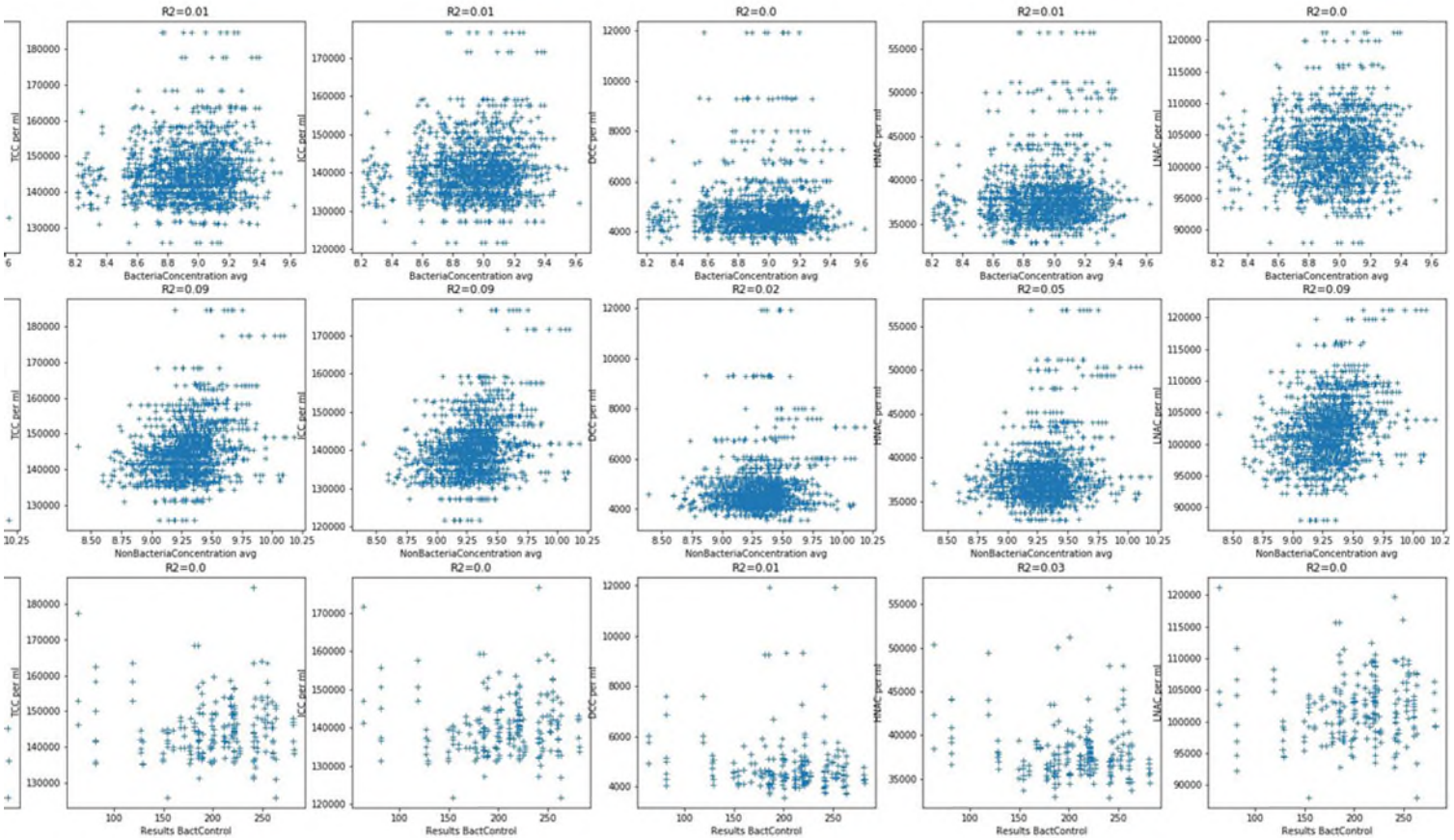


Figure 63. Correlation between BACMON, BACTcontrol and cell number data from the Bactisense (flowcytometer)

5.3 Discussion

5.3.1 Suitability of microbiological sensors for online measurements at the full-scale location

It was not possible to answer the starting question: what causes the growth of coliforms that are sometimes observed on production site Havelterberg? Especially the BACMON sensor seems suitable to get more insight in the process steps and the influence of these process steps on the microbiology of the produced drinking water. However, more information regarding the occurrence of coliforms cannot be gathered.

The incoming Ruinerwold water contains less cells compared to the Steenwijkerwold water and also less variation is observed. In addition, the BACTcontrol results of the Ruinerwold water seem to be lower and is more stable. The number of cells in the Steenwijkerwold water, and the variation herein, seems to be unrelated from the Ruinerwold water. In addition, most peaks from the BACMON measuring the outgoing water to Steenwijkerwold were explained by process changes.

During the test period no coliforms were found by the normal monitoring program of Vitens. Although the sensors give information about the production process at Havelterberg and the microbiological quality of the incoming water of Ruinerwold, no possible cause of the presence of coliform bacteria in the outgoing water to Steenwijkerwold could be found.

5.3.2 Conclusions

Microbial sensors give us many extra insights into the effects on bacterial number or activity of certain processes at the production site. Most often the BACMON peak results correlated with filter backwashes of the treatment process, showing merit value of the BACMON sensor. Still, not all filter backwashes could be detected by the BACMON and not all BACMON peaks could be traced back to operational management of the treatment process. This could be due to other factors having an impact on bacterial or particle numbers that are measured or missed with the BACMON sensor. The BACTcontrol sensor seemed to be less reliable in predicting the peak events resulting from filter back wash operation. Overall, it is concluded that it remains difficult to take or implement direct actions based on the (real-time) results of the sensors.

5.3.3 Lessons learned from the full-scale location

Peaks detected with the BACMON can often be linked to process steps or operational parameters and, therefore, the BACMON can give extra information on the influence of certain treatment processes or operational parameters on the general microbiology. However, a specific process step will not always yield a peak in the BACMON and not all peaks could be explained, probably because there are many options that can cause a peak.

During the test several challenges regarding the implementation of sensors for routine usage were encountered:

- Use the system as an early warning system
  - Which operational process or operational management causes the observed peak in (one of) the sensors results?

  - What to do with the peaks that are caused by (standard) process steps or operational procedures?
- How to make it useful for the process operators?
- How can the added value on the water quality be determined.

6 Full-scale location at WRK, HWL

6.1 Description full-scale location

6.1.1 General introduction

At Waternet, location WRK/WCB Nieuwegein, water is abstracted from the Lekkanaal (side branch of the river Lek/Rhine) and treated with ferric chloride for removal of particles. After this coagulation/sedimentation process and rapid sand filtration (Figure 64), the treated water is transported to the dune area near Vogelenzang. There the water infiltrates into the dunes. The water obtained after dune infiltration is further treated to drinking water using rapid sand filters, disinfection with ozone and active carbon and slow sand filtration. From there, Waternet delivers drinking water to the city of Amsterdam.

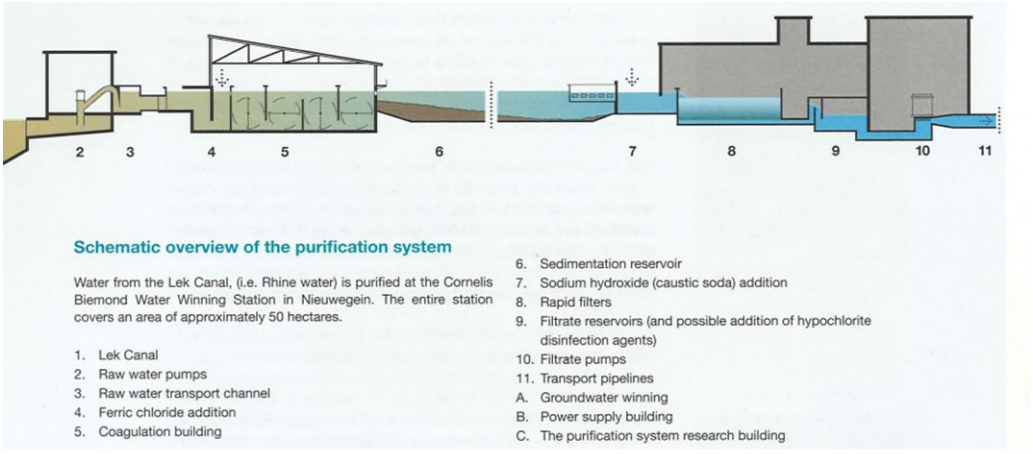


Figure 64. Schematic of the pilot location

6.1.2 Location

The BACTcontrol and BACMON were installed in a cabin at the WRK intake of the Lekkanaal water (point 1 displayed in Figure 64) in Nieuwegein (Figure 65). The Lekkanaal water is already monitored using early warning systems, so connection to the existing network of water pipelines and digital data transport was performed easily.

Figure 65. Location of the intake/production plant WRK Waternet

Next to the intake, both sensor instruments were installed at the end of the pretreatment after the rapid sand filtrate pumps (point 2 in Figure 66) at the WRK. A turbidity sensor, the BACMON and BACTControl sensors and the autosampler were placed at both locations. After several weeks monitoring at the intake, the BACMON was moved to a location where the water was treated with ferric chloride (location 3 in Figure 66) to monitor the water quality at this point for several days.

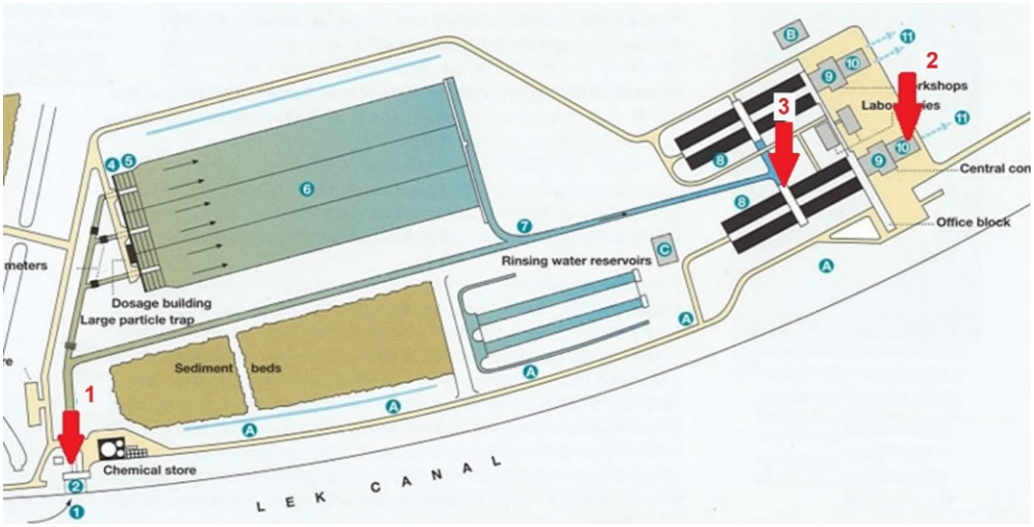


Figure 66. Location of the sensors.

6.1.3 Study aim, hypothesis and how to test this

The study aim is to evaluate the performances of the BACMON and BACTcontrol on river water used for drinking water production before and after the first treatment step ferric chloride coagulation and after rapid sand filtration. In respect to the amount of particles, a significant reduction in the amount is expected in the treated water compared to the river water (80 % removal by coagulation and 90% of the remaining particles by rapid sand filters). In respect to bacteria, historic measurements did present results in which both treatments steps give a reduction of 2 log units in heterotrophic plate counts (Figure 67). It is not known, what the variation will be in water quality on both sampling locations.

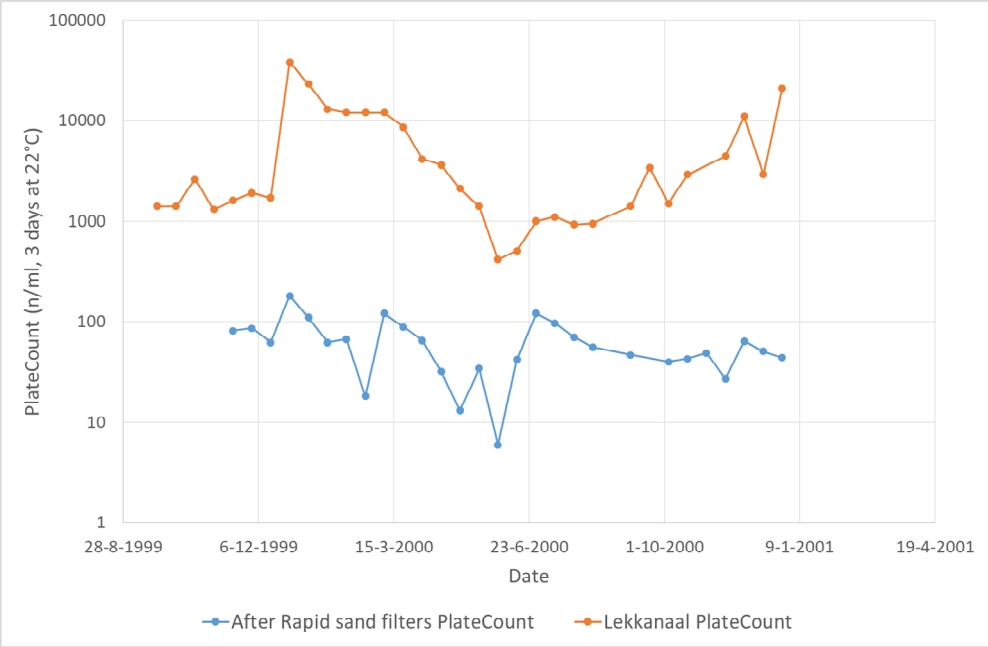


Figure 67. Indication of the number of heterotrophic plate counts at different locations of the first treatment

6.2 Schematic of full-scale location

6.2.1 Overview production plant

The maximum amount of water that can be abstracted from Lekkanaal is around 25,000 m^3 per hour. The coagulation plant consists of three coagulation units, each comprising:

- Flake formation compartment with dimensions of 18.3x16.6x4.1 meters
- Diameter of agitators of 2.5 m with a speed to max 4 rpm
- Sedimentation reservoir with dimensions 300x120x2.5 meters

The dosage of ferric chloride is approximately 3 mg/l.

There are two lines of 40 rapid filters connected in parallel in which the surface area of one filter is 12x4.5 meters. The thickness of the sand bed is 1.2 meters and the maximum filtration rate is 5 meters per hour.

Over time, the sand beds become contaminated by the sedimentary residue. They must be back-washed every three to seven days, depending on the quality of the untreated water and the level of production. For seven minutes, large quantities of air and water are forced through the sand beds in the reverse direction to the normal flow. The rinsing water flows through special pipelines into the rinsing water reservoirs.

6.2.2 Installation of microbiological sensors

In Figure 68 a schematic drawing of the four installed sensors is given, including information on the pipe material, pipe diameter, pressure and flow controller.

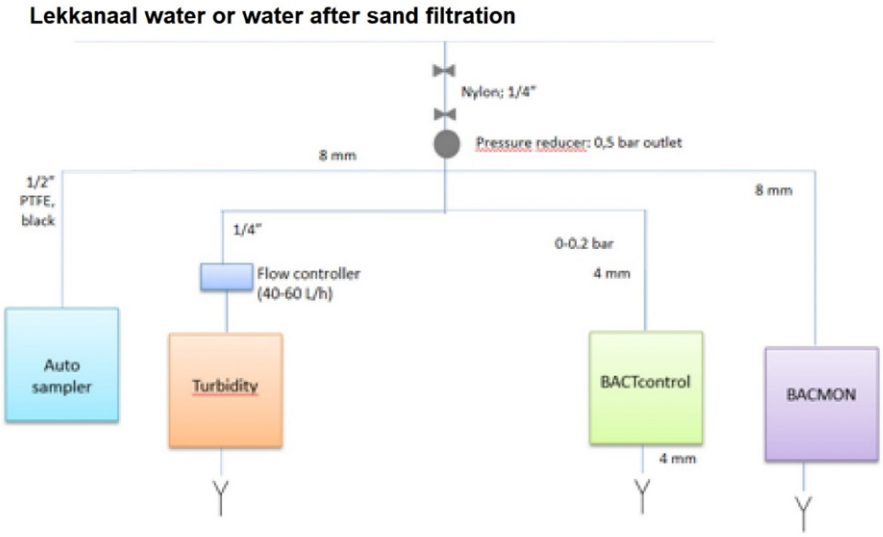


Figure 68. A schematic drawing of the four installed sensors directly after the raw water (Lekkanaal water) intake or after rapid sand filtration.

6.3 Results

6.3.1 Results of sensors (BACMON, BACTcontrol, turbidity)

In Figure 69 sometimes the change of filters or cleaning the chamber on the BACTcontrol has an impact on the measurements afterwards compared to the measurements before maintenance. The decline on activity from 18th of July till 23th of July (directly after cleaning and filter exchange) differs from the other cleaning/filter exchange moments, where a (small) peak concentration was observed. An explanation for this apparent difference, is the injection of insufficient volume of reagents. No explanation could also be given for the peak at the 20th of August, although in the measurements after the peak, the blank values were too high. Another explanation could be that the reagents were changed to a reagent from another supplier.

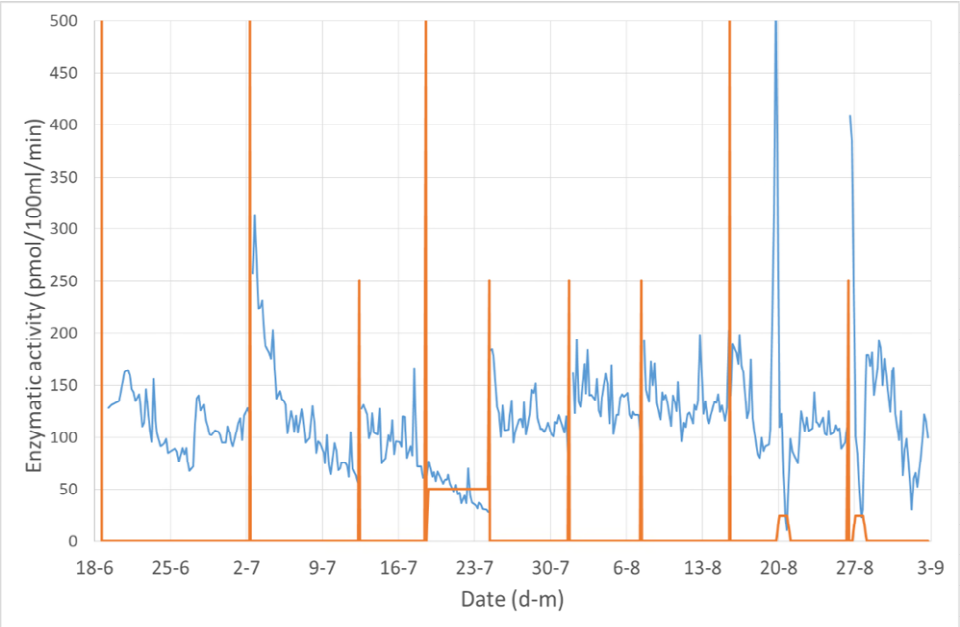


Figure 69. Results of the BACTcontrol at Lekkanaal (blue) and time of maintenance (orange:25 is result not valid (blank value too high), 50 is result not valid (volume reagens too low), 250 is cleaning, 500 is cleaning and filter exchange)

The enzymatic activity measured with the BACTcontrol remains relatively stable in water after rapid sand filtration (Figure 70). An exception to this is the decline in activity observed from the 23rd till the 29th of July, but this decline did not coincidence with any operational parameter. It might be that the micropump did not work correctly, causing air bubbles to be trapped in the tubing of the BACTcontrol, which disturbs the enzymatic measurements.

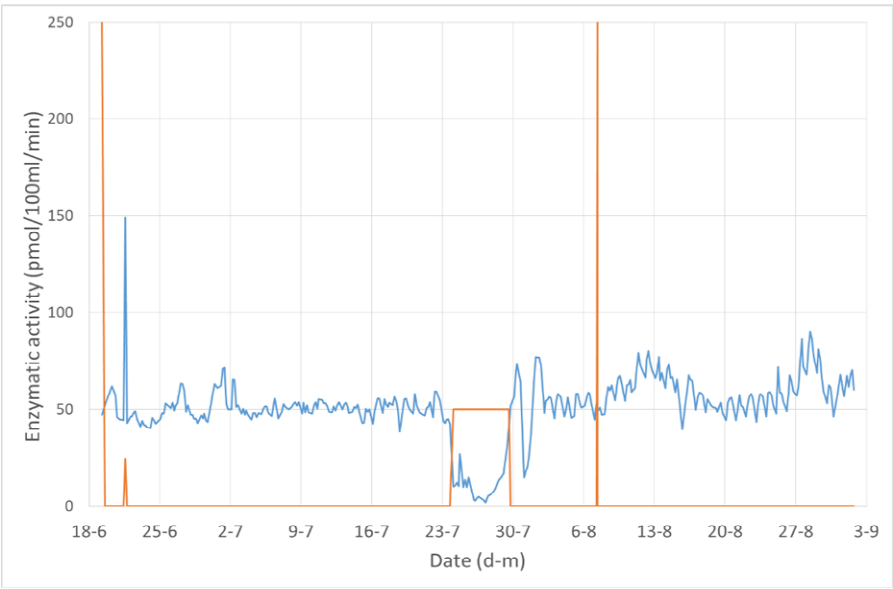


Figure 70. Results of the BACTcontrol after rapid sandfilters (blue) and time of maintenance (orange: 50 is result not valid (volume reagens too low), 250 is cleaning).

In Figure 71, results of the BACMON sensor on the Lekkanaal water are presented. On two occasions (12-7-2019 09:14 and 19-7-2019 20:10), the instrument presented an error in which during the error period no results were obtained. In general, the number of non-bacteria particles in Lekkanaal water is always higher than the number of bacteria. The high number of particles was a challenge to the instrument in a way that only a part of the volume in the inlay could be measured (on average 15% of volume). To solve this problem, the instrument was moved on the 24th of July to a location in the production plant where the water was treated with ferric chloride. On this location the number of particles was much lower than for the Lekkanaal water and the volume of the inlay monitored at this location was on average 98%.

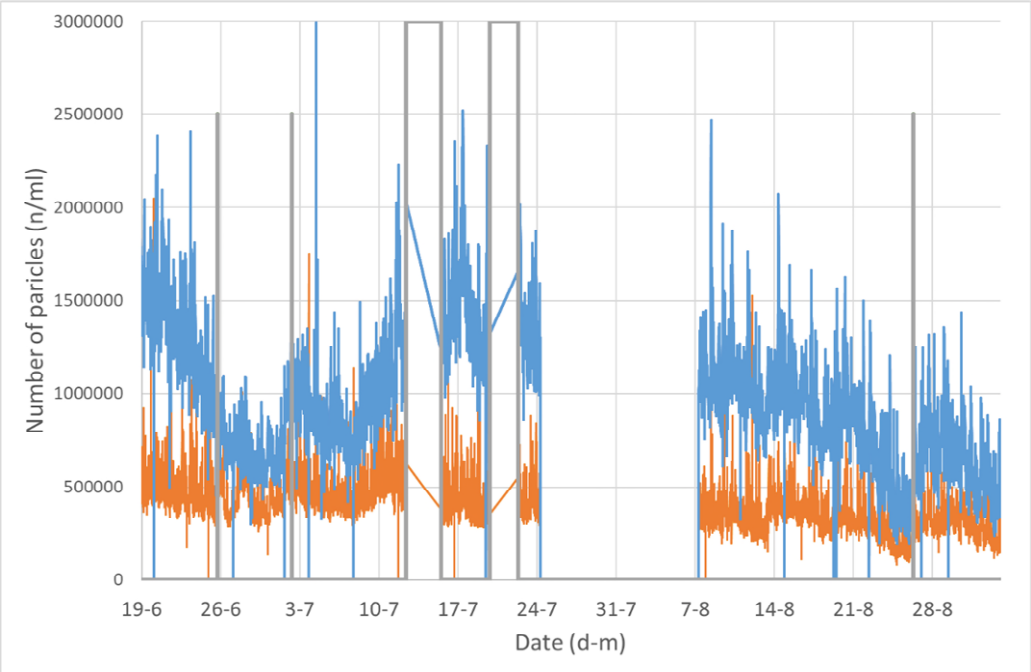


Figure 71. Results of the BACMON at Lekkanaal (orange: bacteria, blue: non-bacteria) and time of maintenance/error (grey: 2500000 is inlay change, 3000000 is error)

Figure 72 presents the results of the BACMON. The drop of number of particles from the 4th of August till the 7th was due to blockage or bad connection of the sample tube in the water stream. The number of bacteria is in general slightly higher than the number of non-bacteria particles. The data presents a more or less regularly pattern, in which sometimes at night the number was two times higher than at the end of the day. The same pattern is also visible with data from the turbidity sensor of Waternet (Figure 73). This kind of pattern was not visible on data of the Lekkanaal water, when the BACMON sensors was placed back at that location on August 8 (Figure 71). In addition, during the same period, the flow of surface water into the coagulation basin and the flow setting of ferric chloride is presented in Figure 74.

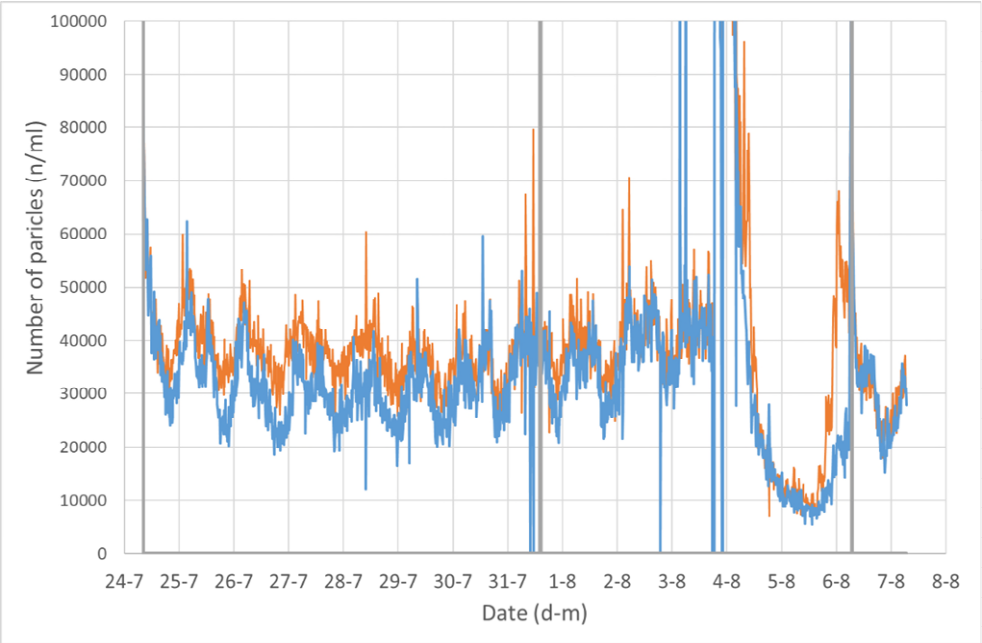


Figure 72. Results of the BACMON on water after coagulation/sedimentation (orange: bacteria, blue: non-bacteria) and time of maintenance/error (grey: 100000 is inlay change)

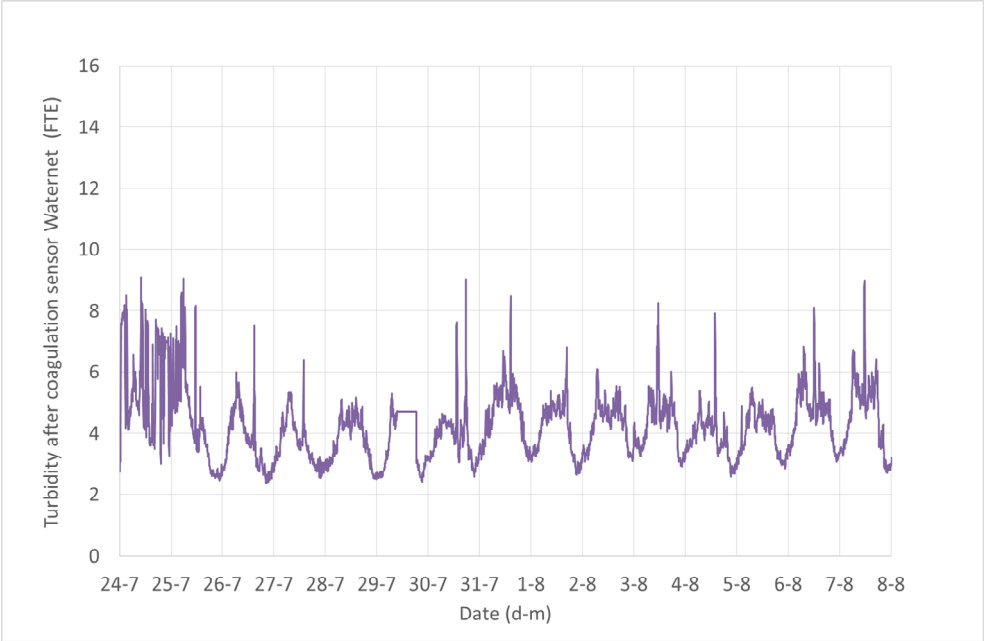


Figure 73. Turbidity data of the water after coagulation/sedimentation.

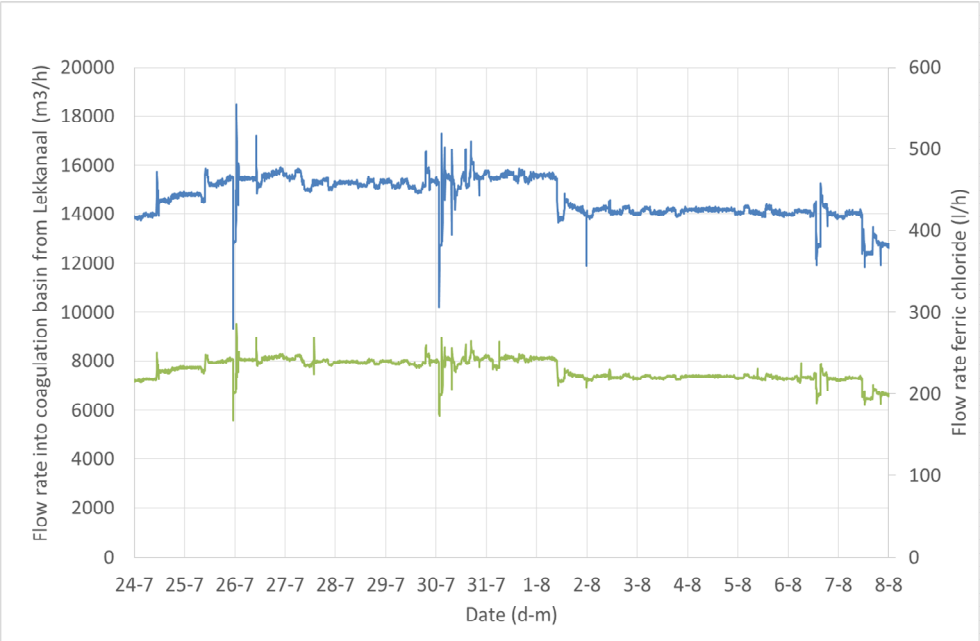


Figure 74. Flow rate of the surface water into the coagulation basin and flow rate ferric chloride.

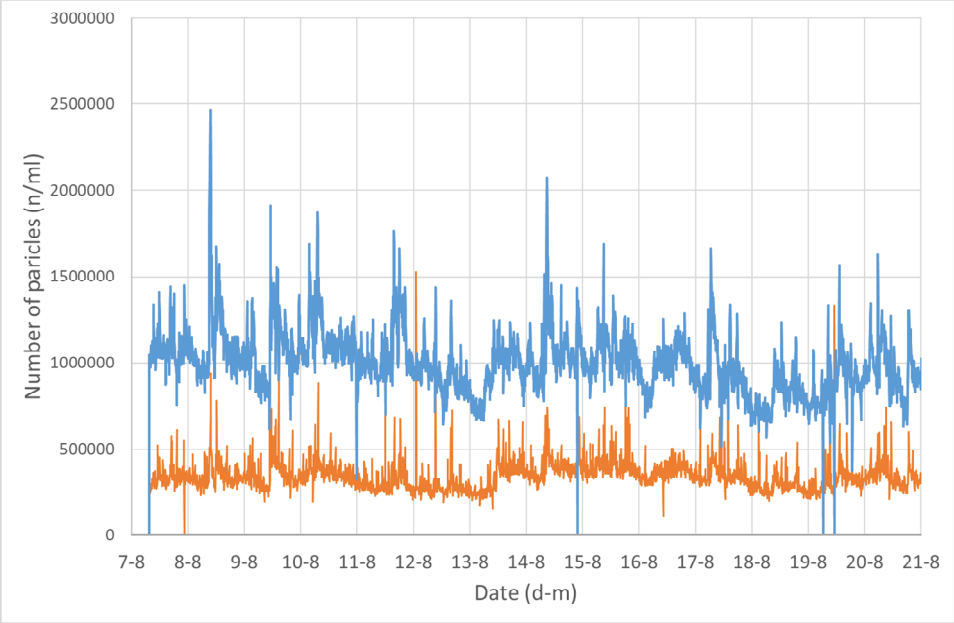


Figure 75. Results of the BACMON on lekkanaal water (orange: bacteria, blue: non-bacteria) in higher resolution (2 weeks period)

In Figure 76 the results of the BACMON on water after the rapid sand filters is presented for the whole monitoring period. The number of bacteria is in general higher compared to the number of particles. The number of bacteria is around 2500 per ml and the number of particles is around 1250 per ml. As with the data presented at location before the rapid sand filters, a more regular pattern is visible in the number of bacteria in water after the rapid sand filters (Figure 75).

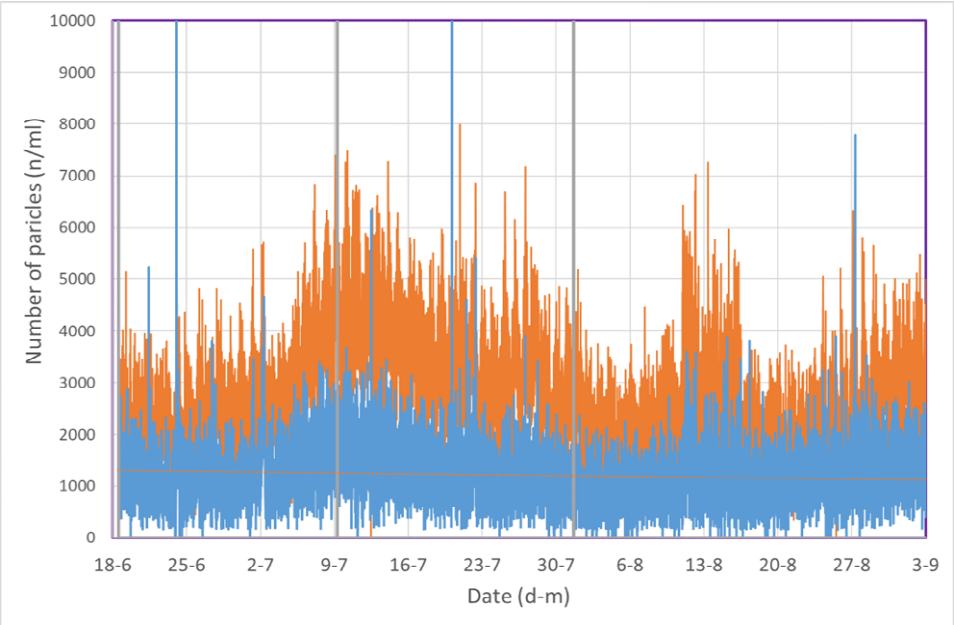


Figure 76. Results of the BACMON on water after the rapid sand filters (orange: bacteria, blue: non-bacteria)

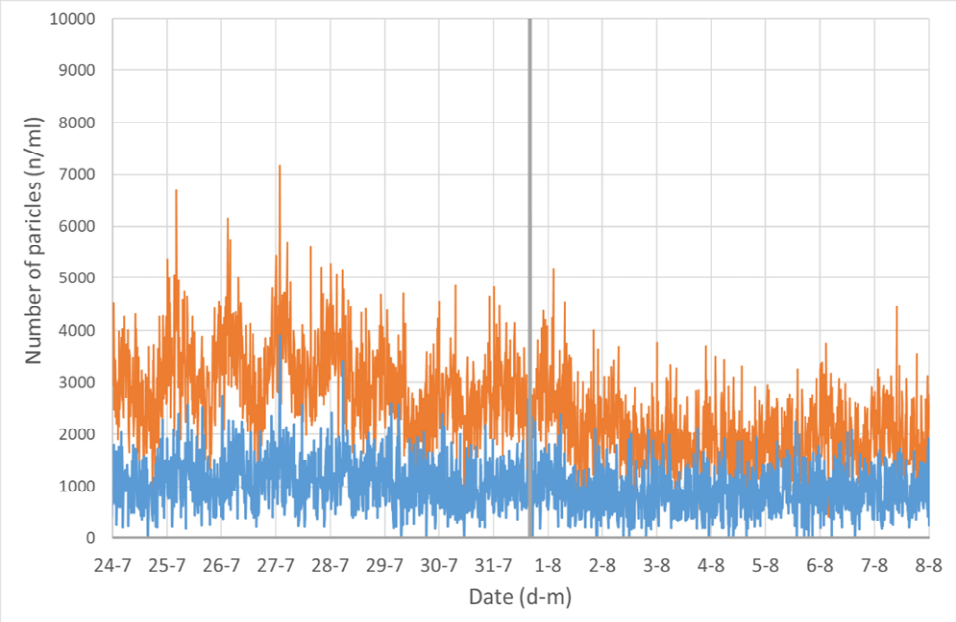


Figure 77. Results of the BACMON on water after rapid sand filters (orange: bacteria, blue: non-bacteria) in higher resolution (2 weeks period) (grey is time when inlay was changed)

In Figure 78, the BACMON presented a reduction of around 3 log units for non-bacteria in water after rapid sand filters compared to Lekkanaal water. For bacteria, a reduction of around 2 log units was measured. These numbers were higher compared to 0.3 to 0.5 log units measured with the BACTcontrol.

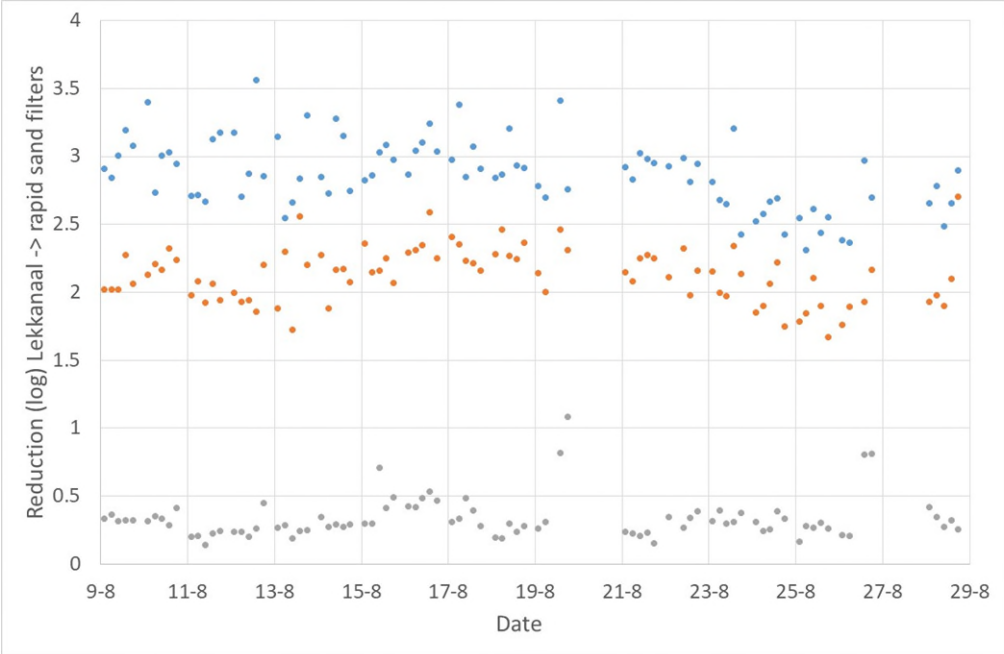


Figure 78 Reduction (log scale) of non-bacteria (blue dots), bacteria (orange) measured with the BACMON and bacteria (grey) measured with the BACTcontrol.

6.3.2 Comparison of data from the three sensors to each other

In Figure 79, the log-transformed values of the enzymatic activity measured with the BACTcontrol, is presented against the log-transformed values of the number of bacteria counted with the BACMON. The sample location was after rapid sand filtration. There is no correlation between the data obtained from the two instruments.

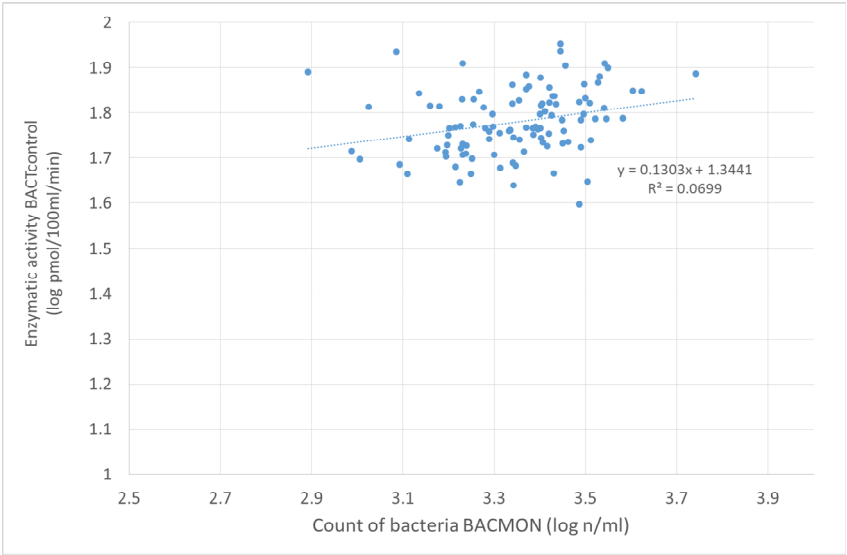


Figure 79. Comparison bacteria between instrument BACTcontrol and BACMON after rapid sandfiltration (period from 9-8-2019 till 30-8-2019)

In Figure 80, the turbidity data in FTU is presented against the number of Non bacteria counted with the BACMON. The sample location was after rapid sand filtration. There is no correlation between the two instruments. However, the sensitivity of the turbidity sensor is too low for this kind of waters.

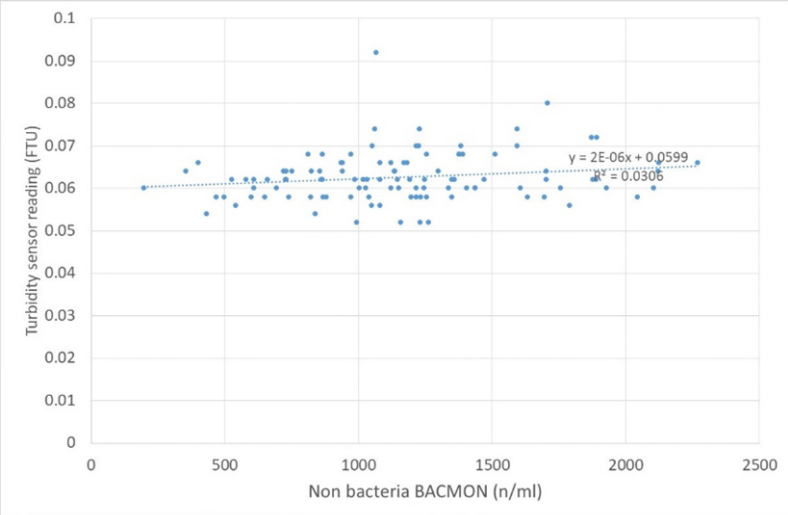


Figure 80. Comparison non-bacteria between instrument Turbidity sensor and BACMON after rapid sandfiltration (period from 9-8-2019 till 30-8-2019)

In Figure 81, the log-transformed values of the enzymatic activity measured with the BACTcontrol, is presented against the log-transformed values of the number of bacteria counted with the BACMON. The sample location was Lekkanaal. There is no correlation between the data from the two instruments.

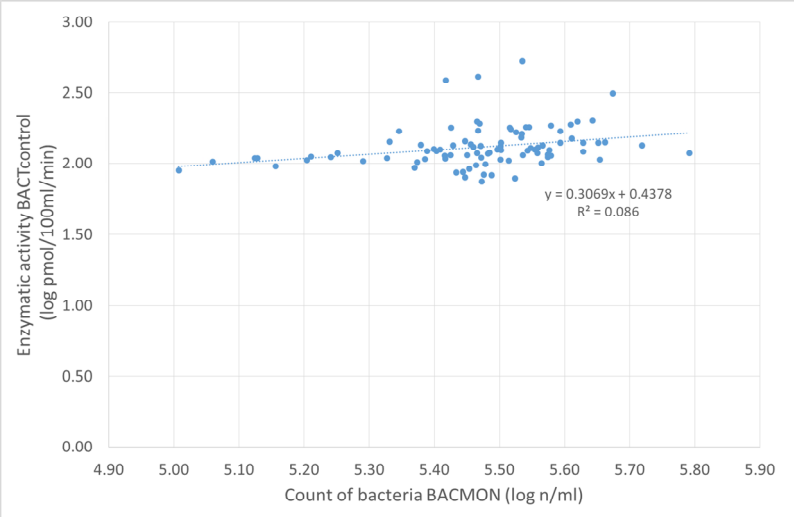


Figure 81. Comparison bacteria between instrument BACTcontrol and BACMON lekkanaal (period from 9-8-2019 till 30-8-2019).

In Figure 82, the turbidity data in FTU is presented against the number of Non bacteria counted with the BACMON. The sample location was Lekkanaal. There is a bad correlation between the data from the two instruments.

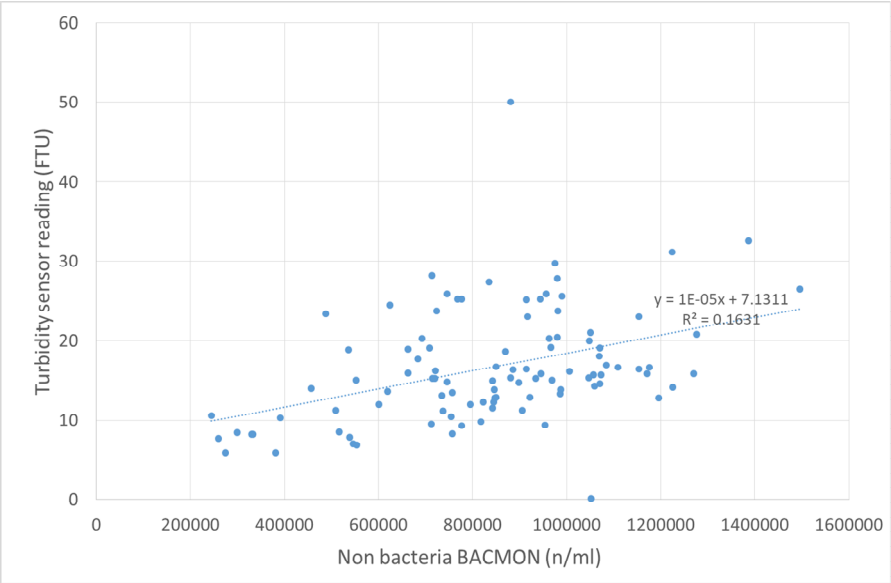


Figure 82. Comparison non-bacteria between instrument Turbidity sensor and BACMON lekkanaal (period from 9-8-2019 till 30-8-2019).

6.3.3 Comparison to laboratory analyses (if applicable)

The log-transformed values of both the BACMON and the BACTcontrol presents no correlation to the log-transformed plate count values (Figure 83). More data is presented 7.2.4.

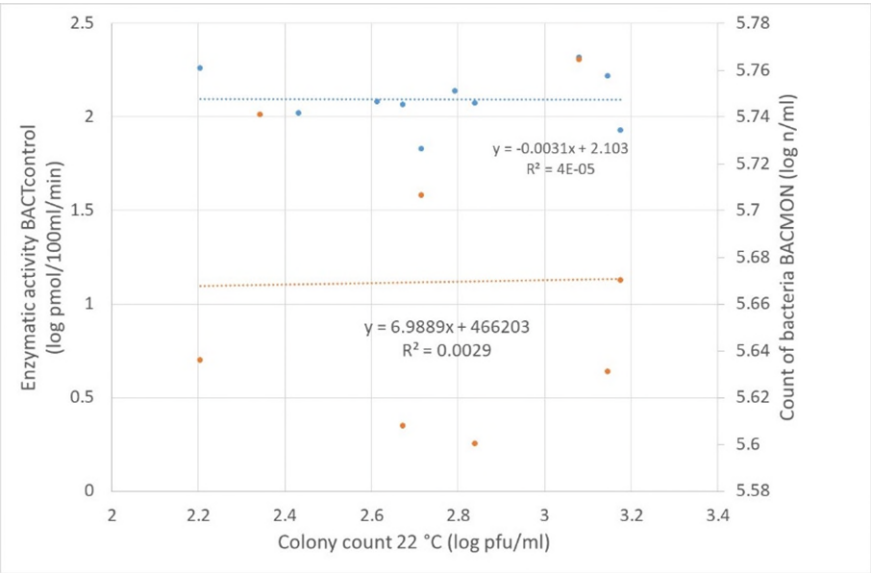


Figure 83. Lekkanaal (blue: BACTcontrol, orange: BACMON)

6.4 Discussion

The BACMON and BACTcontrol sensors provide different outputs (cell numbers versus enzymatic activity) which makes a comparison between these sensors difficult to make. The data measured on activity and the cell number on Lekkanaal water during the test period, do not present data that is useful for the operation at this particular production site. There is no variation of bacteria measured with the BACMON. The BACTcontrol presents sometimes higher activity. However, it seems that the higher activity is the result of performing some maintenance on the system prior to the higher enzymatic activities. Furthermore, it would be useful to have some kind of check-up that each sensor is still working correctly when used on river water like Lekkanaal.

The BACMON could only count particles in 5% of volume that is present in the inlay of the sensor. It remains unknown if the results have the same level of detection when high variation of sludge is present in the water. The instrument did provide a status signal (declining from 100 to 30 % in 4 days on average), but no warning is presented in the data when the measurement becomes too inaccurate. For the BACTcontrol, the measurements can be influenced by the particles present in the water during operation time after maintenance is performed. Sometimes, a high activity is measured directly after maintenance compared to measurements several days later. A regular check, if the instrument provide the same kind of signal during operation time, is needed for this kind of monitoring location. Maintenance was needed every week on both systems, but is easy to perform.

The bacterial water quality after rapid sand filtration can be monitored with both systems. The frequency of maintenance is lower compared to systems operated on water from Lekkanaal. A pattern at the BACMON data is visible in the numbers of bacteria and is more prominent compared to the number of non-bacteria for the water after rapid sand filtration.

No correlation was observed between bacterial counts measured with the BACMON and bacterial activity measured with the BACTcontrol at both monitoring locations, indicating that these parameters were not correlated to each other. In addition, no correlation was observed between turbidity, measured with a dedicated sensor, and the non-bacteria measured with the BACMON at samples from the Lekkanaal and from water after sand filtration. An explanation can be that number of particles in the Lekkanaal were too high to detect them accurately with the BACMON and the number of particles in water after sand filtration became too low for accurate measurement with a turbidity sensor.

7 Correlation of sensor results to biomass/cell count parameters at full-scale locations

7.1 Experimental set-up

7.1.1 Sampling campaign Oud-Turnhout, Pidpa

The cell number in drinking water from the production location Oud-Turnhout varies depending on which of the two treatment trains is used. Shortly after switching from one to the other treatment train the cell number in the drinking water increases, creating a small peak with the BACMON sensor (Chapter 3). A larger peak was created by delaying the switch between the two treatment trains for in total 48 hours. Just before the drinking water production was switched from one treatment to the other (at 8:00) the auto sampler was started (at 7:00) and every hour a water sample was taken and analysed in the laboratory.

Next to the drinking water at the production location, drinking water was also sampled at the water tower of Ravels. The estimated residence time between the production location in Oud-Turnhout and the water tower of Ravels was estimated to be about 16 hours. To ensure that the water samples were taken before the peak arrived, the auto sampler was turned on 14 hours after the switch (at 21:00). A time schedule for this experiment is given in Figure 84.

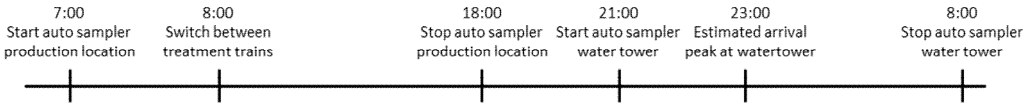


Figure 84. Time schedule of sampling campaign at production location Oud-Turnhout and the water tower in Ravels on 29 and 30 November 2018.

Besides the BACMON and BACTcontrol results, the water samples were studied for membrane-intact and total cell counts using flow cytometry (TCC-FCM), total cell count using microscopy (TCC-MS) and ATP concentration. These results and results from the correlation analysis between all parameters are discussed in chapter 7.2.1.

7.1.2 Sampling campaign cooling water, Uniper

No apparent pattern in cell numbers or enzymatic activity was observed for the cooling tower and surface water with the BACMON and/or BACTcontrol (Chapter 4). Consequently, it was not possible to create a peak event for the cooling tower. Therefore, the auto samplers were turned on at a normal day and samples were taken every 1.5 hour. The auto samplers were started at 13:00 at 13 March 2019 and the last samples were taken at 5:30 at 14 March 2019. The BACTcontrol was only applied to the incoming surface water, but not for the cooling tower water. Besides the BACMON and BACTcontrol results, the water samples were studied for membrane-intact and total cell counts using flow cytometry (TCC-FCM), total cell count using microscopy (TCC-MS) and

ATP concentration. These results and results from the correlation analysis between all parameters are discussed in chapter 7.2.2.

7.1.3 Autosampling campaign Havelterberg, Vitens

At drinking water production location Havelterberg peaks in cell numbers were observed with the BACMON and BACTcontrol after flushing or refilling the marble filters (Chapter 5). Therefore, the auto samplers were started during the refilling of a marble filter (filter number 11). Following this refilling another marble filter (filter number 12) was flushed. Hourly samples were taken from 9:00 until 14:00, after which samples were taken at 1-4 hours interval until 6:00 when the last water sample was taken. The time schedule is displayed in Figure 85. Besides the BACMON and BACTcontrol results, the water samples were studied for membrane-intact and total cell counts using flow cytometry (TCC-FCM), total cell count using microscopy (TCC-MS) and ATP concentration. These results and results from the correlation analysis between all parameters are discussed in chapter 7.2.3.

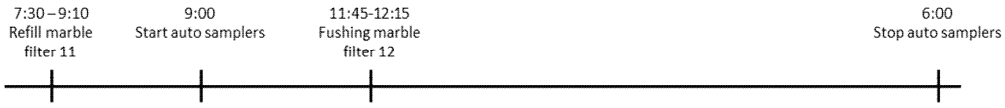


Figure 85. Time schedule of sampling campaign at production location Havelterberg on 21 and 22 May 2019.

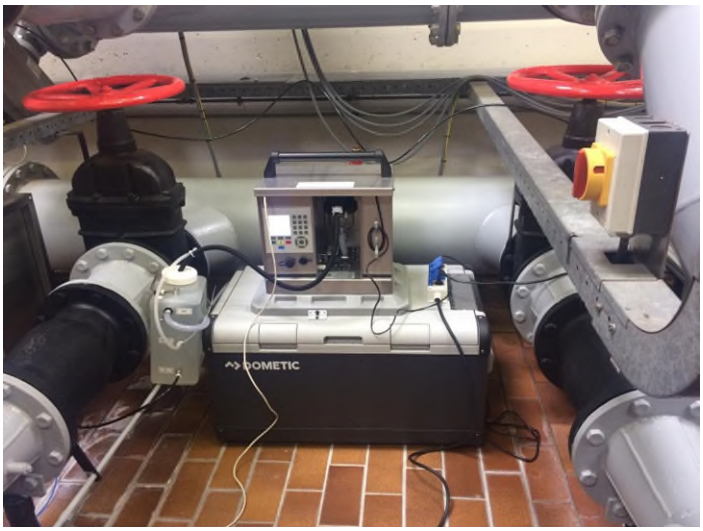


Figure 86. Auto sampler installed at production location Havelterberg.

7.1.4 Sampling campaign water of drinking water production, WRK

At the raw water intake point for drinking water production from the Lekkanaal, no apparent pattern in cell numbers or enzymatic activity was observed with the BACMON and/or BACTcontrol. In the effluent of the rapid sand filters treating the raw Lekkanaal water, sometimes a 24-hour cycle was observed with the BACMON, however, this pattern was not always visible (Chapter 6). Water was sampled every two hours over the duration of 24 hours, samples were taken at the intake and after rapid sand filtration. Besides the BACMON and BACTcontrol results, the water samples were studied for membrane-intact and total cell counts using flow cytometry (TCC-FCM), total cell count

using microscopy (TCC-MS) and ATP concentration. These results and results from the correlation analysis between all parameters are discussed in paragraph 7.2.4.

7.2 Results

7.2.1 Oud-Turnhout, Pidpa

The results from the BACMON on 29 and 30 November 2018 (the period the auto samplers were sampling; chapter 7.1.1) show that at the production location a peak in cell numbers (about 5-fold increase) was visible (Figure 87). The increase started 1 hour after the switch between the treatment trains at 8:00 on 29 November and maximum cell numbers were obtained about 6-7 hours later. At the water tower no increase was visible within the sampling time frame. However, just after the sampling time frame (at around 9:00 on 30 November) the cell numbers started to increase. Because the residence time was estimated to be much shorter, the samples taken at the water tower were taken before the peak in cell numbers reached the water tower.

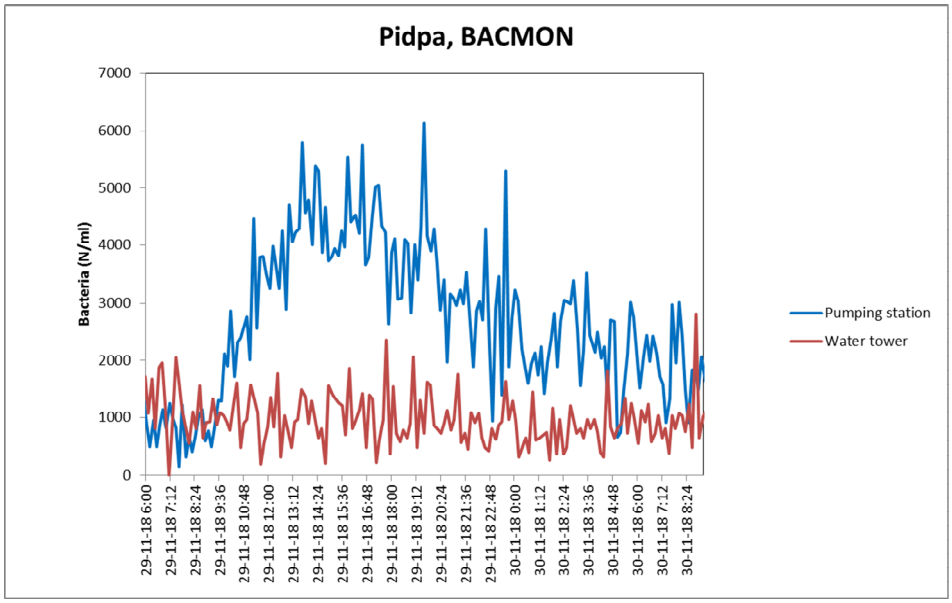


Figure 87. BACMON results in the period of the sampling campaign at Oud-Turnhout.

During the peak of bacterial numbers that is visible with the BACMON at the production location, every hour water samples were taken and analysed using ATP, TCC-FCM and TCC-MS. The peak is also observed with ATP measurements and TCC-MS (Figure 88), but the shape of the peak differs as the BACMON results levelled off at around 11:00, whereas the ATP levels and TCC-MS increased until 14:00 to 15:00. The other cell counts methods (TCC-FCM) did not show a peak within the sampling time frame. This suggests that the BACMON, ATP and TCC-MS measurements are more sensitive in detecting small changes in cell numbers compared to TCC-FCM analyses which are not sensitive enough to detect these small differences. The BACTcontrol only measured the water three times at the production location and no peak is visible. One measurement was performed at the maximum of the peak and the enzymatic activity at that moment was slightly higher than the other two measurements.

Furthermore, the BACMON results are 5 to 50 times lower compared to TCC-MS and 10 to 100 times lower compared to TCC-FCM. The TCC-MS also consistently yields lower cell numbers than TCC-FCM.

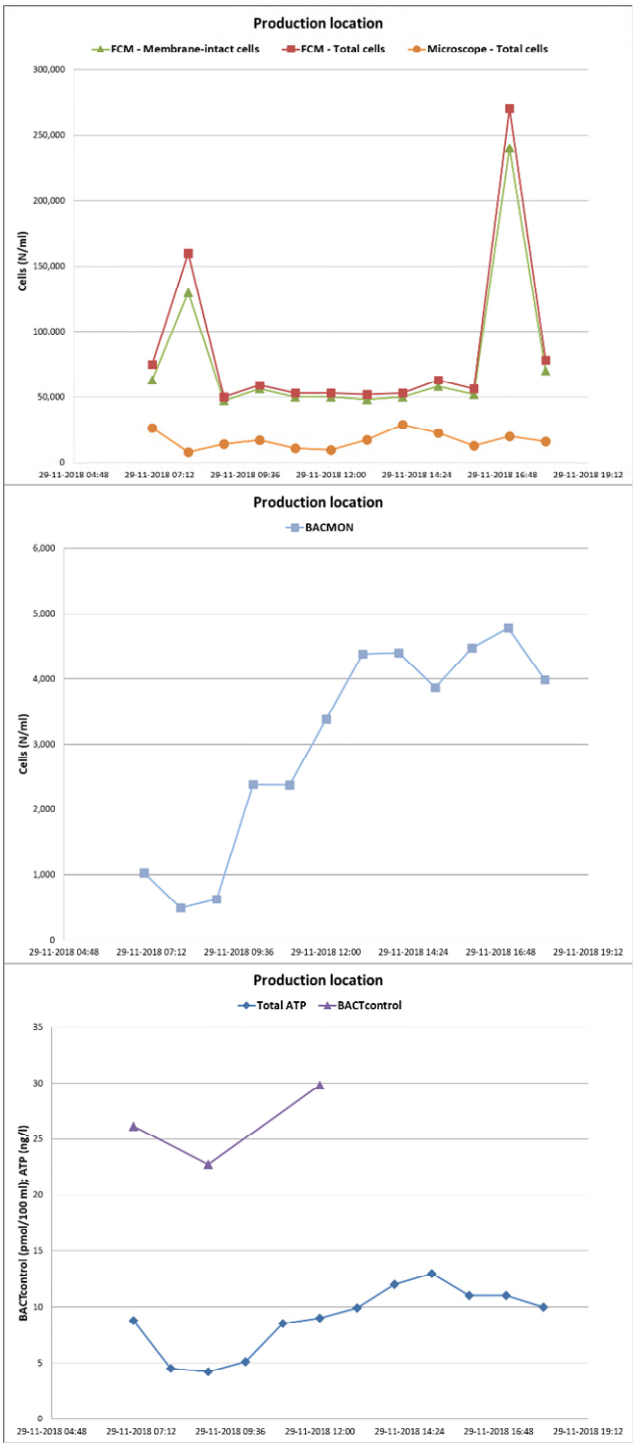


Figure 88. Results of sampling at the full-scale location at the pumping station in Oud-Turnhout of Pidpa. The BACMON results are the average of three measuring points around the autosampling point.

At the water tower no peak in cell numbers was observed with any of the analyses (Figure 89). This agrees with BACMON results which did not observe a peak in cell numbers within the sampling time frame. The ATP concentration fluctuates around 3.3 ng/l, but at 1:00 a higher ATP concentration (7.7 ng/l) was observed. The same pattern

was not visible for the other parameters, indicating that this ATP value might be an outlier.

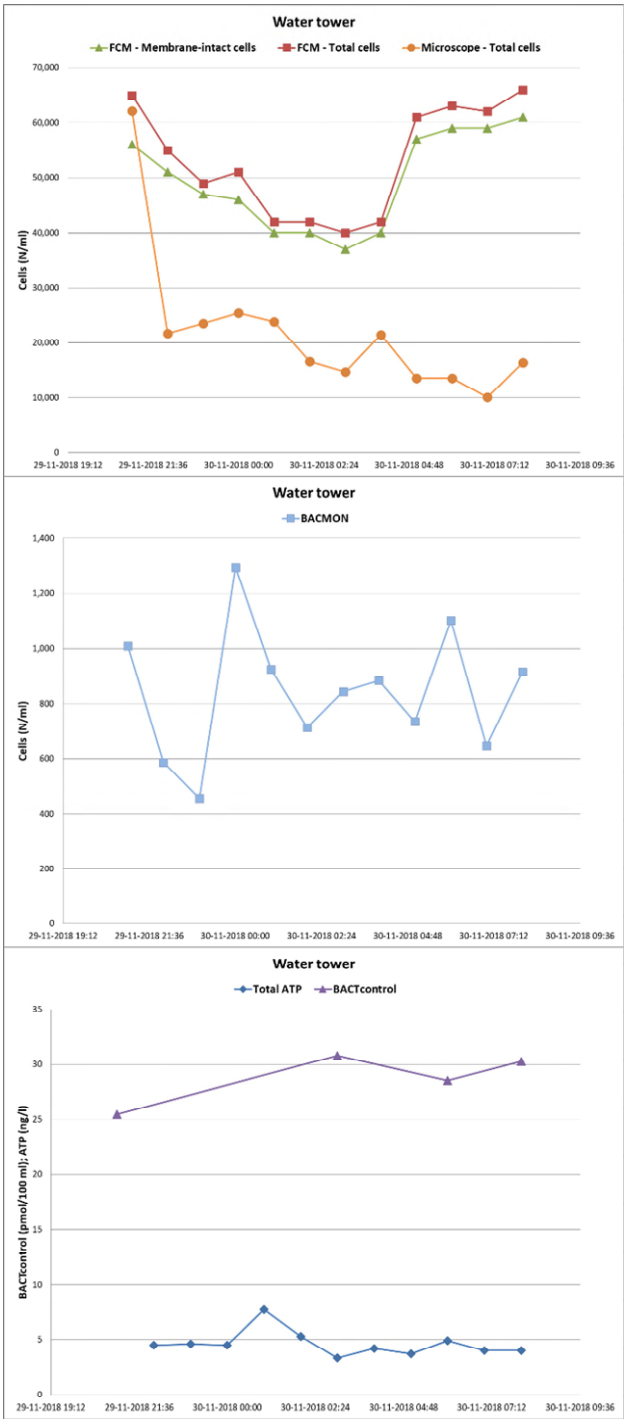


Figure 89. Results of sampling at the full-scale location at the water tower in Ravels of Pidpa. The BACMON results are the average of three measuring points around the autosampling point.

Regression analysis shows that only the correlation between the BACMON and ATP results at the production location are significant (Table 14). The lack of significant correlations between most of the parameters is partly caused by the limited number of measurements (BACTcontrol) and by the fact that no large differences in cell numbers were measured at Ravels with any of the methods and with the TCC-MS and TCC-FCM methods at the production location.

Table 14. Linear regression analysis of the sampling campaign at the production location of Oud-Turnhout and the water tower in Ravels. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p < 0.05$) and if so the r^2 is given.

Water source	Parameter 1	Parameter 2	N	p<0.05	r^2	
Production location	BACMON	ATP	12	Yes	0.69	Moderate
		TCC-MS	12	No		
		TCC-FCM, living	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	5	No		
	BACTcontrol	ATP	3	No		
		TCC-MS	3	No		
		TCC-FCM, living	3	No		
		TCC-FCM, total	3	No		
Water tower	BACMON	ATP	11	No		
		TCC-MS	12	No		
		TCC-FCM, living	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	4	No		
	BACTcontrol	ATP	3	No		
		TCC-MS	4	No		
		TCC-FCM, living	4	No		
		TCC-FCM, total	4	No		
Production location + water tower	BACMON	ATP	23	Yes	0.79	Good
		TCC-MS	24	No		
		TCC-FCM, living	24	No		
		TCC-FCM, total	24	No		
		BACTcontrol	7	No		
	BACTcontrol	ATP	6	No		
		TCC-MS	7	No		
		TCC-FCM, living	7	No		
		TCC-FCM, total	7	No		

7.2.2 Uniper

The data from both bacterial sensors did not indicate specific events in the incoming surface water and cooling tower water. Therefore, it was chosen to take samples with the autosampler on a regular day (13 till 14 March 2019). The first 9.5 hours the BACMON data was relatively stable, but at 22:30 both bacterial and non-bacterial numbers increased and from 2:00 (14 March) bacterial and non-bacterial numbers started to fluctuate between 0 and high numbers (up to 1.5 x 10^6 N/ml for bacteria and 2.2 x 10^6 N/ml for non-bacteria; Figure 90. This is probably caused by sudden fouling of the flow cell, indicating that the bacterial water quality has changed.

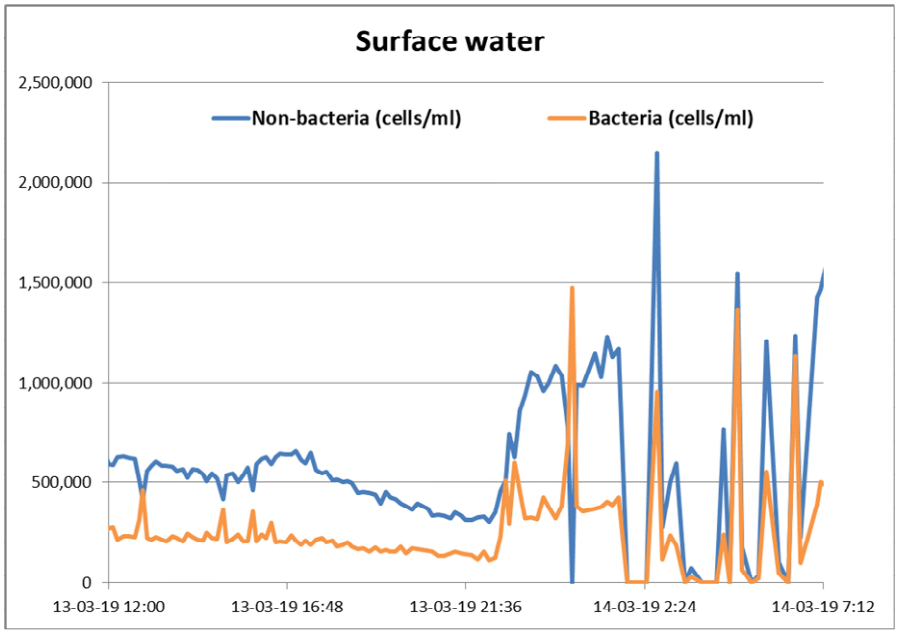


Figure 90. BACMON results of the incoming surface water at Uniper

It was observed that the bacterial numbers enumerated with the BACMON sensor were 10 to 20 times lower than the numbers counted with the flow cytometer or microscope (Figure 90). Furthermore, the bacterial numbers enumerated with the flowcytometer also increased somewhere between 22:00 and 23:30 and remained high till the end of the measuring period with the autosamplers (Figure 91, top). The bacterial counts determined with microscopy also showed an increase, but this increase was somewhat delayed (between 1:00 and 2:30) compared to the BACMON and flowcytometry data. Remarkably, the ATP concentration showed a peak concentration at 14:30, but remained at a stable concentration thereafter, whereas the BACTcontrol also showed an increase in activity at the last measuring point (Figure 91, bottom). These results indicate that the microbiology of the incoming surface water changed in the evening of March 13 and that most parameters, with the exception of ATP, demonstrated this change in microbiological water quality.

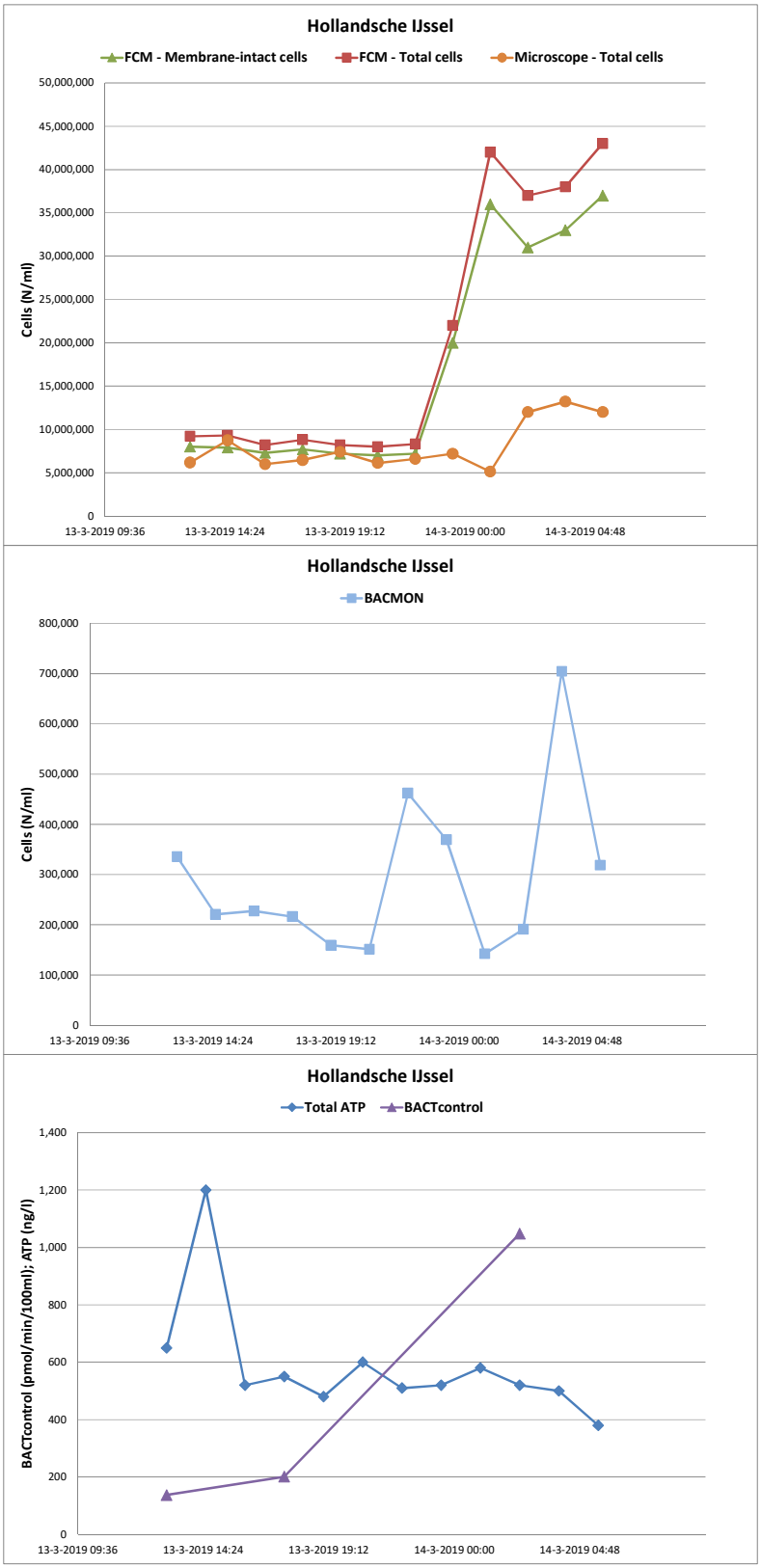


Figure 91. Results from surface water samples taken with the autosampler at Uniper. The BACMON results are the average of three measuring points around the autosampling point.

A correlation analysis between the BACMON data and the other parameters for this period demonstrated that the BACMON data did not significantly ($p>0.05$) correlate with the other parameters measured (Table 15). This seems to contradict the previous paragraph where it was concluded that the BACMON, flow cytometry and microscope data all identified a rather dramatic change in water quality in the evening of 13 March. The main reason that significant and strong correlations were not observed between BACMON and the other parameters was caused by the fluctuating BACMON data during this time period (Figure 92). Although this fluctuating data shows that something happened with the water quality in the evening of 13 March, the BACMON data did not consistently enumerate high bacterial numbers during this event, while the other parameters were consistently high.

The cooling tower water was also sampled in the same period (13 till 14 March 2019) with the autosamplers and these samples were also analysed for ATP and cell numbers. The BACMON data of the cooling tower water for this period is shown in Figure 92. The bacterial numbers enumerated with the cooling tower water remained stable during this period, whereas the non-bacterial numbers showed small peaks at two moments (13 March around 23:00 and 14 March at 6:15-8:00). The bacterial numbers determined with the BACMON sensor were in general 10 to 40 times lower than the bacterial numbers measured with the flow cytometer or microscope (Figure 93). The bacterial numbers enumerated with flowcytometry or microscopy and the ATP concentrations decreased considerably from 13 March 13:00 till 13 March 19:00, after which cell numbers and ATP concentration remained relatively stable Figure 93). The correlation analysis showed that the BACMON data was not significantly ($p>0.05$) correlated to the bacterial cell numbers, enumerated with flowcytometry or microscopy, and ATP concentration (Table 15), whereas the other parameters were strongly correlated with each other ($p<0.05$, R^2 between 0.51 and 0.87). This, thus, shows that the BACMON data is not in agreement with the other parameters from the cooling tower water during the measuring period.

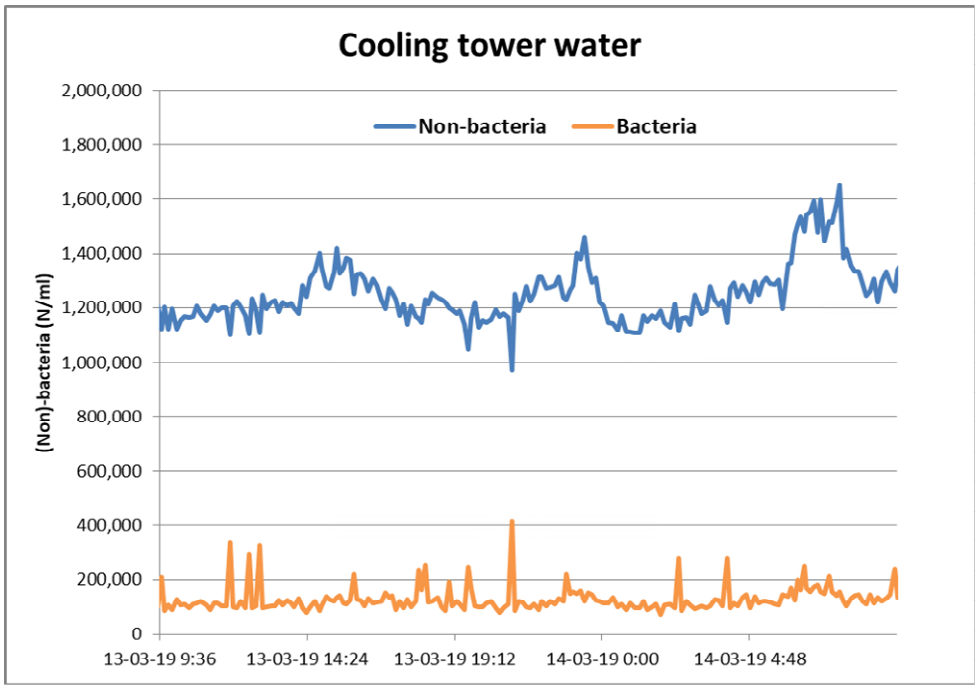


Figure 92. BACMON results of the cooling tower water at Uniper during the sampling campaign during the sampling campaign.

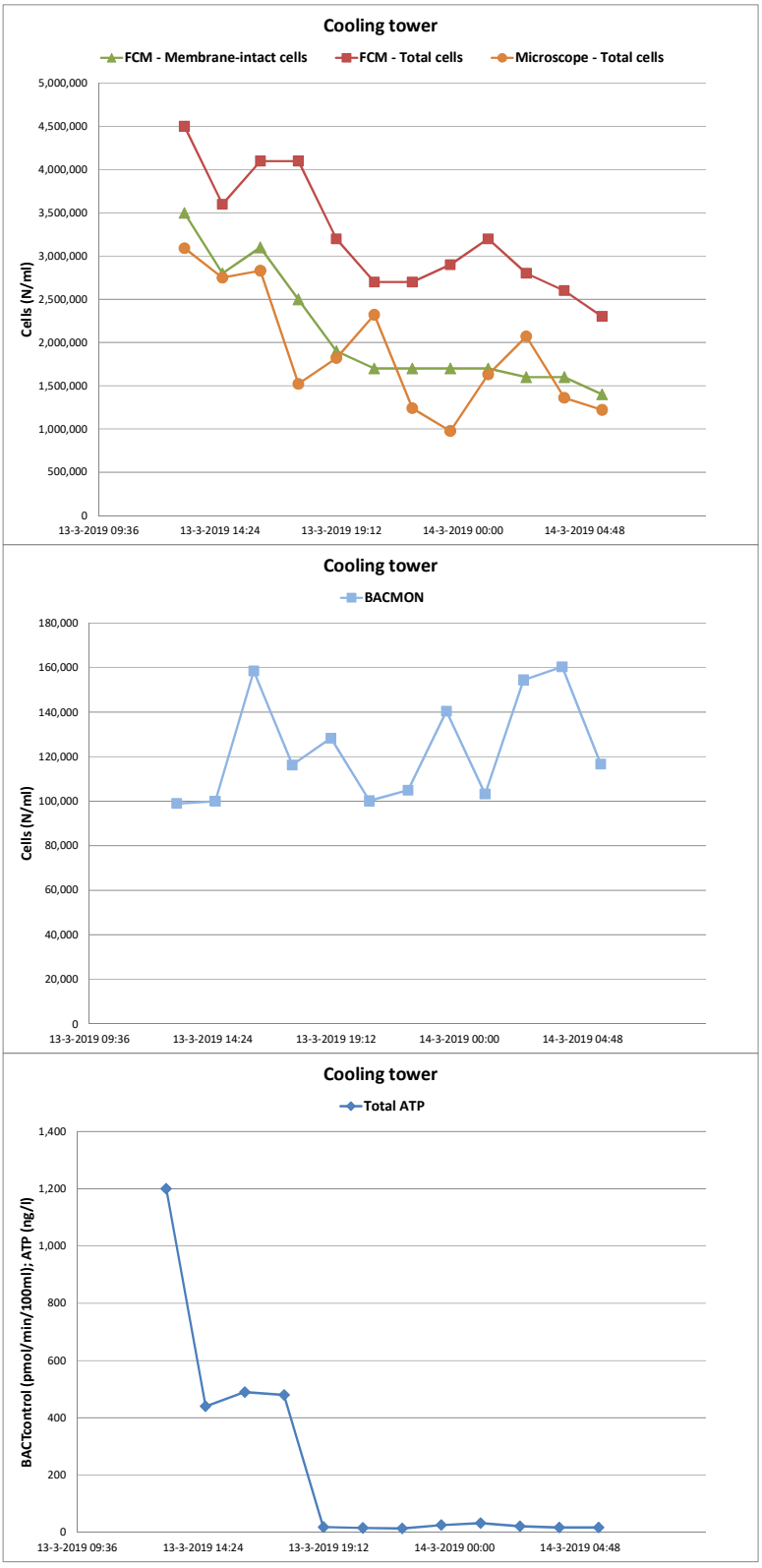


Figure 93. Results from cooling tower water samples taken with the autosampler at Uniper. The BACMON results are the average of three measuring points around the autosampling point.

Table 15. Linear regression analysis of the sampling campaign at Uniper. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p < 0.05$) and if so the r^2 is given.

Water source	Parameter 1	Parameter 2	N	p<0.05	r^2	
Surface water (Hollandse IJssel)	BACMON	ATP	12	No		
		TCC-MS	12	No		
		TCC-FCM, intact	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	3	No		
	BACTcontrol	ATP	3	No		
		TCC-MS	3	Yes	1.0	Excellent
		TCC-FCM, intact	3	Yes	0.99	Excellent
		TCC-FCM, total	3	Yes	0.99	Excellent
Cooling tower	BACMON	ATP	12	No		
		TCC-MS	12	No		
		TCC-FCM, intact	12	No		
		TCC-FCM, total	12	No		
Surface water + cooling tower	BACMON	ATP		No		
		TCC-MS		No		
		TCC-FCM, living		No		
		TCC-FCM, total		No		

7.2.3 Havelterberg, Vitens

At the full-scale location at Havelterberg the water samples were taken during refilling of a marble filter (7:30 – 9:10 on 21 May 2019) and flushing of another marble filter on the same day (11:45 – 12:15; Figure 85, chapter 7.1.3). Effects of refilling and flushing on the sensors and biomass parameters, if any, should be seen in the Steenwijkerwold-water. A peak in the cell numbers, measured by the BACMON, is visible between 10:00 and 11:00, but afterwards the cell numbers remain relatively stable (blue line, Figure 94).

The water from Ruinerwold shows hardly any variation, which was expected since flushing and refilling the marble filter at Havelterberg does not affect the water of Ruinterwold.

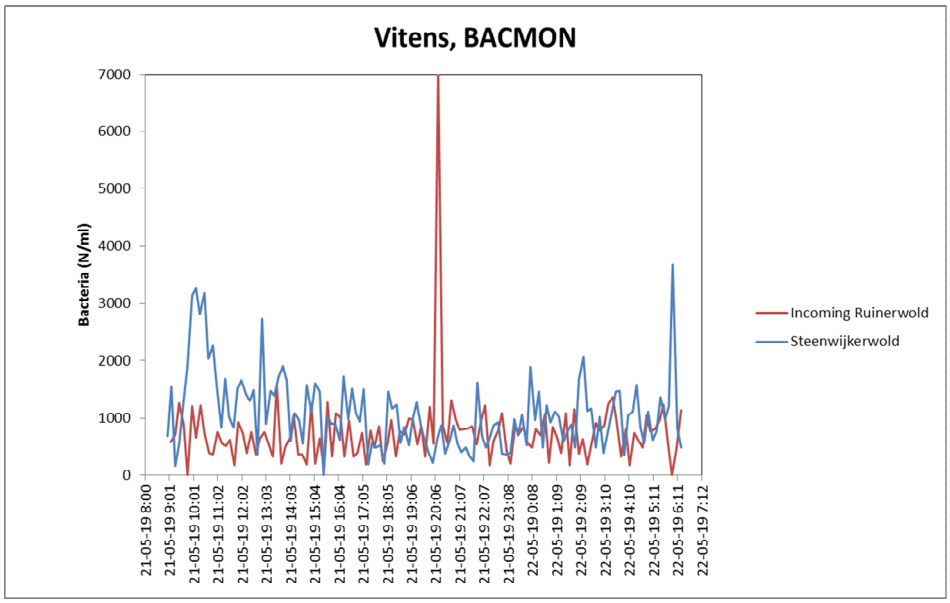


Figure 94. BACMON results in the period of the sampling campaign at production location Havelterberg.

The near lack of variation in cell numbers can also be seen at the results of other biomass parameters for Ruinerwold (Figure 95) and Steenwijkerwold (Figure 96). In Ruinerwold only the ATP levels vary to some extent, but this cannot be related to any of the other parameters.

In drinking water from Ruinerwold the cell numbers measured with TCC-FCM and TCC-MS and the ATP concentrations are slightly higher than Steenwijkerwold drinking water. At both sampling locations BACMON results are 100 to 200 times lower compared to TCC-FCM and TCC-MS for the Ruinerwold water and 80 to 100 times lower for the Steenwijkerwold water.

The enzymatic activity measured with the BACTcontrol differs largely between Ruinerwold and Steenwijkerwold drinking water: 8.75 – 10.15 versus 55.88 – 100.77 pmol/min/100 ml. An apparent cause for this large difference could not be found.

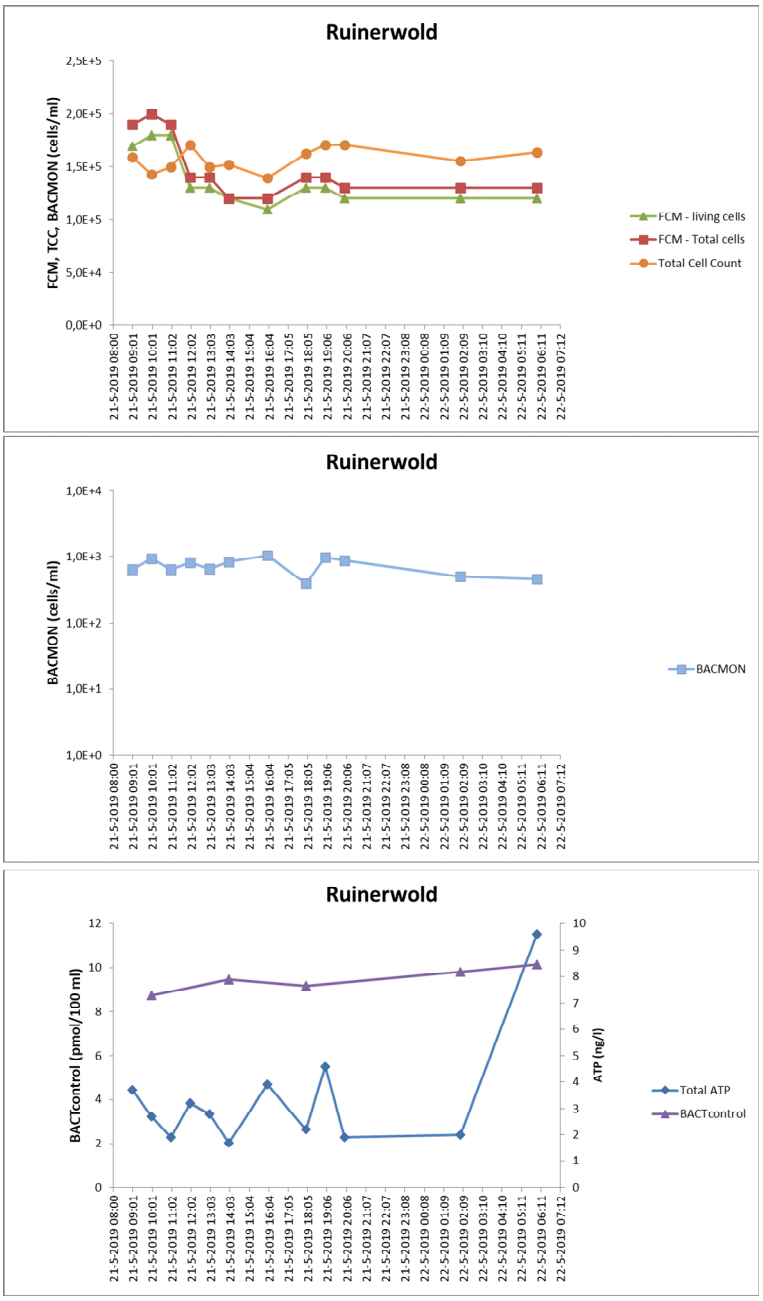


Figure 95. Results of biomass analyses on sampled water at the full-scale location at Havelterberg (Vitens). Incoming drinking water from production location Ruinerwold was sampled. The BACMON results are the average of three measuring points around the autosampling point.

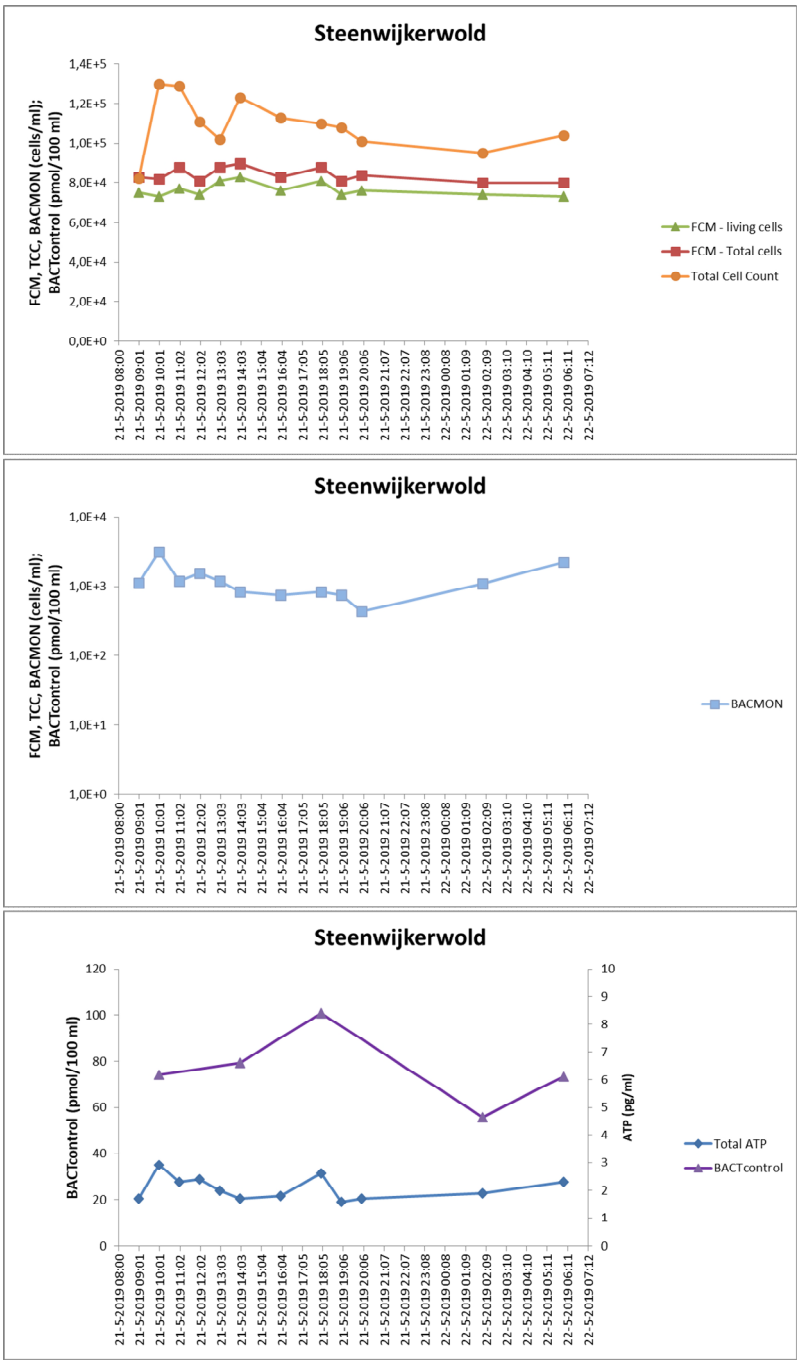


Figure 96. Results of biomass analyses on sampled water at the full-scale location at Havelterberg (Vitens). Produced drinking water to be transported to Steenwijkerwold was sampled. The BACMON results are the average of three measuring points around the autosampling point.

Table 16. Linear regression analysis of the water samples taken at Havelterberg from the incoming water from Ruinerwold and the produced drinking water to be transported to Steenwijkerwold. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p < 0.05$) and if so the r^2 is given. The linear regression was performed on non-log transformed data.

Water source	Parameter 1	Parameter 2	N	p<0.05	r^2	
Ruinerwold	BACMON	ATP	12	No		
		TCC-MS	12	No		
		TCC-FCM, living	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	5	No		
	BACTcontrol	ATP	5	No		
		TCC-MS	5	No		
		TCC-FCM, living	5	No		
		TCC-FCM, total	5	No		
Steenwijkerwold	BACMON	ATP	12	Yes	0.55	Moderate
		TCC-MS	12	No		
		TCC-FCM, living	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	5	No		
	BACTcontrol	ATP	5	No		
		TCC-MS	5	No		
		TCC-FCM, living	5	No		
		TCC-FCM, total	5	No		
Steenwijkerwold + Ruinerwold	BACMON	ATP	24	No		
		TCC-MS	24	No		
		TCC-FCM, living	24	Yes	0.17	Bad
		TCC-FCM, total	24	No		
		BACTcontrol	10	No		
	BACTcontrol	ATP	10	No		
		TCC-MS	10	Yes	0.68	Moderate
		TCC-FCM, living	10	Yes	0.66	Moderate
		TCC-FCM, total	10	Yes	0.60	Moderate

Linear regression analysis shows hardly any correlation between the sensors and biomass biomass parameters (

Table 16). Only in the Steenwijkerwold water the BACMON is moderately correlated to ATP, however no correlation is apparent for the Ruinerwold water. The lack of variation in the parameters analysed during the sampling frame is a probable cause for the absence of significant correlations. When all results from Ruinerwold and Steenwijkerwold are combined, the BACTcontrol is moderately correlated to TCC-FCM and TCC-MS. However, the relevance of this correlation is considered to be low due to the very low variation in cell numbers and enzymatic activities.

7.2.4 WRK, HWL

At the WRK water samples were taken during a 24 h time frame with the autosampler sampling the incoming Lekkanaal water and the water after rapid sand filtration every 2 h (28 August 2019, 10:00 till 29 August 2019, 7:00).

The cell numbers measured with the BACMON showed different small peaks in the incoming Lekkanaal water during the 24 h sampling campaign (Figure 97, upper graph). No clear high peaks were observed with the BACMON during the same sampling campaign of the water after rapid sand filtration (Figure 97, lower graph).

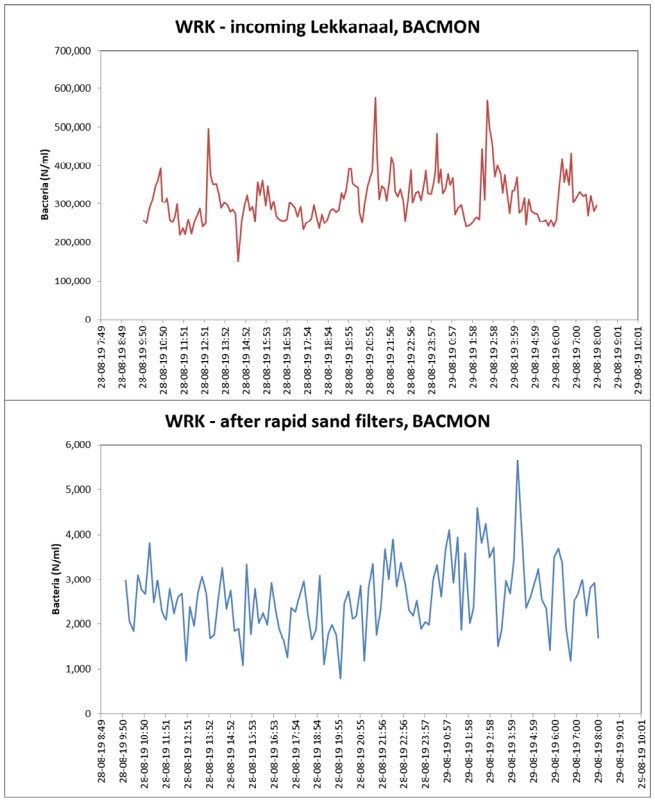


Figure 97. BACMON results from the incoming surface water from the Lekkanaal en the water after rapid sand filtration.

The results from the parameters that were measured on the samples taken with the autosampler as well as the BACMON and BACTcontrol data on these sampling times are given in Figure 98 for the Lekkanaal water and in Figure 99 for the water after rapid sand filtration. For the Lekkanaal water, the cell numbers determined with flow cytometry, showed a decreasing trend in the measuring period, with the exception of a peak value at 28 August 14:00. A similar pattern, although more capricious, was also observed for the cell numbers enumerated with microscopy and the ATP concentration. The BACMON data also showed a small peak around this time, but the BACMON showed a larger peak later on (28 August 22:00), that was not observed with the other cell number parameters or ATP. The BACTcontrol value at 28 August 14:00 was slightly higher than the previous measurement at 10:00, but increasing values were observed towards the end of this measuring period and which did not coincidence with the other parameters. A consequence of these results is that there are no significant ($p>0.05$) correlations between the BACMON or BACTcontrol data and the flow cytometry, microscope or ATP data. In addition, the BACMON and BACTcontrol data were also not significantly correlated (Table 17). From the other parameters only the flow cytometry data showed a significant ($p<0.05$) and moderate correlation ($R^2 \sim 0.65$) with ATP. Cell numbers enumerated with microscopy were not significantly ($p>0.05$) correlated with the other parameters measured (data now shown).

The cell numbers in the water after rapid sand filters, determined with flow cytometry, remained relatively stable during the measuring period on 28/29 August (Figure 99). Only the last value seemed to indicate a clear increase in cell numbers. The cell numbers enumerated with the microscope showed small peak values at three different moments. After the last small peaks, numbers decreased until the last measuring moment. The cell numbers enumerated with the BACMON sensor remained relatively stable as well, although numbers increased slightly from 29 August 00:00 till 29 August 6:00. Moreover, these numbers were 400 times lower than the cell numbers counted with flow cytometry or microscopy. The ATP concentration showed a clear peak on 28 August 16:00, but was relatively stable before and after this peak. The enzymatic activity measured with the BACTcontrol decreased slightly during the measuring period, except for the last time point, which showed a slightly higher value. Overall, this data showed that most parameters behaved differently during the measuring period, although the variation in the data remained small. The correlation analyses showed that the BACMON data nor the BACTcontrol data were significantly correlated with any of the other parameters measured or between each other (Table 17). Furthermore, also the cell numbers enumerated with flow cytometry, cell numbers counted with microscopy and the ATP concentrations did not show significant correlations with each other. A possible explanation for this lack of significant correlations between the different parameters is that the variation in the data was too low.

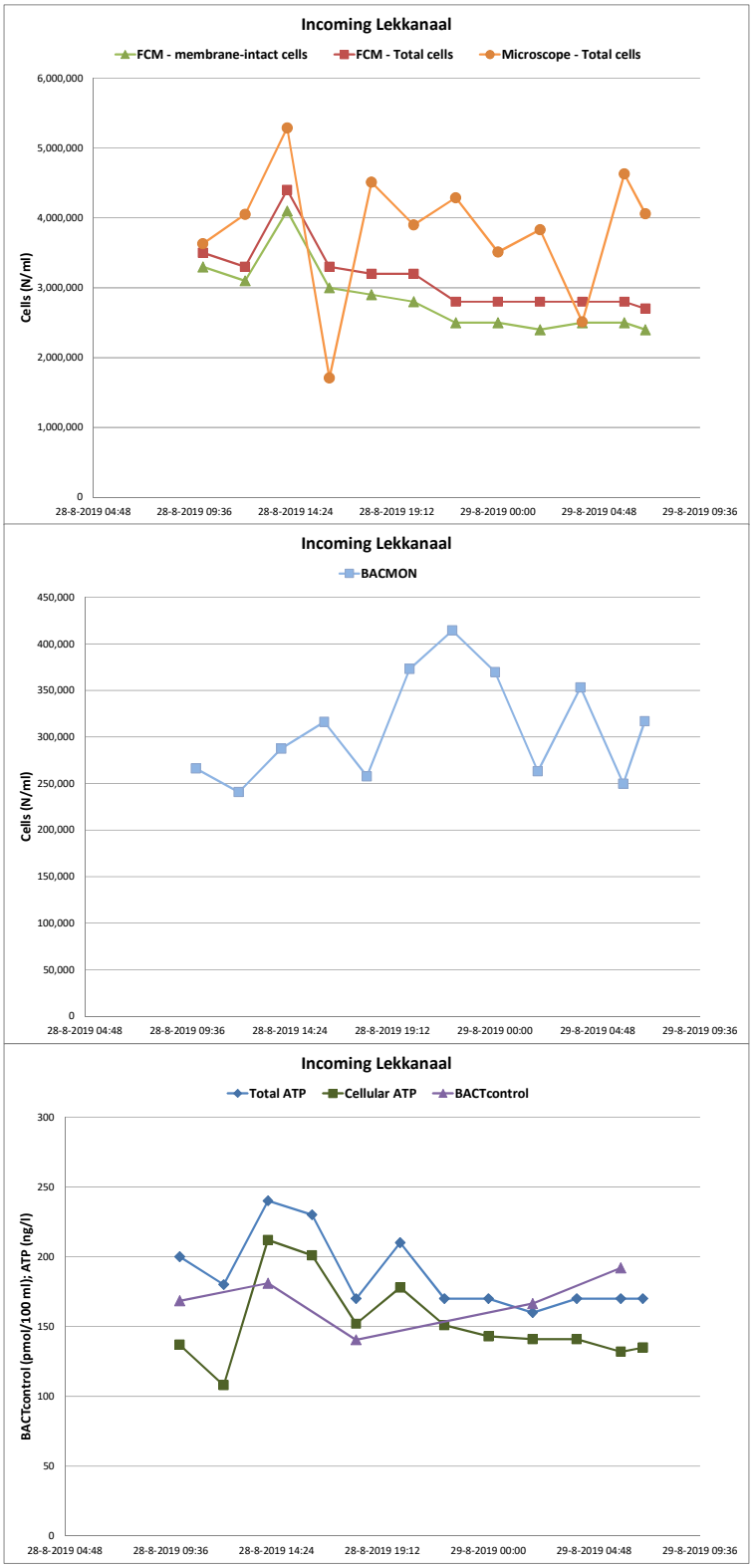


Figure 98. Results of biomass analyses on water samples of the incoming surface water of the Lekkanaal at the WRK. The BACMON results are the average of three measuring points around the autosampling point.

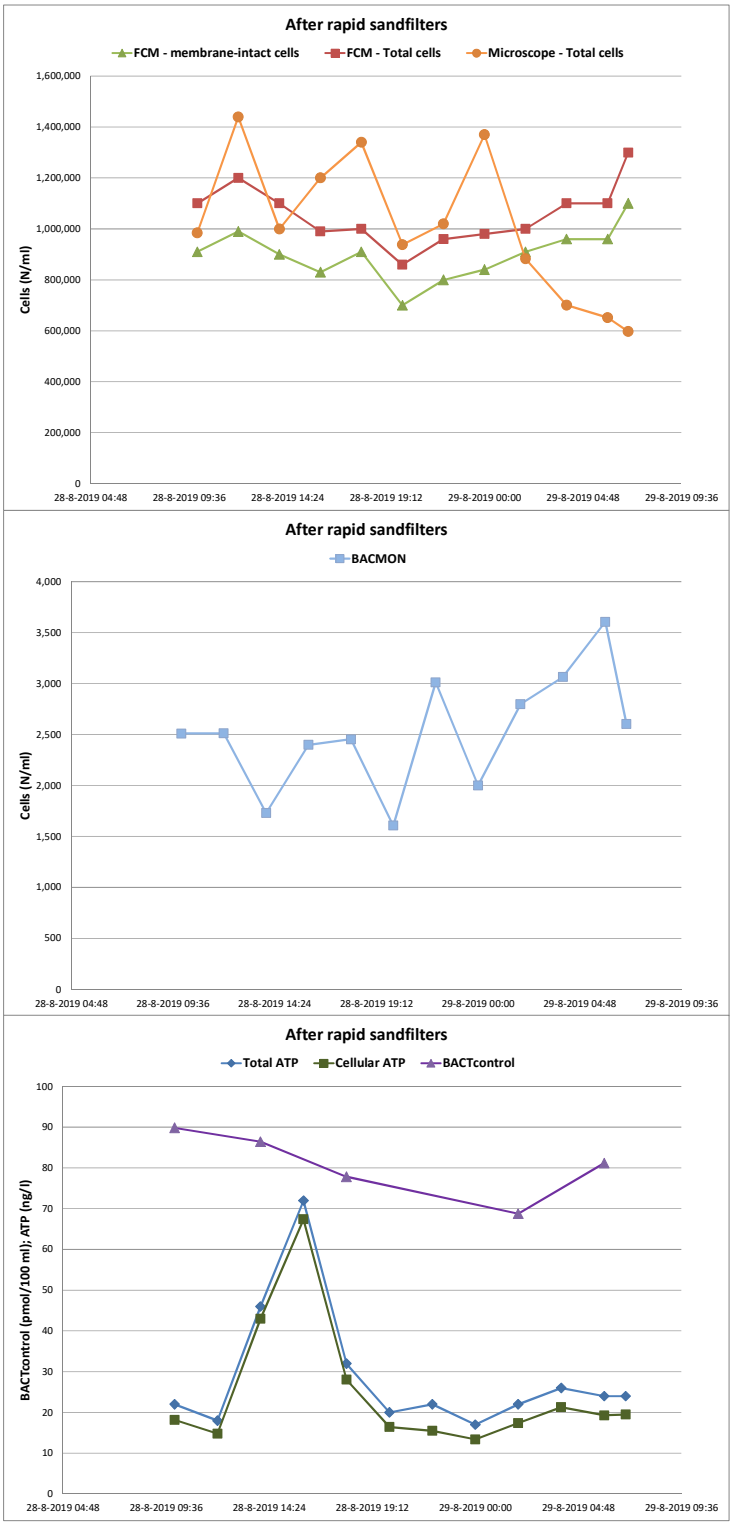


Figure 99. Results of biomass analyses on water sampled after rapid sand filtration at the WRK. The BACMON results are the average of three measuring points around the autosampling point.

Table 17. Linear regression analysis of the water samples taken at the WRK from the incoming surface water from the Lekkanaal and the water after rapid sand filtration. The BACMON and BACTcontrol are compared to the other biomass parameters. Given is the number of measurements (N), whether the linear regression is significant (yes: $p< 0.05$) and if so the r^2 is given. The linear regression was performed on non-log transformed data.

Water source	Parameter 1	Parameter 2	N	p<0.05	r^2	
Lekkanaal	BACMON	ATP	12	No		
		TCC-MS	12	No		
		TCC-FCM, living	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	5	No		
	BACTcontrol	ATP	5	No		
		TCC-MS	5	No		
		TCC-FCM, living	5	No		
		TCC-FCM, total	5	No		
After rapid sand filtration	BACMON	ATP	12	No		
		TCC-MS	12	No		
		TCC-FCM, living	12	No		
		TCC-FCM, total	12	No		
		BACTcontrol	5	No		
	BACTcontrol	ATP	5	No		
		TCC-MS	5	No		
		TCC-FCM, living	5	No		
		TCC-FCM, total	5	No		

7.2.5 All samples combined

The significant correlations observed between BACMON, BACTcontrol, ATP, flow cytometer bacterial cell numbers and microscope bacterial cell numbers for all eight water types are shown in Table 18. It was expected that the bacterial numbers counted with the BACMON sensor, flow cytometer and microscope would be correlated and that the microbial activity determined with the BACTcontrol sensor and ATP would be correlated. However, the bacterial numbers determined with the BACMON sensors was not significantly correlated with any of the other parameters for six of the eight tested water types. In the other two water types (drinking water from production location of Pidpa and Vitens), a significant correlation was only observed between BACMON data and ATP. In addition, although significant correlations between the BACMON data and other parameters was not observed for the incoming surface water at Uniper, all parameters (except ATP) identified a sudden decrease in the microbial water quality at the same moment. The BACTcontrol data was not significantly correlated with any of the other parameters for six of the water types as well. Since no BACTcontrol data was obtained from one water type, only one water type (incoming surface water cooling

Table 18. Summary of linear regression analyses between the sensor data (BACMON, BACTcontrol), cell numbers (flow cytometry, microscopy) and ATP of the water samples taken for each pilot location studied. The orginal data are shown in Table 14 - Table 17.

Company	Water type	Parameter 1	Parameter 2	r^2	Company	Water type	Parameter 1	Parameter 2	r^2
Pidpa	Production location	BACMON	ATP	0.69	Vitens	Ruinerwold	BACMON	None	
		BACTcontrol	None				BACTcontrol	None	
		ATP	BACMON	0.69			ATP	None	
		FCM counts	None				FCM counts	None	
		MS counts	None				MS counts	None	
	Water tower	BACMON	None			Steenwijker-wold	BACMON	ATP	0.55
		BACTcontrol	None				BACTcontrol	None	
		ATP	None				ATP	BACMON	0.55
		FCM counts	None				FCM counts	None	
		MS counts	None				MS counts	None	
Uniper	Surface water	BACMON	None[a]		WRK	Lekkanaal	BACMON	None	
		BACTcontrol	FC counts[b], MS counts[b]	0.99 1.0			BACTcontrol	None	
		ATP	None				ATP	FCM counts	0.65
		FC counts	MS counts, BACTcontrol[b]	0.38 0.99			FC counts	ATP	0.65
		MS counts	FC counts, BACTcontrol[b]	0.38 1.0			MS counts	None	
	Cooling tower	BACMON	None			Rapid sand filtrate	BACMON	None	
		BACTcontrol	Not measured				BACTcontrol	None	
		ATP	FC counts, MS counts	0.78 0.51			ATP	None	
		FC counts	ATP, MS counts	0.78 0.45			FCM counts	None	
		MS counts	ATP, FC counts	0.51 0.45			MS counts	None	

[a] Although no significant correlations were observed, the BACMON, BACTcontrol, cell counts with flow cytometry and microscopy all detected a dramatic change in microbial water quality.
[b] These correlations were based on only three observations and, consequently, less reliable.

tower) showed significant and strong correlation between BACTcontrol data and cell numbers determined with flow cytometry and microscopy. These correlations were, however, based on only three observations and, consequently, not very reliable.

In addition to the general absence of significant correlations between the BACMON or BACTcontrol data and the other microbiological parameters, these other microbiological parameters (ATP, cell numbers determined with flow cytometry or microscopy) did not show significant correlations with each other in five of the eight tested water types. These results are in contrast with previous research, which has shown that these microbiological parameters are often significantly correlated (van der Wielen & van der Kooij, 2010; van der Wielen & van der Kooij, 2011). We believe that the absence of correlations in our study is caused by the low change in the microbiological water quality during the measuring periods. Consequently, the correlations are mostly based on the noise of the observed data, rather than on the true changes in concentrations of microbial numbers or activity.

8 Conclusions and recommendations

8.1 Discussion/Conclusion

8.1.1 Performance BACMON and BACTcontrol on different water types

In general, the BACMON sensor performed well with most of the different water types tested. The BACMON sensor only performed poor on groundwater with relatively high concentrations of iron oxides. In addition, the BACMON sensor also performed less well with one of the two surface water types, which was due to high concentrations of non-bacterial particles that caused only a small volume (on average 15% of the normal volume) to be analyzed with the BACMON sensor. Furthermore, a few malfunctions of the BACMON sensor were observed during some of the monitoring periods on the other water types, but those were related to a disconnection between the sensor and computer or, in case of surface water, were due to fouling of the flow cell (a technical error) of the sensor. Furthermore, the detection limit of the sensor we observed with the different water types is relatively low (300 – 500 bacterial cells/ml), which makes the sensor suitable for the water types tested within the project. **Based on these characteristics, it is concluded that the BACMON sensor performs technically well on drinking water, cooling tower water and surface water after rapid sand filtration.**

The BACTcontrol sensor could reliably measure enzymatic activity in drinking water and surface water, but not in anoxic groundwater, oxic groundwater (with low number of bacteria) or cooling tower water. Although enzymatic activities could be measured in these water types, technical errors with the BACTcontrol sensor were often observed: two different BACTcontrol sensors showed different results in the lab validation study, pressure was too high, detection fault, injection volumes of reagents incorrect, air bubbles in system, influence on activity after reagents are changed, unknown malfunctions and higher enzymatic activity values directly after sensor maintenance. Furthermore, the detection limit corresponds to around 1×10^4 bacterial cells/ml, which is around the total number of cells that can be present in some drinking water types in the Netherlands (van der Wielen & van der Kooij, 2010, 2011). **Based on these characteristics, it is concluded that currently the BACTcontrol sensor cannot be reliably used to continuously measure the bacterial activity in water.** It is, therefore, recommended to first solve the technical issues observed with the BACTcontrol sensor as well as to lower the detection limit of the sensor, before the BACTcontrol sensor is used as an inline system to monitor the bacterial water quality.

8.1.2 Relation of the BACMON and BACTcontrol data with other microbiological parameters

The relation between the bacterial numbers obtained with the BACMON sensor, the enzymatic activity obtained with the BACTcontrol sensors, ATP and cell numbers determined with flow cytometry or microscopy was investigated in different ways.

When the relationship was determined with dilution series of bacteria grown in rich media or with dilution series of different water types, than in general the BACMON data correlated well with ATP and the bacterial cell numbers determined with flow cytometry or microscopy. Despite the good correlation between the bacterial numbers measured

with the BACMON sensor, flow cytometry and microscopy, the bacterial numbers obtained with the BACMON were 1 to 2 log units lower than bacterial numbers obtained with the other two methods. This apparent difference is probably caused by an underestimation of the total bacterial cells by the BACMON sensor in combination with a possible overestimation of bacterial numbers by flow cytometry and microscopy. The overestimation can be caused by counting non-bacterial particles that show autofluorescence or that bind to the fluorescent dye. The underestimation by the BACMON sensor can be due to that the sensor does not detect certain bacteria as a bacterial cell, because the cell characteristics of each bacterial species is not known.

When the relationship was determined by comparing the results from different water samples from the same water type, correlations between the BACMON sensor and ATP or bacterial cell numbers determined with flow cytometry or microscopy were generally not observed, but also ATP, cell numbers determined with flow cytometry and cell numbers determined with microscopy were not correlated for most water types. This was a surprising result, because it was expected that bacterial cell numbers determined with the three different methods would show the same patterns. Apparently, each method determines a different part of the bacterial cell numbers. In addition, bacterial numbers obtained with the BACMON were again 1 to 2 log units lower than those obtained with flow cytometry or microscopy.

It is concluded from these results that bacterial numbers, quantified with the BACMON sensor, flow cytometry and microscopy, in dilutions of the same water sample correlate well with each other. However, bacterial numbers that are present in different water samples and quantified by the BACMON sensor do not correlate with the bacterial numbers quantified by flow cytometry or microscopy. Each method seems to determine another part of the bacterial numbers present in the water. Furthermore, the BACMON sensor seems to underestimate the total number of bacteria in the different water types tested, whereas flow cytometry and fluorescence microscopy might overestimate these numbers.

The BACTcontrol data from the dilution series of a surface water sample showed good correlations with the other biomass or cell number data, but such correlations were not observed for the dilution series of other water types. In addition, the BACTcontrol data obtained from different water samples did in general not show any correlations with the other biomass or cell number data. It was expected that the BACTcontrol data and the ATP data would be related, since both parameters determine the concentration of active biomass. However, it still can be that the enzymatic phosphatase activity determined with the BACTcontrol sensor is fundamentally different from the ATP pool that bacteria keep in their cell.

Based on these results, we conclude that in general the enzymatic activity determined with the BACTcontrol in water does not correlate with other biomass or cell number parameters. It remains uncertain what the cause for the lack of this correlation was.

8.1.3 Event detection with the BACMON and BACTcontrol sensor

Besides the validation study of the sensors and the correlation study with other parameters, both sensors were also used in full-scale installations to determine whether changes in water quality can be observed with the BACMON or BACTcontrol sensor.

The data of the BACMON sensor on different drinking water types showed that in general aberrant BACMON data (normally reflected as peaks in bacterial or non-bacterial numbers) relates to operational parameters, such as change in decanter used or back-flushing of sand filters. Furthermore, a forced peak event at one of these locations also resulted in higher bacterial numbers detected by the BACMON sensor. The BACMON sensor was not able to detect major events on surface water and cooling tower water. Probably no major events happened during the monitoring period on these water types, because the physical/chemical parameters that were determined as well, did also not show events or aberrant water quality during this monitoring period. Finally, the BACMON data from surface water and surface water treated with rapid sand filtration showed regular repetitive day/night patterns, which was also observed with the turbidity sensor. This indicates that, like the turbidity sensor, the BACMON sensor was capable in detecting water quality changes that occurred during 24 hours. Still, there were also peaks observed in bacterial or non-bacterial numbers with the BACMON sensor on these water types that did not coincidence with operational or other parameters. It remains uncertain whether those peaks indicate a 'true' event in bacterial water quality or whether these events were false-positives. **Overall, we conclude from these results that a change in bacterial water quality detected with the BACMON sensor is often related to operational parameters or a change in other water quality parameters. This means that, in general, the BACMON sensor is a reliable sensor to detect events that influence bacterial water quality.**

The data from the BACTcontrol sensor on the drinking water types showed no or poor relations with changes in operational parameters (e.g. change in decanters, backwash flushing of sand filters). In addition, a forced peak event in one of these drinking water systems was not detected with the BACTcontrol sensor. It remains uncertain why the BACTcontrol sensor was unable to detect the events related to operational parameters or the forced peak event. It could be that the detection limit of the sensor is still too high and that changes in the bacterial water quality are lower than the background noise. Furthermore, the BACTcontrol showed clear ad random peaks of enzymatic activity with both surface water types, whereas all other parameters (physical/chemical as well as BACMON data) remained stable during the monitoring period or demonstrated a repetitive pattern. This seems to suggest that the peaks observed with the BACTcontrol are false positive events, rather than that de bacterial water quality truly changed. At one of these water types, it was observed that these peaks occurred mainly after maintenance of the sensor, indicating that technical issues of the sensor were responsible for the observed peaks. Still, at certain moments the BACTcontrol sensor seems to show similar results as the BACMON sensor, suggesting that an improved and optimized BACTcontrol sensor might be capable of detecting events in surface water quality. **Based on the BACTcontrol results from different full scale locations, we conclude that for now the BACTcontrol sensor is not suitable to reliably detect changes in water quality that relate to operational parameters or other water quality parameters.**

8.1.4 Experiences users with the BACMON and BACTcontrol sensor

Three of the four end users were positive about the BACMON sensor and especially mentioned that the system is ease of use, detects peaks that, for a large part, can be related to operational processes. One user mentioned that at one location too much variation was observed between a few consecutive measurements and would like to see a higher signal to noise ratio. The users were less positive about the BACTcontrol sensor, mainly because of the technical issues that were observed with this sensor at the full-scale locations. **It is concluded from these experiences that most of the**

users preferred the BACMON sensor over the BACTcontrol sensor due to the technical errors that regularly occurred with the BACTcontrol sensor.

A point raised by the users is that it remains difficult to judge when a sensor provides a peak. Due to background noise, a peak in bacterial numbers might not always been detected (false-negative) or a detected can still be background noise (false-positive). Defining threshold or guideline values for peak concentrations could be helpful in this matter and these can be based on statistical methods (for example the average ± 3 x standard deviation) or on linking true events to peak concentrations. Another point raised is that it also remains unclear to the users which sensor is the best indicator for microbial water quality, since there is limited correlation between the different microbial parameters measured within this TKI-project. This might also indicate that a suite of sensors is necessary to reliable measure the microbial water quality online. **It is, therefore, concluded that threshold or guidelines values for peak detection will be very helpful for the application of these sensors and that information about the best sensor(s) for monitoring the water quality is warranted.**

8.2 Recommendations BACMON and BACTcontrol sensor

8.2.1 BACMON

During this TKI project, Grundfos has decided to stop with the BACMON sensor. Since they also have not found another company that wanted to take over the BACMON sensor, the BACMON sensor is currently no longer available. Consequently, no further recommendations regarding the BACMON sensor will be made in this report.

8.2.2 BACTcontrol

The research within this TKI project showed that the BACTcontrol sensor regularly showed technical errors and malfunctions, making the current version of the BACTcontrol unsuitable for reliable bacterial water quality monitoring. It is, therefore, recommended to improve and optimize the BACTcontrol sensor in order to significantly reduce the technical errors, malfunction and reduce the detection limit of the sensor. Subsequently, such an improved sensor should then be tested again to identify whether it can reliable detect events that disturb the bacterial water quality.

8.2.3 Overall

The lack of correlation between the different microbial parameters in water determined within this TKI-project, raises the question which sensor(s) are best suitable to monitor the microbial water quality online. Another aspect is that attention should also be given to when (at which value) a peak is a true peak and should result in action to prevent water quality issues. It is, therefore, recommended to test possible microbial water quality sensors not only for their performance on different water types or relation with other general microbial parameters, but also for their capability to warn for microbial water quality issues that should be prevented and to identify at which value exceedance such water quality issues occur.

9 References

Albrechtsen, H.-J., Arnedo, M.J., Appels, J., Baquero, D., Lindhardt, B., Lee, C.O., Wagner, F.B. "On-line monitoring of microbial drinking water quality – on site tests" Nordic Drinking Water Conference, Oslo, June 2018

Allen, M. J., Edberg S. C., Reasoner D. J. Heterotrophic plate count bacteria—what is their significance in drinking water? *Int. J. Food Microbiol.* **92**, 265– 274 (2004).

Appels, J., Baquero, D., Galofré, B., Ganzer, M., van den Dries, J., Juárez, R., Puigdomènech, C., van Lieverloo, J.H.M. "Safety and quality control in drinking water systems by online monitoring of enzymatic activity of faecal indicators and total bacteria" in Skovhus, T. and Højris, B. (eds) "Microbiological Sensors for the Drinking Water Industry", IWA Publishing, London 2018

Banna, M.H., Imran, S., Francisque, A., Najjaran, H., Sadiq, R., Rodriguez, M., Hoorfar, M. 2014 Online Drinking Water Quality Monitoring: Review on Available and Emerging Technologies. *Crit. Rev. Environ. Sci. Technol.* **44**, 1370–1421.

Bertelli, C., Courtois, S., Rosikiewicz, M., Piriou, P., Aeby1, S., Robert, S., Loret, J.-F., and Greub, G. "Reduced Chlorine in Drinking Water Distribution Systems Impacts Bacterial Biodiversity in Biofilms" Front. Microbiol. **9** https://doi.org/10.3389/fmicb.2018.02520 (2018)

Besmer, M.D., Hammes, F. 2016 Short-term microbial dynamics in a drinking water plant treating ground water with occasional high microbial loads. *Wat. Res.* **107**, 11–18.

Byrd J. J., Xu H.-S., Colwell, R. R. Viable but nonculturable bacteria in drinking water. *Appl. Environ. Microbiol.* **57** (3), 875-878 (1991).

Chowdhury, S. Heterotrophic bacteria in drinking water distribution system: A review. *Environ. Monit. Assess.* **184** (10), 6087-6137 (2012).

Fernley, HN and Walker PG. Studies on alkaline phosphatase. 1967. Biochemistry Journal, vol 104, pp 1011-1018.

Friedberg, I and Avigad, G. Some properties of alkaline phosphatase of Pseudomonas fluorescens. 1967. European Journal of Biochemistry, vol 1, pp 193-198.

Højris, B., Christensen, S.C.B., Albrechtsen, H.-J., Smith, C., Dahlqvist, M. 2016 A novel, optical, on-line bacteria sensor for monitoring drinking water quality. *Sci. Rep.* | 6:23935 | DOI: 10.1038/srep23935.

Højris, B.H., Kornholt, S.N., Christensen S. C. B., Albrechtsen, H.-J. and Olesen, L.S. "Detection of drinking water contamination by an optical real-time bacteria sensor" H2Open Journal **1**(2) doi: 10.2166/h2oj.2018.014 (2018)

Lee, A., *et al.* Online monitoring of drinking water quality in a distribution network: a selection procedure for suitable water quality parameters and sensor devices. *Int. J. Syst. Assur. Eng. Manag.* **3** (4), 323-337 (2012).

Li L., Mendis N., Trigui H., Oliver J.D., Faucher S.P. (2014) The importance of the viable but non-culturable state in human bacterial pathogens, *Front. Microbiol.* **5**, 258, https://www.frontiersin.org/article/10.3389/fmicb.2014.00258 (accessed 10 April 2018).

Lopez-Roldan, R., Tusell, P., Courtois, S., Cortina, J. L. 2013 On-line bacteriological detection in water. *Trends Anal. Chem.* **44**, 46-57.

Olesen, L.S., Højris, B.H., Folia, N.B. "Counting totally matters – using GRUNDFOS BACMON for network monitoring" in Skovhus, T. and Højris, B. (eds) "Microbiological Sensors for the Drinking Water Industry", IWA Publishing, London 2018

Pinto, A. J., Schroeder, J., Lunn, M., Sloan, W., Raskin, L. Spatial-Temporal Survey and Occupancy-Abundance Modeling to Predict Bacterial Community Dynamics in the Drinking Water Microbiome. *mBio* **5**(3) e01135-14 (2014).

Prest, E.I., Hammes, F., Kötzsch, S., van Loosdrecht, M.C.M., Vrouwenvelder, J.S. 2013 Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Wat. Res.* **47**(19), 7131–7142.

Raich, J. 2013 Review of sensors to monitor water quality, ERNCIP thematic area, Chemical & Biological Risks in the Water Sector, Deliverable D1 - Task 1, Report EUR 26325 EN. https://erncip-project.jrc.ec.europa.eu/documents/review-sensors-monitor-water-quality (accessed April 23, 2018).

Skovhus, T. and Højris, B. (eds) "Microbiological Sensors for the Drinking Water Industry", IWA Publishing, London 2018

Storey M. V., van der Gaag, B., Burns, B. P. Advances in on-line drinking water quality monitoring and early warning systems. *Water Res.* **45**, 741-747 (2011).

van der Kooij, D. Assimilable organic carbon as an indicator of bacterial regrowth. 1992. JAWWA, vol 84 (2), pp 57-65.

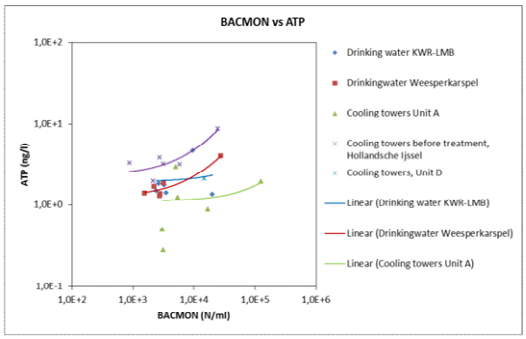
van der Wielen, P. W.; van der Kooij, D. 2010. Effect of water composition, distance and season on the adenosine triphosphate concentration in unchlorinated drinking water in the Netherlands. *Wat. Res.* **44** (17), 4860-4867.

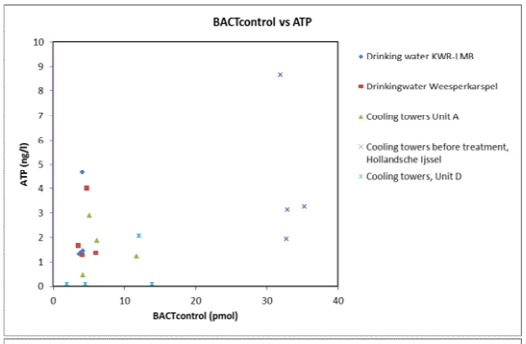
van der Wielen, P. W.; van der Kooij, D. 2011. Opportunistische ziekteverwekkende micro-organismen in drinkwater. BTO 2011.035, KWR Watercycle Research Institute, Nieuwegein, the Netherlands.

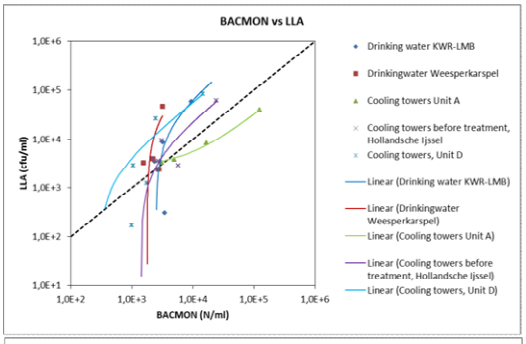
Vang, Ó.V., Corfitzen, C.B., Smith, C., Albrechtsen, H.-J. 2014 Evaluation of ATP measurements to detect microbial ingress by wastewater and surface water in drinking water" *Wat. Res.* **64**(1), 309-320.

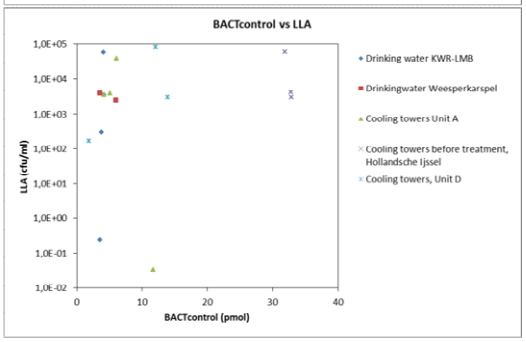
Wang, Z., Han, T., Jeon, T.-J., Park, S., Kim, S. M. Rapid detection and quantification of bacteria using an integrated micro/nanofluidic device. *Sens. Actuators, B* **187**, 683-688 (2013).

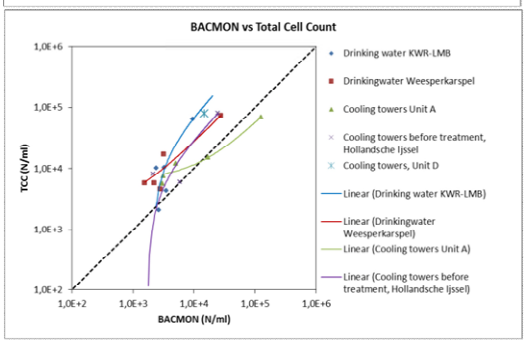
Appendix I Dilution series of bacteria cultured in mineral medium

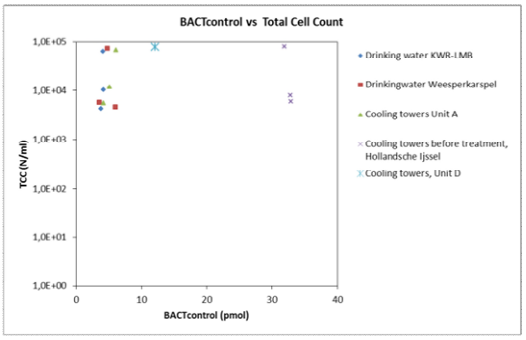


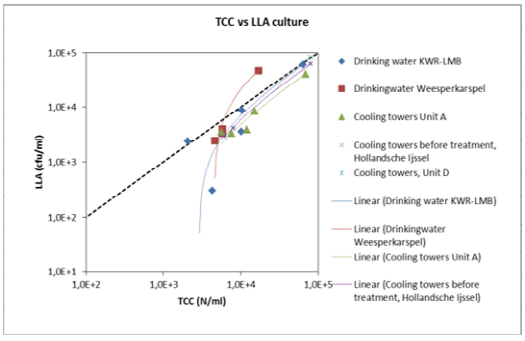




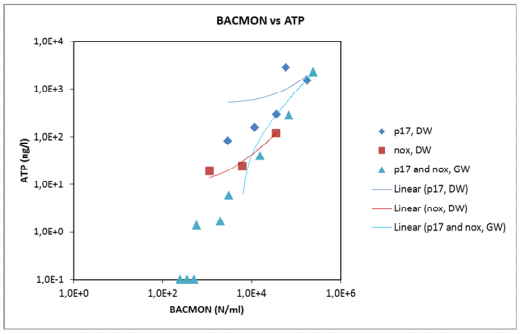


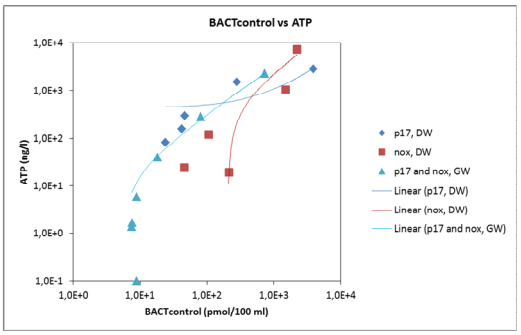


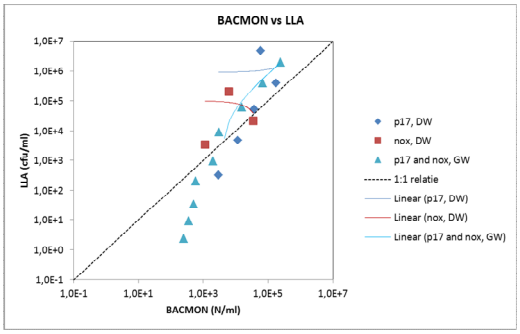


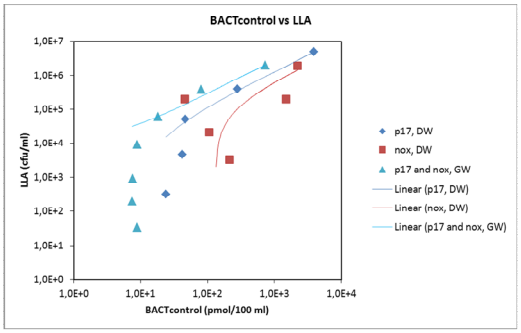


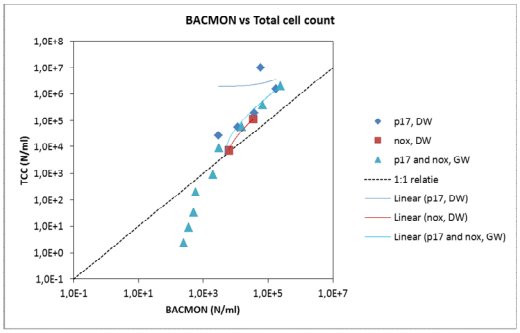
Appendix II Dilution series of bacteria cultured in rich medium

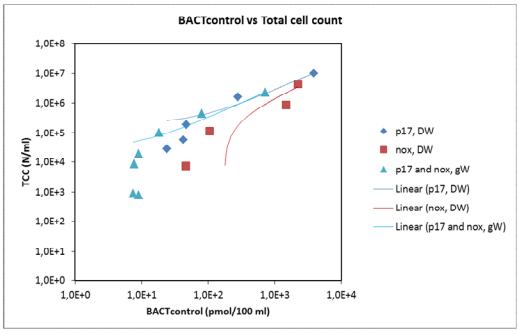


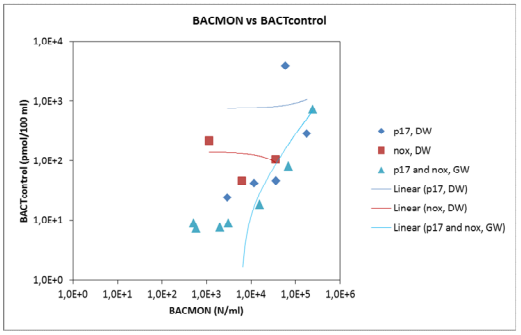


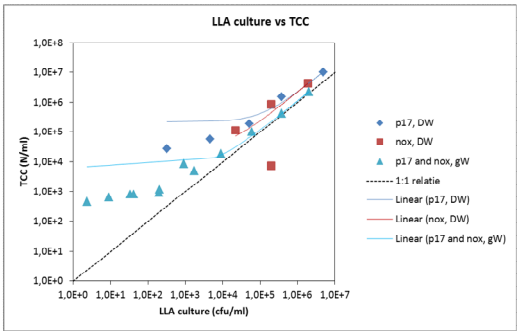




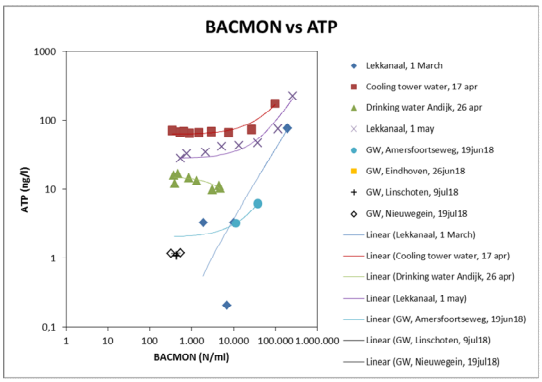


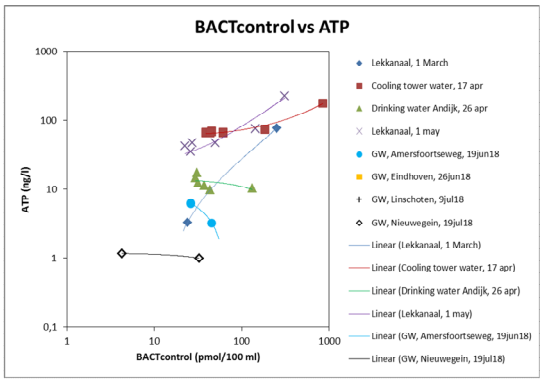


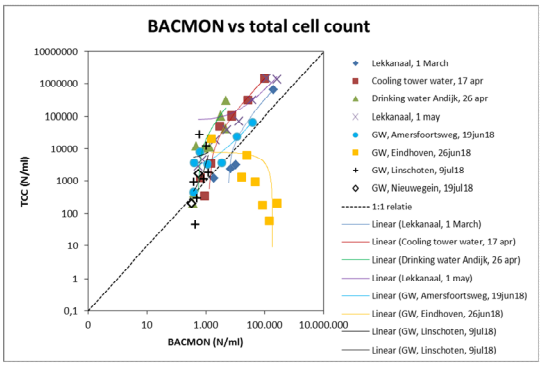


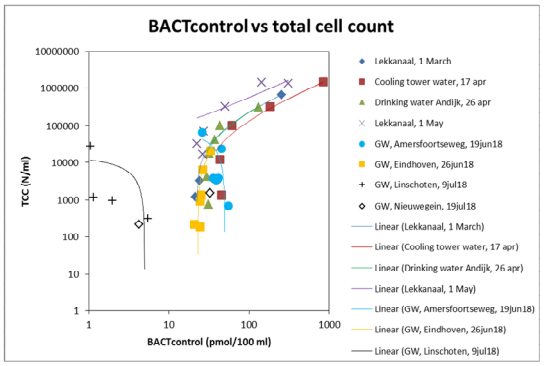


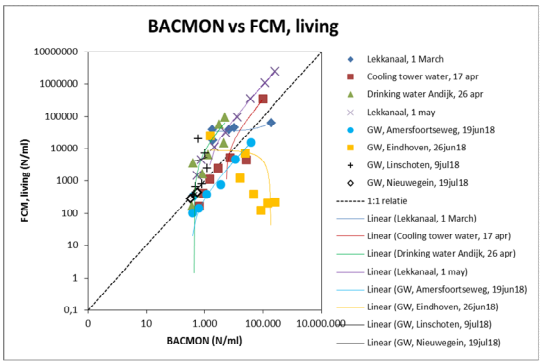
Appendix III Dilution series of 'natural bacteria'

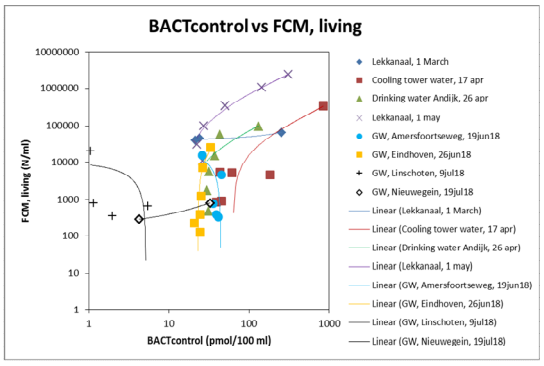


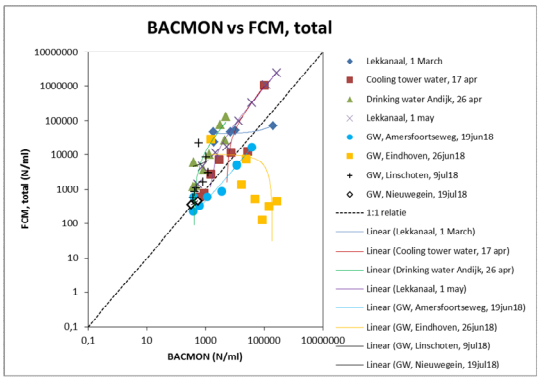











BACMON vs FCM, total
FCM, total (N/ml)
BACMON (N/ml)
Lekkanaal, 1 March
Cooling tower water, 17 apr
Drinking water Andijk, 26 apr
Lekkanaal, 1 may
GW, Amersfoortseweg, 19jun18
GW, Eindhoven, 26jun18
GW, Linschoten, 9jul18
GW, Nieuwegein, 19jul18
1:1 relatie
Linear (Lekkanaal, 1 March)
Linear (Cooling tower water, 17 apr)
Linear (Drinking water Andijk, 26 apr)
Linear (Lekkanaal, 1 may)
Linear (GW, Amersfoortseweg, 19jun18)
Linear (GW, Eindhoven, 26jun18)
Linear (GW, Linschoten, 9jul18)
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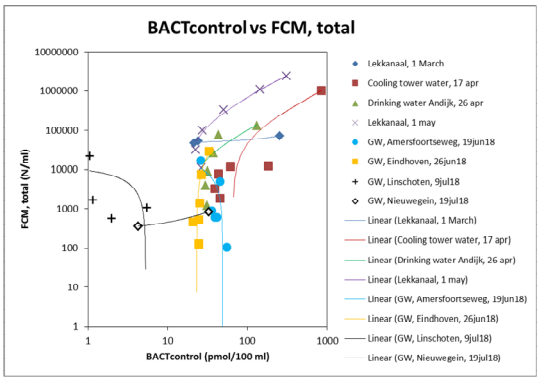
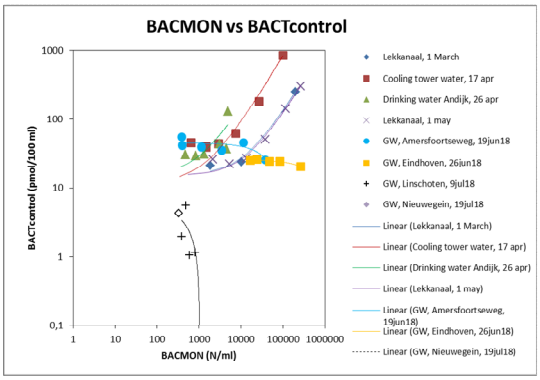
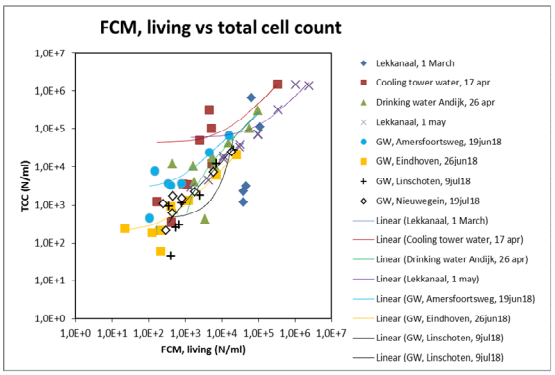
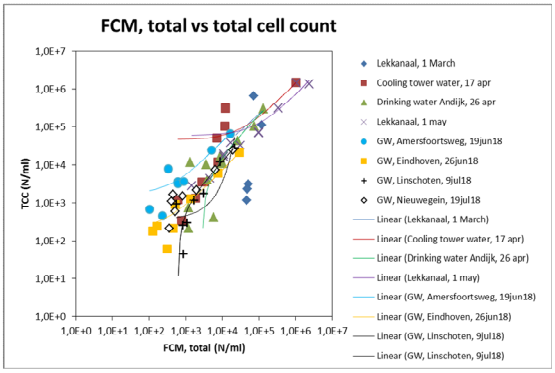
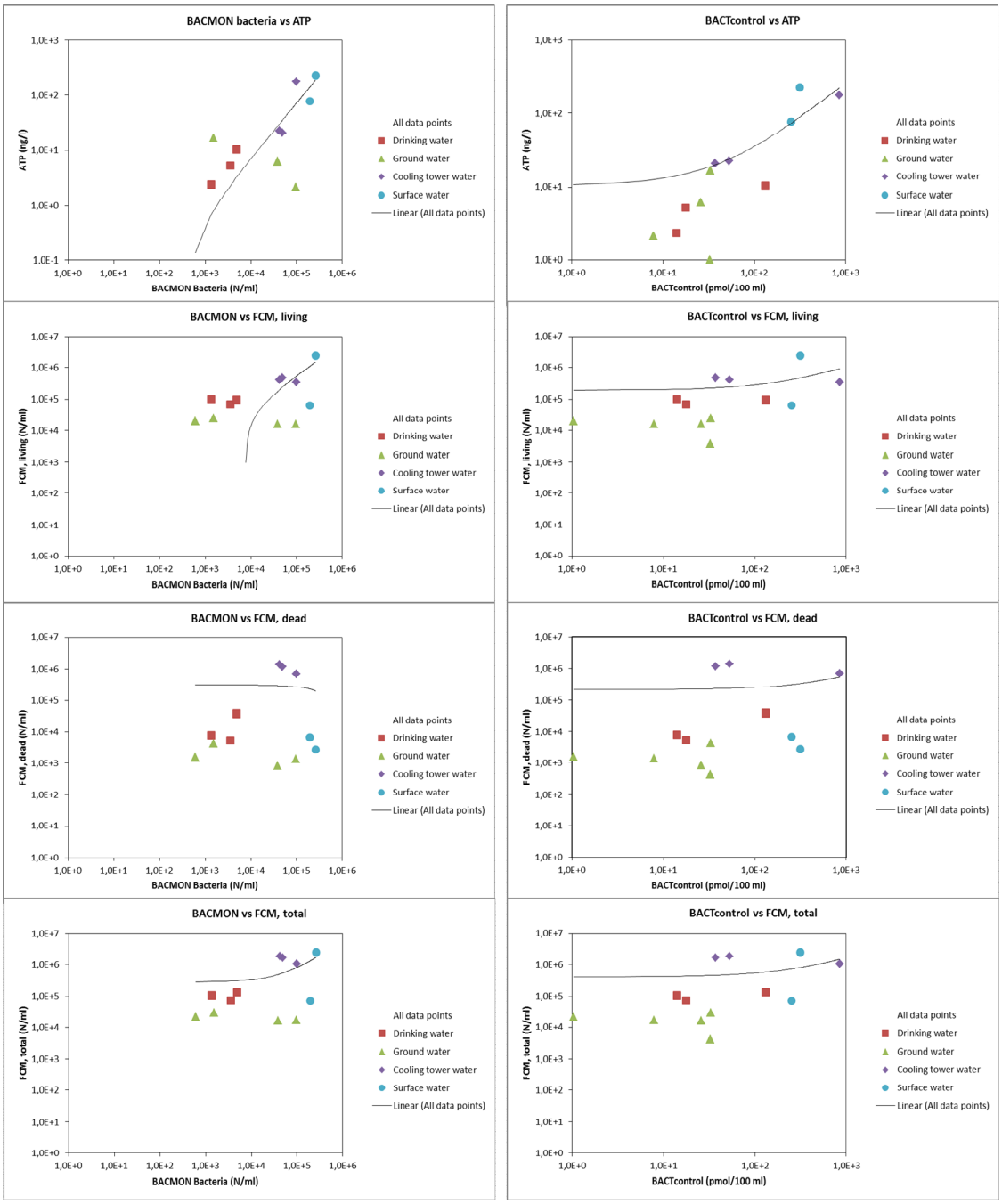

BACTcontrol vs FCM, total
FCM, total (N/ml)
BACTcontrol (pmol/100 ml)
Lekkanaal, 1 March
Cooling tower water, 17 apr
Drinking water Andijk, 26 apr
Lekkanaal, 1 may
GW, Amersfoortseweg, 19jun18
GW, Eindhoven, 26jun18
GW, Linschoten, 9jul18
GW, Nieuwegein, 19jul18
Linear (Lekkanaal, 1 March)
Linear (Cooling tower water, 17 apr)
Linear (Drinking water Andijk, 26 apr)
Linear (Lekkanaal, 1 may)
Linear (GW, Amersfoortseweg, 19jun18)
Linear (GW, Eindhoven, 26jun18)
Linear (GW, Linschoten, 9jul18)
Linear (GW, Nieuwegein, 19jul18)

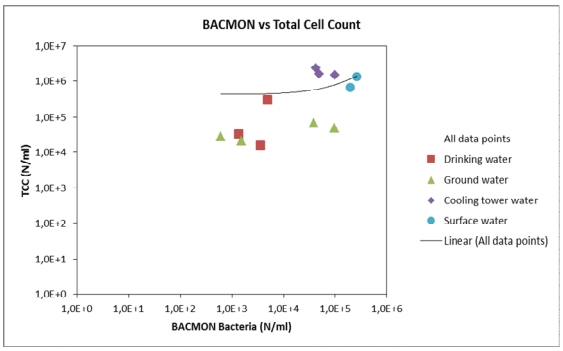

BACMON vs BACTcontrol
BACTcontrol (pmol/100 ml)
BACMON (N/ml)
Lekkanaal, 1 March
Cooling tower water, 17 apr
Drinking water Andijk, 26 apr
Lekkanaal, 1 may
GW, Amersfoortseweg, 19jun18
GW, Eindhoven, 26jun18
GW, Linschoten, 9jul18
GW, Nieuwegein, 19jul18
Linear (Lekkanaal, 1 March)
Linear (Cooling tower water, 17 apr)
Linear (Drinking water Andijk, 26 apr)
Linear (Lekkanaal, 1 may)
Linear (GW, Amersfoortseweg, 19jun18)
Linear (GW, Eindhoven, 26jun18)
Linear (GW, Nieuwegein, 19jul18)

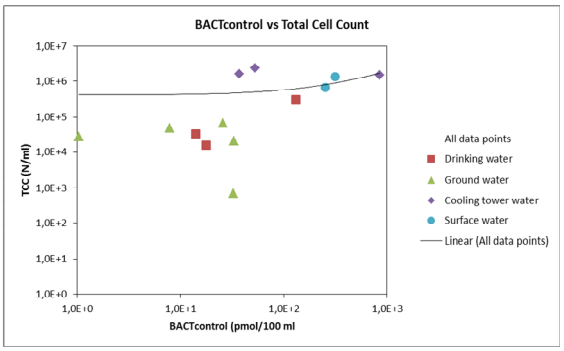

FCM, living vs total cell count
TCC (N/ml)
FCM, living (N/ml)
Lekkanaal, 1 March
Cooling tower water, 17 apr
Drinking water Andijk, 26 apr
Lekkanaal, 1 may
GW, Amersfoortseweg, 19jun18
GW, Eindhoven, 26jun18
GW, Linschoten, 9jul18
GW, Nieuwegein, 19jul18
Linear (Lekkanaal, 1 March)
Linear (Cooling tower water, 17 apr)
Linear (Drinking water Andijk, 26 apr)
Linear (Lekkanaal, 1 may)
Linear (GW, Amersfoortseweg, 19jun18)
Linear (GW, Eindhoven, 26jun18)
Linear (GW, Linschoten, 9jul18)
Linear (GW, Linschoten, 9jul18)

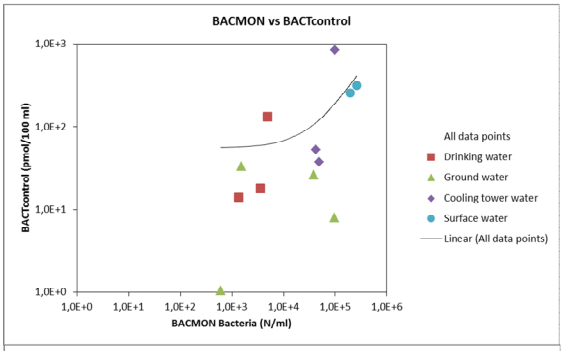

FCM, total vs total cell count
TCC (N/ml)
FCM, total (N/ml)
Lekkanaal, 1 March
Cooling tower water, 17 apr
Drinking water Andijk, 26 apr
Lekkanaal, 1 may
GW, Amersfoortseweg, 19jun18
GW, Eindhoven, 26jun18
GW, Linschoten, 9jul18
GW, Nieuwegein, 19jul18
Linear (Lekkanaal, 1 March)
Linear (Cooling tower water, 17 apr)
Linear (Drinking water Andijk, 26 apr)
Linear (Lekkanaal, 1 may)
Linear (GW, Amersfoortseweg, 19jun18)
Linear (GW, Eindhoven, 26jun18)
Linear (GW, Linschoten, 9jul18)
Linear (GW, Linschoten, 9jul18)

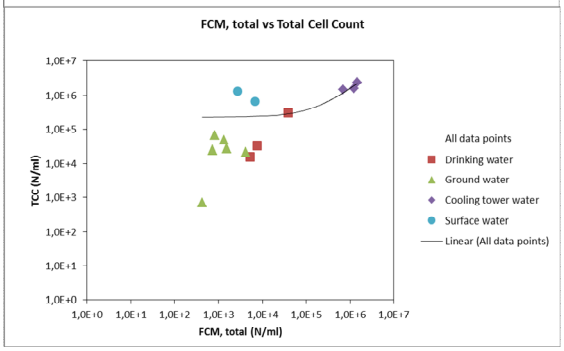
Appendix IV Untreated water samples

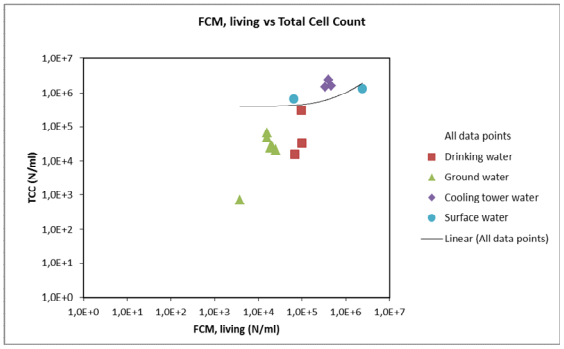



BACMON vs Total Cell Count
TCC (N/ml)
BACMON Bacteria (N/ml)
All data points
Drinking water
Ground water
Cooling tower water
Surface water
Linear (All data points)


BACTcontrol vs Total Cell Count
TCC (N/ml)
BACTcontrol (pmol/100 ml
All data points
Drinking water
Ground water
Cooling tower water
Surface water
Linear (All data points)


BACMON vs BACTcontrol
BACTcontrol (pmol/100 ml)
BACMON Bacteria (N/ml)
All data points
Drinking water
Ground water
Cooling tower water
Surface water
Linear (All data points)


FCM, total vs Total Cell Count
TCC (N/ml)
FCM, total (N/ml)
All data points
Drinking water
Ground water
Cooling tower water
Surface water
Linear (All data points)


FCM, living vs Total Cell Count
TCC (N/ml)
FCM, living (N/ml)
All data points
Drinking water
Ground water
Cooling tower water
Surface water
Linear (All data points)