KWR 2025.079 | August 2025

Rapid inline monitoring of microbiological water quality

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Rapid inline monitoring of microbiological water quality

KWR 2025.079 | August 2025

Project number
403490

Project manager
Astrid Reus

Collaborating partners
Evides, Oasen, Vitens, Dow Benelux, BASF Antwerpen, H2O Biofouling Solutions, Applied Process Technology, MicroLAN, Milispec, Mastiline

Authors
Nikki van Bel, Nienke Koeman-Stein, Frank Oesterholt, Marcelle van der Waals

Quality Assurance
Paul van der Wielen

Sent to
Collaborating partners

This activity was co-financed with PPP funding from the Top Consortia for Knowledge and Innovation (TKIs) of the Dutch Ministry of Economic Affairs and Climate and the results are made public.

Keywords

Sensor, microbiological water quality, drinking water, cooling water

Year of publishing
2025

More information
Nikki van Bel, PhD
T +31 (0)30 60 69 516
E Nikki.van.Bel@kwrwater.nl

PO Box 1072
3430 BB Nieuwegein
The Netherlands

T +31 (0)30 60 69 511
E info@kwrwater.nl
I www.kwrwater.nl

Summary

Introduction

Water is essential for a wide range of applications, from drinking and bathing to industrial processes and agriculture. Despite the varying quality requirements for each use, maintaining high water quality is critical for public health, environmental protection, and operational efficiency. Two key indicators of water quality are the microbial biomass and microbial growth potential of the water. An increase in microbial biomass often signals a change in the water system, while a high growth potential can lead to the proliferation of pathogens or cause aesthetic and technical issues.

Traditional methods for assessing microbial biomass and growth potential are labor-intensive, slow, and not suitable for continuous monitoring. In response, several inline sensors have been developed to provide real-time data. These include the BactoSense, which uses flow cytometry to count bacteria; the BugCount Online and Guardian, which measure ATP as a proxy for active biomass; and the BACTcontrol, which detects microbial enzymatic activity. Additionally, the Continuous Biofilm Monitor (CBM) assesses the potential for biofilm formation by measuring the biomass (ATP) accumulation rate on glass beads.

These sensors offer the advantage of high-frequency, inline monitoring, enabling faster responses to change(s) in water quality and reducing the risk of product quality issues or water waste. However, most of these sensors were originally designed for clean drinking water and may not perform as well in water with higher biomass, nutrient concentration or particulate content, such as industrial cooling or process water. As some of these sensor technologies are relatively new or not yet tested in more complex water matrices, further research is needed to determine their reliability and predictive value in diverse water systems.

Research goal

This report describes the results of the TKI Water Technology project *Rapid Inline Monitoring of Microbiological Water Quality*. The project aimed to evaluate the performance and applicability of four microbiological sensors—BACTcontrol, BactoSense, BugCount Guardian, and the CBM—for real-time, inline monitoring of microbial water quality in various water types, including drinking water, surface water, and industrial cooling water.

The overarching goal was to determine whether these sensors could reliably detect microbial biomass or biofilm formation potential and changes during operation, compare their performance to traditional laboratory methods, and assess their practical use in operational settings. The project also sought to establish signal values for each sensor to support early warning systems and operational decision-making.

Validation Studies

Each sensor was tested under laboratory settings and compared to conventional microbiological methods applied in the laboratory, such as flow cytometry, fluorescence microscopy, and ATP analysis. The validation focused on their detection limits, correlation with laboratory results, and suitability for different water matrices.

The BACTcontrol measures enzymatic activity (alkaline phosphatase) as an indicator of active microbial biomass. It showed reliable results in drinking water and surface water with moderate to high biomass but was less suitable for drinking water samples with low biomass, in which enzymatic activity was often close or below the detection limit. Its performance in cooling water was more variable, but especially the presence of a disinfectant (ozone or chlorine) resulted in different degrees of living, dying, dead and decaying cells and thereby influenced the correlation. Also particulate matter interfered with the measurement.

The BactoSense uses inline flow cytometry to count bacterial cells and distinguishes between intact and damaged cells. It performed well across all water types, particularly in drinking and surface water, where it showed an excellent correlation with laboratory flow cytometry. The correlation with laboratory methods for cooling water was limited due to the presence of a disinfectant (ozone or chlorine). In high-biomass environments like surface and cooling water, it occasionally exceeded its upper detection limit, suggesting the need for inline dilution.

The BugCount Guardian measures ATP concentrations as an indication of active biomass. It was effective in surface and cooling water but less suitable for drinking water due to its high detection limit of 100 ng ATP/l. Similar to the BACTcontrol and BactoSense, the presence of a disinfectant in cooling water led to low correlations with data from cooling tower waters using the laboratory methods.

The CBM assesses the biofilm formation potential biweekly (on drinking water) or weekly (on cooling or surface water) by measuring ATP in biofilms grown on glass beads or coupons. Two methods to measure biofilm ATP were validated. Both the KWR laboratory ATP method and the Milispec/LuminUltra field kit were statistically equivalent under ISO 16140-2 standards. However, the field kit consistently yielded higher ATP values, due to which it is not possible to directly compare the results from the Milispec/LuminUltra-method to historical data obtained with the KWR laboratory method. For application on more turbid waters, such as surface water, pre-filtration was required to prevent clogging of the CBM system.

Pilot Locations

At Evides, sensors were installed on drinking water before and after ultrafiltration membrane, and after the clear water reservoir. The results from the BACTcontrol, BactoSense and Milispec-CBM show respectively reduced bacterial enzyme activity, bacterial cell numbers and biomass accumulation rate in the drinking water after the clear water reservoir compared to drinking water before the ultrafiltration. This matches the historical data of Evides. Whereas the BactoSense result indicated regrowth in the clear water reservoir, which is confirmed by measurements done by Evides, the BACTcontrol did not indicate regrowth as a clear decrease in enzymatic activity was observed.

At Oasen, sensors were tested at two drinking water production locations with different treatment technologies: conventional treatment (De Hooge Boom) and full stream reverse osmosis (Nieuw Lekkerland). Differences in microbial quality were observed between the sites: higher cell numbers and microbial activity at De Hooge Boom compared to Nieuw Lekkerland, which matched historical knowledge on biological stability at these two production locations. The BactoSense and BACTcontrol showed high variations in cell counts and enzymatic activity in the produced drinking water of Nieuw-Lekkerland, which coincided with operational changes or increases in methane or ammonium in the water in between treatment steps. Both methane and ammonium are substrates for microbial growth, explaining increased cell counts and enzymatic activity measured with the sensors. The CBM showed high biofilm formation potential at both locations.

At Vitens, sensors were deployed at two drinking water production locations: Spannenburg and Noardburgum. Microbial activity and cell counts were higher in Spannenburg than in Noardburgum. The BactoSense detected daily fluctuations in cell numbers that seemed to correlate with differences in water demand; these fluctuations were not detected with the BACTcontrol. The CBM demonstrated comparable biofouling potential for both drinking waters.

In a setup of H_2O Biofouling Solutions, which simulated a cooling tower using surface water, it was tested whether the sensors could detect dosing of a cleaning agent (CMIT) or biofilm cleaning agent (Aquafinesse) to the system. The online sensors were able to measure the effect of the used water conditioning products and to measure the effect of water conditioning products on surface water. However, more experiments are required to be able to draw definite conclusions. The CBM showed difficulties with the turbid water due to clogging.

At Dow Terneuzen, the sensors monitored cooling water. The BACTcontrol and BugCount Guardian showed (very) low microbial activity. In contrast, the BactoSense showed high cell numbers. The BactoSense detected changes in cell numbers which coincided with condensate addition, but these were not detected with the other sensors. The CBM indicated low biofilm formation potential, likely due to the presence of chlorine in the cooling water.

At BASF Antwerp, the sensors were tested on brackish cooling water. The BactoSense recorded high cell counts, while the enzymatic activity was often below the lower detection limit of the BACTcontrol and the BugCount Guardian showed fluctuating ATP levels. The CBM faced clogging issues and did not produce stable results. The different sensors did, in general, not show a change in the microbiological water quality once the chlorine dosing frequency and concentration was changed, probably because the free chlorine remained stable during the whole monitoring period. The short hydraulic retention time in the cooling water system limited the possibility to control the microbial water quality in the system based on sensor results.

Setting signal values

An important reason to implement sensors for monitoring microbiological water quality is that the end users can act when a measurement value of the sensor exceeds a signal value. Such a signal value indicates when a measurement result exceeds the normal variation. Several different methods for setting signal values are available, each with their advantages and disadvantages. The 'best' method depends on the goal for which the sensors are to be used. A step-by-step plan on how to set signal values for individual drinking water applications was set up in collaboration with the drinking water utilities and technology suppliers. For each pilot location, signal values were calculated using the statistical approach of average ± 3× standard deviation. The large differences of the individual pilot locations show that these values should be established for each individual pilot location, regardless of the water matrix (drinking water, cooling water and surface water).

As the microbial water quality fulfilled the legislative parameters for the three drinking water pilot locations, it seems unlikely that the normal variation in bacterial cell numbers and enzymatic activity results in microbiological water quality issues (e.g. public health, aesthetical or technical complaints) at the consumers tap. The results, thus, suggest that most of the observed variation could be considered as normal variation that did not impact the microbial water quality in such a way that problems occurred. However, the data can be used for better understanding the effect of operational changes on the produced drinking water quality.

The two cooling towers operated stable during the time period of the pilot and showed very little exceedances. Ideally, the signal value would be correlated to operational parameters, for example changes or unwanted situations therein, but this was not possible in this project due to the stable measurements and operation. Alternative ways to determine the signal value may be more valuable or have more relation to the real practical situation, but additional research into this aspect is required.

Overall Conclusions and Recommendations

For all possible applications, either in drinking water, industrial water or other water types, it is important to determine on forehand the goal to be achieved by monitoring the microbial water quality and whether that goal can be achieved by the application of a sensor or by using other microbial water quality monitoring methods (i.e. laboratory-based methods).

When the sensors were compared to traditional laboratory parameters, the correlation was strong for the BactoSense compared to the laboratory flowcytometry and microscopy cell counting for drinking water. The correlations with the other sensors were less strong.

Changes in process conditions that influence the microbial drinking water quality can be detected with the sensors, which would have been missed by regular laboratory-based monitoring, because the measurement frequency of the sensors is much higher.

The handling of the sensors is easy and does not require much time or attention. The result of the measurement is also much faster available compared to the result of the offline traditional measurements in the laboratory. This gives end users, especially drinking water companies, the opportunity to act faster on changes measured by inline sensors.

Upon implementation of the sensors, it is recommended to first establish baseline values by measuring the water quality with the sensor for a relatively long time to gain experience with the variations that can be expected. After this period a means for setting an alarm that is appropriate for the local situation can be determined in combination with operational measures.

The microbial quality of cooling water varies significantly over time. It was possible to determine the effectiveness of disinfection and a change in incoming water quality. It was not possible to determine whether problems in the cooling tower could be noticed using the tested sensors. However, monitoring the quality of the incoming water, or using sensors on more stable process water might be useful applications for these sensors on industrial water. In industry, microbiological measurements are not done frequently. The inline sensor data of industrial water can, therefore, not be compared easily to the traditional laboratory-based methods. A measurement campaign using both inline sensors and laboratory measurements could increase insight into this aspect.

Contents

1 Introduction

1.1 Background

Water is used for many different purposes, for example to drink, shower, swim, cool, wash or grow plants or as process water in industrial processes. Although water quality requirements differ between these different uses of water, good water quality in all these applications is extremely important for the health of humans, animals, plants and for the environment, the production process and operational security. As a result, water quality problems can lead to social and financial consequences. For example, consumer confidence in drinking water may be undermined, leading them to switch to the expensive and less sustainable use of bottled water, harvesting in the greenhouse horticulture sector may fail due to the growth of plant pathogenic microorganisms in the water system, or high costs may be incurred by (industrial) sectors because certain (industrial) processes have to be stopped due to water quality problems. Important water quality parameters in all these applications are the microbial biomass and the growth potential of the water for microorganisms. A change in the microbial biomass is an indication that somewhere in the water system a change has taken place, as a result of which the biomass has increased or decreased. In addition, a high growth potential of the water can lead to the multiplication of (opportunistic) pathogens in the water due to the presence of high nutrient concentrations, the multiplication of (micro)organisms that can lead to aesthetic complaints (e.g. odor and taste complaints, turbidity) or technical complaints (clogging of pipes, drippers, water meters, membranes or corrosion). However, current methods to determine the growth potential of a water sample are cumbersome, time consuming and, like most methods to determine microbial biomass, cannot be used in-line (continuously and directly in the water stream).

However, several sensors that measure the microbiological water quality have come onto the market in recent years, which can be used to determine the number of bacteria (BactoSense), ATP (BugCount Online or BugCount Guardian) or the enzymatic activity of bacteria (BACTcontrol) as a measure for biomass. In addition, a continuous biofilm monitor (CBM) has been developed to determine the growth potential of water, which represents the amount of nutrients in the water that can be used by microorganisms to form biofilm. In principle, these in-line sensors are suitable for monitoring the microbiological water quality with a high frequency and thus quickly detect changes in the microbiological water quality. Companies can then take quicker action upon signs of a deteriorating water quality. This will lead to less problems with a (too) low product quality due to microbiology related quality issues and will lower the amount of wasted water.

These sensors were in most cases developed for drinking water matrices and not for water matrices with higher biomass and particle concentrations. An interesting question is whether and how these sensors can be applied in different water types (e.g. drinking water, industrial cooling water and process water). Some of these microbiological sensors have only recently become available or have not been applied on water matrices with higher biomass concentrations, which makes it unclear to what extent these microbiological sensors are predictive for water quality problems in the system.

1.2 Project setup and goals

In this project the performance and applicability of three sensors (BACTcontrol, BactoSense, BugCount Online or BugCount Guardian) and the CBM were determined for drinking water and industrial water. First, sensor results were compared to known laboratory methods in a validation study. Following this, the sensors were tested in-line at multiple pilot locations using either drinking or industrial water.

The objective of this project was to develop a new service for the quality control of drinking water and industrial water (process and cooling water) using sensors. To this end the following main research questions were set up:

1. How do the microbiological sensors relate to traditional laboratory techniques and to the other sensors in the project?
2. Which sensor is most suitable in which situation?
3. Can, and if yes, how, the sensor results be translated into specific water quality problems?
4. Can the sensors predict and/or determine the effect of control measures (e.g. disinfection or adaptation of operational management) on microbial biomass and/or the problem organisms? Do the sensors perform better in this respect than traditional laboratory or on-site analyses?
5. Can the microbiological sensors predict different water quality problems before the problem occurs (and therefore there is time to avert problems in time) and what action values are associated with this?
6. Does a combination of different sensors provide added value compared to using only the individual sensors?
7. Can we convert successful applications into a new standard protocol to be set up so that it can be used in more locations?
8. Are adjustments to some sensors necessary so that they perform better on location and in-line? And if so, is this possible?

1.3 Project description and reader's guide

Three in-line sensors (BugCount Online/BugCount Guardian, BactoSense, BACTcontrol) and the in-line CBM monitor were tested. An introduction to these sensors and their working principles is given in **chapter 2.**

To get a better insight in the performance characteristics of the sensors and to test whether the sensors can be used with various water matrices, the sensors were tested in a laboratory on drinking water, process water and cooling water **(chapter 3).** Two types of experiments were set up: 1) Dilution series of different water matrices were made and analyzed with the BugCount Online/BugCount Guardian, BactoSense and BACTcontrol and the results were compared to laboratory analyses (ATP, cell count with flow cytometry and cell count with microscopy). 2) The CBM was installed on drinking water and on surface water and the amount of biomass formed was determined with the mobile ATP-kit of LuminUltra and with the laboratory ATP-method of KWR. Both types of experiments provided insight in whether certain sensors needed optimization before they could be applied for the continuous in-line measurements of the various pilot locations **(chapter 4 – 9).** The results and conclusions of each individual pilot are described in their own chapters.

From April to September 2021 the sensors were tested at drinking water utility Evides, at a pilot treatment plant located in the Hoeksche Waard, the Netherlands **(chapter 4).** The sensors were installed at three locations; the incoming drinking water, directly after ultrafiltration (UF) treatment, and after the following clear water reservoir before distribution to the customers. The main research question was whether the sensors can measure differences in the microbial water quality caused by the UF treatment of the drinking water.

In March – May 2022 and October 2024 – January 2025 the sensors were tested at drinking water utility Oasen, at production location De Hooge Boom (2022) and Nieuw-Lekkerland (2024), the Netherlands **(chapter** Error! Reference source not found.**)**. Sensors were installed after the treated water reservoir to test whether the sensors can measure differences in the microbial water quality of the produced drinking water of production locations De Hooge Boom (conventional treatment plant) and Nieuw-Lekkerland (full-stream reverse osmosis (RO) treatment). By doing so, the sensors were also used to better understand the operation of an RO system and gain more experience with this. In addition, it was tested whether local alarm values could be set for the local operator of Oasen to act upon.

In June – Augustus 2022 the sensors were tested at drinking water utility Vitens, at drinking water production location Spannenburg and Noardburgum, the Netherlands (chapter 6). Sensors were installed after the treated water reservoirs at these production locations. The main research question was whether the sensors can measure differences in the microbial water quality of the produced drinking water of the two production locations and see whether that can be related to differences in treatment steps of the production locations and/or the quality and composition of the untreated ground water from which drinking water is produced. In addition, it was tested whether local alarm values could be set for the local operator of Vitens to act upon.

In June-August 2023 the sensors were tested on simulated cooling water at KWR, in Nieuwegein (chapter Error! Reference source not found.). A mixture of drinking water and surface water was recirculated over the set-up. The main research question was if the sensors would detect the effect of disinfectant dosage, and of a product that was supposed to loosen the biofilm from the tubes and increase the effectiveness of the disinfectant.

In February – May 2024 the sensors were tested on cooling water at industrial utility Dow, the Netherlands (chapter 8). The sensors were installed at the point where the water leaves the open recirculating cooling water system and goes to the process installations to cool these installations. The main research question was whether the sensors were able to work in an industrial environment and with cooling water. The second question was whether variations in chlorine dosage would lead to a change in microbial activity that could be measured by the sensors.

In July – September 2024 the sensors were tested on cooling water at industrial utility BASF, Belgium (chapter 9). This is a brackish water flow-through system and the main research question was whether the sensors could work in an industrial setting with brackish water. Also, the frequency and amount of dosing disinfectant was varied during the test-period and the question was also if the sensors could detect the effect of this changing disinfectant frequency and amount.

For several pilot locations one of the goals was to set signal values. For each individual pilot one method to set signal values was applied, and the method and results of it are described in their respective chapters. In addition, chapter Error! Reference source not found. includes a guidance plan on how to set signal values for application of the sensors for the purpose of drinking water production or distribution in general and how the sensors could be used to for industrial waters.

In chapter 0 the combined conclusions of the validation studies and pilot locations are given.

2 Sensors for microbiological water quality

2.1 Working principle sensors

2.1.1 BactoSense

The BactoSense of bNovate, and distributed in the Netherlands by APT BV, is an online and continuous flow cytometer system (Figure 2-1, left). Every 30 minutes the sensor automatically takes a water sample (Table 2-1). The water sample is stained with two dyes: propidium iodide (stains DNA of bacteria with a permeable membrane) and SYBR green (stains DNA of all bacteria) (Figure 2-2, right). Every individual cell is measured and represented in a dot plot (Figure 2-2, left).

Depending on the dye used for staining the DNA, the software of the BactoSense will determine whether the bacterial cell is probably alive (intact membrane, only SYBR green stain) or dead (not intact cell membrane, both, propidium iodide and SYBR green stains, Figure 2-2 right). The red lines (Figure 2-2, left) are the gates that have to be set manually for each water type thereby defining the different categories (cells with intact or permeable membranes and debris). The gates have to be manually adjusted to each new water type.

It is possible that bacteria with an intact cell membrane are dead or are in a vegetative state and metabolically not (very) active. This is for example the case when a water sample is treated with UV-light. UV-light damages the DNA thereby killing the cell, but it does not affect the cell membrane [1]. As a consequence, the effect of UV-disinfection on the viability of bacteria cannot be monitored with the BactoSense. Chemical disinfection methods (e.g. chlorine, ozone) affect the entire bacterial cell, including the cellular membrane and the effect should, therefore, be measurable with the BactoSense and flow cytometry in general [1].

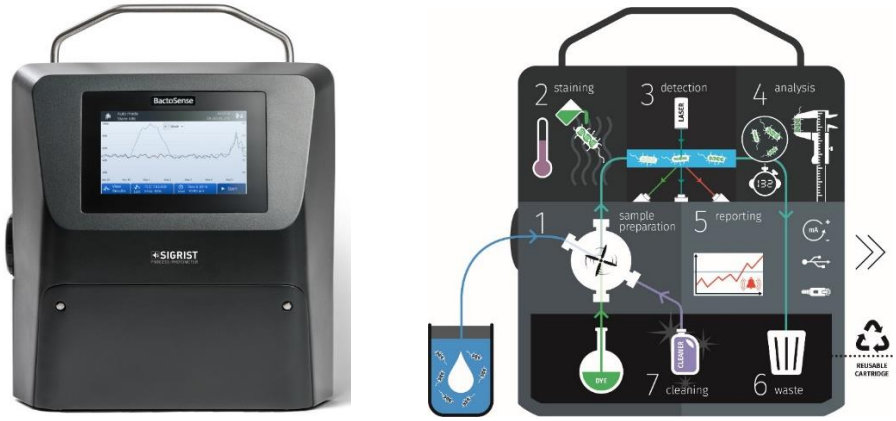


Figure 2-1. BactoSense sensor (left) and a schematic of the internal mechanisms (right).

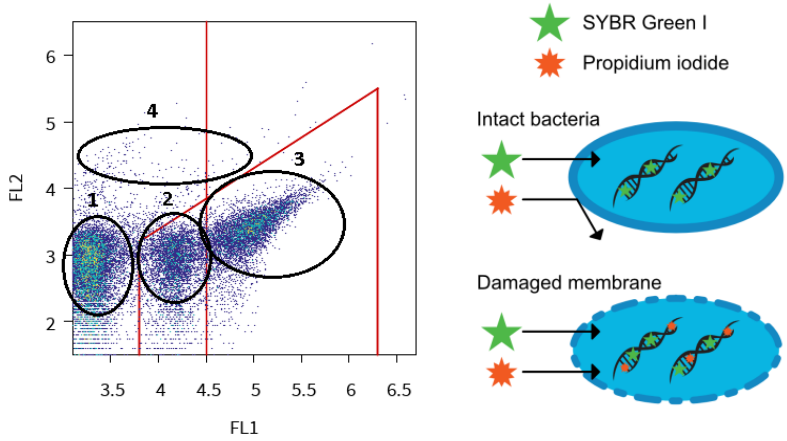


Figure 2-2. Example of a result of the BactoSense (left) and explanation of the principle behind staining cells with SYBR Green and propidium iodide dyes (right). In the dot plot every dot represents one bacterial cell. The place of the dot in the graph depicts whether the bacterial cell is dead (4), probably alive (2, 3) or the cell is disintegrated and forms noise or a background signal (1).

The reagents of the BactoSense are inserted in the machine via a cartridge, the chemical waste is also collected in this cartridge. The cartridge has enough reagents for 900 reactions after which it has to be replaced. The only waste of the BactoSense is the continuous flow-through of the water to be analyzed.

The lower detection limit of the BactoSense is reported by the manufacturer to be 100 cells/ml, whereas the upper detection limit is reported to be around $5x10^6$ cells/ml (Table 2-1). Above and below the detection limit the BactoSense will give a warning that the obtained result is not reliable. The BactoSense was originally developed for measuring drinking water, a relatively clean water type, but was hardly ever tested before on more dirty water types with higher biomass levels (e.g. surface water, cooling water, etc.). Adaptation of the BactoSense to these more dirty water types may, therefore, be necessary.

The BactoSense does not require any calibration during its lifetime. The producer bNovate performs, at the end of the production process, a full validation using beads, sterile water and a reference solution, as well as other thorough testing to make sure the instruments are perfectly aligned with the specifications before they get shipped to the end users. In addition, at the end of any service activities a service validation process is performed using a service validation kit of sterile water and a reference solution, available for service trained people. This is to hand the instrument back to the customer in a validated working state. The validation kit is available for customers, but it is not mandatory from bNovate.

Results can be viewed on the BactoSense sensor itself, or via a password-protected website. Measurements with the BactoSense can only be started or stopped on the sensor itself and not via the website. Also changes to the measurement settings have to be made on the sensor itself.

2.1.2 BugCount Online and BugCount Guardian

The BugCount Online and BugCount Guardian from LuminUltra measure the ATP concentration of a water sample. ATP is present in all living microbial cells and can thus be used as an indicator for the amount of active biomass that is present in a water sample. Both systems automatically take a water sample and in the reaction chamber a lysis buffer and reagents are added. The reagents contain the enzyme luciferase and the substrate luciferine. In the presence of ATP luciferase reacts with luciferine thereby producing light (relative light units: RLU). The amount of light produced is directly proportional to the ATP concentration and thus active biomass present in the sample. The measured ATP concentrations are automatically uploaded to a password-protected website (www.bugcount.luminultra.com) via which the results can be monitored and downloaded. One measurement takes a few minutes. Information on the sensors is summarized in Table 2-1.

Figure 2-3. BugCount Online sensor of LuminUltra. This version was used for validation studies on cooling water and surface water in 2021.

From literature it is known that disinfection of bacteria with UV only has a small effect on the ATP concentration of a water sample and effects are mainly seen at high UV doses (>80 mJ/cm^2) [1, 2]. Chlorine disinfection (already from 0,1 – 1 mg/L for 30 minutes) and ozonation lower the cellular ATP concentration, but not the total ATP concentration which sometimes even increases. Chlorination and ozonation thus cause the release of ATP from bacterial cells and becomes free ATP which can be easier to detect thereby causing the increase in total ATP [1, 3]. As a consequence, the effect of UV disinfection on the viability of bacteria cannot be monitored with the BugCount Online and BugCount Guardian.

Version of BugCount Online used for validation studies

During the course of the project a new version of the BugCount Online was released by LuminUltra, the BugCount Guardian. The first part of the validation (cooling and surface water) was performed with the BugCount Online. The measurement principle of both versions is similar, the differences are described below.

BugCount Online: validation of cooling and surface water

After every measurement a cleaning procedure is performed and the tubes and reaction chamber are flushed with the sample water before a new water sample is taken.

The lower detection limit was reported by LuminUltra to be 100 ng/l (Table 2-1), water samples with a lower ATP-concentration thus yield a result of 0 ng ATP/l and was, therefore, not used to measure drinking water. The upper detection limit was reported to be 10.000.000 ng/l.

Although the BugCount Online performed a cleaning procedure after each measurement, this procedure proved to be not stringent enough. The results with the BugCount Online should therefore be regarded with some precautions.

BugCount Guardian: validation of drinking water and field studies

The BugCount Online and BugCount Guardian take calibration readings for every sample measurement that is conducted. This is done by measuring the background signal and the signal after addition of a known quantity of ATP after sample measurement).

Before each measurement, the BugCount Guardian and BugCount Online measure the ‘background RLU’ using the photomultiplier inside the device. Next the sample is measured which determines and produces a ‘sample RLU’. Then, a small amount of ATP standard is added to the just-measured sample and the RLU is calculated again. These three RLU readings create two controls (high and low) against which the sample measurement is compared.

The new BugCount Guardian can now perform these measurements at different dilution ratios, depending on the sample. This method of calculation effectively calibrates the device with every measurement; as the standard RLU is a known quantity and is generally many times higher than the sample being tested and the 'background RLU' is effectively the zero point. The measurement of both background and ATP standard allows the sample RLU to always be compared to two control methods. These tests and the outputs are all done automatically through the devices sample measurement process and reported through the device software. The quality process tests the device against various concentrations of ATP standard to ensure the unit is operating linearly across a range of ATP concentrations, while the method described above calibrates the device with every measurement.

Figure 2-4. BugCount Guardian sensor of LuminUltra. This version was used for validation studies on drinking water in 2024 and pilot studies in 2023 and 2024.

Table 2-1. Summary of sensor characteristics

	Measurement principle	Lower detection limit	Upper detection limit	Measurement duration
BugCount Online	ATP	100 ng ATP/l	10.000.000 ng ATP/l	Few minutes
BugCount Guardian	ATP	100 ng ATP/l	10.000.000 ng ATP/l	Few minutes
BactoSense	Flow cytometry	100 cells/ml	$5x10^6$ cells/ml	30 minutes
BACTcontrol	Enzymatic activity	0 pmol MUF/min/volume	NA	2 – 2.5 hours

2.1.3 BACTcontrol

The BACTcontrol of microLAN detects microbiological activity in water samples using an enzymatic reaction via which all bacteria can be detected using fluorescence. This is the 'Total activity' analysis in which the presence of the alkaline phosphatase enzyme is measured. This enzyme is present in all bacteria and is thus an indicator for the amount of active bacteria that are present in a water sample.

The BACTcontrol monitors the presence of the alkaline phosphatase enzyme by addition of a substrate containing a fluorescent label 4-methylumbelliferone (MUF). The alkaline phosphatase enzyme releases this fluorescent group from the rest of the substrate, thereby creating free MUF which fluoresces after excitation via UV irradiation (λ_{ex} 360 nm; λ_{em} 450 nm).

The BACTcontrol analyser consists of a reactor (volume: 2 ml) with two chambers that are separated by a reusable ceramic filter with a pore size of 0.45 µm (Figure 2-5). In the reactor the water sample is concentrated by the filter and the temperature (45 ± 0.1 °C) is stabilized. While the concentrated water sample is constantly stirred by a magnetic stirrer, a buffer and the fluorescently labelled substrate are added. This starts the enzymatic reaction and the produced fluorescent light is measured by a fluorescence detector that is included in the BACTcontrol analyser.

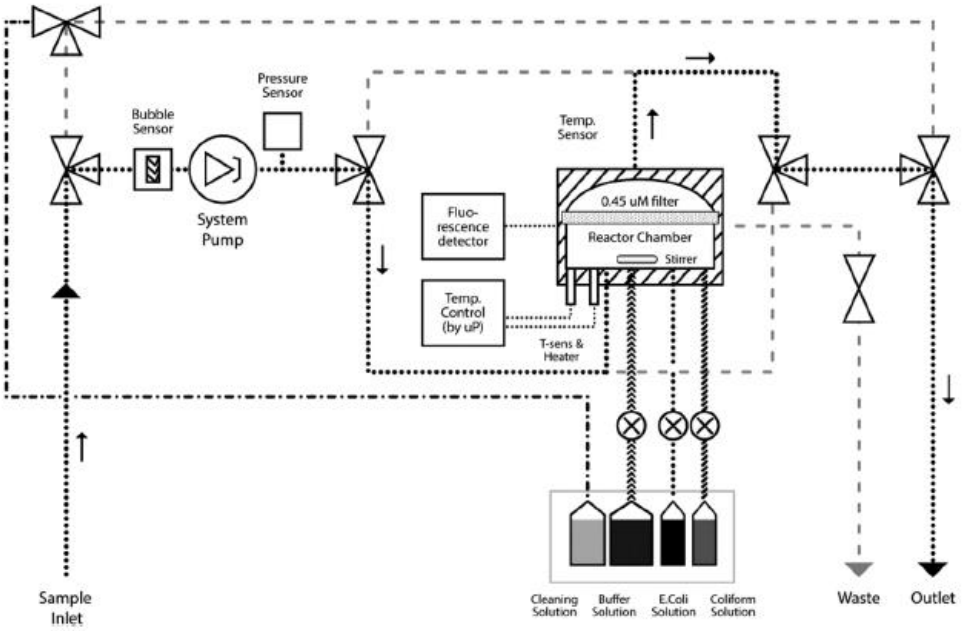


Figure 2-5. Schematic overview of the BACTcontrol analyser

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 total filtration time depends on the volume that has to be filtered and the condition, or fouling, of the filter. The sampled water volume is measured by the pump during this process. One measurement takes around 2 – 2.5 hours to be completed (Table 2-1).

In more detail, after setting the temperature inside the reaction chamber and addition of the buffer and reagents, a short stabilization period starts which is followed by the actual measurement of the fluorescence intensity during a 20-minute incubation period. The fluorometer has been calibrated using a standard solution 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 and thus the amount of enzyme (alkaline phosphatase) and bacteria that are present. The fluorescence intensity is converted into MUF production per time and volume (pmol MUF * min * filtrated volume).

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 for each measurement performed. The measurement is regarded as statistically significant if the average signal during the measurement exceeds the standard deviation by threefold in relation to the theoretical zero line of the reaction. The limit of detection is calculated with the data obtained after the stabilization period: the slope of the regression curve is determined until the end of the measurement phase.
After each measurement, a cleaning procedure is performed by the device. A solution with hydrogen peroxide (3%) and peracetic acid (0.11%) is injected in the reactor chamber and circulated through the internal tubing. At the same time the water is heated to eliminate residues of the measuring process within the system. The BACTcontrol has two waste streams: cleaning and analysis reagents and the water sample to be tested.

The effect of disinfectants or UV disinfection on the phosphatase activity of bacteria is not known. However, it seems most likely that the effect of UV and disinfectants are comparable to their effect on ATP measurements. Both molecules (ATP and phosphatase) are intracellular compounds that are not affected by UV (which mainly affects RNA and DNA). Both molecules are likely to be affected by chlorine or ozonation as this damages the cell membrane and thus releases the compounds and makes them vulnerable for the disinfectants. If this is indeed the case, than the effect of UV disinfection will not (or only to a limited extent) be detected by the BACTcontrol, whereas the effect of chlorination and ozonation are measurable.

Results can be viewed on the BACTcontrol sensor itself, or via an online connection using TeamViewer. In addition, measurements can be started or stopped via the online connection, as well as changes to the settings can be done online.

2.1.4 Continuous Biofilm Monitor with ATP-kit

CBM

The Continuous Biofilm Monitor (CBM) was designed by KWR to measure the biofilm formation potential of drinking water [4, 5]. Biofilm formation in this system is influenced by the amount and type of nutrients that are present in the water. High nutrient levels will generally lead to high levels of biofilm formation.

The CBM consists of four parallel cuvettes (Figure 2-6, left) filled with glass beads (Figure 2-6, right) or a small glass coupon. The water flow over each cuvette is 10 liter water per hour. When the CBM is installed on a drinking water flow, the CBM is sampled every 14 days. For every measurement two (of the total of four) cuvettes are sampled and the amount of biofilm formed on the glass beads is analysed by measuring the ATP concentration. The two cuvettes are replaced by new cuvettes and flow continues. Each cuvette is present in the CBM for 28 days before being sampled and measured. Using this setup, the biofilm formation can be determined every 14 days.

For drinking water application of the CBM normally glass beads are used, while for more dirty water types (e.g. cooling or surface water) glass coupons are used. As glass beads can act as a filter, clogging will occur much faster compared to when using a glass coupon. With both glass coupons and glass beads the biofilm formation on glass is measured. However, with glass beads the interaction between water and biofilm is more intense due to a higher Reynold's number, which results in a better transfer of nutrients from the water to the biofilm. Furthermore, the glass beads may act as a filter resulting in the accumulation of biomass on the beads. This means that not only biofilm formation is measured, but also biofilm/biomass deposited on the beads.

Figure 2-6. Continuous Biofilm Monitor (CBM) of Milispec (left) and a close-up of a cuvette with glass pearls (right)

The ATP-concentration on the glass beads or coupons was, up until now, always measured in a laboratory. However, in this project the field kit of LuminUltra, distributed in the Netherlands by Milispec, for ATP-measurements will be tested. In addition, due to differences in the design of the Milispec-CBM and the KWR-CBM, sampling of the cuvettes of the Milispec-CBM is easier and can be done by people who have had only a short instruction. With the Milispec-CBM combined with the ATP-field kit, the biofilm formation can be measured on site and a laboratory is not needed anymore.

ATP measurement of CBM with KWR-method

The ATP-method of KWR is performed as described earlier [6].

In short, the glass beads (or coupons) of the CBM are sonicated in sterile drinking water to release the biofilm. To do this, glass beads or glass coupons from the CBM are aseptically transferred to a 15-ml tube containing 10 ml sterile drinking water. This tube is sonicated for two minutes. The sonication procedure is performed three times and every time fresh 10 ml sterile drinking water is used. The biofilm will detach from the glass and is resuspended in the water. After sonication the 3x 10 ml with detached biofilm are pooled and the ATP-concentration of the water with suspended biofilm is measured.

The ATP-concentration of the dissolved biofilm solution is measured in a luminometer by automatic addition of the substrate luciferine and enzyme luciferase. In the presence of ATP luciferine is processed by luciferase, thereby releasing light. The amount of light formed is measured by the luminometer. The whole procedure can be performed within minutes.

Of each cuvette the ATP-concentration of the released biofilm is measured twice with the luminometer. The average of these two measurements is given in the results.

ATP measurement of CBM with Milispec/LuminUltra-method

In the Netherlands, Milispec distributes the ATP-kit that was used in this project. With the DSA-kit of LuminUltra the ATP-concentration of the biofilm formed on the glass beads or coupons of the CBM can be measured in the field. All materials needed to perform the ATP-analysis, except for reagents, are assembled together in a hard suitcase (Figure 2-7).

Figure 2-7. ATP-field kit to determine the ATP-amount, as indicator for the amount of biomass present in the biofilm on the glass pearls or glass plates of the CBM.

Measurements were performed according to the manufacturer's protocol. In short, this protocol has the following steps:

- Preparation:
  - Dissolve the freeze-dried Luminase enzyme in the buffer and wait 5 minutes for the enzyme to dissolve (Luminase-solution can be stored at 4°C for 3 months or -20°C for 6 months).
  - Check the activity of the Luminase enzyme by adding 100 µl Luminase enzyme to 2 drops UltraCheck 1 solution. Measure the ATP-concentration within 10 seconds.
- Measuring ATP in biofilm:
  - Harvest a CBM-cuvette with glass beads or a coupon. Gently shake off excess liquid and aseptically transfer the beads (pouring) or coupon (with forceps) to a tube with 5 ml UltraLyse7 buffer.
  - Shake and mix the tube well and incubate for a minimum of 5 minutes to allow for lysis of the bacterial cells and the ATP to be released.
  - Transfer 1 ml from the UltraLyse7 tube to 9 ml UltraLute buffer and mix the contents.
  - Add 100 µl Luminase to a clean test tube and measure immediately, this is the background signal.
  - Add 100 µl of the UltraLute-mixture to the Luminase and measure immediately in the luminometer. The result is available within a few seconds.
- Processing results:
  - The raw results of the luminometer can be entered into the 'myLuminUltra'-app to calculate the ATP-concentration of the biofilm.
  - The results can be stored in the app and long-term trends can be visualized.

Previous studies have shown no difference between the luminometers of KWR and Milispec/LuminUltra [7].

Calculation of BAR and BFR values

The BAR (biofilm accumulation rate) and BFR (biofilm formation rate) values are calculated by dividing the ATP-concentration on the glass pearls (BAR) or plates (BFR) in pg ATP/cm^2 by the number of days during which the biofilm was formed (typically 28 days for drinking water, but might vary for other water types). This gives the amount of biofilm formed per day (pg ATP/cm^2.d).

By using this calculation, the biofilm formation during different periods can be compared to each other. This can be used to study, for example, seasonal differences in water quality. These measurements have been performed for a long time on drinking water at different production locations. Information is therefore available on what normal values are for biologically stable (low nutrient level) and less biologically stable (high nutrient level) drinking water and comparison of many studies resulted in 30 pg ATP/cm^2.d as the cut off value above which water is considered less biologically stable [8]. For other water types this information is not yet available.

3 Validation microbiological sensors

3.1 Introduction

3.1.1 Goal of validation

The BACTcontrol, BactoSense and BugCount Online are rapid, online methods that determine total microbial biomass using different techniques, as described in chapter 2. To assess whether these sensors can be reliably used for online monitoring of the microbiological water quality, including water quality changes caused by disturbances, disinfection or control measures, an initial validation study on different water types (drinking water, cooling water, surface water) was performed. In the validation study the microbiological sensors are compared to proven, conventional laboratory-based methods (i.e. flow cytometry (FCM) or fluorescence microscopy to count (different types of) bacterial cells and an enzymatic method to measure ATP concentrations).

By performing the validation on drinking, cooling and surface water sampled from pilot locations, it is tested whether the sensors can be used reliably on all these water types and whether the water matrix interferes with the measurements. Measuring dilution series of these water samples gives information on the detection range and detection limit of each sensor.

Besides these three sensors, another inline method, the Continuous Biofilm Monitor (CBM) that determines the biofilm formation rate (BFR) and biofilm formation potential of the water, is tested as well; the CBM is described in more detail in chapter 2. The biofilm formation rate and potential are determined by measuring the amount of ATP in the biofilm grown on the glass beads (drinking water) or glass coupons (surface and cooling water) of the CBM using the Milispec/LuminUltra ATP method at site compared to the ATP analysis performed at the laboratory of KWR. Within this study the two ATP methods are compared to each other to test whether the field kit of Milispec/LuminUltra yields similar results as the ATP method of the laboratory of KWR.

3.1.2 Laboratory validation versus pilot location

The validation study and pilot location studies yield complimentary information. By performing validation studies in a laboratory, the sensors can be compared to proven and well-known laboratory methods. However, the results can be seen as a snapshot, as water samples of maximally 36 hours old and that have been transported to the laboratory are used, but the online or near-real time function is not tested. Additional tests on the pilot locations are subsequently performed to i) compare the sensors to each other during normal plant operation procedures, ii) measure freshwater samples directly, without transportation and storage after sampling and iii) get the local process operators or others to gain experience with the sensors and experience themselves if and how the sensors can be used in their situation.

3.2 Materials and Methods

3.2.1 Microbiological sensors

3.2.1.1 BACTcontrol, BactoSense and BugCount Online

The validation experiments were performed using the BACTcontrol, BactoSense and BugCount Online sensors which have been described in detail in chapter 2. The results of the BACTcontrol and BactoSense were quality controlled by the sensor suppliers (microLAN for BACTcontrol and APT BV and bNovate for the BactoSense).

For the BactoSense the Live-Dead-Cartridge (LDC) was used for the experiments with industrial water and surface water, the Intact-Cell-Cartridge (ICC) was used for drinking water experiments. The LDC measures the membrane-intact cell count (ICC) and the membrane-damaged cells (DCC), thereby also giving a result for the total cell count (TCC), whereas the ICC cartridge only measures the membrane-intact cell count (ICC). The water sample is dyed with SYBRgreen, which binds to all DNA, and with Propidium Iodide (PI), which can only enter a bacterial cell when the membrane is damaged and then bind the DNA. Cells to which SYBRgreen and PI are bound are categorized as DCC, cells with only SYBRgreen are ICC, and the sum of DCC and ICC is TCC. However, PI can also bind debris and if there is no clear separation between debris/noise and damaged cells, the DCC can be overestimated and thereby also causes overestimation of the TCC. This depends on the composition of the water type, the microbial population and the gating. The measurement results of the BactoSense were analysed together with APT (distributor of BactoSense) and bNovate (producer of BactoSense) to evaluate and, if necessary, to adapt the gates of the BactoSense for this specific water type. Based on the knowledge and experience of bNovate ICC, HNAC and LNAC were considered reliable and the absolute values, thus, can be used.

The BugCount Guardian was not available at the start of the validation study and therefore the BugCount Online was tested on surface water and industrial water. Due to the high detection limit (100 ng/l), the BugCount Guardian is not suitable for drinking water applications and therefore not included in the drinking water validation study.

The measurement results of the BACTcontrol were evaluated together with microLAN (producer of BACTcontrol) to conclude whether the results were reliable.

3.2.1.2 CBM

Validation of the CBM with the ATP kit on site was performed using the CBM of Milispec and the CBM from KWR. Both the CBM and the ATP-analyses using the Milispec/LuminUltra on site method are described in more detail in chapter 2.

3.2.2 Laboratory analyses

3.2.2.1 Flow cytometry

With flow cytometry the number of bacterial cells in a water sample is counted and a difference can be made between cells with an intact membrane (as an indication for living cells) and a compromised membrane (as an indication for dead cells).

1 ml of the water sample was incubated with propidium iodide (only stains DNA of bacteria with a permeable membrane) and SYBR Green (stains DNA of all bacteria) and measured with a BD FACS Calibur according to KWR-protocol LMB-071. The lower detection limit is 1000 cells/ml.

3.2.2.2 Fluorescence microscopy

With fluorescence microscopy the total number of cells is counted. The water sample was filtrated on a black 0.22 µm polycarbonate filter. After filtration the bacteria are stained with acridine orange which stains bacterial DNA and RNA of all cells (living and death). Using a fluorescence microscope, the number of bacteria was counted. The microscopic preparations were stored at -20°C and analyzed within 1 month after they were frozen.

3.2.2.3 ATP

Water samples

The ATP method of KWR on water is performed according to KWR protocol LMB-002.

In short, in the luminometer the substrate luciferine and enzyme luciferase (Celsis) are automatically added to the water sample. In the presence of ATP, the added luciferine is processed by luciferase, thereby releasing light. The amount of light formed is measured by the luminometer. Subsequently the ATP concentrations are calculated from the produced light using a calibration curve of ATP concentrations against light intensity.

Biofilm samples

The ATP-concentration of the biofilm was measured as described in chapter 2.

3.2.3 Set up validation experiments

3.2.3.1 BugCount Online, BactoSense, BACTcontrol

Water samples for the validation studies were sampled by the project partners from their own water systems and directly shipped to KWR at 4°C. Uniper was sampled by KWR and also directly transported to KWR at 4°C. The water samples were stored at 4°C until the measurements were performed within 3 – 36 hours. The sampling points are shown in Table 3-1.

Table 3-1. Water types and sampling location that were used for validation studies

Project partner	Water type	Sampling location	Date
H_2O Biofouling solution	Cooling water	Uniper	17 February 2021, 1 March 2021, 5 April 2021
H_2O Biofouling solution	Surface water	Rhine river near Elst	17, 22, 28 February 2021, 14 March 2021, 13 April 2021
BASF	Process water	'Fabrikatiewater' entering BASF plant	22, 28 February 2021
BASF	Cooling water	G500	22, 28 February 2021, 16 March 2021
Dow	Cooling water before and after chlorine dosing	Large cooling tower	21, 29 March 2021, 6, 11 April 2021
Evides	Drinking water	Distribution system, Kralingen	4 September 2023
	Drinking water	Distribution system, Zuid-Beijerland	11 September 2023
Vitens	Drinking water	Production location, Groenekan	17 August 2023
		Production location, Soestduinen	29 August 2023
Oasen	Drinking water	Production location, Nieuw-Lekkerland	21 August 2023

The cooling water and surface water samples were measured with three of the sensors (BactoSense, BACTcontrol, BugCount Online), drinking water was measured with two sensors (BactoSense and BACTcontrol) and all water samples were measured with the three lab analyses (flow cytometry, ATP, fluorescence microscopy). Each analysis was performed 2-3 times per water sample and the average results are given in Appendix I.

For measurements with the BactoSense some cooling water and surface water samples were 1:10 or 1:100 diluted in PBS buffer (phosphate buffered saline) if the undiluted water sample did not yield a reliable result (due to exceedances of the upper detection limit or because air bubbles were formed). All averaged results are given in Appendix I. Results used for statistical analysis were always corrected for the 1:10 or 1:100 dilution.

BACTcontrol measurements of cooling and surface water were performed without the ceramic 0.22 µm filter as from previous studies it was known that cooling water will clog the filter very rapidly. In addition, concentration of the water sample is not required as it is expected that cooling water contains high biomass levels. The BACTcontrol results for cooling water (2 ml reaction volume) were extrapolated to 100 ml (the reaction volume for surface water and drinking water) before the results were used for statistical analysis. For measurements of drinking water, a sample volume of 100, 50, 25 or 12 ml was concentrated by the BACTcontrol.

The BugCount Online sensor was only used for validation with surface water and cooling water (February – April 2021). As described in chapter 2, LuminUltra reported some problems with this version due to which the results should be interpreted with caution. Also, they might not be representative for the newer version of the BugCount Online, the BugCount Guardian (used from January 2022 and later).

3.2.3.2 Dilution series

To allow for testing different amounts of biomass per water sample several dilution series were made. To this end, the surface water and cooling water samples were ten-fold diluted in phosphate-buffered saline buffer (PBS) 1:1 – 1:1.000.000, depending on the water sample. If two consecutive dilutions were below the detection limit of a specific sensor or laboratory method, no further dilutions were analysed with this specific sensor or laboratory method. Drinking water was diluted twofold each time from 1:2 to 1:16 in PBS (Groenekan dilution series) or drinking water of KWR (other dilution series) that was first autoclaved (to kill all bacteria present and destroy enzymes) followed by filtration over a 0.22 µm filter to remove dead cells and cell debris. Due to interference of the PBS buffer with the BACTcontrol measurements (in Groenekan dilution series), especially for lower biomass samples, a different diluent (autoclaved and filtrated KWR drinking water) was chosen for the remaining dilution series.

3.2.3.3 CBM with ATP-kit

Previous studies showed that the CBM of Milispec is comparable to the CBM of KWR [7]. In the current project two ATP-methods to measure the amount of biofilm formed in the CBM are compared: the ATP-kit of Milispec/LuminUltra (DSA-kit) and the ATP-method of KWR. This comparison was performed on biofilms sampled from the CBMs that received drinking water of KWR or surface water of the river Meuse at the location of Keizersveer.

In the NEN-EN-ISO 16140-2:2016 standard it is described how an alternative method should be compared to the reference method [9]. This standard was used as a guideline to set up the validation experiments with the CBM of KWR and Milispec and the two ATP-methods of KWR (reference method) and Milispec/LuminUltra (alternative method). These validation experiments are described in short, further details and information of the study design can be found in NEN-EN-ISO 16140-2:2016 [9]. The two ATP-methods are quantitatively compared and two aspects are tested:

- ‘Relative trueness study’ (paragraph 6.1.2 in the standard [9]) is a comparative study between the results obtained with the reference method (KWR ATP-method) and the alternative method (Milispec/LuminUltra ATP-method). This study should ideally be performed on naturally contaminated samples and on several ‘types’ of biofilm. Therefore, biofilms were formed in CBMs that received one of the two water types: drinking water of KWR or surface water of the river Meuse. Each sample (glass beads or glass coupons from one CBM-cuvette) is measured once with each method.

- ‘Accuracy profile study’ (paragraph 6.1.3 in the standard [9]) is used to test the requirement that the alternative method differs from the reference method by less than a certain acceptability criterion. In addition, the repeatability (or variation) of each method is tested. Normally, artificially contaminated samples with different levels of contamination are used for this study. However, as artificial contamination of biofilm samples grown in the CBM is not possible it was chosen to use a naturally grown biofilm, but to vary the

number of days of biofilm growth. This results in biofilms with different amounts of biomass. For the 'Accuracy profile study' normally one sample should be measured five times with the reference and the alternative method, but this is not possible with the cuvettes of the CBM due to the nature of the two ATP methods. To mimic the set-up of measuring one sample five times, three CBMs were placed on drinking water and three CBMs on surface water and these three CBMs at one water type were considered as a triplicate of each other. At every sample day all cuvettes from the three CBMs (twelve cuvettes in total) were sampled. Of each CBM two cuvettes were analysed with the reference (KWR) ATP-method and two cuvettes were analysed with the alternative (Milispec/LuminUltra) ATP-method.

To perform these two studies, three CBMs were installed on drinking water and three CBMs were installed on surface water. Two water types were used for the validation as differences in water quality will lead to differences in the amount and composition of the biofilm that is formed. Both may affect the ATP-measurements and using different water types thus allows testing of the ATP-kits under different conditions. For the drinking water measurements glass beads were used in the CBM. For the surface water measurements, the glass beads were replaced with glass coupons due to clogging of the beads. In addition, a candle filter was installed between the sample point and the CBM's to remove larger particles and prevent clogging of the CBM (Figure 3-1). The candle filters were replaced twice a week.

Figure 3-1. Installation of three CBMs on surface water at Keizersveer. Left: the surface water is first filtrated over a candle filter. Right: Three CBMs (of KWR) that receive the filtrate of the candle filter.

The cuvettes were sampled at different time intervals (3 – 34 days;

Table 3-2) so that the amount of biofilm formed varies and the ATP-methods are tested on different biofilm concentrations. ATP measurements with the Milispec/LuminUltra method and the KWR method were performed at the KWR laboratory. The glass coupons of the CBM's on surface water were sampled on site, added to the first buffer and transported to KWR at 4°C. The ATP-analysis was performed on these surface water samples within 60-90 minutes after sampling. Experiments with the CBMs receiving drinking water were performed at KWR and thus no transport time to the laboratory was involved for those samples.

Table 3-2. Sampling days of CBM's receiving drinking water at KWR or surface water at Keizersveer.

Drinking water KWR		Surface water Keizersveer	
Start	8-12-2020	Start	21-12-2020
Day 34	11-1-2021	Day 21	11-1-2021
Day 7	18-1-2021	Day 7	18-1-2021
Day 14	1-2-2021	Day 14	1-2-2021
Day 10	11-2-2021	Day 10	11-2-2021
Day 21	4-3-2021	Day 18	1-3-2021
Day 28	1-4-2021	Day 3	4-3-2021

3.2.4 Statistical analysis BACTcontrol, BugCount Online and BactoSense

The average of two or three measurements of the same water sample was calculated and this average value was used for further statistical analysis and visualization in scatter plots. The average with standard deviation of each sample and each sensor is given in Appendix I. If one of the two or three results was below the lower detection limit, the value of this detection limit was used to calculate the average and standard deviation. If one of the two or three results was above the upper detection limit the measured value was used to calculate the average and standard deviation.

3.2.4.1 Scatter plots

To generate scatter plots all averaged results above the reported upper detection limit were removed from the dataset and not visualized in the scatter plots. Results below the lower detection limit were replaced by a value similar to the detection limit or by a value of '0.1' if the lower detection limit equals '0', to allow for generation of graphs with a log-scale axis. Scatter plots were made using R software (version 4.1.0) and are shown in Appendix II.

3.2.4.2 Linear correlation analysis

To test whether the results from the BACTcontrol, BugCount Online and BactoSense and/or one of the laboratory biomass parameters are significantly correlated to each other, a linear correlation analysis (Pearson correlation) was performed.

All results above or below the reported upper and lower detection limit, respectively were removed from the dataset. The average value of two or three measurements per water sample was Log_{10}-transformed and used for linear correlation analysis using Pearson correlation. Results of all linear correlation analyses are shown in Appendix V.

Correlations between two tested parameters were considered to be statistically significant when $p < 0.05$ (green in Appendix V). Only for the correlations that were statistically significant, the r^2-value of the correlation is given in the tables of Appendix III. The r^2-value shows how much variation in one parameter is explained by the other parameter. A r^2-value of 0.75, for instance, shows that 75% of the variance in one parameter is explained by the variance in the other parameter. As such, the r^2-value is an indicator of how well two parameters are correlated with each other. The r^2-values were arbitrarily divided into four categories: $r^2 > 0.9$ excellent correlation, $0.7 < r^2 < 0.9$ good correlation, $0.5 < r^2 < 0.7$ moderate correlation, $r^2 < 0.5$ no or bad correlation.

3.2.5 Statistical analysis CBM

3.2.5.1 According to NEN-EN-ISO 16140-2:2016

To compare the CBM values obtained by the LuminUltra/Milispec ATP kit on site with the CBM values obtained by the reference ATP method in the KWR laboratory, the NEN-EN-ISO 16140-2:2016 standard was used. This standard describes in detail how the results should be statistically analyzed. This description was followed, including the display of the results in graphs and tables. A short description of how the statistical analysis was performed can be found below. More details can be found in NEN-EN-ISO 16140-2:2016 [9].

3.2.5.1.1 Relative trueness study

Scatter plot of reference-method versus alternative-method

The results are analyzed using the Bland-Altman method. To this end, the data of each sample are Log_{10}-transformed and plotted in a scatter plot. The results of each water type are displayed with a different symbol.

Calculations

Next the difference between the two methods for each sample is calculated. This also gives an average difference and standard deviation for all samples of one biofilm/water-type. These results are summarized according to Table 3-3. How the calculations were performed can be found in the standard.

Table 3-3. Summarized results for all categories, according to Table 13 from NEN-EN-ISO 16140-2:2016.

Category	Type	Sample	Log_{10} cfu		Mean	Difference
			Reference-method result	Alternative-method result		
1	1	1	R1	A1	(R1 + A1)/2	D1 = A1 – R1
		2	R2	A2	(R2 + A2)/2	D2 = A2 – R2
		3	R3	A3	(R3 + A3)/2	D3 = A3 – R3
		4	R4	A4	(R4 + A4)/2	D4 = A4 – R4
		5	R5	A5	(R5 + A5)/2	D5 = A5 – R5
1	2	6	R6	A6	(R6 + A6)/2	D6 = A6 – R6
		7	R7	A7	(R7 + A7)/2	D7 = A7 – R7
		8	R8	A8	(R8 + A8)/2	D8 = A8 – R8
		9	R9	A9	(R9 + A9)/2	D9 = A9 – R9
		10	R10	A10	(R10 + A10)/2	D10 = A10 – R10
1	3	...	...	...	...	...
Average category 1				$\overline{D}_1$		
Standard deviation category 1				s_{D1}		
...	...	...	...	...	...	...
x4			Rx	Ax	(Rx + Ax)/2	Dx = Ax - Rx
Average category x				$\overline{D}_x$		
Standard deviation category x				s_{Dx}		
Average all categories				$\overline{D}_{all}$		
Standard deviation all categories				s_{Dall}		

Bland-Altman difference plot

The differences between the two methods for each sample (last column in Table 3-3) are then plotted against the mean value of both methods (second-last column in Table 3-3). This gives a visual representation of the degree of bias and the agreement between the two methods. It is expected that no more than one in 20 data values will lie outside the confidence limits.

3.2.5.2 Accuracy profile study

The results of both the reference as alternative method were Log_{10}-transformed and tabulated according to Table 3-4, with the exception that per sample only three instead (t.p. 1 – 3) of five (t.p. 1 – 5) measurements were performed.

Table 3-4. Summarized results for all categories of the accuracy study, according to Table 14 from NEN-EN-ISO 16140-2:2016.

Category	Type	Item (level)	Reference method					Alternative method				
			t.p.[a] 1 (x_1)[b]	t.p. 2 (x_2)	t.p. 3 (x_3)	t.p. 4 (x_4)	t.p. 5 (x_5)	t.p. 1 (y_1)[c]	t.p. 2 (y_2)	t.p. 3 (y_3)	t.p. 4 (y_4)	t.p. 5 (y_5)
Category 1	Type 1	Sample 1 (low)										
		Sample 2 (low)										
		Sample 3 (intermediate)										
		Sample 4 (intermediate)										
		Sample 5 (high)										
		Sample 6 (high)										
...	...	...										
Category x	Type x	Sample 1 - 6										

[a] t.p. = test portion.

[b] (x_a) = log10 test result for the reference method (x) for test portions 1 to 5.

[c] (y_a) = log10 test result for the alternative method (y) for test portions 1 to 5.

Next several statistical parameters are calculated and summarized according to

Table 3-5. Details on how the calculations were performed can be found in NEN-EN-ISO 16140-2:2016 [9], but are summarized below.

X_i and Y_i

Median value of reference (X_i) and alternative (Y_i) method per sample

B_i

Bias: $Y_i - X_i$

Upper and Lower β-ETI (U_i and L_i)

Interval where the expected results will fall.

Calculate the standard deviation for each sample:

$$s_{\text{alt},i} = \sqrt{\frac{1}{n-1}\sum^{n}(y_{ij} - \bar{y}_i)^2}$$

Calculate the combined standard deviation:

$$s_{\text{alt}} = \sqrt{\frac{1}{q}\sum_{i=1}^{q} s_{\text{alt},i}^2}$$

Calculate the U_i $$U_i = B_i + T \cdot s_{\text{alt}}\sqrt{1+\frac{1}{n}}$$

Calculate the L_i:

$$L_i = B_i - T \cdot s_{\text{alt}}\sqrt{1+\frac{1}{n}}$$

Where T is set at the 80th percentile of $T\left(\frac{1-\beta}{2}\right);q(n-1)$ a Student-t-distribution:

AL:

The Acceptability Limits are set at 0.5 and -0.5 Log_{10}.

AL_S:

New Acceptability Limits calculated as a function of the standard deviation: $\text{AL}_s = 4 \cdot s_{\text{ref}}$

Table 3-5. Statistical results of the accuracy study, according to Table 15 from NEN-EN-ISO 16140-2:2016

Category	**Sample**	**Central value (Ref)**	**Central value (Alt)**	**Bias**	**Upper** β-ETI	**Lower** β-ETI	**Upper AL**	**Lower AL**
Category 1	Sample 1	X_i	Y_i	B_i	U_i	L_i	+AL	-AL
	Sample 2							
	Sample 3							
	Sample 4							
	Sample 5							
	Sample 6							

The results of

Table 3-5 are visualized in a graph (Figure 3-15). The Bias (B_i), Upper and Lower β-ETI (U_i and L_i) are plotted against Reference median (X_i). In the same graph the AL and -AL are plotted. If $U_i \leq$ AL and $L_i \geq$ -AL, the alternative method is accepted as being equivalent to the reference method for both the individual categories (biofilm grown on drinking water and surface water) and the combined categories (biofilm grown on water).

If any of the U_i or L_i exceeds the AL or -AL, new Acceptability Limits are calculated (AL_s). If all $U_i \leq AL_s$ and $L_i \geq$ -AL the alternative method is still accepted as being equivalent to the reference method, but only for the given water type (biofilm grown on only drinking water or on only surface water).

3.2.5.3 Wilcoxon signed-Rank test for paired values

As the ATP-dataset (in pg/cm^2, Table 12-4 and

Table 12-5) is not always normally distributed, and the Log_{10}-transformed dataset is also often not normally distributed, the Wilcoxon signed-Rank test for paired values was performed.
The test was performed using the calculator on the following website:
https://www.socscistatistics.com/tests/signedranks/default2.aspx

3.3 Validation BugCount Online, BACTcontrol and BactoSense on surface and cooling water

The three sensors (BugCount Online, BACTcontrol and BactoSense) were validated using surface water and cooling water from different locations, followed by validation of the BACTcontrol and BactoSense on drinking water (Table 3-1). In the tests at the pilot locations (chapters 4 - 9) ideally undiluted water will be measured with the sensors, and therefore it was tested whether undiluted water can indeed be measured with the sensors. In addition, variation in cell numbers in water of the pilot locations should be reliably measured, therefore several dilution series were prepared and measured with the sensors and laboratory analyses.

3.3.1 Results

3.3.1.1 Surface water

Surface water from the Rhine River was tested twice (Figure 3-2). Undiluted surface water contained a high number of cells (2.7 – 3.3 $\times 10^6$ cells/ml, obtained with FCM and fluorescence microscopy at the KWR laboratory) and ATP (total: 913 – 1433 ng ATP/l, also measured at the KWR laboratory). These cell numbers seem to be comparable to the cell numbers measured with the BactoSense. In contrast, the ATP concentration measured in the laboratory was lower than the ATP concentration measured with the BugCount Online (1911 – 2630 ng ATP/l). The BACTcontrol measured enzymatic activities in the range from 837 – 1831 pmol/min/100 ml. The number of cells in surface water was thus at the upper end of what the BactoSense can reliably measure, the BACTcontrol and BugCount Online did not have this potential problem. The Rhine River water contained mainly living cells, as the total number of cells was often similar to, or only slightly higher, than the number of intact cells measured with FCM.
The tenfold dilution steps were measured with all methods, but to different extents. The ATP concentrations and cell numbers determined with the laboratory methods ATP, fluorescence microscopy and FCM showed relatively good tenfold reductions with the tenfold dilution series until the number of cells or ATP drops below the detection limit. The three lowest dilutions (undiluted, 10x and 100x diluted) also showed tenfold reduction in cell numbers with the BactoSense. However, the tenfold reduction in cell numbers was not observed with the BactoSense for the 1,000 = dilution of Rhine water of 15 March 2021 (5 times reduction) and 100,000x dilution of 14 April (7 times reduction). The BACTcontrol measured enzyme activity in the undiluted and 10x diluted sample, but when dilutions became 100x or higher, no enzyme activity could be measured anymore. Furthermore, the reduction in enzyme activity in the 10x diluted sample was higher than expected from the dilution. The BugCount Online only yielded a value for the undiluted and one of the two 10x diluted samples. In all other samples the ATP concentration was below the detection limit of 100 ng ATP/l (* in Figure 3-2).

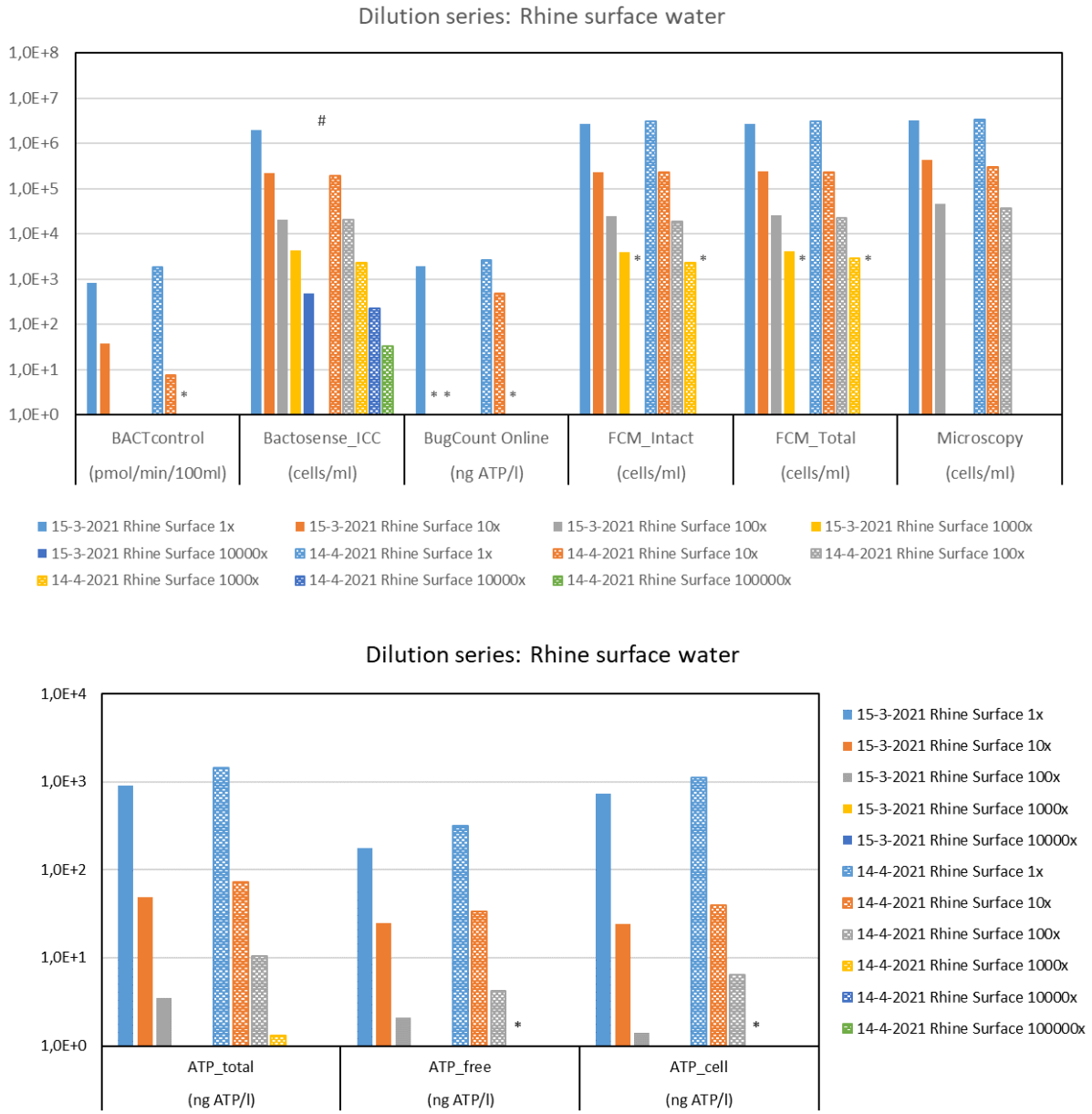


*Figure 3-2. Dilution series of surface water from the Rhine river, the dilution factor is given. Given are the average results from the sensors and laboratory analyses. # value above the detection limit, * value below detection limit. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.*

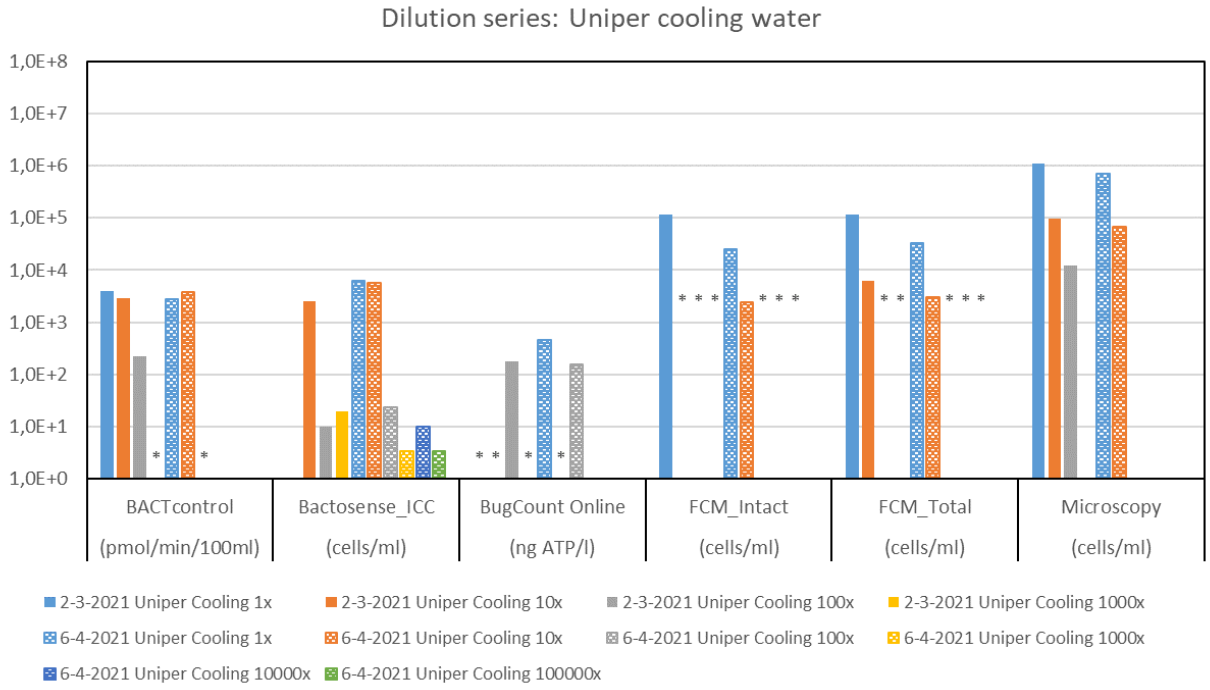
3.3.1.2 Cooling water

Cooling water from the cooling towers of Uniper was tested twice (Figure 3-3). Undiluted water yielded a result with the BACTcontrol (2805 – 4040 pmol MUF/min/100 mL), FCM (total: 0.33 – 1.2×10^5 cells/ml, intact: 0.25 – 1.2×10^5 cells/ml), microscopic counts (0.69 – 1.1×10^6 cells/ml) and ATP (total: 11 – 33 ng ATP/l, free: 11 – 33 ng ATP/l). One of the two undiluted cooling tower water samples yielded no result with the BugCount Online (other sample: 459 ng ATP/l) and both undiluted samples yielded results above the detection limit of the BactoSense. The BactoSense gave an error that bubbles were detected in the water, whereas no error code was available for the BugCount Online.

A tenfold reduction in cell numbers in the tenfold dilutions was only observed with the FCM and fluorescence microscope analyses (Figure 3-3). The ATP concentrations, measured with the laboratory method or the BugCount Online, quickly dropped below the detection limit. The BACTcontrol showed lower enzymatic activity with

increasing dilution, but this activity did not follow the tenfold dilution. Furthermore, the numbers were not tenfold reduced when the dilution series goes from undiluted to 100x diluted.

Almost no cellular ATP was measured with the laboratory method and all the detected ATP was free (or extracellular) ATP. This is most likely caused by the ozone disinfection at Uniper, which destroys a large part of the cells and thereby at the same time releases the ATP from the cells which is diluted in the surrounding water or degraded.



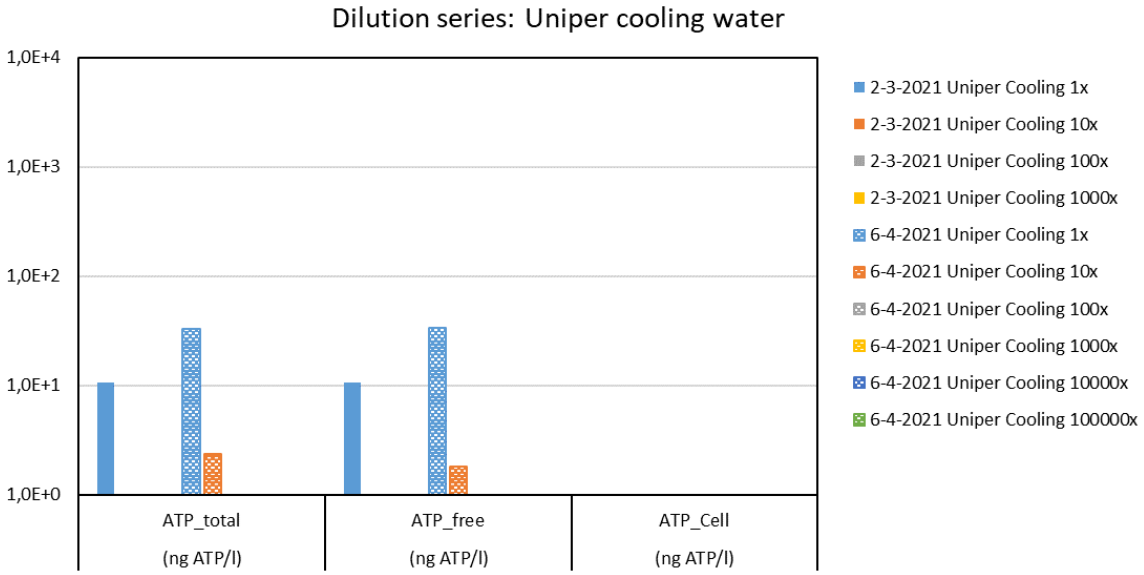
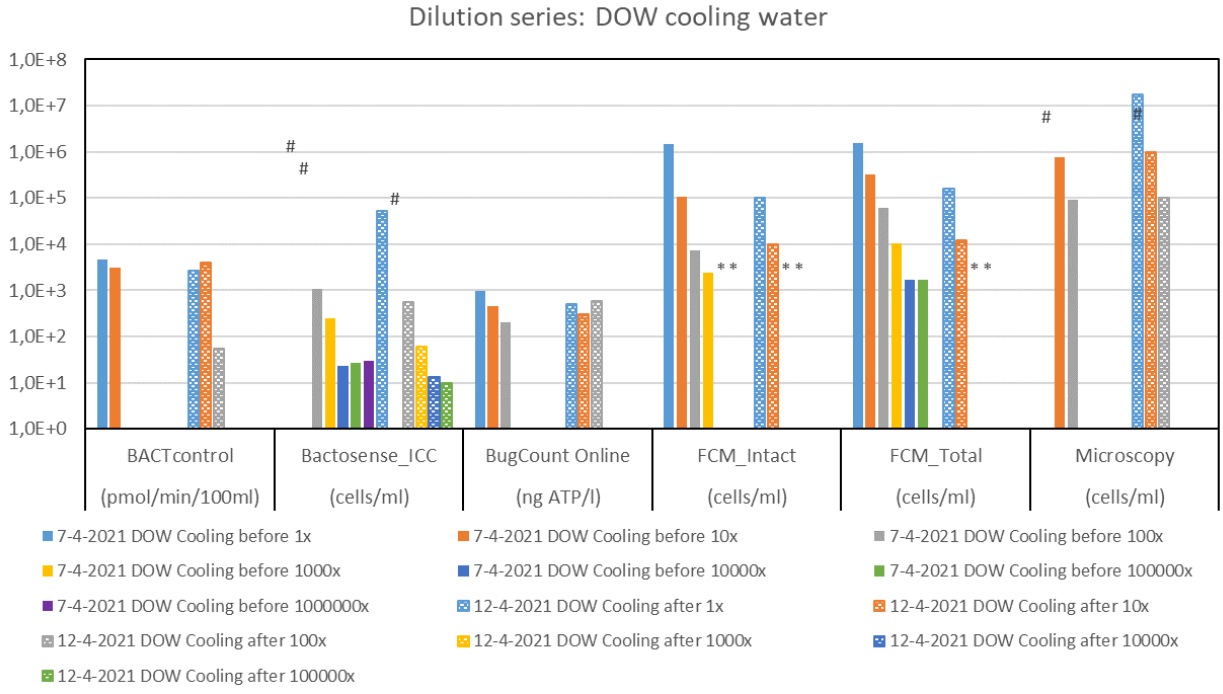


*Figure 3-3. Dilution series of cooling water from Uniper, the dilution factor is given. Given are the average results from the sensors and laboratory analyses. # value above the detection limit, * value below detection limit.*
An error ('bubbles detected') was obtained upon measuring the undiluted water sample of 2-3-2021 with the BactoSense. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.

Cooling water from Dow was tested twice, the water sample was taken before and after shock chlorination (Figure 3-4). Undiluted water yielded a result with the BACTcontrol (2603 – 4578 pmol MUF/min/100 ml), BugCount Online (512 – 955 ng ATP/l), FCM (total: 0.16 – 1.5×10^6 cells/ml; intact: 1.0 – 1.4×10^6 cells/ml) and the ATP laboratory method (total: 183 – 263 ng ATP/l; cellular: <1 – 10 ng/l). The BactoSense measured none of the undiluted samples (intact: 2.3×10^7 cells/ml), as the number of cells in the samples was above the upper detection limit. The undiluted water could also not be reliably measured with fluorescence microscopy due to the high number of cells.

The tenfold dilutions clearly resulted in tenfold decrease in cell numbers when measured with FCM or fluorescence microscopy and in tenfold decrease in ATP concentrations when measured with the laboratory method. However, the sensors did not consistently show tenfold reduction with the tenfold dilutions. Sometimes a reduction was visible (BACTcontrol and BactoSense on 12-4-2021 and BugCount Online on 7-4-2021), but this reduction was not tenfold and, thus, did not follow the tenfold dilution series. Comparable to the measurements of Uniper cooling water, the BactoSense seemed to reach a plateau at around 1x10^4 cells/ml at 10,000x to 1,000,000x dilution.

No clear difference was visible between the water samples taken before or after shock chlorination. This suggests that the microorganisms in the water sample taken before shock chlorination (no free chlorine present) were still damaged by the chlorine of the previous shock chlorination, and that there was no or only limited difference with the water sample taken directly after shock chlorination.



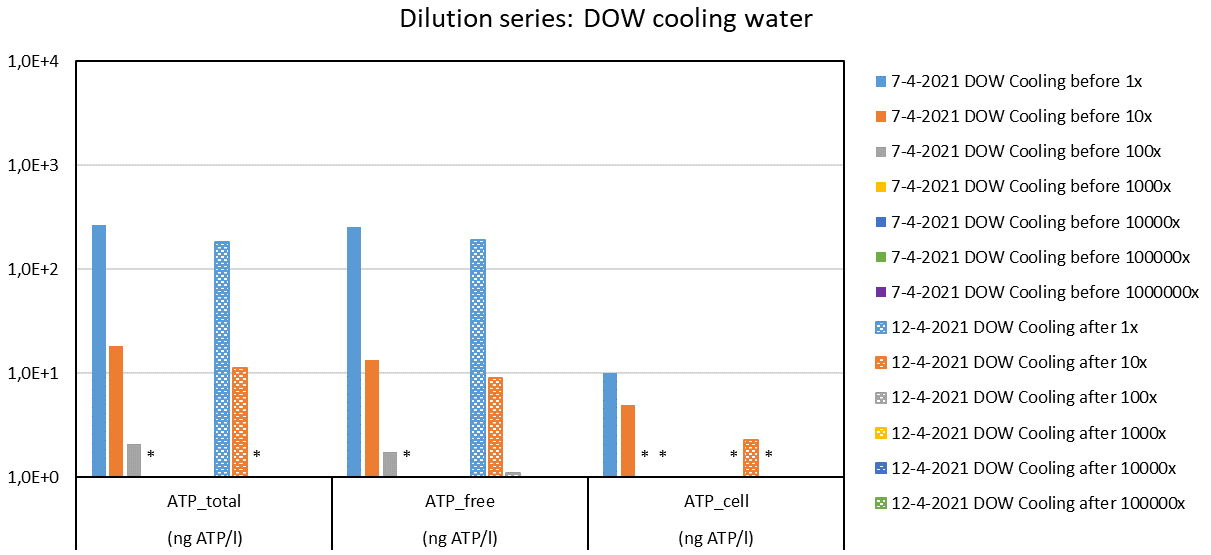
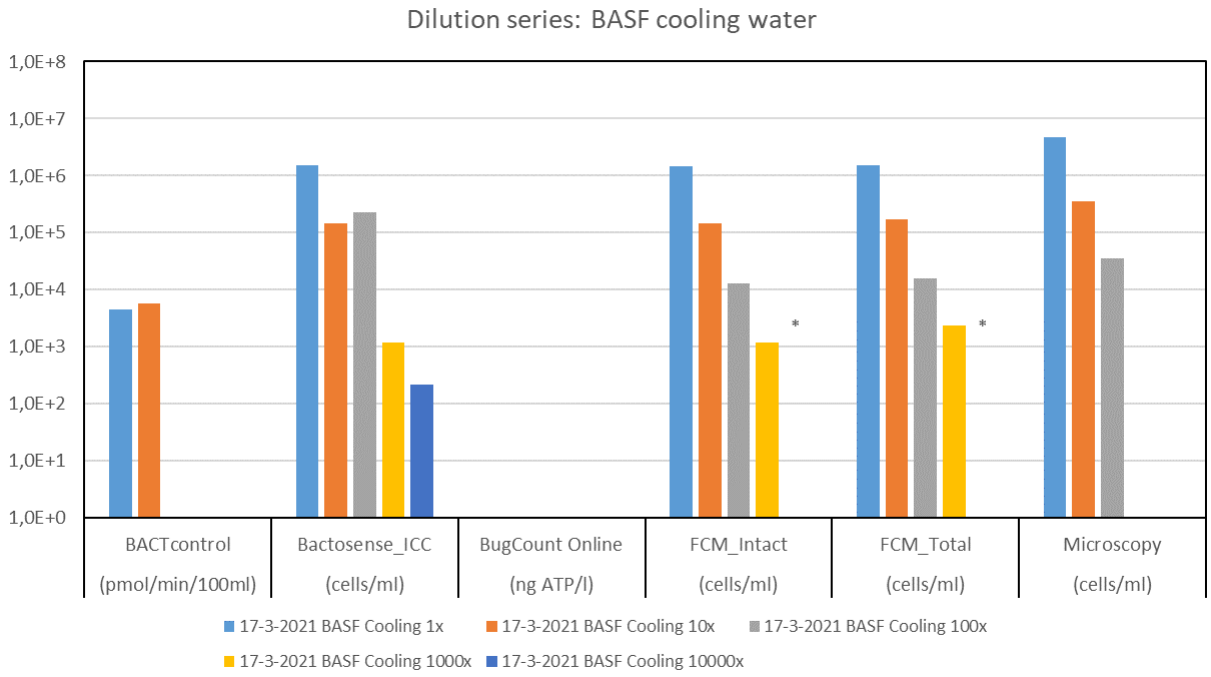


*Figure 3-4. Dilution series of cooling water from Dow, the dilution factor is given. Given are the average results from the sensors and laboratory analyses. # value above the detection limit, * value below detection limit. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.*

Cooling water of BASF was tested once in a dilution series (Figure 3-5). The undiluted water yielded results with all parameters (BACTcontrol: 4473 pmol MUF/min/100 ml; BactoSense_ICC: 1.5×10^6 cells/ml; FCM_intact and total: 1.5×10^6 cells/ml; fluorescence microscopy: $4{,}8\times10^6$ cells/ml; ATP total: 48 ng ATP/l; ATP cellular: 39 ng ATP/l). The BugCount Online could not be tested with this water type due to technical problems not related to the water source. These problems are solved in the new version of the BugCount Online which is named the BugCount Guardian.

A ten times reduction in enzyme activity, cell numbers or ATP in the tenfold dilutions was only visible for the laboratory analyses FCM, fluorescence microscopy and ATP, but not for the data from the BACTcontrol and BactoSense. The BactoSense results showed a strange pattern of varying cell numbers not related to the tenfold dilution series. No abnormalities were observed while performing the experiment that can explain these aberrant results.



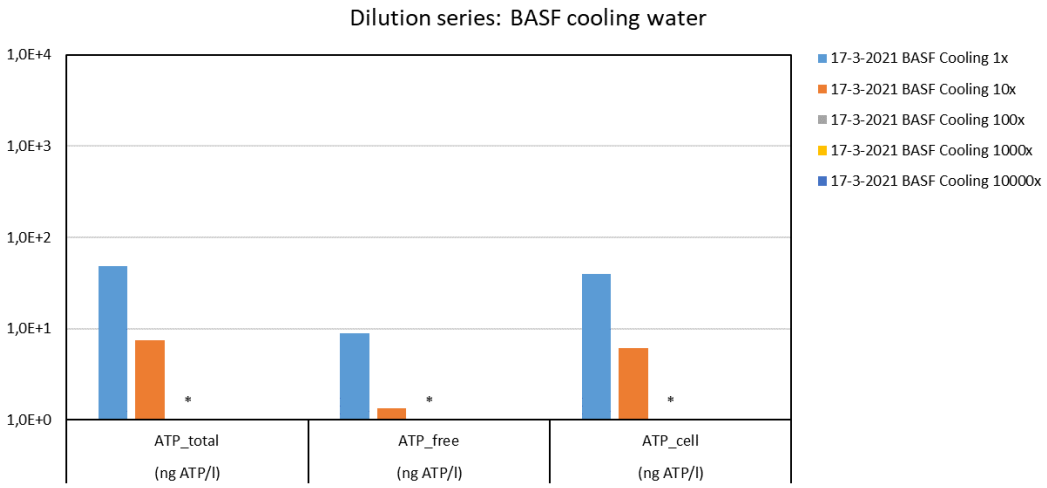


*Figure 3-5. Dilution series of cooling water from BASF, the dilution factor is given. Given are the average results from the sensors and laboratory analyses. # value above the detection limit, * value below detection limit. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.*

3.3.1.3 Drinking water

Drinking water from five different locations was tested (Figure 3-6 - Figure 3-9). Undiluted drinking water yielded comparable cell numbers for the two laboratory methods, FCM ($1.5x10^4$ – $2.8x10^5$ total cells/ml) and microscopy ($7.5x10^3$ – $1.6x10^5$ cells/ml), but the variation between the individual undiluted drinking waters is relatively large. The number of intact cells was comparable with FCM ($1.5x10^4$ – $1.2x10^5$ cells/ml) and BactoSense ($2.3x10^4$ – $1.5x10^5$ cells/ml). The percentage of intact cells with FCM was high for Groenekan, Nieuw-Lekkerland and Soestduinen (92-98%), indicating that the water contained mainly membrane-intact cells, and thus likely mainly living cells. For Kralingen and Zuid-Beijerland the percentage intact cells was lower (41-60%), which is caused by the

dosage of low concentrations chlorine dioxide disinfectant at the final step in the drinking water production. ATP concentrations were <1 ng/l for Groenekan, Soestduinen and Kralingen, indicating that flow cytometry is a more sensitive method in these instances. In Groenekan and Soestduinen this shows that the water contains very low concentrations active biomass, whereas at Kralingen it might be due to the chlorine dioxide dosing. However, in Zuid-Beijerland an ATP concentration could be measured (1.7 ng/l) as well as for Nieuw-Lekkerland (3.4 ng/l). The enzymatic activities measured with the BACTcontrol varied between 0.9 and 66.9 pmol/min/100 ml. Enzymatic activity was low for Soestduinen and Nieuw-Lekkerland (0.9 – 9.7 pmol/min/100 ml) and high for Zuid-Beijerland and Kralingen (27.2 – 66.9 pmol/min/100 ml). Despite the chlorine dioxide addition at Kralingen, the enzymatic activity is still high. This might indicate that the alkaline phosphatase enzyme is no only intracellularly present, but also outside of the cell.

The two-fold dilution steps were measured with all methods, but to different extents. The dilution steps were hardly visible for the ATP methods as the concentration in the undiluted water was already low, even below the detection limit for Groenekan, Soestduinen and Kralingen. For the other locations, an additional two-fold dilution would yield ATP concentrations at or below the detection limit. The FCM and BactoSense showed relatively good twofold reductions. For Nieuw-Lekkerland and Soestduinen (FCM) and Soestduinen, Kralingen (BactoSense) the furthest diluted water samples (8x and 16x) did not result in twice an additional twofold reduction, but a 1.2 – 1.6 fold reduction. The microscopy method showed more variation and between 0.7 – 2.4 fold dilutions per dilution step were measured. The BACTcontrol showed no clear dilution curve for the several drinking waters. The enzymatic activity in the undiluted water of Nieuw-Lekkerland and Soestduinen was already low and close to the detection limit. For Kralingen and Zuid-Beijerland, both with the highest enzymatic activity in the undiluted drinking water, the results varied. The twofold dilutions were observed to some extent, but were not consistent and the fourfold diluted drinking water from Zuid-Beijerland yielded even higher enzymatic activity than the undiluted and twofold diluted water samples, indicating that the enzyme activity might be inhibited in the undiluted drinking water.

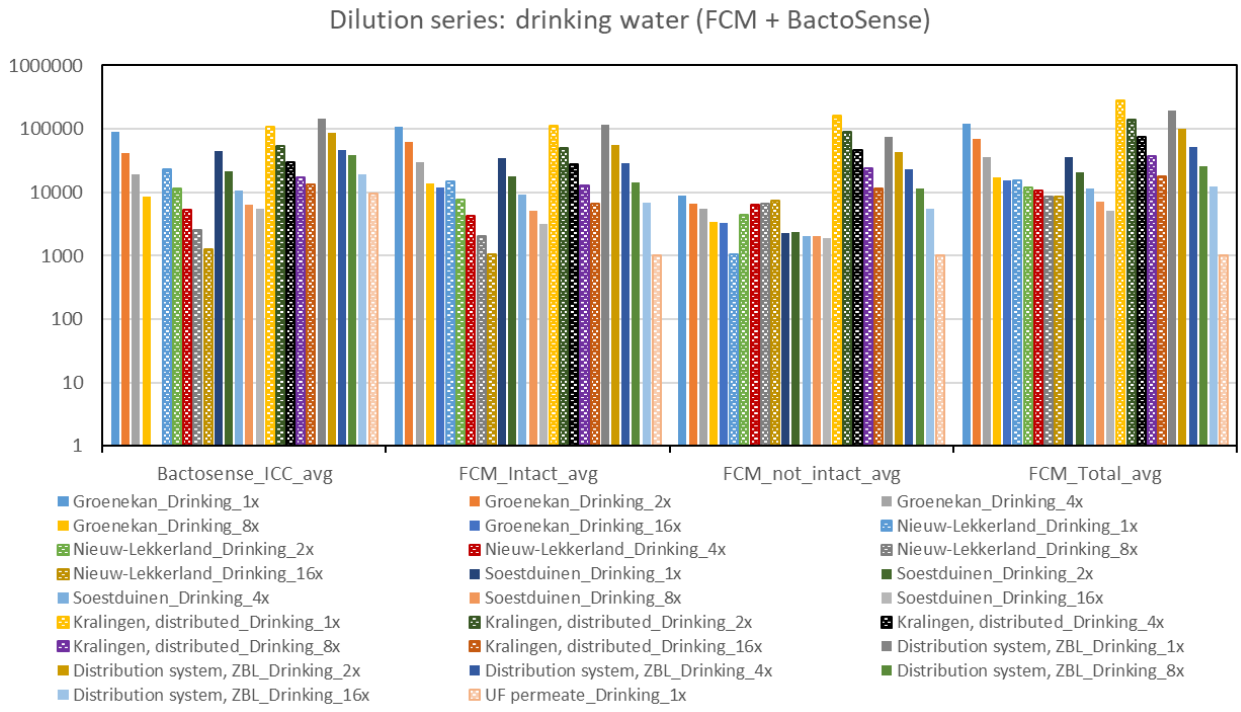


Figure 3-6. Dilution series of drinking water, the dilution factor is given. Given are the average results of FCM and BactoSense sensor. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.

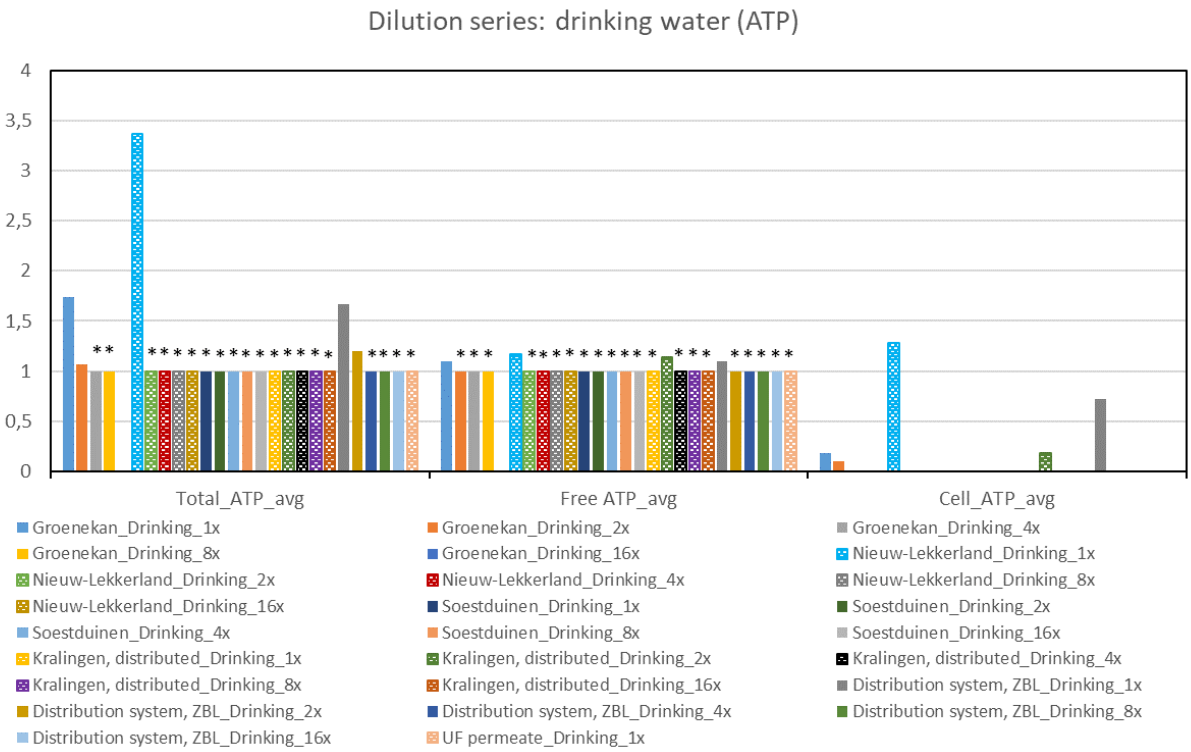


*Figure 3-7. Dilution series of drinking water, the dilution factor is given. Given are the average ATP-results. * value of Total_ATP or Free_ATP below detection limit. As Cell_ATP was calculated, the calculated value is given, without marking with *. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.*

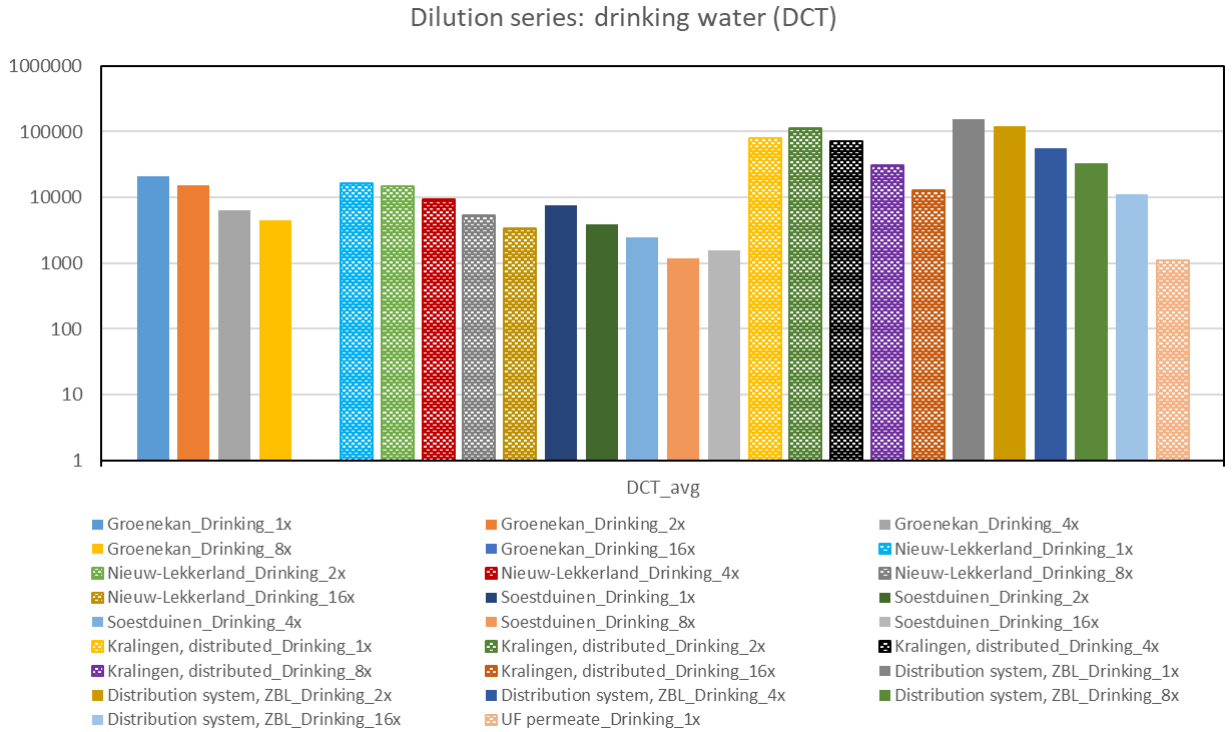


Figure 3-8. Dilution series of drinking water, the dilution factor is given. Given are the average DCT-results. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.

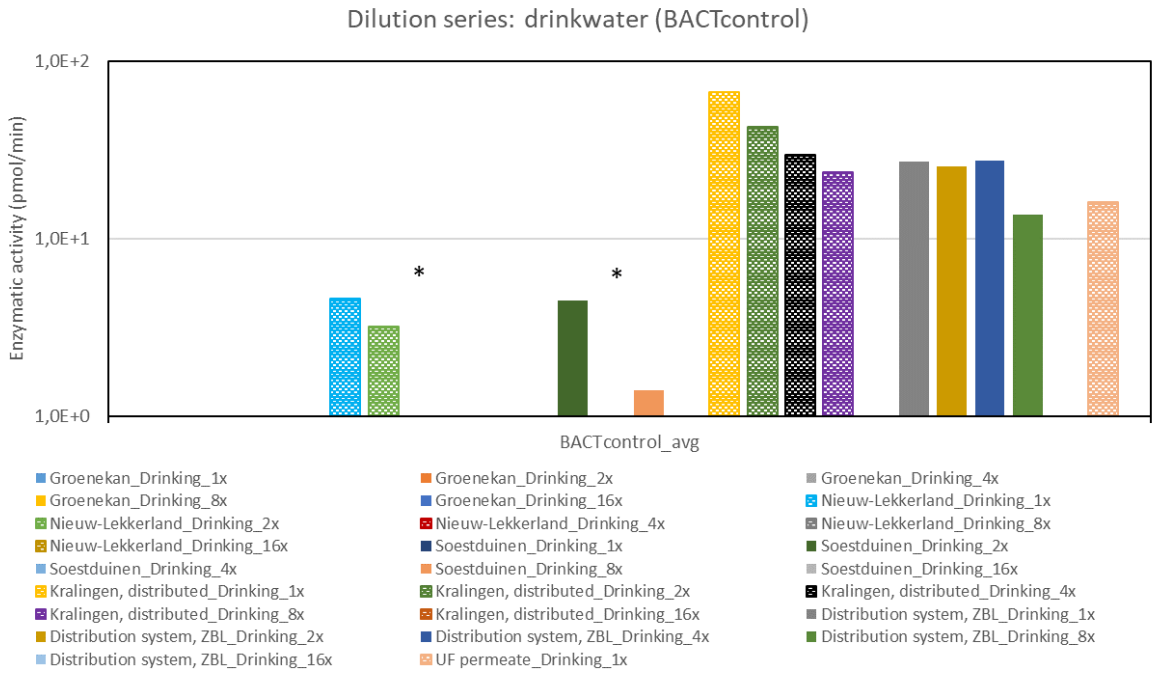


*Figure 3-9. Dilution series of drinking water, the dilution factor is given. Given are the average results of the BACTcontrol sensor. # value above the detection limit, * value below detection limit. Results are shown in Table 12-1, Table 12-2, Table 12-3 in appendices I.I and I.II.*

3.3.1.4 Correlation sensors with biomass parameters

Linear correlation analyses were performed on the log-transformed values of the above-described validation results to test for statistic significant correlations between the sensors and laboratory methods (Table 3-7). The results showed good to excellent correlations between all sensors and laboratory methods for surface water ($R^2 > 0.7$). In contrast, for all cooling waters only a few moderate or good correlations between the sensors and laboratory methods were found. Although the BactoSense and FCM use the same measuring principle, a significant correlation between the results from these two methods (BactoSense_ICC and FCM_intact) was only found for surface water of the river Rhine. This apparent discrepancy was probably caused by the differences obtained with the tenfold dilution series. With the BactoSense a tenfold reduction in cell numbers with these tenfold dilutions was less often observed than with the FCM. In addition, the detection limit of the BactoSense was lower than the detection limit of FCM, which affected the correlation. The BACTcontrol correlated excellently with the BactoSense for cooling water of Uniper, and the BugCount Online correlated excellent with FCM and total ATP concentration for cooling water of Dow.

A possible explanation for the poor correlation between the results from the different detection methods with the different cooling waters is that the disinfection measures that are used with these water types affect the results of some methods. In the cooling towers ozone (Uniper) or chlorine (Dow, BASF) is added as disinfectant, combined with the transport and storage time of the water samples to the KWR laboratory, this most likely yields different degrees of dead, dying or decaying cells and biomass. It is possible that the different viability states of the microorganisms affect the different tested methods in a different way, which results in poor relationships between the results of the different methods.

Table 3-6. Results of linear regression analyses on log transformed measurements of cooling and surface water. Given is de R^2-value if the p-value of the linear regression analysis was below 0.05 (significant correlation) and at least 3 measurements were available. If the p-value was above 0.05, no R^2-value is given. The R^2-values were graded as follows: <0.5 weak correlation between two parameters; 0.51 – 0.7 moderate correlation; 0.7 – 0.9 good correlation; >0.9 excellent correlation. NM: not measured. ND: not enough (reliable) BACTcontrol results. All water types: drinking water, cooling water, surface water.

BACTcontrol								
	All watertypes	Cooling water	BASF	CW_after	CW_before	DOW	Uniper	Surface Water/Rhine
BACTcontrol								
BactoSense_ICC		0,62		0,96				0,70
BugCount			NM					0,97
ATP_Cell								0,89
ATP_Free								0,77
ATP_Total								0,89
FCM_Intact	0,17							0,92
FCM_Not_Intact								0,92
FCM_Total						0,69		0,92
Microscopy	0,45							0,89
BactoSense								
	All watertypes	Cooling water	BASF	CW_after	CW_before	DOW	Uniper	Surface Water/Rhine
BACTcontrol		0,62		0,96				0,70
BactoSense_ICC								
BugCount	0,62							
ATP_Cell					1,00			0,85
ATP_Free	0,40							0,89
ATP_Total	0,49							0,91
FCM_Intact	0,79	0,79						0,92
FCM_Not_Intact	0,39	0,69			0,76	0,62		
FCM_Total	0,72	0,81			0,82	0,64	0,88	0,92
Microscopy	0,41	0,51					0,78	0,77
BugCount Online								
	All watertypes	Cooling water	BASF	CW_after	CW_before	DOW	Uniper	Surface Water/Rhine
BACTcontrol								0,97
BactoSense_ICC	0,62							
BugCount								
ATP_Cell	0,50							0,94
ATP_Free	0,60		NM			0,75		
ATP_Total	0,80				0,99			0,91
FCM_Intact	0,85				1,00	1,00		0,85
FCM_Not_Intact								0,76
FCM_Total	0,51				1,00			0,85
Microscopy	0,33							0,95

The linear correlation analysis showed excellent correlations between intact cells measured with the BactoSense and the laboratory flow cytometry method ($r^2 > 0.9$; Table 7), which is not surprising since both methods are based on the same measurement principle. Correlation was best for each individual drinking water ($r^2 > 0.9$; Table 3-7) and somewhat lower, but still moderate to good for the combined drinking waters and all combined water types ($r^2 > 0.66$). Similar results were obtained for the correlation between the BactoSense with microscopy. Comparison of the actual measured values (Figure 12-9 in appendix III) shows that these are also comparable between BactoSense_ICC and FCM_Intact. For the Groenekan dilution series the BactoSense_ICC yields about 0.5 $\log_{10}$ lower results compared to FCM_Intact, whereas this is the opposite for the Zuid-Beijerland dilution series.

For the BACTcontrol, the correlation analysis was only performed for Kralingen and Zuid-Beijerland, for which enough reliable data points were available. This data showed an excellent correlation with the BactoSense and laboratory flow cytometry parameters ($r^2 > 0.9$; Table 3-7), but for Zuid-Beijerland these correlations were absent. Both BactoSense and BACTcontrol did not correlate with ATP, but this is caused by the limited number of measurements of ATP above the detection limit.

Table 3-7. Results of linear regression analyses on log transformed measurements of drinking water. Given is de R^2-value if the p-value of the linear regression analysis was below 0.05 and at least 3 measurements were available. If the p-value was above 0.05, no R^2-value is given. The R^2-values were graded as follows: <0.5 weak correlation between two parameters; 0.51 – 0.7 moderate correlation; 0.7 – 0.9 good correlation; >0.9 excellent correlation. NM: not measured. ND: not enough (reliable) BACTcontrol results.

BACTcontrol							
	All watertypes	Drinking water	Groenekan	Kralingen	Nieuw-Lekkerland	Soestduinen	Zuid-Beijerland
BACTcontrol							
BactoSense_ICC				0,99			
BugCount		NM		NM			NM
ATP_Cell							
ATP_Free			ND		ND	ND	
ATP_Total							
FCM_Intact	0,17			0,98			
FCM_Not_Intact		0,83		0,97			
FCM_Total		0,69		0,98			
BactoSense							
	All watertypes	Drinking water	Groenekan	Kralingen	Nieuw-Lekkerland	Soestduinen	Zuid-Beijerland
BACTcontrol			ND	0,99	ND	ND	
BactoSense_ICC							
BugCount	0,62	NM	NM	NM	NM	NM	NM
ATP_Cell							
ATP_Free	0,40						
ATP_Total	0,49						
FCM_Intact	0,79	0,84	1,00	0,98	1,00	0,97	0,98
FCM_Not_Intact	0,39	0,32	0,97	0,96			0,97
FCM_Total	0,72	0,66	1,00	0,97	0,94	0,99	0,98
Microscopy	0,41	0,57	0,96		0,96	0,95	0,96

3.3.2 Discussion

3.3.2.1 Suitability for pilot locations

Surface water

The BACTcontrol, BactoSense and BugCount Online were able to measure undiluted surface water reliably. It was observed that surface water contained a high number of cells, which was around the upper detection limit of the BactoSense. This means that for online tests on surface water at the pilot locations, an inline predilution step might be necessary for the BactoSense to prevent missing data when cell numbers are above the upper detection limit. The BACTcontrol and BugCount Online do not have this potential problem.

Cooling water

The water matrix of the Uniper cooling water, cooling water with ozone disinfection, seemed to interfere with especially the BactoSense measurements. Ozonation seemed to create cell debris (or other particles) that were detected by the BactoSense and wrongly identified as bacterial cells. Others have shown in drinking water that ozone and chlorine disinfection decreased the total number of cells as measured with FCM and that the disinfection effects may differ between different types of cells [10]. Such an effect of disinfection apparently also occurred when surface water with disinfectant was studied. Another problem observed with Uniper water was that bubbles interfered with the BactoSense. This 'bubble error' was most likely caused by storage of the water sample between sampling and measuring (max 20 hours). Upon arrival of the water samples at KWR no bubbles were present, however, after storage and upon measurements bubbles were visible, suggesting the bubbles were formed during storage and/or dilution of the water samples. As the BactoSense will sample and measure the water directly when installed at Uniper, this type of errors seems unlikely to happen on location.

Based on the results obtained from the validation study, the other two sensors (BACTcontrol and BugCount Online) seemed to be able to measure undiluted cooling water from Uniper reliably.

The BACTcontrol was able to measure the cooling water of BASF and Dow reliably and the BugCount Online can also measure Dow cooling water reliably. The BactoSense has no 'bubble error' for these two cooling water types, but the cell number is around the upper detection limit. Similar to the surface water a predilution step might be necessary for the BactoSense upon inline operation at a cooling water pilot location.

Effect of dilution on measurements for cooling water and surface water
For the 1:10 dilution series the dilutions were made in a buffer solution without particles. Therefore, not only the number of cells was diluted but also the number of particles. This low particle content may make it easier for the sensors to measure the water samples. All the abovementioned factors will have to be tested during the on-site and online measurements at the pilot locations.

Drinking water
In general, the BACTcontrol and BactoSense measured the drinking water from different locations reliably, which was expected as both sensors were developed for this water matrix. However, the dilution series of drinking water from three locations did not yield reliable results with the BACTcontrol, but this was caused by a technical problem with the diluent applied, and not because of the BACTcontrol. It was observed that for undiluted drinking waters of Soestduinen and Nieuw-Lekkerland the enzymatic activity as measured with the BACTcontrol was low and close to zero, and consequently, the twofold dilutions could not be measured correctly as the values dropped below the detection limit. At the pilot locations with a low enzymatic activity baseline, this might mean that fluctuations, and especially dips in enzymatic activity, might not be detected. The BactoSense did show the twofold dilutions for most measurements, regardless of the source of the drinking water. However, the 8x and 16x dilutions sometimes showed lower dilutions, most likely because the number of cells was approaching the lower detection limit.

3.3.3 Conclusions

Bacterial cell numbers or microbial biomass in surface water of the Rhine River could be reliably measured with the sensors BactoSense, BACTcontrol and BugCount Online and the results of these sensors with surface water of the Rhine River correlated well with the laboratory methods.

Good results were obtained with the tenfold dilution series on surface water, especially for the laboratory methods the tenfold dilutions were visible in the results. The tenfold dilutions were also visible for the sensors, but the reduction was not always tenfold upon higher dilutions. The tenfold dilutions were not, or only to a limited extent, visible for the different cooling tower waters. It was concluded that the data coming from these sensors with cooling tower water should be interpreted with care.

For cooling water, correlations between cell numbers or biomass obtained with the sensors and with laboratory methods were in general not significant. This was most likely caused by the presence of a disinfectant (ozone or chlorine) in the water and the time between sampling at the plant and measuring in the KWR laboratory which resulted in different degrees of living, dying, dead and decaying cells. This is in line with the conclusion that the data coming from these sensors with cooling tower water should be interpreted with care.

It is expected that the sensors will perform better when installed directly onto the cooling water at the pilot location. As the sensors are installed directly onto the water stream and thus measure the water directly, without transport or storage time involved, water quality changes or errors caused by transport and storage will most likely be avoided. The sensors can thus be applied and tested in the pilot location with cooling water.

The number of cells in cooling water, especially the Dow cooling water, was high and close to or above the upper detection limit of the BactoSense. When the BactoSense is applied in the field with this cooling water, a predilution step might have to be included for the BactoSense as, otherwise, it may not result in reliable results. The other sensors did not show problems with the high number of cells. The ICC of the BactoSense is not overestimated and the absolute numbers of this parameter can be used reliably to compare between samples.

Bacterial cell numbers or microbial biomass in drinking water could be reliably measured with the BactoSense and to a lesser extent with the BACTcontrol. The BACTcontrol measured drinking waters with a higher biomass more reliably. The twofold dilutions were especially visible for the BactoSense sensor and the flow cytometry laboratory method. The other methods (ATP, microscopy, BACTcontrol) did show a dilution, but this was more variable (microscopy) or not consistent (BACTcontrol, ATP), most likely because the undiluted drinking water from some locations was close to the lower detection limit. For locations with low biomass concentration, the BACTcontrol might not show all the variation in microbial biomass in the drinking water, as possible dips can be missed. Based on the results it is concluded that both BactoSense and BACTcontrol can be applied in the pilot locations with drinking water.

3.4 Validation of CBM and ATP-kit

In previous studies it was shown that the CBM of Milispec is comparable to the CBM of KWR [7]. In this validation study it was investigated whether ATP concentrations of the biofilm obtained with the onsite biofilm DSA-swab kit of LuminUltra (Milispec/LuminUltra ATP method) was comparable to the ATP concentrations of the biofilm obtained with the KWR laboratory ATP method (KWR ATP method).

3.4.1 Results

Analysis of the ATP concentration in the biofilm that was formed in the CBM shows that the Milispec/LuminUltra ATP method consistently yields higher results than the KWR ATP method (Figure 3-10 and Table 12-4,

Table 12-5 in Appendix VI). The difference in biofilm ATP concentrations between both ATP-methods is visible for biofilm formed in the CBM fed with drinking water and surface water. The difference between ATP concentrations measured with the Milispec/LuminUltra method and KWR method is more pronounced when more biofilm, and thus a higher ATP-concentration, was present. The difference between ATP concentrations measured with a LuminUltra ATP drinking water kit and the ATP method that KWR uses, was already known from a previous project [7], but the same trend is thus visible between the KWR method for ATP-analysis in biofilm and the DSA-swab kit of Milispec/LuminUltra for biofilm analysis which was used in this project. The difference between the two ATP-methods varies within and between water types (Figure 3-10). On biofilm formed in the CBM fed with drinking water the Milispec/LuminUltra ATP-kit yields on average 1.9 ± 0.8 times higher ATP concentrations compared to the KWR ATP-method (Table 12-4 in Appendix VI), whereas for biofilm formed in the CBM fed with surface water this difference is larger (factor 6.0 ± 4.5;

Table 12-5 in Appendix VI).

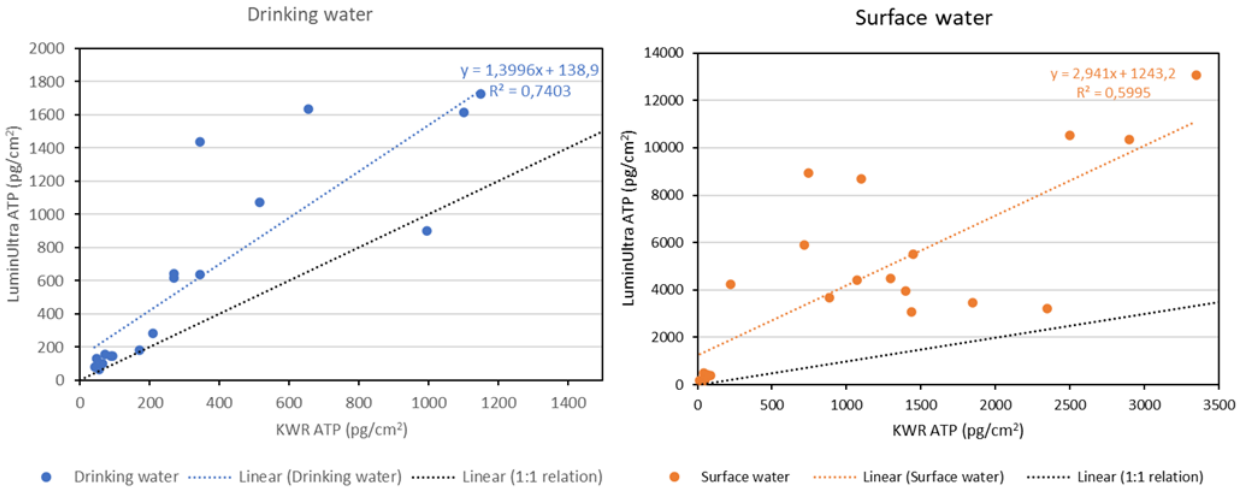


Figure 3-10. ATP-concentrations of the biofilm formed in CBMs on KWR drinking water (left) and surface water of the river Meuse at Keizersveer (right). The ATP content of the biofilm was measured with the KWR-method and DSA-swab method of LuminUltra. Shown are the individual data points of three CBMs per sample day.

The ATP concentrations of the biofilm can be used to calculate the biomass accumulation rate (BAR) on drinking water and the biofilm formation rate (BFR) on surface water (Figure 3-11). By doing so, the nutrient concentration in water responsible for biofilm formation can be compared between different measurement periods and locations. The BAR of biofilm formed on KWR drinking water varies largely during the measurement period (KWR: 4 – 77 pg ATP/cm^2.d, Milispec/LuminUltra: 7 – 111 pg ATP/cm^2.d; Figure 3-11). These BAR-values are also much higher than in previous studies [8] in which the BAR on KWR drinking water was around 10 pg ATP/cm^2.d. This has likely to do with the duration of which the glass pearls were present in the CBM. This duration was previously optimized for drinking water (28 days; [4, 5]), but to perform the validation study longer and shorter time periods were chosen to obtain more or less biofilm. However, this might cause extra variation.

As a consequence of the higher ATP concentrations measured with the Milispec/LuminUltra-method, the Milispec/LuminUltra ATP-method also gives higher BAR-values than the KWR-method. The correlation between both methods varies when individual samples are compared (blue dots in Figure 3-11), but if the average is calculated of the three CBMs that were simultaneously sampled, the variation between the two methods is smaller. However, the BAR remains high (KWR: 5 – 48 pg ATP/cm^2.d, Milispec/LuminUltra: 8 – 88 pg ATP/cm^2.d).

The BFR on surface water is even more variable, most likely due to large variations in the surface water (rain, temperature, particles, etc). It is expected that the water quality and composition at the non-drinking water pilot locations will be more stable (as weather influences are absent and temperature and particles composition of the water is less variable) and that the variation in the CBM results from the pilot locations is smaller compared to this study.

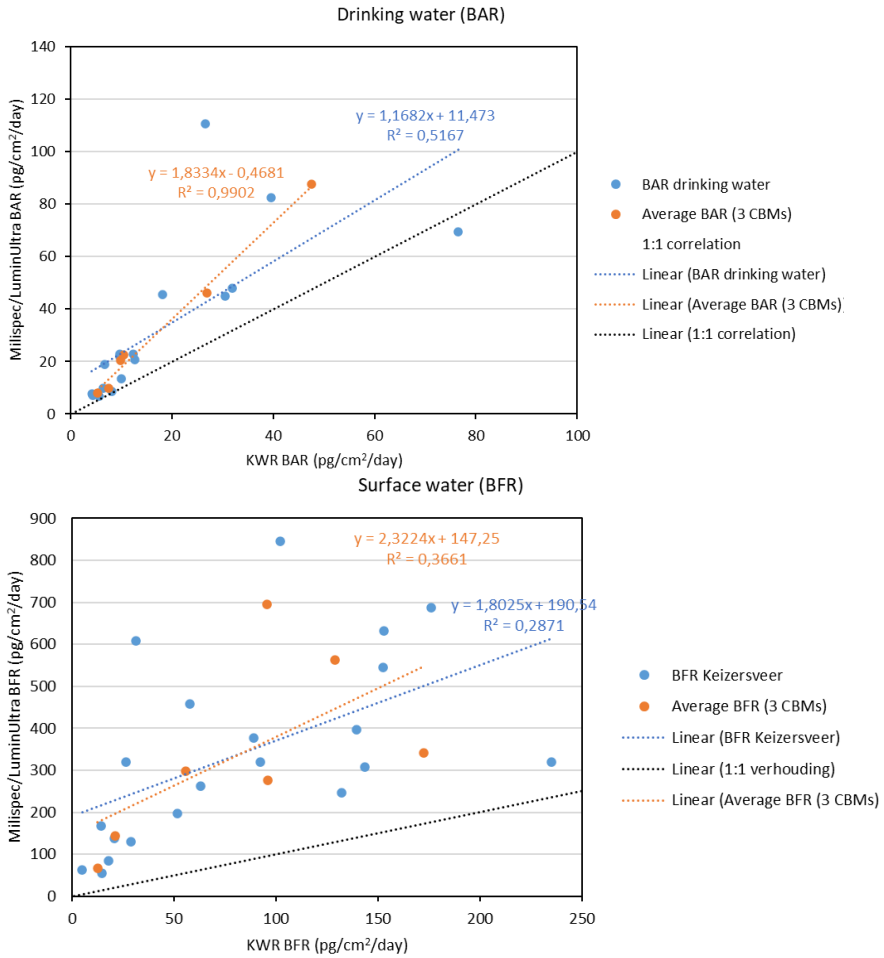


Figure 3-11. BAR-values of KWR drinking water (top) and BFR-values of river Meuse surface water at Keizersveer (bottom). The ATP content of the biofilm was measured with the KWR- and Milispec/LuminUltra-method. Shown are the individual data points of three CBMs and the average of three CBMs (6 cuvettes) per sample day.

The BFR can be influenced by seasonal differences such as water quality (nutrient level and composition) and water temperature, as at higher nutrient levels and higher temperature, within a temperature range of 0 to 15°C, more biofilm is likely to be formed. In Figure 3-12 the BFR-values are compared to the water temperature at Keizersveer. The differences in water temperature are limited (2.6 – 12.6°C) and do not seem to influence the BFR. The influence of the temperature may be higher when the temperatures increase further, as microbiological activity in general is limited at these low temperatures.

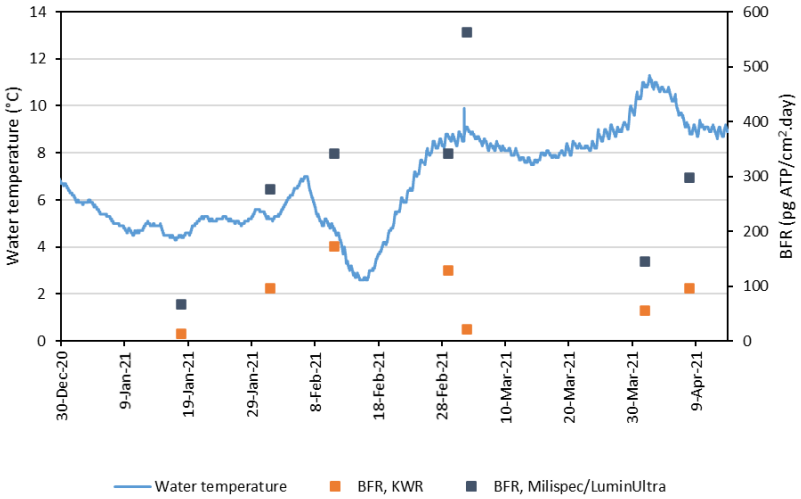


Figure 3-12. Average BFR values (of 3 CBMs) and water temperature of the river Meuse at Keizersveer where the CBMs were installed. Information on water temperature was derived from Rijkswaterstaat (www.waterinfo.rws.nl/).

3.4.2 Statistical analysis

3.4.2.1 According to NEN 16140-2:2016

Relative trueness study

In Figure 3-13 the Log_{10}-transformed results of the ATP concentration on the glass coupons (surface water) and glass beads (drinking water) are shown in a scatter plot. This plot provides a rapid visual assessment to see to which extent the methods agree with each other. As shown earlier (Figure 3-10, with results that were not yet Log_{10}-tranformed) the methods agree quite well with each other, provided that the Milispec/LuminUltra-method yields higher results than the KWR- method.

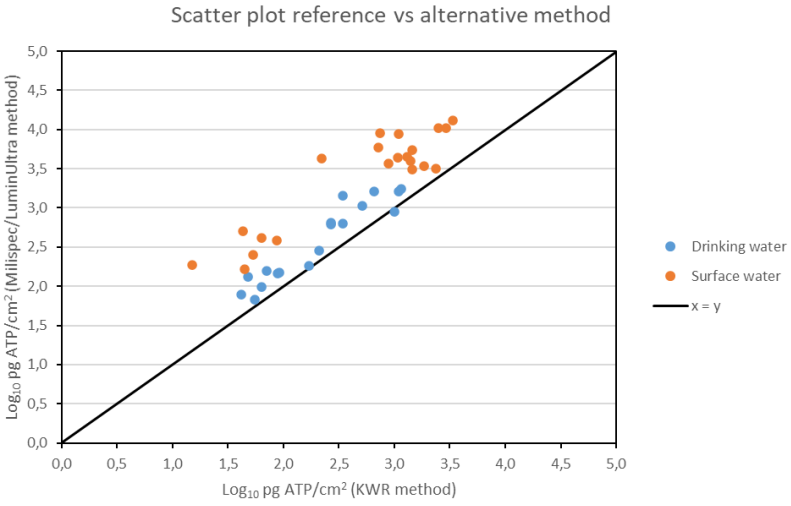


Figure 3-13. Relative trueness study: Scatter plot of Log_{10}-transformed ATP-concentrations of the biofilm measured with the KWR-method (reference method) and with the Milispec/LuminUltra-method (alternative method).

In Figure 3-14 the difference between reference and alternative method are plotted against the mean value of the reference and alternative method in a Bland-Altman graph. It is expected that only one out of 20 data points will lie outside the Limit of Agreement (LoA). In total 18 (drinking water) or 21 (surface water) samples have been measured. If the drinking and surface water results are combined in one graph and the LoA is calculated with both datasets (upper graph in Figure 3-14) many data points lie outside the LoA (5x surface water and 8x drinking water).

If the LoA is calculated per individual water type (lower graphs in Figure 3-14) less data points lie outside the LoA (3x drinking water, 4x surface water), however this is still more than the expected 1 in 20 outliers.

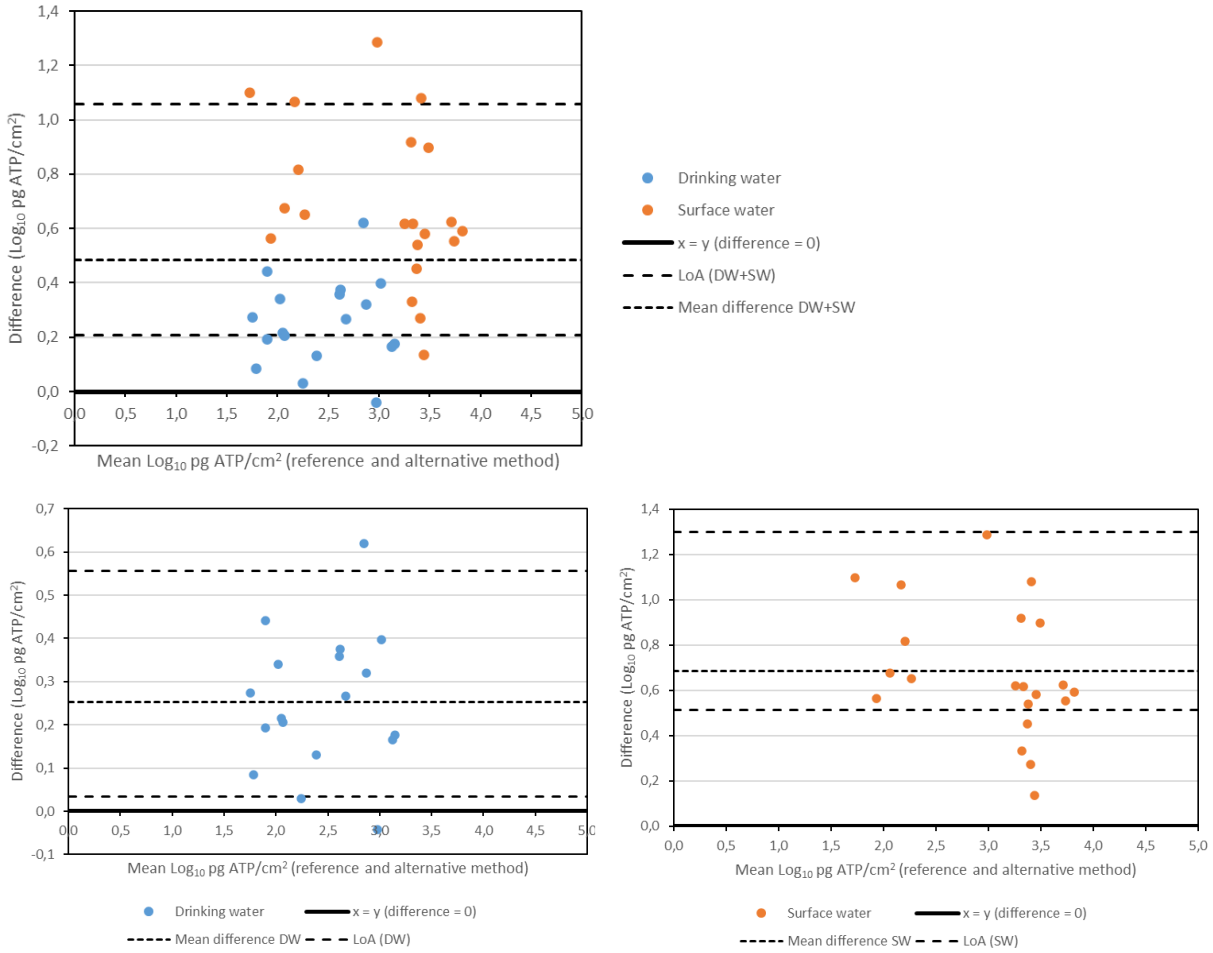


Figure 3-14. Relative trueness study: Bland-Altman difference plots for drinking and surface water

Accuracy profile

A summary of further statistical analysis of the data for the 'Accuracy profile study' is shown in Table 3-8, with more details in Appendix VII.

The parameters bias (the difference between both methods, B_i), upper and lower β-ETI (the interval in which it is expected that 80% of the results will fall, U_i and L_i) are visualized in Figure 3-15 and compared with the Acceptability Limit (AL, set at 0.5 Log_{10}). If both methods (KWR and Milispec/LuminUltra) would give identical results, the bias would be 0. As shown earlier, this is not the case since the Milispec/LuminUltra method yields higher ATP concentrations than the KWR method. This is visualized by the light blue bias line which is always > 0 in Figure 3-15. Further statistical analysis was performed to test whether these differences are acceptable according to the NEN-EN-ISO 16140-2:2016 standard.

The upper and lower β-ETI (U_i and L_i) of drinking water are within the -AL and AL range of -0.5 to 0.5 (all data points are between the two dashed lines in Figure 3-15). However, for surface water nearly all upper β-ETI (U_i) values are above the upper AL of 0.5 (red numbers in Table 3-8 and nearly all light blue data points in bottom left graph of Figure 3-15 are above the upper dashed line). Because some lower and upper β-ETI values are outside of the AL a new AL, named AL_s, was calculated (-1.5 to 1.5 Log_{10}, Table 3-8) conform the standard. There are no requirements on the height of the new AL_s. All upper β-ETI values are within this new AL_s range.

For drinking water taken at KWR, the $U_i \leq$ Upper AL and the $L_i \geq$ Lower AL, and thus the alternative method (Milispec/LuminUltra ATP-method) is regarded as equivalent to the reference method (KWR ATP-method). For surface water the $L_i \geq$ Lower AL, but the U_i does not comply with $U_i \leq$ Upper AL. However, as U_i does comply with $U_i \leq$ Upper AL_s, the alternative method is also accepted as equivalent for the reference method for surface water.

Table 3-8. Summary of statistical results of the 'Accuracy profile' study per individual water type. All results are in Log_{10} units. Italic: values lie outside of the AL and -AL range, but inside the new AL_s-range. More details on the calculations are shown in Appendix VII.

	CBM (days)	Media n ref	Media n alt	Bias	Upper β-ETI	Lower β-ETI	Upper/ Lower AL		Upper/ Lower AL_s	
		X_i	Y_i	B_i	U_i	L_i	AL	-AL	AL_s	- AL_s
Drinking water	10	1.74	1.89	0.15	0.28	0.02	0.5	-0.5		
	7	1.85	2.16	0.31	0.44	0.18	0.5	-0.5		
	21	2.23	2.26	0.03	0.16	-0.10	0.5	-0.5		
	28	2.43	2.80	0.37	0.50	0.25	0.5	-0.5		
	13	2.71	3.03	0.32	0.45	0.19	0.5	-0.5		
	36	3.04	3.21	0.17	0.30	0.05	0.5	-0.5		
Surface water	3	1.65	2.27	0.63	*0.76*	0.49	0.5	-0.5	1.50	-1.50
	3	1.80	2.62	0.82	*0.95*	*0.68*	0.5	-0.5	1.50	-1.50
	7	2.85	3.65	0.79	*0.93*	*0.65*	0.5	-0.5	1.50	-1.50
	14	3.11	3.57	0.45	*0.59*	0.32	0.5	-0.5	1.50	-1.50
	10	3.16	3.51	0.35	*0.49*	0.21	0.5	-0.5	1.50	-1.50
	28	3.16	3.95	0.79	*0.93*	*0.65*	0.5	-0.5	1.50	-1.50
	19	3.46	4.01	0.55	*0.69*	0.41	0.5	-0.5	1.50	-1.50

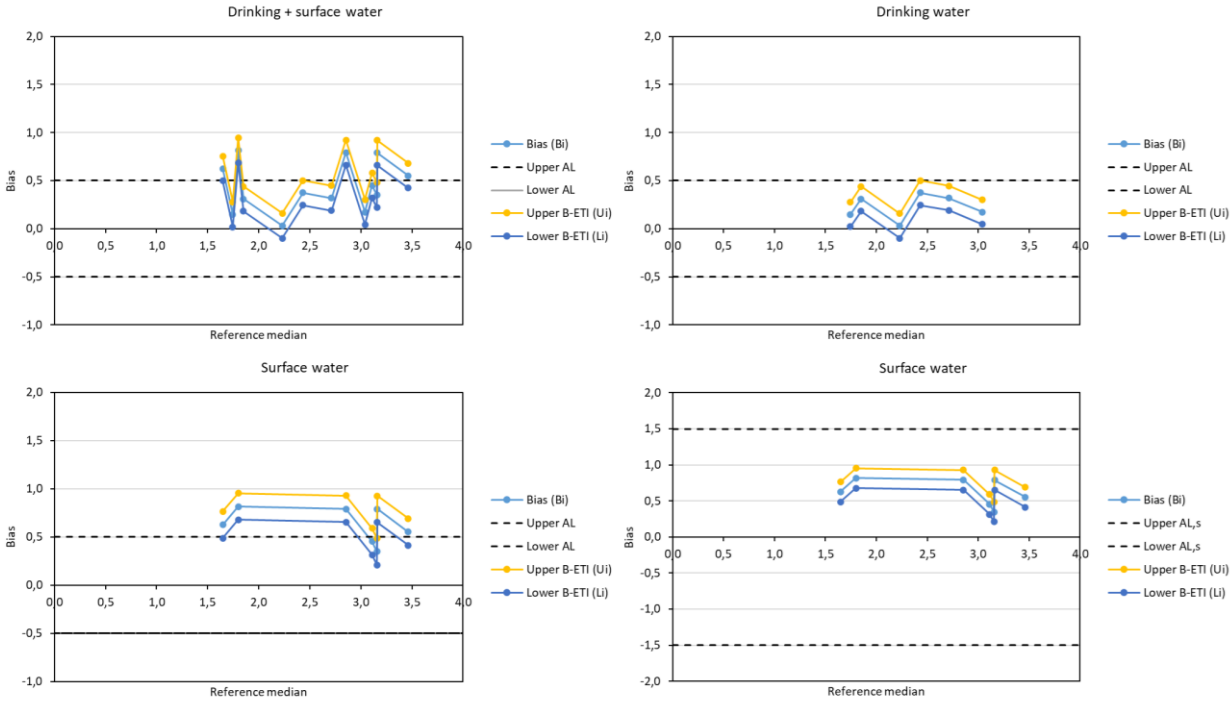


Figure 3-15. Visualization of the accuracy profile of the KWR ATP-method and the Milispec/LuminUltra ATP-method. All results are in Log_{10} units. Top left: drinking + surface water. Top right: drinking water. Bottom left: surface water with AL = 0.5 and -0.5. Bottom right: surface water with AL_s = 1.5 and -1.5.

3.4.2.2 Wilcoxon signed rank test

Comparison of the two ATP-datasets (Table 12-4 and

Table 12-5 of Appendix VII), using the Wilcoxon Signed Rank test for paired values, shows that the two ATP-methods (KWR and DSA-swab) differ significantly from each other ($p < 0.05$) and that the DSA-swab method gives higher ATP concentrations than the KWR method. This holds for when the dataset is split between drinking and surface water, or when both datasets are combined to one dataset.

3.4.3 Discussion

3.4.3.1 Application of CBM on non-drinking water locations

The CBM was originally designed for measuring drinking water, which contains limited number of particles. The tests with the CBM on surface water of the river Meuse show that the CBM has problems when it is fed with more dirty water, containing a high number of particles that can clog the system. To solve this problem, a candle filter was installed before the CBM and this filter was replaced twice a week. Because cooling water can also be quite dirty, it is advised that a candle filter is installed before the CBM, when it is used with cooling water, and that this filter is regularly replaced.

Traditionally, for drinking water every four weeks two cuvettes of the CBM are sampled and analyzed for the amount of biomass formed. The optimal measuring interval for surface water and cooling water is not yet known. Two factors play an important role in this: both water types contain a higher number of biomass and nutrients, which might result in fast biofilm development and a relatively quick stable biofilm concentration. If that is the case, the measuring interval might have to be shortened to produce reliable results. Counteracting this is the presence of disinfectants in cooling water which may inhibit or slow down biofilm formation in the CBM. Consequently, the optimal measuring interval has to be determined on site when the CBM is installed. It is therefore proposed to start with biofilm formation for seven days on these pilot locations, followed by 14 and 21 days. Based upon the biofilm ATP concentrations measured at each individual location, the optimum number of incubation days of the CBM cuvettes has to be set.

3.4.3.2 Replacing the KWR ATP-method with the Milispec/LuminUltra-method

The Milispec/LuminUltra-method yields higher results compared to the KWR-method, but these differences are not considered statistically significant according to the NEN-EN-ISO 16140:2 standard. The acceptable difference between the two methods is set at +0.5 and -0.5 Log_{10} for drinking water and +1.5 and -1.5 Log_{10} for surface water. However, in this study only drinking water from KWR has been tested. It is possible that drinking water with a higher biomass production potential does not fit within the range of +0.5 and -0.5 Log_{10} . It is therefore recommended to test more drinking waters. As no requirements are set for a maximum of the AL_s, the consequence of these large acceptability limits is that the results of the Milispec/LuminUltra-method can be 0.5 Log_{10} (3.16 times) or 1.5 Log_{10} (31.6 times) larger than the KWR-method, but according to the standard are still accepted as being comparable.

On the contrary, statistical comparison between the KWR-method and the Milispec/LuminUltra-method, using the Wilcoxon signed rank test, does show that the two methods differ significantly from each other.

The difference between the two methods is most likely caused by the different lysis buffers that are used to break open the cells and release the ATP. The Milispec/LuminUltra-method uses a stronger lysis buffer than the KWR-method, leading to more ATP release from microorganisms. This phenomenon has been shown before [7, 11].

In practice, a factor of 31.6 is a large difference for CBM-results. This would mean that, for example, a BAR of 40 pg ATP/cm^2/day (KWR-method) is comparable to a BAR of 1264 pg ATP/cm^2/day (Milispec/LuminUltra-method). Normally such a difference would be considered a major difference and indicates that the water 'suddenly' contains a very high amount of nutrients. Therefore, data sets built on the KWR ATP-method cannot be continued with the Milispec/LuminUltra-method without a considerable overlap time during which both methods are used. If no historical data are available, the Milispec/LuminUltra-method can be used without problem although comparison of the data to the historical knowledge built up in the past decennium remains difficult.

3.4.4 Conclusions

Both ATP-methods are suitable to measure ATP in the biofilm on glass beads or coupons of the CBM. However, the Milispec/LuminUltra-method consistently yields higher ATP counts due to a more stringent lysis buffer. This difference is not a constant factor, but is more pronounced for higher ATP concentrations in the biofilm than lower concentrations as well as for biofilms formed in the CBMs fed with surface water than in CBMs fed with drinking water.

Statistical analysis according to NEN 16140-2:2016 of the ATP-results of the KWR and Milispec/LuminUltra-method shows that the methods are considered to be equivalent to each other. However, due to the large acceptability limits (drinking water: +0.5 and -0.5 Log_{10} difference and surface water: +1.5 and -1.5 Log_{10}) replacing the current KWR method with the Milispec/LuminUltra-method may result in a large shift in the results due to which current results cannot be compared one-on-one to historical data. The Wilcoxon signed rank test does show significant differences between the two methods, with the Milispec/LuminUltra-method giving higher results than the KWR-method.

The Milispec/LuminUltra-method gives higher ATP results, but is easier to perform as it does not require a sonification step and the measurement can be performed on site without the requirements of a laboratory. Therefore, if no historical data are available, the data do not have to be compared with available historical data and/or analysis on site by own personnel is wanted, the Milispec/LuminUltra ATP method is suitable for this purpose.

For application of the CBM on non-drinking water locations, it is proposed to measure the ATP concentration in the biofilm after 7, 14 and 21 days of biofilm formation. The results from these analyses can then be used to determine the optimal incubation period of the cuvettes with the glass beads or coupons in the CBM.

4 Monitoring an UF installation and regrowth at Evides

4.1 Introduction test location

4.1.1 Location

In the Hoeksche Waard-West (HWW) Evides distributes drinking water produced from surface water. The surface water is treated at production location Berenplaat and transported to Oud-Beijerland (residence time: 6 – 8 hours), located in the Hoeksche Waard (Figure 4-1). In 2019 the Hoeksche Waard-West has been isolated from the rest of the Evides distribution network and the effect of an extra treatment step (ultrafiltration [UF], pore size: 150 kDa) at Oud-Beijerland on the biological stability of drinking water in the downstream distribution network is being studied. Evides has determined the biological stability of the drinking water in the Hoeksche Waard-West with among others the biomass production potential (BPP) of the drinking water (using ATP and cell count measurements) and the biomass accumulation rate (BAR) using the continuous biofilm monitor (CBM) of KWR. Previous measurements performed on water 'before UF' and after the clear water reservoir ('after CWR') showed that the UF reduces the ATP concentration (from 5.9 to 3.6 ng/l), cell counts (from $4.8x10^5$ to $7.4x10^3$ N/ml), biopolymer concentration, BPP and BAR (from 42 to 3.0 pg ATP cm^2 d^{-1}). The UF thus improves the biological stability and the microbiological quality of the drinking water distributed in the Hoeksche Waard-West.

Figure 4-1 Pilot location Evides, Oud-Beijerland. Indicated is the location of production location Berenplaat (open pin), UF in Oud-Beijerland (closed pin) and, schematically, the borders of the Hoeksche Waard-West.

4.1.2 Goal

Within this project it was tested whether the sensors can measure differences in the microbial water quality caused by the UF treatment of the drinking water. For this, the sensors were installed at three locations within the UF pilot plant in Oud-Beijerland (Figure 4-2):

1. Incoming drinking water, produced at treatment plant Berenplaat (BPL), at Oud-Beijerland (before UF);
2. Permeate of the UF at Oud-Beijerland (after UF);
3. Drinking water that leaves the clear water reservoir (after CWR).

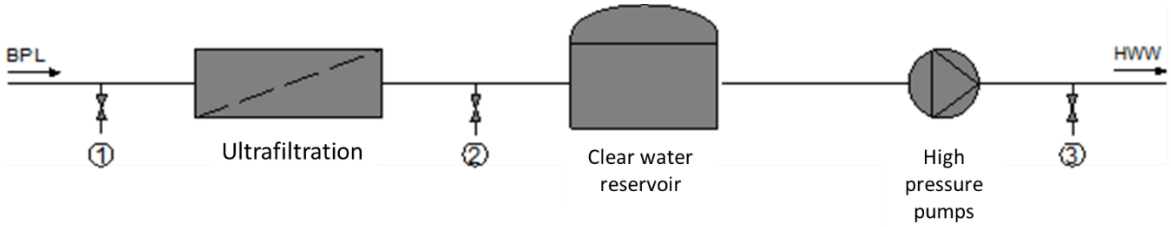


Figure 4-2 The different sampling locations at Evides.

4.2 Monitoring campaign

4.2.1 Experimental setup at Oud-Beijerland

The sensors were installed at the location Oud-Beijerland at three sampling points:

- Before ultrafiltration (QOBL20UFIN1; before UF): number 1 in Figure 4-2
- After ultrafiltration (QOBL45UFPER1.2; after UF): number 2 in Figure 4-2
- After clear water reservoir (QOBL80HDP; after CWR): number 3 in Figure 4-2

The monitoring period varied per location and per sensor. The BACTcontrol and BactoSense were installed for one month 'before UF' and 'after UF' and for two months 'after CWR' (Table 4-1). Two types of CBMs were used in this research: a more user-friendly CBM that was developed by Milispec (Milispec-CBM) which is based on the original CBM developed by KWR (KWR-CBM). The Milispec-CBM was tested at two sampling points for two months: before UF and after CWR. After the CWR, a KWR-CBM was installed for a long period (14 April – 13 October 2021) as part of Evides' own standard monitoring program. Before the UF a KWR-CBM was installed in the same period as the Milispec-CBM (May – July 2021). This enables the comparison between both types of CBMs, sampling of cuvettes and ATP-analyses.

The sensors and CBMs were all installed and replaced at the same time (before UF: 28 April 2021, after UF: 10 June 2021, after CWR: 15 July 2021). However, due to varying reasons, measurement data are not always available for the entire period. This is visible by examining the exact dates shown in Table 4-1.

*Table 4-1 Sampling locations and monitoring times. Given are the installation dates per sensor and for which period measurement data were available. * as part of Evides' own ongoing research*

		Before ultrafiltration (before UF)	After ultrafiltration (after UF)	After clear water reservoir (after CWR)
BactoSense	Installation period	28 April – 10 June 2021	11 June – 14 July 2021	15 July – 7 September 2021
	Data available	28 April – 26 May 2021	11 June – 14 July 2021	26 July – 8 August 2021
BACTcontrol	Installation period	28 April – 10 June 2021	11 June – 14 July 2021	15 July – 7 September 2021
	Data available	4 May – 10 June 2021	11 June – 14 July 2021	16 July – 7 September 2021
CBM Milispec	Installation period	28 April – 14 July 2021		15 July – 7 September 2021
	Data available	26 May – 7 July 2021		4 August – 1 September 2021
CBM KWR	Installation period	28 April – 14 July 2021		14 April – 13 October 2021*
	Data available	26 May – 7 July 2021		28 April – 1 September 2021*

The BactoSense and BACTcontrol sensors and the CBMs were all connected to the same sampling point via a splitter (Figure 4-4, left). This splitter divides the water stream in four separate streams, of which two streams led to the two CBMs, one stream led to the sensor platform (Figure 4-5) and one stream to a water tap for regular sampling and analysis. The sensors were placed on a movable platform with multiple levels (Figure 4-5). A schematic overview of the sensor platform is given in Figure 4-3. The water stream that enters the sensor platform is divided over several connections for the BACTcontrol, BactoSense and some reserve connections (Figure 4-4, right). This splitter has an open end to ensure a continuous water flow.

For connecting the sensors to the water stream every time new tubes, made of Teflon material that does not promote bacterial growth, were used at each sampling location. The splitter was made of stainless-steel material, without the usage of adhesives or other compounds that can promote bacterial growth.

The four-way splitter (Figure 4-4, left) and the splitting station (Figure 4-4, right) were cleaned before installation and after finishing the tests at Evides. Cleaning was done with SDS and citric acid to remove biofilm.

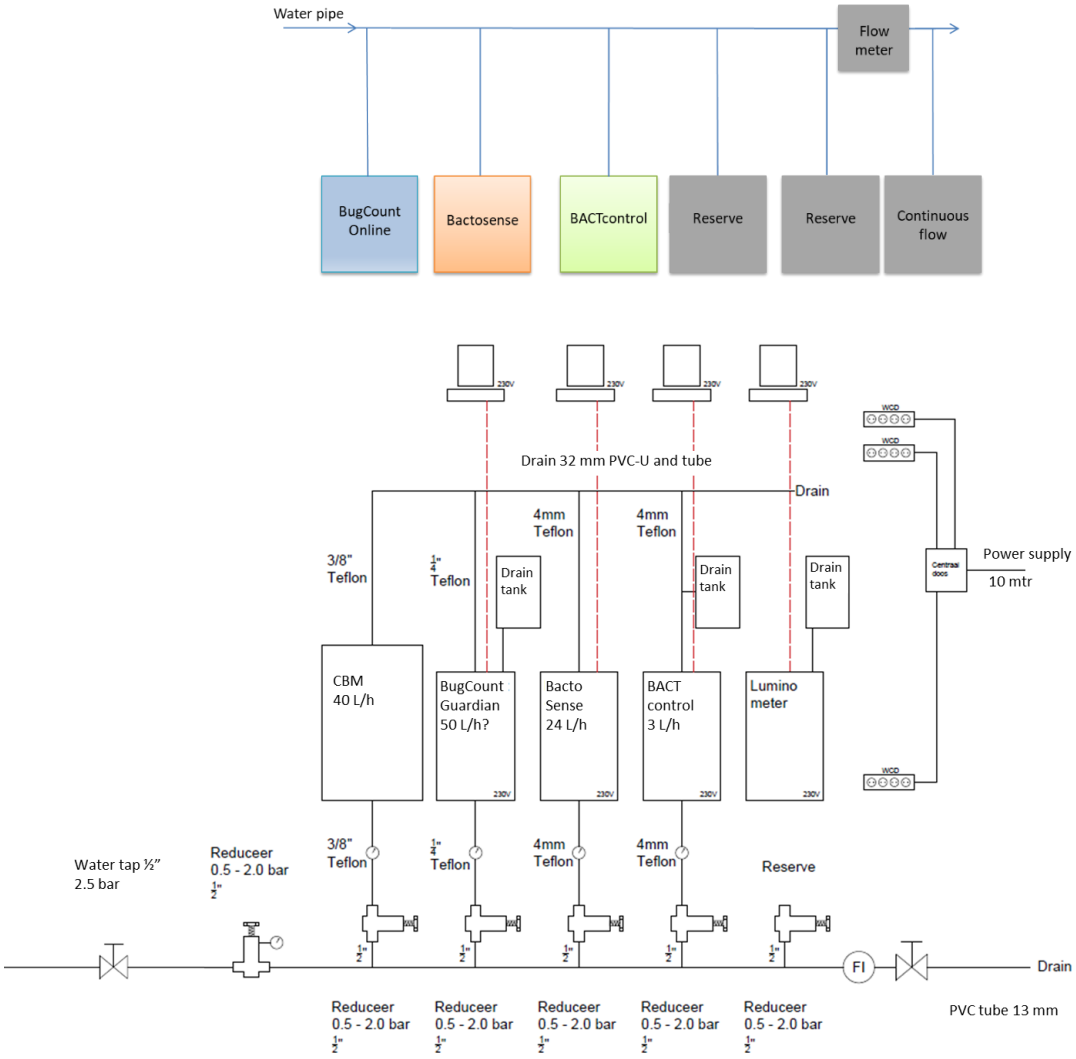


Figure 4-3. Top: Schematic overview of splitter station (photo in Figure 4-4, right) to divide the incoming water over the different sensors. The BugCount Guardian was not active at Evides. Bottom: More detailed schematic of the splitter station.

Figure 4-4. Left: sample point 'before UF' on which a splitting device is installed. The water is split 4-ways, from left to right: sample point for the official monitoring program of Evides, CBM of KWR, CBM of Milispec and connection for another splitter (right figure) to which the BactoSense and BACTcontrol are connected. Right: Splitter to which the sensors BactoSense, BACTcontrol, BugCount Online or Guardian can be connected. A schematic overview of the splitter is given in Figure 4-3.

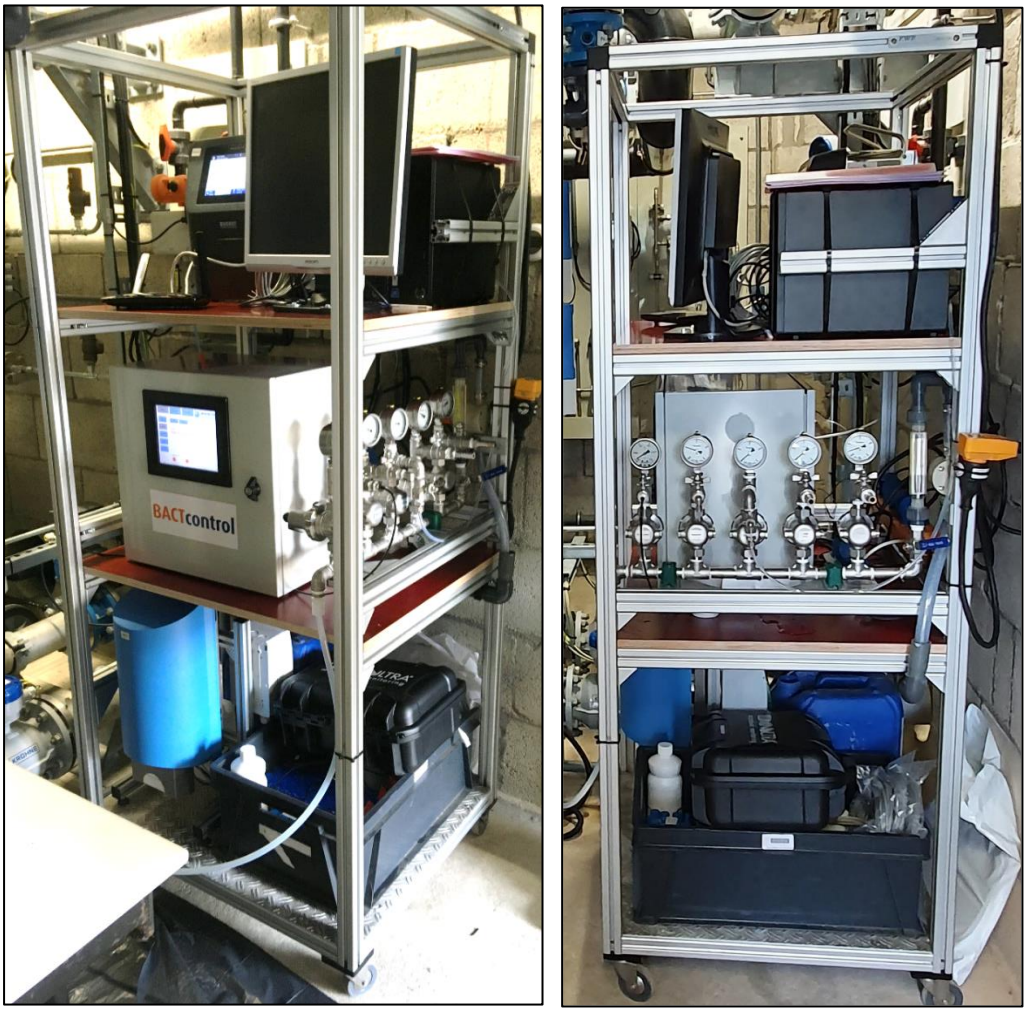


Figure 4-5. Top: Sensor set up at Evides. From top to bottom: BactoSense (partly hidden behind computer screen), BACTcontrol and splitter station, BugCount Online (not active at Evides), crate with reagents, consumable and other items to perform the ATP-analysis of the biofilm formed in the Milispec-CBM.

4.2.2 Monitoring details

The monitoring frequency of the BactoSense and BACTcontrol can be programmed and was set at a 2-hour interval for 'before UF' and 'after UF' drinking water. The interval for 'after CWR' drinking water was 2 hours at the start (from July 26 till August 25, 2021) and changed to a 6-hour interval towards the end of the monitoring period (from August 25 to September 7, 2021).

The Milispec-CBM was sampled every two weeks by Evides. The ATP content of the biofilm was measured with the LuminUltra ATP kit on-site by Evides (according to the protocol of Chapter 2). The KWR-CBM was sampled by KWR and analyzed at the microbiology laboratory of KWR. The sampling of the KWR-CBM and Milispec-CBM was done at the same day to ensure that the ATP content of the biofilm of both CBMs was determined on the same day.

Additional analyses by Evides

Evides performed additional analysis during the measurement period of the sensors. Results of the following measurements are shown in this report:

- KWR-CBM to determine BAR;
- BPP test;
- ATP;
- and cell counts using FCM.

4.2.3 Measurement, data processing and statistics

The measurements performed by the BACTcontrol and BactoSense sensors were done almost at the same time. However, it was not possible to schedule sampling of both sensors in such a way that measurements would be performed at exactly the same time.

BACTcontrol

The measurement results of the BACTcontrol were quality-controlled by microLAN before further data analysis. The first two measurement results after a temporary stop of the BACTcontrol (longer than 38 hours) were removed from the dataset and thus not used in data processing and interpretation. The results from these measurements were often higher than the results from the other measurements, and is caused by a technical aspect, because of biomass build-up during the standstill period of the BACTcontrol (Figure 4-6). Often several internal cleaning cycles of the BACTcontrol were required before measurements returned to a normal level.

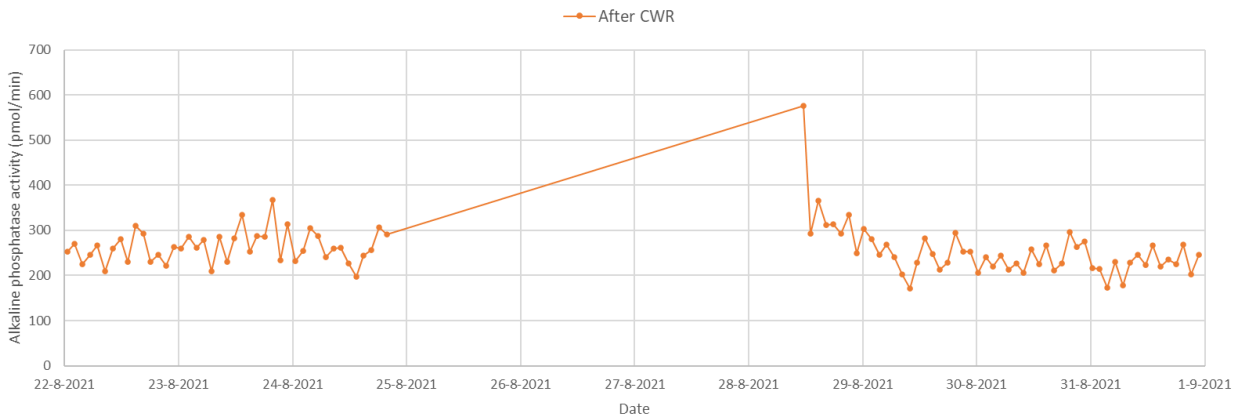


Figure 4-6 Increased activity after shutdown of the BACTcontrol.

BactoSense

The measurement results of the BactoSense were analysed together with APT (distributor of BactoSense) and bNovate (producer of BactoSense) to evaluate and, if necessary, to adapt the gates of the BactoSense for this specific water type. Based on the knowledge and experience of bNovate ICC, HNAC and LNAC were considered reliable and the absolute values, thus, can be used. An example of the results and gates for the three water types (before UF, after UF, and after CWR) is given in Figure 4-7.

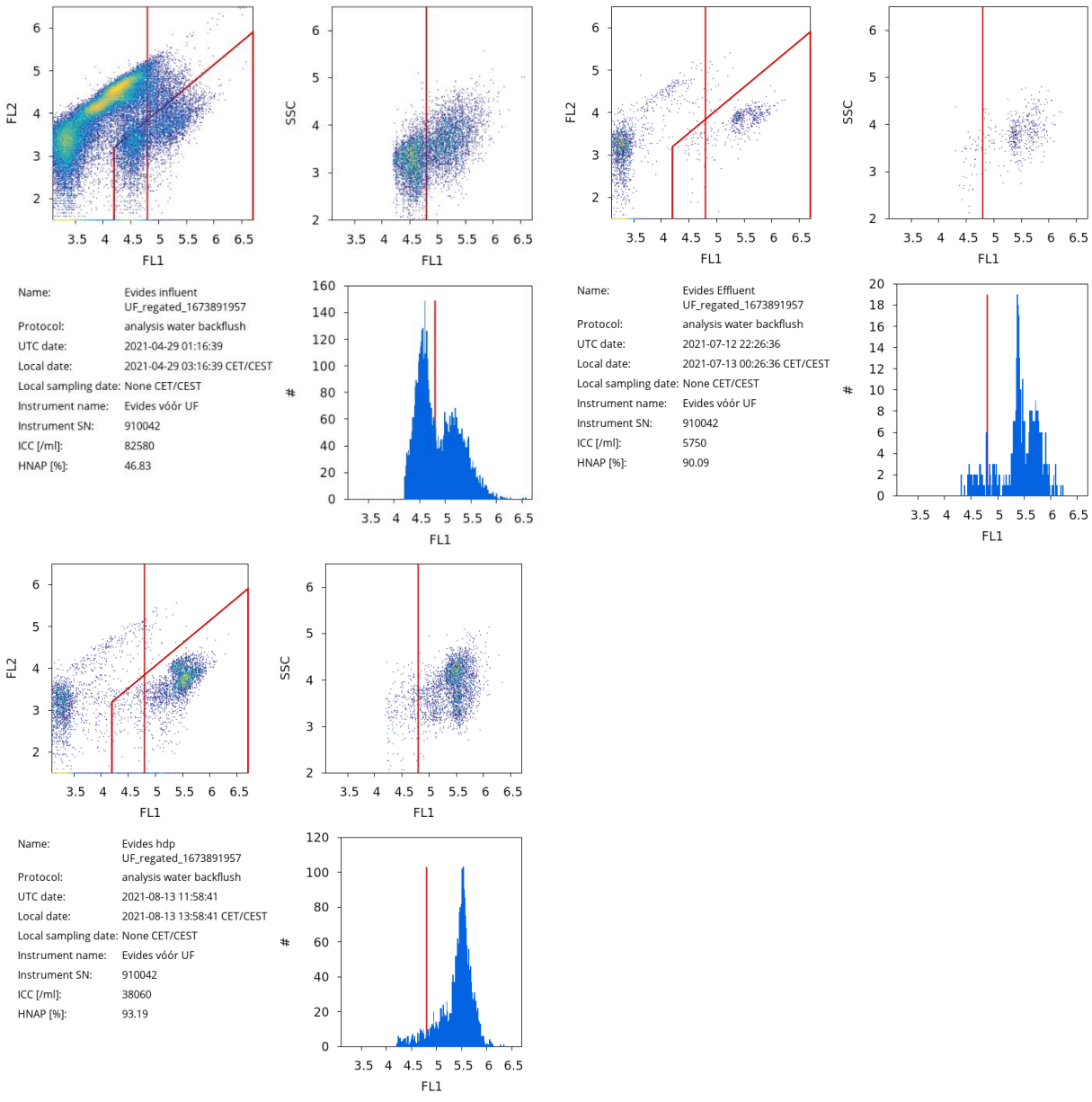


Figure 4-7 BactoSense results and gate examples before UF.

CBM

The Milispec-CBM and KWR-CBM were sampled every two weeks, and on the same date. The Milispec-CBM was sampled and analyzed by Evides. The ATP analysis was performed as described in paragraph 2.1.4. In brief, ATP was released from the bacterial cells of the biofilm on the glass beads using lysis buffer from the LuminUltra ATP-field kit, and the ATP-concentration was measured with a luminometer. The KWR-CBM was sampled and analysed by KWR. The ATP analysis was performed as described in paragraph 2.1.4. In brief, the cuvettes were sampled, and the glass beads were sonicated to release the biofilm. Subsequently, ATP was released from the bacterial cells of the suspended biofilm using the Celsis ATP kit and the ATP concentration of the released cells was measured with a luminometer.

Statistics

With a Shapiro-Wilkinson test it was tested whether the different datasets (BACTcontrol and BactoSense per sampling location) were normally distributed. For both the BACTcontrol and the BactoSense the dataset was not normally distributed. Therefore, a Kruskal-Wallis test, with Mann-Whitney post-hoc, was used to determine whether the results differed significantly ($p<0.05$) between the three measurement locations ('before UF', 'after UF', 'after CWR').

An alarm value was calculated to determine a threshold value to separate between noise (e.g. operational or instrumental) below the baseline, and events defined as measurements above the baseline [12]. The alarm value was calculated as formulated by Favere et al. [12]:

$$Alarm\ value = average\ alkaline\ phosphatase\ activity\ \pm 3 \times standard\ deviation$$

4.3 Results

The results of the tests at Oud-Beijerland are first described per monitoring technique (BACTcontrol, BactoSense and CBM) and are then also compared to each other.

4.3.1 BACTcontrol

The results of the BACTcontrol sensor are shown in Figure 4-8.

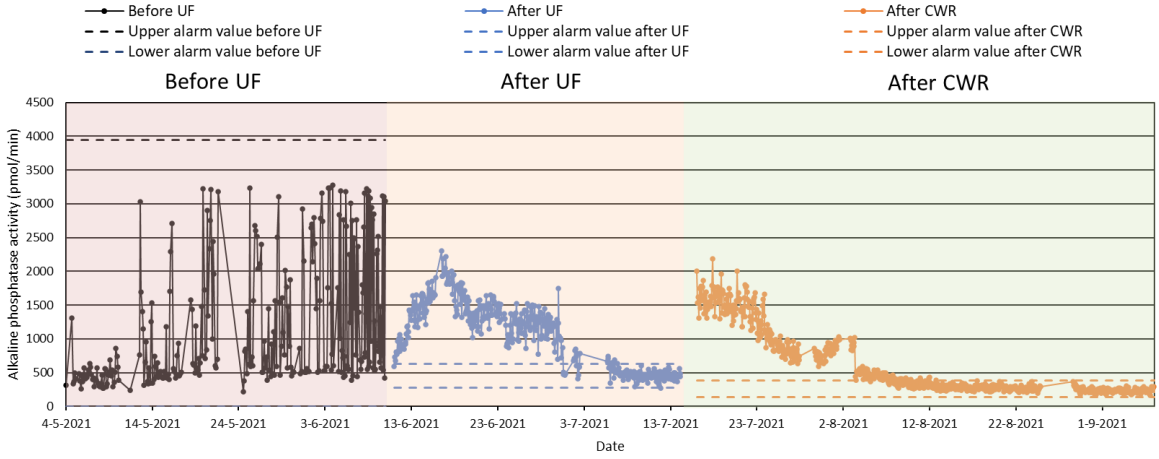


Figure 4-8 BACTcontrol results of measurements before UF (28 April to 9 June 2021), after UF (11 June to 14 July 2021) and after CWR (16 July to 7 September 2021) at Oud-Beijerland, Evides. Alarm values (calculated according to formula described in paragraph 2.3.4) were calculated before UF, after UF at the constant ALP activity from the 7th of July 2021 and after CWR at the constant ALP activity from the 12th of August 2021.

The first results (28th of April – 3rd of May 2021) of the BACTcontrol were not representative and therefore removed from the dataset. There were large fluctuations in the alkaline phosphatase (ALP) activity measured by the BACTcontrol before the UF (black line in Figure 4-8). Enzymatic activity varied between 214 and 3278 pmol/min, without a clear or obvious pattern. The exact cause of these large fluctuations remains unknown, but it could be that backwashing of the UF filter (every 40 minutes, duration 3 minutes) disturbed the water and sediment, causing particles to enter the BACTcontrol which, subsequently, interfere with the enzyme activity measurement.
The ALP activity increased after placement at the location after UF on the 11th of June. The ALP activity increased until 16 June 2021 (2303 pmol/min) after which it slowly decreased until about 400-500 pmol/min on 13 July 2021. After moving the BACTcontrol from 'after UF' to 'after CWR', the ALP activity level was immediately very high (1500-1700 pmol/min), and slowly decreased to 200-300 pmol/min and remained stable at this level after 12 August 2021. The cause of these high values after moving the sensors is not known. Perhaps moving the sensor was the cause, although there is no indication for that. It seems unlikely that the high ALP activity levels were caused by carry over from the previous location ('after UF'). Although the sensors were not cleaned in between the different monitoring locations, the ALP activity level at 'after CWR' is much higher than the ALP level at 'after UF' during the last monitoring days.

A statistical summary of the BACTcontrol measurements can be found in Table 4-2. The drinking water 'before UF' gives significantly higher ALP activity levels compared with drinking water 'after UF' and 'after CWR' (Kruskal-Wallis: $p<0.05$). Drinking water 'After UF' has significantly higher ALP activity levels than drinking water 'after CWR' (Kruskal-Wallis: $p<0.05$).These results suggest that UF removes bacterial activity in drinking water and that the bacterial activity is further reduced during residence in the CWR (Paragraph 4.4.1 and 4.4.2).

*Table 4-2 Statistical summary of BACTcontrol results at Oud-Beijerland, Evides. ALP is stated in pmol/min. * Only the period with a stable, low, signal (12 August – 7 September 2021). SD: Standard deviation. SE: standard error. Count: number of reliable measurements. Alarm values are given in chapter 4.4.4.*

	Mean	Median	Minimum	Maximum	SD	SE	Count
Before UF	1165	663	214	3278	926	55	282
After UF	1059	1135	271	2303	482	24	421
After CWR	594	330	162	2186	476	18	707
After CWR*	261	259	162	431	42	2	345

4.3.2 BactoSense

During the monitoring period at Evides, no data was obtained with the BactoSense during two periods. First, after 26 May 2021 to 10 June 2021 the BactoSense was switched off because the cartridge was empty. Second, after moving the sensors from 'after UF' to 'after CWR' (15 July 2021) problems occurred during start-up. The BactoSense was reset at 26 July 2021, after which measurements continued without problems till 7 September 2021.

The cell numbers in drinking water 'before UF' were variable, but the observed peaks were composed of multiple measurements (Figure 4-9), indicating that these peaks were reliable peaks with elevated cell numbers.
The BactoSense results showed a clear difference in cell numbers between the three monitoring locations (Figure 4-9 and Table 4-3). 'After UF' the cell counts in drinking water were much lower compared to 'before UF' (ICC: $4.3x10^3$ vs $1.0x10^5$ cells/ml). These differences in cell numbers in drinking water 'before UF' and in drinking water 'after UF' showed that UF indeed removed a large fraction of the bacterial cells. With a pore size of 150 kDa (< 0.01 µm), it is expected that the UF removes all bacterial cells as bacterial cells are larger than 0.01 µm, but a low number of cells was still detected. This can be caused by (a low level of) regrowth in the UF-installation and pipes or in the splitting station that was used for installing the sensors. Regrowth in the sensors or connecting tubes itself is unlikely as for each location new tubes were used.

'After CWR' a higher number of ICC and high nucleic acid cells (HNAC) was observed in the drinking water compared to 'after UF' (ICC: $4.0x10^4$ vs $4.3x10^3$ cells/ml; HNAC: $3.8x10^4$ vs $3.8x10^3$ cells/ml)'. The increase in ICC and HNAC showed that regrowth occurred in the CWR (average residence time of 24 hours).

A few outliers are visible in the drinking water 'after CWR' (indicated by the arrows in Figure 4-9). These outliers (each time a single measurement) showed an increased ICC and low nucleic acid cell (LNAC) count. Further inspection of the BactoSense and the operational parameters provided by Evides did not give a clear indication about the cause of these outliers.

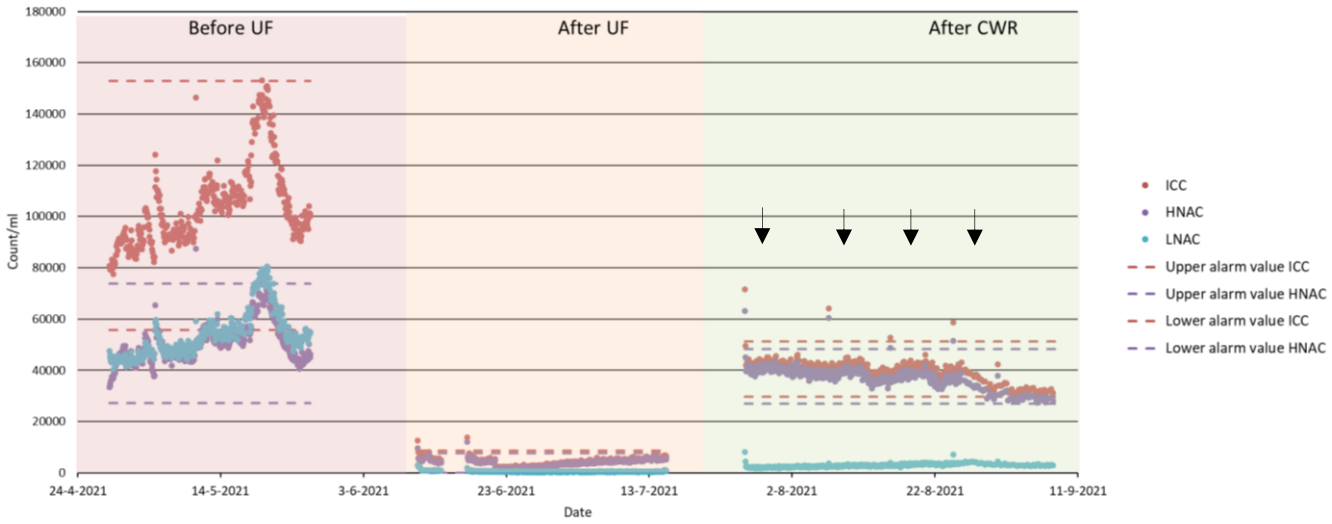


Figure 4-9 BactoSense results of measurements 'before UF' (28 April to 9 June 2021), 'after UF' (11 June to 14 July 2021) and 'after CWR' (16 July to 8 September 2021) at Oud-Beijerland, Evides.

To visualize differences in cell counts in drinking water 'after UF' and 'after CWR', the results are differently visualized in Figure 4-10. Interestingly, a sudden decrease in ICC and HNAC was observed on 21 June 2021 between 08:25 and 11:25 hours (indicated with the black arrow in Figure 4-10). After about two weeks, however, the ICC and HNAC are again stable and at the same level as before 21 June 2021. On 21 June 2021 maintenance work was performed on the pump of the cleaning reagent of the UF. During this day no chemical enhanced backwashing with NaOCl of the UF was performed. It seems that this has decreased the number of bacterial cells immediately in the drinking water 'after UF' and that it took some time before numbers were back to the levels before this incident. It is also possible that unknown changes in the influent concentration occurred causing a decreased number of bacterial cells.

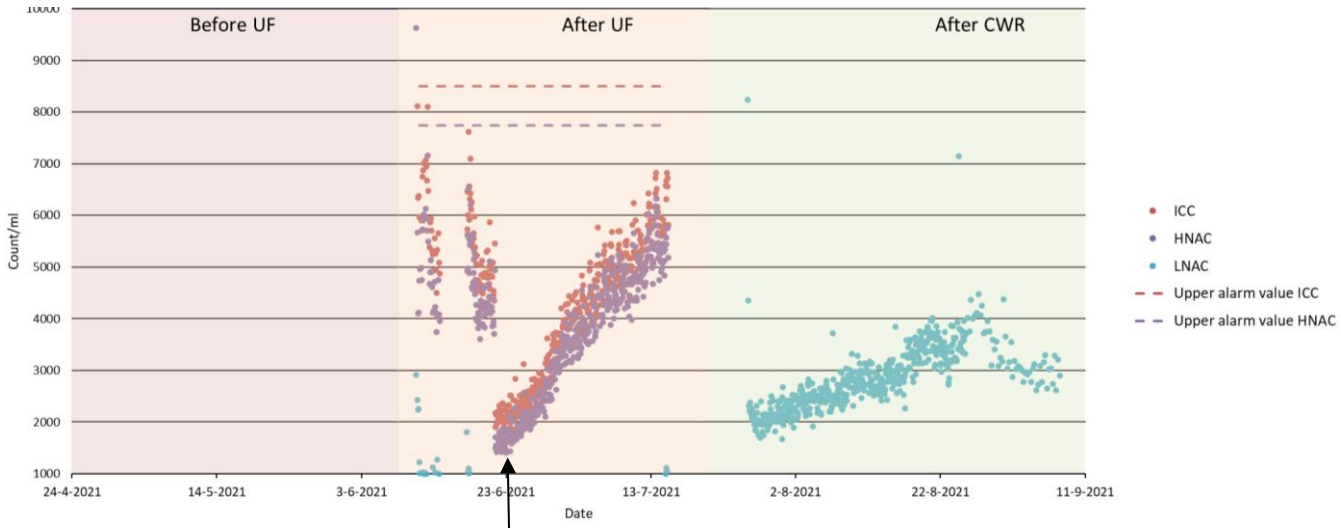


Figure 4-10 BactoSense results of measurements before UF (28 April to 9 June 2021), after UF (11 June to 14 July 2021) and after CWR (16 July to 8 September 2021) at Oud-Beijerland, Evides. The black arrow indicates the point at which the ICC and HNAC counts are suddenly decreased.

The statistical summary of the results is shown in Table 3-2. All types of cells (ICC, HNAC, LNAC) in drinking water 'before UF' are significantly higher than in drinking water 'after UF' and 'after CWR' (Kruskall-Wallis: $p<0.05$, Table 4-3). Furthermore, ICC, HNAC and LNAC in drinking water 'After UF' are significantly lower than in drinking water 'after CWR' (Kruskall-Wallis: $p<0.05$). These results, thus, confirm that UF removed a significant part of the bacterial cells from the drinking water and that regrowth of bacterial cells occurred in the CWR.

Table 4-3 Summary statistics BactoSense. Values are given in count/ml. SD: Standard deviation. SE: standard error. Count: number of reliable measurements. Alarm values are given in chapter 4.4.4.

Cell count	Sampling location	Mean	Median	Minimum	Maximum	SD	SE	Count	95-percentile	99-percentile
ICC	Before UF	104288	101000	77420	153080	16159	761	451	141910	149665
	After UF	4278	4510	1740	13830	1408	1981872	467	6237	7030
	After CWR	40471	40980	29740	71470	3565	12709581	537	43940	45986
HNAC	Before UF	50577	49450	33380	87310	7767	60332299	451	66705	71285
	After UF	3788	4040	1410	12020	1316	1731408	467	5602	6187
	After CWR	37630	38200	27320	63230	3574	12771885	537	41626	43405
LNAC	Before UF	53706	52320	40460	80560	8851	78333933	451	74225	78550
	After UF	486	440	210	1810	185	34260	467	924	1054
	After CWR	2837	2760	1670	8240	631	397954	537	3764	4314

4.3.3 CBM

Milispec-CBM

The biomass accumulation rate (BAR) determined with the Milispec-CBM of the drinking water 'before UF' ranged between 137 and 270 pg ATP cm^{-2} day^{-1} (Figure 4-11). The BAR in the drinking water 'after CWR' was much lower: 10.0, 31 and 9.5 pg ATP cm^{-2} day^{-1}.

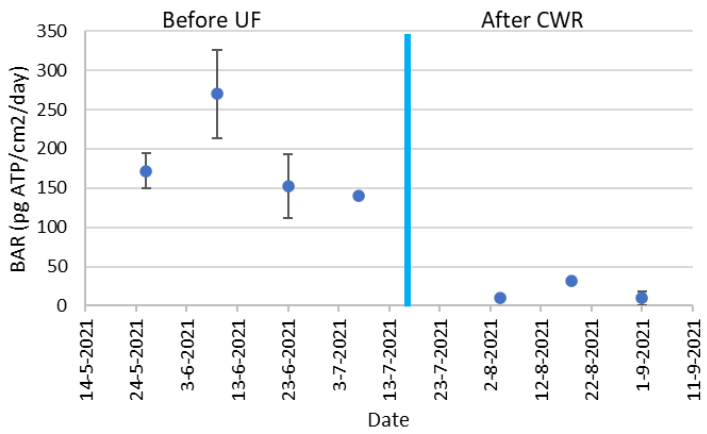


Figure 4-11 The BAR determined with the Milispec-CBM on drinking water 'before UF' and 'after CWR' at location Oud-Beijerland, Evides. Data are shown as average values ± standard deviation.

KWR-CBM

The BAR determined by the KWR-CBM of the drinking water 'before UF' ranged between 33.0 and 48.4 pg ATP cm^{-2} day^{-1} (Figure 4-12). The BAR of the drinking water 'after CWR' ranged between 3.0 and 26.9 pg ATP cm^{-2} day^{-1} (Figure 4-12).

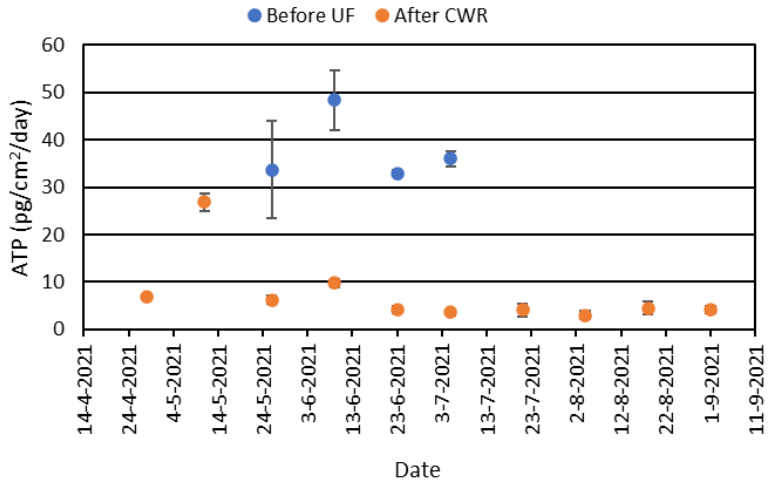


Figure 4-12 The BAR determined with the KWR-CBM on drinking water 'before UF' and 'after CWR' at location Oud-Beijerland, Evides. The data are shown as average values ± standard deviation.

Comparison KWR-CBM and Milispec-CBM

In Figure 4-13 and Figure 4-14 the KWR-CBM and Milispec-CBM results are compared, respectively 'before UF' and 'after CWR'. The ATP concentrations, and thus the BAR, measured with the Milispec-CBM are on average 4 to 5 times higher than the KWR-CBM, which seems to be in line with the results from the validation study. The fluctuations of the BAR that were observed during the monitoring period (trends), were comparable between the KWR and Milispec CBM-system on drinking water 'before UF', but on drinking water 'after UF' the Milispec-CBM yields one higher BAR value at August 18 compared to the other two monitoring dates, whereas the BAR values of the KWR-CBM remained constant during the whole monitoring period.

The high value on 18 August 2021 (31 pg cm^{-2} day^{-1} compared to approximately 10 pg cm^{-2} day^{-1} at the other two monitoring dates) was obtained from a single measurement (only one cuvette was sampled) as the other cuvette broke during sampling. It can therefore not be investigated whether this is a reliable value or it is too high because of technical reasons while performing the ATP measurement.

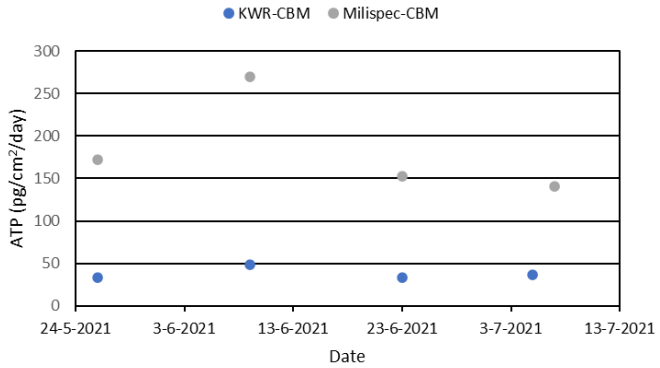


Figure 4-13 Data comparison of the BAR values obtained with the KWR-CBM and Milispec-CBM on drinking water 'before UF'.

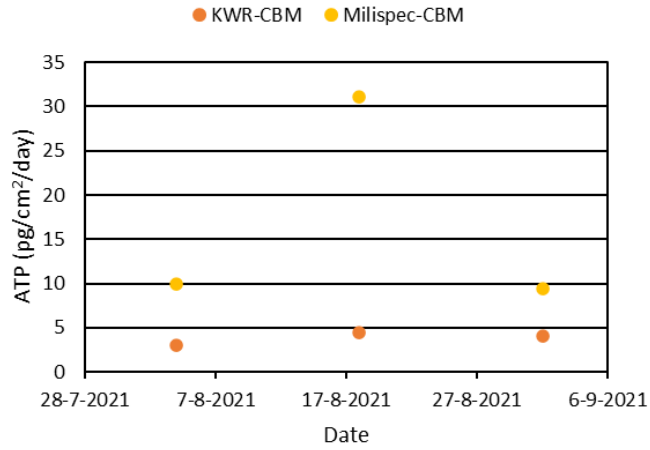


Figure 4-14 Data comparison of the BAR values obtained with the KWR-CBM and Milispec-CBM on drinking water 'after CWR'.

4.4 Discussion

4.4.1 Comparison BACTcontrol with BactoSense

Ideally, sensors for microbiological water quality that measure the same water should give similar trends. In this paragraph the results of the BACTcontrol are compared to the BactoSense results. The BACTcontrol and BactoSense results for drinking water 'before UF', 'after UF' and 'after CWR' are shown in respectively Figure 4-15, Figure 4-16 and Figure 4-17. Comparison of the BACTcontrol and BactoSense results of drinking water 'before UF' shows that the BACTcontrol results fluctuates highly with peaks composed of only one or two measurements, whereas the BactoSense ICC measurements showed more gradual fluctuations with peaks often composed of a relatively large number of measurements. It is assumed that the fluctuations in the BACTcontrol results were caused by the backflush regime of the UF which largely affects the pressure and water flow. The influence of these backwashes on the BactoSense results seems to be much lower as this high frequency of fluctuations were not observed with the BactoSense.

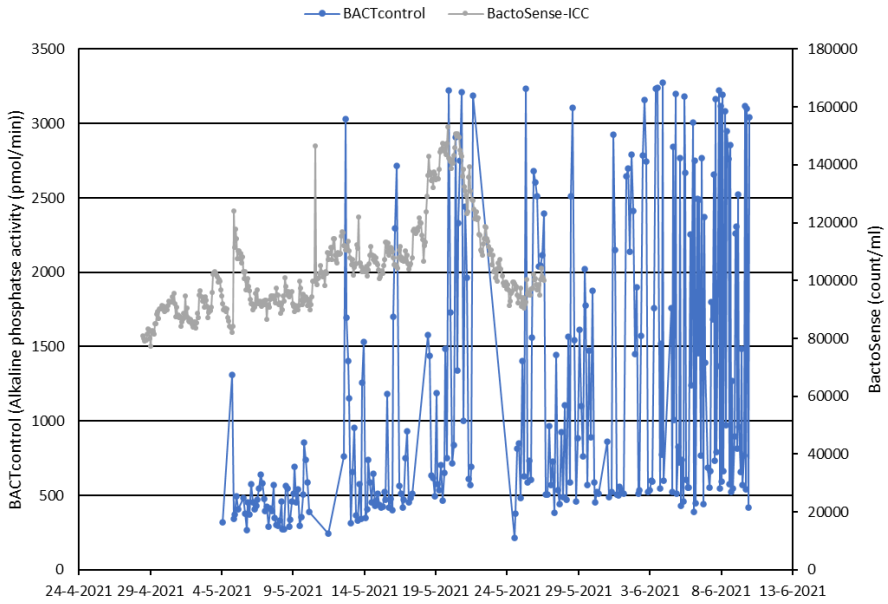


Figure 4-15 BACTcontrol and BactoSense results of drinking water 'before UF'. The first results of the BACTcontrol were not representative and therefore removed from the dataset.

In drinking water 'after UF', a more stable pattern for the BACTcontrol and BactoSense results was visible (Figure 4-16 and Figure 4-10). However, the changes in the microbial water quality are not comparable between both sensors. The enzymatic activity measured with the BACTcontrol increased until mid-June 2021 (up to 2000 – 2200 pmol/min) after which it gradually declined to 400 – 500 pmol/min in the beginning of July 2021, and the enzymatic activity seemed to stabilize. The BactoSense is more stable during the monitoring period. From the start, at 11 April, until 21 June 2021 the cell numbers were relatively stable. After the decrease in cell numbers on 21 June 2021 (described in paragraph 4.3.2) it takes about two weeks before the cell numbers are again stable and at the same level as before 21 June 2021. This drop in cell numbers measured with the BactoSense on 21 June 2021 was not visible with the BACTcontrol. The drop in bacterial cells measured with the BactoSense may be related to an operational aspect of the UF membrane (no chemical enhanced backwashing performed on this day), but this action did not result in a drop of the enzyme activity measured with the BACTcontrol.

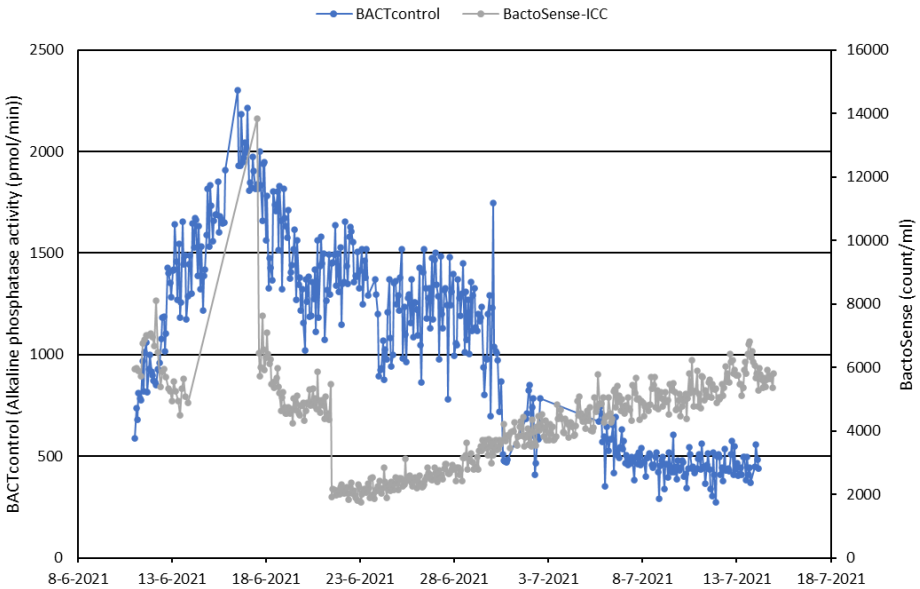


Figure 4-16 BACTcontrol and BactoSense results 'after UF'.

The ALP measured by the BACTcontrol in drinking water 'after CWR' started high (1500 – 1700 pmol/min) and decreased up to a stable activity of about 250 pmol/min in mid-August 2021. The cell numbers (ICC: $2x10^3$ cells/ml) obtained with the BactoSense in this water type were relatively stable over time until the end of August 2021. During the last two weeks of the monitoring period the cell numbers decreased slightly to about $3.2x10^4$ cells/ml (ICC) with a few outliers as was previously described (paragraph 4.3.2). Again, the results of the BACTcontrol and BactoSense do not match.

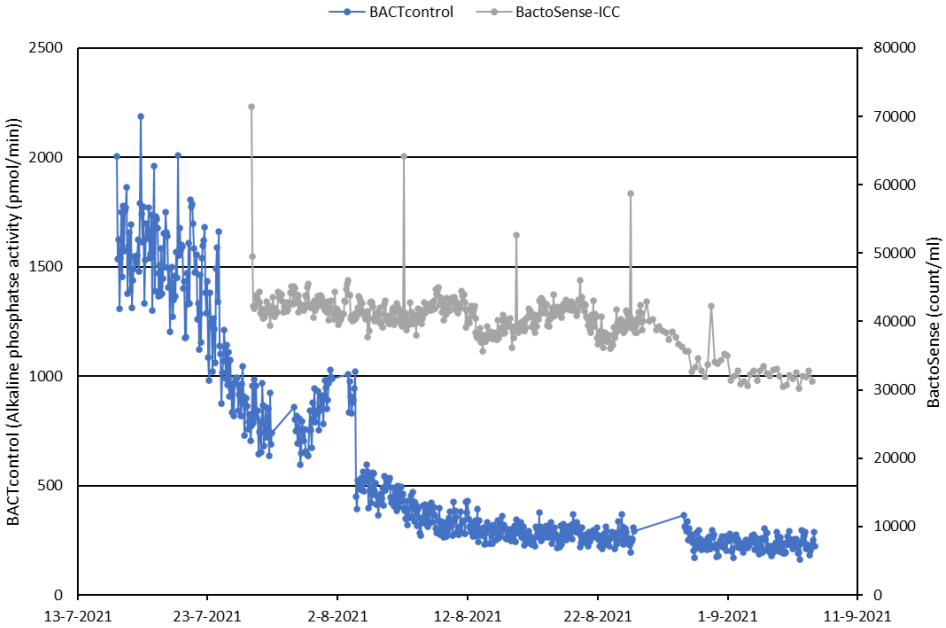


Figure 4-17 BACTcontrol and BactoSense results after CWR.

For none of the locations ('before UF', 'after UF', 'after CWR') the trend of BACTcontrol- and BactoSense-results in time overlap. Next to that also the trend between the locations do not always overlap. Both results from the BACTcontrol and BactoSense showed highest values in drinking water 'before UF', but the BACTcontrol showed the lowest values in drinking water 'after CWR', suggesting that the bacterial activity decreased during residence in the CWR. In contrast, the BactoSense showed the lowest results in the drinking water 'after UF', suggesting that bacterial growth occurred during residence in the CWR. The data from the regular monitoring programme of Evides (described in more detail in paragraph 4.4.2) also showed that the cell numbers (Figure 4-19) and ATP (Figure 4-18) concentrations were lower in the drinking water 'after UF' than in the drinking water 'after CWR', confirming that growth occurred in the CWR.

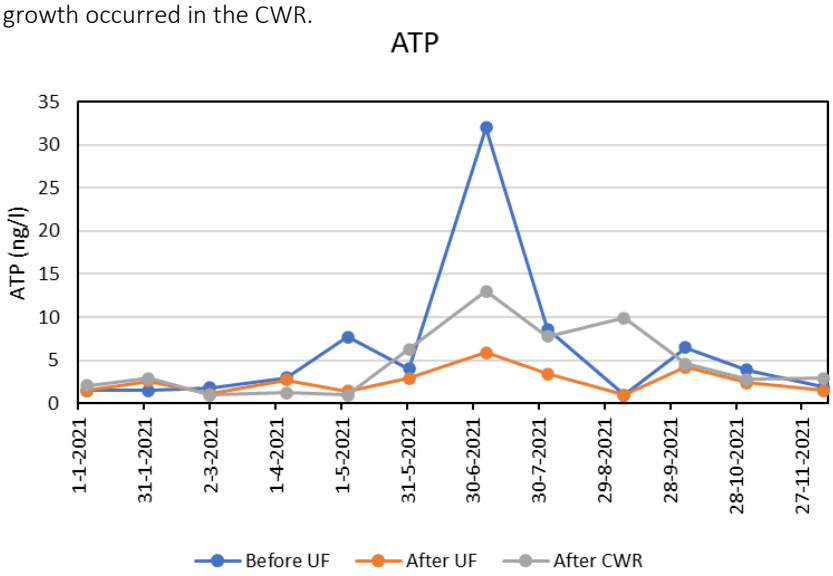


Figure 4-18 ATP concentration in water 'before UF', 'after UF' and 'after CWR'. Measurements were performed by Aqualab Zuid for Evides.

4.4.2 Comparison BactoSense FCM method with laboratory FCM method

On regular basis manual water samples were taken as part of the regular monitoring program of Evides and FCM measurements in the laboratory (laboratory FCM) were performed. The results showed that the TCC decreased significantly between drinking water ‘before UF’ and drinking water ‘after UF’ (Figure 4-19 and

Table 4-4, 3.5 to 4.5x10^5 vs 2.5 to 4.7x10^3 cells/ml) and that these numbers increased ‘after CWR’ (average TCC: 3.2x10^4 cells/ml). ‘Before UF’ the ICC is only about 5-6% of the TCC, which increased to 21.3 – 34.4% ‘after CWR’ (

Table 4-4).

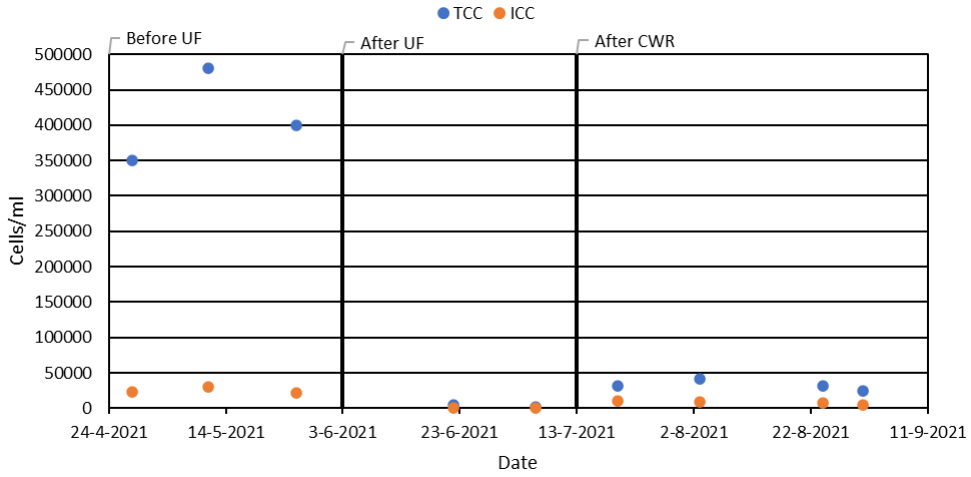


Figure 4-19 TCC and ICC based on FCM performed by Aqualab Zuid for Evides.

The TCC and ICC cell counts are comparable between the BactoSense results and the FCM results obtained from the regular monitoring program of Evides (Figure 4-20 and

Table 4-4). The same pattern (decrease in ICC by ultrafiltration and an increase 'after CWR') was observed with the laboratory FCM method compared with the BactoSense FCM method, even though the number of data points was limited.

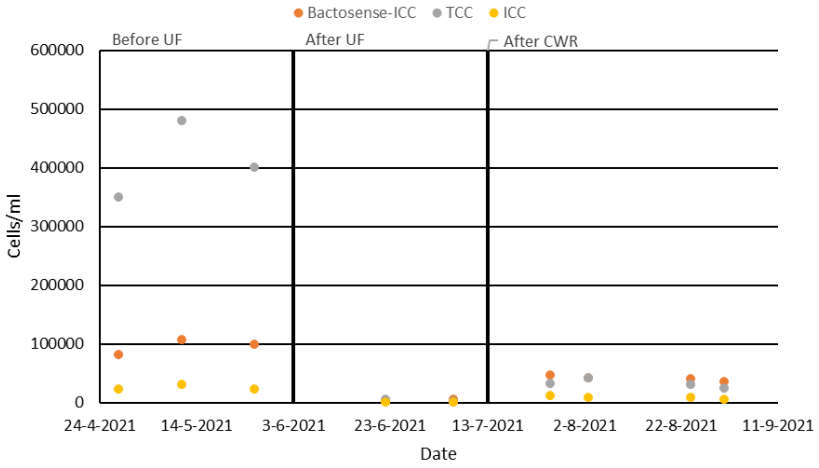


Figure 4-20 Comparison between the cell numbers obtained with the BactoSense FCM and the laboratory FCM.

Table 4-4 Cell counts from the BactoSense FCM method and the laboratory FCM. Values are given in count/ml. The BactoSense values are the averages of that specific day.

		BactoSense	FCM-laboratory		
		ICC	TCC	ICC	%ICC
Before UF	28-4-2021	80729	350000	23000	6,6
	11-5-2021	107076	480000	30000	6,3
	26-5-2021	99274	400000	22000	5,5
After UF	22-6-2021	2098	4700	180	3,8
	6-7-2021	4784	2500	410	16,4
After CWR	26-7-2021	46462	32000	11000	34,4
	3-8-2021	41479	42000	9100	21,7
	24-8-2021	40941	31000	8000	25,8
	31-8-2021	34600	24000	5100	21,3

Overall, the BactoSense FCM and the laboratory FCM, thus, showed comparable trends, which indicates that the usage of BactoSense to determine cell counts could be a good alternative for the laboratory FCM method. Nevertheless, the ICC measured with the BactoSense are much higher compared with the laboratory FCM method. However, additional studies on comparing both methods should be performed before a reliable and definite conclusion can be drawn. When both methods are comparable, switching from laboratory FCM to BactoSense FCM is possible without losing the possibility to compare current and future results to historical data.

4.4.3 CBM

Since biofilm formation in drinking water processes is a relatively slow process because of the low nutrient concentration, the CBM can only provide a reliable BAR value after glass beads have been exposed to the drinking water for four weeks. Consequently, due to this aspect and the nature of the CBM, only one BAR value can be obtained every two weeks, which is a very limited number of measurements compared to the BACTcontrol or BactoSense. Nevertheless, the limited CBM dataset does show the effect of UF on the microbiological water quality, as the BAR of drinking water is considerably lower 'after CWR' than 'before UF' (Figure 4-11 and Figure 4-12).
The trend of the BAR-values was similar for the Milispec-CBM and KWR-CBM, despite that different methods were used to measure the ATP-content of the formed biofilm on the glass beads. After improving the removal of the cuvettes from the CBM, the Milispec-CBM on-site seems to be a good alternative for the KWR-CBM for which the ATP-concentration of the biofilm must be analyzed in a laboratory. The advantage of the Milispec-CBM is that the user can install, sample and analyze the CBM himself and is not dependent on KWR. Which makes the analysis cheaper and easier to perform. However, due to differences in the ATP measurement procedure the BAR values obtained with the Milispec-CBM were four to five times higher compared to the BAR values obtained with the KWR-CBM. This was also seen in the validation of Milispec-CBM (chapter 3, paragraph 4.1). Therefore, switching from KWR-CBM to Milispec-CBM will give similar trends, but the new results obtained with the Milispec-CBM cannot directly be compared to historical data obtained with the KWR-CBM. It might be that a constant correction factor can be used to calculate BAR values from the KWR-CBM to BAR values obtained by the Milispec-CBM, but more data from both systems are required to be able to determine this reliably.

4.4.4 Alarm values

For both the BACTcontrol and BactoSense alarm values were calculated per monitoring location (i.e. 'before UF', 'after UF', 'after CWR') with the formula average ± 3×SD as described in chapter 0. Measurements below the lower alarm value can indicate a (sudden) reduction in bacteria or bacterial activity, whereas measurements above the upper alarm value can indicate a sudden increase of bacteria or bacterial activity. Both alarms, thus, could indicate that something is wrong with the water quality and that action might be needed.

The alarm values for the BACTcontrol were calculated for drinking water 'before UF', 'after UF', 'after UF' at the constant ALP activity from the 7th of July 2021, 'after CWR' and 'after CWR' at the constant ALP activity from the 12th of August 2021 (indicated with the dashed line in Figure 4-8). The calculated alarm values are given in Table 4-5. The calculated lower alarm values were always lower than the lowest value obtained for each water type (sometimes ALP activity of the lower value is below zero) and the higher alarm values were in general higher than the maximum values observed. Only in the drinking water 'after CWR' the higher alarm value was a little bit lower than the maximum ALP activity. As a consequence, only very few measurements exceeded these alarm values. The very low and very high alarm values for the BACTcontrol are caused by the high variation of the measurement data as these alarm values are based on the standard deviation.

Although the alarm values are best calculated using measurements from a stable period, also the alarm value of the entire measurement period per location was calculated to point out the difference between the two. When the entire measurement period was used, the alarm values are very high for 'after UF' and 'after CWR'. This is caused by the large variation in the dataset and after moving the BACTcontrol the alarm value seems strongly overestimated. These high values are therefore not useful for usage as an early warning system. In chapter 11 and 12 other methods to calculate an alarm value, to be used as an early warning system, are described.

*Table 4-5. The lower and higher alarm values count and number of exceedances of the BACTcontrol in drinking water 'before UF', 'after UF' and 'after CWR'. * Only the period with a stable, low, signal ('after UF': 7-14 July 2021 and 'after CWR': 12 August – 7 September 2021). Count: number of reliable measurements. Exceedances: number of measurements that exceeds the calculated alarm value. Alarm values in pmol ALP/min. SD: Standard deviation*

	Count	Mean ± SD	Lower alarm value	Exceedances lower alarm value	Upper alarm value	Exceedances upper alarm value
Before UF	282	1165±926	0[1]	0	3943	0
After UF	421	1059±482	0[2]	0	2506	0
After UF*	106	456±59	279	0	633	0
After CWR	707	594±476	0[2]	0	2023	1
After CWR*	345	261±42	136	0	386	2

[1]*Calculated lower alarm value of -1613*
[2]*Calculated lower alarm value of -388*
[3]*Calculated lower alarm value of -833*

For the BactoSense, lower and higher alarm values were calculated for ICC and HNAC and these values are given in

Table 4-6. Depending on the parameter and measurement location 0 to 3 exceedances (lower alarm value) and 1 to 10 exceedances (upper alarm value) were recorded and thereby indicate a significant deviation of the microbiological water quality from the baseline (Figure 4-9). Whether or not these exceedances directly or indirectly result in undesired water quality problems was not part of this research and must be investigated in future studies.

Interestingly, the drop in cell numbers in drinking water 'after UF' on 21 June 2021 does not cause an exceedance of the alarm value when the current calculations are used. This is caused by the fact that it took about two weeks before cell numbers were restored, which affected the average and standard deviation used to calculate the alarm value. Consequently, it can be better to calculate these alarm values from data that do not contain sudden increases or decreases of cell numbers and the time period required to return to the baseline level and/or a longer stable history.

*Table 4-6. The lower and higher alarm values, counts and number of exceedances of BactoSense in drinking water 'before UF' (28 April – 26 May 2021), 'after UF' (11 June – 14 July 2021) and 'after CWR' (from the 26th of July 2021 onwards. * Only the period with a stable, high, signal ('after UF': 11 June - 4 July 2021) Count: number of reliable measurements. Exceedances: number of measurements that exceeds the calculated alarm values. Alarm values: cells/ml.*

	Count	Mean ± SD		Lower alarm value		Exceedances (lower alarm value)		Upper alarm value		Exceedances (upper alarm value)	
		ICC	HNAC	ICC	HNAC	ICC	HNAC	ICC	HNAC	ICC	HNAC
Before UF	451	104288±16159	50577±7767	55811	27275	0	0	152764	73879	1	1
After UF	467	4278±1408	3788±1316	54	0[1]	0	0	8501	7735	1	1
After UF*	251	5317±865	4758±762	2721	2471	0	0	7912	7045	2	2
After CWR	537	40471±3565	37630±3574	29776	26909	1	0	51167	48352	4	4

[1]*Calculated lower alarm value of -160*

4.5 Location-specific conclusions

After the modification of the sampling of the cuvettes, the Milispec-CBM was successfully operated by Evides. The trends in the BAR values obtained with Milispec-CBM match the trends in the BAR values observed with the KWR-CBM, although the values obtained with the Milispec-CBM are four to five times higher than the values obtained with the KWR-CBM.

The results from the BACTcontrol, the BactoSense and the Milispec-CBM show respectively reduced bacterial enzyme activity, bacterial cell numbers and biomass accumulation rate in the drinking water 'after CWR' compared to drinking water before 'UF'. Reduced biomass and improved biological stability have also been shown for drinking water 'after CWR' compared to 'before UF' by historical data of Evides.

The BactoSense results showed higher cell numbers in drinking water 'after CWR' compared to drinking water 'after UF', which indicates bacterial regrowth in the CWR. In contrast, the data from the BACTcontrol showed a clear decrease in bacterial enzyme activity in drinking water 'after CWR' compared to drinking water 'after UF', which indicates inactivation of bacterial activity during residence in the CWR. It is, therefore, concluded from these results that different sensors of microbial water quality can give conflicting results on the microbial water quality.

Historical data obtained by Evides on cell numbers, ATP concentration and the microbial growth potential of the drinking water have shown that regrowth does occur in the CWR. Consequently, it can be concluded that of the two sensors tested on these two water types, only the BactoSense was able to detect the regrowth in the CWR. The BactoSense and BACTcontrol yield different trends during the monitoring period of each water type, demonstrating again that cell numbers and enzymatic activity in drinking water do not give comparable responses. For instance, a sudden drop in cell numbers and slow restoration of cell numbers was measured with the BactoSense, but this could not be seen with the BACTcontrol. This drop in cell numbers was observed on the same day maintenance was performed on the UF, which might have been the cause for this drop in cell numbers.

5 Monitoring drinking water production with conventional and RO treatment at Oasen

5.1 Introduction test location

5.1.1 Location

Two different drinking water production locations at Oasen were used for this study: De Hooge Boom at Kamerik and Nieuw-Lekkerland (Figure 4-1). Both production locations produce drinking water from groundwater and infiltrated surface water. A conventional treatment (rapid sand filtration, softening, carry-over filtration, activated carbon filtration) has been used at Kamerik till 2024 and was measured in 2022 with the sensors in this project. At the end of 2024 this conventional treatment has been replaced by full-stream reverse osmosis (RO), followed by ion exchange, calcite filtration, aeration and remineralization. In Nieuw-Lekkerland, a comparable new treatment plant, full-stream RO with remineralization, has been in commission since 2023. Sensors were placed at this location in 2024.

In the coming future Oasen will renovate several conventional treatment plants where full-stream RO is the main treatment step, which enables Oasen to deal with future quality changes of the groundwater which is expected to become more brackish and contain more (emerging) contaminants. In addition to removing these substances with RO, it is also expected that the full-stream RO will produce drinking water with a higher biological stability, that contains less nutrients for microbiological regrowth in the distribution system. Drinking water production using full-stream RO is relatively new for Oasen, and experience still has to be enlarged.

Figure 5-1 Pilot locations Nieuw-Lekkerland (red dot) and Kamerik (black triangle), Oasen.

5.1.2 Goal

Within this project it was tested whether the sensors can measure differences in the microbial water quality of the produced drinking water of production locations De Hooge Boom and Nieuw-Lekkerland. These differences might be caused by differences in treatment steps of the production locations (conventional treatment compared to full-stream RO treatment) and/or the quality and composition of the untreated ground water from which drinking water is produced. It is hypothesized that the water quality of De Hooge Boom will contain more microbiological biomass and nutrients compared to the new production plant at Nieuw-Lekkerland.

The sensors will also be used to better understand the full stream RO-treatment concept. Are there, unknown up until now, specific RO-related dynamics or events that influence the microbiological water quality of the produced drinking water? And can the sensor results be linked to (legislative) microbiological parameters?

In addition, it was tested whether local signal values could be set for the local operator of Oasen to act upon.

5.2 Monitoring campaign

5.2.1 Experimental set up at production locations De Hooge Boom and Nieuw-Lekkerland

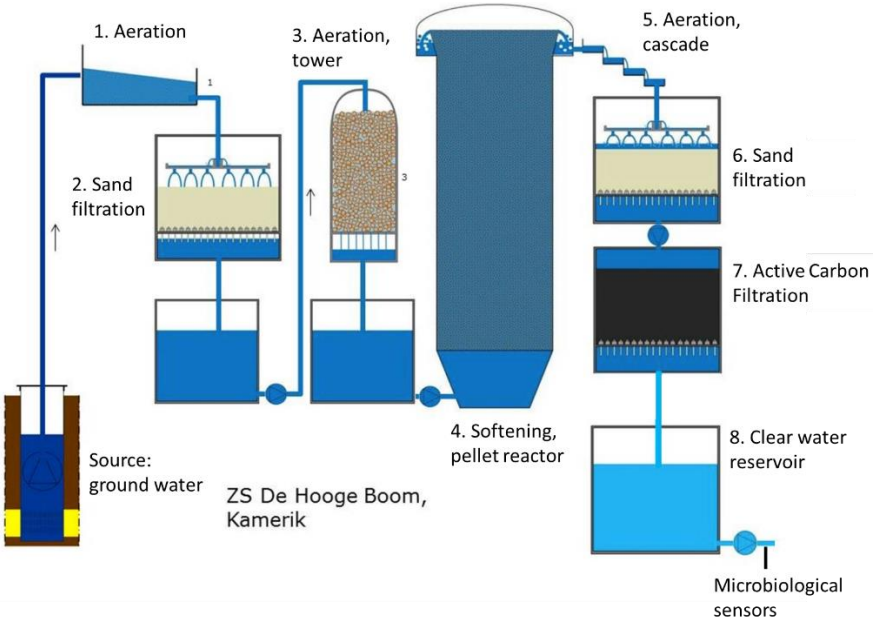
The sensors were installed after the treated water reservoirs at both production locations De Hooge Boom and Nieuw-Lekkerland. Biomass monitoring using the BactoSense and BACTcontrol sensors, and measuring the biofilm formation using the CBM was done at De Hooge Boom for two months (21 March – 23 May 2022) and at Nieuw-Lekkerland for three months (11 October 2024 – 15 January 2025; Table 4-1). Two types of CBMs were used in the research: a more user-friendly CBM that was developed by Milispec (Milispec-CBM) which is based on the original CBM developed by KWR (KWR-CBM) and the original KWR-CBM, that is less user-friendly. At De Hooge Boom only the Milispec-CBM was installed, whereas at Nieuw-Lekkerland the Milispec-CBM was initially installed (11 October 2024 – 15 January 2025), but due to technical issues it had to be cleaned an additional time and was reinstalled again two weeks later (25 October 2024; described further below). In addition, a KWR-CBM was installed (3 December 2024 – 15 January 2025).

*Table 5-1 Sampling locations and monitoring times. * The Milispec-CBM was installed on 11 October 2024, but was cleaned and reinstalled on 25 October 2024. The latter date should be considered the first day of the measurement campaign.*

	De Hooge Boom	Nieuw-Lekkerland
BactoSense	21 maart – 23 mei 2022	11 October 2024 – 15 January 2025
BACTcontrol	21 maart – 23 mei 2022	11 October 2024 – 14 January 2025
CBM Milispec	21 maart – 23 mei 2022	11/25 October 2024 – 15 January 2025*
CBM KWR	-	3 December 2024 – 15 January 2025

A schematic overview of both production locations is given in Figure 5-2.

Production location De Hooge Boom: conventional treatment plant



Production location Nieuw-Lekkerland: full-stream RO treatment plant

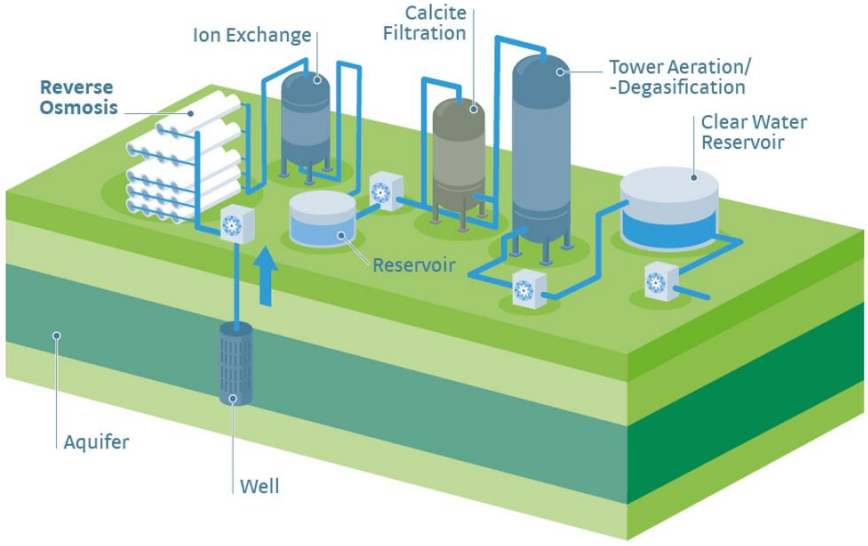


Figure 5-2 Overview production location De Hooge Boom (top) and Nieuw-Lekkerland (bottom). Sensors were placed after the treated water reservoir.

The CBM, BactoSense and BACTcontrol were all connected to the same sampling point via a splitter (Figure 4-4 in Chapter 4, right). This splitter has an open end to ensure a continuous water flow. A schematic overview of the sensor platform is given in Figure 4-3 of Chapter 4). The splitting station (Figure 4-4 in Chapter 4, right) was cleaned before installation and after finishing the measurements at Oasen. Cleaning was performed with SDS and citric acid to remove biofilm build up. Before the splitter was used at Nieuw-Lekkerland, it was in operation on the cooling water of BASF (Chapter 9). Initial results of the BACTcontrol and BactoSense after installation of the splitter at Nieuw-Lekkerland showed that the normal cleaning procedure between BASF and OASEN was not sufficient to remove all biomass as the sensor results indicated much higher biomass levels than expected. Since an extra cleaning procedure did not solve the problem, the splitter was replaced after one week by a newly constructed, clean, splitter (18 October 2024). Due to this issue, the Milispec-CBM had received contaminated water for one week and, therefore, the Milispec-CBM was also cleaned an additional time and reinstalled with new, clean tubing (25 October 2024).

The Milispec-CBM, BactoSense and BACTcontrol were placed in a cart at both De Hooge Boom and Nieuw-Lekkerland. At Nieuw-Lekkerland the KWR-CBM was installed next to the cart (Figure 6-3).
For connecting the sensors to the drinking water network, new tubes, made of Teflon material that does not promote bacterial regrowth, were used. The splitter was made of stainless-steel material, without the use of adhesives or other compounds that can promote bacterial growth.

Figure 5-3. Installation of BACTcontrol, BactoSense, KWR-CBM and Milispec-CBM at production location Nieuw-Lekkerland, Oasen.

5.2.2 Monitoring details

The measurement frequency of the BactoSense and BACTcontrol can be programmed and was set at a 2- to 6-hour interval. The BactoSense at De Hooge Boom initially used a 2-hour frequency and this was changed to a 6-hour interval for the last three weeks of the two-month measurement period. At Nieuw-Lekkerland the 2-hour interval was maintained for the three-month measurement period. The BACTcontrol performed measurements approximately every 1.5 – 2 hours in 2022 at De Hooge Boom and was scheduled to perform a measurement every 4 hours in 2024 at Nieuw-Lekkerland.

For the BactoSense two types of cartridges were used: the Life-Dead-Count (LDC) in 2022 at De Hooge Boom and the Intact-Cell-Count (ICC) in 2024 at Nieuw-Lekkerland. Although the LDC cartridge measured both the total number of cells (TCC) and the number of intact cells (ICC), only the ICC results were used as the TCC measurements were considered not reliable enough by bNovate.

Oasen operated the BactoSense and BACTcontrol during the monitoring period.

Every two weeks, two cuvets of the Milispec-CBM and KWR-CBM were sampled by Oasen, KWR or Milispec and the ATP content of the biofilm was measured with the LuminUltra ATP DSA-kit on-site (according to the protocol of Chapter 2). The sampling of the KWR-CBM and Milispec-CBM was done at the same day.

5.2.2.1 Additional analyses by Oasen

No additional analyses were performed during the measurement period in 2022 at De Hooge Boom.

Oasen performed additional analyses on the produced drinking water at the production plant during the measurement period in 2024 at Nieuw-Lekkerland. These analyses were outsourced to the Vitens laboratory. Three times a week the number of cells (flow cytometry), ATP and Heterotrophic Plate Count at 22°C (HPC22) were determined by the Vitens laboratory. Analyses by the Vitens laboratory were also performed for HPC22, methane, manganese, iron and ammonium on water samples taken at the following steps in the production plant: Well pump, Groundwater combined, Candle filter, RO-permeate, IEX filtrate, IEX combined, IEX out reservoir, Calcite filter before bypass, Calcite filter after bypass, Aeration tower out, $MgCl_2$ dosing out, Treated water reservoir. These analyses were performed at irregular intervals, and not all parameters were analysed at all sampling points.

5.2.3 Measurement, data processing and statistics

5.2.3.1 BACTcontrol

The measurement results of the BACTcontrol were quality-controlled by microLAN before further data analysis. In addition, the first three to five measurements after a temporary stop of the BACTcontrol (longer than 6 hours) were removed from the dataset and thus not used in data processing and interpretation. The results from these measurements were often higher than the results from the other measurements. This is likely caused by a technical aspect, as biomass build-up occurred during the standstill period of the BACTcontrol, often several internal cleaning cycles of the BACTcontrol were required before measurement values returned to a normal level.

The BACTcontrol measurements between 14 and 19 April 2022 were discarded as many ‘no water’-errors were observed. This was due to a leak supply tube for the reaction chamber. After this was solved, no errors were detected.

5.2.3.2 BactoSense

The measurement results of the BactoSense were analysed together with bNovate (producer of BactoSense) to evaluate and, if necessary, to adapt the gates of the BactoSense for this specific water type. An example of the results and gates for the two production locations (De Hooge Boom and Nieuw-Lekkerland) is given in Figure 6-5.

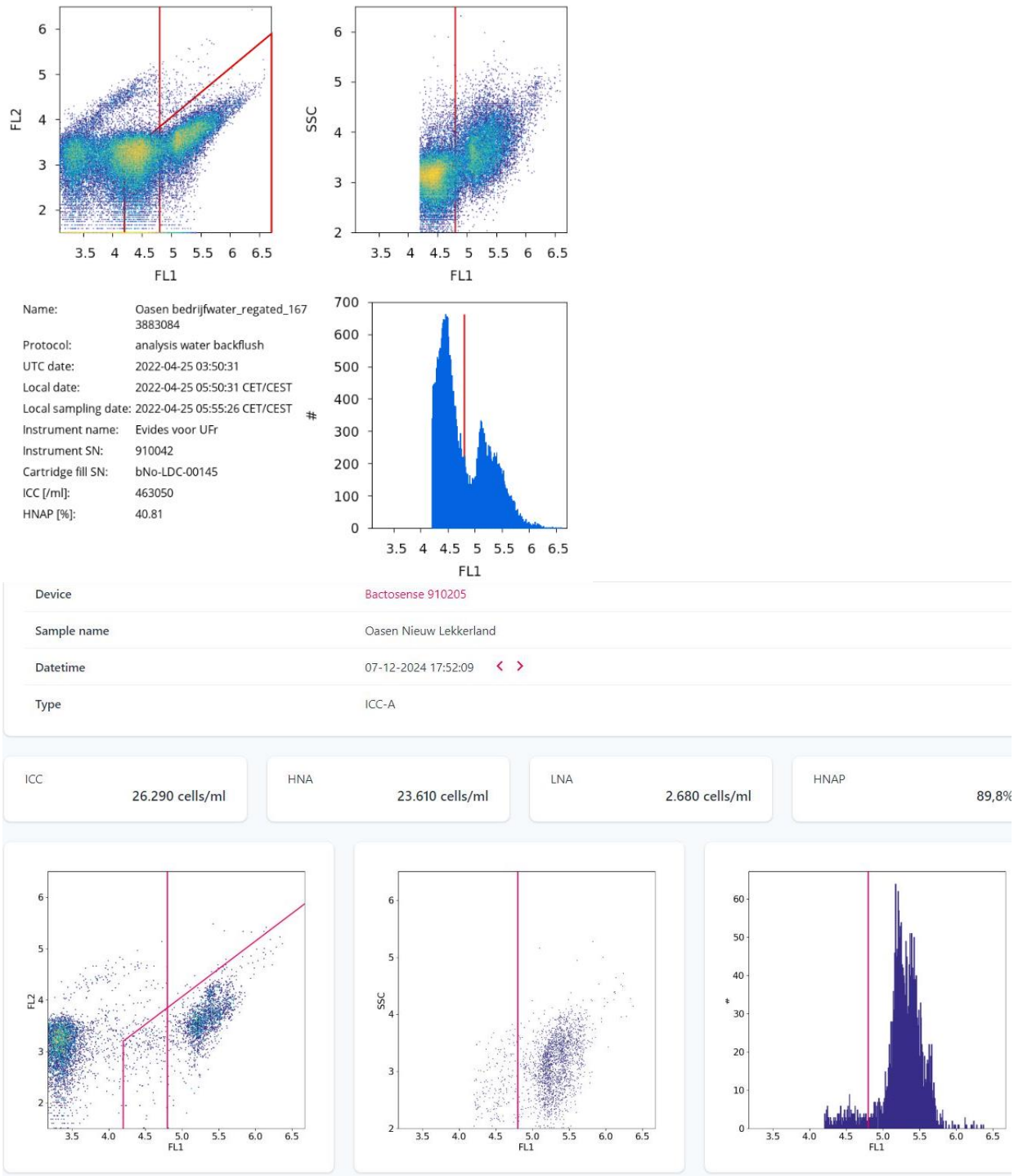


Figure 5-4. BactoSense results and gate examples at De Hooge Boom (top) and Nieuw-Lekkerland (bottom).

5.2.3.3 CBM

The Milispec-CBM and KWR-CBM were sampled and analyzed by Oasen, KWR and/or Milispec every two weeks on the same date. The ATP analysis was performed as described in paragraph 2.1.4 (Chapter 2). In brief, ATP was released from the bacterial cells in the biofilm on the glass beads using the lysis buffer from the LuminUltra ATP-field kit and the ATP-concentration was measured with a luminometer.

5.2.3.4 Statistics

With a Shapiro-Wilkinson test it was tested whether the different datasets (BACTcontrol and BactoSense per sampling location) were normally distributed. For both the BACTcontrol and the BactoSense the dataset was not normally distributed. Therefore, the non-parametric Kruskal-Wallis test, with Mann-Whitney post-hoc, was used to determine whether the results differed significantly ($p<0.05$) between the two measurement locations (De Hooge Boom and Nieuw-Lekkerland).

An signal value was calculated to determine a threshold value to separate between noise (e.g. operational and instrumental noise) considered to be normal variation, and events defined as measurements above or below the signal value [12]. The signal value was calculated as formulated by Favere et al. [12] for the cleaned datasets.

The signal values were calculated as follows:

$$Alarm\ value = average \pm 3 \times standard\ deviation$$

This formula is based on the assumption that the dataset follows a normal distribution and that 99.7% of all data points falls within the $average + 3 \times SD$ and $average - 3 \times SD$ boundaries.

5.3 Results

In this chapter, the results at De Hooge Boom (2022) and Nieuw-Lekkerland (2024) are first described per monitoring technique (BACTcontrol, BactoSense and CBM) and the two locations are compared to each other for each monitoring technique. In paragraph 6.4 the results of the BACTcontrol, BactoSense and CBM are compared to each other (paragraph 0), results of laboratory parameters (5.4.1 and 0) and to operational processes (paragraph 0). Signal values (that are already shown in the figures of the chapter are calculated and discussed in paragraph 4.4.4.

5.3.1 BACTcontrol

The results of the BACTcontrol sensor are shown in Figure 6-6 and Figure 6-7. As described in paragraph 0., the first three to five measurements after a period in which the BACTcontrol did not perform measurements were removed from the dataset. At De Hooge Boom this occurred after a temporary water supply stop by Oasen (28 March 2022), after a stagnant period (9 – 11 April 2022) and after a leakage in the supply tube of the reaction chamber (14 – 19 April 2022). Another 7 measurement results were removed as advised by microLAN as they did not meet the quality control standards. For Nieuw-Lekkerland, five measurement results were removed after a stagnant period (22 – 28 October 2024, 29 October – 1 November 2024, 2 – 4 November 2024, 24 November – 3 December, 6 – 30 December 2024). Another 24 measurement results were removed as advised by microLAN as they did not meet the quality control standards. For Nieuw-Lekkerland the results are displayed twice, with two different scales on the y-axis. This was done because the high BACTcontrol results in January do fulfil the quality control standards, but these high values make the other results less visible in the graphs.

The enzymatic activity varied between 188 – 699 pmol/min for De Hooge Boom (2022) and 47 – 3499 pmol/min for Nieuw-Lekkerland (2024). At De Hooge Boom the enzymatic activity increased in the first week (21 – 28 March 2022) after which it stabilized between 400 – 500 pmol/min. After approximately 6 weeks the enzymatic activity dropped to a lower and more stable level of 250 – 350 pmol/min. At Nieuw-Lekkerland many data points are missing due to several stagnant periods. At the start of the monitoring period the enzymatic activity fist peaked to 730 pmol/min between 20 – 22 October 2024 and peaked again to 3,499 pmol/min between 2 – 10 January 2025. In November the enzymatic activity was stable and low between 47 - 147 pmol/min.

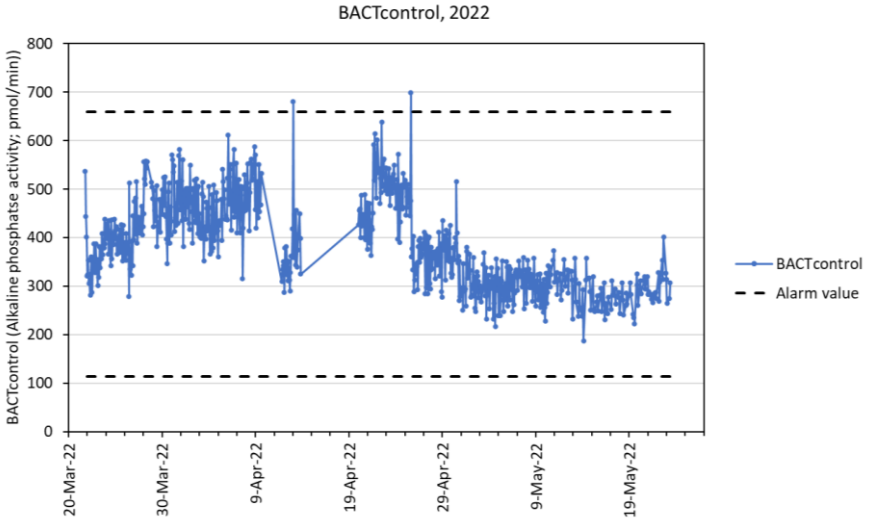


Figure 5-5 BACTcontrol results of production location De Hooge Boom, Oasen (measurements 21 March to 23 May 2022). Signal values (calculated according to formula described in paragraph 2.3.4) were calculated at the ALP activity from 21 March to 23 May 2022.

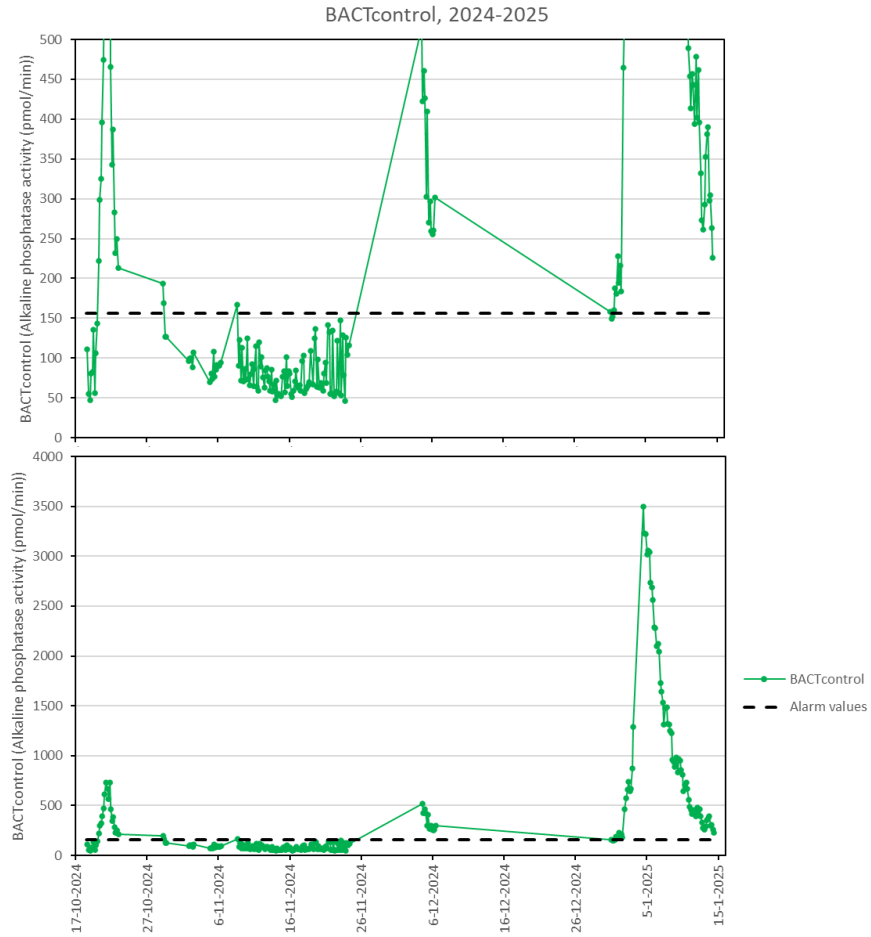


Figure 5-6 BACTcontrol results of production location Nieuw-Lekkerland, Oasen. Shown are the measurements for the entire period (top: 18 October 2024 to 14 January 2025) and without the major peak in the first week of January (bottom: 18 October – 31 December 2024). Signal values (calculated according to formula described in paragraph 2.3.4) were calculated at the ALP activity from 9 – 24 November 2024.

A comparison of the BACTcontrol measurements from production plant De Hooge Boom, with a conventional treatment, to Nieuw-Lekkerland, with full-stream reverse osmosis treatment, is shown in Table 4-2 and Figure 5-7. For Nieuw-Lekkerland, the baseline level was determined for the period between 9 – 24 November 2024, during which period no known abnormalities in the production plant occurred and laboratory parameters were normal (described in paragraph 5.4.1 and 0). The baseline level of enzymatic activity of the drinking water from Nieuw-Lekkerland is lower (47 - 147 pmol/min) compared to De Hooge Boom (300 – 500 pmol/min). Whereas in Nieuw-Lekkerland two or three peaks are visible, these are not clearly visible for De Hooge Boom. The average enzymatic activity in the drinking water of De Hooge Boom (2022, entire monitoring period) was significantly higher than the average enzymatic activity in the drinking water of Nieuw-Lekkerland (9 – 24 November 2024; 386 vs 80 pmol/min; Kruskal-Wallis: $p<0.05$). This difference in baseline level is clearly visible in Figure 5-7.

Table 5-2 Summary of BACTcontrol results at De Hooge Boom (2022) and Nieuw-Lekkerland (2024). ALP is stated in pmol/min. SD: Standard deviation. SE: standard error. Count: number of reliable measurements. Calculated signal values are given in chapter 4.4.4.

	Mean	Median	Minimum	Maximum	SD	SE	Count
De Hooge Boom (21 March – 23 May 2022)	386	378	188	699	91	3	704
Nieuw-Lekkerland (9 – 24 November 2024)	80	71	47	147	25	3	88

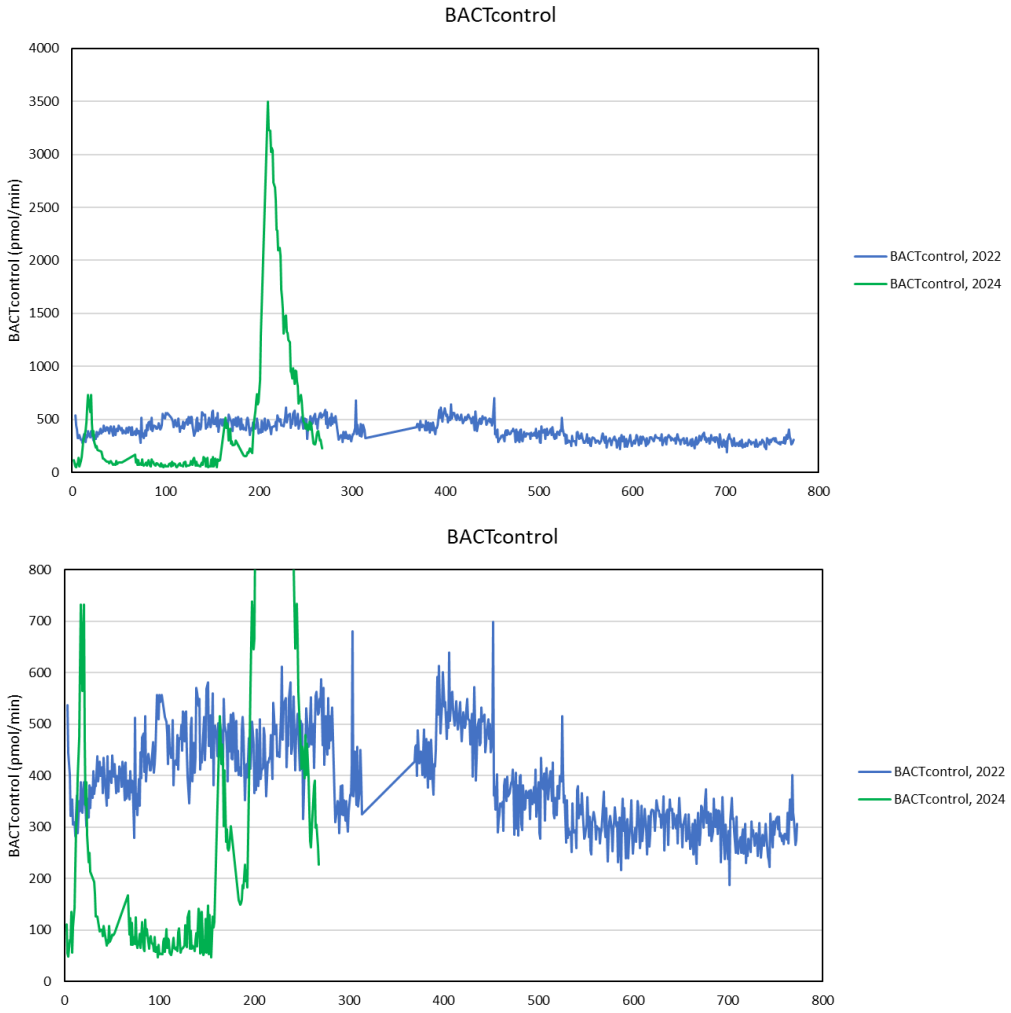


Figure 5-7 BACTcontrol results of production location De Hooge Boom (2022) and Nieuw-Lekkerland (2024). Shown are the measurements for the entire period with top and bottom figures having different scales on the y-axis for a more detailed view of the results and to enable easy comparison of both measurement periods. On the x-axis the number of measurements is shown.

5.3.2 BactoSense

The BactoSense results from the individual locations are shown in Figure 6-8 and Table 6-3. The number of intact cells (ICC) in the drinking water from De Hooge Boom (2022) varied between 3.8 $\times 10^5$ – 7.2$\times 10^5$ count/ml. The lower values were caused by eleven low measurements (out of 585 measurements). Without these eleven measurements, the minimum number of ICC was 5.3$\times 10^5$ count/ml. The HNAC-count varied between 1.3$\times 10^5$ – 2.1$\times 10^5$ count/ml. Most changes in ICC numbers were gradual except for three more sudden changes in cell number, with an increase of approximately 6$\times 10^4$ – 8$\times 10^4$ count/ml on 29 – 30 April 2022, 6 – 7 May 2022 and 10 – 11 May 2022. Approximately 23 – 41% of the ICC is HNAC and in general the dynamics in the HNAC count matched the dynamics in ICC count, although the changes were less drastic.

At Nieuw-Lekkerland (2024) the ICC count varied between 1.1$\times 10^4$ – 4.4$\times 10^4$ count/ml. The HNAC varied between 8.0$\times 10^3$ – 3.9$\times 10^4$ count/ml and the majority of ICC consisted of HNAC (74 – 91%). During the three-month measurement period several peaks in cell count were detected, these peaks varied in length from less than a day to a few days.

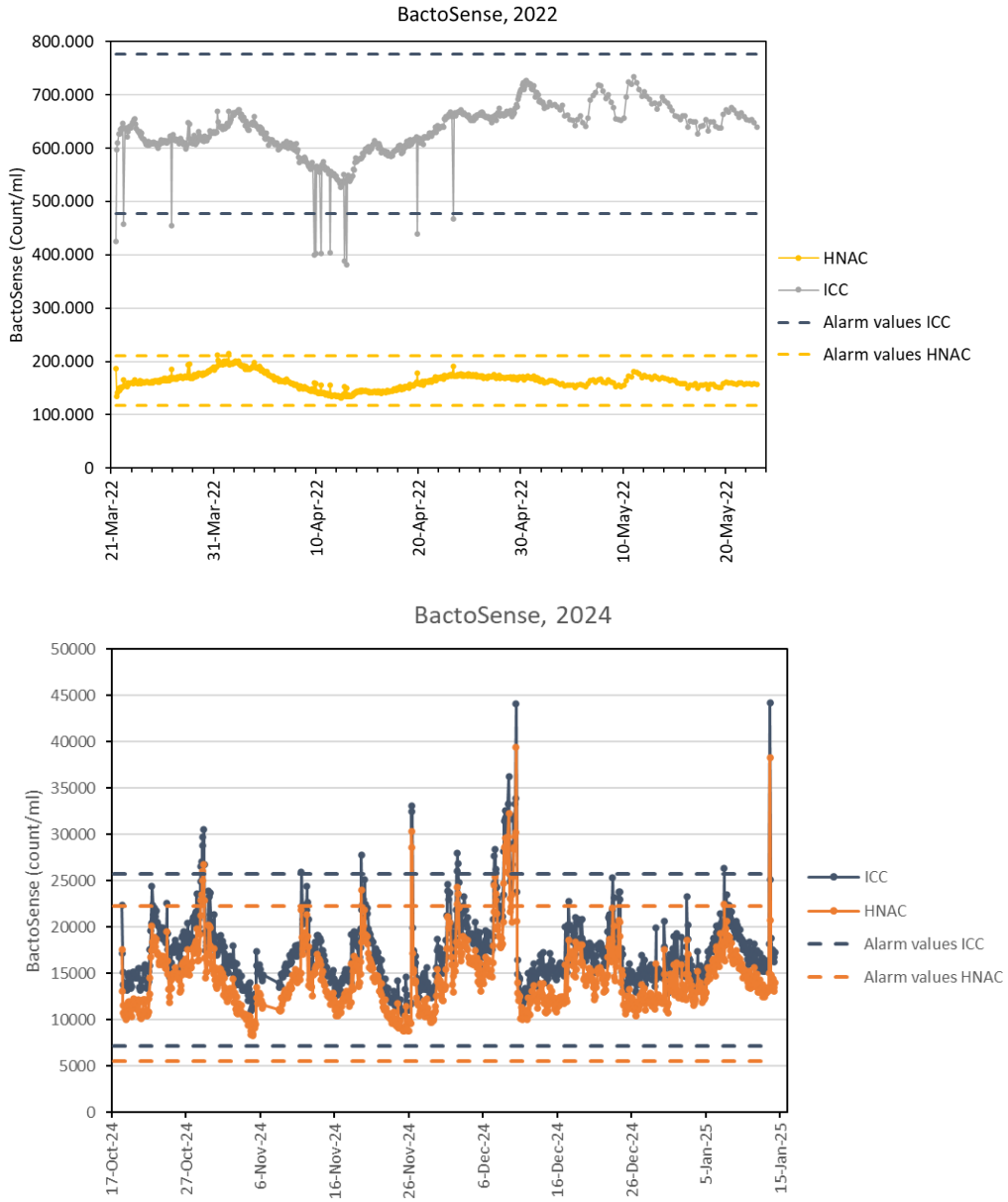


Figure 5-8. BactoSense results of measurements from De Hooge Boom (top; 21 March – 23 May 2022) and Nieuw-Lekkerland (bottom; 18 October 2024 – 15 January 2025) at Oasen. Signal values (calculated according to formula described in paragraph 2.3.4) were calculated using the cell numbers from 21 March – 23 May 2022 or 9 – 24 November 2024.

A comparison of the BactoSense results from production plant De Hooge Boom (conventional treatment) with those from plant Nieuw-Lekkerland (full-stream reverse osmosis treatment) is shown in Table 6-3 and Figure 5-9. The median ICC count in the drinking water of De Hooge Boom (2022) was significantly higher than in the drinking water of Nieuw-Lekkerland (6.3×10^5 vs 1.8×10^4 counts/ml; Kruskal-Wallis: $p<0.05$). Consequently, the difference in HNAC between the two locations was also significant (1.6×10^5 vs 1.4×10^4 counts/ml; Kruskal-Wallis: $p<0.05$). In Figure 5-9**Error! Reference source not found.** this significant difference is also clearly visible. The percentage of HNAC of the ICC count was significantly higher for Nieuw-Lekkerland than De Hooge Boom (26% vs 83%; Kruskal-Wallis: $p<0.05$).

Table 5-3. Summary statistics of BactoSense measurements at De Hooge Boom (21 March – 23 May 2022) and Nieuw-Lekkerland (18 October 2024 – 15 January 2025). Values are given in count/ml. SD: Standard deviation. SE: standard error. Count: number of reliable measurements. Calculated signal values are given in chapter 4.4.4.

	Mean	Median	Min.	Max.	SD	SE	Count	95-percentile	99-percentile
ICC									
De Hooge Boom (2022)	6.3×10^5	6.3×10^5	3.8×10^5	7.3×10^5	5.0×10^4	2.1×10^3	585	7.0×10^5	7.2×10^5
Nieuw-Lekkerland (2024)	1.7×10^4	1.8×10^4	1.1×10^4	4.4×10^4	3.9×10^3	1.2×10^2	1044	2.4×10^4	3.2×10^4
HNAC									
De Hooge Boom (2022)	1.6×10^5	1.6×10^5	1.3×10^5	2.1×10^5	1.5×10^4	6.4×10^2	585	1.9×10^5	2.0×10^5
Nieuw-Lekkerland (2024)	1.4×10^4	1.4×10^4	8.3×10^3	3.9×10^4	3.6×10^3	1.1×10^2	1044	2.0×10^4	2.9×10^4

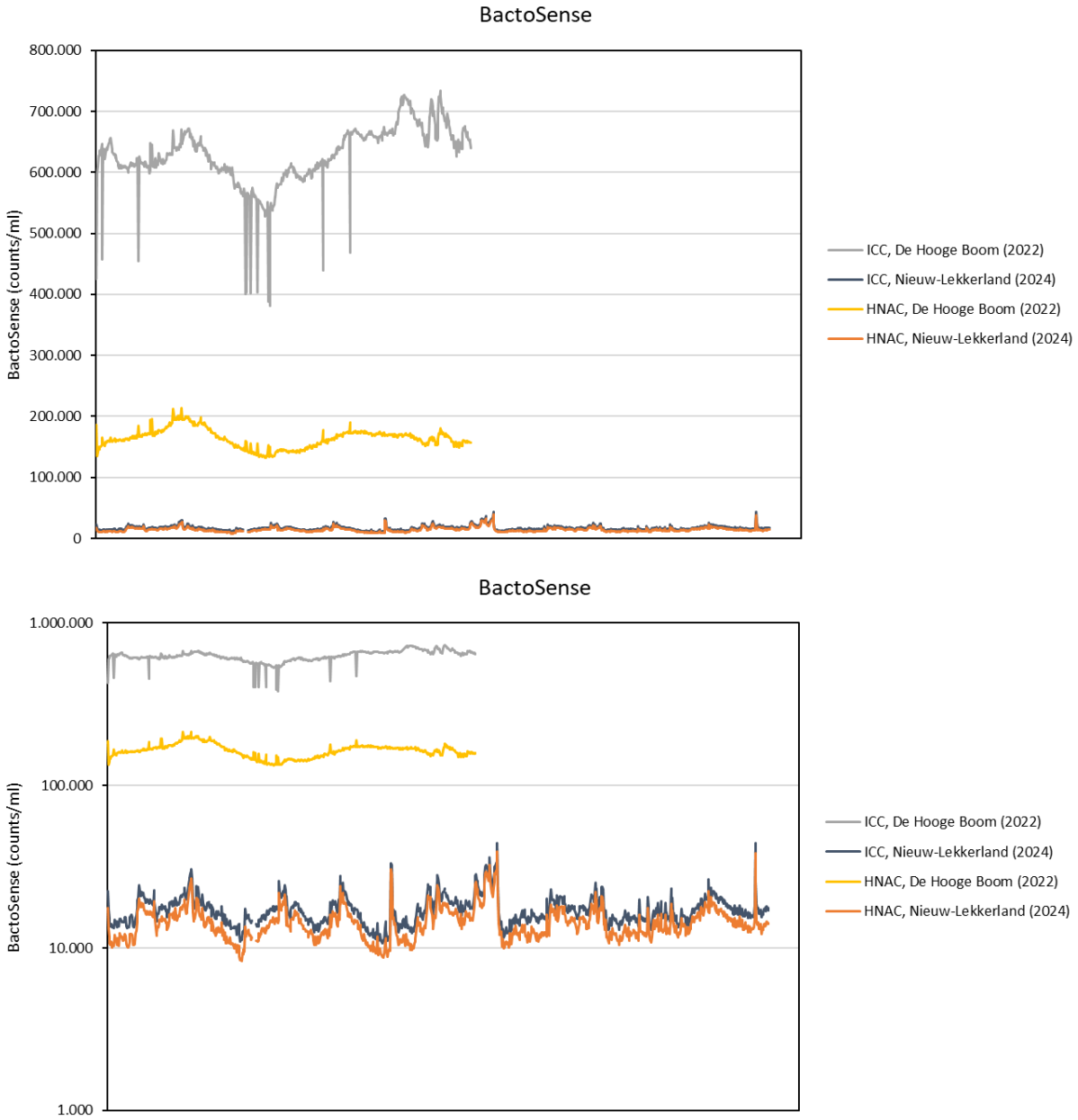


Figure 5-9. BactoSense results (ICC and HNAC) from De Hooge Boom (2022) and Nieuw-Lekkerland (2024). Given are the results on a linear scale (top) and on a Log_{10}-scale (bottom) for better visualization.

5.3.3 CBM

The biomass accumulation rate (BAR) determined with the Milispec-CBM of the drinking water at De Hooge Boom in 2022 was high, variable and ranged between 285 – 1,431 cm^{-2} day^{-1} (Figure 5-10). At production location Nieuw-Lekkerland the BAR was determined with the Milispec-CBM and the KWR-CBM, which were equally variable and ranged between respectively 255 – 1,187 and 311 – 1,387 pg ATP cm^{-2} day^{-1}. Recently, a guideline BAR- value of 30 pg ATP cm^{-2} day^{-1} was proposed for drinking water at the production plan. Produced drinking water with lower values are considered biologically stable with respect to regrowth of *Aeromonas* and HPC22 in the distribution system [13]. This guideline, however, was based on the Celsis ATP-method of the KWR-laboratory. Previous comparison between the LuminUltra ATP-method (used in this study on site) and the Celsis ATP-method at the KWR laboratory showed that the LuminUltra ATP-method resulted in higher values than the Celsis ATP-method, but that this was not a constant factor. This makes it difficult to compare the guideline BAR-value with the results at the Oasen sites (Chapter 3).

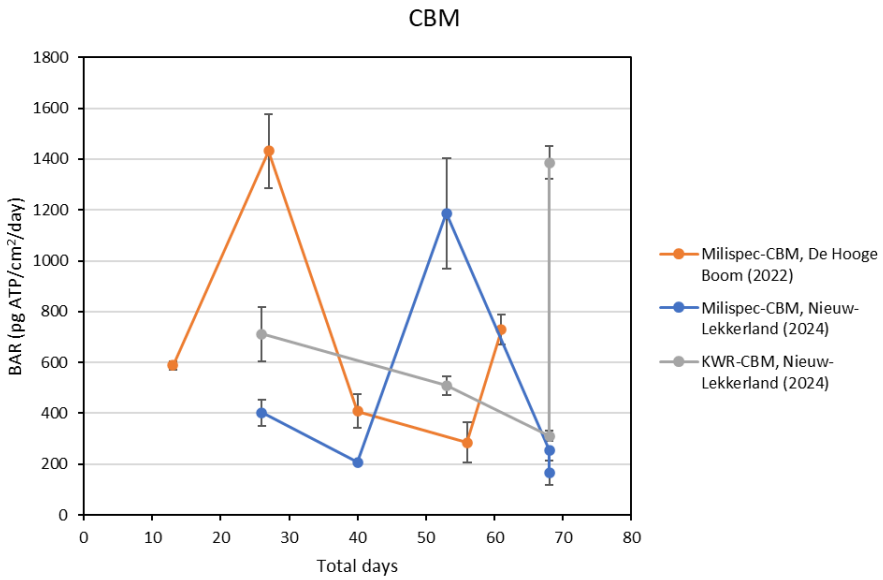


Figure 5-10 The BAR determined with the Milispec-CBM at production locations De Hooge Boom (2022) and Nieuw-Lekkerland (2024) and the KWR-CBM at Nieuw-Lekkerland (2024). For both CBMs the ATP-analysis was performed with the LuminUltra kit. The data are shown as average values ± standard deviation.

5.4 Discussion

5.4.1 Laboratory parameters Nieuw-Lekkerland

Several additional parameters were analysed for the produced drinking water from Nieuw-Lekkerland during the monitoring period with the sensors:

- pH, electrical conductivity (EC) and temperature; every two weeks
- cell counts with flow cytometry in the laboratory, ATP and HPC22; three times a week (Figure 5-12)
- to control the performance of several treatment processes, Oasen sampled water after different treatment processes at irregular intervals. These water samples were analyzed for ammonium (NH_4), iron (Fe), manganese (Mn), methane and heterotrophic plate counts at 22°C (HPC22).

The temperature, pH and electrical conductivity (EC) were stable during the monitoring period (Figure 5-11).

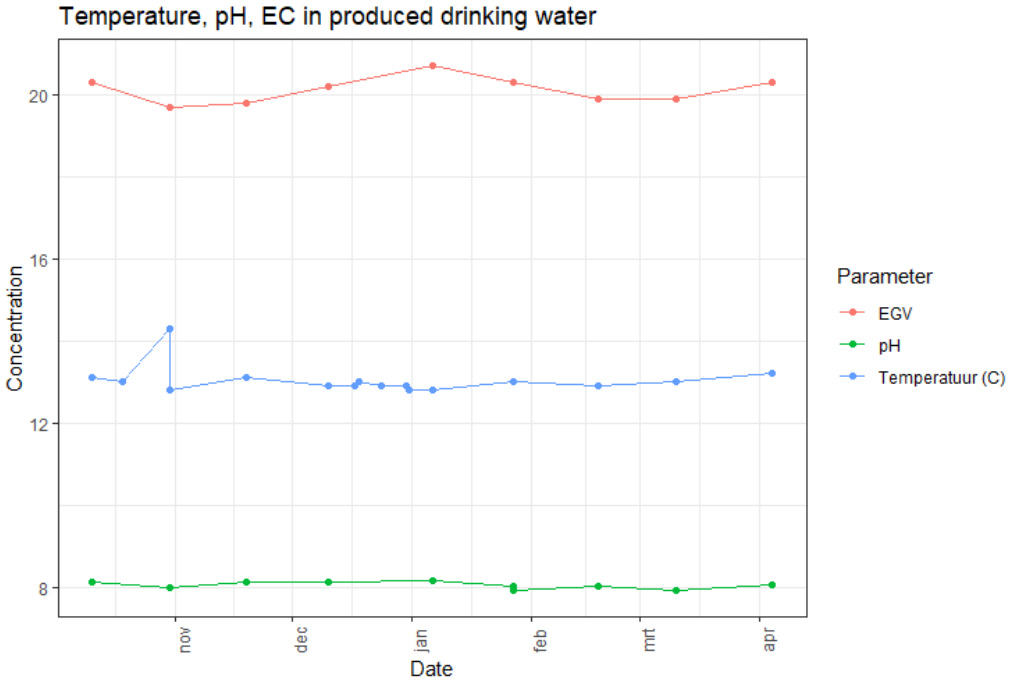


Figure 5-11. Temperature, conductivity (EC) and pH of produced drinking water at production location Nieuw-Lekkerland (2024 – 2025).

In the produced drinking water from Nieuw-Lekkerland the HPC22 varied between 0.1 – 312 cfu/ml, but was below 50 cfu/ml most of the time. For four out of 36 measurements (11.1%) HPC22 was above 100 cfu/ml (a yearly mean of 100 cfu/ml is the legal guideline). The number of total and intact cells (measured with FCM in the laboratory) varied approximately 1 $\log_{10}$ during the measurement period: 1.5 $\times 10^4$ – 2.1$\times 10^5$ cells/ml (total cells) and 1.4$\times 10^4$ – 1.7$\times 10^5$ cells/ml (intact cells).

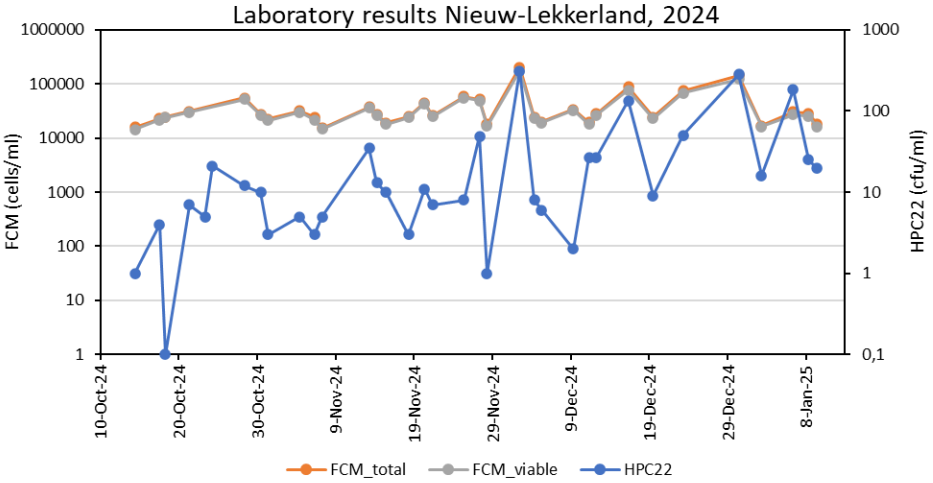


Figure 5-12. Flow cytometry (FCM) and heterotrophic plate count 22°C (HPC22) laboratory analyses performed on the produced drinking water of Nieuw-Lekkerland (2024).

5.4.1.1 Comparison BACTcontrol with laboratory parameters on produced drinking water

Comparison of the laboratory results to the BACTcontrol (Figure 5-13) does not yield clear comparable trends. The continuous low HPC22-levels in October and November coincide with relatively low BACTcontrol results, but the BACTcontrol peak on 20 October 2024 is not visible in HPC22. At the time of the higher HPC22 numbers in December 2024 no BACTcontrol data are available, whereas the HPC22-peak in beginning of January 2025 is matched by high enzymatic activity as measured with the BACTcontrol. This is comparable to the laboratory FCM-results, however, in January 2025 the number of cells is low whereas enzymatic activity is high. An important point is the difference in measurement principle between HPC22 (culture) and the BACTcontrol (enzymatic activity) which can explain the partial lack in correlation between the BACTcontrol and HPC22 results. Peaks in the ATP concentration match with a peak in the BACTcontrol on 20 – 21 October 2024, but the other peaks in ATP do not match the BACTcontrol results.

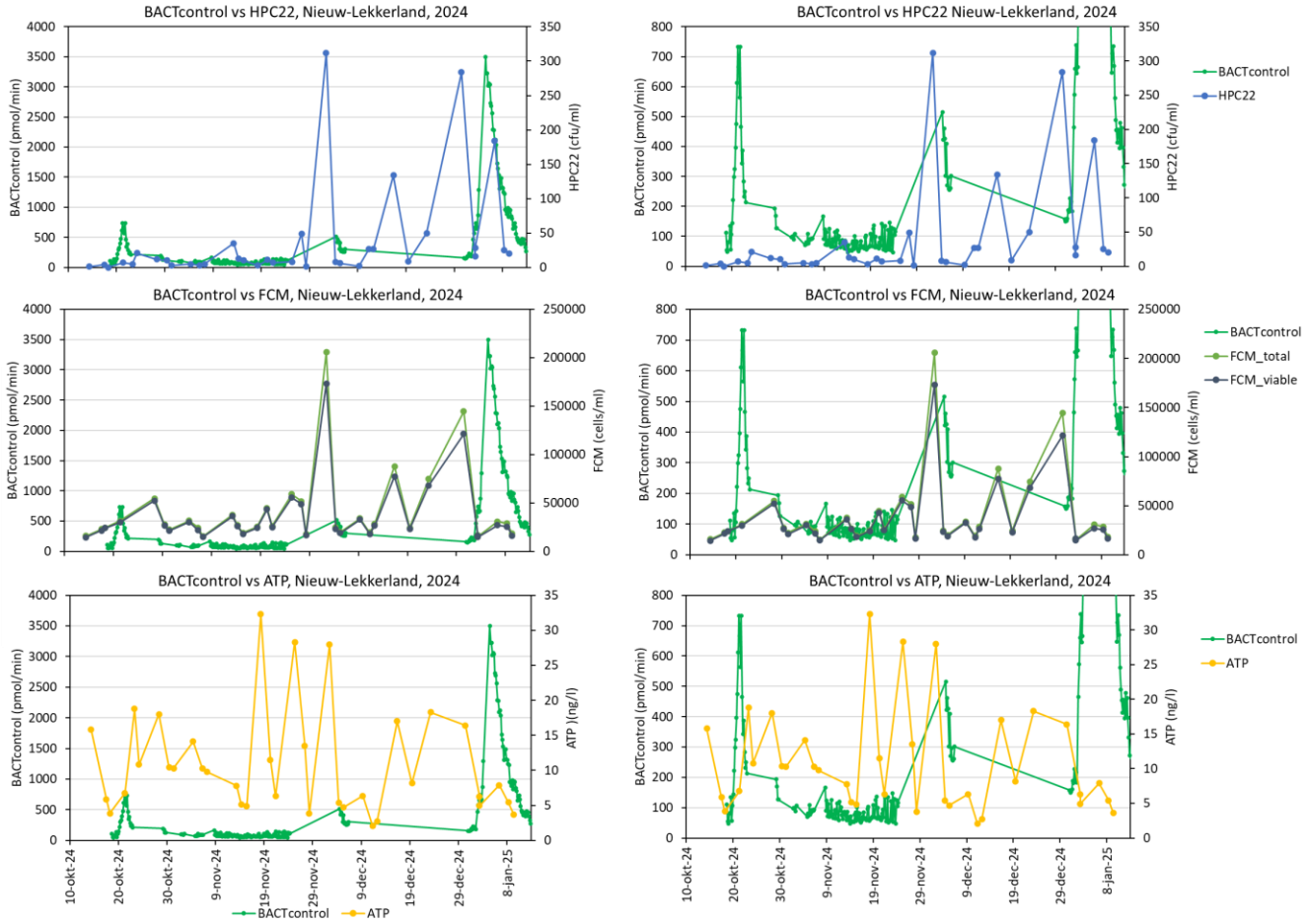


Figure 5-13. Comparison of flow cytometry (FCM), HPC22 and BACTcontrol results of the produced drinking water of Nieuw-Lekkerland (2024). Left: all available data are shown. Right: The y-axis of the BACTcontrol has been adapted to better visualize the datapoints with lower results.

5.4.2 Comparison BactoSense with laboratory parameters on produced drinking water

The number of intact cells measured with the laboratory FCM method is 0.7 – 8.8 times (average: 2.3 times) higher than the number of intact cells measured with the BactoSense. Comparison of the laboratory and in-line FCM methods (BactoSense) does not clearly show comparable trends in the increase of decrease of cell numbers (Figure 5-14; top). Although some trends of increasing or decreasing cell numbers are shown with both methods (28 October, 6, 13, 20 and 27 November 2024) others are not (December 2024 and January 2025). This suggests a relatively large variation between the FCM methods in these samples. This is in contrast to the validation study (chapter 3) where the correlation for drinking water between the two methods was high (r^2: 0.79 – 1.0) and the variation was smaller and the laboratory FCM method yielded 0.1 – 1.6 times (average: 0.8 times) higher numbers that the intact cells measured with the BACTcontrol. One of the reasons could be that for the laboratory FCM analysis, water samples were taken at the treatment plant, stored and transported to the laboratory before the analysis could be performed. Although a 24-hour storage at 4°C is allowed for this, it may be possible that some changes in the cell numbers occur. The BactoSense directly analyses the drinking water. In addition, the flow cytometry method (staining cells, gating, and instrumental parameters) differs between the laboratory FCM method and the BactoSense which all can influence the analysis result.

Comparison of the BactoSense with HPC22 also does not give comparable trends. Whereas the HPC22 count is low and stable in October and November 2024, the BactoSense varies between approximately 9,000 and 30,000 cells/ml. In addition, the HPC22 peaks in December 2024 and January 2025 are not consistently matched by increased cell numbers at the same time. A reason for this could be that bacteria that are counted with the HPC22-method are only a small percentage (<1%) of the total number of cells present in drinking water, an increase can thus remain invisible for the BactoSense. Furthermore, a lack of correlation between HPC22 and FCM-data has been observed more often [14, 15].

Comparison of the BactoSense with ATP sometimes shows similar trend, but not always. Whereas the peaks of the BactoSense and ATP in October and November 2024 match relatively well, the ATP peaks in December 2024 and January 2025 are not consistently matched by increased cell numbers at the same time.

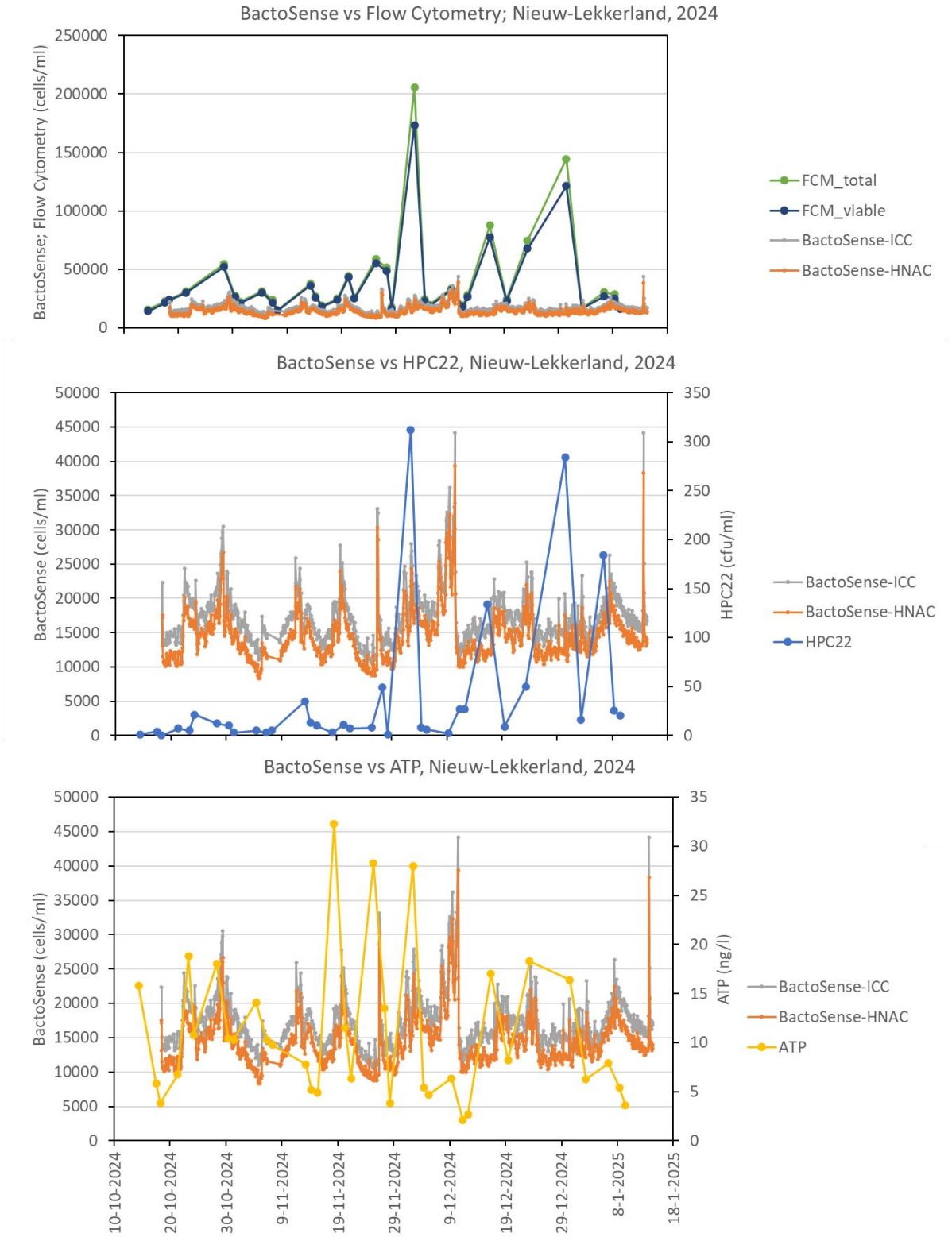


Figure 5-14. Comparison of flow cytometry (FCM), HPC22, ATP and BactoSense results of the produced drinking water of Nieuw-Lekkerland (2024).

5.4.3 Influence of operational production processes on measurements with BACTcontrol and BactoSense

Changes in operational parameters may potentially influence enzymatic activity or cell numbers in the produced drinking water and might therefore be detected by the sensors. To study this, results of the BactoSense and BACTcontrol were compared to known changes in operational parameters and to laboratory measurements of water samples taken throughout the production plant. Ideally, sensors for microbiological water quality that measure the same water should give comparable results. In other words when the cell numbers (measured with BactoSense) increase, also the enzymatic activity (measured with BACTcontrol) should increase and vice versa. Due to several changes in the operator personnel at both production plants during and after both testing periods, not all changes in operational parameters can be retrieved. Especially from the tests in 2022 at De Hooge Boom no information is available.

At several moments during the three-month monitoring period at production location Nieuw-Lekkerland, several laboratory parameters were measured in the produced drinking water or after one of the treatment steps as well as the inline sensor methods. Operational changes, that occurred during the monitoring period at Nieuw-Lekkerland in 2024 – 2025 and which might have an effect on the laboratory parameters and/or the sensors, are described below:

- 19 – 21 October 2024:
    - The previous regeneration of the ion exchange filters (IEX) was performed too late, due to which ammonium removal was not optimal in this period. This led to an increase in ammonium concentration, but not exceedance, in the water leaving the IEX on 21 October 2024 (IEX_out and IEX_combined: 0,031 – 0,038 µg/l). No data were available on this date of ammonium in the produced drinking water.
    - The laboratory measurements (HPC22, iron, methane, manganese) for this period showed no abnormalities.
    - The BACTcontrol detected an increase in enzymatic activity starting at 19 October of approximately 55 pmol/min, with a peak on 21 October of 733 pmol/min. The BactoSense peaked one day later (22 October) with and increase from approximately 14,000 cells/ml to 24,000 cells/ml.
    - Conclusion: Ammonium in water is known to serve as a nutrient for microbial growth. This is visible first in the increased enzymatic activity measured with the BACTcontrol and one day later in higher cell numbers with the BactoSense. Probably the enzyme activity per cell will first increase, until the bacterial cells have established enough energy to divide into more cells a day later.

- 30 October 2024:
    - Two very high HPC22 values were measured in the water after IEX of two parallel IEX filters (IEX_combined: twice 1,000 cfu/ml). HPC22 values in produced drinking water remained below 100 cfu/ml, but one out of three HPC22 samples was slightly higher (5 - 66 cfu/ml), instead of 5 – 21 cfu/ml a few days prior.
    - This coincided with higher iron (0.015 mg/l) and manganese (0.0151 mg/l) concentrations in drinking water.
    - There were no results available for the other laboratory measurements (ammonium and methane) for this period.
    - A few measurements of the BACTcontrol showed increased enzymatic activity on 29 October at around 125 - 200 pmol/min. The BactoSense also peaked on 29 October with an increase to almost 30,000 cells/ml.
    - Conclusion: Iron and manganese can serve as nutrients for bacteria, but as the metabolization process is slow, higher iron or manganese levels in water do not directly lead to microbial growth. In addition, it can adsorb to biofilm and subsequently immobilize difficult to degrade organic

carbon by adsorption. Consequently, the bacteria in the biofilm have more time to process these compounds. As all laboratory and sensor measurements were performed in water, it seems unlikely that the iron and manganese increase directly caused a microbial increase in the water. Another possibility is that the ammonium levels in the water after the IEX (which were high on 21 October) stayed elevated for several days and thereby allowing microbial growth (no laboratory measurements are available to support this hypothesis). The BactoSense results do seem to support this hypothesis as the cell count decreased after the peak of 22 October.

- End of October to 6 December 2024:
    - Aeration tower 4 did not work optimally to remove methane. On 6 December the rings in the aeration tower were cleaned by an external company and aeration tower 4 itself was also cleaned which solved the problems.
    - At the beginning of November (6 and 7 November), measurements showed increased HPC22 in the water after aeration tower 3 (256 and 204 cfu/ml) but low levels in the produced drinking water (3 and 5 cfu/ml).
    - In the entire period (end of October – 6 December) HPC22 in the produced drinking water was below 66 cfu/ml, except for 312 cfu/ml on 2 December.
    - Iron concentration was elevated on 4 November (0.0034 and 0.0112 mg/l) in both water streams after the IEX (IEX_out), and was low again at the next measurement on 19 November.
    - The other laboratory measurements (ammonium, manganese, methane) for this period showed no abnormalities.
    - The BACTcontrol measured a continuously low enzymatic activity between 47 – 147 pmol/min, except for higher values on 4 – 6 December (increase to 460 pmol/min, decreasing thereafter to 260 pmol/min). Between 24 November and 4 December no results are available. The BactoSense showed a different pattern with variable cell counts between 9,000 cells/ml and 32,000 cells/ml. Several peaks were observed (on 12, 19, 26 November and 1-2 December) during the period in which the aeration tower 4 did not work optimally. The peak that was observed directly after the cleaning (7 – 10 December) could be caused by release of dislodged biofilm (due to cleaning) into the drinking water.
    - Conclusion: The BACTcontrol and BactoSense patterns did not match with each other during this period (end of October to 6 December 2024). The low levels of the BACTcontrol do match the, in general, low HPC22. The variation of the BactoSense was not observed with HPC22, except for an overlapping peak on 1 – 2 December.

- 16 December 2024:
    - No known operational changes occurred in this period.
    - On 16 December, HPC22 was high in one of the produced drinking water streams (134 cfu/ml), but remained low in the other (19 cfu/ml).
    - Ammonium, iron, methane and manganese showed no abnormalities
    - No BACTcontrol results were available for 16 December. The BactoSense detected low cell counts (approximately 14,000 cells/ml).
    - Conclusion: The increase in HPC22 was not matched by the BactoSense. As the sensors were measuring water from the treated water reservoir, the high HPC22-levels in one (out of three) drinking water streams could have been diluted or absent in the treated water reservoir itself and therefore not visible with the BactoSense.

- 17 and 24 December 2024:
    - Aeration towers 1 and 4 were flushed (with drinking water) on 17 and 24 December. The flushing water was discarded and not distributed to the treated water reservoir.
    - Whereas on 16 December the HPC22 was high in drinking water (134 cfu/ml), it was low on 17 December (15 and 19 cfu/ml). On 24 December HPC22 was even lower (1 and 5 cfu/ml).
    - The parameters ammonium, iron, methane and manganese were not measured on 17 December and/or showed no abnormalities on 24 December.
    - No BACTcontrol results were available for 17 December. The BactoSense detected a small but sharp increase in cell counts (approximately from 15,000 to 20,000 cells/ml) which lasted until about 20 December. The flushing on 24 December also caused a sharp increase (approximately from 17,000 to 23,000 cells/ml).
    - Conclusion: Both BactoSense and HPC22 showed a (small) increase. Whereas HPC22 rapidly decreased to background levels, the duration of the BactoSense peak was longer. This could be caused by the different subset of bacteria that is detected by the respective methods. The HPC22 method detects bacteria that grow at high substrate concentrations, whereas the BactoSense detects all bacteria. It is possible that the first increase in bacteria is caused by bacteria that grown in substrate-rich conditions, and is detected by both methods. After this initial growth, the bacterial growth is caused by bacteria that grow under normal substrate conditions and are thus no longer detected with the HPC22-method, but are detected with the BactoSense. In addition, the increase in cell numbers on 24 December detected with BactoSense could include bacteria that were released from dislodged biofilm (due to cleaning) into the drinking water.

- 30 December 2024:
    - HPC22 was high in the three separate produced drinking water streams after the treated water reservoir (39, 157 and 284 cfu/ml).
    - No abnormalities were found for ammonium and no data were available for the other laboratory parameters (iron, methane, manganese) for this date.
    - No BACTcontrol results were available for 30 December. The BactoSense levels were relatively average at around 14,500 – 20,000 cells/ml. A closer look at the BactoSense results revealed a peak on 30 December around 10:00 AM (Figure 5-15). Although no sampling time is available of the grab samples that were taken for HPC22, it could be possible that the increase in HPC22 is also visible with the BactoSense.

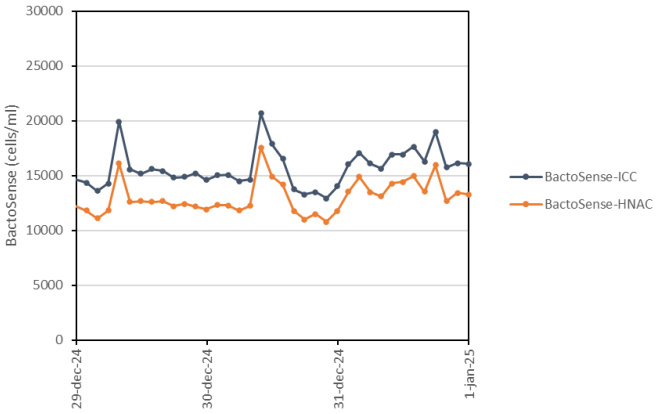


Figure 5-15. BactoSense and HPC22 results on 30 December 2024.

    - Conclusion: The increase in HPC22 is not matched by the BactoSense.

- Beginning of January 2025:
  - Aeration tower 3 was not working optimally.
  - On 6 January, HPC22 levels varied in the three separate water streams of produced drinking after the treated water reservoir, including three values above 100 cfu/ml (54, 116, 128 and 184 cfu/ml). But levels were low in the water after the aeration tower (1 - 58 cfu/ml).
  - Ammonium: no abnormalities.
  - An increase in iron and manganese was observed on 6 and 8 January in water after the calcite filters: 0.012 – 0.016 mg/l (iron) and 0.013 – 0.015 mg/l (manganese). Manganese, but not iron, was high in the produced water before the treated water reservoir (0.0126 and 0.0133 mg/l).
  - Methane was high in the water after the aeration tower on 6 January (6.3 – 8.1 mg/l), but not on 8 January.
  - The BACTcontrol detected a major increase in enzymatic activity up to 3,400 pmol/min, starting at 2 January and peaking on 4 January after which the enzymatic activity slowed decreased. The BactoSense showed increased cell number starting at 4 January with the peak on 7 January.
  - Conclusion: Methane is known to serve as an energy-rich substrate for microorganisms and should, therefore, always be completely removed during drinking water production. However, methane was not completely removed by aeration on 6 January, and was perhaps already earlier present in the water (although there are no laboratory measurements to prove this). The methane promoted significant microbial growth which led to the increase in enzymatic activity first, followed by an increase in cell numbers. Comparable to the situation on 19 – 21 October in which increased ammonium levels caused microbial growth.

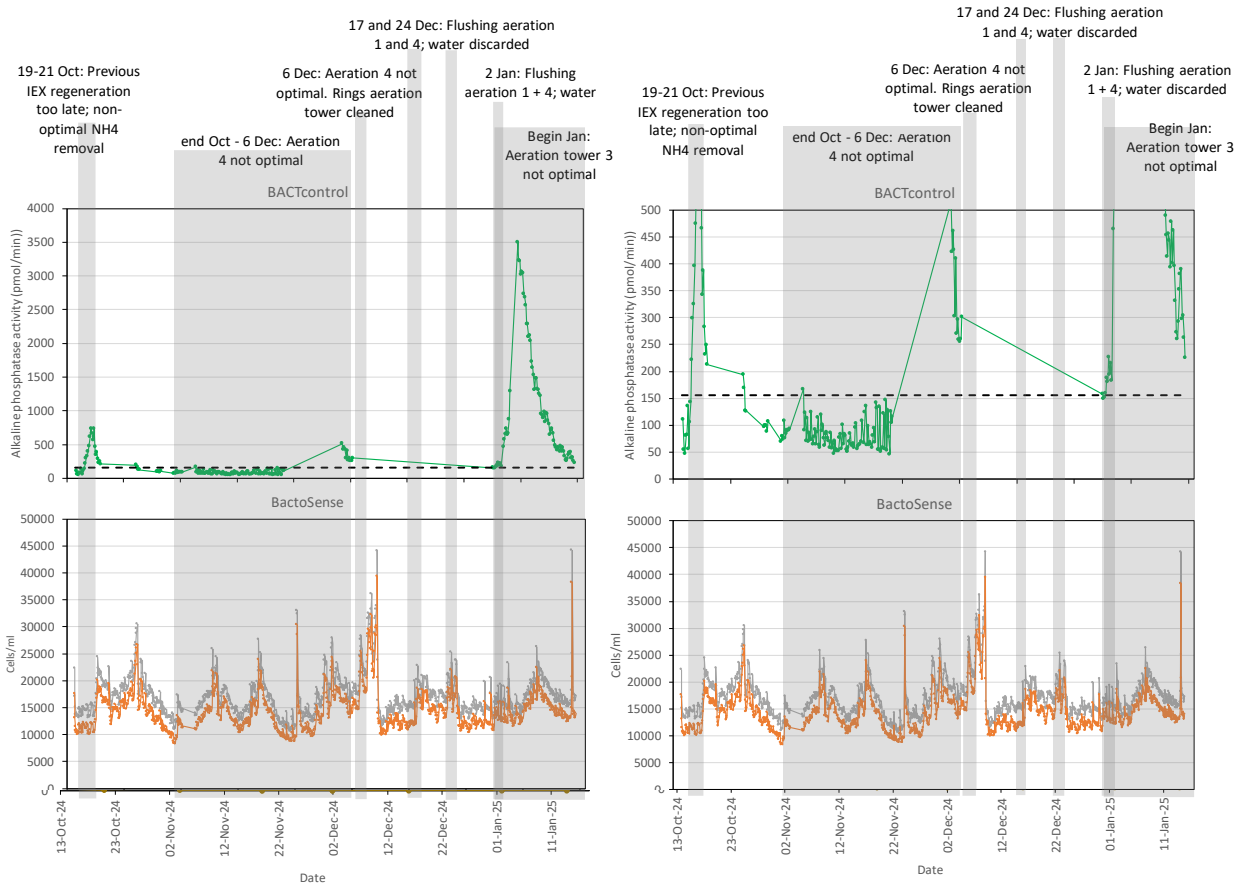


Figure 5-16. Results from sensors (BACTcontrol, BactoSense) at several steps in the drinking water production plant of Nieuw-Lekkerland. In grey is indicated during which period operational changes occurred, as indicated by Oasen. Left: all data. Right: adapted y-axis to also show the results in the lower ranges.

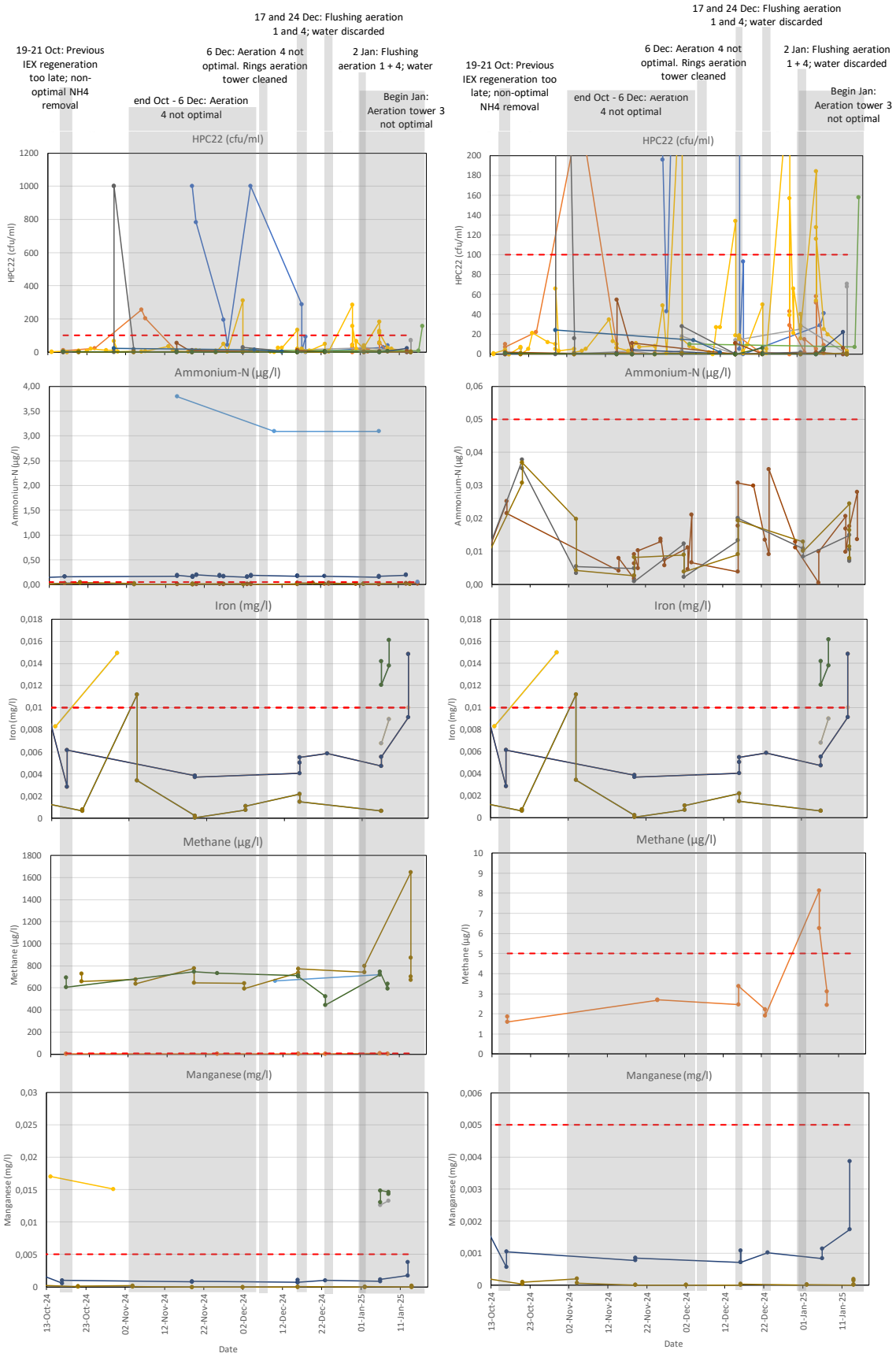


Figure 5-17. Results from laboratory parameters (HPC22, iron, manganese, ammonium, methane) at several steps in the drinking water production plant of Nieuw-Lekkerland. In grey is indicated during which period known operational changes occurred. Left: all data. Right: adapted y-axis to also show the results in the lower ranges.

5.4.4 CBM

Biofilm formation in drinking water processes is a relatively slow process due to the low nutrient concentration in drinking water. Therefore, the CBM can only provide a reliable BAR-value after glass beads have been exposed to the drinking water for four weeks. Consequently, due to this aspect and the nature of the CBM, only one BAR value can be obtained every two weeks.

The BAR-values obtained for the water from De Hooge Boom and Nieuw-Lekkerland were very high for drinking water. As these high BAR-values were measured at Oasen, but not at the other two drinking water locations (Evides, chapter 4 and Vitens, chapter 6), it is unlikely that the setup of the sensors and used materials (as described in chapter 2) caused the high BAR-values. Although the iron and manganese concentrations in the produced drinking water were below the detection limit, iron or manganese deposits were observed in the cuvettes upon sampling and analysis and in the tube between the drinking water tap point of Oasen and the sensor setup (Figure 5-18). Accumulation of dissolved iron and/or manganese on the glass pearls can serve as nutrients for microbial growth or it can immobilize difficult to degrade DOC in the biofilm once adsorbed to the biofilm. The microorganisms in the biofilm on the glass pearls will then have enough time to degrade this DOC, leading to higher ATP levels.

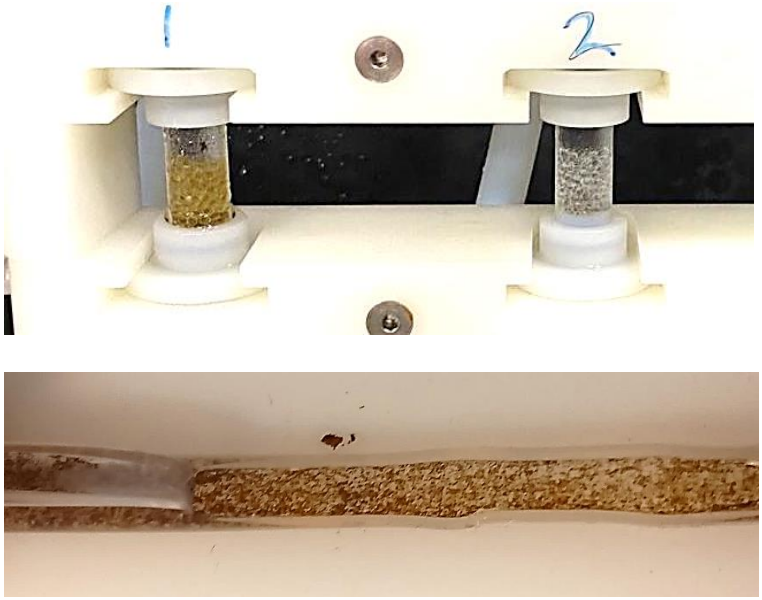


Figure 5-18 Iron or manganese deposits on the glass pearls of the Milispec-CBM (top photo). Shown are two cuvets with (left, 1) and without (right, 2) deposits. Bottom photo: iron or manganese deposits on the tube connecting the Oasen tap point to the sensor setup at Nieuw-Lekkerland (2024).

5.4.5 Signal values

For both the BACTcontrol and BactoSense signal values were calculated for each production location with the formula $Signal\ value = average \pm 3 \times standard\ deviation$ as described in chapter 0. Measurements below the lower signal value can indicate a (sudden) reduction in bacterial numbers or activity, for example due to sudden die-off of bacteria. Whereas measurements above the upper signal value can indicate an increase of bacterial counts or activity, for example due to growth, changes in operational parameters or other processes. Both alarms, thus, could indicate that something happened with the microbial water quality and that action might be needed.

At De Hooge Boom (2022) the signal values were calculated using all reliable results from the monitoring period (21 March to 23 May 2022). For Nieuw-Lekkerland, the results from 9 – 24 November 2024 were used for both BACTcontrol and BactoSense. This period was chosen as the laboratory parameters were within normal limits, indicating normal operational procedures and drinking water production. The observed dynamics for the BACTcontrol and BactoSense are therefore considered normal variation of the drinking water produced at Nieuw-Lekkerland.

The calculated signal values for the BACTcontrol are given in Table 4-5 and are indicated with the dashed lines in Figure 6-6 and Figure 6-7 (chapter 3 of this report). The calculated lower signal values for De Hooge Boom and Nieuw-Lekkerland were always lower than the lowest value obtained for each water type. The calculated upper signal value for De Hooge Boom was exceeded twice. This was caused by two individual high measurements (Figure 6-6) which did not seem to indicate real changes in microbial water quality. For Nieuw-Lekkerland the upper signal value was exceeded 107 times (Figure 6-7), this was caused by three major peaks as a consequence of higher ammonium and methane levels and thus microbial growth (paragraph 0). In this case, the exceedances thus indeed indicated a change in microbial water quality originating from a change in chemical water quality (i.e. enhanced ammonium and methane concentrations).

Table 5-4. The lower and upper signal values, counts and number of exceedances of the BACTcontrol in drinking water at De Hooge Boom (2022) and Nieuw-Lekkerland (2024). The signal values were calculated for the periods 21 March – 23 May 2022 (De Hooge Boom) and 9 – 24 November 2024 (Nieuw-Lekkerland). Exceedances: number of measurements that exceeds the calculated signal value during the entire monitoring period.

	Lower signal value (pmol/min)	Exceedances lower signal value	Upper signal value (pmol/min)	Exceedances upper signal value
De Hooge Boom (2022)	114	0	659	2
Nieuw-Lekkerland (2024)	3	0	156	107

For the BactoSense, signal values were calculated for ICC and HNAC and these values are given in Table 6-5. For De Hooge Boom the lower ICC signal value (4.8×10^5 cells/ml) was exceeded eleven times. These exceedances are the outliers pointed out in Figure 6-8 (in chapter 3 of this report) for which no explanation could be found. Other than those outliers, the daily fluctuations in ICC and HNAC did not exceed the lower or upper signal values at De Hooge Boom. The data from both the BactoSense and BACTcontrol, thus, show that during the monitoring period the microbial water quality at De Hooge Boom was normal for that location.

At Nieuw-Lekkerland the upper signal values of both ICC (2.6×10^4 cells/ml) and HNAC (2.2×10^4 cells/ml) were exceeded respectively 37 and 36 times. Whereas some of the exceedances coincide with known operational changes and deviating results of the laboratory parameters, others do not. This means that part of the exceedances relates to aberrations in microbial water quality that originated from changes in chemical water quality. However, another part of the exceedances seems to relate to normal variation in cell numbers of drinking water and is no indication for problems with the microbial water quality.

Table 5-5 The lower and higher signal values and number of exceedances of BactoSense in drinking water at De Hooge Boom (2022) and Nieuw-Lekkerland (2024). Signal values were calculated for the periods 21 March to 23 May 2022 and 9 – 24 November 2024. Exceedances: number of measurements that exceeded the calculated signal value during the entire monitoring period.

	Lower signal value				Upper signal value			
	Cells/ml		Exceedances		Cells/ml		Exceedances	
	ICC	HNAC	ICC	HNAC	ICC	HNAC	ICC	HNAC
De Hooge Boom (2022)	$4.8\text{x}10^5$	$1.2\text{x}10^5$	11	0	$7.8\text{x}10^5$	$2.1\text{x}10^5$	0	2
Nieuw-Lekkerland (2024)	$7.2\text{x}10^3$	$5.6\text{x}10^3$	0	0	$2.6\text{x}10^4$	$2.2\text{x}10^4$	37	36

In chapter 10 more methods to calculate signal values are described, for example to take the daily fluctuations into account.

5.5 Location-specific conclusions

The BAR values for the treated water of De Hooge Boom and Nieuw-Lekkerland are high. The cause of the high BAR values is probably the iron and/or manganese present in the drinking water which can be used for microbial growth when it is encapsulated in the biofilm.

The cell numbers, as measured with the BactoSense, are significantly higher in drinking water from De Hooge Boom compared to Nieuw-Lekkerland. Cell numbers varied at both locations, but the changes at Nieuw-Lekkerland were shorter and more sudden compared to the more gradual changes in De Hooge Boom. Part of the peaks in cell numbers can be linked to changes in the operational control of the treatment plant of Nieuw-Lekkerland and/or can be linked to levels of HPC22, iron, manganese, ammonium or methane. Interestingly, some, but not all, of these peaks exceeded the signal value which would indicate a significant change in microbial water quality. From De Hooge Boom no information was available on operational parameters or from additional laboratory measurements.

The enzymatic activity, as measured with the BACTcontrol, was significantly higher in drinking water from De Hooge Boom compared to Nieuw-Lekkerland. Changes in enzymatic activity at De Hooge Boom were minimal and gradual. At Nieuw-Lekkerland three major peaks were observed which coincided with operational changes or increases in methane or ammonium, which are both substrates for microbial growth.

The three peaks of the BACTcontrol were also visible with the BactoSense, although the peak in enzymatic activity was often one or two days earlier compared to the cell numbers. However, a two-week period during which the BACTcontrol measured low and stable enzymatic activity was not matched by the highly variable cell numbers measured with the BactoSense. The two sensors thus sometimes showed comparable trends in measurement values, but not always and consequently they can give conflicting insights into the microbial water quality.

Part of the variation in enzymatic activity and cell numbers could be explained by changes in operational parameters and/or laboratory measurements in the treatment plant. However, it seems unlikely that the normal variation in bacterial numbers and activity in drinking water results in microbiological water quality issues (e.g. public health, aesthetical or technical complaints) at the consumers tap. The results, thus, suggest that most of the observed variation could be considered as normal variation that did not impact the microbial water quality in such a way that problems occurred.

The sensors did not give (new) insights into the performance of the RO treatment step, but gave new insight into the performance of the whole treatment train. To study the performance of the RO treatment solely, the sensors have to be placed directly after the RO to ensure that other treatment steps (such as IEX and aeration) do not potentially influence the microbiological water quality.

The goal of Oasen, to set local signal values and determine at which alarm or trigger value action should be taken, was partly met. Signal values were set and some exceedances clearly indicated problems with water quality issues (mainly methane and ammonia breakthrough), but not all changes in operational parameters led to an exceedance and sometimes the signal value was exceeded, but the monitored processes were not the cause for the exceedances.

6 Monitoring drinking water production at Vitens

6.1 Introduction test location

6.1.1 Location

Two different drinking water production locations at Vitens were used for this study: Spannenburg and Noardburgum (Figure 4-1). Both production locations produce drinking water from groundwater. From historical data and experiences it is known that Noardburgum produces more biologically stable water than Spannenburg, meaning that drinking water from Noardburgum contains less nutrients in water for bacterial growth than the drinking water from Spannenburg. However, most biological stability measurements at Spannenburg have been done before they installed Ion exchange as last treatment step that probably also improves the biological stability of the drinking water. Furthermore, in the beginning of 2021 the resin in the ion exchanger in Spannenburg has been replaced, that probably improves the biological stability. But not much data is available on this.

Vitens experiences *Aeromonas* regrowth in the drinking water distribution system of Spannenburg and Noardburgum, especially in the 'mix zones' where alternately water of production locations Spannenburg or Noardburgum is delivered. The cause of this regrowth problem is not entirely known, but probably relates to the biological stability of both water types.

Figure 6-1 Pilot locations Spannenburg (red dot) and Noardburgum (black triangle), Vitens

6.1.2 Goal

Within this project it was tested whether the sensors can measure differences in the microbial water quality of the produced drinking water of production locations Spannenburg and Noardburgum. These differences might be caused by differences in treatment steps of the production locations and/or the quality and composition of the untreated ground water from which drinking water is produced. It is hypothesized that differences in water quality will be detected between the two produced drinking water at the production plant, and that this may explain the *Aeromonas* regrowth in the distribution system. In addition, it was tested whether local alarm values could be set on which the local operator of Vitens can act.

6.2 Monitoring campaign

6.2.1 Experimental set up at production locations Spannenburg and Noardburgum

The sensors were installed after the clear water reservoirs at both Spannenburg and Noardburgum. Biomass monitoring using the BactoSense and BACTcontrol sensors, and biofilm formation using the CBM was done at Spannenburg and Noardburgum for 2.5 months (10 June – 24 August 2022; Table 6-1). Two BACTcontrol sensors were installed simultaneously, one at production location Spannenburg and one at production location Noardburgum. Due to technical problems, data are only available for the period 21 July – 24 August. One BactoSense system was available during the monitoring period and was first placed at Spannenburg (10 June – 3 August 2022) and then moved to Noardburgum (3 August – 24 August 2022). Two types of CBMs were used in the research: a more user-friendly CBM that was developed by Milispec (Milispec-CBM) which is based on the original CBM developed by KWR (KWR-CBM) and the original KWR-CBM, that is less user-friendly. The Milispec-CBM and KWR-CBM were installed simultaneously at both locations (10 June – 24 August 2022). This enabled the comparison between both types of CBMs, sampling of cuvettes and ATP-analyses.

Table 6-1 Sampling locations and monitoring times. Given are the installation dates per sensor and for which period measurement data were available.

		Spannenburg	Noardburgum
BactoSense	Installation period	10 June – 3 August 2022	3 August – 24 August 2022
	Data available	10 June – 3 August 2022	3 August – 24 August 2022
BACTcontrol	Installation period	10 June – 24 August 2022	10 June – 24 August 2022
	Data available	20 July – 24 August 2022	20 July – 24 August 2022
CBM Milispec	Installation period	14 June – 24 August 2022	-
	Data available	14 June – 24 August 2022	-
CBM KWR	Installation period	-	14 June – 24 August 2022
	Data available	-	14 June – 24 August 2022

A schematical overview of both production locations is given in Figure 6-2. The installation site of the sensors is indicated in red.

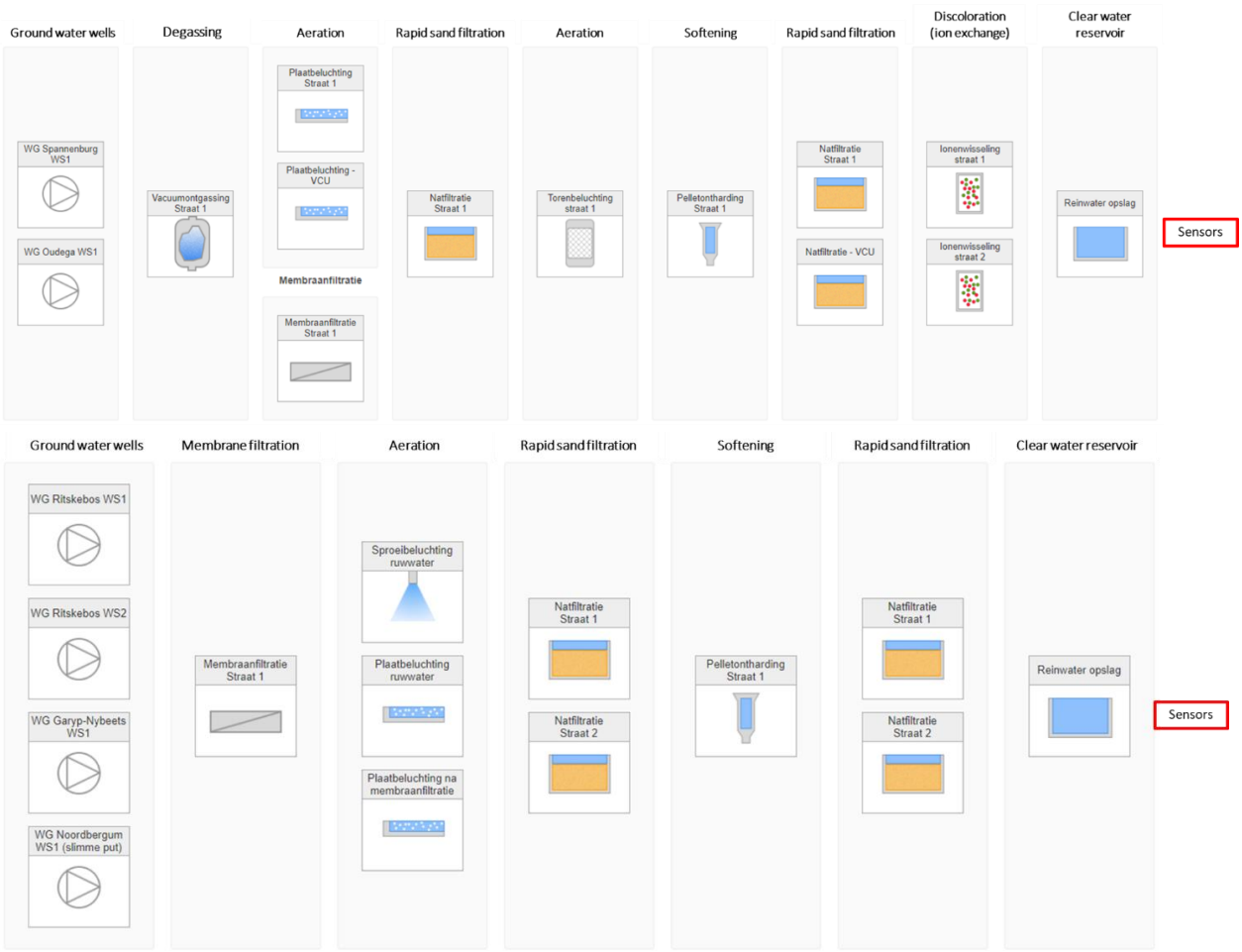


Figure 6-2 Overview production location Spannenburg (top) and Noardburgum (bottom). Sensors were placed after the clear water reservoir.

The CBM, BactoSense and BACTcontrol were all connected to the same sampling point via a splitter (Figure 4-4 in Chapter 4, right). This splitter has an open end to ensure a continuous water flow. A schematic overview of the sensor platform is given in Figure 4-3 of Chapter 4). The splitting station (Figure 4-4 in Chapter 4, right) was cleaned before installation and after finishing the tests at Vitens. Cleaning was performed with SDS and citric acid to remove biofilm build up.

The CBM, BactoSense and BACTcontrol were placed on tables, with extra space to perform the ATP analyses (Figure 6-3).

Figure 6-3. Installation of BACTcontrol, BactoSense and Milispec-CBM at production location Spannenburg, Vitens. The splitting station was placed between the BactoSense and the CBM.

For connecting the sensors to the drinking water network, new tubes, made of Teflon material that does not promote bacterial regrowth, were used. The splitter was made of stainless-steel material, without the usage of adhesives or other compounds that can promote bacterial growth. The KWR-CBM at Noardburgum was inadvertently not covered after installation, which is required to prevent light reaching the water. This caused algae growth which became visible at the end of June 2022. On 3 August a dark bag was placed over the KWR-CBM. This caused the amount of algae to reduce over time. Nevertheless, the algae were not completely removed and influenced the ATP measurements of the glass pearls of the KWR-CBM. The Milispec-CBM in Spannenburg was covered from the start of the measurement period and, therefore, did not develop algae growth.

6.2.2 Monitoring details

The measurement frequency of the BactoSense and BACTcontrol can be programmed and was set at a 2-hour interval. The measurements performed by the BACTcontrol and BactoSense sensors were done almost at the same time. However, it was not possible to schedule sampling of both sensors in such a way that measurements would be performed at exactly the same time.

Vitens operated the BactoSense and BACTcontrol during the measurement period.

The Milispec-CBM was installed at Spannenburg and a KWR-CBM was installed at Noardburgum. Every two weeks, both CBMs were sampled by Vitens and the ATP content of the biofilm was measured with the LuminUltra ATP kit on-site (according to the protocol of Chapter 2). The sampling of the KWR-CBM and Milispec-CBM was done at the same day.

<u>Additional analyses by Vitens</u>

During the measurement period of the sensors, Vitens performed additional analyses on the produced drinking water at the production plant: turbidity, Heterotrophic Plate Count (22°C) and *Aeromonas*. These results are also shown in this report.

6.2.3 Measurement, data processing and statistics

BACTcontrol

The measurement results of the BACTcontrol were quality-controlled by microLAN before further data analysis. After the quality control, the first three measurement results after a temporary stop of the BACTcontrol (longer than 27 hours) were removed from the dataset and thus not used in data processing and interpretation. The results from these measurements were often higher than the results from the other measurements. This is likely to be caused by a technical aspect, as biomass build-up occurred during the standstill period of the BACTcontrol (Figure 6-4). Often several internal cleaning cycles of the BACTcontrol were required before measurement values returned to a normal level.

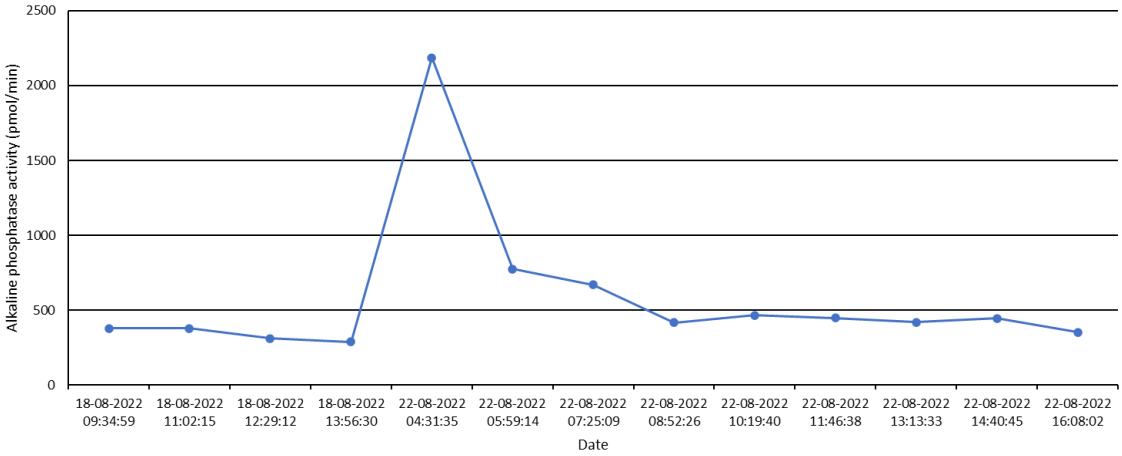


Figure 6-4 Increased ALP activity (between 05:59:14 and 08:52:26 on 22 August 2022) after shut down of the BACTcontrol after 13:56:30 on 18 August 2022.

Due to technical issues and a lack of software update, the results from the BACTcontrol between installation on 10 June and 20 July were not reliable and therefore these results are omitted from this report. The different issues were solved on 20 July by microLAN.

BactoSense

The measurement results of the BactoSense were analysed together with APT (distributor of BactoSense) and bNovate (producer of BactoSense) to evaluate and, if necessary, to adapt the gates of the BactoSense for this specific water type. Based on the knowledge and experience of bNovate the gates were adapted twice after which the ICC, HNAC and LNAC were considered reliable and the absolute values were used. The TCC could not be reliably determined. An example of the results and gates for the two production locations (Spannenburg and Noardburgum) is given in Figure 6-5.

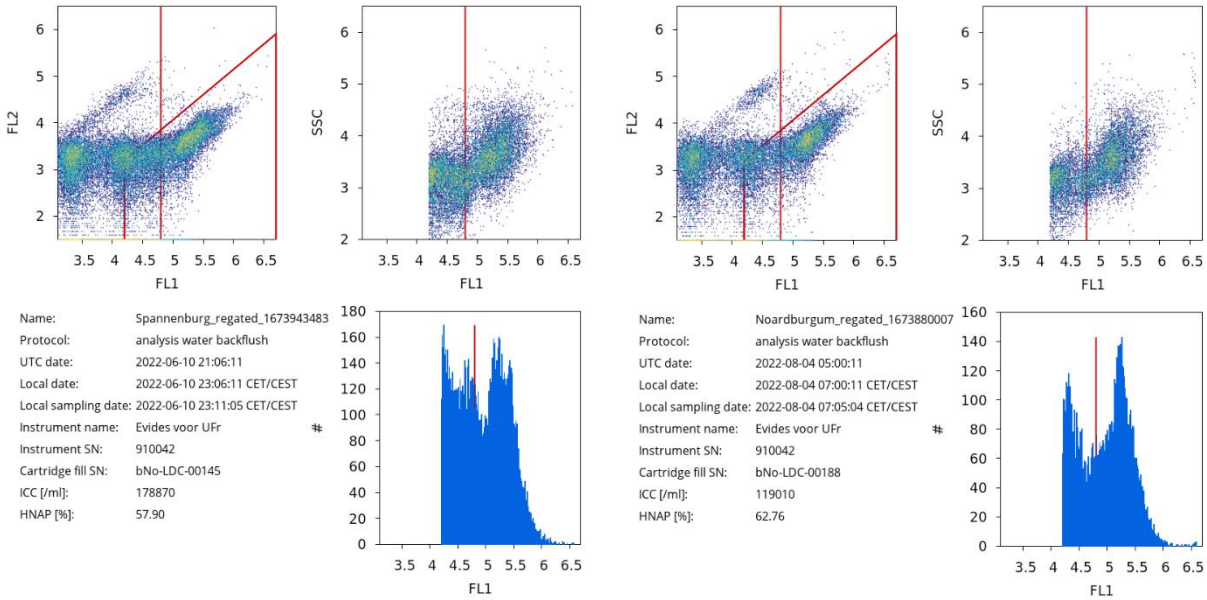


Figure 6-5. BactoSense results and gate examples Spannenburg and Noardburgum.

CBM

The Milispec-CBM and KWR-CBM were sampled and analyzed by Vitens every two weeks on the same date. The ATP analysis was performed as described in paragraph 2.1.4 (Chapter 2). In brief, ATP was released from the bacterial cells in the biofilm, that was formed on the glass beads, using the lysis buffer from the LuminUltra ATP-field kit and the ATP-concentration was measured with a luminometer.

Statistics

With a Shapiro-Wilkinson test it was tested whether the different datasets (BACTcontrol and BactoSense per sampling location) were normally distributed. For both the BACTcontrol and the BactoSense the dataset was not normally distributed. Therefore, the non-parametric Kruskal-Wallis test, with Mann-Whitney post-hoc, was used to determine whether the results differed significantly ($p<0.05$) between the two measurement locations (Spannenburg and Noardburgum).

An alarm value was calculated to determine a threshold value to separate between noise (e.g. operational and instrumental noise) considered to be normal variation, and events defined as measurements above or below the alarm value [12]. The alarm value was calculated as formulated by Favere et al. [12] for the datasets. From this dataset some BACTcontrol measurements were removed as described above.
The value was calculated as follows:

$$Alarm\ value = average \pm 3 \times standard\ deviation$$

This formula is based on the assumption that the dataset follows a normal distribution and that 99.7% of all data points falls within the $average + 3 \times SD$ and $average - 3 \times SD$ boundaries. This is a generally accepted calculation.

6.3 Results

The results of the tests at Spannenburg and Noardburgum are first described per monitoring technique (BACTcontrol, BactoSense and CBM) and are then also compared to each other.

6.3.1 BACTcontrol

The results of the BACTcontrol sensor are shown in Figure 6-6 and Figure 6-7. As described in paragraph 0., after a period that the BACTcontrol was turned off, the first three measurements were removed from the database. In total four ALP activity peaks were removed (two at Spannenburg and two at Noardburgum). The results shown in Figure 6-6, Figure 6-7 and Table 4-2 are based on this updated dataset.

The enzymatic activity varied between 413 – 1212 pmol/min for Spannenburg and 60 – 1001 pmol/min for Noardburgum. At Spannenburg, the enzymatic activity was higher at the start of the BACTcontrol measurements (20 July). After 7 days the activity stabilized to an average value of 653±84 pmol/min. At Noardburgum the enzymatic activity fluctuated more over the measurement period, with a clear rise in enzyme activity at the beginning of Augustus, after which the activity decreased again to a more stable level at the end of August.

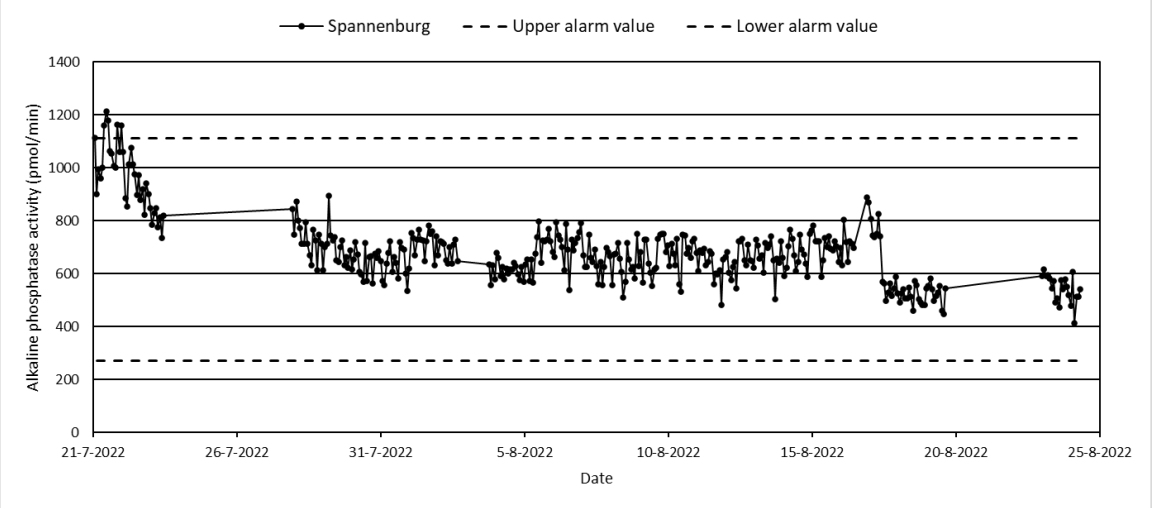


Figure 6-6 BACTcontrol results of measurements (20 July to 24 August 2022) at Spannenburg, Vitens. Alarm values (calculated according to formula described in paragraph 2.3.4) were calculated at the ALP activity from 20 July to 24 August 2022.

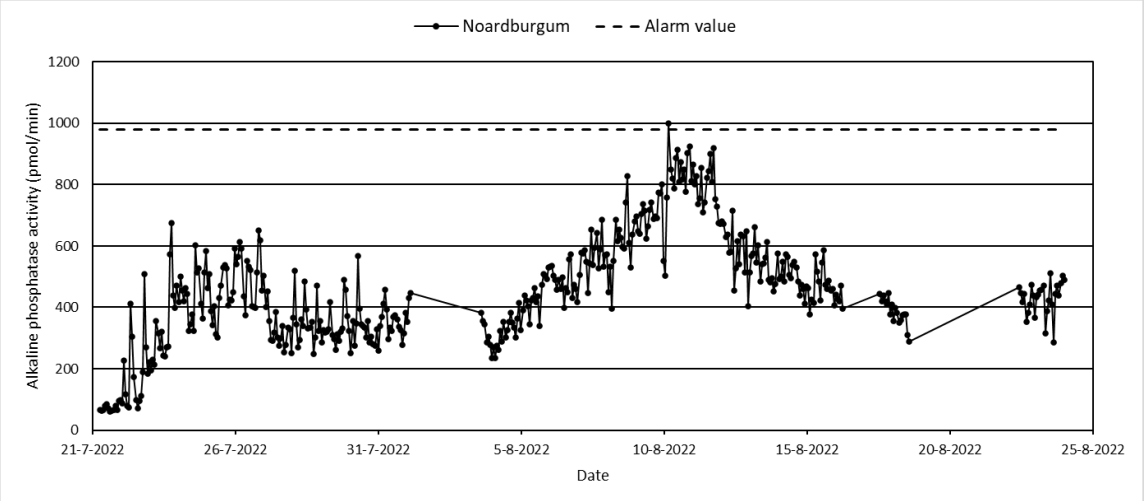


Figure 6-7 BACTcontrol results of measurements (21 July to 23 August 2022) at Noardburgum, Vitens. Alarm values (calculated according to formula described in paragraph 2.3.4) were calculated at the ALP activity from 21 July to 23 August 2022. The lower alarm value is negative and not displayed.

A summary of the BACTcontrol measurements, to compare the results from Spannenburg with those from Noardburgum, is shown in Table 6-2. The average enzymatic activity in the drinking water of Spannenburg was significantly higher than the average enzyme activity in the drinking water of Noardburgum (691 vs 454 pmol/min; Kruskal-Wallis: $p<0.05$).

Table 6-2 Summary of BACTcontrol results at Spannenburg and Noardburgum, Vitens. ALP is stated in pmol/min. SD: Standard deviation. SE: standard error. Count: number of reliable measurements. Calculated alarm values are given in chapter 4.4.4.

	Mean	Median	Minimum	Maximum	SD	SE	Count
Spannenburg	691	672	413	1212	140	7	386
Noardburgum	454	443	60	1001	175	8	439

6.3.2 BactoSense

The BactoSense results showed a clear difference in cell numbers in drinking water between Spannenburg and Noardburgum (Figure 6-8 and Table 6-3). The intact cell numbers (ICC) were relatively constant for both Noardburgum and Spannenburg, but the average ICC and HNA cell numbers were slightly higher in Spannenburg (ICC: $1.8x10^5$ and HNAC: $1.0x10^5$ cells/ml) compared to Noardburgum (ICC: $1.1x10^5$ and HNAC: $6.9x10^4$ cells/ml; Table 6-3). The percentage of HNAC among the ICC was more or less comparable between Spannenburg (56%) and Noardburgum (64%). A few outliers were visible at both production locations (indicated by the arrows in Figure 6-8), showing an increased ICC and occasionally increased HNAC. On 29 June the outlier coincided with a higher pH of the water (further described in chapter 6.4.1). Further inspection of the specific BactoSense measurements did not give a clear (technical) indication for the cause of these outliers.

At 4 July 2022 (indicated with * Figure 6-8) the cartridge of the BactoSense (containing all the reagents) was replaced, which resulted in a small trend break. Slight differences between different cartridges are known to bNovate, but these differences are always small.

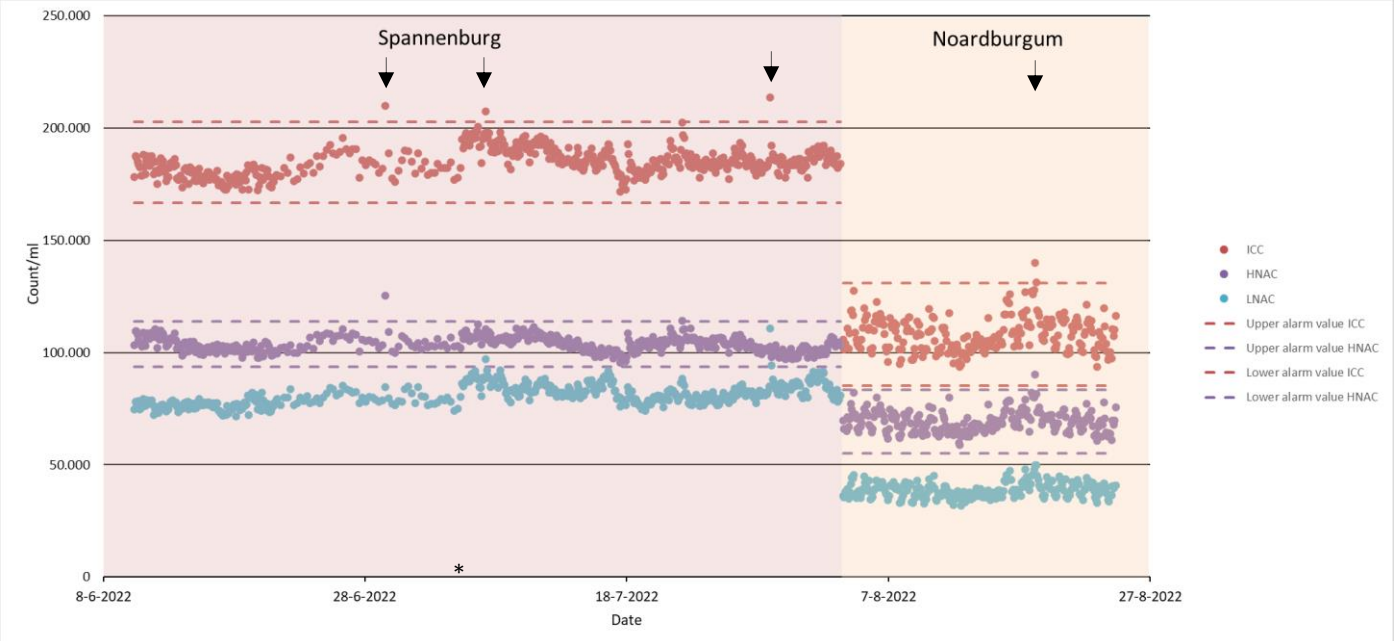


*Figure 6-8. BactoSense results of measurements from Spannenburg (10 June – 3 August 2022) and Noardburgum (3 – 24 August 2022) at Vitens. *: replacement of cartridge*

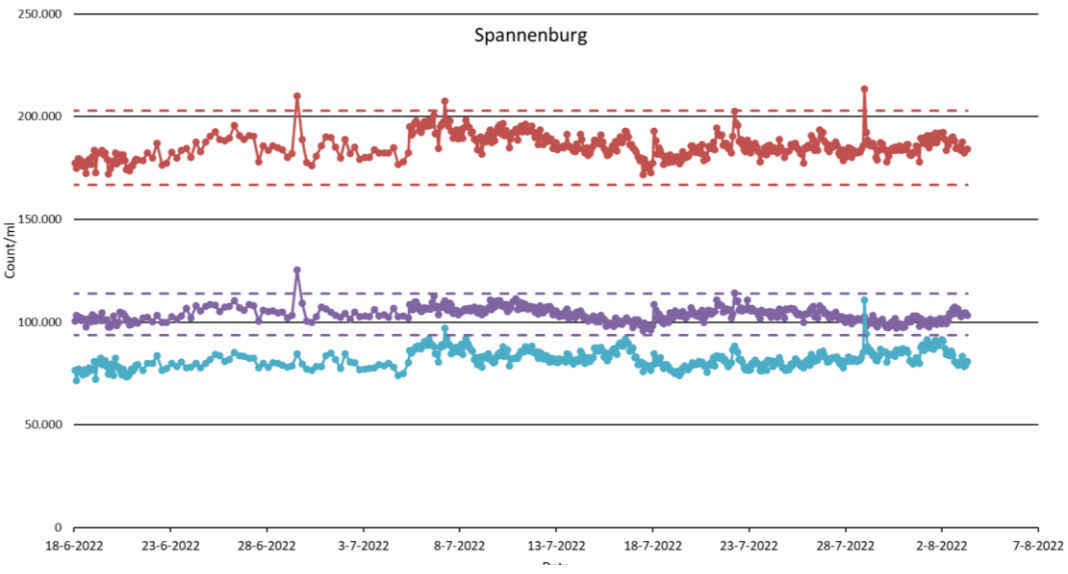
A summary of the results is shown in Table 6-3. All types of cells (ICC, HNAC and LNAC) in drinking water at Spannenburg were significantly higher than in drinking water at Noardburgum (Kruskal-Wallis: $p<0.05$, Table 6-3).

Table 6-3. Summary statistics BactoSense. Values are given in count/ml. SD: Standard deviation. SE: standard error. Count: number of reliable measurements. Calculated alarm values are given in chapter 4.4.4.

Cell count	Sampling location	Mean	Median	Min.	Max.	SD	SE	Count	95-percentile	99-percentile
ICC	Spannenburg	$1.8x10^5$	$1.8x10^5$	$1.7x10^5$	$2.1x10^5$	$6.0x10^3$	$2.6 x10^2$	536	$2.0x10^5$	$2.0x10^5$
	Noardburgum	$1.1x10^5$	$1.1x10^5$	$9.4x10^4$	$1.4x10^5$	$7.6x10^3$	$4.8 x10^2$	251	$1.2x10^5$	$1.3x10^5$
HNAC	Spannenburg	$1.0x10^5$	$1.0x10^5$	$9.6x10^4$	$1.3x10^5$	$3.4x10^3$	$1.5 x10^2$	536	$1.1x10^5$	$1.1x10^5$
	Noardburgum	$7.5x10^4$	$7.4x10^4$	$6.4x10^4$	$9.8x10^4$	$5.1x10^3$	$3.2 x10^2$	251	$7.8x10^4$	$8.2x10^4$
LNAC	Spannenburg	$1.3x10^5$	$1.3x10^5$	$1.2x10^5$	$1.8x10^5$	$8.0x10^3$	$3.5 x10^2$	536	$8.9x10^4$	$9.2x10^4$
	Noardburgum	$5.8x10^4$	$5.8x10^4$	$4.7x10^4$	$7.4x10^4$	$5.1x10^3$	$3.2 x10^2$	251	$4.5x10^4$	$4.8x10^4$

Closer inspection of the results of the BactoSense revealed a regular pattern in cell numbers of the three different cell types (ICC, HNAC, LNAC) at Noardburgum, except for the period between 12 and 15 August 2022 (Figure 6-9, bottom). The daily pattern often showed small dips in cell numbers between 17:00 and 23:00, with peaks during the morning and, therefore, probably caused by the higher water flow at peak moments. At Spannenburg no regular pattern was observed (Figure 6-9, top) and this difference is most likely caused by differences in the last treatment step of drinking water production: rapid sand filtration in Noardburgum and ion exchange in Spannenburg.

In the discussion chapter (chapter 6.4) the results of the BactoSense will be compared to changes in operational parameters and water demand of the production plant.



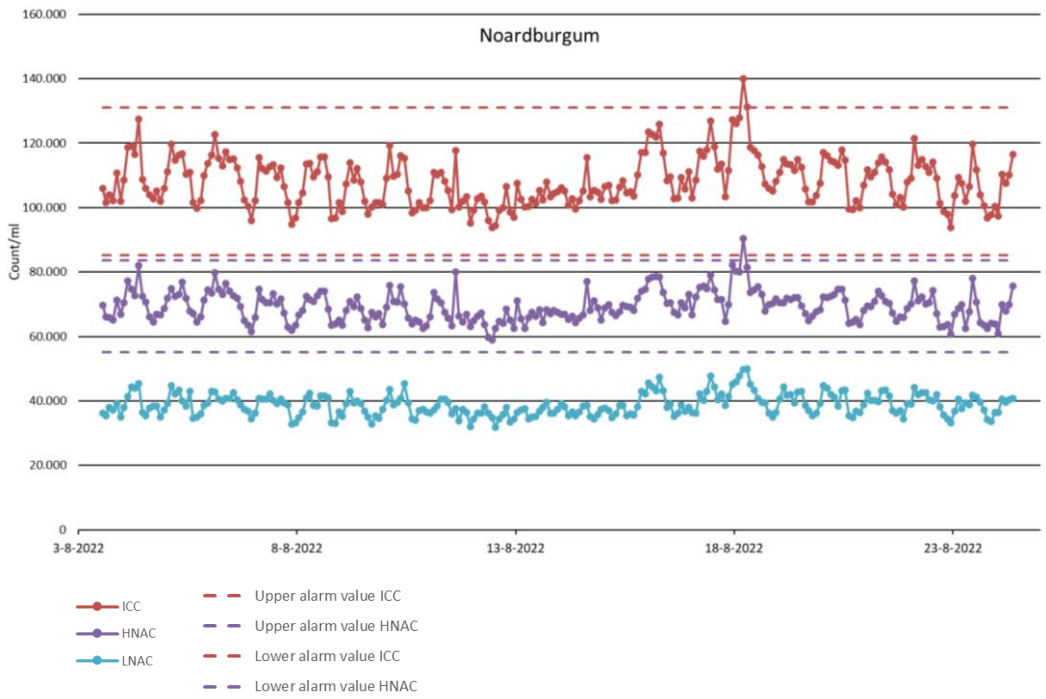


Figure 6-9. BactoSense results of measurements from Spannenburg (10 June – 3 August 2022; top) and Noardburgum (3 – 24 August 2022; bottom) at Vitens.

6.3.3 CBM

The biomass accumulation rate (BAR) determined with the Milispec-CBM of the drinking water at Spannenburg was stable and ranged between 2.7 and 7.1 pg ATP cm^{-2} day^{-1} (Figure 6-10). The BAR determined with the KWR-CBM of the drinking water at Noardburgum varied much more and ranged between 3.8 and 22.7 pg ATP cm^{-2} day^{-1}. However, in the KWR-CBM at Noardburgum algae were observed from the start of the experiment making the results less reliable, although visible inspection suggested that the algae decreased over time. Especially the first few measurements will overestimate the BAR due to the presence of ATP from algae and this, thus, resulted in decreasing BAR values over time. At Spannenburg the BAR fluctuated around 5 pg ATP cm^{-2} day^{-1} (Figure 6-10) and at the last measurement points, the BARs in the drinking water of Spannenburg and Noardburgum were comparable.

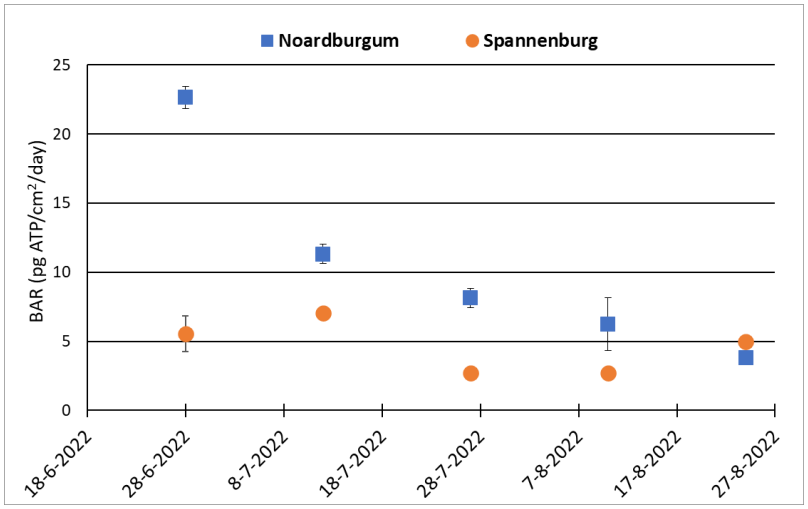


Figure 6-10 The BAR determined with the KWR-CBM in Noardburgum and the Milispec-CBM in Spannenburg, Vitens. The data are shown as average values ± standard deviation.

6.4 Discussion

6.4.1 Influence of operational production processes on measurements with BACTcontrol and BactoSense

Results of the BactoSense and BACTcontrol were compared to changes that occurred in the treatment plant as changes in operational parameters might influence microbial activity or cell numbers in the produced drinking water and might, therefore, be detected. The following operational changes occurred during the monitoring period:

- Spannenburg:
  - Rapid sand filters are flushed every 2h20min (prefilters) or every 7h20min (final filters);
  - 19 June 2022: slightly lower water demand pattern and increase in water level in the clear water reservoirs;
  - 29 June 2022, 6:00: increase pH of drinking water.
- Noardburgum:
  - 9 – 16 August 2022: higher demand of drinking water by consumers;
  - 11 – 15 Augus 2022: extra ground water was abstracted from two (varying) ground water wells. As a consequence more drinking water can be produced, the water passes the treatment plant more quickly and the rapid sand filters have to flush more often;
  - 12 August 2022, 9:30 – 13:30: more ground water was abstracted from two (varying) ground water wells, in addition to the already increased groundwater extraction between 11 – 15 August. Due to the very high water production (due to the high demand), a small part of the water went through the bypass (using UV disinfection) resulting in harder drinking water than normally.

During the first two changes in operational parameters at Spannenburg (19 and 29 June 2022), the BACTcontrol experienced technical issues and no results were available. The BactoSense was operational at that time and the pH increase on 29 June on 6:00 coincides with the outlier value from the BactoSense at 29 June on 13:30 (most left arrow in Figure 6-8). It is uncertain whether this outlier is indeed caused by the pH increase, and if so, why the pH increase results in higher cell numbers.

The higher water demand in Noardburgum between 9 and 16 August, resulting in abstraction of more groundwater can be seen in the BactoSense and BACTcontrol results. The daily pattern that is visible in the BactoSense results (

Figure 6-11) disappears between 12 and 15 August 2022 and the cell numbers are slightly lower in this period. Lower cell number can be expected in these circumstances, as the filters treat more water and this water is mixed with a small water stream that is UV disinfected and not filtrated. In contrast, the BACTcontrol showed higher enzymatic activity compared to the rest of the measurement period. However, the enzymatic activity already increased from 4 to 10 August 2022, which was before the higher water demand. This seems to indicate that the changes in the BACTcontrol results were not related to the higher water demand.

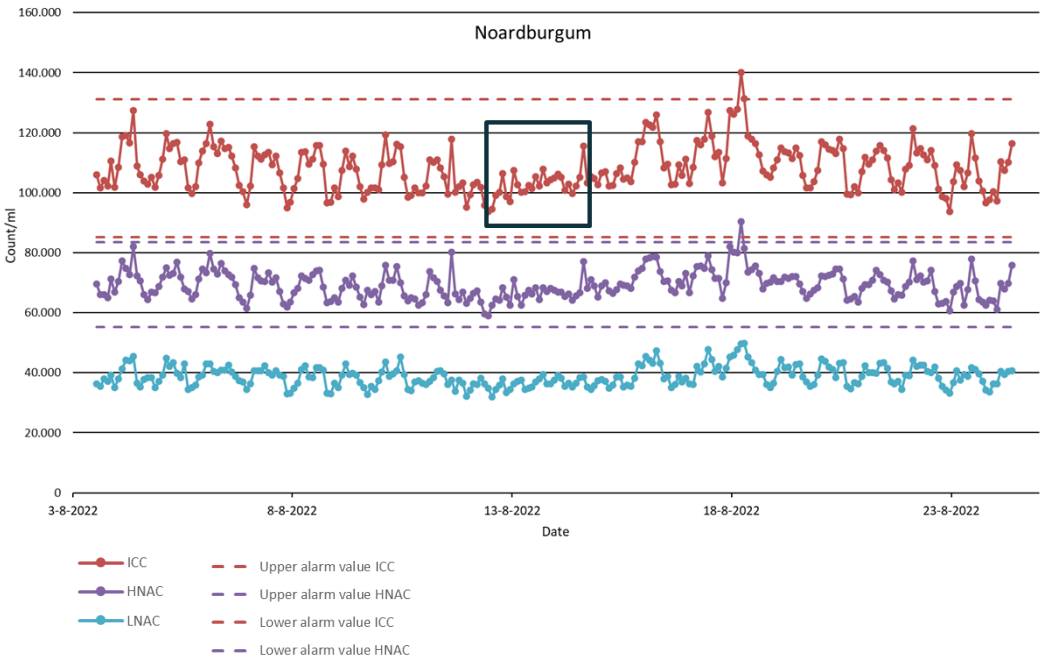


Figure 6-11. BactoSense results of drinking water at Noardburgum. Indicated is the period of operational changes of extra ground water abstraction due to the higher water demand (11-15 August).

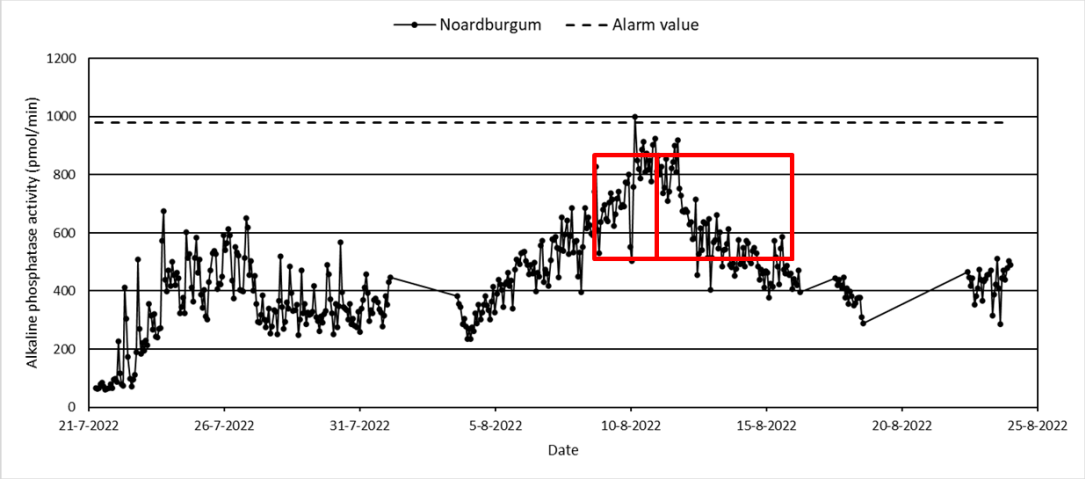


Figure 6-12. BACTcontrol results of drinking water at Noardburgum. Indicated is the period of higher water demand between 9-16 August (large red rectangle) and the period during which extra ground water was extracted (11-15 August, right half of the rectangle).

The BACTcontrol and BactoSense did not detect an effect of flushing of the rapid sand filters on the biomass (cell numbers or enzymatic activity) in the produced drinking water.

The (daily) variation measured with the BactoSense and BACTcontrol raised the question on the relevance of fluctuating biomass levels for the microbiological drinking water quality and issues that relate to microbial growth in the distribution system. The treated water at Spannenburg and Noardburgum fulfills the legal requirements, but the *Aeromonas* numbers in distributed drinking water exceed the legal requirements, especially in the distribution system of Spannenburg. Furthermore, it is known that the biological stability parameters for drinking water of Spannenburg do not fulfil the guidance values. As far as is known to the operators at Spannenburg and Noardburgum, no problems occurred at these treatment plants which could have affected the water quality negatively. It remains uncertain in what way the observed variation in enzyme activity and cell numbers, measured with the BACTcontrol and BactoSense affects the *Aeromonas* numbers in the distribution system.

6.4.2 Comparison BACTcontrol with BactoSense

Ideally, sensors for microbiological water quality that measure the same water should give comparable results. In other words when the cell numbers, measured with BactoSense, increase also the enzymatic activity (measured with BACTcontrol) should increase and vice versa. To this end, results of the BACTcontrol were compared to the BactoSense results for Spannenburg (Figure 6-13) and Noardburgum (Figure 6-14Figure 4-16).

The ALP measured by the BACTcontrol at Spannenburg started high (940-1140 pmol/min) and decreased to an activity of 480-620 pmol/min by the end of August 2022. The cell numbers (ICC: 1.8x10^5 cells/ml) obtained with the BactoSense at Spannenburg were relatively stable over time until the end of August 2022 with a few outliers as was previously described (Paragraph 6.3.2). The results of the BACTcontrol and BactoSense, thus, did not show such a comparable trend.

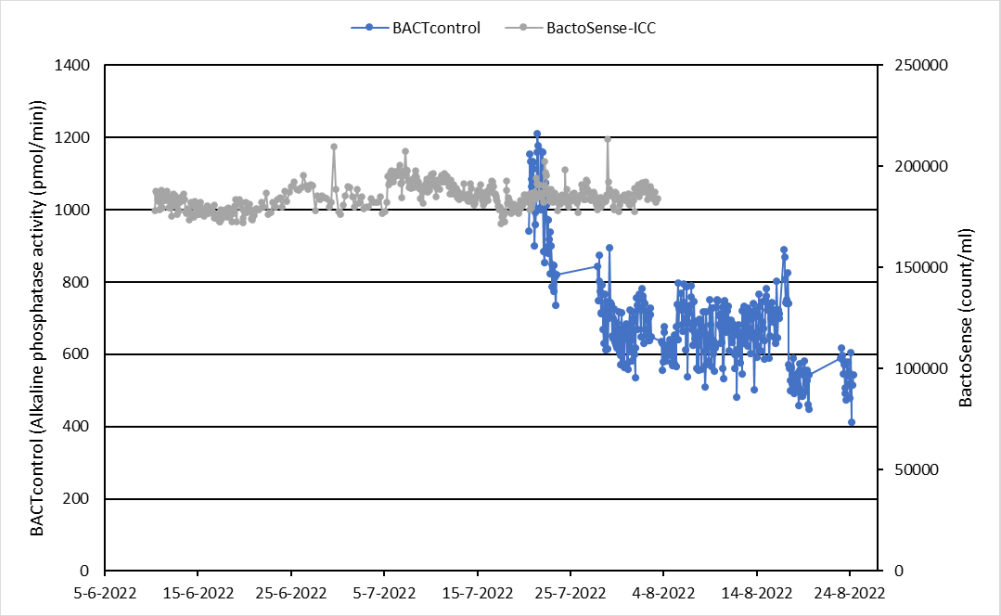


Figure 6-13. BACTcontrol and BactoSense results of drinking water at Spannenburg.

The ALP measured by the BACTcontrol at Noardburgum is fluctuating over time, on 20 July 2022 the ALP was 65 pmol/min and increased up to about 900 pmol/min on 10-11 August 2022 after which it decreased to about 500 pmol/min at the end of August 2022. The cell numbers obtained with the BactoSense at Noardburgum were relatively stable (ICC: 1.1x10^5 cells/ml) over time until the end of August 2022 with a few outliers as was previously described (chapter 6.3.2). Again, also for Noardburgum the results of the BACTcontrol and BactoSense do not match.

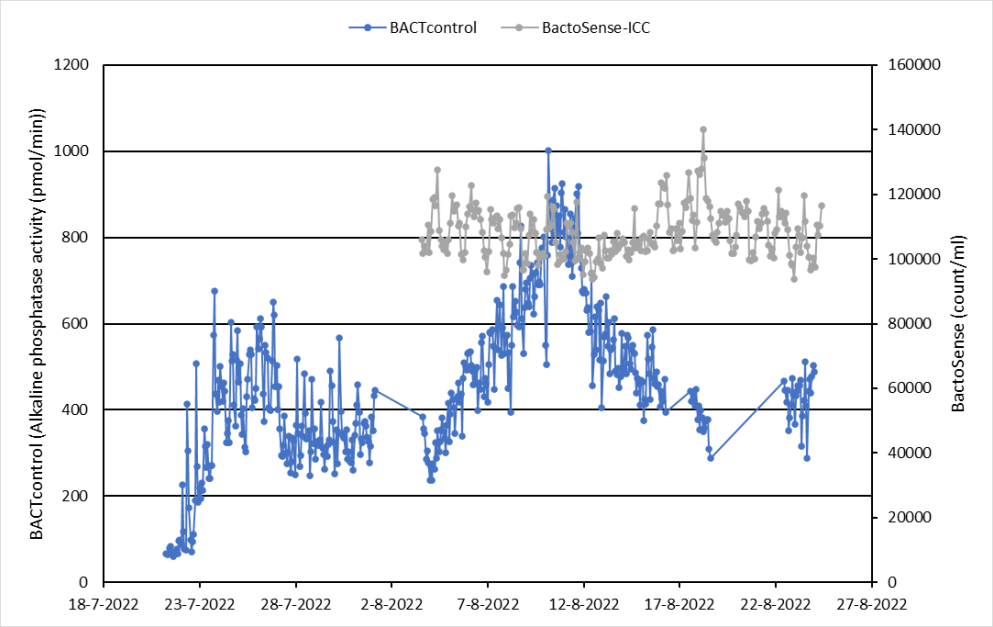


Figure 6-14. BACTcontrol and BactoSense results of drinking water at Noardburgum.

Comparison of the BACTcontrol and BactoSense results of drinking water at Spannenburg and Noardburgum showed that the BACTcontrol results fluctuate, whereas the BactoSense ICC measurements showed a more constant result. It is not known why the BACTcontrol and BactoSense do not show comparable results.
At Noardburgum, the total cell counts (ICC and HNAC) and enzymatic activity were lower compared to Spannenburg. This was in line with the fact that Noardburgum has more biologically stable water than Spannenburg as well as lower *Aeromonas* number in the distribution system.

The daily pattern of ICC, HNAC and LNAC was more pronounced at Noardburgum compared with Spannenburg. This regular pattern could be due to the differences in the final treatment step: rapid sand filtration in Noardburgum and ion exchange in Spannenburg. The ion exchange at Spannenburg might bind bacteria, thereby stabilizing the fluctuations. However, it cannot be excluded that other factors can also play a role in this.

6.4.3 CBM

Biofilm formation in drinking water processes is a relatively slow process due the low nutrient concentration in drinking water. Therefore, the CBM can only provide a reliable BAR value after glass beads have been exposed to the drinking water for four weeks. Consequently, due to this aspect and the nature of the CBM, only one BAR value can be obtained every two weeks.

The BAR values at Spannenburg (2.7 – 7.1 pg/cm^2/day) were on average lower than those for Noardburgum (3.8 – 22.7 pg/cm^2/day), but the high BAR values at Noardburgum were caused by algae growth in the KWR-CBM. After covering the KWR-CBM to prevent algae growth, BAR values of the drinking water at Noardburgum were comparable to those of Spannenburg.

6.4.4 Laboratory methods Vitens

Turbidity, heterotrophic plate count at 22°C and *Aeromonas* were measured by Vitens in the produced drinking water from both Noardburgum and Spannenburg (Figure 6-15). The turbidity at Spannenburg (average: 0.055 FTE) is lower compared to Noardburgum (average: 0.41 FTE). On 15 August between 15:40 and 15:44 the turbidity increased until 3 FTE, which coincided with the final day of increased water demand. On this day stable BactoSense results were recorded, without the daily fluctuations, but the BACTcontrol detected a small increase in enzymatic activity at this time period. Due to this small increase it is difficult to conclude whether this small increase was caused by normal variation of the BACTcontrol or that it was caused by the process that also caused the turbidity increase.

The heterotrophic plate count is higher for Spannenburg (average: 15.7 CFU/ml) compared with Noardburgum (average: 3.5 CFU/ml). On 26 July 2022 in Spannenburg a value of 98 CFU/ml was measured. On this day no aberrant measurements with the BactoSense were recorded and no data for the BACTcontrol was available due to technical issues.

No *Aeromonas* was detected on 9 June 2022, the only time that *Aeromonas* was measured in the experimental time period at Noardburgum. In Spannenburg the *Aeromonas* count was below 30 CFU/100 ml for the three measurements performed.

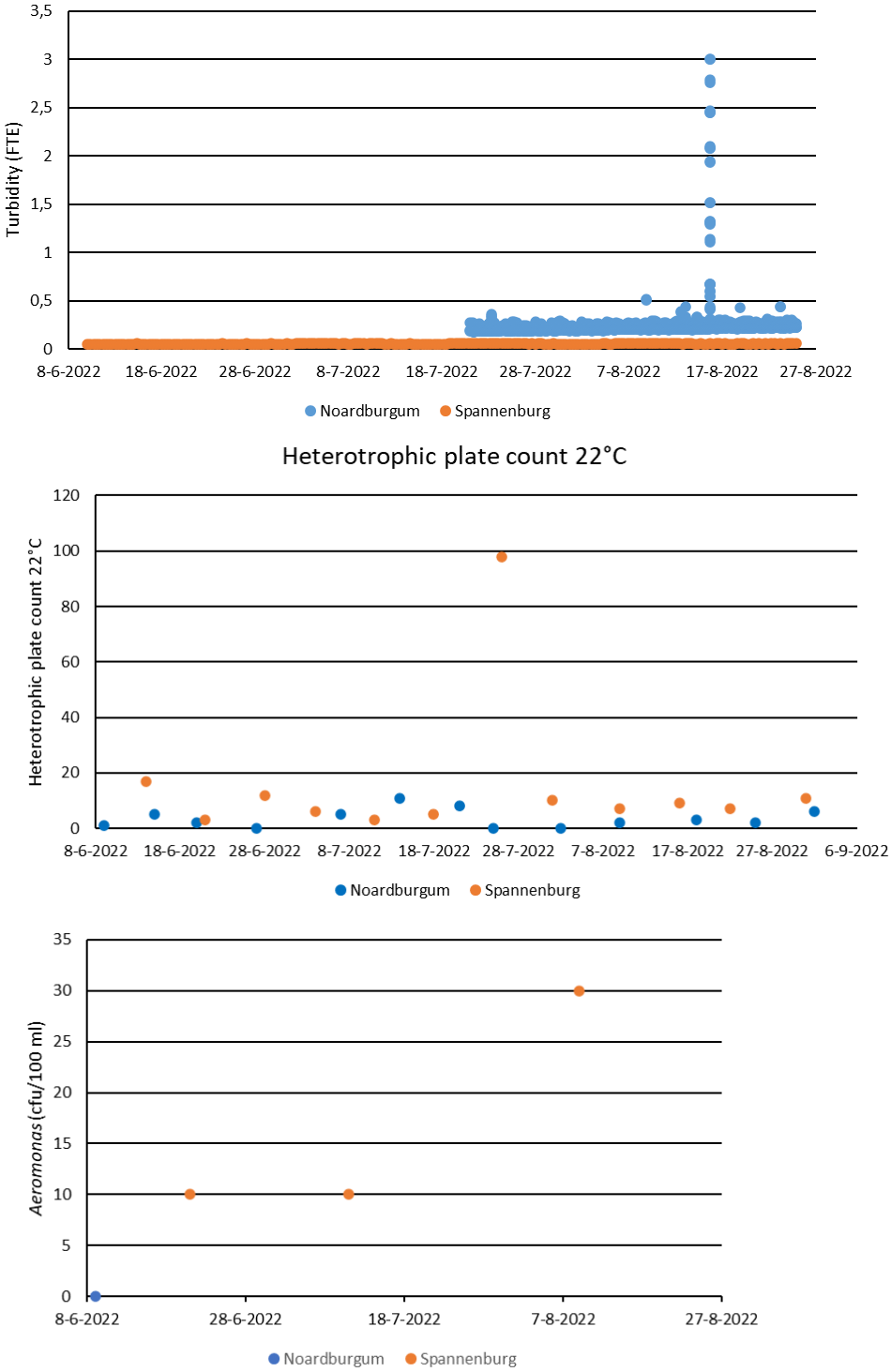


Figure 6-15. Turbidity (top, heterotrophic plate count 22°C (middle) and Aeromonas (bottom) analyses performed by Vitens on the produced drinking water of Noardburgum and Spannenburg.

6.4.5 Alarm values

For both the BACTcontrol and BactoSense alarm values were calculated per production location with the formula

$$Alarm\ value = average \pm 3 \times standard\ deviation$$

Measurements below the lower alarm value can indicate a (sudden) reduction in bacteria or bacterial activity, for example due to sudden die-off of bacteria. Whereas measurements above the upper alarm value can indicate an increase of bacteria or bacterial activity due to, for example, regrowth, changes in operational parameters or other processes. Both alarms, thus, could indicate that something happened with the water quality and that action might be needed.

The calculated alarm values for the BACTcontrol are given in Table 6-4 and are indicated with the dashed lines in Figure 6-6 and Figure 6-7 (chapter 6.3). The calculated lower alarm values were always lower than the lowest value obtained for each water type (sometimes ALP activity of the lower value is below zero). The calculated higher alarm values were exceeded ten times (Spannenburg) or once (Noardburgum).

Table 6-4. The lower and higher alarm values, counts and number of exceedances of the BACTcontrol in drinking water at Spannenburg and Noardburgum. Count: number of reliable measurements. Exceedances: number of measurements that exceeds the calculated alarm value. Alarm values in pmol ALP/min. SD: Standard deviation

	Count	Mean ± SD (pmol/min)	Lower alarm value (pmol/min)	Exceedances lower alarm value	Upper alarm value (pmol/min)	Exceedances upper alarm value
Spannenburg	386	691±140	272	0	1111	10
Noardburgum	439	454±175	0[1]	0	980	1

[1]*The calculated lower alarm value of -72 pmol/min was set to 0 pmol/min.*

For the BactoSense, alarm values were calculated for ICC and HNAC cell types and these values are given in Table 6-5. For Spannenburg the upper ICC alarm value ($2.0x10^5$ cells/ml) was exceeded three times, whereas in Noardburgum the upper ICC alarm value ($1.3x10^5$ cells/ml) was exceeded once. These exceedances are the outliers pointed out in Figure 6-8 (in chapter 3 of this report) for which no technical explanation could be found. The daily fluctuations did not exceed the alarm values.

Table 6-5 The lower and higher alarm values, counts and number of exceedances of BactoSense in drinking water at Spannenburg (10 June – 3 August 2022) and Noardburgum (3 August – 24 August 2022). Count: number of reliable measurements. Exceedances: number of measurements that exceeds the calculated alarm values.

	Count	Mean ± SD (cells/ml)		Lower alarm value (cells/ml)		Exceedances (lower alarm value)		Upper alarm value (cells/ml)		Exceedances (upper alarm value)	
		ICC	HNAC	ICC	HNAC	ICC	HNAC	ICC	HNAC	ICC	HNAC
Spannenburg	536	$1.8x10^5$ ± $6.0x10^3$	$1.0x10^5$ ± $3.4 x10^3$	$1.7x10^5$	$9.4x10^4$	0	0	$2.0x10^5$	$1.1x10^5$	3	2
Noardburgum	251	$1.1x10^5$ ± $7.6x10^3$	$6.9x10^4$ ± $4.7 x10^3$	$8.5x10^4$	$5.5x10^4$	2	0	$1.3x10^5$	$8.4x10^4$	1	1

In chapter 10 more methods to calculate alarm values are described, for example to take the daily fluctuations into account.

6.5 Location-specific conclusions

The BAR values for the treated water of Noardburgum, obtained when algae growth was diminished, are comparable to those for the treated water of Spannenburg. The biofouling potential of the drinking water, thus, seems to be the same between these two water types.

The results from the BactoSense showed slightly lower cell numbers in the treated water of Noardburgum than in the treated water of Spannenburg. In addition, in Noardburgum, but not in Spannenburg, daily fluctuations in drinking water cell numbers were visible. These variations seem to correlate to the water demand differences during day and night time. The BACTcontrol at Spannenburg initially showed a decrease in enzymatic activity followed by more stable values. In Noardburgum the enzyme activity showed more fluctuations. The results of the BactoSense and BACTcontrol did not show comparable trends in measurement values and these two different sensors to monitor microbial water quality, thus, seem to give conflicting insights into the microbial water quality.

All types of cells (ICC, HNAC and LNAC) and enzymatic activity in drinking water at Spannenburg were significantly higher than in drinking water at Noardburgum. The microbial quality of the treated water at Noardburgum, thus, seems to be better than the microbial quality of the treated water at Spannenburg.

The effect of higher water demand and the changes in operational parameters to produce more drinking water coincided with a loss in the daily fluctuation of the cell numbers in the drinking water measured with the BactoSense. The BACTcontrol showed higher enzymatic activity compared to the rest of the measurement period. However, the enzymatic activity already increased before the higher water demand. This seems to indicate that the changes in the BACTcontrol results were not related to the higher water demand.

Comparison of the results of the CBM, BactoSense and BACTcontrol with the parameters measured by Vitens (*Aeromonas*, HPC22, turbidity) can explain some of the (limited) differences in *Aeromonas* and HPC.

The (daily) variation measured with the BactoSense and BACTcontrol raises the question whether fluctuating biomass levels negatively impact the microbiological water quality at the consumers tap. As no problems were reported during the measurement period by Vitens, this suggests that the observed variation can be considered as normal variation that does not impact the microbial water quality in such a way that problems occur.

The goal of Vitens, to set local alarm values and determine at which alarm or trigger value action should be taken, was not met due to several technical issues. The most important issue was that not enough data was available from the BACTcontrol, BactoSense and CBM that operating simultaneously at both locations.

7 Monitoring of surface water – H_2O Biofouling solutions

7.1 Microbiological situation

7.1.1 Background and goal

In the project 'rapid inline monitoring of microbiological water quality' sensors for the presence of bacteria in water are tested on several water types. Four sensors are developed by technology providers, and validated by KWR. H_2O Biofouling solutions has a test setup in which an open recirculating cooling system can be simulated. It contains 6 heat tubular exchangers in series, in transparent polycabonate tubes. In this way, visual observations can be made of the effect of conditioning methods.

Cooling water conditioning focusses on three aspects: prevention of scaling, corrosion and microbiology. The tests during this pilot are focused on the measurement of microbiology, and whether the effect of conditioning methods on the microbiology can be measured. The effectiveness of the conditioning chemicals in itself was not the topic of this research.

7.1.2 Location

The tests took place at KWR, in the experimental hall, simulating a cooling tower that is made up with surface water. The surface water (Lek canal Nieuwegein)) was not pretreated and, thus, contained relatively high microbial activity and was circulated over the system, with the goal to have biofilm buildup in the test system. This could be visually observed in the transparent tubes that were in the circulation system (Figure 7-1).

Figure 7-1: transparant tube for observation of biofilm growth.

7.2 Monitoring campaign

7.2.1 Experimental set up at KWR

The experimental setup consists of a rack with six transparent condenser pipes, in order to visually observe biofilm development. The condenser rack is connected to a 400 L basin which is filled with surface water, collected from the Lek canal, supplemented with tapwater (200L each), and replaced with fresh Lek canal/tap water after the first experiment. A solution of nutrients was added to promote biofilm growth (Pokon Bio kamerplantenvoeding. Organische minerale meststof NPK 3-2-5). Water is recirculated through the condenser rack and heated to approximately 25°C in order to allow microorganisms to grow and biofilm to develop. The KWR biofilm monitor (CBM) and Milispec biofilm monitor (Milispec) do not affect the water, so it recirculates back into the water basin (figure 1). The BACTcontrol, BugCount Guardian and BactoSense use chemicals (e.g. dyes, enzymes, cleaning agents), so the water taken up by these systems is discharged from the recirculation system. Water to the BactoSense is diluted 10x with reverse osmosis (RO) treated water to ensure all values are within measuring range (Figure 7-2).

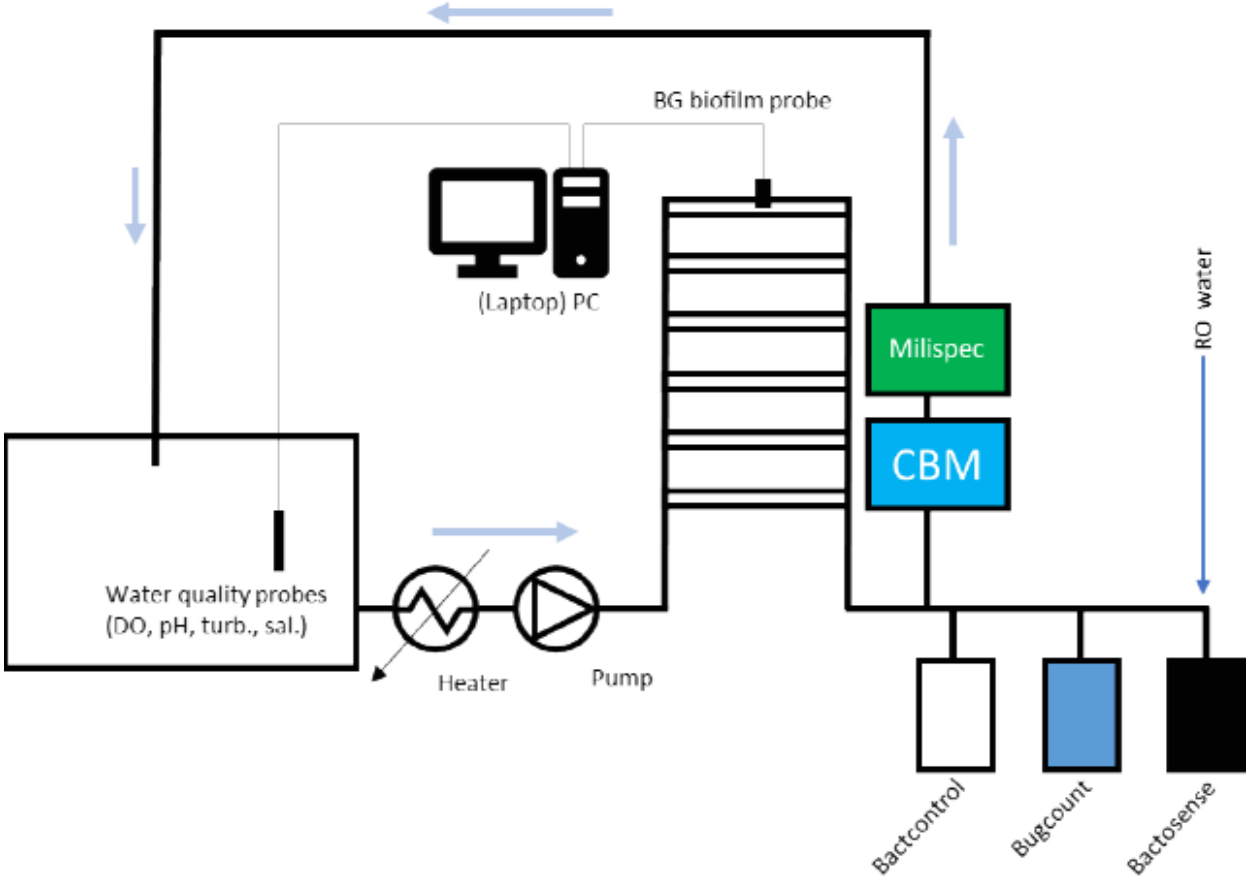


Figure 7-2: Schematic picture of the test set-up

After startup, the surface water in the system is recirculated to allow microorganisms to grow and biofilm to develop. Microbial growth is observed and measured by BACTcontrol, BugCount and BactoSense (Figure 7-3). The dosing strategy consists of two steps. First AquaFinesse (AF), a product that releases biofilm from surfaces, is dosed. The product AF itself is not a biocide, therefore CMIT (a non-oxidizing biocide) is dosed as a second step two days after AF dosage, in order to eliminate microorganisms.

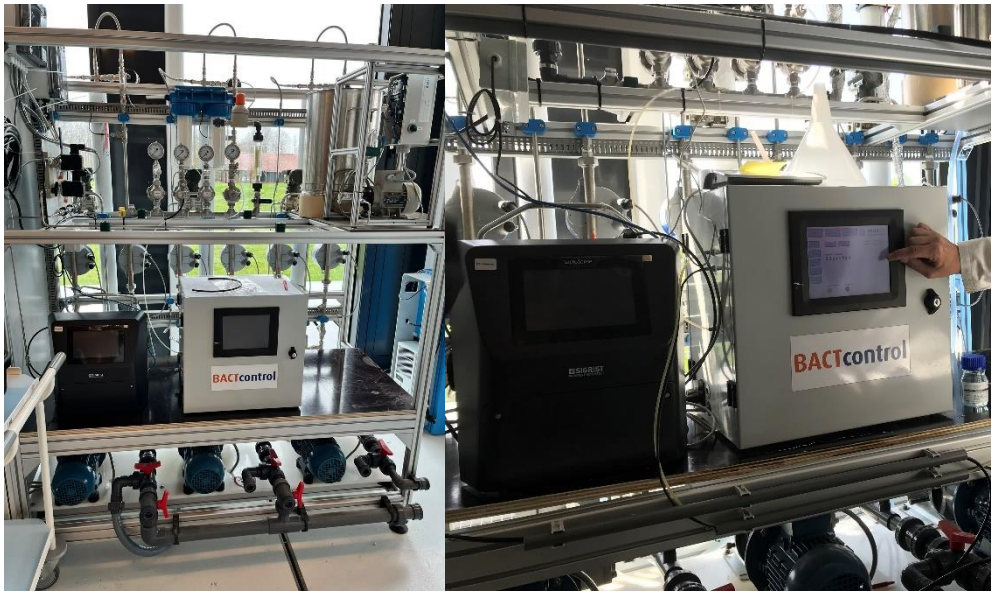


Figure 7-3: BactoSense and BACTcontrol on the trolly with sensors.

7.2.2 Monitoring details

The set-up was installed in March 2023. The start-up took a while before the operation was stable. The testing period ran from June 3 to August 7, 2023.

The monitoring frequency was every 90 minutes for the BACTcontrol, once every four hours for the BactoSense, and once every four hours for the BugCount guardian. The CBM was measured following the scheme in Table 7-1.

7.2.2.1 Additional analyses

No additional analyses were done.

7.2.3 Measurements, data processing

7.2.3.1 BACTcontrol

The raw data were acquired. Datapoints where the value was below the detection limit were discarded, and datapoints where the sensor itself indicated an erroneous measurement were discarded.

7.2.3.2 BactoSense

On July 12, the reported data were very high and the BactoSense gave the following error: TCC out of range, bubbles detected. Hence, these datapoints were discarded.

7.2.3.3 Bugcount Guardian

The BugCount Guardian took a sample to measure the dATP, and approximately 10 minutes later a new samples is taken to measure the tATP. These two samples were treated as one sample to calculate the cATP, which is shown in the graphs.

7.2.3.4 KWR CBM and Milispec CBM

There was a total of 12 cuvettes, 8 in the KWR CBM and 4 in the Milispec CBM. They were all analysed using the same method using the Luminultra ATP kit. Table 7-1 shows the dates that samples were taken. Normally, the CBM is used to measure the biomass accumulation or biofilm rate, by dividing the ATP concentration of the biofilm measured after 28 days in the CBM with the number of days that the cuvette was in the CBM (pg ATP cm^{-2} dag^{-1}). During the trials with the pilot systems, however, the biofilm concentration in the biofilm was followed by sampling cuvettes after short time intervals. Consequently, results are shown in pg ATP/cm^2.

Table 7-1: Schedule of dosing trials. AF = AquaFinesse, a product that releases biofilm. CMIT = Chlorometyhlisothiozolinone

Date	Action	Comments
5-6-2023	Analysis 1x Milispec CBM cuvette Analysis 2x KWR CBM cuvette Dosage of AF, 4 mg/L	Milispec CBM contains 4 cuvettes total KWR CBM contains 8 cuvettes total
7-6-2023	Analysis 1x Milispec CBM cuvette Analysis 2x KWR CBM cuvette Dosage of CMIT, 10 mg/L	
12-6-2023	Analysis 1x Milispec CBM cuvette Analysis 2x KWR CBM cuvette Dosage of CMIT, 20 mg/L	
15-6-2023	1st trial finished Test setup flushed to remove any residual conditioning product (AF and/or CMIT) Water basin refilled with “fresh” surface water for 2nd trial	
7-7-2023	Analysis 1x Milispec CBM cuvette Analysis 2x KWR CBM cuvette Dosage of AF, 4 mg/L	
10-7-2023	Analysis 1x Milispec CBM cuvette Analysis 2x KWR CBM cuvette Dosage of CMIT, 20 mg/L	
14-7-2023	Analysis 1x Milispec CBM cuvette Analysis 2x KWR CBM cuvette	
17-7-2023	Analysis 1x Milispec CBM cuvette Analysis 1x KWR CBM cuvette Placement of new cuvettes in both CBM	No dosing to allow regrowth of fouling 4x KWR + 1x Milispec cuvettes available
27-7-2023	Nutrients added to stimulate growth	
3-8-2023	Analysis 2x KWR CBM cuvette Dosage of CMIT, 20 mg/L Analysis 2x KWR CBM cuvette	2x cuvette analysis before CMIT dosage 2x cuvette analysis 1hr after CMIT dosage
7-8-2023	Analysis of Milispec CMB cuvette	Only a single Milispec cuvette was available
	End of trial	

7.3 Results

7.3.1 BACTcontrol

Figure 7-4 and Figure 7-5 show the BACTcontrol results from the different dosing trials, with the first trial running from June 3rd to June 15th 2023, the second from June 15th to July 17th, 2023 and the third from July 17th to the end of the field test period on August 7th, 2023. Between the first and second trial the system was flushed and filled with fresh water from the Lek canal, whereas the system was not flushed between the second and third trial.

Dosing of 4 mg/L AF gave a slight increase in the activity in the water phase, probably because biofilm was released into the water by the effect of AF. The addition of 10 mg/L CMIT did not affect the activity, but adding 20 mg/l CMIT dosing, the activity increased. Although CMIT is a disinfectant, an increased concentration in the water phase may be measured as an increased activity. However, the effect may not be significant.

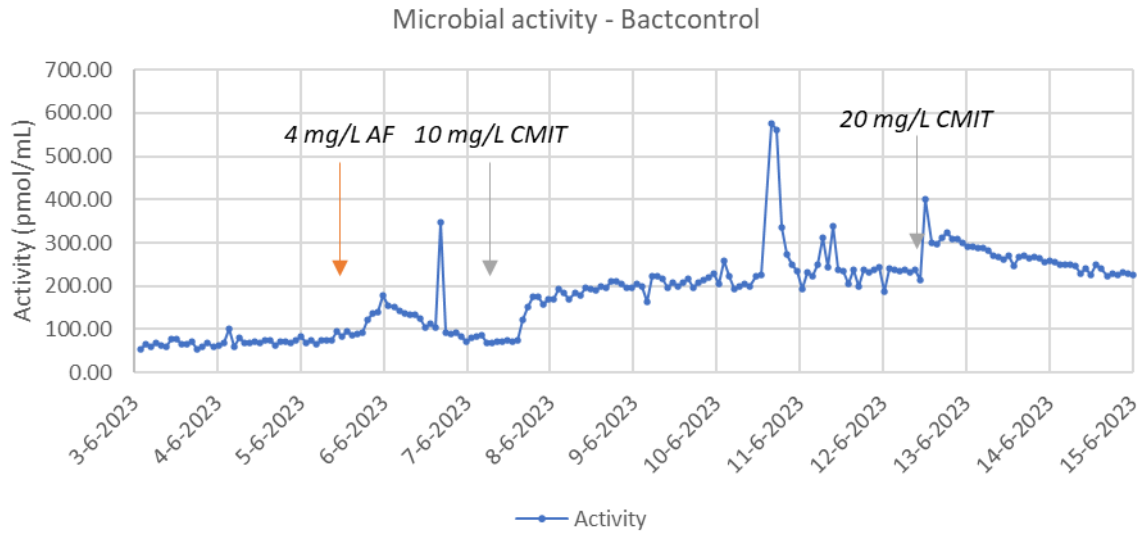


Figure 7-4: Microbial activity (pmol/mL) as measured by the BACTcontrol analyser during the first dosing trial. The orange arrow indicates dosage of AquaFinesse (AF), the grey arrows indicate dosage of CMIT.

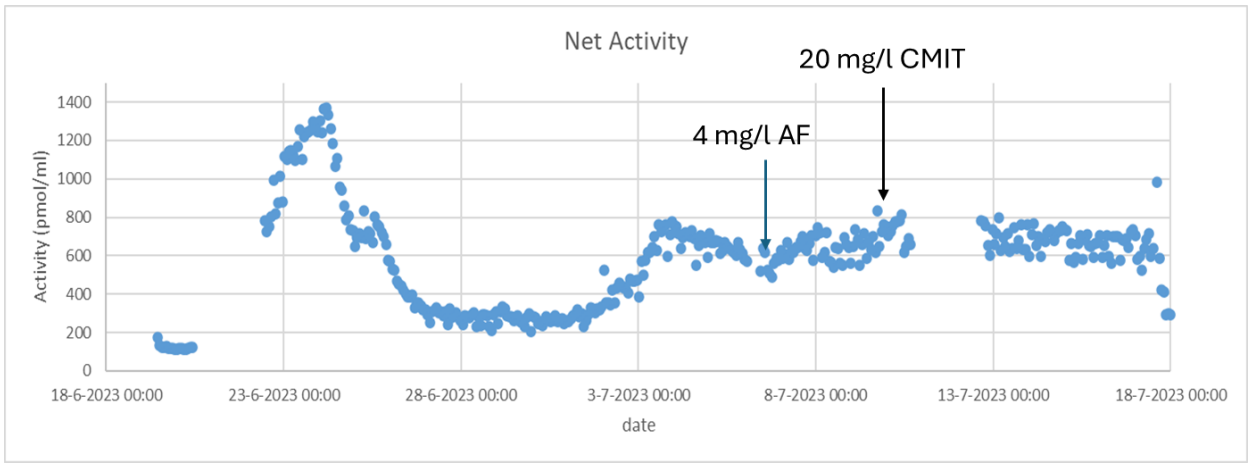


Figure 7-5: Microbial activity (pmol/mL) as measured by the BACTcontrol analyser during the second dosing trial. The orange arrow indicates dosage of AquaFinesse (AF), the grey arrow indicates dosage of CMIT. Due to a blocked discharge channel within the analyser, no measurements were performed from 15-6 to 19-6 and from 21-6 to 23-6. From 11-7 to 14-7 no measurements were performed due to a delayed shipment of chemicals.

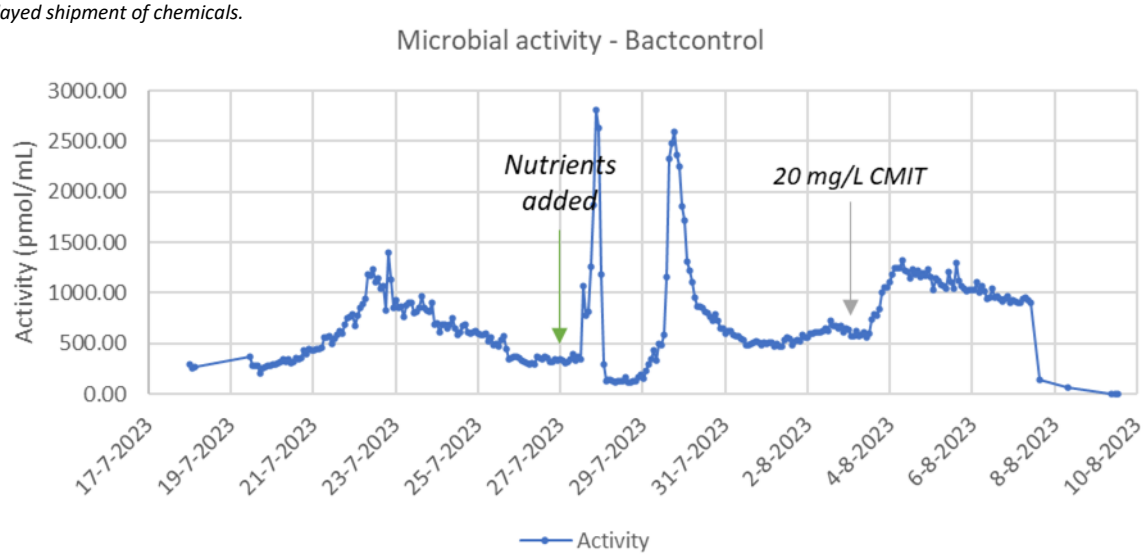


Figure 7-6: Microbial activity (pmol/mL) as measured by the BACTcontrol analyser during the third dosing trial. The green arrow indicates dosage of nutrients to stimulate microbial growth, the grey arrow indicates dosage of CMIT.

7.3.1.1 General comments BACTcontrol

The BACTcontrol analyser was operational during most of the experimental period. In June, a leakage of the incubation occurred due to a blocked discharge channel. When this issue was resolved, the analyser performed without any major issues.

Within a day after dosage of AF, an increase in microbial activity in the water phase was picked up by the BACTcontrol, which may indicate detachment from wall surfaces and transfer to the water phase of active microorganisms. However, this is not consistent throughout all the trials performed so it is not possible to definite conclude that detachment occurs in the pilot system due to dosage of AF. Dosage of CMIT did not significantly decrease the microbial activity in the water phase, indicating that the dosed concentrations may have been insufficient to effectively reduce microbial growth. In fact, sometimes activity increased after CMIT dosing, indicating that some biofilm may detach and contain some active biomass, which is then measured as an increased activity.

Nutrients were added to the water basin on July 27th to stimulate microbial growth. A peak in activity was seen on July 28th and July 30th, which is likely to be attributed to microbial growth. However, comparing the data with the other sensors, this peak was not observed with the BactoSense on July 28th. A consecutive dosage of 20 mg/L CMIT on the 3rd of August did not decrease microbial activity, but appeared to increase activity instead. The dosage of CMIT could have been too low to see an effect of disinfection.

7.3.2 BactoSense

7.3.2.1 Results BactoSense from the three trials

The BactoSense results from the three dosing trials are shown in Figure 7-7 to Figure 7-9. Dosing of 4 mg/l AF resulted in a small increase in intact cell numbers measured with the BactoSense. CMIT dosing did not result in a decrease in intact cell numbers, but when the highest CMIT-dose was administered the ICC counts increased. Dosing of nutrients resulted in an increase of ICC, demonstrating bacterial growth due to the nutrients. Finally, none of the dosing affected the HNAP%, demonstrating that this BactoSense parameter is less informative than ICC.

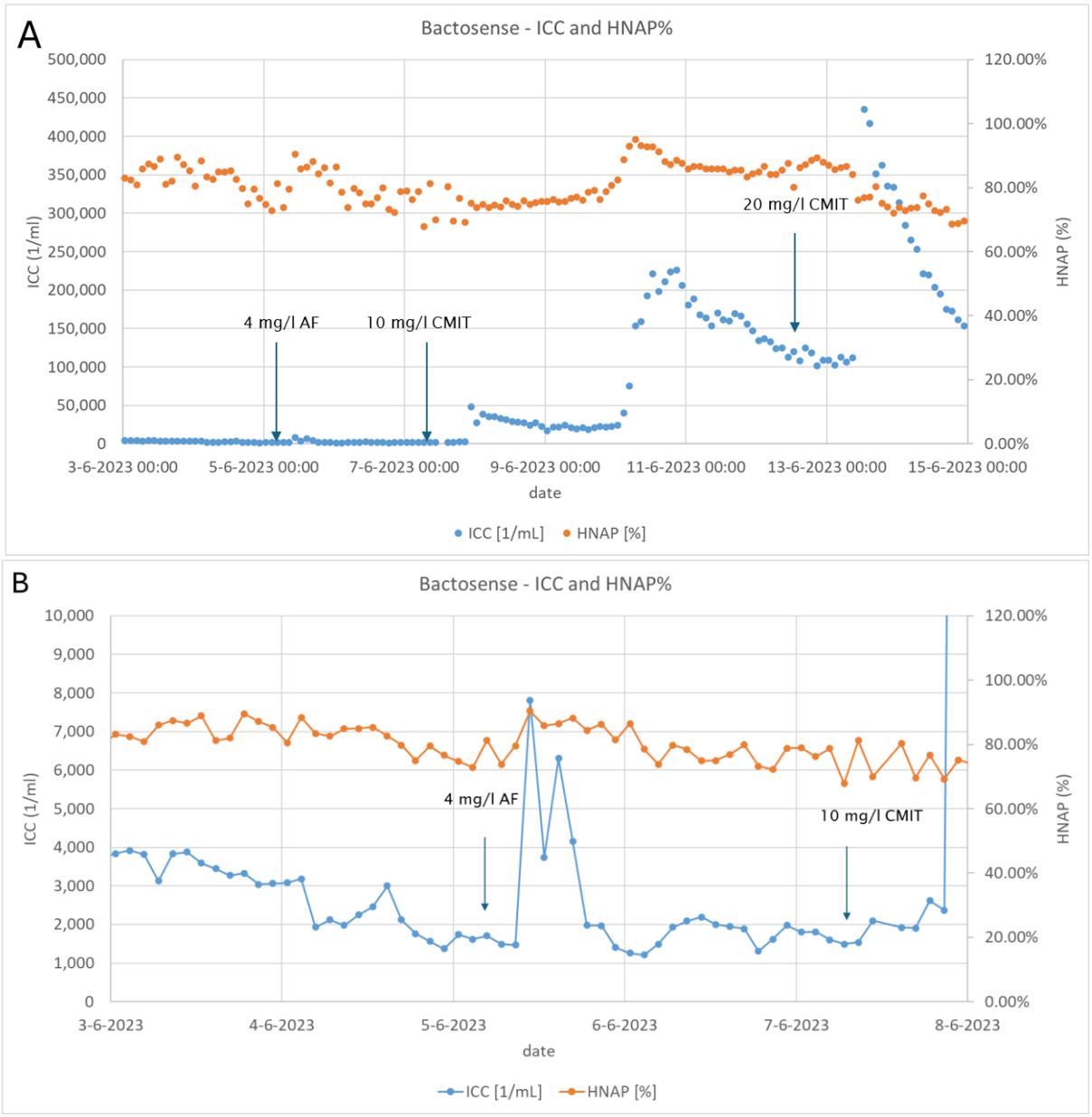


Figure 7-7: : Intact microbial cell count (ICC) as measured by the BactoSense analyser during the first dosing trial. The orange arrow indicates dosage of AF, the grey arrows indicate dosage of CMIT. A: ICC overview of the entire dosing trial. B: zoomed in ICC overview from 3-6-2023 to 6-8-2023

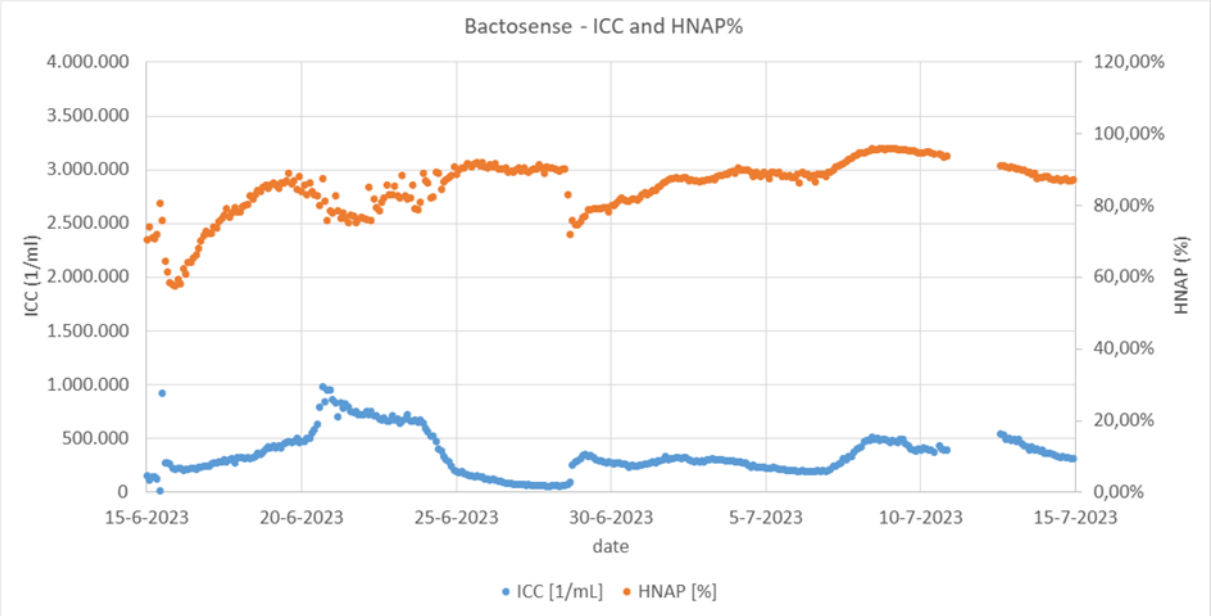


Figure 7-8: ICC as measured by the BactoSense analyser during the second dosing trial. The s arrows indicate dosage of AF and CMIT. Between July 11th and July 14th the readings were out of range due to air bubbles present in the measurement chamber of the analyser

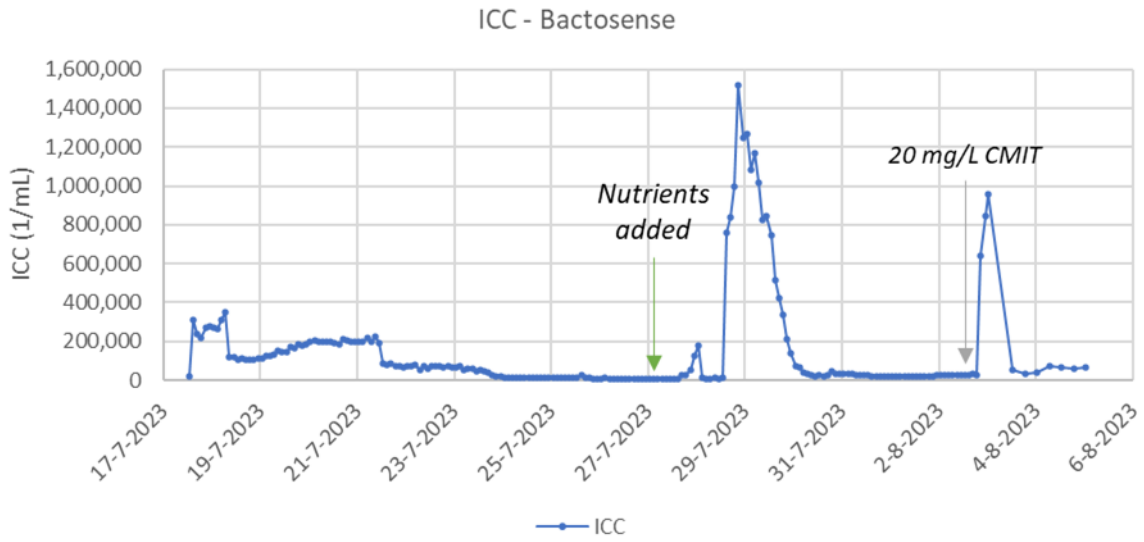


Figure 7-9: ICC results from the BactoSense during the third dosing trial. Green arrow indicates addition of nutrients, the grey arrow indicates CMIT dosage.

7.3.2.2 General comments BactoSense

The BactoSense analyser operated without major issues during most of the experimental period. On July 5th the analyser stopped measuring because the cartridge was depleted. Before starting the experiment, it was expected that the bacterial cell numbers in the surface water were higher than the maximum numbers that can be measured using the BactoSense. To ensure that all measurements were within the BactoSense measuring range, the water samples were diluted to a ratio of 1:9 with demineralized water. Between July 11th and July 14th some of the readings were out of range due to detection of air bubbles within the measurement chamber and that disturbed reliable detection of bacterial cells with the BactoSense.

As described, within a day after dosing AF, a peak in ICC was measured by the BactoSense, indicating release of (living) microorganisms from the wall surface. This peak coincides with the microbial activity peak as measured by the BactoSense (chapter 7.3.1). Dosage of CMIT did not significantly decrease the intact cell numbers in the water phase, indicating that the dosed concentrations may have been insufficient to effectively reduce bacterial numbers. Nutrients were added to the water basin on July 27th to stimulate microbial growth. This led to a small increase in ICC on July 28th and a large increase in ICC on July 30th. A consecutive dosage of 20 mg/L CMIT on 3th of August led to an additional increase in ICC. These results are comparable to the results of the BACTcontrol and can, thus, be explained by the same mechanisms as described in paragraph 7.3.2.1.

7.3.3 BugCount Guardian

7.3.3.1 Results BugCount Guardian with the three trials

Figure 7-10 to Figure 7-12 show the BugCount results from the three dosing trials. Dosing of AF resulted in an increase in the cATP-concentration in the water. In general, CMIT dosing did not result in an increase of decrease of the cATP concentration. An exception to this was the decrease in cATP concentration after dosing 20 mg/l CMIT in the second trial. The nutrients dosing seem to result in an increase of cATP, but this was difficult to interpret, because the BugCount Guardian was offline during most of this period.

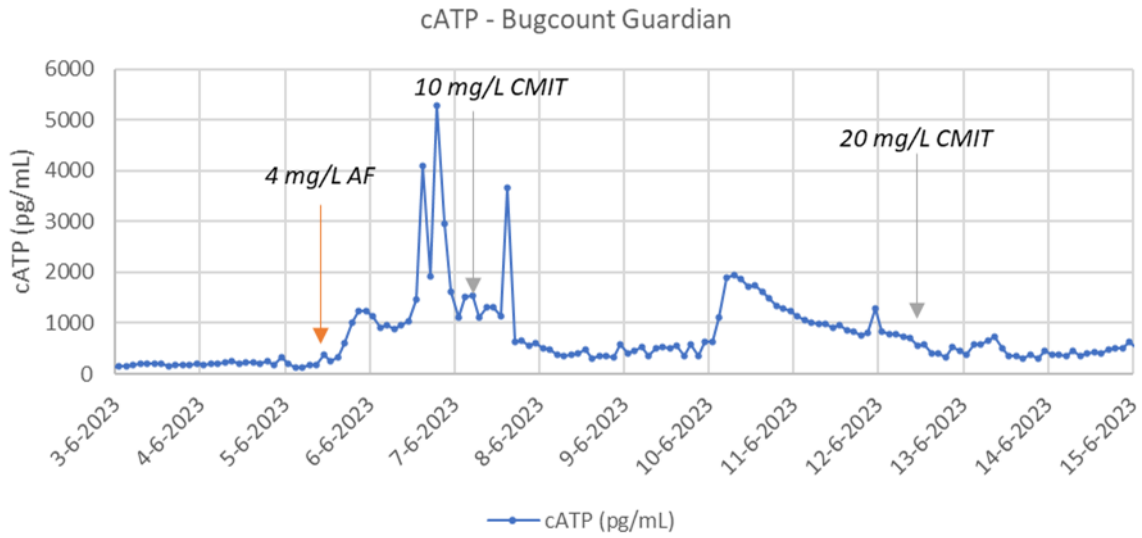


Figure 7-10: cATP (cellular ATP) as measured by the BugCount analyser during the first dosing trial. The orange arrow indicates dosage of AquaFinesse (AF), the grey arrows indicate dosage of CMIT.

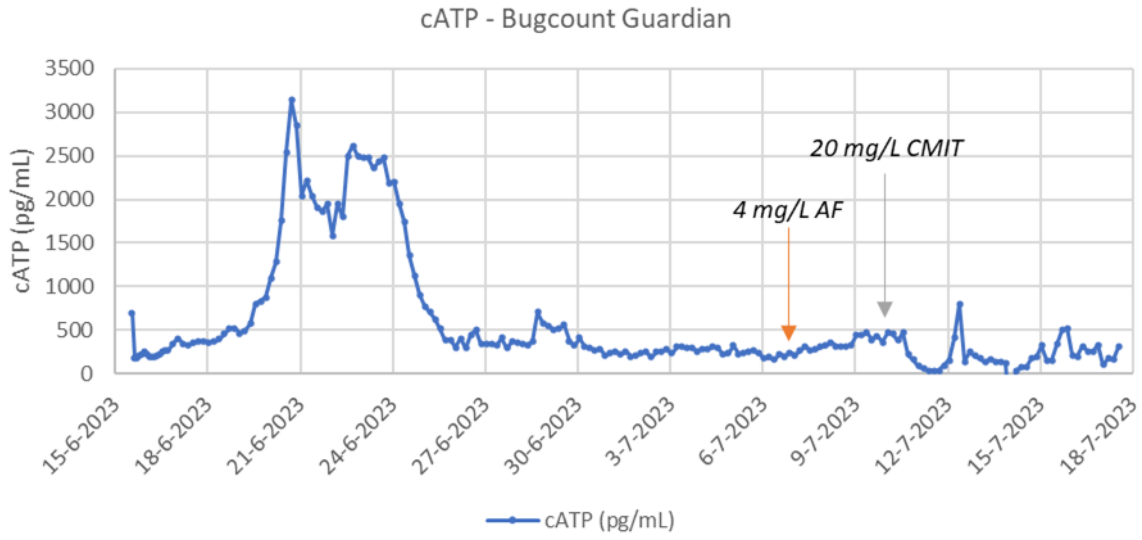


Figure 7-11: cATP (cellular ATP) as measured by the BugCount analyser during the second dosing trial. The orange arrow indicates dosage of AquaFinesse (AF), the grey arrow indicates dosage of CMIT.

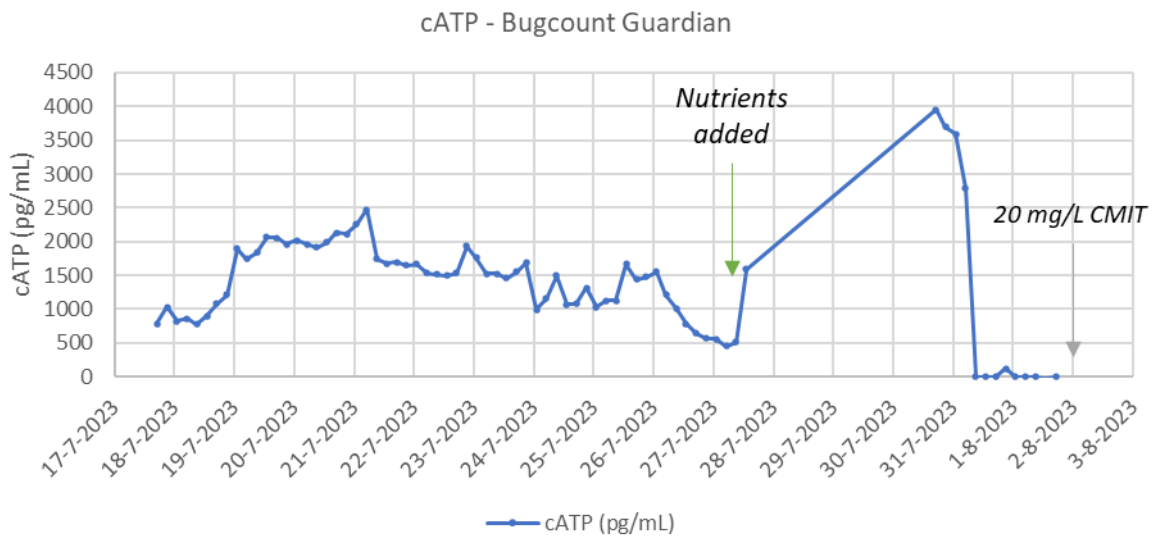


Figure 7-12: cATP (cellular ATP) resultsfrom the BugCount Guardian during the third dosing trial. Green arrow indicates addition of nutrients, the grey arrow indicates CMIT dosage. No results were reported between July 28th and July 31st.

7.3.3.2 General comments BugCount Guardian

The BugCount Guardian operated without major issues for most of the experimental period. No results were, however, reported between July 28th and July 31st, the exact reason for this error remains unclear. From July 31st onwards, the ATP results were below the detection limit, although the device was sufficiently stocked with reagents.

Similar to the BactoSense and BACTcontrol results, a cATP peak was picked up by the BugCount approximately one day after dosage of AF. This may indicate detachment from wall surfaces and transfer to the water phase of microorganisms, although this was not consistently observed for the BugCount throughout all the trials performed. Therefore, it is not possible to conclude that detachment is happening due to dosage of AF based on BugCount data alone. No significant effects were observed after CMIT dosage, except for the second trial, indicating that the CMIT concentration was ineffective in eliminating microbial growth. That result from the BugCount was comparable to the results from the BACTcontrol and BactoSense. It was expected that the combination of AF and CMIT would result in a high increase in suspended dead biomass, which would show as an increased cATP measurement in the BugCount.

Nutrients were added to the water basin on July 27th to stimulate microbial growth. Unfortunately, no data from the BugCount was available from July 28th until July 31st. However, on July 31st an increase in the cATP concentration was picked up by the BugCount. This is comparable to the results from the BACTcontrol and BactoSense.

7.3.4 CBM

7.3.4.1 Results CBM Millispec and KWR

Figure 7-13 to Figure 7-15 represents the biofilm concentration, measured as pg ATP/cm^2, from the Millispec and KWR CBM during the three dosing trials. The results showed that the biofilm concentrations varied during the courses of the three trials, probably under influence from the different dosing strategies.

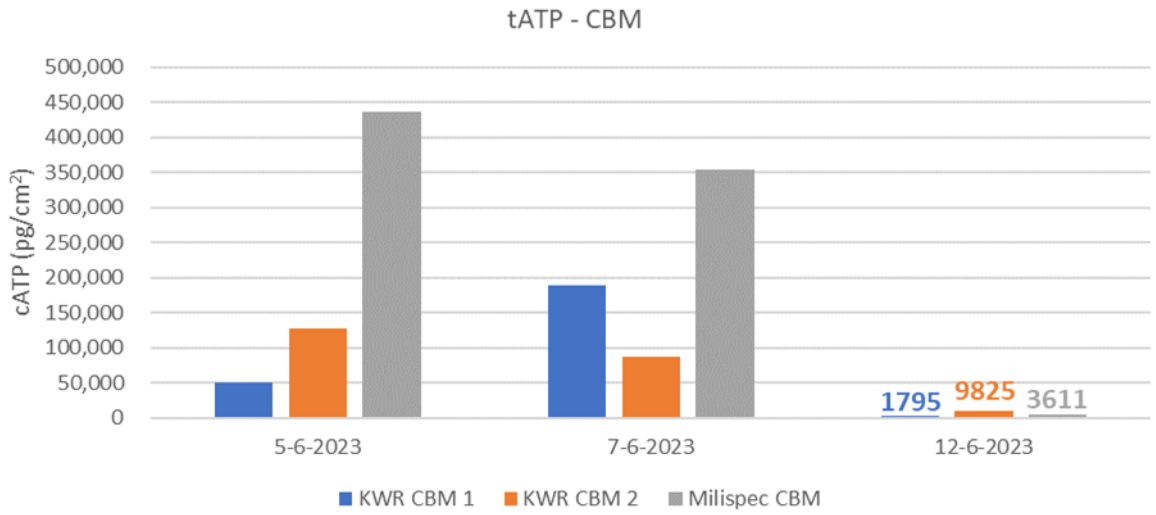


Figure 7-13: Biofilm tATP results from the Milispec and KWR CBM. Each sampling session, 2 cuvettes of the KWR CBM and 1 cuvette of the Milispec CBM were analysed. 4 mg/l AF was dosed on 05-06-2023, 10 mg/l CMIT on 07-06-23 and 20 mg/l CMIT on 14-06-2023 after cuvettes had been sampled on 05-05 and 07-06.

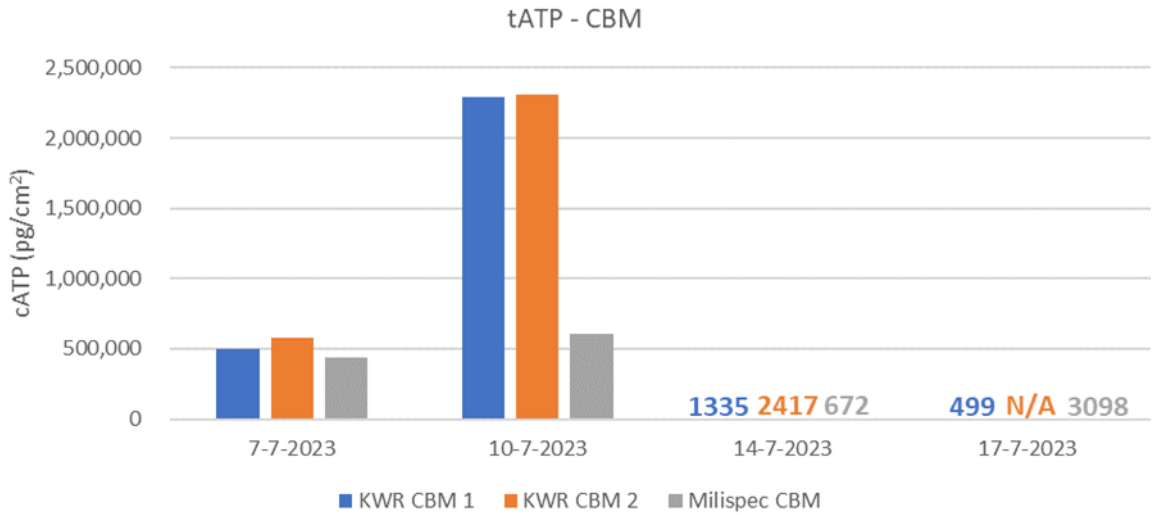


Figure 7-14: Biofilm tATP results from the Milispec and KWR CBM. Each sampling session, 2 cuvettes of the KWR CBM and 1 cuvette of the Milispec CBM were analysed. N/A indicates there was no cuvette available for analysis. 4 mg/l AF was dosed on 07-07-2023 en 20 mg/l CMIT on 10-07-2023 after cuvettes had been sampled on these days.

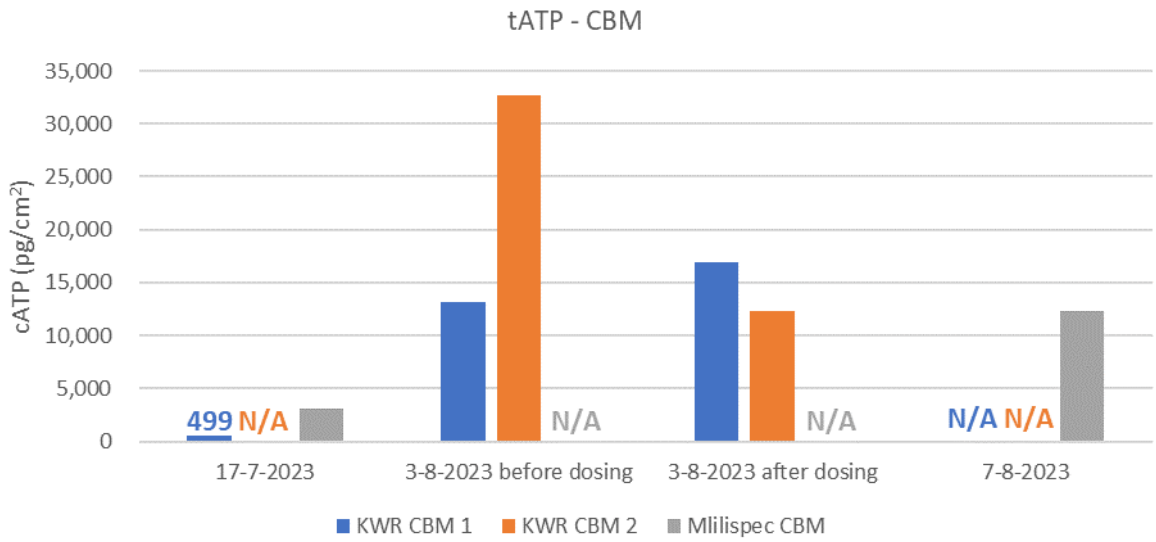


Figure 7-15: Biofilm tATP results from the Milispec and KWR CBM. On 27-07-2023 nutrients were dosed and on 03-08-2023, KWR cuvettes were collected before 20 mg/l CMIT dosage and 1 hr after this CMIT dosage. N/A indicates there was no cuvette available for analysis

7.3.4.2 General comments CBM

For the duration of the experiment, both CBM's operated without major issues, except that during daily checks it was observed that the flow through both monitors had to be adjusted regularly, since the flow reduced over time. This was caused by debris (biofilm/suspended particles in the Lek water) blocking the flow control valves.

The results indicate that at some time points the two cuvettes from the KWR CBM gave the same value, whereas at other time points the variation between the two KWR cuvettes sampled at the same time gave large variation, indicating different biofilm buildup between each specific glass coupon at those moments. This variation may be caused by the varying flow conditions within the monitor due to fouling of the flow control valves. It is, therefore, necessary to prevent clogging of the flow control valves for accurate quantification of biofilm with the CBM when fed with more dirty water like surface water.

Due to the problem with the control valve, it is difficult to draw reliable conclusions from the effect of AF or CMIT on the biofilm. However, in general, it was observed that the samples taken after CMIT dosing showed much lower biofilm values than before CMIT dosing. This is in contrast with the results from the other sensors, probably because the CBM is the only apparatus that measure biofilm formation. The other sensors only measure the water quality and not what the effect of the AF or CMIT dosing is on biofilm.

7.4 Discussion

7.4.1 Comparison of the sensors

The three sensors BACTcontrol, BactoSense and BugCount Guardian showed similar trends during the experimental period. All three sensors measured a small increase in their parameter (either intact cell numbers or activity) was on June 10 and in increase between June 20-24 (Figure 7-16). There were no process changes in those periods that may have caused this, and since the water was recirculated, major changes in the water quality are not expected. It, therefore, remains unknown what the cause was for these changes.

Although the effect of the treatment was not seen, it was observed that the values of the sensor parameter all increased on June 10, and in the period 15-21 June, and decreased after June 23. The same accounts for the period around July 23. This cannot be explained by changes in the processes; however all three sensors show this change. The BugCount Guardian and the CBM both measure microbial activity as ATP. However, the BugCount measures the ATP concentration of the water phase, whereas the CBM measures the ATP concentration of the biofilm phase. Therefore, these ATP concentrations cannot be compared with each other.

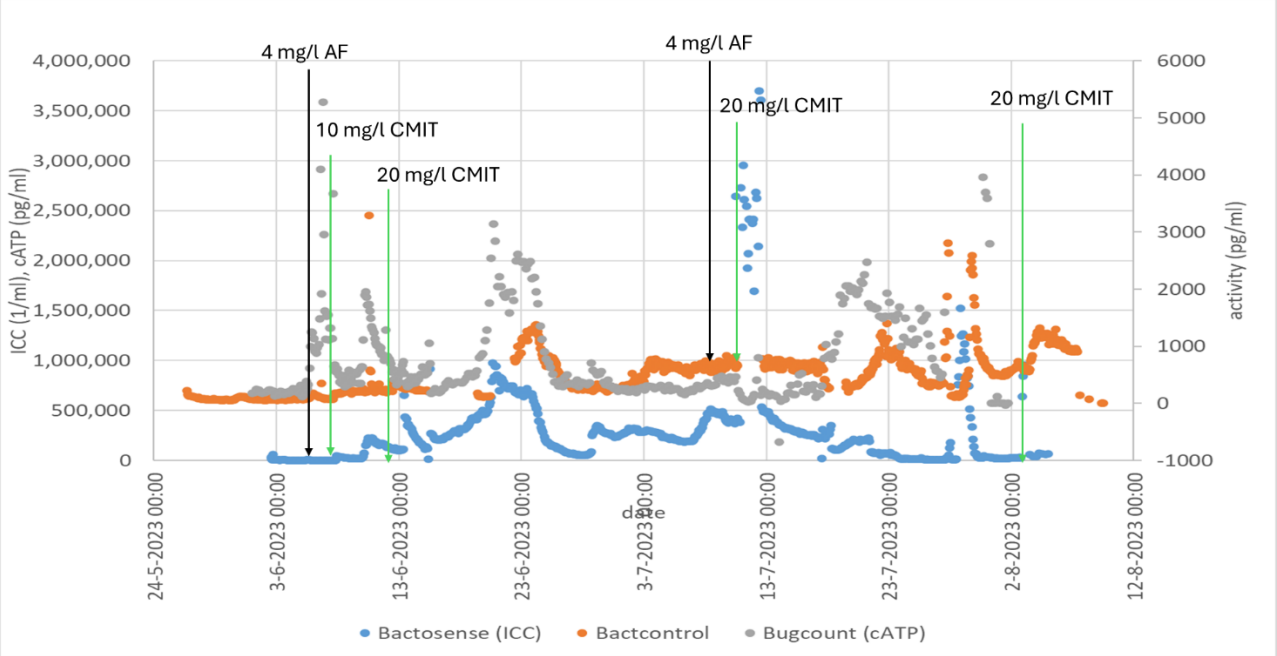


Figure 7-16: All three sensors over the whole experimental period

No considerable effect of dosing of Aquafinesse or CMIT was observed for the data from the BactoSense and BugCount Guardian, while a small increase was observed in two of the trials with the BACTcontrol after dosage of CMIT. This increase, however, is likely not statistically significant compared to the background variation of the sensor values. Although the duplicate values differed sometimes considerably for the CBM, the CBM did measure a large effect of CMIT dosing on the active biofilm concentration. This apparent discrepancy between the three sensors and the CBM is most likely caused by the fact that the sensors measure intact cell numbers and activity in the water phase, whereas the CBM determines the effect on the active biofilm concentration. This would mean that CMIT-dosing has a large effect on the biofilm, but the impact on the microorganisms in the water phase is less pronounced, maybe due to continuous flow and recirculation of the water.

7.5 Conclusions and recommendations

7.5.1 Conclusions

From the results obtained during the experiment performed at KWR the following conclusions can be drawn:

- The online sensors BACTcontrol, BactoSense and BugCount Guardian operated without major issues for the duration of the experiment.

- Although the variation in values of the different sensor parameters was small during the trials, the variation in results from BACTcontrol, BactoSense and BugCount Guardian follow similar trends. This indicates that these devices can be applied to monitor the microbial water quality in a water system containing surface water.

- The upper detection limit of the BactoSense is such that the water from the Lek canal needed to be diluted at least twice. In heavily fouled waters it may be necessary to increase the dilution factor to stay within the measuring range of the BactoSense.

- It was found that there is no considerable daily variation in microbial activity or bacterial cell numbers in the water. This implies that 3-4 measurements per day is sufficient for water quality monitoring under the applied conditions and pilot setup.

- The CBM's showed strong variability in biofilm buildup in a water system consisting of surface water, which could be due to difference in flow regime caused by clogging of the flow valves of the CBM. Consequently, it is difficult to accurately quantify the potential biofilm formation of surface water by using the current CBM. Therefore, Continuous Biofilm Monitoring is for now more suitable to establish either the presence or absence of biofilm.

- The water conditioning products AF and CMIT at concentrations as applied during this test do not have a negative impact on the operation of the online sensors.

- Dosage of AF resulted in an increase in microbial activity or cell count in the water phase, although the increase was small and comparable to some of the natural variation observed during the monitoring period. These results indicate that the online sensors might be able to measure the effect of water conditioning products on surface water, but more experiments are required to be able to draw definite conclusions.

- CMIT dosage at a concentration of 20 mg/L is not sufficient to kill microorganisms in the water phase in this system as no considerable changes were observed by the online sensors and cell counts or microbial activity remained relatively high after CMIT dosing. The CMIT dosing, however, showed a clear reduction in the biofilm concentration measured with the CBM, indicating that might be able to eradicate a considerable amount of biofilm.

- The BACTcontrol, BactoSense and BugCount Guardian showed an increase in microbial activity or cell counts after addition of nutrients, as was expected. These results again indicate that the online sensors are able to measure the effect of water conditioning products.

7.5.2 Recommendations

Continuous dilution of the water with demi-water is not a practical solution for water types with high cell numbers, such as surface water. It is, therefore, recommended to increase the upper detection limit of the BactoSense, or only dilute the water that needs to be sampled. This requires a different internal design of the sensor.

In water with relatively high suspended solids the needle valves of the CBM block quite rapidly, reducing or stopping the flow through the CBM system, affecting the reliability of the measurements. Installing an upstream filter, which traps particles, may increase operational reliability, but also filters out nutrients for biofilm formation. Another option might be using broader needle valves that are less prone to clogging.

To effectively monitor and control microbial growth in the water of industrial water systems, a measurement interval of four times per day is sufficient. Increasing the number of measurements per day does not necessarily improve the ability to control microorganisms effectively, however it does increase expenditure of reagents/chemicals. Furthermore, to monitor the effectiveness of water conditioning products on the microbiology in water systems, measurements of both the water and biofilm phase should be applied.

8 Monitoring cooling water at Dow Terneuzen

8.1 Microbiological situation

8.1.1 Background and goal

Dow would like to use the sensors to get more information on the short-term variation of the microbiological cooling water quality and on the effectiveness of their disinfection system. The sensors can also be used to optimize disinfection of the cooling water when a different water source is used, thereby increasing the flexibility of their water systems.

The goal of the pilot test was to see the applicability of the sensors in an industrial environment. This means that they should be able to run smoothly, with limited maintenance, for at least three months. Experience with sampling, pretreatment and other maintenance efforts should be in line with industrial standards. The goal was also to see if the sensors are affected by plant conditions and are able to pick up chances such as different water sources or change in biocide dosing. The last goal was to see if the results of the sensors are comparable or complementary.

8.1.2 Location

The test took place at the cooling tower of the Elsta Cogen plant at Dow Terneuzen (NL)(Figure 8-1 and Figure 8-2). This plant produces both 90 bar steam and electricity for the production plants at the Dow Terneuzen Industry Park.

The induced draft cooling tower consists of five identical cells with a total make-up waterflow of ~175m^3/hr. Surface water is used as suppletion water in combination with a minor amount of recycle condensate. The cooling tower is operated at around five cycles of concentration and a chemical treatment program is applied to minimize scaling, corrosion, biofouling and Legionella development. The pH is adjusted to pH 7.5 -7.7 and scaling and corrosion inhibitors and a dispersant are dosed. Bleach is dosed to maintain a continuous free oxidant (FO) concentration between 0.2 and 0.3 mg/l. The setpoint was set on 0.2 mg/l Cl_2 up to March 19. From 20-22 March the setpoint was lowered to 0.15 mg/l Cl_2 and from March 23-25 the setpoint was set to 0.25 mg/l Cl_2. After March 25, the setpoint was returned to 0.2 mg/l Cl_2 for the remaining monitoring period.

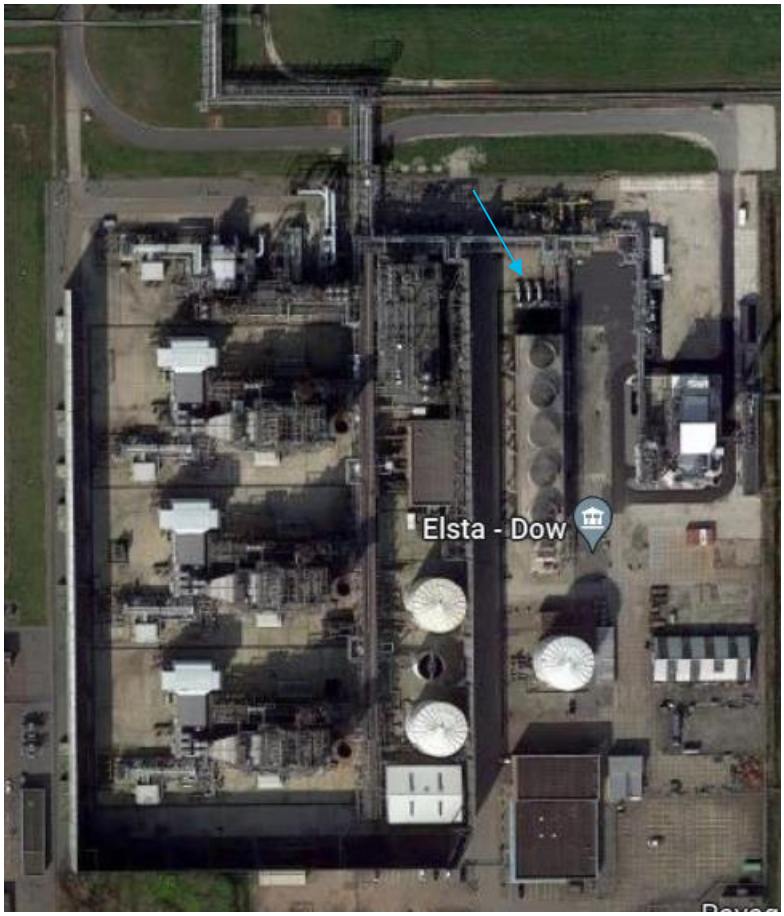


Figure 8-1: Top view of Elsta cooling tower. The arrow indicates where the water is supplied to the plants

Figure 8-2: Side view of the Elsta cooling tower where the cooling water is supplied to the plants. This is the side that is on the upper side in the previous figure.

Seasonal variations and the source of the supplied surface water may have an impact on the microbiological water quality. During the test period (February-May 2024), seasonal influence may be present. The effect of the additional stream of condensate was not expected, but results showed that it turned out to have a short term effect on the microbial quality.

To minimize the use of chemicals in cooling water treatment, Dow is looking for monitoring technologies that can result in more controlled and thus reduced dosing of treatment chemicals. Sensors that can measure or predict the microbiological water quality could possibly help in a reduction of bleach dosing. Used sensor technology should be reliable and produce data that makes sense at a frequency that allows dosing control based on demand.

8.2 Measurements

8.2.1 Experimental set-up at Dow Terneuzen

The sensors were placed in a 20ft container that was next to the cooling tower. Water was taken from the pressure side of a small pump continuously pumping water from the basin into the cooling water system in the plant. The container is insulated, equipped with air-conditioning and a ventilation system and has a smoke alarm. The air is monitored for hydrocarbons.

Water enters the container with a pressure of ~5.5 bars, and this is lowered to ~2 bars using a pressure reduction valve (Figure 8-3 and Figure 8-4). A pressure reduction valve is installed prior to each sensor to ensure the sensor specific pressure and flow.

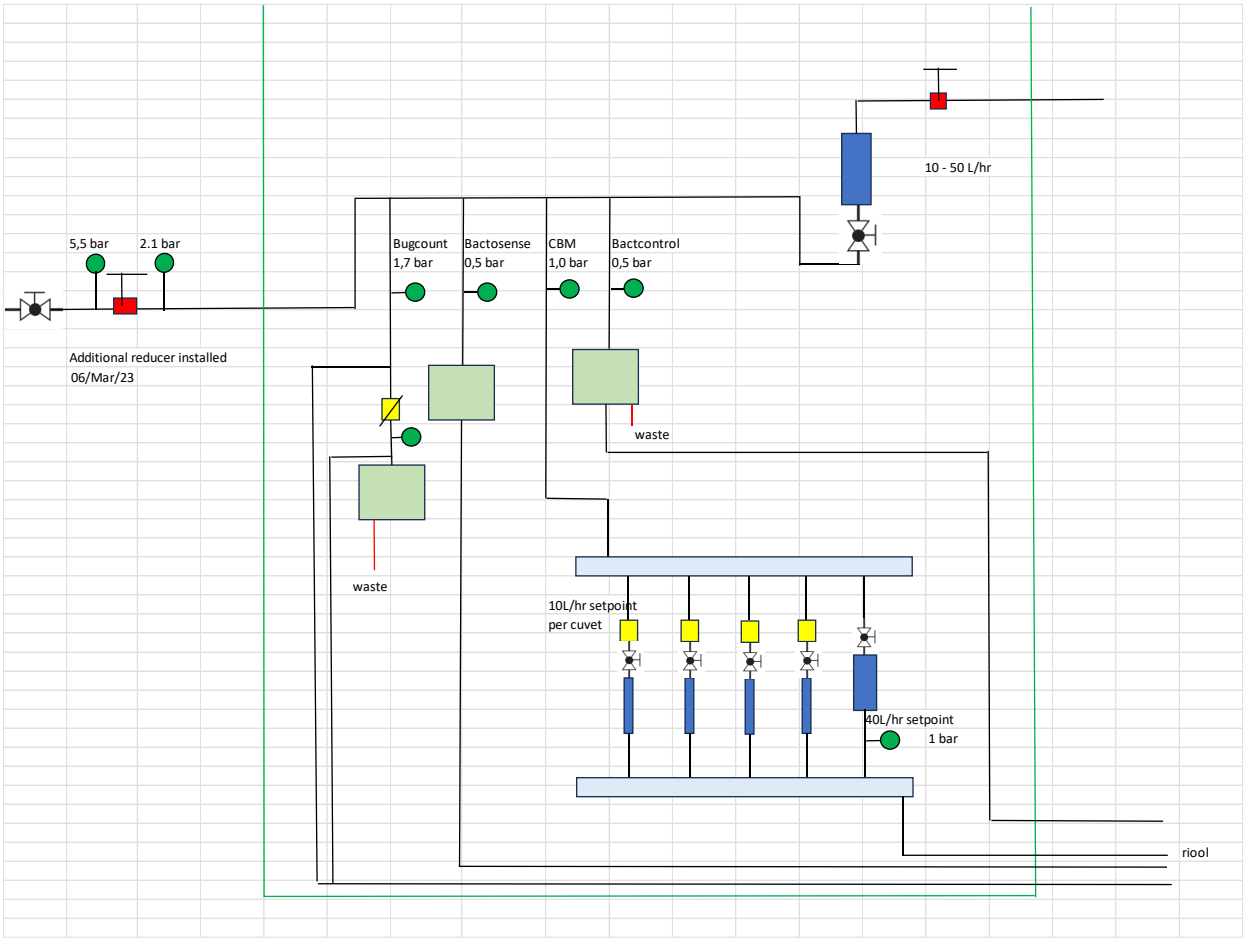


Figure 8-3: Schematic overview of sensor set-up

Figure 8-4: Several pictures that show the inside of the container with the pressure reduction valve (5-->2 bars) and the reduction valves for each individual sensor

The sensors are placed on a trolly in the container (Figure 8-5). Excess water is led to the sewer system. Water containing chemicals used for the measurements is gathered in a separate waste container.

Figure 8-5: The sensors and the set-up in the container. Upper figures show the BactoSense, BACTcontrol and the BugCount Guardian. The lower picture shows the CBM.

8.2.2 Measurement Frequency

The sensors were installed in the period 16 February – 13 May 2024. The BACTcontrol measured microbial enzyme activity every 3 or 5 hours. The measurement frequency of the intact cell numbers by the BactoSense was set at 1 per hour. The BugCount Guardian measured the total and dissolved ATP concentration every 2,4 or 6 hours. Every two weeks, the CBM was sampled by Dow and the ATP content in the biofilm was measured with the LuminUltra ATP kit on-site (according to the protocol of Chapter 2). An overview of the applied sensors with their measurement frequency is given in Table 8-1.

Table 8-1: Overview of applied sensors with their measurement frequency

	Measurement frequency	Measurement period
BACTcontrol	1 per 3 hr 1 per 5 hr	
BactoSense	1/hr	16-2 to 24-3 13-4 to 13-5
BugCount Guardian	1 per 2, 4 or 6 hrs	The frequency was determined by the time span between reagents replacement.
CBM Milispec	7 tests performed on coupons	

8.2.2.1 Additional analysis by Dow

Dow analysed pH, electrical conductivity and free chlorine. Legionella was also analysed twice during the pilot period but not detected. The ATP kit from LuminUltra was also used to measure the ATP concentration in the cooling water on three moments in March 2024.

8.2.3 Data processing

8.2.3.1 BACTcontrol

The BACTcontrol had multiple errors that caused the sensor to stop measuring (MODBus error). This seemed to be due to the variation in voltage at the site. The first two datapoints after the sensor stopped for more than 24 hours were discarded. Stopping caused the sensor to foul and gave unreliable data.

The enzyme activity values measured were very low. However, they were in almost all cases higher than the detection limit.

The sensor itself also gave an error message (erroneous measurement ON/ OFF). When it gave ON, this datapoint was discarded.

8.2.3.2 BactoSense

The data from the BactoSense is reviewed. From each measurement the ICC and HNAP are calculated. In the period March 24 to April 12 there were no measurements due to an empty cartridge. The gatings were preset by the sensor supplier and not adjusted during or after the pilot period.

8.2.3.3 BugCount Guardian

The data from the BugCount Guardian were reviewed and the data between April 19 and April 28 are discarded. At this moment there were problems with the waterflow towards the sensor and the data don't seem to be reliable. Moreover, on May 8 the peak seems to be caused by an error in the standard measurement. The rest of the data is used in the data analysis.

8.2.3.4 CBM

Every week two cuvettes from the CBM were measured, which gave a two-week biofilm development time on the coupons in each cuvette. The ATP analysis was performed as described in paragraph 2.1.4 of the overall report. Briefly, the bacterial cells and the ATP was released from the glass plates using the LuminUltra ATP-field kit.

8.3 Results

8.3.1 Chlorine dosing

Chlorine dosing was set at 0.2 mg/l Cl_2 for the period from the start of the pilot to March 19. In the period between March 19 to March 22, the dosing was lowered to 0.15 mg/l Cl_2, and from March 22 to March 24 the dosing was increased to 0.25 mg/l Cl_2. After March 24 the concentration was set at 0.2 mg/l Cl_2 again. The real Cl_2 concentration in the system is also measured and follows the dosing. However, it varies as the load (thermal and organic) in the cooling tower varies, and the recycle-condensate stream is added regularly, also causing fluctuations in the residual chlorine concentration, see Figure 8-6.

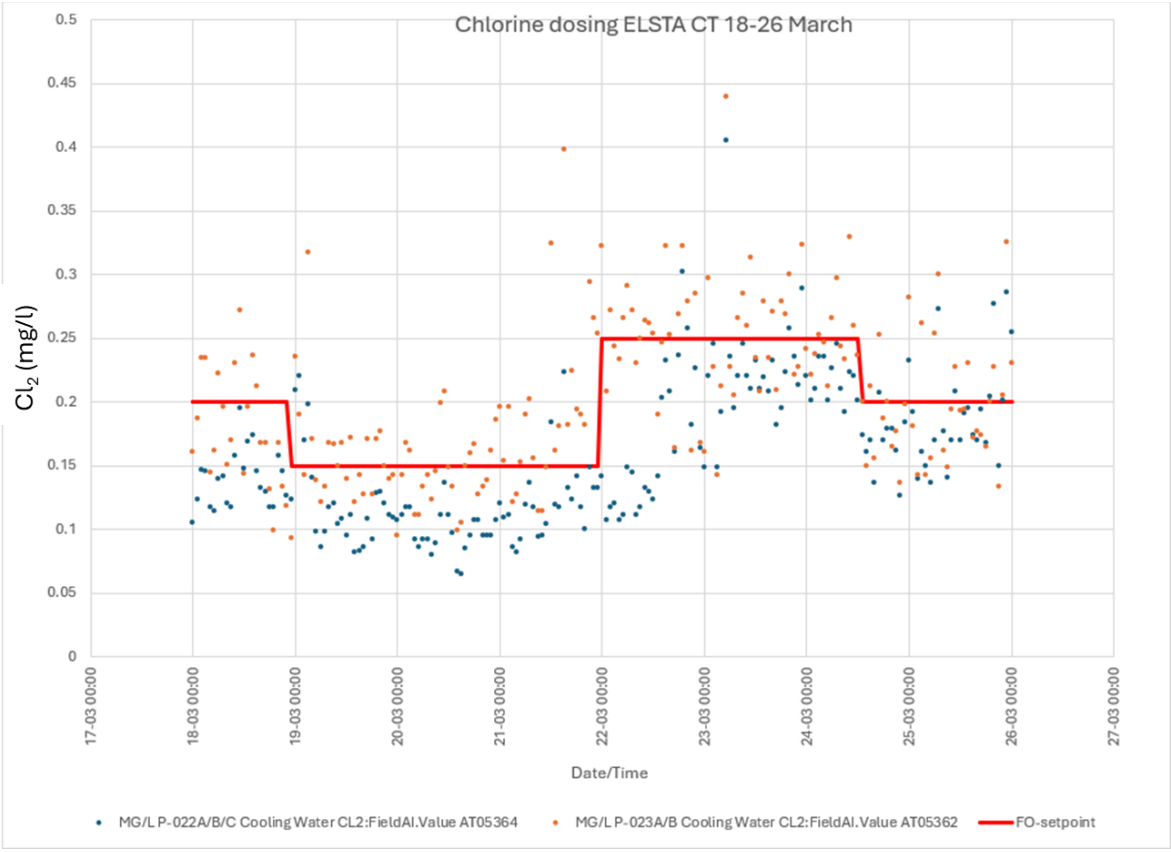


Figure 8-6: Chlorine dosing and measured concentration in the period March 18 - 26, 2024

8.3.2 BACTcontrol

The enzymatic activity of the water, measured with BACTcontrol, varies between ~15 and ~60 pmol/min (Figure 8-7 This is in the lower part of the detection range. As the water is disinfected, it can be expected that the enzymatic activity is low. Only the measurements that did not gave an error are shown.

In the period that the chlorine dosing varied, there were no valid measurements of the BACTcontrol, so the effect of altering the chlorine dosing on the enzyme activity could not be determined.

The source water up to April 1 is surface water from Belgium; from April 1 onwards the source water was Biesbosch water. The results from the BACTcontrol showed no significant difference (t-test; P>0.05) in microbial activity between the two source waters.

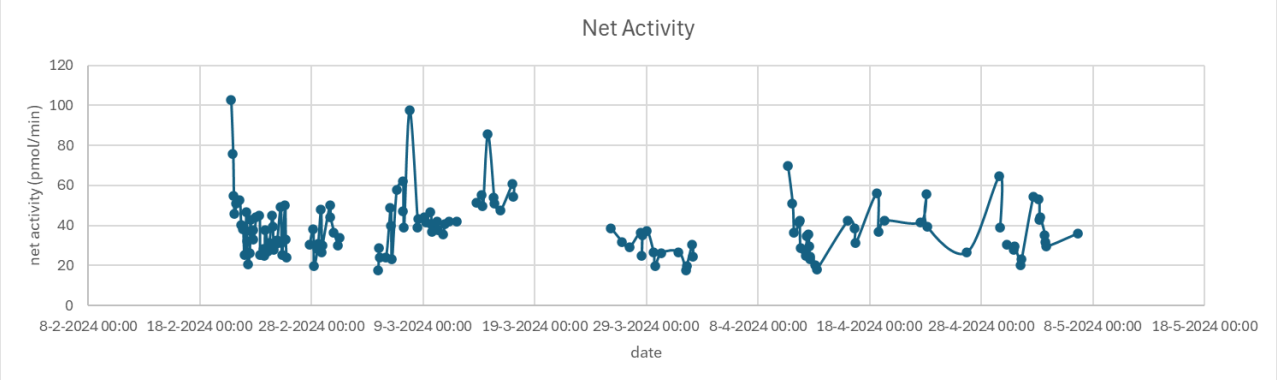


Figure 8-7: BACTcontrol results of measurements (18 Feb – 8 May 2024) at Dow, Terneuzen

8.3.3 BactoSense

The BactoSense measured the ICC (intact cell count), the high nucleic acid cells (HNAC)) and the low nucleic acid cells (LNAC) based on the gates set for the BactoSense. From March 25 to April 13 there are no measurements as the cartridge was finished and it took a while before it was replaced. There is a clear difference between variability in results in the period up to April 1 and the period after April 13.. The variation in ICC for the first period is higher than for the second period (Figure 8-8). There is a significant difference between the two periods regarding the value of ICC and HNAC (t-test; $p<0.05$).

Looking at the off-line and on-line monitoring results for the CT, there is no clear explanation for this difference. There is a small shift in the conductivity in the circulating water when the two periods are compared. Average 3000 µS/cm in the first period vs. 2850 µS/cm in the second period. Feedwater quality (surface water from large storage basin) did not change significantly based on the weekly feedwater analyses.

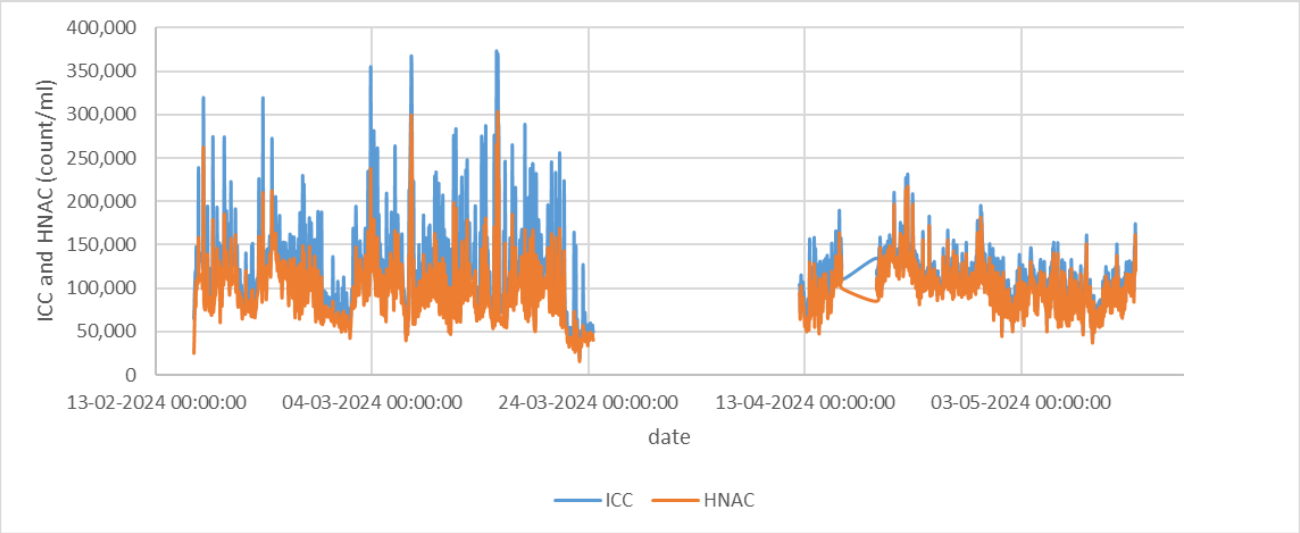


Figure 8-8 BactoSense results of the Dow pilot: ICC and HNAC

The HNAC is also used to calculate the percentage of high nucleic acid cells (HNAP: high nucleic acid percentage) and shown in Figure 8-9. The HNAP is on average between 60 and 80% in the first period, and close to 90% in the second period. This difference is also significant (t-test, $p<0.05$).

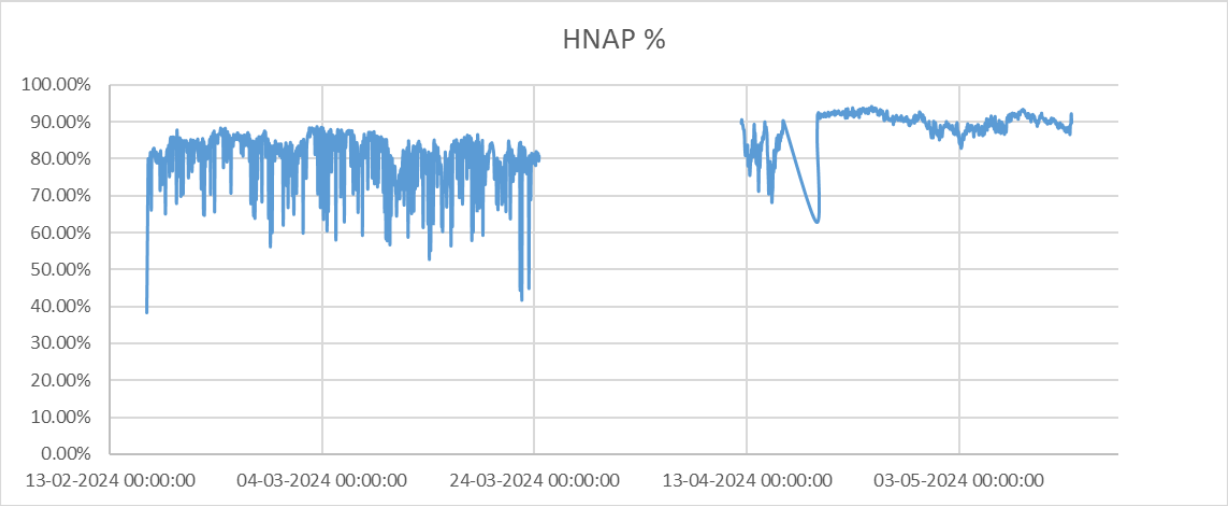


Figure 8-9: BactoSense high nucleic acid cells as a percentage of the total intact cell count (HNAP)

When zooming in on a small period, a peak in ICC be seen every few datapoints (Figure 8-10). This peak only lasts one measurement, indicating a frequent short-change in water quality. It is hypothesized that this is due to the addition of a large volume of recycle condensate water in the cooling tower-basin. The addition point of the condensate water is close to the point where the sensors are installed, and it is expected that there is poor mixing of this batch-wise added condensate with basin water. Additional experiments are, however, needed to confirm or reject this hypothesis.

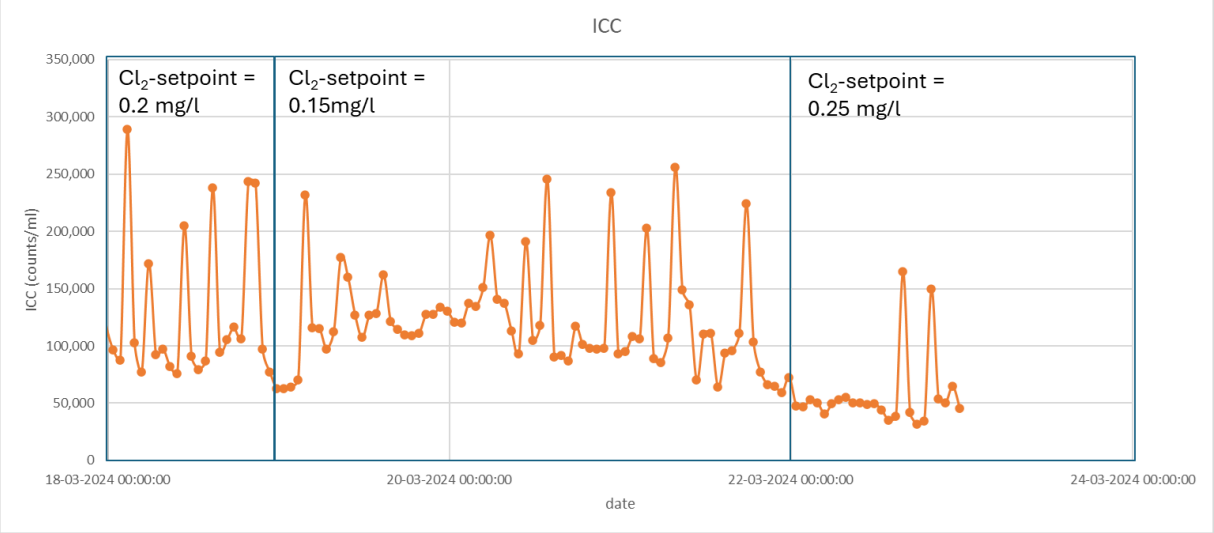
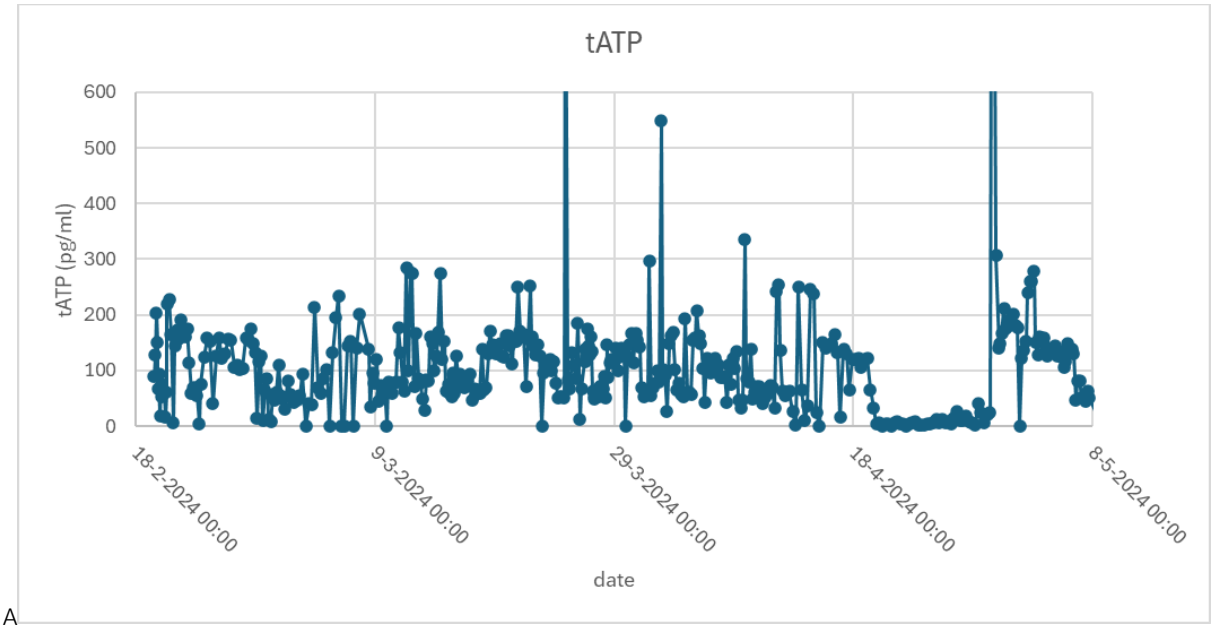


Figure 8-10: ICC of Dow pilot, measured by the BactoSense

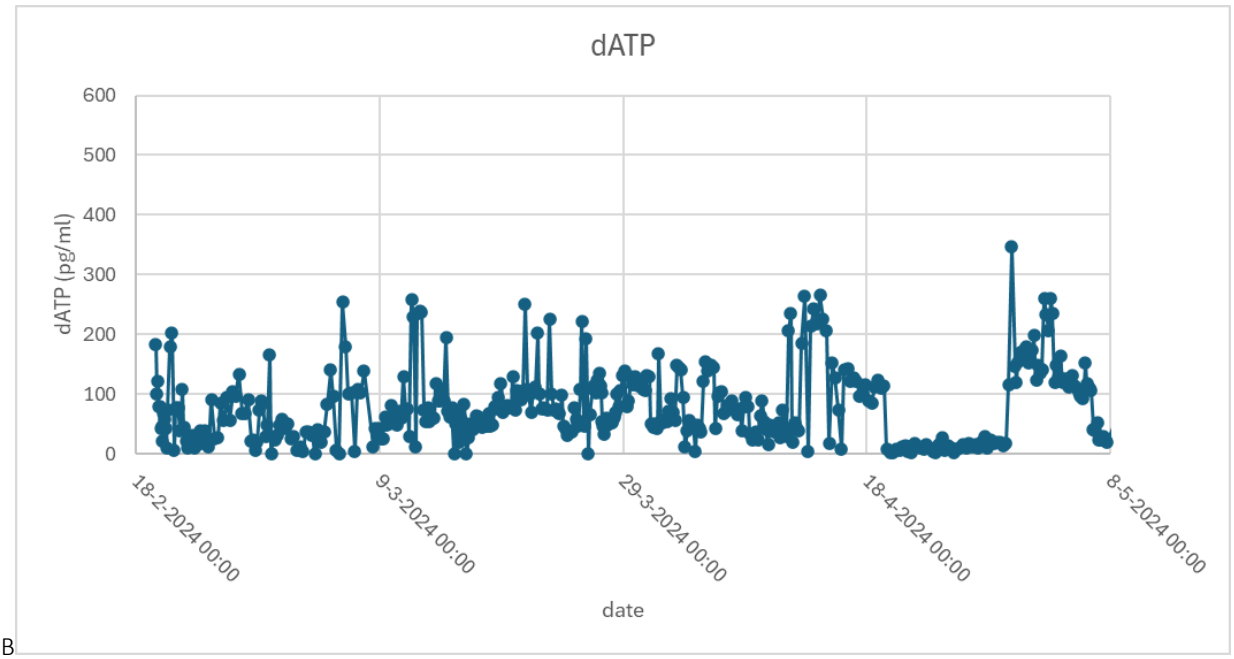
From March 19 to March 25, the chlorine dosing was changed. Lowering the setpoint chorine does not seem to give a change in ICC (see Figure 8-10). There was no significant difference between the period March 16-March 18 and the period March 19-March 21 (t-test, $p>0.05$). When the setpoint is increased, on March 22, the ICC is in the lower end of the measurement range. This decrease is significant compared to the period when the setpoint was 0.2 mg/l Cl_2. Also here, peaks can be found, that may be attributed to the addition of the condensate. There are no measurements of the BactoSense when the dosing was brought back to the chlorine setpoint of 0.2 mg/l, so it cannot be seen if the ICC returns to values measured at the start of the monitoring period.

8.3.4 BugCount Guardian

The BugCount Guardian measures the dissolved ATP (dATP) and the total ATP (tATP) in two samples that are taken within 10 minutes from each other. They are therefore regarded as one sample. Both parameters are also used to calculate the cellular ATP (cATP) by subtracting the dATP from the tATP. cATP is a measure for active microbial biomass in the system and dATP is a measure for death biomass. The total ATP (tATP) is shown in Figure 8-11A, the dATP in Figure 8-11B and cATP in Figure 8-13.



A



B

Figure 8-11: tATP and dATP concentrations in the water measured with the BugCount Guardian at Dow

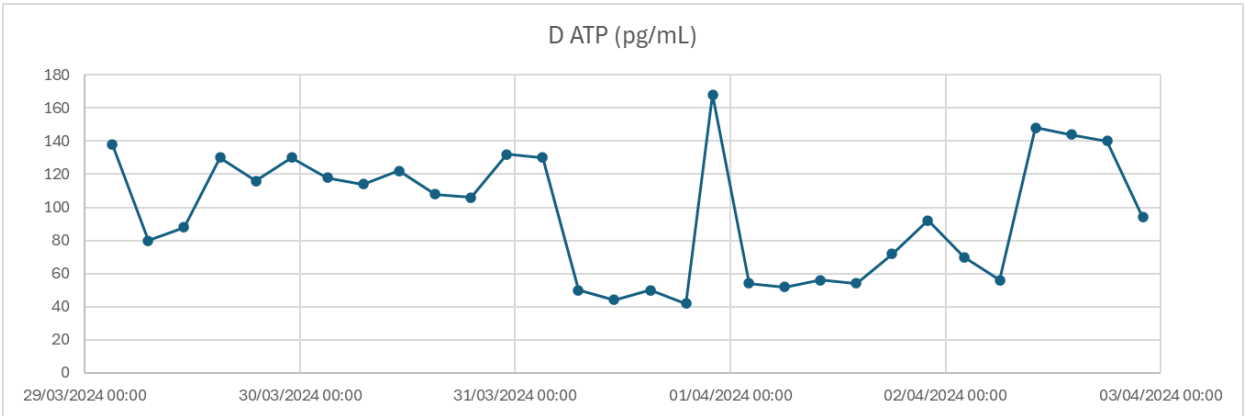


Figure 8-12: Zoom in dATP at Dow

Both dATP and dATP vary considerable during the monitoring period (between 0 to > 500 pg/ml). The cATP concentration in the water is around zero (Figure 8-13). Some values are negative but this is because dATP and cATP are measured in two different samples and not from one, and this can result in small variations in amount of ATP, resulting in a calculated cATP that may be negative.

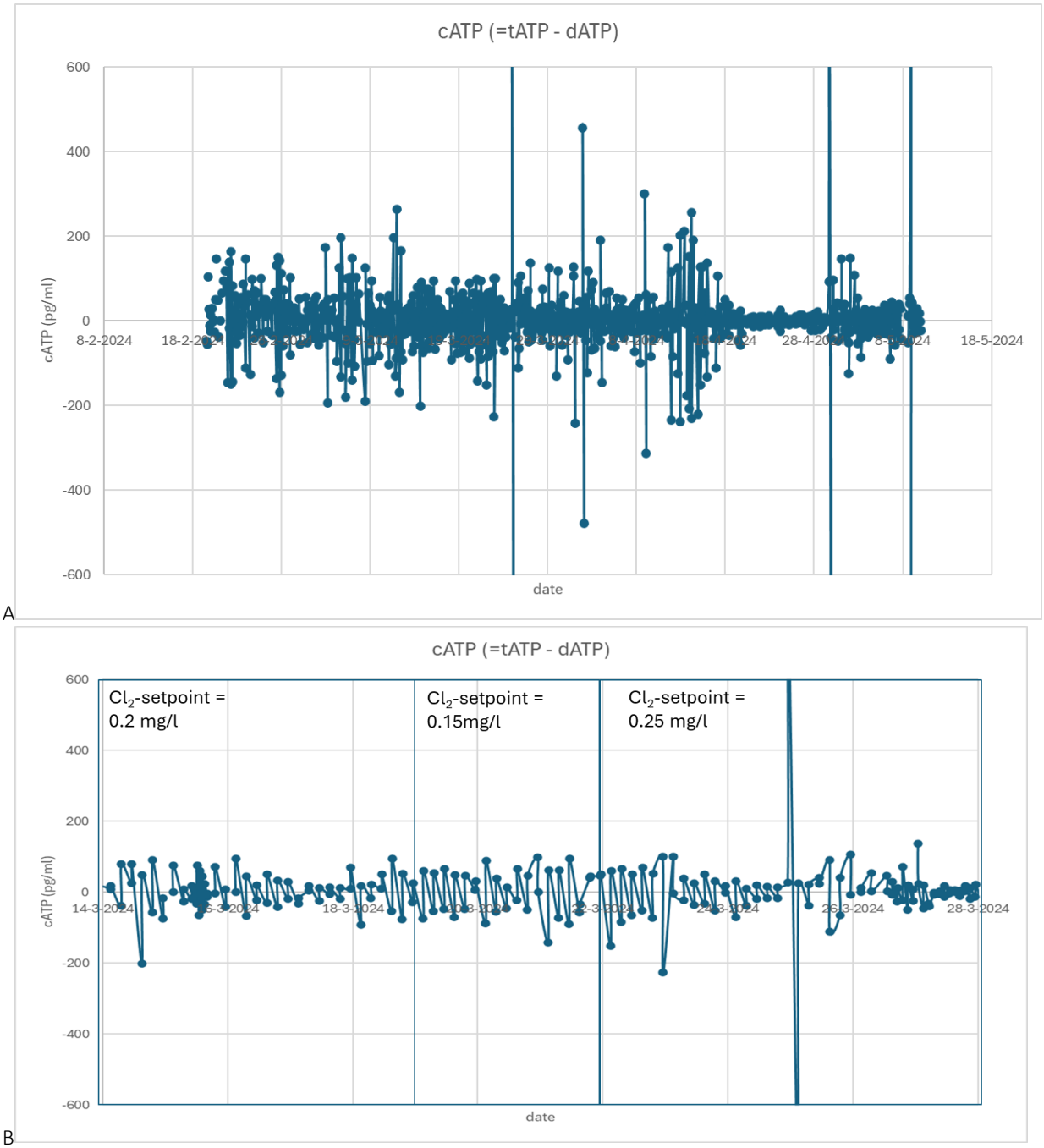


Figure 8-13: The cATP concentration in water calculated from the tATP and dATP concentrations, at Dow. A: whole monitoring period, B, zoom in on 14-19 March.

As the cATP concentration is around zero, no effect of a change in chlorine could be seen.

8.3.5 CBM

The CBM was used to measure the biofilm formation potential. Two cuvettes were measured at the same moment, and the average is calculated, see Figure 8-14. The error bars give the variation from the average.

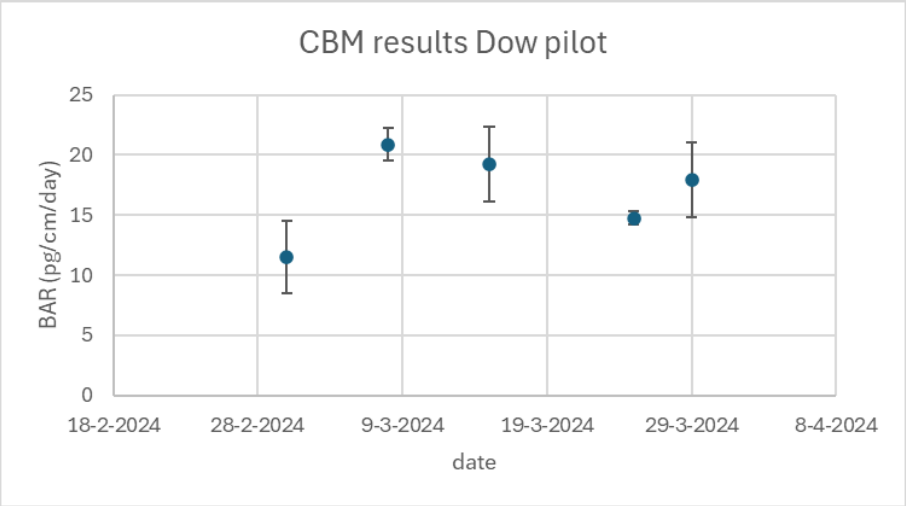


Figure 8-14: CBM results from pilot at Dow. BAR = biomass accumulation rate

Two cuvettes that were measured at the same moment, had a similar result. Although the waterflow decreased regularly in the cuvettes due to blocking of the needle valves, the duplicates had similar values. Because the change in chlorine dosing was only during a small period of days (19 March to 24 March), the effects were not seen on the CBM values, since the biomass accumulation rate is based on biofilm formation during a two-week period.

8.4 Discussion

8.4.1 BACTcontrol

At Dow the cooling water is chlorinated, resulting in bacterial levels below the detection limit. The reliability of the data is, therefore, low. The chlorine concentration is controlled but chlorine measurements demonstrate that the real-world concentrations vary, and this variation, thus, affect the measurement results of the BACTcontrol. As a result, the effect of switching from water source or adjusting the chlorine dosing on the microbiology could not be reliable determined.

8.4.2 BactoSense

The BactoSense did not give significantly different measurement values when two periods of measurements (before April 1 and after April 14) but the fluctuations in the BactoSense values were clearly lower in the second period. The fluctuations in the BactoSense data were large, but this was probably caused by the periodical addition of the condensate water. The effect of changing the chlorine dosing did not have a clear effect on the BactoSense values, maybe because the change in chlorine concentration was too low to affect the microbial viability.

8.4.3 BugCount Guardian

The tATP and dATP concentrations were measured with about 10 minutes difference. A new sample was taken for the measurement. When the difference between the tATP and dATP is calculated, this would result in the cellular ATP, but this gives many negative values. This means that the biomass concentration fluctuated within this ten minute timeframe, and as a result these two samples cannot be regarded as one to calculate the cATP. However, the dATP and tATP values are in the same range, suggesting that the cATP concentration is very low, or around zero. This is also expected as the water is chlorinated, which will eradicate the microorganisms and leaving only dead biomass, for which dATP is a measure.

8.4.4 Comparison of the three online sensors

During the pilot period, three online sensors were used and the results from all three sensors are visualized in . The different sensors measure different microbiological parameters, making it is hard to compare the results directly. However, similar trends are expected. Although there seems to be a step change in conductivity in the circulating water, indicating a possible difference in feedwater quality or CT-operations, the effect on the bacteriological sensors is negligible except for the variability in BactoSense ICC and HNA%

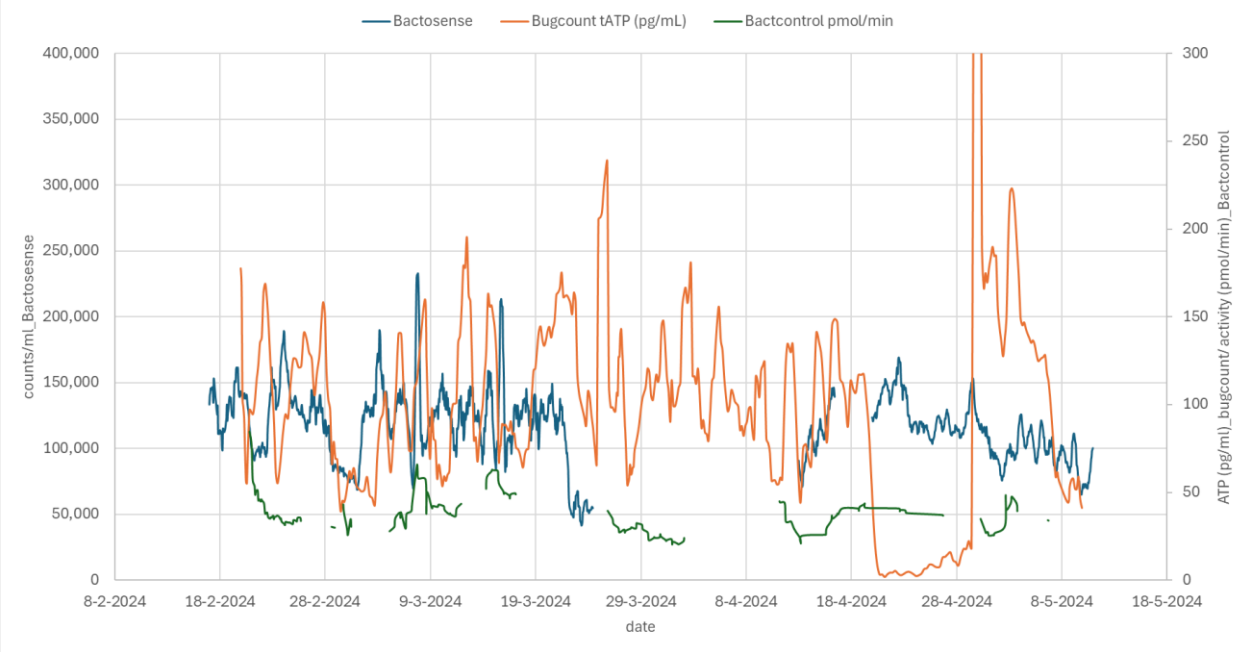


Figure 8-15: Results from the three online sensors during the monitoring period at Dow. 24-hour moving average.

There are no clear trends that can be observed in all three sensors, or specific operational factors that would cause a change.

8.4.5 CBM

The duplicates of the CBM gave similar results, indicating that the CBM seems to give reproducible results. However, clogging of the valves of the flow meters and the cuvettes were observed, which impacted the flow over the cuvettes. This effect of clogging on the flow rate might also impact the biofilm formation on the coupons in the cuvettes of the CBM and, therefore, it remains uncertain how reliable the CBM-values are.

The timespan for the development of the biofilm on the cuvettes was 14-17 days, which is around 14 days shorter than the timespan normally used to determine the BAR for drinking water (see chapters 4 - 6). Reducing the time span to 14 to 17 days for the dirtier surface water than drinking water gave results that were within the measurement limits of the sensor/ ATP kit. This shows that by adapting the time span in the sampling protocol from drinking water to different water types is possible and give results that are within the detection limit.

8.5 Conclusions and recommendations

8.5.1 Conclusions

The sensors have been tested on a cooling tower which has a stable (bacteriological) quality of feed water (effect of the recycle condensate addition is limited on the whole system). With the applied biocide dosing, the cooling tower has shown to be ‘in control’ with respect to biological quality, making the use of sensors on this system less essential. Systems with more variability on feed water, having frequent biological problems or strict limitation on biocide usage can definitely benefit from the data generated by the sensors.

The BactoSense was able to detect the impact of the frequent (batchwise) suppletion of recycle condensate in the CT basin. The microbial quality in the overall cooling tower system was not significantly impacted but the suppletion was done close to the sample take-off point for the sensors. Therefore it had a short effect on the local quality which could be detected by the BactoSense. The frequency of analysis (once per hour) leads to a higher probability peaks are caught.

The BACTcontrol was not able to produce useful results for this stream, as the data were below the detection limit or were erroneous.

Both the BugCount Guardian and BACTcontrol showed that there very few active cells in the surface water during the whole monitoring period. In contrast, the intact cell concentration determined with the BactoSense were relatively high. It can be concluded from these results that the chlorination applied diminished the activity of the cells but left the cell membrane of the cells for a large part intact.

It was not possible to see the effect of the change in chlorine setpoint on the parameter values of the three sensors tested. The chlorine setpoint was only increased or decreased by 25%, but with all chlorine setpoints the inactivation of microorganisms seems to be high. The applied increase in chlorine, thus, did not give an extra decrease in active cells.

The three online sensors didn't need much maintenance or calibration efforts during the three-months period. As for all on-line monitoring devices, regular checks on sample flows, internal leakages, electronic errors, waste containers and reagent levels are needed. The sensors are suitable for use in an industrial environment if placed inside a cabinet, analyzer house or inside a building. Besides power no additional utilities are needed.
The CBM showed low BAR-values, comparable to drinking water, demonstrating that the chlorination also prevents considerable biofilm formation in the system.

Clogging of the valves of the flow meters in the CBM resulted in unequal flow during the monitoring period and between cuvettes, which could affect the CBM results.

8.5.2 Recommendations

It is important to select an appropriate and representative sample take-off point in the process. Sample stream should be taken from a well-mixed zone. Depending on the control strategy, the sensors can be placed in the feedwater to proactively check the suppletion water and adjust/control biocide dosing or in the circulating water to check the effectiveness of the disinfection.

It is important to design and build the sampling system in front of the sensors in such a way that sufficient flow is guaranteed, pressure ranges are respected and algae growth in tubing is prevented. The system should have easy drain and flush possibilities to minimize build-up of biomass/debris that could block flowmeters/pressure regulators.

As the valve of the flow meters in the CBM clogged easily, it is recommended to use other valves that are less prone to clogging. Using a filter before the sensor could also decrease the clogging but this will also remove potential nutrients and can serve as a growth medium. This is unwanted as it will affect the result from the CBM, since the CBM measures biofilm growth potential, based on the amount of nutrients available in the system.

9 Monitoring cooling water production at BASF Antwerp

9.1 Microbiological problem/situation

9.1.1 Goal

The inline sensors have not yet been tested on brackish water that is used for cooling purposes in an industrial setting. BASF uses this type of water for cooling purposes in their industry. The goal of the pilot was, therefore, to evaluate whether the sensors are applicable in an industrial setting in terms of following the microbial water quality and robustness. In addition, BASF wants to get experience with the sensors and evaluate the effort that is required to keep the sensors operational when running with brackish water. As a result, the experiments at BASF were done to find answers to the following questions:

- How is the sampling done and what is required to get the water to the sensors?
- Is pretreatment/filtration needed?
- How much maintenance is required?
- How much effort is required for calibration?
- How much reagents are used and is there a production of waste streams?

Furthermore, BASF wants to obtain knowledge on the microbiological quality in their brackish water and the impact of the biocide conditioning on it. Finally, BASF also wants to investigate whether there is a correlation between increased dosing and the sensor results.

9.1.2 Location

The pilot was performed at BASF Antwerp. For cooling purposes, BASF uses brackish water that is taken in from the docks. This water is supplied to the production plants via a pressurized grid. The production plants take in cold water from the pressurized brackish water grid based on their cooling needs and return it afterwards to a gravitational grid. Several cooling towers are positioned at strategic positions on the site. These cooling towers take in (warm) water from the gravitational grid, cool it and pump it back into the cold water pressurized grid. The gravitational grid – the part which is not fed to a cooling tower – flows back to the docks. Hence, the cooling water at BASF is a flow-through system and not a closed loop system. The cycles of concentration of the cooling water (how much water is evaporated and thus how much the remaining water is concentrated) vary depending on the operation of the cooling towers and the pumps at the intake stations.

The test was performed at cooling tower E500 (CT E500), a forced draft cooling tower with four cells of roughly 3 500 m^3/h each (Figure 9-1). CT E500 is fed with water from a singular production plant (stable flow and temperature), the cooling tower is conditioned with NaClO at either the warm or the cold side through a batch dosing system. During the test, all NaClO was dosed in the cold-water basin. At the D-cell (westernmost cell), an in-line ClO_2 dosing system is used for conditioning as well.

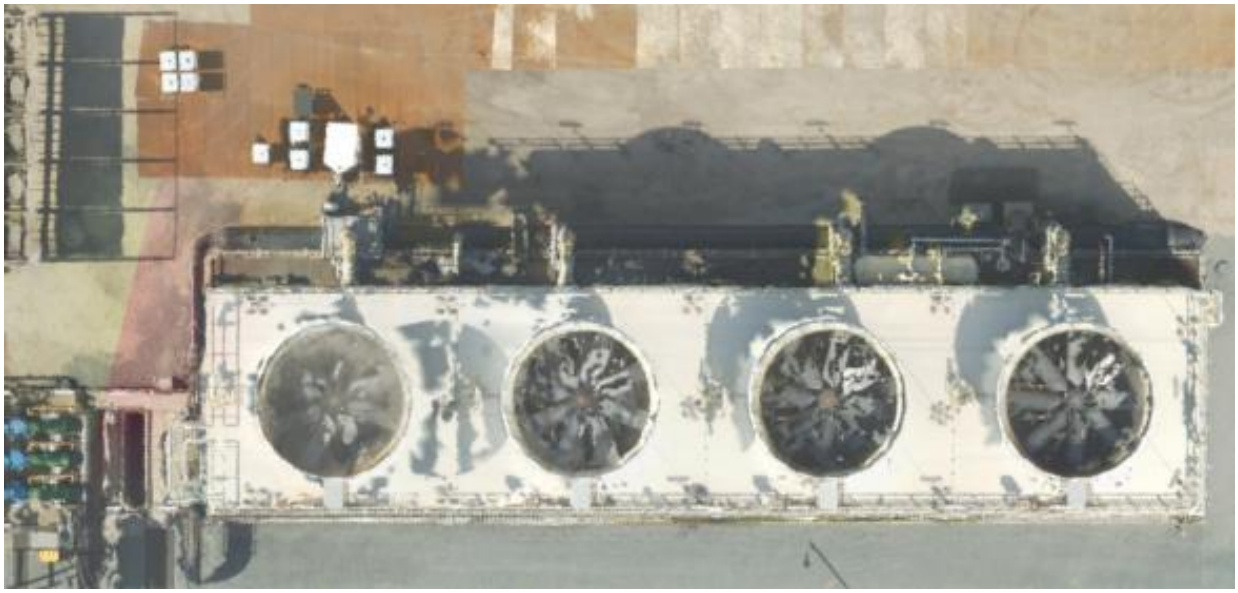


Figure 9-1: Top view of the cooling towers at the BASF location in Antwerp

Figure 9-2: container with the sensors inside

The BactoSense, BACTcontrol, BugCount Guardian and Continuous Biofilm Monitor (CBM) were stationed inside a container to shield them from weather effects (Figure 9-2). The BioGeorge was positioned outside the container (Figure 9-3).

Figure 9-3: Connections to the container. The tube with the BioGeorge is situated on the outside of the container. On the floor left in the figure.

In the cold-water basin, a submerged pump was placed with a bypass circulation back to the basin (to control the pressure and flow rate to the sensors). The water was first sent through a 250 µm filter (redundant setup) and then to the sensors (Figure 9-4). A total flow of roughly 1.1 m³/h was used, roughly 400 l/h to the BioGeorge, 700 l/h to the sensors inside the container.

Figure 9-4: Water is taken from the basin (Left). Filter set-up (right)

9.2 Measurements

9.2.1 Experimental set up at BASF Antwerp

Biomass monitoring using the BactoSense, BACTcontrol, BugCount Guardian, CBM and BioGeorge was done at BASF Antwerp site for 2.5 months (Table 9-1). The measurement period was from July 11 up to September 13, 2024.

Table 9-1 Sampling locations and monitoring frequency. The piloting period was in 2024.

	Measurement period	Measurement frequency
BactoSense	11-7 to 13-9	30 min 2 h
BACTcontrol	11-7 to 13-9	92 min
BugCount Guardian	11-7 to 13-9	120 min
CBM	11-7 to 13-9	~1 time per two weeks
BioGeorge	11-7 to 13-9	Generated current (biofilm activity) every 10 minutes. Applied current (biofilm thickness) every 6 hours.

9.2.2 Measurement frequency

The measurement frequency of the BactoSense, BACTcontrol and BugCount Guardian can be programmed and was set at an interval of 30 minutes or 2 hours. BASF was responsible for handling the BactoSense and BACTcontrol. The Milispec-CBM was installed and sampled by BASF every one to two weeks and the ATP content in the biofilm was measured with the LuminUltra ATP kit on-site (according to the protocol of Chapter 2).

9.2.2.1 Additional analyses by BASF

BASF measured the free chlorine concentration and logged the biocide dosing. This dosed biocide was sodium hypochlorite in cooling tower E500 and chlorine dioxide in D300, which is upstream of E500.

9.2.3 Data processing and statistics

9.2.3.1 BACTcontrol

The first three measurement results after a temporary stop of the BACTcontrol (longer than several hours) were removed from the dataset and thus not used in data processing and interpretation. The results from these measurements were often higher than the results from the other measurements. This is likely to be caused by a technical aspect, as biomass build-up occurred during the standstill period of the BACTcontrol. When the net enzyme activity was lower than the detection limit, the datapoint was also disregarded.

9.2.3.2 BactoSense

The gating of the data was set by bNovate, based on their experience. This gating was not adjusted afterwards. The results calculated for ICC, HNAP and LNAP were used for data processing.

9.2.3.3 BugCount Guardian

The BugCount Guardian measures the dissolved ATP (dATP) concentration and then approximately 10 minutes later the total ATP (tATP) concentration. These two measurement points are regarded as one measurement and are used to calculate the cellular ATP (cATP) concentration, by subtracting the dATP concentration from the tATP concentration.

9.2.3.4 CBM

The CBM contained four cuvettes and at each date, two cuvettes were sampled and replaced by new ones. The time between the measurements was 14-20 days.

9.3 Results

9.3.1 Chlorine dosing

Chlorine was dosed in cooling tower E500 where the sensors are situated. Moreover, chlorine is also dosed in cooling tower D300, which is upstream from E500 (around 30 min residence time) and, thus, could also affect the chlorine concentration in cooling tower E500. The dosing to E500 is batch-wise, and the total dosed amount per day is shown in Figure 9-5.

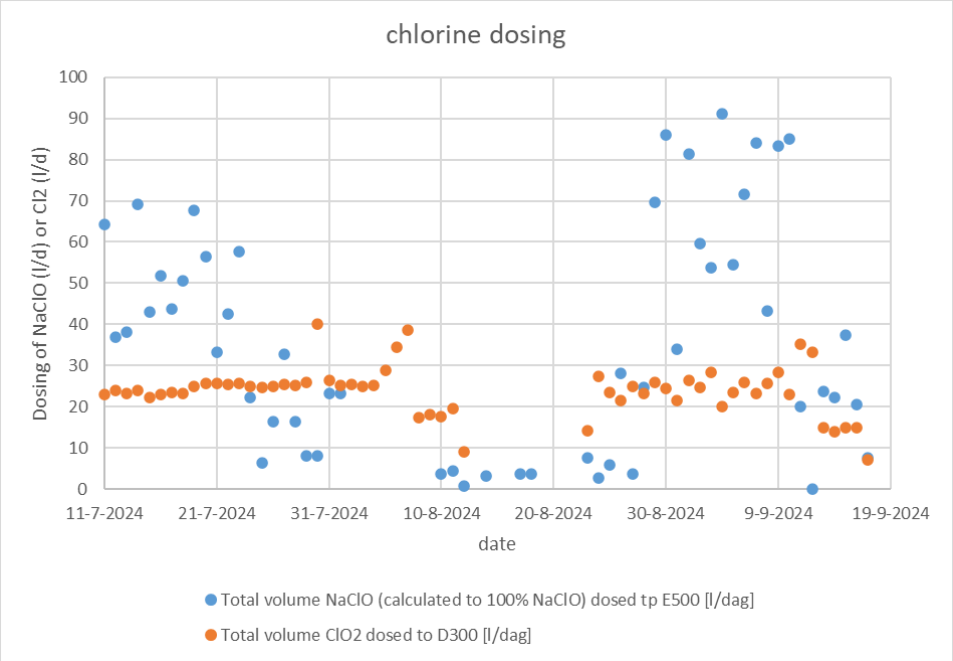


Figure 9-5: chlorine dosing to cooling towers D300 and E500. The sensors are situated at cooling tower E500, and cooling tower D300 is located upstream of E500.

In the period 8-20 August 2024 the frequency of dosing lowered from 4 times a day 2 min dosing to 2 times a day 2 min dosing in cooling tower E500. In the period 21-23 August 2024 the frequency was increased to 10-34 times a day 2 minutes dosing. On 24-27 August 2024 the frequency was brought back to 4 times a day 2 minutes dosing. From 17 August to 11 September 2024 the frequency was increased to 68 times a day 1 minute dosing. After Sept 11, the frequency was brought to 28 times a day 1 minute dosing.

Upstream of E500 is cooling tower D300. In this cooling tower, chlorine dioxide is dosed. In the period 11 July – 8 August this was 2 times a day 120 min, resulting in approximately 23-25L/day. From 8 August - 12 August there was dosed for 6 times/day 30 min, resulting in 17-19L/day. From 13 to 22 August, there were operational problems and chlorine dioxide could not be dosed on the cold side of this cooling tower. During this period more frequent and more biocide was dosed on the warm side of the tower, but this will be less effective than dosing on the cool side of the tower. Dosing in the cold side started again on August 23 with 5 times/day 30 minutes and from August 24 onwards 9-10 times/day 30 minutes dosing, resulting in 20-28L/day.

The free chlorine is measured every minute and the effect of the batchwise dosing on the free chlorine concentration is clearly seen, see Figure 9-6

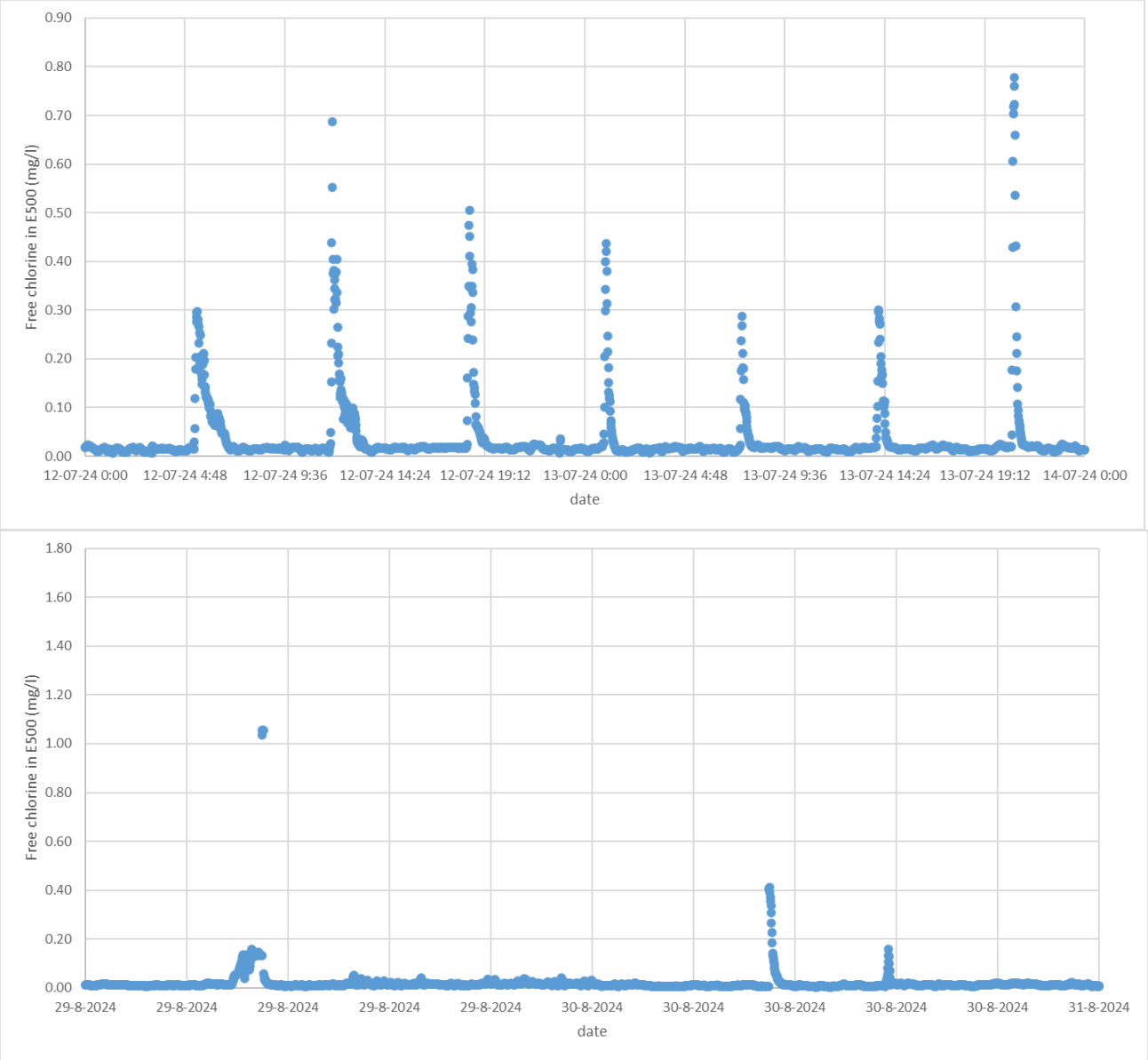


Figure 9-6: Free chlorine concentration in cooling tower E500. Top: chlorine concentration during low dosing frequency (4x/day, 2min), Bottom: chlorine concentration during high dosing frequency (68x/d 1 min).

9.3.2 BACTcontrol

The results of the BACTcontrol sensor are shown in Figure 9-7. The sensor had several periods that there no valid data were obtained from the BACTcontrol, either due to erroneous measurements, or because there were no measurements done. In the period from 19-22 August 2024, there was no cleaning liquid left. This resulted in increased values. From August 27 onwards, the amount of chlorine dosed was higher, and this resulted in lower enzyme activity values in the water, as can be seen by a decreasing line in Figure 9-7.

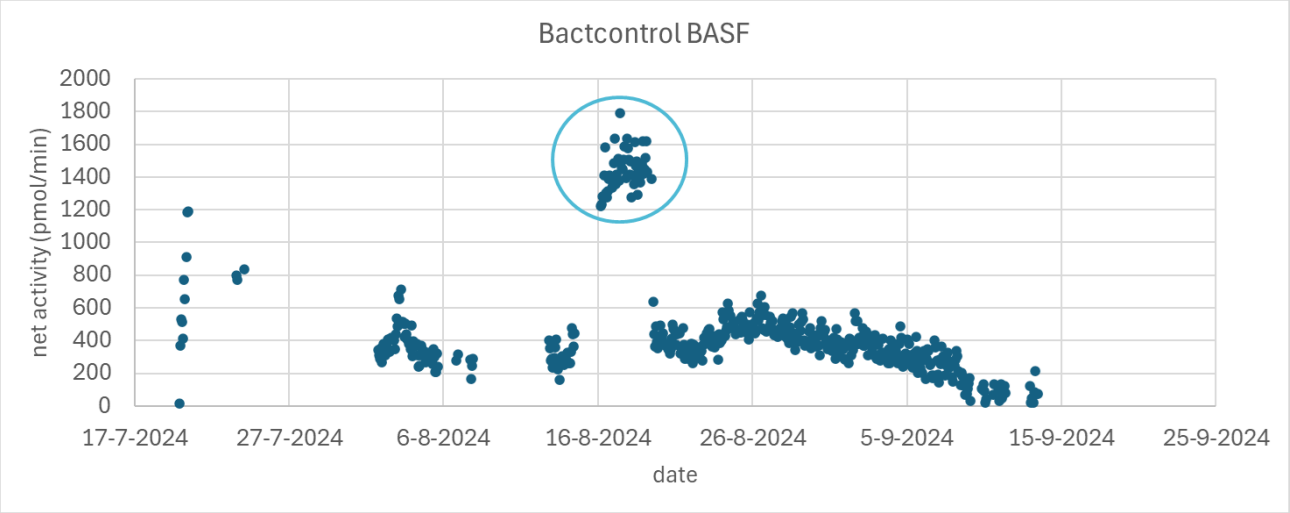


Figure 9-7: BACTcontrol measurements at BASF. The values in the blue circle were obtained when there was no cleaning liquid left in the sensor.

9.3.3 BactoSense

The intact cell counts are shown in Figure 9-8. The detection is reliable up to 2,000,000 cells/ml. In the period 7-27 July and 1-5 September 2024 cell numbers were higher than this upper limit value, resulting in less reliable cell numbers. On July 23 more than 10% of the volume were bubbles in the water, which resulted in low cell count values and can, therefore, not be trusted. Between 9 and 20 August, the water was diluted by a factor 2. The measured value was then below the detection limit. The real value is given in the graph, and is above 2 million, but reliable. There is a sudden jump in cell count on 9 August, indicating an effect of the dilution.

Most of the measurements showed intact cell counts between 1 and 2 million cells/ml (or even higher). The lower chlorine dosing (13-20 August 2024) resulted in an increase of cell number from 2.5 million on August 10 to 3,2 million on August 13 (24 hour moving average). The dilution was stopped on August 20 and higher chlorine was dosed. The cellnumbers went back to the values from before August 9. An increased chlorine dosing thus didn't show an effect on the intact cell counts and the lower cell number seemed to be an effect of the dilution rather than of the increased chlorine dosage, although the concentration of cells seems to decrease slowly after September 6. Furthermore, the percentage of bacteria with a high nucleic acid content (HNAP, Figure 9-9) is not changing significantly when the chlorine dosing changed. It is in general between 40 and 80%.

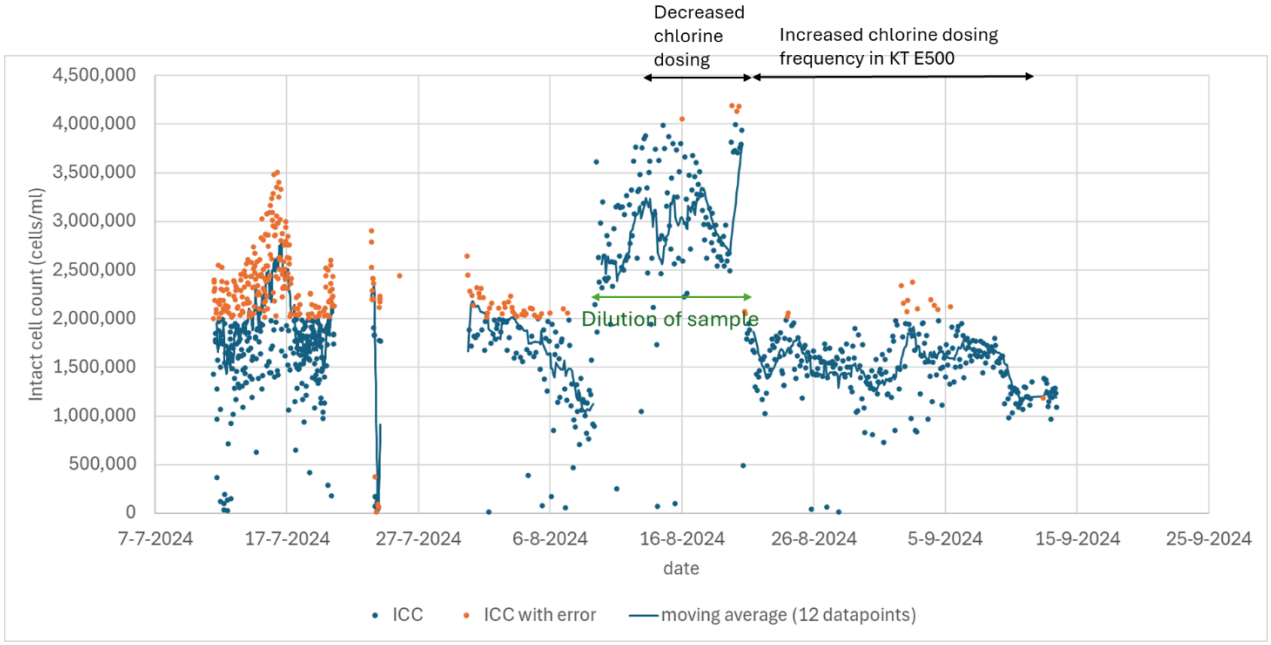


Figure 9-8: ICC measurement with the BactoSense at BASF. Orange dots: measurements with error message. Blue dots: Reliable measurements

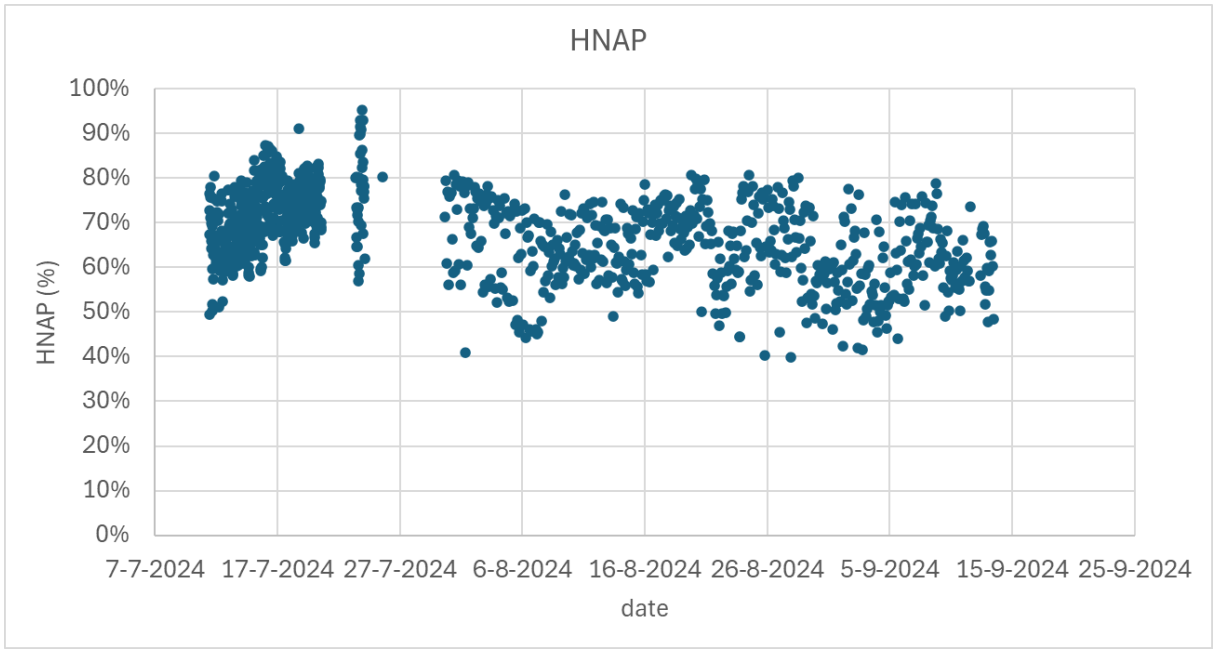


Figure 9-9: Percentage high nucleic acids cells (HNAP) obtained with the Bactosense at BASF

9.3.4 BugCount Guardian

The BugCount measures the total ATP concentration (tATP) and the dissolved ATP concentration (dATP). The dATP is on average between 0 and 200 pg ATP/ml, while the tATP varies between 0 and 1200 pg ATP/ml (Figure 3-6 and 3-7). This means that most of the ATP is present as cellular ATP (cATP). The ATP concentrations vary very strongly over the monitoring period.

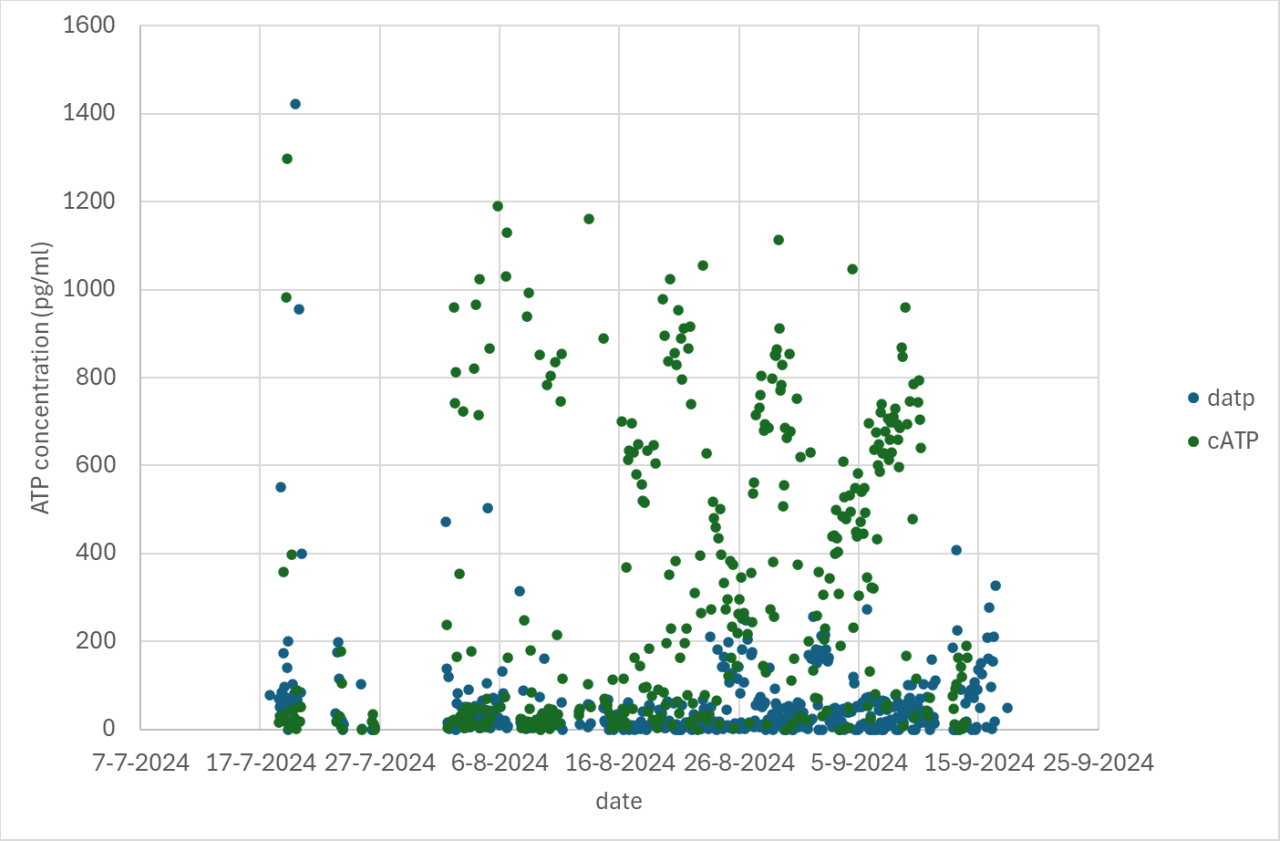


Figure 9-10: ATP concentration measurements by the BugCount Guardian at BASF

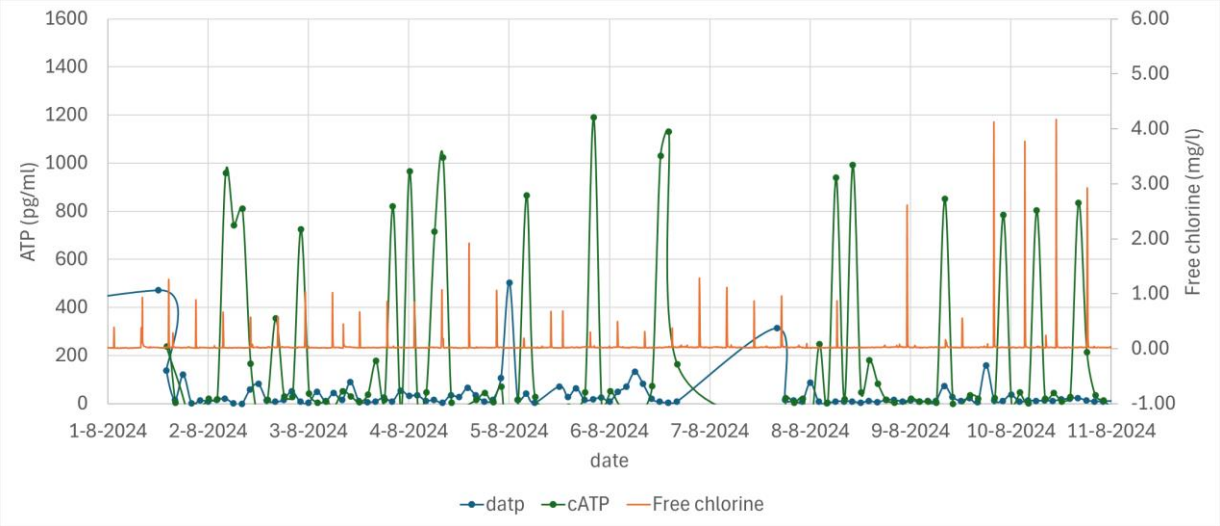


Figure 9-11: Zoom in of cATP and dATP measured at BASF, including free chlorine concentrations.

When zooming into the period 1-11 August 2024, we see that that every few measurements, the cATP concentration is near zero, but at many other moments the cATP concentration peaks. Right after the chlorine peak, the cATP is mostly near zero. It can, subsequently, increase, because the cooling tower has a flow through system, and the microorganisms in the water in the next measurements have had the time to grow between the previous chlorination point (D300) and this cooling tower E500. However, this increase in cATP between two chlorine peaks is not always seen.

9.3.5 Comparison BACTcontrol, BactoSense and BugCount Guardian

The three online sensors that measure the microbiological water quality are compared in Figure 9-12. There are no clear similar trends that are seen in all three sensors. From 27 August 2024 onwards the chlorine frequency and amount was increased, but this change did not result in a change in the sensor data, except for the BACTcontrol, which showed decreasing enzyme activity after 27 August. A lack of clear trends when the disinfection frequency and doses changed, might have been caused by the fact that the cooling tower has a flow through system with highly varying conditions of the incoming water quality. Another reason for the lack in changing trend may be that the sensors are not sensitive enough to pick up changes caused by the change in dosing of chlorine frequency and amount. There is no clear evidence that confirms either hypothesis.

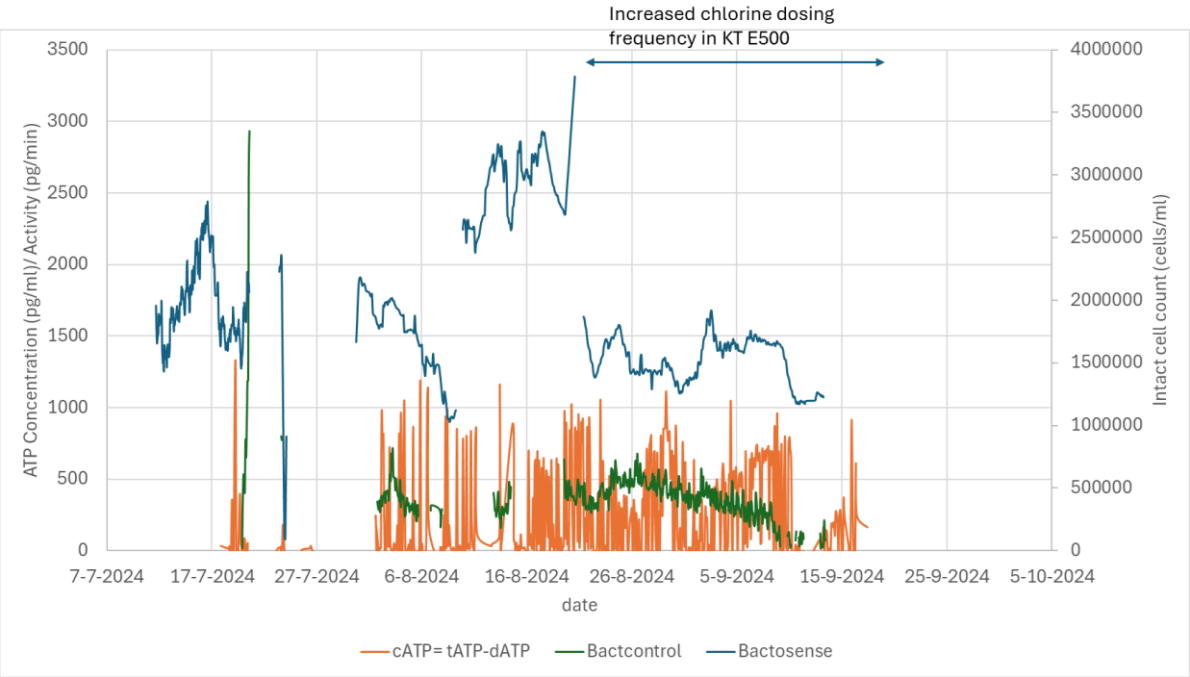


Figure 9-12: cATP (BugCount Guardian), bacterial activity (BACTcontrol) and intact cell count (BactoSense) at BASF. 24h moving average plotted for all sensors.

9.3.6 BioGeorge

The BioGeorge can measure biofilm thickness and biofilm activity by determining the applied current and the generated current, respectively.

From Figure 9-13 and Figure 9-14 it can be deduced that an alarm was generated on 24 July 2024 at the applied current (biofilm thickness) because one of the measured values was more than 100% of the baseline. As the alarm is not reset, the alarm stayed on, but there is no indication that an alarm was necessary. The values remained fairly stable until 30 July 2024. On 30 July 2024 a strong increase in the applied current (biofilm thickness) and a strong decrease in the generated current (biofilm activity) was measured. An increase in applied current is an indication of biofilm growth. However, given the abrupt increase on 30 July and the relatively high applied current value, this could also have been caused by fouling of the probe. Normally, the applied current rises much more gradually instead of such an abrupt increase. An alarm reset was performed on 2 August 2024, but most likely the probe has not been cleaned before this alarm reset, still causing high applied values. This problem prevents the establishment of a stable baseline, which is why the data points are marked yellow until the end. Based on the BioGeorge results, it is suspected that there was a lot of sediment and/or organic contamination in the water, that interfere with the current measurements with the probe. The visual observation that a lot of debris was present in the water confirmed this hypothesis. Therefore, it is not possible to draw a reliable conclusion regarding the biofilm formation using the BioGeorge in this situation.

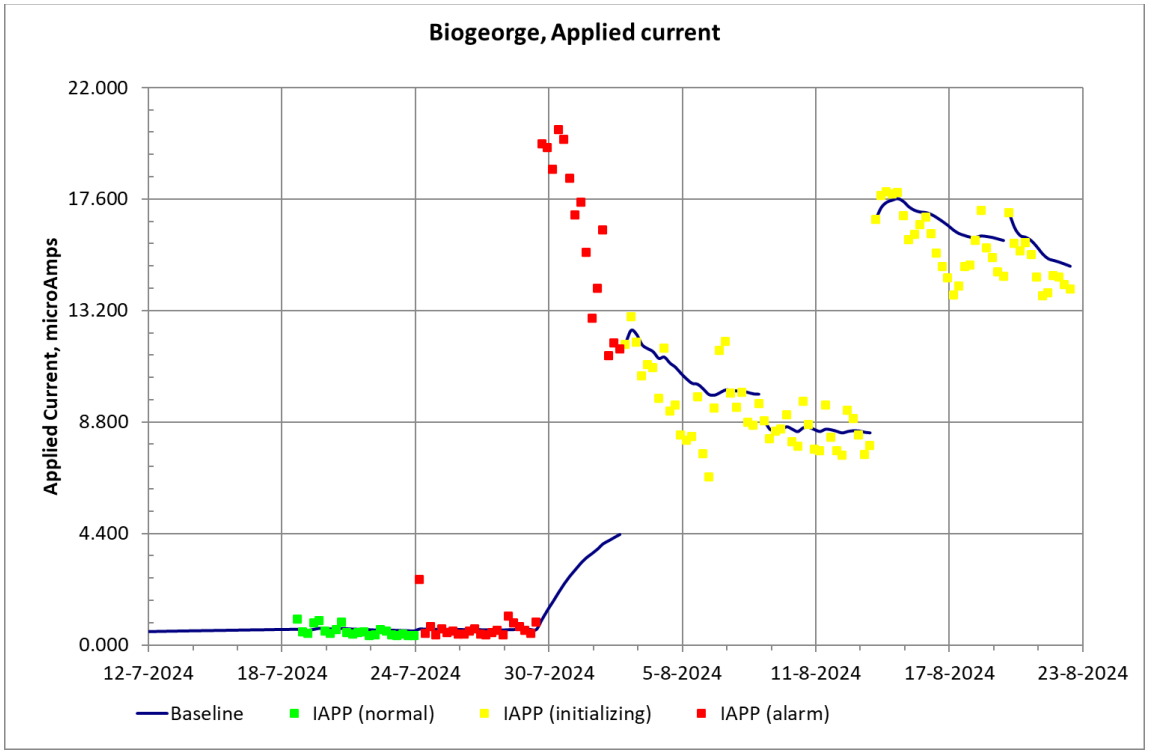


Figure 9-13: The applied current results from the BioGeorge measurement at BASF. Applied current is a measure for the biofilm thickness.

The Iapp is an indication of the “thickness” of the biofilm (EPS). This value is always positive.
The Igen is an indication of the “activity” of the bacteria in the biofilm. This value can be both positive and negative and depends, among other things, on the type of bacteria in the biofilm.

The colors in the graphs mean the following:

- Green = normal; no biofilm activity
- Red = alarm; biofilm is formed. This is set by default when the Iapp or Igen value deviates more than 100% from “normal”, but can be adjusted by the user. An alarm must always be reset manually, otherwise everything remains red.
- Yellow = initialization; After a manual reset, the BioGeorge attempts to generate a new baseline.

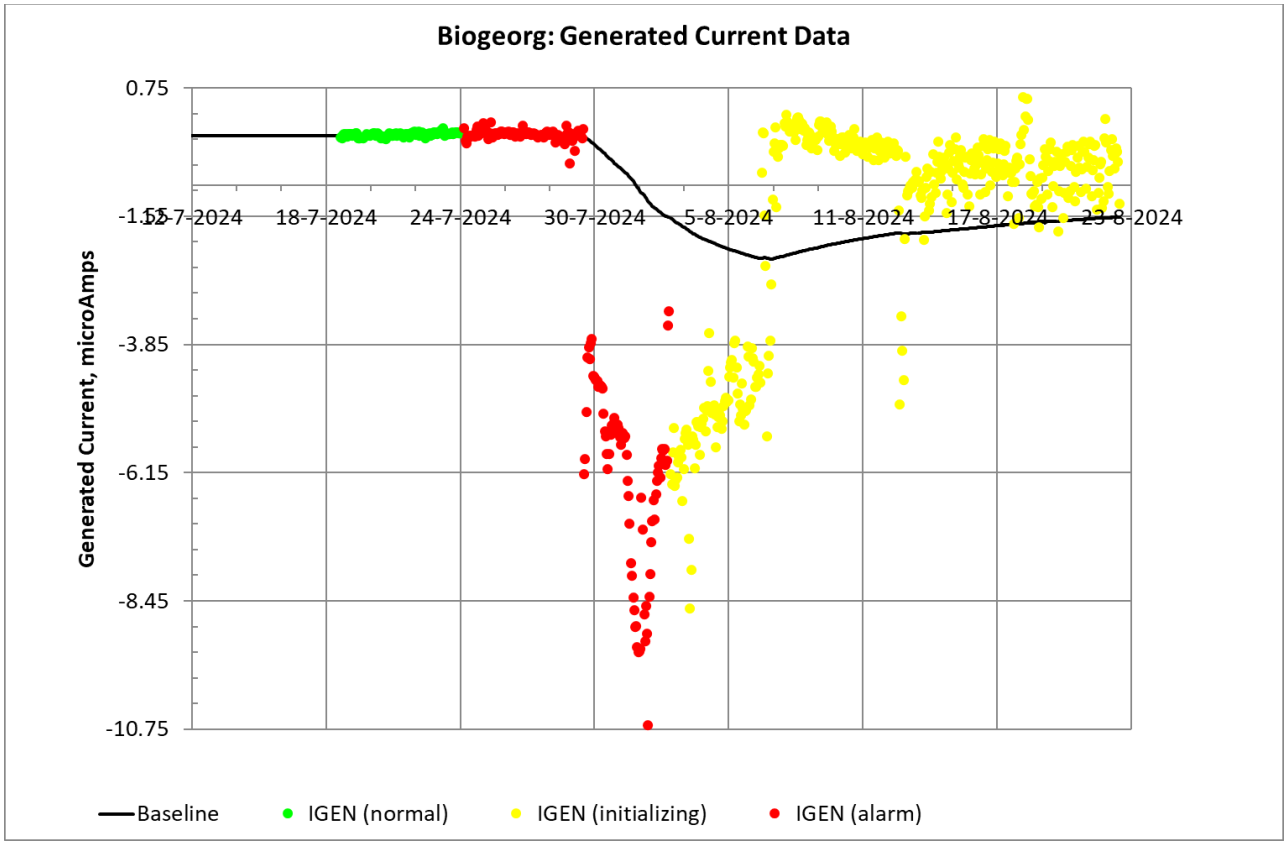


Figure 9-14: The generated current results from the BioGeorge at BASF. Generated current is a measure for the biofilm activity.

9.3.7 CBM

The CBM that was used, contained four cuvettes that each contained a glass coupon. At each moment a measurement was done, two cuvettes were removed and measured. The other two were taken at the next sampling moment. The water ran through the CBM from 11 July to 17 September, 2024.

The flow of the CBM had to be recovered every day, as after a few hours the flow was recovered the flow goes down again, due to clogging of the needle valves. A filter of 250 µm that was installed to prevent clogging, but the sieve size was not fine enough to prevent this. This was also visually observed, as some brown debris was seen on the cuvette (Figure 9-15). 6 August, a finer filter of 50 µm was installed. This resulted in a more stable flow of the CBM, but the flow still decreased after several days.

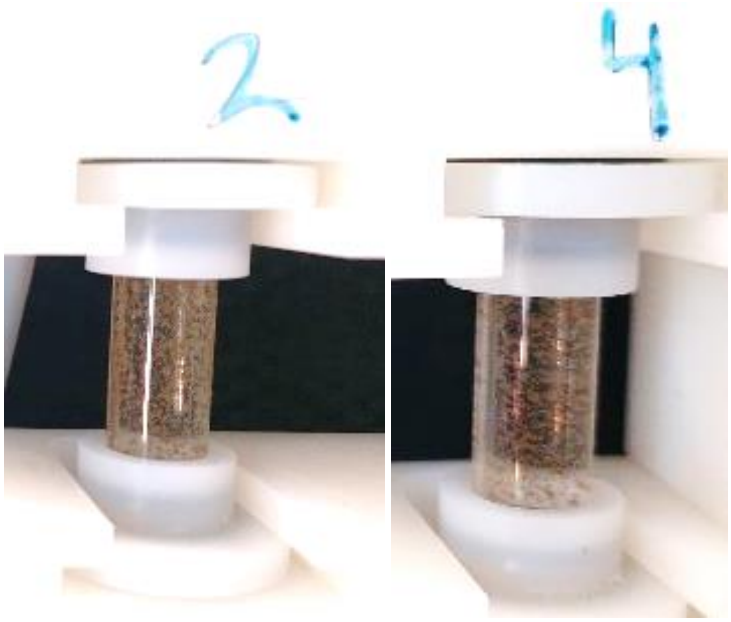


Figure 9-15: brown debris in the cuvettes on July 30, 2024

9.3.7.1 ATP concentrations

The results from the CBM are shown in Figure 9-16. The biomass accumulation rate (BAR), a measure for biofilm formation, varied considerable between values around 1000 to 100,000 pg ATP cm^{-2} day^{-1}. The highest BAR values were obtained when the disinfection frequency was the highest (from 20 August 2024), which was unexpected as a higher frequency should better eradicate micro-organisms and biofilms from the system. The measured values at Sept 2, 4 and one of the measurements at Sept 17 were above the detection limit of 10 million RLU, resulting in unreliable measurements. They are, however, shown in the graph.

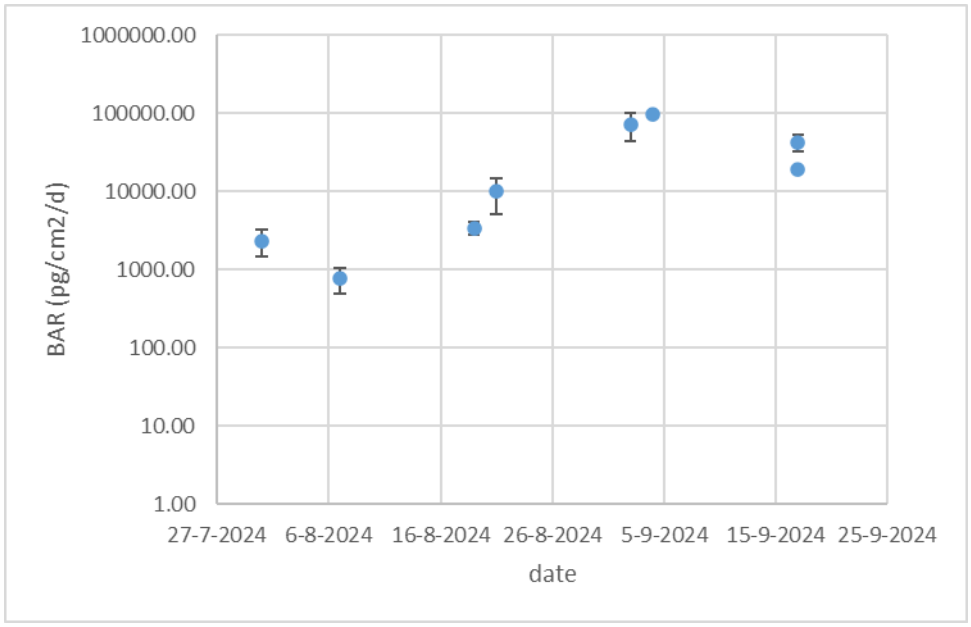


Figure 9-16: The biomass accumulation rate measured with the CBM at BASF

9.4 Discussion – comparison of Dow and BASF results

At location Dow (chapter 8) and location BASF, described in this chapter, the sensors were installed on disinfected surface water. At the Dow location, the water was continuously disinfected, whereas at BASF the water was treated frequently during a day. The difference in observation between these two disinfection strategies on the sensor results will be discussed below for each sensor.

The only operational parameter that was changed during the pilot period at BASF was the chlorine dosing frequency and amount. However, as the frequency and amount of dosing increased, there was no increase in free chlorine concentration. This indicates that the water may have been more loaded by organics and microbiology during that period.

9.4.1 BACTcontrol

The results of the BACTcontrol at the Dow location were not reliable, probably because the continuous disinfection of the water affected the fluorescence and enzyme in the assay. However, at the BASF location, nearly all measurements were valid (not erroneous), suggesting that the effect of chlorine on the fluorescence or enzyme were not problematic. This is probably caused by the fact that the brackish water at BASF was not continuously disinfected, resulting in measurements where chlorine was too low or absent to affect the enzyme assay in the sensor. Furthermore, the BACTcontrol was the only sensor that showed lower enzyme activity when the chlorine dosing frequency was increased, which was expected. It, thus, seems that the BACTcontrol can perform on surface water that is disinfected with short time intervals.

9.4.2 BactoSense

The intact cell counts were up to ten times higher in the discontinued chlorine-dosed water at BASF than in the continuously chlorine-dosed water at Dow, which again seems to demonstrate that discontinued chlorine-dosing results in higher active biomass and intact cell numbers than continuous chlorination, although the used watersource can also be the cause of this difference. The increased chlorine dosing frequency did not result in lower intact cell numbers and was, thus, opposite to the results of the BACTcontrol.

9.4.3 BugCount Guardian

The continuous chlorine-dosed water at Dow showed cellular ATP-concentrations that were below the detection limit. With the discontinuous chlorination of the water at BASF, the cellular ATP-concentration was also often below the detection limit, but periodically relatively high peak concentrations of cellular ATP were observed. These peaks could have been measured at moments when the chlorine concentration in the brackish water was low, because measurements were between two dosing moments.

The results from all three sensors that measure water quality, thus, demonstrated that discontinuous chlorine dosing, as done at BASF, results in more active biomass or intact cell numbers than continuously dosed chlorine that was done at Dow. It, however, remains difficult to draw this conclusion as other factors (e.g. water source, water quality, cooling tower setup) are also different between the two locations. However, it still provides an indication that continuous chlorination might be more effective in reducing microbial activity in water than discontinuous chlorination.

9.4.4 CBM

The BAR values obtained with the CBM were very high with the discontinuous chlorinated water at BASF. These values were 100 to 10,000 times higher than the values obtained with the continuous chlorinated water at Dow. This is another indication that discontinuous chlorination doesn’t seem to be very effective in reducing the microbiology in cooling tower systems. However, care should be taken with interpreting the CBM-results, because clogging of the flow meters affected the water flow, which could have an effect on the BAR-values.

9.4.5 Answers to the research questions

BASF had posed several questions for which they wanted to find an answer with this study. First, they wanted to know whether pretreatment/filtration is needed for the sensors can be applied to the water. The results demonstrated that pretreatment is required for the CBM, but such pretreatment has to be intense and is likely to affect the CBM results. Instead of using pretreatment, it is necessary that the CBM system is changed in such a manner that the flow meters are less prone to clogging. Due to the high intact cell counts measured with the BactoSense, the water should be diluted before measured with the BactoSense to stay within the measurement range of the device. However, there seems to be an effect of dilution on the results. Therefore it is more usefull to look at trends than at real numbers. The other question was how many maintenance is required and from the experiences of BASF it is concluded that most sensors required low maintenance, with the exception of the CBM due to the fast flow rate problems after the correct flow rate was manually set again. BASF also hoped to see that altering the chlorine dosing frequency affect the microbial water quality determined with the sensors, but in general no effect on the water quality was recorded with the sensors when disinfection dosing was altered. The only exception was the BACTcontrol, who showed an expected lower enzyme activity when dosing frequency increased and could, therefore, be used to determine the effect of discontinuous chlorine dosing to surface water. However, as the other sensors did not detect a change, it remains uncertain whether the lower enzyme activity with the BACTcontrol was caused by lower active biomass in the water or that the increased dosing frequency affected the enzyme/fluorescence assay as was observed with the continuous chlorine dosing at Dow.

9.5 Conclusions and recommendations

9.5.1 Conclusions

The system at BASF is a flow-through system with brackish water. This setup probably results in a varying water quality that might cause fluctuations in the measurements with the sensors.

The different sensors did, in general, not show a change in the microbiological water quality once the chlorine dosing frequency and amount was changed, probably because the free chlorine remained stable during the whole monitoring period. The only exception was a lower enzyme activity measured with the BACTcontrol when chlorine dosing frequency increased, but it remains difficult to conclude whether that was a real effect on the microbial water quality or that it was caused by interference with the enzyme/fluorescence assay of the BACTcontrol.

The system at BASF is a flowthrough system. Therefore, it is difficult to control the biocide dosing by the measurements of the sensors as the residence time of the water is very short. Although the CBM needs adaptations to prevent clogging, the other sensors gave stable results. No effect was seen with the sensors from the changes in chlorine dosing strategy. This may indicate that the sensors are not sensitive enough to pick up these changes, or that a changed strategy was not effective in changing the microbiological activity in the water significantly.

9.5.2 Recommendations

As the valve of the flow meters in the CBM clogged easily, it is recommended to use other valves that are less prone to clogging.

10 Establishing signal values for sensors using different methods

10.1 Signal values for application of inline microbiological methods in practice

An important reason to implement sensors for monitoring the microbiological water quality is that the end users can act when a measurement value of the sensor exceeds a signal value. Such a signal value indicates when a measurement result exceeds the normal variation. Sensors that are able to monitor the microbiological water quality can be used for different purposes, and the purpose for which a specific sensor will be used, largely determines how signal values can be set. However, not all end users involved in the project (drinking water utilities, companies with industrial water) already have a clear goal for application of microbiological water quality sensors.

In this chapter, possible methods for setting signal values are described. Together with the project partners (drinking water utilities, companies with industrial water and technology suppliers) the most appropriate methods for setting signal values for the sensors tested in this project, and the possible applications that the drinking water utilities envision, were discussed and are described here.

10.1.1 Methods for setting signal values

Signal values for sensors that measure the microbiological water quality can be deduced with different methods. Each method with its own advantages and disadvantages (Table 10-1). As described above, the 'best' method depends on the goal for which the sensors are to be used.

Table 10-1. Methods for setting signal values with advantages and disadvantages. 'bNovate-method': one-month monitoring with BactoSense combined with analysis of grab samples for legal parameters. Based on this and 99% confidence interval the first signal values are set, but this is an continuous process of validation, review and update.

General		
Method	Advantage	Disadvantage
Historical knowledge (laboratory) parameters drinking water quality	Much real-life experience. Link to water quality, process parameters.	Difficult to apply to sensors that monitor new parameters. Setting up large dataset and knowledge takes time. Comparison of sensors to laboratory parameters can be difficult to perform and correlation can vary strongly.
Comparison study between new method/sensor and laboratory parameters	Compare sensor with large set of already proven parameters, including legal parameters. Relate sensor results to well-known laboratory parameters and their guideline values.	Not possible for every new method/sensor. Elaborate and costly comparison preferred. Comparison of sensor to laboratory parameter seems to differ from laboratory tests to field tests. Sensors can measure a new parameter, to which laboratory parameter should it be compared?
Absolute lower and upper limit per new method/sensor	Indication water quality change is severe. Applicable for parameters with low natural variation. Similar for all drinking water locations.	Not flexible, might miss incidents or abnormal variation that is within limit.

Based on results pilot studies		
Method	Advantage	Disadvantage
Average ± 3x SD (standard deviation)	Well known and standard method to set signal values. Easy. Set site specific signal value (specific for each situation).	Does not account for (deviations from) daily fluctuations. First a baseline has to be established. Set site specific signal values (sensor not directly applicable). Always 0.3% exceedances, regardless of whether they are relevant. No direct link to legal parameters.
Moving average ± 3x SD (standard deviation)	Standard method to set signal values. Outliers are ignored. Accounts for daily fluctuations.	May miss small, but relevant, peaks. Set site specific signal values. First a baseline has to be established. No direct link to legal parameters.
95/99 percentile	Easy. Set site specific signal value.	Not flexible, might miss incidents or abnormal variation that is within limit. First a baseline has to be established. Always 5% or 1% exceedances, regardless of whether they are relevant. No direct link to legal parameters. Set site specific signal value.
Algorithm	Correlation with different parameters (biological, physico-chemical) and operational parameters.	Large dataset needed, including incidents. Set site specific signal value.
Challenging tests (induce known changes in water quality, such as operational changes or exceedance of legal parameters)	Signal values linked to real aberrations in microbiological water quality.	May be difficult to achieve, as deliberate spikes or changes in microbial water quality or operational parameters should be introduced. May take a long time before signal values are set.
bNovate-method (one-month BactoSense monitoring and analysis of grab samples for legal parameters. Based on this and 99% confidence interval initial signal values are set, followed by continuous validation, review and update).	Comparison with legal parameters.	Takes time to establish. Does not account for (deviations from) daily fluctuations. Set site specific signal value.

10.1.2 Drinking water

The goals for applying sensors to continuously monitor the microbiological drinking water quality can for instance be (not all were studied in this project):

- To predict or warn for possible microbial water quality aberrations that leads to aesthetic, technical or public health issues
- To safeguard the function of specific treatment processes in the drinking water production plant (e.g. rapid sand filtration, membrane filtration, etc)
- To monitor the dynamics of microbial biomass in produced drinking water
- To determine the effect of production steps, water towers, or other processes on the produced or distributed water quality

- To warn that growth of *Aeromonas* in the distribution system occurs so that measures can be taken to prevent *Aeromonas* from exceeding the legislative values
- To warn that growth of *Legionella* in the distribution system or premise plumbing system occurs so that measures can be taken to prevent *Legionella* from exceeding the legislative values

In addition, a difference can be made in using the sensors for (continuous) monitoring of the produced drinking water quality or as a research tool, for instance to better understand the effect of specific steps of the drinking water production plant on the water quality.

Discussions with the drinking water utilities during the project, indicated the following areas for possible application of the sensors, or not:

- Monitoring different treatment processes during a longer period: improve understanding of the performance of the treatment process and its effect on the water quality. Examples include a better understanding of (conventional) production plants by measuring after each treatment step or a better understanding of new treatment processes in the production plant (such as reverse osmosis), and what the result is of operational changes on the microbial water quality.
- Safeguarding of surface water intake and to be warned when to temporarily stop the intake. A comparison to legal parameters (index pathogens for the QMRA) during e.g. a year would be needed for this application.
- No general role for using sensors in the distribution system.
- Use after maintenance as a relative fast method to indicate whether the water quality is back to normal in the system.

The experiences with the microbiological sensors in the project and their intended application in real-life were discussed with the drinking water utilities and technology suppliers. This led to a step-by-step plan on how to set signal values for individual drinking water applications. A step-by-step plan for application of the BACTcontrol and BactoSense on drinking water is given in paragraph 10.1.2.1. For the CBM, guideline values have been set before, this is summarized in paragraph 10.1.2.2.

10.1.2.1 Step-by-step plan for BACTcontrol, BactoSense

Monitoring source water intake (surface water)

Monitoring the bacterial quality of raw water for drinking water production (surface water) at the point of intake can be used to guide decision making to temporarily stop the intake of raw water in case of a high fecal load, and thus fecal pathogens.

To be able to link the sensor results to a low or high fecal load, a monitoring program of the surface water for at least one year should be performed. Such a monitoring program should consist of sampling large volumes (100 – 500 L) of the surface water and analysis of the legislative parameters that are mentioned in the Dutch QMRA guidelines ('Richtsnoer AMVD' [16]): index pathogens *Campylobacter jejuni*, *Cryptosporidium*, *Giardia*, (entero)viruses; and the fecal indicators *E. coli* and enterococci. These results can be compared to results of the continuously measuring BACTcontrol or BactoSense. Ideally these large volume samples should be taken at moments of high and low BACTcontrol or BactoSense results, although this might be difficult to arrange logistically and requires high flexibility of the involved laboratory. Coupling of the sensor to an auto sampler allows for automatic sample collection at certain intervals, or at specific high or low sensor results. These samples (typically <1 L) could then be analyzed for *E. coli* and enterococci to get an indication of fecal contamination and to compare the *E. coli* and enterococci concentrations to the sensor results. Such studies have been done with a β-D-glucuronidase sensor (enzyme specific for *E. coli* bacteria) that triggered an auto sampler, followed by fecal pathogen and *E. coli* analysis [17]. This same principle could be applied here. However, the correlation of *E. coli* and enterococci with fecal pathogens in surface water varies depending on many variables (e.g. source fecal pollution, location, seasonal

influences). In addition, little to no information is available on how well total bacterial cells (BactoSense) or enzymatic activity (BACTcontrol) correlate with *E. coli*. Also this correlation is expected to be variable as i) *E. coli* is only a very small part of the total bacterial number in surface water. A major increase in *E. coli* might still not be detected within the total number of bacteria as it is still a small part; ii) when nutrient concentrations are high, bacteria will grow and the total number of cells will increase, even if there is no link to the fecal load.

It should therefore be tested whether the sensors could be set up to temporarily stop the intake of raw water in case of a high fecal load. Taking into account the above mentioned comparisons, to the index pathogens and/or *E. coli*, and the variability in correlation between sensors and fecal load.

Drinking water production treatment steps

Monitoring before and after an individual treatment step during drinking water production can give insight into and understanding of the effect of the specific step on the microbial water quality. As such, the sensors are then used as a research tool. The required time for monitoring the effect of a treatment step on the water quality may vary per treatment step. Measuring the effect of backflushing rapid sand filters or membranes could be done for a relatively short period (week-month), as these filters are backwashed multiple times a week. However, measuring the effect of e.g. temperature on active carbon filtration takes months, as the temperature is seasonally dependent. The short-term effect of regeneration of activated carbon on the water quality can be investigated within a short period, e.g. measuring one week before and after regeneration will give insight into how the regeneration itself affects water quality. Longer monitoring is needed to monitor what happens with the water quality in between two regeneration cycles.

Measuring the effect of removing the Schmutzdecke on the efficiency of slow sand filters might also take weeks to months, as regrowth of the Schmutzdecke is a slow process. However, also in this situation measuring one week before and after removal of the Schmutzdecke will give insight in the direct effect on the water quality. Ideally, the sensor measurements should be combined with a (limited) laboratory monitoring program for legal (HPC or *Aeromonas* as regrowth indicators for the distribution system) or other microbiological parameters (ATP, flow cytometry) or with currently used physicochemical parameters. These parameters are more familiar for the drinking water utilities and combining these results with the sensor results will help gaining experience, understanding and feeling of the sensor results.

In addition to comparing sensor results to a monitoring program for legal or other microbiological parameters, extra information and insight can be gained by coupling the sensor results to the performance characteristics of the specific treatment step and changes in the operational procedure. If dynamics in the sensor results can be (further) explained in this manner, and indeed adds additional value to, and insight in, the process, this allows for faster and improved implementation of the sensor in daily practice.

Drinking water leaving the treatment plant

Monitoring the drinking water leaving the treatment plant can be used as a final safeguard of the water quality before it enters the distribution system. As abnormalities in the microbial quality of the produced drinking water at the plant are very rare, it is easy to establish a baseline with normal variation and signal values. A way to get a reliable baseline is to measure the drinking water leaving the plant for a prolonged period, for example one year, and combine this with frequent measurements of the legal and guideline parameters in the drinking water (biological stability parameters: HPC22, *Aeromonas*, and coliforms; fecal indicators: *E. coli* and enterococci).

Exceedances in the legal parameters can than perhaps be linked with the sensor results. In addition, the sensor results can be combined with legal parameters HPC22 and *Aeromonas* in the distribution system, especially distribution systems with a lower biological stability, to explore whether the sensor results can be used to predict nuisance regrowth conditions in the distribution system.

Conclusions

It is concluded that more research is needed to determine the best method for setting signal values for drinking water. Furthermore, it will be difficult to set signal values for drinking water in the Netherlands because the drinking water has a very high quality and hardly any exceedances of legal parameters occur. It will be easier to set signal values for sensors on source water that is used for drinking water production, as the microbial load in source water varies much more, especially when surface water is used as source for drinking water. However, linking this to the legal parameters might be challenging as the required laboratory analyses, to compare the sensor results to, are expensive and not very frequently performed and, thus, requires additional research as well.

10.1.2.2 CBM

Recently, a guidance value for the BAR has been set for application of the CBM as a biological stability parameter for drinking water produced from groundwater [13]. If the BAR-values of the drinking water leaving the treatment plant is below this guidance value, the legislative regrowth parameters HPC22 and *Aeromonas* remain below the legislative values in the (unchlorinated) drinking water distribution system [8, 13]. This guidance value was 30 pg ATP/cm^2/day (established with the KWR laboratory ATP method) and was based on monitoring the BAR in treated drinking water at the production plant and HPC22 and *Aeromonas* in the distribution system of 34 production plants in the Netherlands. For drinking water produced from surface water, no statistically significant correlation was found between the BAR and the legal parameters HPC22 and *Aeromonas*. However, the iron accumulation rate (FeAR) that also can be measured with the CBM showed a significant correlation with HPC22 and *Aeromonas* in the distribution system and a guidance value of 0.34 mg Fe/m^2/day was deduced for that parameter.

I should be taken into account that this guidance value was set with the KWR laboratory ATP method, and not with the LuminUltra method that was used in this project and which was shown to yield higher ATP levels. Therefore, this guidance value cannot be used directly in this project.

10.1.2.3 Recommendations for follow-up studies

In addition to the above-mentioned application areas that were discussed with the drinking water utilities, the sensors can also be used for other purposes. A few examples are given below with which the focus of the study has a broader scope:

- What is the effect of different treatment steps of one production location, or similar treatment steps of different production locations, on the microbiological water quality? This allows for comparison between the performance of treatment steps and/or production locations to each other which can then be optimized if needed. For example, comparing measurements after rapid sand filters on e.g. 20 ground water production locations in the pre- and posttreatment will yield information on the effect of rapid sand filters on the water quality and whether this differs between production locations. Comparison of these results to rapid sand filters of surface water production locations, gives information on their performance of rapid sand filters and whether their effect on the microbiological water quality is comparable or different when the raw water source varies.
- By measuring all or many treatment steps in one production location, it can be studied at which step bacterial growth occurs or when bacteria removed. This leads to a better understanding of the treatment plant and allows for optimized operational control of the treatment train in the plant.

10.1.3 Cooling water and surface water

10.1.3.1 BACTcontrol, BactoSense, BugCount Guardian

Like for drinking water, the goal for using the sensors at the industrial sites varies as well.

Monitoring effect of disinfection

At cooling towers, a disinfection step is applied to prevent growth of *Legionella* in these systems, and growth of a biofilm, which lowers heat transfer. This disinfection is usually based on a chlorine setpoint, because at least a certain amount of free chlorine must be present to make sure that the disinfection is sufficient. However, dosing too much chlorine is unnecessary and non-sustainable. Sensors could be used to control disinfectant dosing in these cases by establishing a certain baseline of microbial activity. When the activity drops too much, the disinfectant dosing could be lowered. Also, when the disinfectant dose is too low, the free chlorine concentration drops and, as a result, the microbial activity increases which can be detected by the sensors and disinfectant dosing can be increased. When the chlorine concentration changes significantly, the response in the sensors results can then be used to set a signal value. Furthermore, it is possible to set an alarm for exceedances under normal operation, e.g. by the average ± 3x the standard deviation. However, results above the signal value does not specifically indicate a situation in which action needs to be taken, this can only be established by linking signal values to 'off spec' situations. It can, however, take a long monitoring period to establish such a signal value.

Another application, where the sensors are used as a research tool, is to measure the effect of different disinfection products or concentrations. When a disinfection product is dosed, a change in microbial activity should be seen. The sensors can be used to determine the effect of different (combinations of) products and their dosed concentrations. An experimental procedure with spikes of disinfection products is required in this situation and this may not be possible inline in the larger system but requires a test outside the main system.

Effect of water source or leakages

When the water source for a process such as cooling is changed, the microbial activity might be affected. Either due to the higher (or lower) concentration of bacterial cells present in the source, or due to the higher concentration of nutrients that allows for more growth. In a cooling system, leakages can occur, which may also cause a change in microbial numbers or activity. An indication of leakages can be observed when the microbial numbers or activity in the main source is not changed, but the microbial numbers or activity in the system are.
To be able to detect this with the sensors, the frequency that the source is changed is important. In other words, a baseline of one source needs to be established, to be able to detect a change to another source.

Application on process water

The water quality of surface water and cooling water varies a lot, and microbial numbers and activity are high. Application of the sensors on cleaner water, such as process water, could also be beneficial. The effect of treatment steps on the microbial numbers and activity can be monitored just as described for drinking water and changes in process conditions would lead to changes in microbial numbers and activity that can be monitored.

Location of the sensors

The purpose of using the sensors and the location where to install the sensors are very much related. In the case of Dow, the addition of a batch of condensate water could be seen back in the measurements with the online sensors as the water sources were not well mixed before reaching the sensors. In the case of BASF, the effect of disinfection could be monitored but not controlled as the residence time of the water was very short and the measurement time of the sensors was longer than this residence time. This still expresses the need for sensors with shorter measurement times.

10.1.3.2 CBM

The CBM is meant to measure the potential for biofilm growth. The water sources used in industry, surface water or cooling water, contained many particles that clogged the valves in the sensor. Therefor the BAR could not be determined reliably as the water content that passed the CBM was not constant. However, it could be concluded that biofilm growth took place in the CBM. When the CBM-design can be improved (e.g. use valves less prone for clogging), the CBM can be used to determine the BAR. When the disinfectant concentration in the system is high enough, it is expected that biofilm growth is limited. Consequently, when the BAR of the CBM is high, it is a sign that the disinfectant is not active in the system. This can thus only be applied when an active disinfectant, such as chlorine-based, is dosed. In clean water, such as process water, the BAR can give information on the biofilm formation potential and regrowth in the distribution system, comparable to drinking water. However, to establish a signal value for the BAR is probably a lot of work. In those cases, it is suggested to take the alarm value of 30 pg/cm^2/day (the same as for drinking water).

10.2 Comparison pilot locations

10.2.1 Comparison signal values between drinking water pilot locations

The BactoSense, BACTcontrol and CBM have been installed at three drinking water pilots: Evides (chapter 4), Oasen (chapter 5) and Vitens (chapter 6). For all these locations signal values were calculated using the formula $Signal\ value = average \pm 3 \times standard\ deviation$ and these values were shortly discussed in their respective report chapters. In this chapter, the signal values are compared between the different pilot locations.

10.2.1.1 BactoSense

A summary of the measurement results of the BactoSense at the drinking water locations is given in

Table 10-2. The upper signal value of the BactoSense for drinking water varied considerably between the different locations, from 8.5×10^3 to 7.8×10^5 cells/ml. Drinking water produced by conventional treatment plants (Evides – Before UF, Oasen – De Hooge Boom and both Vitens locations) showed higher average cells numbers and, thus, higher upper signal values compared to drinking water produced by treatment plants that have membrane filtration in their treatment (Evides – After UF and After CWR, Oasen – Nieuw Lekkerland). Concomitant, the lower signal value also varied considerably, from 5.4×10^1 to 1.7×10^5 cells/ml. The lowest signal value was determined for Evides – After UF which was measured directly after the UF step in which cells are removed and (re)growth could not yet have occurred.

The difference between the lower and upper signal values is the normal range for cell numbers in the studied drinking water. This difference is small for both Vitens locations (Spannenburg and Noardburgum) and Evides – After CWR. This shows that the cell numbers in drinking water at these locations were relatively stable and normal daily variation falls within this small range. Whereas at the other locations, the difference between lower and upper signal values is larger, implying that bacterial cell numbers are less stable at these locations. However, as the microbial water quality fulfilled the legislative parameters for the three drinking water pilot locations, it seems unlikely, but not impossible, that this normal variation in bacterial cell numbers in drinking water results in microbiological water quality issues (e.g. public health, aesthetical or technical complaints) at the consumers tap. The results, thus, suggest that most of the observed variation can be considered as normal variation that did not impact the microbial water quality in such a way that problems occurred.

Table 10-2. Statistical summary and signal values of BactoSense_ICC of all drinking water pilot locations. Part of these results are also shown in the respective chapter of the pilot locations: Evides (chapter 4), Oasen (chapter 5) and Vitens (chapter 6). For the Evides locations only the period with a stable, low, signal ('after UF': 7-14 July 2021 and 'after CWR': 12 August – 7 September 2021) was used. For the Oasen (Nieuw-Lekkerland; 2024) location, the signal values were calculated for the period with a stable, low signal (9 – 24 November 2024). The number of exceedances of the lower or upper signal value was counted for the entire monitoring period of the pilot.

	Evides			Oasen		Vitens	
BactoSense_ ICC	Before UF	After UF	After CWR	De Hooge Boom (2022)	Nieuw-Lekkerland (2024)	Noardburgum	Spannenburg
Mean	1.0×10^{5}	4.3×10^{3}	4.0×10^{4}	6.3×10^{5}	1.7×10^{4}	1.1×10^{5}	1.8×10^{5}
Median	1.0×10^{5}	4.5×10^{3}	4.1×10^{4}	6.3×10^{5}	1.7×10^{4}	1.1×10^{5}	1.8×10^{5}
Minimum	7.7×10^{4}	1.7×10^{3}	3.0×10^{4}	3.8×10^{5}	1.1×10^{4}	9.4×10^{4}	1.7×10^{5}
Maximum	1.5×10^{5}	1.4×10^{4}	7.2×10^{4}	7.3×10^{5}	3.1×10^{4}	1.4×10^{5}	2.1×10^{5}
Standard Deviation	1.6×10^{4}	1.4×10^{3}	3.6×10^{3}	5.0×10^{4}	3.4×10^{3}	7.6×10^{3}	6.0×10^{3}
Count	451	467	537	585	2.3×10^{2}	251	536
90-percentile	1.3×10^{5}	5.8×10^{3}	4.3×10^{4}	6.8×10^{5}	2.1×10^{4}	1.2×10^{5}	1.9×10^{5}
95-percentile	1.4×10^{5}	6.2×10^{3}	4.4×10^{4}	7.0×10^{5}	2.3×10^{4}	1.2×10^{5}	2.0×10^{5}
Upper signal value	1.5×10^{5}	8.5×10^{3}	5.1×10^{4}	7.8×10^{5}	2.8×10^{4}	1.3×10^{5}	2.0×10^{5}
Exceedances upper signal value	1	1	4	0	0	2	3
Lower signal value	5.6×10^{4}	5.4×10^{1}	3.0×10^{4}	4.8×10^{5}	6.9×10^{3}	8.5×10^{4}	1.7×10^{5}
Exceedances lower signal value	0	0	1	10	0	0	0
Difference lower-upper signal value	9.7×10^{4}	8.5×10^{3}	2.1×10^{4}	3.0×10^{5}	2.1×10^{4}	4.6×10^{4}	3.6×10^{4}

10.2.1.2 BACTcontrol

A summary of the measurement results of the BACTcontrol at the drinking water locations is given in Table 10-3. Like the BactoSense, the upper signal value of the BACTcontrol for drinking water also varied considerably between locations, from 156 – 3914 pmol/min. The very high signal value for drinking water from Evides – Before UF (3914 pmol/min) is hypothesized to be caused by pressure changes in the downstream UF installation. Without this high signal value, the range for the upper signal values remains still large, from 156 – 1111 pmol/min. Comparable to the BactoSense, the higher upper signal values for the BACTcontrol were also deduced for the locations with a conventional treatment plant (Oasen – De Hooge Boom and both Vitens locations). For the locations including membrane filtration (Evides – After CWR, Oasen – Nieuw Lekkerland) the upper signal value is lower. However, directly after membrane filtration at Evides (Evides – After UF), a relatively high upper signal value was obtained. This might be caused by carry over, as the sensors first measured drinking water at Evides before the UF (with high enzymatic activities) followed by measurement after the UF. The lower signal value also varied largely between the different locations, from 0 – 279 pmol/min. There is no clear relation between treatment plant and the lower signal value.

The difference between the lower and upper signal values, the normal range for enzymatic activity in the drinking water at a location, is smaller for the locations with membrane filtration (Evides – After UF and After CWR, Oasen – Nieuw Lekkerland) compared to the locations with conventional treatment (Oasen – De Hooge Boom and both Vitens locations). The enzymatic activity in the drinking water of the membrane-filtration locations is thus more

stable compared to the other locations. As the microbial water quality fulfilled the legislative parameters for the three drinking water pilot locations, it seems unlikely, but not impossible, that this normal variation in enzymatic activity in drinking water results in microbiological water quality issues (e.g. public health, aesthetical or technical complaints) at the consumers tap. The results, thus, suggest that most of the observed variation could be considered as normal variation that did not impact the microbial water quality in such a way that problems occurred.

*Table 10-3. Statistical summary and signal values of BACTcontrol of all drinking water pilot locations. Part of these results are also shown in the respective chapter of the pilot locations: Evides (chapter 4), Oasen (chapter 5) and Vitens (chapter 6). For the Evides locations only the period with a stable, low, signal ('after UF': 7-14 July 2021 and 'after CWR': 12 August – 7 September 2021) was used. For the Oasen (Nieuw-Lekkerland; 2024) location, the signal values were calculated for the period with a stable, low signal (9 – 24 November 2024). The number of exceedances of the lower or upper signal value was counted for the entire monitoring period of the pilot. * A negative lower signal value was set at 0 pmol/min.*

BACTcontrol	Evides			Oasen		Vitens	
	Before UF	After UF (stable)	After CWR (stable)	De Hooge Boom (2022)	Nieuw-Lekkerland (2024)	Noardburgum	Spannenburg
Mean	1158	456	261	389	448	454	691
Median	676	455	259	378	137	443	672
Minimum	214	271	162	188	47	60	413
Maximum	3278	608	431	1550	3499	1001	1212
Standard Deviation	918	59	42	104	685	175	140
Count	292	106	345	706	225	439	386
90-percentile	2765	518	314	515	1275	690	861
95-percentile	3112	549	763	544	2115	801	1012
Upper signal value	3914	633	386	702	156	980	1111
Exceedances upper signal value	0	0	2	2	107	1	10
Lower signal value	0*	279	136	76	3	0*	272
Exceedances lower signal value	0	1	0	0	0	0	0
Difference lower-upper signal value	3914*	355	250	626	153	980*	839

10.2.1.3 CBM

A summary of the measurement results of the CBM (with ATP measurement of LuminUltra that can be performed on-site) at the drinking water locations is given in Table 10-4. As a guidance value for biologically stable drinking water has been set for the CBM [8, 13], signal values were not calculated, instead the CBM results are compared between the locations and with the validation study (Chapter 3).

The average BAR-values are low for both Vitens locations and Evides-After CWR. The three CBMs that were installed at Oasen all yielded high BAR-values, most likely caused by the encapsulation of iron and manganese in the biofilm that enables higher microbial growth [18].

A guidance value for biologically stable drinking water (30 pg ATP cm^{-2} day^{-1}) has been set for the CBM, but only in combination with the laboratory ATP-method of KWR [8, 13]. The validation study in this report (Chapter 3, paragraph 3.4.1) compared the two ATP-methods (KWR laboratory and LuminUltra) to each other for application in combination with the CBM. This showed that the LuminUltra kit yields on average 1.9 ± 0.8 times higher ATP, and thus BAR values than the KWR laboratory method. The guidance value for the BAR would then be approximately 33 – 81 pg ATP/cm^2/day, thus a guidance value for the BAR with the LuminUltra kit should be regarded with caution due to the large variation.

*Table 10-4. Statistical summary of the BAR-values (pg ATP/cm^2/day) of the CBM of all drinking water pilot locations. Part of these results are also shown in the respective chapter of the pilot locations: Evides (chapter 4), Oasen (chapter 5) and Vitens (chapter 6). Only the results obtained with the ATP-kit from LuminUltra (and not the ATP-method of the KWR laboratory) are shown. *KWR-CBM with ATP-LuminUltra method.*

	Evides		Oasen			Vitens	
CBM	Before UF	After CWR	De Hooge Boom (2022)	Nieuw-Lekkerland (2024)	Nieuw-Lekkerland (2024)*	Noardburgum*	Spannenburg
Mean	174	17	688	443	729	10	5
Median	153	10	588	255	610	8	5
Minimum	137	9	285	165	311	4	3
Maximum	270	31	1431	1187	1387	23	7
Standard Deviation	55	12	448	425	468	7	2
Count	5	3	5	5	4	5	5

10.2.2 Industrial locations

The pilots at Dow and BASF were done in periods when the cooling systems were running well and there were no situations that lead to alarms at the cooling tower. This may be because no alarm situations were occurring, or because alarm situations are missed with the currently used measurement methods. The sensors may give additional information about the bacteriological situation of the cooling systems. In the following paragraphs, the average values per period were calculated, as well as the signal values by the proposed method *signal value = mean ±3 × Standard deviation*.

Very little microbiological measurements using laboratory methods were done at the pilot locations and there is thus also limited information about the day-to-day situation. The correlation between the laboratory methods and the online methods was too small to use these methods to compare the (limited) data available to the sensor data.

10.2.2.1 BACTcontrol

A summary of the measurement results of the BACTcontrol at the industrial water locations is given in Table 10-5. The standard deviation is in the same order of magnitude as the mean for all the locations and measurements periods. This leads to lower signal values between 0 and 0.76 pmol/min with no cases that exceed the lower signal value. The upper signal values ranged from 71.08 to 1784.5 pmol/min and was exceeded multiple times.

The BACTcontrol values of Dow are much lower than the values of BASF, and the upper signal values of Dow are, thus, also much lower than for BASF. The lower signal value is (near) zero for both locations. There is also a large difference between the two measurement periods at BASF. Although the mean of the first period (Peak chlorine dosing) is only a factor 1.5 higher than during the second period (More frequent dosing), the maximum is a factor 4.3 higher. This leads to a much higher upper signal value (1784 pmol/min) in the first period compared to the second period (743 pmol/min). The standard deviation in the second period is much smaller and exceedances of the upper signal values are noticed much more often: 49 in the second period versus 6 in the first period.

Table 10-5: Statistical summary and signal values of BACTcontrol of the industrial water pilot locations

	Dow		BASF	
	Period 1	Period 2	Peak chlorine dosing	More frequent dosing
Mean	37.80	35.92	496.5	321.9
Median	36.50	35.60	379.0	339.1
Minimum	0.00	11.40	206.5	20.9
Maximum	102.70	70.00	2932.3	676.0
Standard Deviation	15.01	11.72	429.3	140.6
Standard Error	1.17	1.46	49.6	9.0
Count	165.00	65.00	76.0	247.0
90-percentile	54.10	54.00	772.9	481.3
95-percentile	61.96	55.78	993.5	522.1
Upper signal value	82.84	71.08	1784.5	743.8
Exceedances upper signal value	3	0	6	49
Lower signal value	0.00	0.76	0.00	0.00
Exceedances lower signal value	0	0	0	0
Difference lower-upper signal value	82.84	70.31	1784.5	743.8

10.2.2.2 BactoSense

A summary of the measurement results of the BactoSense at the industrial water locations is given in

Table 10-6. The standard deviation is 1 order of magnitude smaller than the mean value. This is a relative standard deviation of 10%. The mean at Dow is much lower than at BASF (comparable to BACTcontrol results) and this resulted in higher upper signal values for BASF compared to Dow (also comparable to BACTcontrol). The exceedances of the upper signal values were quite different from BACTcontrol, because now only period 1 of Dow exceeded upper signal values 11 times, whereas upper signal values of BASF were never exceeded.

Table 10-6: Statistical summary and signal values of BactoSense of the industrial water pilot locations

	Dow		BASF	
	Period 1	Period 2	Peak chlorine dosing	More frequent dosing
Mean	1.20×10^{5}	1.11×10^{5}	1.99×10^{6}	1.49×10^{6}
Median	1.11×10^{5}	1.10×10^{5}	2.02×10^{6}	1.50×10^{6}
Minimum	1.97×10^{4}	4.01×10^{4}	1.19×10^{4}	1.09×10^{4}
Maximum	3.73×10^{5}	2.32×10^{5}	4.06×10^{6}	2.38×10^{6}
Standard Deviation	5.04×10^{4}	2.70×10^{4}	7.37×10^{5}	3.34×10^{5}
Standard Error	1.70×10^{3}	1.05×10^{3}	2.86×10^{4}	2.30×10^{4}
Count	8.83×10^{2}	6.70×10^{2}	6.65×10^{2}	2.12×10^{2}
90-percentile	1.77×10^{5}	1.45×10^{5}	2.86×10^{6}	1.88×10^{6}
95-percentile	2.26×10^{5}	1.53×10^{5}	3.25×10^{6}	1.98×10^{6}
Upper signal value	2.71×10^{5}	1.92×10^{5}	4.20×10^{6}	2.49×10^{6}
Exceedances upper signal value	11	0	0	0
Lower signal value	0	2.98×10^{4}	0	4.89×10^{5}
Exceedances lower signal value	0	0	0	0
Difference lower-upper signal value	2.71×10^{5}	1.92×10^{5}	4.20×10^{6}	2.00×10^{6}
Number of measurements	552	669	664	211

10.2.2.3 BugCount Guardian

A summary of the measurement results of the BugCount Guardian at the industrial water locations is given in

Table 10-7. The standard deviation is 1 order of magnitude smaller than the mean value, also indicating that the variation in the signal is relatively low compared to the mean, as the relative standard deviation is 10% of the mean. There were little exceedances of the signal value. Again, means are lower for Dow than BASF, resulting in higher upper signal values for BASF, but exceedances remain low (maximum 3, which is different for BACTcontrol and BactoSense).

Table 10-7: Statistical summary and signal values of BugCount Guardian of the industrial water pilot locations

	Dow		BASF	
	Period 1	Period 2	Peak dosing	High freq dosing
Mean	88.20	91.08	210.38	340.57
Median	74.00	72.00	58.00	278.00
Minimum	0.00	0.00	0.00	-7.00
Maximum	952.00	1596.00	1438.00	1184.00
Standard Deviation	75.44	111.46	334.37	314.44
Standard Error	3.16	5.69	33.78	20.56
Count	572.00	385.00	99.00	235.00
90-percentile	161.80	177.20	815.60	770.00
95-percentile	202.00	236.00	1029.00	842.60
Upper signal value	315	425	1213.51	1283.89
Exceedances upper signal value	3	1	1	0
Lower signal value	0	0	0	0
Exceedances lower signal value	0	0	0	0
Difference lower-upper signal value	315	425	1213.51	1283.89
Number of measurements	257	187	98	234

10.2.3 Overall conclusions pilot locations

Several different methods for setting signal values are available, each with its advantages and disadvantages. The 'best' method depends on the goal for which the sensors are to be used. More research is needed to determine the best method for setting signal values, especially for drinking water as legal parameters are hardly ever exceeded. The large difference in the lower and upper signal values of the individual pilot locations, shows that these values should be established for each individual pilot location, regardless of the water matrix (drinking water, cooling water and surface water). Consequently, signal values obtained for drinking water from one treatment plant cannot be applied to drinking water from another treatment plant. In this project the role of seasonal influences was not studied, but as temperature may influence microbial water quality, it is possible that the signal value may not only differ per location but also between seasons at one treatment plant.

A step-by-step plan on how to set signal values for individual drinking water applications was set up in collaboration with the drinking water utilities and the technology suppliers. This was done for:

- Drinking water production treatment steps: Monitoring before and after an individual treatment step during drinking water production and coupling these results to laboratory analyses, operational parameters and performance characteristics.
- Produced drinking water (leaving the treatment plant): problematic abnormalities in produced drinking water are likely very rare, due to which non-problematic variation is mainly monitored with the sensors. As a result, it might take a long time to establish a reliable baseline and to explore whether signal values are exceeded.

As the microbial water quality fulfilled the legislative parameters for the three drinking water pilot locations, it seems unlikely that the normal variation in bacterial cell numbers and enzymatic activity results in microbiological water quality issues (e.g. public health, aesthetical or technical complaints) at the consumers tap. The results, thus, suggest that most of the observed variation could be considered as normal variation that did not impact the microbial water quality in such a way that problems occurred.

The two cooling towers operated stable at the time period of the pilot. There were also very little exceedances. It would be better to find an operational limit for the sensors to give an signal, which was not possible in this stable period. Other ways to determine the signal value may be more valuable or have more relation to the real practical situation.

11 Conclusions and recommendations

11.1 Conclusions

11.1.1 Validation studies

11.1.1.1 Drinking water

Bacterial cell numbers in drinking water could be measured with the BactoSense. The active microbial biomass could be determined with the BACTcontrol. The detection limit of the BugCount Guardian was not low enough to allow drinking water measurements.

The BACTcontrol measured the active biomass more reliably in drinking waters with a higher biomass concentration (e.g. drinking water with a lower biological stability) than with a lower biomass concentration. The BactoSense measured the bacterial cell numbers reliably in drinking water with a high or low biomass concentration. The dilution series showed good results for the BactoSense and the flow cytometry laboratory method. The dilution series results were more variable with the microscopy laboratory method or not consistent for the BACTcontrol and the ATP laboratory method - most likely because the undiluted drinking water from some locations was already close to the lower detection limit of these methods.

Both the KWR laboratory and the mobile Milispec/LuminUltra ATP-methods are suitable to measure ATP of the biofilm on glass beads or coupons in the CBM. However, the Milispec/LuminUltra-method consistently yields higher ATP concentrations than the KWR laboratory method, but this factor is not constant.

The Milispec/LuminUltra-method for ATP is easier to perform than the KWR laboratory method. If no historical data are available, the data do not have to be compared with available historical data and/or analysis on site by own personnel is wanted, the Milispec/LuminUltra ATP-method is suitable for this purpose. However, historical results obtained with the ATP laboratory method cannot be compared to the results obtained with the Milispec/LuminUltra ATP-method.

11.1.1.2 Surface water and industrial water

Bacterial cell numbers or microbial biomass in surface water or industrial water could, in general, be measured with the BactoSense, BACTcontrol and BugCount Online. For surface water the results also correlated well with the laboratory methods. For cooling water, correlations between cell numbers or biomass obtained with the sensors and with laboratory methods were in general not significant. This was most likely caused by the presence of a disinfectant (ozone or chlorine) in the water and the time between sampling at the plant and measuring the microbiological parameters with the sensors and laboratory methods at KWR. During that transportation time different degrees of living, dying, dead and decaying cells could have been formed. It was concluded from the results that data coming from the sensors monitoring cooling tower water can probably not be compared to the laboratory data reliably, as these may be inconsistent.

The cell numbers in the cooling or surface water are high and close to or above the upper detection limit of the BactoSense. When the BactoSense is applied in the field a predilution step might need to be included as, otherwise, it may not result in reliable results. The other sensors did not show problems with the high cell number of microbial biomass.

For application of the CBM on non-drinking water locations, it is proposed to measure the ATP concentration in the biofilm after 7, 14 and 21 days of biofilm formation. The results from these analyses can then be used to determine the optimal incubation period of the cuvettes with the glass beads or coupons in the CBM. Furthermore, replacing the current KWR method with the Milispec/LuminUltra-method will result in a large shift in the results due to which current results cannot be compared one-on-one to historical data obtained with the ATP laboratory method.

11.1.2 Case studies drinking water locations

11.1.2.1 Data

It is concluded from testing the sensors at three different drinking water locations that the BACTcontrol and BactoSense can show comparable or different trends. Cell numbers and enzymatic activity in drinking water, thus, sometimes give comparable responses for certain locations, but also regularly differ at other locations, meaning that cell numbers and enzymatic activity measure different aspects of the total microbial biomass.

The enzymatic activity measured with the BACTcontrol at the drinking water locations was significantly different between locations. Only some dynamics in enzymatic activity observed at the Evides and Vitens locations could be explained by known changes in water quality or by complimentary laboratory analyses. However, most of the dynamics in enzymatic activity could not be explained by other data gathered from these locations. In the Oasen pilot, most of the dynamics could be explained, as the operation of the production plant was less stable at the time. Still, the drinking water always fulfilled the legal parameters. In addition, more information was available on the operational parameters and laboratory measurements (HPC22, iron, manganese, ammonium or methane) for the Oasen location than the Evides and Vitens location, which better linked BACTcontrol results to these parameters.

The cell numbers measured with the BactoSense at the drinking water locations significantly differed from each other. Removal of bacteria by UF and regrowth in the clear water reservoir were successfully detected at Evides. For one of the Vitens locations daily fluctuations in drinking water cell numbers were visible, which corresponded with water demand. Part of the peaks in cell numbers at the Oasen location Nieuw-Lekkerland could be linked to changes in the operational control of the treatment plant of Nieuw-Lekkerland and/or could be linked to levels of HPC22, iron, manganese, ammonium or methane.

The BAR values measured with the Milispec-CBM varied between the pilots, but in general correlated with historical knowledge on the biological stability of these different drinking waters.

Signal values for each sensor have been set for each drinking water location, using the formula $\text{average} \pm 3 \times \text{SD}$. Interpretation of exceedances of these signal values is, however, difficult for various reasons: hardly any variation in drinking water quality yielding stable sensor results, peak(s) that cannot be explained by operational changes or other causes, and not enough available data. It is, therefore, concluded that useful signal values cannot be deduced from sensor data collected during a stable three-months monitoring period at a drinking water location.

As no microbial water quality problems were reported during the measurement periods at the drinking water utilities, it is also concluded that the variation observed with the BactoSense and the BACTcontrol can be considered as normal variation that does not impact the microbial water quality in such a way that problems occur.

11.1.2.2 Experiences by the drinking water companies

As drinking water contains low levels of nutrients and bacteria (compared to other water matrices), it is especially important that all materials used for the sensor measurements are either new or cleaned thoroughly. This prevents incorrect measurements results.

During the monitoring periods, the BACTcontrol gave error messages on several occasions that were caused by several reasons. After an error, the BACTcontrol stopped measuring until the error was manually solved and measurements were restarted. The drinking water companies thus had difficulties judging the added value of the BACTcontrol, and the lower number of datapoints also made it more challenging to come to signal values.

The BactoSense required minimal hands-on time and yielded no errors. However, some datapoints were lost as it was noted too late that the cartridge had to be replaced. It is, therefore, concluded that the drinking water utilities need to remain proactive to replace this cartridge in time.

Although most manual steps in the ATP measurement were relatively easy to perform, especially taking out the cuvettes sometimes proved to be difficult. This was improved during the project after which sampling was relatively easy.

11.1.3 Case studies industrial water locations

11.1.3.1 Data

The BACTcontrol can be applied in an industrial setting to determine the active microbial biomass in water. When the chlorine concentration was above the detection limit (successful disinfection), the amount of microbial biomass present (and thus the enzymatic activity) was below the detection limit. Thus, the BACTcontrol can be used to determine the effectiveness of water disinfection. When no chlorine could be detected, the BACTcontrol worked stable and the data at BASF and the pilot at KWR could be used to follow the microbial water quality.

The BactoSense can be applied in an industrial setting to determine the bacterial cell numbers in water. The BactoSense gave stable results and dynamics in cell numbers in industrial water and dynamics could be linked to changes in the water source. Thus, the BactoSense is able to detect microbial water quality changes. An effect of a change in chlorine dosage on cell numbers was not detected with the BactoSense for all three locations. The BactoSense, thus, seems to be less suitable to determine the effectiveness of water disinfection.

The BugCount Guardian can be applied in an industrial setting to determine the ATP-concentrations, as a measure of active biomass, in water. The measured cellular ATP concentrations by the sensor at both BASF and Dow were below the detection limit. This is probably due to the presence of chlorine for disinfection and the relatively high detection limit of 100 ng ATP/l. This, thus, resulted in cellular ATP concentration below detection limit, whereas the concentrations of both total and dissolved ATP were above the detection limit. These results indicate that most of the biomass was present as inactive (dead) cells and the ATP was released as free (thus dissolved) molecules into the water matrix. It, thus, seems possible to determine the effect of disinfection on the active microbial biomass with the BugCount Guardian.

In general, the microbial water quality data obtained from the sensors at the industrial pilots were highly variable making data interpretation difficult. Changes in microbial water quality detected with one sensor were not always seen with another sensor. In addition, changes could not always be related to operational changes such as chlorine dosage. It was not possible to deduce reliable and useful alarm values, using the mean ± 3×standard deviation, from the sensor data at the industrial locations.

It was the first time that the sensors were applied on industrial water types instead of drinking water. Because the water quality of industrial water types differs from drinking water, several challenges were raised during the project. The results did not yet allow to check whether a combination of sensors would give additional value compared to using only an individual sensor.

It is expected that the sensors will perform better when installed directly onto the cooling water at the pilot location than observed from the validation study. As the sensors are installed directly onto the water stream and thus measure the water directly, without transport or storage time involved (which is the case for the validation study at the laboratory), water quality changes or errors caused by transport and storage will most likely be avoided.

The results of the CBM could not reliable be used on industrial water as the CBM clogged many times due to the high organic/particle load of industrial water. Still, it was shown that, even though the water was disinfected, biofilm could form with the cooling tower water of BASF. In contrast, at Dow, the measured amount of biofilm in the CBM fed with cooling tower water was very low. This showed that the effectiveness of disinfection could also be determined using the CBM.

11.1.3.2 Practical

The sensors could be applied on industrial water and they were easy to work with. Even in brackish water, there were no major problems.

In general, no major problems were observed with the BACTcontrol, BactoSense and BugCount Guardian, although the industrial environment can sometimes be challenging for the practical application of the sensors. The electricity output can vary at these locations and sometime for very short moments there could be a power shortage, resulting in malfunctioning sensor. However, simple measures could be taken (using an UPS) to tackle this problem. Another problem with these types of water, can be the high concentrations of cells, which may be tackled by diluting the water. Also, the water may contain particles, and this can cause clogging of the system, especially in the CBM. Using different valves or larger tubing, may solve this problem. It is therefore concluded that the sensors could be used in an industrial setting after taking some extra practical measures.

11.1.4 Overall

When the sensors were compared to traditional laboratory parameters, the correlation was strong for the BactoSense compared to the flowcytometry and microscopy for drinking water. The correlations with the other sensors were less strong.

Changes in process conditions that influence the microbial water quality can be detected with the sensors, which would have been missed by regular laboratory-based monitoring, because the measurement frequency is much higher.

The handling of the sensors is easy and does not require much time and attention. The result of the measurement is also much faster available compared to the result of the traditional, offline measurements. This gives end users, especially drinking water companies the opportunity to act on changes measured by the inline sensors.

Which sensor is the most suitable in a particular situation depends on the location and the purpose of the sensor. For the industrial locations (i.e. cooling water), the detection limit could be a limiting factor.

11.2 Recommendations

Upon implementation of the sensors, it is recommended to first establish baseline values by measuring the water quality with the sensor for a relatively long time, also to gain experience with the variations that can be expected. This should be done for each of the tested inline sensors (BACTcontrol, BactoSense and BugCount Guardian). After this period a method for setting an alarm that is appropriate for the local situation, can be determined. The baseline values in combination with operational measures can be used for this.

The water quality of cooling water varies significantly. It was possible to determine the effectiveness of disinfection and a change in quality of incoming water. However, it was not possible to determine whether problems in the cooling tower could be noticed using the tested sensors. However, monitoring the quality of the incoming water, or using the sensors on more stable process water might be useful applications for these sensors on industrial water. In industry, microbiological measurements are not done frequently. The inline sensors can therefore not be compared easily to the traditional methods for industry. A measurement campaign using both inline sensors and laboratory measurements could increase insight in this.

For all possible applications, either in drinking water, industrial water or other water types, it is important to determine on forehand the goal to be achieved by monitoring the microbial water quality and whether that goal can be achieved by the application of a sensor or other microbial water quality monitoring methods.
Follow-up studies can be performed for different application areas:

- What is the effect of different treatment steps on the microbiological water quality at one drinking water production location, or similar treatment steps at different drinking water production locations? By applying sensors, it is possible to detect more aberrations in water quality in time than routine laboratory monitoring. Such an approach also allows for comparison between the performance of treatment steps and/or production locations to each other, which can be used to optimize specific treatment steps if needed. Gathering operational treatment data is then needed to be able to compare sensor results with operational issues.
- By measuring all or many treatment steps in one production location, sensors can provide more detailed information about at which step bacterial growth occurs or where bacteria are removed than routine laboratory analysis. This detailed information leads to a better understanding of the treatment plant and allows for optimized operational control of the treatment processes in the plant.
- Monitoring the produced drinking water, leaving the treatment plant, can be used as a final safeguard of the water quality. However, as abnormalities in the drinking water quality are very rare, it might be difficult to establish a baseline with normal variation and signal values. Furthermore, such applications only seems logical for treatment plants that have significant regrowth issues (regular exceedance of HPC22 or *Aeromonas*, growth of opportunistic pathogens, consumer complaints) in their distribution system and are investigating the cause and/or implementing measures to improve the situation. The effect of which can be monitored with the sensors.

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I Results drinking, cooling and surface water

I.I Results sensor analyses

Table 12-1. Results of sensor measurements in validation experiments on drinking, cooling and surface water. ICC: intact cell count, Avg: average, SD: standard deviation, NA: not available

Date	Sample location	Water type	Dilution	BACTcontrol (pmol/min/100 ml)		BactoSense_ICC (cells/ml)		BugCountOnline (ng ATP/l)	
				Avg	SD	Avg	SD	Avg	SD
18-02-2021	Uniper	Cooling water	1	3473	1015	NA	NA	0	0
02-03-2021	Uniper	Cooling water	1	3060	184	2.7E+04	7.1E+03	0	0
02-03-2021	Uniper	Cooling water	1	4040	NA	NA	NA	0	0
02-03-2021	Uniper	Cooling water	10	2855	NA	2.5E+04	1.1E+04	0	0
02-03-2021	Uniper	Cooling water	100	225	NA	1.0E+01	1.5E+02	181	314
02-03-2021	Uniper	Cooling water	1000	0	NA	2.0E+01	9.0E+01	0	0
15-3-2021	Rhine	Surface water	1	824	176	2.3E+06	8.2E+04	2470	456
15-3-2021	Rhine	Surface water	1	837.8	297.6	2.3E+06	7.9E+04	1911	931
15-3-2021	Rhine	Surface water	10	38.2	25.2	2.2E+05	1.9E+04	0	0
15-3-2021	Rhine	Surface water	100	0	0	2.1E+04	1.7E+03	0	0
15-3-2021	Rhine	Surface water	1000	NA	NA	4.4E+03	4.1E+03	NA	NA
15-3-2021	Rhine	Surface water	10000	NA	NA	4.8E+02	6.2E+02	NA	NA
17-3-2021	BASF	Cooling water	1	4473	67	1.5E+06	1.1E+04	NA	NA
17-3-2021	BASF	Cooling water	10	5718	357	1.5E+05	2.7E+04	NA	NA
17-3-2021	BASF	Cooling water	100	0	0	2.2E+05	2.7E+05	NA	NA
17-3-2021	BASF	Cooling water	1000	NA	NA	1.2E+03	1.6E+02	NA	NA
17-3-2021	BASF	Cooling water	10000	NA	NA	2.2E+02	4.6E+03	NA	NA
21-03-2021	Dow	Cooling water, before	1	8185	1428	3.9E+04	2.6E+03	0	0
22-03-2021	Dow	Cooling water, after	1	1913	88	4.2E+04	1.0E+04	271	469
06-04-2021	Uniper	Cooling water	1	2805	42	6.2E+03	6.9E+04	459	796
06-04-2021	Uniper	Cooling water	10	3820	134	5.7E+03	8.2E+03	0	0
06-04-2021	Uniper	Cooling water	100	0	0	2.3E+01	1.2E+02	158	274
06-04-2021	Uniper	Cooling water	1000	NA	NA	3.3E+00	5.9E+01	NA	NA
06-04-2021	Uniper	Cooling water	10000	NA	NA	1.0E+01	7.0E+01	NA	NA
06-04-2021	Uniper	Cooling water	100000	NA	NA	3.3E+00	6.4E+00	NA	NA
07-04-2021	Dow	Cooling water, before	1	4578	598	NA	NA	955	516
07-04-2021	Dow	Cooling water, before	10	3128	251	NA	NA	449	393
07-04-2021	Dow	Cooling water, before	100	0	0	1.1E+03	1.5E+04	204	353
07-04-2021	Dow	Cooling water, before	1000	NA	NA	2.4E+02	1.2E+03	NA	NA
07-04-2021	Dow	Cooling water, before	10000	NA	NA	2.3E+01	1.8E+03	NA	NA
07-04-2021	Dow	Cooling water, before	100000	NA	NA	2.7E+01	3.9E+03	NA	NA
07-04-2021	Dow	Cooling water, before	1000000	NA	NA	3.0E+01	1.7E+03	NA	NA
12-04-2021	Dow	Cooling water, after	1	2603	675	5.2E+04	1.6E+06	512	457
12-04-2021	Dow	Cooling water, after	10	3950	170	3.1 E+05	NA	303	525

Date	Sample location	Water type	Dilution	BACTcontrol (pmol/min/100 ml)		BactoSense_ICC (cells/ml)		BugCountOnline (ng ATP/l)	
12-04-2021	Dow	Cooling water, after	100	55	78	5.6E+02	2.4E+03	573	609
12-04-2021	Dow	Cooling water, after	1000	NA	NA	6.0E+01	8.7E+02	NA	NA
12-04-2021	Dow	Cooling water, after	10000	NA	NA	1.3E+01	8.2E+02	NA	NA
12-04-2021	Dow	Cooling water, after	100000	NA	NA	1.0E+01	3.6E+02	NA	NA
14-04-2021	Rhine	Surface water	1	1831.8	89.4	NA	NA	2631	1358
14-04-2021	Rhine	Surface water	10	7.5	4.9	2.0E+05	7.9E+03	470	815
14-04-2021	Rhine	Surface water	100	0	0	2.1E+04	6.9E+02	0	0
14-04-2021	Rhine	Surface water	1000	NA	NA	2.3E+03	4.6E+02	NA	NA
14-04-2021	Rhine	Surface water	10000	NA	NA	2.2E+02	7.0E+02	NA	NA
14-04-2021	Rhine	Surface water	100000	NA	NA	3.3E+01	2.1E+02	NA	NA
17-08-2023	Groenekan	Drinking	1	NA	NA	9.1E+04	9.1E+04	NA	NA
17-08-2023	Groenekan	Drinking	2	NA	NA	4.2E+04	4.2E+04	NA	NA
17-08-2023	Groenekan	Drinking	4	NA	NA	1.9E+04	1.9E+04	NA	NA
17-08-2023	Groenekan	Drinking	8	NA	NA	8.6E+03	8.6E+03	NA	NA
17-08-2023	Groenekan	Drinking	16	NA	NA	NA	NA	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	1	0.9	NA	2.3E+04	2.3E+04	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	2	4.6	NA	1.2E+04	1.2E+04	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	4	3.2	NA	5.3E+03	5.3E+03	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	8	4.0	NA	2.6E+03	2.6E+03	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	16	NA	NA	1.3E+03	1.3E+03	NA	NA
29-08-2023	Soestduinen	Drinking	1	9.7	NA	4.4E+04	4.4E+04	NA	NA
29-08-2023	Soestduinen	Drinking	2	4.5	NA	2.2E+04	2.2E+04	NA	NA
29-08-2023	Soestduinen	Drinking	4	-2.6	NA	1.1E+04	1.1E+04	NA	NA
29-08-2023	Soestduinen	Drinking	8	1.4	NA	6.5E+03	6.5E+03	NA	NA
29-08-2023	Soestduinen	Drinking	16	NA	NA	5.5E+03	5.5E+03	NA	NA
04-09-2023	Kralingen, distributed	Drinking	1	66.9	NA	1.1E+05	1.1E+05	NA	NA
04-09-2023	Kralingen, distributed	Drinking	2	42.5	NA	5.5E+04	5.5E+04	NA	NA
04-09-2023	Kralingen, distributed	Drinking	4	29.7	NA	2.9E+04	2.9E+04	NA	NA
04-09-2023	Kralingen, distributed	Drinking	8	23.7	NA	1.7E+04	1.7E+04	NA	NA
04-09-2023	Kralingen, distributed	Drinking	16	NA	NA	1.3E+04	1.3E+04	NA	NA
11-09-2023	Distribution system, ZBL	Drinking	1	27.2	12.8	1.5E+05	1.4E+04	NA	NA
11-09-2023	Distribution system, ZBL	Drinking	2	25.4	NA	8.8E+04	1.6E+04	NA	NA
11-09-2023	Distribution system, ZBL	Drinking	4	27.4	NA	4.6E+04	7.8E+03	NA	NA
11-09-2023	Distribution system, ZBL	Drinking	8	13.7	NA	3.9E+04	2.2E+04	NA	NA
11-09-2023	Distribution system, ZBL	Drinking	16	NA	NA	1.9E+04	3.6E+03	NA	NA
14-09-2023	UF permeate	Drinking	1	15.3	NA	9.4E+03	1.7E+03	NA	NA

I.II Results laboratory analyses

Table 12-2. Results of laboratory measurements in validation experiments on drinking, cooling and surface water. FCM: flow cytometry, Avg: average, SD: standard deviation, NA: not available.

Date	Sample location	Water type	Dilution	FCM_Intact (cells/ml)		FCM_Not_Intact (cells/ml)		FCM_Total (cells/ml)		Fluorescence microscopy cells/ml)	
				Avg	SD	Avg	SD	Avg	SD	Avg	SD
18-02-2021	Uniper	Cooling water	1	3.0E+06	5.7E+05	8.3E+05	4.1E+05	3.8E+06	9.3E+05	1.9E+07	2.6E+06
18-02-2021	Rhine	Surface water	1	1.7E+06	1.0E+05	<1.0E+05	NA	1.7E+06	1.0E+05	7.8E+06	2.9E+06
23-02-2021	Rhine	Surface water	1	9.6E+05	4.5E+04	<1.0E+05	NA	1.0E+06	9.1E+04	5.5E+06	7.7E+05
23-02-2021	BASF	Cooling water	1	1.4E+06	1.7E+05	<1.0E+05	NA	1.4E+06	1.7E+05	2.7E+06	3.1E+05
23-02-2021	BASF	Proces water	1	4.9E+04	8.1E+03	3.4E+05	2.1E+04	3.9E+05	2.5E+04	6.5E+05	3.6E+04
01-03-2021	BASF	Cooling water	1	2.9E+06	1.5E+05	<1.0E+05	NA	2.9E+06	1.5E+05	6.6E+06	1.1E+06
01-03-2021	BASF	Proces water	1	2.5E+05	1.5E+04	2.0E+04	6.8E+03	2.7E+05	2.0E+04	9.2E+06	1.7E+06
01-03-2021	Rhine	Surface water	1	8.0E+05	1.2E+05	<1.0E+05	NA	8.0E+05	1.2E+05	5.7E+06	8.1E+05
02-03-2021	Uniper	Cooling water	1	1.2E+05	5.8E+03	<1.0E+04	NA	1.2E+05	5.8E+03	8.7E+06	5.5E+06
02-03-2021	Uniper	Cooling water	1	1.2E+05	5.8E+03	<1.0E+04	NA	1.2E+05	5.8E+03	1.1E+06	2.9E+05
02-03-2021	Uniper	Cooling water	10	<1.0E+03	NA	6.0E+03	5.8E+01	6.1E+03	1.2E+02	9.5E+04	2.5E+04
02-03-2021	Uniper	Cooling water	100	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	1.2E+04	1.8E+03
02-03-2021	Uniper	Cooling water	1000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
15-3-2021	Rhine	Surface water	1	1.50E+06	1.73E+05	<1.0E+05	NA	1.50E+06	1.73E+05	5.99E+06	4.95E+05
15-3-2021	Rhine	Surface water	1	1.5E+06	1.7E+05	<1.0E+05	NA	1.5E+06	1.7E+05	6.0E+06	4.9E+05
15-3-2021	Rhine	Surface water	1	2.7E+06	2.1E+05	<1.0E+05	NA	2.7E+06	2.1E+05	3.3E+06	7.8E+05
15-3-2021	Rhine	Surface water	10	2.3E+05	5.8E+03	<1.0E+04	NA	2.4E+05	5.8E+03	4.2E+05	6.3E+04
15-3-2021	Rhine	Surface water	100	2.5E+04	5.8E+02	<1.0E+03	NA	2.5E+04	5.8E+02	4.7E+04	3.6E+03
15-3-2021	Rhine	Surface water	1000	3.9E+03	1.1E+03	<1.0E+03	NA	4.1E+03	1.3E+03	NA	NA
15-3-2021	Rhine	Surface water	10000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
17-3-2021	BASF	Cooling water	1	1.5E+06	1.5E+05	<1.0E+05	NA	1.5E+06	2.0E+05	4.8E+06	1.9E+06
17-3-2021	BASF	Cooling water	10	1.4E+05	5.8E+03	3.2E+04	1.0E+03	1.7E+05	5.8E+03	3.5E+05	5.4E+04
17-3-2021	BASF	Cooling water	100	1.3E+04	1.2E+03	3.5E+03	6.6E+02	1.6E+04	1.0E+03	3.6E+04	6.0E+03
17-3-2021	BASF	Cooling water	1000	1.2E+03	1.7E+02	1.5E+03	7.1E+01	2.4E+03	3.6E+02	NA	NA
17-3-2021	BASF	Cooling water	10000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
21-03-2021	Dow	CW, before	1	1.3E+06	2.0E+05	1.4E+05	3.1E+04	1.5E+06	2.0E+05	1.1E+07	2.5E+06
22-03-2021	Dow	CW, after	1	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	1.1E+07	6.8E+05
29-03-2021	Dow	CW, before	1	1.4E+06	5.8E+04	2.0E+06	1.2E+05	3.4E+06	1.7E+05	1.9E+07	2.7E+06
30-03-2021	Dow	CW, after	1	1.2E+05	<5.1E+05	1.0E+06	8.5E+05	1.3E+06	1.2E+06	1.4E+07	9.6E+05
06-04-2021	Uniper	Cooling water	1	2.5E+04	1.8E+04	1.1E+04	5.8E+02	3.3E+04	1.9E+04	6.9E+05	1.1E+05
06-04-2021	Uniper	Cooling water	10	2.4E+03	1.4E+03	1.3E+03	3.5E+02	3.0E+03	1.6E+03	6.7E+04	6.3E+03
06-04-2021	Uniper	Cooling water	100	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
06-04-2021	Uniper	Cooling water	1000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
06-04-2021	Uniper	Cooling water	10000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA

Date	Sample location	Water type	Dilution	FCM_Intact (cells/ml)		FCM_Not_Intact (cells/ml)		FCM_Total (cells/ml)		Fluorescence microscopy cells/ml)	
06-04-2021	Uniper	Cooling water	100000	NA	NA	NA	NA	NA	NA	NA	NA
07-04-2021	Dow	CW, before	1	1.4E+06	1.2E+05	1.1E+05	5.8E+03	1.5E+06	1.2E+05	1.4E+07	6.1E+05
07-04-2021	Dow	CW, before	10	1.1E+05	2.2E+04	2.2E+05	1.0E+04	3.3E+05	3.1E+04	7.7E+05	2.2E+05
07-04-2021	Dow	CW, before	100	7.4E+03	1.2E+03	5.4E+04	3.5E+03	6.2E+04	3.5E+03	9.1E+04	1.4E+04
07-04-2021	Dow	CW, before	1000	2.4E+03	1.3E+03	8.2E+03	1.6E+03	1.1E+04	3.0E+03	NA	NA
07-04-2021	Dow	CW, before	10000	<1.0E+03	NA	1.5E+03	2.5E+02	1.7E+03	3.6E+02	NA	NA
07-04-2021	Dow	CW, before	100000	<1.0E+03	NA	1.3E+03	0.0E+00	1.7E+03	2.1E+02	NA	NA
07-04-2021	Dow	CW, before	1000000	NA	NA	NA	NA	NA	NA	NA	NA
12-04-2021	Dow	CW, after	1	<1.0E+05	NA	1.0E+05	0.0E+00	1.6E+05	2.6E+04	1.8E+07	2.2E+06
12-04-2021	Dow	CW, after	10	<1.0E+04	NA	1.0E+04	0.0E+00	1.2E+04	2.2E+03	9.9E+05	2.9E+05
12-04-2021	Dow	CW, after	100	<1.0E+03	NA	<1.0E+03	NA	1.0E+03	0.00E+00	9.9E+04	1.1E+04
12-04-2021	Dow	CW, after	1000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
12-04-2021	Dow	CW, after	10000	NA	NA	NA	NA	NA	NA	NA	NA
12-04-2021	Dow	CW, after	100000	NA	NA	NA	NA	NA	NA	NA	NA
14-04-2021	Rhine	Surface water	1	3.1E+06	2.6E+05	<1.0E+05	NA	3.1E+06	2.6E+05	3.3E+06	9.1E+05
14-04-2021	Rhine	Surface water	10	2.3E+05	1.5E+04	<1.0E+04	NA	2.3E+05	1.5E+04	3.0E+05	1.6E+04
14-04-2021	Rhine	Surface water	100	1.9E+04	2.1E+03	4.3E+03	1.5E+03	2.3E+04	1.5E+03	3.8E+04	4.0E+03
14-04-2021	Rhine	Surface water	1000	2.3E+03	1.2E+02	<1.0E+03	NA	2.9E+03	1.2E+02	NA	NA
14-04-2021	Rhine	Surface water	10000	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	NA	NA
14-04-2021	Rhine	Surface water	100000	NA	NA	NA	NA	NA	NA	NA	NA
17-08-2023	Groenekan	Drinking	1	1.1E+05	1.0E+04	8.9E+03	9.9E+02	1.2E+05	1.0E+04	2.1E+04	1.7E+03
17-08-2023	Groenekan	Drinking	2	6.2E+04	7.9E+03	6.6E+03	2.3E+02	6.9E+04	7.9E+03	1.5E+04	8.1E+02
17-08-2023	Groenekan	Drinking	4	3.0E+04	2.3E+03	5.5E+03	6.0E+02	3.6E+04	3.1E+03	6.3E+03	2.7E+02
17-08-2023	Groenekan	Drinking	8	1.4E+04	2.6E+03	3.4E+03	3.6E+02	1.7E+04	2.6E+03	4.6E+03	3.3E+02
17-08-2023	Groenekan	Drinking	16	1.2E+04	1.0E+03	3.2E+03	5.9E+02	1.5E+04	5.8E+02	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	1	1.5E+04	0.0E+00	1.0E+03	5.8E+01	1.5E+04	5.8E+02	1.6E+04	1.3E+03
21-08-2023	Nieuw-Lekkerland	Drinking	2	7.7E+03	5.0E+02	4.4E+03	2.3E+02	1.2E+04	0.0E+00	1.5E+04	1.1E+03
21-08-2023	Nieuw-Lekkerland	Drinking	4	4.3E+03	3.1E+02	6.4E+03	3.6E+02	1.1E+04	5.8E+02	9.3E+03	1.3E+03
21-08-2023	Nieuw-Lekkerland	Drinking	8	2.1E+03	4.0E+02	6.5E+03	9.2E+02	8.6E+03	6.0E+02	5.3E+03	6.3E+02
21-08-2023	Nieuw-Lekkerland	Drinking	16	1.1E+03	1.2E+02	7.5E+03	2.6E+02	8.5E+03	4.6E+02	3.3E+03	9.7E+02
29-08-2023	Soestduinen	Drinking	1	3.4E+04	1.0E+03	2.3E+03	8.3E+02	3.6E+04	1.7E+03	7.5E+03	4.3E+02
29-08-2023	Soestduinen	Drinking	2	1.8E+04	1.0E+03	2.4E+03	4.6E+02	2.1E+04	1.2E+03	3.9E+03	6.8E+02
29-08-2023	Soestduinen	Drinking	4	9.4E+03	9.3E+02	2.1E+03	5.5E+02	1.1E+04	5.8E+02	2.5E+03	6.5E+02
29-08-2023	Soestduinen	Drinking	8	5.1E+03	2.9E+02	2.0E+03	2.3E+02	7.2E+03	4.5E+02	1.2E+03	6.1E+02
29-08-2023	Soestduinen	Drinking	16	3.2E+03	1.7E+02	1.9E+03	4.0E+02	5.0E+03	2.1E+02	1.6E+03	5.1E+02
04-09-2023	Kralingen	Drinking	1	1.1E+05	5.8E+03	1.6E+05	5.8E+03	2.8E+05	5.8E+03	7.8E+04	3.0E+03
04-09-2023	Kralingen	Drinking	2	5.0E+04	2.0E+03	9.0E+04	2.3E+03	1.4E+05	0.0E+00	1.1E+05	4.9E+03

Date	Sample location	Water type	Dilution	FCM_Intact (cells/ml)		FCM_Not_Intact (cells/ml)		FCM_Total (cells/ml)		Fluorescence microscopy cells/ml)	
04-09-2023	Kralingen	Drinking	4	2.7E+04	1.5E+03	4.7E+04	1.0E+03	7.4E+04	2.6E+03	7.2E+04	3.4E+03
04-09-2023	Kralingen	Drinking	8	1.3E+04	1.0E+03	2.4E+04	1.5E+03	3.7E+04	1.7E+03	3.0E+04	1.1E+03
04-09-2023	Kralingen	Drinking	16	6.5E+03	6.2E+02	1.1E+04	5.8E+02	1.8E+04	1.0E+03	1.3E+04	2.4E+02
11-09-2023	Zuid-Beijerland	Drinking	1	1.2E+05	5.8E+03	7.6E+04	1.5E+03	1.9E+05	1.2E+04	1.6E+05	9.9E+03
11-09-2023	Zuid-Beijerland	Drinking	2	5.6E+04	1.5E+03	4.4E+04	2.5E+03	9.9E+04	2.3E+03	1.2E+05	2.5E+03
11-09-2023	Zuid-Beijerland	Drinking	4	2.9E+04	0.0E+00	2.3E+04	1.5E+03	5.2E+04	1.5E+03	5.6E+04	2.7E+03
11-09-2023	Zuid-Beijerland	Drinking	8	1.4E+04	1.2E+03	1.1E+04	5.8E+02	2.6E+04	2.0E+03	3.3E+04	1.9E+03
11-09-2023	Zuid-Beijerland	Drinking	16	7.0E+03	3.5E+02	5.4E+03	3.5E+02	1.2E+04	5.8E+02	1.1E+04	5.1E+02
14-09-2023	UF permeate	Drinking	1	<1.0E+03	NA	<1.0E+03	NA	<1.0E+03	NA	1.1E+03	3.1E+02

Table 12-3. Results of ATP measurements in validation experiments on drinking, cooling and surface water. Avg: average, SD: standard deviation, NA: not available.

Date	Sample location	Water type	Dilution	Total_ATP (ng ATP/l)		Free_ATP (ng ATP/l)		Cell_ATP (ng ATP/l)	
				Avg	SD	Avg	SD	Avg	SD
18-02-2021	Uniper	Cooling water	1	61.7	2.5	34.3	4.9	27.3	7.4
18-02-2021	Rhine	Surface water	1	176.7	15.3	11.9	6.1	164.7	15.6
23-02-2021	Rhine	Surface water	1	126.7	37.9	15.3	5.1	111.3	33.3
23-02-2021	BASF	Cooling water	1	21.0	1.0	17.0	1.0	4.0	1.0
23-02-2021	BASF	Proces water	1	<1.0	0.0	<1.0	<0.2	<1.0	0.0
01-03-2021	BASF	Cooling water	1	53.7	0.6	18.3	0.6	35.3	0.6
01-03-2021	BASF	Proces water	1	<1.0	0.0	<1.0	0.0	<1.0	0.0
01-03-2021	Rhine	Surface water	1	160.0	10.0	31.3	4.0	128.7	8.1
02-03-2021	Uniper	Cooling water	1	9.0	0.4	8.9	0.4	<1.0	0.8
02-03-2021	Uniper	Cooling water	1	10.6	0.8	10.7	0.6	<1.0	0.2
02-03-2021	Uniper	Cooling water	10	<1.0	0.0	<1.0	0.0	<1.0	0.0
02-03-2021	Uniper	Cooling water	100	<1.0	0.0	<1.0	0.0	181.3	314.1
02-03-2021	Uniper	Cooling water	1000	<1.0	0.0	<1.0	0.0	<1.0	0.0
15-3-2021	Rhine	Surface water	1	673	75	93	15	580	61
15-3-2021	Rhine	Surface water	1	673.3	75.1	93.3	15.3	580.0	60.8
15-3-2021	Rhine	Surface water	1	913.3	172.4	176.7	72.3	736.7	230.3
15-3-2021	Rhine	Surface water	10	49.3	5.9	25.0	1.7	24.3	7.1
15-3-2021	Rhine	Surface water	100	3.5	1.0	2.1	0.5	1.4	0.7
15-3-2021	Rhine	Surface water	1000	NA	NA	NA	NA	NA	NA
15-3-2021	Rhine	Surface water	10000	NA	NA	NA	NA	NA	NA
17-3-2021	BASF	Cooling water	1	48.3	0.6	8.9	3.4	39.4	3.0
17-3-2021	BASF	Cooling water	10	7.4	0.7	1.3	0.6	6.1	1.1
17-3-2021	BASF	Cooling water	100	<1.0	0.0	<1.0	0.0	<1.0	0.0
17-3-2021	BASF	Cooling water	1000	NA	NA	NA	NA	NA	NA

Date	Sample location	Water type	Dilution	Total_ATP (ng ATP/l)		Free_ATP (ng ATP/l)		Cell_ATP (ng ATP/l)	
17-3-2021	BASF	Cooling water	10000	NA	NA	NA	NA	NA	NA
21-03-2021	Dow	Cooling water, before	1	85.0	1.0	86.3	2.1	<1.0	2.1
22-03-2021	Dow	Cooling water, after	1	91.7	5.5	24.7	1.5	67.0	4.4
29-03-2021	Dow	Cooling water, before	1	153.3	5.8	133.3	5.8	20.0	10.0
30-03-2021	Dow	Cooling water, after	1	110.0	0.0	110.0	0.0	<1.0	0.0
06-04-2021	Uniper	Cooling water	1	33.0	1.0	33.7	1.2	<1.0	1.5
06-04-2021	Uniper	Cooling water	10	2.4	0.2	1.8	0.2	0.6	0.3
06-04-2021	Uniper	Cooling water	100	<1.0	0.0	<1.0	0.0	<1.0	0.0
06-04-2021	Uniper	Cooling water	1000	NA	NA	NA	NA	NA	NA
06-04-2021	Uniper	Cooling water	10000	NA	NA	NA	NA	NA	NA
06-04-2021	Uniper	Cooling water	100000	NA	NA	NA	NA	NA	NA
07-04-2021	Dow	Cooling water, before	1	263.3	5.8	253.3	5.8	10.0	0.0
07-04-2021	Dow	Cooling water, before	10	18.3	1.2	13.3	0.6	5.0	1.0
07-04-2021	Dow	Cooling water, before	100	2.1	0.3	1.8	0.5	<1.0	0.4
07-04-2021	Dow	Cooling water, before	1000	<1.0	0.0	<1.0	0.0	<1.0	0.0
07-04-2021	Dow	Cooling water, before	10000	NA	NA	NA	NA	NA	NA
07-04-2021	Dow	Cooling water, before	100000	NA	NA	NA	NA	NA	NA
07-04-2021	Dow	Cooling water, before	1000000	NA	NA	NA	NA	NA	NA
12-04-2021	Dow	Cooling water, after	1	183.3	5.8	190.0	0.0	<1.0	5.8
12-04-2021	Dow	Cooling water, after	10	11.3	0.6	9.1	0.6	2.3	1.0
12-04-2021	Dow	Cooling water, after	100	<1.0	0.0	<1.0	<0.2	<1.0	<0.2
12-04-2021	Dow	Cooling water, after	1000	NA	NA	NA	NA	NA	NA
12-04-2021	Dow	Cooling water, after	10000	NA	NA	NA	NA	NA	NA
12-04-2021	Dow	Cooling water, after	100000	NA	NA	NA	NA	NA	NA
14-04-2021	Rhine	Surface water	1	1433.3	57.7	320.0	91.7	1113.3	98.7
14-04-2021	Rhine	Surface water	10	73.3	4.0	33.7	2.5	39.7	6.4
14-04-2021	Rhine	Surface water	100	10.5	1.3	4.2	0.3	6.4	1.1
14-04-2021	Rhine	Surface water	1000	1.3	0.5	<1.0	0.0	<1.0	0.5
14-04-2021	Rhine	Surface water	10000	NA	NA	NA	NA	NA	NA
14-04-2021	Rhine	Surface water	100000	NA	NA	NA	NA	NA	NA
17-08-2023	Groenekan	Drinking	1	1.7	0.3	1.1	0.2	0.2	0.0
17-08-2023	Groenekan	Drinking	2	1.1	0.1	1.0	0.0	0.1	0.1
17-08-2023	Groenekan	Drinking	4	<1	0.0	<1	0.0	<1	0.0
17-08-2023	Groenekan	Drinking	8	<1	0.0	<1	0.0	<1	0.0
17-08-2023	Groenekan	Drinking	16	NA	NA	NA	NA	NA	NA
21-08-2023	Nieuw-Lekkerland	Drinking	1	3.4	0.3	1.2	0.2	1.3	1.6
21-08-2023	Nieuw-Lekkerland	Drinking	2	<1	0.0	<1	0.0	<1	0.0
21-08-2023	Nieuw-Lekkerland	Drinking	4	<1	0.0	<1	0.0	<1	0.0

Date	Sample location	Water type	Dilution	Total_ATP (ng ATP/l)		Free_ATP (ng ATP/l)		Cell_ATP (ng ATP/l)	
21-08-2023	Nieuw-Lekkerland	Drinking	8	<1	0.0	<1	0.0	<1	0.0
21-08-2023	Nieuw-Lekkerland	Drinking	16	<1	0.0	<1	0.0	<1	0.0
29-08-2023	Soestduinen	Drinking	1	<1	0.0	<1	0.0	<1	0.0
29-08-2023	Soestduinen	Drinking	2	<1	0.0	<1	0.0	<1	0.0
29-08-2023	Soestduinen	Drinking	4	<1	0.0	<1	0.0	<1	0.0
29-08-2023	Soestduinen	Drinking	8	<1	0.0	<1	0.0	<1	0.0
29-08-2023	Soestduinen	Drinking	16	<1	0.0	<1	0.0	<1	0.0
04-09-2023	Kralingen	Drinking	1	<1	0.0	<1	0.0	<1	0.0
04-09-2023	Kralingen	Drinking	2	<1	0.0	1.1	0.2	0.2	0.2
04-09-2023	Kralingen	Drinking	4	<1	0.0	<1	0.0	<1	0.0
04-09-2023	Kralingen	Drinking	8	<1	0.0	<1	0.0	<1	0.0
04-09-2023	Kralingen	Drinking	16	<1	0.0	<1	0.0	<1	0.0
11-09-2023	Zuid-Beijerland	Drinking	1	1.7	0.6	1.1	0.2	0.7	0.6
11-09-2023	Zuid-Beijerland	Drinking	2	1.2	0.2	1.0	0.0	0.0	0.0
11-09-2023	Zuid-Beijerland	Drinking	4	<1	0.0	<1	0.0	<1	0.0
11-09-2023	Zuid-Beijerland	Drinking	8	<1	0.0	<1	0.0	<1	0.0
11-09-2023	Zuid-Beijerland	Drinking	16	<1	0.0	<1	0.0	<1	0.0
14-09-2023	UF permeate	Drinking	1	<1	0.0	<1	0.0	<1	0.0

II Scatter plots cooling and surface water

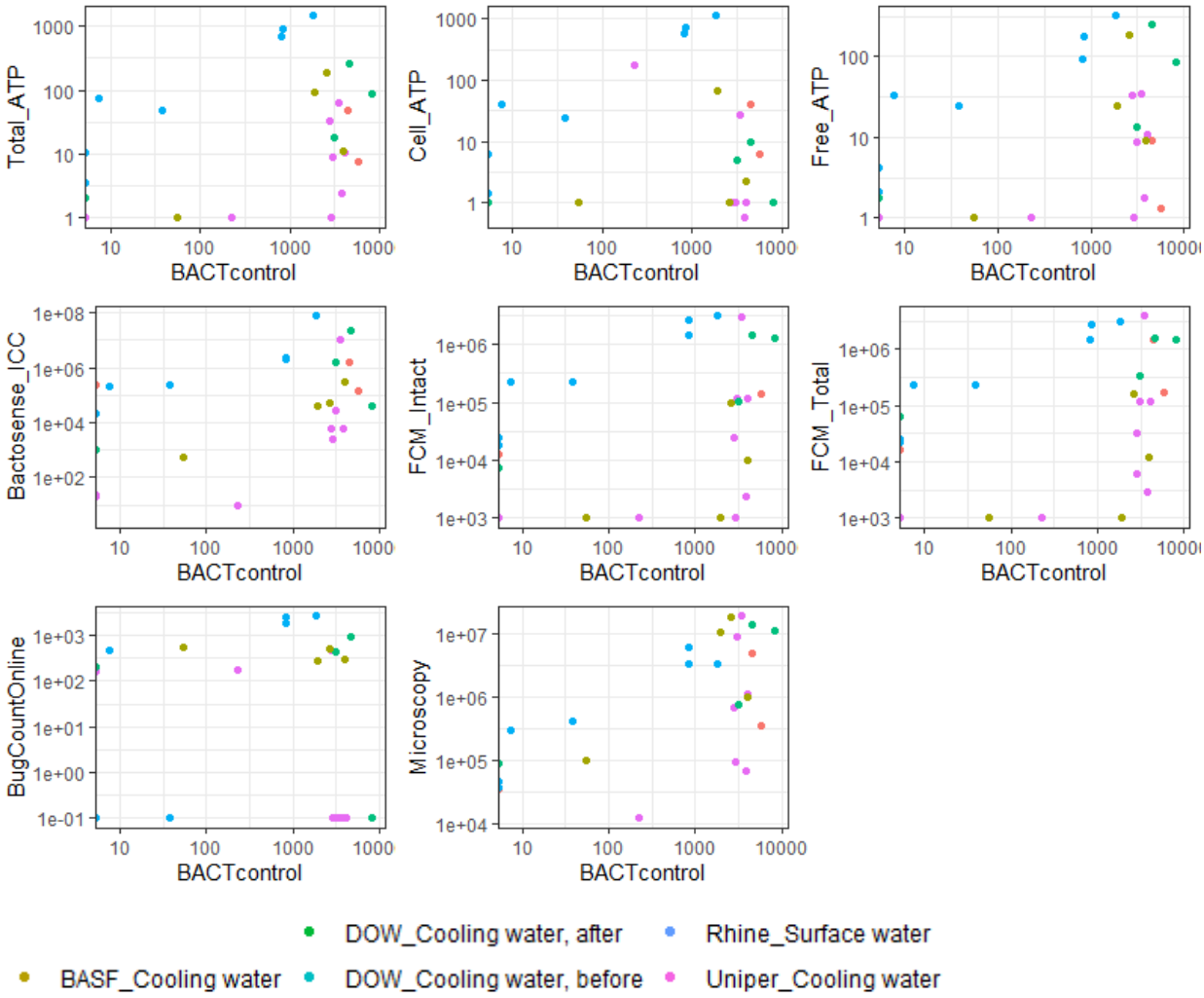

Figure 12-1. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of the BACTcontrol to the other sensors and laboratory parameters.

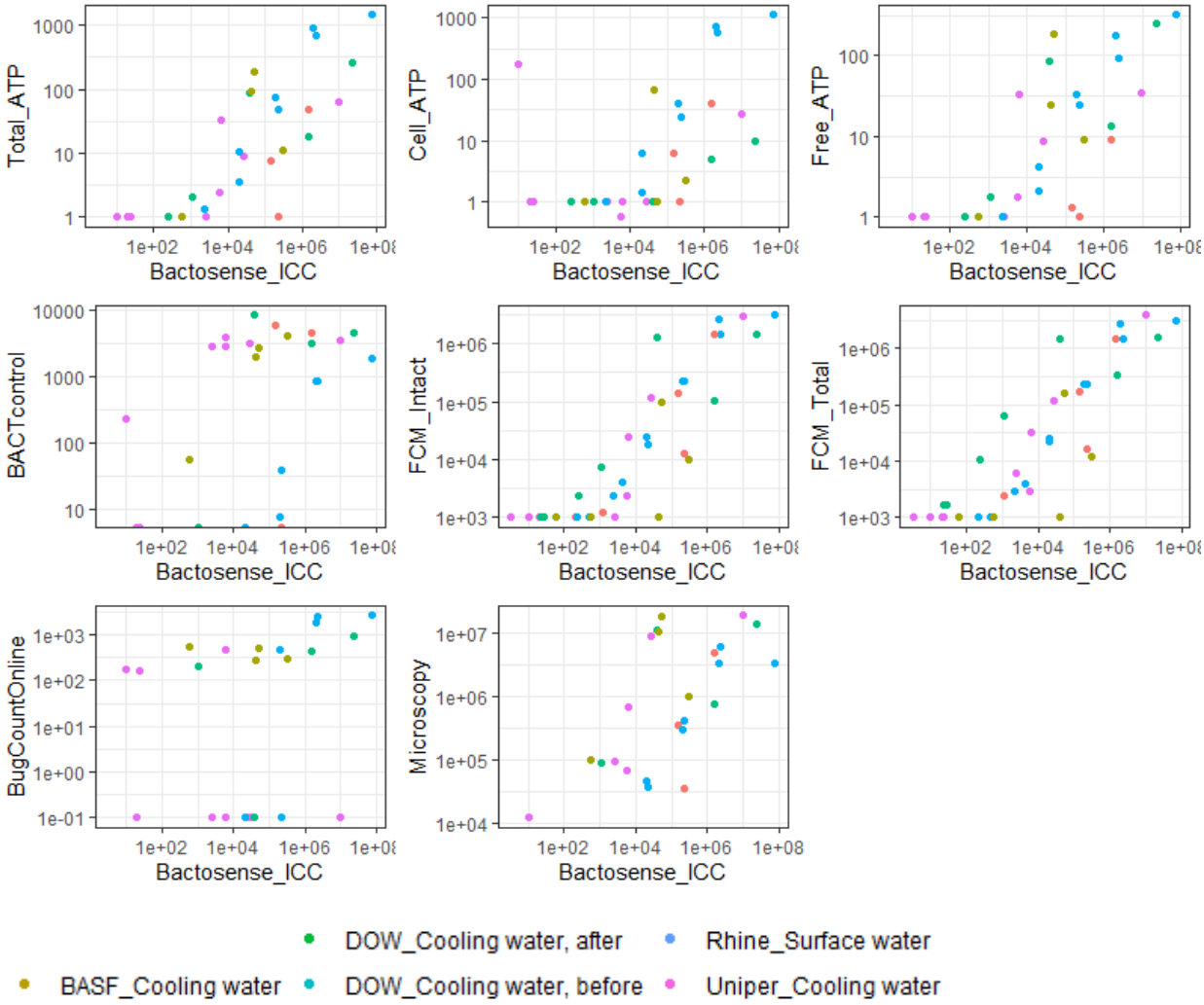

Figure 12-2. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of the BactoSense (intact cells) to the other sensors and laboratory parameters.

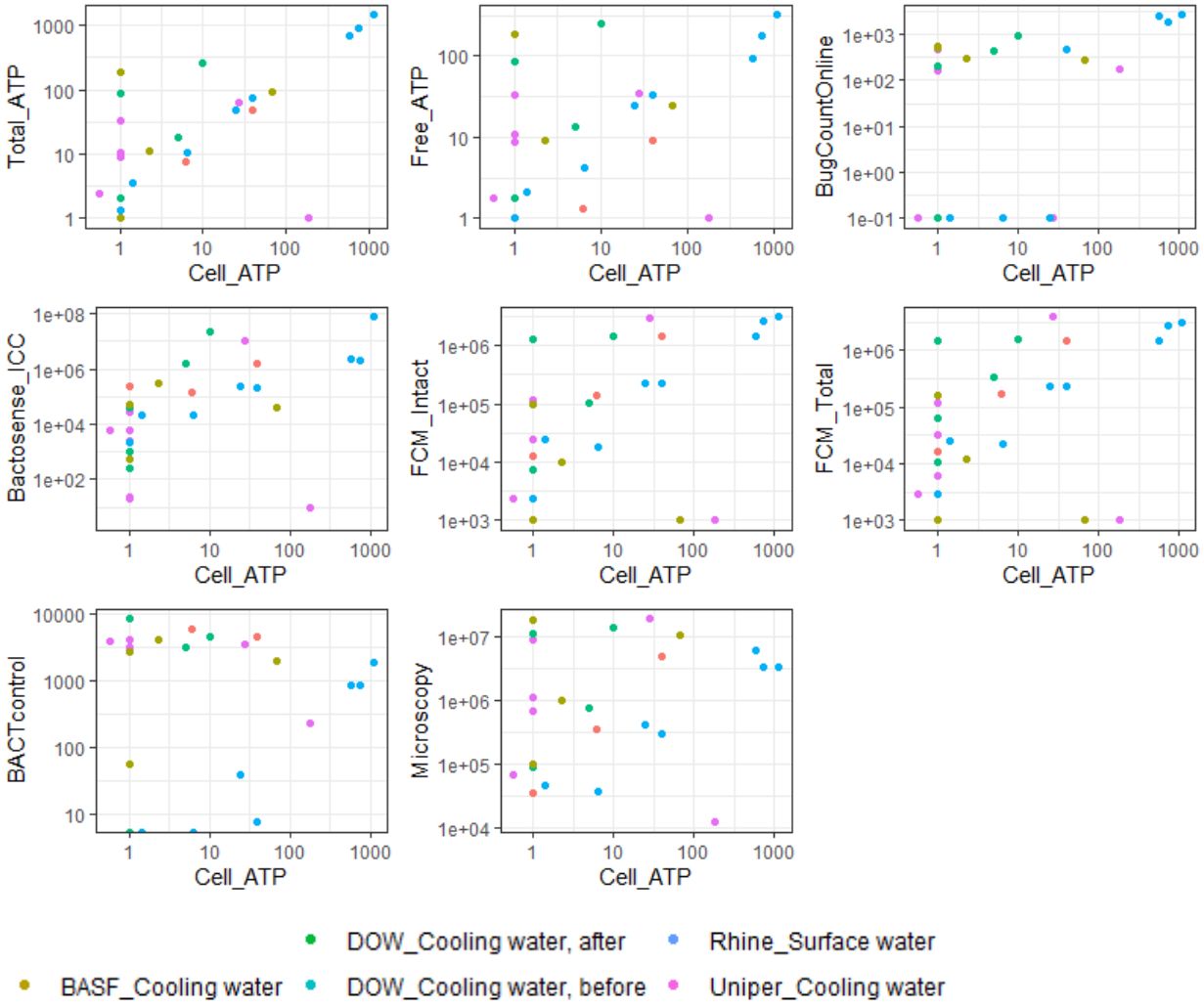

Figure 12-3. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of cellular ATP measurements to the sensors and laboratory parameters.

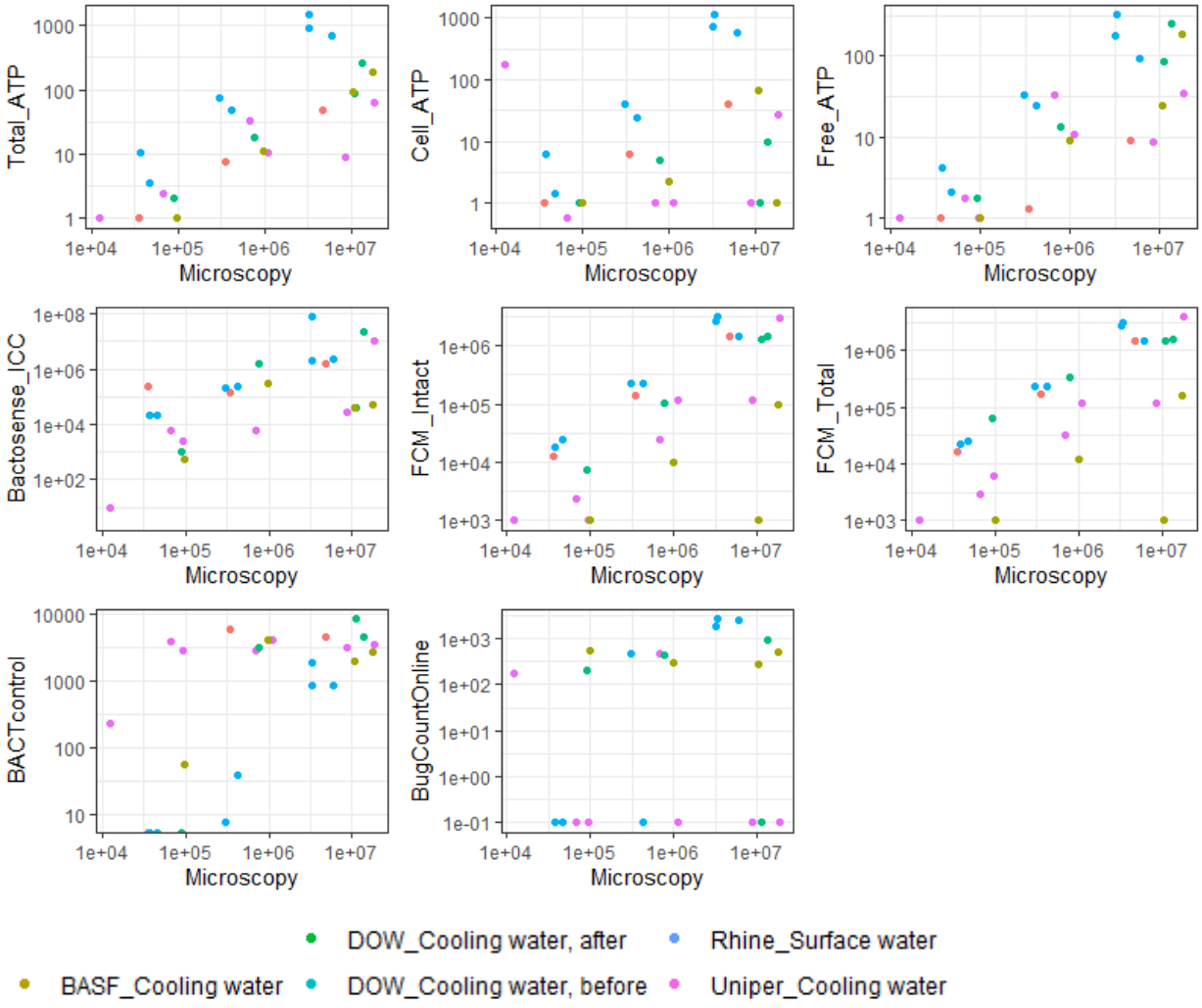

Figure 12-4. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of direct cell count (microscopy) measurements to the sensors and laboratory parameters.

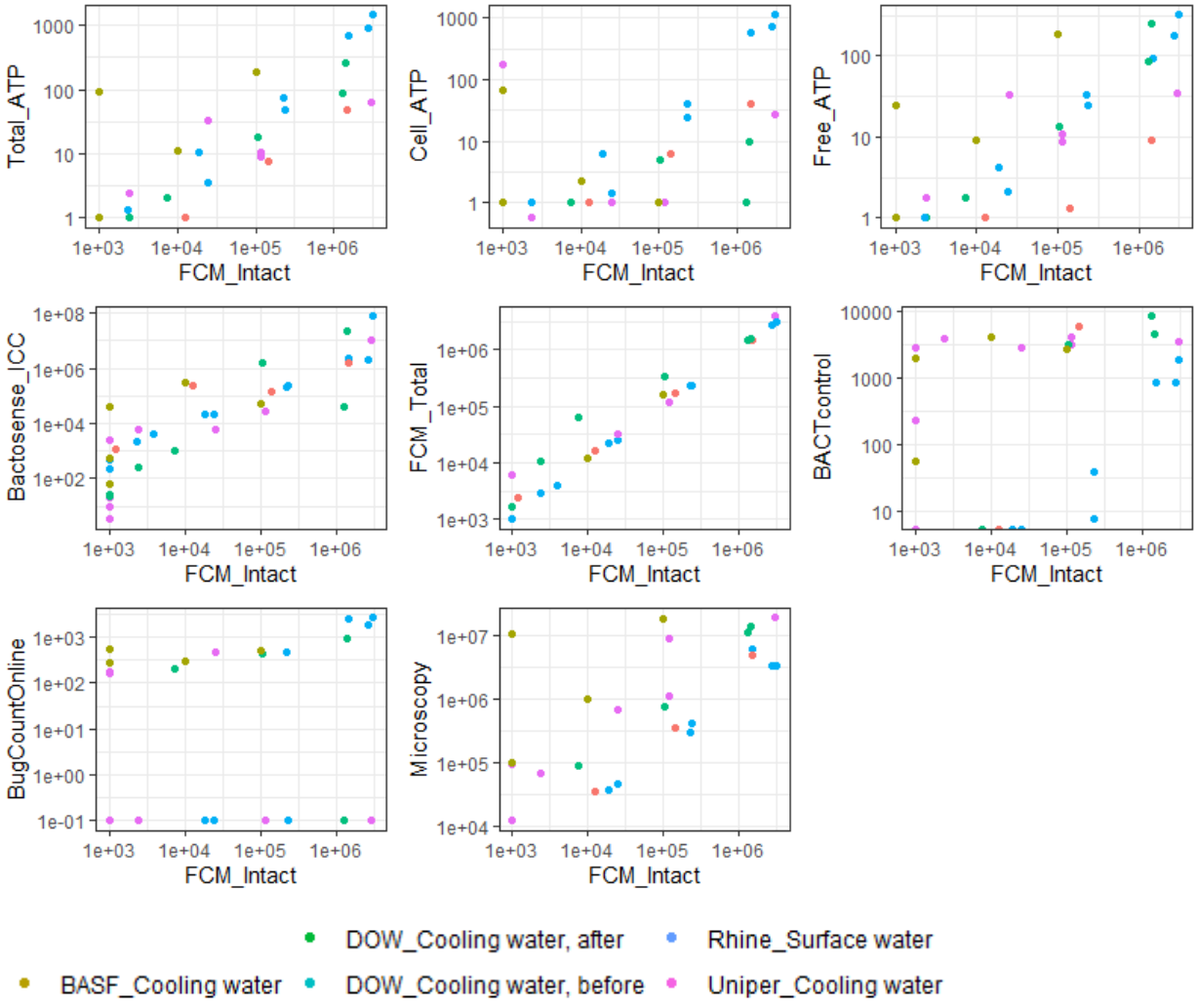

Figure 12-5. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of flow cytometry (intact cells) measurements to the sensors and laboratory parameters.

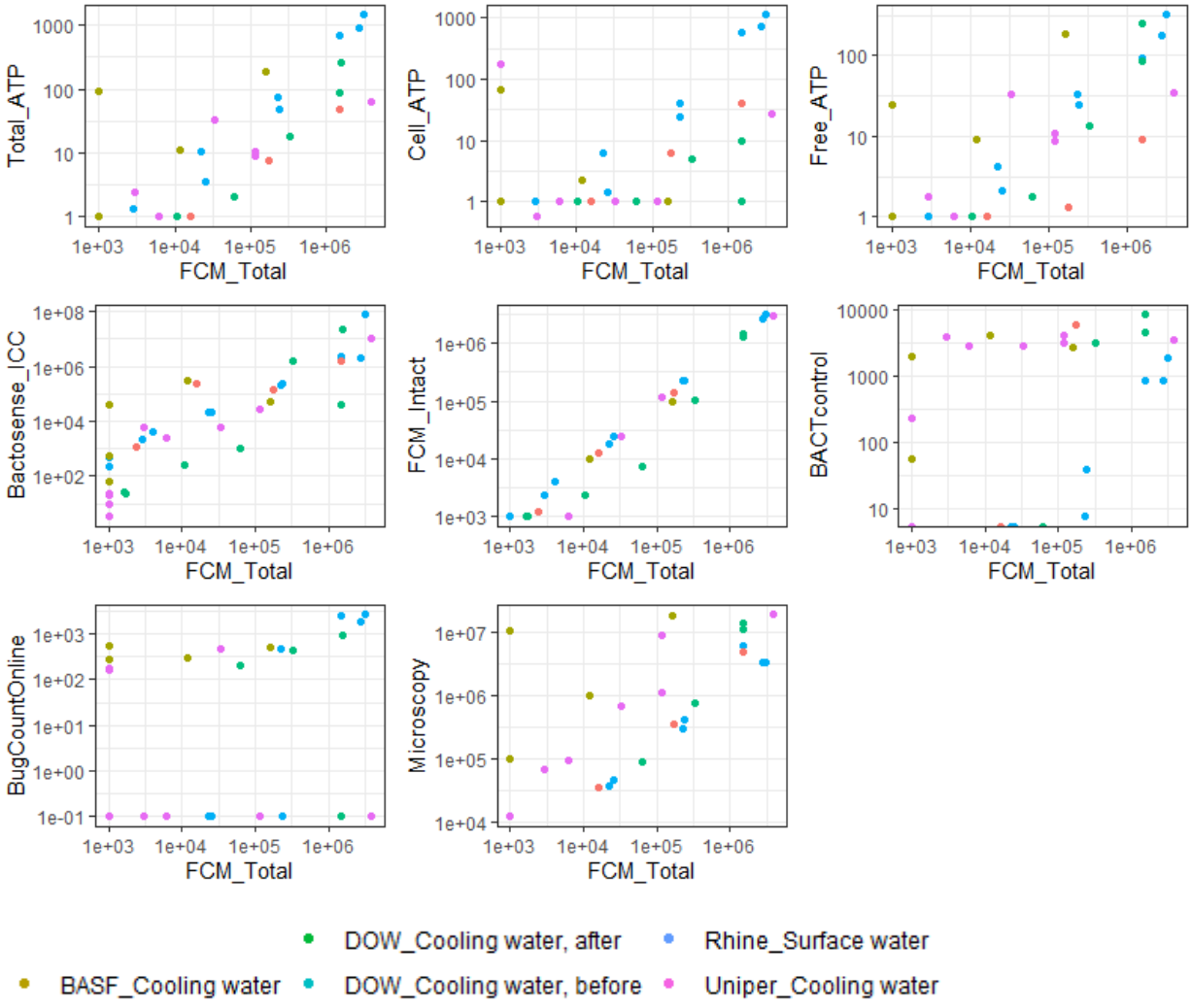

Figure 12-6. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of flow cytometry (total cells) measurements to the sensors and laboratory parameters.

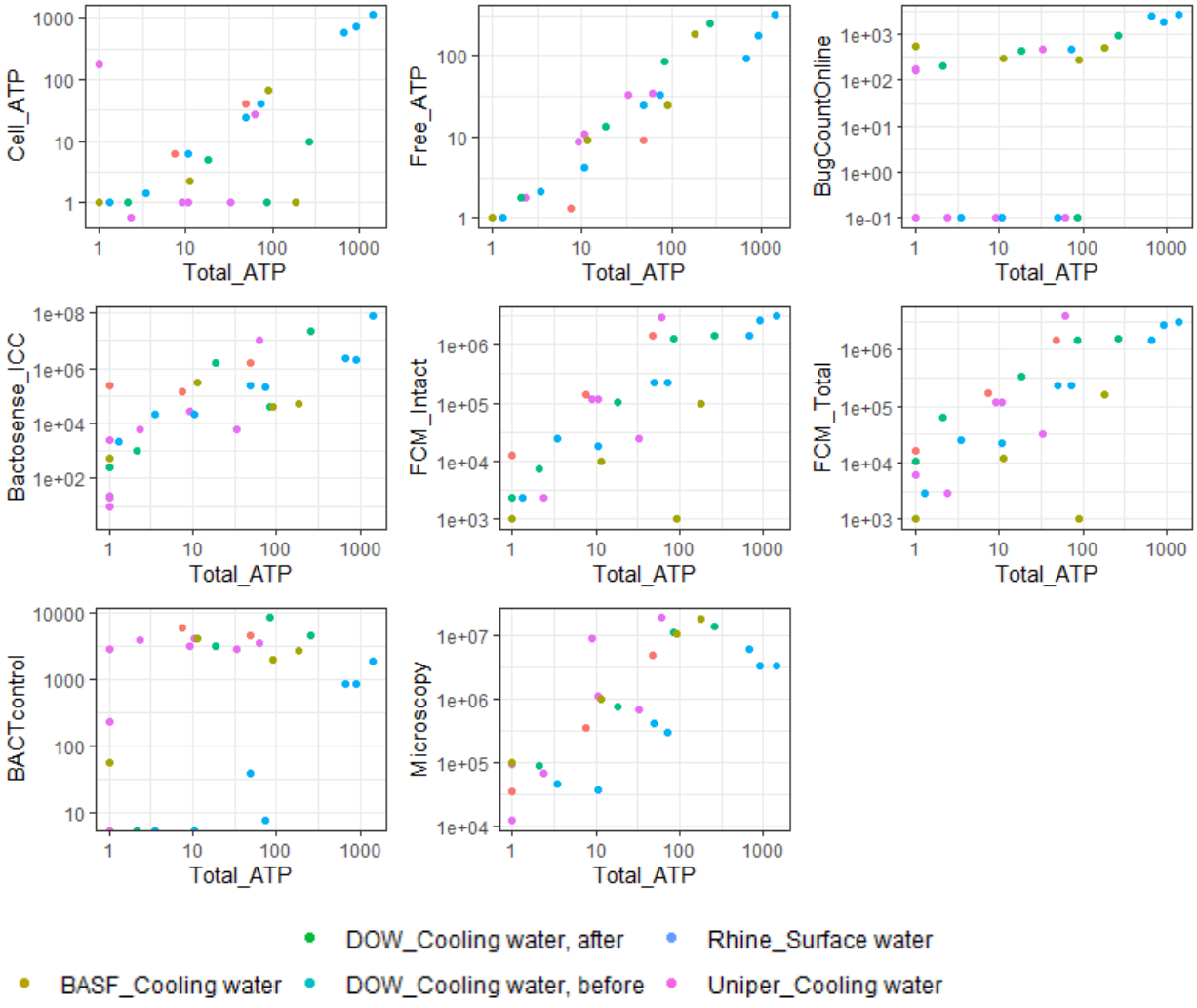

Figure 12-7. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of total ATP measurements to the sensors and laboratory parameters.

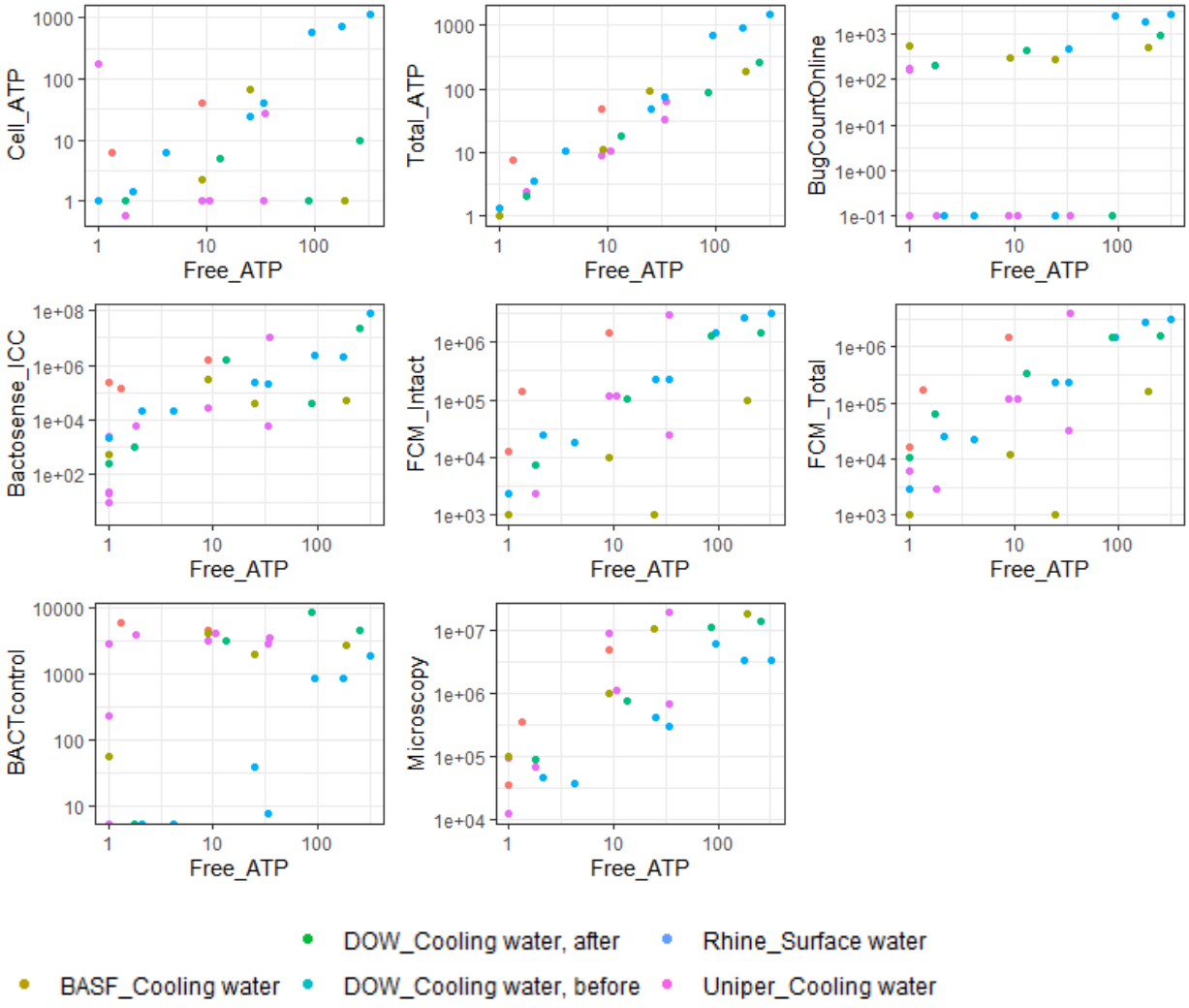

Figure 12-8. Scatter plots of results of validation of surface water and cooling water. Shown is the comparison of free ATP measurements to the sensors and laboratory parameters.

III Scatter plots drinking water

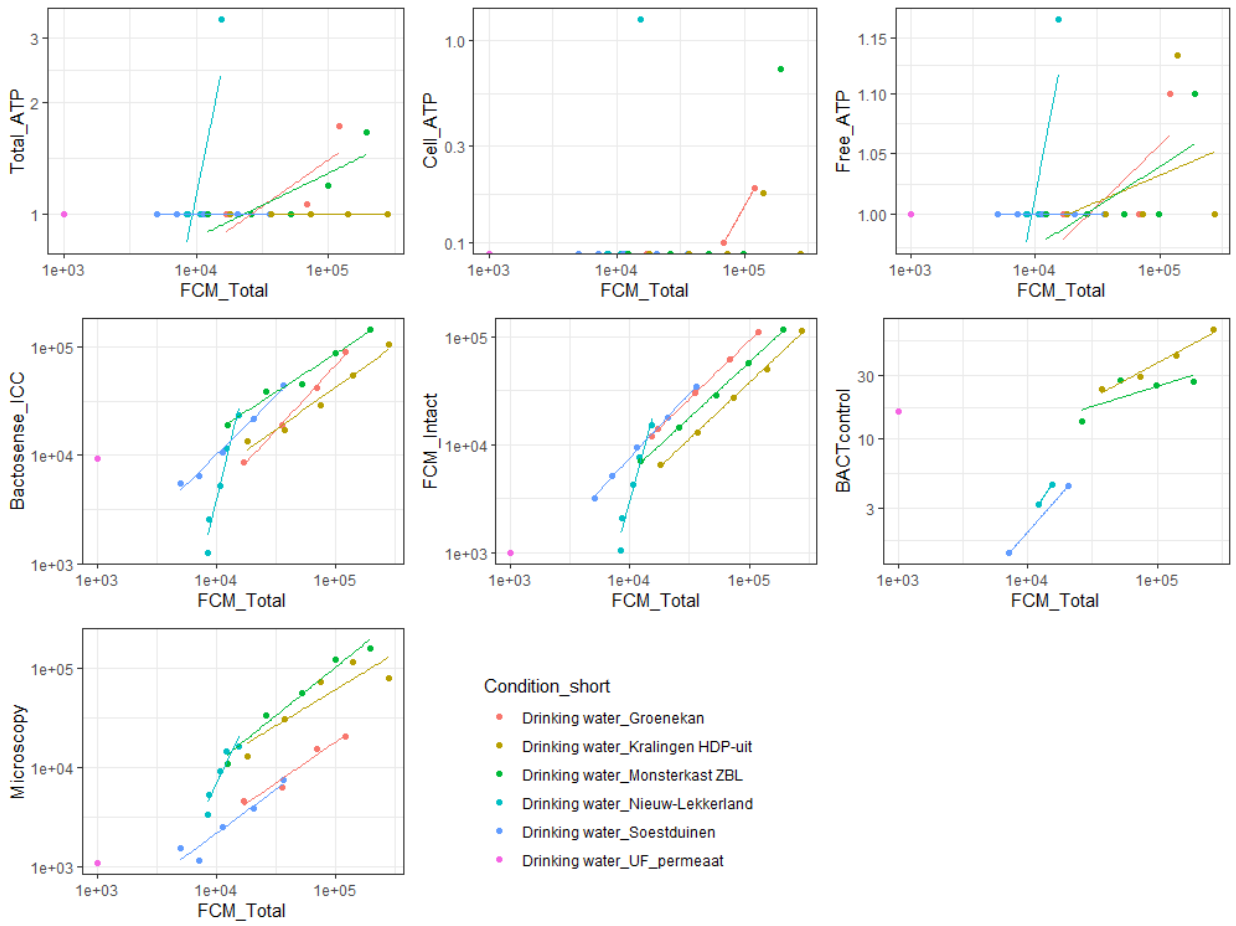

Figure 12-9. Scatter plots of results of validation of drinking water. Shown is the comparison of the FCM_Total to the other sensors and laboratory parameters.

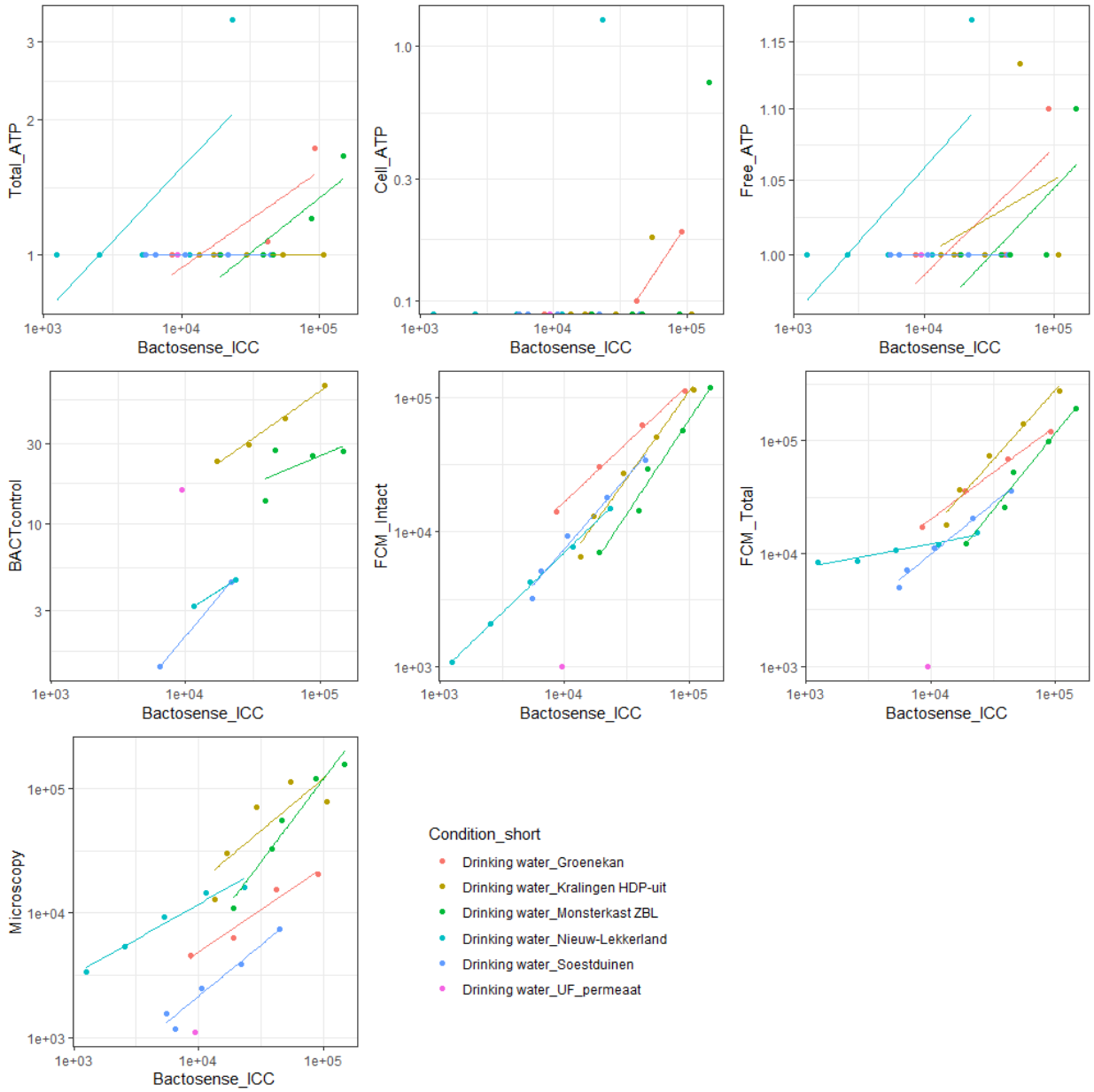

Figure 12-10. Scatter plots of results of validation of drinking water. Shown is the comparison of the BactoSense_ICC to the other sensors and laboratory parameters.

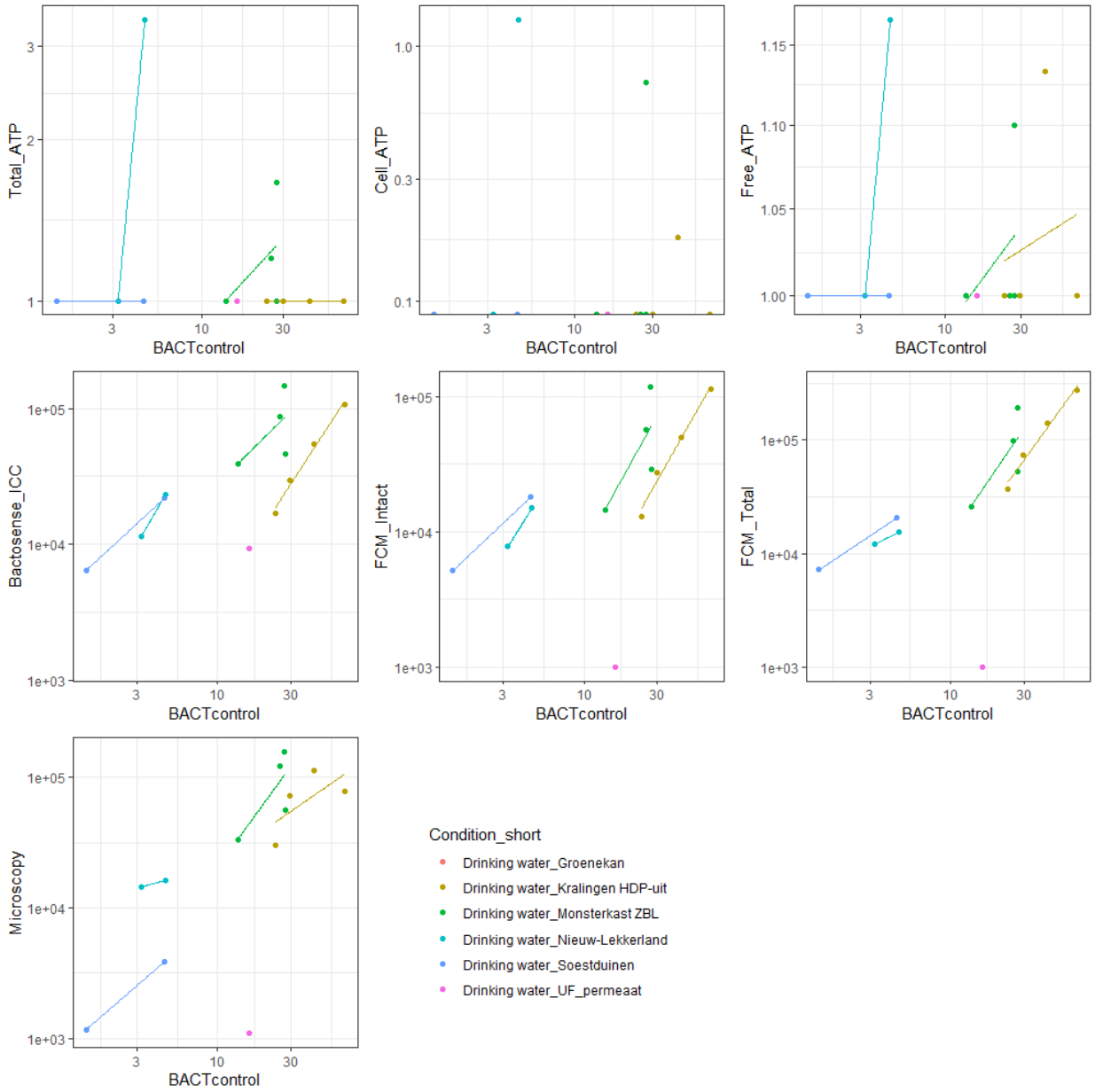

Figure 12-11. Scatter plots of results of validation of drinking water. Shown is the comparison of the BACTcontrol to the other sensors and laboratory parameters.

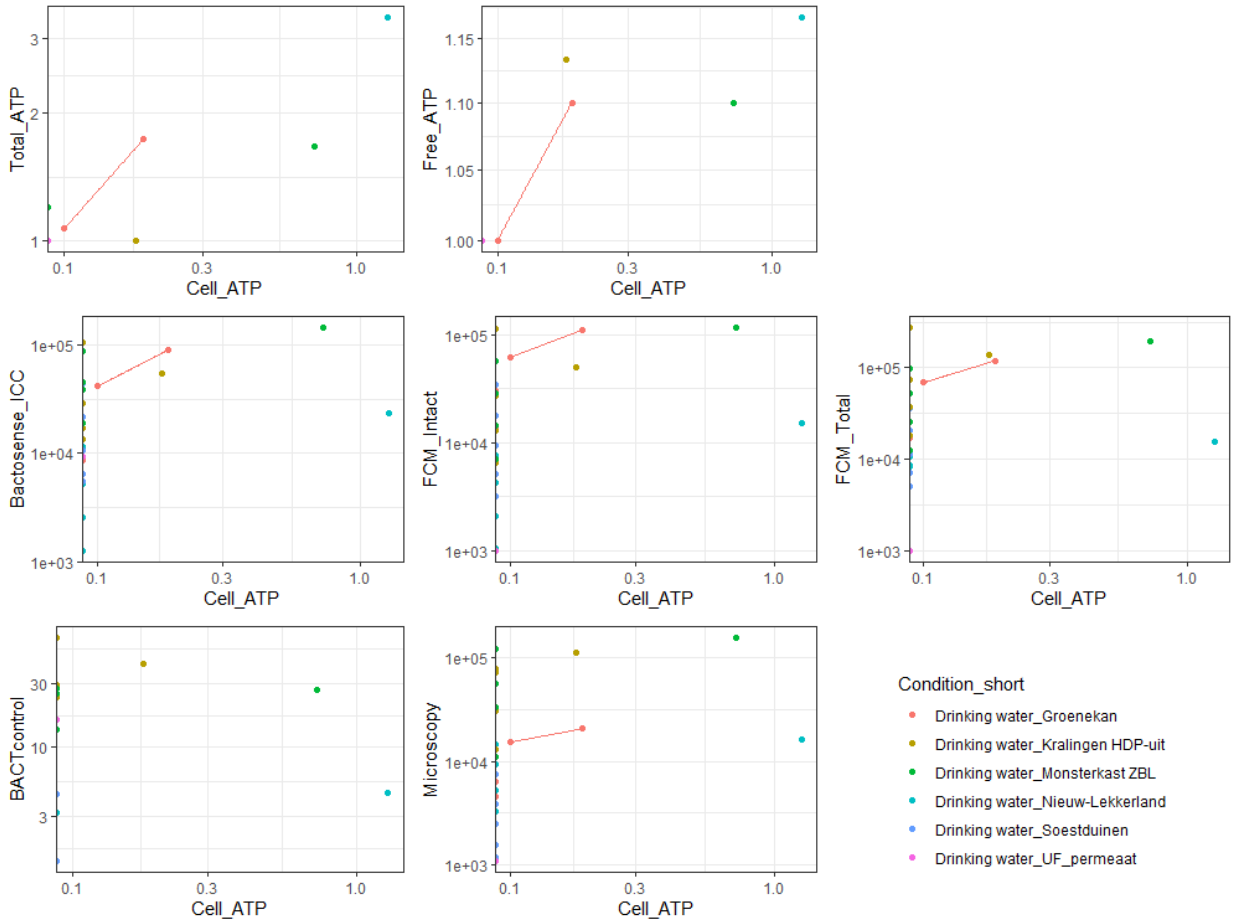

Figure 12-12. Scatter plots of results of validation of drinking water. Shown is the comparison of Cell_ATP to the other sensors and laboratory parameters.

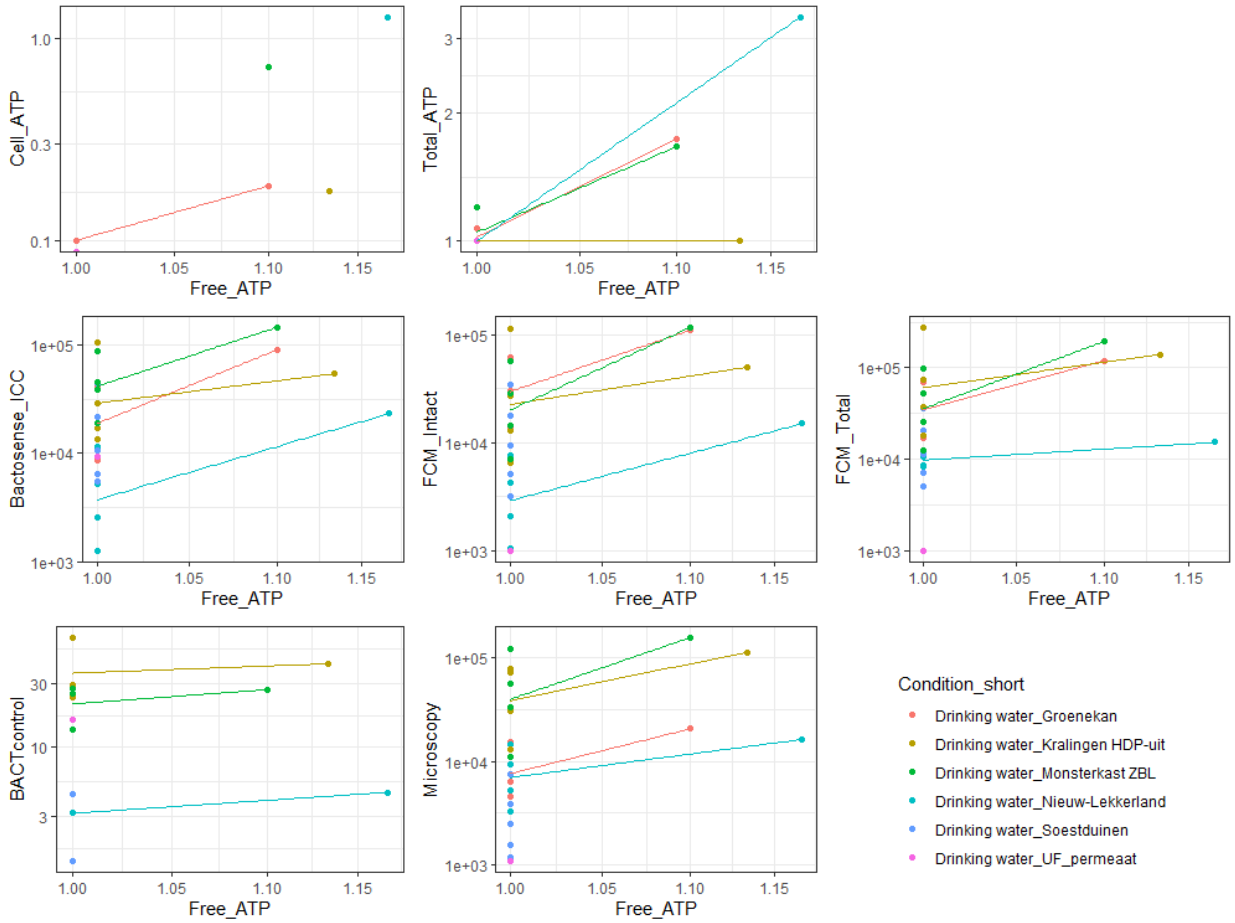

Figure 12-13. Scatter plots of results of validation of drinking water. Shown is the comparison of the Free_ATP to the other sensors and laboratory parameters.

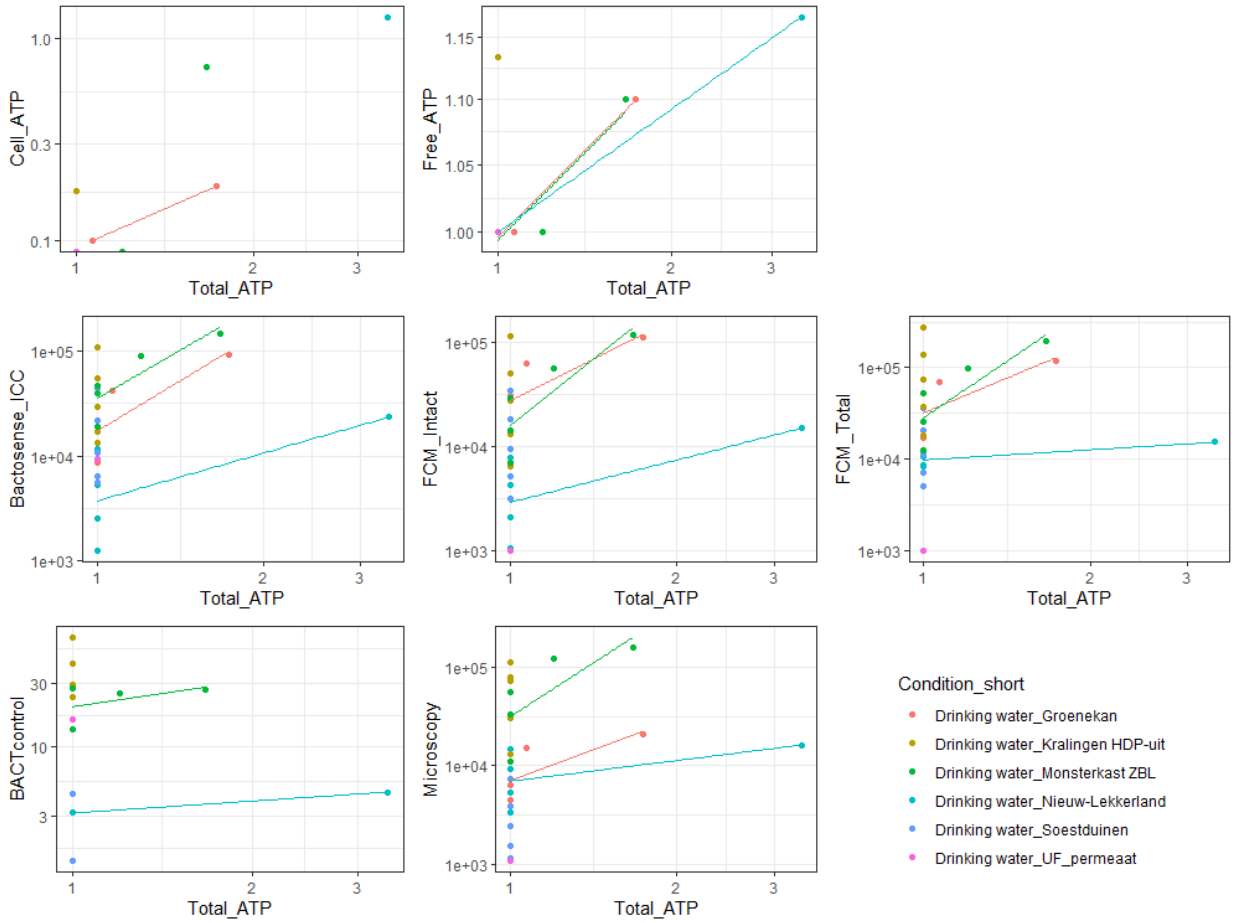

Figure 12-14. Scatter plots of results of validation of drinking water. Shown is the comparison of the Total_ATP to the other sensors and laboratory parameters.

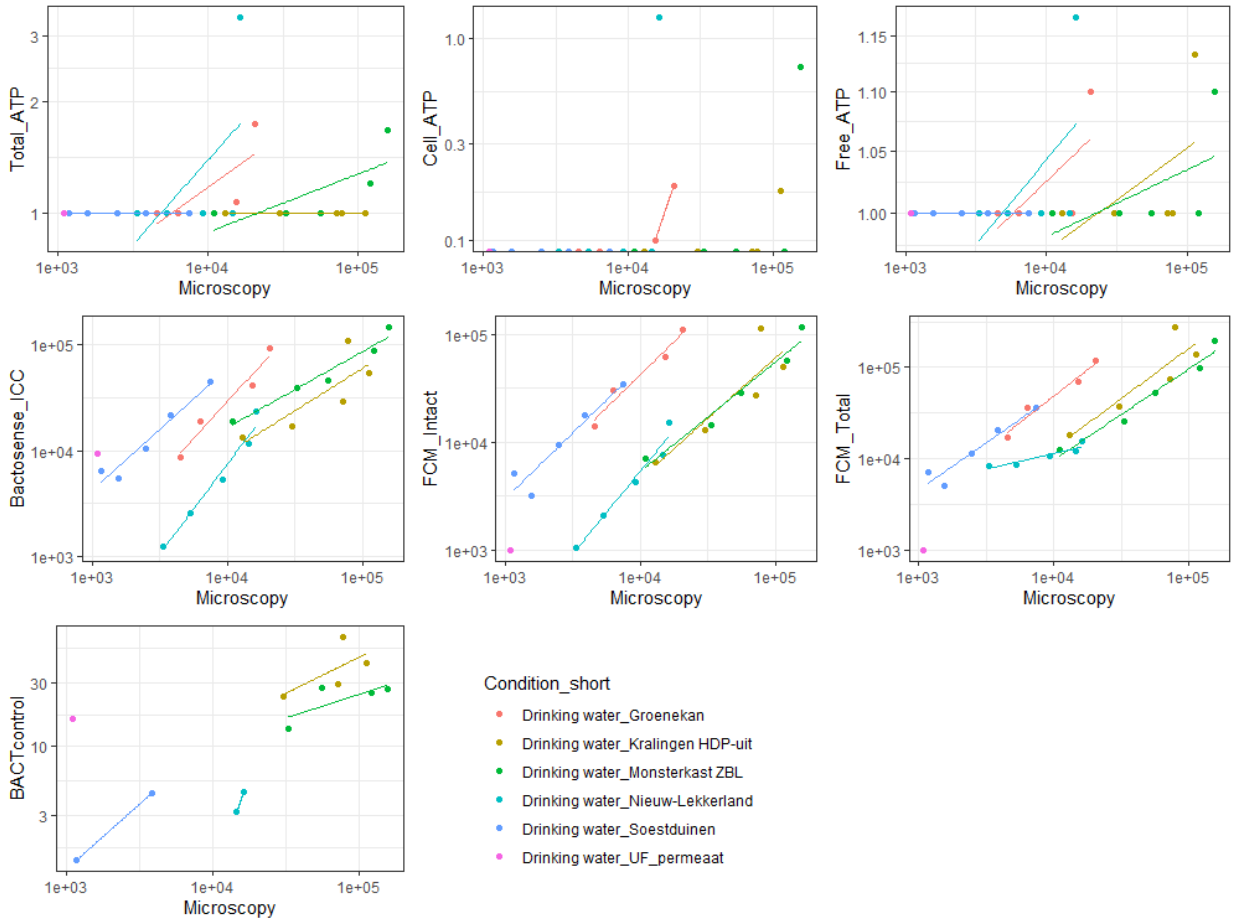

Figure 12-15. Scatter plots of results of validation of drinking water. Shown is the comparison of the Microscopy method to the other sensors and laboratory parameters.

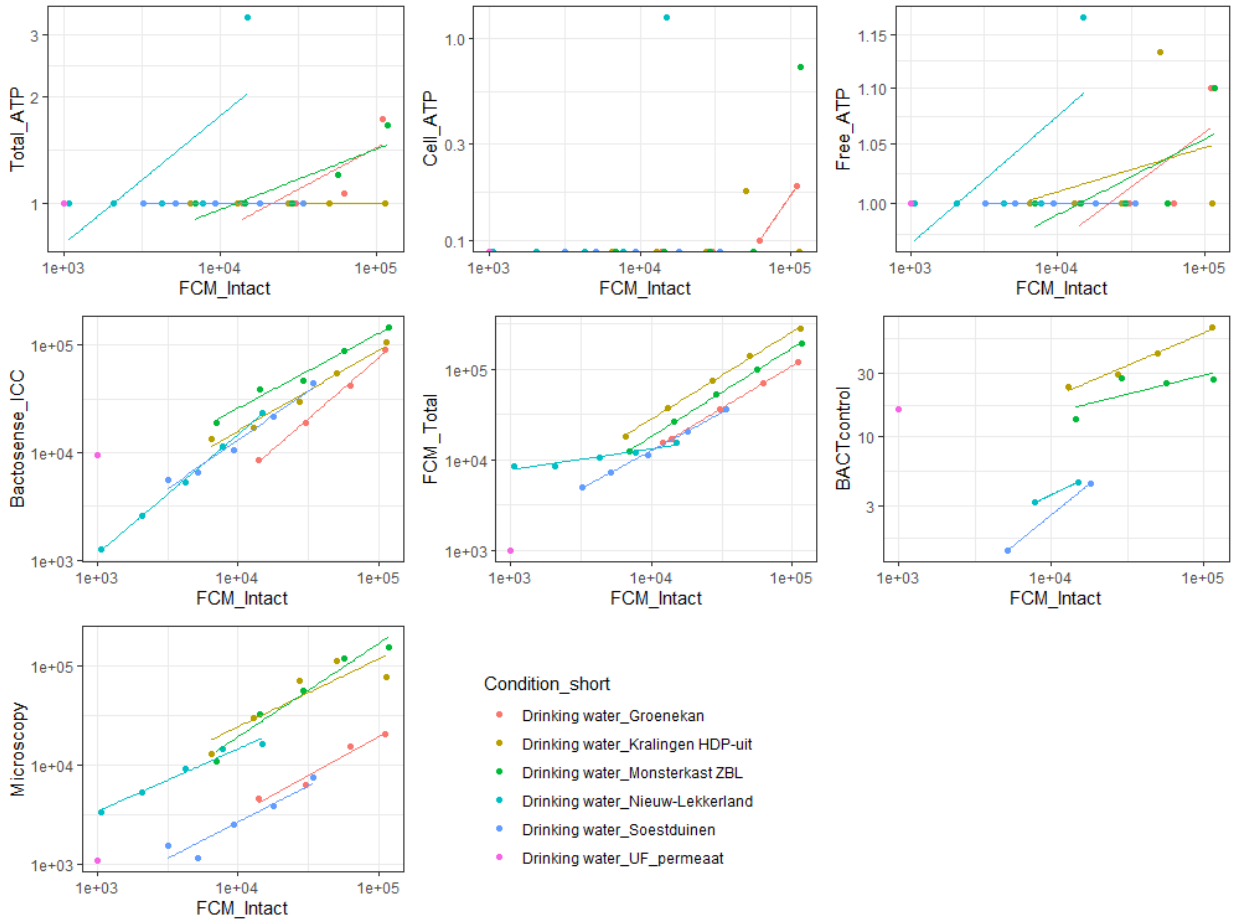

Figure 12-16. Scatter plots of results of validation of drinking water. Shown is the comparison of the FCM_Intact method to the other sensors and laboratory parameters.

IV Scatter plots all water matrices

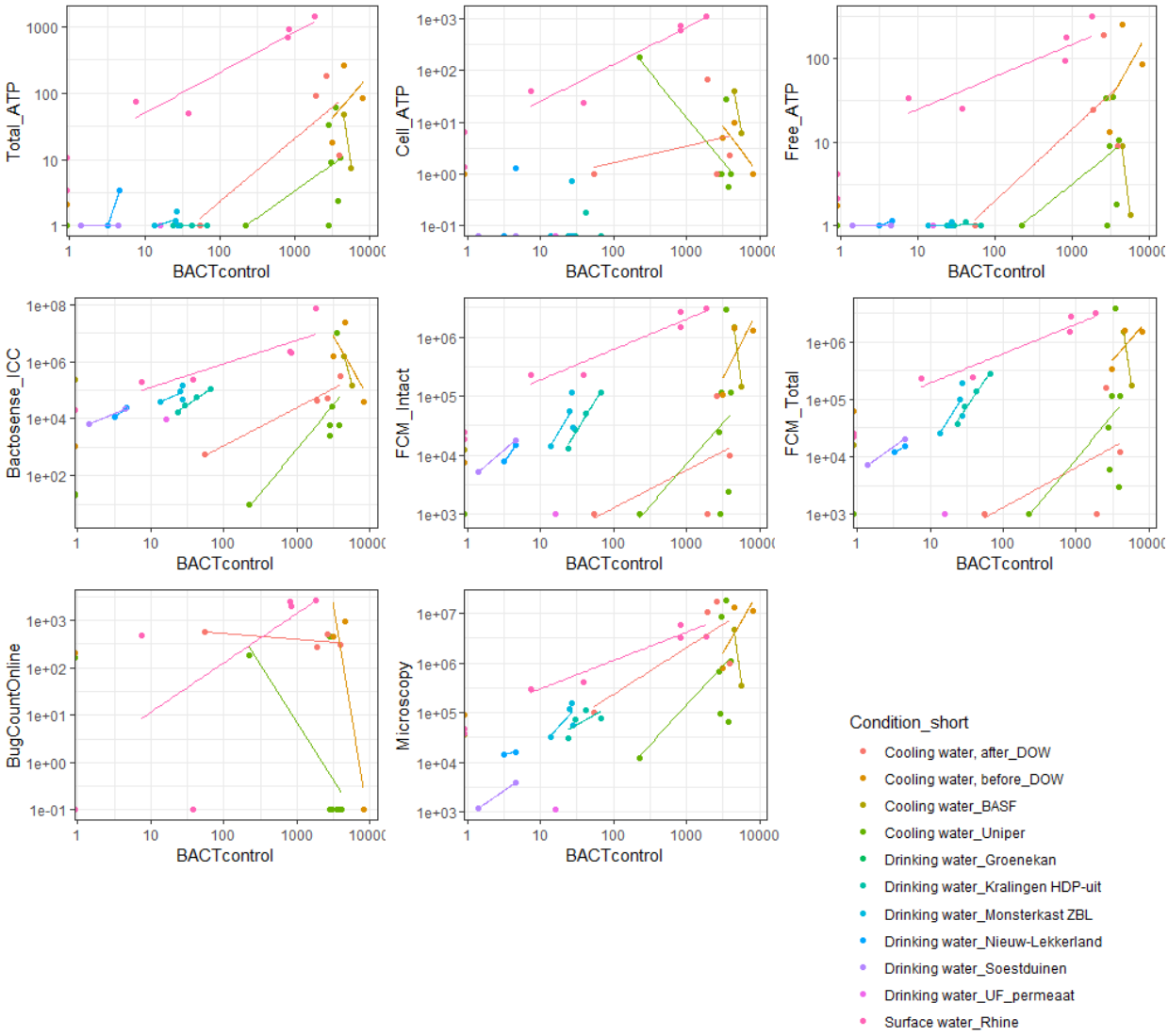

Figure 12-17. Scatter plots of results of validation all water matrices. Shown is the comparison of the BACTcontrol the other sensors and laboratory parameters.

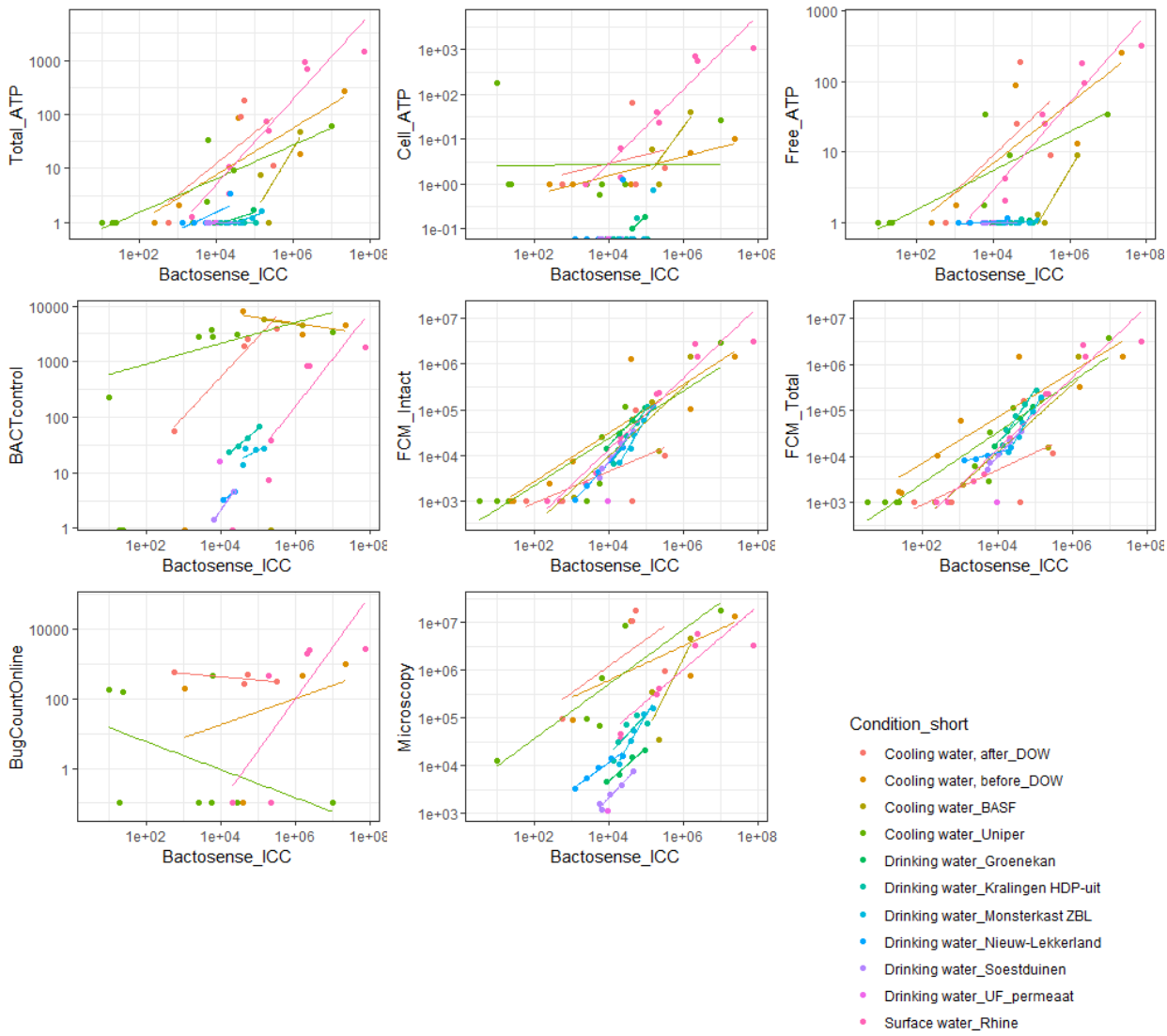

Figure 12-18. Scatter plots of results of validation of all water matrices. Shown is the comparison of the BactoSense_ICC to the other sensors and laboratory parameters.

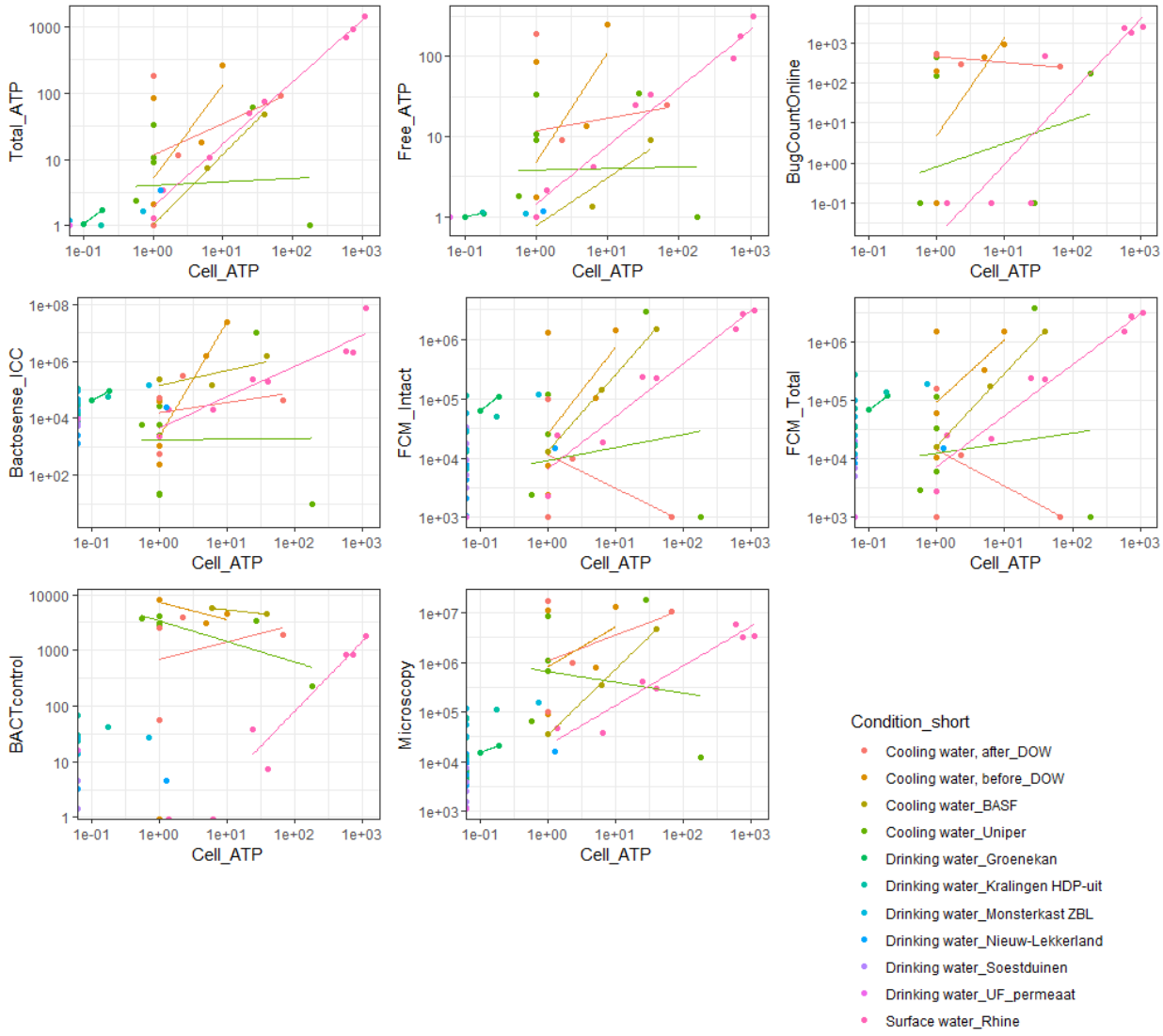

Figure 12-19. Scatter plots of results of validation all water matrices. Shown is the comparison of Cell_ATP to the other sensors and laboratory parameters.

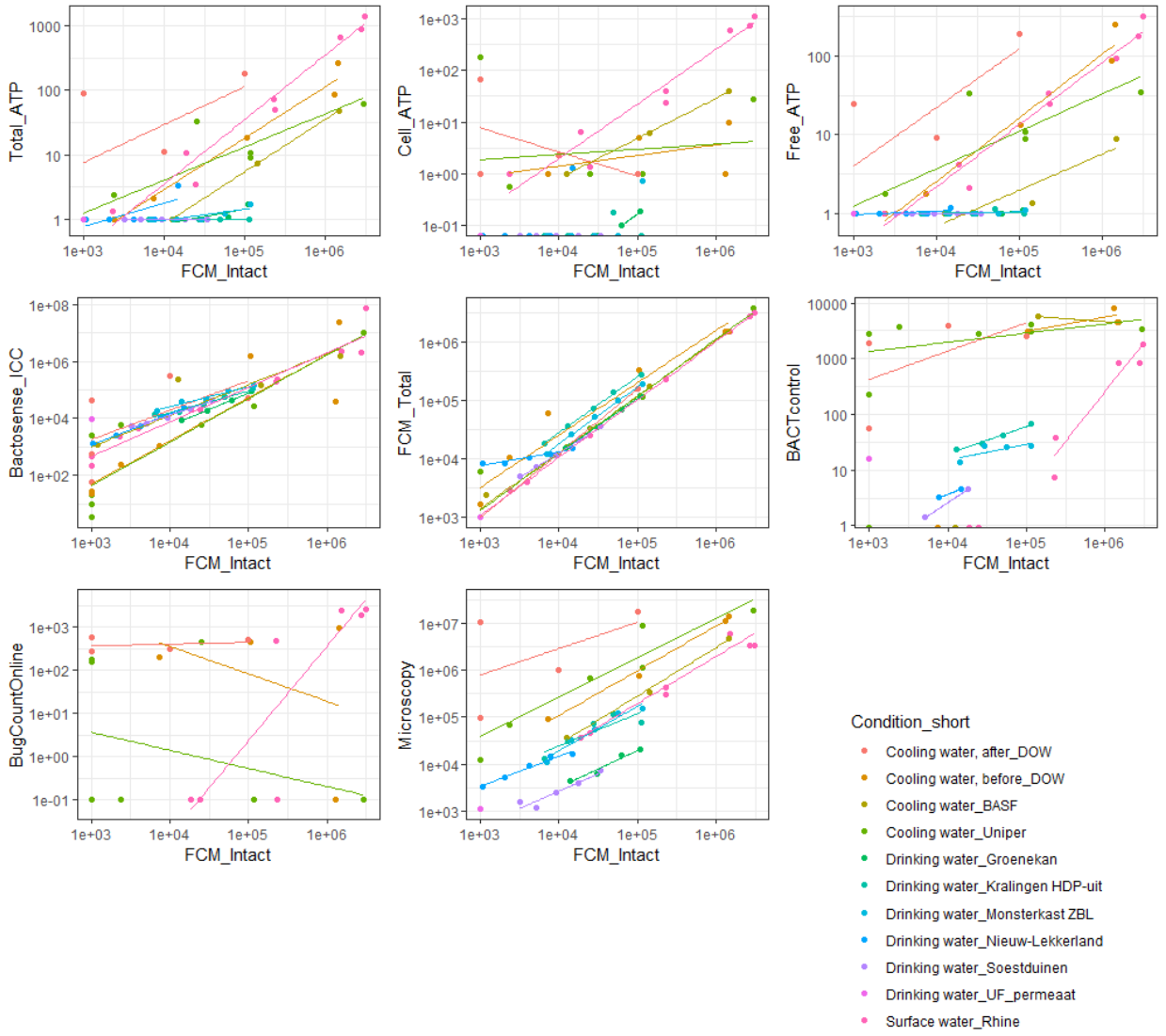

Figure 12-20. Scatter plots of results of validation of all water matrices. Shown is the comparison of the FCM_Intact method to the other sensors and laboratory parameters.

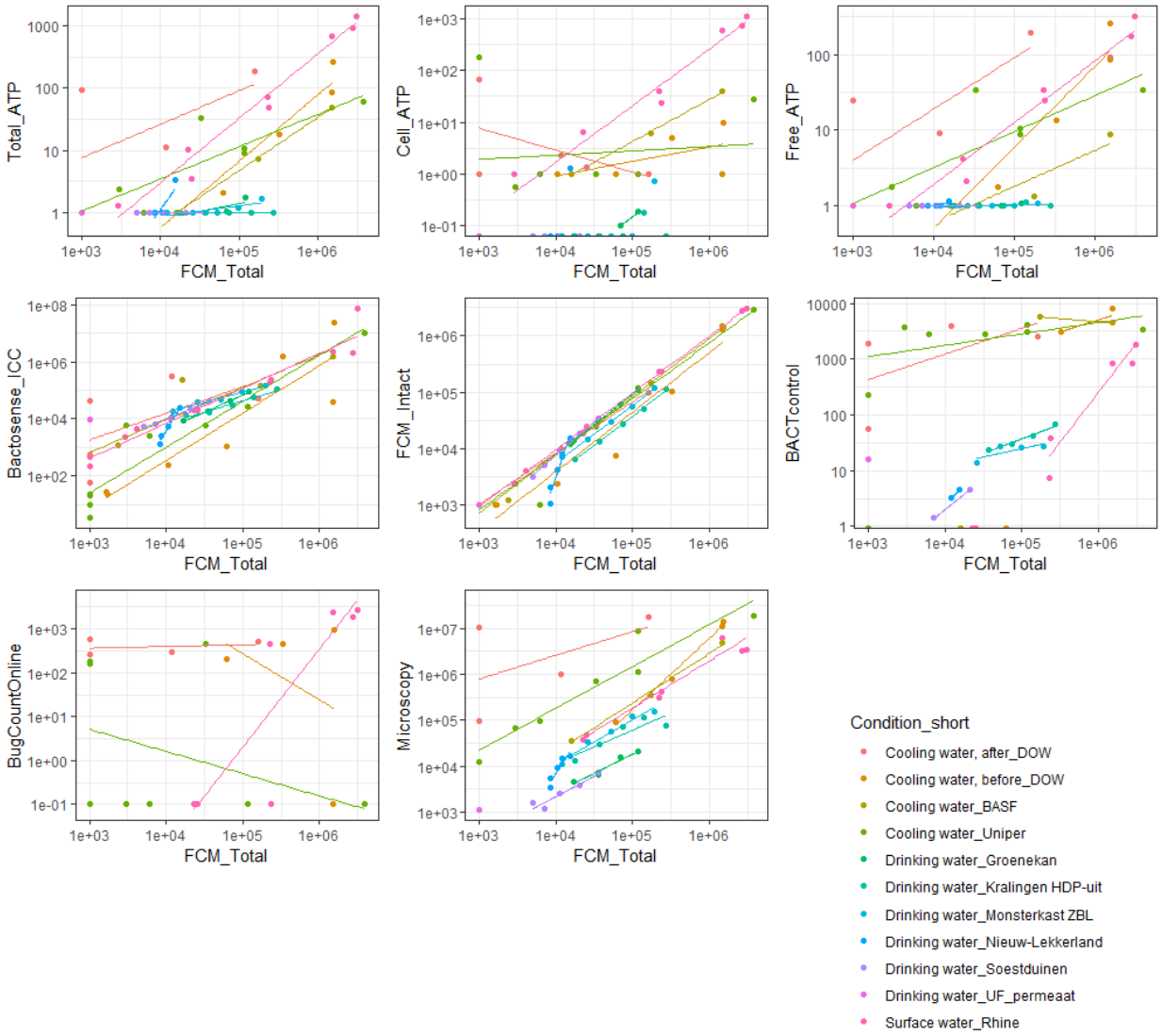

Figure 12-21. Scatter plots of results of validation of all water matrices. Shown is the comparison of the FCM_Total method to the other sensors and laboratory parameters.

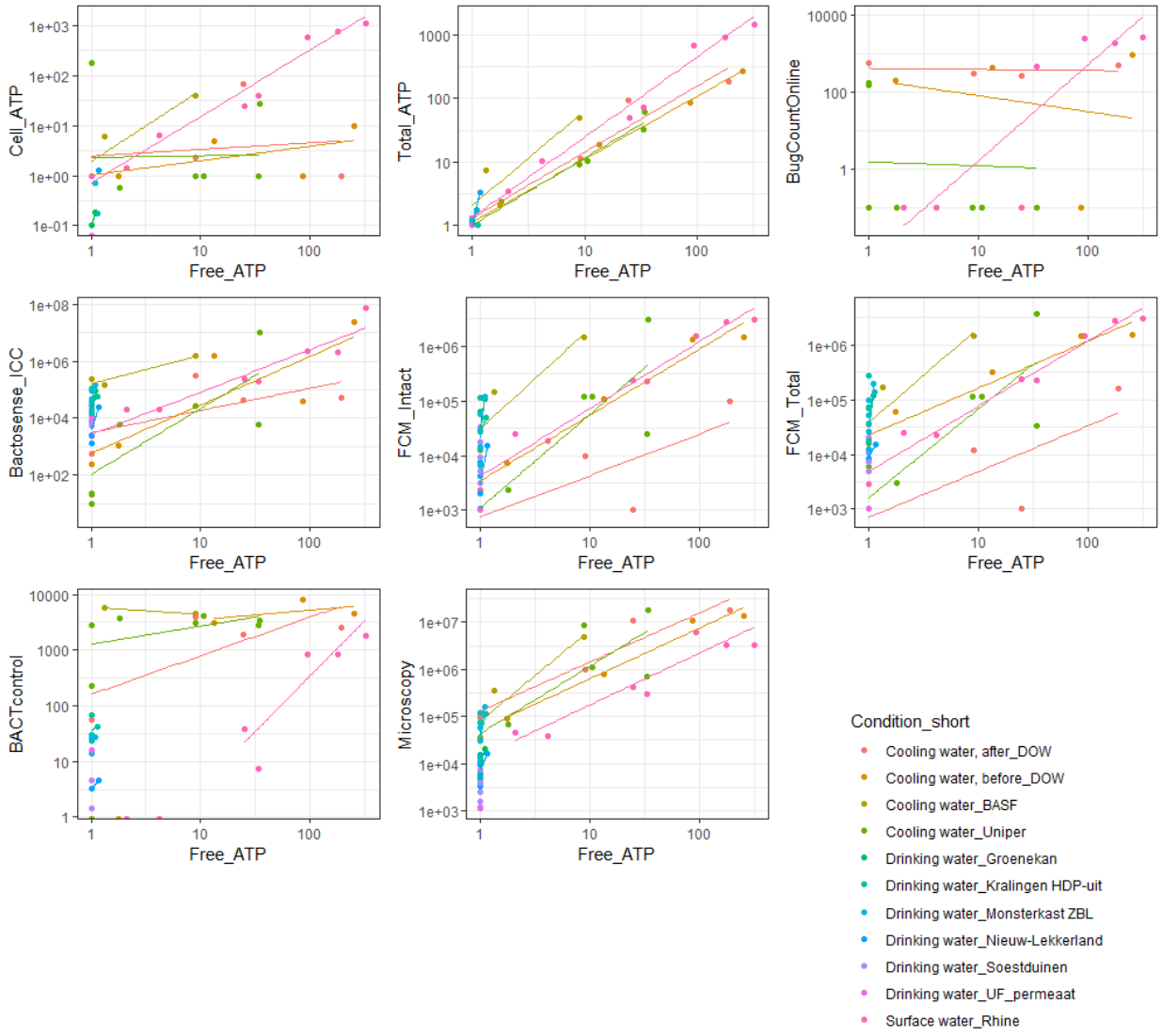

Figure 12-22. Scatter plots of results of validation of all water matrices. Shown is the comparison of Free_ATP to the other sensors and laboratory parameters.

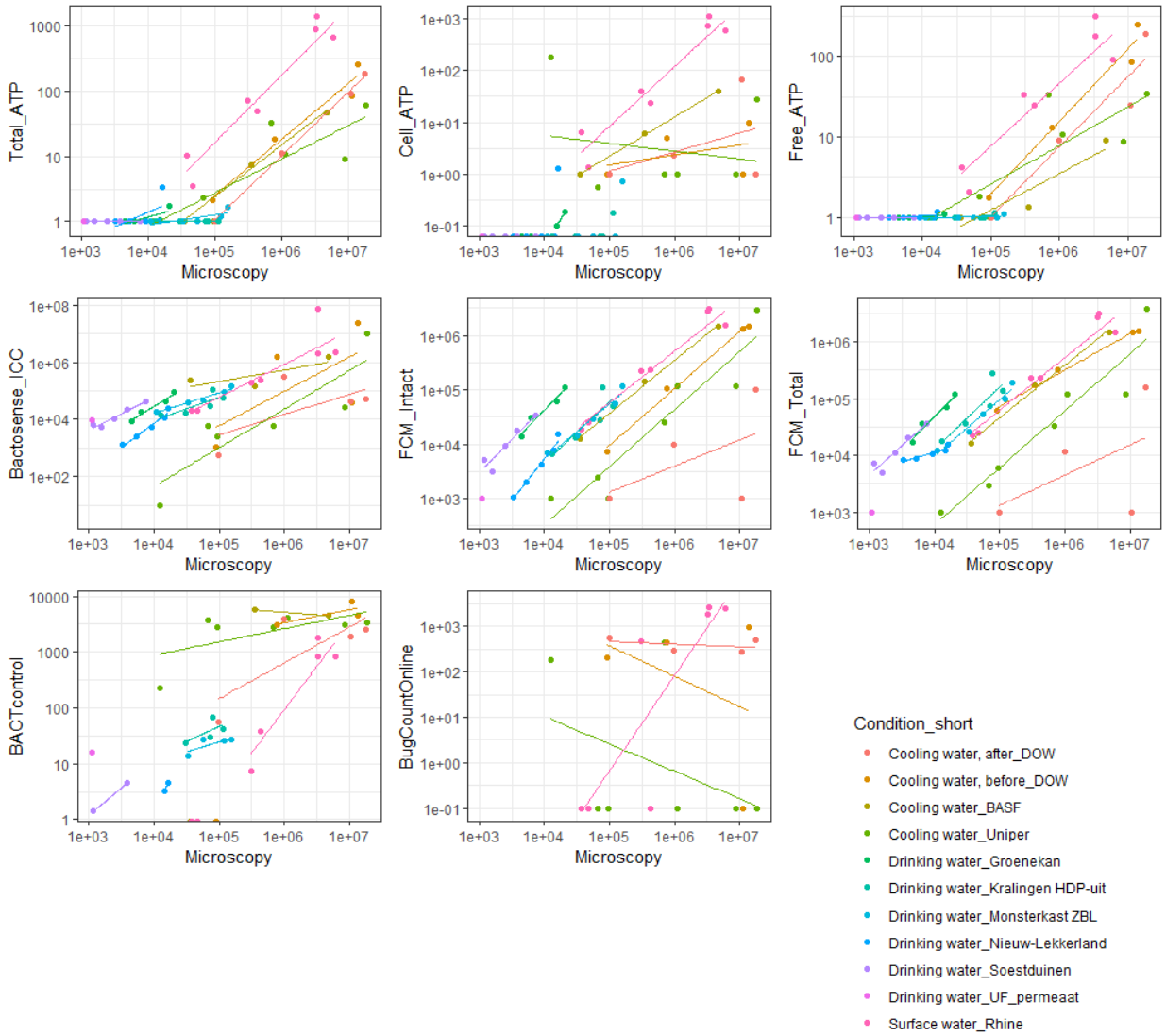

Figure 12-23. Scatter plots of results of validation of all water matrices. Shown is the comparison of the Microscopy method to the other sensors and laboratory parameters.

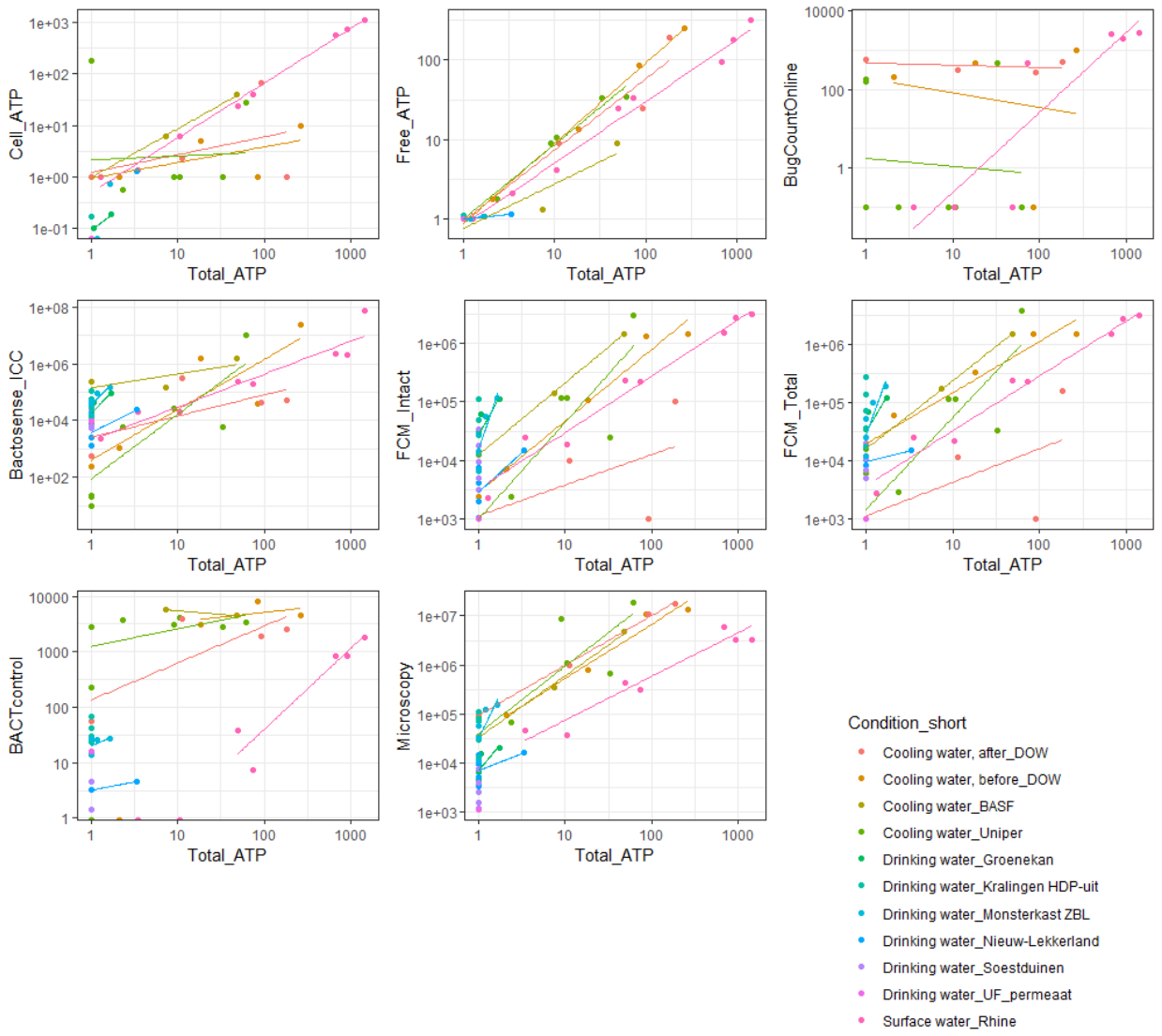

Figure 12-24. Scatter plots of results of validation of all water matrices. Shown is the comparison of the Total_ATP method to the other sensors and laboratory parameters.

V Statistical analysis

		BACTcontrol			BactoSense			BugCount Online		
		p-value	R2	N	p-value	R2	N	p-value	R2	N
All watertypes	BACTcontrol				0,18	0,06	29	0,73	0,01	13
	BactoICC	0,18	0,06	29				0,00	0,62	15
	BugCount	0,73	0,01	13	0,00	0,62	15			
	Cell_ATP				0,07	0,16	21	0,02	0,50	11
	FCM_intact	0,04	0,17	24	0,00	0,79	48	0,00	0,85	9
	FCM_not_intact	0,96	0,00	18	0,00	0,39	42	0,47	0,13	6
	FCM_Total	0,21	0,06	28	0,00	0,72	54	0,01	0,51	12
	Free_ATP	0,49	0,03	19	0,00	0,40	23	0,00	0,60	12
	Microscopy	0,00	0,45	30	0,00	0,41	50	0,03	0,33	14
	Total_ATP	0,44	0,03	21	0,00	0,49	26	0,00	0,80	12
BASF	BACTcontrol					1,00	2	Not measured		
	BactoICC		1,00	2						
	BugCount									
	Cell_ATP		1,00	2		1,00	2			
	FCM_intact		1,00	2	0,11	0,79	4			
	FCM_not_intact		0,00	1	0,54	0,44	3			
	FCM_Total		1,00	2	0,13	0,76	4			
	Free_ATP		1,00	2		1,00	2			
	Microscopy		1,00	2	0,42	0,63	3			
	Total_ATP		1,00	2		1,00	2			
Cooling water	BACTcontrol				0,02	0,62	8		1,00	2
	BactoICC	0,02	0,62	8				0,15	0,94	3
	BugCount		1,00	2	0,15	0,94	3			
	Cell_ATP	0,30	0,26	6	0,84	0,01	6		0,00	1
	FCM_intact	0,68	0,04	7	0,00	0,79	8		0,00	1
	FCM_not_intact	0,84	0,02	5	0,02	0,69	7		0,00	1
	FCM_Total	0,41	0,11	8	0,00	0,81	9		0,00	1
	Free_ATP	0,08	0,50	7	0,55	0,09	6		0,00	1
	Microscopy	0,10	0,34	9	0,03	0,51	9		1,00	2
	Total_ATP	0,62	0,05	7	0,14	0,46	6		0,00	1
Cooling water_after	BACTcontrol				0,02	0,96	4	0,35	0,43	4
	BactoICC	0,02	0,96	4				0,31	0,48	4
	BugCount	0,35	0,43	4	0,31	0,48	4			
	Cell_ATP		1,00	2		1,00	2		1,00	2
	FCM_intact	0,43	0,33	4	0,29	0,36	5	0,76	0,06	4
	FCM_not_intact		1,00	2		1,00	2		1,00	2
	FCM_Total	0,40	0,66	3	0,52	0,47	3	0,90	0,02	3
	Free_ATP	0,74	0,16	3	0,52	0,47	3	0,31	0,78	3
	Microscopy	0,22	0,60	4	0,37	0,40	4	0,63	0,14	4
	Total_ATP	0,43	0,61	3	0,22	0,89	3	0,62	0,32	3
Cooling water_before	BACTcontrol				0,53	0,45	3		1,00	2
	BactoICC	0,53	0,45	3				0,16	0,94	3
	BugCount		1,00	2	0,16	0,94	3			
	Cell_ATP		1,00	2	0,04	1,00	3	0,20	0,90	3
	FCM_intact	0,43	0,61	3	0,09	0,66	5	0,00	1,00	3
	FCM_not_intact	0,66	0,26	3	0,01	0,76	7	0,65	0,27	3
	FCM_Total	0,42	0,63	3	0,00	0,82	7	0,01	1,00	3
	Free_ATP	0,64	0,28	3	0,24	0,57	4	0,08	0,99	3
	Microscopy	0,45	0,58	3	0,34	0,43	4	0,06	0,99	3
	Total_ATP	0,69	0,22	3	0,21	0,62	4	0,05	0,99	3
DOW	BACTcontrol				0,07	0,51	7	0,84	0,01	6
	BactoICC	0,07	0,51	7				0,23	0,27	7
	BugCount	0,84	0,01	6	0,23	0,27	7			
	Cell_ATP	0,23	0,59	4	0,45	0,20	5	0,57	0,12	5
	FCM_intact	0,43	0,61	3	0,09	0,66	5	0,00	1,00	3
	FCM_not_intact	0,92	0,00	5	0,01	0,62	9	0,40	0,24	5
	FCM_Total	0,04	0,69	6	0,01	0,64	10	0,54	0,10	6
	Free_ATP	0,63	0,06	6	0,20	0,31	7	0,03	0,75	6
	Microscopy	0,06	0,54	7	0,12	0,35	8	0,40	0,15	7
	Total_ATP	0,99	0,00	6	0,22	0,29	7	0,08	0,59	6
Drinking water	BACTcontrol				0,32	0,16	8			
	BactoICC	0,32	0,16	8						
	BugCount	Not measured			Not measured					
	Cell_ATP	0,84	0,00	21		1,00	2	Not measured		
	FCM_intact	0,06	0,47	8	0,00	0,84	25			
	FCM_not_intact	0,00	0,83	8	0,00	0,32	25			
	FCM_Total	0,01	0,69	8	0,00	0,66	25			
	Free_ATP	0,94	0,00	21		1,00	2			
	Microscopy	0,25	0,22	8	0,00	0,57	25			
	Total_ATP		1,00	2	0,17	0,69	4			
Groenekan	BACTcontrol	Not measured			Not measured			Not measured		
	BactoICC									
	BugCount				Not measured					
	Cell_ATP					0,00	1			
	FCM_intact				0,00	1,00	4			
	FCM_not_intact				0,02	0,97	4			
	FCM_Total				0,00	1,00	4			
	Free_ATP					0,00	1			
	Microscopy				0,02	0,96	4			
	Total_ATP					0,00	1			

		BACTcontrol			BactoSense			BugCount Online		
		p-value	R2	N	p-value	R2	N	p-value	R2	N
Kralingen	BACTcontrol				0,00	0,99	4	Not measured		
	BactoICC	0,00	0,99	4						
	BugCount	Not measured			Not measured					
	Cell_ATP	0,75	0,06	4	0,59	0,11	5			
	FCM_intact	0,01	0,98	4	0,00	0,98	5			
	FCM_not_intact	0,02	0,97	4	0,00	0,96	5			
	FCM_Total	0,01	0,98	4	0,00	0,97	5			
	Free_ATP	0,75	0,06	4	0,59	0,11	5			
	Microscopy	0,34	0,44	4	0,09	0,67	5			
	Total_ATP		0,00	4		0,00	5			
Nieuw-Lekkerland	BACTcontrol	Not measured			Not measured			Not measured		
	BactoICC									
	BugCount				Not measured					
	Cell_ATP					0,00	1			
	FCM_intact				0,00	1,00	5			
	FCM_not_intact				0,07	0,71	5			
	FCM_Total				0,01	0,94	5			
	Free_ATP					0,00	1			
	Microscopy				0,00	0,96	5			
	Total_ATP					0,00	1			
Rhine	BACTcontrol				0,04	0,70	6	0,00	0,97	5
	BactoICC	0,04	0,70	6				0,14	0,57	5
	BugCount	0,00	0,97	5	0,14	0,57	5			
	Cell_ATP	0,00	0,89	6	0,00	0,85	8	0,01	0,94	5
	FCM_intact	0,00	0,92	6	0,00	0,92	10	0,03	0,85	5
	FCM_not_intact	0,00	0,92	8		0,00	1	0,00	0,76	8
	FCM_Total	0,00	0,92	6	0,00	0,92	10	0,03	0,85	5
	Free_ATP	0,02	0,77	6	0,00	0,89	8	0,12	0,61	5
	Microscopy	0,01	0,89	6	0,00	0,77	8	0,01	0,95	5
	Total_ATP	0,00	0,89	6	0,00	0,91	9	0,01	0,91	5
Soestduinen	BACTcontrol	Not measured			Not measured			Not measured		
	BactoICC									
	BugCount				Not measured					
	Cell_ATP					0,00	5			
	FCM_intact				0,00	0,97	5			
	FCM_not_intact				0,06	0,75	5			
	FCM_Total				0,00	0,99	5			
	Free_ATP					0,00	5			
	Microscopy				0,00	0,95	5			
	Total_ATP					0,00	5			
Surface Water	BACTcontrol				0,04	0,70	6	0,00	0,97	5
	BactoICC	0,04	0,70	6				0,14	0,57	5
	BugCount	0,00	0,97	5	0,14	0,57	5			
	Cell_ATP	0,00	0,89	6	0,00	0,85	8	0,01	0,94	5
	FCM_intact	0,00	0,92	6	0,00	0,92	10	0,03	0,85	5
	FCM_not_intact	0,00	0,92	8		0,00	1	0,00	0,76	8
	FCM_Total	0,00	0,92	6	0,00	0,92	10	0,03	0,85	5
	Free_ATP	0,02	0,77	6	0,00	0,89	8	0,12	0,61	5
	Microscopy	0,01	0,89	6	0,00	0,77	8	0,01	0,95	5
	Total_ATP	0,00	0,89	6	0,00	0,91	9	0,01	0,91	5
Uniper	BACTcontrol				0,08	0,58	6		1,00	2
	BactoICC	0,08	0,58	6				0,15	0,94	3
	BugCount				0,15	0,94	3			
	Cell_ATP	0,51	0,62	20	0,73	0,07	4		0,00	1
	FCM_intact	0,93	0,00	5	0,08	0,84	4		0,00	1
	FCM_not_intact	0,97	0,00	4	0,07	0,87	4		0,00	1
	FCM_Total	0,78	0,02	6	0,02	0,88	5		0,00	1
	Free_ATP	0,36	0,28	5	0,48	0,27	4		0,00	1
	Microscopy	0,14	0,38	7	0,02	0,78	6		1,00	2
	Total_ATP	0,45	0,20	5	0,34	0,44	4		0,00	1
Zuid-Beijerland	BACTcontrol				0,39	0,38	4	Not measured		
	BactoICC	0,39	0,38	4						
	BugCount	Not measured			Not measured					
	Cell_ATP	0,63	0,14	4	0,17	0,52	5			
	FCM_intact	0,24	0,58	4	0,00	0,98	5			
	FCM_not_intact	0,21	0,62	4	0,00	0,97	5			
	FCM_Total	0,23	0,60	4	0,00	0,98	5			
	Free_ATP	0,63	0,14	4	0,17	0,52	5			
	Microscopy	0,24	0,58	4	0,00	0,96	5			
	Total_ATP		1,00	2		1,00	2			

VI CBM results

Table 12-4. CBM results on drinking water at KWR. Results of the three individual CBMs, including the average and SD of the three CBMs are given.

		KWR			DSA-swab (LuminUltra)			DSA-swab / KWR
Sampling date	Run time CBM (days)	ATP (pg/cm²)	ATP, SD	BAR (pg/cm²/dag)	ATP (pg/cm²)	ATP, SD	BAR (pg/cm²/dag)	Factor
12-1-2021	36	1150	71	32	1728	143	48	1.5
		1100	0	31	1612	418	45	1.5
		655	64	18	1638	513	45	2.5
	Average	968		27	1659		46	
	SD	273		8	61		2	
25-1-2021	13	995	148	77	903	182	69	0.9
		515	64	40	1074	99	83	2.1
		345	134	27	1438	124	111	4.2
	Average	618		48	1138		88	
	SD	337		26	273		21	
1-2-2021	7	71	18	10	155	42	22	2.2
		89	6	13	145	54	21	1.6
		48	6	7	131	12	19	2.8
	Average	69		10	144		21	
	SD	21		3	12		2	
11-2-2021	10	56	35	6	68	10	7	1.2
		64	21	6	99	21	10	1.6
		42	4	4	78	31	8	1.9
	Average	54		5	82		8	
	SD	11		1	16		2	
4-3-2021	21	170	28	8	182	9	9	1.1
		210	57	10	284	36	14	1.4
		93	8	4	148	22	7	1.6
	Average	158		8	205		10	
	SD	60		3	70		3	
1-4-2021	28	345	21	12	637	228	23	1.8
		270	42	10	615	85	22	2.3
		270	42	10	641	150	23	2.4
	Average	295		11	631		23	
	SD	43		2	14		1	

Table 12-5. CBM results on surface water of the river Meuse at Keizersveer. Results of the three individual CBMs are given.

		KWR			DSA-swab (LuminUltra)			DSA-swab / KWR
Sampling date	Run time CBM (days)	ATP (pg/cm²)	ATP, SD	BFR (pg/cm²/dag)	ATP (pg/cm²)	ATP, SD	BFR (pg/cm²/dag)	Factor
18-1-2021	3	45	6	15	163	20	54	3.7
		15	0	5	188	1	63	12.6
		54	21	18	253	16	84	4.7
	Average	38		13	201		67	
	SD	20		7	46		15	
1-2-2021	14	1850	71	132	3453	1847	247	1.9
		1295	714	93	4484	859	320	3.5
		885	728	63	3674	1967	262	4.2
	Average	1343		96	3871		276	
	SD	484		35	543		39	
11-2-2021	10	2350	778	235	3202	1209	320	1.4
		1395	573	140	3958	1176	396	2.8
		1435	940	144	3075	755	308	2.1
	Average	1727		173	3412		341	
	SD	540		54	477		48	
1-3-2021	19	1100	0	58	8692	3570	457	7.9
		2900	1273	153	10342	5038	544	3.6
		3350	354	176	13055	12635	687	3.9
	Average	2450		129	10696		563	
	SD	1191		63	2203		116	
4-3-2021	3	43	33	14	501	167	167	11.6
		87	6	29	389	51	130	4.5
		63	4	21	412	40	137	6.5
	Average	64		21	434		145	
	SD	22		7	59		20	
1-4-2021	28	745	502	27	8935	2140	319	12.0
		1450	495	52	5515	3905	197	3.8
		2500	1273	89	10534	4911	376	4.2
	Average	1565		56	8328		297	
	SD	883		32	2564		92	
8-4-2021	7	1070	467	153	4421	2594	632	4.1
		220	14	31	4253	570	608	19.3
		715	544	102	5915	1489	845	8.3
	Average	668		95	4863		695	
	SD	427		61	915		131	

VII Statistical analysis CBM according to NEN 16140-2

VII.I Accuracy profile, drinking and surface water combined

Table 12-6: Accuracy profile CBM

Run time CBM (days)		3	3	7	10	14	19	28	7	10	13	21	28	36
Reference method		45	43	1070	2350	1850	1100	745	71	56	995	170	345	1150
		15	87	220	1395	1295	2900	1450	89	64	515	210	270	1100
		54	63	715	1435	885	3350	2500	48	42	345	93	270	655
Alternative method		163	501	4421	3202	3453	8692	8935	155	68	903	182	637	1728
		188	389	4253	3958	4484	10342	5515	145	99	1074	284	615	1612
		253	412	5915	3075	3674	13055	10534	131	78	1438	148	641	1638
Reference method (Log_{10} transformed)		1.65	1.63	3.03	3.37	3.27	3.04	2.87	1.85	1.74	3.00	2.23	2.54	3.06
		1.18	1.94	2.34	3.14	3.11	3.46	3.16	1.95	1.80	2.71	2.32	2.43	3.04
		1.73	1.80	2.85	3.16	2.95	3.53	3.40	1.68	1.62	2.54	1.97	2.43	2.82
Step 1	X_i	1.65	1.80	2.85	3.16	3.11	3.46	3.16	1.85	1.74	2.71	2.23	2.43	3.04
Alternative method (Log_{10} transformed)		2.21	2.70	3.65	3.51	3.54	3.94	3.95	2.19	1.83	2.96	2.26	2.80	3.24
		2.27	2.59	3.63	3.60	3.65	4.01	3.74	2.16	2.00	3.03	2.45	2.79	3.21
		2.40	2.62	3.77	3.49	3.57	4.12	4.02	2.12	1.89	3.16	2.17	2.81	3.21
Step 2	Y_i	2.27	2.62	3.65	3.51	3.57	4.01	3.95	2.16	1.89	3.03	2.26	2.80	3.21
Step 3	$s_{alt,i}$	0.097	0.057	0.078	0.059	0.059	0.089	0.146	0.037	0.084	0.102	0.144	0.010	0.016
	n	3	3	3	3	3	3	3	3	3	3	3	3	3
	$\frac{1}{n-1}$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	$\bar{Y}_i$	2.30	2.63	3.68	3.53	3.59	4.02	3.91	2.16	1.91	3.05	2.29	2.80	3.22
	$\sum(Y_{ij} - \bar{Y}_i)^2$	0.02	0.01	0.01	0.01	0.01	0.02	0.04	0.00	0.01	0.02	0.04	0.00	0.00
Step 4	s_{alt}	0.085												
	q	13	13	13	13	13	13	13	13	13	13	13	13	13
	$\frac{1}{q}$	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077
	${s_{alt}}^2$	0.009	0.003	0.006	0.003	0.004	0.008	0.021	0.001	0.007	0.010	0.021	0.000	0.000
Step 5	$s_{ref,i}$	0.298	0.153	0.357	0.127	0.160	0.263	0.263	0.137	0.094	0.232	0.185	0.061	0.136
	n	3	3	3	3	3	3	3	3	3	3	3	3	3
	$\frac{1}{n-1}$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	$\bar{X}_i$	1.52	1.79	2.74	3.22	3.11	3.34	3.14	1.82	1.72	2.75	2.17	2.47	2.97
	$\sum(X_{ij} - \bar{X}_i)^2$	0.18	0.05	0.25	0.03	0.05	0.14	0.14	0.04	0.02	0.11	0.07	0.01	0.04
	$\frac{1}{n}$	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
	${s_{ref,i}}^2$	0.089	0.023	0.127	0.016	0.026	0.069	0.069	0.019	0.009	0.054	0.034	0.004	0.018
	s_{ref}	0.431												
Step 6	$B_i = Y_i - X_i$	0.63	0.82	0.79	0.35	0.45	0.55	0.79	0.31	0.15	0.32	0.03	0.37	0.17

Run time CBM (days)		3	3	7	10	14	19	28	7	10	13	21	28	36
Step 7	$s_{alt}\sqrt{1+\frac{1}{n}}$	0.099												
	$T_{\left(\frac{1-\beta}{2};q(n-1)\right)}$	1.315												
	$T \cdot s \cdot \sqrt{}$	0.130												
	U_i	0.76	0.95	0.92	0.48	0.58	0.68	0.92	0.44	0.28	0.45	0.16	0.50	0.30
	L_i	0.50	0.69	0.66	0.22	0.32	0.42	0.66	0.18	0.02	0.19	-0.10	0.24	0.04

VII.II Accuracy profile, drinking water

Table 12-7: Accuracy profile CBM drinking water

Run time CBM (days)		7	10	13	21	28	36
Reference method		71	56	995	170	345	1150
		89	64	515	210	270	1100
		48	42	345	93	270	655
Alternative method		155	68	903	182	637	1728
		145	99	1074	284	615	1612
		131	78	1438	148	641	1638
Reference method (Log_{10} transformed)		1.85	1.74	3.00	2.23	2.54	3.06
		1.95	1.80	2.71	2.32	2.43	3.04
		1.68	1.62	2.54	1.97	2.43	2.82
Step 1	X_i	1.85	1.74	2.71	2.23	2.43	3.04
Alternative method (Log_{10} transformed)		2.19	1.83	2.96	2.26	2.80	3.24
		2.16	2.00	3.03	2.45	2.79	3.21
		2.12	1.89	3.16	2.17	2.81	3.21
Step 2	Y_i	2.16	1.89	3.03	2.26	2.80	3.21
Step 3	$s_{alt,i}$	0.037	0.084	0.102	0.144	0.010	0.016
	n	3	3	3	3	3	3
	$\frac{1}{n-1}$	0.5	0.5	0.5	0.5	0.5	0.5
	$\bar{Y}_i$	2.16	1.91	3.05	2.29	2.80	3.22
	$\sum(Y_{ij} - \bar{Y}_i)^2$	0.00	0.01	0.02	0.04	0.00	0.00
Step 4	s_{alt}	0.082					
	q	6	6	6	6	6	6
	$\frac{1}{q}$	0.167	0.167	0.167	0.167	0.167	0.167
	${s_{alt}}^2$	0.001	0.007	0.010	0.021	0.000	0.000
Step 5	$s_{ref,i}$	0.137	0.094	0.232	0.185	0.061	0.136
	n	3	3	3	3	3	3
	$\frac{1}{n-1}$	0.5	0.5	0.5	0.5	0.5	0.5
	$\bar{X}_i$	1.82	1.72	2.75	2.17	2.47	2.97
	$\sum(X_{ij} - \bar{X}_i)^2$	0.04	0.02	0.11	0.07	0.01	0.04
	$\frac{1}{n}$	0.33	0.33	0.33	0.33	0.33	0.33
	${s_{ref,i}}^2$	0.019	0.009	0.054	0.034	0.004	0.018
	s_{ref}	0.215					
Step 6	$B_i = Y_i - X_i$	0.31	0.15	0.32	0.03	0.37	0.17

Run time CBM (days)		7	10	13	21	28	36
Step 7	$s_{alt}\sqrt{1+\frac{1}{n}}$	0.094					
	$T_{\left(\frac{1-\beta}{2};q(n-1)\right)}$	1.356					
	$T \cdot s \cdot \sqrt{}$	0.128					
	U_i	0.44	0.28	0.45	0.16	0.50	0.30
	L_i	0.18	0.02	0.19	-0.10	0.25	0.05

VII.III Accuracy profile, surface water

Table 12-8: Accuracy profile CBM surface water

		Surface water						
Item (days)		3	3	7	10	14	19	28
Reference method		45	43	1070	2350	1850	1100	745
		15	87	220	1395	1295	2900	1450
		54	63	715	1435	885	3350	2500
Alternative method		163	501	4421	3202	3453	8692	8935
		188	389	4253	3958	4484	10342	5515
		253	412	5915	3075	3674	13055	10534
Reference method (Log_{10} transformed)		1.65	1.63	3.03	3.37	3.27	3.04	2.87
		1.18	1.94	2.34	3.14	3.11	3.46	3.16
		1.73	1.80	2.85	3.16	2.95	3.53	3.40
Step 1	X_i	1.65	1.80	2.85	3.16	3.11	3.46	3.16
Alternative method (Log_{10} transformed)		2.21	2.70	3.65	3.51	3.54	3.94	3.95
		2.27	2.59	3.63	3.60	3.65	4.01	3.74
		2.40	2.62	3.77	3.49	3.57	4.12	4.02
Step 2	Y_i	2.27	2.62	3.65	3.51	3.57	4.01	3.95
Step 3	$s_{alt,i}$	0.097	0.057	0.078	0.059	0.059	0.089	0.146
	n	3	3	3	3	3	3	3
	$\frac{1}{n-1}$	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	$\bar{Y}_i$	2.30	2.63	3.68	3.53	3.59	4.02	3.91
	$\sum(Y_{ij} - \bar{Y}_i)^2$	0.02	0.01	0.01	0.01	0.01	0.02	0.04
Step 4	s_{alt}	0.089						
	q	7	7	7	7	7	7	7
	$\frac{1}{q}$	0.143	0.143	0.143	0.143	0.143	0.143	0.143
	${s_{alt}}^2$	0.009	0.003	0.006	0.003	0.004	0.008	0.021
Step 5	$s_{ref,i}$	0.298	0.153	0.357	0.127	0.160	0.263	0.263
	n	3	3	3	3	3	3	3
	$\frac{1}{n-1}$	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	$\bar{X}_i$	1.52	1.79	2.74	3.22	3.11	3.34	3.14
	$\sum(X_{ij} - \bar{X}_i)^2$	0.18	0.05	0.25	0.03	0.05	0.14	0.14
	$\frac{1}{n}$	0.33	0.33	0.33	0.33	0.33	0.33	0.33
	${s_{ref,i}}^2$	0.089	0.023	0.127	0.016	0.026	0.069	0.069
	s_{ref}	0.374						
Step 6	$B_i = Y_i - X_i$	0.63	0.82	0.79	0.35	0.45	0.55	0.79

		Surface water						
Item (days)		3	3	7	10	14	19	28
Step 7	$s_{alt}\sqrt{1+\frac{1}{n}}$	0.102						
	$T_{\left(\frac{1-\beta}{2};q(n-1)\right)}$	1.345						
	$T \cdot s \cdot \sqrt{}$	0.138						
	U_i	0.76	0.95	0.93	0.49	0.59	0.69	0.93
	L_i	0.49	0.68	0.65	0.21	0.32	0.41	0.65