# Reducing bacterial growth in cold water dispensers

F.N.J. Huijgens

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# Reducing bacterial growth in cold water dispensers

by

F.N.J. Huijgens

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| Thesis committee: | Prof. dr. ir. J.P. van der Hoek, | TU Delft, Drinking water |
| | Dr. Ir. L. Zlatanovic, | TU Delft, Drinking water |
| | Dr. Ir. H. Spanjers, | TU Delft, Industrial water |




TU Delft

# Abstract

Cold and Granular Activated Carbon filtered Water Dispensers (CGWDs) have been marketed as the solution for those who have access to drinking water of proper quality but wish to have immediately available cold water with a better taste than drinking water. CGWDs are devices that are i) directly connected to the drinking water network, ii) equipped with a Granular Activated Carbon (GAC) filter, and iii) equipped with a cooling reservoir. CGWDs are widely used and are becoming even more popular. However, in previous studies, CGWDs appeared to be vulnerable to bacterial growth, i.e. the bacterial quantities in the effluent had increased compared to those in the influent. More alarming, however, was the presence of opportunistic and pathogenic bacteria in the effluent, while being absent in the influent. The objectives of this study are to assess whether suggested measures can decrease the bacterial growth within CGWDs and to examine whether the free chlorine and organic matter removal performances are affected by the implementation of these measures.

In experiment 1, three different configurations of CGWDs were examined in an experimental form. The first configuration was that of a conventional CGWD (CGWD-C). In the second configuration, a boiler was implemented as pre-treatment of the influent and in addition, the GAC filter was placed into the boiler (CGWD-B). The third configuration was similar to the second configuration, except the CGWDs were first exposed to steam prior to the experiment (CGWD-B&S). On every weekday, during a period of 56 days, 2 liters of water was drawn from the CGWDs. The water quality of the influent was similar to that of typical English drinking water. On eight different days during the experiment, samples from the influent and effluent were microbiologically analyzed. Intact cell count (ICC), total cell count (TCC), heterotrophic plate count at 22°C (HPC22), and cellular ATP (cATP) were enumerated. The ICC in the effluent of CGWD-C was significantly higher than the ICC in the influent. In contrast, no significant difference was observed between the ICC in the effluent of CGWD-B and the ICC influent, while the ICC in the effluent of the CGWD-B&S was significantly lower than the ICC in the influent. The maximum values of ICC in the effluent of CGWD-C, CGWD-B, and CGWD-B&S were, respectively, 960%, 84%, and 49% higher than the ICC in the influent. The maximum values of HPC22 in the effluent of CGWD-C, CGWD-B, and CGWD-B&S during the experiment were, respectively, $6.8 \times 10^4$, $7.6 \times 10^5$, and $1.2 \times 10^5$ cfu/mL, which was above the applicable quality standard of 100 cfu/mL from the European Drinking Water Directive. However, the HPC22 in the effluent of CGWD-B&S was significantly lower than the HPC22 in the effluent of CGWD-C and CGWD-B. Moreover, CGWD-B&S was the only configuration being compliant to the water quality standards during the first 37 hours. The cATP concentration in the effluent of CGWD-B&S1 was similar to that of chlorinated drinking water, whereas the cATP concentration of CGWD-B and CGWD-C increased. At the end of the experiment, the bacterial activity in the filter bed material has been analyzed using High Energy Sonification followed by ATP analysis. The measured tATP concentrations in the treated samples of CGWD-B and CGWD-B&S were below the detection limit of 1 ng ATP/L, whereas the samples of the CGWD-C contained $2.5 \times 10^3$ pg ATP / g GAC.

In experiment 2, the TOC and chlorine removal performances of GAC filters treating room temperature water were compared to that of boilers with an integrated GAC filter. During a period of 312 hours, the filters were operated continuously with an average flow rate of 1.7 L/min. The free chlorine and total organic carbon (TOC) concentrations were measured in respectively, 95 and 50 samples. The TOC and chlorine removal efficiency of the boilers with integrated GAC was significantly higher than that of the boilers treating room temperature water.

The degree of bacterial growth in CGWDs could be decreased with the implementation of the boiler with an integrated GAC filter. By exposing the CGWD to steam prior to the experiment the degree of bacterial growth in the CGWD could be reduced even more. Placing the GAC filter into the boiler prevented the bacteria from growing onto the filter material. On the other hand, it was not enough to keep the heterotrophic bacteria from growing to numbers above the applicable standard. However, with the application of steam, it was possible to reduce the numbers of HPC22 below the detection limit during the first 37 hours. This shows a potential for periodic steam sterilization of CGWDs. Moreover, placing the GAC filter into the boiler increased the TOC removal efficiency of the CGWDs. Implementing a boiler as pre-treatment ensured that the effluent of the CGWDs contained no free chlorine.



Keywords: Drinking water, Point-of-Use, Granular Activated Carbon, Bacterial growth, Sterilization

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While looking back at the graduation process, I can say that I took a leap in faith and dived into the lab to discover the ins and outs of bacterial growth and cold water dispensers. My goal for this thesis was to conduct research that will contribute to scientific knowledge and at the same time provide actual usefulness to current practice. I hope succeeded in this goal, and I hope you will enjoy reading my graduation thesis.

Frank Huijgens

*Rotterdam, January 2021*

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# Acronyms

| | |
|---|---|
| **AOC** | Assimilable Organic Carbon |
| **ATP** | Adenosine Triphosphate |
| **cATP** | Cellular Adenosine Triphosphate |
| **CDS** | Chemical Dosing System |
| **CGWD** | Cold and Granular Activated Carbon filtered Water Dispenser |
| **DCC** | Dead Cell Count |
| **DWTP** | Drinking Water Treatment Plant |
| **fATP** | Free Adenosine Triphosphate |
| **FCM** | Flowcytometry |
| **GAC** | Granular Activated Carbon |
| **HES** | High Energy Sonifier |
| **HPC22** | Heterotrophic plate counts at 22 degree Celsius |
| **ICC** | Intact Cell Count |
| **NPOC** | Non-Purgeable Organic Carbon |
| **PAC** | Powdered Activated Carbon |
| **PoU** | Point of Use |
| **RLU** | Relative Light Units |
| **tATP** | Total Adenosine Triphosphate |
| **TCC** | Total Cell Count |
| **TMS** | Temperature Management System |
| **TOC** | Total Organic Carbon |

# 1 Introduction

## 1.1. Drinking water: from the tap, bottle, or PoU device

Roughly said, there could be two reasons why people are not willing to drink water from the tap. First of all, potential health hazards due to poor water quality could be a reason for not drinking water from the tap. The presence of contaminants such as pathogens, heavy metals, pesticides, pharmaceutical residues, and complex industrial chemicals, like PFASs, are potential hazards for health (WHO, 2003). However, if health hazards are not an issue, a second reason could be the poor taste of the water, for example, caused by organic matter and chlorine (US EPA, 2006).

Both reasons, potential health hazards and poor taste ensured the demand for alternatives for tap water. For instance, in Southern Asia, 65% of the population used other improved sources for drinking water, rather than piped water on premises in 2012 (United Nations, 2014). One of the most popular alternatives for tap water is bottled water. In 2017 bottled water became the most popular beverage in the United States (Karg, 2018). However, Evian, one of the largest producer of bottled water in the United States of America, experienced a backlash in 2018 as consumers started to avoid disposable single-use plastics due to more strict governmental regulations (Saabira Chaudhuri, 2018). Point-of-Use (PoU) devices have been marketed as the solution for those who would like to drink water that is safe for health, tasting better, and readily available. PoU devices treat the water locally at the point of use, i.e. under or around the sink, just before consumption.

## 1.2. Problem statement

The global market share of PoU devices is expected to grow from 15 billion US dollars in 2020 to 23.4 billion US dollars by 2025, with an annual growth of almost 10% (Markets and Markets, 2020). While PoU devices are increasingly used around the world, concerns have been raised about increased risks of water-borne illness as a result of consuming water from PoU devices (Reynolds et al., 2008; Sacchetti et al., 2015).

During long periods of stagnation and in case of inadequate maintenance, PoU devices appeared to be vulnerable to bacterial growth (Zanetti et al., 2009). Previous studies showed that building plumbing systems are more vulnerable to bacterial growth than distribution pipelines, because of a higher ratio of surface area to volume, higher decay rates of disinfectant, and longer stagnation times with higher temperatures (Lautenschlager et al., 2010; Wen et al., 2015; Zlatanović et al., 2017). The same conditions are expected to increase bacterial growth within PoU devices (Park et al., 2019). It is known that bacteria can easily adhere to narrow polymeric tubes and connecting parts (Walker & Marsh, 2007). These kind of materials are also widely applied in PoU devices. After the adhering of the bacteria, a layer of extracellular polymeric substance will be formed on the surfaces (Szymańska, 2005). Within the formed layer, the so-called biofilm, bacteria will cultivate and eventually grow to higher quantities. At a certain point in time, a part of the bacteria will detach from the biofilm and immerse into the water. After which the process of adhering and immersing will repeat itself on new surfaces, and thus the bacterial quantities will increase over time (Girolamini et al., 2019). This cyclic process is known to promote the growth of opportunistic and pathogenic bacteria to unacceptable levels that may pose harm to health (e.g. *Pseudomonas aeruginosa*, *Legionella*, and *Mycobacterium*) (Zanetti et al., 2009).

Worldwide drinking water directives have been drawn up that require the drinking water to be free of pathogenic bacteria in order to protect its costumers (e.g. European Drinking Water Directive and the Safe Drinking Water Act in the United States) (European Council Directive, 1998). In addition, many European countries have developed (non-mandatory) certification tests (e.g. the BPP test in The Netherlands, the W270 in Germany, and the MDOD test in the United Kingdom) (Park et al., 2019) and national legislation (e.g. the Legislative Decree no. 2 of 98/83/CE in Italy) to deal with bacterial growth in PoU devices (Zanetti et al., 2009). Despite applicable law, in numerous recent studies, it was concluded that the consumption of the effluent of PoU devices was not compliant to the local regulations, inducing health risks (Chen et al., 2020; Girolamini et al., 2019; Liguori et al., 2010; Nriagu et al., 2018; Rezaeinia et al., 2018; Sacchetti et al., 2014; Sacchetti et al., 2015). Girolamini et al. (2019) and Park et al. (2019) concluded that there is limited awareness in society of the potential health risks of bacterial growth in PoU devices. In addition to that is argued that this lack of information is mainly because of the common use of the PoU devices (without a designated person in charge) and a lack of control performed by health authorities.

## 1.3. Scope

There are three factors that play an important role in the design of PoU devices. The first factor is the applicable legislation that deals with the drinking water quality in a certain country, and thus determines the composition of the influent of PoU devices. In the European Union, for example, the drinking water must be compliant with the European Drinking Water Directive (European Commission, 2016). The second factor is the applicable legislation on the water quality of the effluent of PoU devices in a country to which the manufacturer must comply. In some countries, regulations have been drawn up to deal with the taste, odor, color, and hazardous contaminants (e.g. heavy metals and organic chemicals) released into the water from materials used in PoU devices (e.g. KTW Guideline in Germany, BS 6920 in the United Kingdom, and EN 1420, 12873 in the European Union) (Park et al., 2019). The third factor is the customer's wish in terms of taste, temperature, comfort, and safety. Since the aforementioned factors are not universally equal around the world, there are many different types of PoU devices available.

This study focuses on bacterial growth in point-of-use devices that are 1) directly connected to the drinking water network, 2) equipped with a Granular Activated Carbon GAC filter, and 3) equipped with a cooling reservoir. This type of device is hereinafter referred to as 'Cold and Granular Activated Carbon filtered Water Dispenser' (CGWD). It is widely presumed that GAC filters will remove free chlorine and organic matter, responsible for the deteriorated taste and odor of the water (Rogers,2001). The configuration of a conventional CGWD is shown in Figure 1.1.

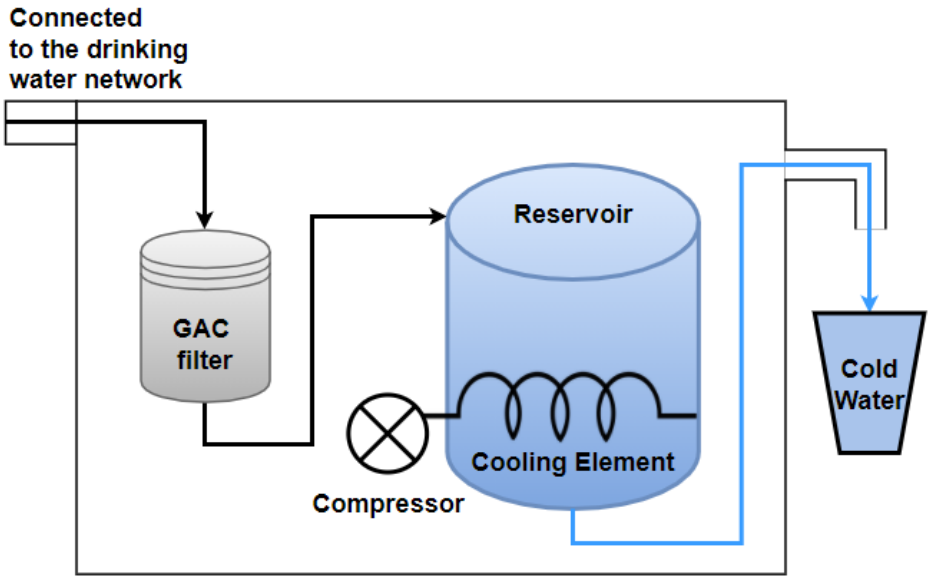


Figure 1.1: The configuration of a conventional CGWD

CGWDs are specially designed for areas where 1) the tap water is of proper quality and safe to drink, 2) the tap water contains chlorine and organic matter, and 3) the instant availability of cold water is desired.

Girolamini et al. (2019) stated that there should be more concern about bacterial growth in CGWDs and that there is a need for long-term and periodic procedures in order to be compliant with water quality standards. One of the most common measures against bacterial growth in CGWDs devices are periodic disinfection. However, Zanetti et al. (2009) concluded that disinfection with peracetic acid and hydrogen-peroxide only had a temporary effect on bacterial growth in CGWDs and that it was difficult to remove the opportunistic bacteria *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is capable of developing a certain degree of resistance to disinfectants (Tuttlebee et al., 2002).

## 1.4. Research objective

Based on an extensive literature review (see section 2.4), new measures against bacterial growth in CGWDs are suggested in this study. The first suggested measure is to pretreat the influent in a boiling water reservoir to inactivate the bacteria being present in the influent. The second suggestion is to displace the GAC filter into the boiling water reservoir so that bacterial growth on the material of the GAC filter would be minimized because of the high temperatures. Thirdly, it is suggested to use the boiler to generate steam to sterilize all downstream parts of the boiler (all the parts to be sterilized are marked red in Figure 1.2) prior to commissioning. By doing this, the number of bacteria added during the assembling, transporting, or installing phase could be decreased.

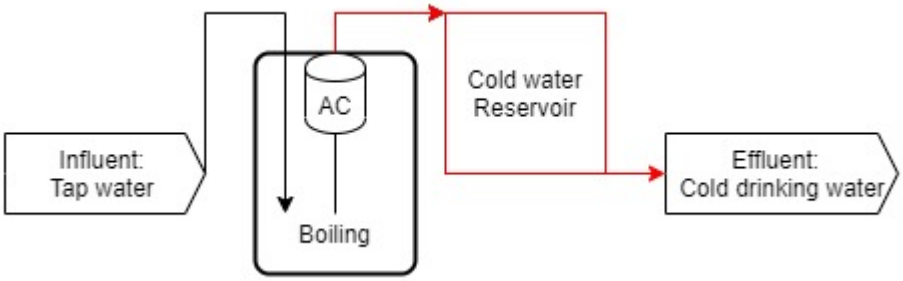


Figure 1.2: The suggested measures implemented in the conceptual design of a CGWD

The primary reason for implementing the GAC filter is the removal of chlorine and organic matter from the water. Usually, the treated water has a temperature between 20 and 25 °C during GAC filtration. When the GAC is integrated within the boiling reservoir, the temperature will lie around 105 °C during the filtration process. However, there have been no controlled studies that compare the removal efficiency between normal GAC filters with the removal efficiency of boilers with integrated GAC filters.

Therefore, the objectives of this study are to assess whether suggested measures can decrease the bacterial growth within CGWDs and to examine whether the free chlorine and organic matter removal performances are affected by the implementation of these measures.

## 1.5. Research questions

The following research questions will be answered in this study to achieve the research objective:

- Could the bacterial growth in CGWDs be decreased if the water is heated to 105 °C by replacing the GAC filter into the boiler water reservoir?
- Could the bacterial growth in CGWDs be further decreased if the device is additionally exposed to steam prior to commissioning?
- Would placing the GAC filter into a boiling water reservoir, affect the organic matter removal efficiency of the CGWD?
- Would placing the GAC filter into a boiling water reservoir, affect the chlorine removal efficiency of the CGWD?

The exact way in which the research questions will be answered is described in detail in chapter 3.

## 1.6. Relevance of the study

This study, which assesses the suitability of suggested measures against bacterial growth in CGWDs, is of paramount importance to both science and society. This section will underline this importance by summarizing the previous sections.

CGWDs are widely used and are becoming even more popular. However, CGWDs appeared to be vulnerable to bacterial growth. It is concluded that bacterial growth could also lead to the presence of pathogenic bacteria in the effluent water. The presence of pathogenic bacteria in the effluent of the CGWD induces health risks. A long-term and cyclic measure against bacterial growth is needed to be compliant with water quality standards. Disinfectants, however, turned out to be only partially effective and merely for limited periods of time. As an alternative, a set of measures against bacterial growth is suggested. It is expected that the degree of bacterial growth could be reduced by implementing the aforementioned measures. However, this hypothesis is yet to be confirmed by scientific research. Furthermore, the refinement of tap water using GAC filters integrated into boiling water reservoirs is unusual and therefore not described in literature.

## 1.7. Structure of the report

This report is composed of 7 chapters. In Chapter 2, the results of the literature review are presented. Chapter 3 describes the methodologies that were followed during the experiments. Chapter 4 deals with the results of the performed experiments, which are then discussed in Chapter 5. In Chapter 6 conclusion will be drawn and the research question will be answered, whereas in Chapter 7 recommendations will be made for further research.

# 2

# Literature review

In this chapter, the knowledge gathered by the literature study will be presented. This will give insight into the theory and induce a practice-oriented study. In the first two sections, more information will be provided on organic matter and free chlorine in drinking water. The third section provides a general understanding of the working principle of the GAC. The fourth section amplifies the enhancing factors of bacterial growth in CGWDs.

## 2.1. Organic matter in drinking water

Organic matter, organic material, or natural organic matter is the fraction that contains all compounds that contain carbon-hydrogen bonds, so-called organic compounds (Rogers, 2001). Organic matter is present to varying degrees in all sources of drinking water, due to the interaction between the hydrological cycle and the geosphere and biosphere (Matilainen & Sillanpää, 2010). The presence of organic matter in drinking water can cause several problems. Multiple organic compounds are related to the off-taste of drinking water (Rogers, 2001). Furthermore, organic matter reacts with disinfection agents forming disinfection-by-products (DPBs) (Matilainen & Sillanpää, 2010). DBPs have been associated with adverse health effects (Richardson, 2003). Additionally, organic matter promotes bacterial growth within the drinking water network (Matilainen & Sillanpää, 2010). Organic matter is the result of a complex set of various reactions and so the composition of organic matter is very variable. This makes the removal of the organic matter very challenging for drinking water companies (Sillanpää et al., 2018). The fact that an increasing trend of organic matter concentration in source water is observed over the last years, makes it even more challenging (Matilainen & Sillanpää, 2010).

## 2.2. Chlorine in drinking water

Chlorine is a chemical element and at room temperature chlorine is the yellow-green gas Cl2. However, chlorine is a very reactive and strong oxidizer and is naturally not present in nature (Lenntech, n.d.). When one talks about the taste and odor of chlorine in the drinking water, one does not simply taste or smell the chlorine gas itself, but one does taste and/or smell hypochlorite (OCl-) and hypochlorous acid (HOCl) (Rogers, 2001). Hypochlorite and hypochlorous acid are both powerful bactericides (Brandt et al., 2017) and are widely used as a disinfectant. The most common ways to add these chlorine compounds to the drinking water is by solving sodium hypochlorite (NaOCl) in water or by dosing chlorine gas. When sodium hypochlorite is dissolved in water, Na+ and OCl- ions will be formed. In the case of dosing chlorine gas, the chlorine gas will rapidly react with $H_20$ to form hypochlorous acid (HOCl-) and hydrogen chloride (HCl). Depending on the pH of the water, hypochlorite is present as hypochlorous acid. At a pH of 7.5, both forms are in balance (Potwora, 2009).

The sum of the hypochlorite and hypochlorous acid concentrations is called free chlorine. The free chlorine concentration will decrease due to the reactions with certain compounds (CDC, 2014). In many countries, minimum levels of free chlorine are set as a requirement for the effluent of the tap at the customer in order to prevent bacterial growth. The guideline of the WHO for drinking water prescribes a maximum concentration of 5 mg of free chlorine per liter (WHO, 2003).

To reach a certain level of free chlorine, first, the chlorine demand must be exhausted. The chlorine demand is the result of a combination of multiple complex reactions between chlorine and organic matter in the water that form chlorinated organic chemicals (CDC, 2014). These chlorinated organic chemicals have no disinfection capacity. After the dosing of chlorine to the drinking water, the free chlorine is equal to the total dosed chlorine minus the chlorine demand (CDC, 2014). After the chlorine demand is exhausted, the hypochlorite and hypochlorous acid could also react with ammonia forming chloramines. Chloramines have a certain disinfection capacity, however, this is 20-fold lower than the disinfection capacity of hypochlorite and hypochlorous acid (Rogers, 2001). The total concentration of the formed chloramines is the so-called combined chlorine. The sum of free and combined chlorine is called total chlorine (Brandt et al., 2017). However, in drinking water with low ammonia concentrations, the amount of combined chlorine will be negligible.

Free chlorine is known to add unfavorable undesirable taste and odor to the water. The odor and taste thresholds of hypochlorite are, respectively, 0.36 and 0.30 mg/L. Whereas the taste and odor thresholds of hypochlorous acid are, respectively, 0.28 and 0.24 (Rogers, 2001). Moreover, concerns have been raised about the forming of disinfection by-products, as described in section 2.1. Instead of using free chlorine, another strategy against bacterial growth, which is applied in the Netherlands, could be to improve the biological stability of the drinking water. By decreasing the AOC concentration of the drinking water to less than 10 µg acetate-C/L, bacterial growth can be limited in the drinking water network without using free chlorine (Smeets et al., 2009).

## 2.3. Granular Activated Carbon

Activated carbon can be produced by physically or chemically activating carbonaceous materials such as wood, charcoal, bamboo, coconut, etc. Activated carbon is a highly porous material and therefore has a large surface area and thus is a very effective adsorbent (O'Connor et al., 2009). Therefore, activated carbon filtration is the most applied purification technique used in PoU devices (Moreno-Castilla, 2004). Over time the GAC filter bed will become saturated with the adsorbed compounds and the removal efficiency will decrease. Normally the dimensions of the GAC filter and time before replacement are selected in such a way that the GAC delivers the desired effluent concentration in a predetermined period of time. Activated carbon is available in two forms, Granular Activated Carbon (GAC) and Powdered Activated carbon (PAC). Both forms are made from the same materials, however GAC has a relatively larger particle size compared to PAC and is installed in a fixed bed container and can be exposed continuously. Whereas PAC is dosed as a powder with small particle size and is mostly used in batch processes (Sheth & Soni, 2005). Due to the smaller particle size, PAC has a larger surface area and thus faster adsorption kinetics than GAC (Kårelid et al., 2017). On the other hand. GAC is contained in a fixed column so that the saturation of the activated carbon can be reached, whereas for PAC longer periods of recirculation are needed to reach the adsorption equilibrium (Kårelid et al., 2017).

GAC is very effective in removing free chlorine from water (Naseer et al., 2012). During the dechlorination process of activated carbon the surface of the activated carbon is oxidized by chlorine. The decomposition reactions of hypochlorite and hypochlorous acid are shown below (Li et al., 2010):

$$C^* + HOCl \longrightarrow C^*O + H^+ + Cl^- \tag{2.1}$$

$$C^* + OCl^- \longrightarrow C^*O + Cl^- \tag{2.2}$$

Where C* is the reactive site of the activated carbon and where C*O is the oxidized site of activated carbon. In this way, the free chlorine has been reduced to the chloride ion (Cl-). The oxidized sites of the activated carbon, however, are very unstable and decompose to form carbon dioxide, which is shown in the reaction below (Li et al., 2010):

$$C^*O + C \longrightarrow CO_2 \tag{2.3}$$

In general, it is thought that PoU GAC removes organic compounds responsible for taste and odor removal (Rogers, 2001). However, a survey among major manufacturers of PoU filters showed that the correctness of this assumption has not been confirmed by experiments. Instead, major manufacturers have taken the results of the chlorine removal performance tests as a measure of the overall performance of the PoU GAC. (Rogers, 2001)

## 2.4. Factors enhancing bacterial growth in PoU devices

In order to suggest measures against bacterial growth in CGWDs in this study, firstly the enhancing factors of bacterial growth in CGWDs have been examined in a literature review. This section amplifies the most important findings of this literature review.

The biological stability of tap water strongly affects the degree of bacterial growth. As described in section 2.2, there are two common strategies in dealing with bacterial growth in drinking water systems: 1) applying disinfection or 2) improving the bacteriological quality of the drinking water without applying any disinfection. The biological stability of tap water can be measured using the Assimilable Organic Carbon (AOC) concentration: the lower the AOC concentration in the tap water, the higher the biological stability and thus the less bacterial accumulation (X. Liu et al., 2015). In areas where free chlorine is dosed, AOC concentrations are still relatively high in the drinking water. Deshommes et al. (2012) imputed the bacterial growth in PoU devices due to the fact that the residual chlorine is removed by the GAC filter, and that if the water is not biologically stable at forehand biofilms will be formed more easily downstream of the GAC filter.

Eckner (1992) concluded that the way of assembling could affect bacterial accumulation in PoU devices that extract the water from large 20-liter polyethylene terephthalate (PET) bottles. PET bottles with non-airtight connectors and screw caps, that had to be touched by the installer during assembling, appeared to have higher bacterial quantities in the effluent. In CGWDs, bacteria could be introduced during the assembling of the device in the factory, or during the installing of the device by the customer.

Another enhancing factor of bacterial growth in CGWDs could be the GAC filter. Chaberny et al. (2006) concluded that a GAC filter increases the bacterial activity of the effluent water if there are relatively long periods of water stagnation. Chaberny et al. (2006) analyzed the bacterial activity of a PoU device equipped with a GAC filter and observed a decrease in the bacterial activity after the GAC filter was permanently removed from the device. Nriagu et al. (2018) suggests that during water stagnation, the filter bed, filled with organic matter and at an appropriate temperature, becomes the ideal environment for bacteria and biofilms are formed. When the device is used again, parts of the biofilm will detach from the GAC filter and end up in the effluent.

However, GAC is not the only ideal environment for the forming of biofilms. In literature, several other causes are mentioned. For example, in addition to the GAC filter, the water comes into contact with several parts such as pipes, valves, and the reservoir, some of these parts are made from polymeric plastics. During laboratory experiments, Connell (2014) observed a release of AOC into the drinking water from certain polymeric plastics, and as described before AOC increases the biological instability of the water. Park et al. (2019) confirmed that several types of polymeric plastics in PoU devices appeared to be more vulnerable for biofilm forming. Walker and Marsh (2007) concluded that dentist equipment was more vulnerable to bacterial growth due to the presence of narrow and long polymeric tubes.

Moreover, the operational parameters of the PoU devices appeared to play an important role in bacterial growth in PoU devices equipped with GAC filters. (Su et al., 2009) observed that PoU GAC filters, operated with lower flow rates, longer stagnation periods and higher filter temperatures were more vulnerable to bacterial growth. It was concluded that with higher flow rates, the biofilm could be washed away more easily and shorter stagnation periods and lower filter temperatures could slow down the biofilm-forming within the device.

# 3

# Methodology

This chapter begins by interpreting the load case that was used during the experiments. It will then go on to the first experiment and its experimental set-up is described. Additionally, the second experiment will be laid out using the same structure as used for describing the first experiment.

## 3.1. Load case

As described before, CGWDs are specially designed for areas where the tap water is of proper quality and safe to drink, but also where the taste of the water could be improved by removing chlorine and organic matter. According to the literature review on facilitating factors on bacterial growth in CGWDs (see section 2.4), CGWDs are more vulnerable in those areas where residual disinfectant is applied, instead of making the drinking water biologically stable by removing AOC. A country where CGWDs are widely applied to remove chlorine and organic matter from the tap water, but also where the tap water is relatively biologically unstable, is England. Therefore, it was decided to expose the CGWDs to water that has similar AOC, TOC, and free chlorine concentrations as typical English water. Since both experiments were executed in the Netherlands, the Dutch drinking water had to be modified in such a way that the water had a similar water quality as English tap water. In general, English tap water contains high AOC concentrations, with maximum concentrations up to µg 250 acetate-C/L in some regions (Pick et al., 2019). The average concentration of free chlorine in English tap water is 0.5 mg $Cl_2$/L and the TOC concentration varies between 2.0 and 3.0 mg C/L (Ascott et al., 2019). The dutch tap water was originated from the drinking water treatment plant (DWTP) 'Kralingen' operated by the drinking water company Evides, Netherlands. The drinking water from this particular DWTP has a similar TOC concentration to that of English tap water (Zhou et al., 2020), whereas the AOC concentration is below 10 µg acetate-C/L and no residual disinfectant is applied (Smeets et al., 2009). Therefore, it was decided to increase the concentrations of AOC and free chlorine to the maximum concentrations, being, respectively, µg 250 acetate-C/L and 0.5 mg $Cl_2$/L in both experiments.

## 3.2. Experiment 1 - Analyzing measures against bacterial growth

The goal of experiment 1 is to answer the following research questions:

- *Could the bacterial growth in CGWDs be decreased if the water is heated to 105°C by replacing the GAC filter into the boiler water reservoir?*
- *Could the bacterial growth in CGWDs be decreased even more if the device is additionally exposed to steam prior to commissioning?*

In order to answer these questions, three different configurations of CGWDs were tested. The first configuration was that of a conventional CGWD. In the second configuration, a boiler with an integrated GAC filter was implemented. The third configuration was similar to the second configuration, but additionally, the devices were exposed to steam prior to the experiment. The degree of bacterial growth was observed by analyzing the bacterial quality of the effluent over time and comparing this with the bacterial quality of the influent. Heterotrophic plate count at 22°C, ATP and flowcytometry were used

as microbial analysis. The duration of the experiment was 56 days. This section will describe in detail which methodology was followed during the experiment.

### 3.2.1. Experimental set-up

The experimental set-up was operated in the test room of Quooker B.V., located in Ridderkerk (Netherlands), from 29-09-20 until 24-11-2020. The experimental set-up consisted of a chemical dosing system (CDS) and 6 CGWDs. The experimental set-up is shown in Figure 3.1.

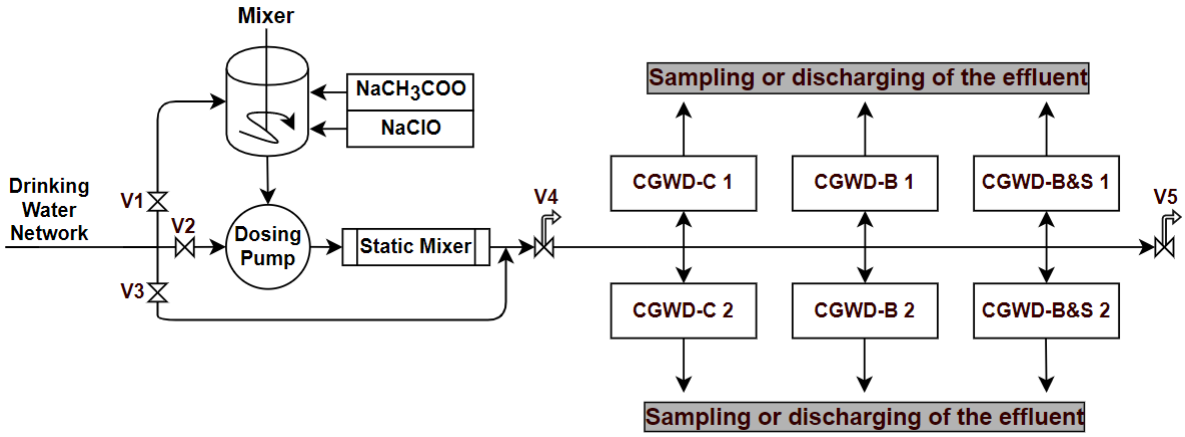


Figure 3.1: The experimental set-up of experiment 1

*Chemical dosing system*

Via the main pipe, the experimental set-up was connected to the drinking water network. Directly after the connection with the drinking water network, the chemical dosing system (CDS) was implemented. The CDS consisted of a dosing pump, a stock solution tank with a mixer, a static mixer, and a bypass system. The CDS can be operated using three valves. The drinking water was conveyed from the water treatment plant 'Kralingen' operated by the drinking water company Evides, Netherlands. After the connection with the main tube, the chemical dosing system is situated. Using a dosing pump (Aquablend Xtreme - Hydro Systems), a flow equal to 2% of the passing flow was extracted from the stock solution tank and added to the drinking water. The stock solution was filled with Dutch tap water using valve 1 (V1). Hereafter, anhydrous sodium acetate was solved into the water of the stock solution tank, whereas a sodium hypochlorite solution was added to the stock solution tank. The theoretical concentration of AOC in the influent of the CGWDs was thereby increased from 1.8 µg acetate-C/L to 250 µg acetate-C/L. The theoretical concentration of free chlorine in the influent of the CGWDs was thereby increased from 0.00 to 1.10 mg $Cl_2$/L. In reality, however, the free chlorine concentration was between 0.4 - 0.6 mg $Cl_2$/L, the difference was caused by the chlorine demand of the water and degradation of free chlorine in the stock solution tank.

In the stock solution tank, a mixer was placed to fully mix the chemicals over the tank. At the end of the chemical dosing system, a static mixer (6 diagonal panels, diameter 15 mm, PRIMIX) was placed to distribute the chemical over the whole section of the pipe. The chemical dosing system could be bypassed by the closing of valve 2 (V2) and the opening of valve 3 (V3).

*CGWDs*

Directly after the chemical dosing system, six CGWDs were connected to the system. Each CGWD consisted of a boiler with an integrated GAC filter and a cooling water device. A sectional drawing of the boiler is shown in Figure 3.2. After the CDS, the pressurized tap water entered the boiler at the top (A), and via a tube, the water was led to the bottom of the reservoir (B). Within the reservoir, a heating element (C) and temperature sensors (D) were situated. The heating element and temperature sensors together with temperature management software, form the temperature management system (TMS). Before the water left the reservoir, the water entered the GAC filter at the top (E) and is led through the filter bed material to the bottom of the GAC filter. From there, the water left the boiler via a small tube in the middle of the GAC filter (F). The GAC filter had the same temperature as the water in the

reservoir itself. The filter packaging of the GAC filter was made from PPSU (Polysulfone) and the bed filter material was of the type NORIT® ROX 0.8. The density of the filter bed material is 355 g/L. For each boiler, the TMS could be switched on or off. In case the TMS was turned on, and the temperature of the water within the reservoir was below 103°C, the heating element started to heat. If the water reached a temperature of 108°C, the heating element went into standby mode until the temperature was below 103°C again. After the boiler, the water was led to the cooling water device. The water was cooled down to a temperature of 8°C in the reservoir of the cooling water device.

The volumes of the reservoir of the boiler, the GAC filter, and the reservoir of the cooling water device were respectively 2.66, 0.34, and 2.0 liters. The total volume of the CGWDs was 5 liters. Every cycle of tapping, 2 liters of water was tapped from the CGWDs so that the water in the CGWD was renewed every 3 cycles. The tapping cycles were executed on every weekday at 10:00 AM. This means that the time until the next cycle was 24 hours from Tuesday to Friday, while the time until the tapping cycle on Friday was 72 hours. So the stagnation time of the water, depending on the day of tapping was between 72 and 120 hours.

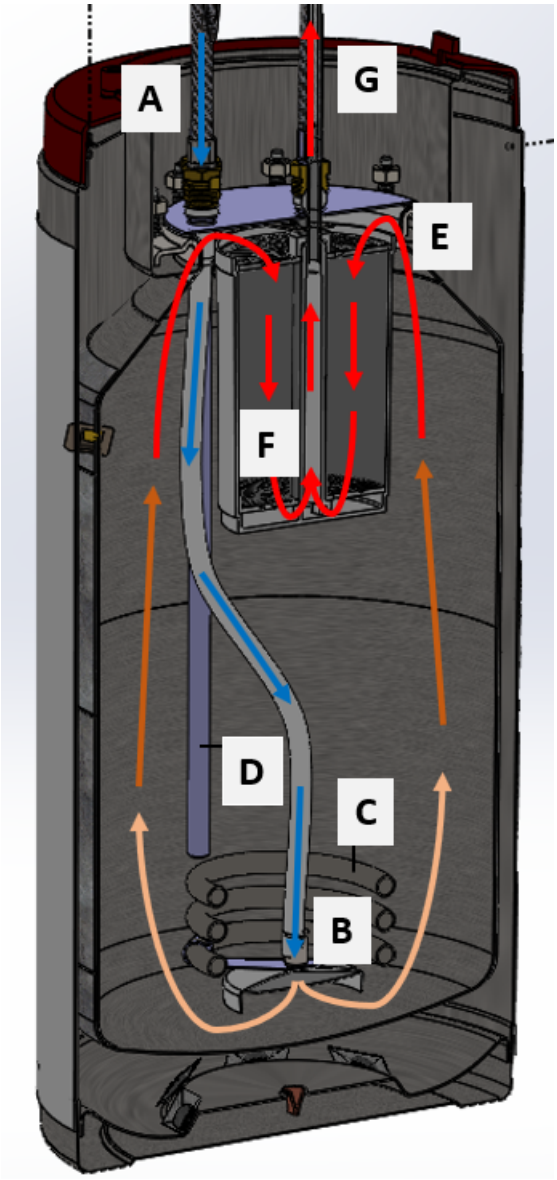


Figure 3.2: A cross-section of the boiler with an integrated GAC filter

During the experiment, 3 different types of CGWDs were tested in duplicate. The three different types are shown in Figure 3.3. The TMS of the boilers of the first two CGWDs, hereinafter referred to

as CGWD-C1 and CGWD-C2 (A), was turned off in order to mimic the configuration of conventional CGWDs. For two other CGWDs, hereinafter referred as CGWD-B1 and CGWD-B2 (B), the TMS was turned on, implementing two measures: boiling the influent water and displacing the GAC filter into the boiling water reservoir. For the latter two CGWDs (C), hereinafter referred to as CGWD-B&S1 and CGWD-B&S2, the TMS were turned on as well. Additionally, another measure was implemented. Namely, the exposure of the cooling water device to steam with a minimum temperature of 105°C for a period of 20 minutes. The three types of CGWDs that were tested in the experiment are shown in Figure 3.3. All six CGWDs were connected with the same type of faucet. By opening the faucet, water started to flow induced by the pressure on the drinking water network in the test room. Before the start of the experiment, all GAC filters were removed and the CGWDs and faucets were flushed with water from the chlorine dioxide dosing skid. The six CGWDs were exposed to water with a free chlorine concentration of 0.2 mg $Cl_2$/L for a period of 40 minutes and afterward the CGWDs and faucets were dried and emptied using compressed air for a period of 15 minutes.

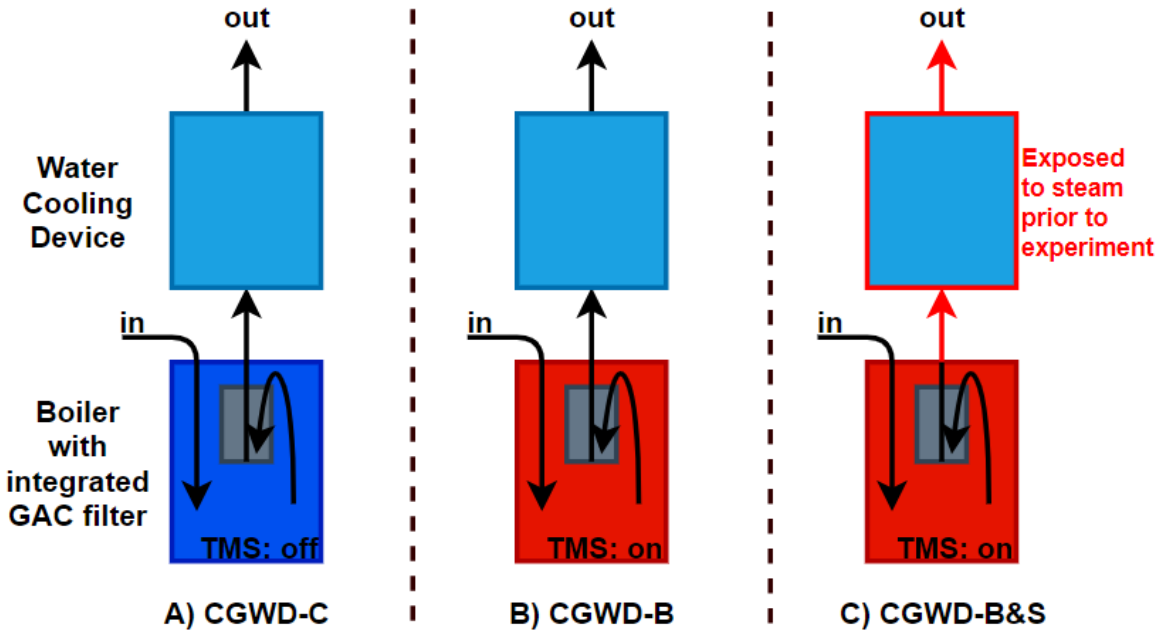


Figure 3.3: The three different types of CGWDs that were tested in duplicate

*Daily activities*
Every weekday, the feeding tank was rinsed twice with tap water en emptied. After this, the feeding tank is filled with tap water, and chemicals are added. Before tapping, 2 liters of water from every CGWD, 2x5 liters of modified tap water was flushed via valve 4 (V4) and valve 5 (V5) to clean the chemical dosing system and the pipe network. This was done to minimize the chance of bacteria being present in the influent, but also to be sure that both the concentration of AOC and free chlorine were correct in the influent. Then the free chlorine concentration in the influent was checked using valve 4 (V4). In case the free chlorine concentration was below 0.4 mg Cl2/L or above 0.6 mg CL2/L, the feeding tank and mixer were rinsed and the previous steps were repeated. In case the free chlorine concentration was indeed between 0.4 and 0.6 mg Cl2/L, the exact concentration was noted and 2 liters of modified tap water was tapped from every CGWD. In case microbial analysis had been executed, some of the water was sampled, otherwise, the water was discharged. After the tapping of the water from the CGWDs, the free chlorine concentration was measured again and noted. Thereafter, the mixer and feeding tank was rinsed and set to dry upside down. At the end of every week, both the feeding tank and mixer were thoroughly cleaned with ethanol.

*Extracting the biofilm from GAC filter*
At the end of the experiment, the boilers were transported to KWR Water Institute, located in Nieuwegein (Netherlands). Here, the GAC filters were removed from CGWD-C1, CGWD-B1, and CGWD-B&S1.

From the middle of each GAC filter, 3 grams of filter material was extracted. 50 mL of purified water was added to the filter material. The three mixtures of purified water and the GAC filter material were placed into the High Energy Sonifier (HES) for a period of 2 minutes at a capacity of 45%. 30 mL of the sonified mixture was then taken as a sample for microbial analysis (see section C for some illustrations). The whole procedure was executed by employees from KWR Water Institute according to the KWR House regulation LMB-013.

### 3.2.2. Sampling

As described before, every weekday 2 liters of water, with a similar quality as English tap water, had to be tapped. On days 0, 1, 2, 7, 14, 28, 42, and 56 of the experiment, a part of the tapped was sampled for microbial analysis. Before the sampling, 50 mL of water was tapped from all devices and discharged. The first flush could contain bacteria that were present in the faucet, while the focus of this thesis is the bacterial accumulation within the CGWD itself. 2x250 mL of the effluent was sampled for total and free ATP analysis, followed by 500 mL of the effluent for heterotrophic plate counts at 22°C (HPC22) and finally 50 mL of effluent for flowcytometry (FCM) analysis. The remaining effluent was discharged. Furthermore, samples were taken from the feeding tank and from the influent of the CGWDs (using Valve 4 - V4) for HPC22 and ATP analysis. All samples were stored at a temperature below 8°C, directly after sampling.

### 3.2.3. Physical analyses

The TMS's of CGWD-B1, CGWD-B2, CGWD-B&S1, and CGWD-B&S2 were daily checked. Furthermore, the temperature of the influent water and effluent water of the CGWDs was measured at the beginning, halfway, and at the end of the experiment using a simple probe thermometer. At the same time, the flow rate was measured using a timer and a measuring cup.

### 3.2.4. Chemical analyses

The free chlorine concentration was measured using a pocket-size photometer with 10 mL cuvettes and corresponding DPD reagents (HANNA Instruments, type: HI701, Netherlands).

### 3.2.5. Microbial analyses

*HPC22 analysis*

Heterotrophic Plate Count (at 22 degrees Celsius) analyses were performed by Synlab, Oosterhout (Netherlands), conform ISO 6222. In epidemiological studies, it is argued that exposure to high HPC22 in water has no significant effect on human health and that the exposure rates are negligible to those via foodstuffs (Allen et al., 2004; Bartram et al., 2003). However, HPC22 indicates that the circumstances are appropriate for bacterial growth, which eventually could pose harm to human health (Allen et al., 2004). While the application of a hard requirement for HPC22 is disputed in literature (Sartory, 2004), maximum counts are laid down in water quality standards all over the world (Allen et al., 2004). Therefore, HPC22 remained a basic indicator parameter to include in the microbial analysis of drinking water.

*FCM analysis*

In addition to HPC22, flowcytometry (FCM) analysis was performed by KWR Water Institute according to KWR House regulation LMB-013. With FCM analysis, the number of intact cells (ICC), dead cells(DCC), and total cells (TCC) was determined. FCM is a reliable and accurate analysis that can be used to determine the degree of bacterial growth in terms of physical cell count (Whalen et al., 2018). While there is a positive correlation between HPC22 and FCM counts, there are also examples in which high values of HPC22 were obtained together with low FCM counts (Cheswick et al., 2019) and vice-versa (Todar K., 2004). Whereas HPC22 analysis only cultivates <1% of the total bacterial population in water (Abushaban et al., 2019), ICC/TCC analysis determines the exact number of (living) bacteria that are present in water. FCM analysis is a quantitative analysis and should not be used to provide information on the bacterial quality of the water (Cheswick et al., 2019). However, the main objective of the experiment was to determine to which degree bacterial growth could be reduced, and thus no distinction of specific bacteria is desired.

*ATP analysis*

The bacterial activity of bacterial cells varies considerably per species. Whereas FCM quantifies the number of cells, FCM provides no information on the total bacterial activity of the biomass. Therefore, in addition to HPC22 and FCM, ATP analysis was performed on the water samples. ATP is the energy-carrying component within cells (Kroll, 2009). The amount of ATP within cells is measured as cATP. With cellular ATP (cATP) analysis the amount of potential energy within living cells is determined and this provides an indirect indication of the total quantity of the living biomass in the water (Whalen et al., 2018). During the die-off phase of cells, the ATP is released into the extracellular environment of the cells. This type of ATP is so-called free ATP (fATP) (Faas et al., 2017). The sum of fATP and cATP is the so-called total ATP (tATP).

In this study free ATP (fATP) and total ATP (tATP) analysis were done with the Luminometer (3M, Clean-Trace LX25, Netherlands) and the corresponding swabs (see Figure 3.4). The results of both analyses were used to calculate the cATP concentration, this is described in detail in the next paragraph. The AQF100 and AQT200 swabs were used for the measurement of fATP and tATP, respectively. For each measurement, one swab was put into the sample and thereafter analyzed in the luminometer. The swabs were used according to the instructions in the manual.


Figure 3.4: The measurement of tATP with the AQT200 swab of 3M

The luminometer measures fATP and tATP in relative light units (RLU). In order to calculate the cATP concentration, first the values of fATP and tATP had to be converted from RLU to ng ATP/L. Lastly, the fATP concentration in ng ATP/L was subtracted from the value of free ATP in ng/L. In order to convert the values from RLU to ng/L, a calibration curve had to be made for both fATP and tATP. Furthermore, the calibration curve could be used to analyze the accuracy of the luminometer. This was done in collaboration with KWR Water institute. An aqueous solution with a concentration of 100 mM ATP (Adenosine triphosphate) and a volume of 0.25 mL (ThermoScientifc) was purchased from VWR (USA). 0.1 mL of the standard ATP solution was added to 1L of tap water. This mixture was diluted again in 4 different ratios of 1:50,000, 1:100,000, 1:200,000, and 1:1,000,000. Additionally, a fifth sample was analyzed as a reference. This sample contained normal drinking water which was heated to 105°C for 1 hour in a boiling water reservoir. The theoretical concentration of total ATP in the 4 solutions was, respectively 100, 50, 25, 5, and 0 ng total ATP/L. The followed procedure was inspired by the procedure described in Ochromowicz and Hoekstra (2005). The concentrations of fATP and tATP in those 5 solutions were measured in RLU using the 3M Clean-Trace Luminometer. The same solutions were also sent to the KWR Water institute where the concentrations of fATP and tATP were measured in ng/L according to KWR House regulation LMB-002.

*Biofilm analysis*

The samples obtained from the biofilm extraction with the HES were analyzed using total ATP measurement by KWR Water Institute. The followed procedure was in accordance with the KWR House regulation LMB-013. Because of the HES treatment, the GAC material was pulverized increasing the turbidity of the samples. Since the ATP assays measure the amount of emitted light after the enzymatic

reaction, the turbidity affects the ATP measurement. For samples with a tATP concentration above 300 ng ATP/L, dilution curve was made to determine the real tATP concentration of the sample. For samples with tATP concentrations below 300 ng ATP/L in the original samples, it was not possible to make a dilution curve, instead, a minimum concentration and the inhibition was determined.

### 3.2.6. Statistical analysis

The measurement results of the chemical analysis have been statistically analyzed using SPSS (Data Analysis and Statistical Software; version 26.0 for Windows; IBM, Chicago, IL, USA). The statistical significance was set to $p < 0.05$. The Kolmogorov-Smirnov exponentiality and normality tests were done to check whether the data was exponentially or normally distributed, respectively. The data from the HPC and FCM measurement appeared to be exponentially distributed, therefore the data was converted into $logx$ to normalize the data. The data from ATP and free chlorine measurement appeared to be normally distributed, so no conversion was needed. The paired t-test was used:

- to compare the HPC22 in the effluent of the CGWDs to the HPC22 in influent.
- to compare the HPC22 in the effluents of the CGWDs with each other.
- to compare the ICC in the effluent of the CGWDs to the ICC in the influent.
- to compare the ICC in the effluents of the CGWDs with each other.
- to compare the cATP concentrations in the effluent of the CGWDs to the cATP concentrations in the influent.
- to compare the cATP in the effluents of the CGWDs with each other.
- to compare the free chlorine concentration in the effluent that was measured before tapping with the free chlorine concentration that was measured after the tapping.

## 3.3. Experiment 2 - Analyzing removal performances of the boilers

The goal of experiment 2 was to answer the following research questions:

- Would placing the GAC filter in a boiling water reservoir, affect the organic matter removal efficiency of the CGWD?
- Would placing the GAC filter in a boiling water reservoir, affect the chlorine removal efficiency of the CGWD?

To answer these questions, the removal performances of two GAC filters treating room temperature water were compared to the removal performances of two boilers with integrated GAC filter. While both types of boilers were continuously flushed, the removal performances were obtained by measuring the concentration of TOC and chlorine in the influent and effluent over time and calculating the difference between both. After 333 hours, experiment 2 was terminated as a power failure caused the pump and heating element to malfunction. The penultimate time point at which samples were taken without malfunctioning of the set-up was at t = 312 hours. Therefore, only the collected data before t = 312 hours was analyzed. This section will describe in detail which methodology was followed during the experiment.

### 3.3.1. Experimental set-up

The experiment was operated in the Water laboratory at the Technical University of Delft from 23-06-2020 until 07-07-2020. The set-up consisted of a chemical dosing system, a constant flow regulation system, 2 parallel cold water lines, and 2 parallel boiling water lines. The experimental set-up is shown in Figure 3.5. The set-up has been operated for 333 hours in sequence, with 3 interruptions (used to refill stock solutions) of 90, 87, and 31 minutes. Originally, it was the plan to terminate the experiment until each boiler had removal efficiency below 50% for both chlorine and TOC. However, the experiment was terminated earlier because of a power failure causing the pump and heat element to malfunction.

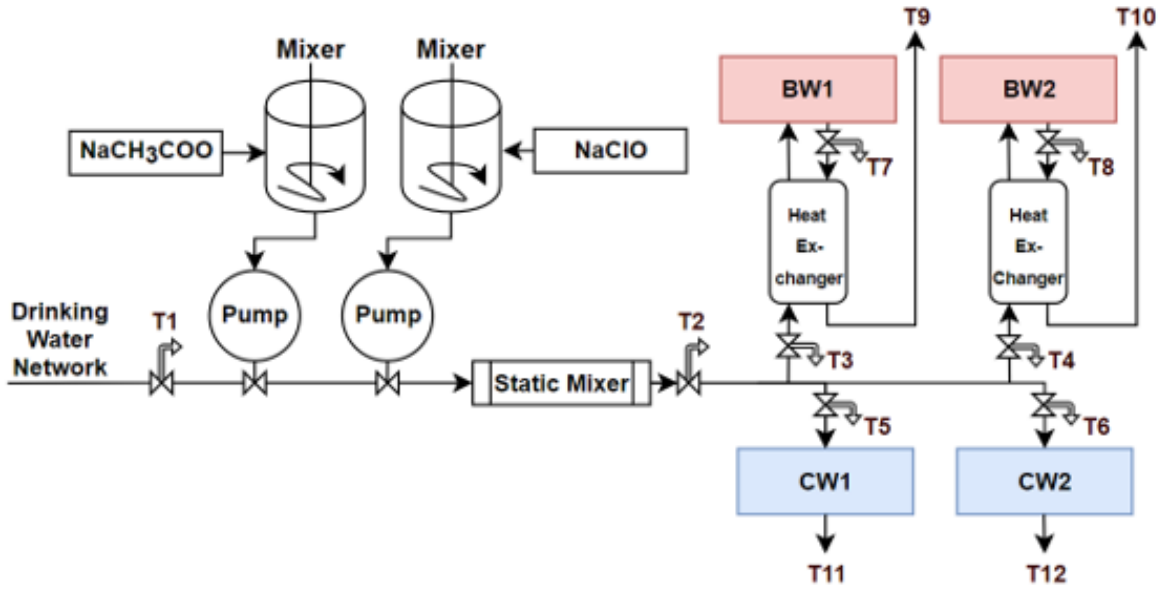


Figure 3.5: An overview of the experimental set-up of experiment 2

*Chemical dosing system*

Via the main pipe, the set-up was connected to the drinking water network. After the connection with the main tube, the chemical dosing system (CDS) is situated. The CDS consisted of 2 peristaltic pumps, 2 stock solution tanks, 2 mixers, and one 1 static mixer. With the first peristaltic pump (Watson-Marlow 120S/DV), 16 mL/min was extracted from the sodium acetate solution tank and dosed to the tap water. The theoretical concentration of AOC in the sodium acetate solution tank was brought to 104.2 mg

acetate-C/L by solving anhydrous sodium acetate in the tap water. The theoretical concentration of AOC in the water was thereby increased from 16 µg/L to 250 µg acetate-C/L. Via the second peristaltic pump (Watson-Marlow 120S/DV), 16 mL/min was extracted from the NaOCL solution tank and dosed to the tap water. The theoretical concentration of free chlorine in the NaOCL solution tank was brought to 282 mg $Cl_2$/L by solving sodium hypochlorite in the tap water. The theoretical concentration of free chlorine in the water was thereby increased to 0.64 mg $Cl_2$/L. In reality, however, the mean free chlorine concentration was 0.51 mg $Cl_2$/L, the difference was caused by the chlorine demand of the water. Both storage tanks had a volume of 120 liters and had to be (partially) refilled 3 times during the experiment. In each storage tank, a mixer was placed to fully mix the chemicals over the tank. After the dosing of the chemicals, a static mixer (6 diagonal panels, diameter 15 mm, PRIMIX) was placed to distribute the chemical over the whole section of the pipe (see section A for the design of the flow regulator). More information on the used chemical is described in .

*Constant flow regulation system*
After the CDS, a pump (DAB E.sy Box mini 3, Italy) was placed to increase the pressure to 3.5 bar to increase the flow rate, without the pump the flow rate appeared to be too low. Furthermore, flow regulators (Neoperl – G LP 2.0) with a maximum flow rate of 2 L/min were implemented in each effluent pipe to maintain the same flow rate in each line (see section B for the design of the flow regulators).

*Four parallel lines*
After the static mixer, the water was equally spread over 4 parallel lines. In each line, a boiler with an integrated GAC filter was placed. The same boilers, equipped with integrated GAC filters, were used as in experiment 1 (see subsection 3.2.1 for a detailed description of the boiler and the GAC filters). The volume of the reservoir in the boiler and the GAC filter were, respectively 6.63 and 0.37 liters. The average flow rate was 1.7 L/min, so the average residence time in the reservoir was approximately 4 minutes, whereas the empty bed contact time of the filter was approximately 13 seconds.

In two lines, the TMS of the boilers, hereinafter referred to as BW1 & BW2, was switched on. Before entering BW1 & BW2, the water first went through a heat exchanger. Due to the heat exchanger, the temperature of the water entering BW1 & BW2 was increased to approximately 90°C. Within BW1 & BW2, the water was further heated to a temperature of approximately 105°C. Since the GAC filters were integrated into the boiler, the water had a temperature of 105°C in both the reservoir and GAC filter. The outlets of BW1 & BW2 were connected to the heat exchanger as well so that the leaving water was cooled down to approximately 35°C. The heat exchanger was implemented to minimize the temperature difference between the influent and effluent water of the boiler. The heat exchangers were purchased from HRALE (China) and are designed to operate with a temperature differential of maximal 15°C. This was necessary because the heating element only had a limited power of 2.2 kW and was not able to increase the temperature of the water from 20°C to 105°C continuously. With a maximum temperature differential of 15°C and a total volume to be heated of 7 liters, the total maximum needed power was 2.03 kW. On the top of BW1 and BW2, LED lights were installed that communicated whether the heating element was functioning or in standby mode. During the preliminary test phase of the experiment, the temperatures in the bottom and top of the boiling water reservoirs were logged with temperature sensors for a duration of 24 hours. The temperatures were hourly checked every time the temperatures in the filter bed were above 103°C. Furthermore, the LED lights were checked using the data of the temperature sensors and the system appeared to be functioning well. In the two other lines, the TMS of the boilers, hereinafter referred to as CW1 & CW2, was switched off so that the water in those boilers, and thus in the GAC, had the same temperature as the water in the drinking water network.

*Sampling points*
In order to take samples, 12 sampling points (T1 – T12) were implemented within the set-up. T1 was situated at the inlet of the system. T2 was situated after the static mixer and thus after the dosing of the chemicals, and was used for the reference samples. T3 – T6 were suited at the beginning of the four lines (before the reservoirs and before the heat exchangers), and were mainly used in the test phase to check whether the chemicals were equally distributed over the 4 lines. T7 and T8 were situated directly after the effluent of the boiling reservoirs and were mainly used during the testing phase to check the temperature of the water at the outlet of the boilers during the calibration. T9 – T12 were situated at the end of the four lines and were used to take samples from the effluent of the 4 lines. All sampling points are shown in Figure 3.5.

*Additional experiment without GAC filters*
Additionally, a brief experiment was executed to investigate the role of GAC filters, to understand the removal mechanisms of free chlorine and TOC by GAC and boiling. At the end of the experiment, on 07-07-2020, the filters were removed from BW1 and CW1 and the experiment was continued for 2 hours in those two lines. The flow rates of the two peristaltic pumps were set to 8 mL/min and the other two lines were disconnected, so that the same concentrations of TOC and chlorine in the influent and the same flow rates were maintained as during experiment 2. Samples were taken 70 and 91 minutes after commissioning, both for TOC and free chlorine analyses.

### 3.3.2. Sampling
All samples were taken on weekdays. For temperature measurement, samples of 50 mL were taken at sampling points T7 – T12 at t = 0, 8, 32, 56, 78, 150, 173, 196, 219, 243, 312 hours. For TOC measurement, 6 samples of 30 mL were taken at sampling points T1, T2 and T9 – T12 after, respectively, 0, 2, 4, 6, 8, 24, 32, 48, 56, 72, 78, 144, 150, 168, 173, 191, 196, 219, 241, 312 hours. At the same time, 5 samples of 10 mL were taken at T2 and T9 – T12 for free chlorine measurement.

### 3.3.3. Physical analyses
The samples for temperature measurement were directly analyzed using a probe thermometer. Furthermore, LED lights were used to regularly monitor the temperature within BW1 & BW2. During sampling, the positions of the volume meters and the time was noted manually. By calculating the difference in time and volume, the flow rates between moments of sampling could be calculated.

### 3.3.4. Chemical analyses
All the chemical analysis during this experiment were performed in the Water laboratory of the Technical University of TU Delft.

*Total Organic Carbon*
In this study, the organic matter removal performance of the CGWD will be assessed using TOC analysis. In experimental form, one could expose GAC filters continuously to a certain concentration of specific organic compounds, like Geosmin and MIB, as was done by Drikas et al. (2009) and Yang et al. (2010). However, the analysis of these organic compounds is rather complex, time-consuming, and costly. Furthermore, it is relatively difficult to draw conclusions from these tests. In reality, organic compounds like Geosmin and MIB are only found sporadically in the tap water, and not continuously. Also, the off-taste in drinking water is not caused by one or two organic compounds, but by many different organic compounds. García-García et al. (2015) and Hozalski et al. (1995) analyzed the reduction of natural organic matter in water by measuring the reduction of Total Organic Carbon (TOC). With TOC analysis the weight of all fractions of organic compounds can be quantified. Measuring the TOC reduction thereby could give a gross measure of the removal of all forms of organic compounds together (Mrayyan & Battikhi, 2005). In that case, the removal of all organic compounds is investigated and more realistic circumstances could be imitated during the experiment in terms of taste and odor improvement.

Directly after sampling, 1.6 mL of hydrochloric acid (2% solution) was added to the samples. The samples were stored in the fridge for a maximum of 7 days. The TOC concentration was analyzed by a TOC analyzer, (TOC-V analyzer, Shimadzu, Japan). After acidifying the samples inorganic compounds are removed, the TOC analyzer measures TOC as non-purgeable organic carbon (NPOC). However,

tap water does not contain any significant amounts of volatile or purgeable organic compounds. Therefore, the TOC concentration is directly determined as NPOC. During each measurement cycle, 1 standard sample and 3 blank samples were added to check the accuracy and background concentration of the device. The mean error during the measurement of the standard and blank was 0.01 mg C/L. In order to compute the removal efficiency of TOC by each boiler, the following formula was used:

$$RE_{TOC,i}(t) = \frac{C_{TOC,in}(t) - C_{TOC,out,i}(t)}{C_{TOC,in}(t)} \cdot 100\%$$

Where $RE_{TOC,i}(t)$ is the removal efficiency of TOC by boiler i at time = t, $C_{TOC,in}(t)$ is the concentration of TOC in the influent (at T2) at time = t and where $C_{TOC,out,i}(t)$ is the TOC concentration at the effluent of boiler i at time = t.

*Assimilable Organic Carbon*
In order to check whether the CDS was dosing the right amount of AOC to the tap water, the AOC concentration had to be determined. The increase in the AOC concentration due to the addition of sodium acetate via the CDS was obtained indirectly by calculating the difference between the TOC concentration in the influent of the concentration of TOC in the influent (before the CDS) of the CGWDs (after the CDS).

The principle of increasing the concentration of AOC by dosing sodium acetate was tested before the experiment. In this test, 69.5 mg of anhydrous sodium acetate was solved in 2L of tap water. In that case, the theoretical concentration of acetate should have been increased by 10.17 mg acetate-C/L, which means that the TOC concentration would also be increased by 10.17 mg C/L. Two samples of the test solution were analyzed and two samples of drinking water were analyzed using the TOC analyzer. The mean TOC concentration of the test solution was 11.8 mg C/L, where the drinking water samples had a mean TOC concentration of 1.8 mg C/L.

*Free chlorine*
In this study, the chlorine removal performance of the CGWDs was assessed using free chlorine analysis. In the case of drinking water, in areas where the tap water is of proper quality, the amount of combined chlorine can be neglected because of the relatively low concentrations of ammonia in drinking water (Pick et al., 2019). In the case of drinking water with low ammonia concentrations, the off-taste is caused by the presence of free chlorine (CDC, 2014).

The concentration of free chlorine was determined using DPD kits (Spectroquant from VWR and a spectrophotometer (NOVA 60, Merck, USA). Measurements below 0.05 ppm and above 2.50 ppm were outside the detection limits. If the free chlorine appeared to be below 0.05 ppm, the chlorine concentration was set to 0.05 ppm. In order to compute the removal efficiency of the boilers for free chlorine, the following formula was used:

$$RE_{fc,i}(t) = \frac{C_{fc,in}(t) - C_{fc,out,i}(t)}{C_{in}(t)} \cdot 100\%$$

Where $RE_{fc,i}(t)$ is the removal efficiency of free chlorine by boiler i at time = t, $C_{fc,in}(t)$ is the free chlorine concentration in the influent (at T2) at time = t and where $C_{fc,out,i}(t)$ is the free chlorine concentration at the effluent of boiler i at time = t.

### 3.3.5. Statistical analysis

All the measurement results of the chemical analysis have been statistically analyzed using SPSS (Data Analysis and Statistical Software; version 26.0 for Windows; IBM, Chicago, IL, USA). The statistical significance was set to $p < 0.05$. The Wilcoxon Signed Ranks Test was used:

- to compare the chlorine and TOC concentrations in the influent to the chlorine and TOC concentrations in the effluent of the four lines
- to compare the chlorine and TOC concentrations in the effluents of the four different lines.
- to compare the removal efficiencies of chlorine and TOC of the four different lines
- to compare the flow rates of the four different lines

# 4

# Results

In this chapter, the results of both experiments are examined. Firstly, an overview of the results of the first experiment including graphs and tables is given. Then, the outcomes of the second experiment will be issued. Lastly, a sum-up of the main findings of both experiments will be given.

## 4.1. Experiment 1 - Analyzing measures against bacterial growth

The measurement data of the FCM, HPC22 and ATP analyses are presented in Appendix E, Appendix F, and Appendix G.

### 4.1.1. Temperature

The TMS of the boilers CGWD-B1, CGWD-B2, CGWD-B&S1, and CGWD-B&S2 were functioning properly during the experiment so that the temperature was always between 103 and 108°C within the boilers. The average temperature of the influent was 21.2°C (n=3). The effluent temperatures of CGWD-C1, CGWD-C2, CGWD-B1, CGWD-B2, CGWD-B&S1 and CGWD-B&S2 were, respectively, 7.0, 7.3, 6.8, 7.3, 7.2 and 6.9°C (n=6x3). No abnormal changes were observed.

### 4.1.2. Free chlorine

Every weekday, the free chlorine concentration in the influent was measured twice. After installing the chemical dosing system, a first sample was taken before water was tapped from the CGWDs. Whenever the free chlorine concentration between 0.4 and 0.6 mg $Cl_2$/L, the concentration was noted and water could be tapped from the CGWDs. These values are shown in Figure 4.2 as 'Prior to tapping'. After 2 liters of water had been tapped from each CGWD, the free chlorine concentration was measured again and noted. These values are shown in Figure 4.2 as 'After tapping'. The difference between the free chlorine concentration before and after tapping is shown as 'difference' in Figure 4.2. It can be observed that on several days, the difference was negative, meaning that the free chlorine concentration after tapping was higher than that of before tapping, this could be caused by inaccuracies during measurement or by fluctuations in the dosing of free chlorine by the CDS.

The mean free chlorine concentration in the influent prior to the tapping of the water was 0.54 ± 0.07 mg $Cl_2$/L. The mean free chlorine concentration in the influent afterwards of the tapping was slightly lower. Namely, 0.53 ± 0.07 mg $Cl_2$/L. The mean free chlorine concentrations and the corresponding standard deviations of the free chlorine concentration prior and after tapping are shown in Figure 4.2. The paired t-test showed no significant difference between the free chlorine concentration in the samples taken before and after the tapping. The means of the free chlorine concentration in the influent are shown in Figure 4.2.

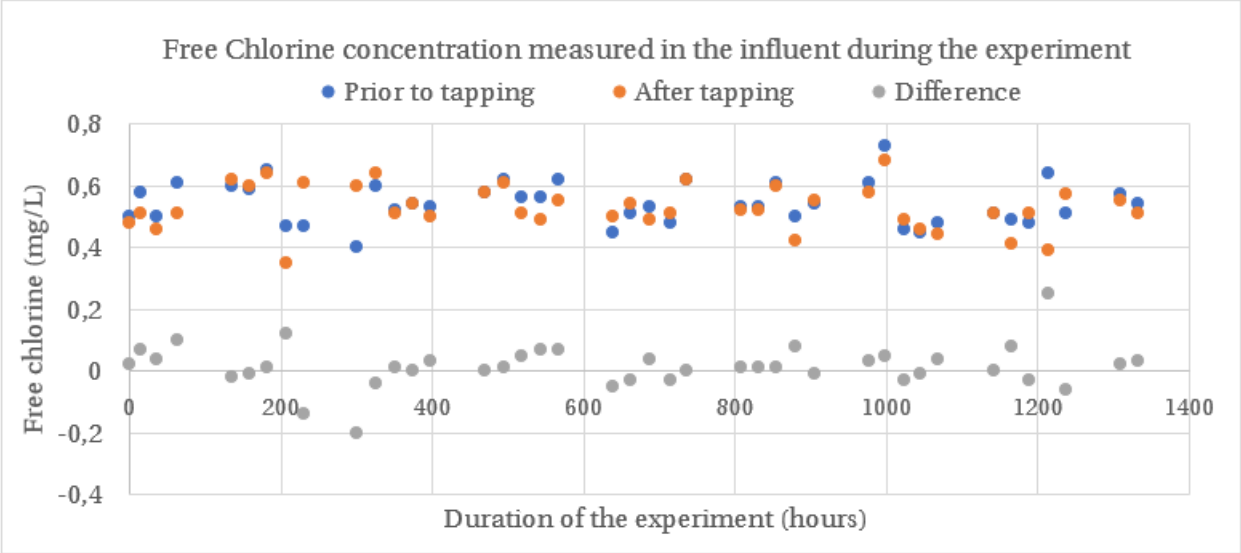


Figure 4.1: The free chlorine concentrations in the influent of the CGWDs before and after tapping along with the difference between before and after

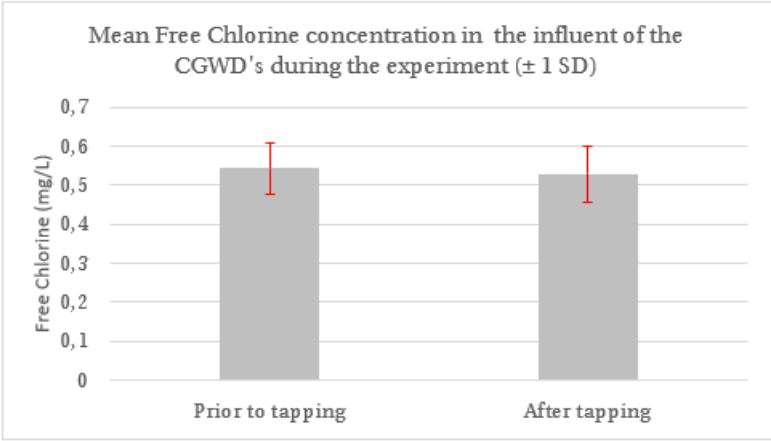


Figure 4.2: The distribution of the free chlorine concentrations before and after tapping water from the CGWDs

### 4.1.3. Flowcytometry analysis

*ICC and TCC in the influent*

ICC and TCC analysis were performed on 8 samples from the influent. The ICC and TCC during the experiment are shown in Figure 4.3. The mean ICC in the influent was $4.0 \times 10^4 \pm 3.5 \times 10^4$ cells/mL and the mean TCC was $2.1 \times 10^5 \pm 5.2 \times 10^4$ cells/mL. The ratio of ICC/TCC in the influent during the experiment is shown in Figure 4.4. The mean ratio between ICC and TCC in the influent was 19 ±16%.

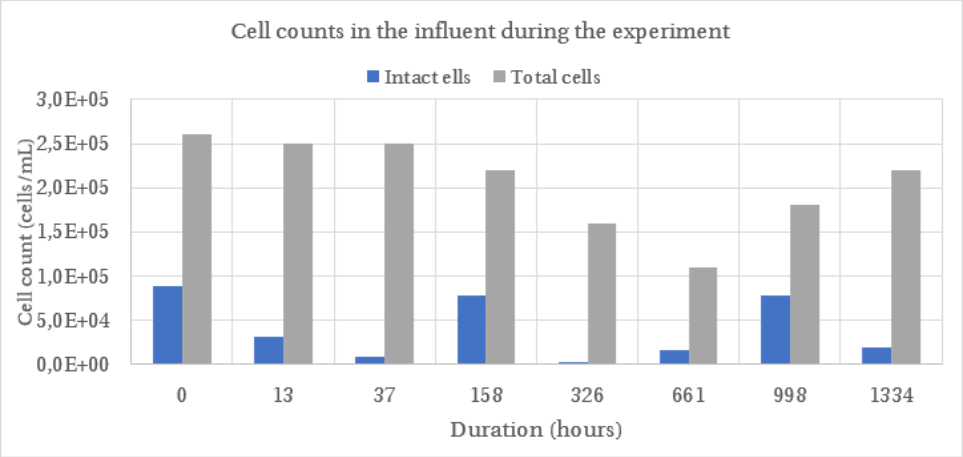


Figure 4.3: The number of intact and total cells in the influent of the CGWDs during the experiment

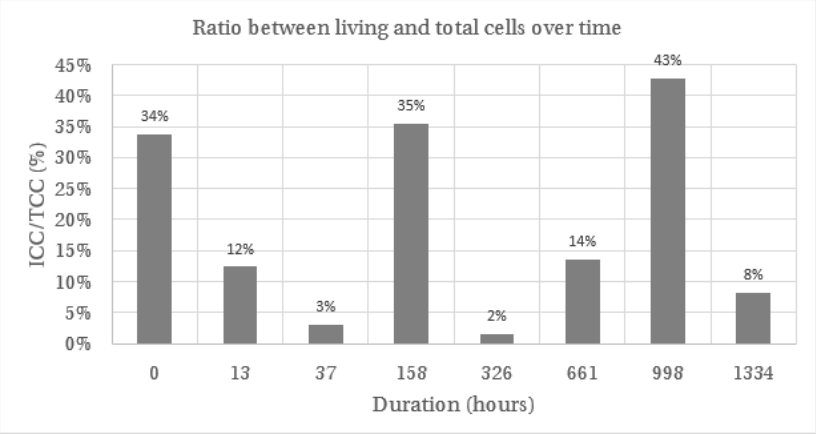


Figure 4.4: The ratio between the number of intact and the number of total cells in the influent of the CGWDs during the experiment

*Differences between duplicates*

Since FCM analysis are relatively costly, it was decided to limit the number of duplicates analysis. At t = 0, 28, and 56 days, samples were taken in duplicate from the CGWDs to compare the values at the beginning, halfway, and at the end of the experiment. At t = 1, 2, 7, 14, and 42 days, the effluent samples of CGWD-C1, CGWD-B1 and CGWD-B&S1 were analyzed only. The values of ICC in the effluent of the CGWD-Cs at t = 0, 38, and 56 days are shown in Figure 4.5. Whereas the ICC values in the effluent of the CGWD-Bs and CGWD-B&Ss are shown in Figure 4.6 and Figure 4.7, respectively. Since only 3 samples were taken per set of duplicates, no accurate statistical analysis could be performed. Instead, a relative difference between the values of CGWD-B1 and CGWD-B2 of 50% was assumed to be acceptable. Whereas the differences between the duplicates of the CGWD-C and CGWD-B&S were acceptable, it could be seen that a larger difference is obtained for the CGWD-B's at t = 56 days (see Figure 4.6).

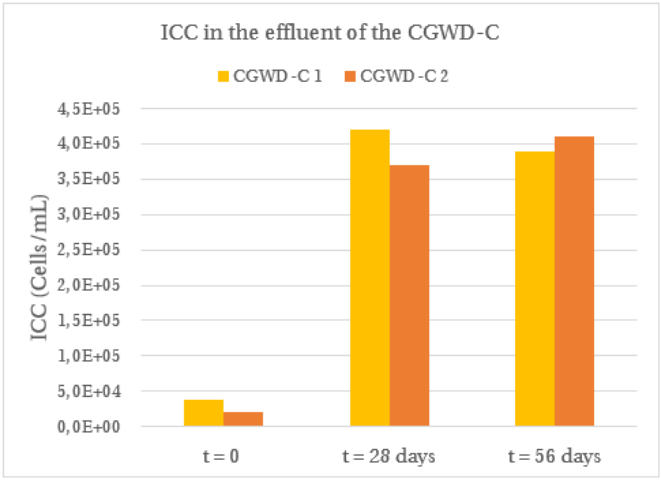


Figure 4.5: Comparison of the number of living cells in the effluent of both CGWD-Cs

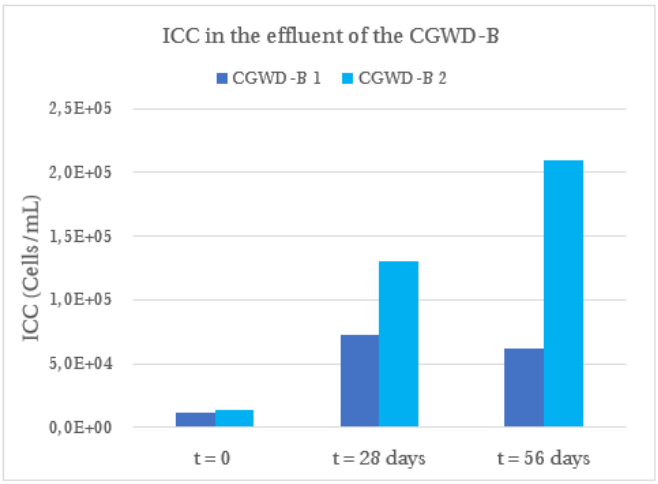


Figure 4.6: Comparison of the number of living cells in the effluent of both CGWD-Bs

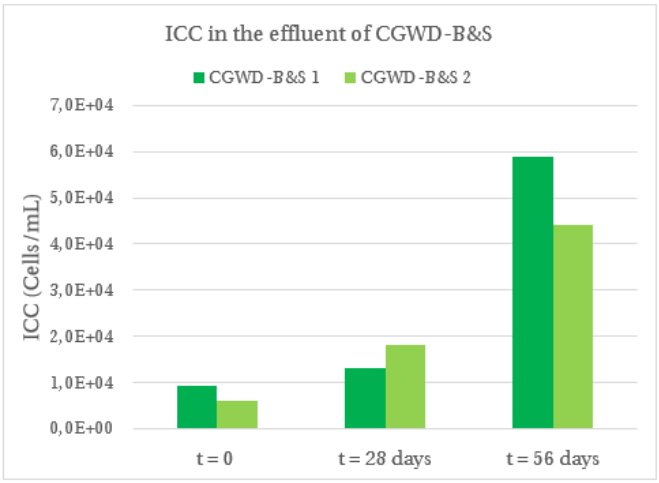


Figure 4.7: Comparison of the number of living cells in the effluent of both CGWD-B&Ss

*ICC and TCC in the effluent*
The ICC and TCC in the effluent of CGWD-C1, CGWD-B1 and CGWD-B&S1 during the experiment are shown in Figure 4.8 and in Figure 4.9.

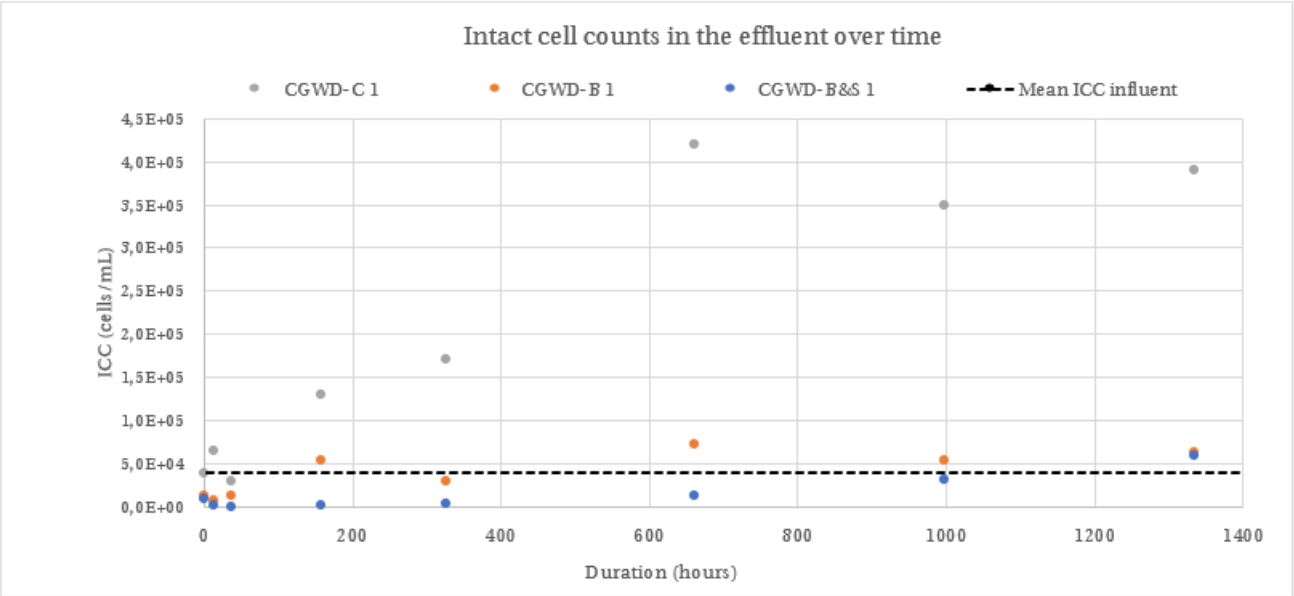


Figure 4.8: The variable amount of living bacterial cells in the effluent of CGWDs during the experiment

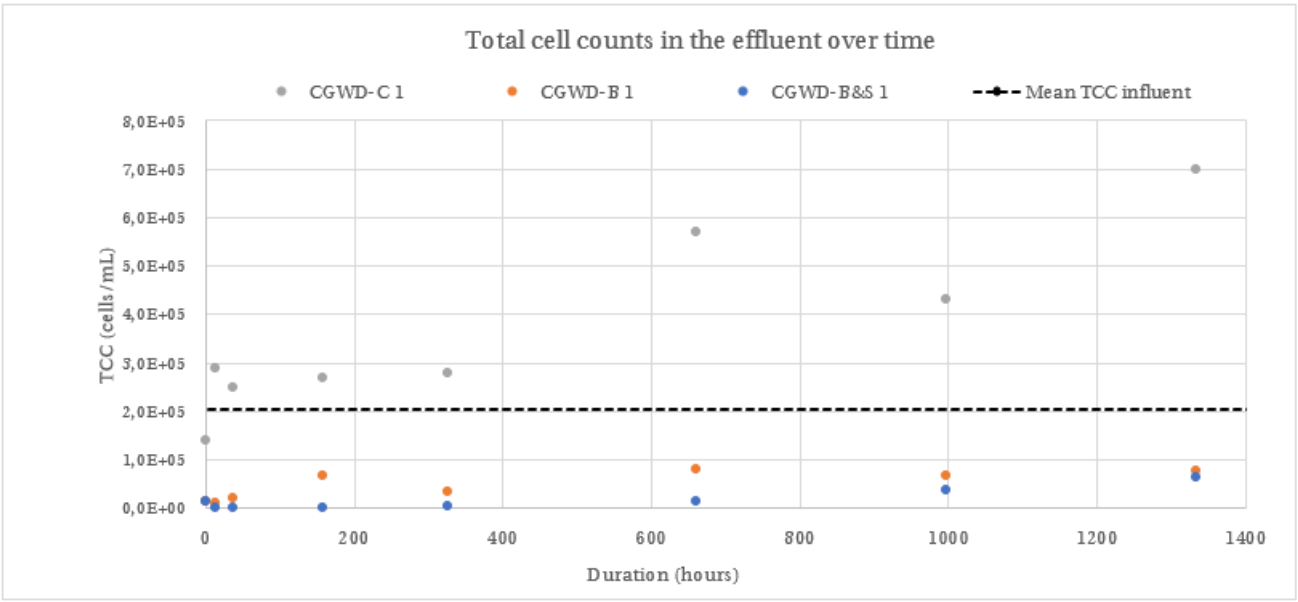


Figure 4.9: The variable amount of living and dead bacterial cells in the effluent of CGWDs during the experiment

The values of the ICC in the effluent of the CGWDs, the maximum values of ICC in the effluent of the CGWDs, and the maximum increase of ICC relative to that of the influent are shown in Table 4.1. In the same way, the values for TCC are presented in Table 4.2.

| | Units | CGWD-C1 | CGWD-B1 | CGWD-B&S1 |
|---|---|---|---|---|
| ICC in Effluent at t=0 | (cells/mL) | 3.8 x $10^4$ | 1.2 x $10^4$ | 9.3 x $10^3$ |
| Max ICC in effluent | (cells/mL) | 4.2 x $10^5$ | 7.3 x $10^4$ | 5.9 x $10^4$ |
| Max ICC in effluent - Mean ICC in influent | (cells/mL) | 3.8 x $10^5$ | 3.3 x $10^4$ | 1.9 x $10^4$ |
| Max increase ICC relative to the influent | (%) | 960% | 84% | 49% |

Table 4.1: Overview of the results of the ICC analyses

| | Units | CGWD-C1 | CGWD-B1 | CGWD-B&S1 |
|---|---|---|---|---|
| TCC in Effluent at t=0 | (cells/mL) | 1.4 x $10^5$ | 1.6 x $10^4$ | 1.4 x $10^4$ |
| Max TCC in effluent | (cells/mL) | 7.0 x $10^5$ | 8.0 x $10^4$ | 6.5 x $10^4$ |
| Max TCC in effluent - Mean TCC in influent | (cells/mL) | 4.9 x $10^4$ | -1,3 x $10^5$ | -1.4 x $10^5$ |
| Max increase TCC relative to the influent | (%) | 31% | -68% | -99% |

Table 4.2: Overview of the results of the TCC analyses

From Table 4.1 and Figure 4.8 it can be observed that the number of living bacteria in the effluent of all configurations increased over time. The maximum values of ICC in the effluent of all configurations were above the mean ICC in the influent. However, the maximum increase between the ICC in the influent and the ICC in the effluent of CGWD-B&S1 was slightly lower than that of CGWD-B1 and much lower than that of CGWD-C1.

The paired t-test showed that the ICC in the effluent of CGWD-C1 and CGWD-B&S1 was significantly higher than the ICC in the effluent ($p < 0.05$). However, no significant difference was found between the ICC in the effluent of CGWD-B1 and the ICC in the influent ($p > 0.05$). Moreover, the mean ICC in the effluent of CGWD-B&S1 was significantly lower than the ICC in the influent ($p < 0.05$). After comparing the ICC values in the effluents of CGWDs to each other, using the paired t-test, it was shown that the ICC in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-B1 ($p < 0.05$). Where as the ICC in the CGWD-B1 were significantly lower than that that of CGWD-C1 ($p < 0.05$).

From Table 4.2 and Figure 4.9 it can be observed that the number of living and dead bacteria in the effluent of all configurations increased over time. The maximum values of TCC in the effluent of CGWD-C1 was above the mean ICC in the influent, whereas the maximum values of the CGWD-B1 and CGWD-B&S1 were below the mean ICC in the influent. The maximum decrease between the ICC in the influent and the ICC in the effluent of CGWD-B&S1 was slightly higher than that of CGWD-B1, whereas for CGWD-C1 an increase in the ICC was observed.

The paired t-test showed that the TCC in the effluent of CGWD-B1 and CGWD-B&S1 were significantly lower than the TCC in the effluent ($p < 0.05$). However, no significant difference was found between the TCC in the effluent of CGWD-C1 and the ICC in the influent ($p > 0.05$). After comparing the TCC values in the effluents of the CGWDs to each other, using the paired t-test, it was shown that the TCC in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-B1 ($p < 0.05$). Whereas the TCC in the CGWD-B1 was significantly lower than that of CGWD-C1 ($p < 0.05$).

### 4.1.4. HPC22 Analysis

*HPC22 in the influent & stock solution tank*
On each sampling day, a sample was taken from the influent and the stock solution tank for HPC22 analysis. The mean HPC22 in the influent was 34.8 ± 87.4 cfu/mL, however this was strongly affected by an outlier of 250 cfu/mL at t = 42 days. During the rest of the experiment, the HPC22 in the influent was below 15 cfu/mL (5 times below the detection limit of 1 cfu/mL). The HPC22 in the stock solution tank was equal to 2 cfu/mL for two times, the rest of the experiment HPC22 was below the detection limit.

*HPC22 in the effluent*
The values of HPC22 in the effluent of CGWD-C1, CGWD-B1 and CGWD-B&S1 during the experiment are shown in Figure 4.10.

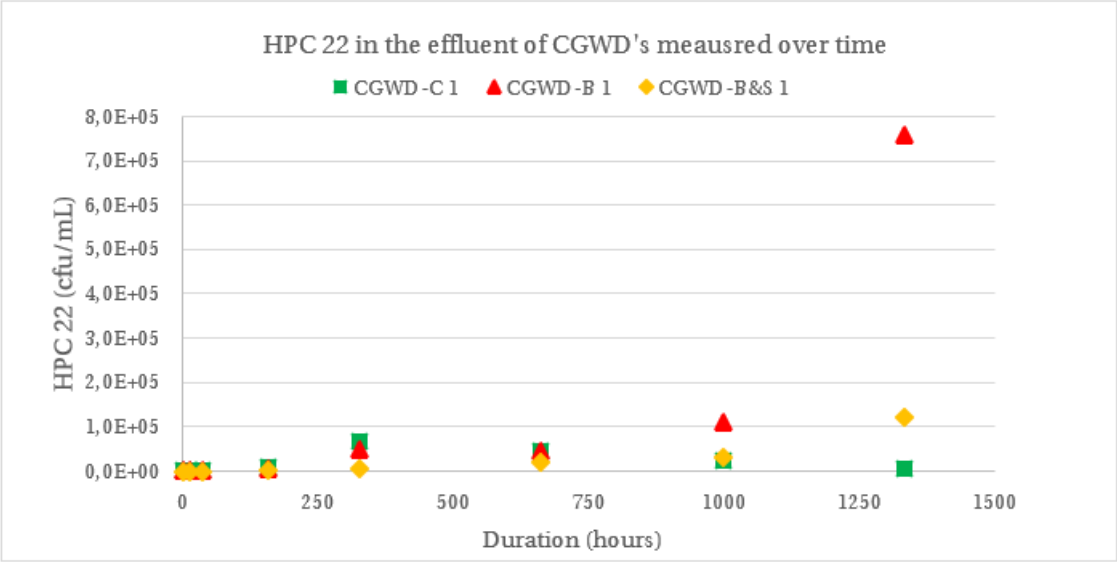


Figure 4.10: The growth pattern of heterotrophic bacteria in the effluent of the CGWDs during the experiment

The key parameters of the HPC22 analysis are shown in Table 4.3

| | Units | CGWD-C1 | CGWD-B1 | CGWD-B&S1 |
|---|---|---|---|---|
| HPC22 in the effluent at t=0 | (cfu/mL) | 430 | 1700 | 1 |
| Maximum HPC22 in the effluent | (cfu/mL) | 6.8 x $10^4$ | 7.6 x $10^5$ | 1.2 x $10^5$ |

Table 4.3: Overview of the results of the HPC22 analyses

From Table 4.3 and Figure 4.10 it can be observed that the HPC22 in the effluent of all CGWDs increased over time. The values of HPC22 in the effluent of all CGWDs were always above the HPC22 in the influent. The maximum value of HPC22 in the effluent was first reached by CGWD-C1, after which the HPC22 slowly decreased.

The paired t-test showed that there were no significant differences between HPC22 in the effluents of the duplicates ($p > 0.05$). Furthermore, a significant increase was shown between HPC22 in the effluents of all CGWDs compared to the HPC22 in the influent ($p < 0.05$). After comparing the HPC22 in the effluents of the CGWDs to each other, using the paired t-test, it was shown that the PC22 in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-B1 and CGWD-C1 ($p < 0.05$). However, no significant difference was shown between the HPC22 in the effluent of CGWD-B1 and that of CGWD-C1 ($p > 0.05$).

### 4.1.5. ATP Analysis

The results of the ATP analysis of the calibration and the corresponding calibration curve and conversion formula are discussed in Appendix D. After the first calibration experiment, it was decided to discard the values of days 0, 1, and 2 due to the improper handling of the measurement equipment leading to inaccurate results.

*ATP in the influent & stock solution tank*

The calculated cATP concentrations in the influent and stock solution tank are shown in Table 4.4. Some of the cATP concentrations in the influent and stock solution tank were below zero, which is impossible. Interestingly, no abnormal values were obtained after calculating the cATP concentration in the effluent of the CGWDs, while the samples were taken and analyzed according to the same procedure. The difference between both types of water is that the influent and stock solution tank had relatively high free chlorine concentrations, while it can be assumed that effluent of the CGWDs contains no free chlorine because of the GAC filter. This led to the suspicion that the free chlorine concentration influenced the ATP measurement of the luminometer. Some minor tests with the luminometer proved that if two of the same samples, but one with and one without free chlorine, were analyzed, that the sample with chlorine had a significantly lower reading of tATP.

| | Cellular ATP (ng ATP/L) | | | | |
|---|---|---|---|---|---|
| Date | 6 Oct | 13 Oct | 27 Oct | 10 Nov | 24 Nov |
| Duration | 158 | 326 | 661 | 998 | 1334 |
| Tank | 1.2 | 1.3 | -4.5 | -3.8 | 0.8 |
| Inlet | -0.7 | 0.7 | -0.5 | -2.7 | 0.7 |

Table 4.4: The bacterial activity of the water in the influent and solution tank during the experiment

*ATP in the effluent*

The cATP concentrations in the effluent of CGWD-C1, CGWD-B1, and CGWD-B&S1 during the experiment are shown Figure 4.11.

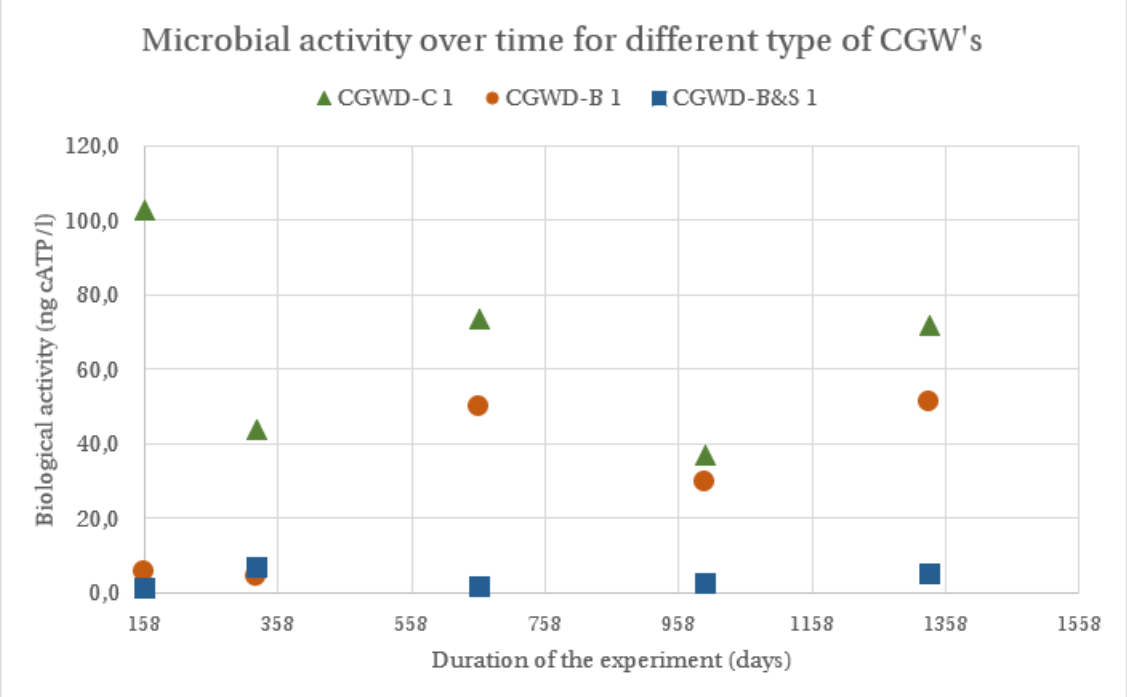


Figure 4.11: The bacterial activity of the water in the effluent of the CGWDs during the experiment

The maximum values of the acrshortcATP concentrations in the effluent of CGWD-C1, CGWD-B1, and CGWD-B&S1 were, respectively, 102.7, 50.9, and 6.8 ng cATP/L.T he paired t-test showed that there were no significant differences between the duplicates ($p > 0.05$). After comparing the cATP concentrations in the effluents of the CGWDs to each other, using the paired t-test, it was shown that the acrshortcATP concentration in the effluent of the CGWD-B&S1 was significantly lower than that of CGWD-B1 ($p < 0.05$). Whereas the cATP concentration in the CGWD-B1 was significantly lower than that of CGWD-C1 ($p < 0.05$).

*Biofilm analysis*
After the experiment was terminated, small fractions of the filter bed material of CGWD-C1, CGWD-B1, and CGWD-B&S1 were treated with a High Energy Sonifier (HES) to extract the biomass. After the treatment with the HES, the three samples were analyzed with ATP analysis. The biomass per unit weight of the filter material in the samples of CGWD-1 was 2.5E+03 pg ATP/g GAC after 2 minutes at a capacity of 45%. The measured values of the tATP concentration in the samples of CGWD-B1 and CGWD-B&S1 were both below the detection limit of 1 pg ATP/L. The values of the biomass per unit weight of the filter material of CGWD-B1 and CGWD-B&S1 were, respectively, <16 pg ATP/g GAC and <13 pg ATP/g GAC.

### 4.1.6. Outline results experiment 1

The first experiment was performed to compare the degree of bacterial growth between three different configurations of CGWDs. The first configuration was that of a conventional CGWD (CGWD-C1). In the second configuration, a boiler with an integrated GAC filter was implemented (CGWD-B1). The third configuration was similar to the second configuration, but additionally, the devices were exposed to steam prior to the experiment (CGWD-B&S1). An overview of the important results is shown in Table 4.5.

| Analysis | Units | n | Influent | CGWD-C1 | CGWD-B1 | CGWD-B&S1 |
|---|---|---|---|---|---|---|
| Temp. effluent | °C | 6 | - | 7.0 | 6.8 | 7.2 |
| Temp. boiler | °C | 6 | - | 21.2 | 105 | 105 |
| Free chlorine influent | mg Cl2/L | 41 | 0.54 ± 0.07 | - | - | - |
| Mean HPC22 | cfu/mL | 8 | 0.48 ± 0.87 | - | - | - |
| HPC22 at t=0 | cfu/mL | 8 | - | 4.3 x $10^2$ | 1.7 x $10^3$ | <1 |
| Max HPC22 | cfu/mL | 8 | - | 6.8 x $10^4$ | 7.6 x $10^5$ | 1.2 x $10^5$ |
| Mean ICC influent | cells/mL | 8 | 4.0 × $10^4$ | - | - | - |
| ICC at t=0 | cells/mL | 8 | - | 3.8 x $10^4$ | 1.2 x $10^4$ | 9.3 x $10^3$ |
| Max ICC | cells/mL | 8 | - | 4.2 x $10^5$ | 7.3 x $10^4$ | 5.9 x $10^4$ |
| Mean TCC influent | cells/mL | 8 | 2.1 x $10^5$ | - | - | - |
| TCC at t=0 | cells/mL | 8 | - | 1.4 x $10^5$ | 1.6 x $10^4$ | 1.4 x $10^4$ |
| Max TCC | cells/mL | 8 | - | 7.0 x $10^5$ | 8.0 x $10^4$ | 6.5 x $10^4$ |
| Max cATP | ng cATP/L | 5 | - | 102.7 | 50.9 | 6.8 |
| tATP in GAC | pg tATP/g GAC | 1 | - | 2500 | <16 | <13 |

Table 4.5: Overview of the results of experiment 1

## 4.2. Experiment 2 - Analyzing removal performances of the boilers

### 4.2.1. Flow rates

The total treated volume by the boilers BW1, BW2, CW1, and CW2 was, respectively, 32,000 L (95,200 bed volumes), 30,800 L (91,500 bed volumes), 34,200 L (101,700 bed volumes) and 34,600 L (102,800 bed volumes). The flow rate for each boiler during the experiment is shown inFigure 4.12.

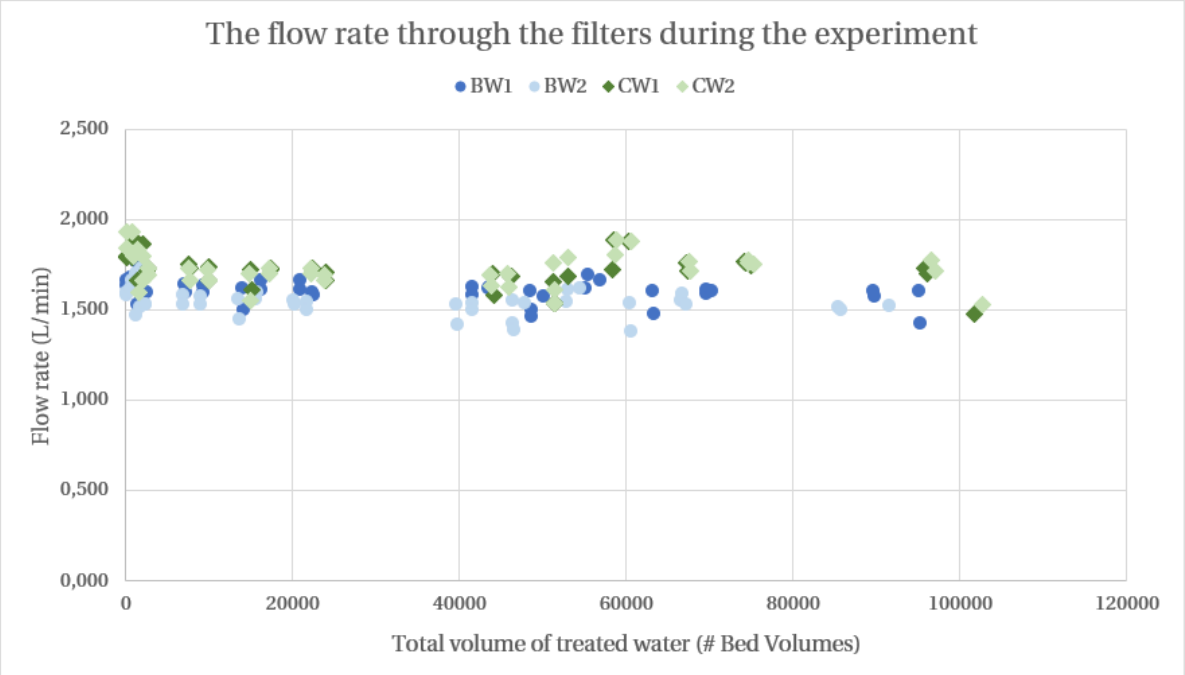


Figure 4.12: The flow rates of the water flushed through the boilers during the experiment

The mean flow rates of BW1, BW2, CW1, and CW2 were, respectively, 1.61 ± 0.06 L/min, 1.55 ± 0.07 L/min, 1.72 ± 0.09 L/min, and 1.74 ± 0.10 L/min. The flow rate of BW1 was significantly higher than the flow rate of BW2 ($p < 0.05$), whereas no significant difference was found between the flow rates of CW1 and CW2 ($p > 0.05$). Furthermore, the flow rates of B1 and BW2 were significantly lower than the flow rates of CW1 and CW2 ($p < 0.05$).

### 4.2.2. Temperatures

The mean temperatures of the water in the top of the reservoirs of BW1 and BW2 were, respectively, 102.9°C (±2.5, n = 11) and 103.1°C (±2.2, n = 11). The temperatures of the effluent from BW1, BW2, CW1, and CW2 were, respectively, 31.2°C (±2.1, n=11), 30.8°C (±2.3, n=11), 21.2°C (±1.1, n=11) and 21.2°C (±0.9, n=11).

### 4.2.3. Free chlorine

During the experiment, the free chlorine concentration was measured in 95 samples. At 19 different moments during the experiment, 5 samples were taken. One sample from the influent and four samples from the effluent of the boilers.

The free chlorine concentrations in the influent and the effluent of BW1 and CW1 are shown in Figure 4.13. The mean free chlorine concentration in the influent water was 0.51 mg/L (Standard deviation = 0.03 mg/L). The free chlorine concentrations in the effluent of BW1, BW2, CW1, and CW2, directly measured after the start of the experiment were, respectively, <0.05, <0.05, 0.07, and 0.09 mg/L. The Wilcoxon Signed Ranks test showed a significant difference between the free chlorine concentration in the effluent of all boilers and the free chlorine concentration in the influent ($p < 0.05$). The Wilcoxon Signed Ranks test showed no significant difference between the free chlorine concentration in the effluent of BW1 and BW2 ($p > 0.05$), and neither between CW1 and CW2 ($p > 0.05$). Throughout the experiment, the free chlorine concentration in the effluent of BW1 and BW2 was always equal or below 0.05 mg/L. However, the free chlorine concentration in the effluent of CW1 and CW2 increased over time. After 102,000 treated bed volumes, the free chlorine concentrations in the effluent of CW1 and CW2 were both 0.18 mg $Cl_2$/L. The Wilcoxon Signed Ranks test showed a significant difference between the free chlorine concentration in the effluent of BW1 and CW1 ($p < 0.05$).

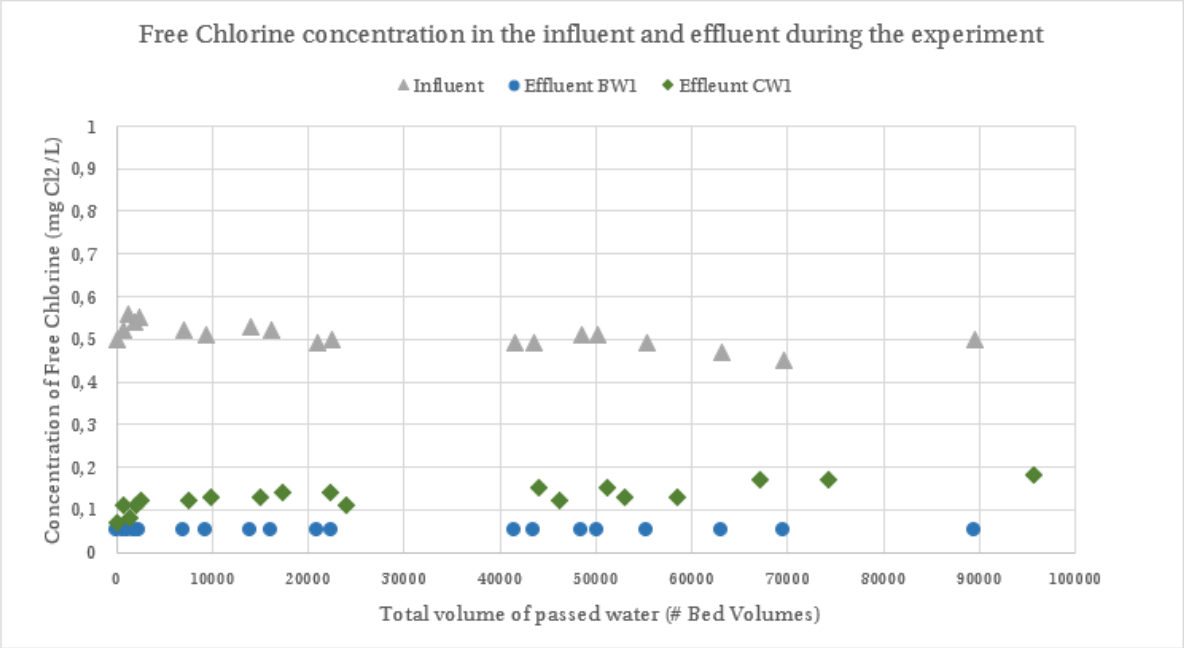


Figure 4.13: The free chlorine concentration in the influent and effluent of BW1 and CW1

The free chlorine removal efficiencies during the experiment of BW1 and CW1 are shown in Figure 4.14. The free chlorine removal efficiency in the effluent of BW1, BW2, CW1, and CW2, directly after the start of the experiment was, respectively, >90%, >90%, 86%, and 82%. However, it is important to note that the free chlorine removal efficiency of BW1 and BW2 could be higher. Whenever the free chlorine concentration in the effluent was below the detection limit of 0.05 mg $Cl_2$/L, the value was set to 0.05 mg $Cl_2$/L. The Wilcoxon Signed Ranks test showed no significant difference between the free chlorine removal efficiency of BW1 and BW2 ($p > 0.05$), and neither between CW1 and CW2 ($p > 0.05$). Whereas the free chlorine removal efficiency of BW1 and BW1 remained unchanged during the experiment (> 90%), the free chlorine removal efficiency of CW1 and CW2 decreased. At the end of the experiment, CW1 and CW2 treated, respectively, 95,500 and 96,600 bed volumes. The free chlorine removal efficiencies of CW1 and CW2 were both 64% at the end of the experiment. The Wilcoxon Signed Ranks test showed a significant difference between free chlorine removal efficiency of BW1 and CW1 ($p < 0.05$).

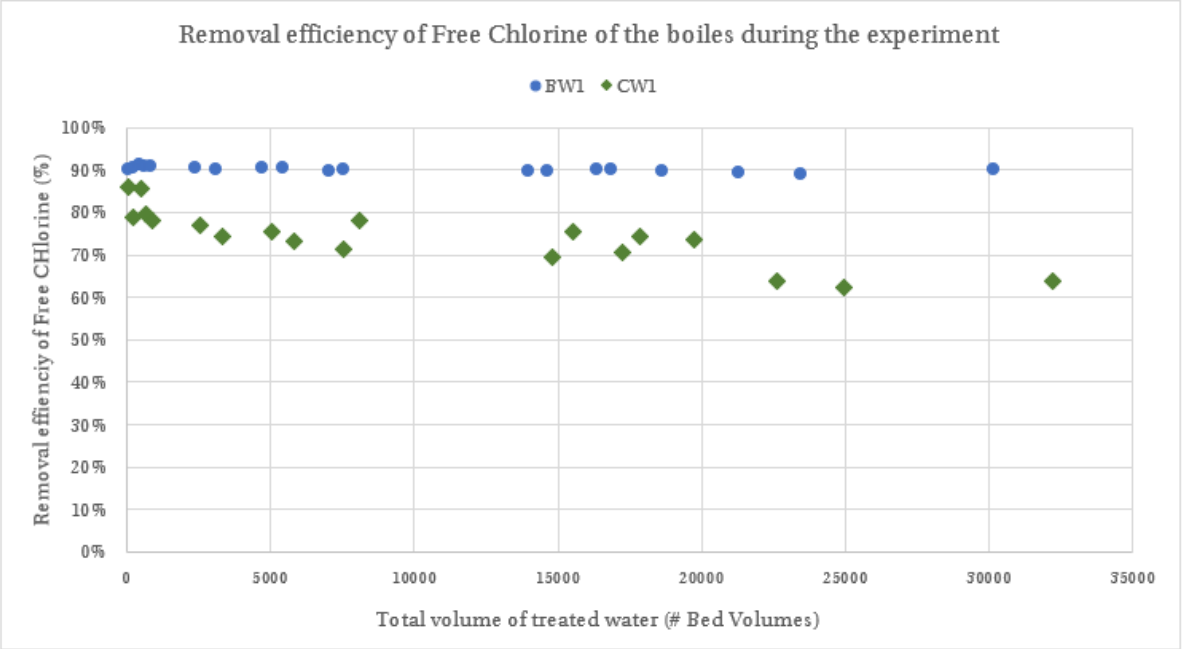


Figure 4.14: The free chlorine removal efficiencies of BW1 and CW1 during the experiment

### 4.2.4. Total organic carbon

After 168 hours it was decided to stop measuring the TOC concentration since the preliminary results showed that the removal efficiencies of all boilers were close to 0%. During the measurement of the samples from 25-06-2020 and 26-06-2020, the TOC analyzer overheated due to a technical defect. While being kept in the TOC analyzer during the whole weekend, the TOC samples were not stored under the right circumstances and no measurements were performed. Therefore, it was decided to discard these samples. In total, the TOC concentration was measured in 50 samples. At 10 different moments during the experiment, 5 samples were taken. One sample from the influent and four samples from the effluent of the boilers.

The TOC concentrations in the influent of the set-up, in the influent of CW1 and BW1, and the effluent of CW1 and BW1 during the experiment are shown in Figure 4.15. The mean TOC concentration in the influent was 1.98 ± 0.18 mg C/L (n=10). The TOC concentration in the effluent of BW1, BW2, CW1, and CW2, directly measured after the start of the experiment was, respectively 0.75, 0.77, 1.15, and 1.22 mg/L. The Wilcoxon Signed Ranks test showed a significant difference between the TOC concentration in the effluent of all boilers and the TOC concentration in the influent ($p < 0.05$). The Wilcoxon Signed Ranks test showed no significant difference between the TOC concentration in the effluent of BW1 and BW2 ($p > 0.05$), and neither between that of CW1 and CW2 ($p > 0.05$). Throughout the experiment, the TOC concentration in the effluent of BW1, BW2, CW1, and CW2 followed an increasing trend. However, The Wilcoxon Signed Ranks test showed a significant difference between the TOC concentration in the effluent of BW1 and CW1 ($p < 0.05$). At the time of the last measurement, BW1, BW2, CW1, and CW2 treated, respectively, 48,600, 46,500, 51,400, and 51,400 bed volumes. The TOC concentrations in the effluent of BW1, BW2, CW1, and CW2 were, respectively, 1.78, 1.79, 1.85, and 1.82 mg C/L at the time of the last measurement.

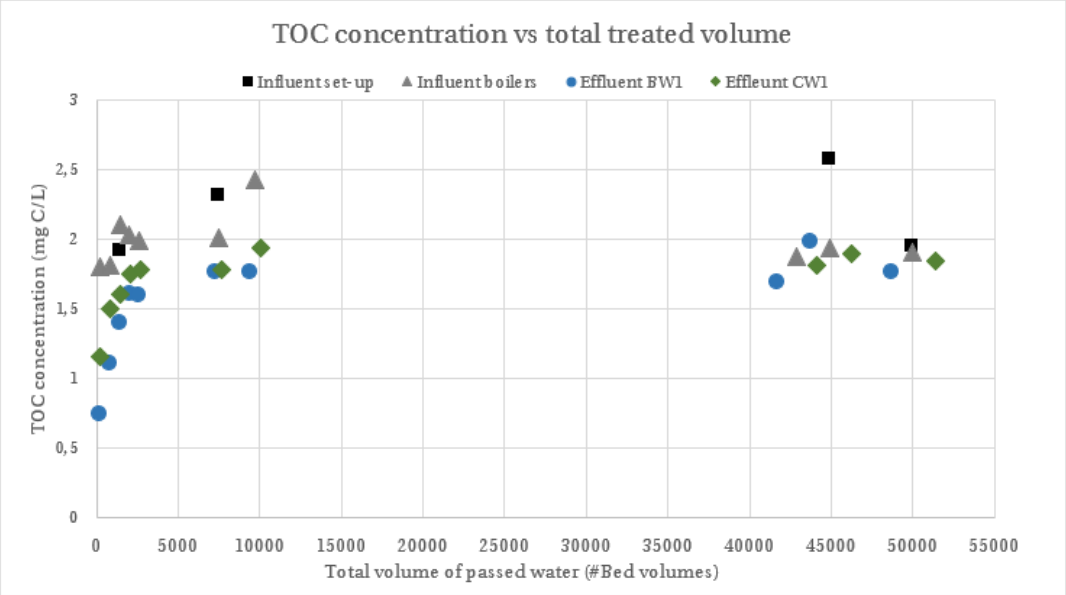


Figure 4.15: Total organic carbon content in the influent and effluent of BW1 and CW1 during the experiment

The TOC removal efficiencies during the experiment of BW1 and CW1 are shown in Figure 4.16. The TOC removal efficiency in the effluent of BW1, BW2, CW1, and CW2, directly after the start of the experiment, was respectively 58.4%, 61.5.0%, 35.7%, and 38.4%. The Wilcoxon Signed Ranks test showed no significant difference between the TOC removal efficiency of BW1 and BW2 ($p > 0.05$), and neither between CW1 and CW2 ($p > 0.05$). The TOC removal efficiency of all boilers followed a decreasing trend. However, the TOC removal efficiency of BW1 and BW2 was always slightly higher compared to that of CW1 and CW2 during the experiment. The Wilcoxon Signed Ranks test showed a significant difference between TOC removal efficiency of BW1 and CW1 ($p < 0.05$). At the time of the last measurement, BW1, BW2, CW1, and CW2 treated, respectively, 48,600, 46,500, 51,400, and 51,400 bed volumes. The TOC removal efficiencies of BW1, BW2, CW1, and CW2 were, respectively, 7%, 10%, 3%, and 8% mg C/L at the time of the last measurement.

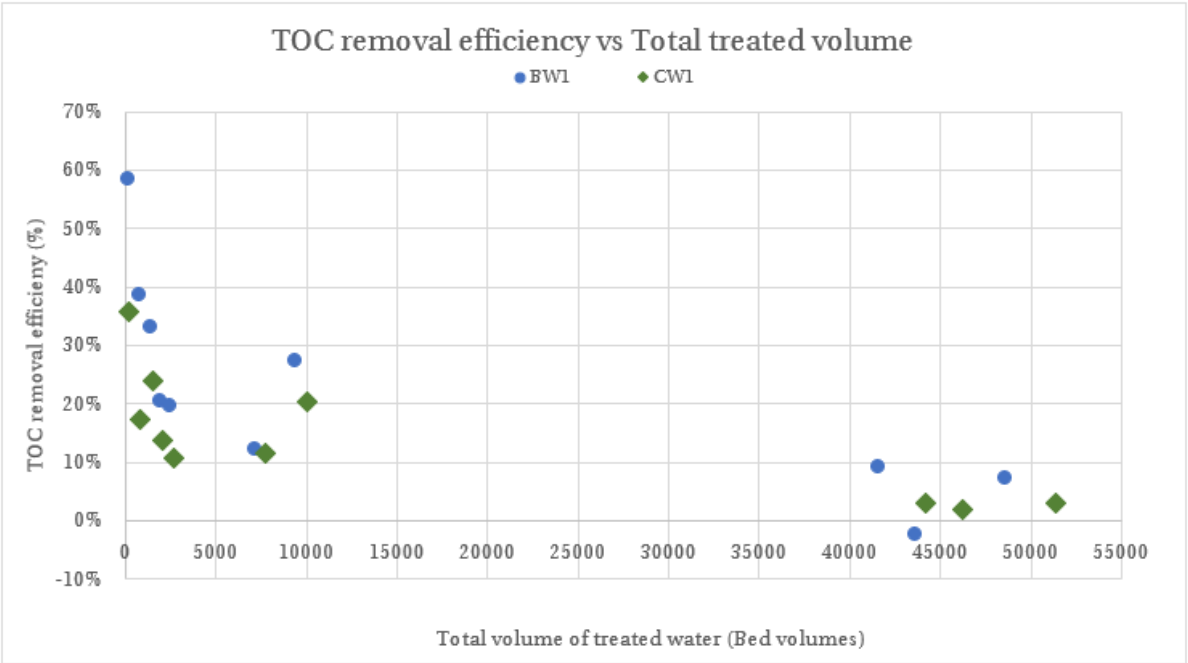


Figure 4.16: The TOC removal efficiencies of BW1 and CW1 during the experiment

Whereas the calculated TOC removal efficiency strongly depends on the TOC concentration in the influent, it was decided to investigate the origin of the differences between the TOC concentration in the effluent. During the measurements with the TOC analyzer, blank and standard solutions were added and measured as a check. The maximum error of the TOC analyzer appeared to be 0.01 mg/L. Therefore, the suspicion aroused that the TOC concentration in the influent of the experimental set-up is subject to relative high fluctuations. To check this, 10 samples from the influent for TOC analysis were taken consecutively, with an interval of 15 seconds. The results are shown in Table 4.6.

| Sample # | Duration (s) | TOC (mg C/L) |
|---|---|---|
| 1 | 15 | 1.903 |
| 2 | 30 | 1.648 |
| 3 | 45 | 1.845 |
| 4 | 60 | 1.633 |
| 5 | 75 | 1.685 |
| 6 | 90 | 1.649 |
| 7 | 105 | 1.631 |
| 8 | 120 | 1.658 |
| 9 | 135 | 1.602 |
| 10 | 150 | 1.663 |

Table 4.6: The variability of the measured TOC concentrations in a short period of time

### 4.2.5. Assimilable organic carbon

In the original lab design, the AOC concentration at the inlet of the reservoir had to be calculated by subtracting the TOC concentration in the effluent of the set-up (before the CDS) from the TOC concentration in the effluent of the boilers (after the CDS). During the experiment, the theoretical concentration of AOC was increased to 250 µg acetate-C/L, which can be measured as 0.25 mg C/L with the TOC analyzer. As described in the previous section, the measured TOC concentrations varied up to 0.3 mg C/L within a time span of 15 seconds. This lead to very inaccurate AOC concentrations, with 3 of the four calculated AOC concentrations being negative.

### 4.2.6. Additional experiment without GAC filters

Additionally, a brief experiment was executed to investigate the role of GAC filters, to understand the removal mechanisms of free chlorine and TOC by GAC and boiling.

For a period of 91 minutes, the water was led through BW1 and CW1 from which the GAC filters were removed. During the experiment, the mean temperature in BW1 was 100.3°C and in CW1 the mean temperature was 22.0°C. The mean flow rate was 1.9 L/min. The average retention time of the water in the reservoirs was approximately 3.5 minutes.

The concentration of free chlorine in the influent of the boilers after 70 and 91 minutes was, respectively, 0.44 and 0.42 mg/L. The concentrations of free chlorine in the effluent of BW1 after 70 and 91 minutes were, respectively, <0.05 and <0.05 mg/L. The concentrations of free chlorine in the effluent of CW1 after 70 and 91 minutes were, respectively, 0.44 and 0.42 mg/L.

The concentration of TOC in the influent of the boilers after 70 and 91 minutes was, respectively, 1.63 and 1.53 mg C/L. The concentrations of TOC in the effluent of BW1 after 70 and 91 minutes were, respectively, 1.54 and 1.94 mg C/L. The concentrations of TOC in the effluent of CW1 after 70 and 91 minutes were, respectively, 1.43 and 1.58 mg C/L.

### 4.2.7. Outline results experiment 2

The second experiment was performed to compare the removal performances of two GAC filters treating room temperature water (CW1 and CW2) to the removal performance of two boilers with integrated GAC filters (BW1 and BW2). An overview of the most important results is shown in Table 4.7.

| | Units | Influent | BW1 | CW1 |
|---|---|---|---|---|
| Mean flow rates | L/min | - | 1.61 ± 0.06 | 1.72 ± 0.09 |
| Mean temperature boiler & GAC | °C | - | 102.9 ± 2.5 | 21.2 ± 1.1 |
| Free chlorine concentration (t = 0) | mg $Cl_2$/L | 0.50 | <0.05 | 0.07 |
| Free chlorine removal efficiency (t = 0) | % | - | >90 | 86 |
| Free chlorine concentration (t = 312 hr) | mg $Cl_2$/L | 0.50 | <0.05 | 0.18 |
| Free chlorine removal efficiency (t = 312 hr) | % | - | >90 | 64 |
| Total treated volume (t = 312 hr) | Bed volumes | - | 95,200 | 101,700 |
| TOC concentration (t = 0) | mg $Cl_2$/L | 1.79 | 0.75 | 1.15 |
| TOC Removal efficiency (t = 0) | % | - | 58 | 36 |
| TOC concentration (t = 168 hr) | mg $Cl_2$/L | 1.90 | 1.78 | 1.85 |
| TOC Removal efficiency (t = 168 hr) | % | - | 7 | 3 |
| Total reated volume (t = 168 hr) | Bed volumes | - | 48,600 | 51,400 |

Table 4.7: Overview of the results of experiment 2

# 5

# Discussion

In this chapter, the results of the experiments will be discussed. Besides comparing the results between the different tested devices, the results will also be compared with results from previous research testing similar devices. Furthermore, the shortcomings of the followed procedures and future directions will be stated.

## 5.1. Experiment 1 - Analyzing measures against bacterial growth

The aim of the experiment was to assess whether the degree of bacterial growth in CGWDs, under a certain load-case, could be decreased by implementing measures. In order to so, three different configurations of CGWDs were tested in duplicate. The first configuration was that of a conventional CGWD (CGWD-C). In the second configuration, a boiler with an integrated GAC filter was implemented (CGWD-B). The third configuration was similar to the second configuration, but additionally, the devices were exposed to steam prior to the experiment (CGWD-B&S).

*Boundary conditions*

I was decided to feed the CGWDs with water that had similar quality as English tap water. England is a country in which CGWDs are widely used and where the drinking water has relatively high AOC concentrations. Since the experiment was performed in the Netherlands, a methodology had to be developed in order to do so. Boundary conditions were formulated to test whether the methodology succeeded in examining the CGWDs under typical English circumstances.

The first boundary conditions were that concentration of free chlorine in the influent of CGWDs had to be around 0.50 mg/L during the tapping of the water. During the experiment, the concentrations of free chlorine before and after the tapping were respectively 0.54 ± 0.07 mg/L and 0.53 ± 0.07 mg/L. Furthermore, no abnormal changes were observed in the results of the free chlorine concentrations in the influent, and the decay of free chlorine during the tapping was not significant.

The second boundary conditions prescribed that the AOC concentration in the influent of the CGWDs had to be 250 µg acetate-C/L. Since AOC analysis are relatively time-consuming and costly, it was decided to not measure the AOC concentration. Although, the concentration of AOC in reality could differ from the theoretical concentration. AOC is known to promote bacterial growth whereby the AOC will be consumed (van der Kooij, 1992). During the test, the bacterial counts in the influent and stock solution tank were analyzed and no signs of bacterial growth were observed. X. Liu et al. (2015) concluded that after the addition of free chlorine the number of intact cells decreased over time, whereas the AOC concentration increased over time due to the oxidation and chlorine substitution on aromatic compounds, whereby chlorinated aromatics and organic compounds with carboxylic and alcohol functionalities were formed. These disinfection-byproducts are considered AOC. High concentrations of chlorine were present in the solution tank, which could have increased the AOC concentration. However, in this study obtained results show no significant decay of free chlorine in the tank or in the pipe network. Based on the results of the HPC22 analysis and the free chlorine measurements, it was concluded that AOC concentration in the influent was similar to that of typical English tap water.

The third boundary condition stated that abnormal changes in the bacterial quality of the inlet water had to be prevented. The HPC22 analysis showed no abnormalities in 7 of the 8 samples, with only two

values above the detection limit of 2 and 14 cfu/mL. However, on day 42, the HPC22 in the sample of the influent was 250 cfu/mL. The European Drinking Water Directive prescribes a maximum of 100 cfu/mL in the effluent of drinking water treatment plants (European Commission, 2016). During preliminary tests, the solution tank appeared to be vulnerable to bacterial growth, because of ineffective chlorine dosing and improper cleaning of the tank. However, the HPC22 in the sample of the solution tank was <1 cfu/mL on day 42. This makes it highly unlikely that the tank was the source of bacteria. Moreover, on day 42, the chlorine concentrations before and after tapping of water were respectively 0.73 and 0.68 mg $Cl_2$/L. In the case of bacterial growth, higher counts were expected in later samples, but this was not the case. Therefore, the high HPC22 value on day 42 is expected to be caused by contamination of the sample during sampling, transport, or analysis in the laboratory. Large variations were observed for ICC and TCC in the influent, as well as the ICC/TCC ratio. The mean ICC in the influent was 4.0 × $10^4$ ± 3.5 × $10^4$ cells/mL (n=8), whereas the TCC in the influent was 2.1 × $10^5$ ± 5.2 × $10^4$ cells/mL (n=8). These values of ICC and TCC were similar to values found in previous research on chlorinated drinking water networks (Nescerecka et al., 2018). G. Liu et al. (2013) observed similar values for C in Dutch drinking water, which was the influent of the set-up. A similar variation of the ICC/TCC ratio was observed in a chlorinated drinking water system in Scotland. Cheswick et al. (2019) concluded that the variation of ICC/TCC was a reflection of the chlorine disinfection efficiency changing over time, induced by changing characteristics of the influent. The cATP analysis of the samples from the influent yielded unlikely results, with 3 of the 5 measured concentrations being negative. The effluent samples were non-chlorinated because of the GAC filters, while the influent and solution tank samples contained free chlorine. The functioning of the ATP analysis is based on an enzymatic reaction and therefore could be disturbed by aggressive chemicals like organic chemicals (Kiuru et al., 2010). Luminometers do not have an internal standard and the measurements will be affected by chlorine. Since the calibration curve was made with non-chlorinated samples, the calibration was not fitted for chlorinated samples of the influent. Therefore, the values of the cATP analysis of the influent and stock solution tank were neglected. Based on the results of the FCM and HPC22, it was concluded that the bacteriological quality of the influent was similar to that of typical English tap water.

*Bacterial growth in CGWDs*

All three different configurations were tested in duplicate as quality control of the conduct of the performed experiment. No significant differences were found with HPC22 and cATP between the duplicates.

The HPC22 in the effluent of all three different configurations increased significantly during the experiment. The maximum values of HPC22 in the effluent of CGWD-C1, CGWD-B1 and CGWD-B&S1 were, respectively, 6.8 × $10^4$, 7.6 × $10^5$, and 1.2 × $10^5$ cfu/mL (n=8). All CGWDs exceeded the norm of <100 cfu/mL after 7 days, while the HPC22 in the influent was compliant to the norm. In previous studies, similar values of  were observed in the effluent of PoU devices treating chlorinated drinking water (Chaidez & Gerba, 2004; Snyder et al., 1995). No significant difference was observed between CGWD-C1 and CGWD-B1. Therefore, it can be concluded that the growth of heterotrophic bacteria could not be decreased with the pre-treatment of a boiler with an integrated GAC. According to the findings of the literature review on enhancing factors of bacterial growth (see section 2.4), it was concluded that the GAC filter material promotes the growth of heterotrophic bacteria. Therefore, it was proposed to place the GAC filter into the boiler in order to limit bacterial growth on the GAC filter, additionally, the bacteria in the influent would be inactivated due to the high temperatures. However, during the experiment, the growth of heterotrophic bacteria in CGWD-B1 could not be decreased by replacing the GAC filter into the boiler. This indicates that in addition to the GAC filter bed and the bacteria in the influent, other factors are causing the growth of heterotrophic bacteria in the CGWD-B1. Interestingly, the HPC22 in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-C1 and CGWD-B1 Moreover, during the first 37 hours, no HPC 22 was found in the effluent of CGWD-B&S1, while the HPC22 in the other devices was already between $10^2$ and $10^4$ cfu/mL. Thus, the degree of heterotrophic bacteria growth can be decreased by exposing the CGWD to steam prior to commissioning. In combination with the results of CGWD-B1, this suggests that heterotrophic bacteria were already present in the CGWD-B1 at the start of the experiment. The growth of heterotrophic bacteria however can not be ceased completely with the application of steam. The reason for this could be the fact that the steam was only partially effective in eliminating heterotrophic bacteria. Secondly, heterotrophic bacteria could survive the boiler in case of short residence times. Even the smallest amount of heterotrophic bacteria could

eventually lead to high counts, certainly with AOC concentrations around 250 mg acetate-C/L (Lautenschlager et al., 2010). It is important to note that HPC22 only cultivates a small fraction of the total bacterial population (<1%) (Abushaban et al., 2019). HPC22 analysis is useful to compare the results with the water quality standards and to compare the results with other research projects. However, the use of HPC22 in assessing the growth of the entire population of bacteria in water has been criticized multitudinous times (Staley & Konopka, 1985).

With FCM analysis, ICC and TCC measurements were done in order to quantify the total (living) bacterial population in the effluent. The maximum ICC and TCC in the effluent of CGWD-C1 were, respectively, $4.2 \times 10^5$ living cells/mL and $7.0 \times 10^5$ total cells/mL. So the maximum increases of ICC and TCC in the effluent of CGWD-C1 relative to the ICC in the influent were, respectively, 960% and 239%. Overall, the ICC and TCC in the effluent of CGWD-C1 were significantly higher than the mean ICC and TCC in the influent. These observations correspond to previous research on the deterioration of the bacteriological quality in PoU devices (Chen et al., 2020).

The maximum ICC in the effluent of CGWD-B1 was $7.3 \times 10^4$ living cells/mL, which is an increase of 84% relative to the ICC in the influent. However, the differences between the ICC in the effluent of CGWD-B1 and the ICC in the influent were not significant. At the beginning of the experiment, the ICC in the effluent of CGWD-B1 was slightly lower than the ICC in the influent. This confirms the hypothesis that the amount of livings cells is reduced by the boiler. However, After 7 days the ICC in the effluent was similar to the ICC in the influent. This implies that bacterial growth occurs within the CGWD and that the pre-treatment with a boiler with an integrated GAC filter only has a temporary effect. The maximum TCC in the effluent of CGWD-B1 was $8.0 \times 10^4$ total cells/mL, which is an decrease of 61% relative to the ICC in the influent. Overall, the TCC of CGWD-B1 was significantly lower than the TCC in the effluent of CGWD-C. After a period of 7 days, TCC in the effluent of CGWD-B1 seemed to stabilize. Whereas the average ICC/TCC was 66% during the first 7 days, the average ICC/TCC increased to 84% during the last 42 days. This could imply that the increase of TCC in CGWD-B is indirectly limited by the availability of dead cells when the GAC filter is not functioning as breeding ground (Chaberny et al., 2006).

The maximum ICC and TCC in the effluent of CGWD-B&S1 were, respectively, $5.9 \times 10^4$ living cells/mL and $6.5 \times 10^4$ total cells/mL. So the maximum value of ICC in the effluent of CGWD-B&S1 was 49% higher than the ICC in the influent, whereas the maximum TCC was 68% lower than the ICC in the influent. Overall, the ICC and TCC in the effluent of CGWD-B&S1 were significantly lower than the ICC and TCC in the influent. This confirms the hypothesis that the application of steam prior to commissioning further decreases the degree of bacterial growth in CGWDs. During the experiment, the ICC in the effluent of CGWD-B&S1 slowly increased and after 42 days the ICC in the effluent was higher than the mean ICC in the influent. This implies that it was not able to completely eliminate bacterial growth. However, both ICC and TCC in the effluent of CGWD-B&S1 were significantly lower than that of the influent and effluent of CGWD-C1 and CGWD-B1.

Additionally, to HPC22 and FCM, ATP analysis was performed. As described before, the cATP analysis of the influent produced erroneous results. In previous research, however, the cATP concentration in chlorinated drinking water networks was between 1.3 and 4.9 ng cATP/L (Nescerecka et al., 2014). The maximum cATP concentration of CGWD-C1, CGWD-B1, and CGWD-B&S1 was respectively 102.7, 50.9, and 6.8 cATP/L (n=5). This indicates that the microbial activity in the effluent of all CGWDs increased compared to the cATP concentration in the influent. However, the cATP concentration in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-B1 and CGWD-C1. This also corresponds to the results of the ICC results, since only limited bacterial growth was observed, and thus low microbial activity would be expected. Furthermore, the bed material from the GAC filter from each CGWD was analyzed with ATP analysis, after the sampled material was treated by the HES. The ATP measurements of the treated samples of CGWD-B1 and CGWD-B&S1 were below the detection limit. The treated sample of the filter material of CGWD-C1 however contained 2500 pg tATP / g GAC, which is similar to reported biomass quantities in GAC filter at drinking water treatment plants (Schwartz et al., 2009). This confirms the hypothesis that the bacterial growth within the GAC filter can be stopped if the GAC filter is placed into the boiler.

*Limitations of the experiment*

In the statistical analysis, the paired t-test was used, which determines whether the mean difference between two different data sets is zero. Since the experiment assesses bacterial growth, the results are very time-dependent. Even the configurations with the highest growth rates eventually will reach a maximum, whereas other configurations could reach this maximum in a later stadium. In the case of different growth rates, the differences will be the largest during the beginning, whereby the mean difference between the sets would be significantly higher than zero. However, if one would continue the experiment for 2 more months, the differences between the different configurations probably could be smaller since all configurations reached the maximum state. Therefore, it is important to note that the experiment only has been executed for 56 days. Furthermore, the power of the statistical analysis was relatively weak due to the small sample sizes. Larger sample sizes are required to improve the power of the statistical analysis of the paired t-test.

Moreover, the tapping frequency and the volume to be tapped have a large influence on the obtained results. Firstly, the stagnation time of the water within CGWDs is determined by tapping frequency. Longer stagnation times are favorable for biofilm-forming (Chen et al., 2020; Lautenschlager et al., 2010). Since the water was only tapped from the CGWDs once per weekday, the stagnation time was between 24 and 72 hours. However, in reality, the stagnation times will depend on the costumers tapping pattern and thus other bacterial growth patterns could be obtained. Secondly, the overall residence time of the water is not only determined by the frequency, but also by the volume that is tapped. In drinking water networks, long residence times were known to be favorable for bacterial growth (Bartram et al., 2003). However, the tapping frequency and volume to be tapped also determine the amount of AOC that is supplied to the bacteria. Longer residence times therefore will decrease the supply of AOC to the bacteria. During the experiment, 2 liters of water was tapped per weekday. Therefore, depending on the day, the overall residence time in the CGWDs was between 72 and 120 hours. In reality, the overall residence time could be different. Furthermore, the tapping pattern affects the efficiency of the inactivation of bacteria in the influent by the boiler. The fundamental idea behind implementing a boiler with an integrated GAC filter was that bacterial growth on the GAC filter would be implemented and that bacteria in the influent would be inactivated. However, the degree of inactivation of the bacteria in the influent within the boiler strongly depends on the residence time of the water in the boiler. The shorter the residence time of the water within the boiler, the higher the change of bacteria surviving the boiler. At the moment of tapping, water with a temperature of 90°C will enter the boiler, while the water being present in the boiler has a temperature of 105°C. In this case, the water will not be mixed, but a thermocline will be formed between the two layers preventing the two layers from mixing. The combined volume of the boiler with integrated GAC filter is 3 liters, whereas every weekday, 2 liters of water were tapped. A thermocline is a thin layer that distinct two bodies of water with large differences in temperature or density, which is also a common phenomenon in the oceans (Fiedler, 2010). Originally it was thought that within the boiler, this thermocline would ensure that the newly arrived water would stay in the water for at least one cycle, before leaving the boiler. An exposure to 105°C for at least 24 hours would inactivate almost all bacteria in the boiler. However, during some additional tests with the CGWD-Bs, it appeared that the thermocline could be easily disrupted by thermal turbulence whenever the heating element started to heat the water. After tapping of approximately 0.5 liters, the TMS registered too low temperatures in the bottom of the boiler and thereby activated the heating element, causing the layers to mix. During the experiment, in one go, 2 liters of water were tapped. This means that during the experiment, the thermocline was disrupted every weekday, and thus the duration of the exposure to high temperatures in fact could be much shorter (in the order size of seconds). The tapping of 2 liters of water in one go is somehow unusual and instead more frequent tapping of smaller amounts would be more realistic. Smaller amounts (below 0.5L) would not trigger the heating element and thereby the duration of exposure would be extended. In that case, there will be a smaller change of bacteria surviving the boiler that could trigger bacterial growth downstream of the boiler. Therefore, the bacterial growth in the CGWD-Bs and CGWD-B&Ss could be aggravated during the experiment because of the followed tapping pattern.

As part of one the measures, the water was heated to a temperature of 105°C by the boiler, after which the water was cooled down again to 8°C. At some point, the water containing high numbers of available biomass as nutrients will pass through the range of 25 - 45°C. These circumstances are known to be favorable for the pathogenic *Legionella*, which may induce additional health risks (Lau & Ashbolt, 2009). However, this prevailing concern was not explored during the study.

## 5.2. Experiment 2 - Analyzing removal performances of the boilers

The aim of the experiment was to assess whether the removal efficiencies of free chlorine and TOC would be affected if the GAC was placed into a boiler. In order to so, the free chlorine and TOC removal performances of two GAC filters treating room temperature water (CW1 and BW1), have been compared to that of two GAC filters placed into boilers in which the water was heated to 105°C (BW1 and BW2).

*Boundary conditions*

In order to compare the removal performances of different GAC filters, it was important that all GAC filters were tested under the same load-case and kinetics. The only difference may be the difference between the temperatures in the boilers. To draw valid conclusions from the results, boundary conditions were formulated.

The first requirement stated that the free chlorine concentration in the influent had to be increased to a level of 0.50 mg $Cl_2$/L by the CDS. During the test, the mean free chlorine concentration in the influent was 0.51 ± 0.03 mg $Cl_2$/L (n=19). Therefore, it was concluded that the CDS increased the free chlorine concentration to an acceptable level.

In the second requirement, it was established that the AOC concentration must be increased to a level of 250 µg acetate-C/L by the CDS. Originally, the increase of the AOC concentration had to be calculated by subtracting the TOC concentration in the water before the CDS from the TOC concentration in the water after the CDS. However, the TOC concentration in the influent appeared to be subject to large variations (differences of 0.3 mg C/L within a time span of 15 seconds). According to the measurement results of the blank solutions that were added as a check, these variations were not a result of the inaccuracy of the measurement equipment. Therefore it is argued that these variations in fact are a reflection of the actual fluctuation of TOC in the drinking water network. These variations made the calculation of AOC with the TOC analyzer very unreliable. The same applies as for experiment 1, AOC is easily assimilated by heterotrophic bacteria (van der Kooij, 1992). Whereas in experiment 1, the sodium acetate was added to the same tank as the hypochlorite, in this experiment both chemicals were added in separate tanks. In the tank without sodium acetate, no free chlorine was present to decrease the level of bacteria growth in the tank. Therefore, in reality, the AOC concentration in the tank could be lower than the theoretical concentration.

Thirdly, the temperature in the boilers of BW1 and BW2 had to be continuously around 105°C. From the temperature measurements and the observations of the TMS, it was concluded that both temperatures were of the desired level.

The last boundary condition was that each boiler was exposed to the same flow rate during the entire experiment. In the set-up, BW1 and BW2 were connected to heat exchangers, where CW1 and CW2 were not. Without a pump in the set-up, BW1 and BW2 would have lower flow rates because of the pressure drops of the heat exchangers. In order to maintain the same flow rates between the different lines, a pressure pump and flow regulators were installed. The idea was to increase the flow rate above 2 L/min with help of the pump and then to limit the maximum flow rate to 2 L/min with the flow regulators. However, the pressure pump could only be operated in cycle mode. Whenever the pressure reached 3.5 bar, the pump stopped working instead of maintaining the right pressure level. If the pressure dropped below 2.5 bar, the pump started again. While the pressure of 3.5 bar was needed to maintain a flow rate above 2 L/min. This caused significantly lower flow rates through BW1 and BW2 compared to CW1 and CW1. This means that the kinetics of the filters to be compared were different, which is important to realize while comparing the results of the chemical analysis.

*TOC and chlorine removal performances*

During the experiment, the free chlorine and TOC concentrations were regularly measured in the influent and effluent of the four different boilers. No significant differences were obtained between the TOC and free chlorine concentrations in the effluent of the duplicates.

The TOC removal efficiency of BW1 was significantly higher than the TOC removal efficiency of CW1 during the experiment. The initial TOC removal efficiencies of BW1 and CW1 were respectively 58% and 36%. Whereas in literature initial TOC removal efficiencies of 90% are reported for GAC (Hatt et al., 2013), the aforementioned values are relatively low. The relatively low removal efficiencies could be a consequence of unfavorable kinetics. The average empty bed contact time in the filters was only 12 seconds (empty bed volume of 360 mL, a flow rate of approx. 1.7 L/min). Another reason could

be the lower adsorptive capacities of the GAC that were used in the experiment. Large differences between the TOC removal efficiencies of different GAC filters were reported in previous research (Hatt et al., 2013).

Additionally to the experiment, a short test was performed in which the influent and effluent concentrations were measured again, but this time the GAC filters within the boilers were removed. In this test, the TOC concentrations in the influent and effluent of BW1 were similar. This implies that adsorption was the leading mechanism in the removal of TOC in BW1 (during the experiment, with the GAC filter). Since the adsorption capacity increased as the temperature increased, the suggestion is that the adsorption was controlled by endothermic processes. For a long time, it was assumed that adsorption controlled by physical interactions was reduced by increased temperatures (Schreiber et al., 2007). However, previous experiments showed that the effect of temperature during GAC adsorption is less predictable (Piai et al., 2020). Whereas the adsorption rates increase with increasing temperatures due to higher diffusion (Taghdiri & Zamani, 2013), it depends on endothermic or exothermic processes whether the overall removal capacity increases or decreases (Gupta et al., 2011).

With regard to the removal of free chlorine by the boilers, the removal efficiency of BW1 was significantly higher than the free chlorine removal efficiency of CW1 during the experiment. The initial free chlorine removal efficiency of BW1 and CW1 are respectively > 90% and 86%. Patil et al. (2013) described similar values for the free chlorine removal efficiencies of PoU GAC filters. More surprisingly, the TOC concentration in the effluent of BW1 was equal to or below the detection limit (0.05 mg $Cl_2$/L) during the entire experiment, even after 90,000 treated bed volumes. In an additional test without GAC filters, it appeared that all the free chlorine was removed from the water in BW1. This indicates that the removal of free chlorine in BW1 was predominantly generated by the boiling of the water. At high temperatures, most of the free chlorine will be present as hypochlorite ($ClO^-$). Bolyard et al. (1993) observed that the hypochlorite will decompose with increasing water temperatures, following the chemical reaction described in eq. 5.1. However, no additional chemical analyses were performed to support this theory.

$$3\,OCl^- \longrightarrow ClO_3{}^- + 2\,Cl^- \tag{5.1}$$

*Limitations of the experiment*

The pores of GAC can be divided into three different classes: micropores (<2 nm diameter), mesopores (2 - 50 nm diameter), and macropores (>50 nm diameter). Macropores function as the main transport channels for the water, whereas the mesopores transport the adsorbates towards the micropores, which represent more than 99% of the internal surface area. The adsorption capacity depends on the accessibility of the micropores (and thus the pore-size distribution) for the adsorbates, but also on the velocity of the water through the pores. During periods of stagnation, the adsorbates in the water will be more easily absorbed via the mesopores onto the micropores (Knezev, 2015). During the experiment, the boilers with integrated GAC filters were flushed continuously with water in order to analyze the chlorine and TOC removal performances over time. In reality, however, the GAC filters are only flushed sporadically and relatively short periods of flow will alternate with longer periods of stagnation. Because of these long periods of stagnation, the removal capacity will be higher in reality.

During the experiment, Dutch tap water was used and the concentrations of free chlorine and AOC were adjusted to levels similar to that of typical English water so that free chlorine and TOC removal performances of the CGWDs in England could be analyzed. However, besides the free chlorine and TOC concentrations the water quality of the Dutch tap water differs in more ways from that of typical English water. Lutes et al. (2010) reported nitrate, sulfate, and chloride as the most importing competing elements to be considered when determining the removal performance of GAC. The mean concentrations of these competing anions in the water from DWTP Kralingen and typical English tap water are shown in Table 5.1 (Ascott et al., 2019; Prest, 2015).

| Parameter | Unit | England | DWTP Kralingen |
|---|---|---|---|
| Sulfate | mg $SO_4$/L | 50 | 52 |
| Nitrate | mg $NO_3$/L | 14 | 12.8 |
| Chloride | mg $Cl^-$/L | 35 | 53.5 |

Table 5.1: The difference between water quality parameters effecting GAC removal in England and The Netherlands

Whereas the sulfate and nitrate levels are of a similar level in both cases, the chloride level is

somehow lower in the water used during the experiment. Because of the higher levels of chloride in the water from the DWTP in Kralingen, the free chlorine and TOC will encounter more competition during the adsorption process. Therefore, the removal efficiencies of the CGWD could have been higher when exposed to real English water.

# 6

# Conclusion

While CGWDs are increasingly used in daily life around the world, concerns raised by the scientific community have raised the need for long-term and periodic measures against bacterial growth. This study showed the potential of the implementation of a boiler with an integrated GAC filter combined with sterilization as a solution to reduce bacterial growth within CGWDs.

The first objective of this study is to assess whether suggested measures can decrease the bacterial growth within CGWDs. In experiment 1, the influent and effluent of three different configurations were microbiologically analyzed. In the conventional configuration, no measures were implemented (CGWD-C). In the second configuration, a boiler with an integrated GAC filter was implemented (CGWD-B). The third configuration was similar to the second configuration, but additionally, the device was exposed to steam (20 minutes > 105 ºC) prior to the experiment (CGWD-B&S). Based on the results of experiment 1, it can be concluded that bacterial growth in CGWDs could be decreased with the implementation of the boiler with an integrated GAC filter. By exposing the CGWD to steam prior to commissioning the bacterial growth in the CGWD could be reduced even more. However, bacterial growth could not be ceased completely. HPC22 analysis showed a growth pattern of heterotrophic bacteria in all three different configurations to a level above the applicable water quality standard of 100 cfu/mL. However, with the application of sterilization prior to the experiment, the growth of heterotrophic bacteria could be delayed for at least 37 hours.

The second objective of this study is to examine whether the free chlorine and organic matter removal performances are affected by the implementation of these measures. During experiment 2, the influent and effluent of two boilers with integrated GAC filter and two GAC filters treating room temperature water were chemically analyzed and compared. From the results of experiment 2, it can be concluded that placing of the GAC filter into the boiler increased the TOC removal efficiency of the CGWDs. Heating the water to a temperature of 105ºC within the boiler for 4 minutes ensuring that the effluent of the CGWDs contained no free chlorine.

The following list describes all the notable conclusions in a detailed manner:

- Conventional CGWDs that were tested in this study appeared to be vulnerable to bacterial growth. The effluent of the CGWD-Cs contained significantly higher ICC than the influent. The maximum value of ICC in the effluent of CGWD-C1 was 4.2 x $10^5$ living cells/mL after 28 days, which was 980% higher than the ICC in the influent.
- The effluent of the CGWD-Cs was always above the applicable water quality standard of 100 cfu/mL, while the influent was compliant with the applicable standard. High HPC22 showed that circumstances are favorable for bacterial growth. The maximum value of HPC22 in the effluent of the CGWD-C1 was 6.8 x $10^4$ cfu/mL after 7 days.
- According to cATP analysis, the bacterial activity of the water was increased by the CGWD-Cs. The maximum cATP concentration in the effluent of CGWD-C1 was 102.7 ng cATP/L after 14 days.

- The degree of bacterial growth in CGWDs could be decreased with the implementation of the boiler with an integrated GAC filter. The ICC in the effluent of CGWD-B was significantly lower than the ICC in the effluent of CGWD-C. In fact, no bacterial growth was observed in the CGWD-Bs since no significant differences observed between the ICC in the effluent of CGWD-B and the ICC in the influent. The maximum value of ICC in the effluent of CGWD-B1 was 7.3 x $10^4$ living cells/mL after 28 days, which was 84% higher than the ICC in the influent.
- No significant differences in HPC22 were observed between the effluent of the CGWD-Bs and the effluent of the CGWD-Cs. Similarly, as for CGWD-C, the HPC22 in the effluent of the CGWD-Bs was always above the applicable standard. The maximum HPC22 in the effluent of CGWD-B1 was 7.6 x $10^5$ cfu/mL.
- The increase in bacterial activity in the effluent could be reduced by the implementation of the boiler with an integrated GAC filter. The cATP concentration in the effluent of CGWD-B1 was significantly lower than the cATP concentration of the CGWD-C1. The maximum cATP concentration in the effluent of CGWD-B1 was 50.9 ng cATP/L after 56 days.
- By exposing the CGWD to steam prior to commissioning, on top of the implementation of the boiler with an integrated GAC filter, the degree of bacterial growth in the CGWD could be reduced even more. Actually, overall the values of ICC in the effluent of the CGWD-B&Ss were significantly lower than that of the influent. The maximum ICC in the effluent of CGWD-B&S1 was 5.9 x $10^4$ living cells/mL after 56 days, which was 48% higher than the ICC in the influent.
- During the first 37 hours of the experiment, the CGWD-B&Ss were compliant with the applicable water quality standard of HPC22 (<100 cfu/mL). After that, the HPC22 in the effluent started to increase. The HPC22 in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-C1 and CGWD-B1. The maximum HPC22 in the effluent of the CGWD-B&Ss was 1.2 x $10^5$ cfu/mL after 56 days.
- The bacterial activity in the effluent of the CGWD-B&Ss was of comparable level to that of chlorinated drinking water systems described in previous research. The cATP concentration in the effluent of CGWD-B&S1 was significantly lower than that of CGWD-B1 and CGWD-C1. The max cATP concentration in the effluent of the CGWD-B&Ss was 6.8 ng cATP/L after 14 days.
- Placing the GAC filter into the boiler prevented the bacteria from growing onto the filter material. The measured tATP concentrations in the treated samples of CGWD-B and CGWD-B&S were below the detection limit. Whereas the samples of the CGWD-C contained 2.5E+03 pg ATP / g GAC.
- Placing the GAC filter into the boiler increased the TOC removal efficiency of the CGWDs in this study. The removal capacity of TOC increased at higher temperatures.
- Placing the GAC filter into the boiler ensured that all free chlorine removal was removed by the CGWD. The effluent of the boilers without filter contained no free chlorine, which implies that due to the heating of the water to 105 ºC all free chlorine is decomposed.

# 7

# Recommendations

Based on the results from the experiments and the conclusions, some recommendations can be made. Firstly, it is recommended to investigate whether it is possible to keep the HPC22 in the effluent below the applicable limit of <100 cfu/mL with the application of periodic sterilization, instead of only once prior to commissioning. In order to do so, first the time until the breakthrough of HPC22 must be determined. In this study, the effluent of CGWD, which was sterilized prior to the experiment, was compliant with the applicable standard of HPC22 during the first 37 hours, whereas the conventional CGWDs were not compliant. Somewhere between 37 and 158 hours after the start, the HPC22 exceeded the norm of 100 cfu/mL. By taking more samples in the first hours, the growth pattern of heterotrophic bacteria could be examined in more detail. If the time of breakthrough is known, one could apply sterilization right before the HPC22 in the effluent exceeds the applicable norm and repeat this process periodically. Thereafter, it should be analyzed whether the sterilization is effective in the removal of the biofilm and whether the breakthrough times change over time. If it could be possible to keep the HPC22 in the effluent below the applicable standards with periodic sterilization, the sterilization process could even be automated. This would be a more robust alternative than replacing the filters and applying disinfectant since it can never be forgotten or neglected.

The second recommendation would be to analyze whether the implementation of the boiler with integrated GAC filter and sterilization also reduce the number of pathogenic bacteria in the effluent. In this study, FCM analysis was performed to determine the growth pattern of the total living bacteria population, whereas ATP analysis was performed to give insights into the total bacterial activity of the water. While this gives a clear insight into the development of the bacterial quantities over time, no specific bacteria were identified. However, different species of bacteria can be more or less resilient to higher temperatures. While this study showed that it was possible to reduce bacterial growth, the results do not provide any insights on whether the growth of pathogenic bacteria and thus health risks, could be reduced. Specific pathogenic bacteria could be unidentified with biochemical identification techniques (e.g. 16S rRNA sequencing, Fluorescence in situ hybridization, and analytical profile index kits).

Thirdly, it is recommended to investigate the effect of the used materials within the CGWDs. From the literature review, it became clear that certain polymeric materials are known to promote the formation of biofilm. However, in this study, this effect was not looked into. By replacing all polymeric parts of the CGWD with parts of material with lower biofilm-forming potential, like stainless steel or copper, the bacterial growth could be analyzed over time and compared to a CGWD with polymeric parts.

The fourth recommendation would be to investigate the effect of boiling water on the taste and odor of the water. The boiler used during the test, are normally used for the production of instantly boiling water. From the results of experiment 2, it is known that all free chlorine is removed within the boiler. Therefore, it can be doubted whether the GAC filter is still necessary for the boiler in areas where the water has a proper taste if only chlorine would be removed. However, during the experiment, some experts suggested that the GAC filter also serves a different purpose. Namely, the removal of com-

pounds that were formed by the reactions in the boiler and add an unfavorable taste to the water. This suspicion could be checked by chemically analyzing the influent of the boiler accompanied by sensory research.

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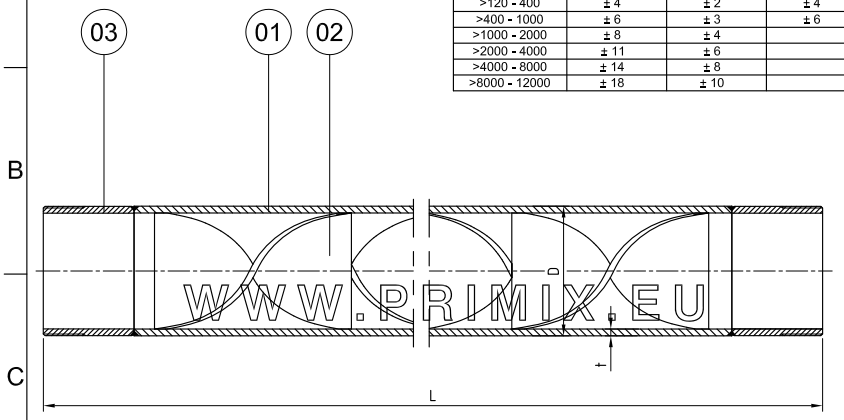
# Appendices

# A. Technical specifications static mixer

| Rev.: | Revision note: | Drawn date: | Checked date: | Approved date: |
|---|---|---|---|---|
| 1 | Tolerance table changed | MS 20/04/09 | RJW 20/04/09 | RJW 20/04/09 |

Tolerance for pressure vessels according DIN 28005-1

| Range [mm] | Vessel length [mm] | Distance nozzle to reference line [mm] | Nozzle facing to reference line [mm] |
|---|---|---|---|
| >30 - 120 | ± 3 | ± 2 | ± 3 |
| >120 - 400 | ± 4 | ± 2 | ± 4 |
| >400 - 1000 | ± 6 | ± 3 | ± 6 |
| >1000 - 2000 | ± 8 | ± 4 | |
| >2000 - 4000 | ± 11 | ± 6 | |
| >4000 - 8000 | ± 14 | ± 8 | |
| >8000 - 12000 | ± 18 | ± 10 | |




SECTION

Grey marked area is QDS program

MIXER DIMENSION & WEIGHT (BSP(M) NIPPLES)

| Size | Outside diameter | Wall thickness | NUMBER OF ELEMENTS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | | 3 | | 4 | | 5 | | 6 | | 8 | |
| | D [mm] | t [mm] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] |
| DN15 / 1/2" | 21.34 | 2.77 | 125 | 0.3 | 150 | 0.3 | 175 | 0.3 | 200 | 0.4 | 225 | 0.4 | 275 | 0.5 |
| DN20 / 3/4" | 26.67 | 2.87 | 149 | 0.4 | 184 | 0.5 | 219 | 0.6 | 254 | 0.6 | 289 | 0.7 | 355 | 0.9 |
| DN25 / 1" | 33.40 | 3.38 | 169 | 0.7 | 214 | 0.8 | 259 | 1.0 | 304 | 1.1 | 349 | 1.2 | 430 | 1.5 |
| DN32 / 1 1/4" | 42.16 | 3.56 | 215 | 1.2 | 275 | 1.4 | 330 | 1.7 | 390 | 2.0 | 445 | 2.2 | 555 | 2.7 |
| DN40 / 1 1/2" | 48.26 | 3.68 | 233 | 1.5 | 303 | 1.9 | 368 | 2.3 | 433 | 2.6 | 498 | 3.0 | 630 | 3.7 |
| DN50 / 2" | 60.33 | 3.91 | 290 | 2.4 | 370 | 3.0 | 455 | 3.6 | 540 | 4.2 | 625 | 4.9 | 795 | 6.1 |
| DN65 / 2 1/2" | 73.03 | 5.16 | 330 | 4.1 | 430 | 5.2 | 530 | 6.2 | 630 | 7.3 | 730 | 8.4 | 935 | 10.6 |
| DN80 / 3" | 88.90 | 5.49 | 390 | 6.3 | 510 | 8.1 | 640 | 10.0 | 760 | 11.8 | 890 | 13.6 | 1140 | 17.3 |
| DN100 / 4" | 114.30 | 6.02 | 500 | 12.4 | 660 | 15.8 | 830 | 19.4 | 990 | 22.8 | 1160 | 26.4 | 1480 | 33.2 |

| Size | Outside diameter | Wall thickness | NUMBER OF ELEMENTS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10 | | 12 | | 14 | | 16 | | 20 | | 24 | |
| | D [mm] | t [mm] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] | L [mm] | W [kg] |
| DN15 / 1/2" | 21.34 | 2.77 | 325 | 0.6 | 375 | 0.6 | 425 | 0.7 | 475 | 0.8 | 580 | 1.0 | 680 | 1.1 |
| DN20 / 3/4" | 26.67 | 2.87 | 420 | 1.0 | 490 | 1.2 | 555 | 1.3 | 625 | 1.5 | 755 | 1.7 | 890 | 2.0 |
| DN25 / 1" | 33.40 | 3.38 | 515 | 1.8 | 600 | 2.0 | 685 | 2.3 | 770 | 2.6 | 940 | 3.1 | 1110 | 3.7 |
| DN32 / 1 1/4" | 42.16 | 3.56 | 670 | 3.2 | 780 | 3.7 | 890 | 4.3 | 1005 | 4.8 | 1230 | 5.8 | 1455 | 6.8 |
| DN40 / 1 1/2" | 48.26 | 3.68 | 760 | 4.4 | 895 | 5.1 | 1025 | 5.8 | 1155 | 6.5 | 1415 | 7.9 | 1680 | 9.4 |
| DN50 / 2" | 60.33 | 3.91 | 965 | 7.3 | 1130 | 8.5 | 1300 | 9.7 | 1465 | 10.9 | 1805 | 13.3 | 2140 | 15.8 |
| DN65 / 2 1/2" | 73.03 | 5.16 | 1135 | 12.8 | 1335 | 14.9 | 1535 | 17.1 | 1740 | 19.3 | 2140 | 23.6 | 2540 | 27.9 |
| DN80 / 3" | 88.90 | 5.49 | 1390 | 20.9 | 1640 | 24.6 | 1890 | 28.2 | 2135 | 31.8 | 2635 | 39.1 | 3135 | 46.4 |
| DN100 / 4" | 114.30 | 6.02 | 1810 | 40.2 | 2135 | 47.1 | 2465 | 54.1 | 2790 | 61.0 | 3445 | 75.0 | 4100 | 88.9 |

| Item No.: | Description: | Reference standard / drawing: | Material: | Mat. Cert.: | No.- off: | Final dimension | Weight: | Rev.: |
|---|---|---|---|---|---|---|---|---|
| 03 | BSP(M) Weld nipple | | 316 | - | 2 | | | |
| 02 | Helical Shaped element | | 1.4404/316L | 2.2 | | | | |
| 01 | Housing | | 1.4404/316L | 3.1 | 1 | | | |

| Drawn by: | Checked by: | Approved by: | Date: | Client ref.: | Project: | Scale: | Format: |
|---|---|---|---|---|---|---|---|
| MR | FP | | 5-11-2015 | | | 1:~ | A 4 |

PRIMIX
PERFORMANCE BY DESIGN
Postbus 220, 3640 AE Mijdrecht
Nijverheidsweg 17F Mijdrecht
The Netherlands www.primix.eu

Projection:

Title: BSP(M) STATIC MIXER

| dwg.no.: | Sheet: | Rev.No.: |
|---|---|---|
| PMS___-___-316L-BSP(M) | 1/1 | 1 |

# B. Technical specifications flow regulator



**regulator housing:**

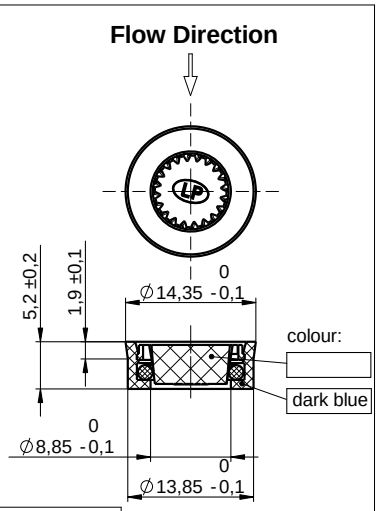
material: POM-Hostaform C9021

**o-ring:**

material: EPDM 50 til 70 shore

**regulator core:**

material: POM-Hostaform C9021




| | | | flowtolerance deviations | flowtolerance pressure range | | |
|---|---|---|---|---|---|---|
| 58.7435.10000 | 7,6 L | lilac 40/6193 | see NEOPERL Template Database | | | ✓ |
| 58.7434.10000 | 14,0 L | pink 40/6187 | | | | ✓ |
| 58.7433.10000 | 13,0 L | olive 70/6195 | | | | ✓ |
| 58.7432.10000 | 12,0 L | red 47 | | | | ✓ |
| 58.7431.10000 | 11,0 L | brown 65 | | | | ✓ |
| 58.7430.10000 | 10,0 L | light blue 80/5504 | | | | ✓ |
| 58.7429.10000 | 9,0 L | orange 40/3738 | | | | ✓ |
| 58.7428.10000 | 8,0 L | natural | | | | ✓ |
| 58.7427.10000 | 7,0 L | green 76 | | | | ✓ |
| 58.7426.10000 | 6,0 L | black 10/5358 | | | | ✓ |
| 58.7425.10000 | 5,0 L | yellow 56 | | | | ✓ |
| 58.7424.10000 | 4,0 L | grey 39 | | | | ✓ |
| 58.7423.10000 | 3,0 L | brown 65 | | | | ✓ |
| 58.7422.10000 | 2,0 L | olive 70/6195 | | | | ✓ |
| 58.7421.10000 | 1,0 L | pink 40/6187 | | | | ✓ |
| PART-NO | L/MIN | COLOUR | + % | - % | [bar] | AVAILABLE |

1:1



**NEOPERL GmbH**
**D-79379 Müllheim**

Change No.: **500000002634**
Date: 06.08.2013
Drawn: A. Welz

Approval of drawing change:
Date: 11.03.2015
Approv.: J. Barnett

**Rev. D**

Surface DIN EN ISO 1302

Part No.:
**58.7421.10000 - 58.7435.10000**

| | Date | Name |
|---|---|---|
| Drawn: | 06.08.2013 | A. WELZ |
| Approv.: | 11.03.2015 | J. BARNETT |

General tolerances DIN ISO 2768-fH

Workpiece edges DIN ISO 13715

Title:
**flow regulator G-LP**

Scale: 2:1

Format: A4

Page 1 of 1

Volume:

Weight:

NEOPERL®
flow, stop and go®

| Material: | Material Description / Tradename: | Colour: |
|---|---|---|
| | | |

Replacement for: 58.7421.1-58.7434.1 REV.C

Origin: .

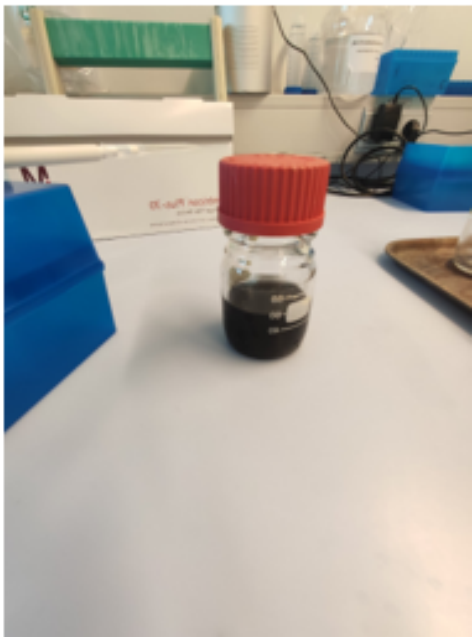
## C. Biofilm extraction from GAC material at KWR Water Institute

Step 1: Extracting 3 gram of the filter material

Step 2: Adding 50 mL purified water

Step 3: extracting biofilm in HES

Step 4: Sampling for microbial analysis



# D. Calibration of the 3M Clean-Trace Luminometer

On day 2 of the experiment, the first calibration solutions were analyzed by the Luminometer and KWR Water Institute, after which a first calibration curve was made. However, the linear regressions through the data points appeared to have very low R-squared values (<0.50). At that point, the manual was examined again thoroughly. From the manual, it appeared that some mistakes were made in handling the luminometer. The swabs were put too long and too deep in the samples. After a sensitivity analysis, it was concluded that the errors had a significant influence on the measurement results. The same applied for the ATP measurement of day 0, 1, and 2. Therefore, it was decided to neglect the ATP results of days 0, 1, and 2, and to construct a second calibration curve and to adjust the procedure of ATP measurement with the luminometer. The results of the ATP analysis during the second calibration are shown in Table D.1.

| Type | Unit | Source | #1 | #2 | #3 | #4 | #5 |
|---|---|---|---|---|---|---|---|
| fATP | RLU | Luminometer | 1163 | 405 | 199 | 73 | 32 |
| | ng/L | KWR | 140 | 59 | 22 | 5.6 | 0 |
| tATP | RLU | Luminometer | 913 | 401 | 160 | 56 | 5 |
| | ng/L | KWR | 150 | 68 | 27 | 8.2 | 0 |

Table D.1: The measured ATP concentrations in the calibration solutions

With the results from Table D.1, the calibration curve for both free ATP and total ATP could be made by plotting the RLU values on the Y-axis, and the ng/L values on the X-axis. The calibration curves for free ATP and total ATP are shown in the Figure D.1.

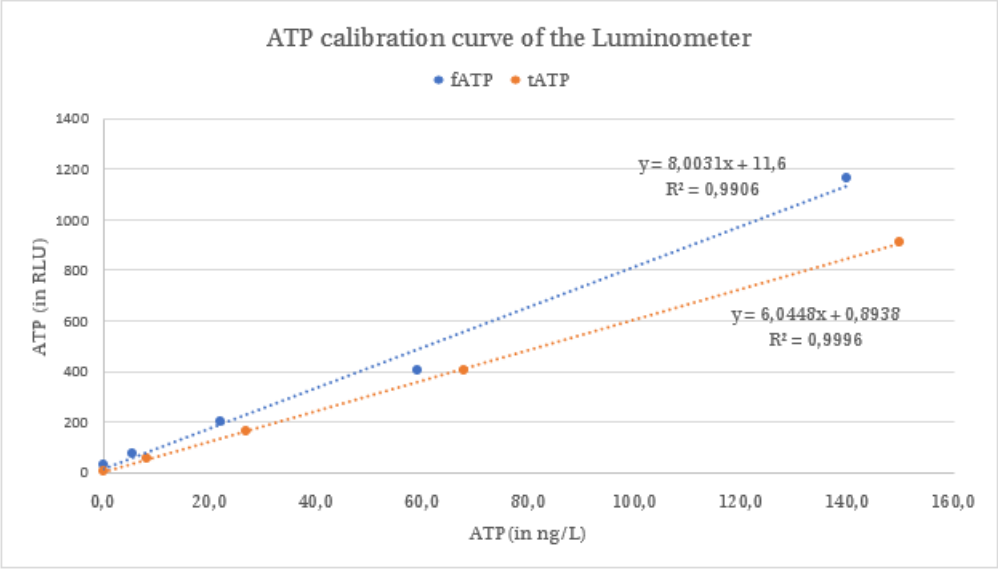



Figure D.1: The calibration curves of fATP and tATP

By using the equation of the trend lines from Figure D.1, the obtained values of free ATP and total ATP in RLU, which were measured with the luminometer during the experiment, were converted to ng/L. By subtracting the value of free ATP in ng/L from the value of total ATP in ng/L, the cellular ATP concentration (in ng/L) was calculated. The R-squared values of the free and total ATP analysis were both above 0.99. The following formula was used to calculate the cellular ATP concentration during the graduation project:

$$cATP = \frac{tATP - 0.8938}{6.0448} - \frac{fATP - 11.6}{8.0031} \quad (1)$$

Where cATP was the cellular ATP concentration in ng/L and where tATP and fATP were, respectively, the total and free ATP values in RLU.

# E. Measurement data Flowcytometry analysis experiment 1

| Date | | 29-sep | 30-sep | 1-okt | 6-okt | 13-okt | 27-okt | 10-nov | 24-nov |
|---|---|---|---|---|---|---|---|---|---|
| Time | | 21:00 | 10:00 | 10:00 | 11:00 | 11:00 | 10:00 | 10:30 | 10:30 |
| Duration (hours) | | 0 | 13 | 37 | 158 | 326 | 661 | 998 | 1334 |
| Location | Type | cell count by flowcytometry [cells/mL] | | | | | | | |
| Influent | ICC | 8,8E+04 | 3,1E+04 | 7,7E+03 | 7,8E+04 | 2,40E+03 | 1,50E+04 | 7,70E+04 | 1,80E+04 |
| | DCC | 1,8E+05 | 2,1E+05 | 2,4E+05 | 1,4E+05 | 1,58E+05 | 9,80E+04 | 1,00E+05 | 2,00E+05 |
| | TCC | 2,6E+05 | 2,5E+05 | 2,5E+05 | 2,2E+05 | 1,60E+05 | 1,10E+05 | 1,80E+05 | 2,20E+05 |
| | ICC/TCC | 34% | 12% | 3% | 35% | 2% | 14% | 43% | 8% |
| CGWD-C 1 | ICC | 3,8E+04 | 6,4E+04 | 2,9E+04 | 1,3E+05 | 1,70E+05 | 4,20E+05 | 3,50E+05 | 3,90E+05 |
| | DCC | 9,8E+04 | 2,2E+05 | 2,2E+05 | 1,4E+05 | 1,10E+05 | 1,50E+05 | 7,40E+04 | 3,10E+05 |
| | TCC | 1,4E+05 | 2,9E+05 | 2,5E+05 | 2,7E+05 | 2,80E+05 | 5,70E+05 | 4,30E+05 | 7,00E+05 |
| | ICC/TCC | 27% | 22% | 12% | 48% | 61% | 74% | 81% | 56% |
| CGWD-C 2 | ICC | 2,1E+04 | | | | | 3,70E+05 | | 4,10E+05 |
| | DCC | 1,1E+05 | | | | | 1,20E+05 | | 2,00E+05 |
| | TCC | 1,3E+05 | | | | | 4,90E+05 | | 6,10E+05 |
| | ICC/TCC | 16% | | | | | 76% | | 67% |
| CGWD-B 1 | ICC | 1,2E+04 | 6,3E+03 | 1,2E+04 | 5,4E+04 | 3,00E+04 | 7,30E+04 | 5,30E+04 | 6,20E+04 |
| | DCC | 4,9E+03 | 6,9E+03 | 7,5E+03 | 1,3E+04 | 5,00E+03 | 6,90E+03 | 1,50E+04 | 1,60E+04 |
| | TCC | 1,6E+04 | 1,3E+04 | 2,0E+04 | 6,7E+04 | 3,50E+04 | 8,00E+04 | 6,80E+04 | 7,80E+04 |
| | ICC/TCC | 75% | 48% | 60% | 81% | 86% | 91% | 78% | 79% |
| CGWD-B 2 | ICC | 1,4E+04 | | | | | 1,30E+05 | | 2,10E+05 |
| | DCC | 5,6E+03 | | | | | 1,30E+04 | | 2,30E+04 |
| | TCC | 2,0E+04 | | | | | 1,40E+05 | | 2,30E+05 |
| | ICC/TCC | 70% | | | | | 93% | | 91% |
| CGWD-B&S 1 | ICC | 9,3E+03 | 1,3E+03 | 3,0E+02 | 1,7E+03 | 3,90E+03 | 1,30E+04 | 3,10E+04 | 5,9E+04 |
| | DCC | 4,4E+03 | 1,7E+03 | 1,0E+03 | 6,0E+02 | 1,50E+03 | 1,50E+03 | 7,30E+03 | 5,7E+03 |
| | TCC | 1,4E+04 | 3,0E+03 | 1,3E+03 | 2,3E+03 | 5,40E+03 | 1,40E+04 | 3,90E+04 | 6,5E+04 |
| | ICC/TCC | 53% | 43% | 23% | 74% | 72% | 93% | 79% | 91% |
| CGWD-B&S 2 | ICC | 5,9E+03 | | | | | 1,80E+04 | | 4,4E+04 |
| | DCC | 2,0E+03 | | | | | 1,90E+03 | | 1,2E+04 |
| | TCC | 7,9E+03 | | | | | 2,00E+04 | | 5,6E+04 |
| | ICC/TCC | 75% | | | | | 90% | | 79% |

# F. Measurement data HPC22 analysis experiment 1

| Date | 29-sep | 30-sep | 1-okt | 6-okt | 13-okt | 27-okt | 10-nov | 24-nov |
|---|---|---|---|---|---|---|---|---|
| Time | 21:00 | 10:00 | 10:00 | 11:00 | 11:00 | 10:00 | 10:30 | 10:30 |
| Duration (hours) | 0 | 13 | 37 | 158 | 326 | 661 | 998 | 1334 |
| Location | CFU/mL | CFU/mL | CFU/mL | CFU/mL | CFU/mL | CFU/mL | CFU/mL | CFU/mL |
| Inlet | <1 | <1 | <1 | <1 | 2,00E+00 | 1,40E+01 | 2,50E+02 | <1 |
| Feeding tank | <1 | <1 | <1 | <1 | <1 | 2,00E+00 | 2,00E+00 | <1 |
| CGWD-C 1 | 4,30E+02 | 1,20E+03 | 1,40E+03 | 1,00E+04 | 6,80E+04 | 4,70E+04 | 2,30E+04 | 5,90E+03 |
| CGWD-C 2 | 2,70E+02 | 3,40E+03 | 1,00E+04 | 5,70E+04 | 5,20E+04 | 3,60E+04 | 5,70E+04 | 1,70E+04 |
| CGWD-B 1 | 1,70E+03 | 3,50E+02 | 2,50E+03 | 6,00E+03 | 4,90E+04 | 4,50E+04 | 1,10E+05 | 7,60E+05 |
| CGWD-B 2 | 2,70E+03 | 1,20E+04 | 2,30E+04 | 1,10E+04 | 2,60E+04 | 2,20E+05 | 4,70E+04 | 1,10E+04 |
| CGWD-B&S 1 | <1 | <1 | <1 | 5,50E+02 | 6,20E+03 | 1,90E+04 | 2,90E+04 | 1,20E+05 |
| CGWD-B&S 2 | <1 | <1 | <1 | 6,50E+02 | 4,70E+03 | 2,70E+04 | 1,40E+05 | 2,70E+04 |

# G. Measurement data ATP analysis experiment 1

| Date | 6-Oct | | | | | | 13-Oct | | | | | | 27-Oct | | | | | | 10-Nov | | | | | | 24-Nov | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | 11:00 | | | | | | 11:00 | | | | | | 10:00 | | | | | | 10:30 | | | | | | 10:30 | | | | | |
| Duration | 158 | | | | | | 326 | | | | | | 661 | | | | | | 998 | | | | | | 1334 | | | | | |
| | fATP | tATP | fATP | tATP | cATP | | fATP | tATP | fATP | tATP | cATP | | fATP | tATP | fATP | tATP | cATP | | fATP | tATP | fATP | tATP | cATP | | fATP | tATP | fATP | tATP | cATP | |
| Type | RLU | RLU | ng/L | ng/L | ng/L | | RLU | RLU | ng/L | ng/L | ng/L | | RLU | RLU | ng/L | ng/L | ng/L | | RLU | RLU | ng/L | ng/L | ng/L | | RLU | RLU | ng/L | ng/L | ng/L | |
| Tank | 14 | 10 | 0,3 | 1,5 | 1,2 | | 21 | 16 | 1,2 | 2,5 | 1,3 | | 53 | 5 | 5,2 | 0,7 | -4,5 | | 49 | 6 | 4,7 | 0,8 | -3,8 | | 20 | 12 | 1,0 | 1,8 | 0,8 | |
| Inlet | 29 | 10 | 2,2 | 1,5 | -0,7 | | 42 | 28 | 3,8 | 4,5 | 0,7 | | 36 | 16 | 3,0 | 2,5 | -0,5 | | 41 | 7 | 3,7 | 1,0 | -2,7 | | 30 | 19 | 2,3 | 3,0 | 0,7 | |
| CGWD-C1 | 40 | 643 | 3,5 | 106,2 | 102,7 | | 21 | 271 | 1,2 | 44,7 | 43,5 | | 333 | 687 | 40,2 | 113,5 | 73,3 | | 215 | 377 | 25,4 | 62,2 | 36,8 | | 71 | 479 | 7,4 | 79,1 | 71,7 | |
| CGWD-C2 | 34 | 111 | 2,8 | 18,2 | 15,4 | | 39 | 506 | 3,4 | 83,6 | 80,1 | | 382 | 499 | 46,3 | 82,4 | 36,1 | | 117 | 417 | 13,2 | 68,8 | 55,7 | | 182 | 493 | 21,3 | 81,4 | 60,1 | |
| CGWD-B1 | 35 | 52 | 2,9 | 8,5 | 5,5 | | 75 | 73 | 7,9 | 11,9 | 4,0 | | 53 | 334 | 5,2 | 55,1 | 49,9 | | 95 | 241 | 10,4 | 39,7 | 29,3 | | 327 | 547 | 39,4 | 90,3 | 50,9 | |
| CGWD-B2 | 72 | 192 | 7,5 | 31,6 | 24,1 | | 40 | 435 | 3,5 | 71,8 | 68,3 | | 82 | 91 | 8,8 | 14,9 | 6,1 | | 60 | 244 | 6,0 | 40,2 | 34,2 | | 76 | 216 | 8,0 | 35,6 | 27,5 | |
| CGWD-B&S1 | 19 | 13 | 0,9 | 2,0 | 1,1 | | 41 | 64 | 3,7 | 10,4 | 6,8 | | 51 | 40 | 4,9 | 6,5 | 1,5 | | 51 | 46 | 4,9 | 7,5 | 2,5 | | 160 | 144 | 18,5 | 23,7 | 5,1 | |
| CGWD-B&S2 | 19 | 48 | 0,9 | 7,8 | 6,9 | | 19 | 55 | 0,9 | 9,0 | 8,0 | | 23 | 100 | 1,4 | 16,4 | 15,0 | | 65 | 81 | 6,7 | 13,3 | 6,6 | | 80 | 55 | 8,5 | 9,0 | 0,4 | |