Ultrasound Disinfection in Domestic Drinking Water System

CIE5050-09 Additional Thesis

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1 | Introduction

Impeccable drinking water quality is the goal of every drinking water company when supplying drinking water to their customers. However, even though the quality of the treated water leaving the drinking water treatment plant might be at the highest level, the quality may deteriorate during the distribution of the drinking water. The change of the drinking water quality is caused by various factors, such as pipe materials, temperature change, stagnant segments, contamination by subsurface water (Lautenschlager, et al., 2013), and microorganism regrowth (van der Kooij, 1999).

Regrowth of microorganisms is the most important water quality change because the bacterial growth can lead to aesthetic changes (taste, odor, and color) (Lautenschlager, et al., 2010), the enhancement of corrosion of plumbing materials (Eboigbodin, et al., 2008), and proliferation of opportunistic bacteria (Liu, et al., 2014; Hammes, et al., 2010; Proctor & Hammes, 2015). Microbial contamination in the drinking water distribution system (DWDS) is indicated by biofilm formation which comprises of attaching bacteria to the pipe walls (Douterelo, et al., 2013). Biofilm in DWDSs is important for the bacteria to protect themselves from the challenging environment in a DWDS with low nutrient concentrations and fluctuating hydraulic (Critchley, Cromar, McClure, & Fallowfield, 2001) conditions (Douterelo, et al., 2014).

In ensuring the microbiological stability in the distribution network, a disinfectant residual (usually chlorine) is maintained in many countries (Lautenschlager, et al., 2013). However, due to the drawback of chlorination, which produces toxic by-products (Lambert, et al., 2010), some countries, especially the Netherlands, have phased out chlorine as disinfectant. They avoid the bacteria growth by limiting the supporting nutrients in the drinking water (Lautenschlager, et al., 2013). Previous studies found that bacterial growth is hindered in distribution networks due to low nutrient concentrations, low temperature, and short residence times. The alteration of the drinking water quality might also occur in household connections, where the temperature is higher, have longer residence times, and there is the possibility of mixing between the water and contaminated water from household environment (Lautenschlager, et al., 2010). Therefore, understanding the microbiology in DWDS, especially in household connections, is significant due to the safety of drinking water to be consumed.

Apart from that, the Netherlands is actively contributing to reduce CO_2 emissions by reducing gas usage in the coming years. This will affect the hot water system in the household since 93.7% of the dwellings use gas for heating purposes (Litjens, et al., 2018), including heating the water. Heat pumps that are driven by renewable energy sources are found to be an alternative method for heating purpose (Ommen, et al., 2014). In order to operate the heat pump system efficiently, the heating only works until sufficient temperature, which is 40°C for hot water that the temperature is considered complying the comfort requirement (Yang, et al., 2016). Furthermore, this heat pump system is beneficial that the hot water system can directly supply hot water with comfortable temperature. These days, the warm water that is obtained from the tap water is heated until 60°C (Roger, et al., 1994) and then combined with cold water until a temperature of water is 40°C. However, the temperature is in the range where *Legionella* is able to survive in water system (UK

Health and Safety, 2013). Therefore, additional approach should be applied together with the application of heat pumps system to prevent *Legionella* proliferation in hot water system.

As previously explained, providing residual disinfection is not an option in the Netherlands. Hence, another way should be invented to avoid Legionella growth in the household hot water system once the heating system using heat pumps works. Ultrasound emerges as a new technology for water disinfection, yet it is still not widely used in drinking water production. It offers a physical disinfection method, which does not change the quality of the water chemically. The principle of this method is killing bacteria using an ultrasound acoustic frequency (20 kHz and higher) (Vasilyak, 2010) that creates a phenomenon called cavitation (formation and collapse of microbubble) which leads to several bacteria killing mechanisms (Gogate, 2007). This method is considered to be free from any hazardous by-products (Hulsmans, et al., 2010) and appears to be a new alternative in disinfecting water in the household connection to compromise the situation once the heating temperature of water is lower in the future.

In accordance with the risk of bacteria regrowth in the household connection in a lower temperature of the future hot water system, and the available ultrasound technology, a study investigating the in-situ ultrasound disinfection in household connection will bring a new perspective. In addition, there is already a contradicting argument between previous study on ultrasound disinfection and a finding from a company. The study found that the ultrasound disinfection occurs in a short distance (Gogate, 2007), while the company claims that the effect of ultrasound can reach up to 40 m (H2O Technics, 2019). The cause of this different argument is assumed to be from the different treatment operation. According to opportunity of the ultrasound to disinfect drinking water and the different argument, an investigation on efficacy of ultrasound disinfection in microbial growth control in household is desired. Therefore, this research is prepared with the objective of:

Inventing a lab-scale experimental set-up to investigate the efficacy of ultrasound disinfection in microbial growth control in household water system providing water of 40°C

2 | Literature Review

2.1 Microbiology in Drinking Water Distribution Systems (DWDSs)

2.1.1 The presence of bacteria in DWDS

Bacteria presence in drinking water has been noticed since the early stage of microbial studies (Proctor & Hammes, 2015). Initially, the study focused on fecal bacteria, however, with various emerging new bacteria, DWDS starts to be a concern to nonfecal opportunistic bacteria, such as *Legionella* and *Mycobacteria*. In fact, the microbiology of DWDS is complex since the whole DWDS (source to tap) offers different habitat conditions for bacteria (Proctor & Hammes, 2015). In general, bacteria are distinguished based on different phases, which are bulk water (flow through the water main), pipe wall biofilm (formed on the inner surface of the pipe), suspended solids (particulate matter transported throughout the network), and loose deposits (particulate matter accumulated/settled on the pipe bottom) (Liu, et al., 2014). The complexity of microbiology is represented in different stages of bacteria community as show in Figure 1.

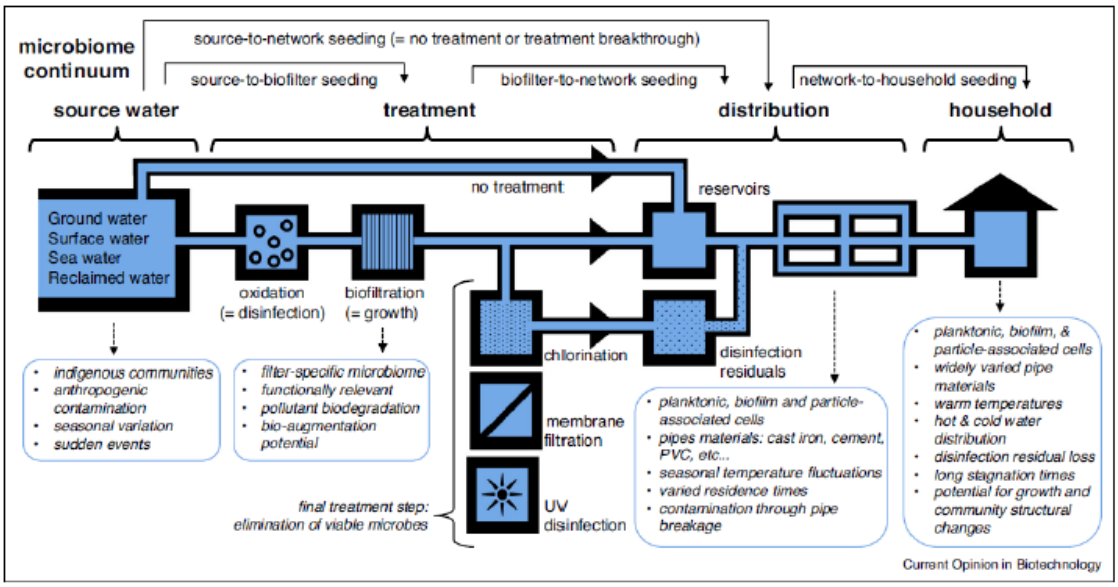


Figure 1. Different microbiology in different segment of drinking water system

(Source: Proctor & Hammes, 2015)

Based on Figure 1, microorganisms in household systems are present as planktonic, biofilm, and particle-associated cells. The presence of biofilms is significant because more than 90% of overall bacteria community can be found in biofilm (Liu, et al., 2016), some references mention around 95% (Douterelo, et al., 2016b; Eboigbodin, et al., 2008; Liu, et al., 2014).

A biofilm is a persistent microbial community which attach and grow on the pipe surface with the size of a few micrometers to a few millimeters. Bacteria communities agglomerate through cells that are embedded in a gelatinous matrix of extracellular polymeric substance (EPS) which they produce themselves (Liu, et al., 2016). EPS is built up from complex heterogenous substances, such

as polysaccharides, proteins, lipids, and nuclei acids, and serves as a cohesive force which enables the bacteria to attach to the pipe surface (Eboigbodin, et al., 2008), and also serves as a protection from adverse external factors (Douterelo, et al., 2016a; Douterelo, et al., 2014), such as disinfectants (Douterelo, et al., 2013). In addition to the bacteria composition in the biofilm, the biofilm poses a major threat in drinking water supply in household connections when biofilm detaches and mobilises within the system since it deteriorates the water quality (Eboigbodin, et al., 2008) along the pipes.

2.1.2 Affecting Factors of Microbial Growth in DWDS

a) Nutrient availability

The nutrients needed for the bacteria to grow are classified into organic and inorganic matter. In general, organic nutrients are analysed through AOC and TOC measurements. AOC represents the carbon that is dissolved in water and readily to be taken up by the bacteria, while TOC includes all carbons that are carried in particles and as dissolved organic matter. Microbial growth in water is sensitive to the organic carbon presence, as found in a study that an increase of 10 μg/L organic carbon consumption by the bacteria can yield an additional formation of 10^4-10^5 cells/ml (Lautenschlager, et al., 2013). Moreover, inorganic matters, such as phosphorus and nitrogen, contribute to biofilm formation in DWDS. Phosphorus is used for cellular metabolism, whilst nitrogen has a role in biofilm development and building blocks for protein and genetic materials (Liu, et al., 2016).

b) Temperature fluctuation

Bacteria grow effectively under optimum growing temperature. As found in a study that investigated the change of microbial abundance between summer and winter time, during summer time, the total the number of cell count (TCC) and intact cell count (ICC) is higher than during the winter time (Zlatanović, et al., 2017). Furthermore, the difference of temperature can affect the ability of microorganisms in producing EPS, therefore bacteria hydrophobicity modification is possible. It is suggested that bacteria modify then their cellular membrane lipid composition (Liu, et al., 2016). Besides influencing EPS, the AOC concentration has a reverse seasonal trend compared to the water temperature, with the lowest AOC concentration at the highest temperature (Zlatanović, et al., 2017).

c) pH fluctuation

Produced drinking water has an adjusted pH to avoid corrosion or scaling in the DWDS. However, there are specific pH ranges for optimum growth of the different bacteria. pH can affect the nutrient availability through shifting chemical equilibrium of the mineral carbon source (Liu, et al., 2016). Also, various chemical reactions are pH specific, therefore, when bacteria live in the optimum pH, they can proliferate effectively due to optimum metabolism.

d) Hydraulic conditions

Based on a study from Douterelo, et al. (2013), different microorganism communities and richness are detected under different hydraulic regimes. From another study it was concluded that turbulency correlates to the thickness of the biofilm, where the biofilm is thicker under increased

flow velocity. Under higher flow rates, tranport of suspended bacteria in the bulk water and nutrient transport increase, which stimulates futher growth (Liu, et al., 2016). However, higher flow rates can also result in detachment of the mature biofilm due to higher shear forces at the biofilm surface (Ohl, et al., 2003; Liu, et al., 2016), although it has also been found that high shear forces boost EPS production that will enhace the adhesion between biofilm and material surface (Liu, et al., 2016).

e) Pipe materials

Certain pipe materials might release substances that are considered as a nutrient source for microorganisms. These substances come from chemicals contained in the raw material and/or used in the making process of the pipe. Plastic pipes might contain phosphorus as found in the study of Lehtola, et al. (2004), and plastic materials commonly contain various additive chemicals, such as antioxidant which mostly consists of organic molecules with phenolic groups. While, in the same study, longer duration was needed for biofilm to be formed in copper pipes. This is because there are some bacteria strains which are sensitive to copper.

2.2 Microbiology Assessment in DWDS

Investigating the microbial environment in the DWDS is a challenge because the pipes are not readily accessible and collecting the samples from the system is difficult. Generally, the analysis of microbiology in drinking water is divided into the bulk water and biofilms.

Having a representative biofilm sample is a challenge because the drinking water microbiome varies over the stages of DWDS (Proctor & Hammes, 2015) as explained in Figure 1. There exist two approaches to analyse the microbiology of the DWDS, which are to cut out pipes and to install a device inserted in the pipe (Douterelo, et al., 2014).

2.2.1 Common Observed Parameters for Microbiology Assessment in DWDS

a) Microorganism abundance

- Heterotrophic Plate Count (HPC)

  Microbial quantification using HPC is widely used to monitor the changes of microbial quality in the bulk water. However, this method limits the analysis to only the heterotrophic and culturable bacteria at a specific temperature (Douterelo, et al., 2014). Moreover, HPC is able to detect only less than 1% of the total bacterial concentration in drinking water (Prest, et al., 2016).

- Flow Cytometry

  A wide range of bacteria types are present in water and they are unlikely to be detected with HPC, therefore culture-independent methods are used to assess the actual microbial diversity. One fast and reliable alternative to assess the microorganism concentration is flow cytometry. This method uses fluorescent dyes to stain the bacteria cells and the cells will be

analysed using a laser beam that scatters the light and excites the dyes (Douterelo, et al., 2014). The advantage of flow cytometry is that it is able to distinguish the intact cells and the damaged one. Commonly, there are two type of stains used to stain the membrane cell integrity to obtain the information of living and dead cells. SYBR Green I and Propidium iodide are mostly used for the staining. Propidium iodide is only able to penetrate through a damaged cell membrane (van Nevel, et al., 2017), therefore, the intact cells would not get stained. This is how flow cytometer can detect the living and dead cells. Furthermore, the result is derived into two subgroups of bacteria, which are High Nuclei Acid (HNA) and Low Nuclei Acid (LNA) bacteria. The distinctions between those two subgroups are the actual nucleic content and cell size that are indicated by the fluorescent intensity and scatter signals (Zlatanović, et al., 2017).

b) Organic Carbon

In DWDS, nutrients in the form of organic carbon is a food source for microorganisms. Therefore, organic carbon is an important factor for controlling the microbial activity in DWDS. The common measured organic carbon is Dissolved Organic Carbon (DOC). Basically, DOC represents the measurement of organic in water and has little actual bacteria growth potential of the water because it is not easily degradable. Therefore, Assimilable Organic Carbon (AOC) is preferably used instead of DOC because AOC is a fraction of DOC that is readily available for microorganisms. Hence, it is more suitable to represent the carbon consumed by the bacteria to live. However, sometimes AOC is converted and represented as biological DOC for the purpose of comparison (Vital, et al., 2012). Furthermore, this method has limitations: i) assuming bacterial growth only limited by organic carbon; ii) quantifying the amount of nutrient instead of the bacteria; and iii) bacteria-type dependent (Douterelo, et al., 2014).

c) Adenosine Triphosphate (ATP)

Measurement of ATP is a method of assessing the microbial activity. It has a wide range of applications to assess bacteria in bulk water, pipe wall biofilm, suspended solids, and loose deposit (Douterelo, et al., 2014). ATP is suggested as an alternative or complimentary parameter for biological stability analysis in drinking water because of its ability to present the viable biomass in water (Lautenschlager, et al., 2013). In addition, ATP analysis is able to detect very low amounts of biomass within a short time analysis (van der Kooij, 1999). Due to different bacteria phases, the ATP measurement is distinguished into free ATP and cell bound ATP (Vital, et al., 2012).

d) Microbial composition

In principle, determining the composition of the bacteria involves Polymerase Chain Reaction (PCR) amplification and DNA extraction (Prest, Hammes, van Loosdrecht, & Vrouwenvelder, 2016). The information obtained from this analysis is essential to detect specific pathogens, bacteria associated in biofilm in DWDS, also to assess the influence of abiotic factors on microbial communities (Douterelo, et al., 2014). Different methods are found to extract the DNA information from the microbial community, such as molecular technique which use ribosomal RNA (rRNA) gene as marker genes and fingerprinting technique that analyse the separated RNA based on their sequence composition (Douterelo, et al., 2014).

2.2.2 Legionella Significance in Domestic Drinking Water System

The presence of Legionella bacteria in household connection is highly important to be analysed due to the risk of Legionnaires' disease. The potentially fatal form of this disease is pneumonia which is caused by *Legionella pneumophila*. These bacteria are commonly found in natural water systems, such as river, lakes, and ponds, yet in low numbers (Health and Safety UK, 2013). Though, the number of Legionella tends to multiply in the heating system in the household. This is triggered by the supporting environmental condition for *Legionella* to thrive, namely increased temperature, products of pipe corrosion, and deposits presence that contain amoeba and biofilms. *Legionella* bacteria can survive at water temperatures in between 0-63°C, pH ranges between 5.0-8,5, and dissolved oxygen concentrations of 0.2-1.5 ppm (Rakić & Štambuk-Giljanović, 2016). Furthermore, a study found that *Legionella pnemophila* proliferate at the most at 40°C (Roger, et al., 1994), which a typical temperature for shower. Therefore, maintaining high temperature water in the hot water system is crucial to avoid Legionella growth. This is supported by another study mentioned that *Legionella* are instantly inactive at temperature above 70°C (Kim, et al., 2002).

Legionnaires' disease is not a water-borne disease, yet it is spread through aerosols or sprays containing Legionella bacteria (PABIAC, 2017). Therefore, the transmission of the disease in DWDS occurs in the household through the plumbing equipment, such as faucets, showerheads, and hot water tanks (Rakić & Štambuk-Giljanović, 2016). Because of that, it is highly important to inhibit the proliferation of *Legionella* bacteria in DWDS, especially in warm water systems.

2.3 Energy Transition in the Netherlands

In the attempt to implement the Paris Agreement, the Dutch government has set a goal to decrease the CO_2 emission up to 95% by 2050 by cutting off the fossil fuel usage to close to zero (Ministry of Economic Affairs of the Netherlands, 2017). This policy will affect the heating in the households in the Netherlands as 93.7% of the total households use natural gas for heating purpose (Litjens, et al., 2018). Therefore, the Dutch government released an Energy Agreement as part of the National Energy Policy for the energy transition towards renewable energy production in aiming the target in 2050 (Ministry of Economic Affairs of the Netherlands, 2017).

Renewable energy sources will replace the fossil fuel in the future, including the building sector for heating and cooling purpose. Over a decade, heat pump appears to be a technology that can increase the use of renewable energy sources (Fraga, et al., 2018) for heating and cooling the buildings. Heat pump is considered as electrification of heat (Litjens, et al., 2018) because it uses electricity to move the heat instead of generate the heat directly (US Department of Energy, 2019a). There are several types of heat pumps based on the heat source: air, water, and geothermal heat pumps (US Department of Energy, 2019a). In the future, the heat pump systems are likely integrated with the future renewable energy sources, for instance solar thermal energy, inudstrial excessive heat, and waste incineration (Yang, et al., 2016). However, air source and ground source systems are the most common application for the heat pump system (Fraga, et al., 2018) in the current systems.

In principle, heat pumps work like a refrigerator but in reverse. Heat pump pulls the heat from the surrounding, depending on the heat source, and transfer it to the space or water heater tank for hot water system (US Department of Energy, 2019b). In the larger scale, heat pumps are implemented in the district heating to supply the heat to the households, which is found to be a cost and energy efficient way to deliver the heat (Yang, et al., 2016; Ommen, et al., 2014).

A major consideration for heat pump is the temperature difference between the source and the sink in the district heating, while the heat capacity in the inlet and outlet is finite (Ommen, et al., 2014). Preferably, the gap of the temperature is not too large, as an illustration, a -15°C of source temperature will struggle to provide a heating of 60°C, but ground source with temperature of 10°C can result a good output for heating of 40°C (ICAX, 2019). Therefore, the heat pump might deliver lower temperature than the hot water system due to this requirement of temperature difference. The heat will be supplied to have sufficient heating for providing the sufficient temperature, both for heating the space and water, so that no excess of heat will be transmitted. Furthermore, the heat pumps will deliver the temperature as required for comfort, which is 40°C and 45°C for kitchen use (Yang, et al., 2016).

This lower heating temperature offers a benefit in hot water supply in the household, which provides a hot water system that has the comfortable temperature to use. Therefore, the customers do not need the storage for hot water or combine the water, instead the hot water with comfortable temperature can be directly used from the tap (Schmidt, et al., 2017). However, the heating system using heat pump rises the concern of *Legionella* presence in the hot water system due to the output temperature is in the range of the environment where *Legionella* could proliferate (Yang, et al., 2016).

2.4 Ultrasound Sonication Application

2.4.1 The Principle of Ultrasound Sonication

The principle of ultrasound sonication is transmitting an ultrasound acoustic frequency (>20 kHz) which creates cavitation in water. Cavitation is defined as the phenomena of formation, growth, and subsequent collapse of microbubbles occurring in extremely small-time interval (Hulsmans, et al., 2010) that can release a large magnitude of energy in a small area that leads to high-energy intensity (Gogate, 2007). The cavitation generated by ultrasound sonication is considered as acoustic cavitation because the ultrasonic generates acoustic streaming in the liquid (Ashokkumar, 2011). Besides acoustic cavitation, hydrodynamic cavitation (pressure variation through velocity variation), optic cavitation (produced by photons of high-intensity light), and particle cavitation (resulted by beam of the elementary particles) also exist (Gogate, 2007).

Specific to acoustic cavitation, the bubbles will oscillate and collapse then resulting physical effects, such as shock waves, microjets, turbulence, and shear forces (Ashokkumar, 2011). Moreover, the ultrasound generates mechanical agitation that could be applied for surface cleaning (Yusof, et al., 2016). This is possible because the collapse of the cavitation bubble near the surface could result a powerful jet and shock wave directed to the surface which is able to release the dirt and bacteria

from the surface (Mason, 2016; Yusof, et al., 2016). Therefore, ultrasound emerged as an alternative for cleaning in industrial machinery in the 1950s due to its advantage to dislodge the surface contaminant deeply into the cavities (Mason, 2016).

Later, ultrasound cavitation is adopted for the beverage and food processing industry. Milk industry is one of the examples, which the ultrasound cavitation is utilized to homogenise that fat globules size to nano-scale particle size (Crudo, et al., 2014). Furthermore, through control manner (i.e. frequency, power, distance), the generated force from cavitation bubbles is sufficient to cause lethal cellular damage (Svendsen, et al., 2018). Ultrasound cavitation showed a potency for disinfection, and the implementation of the disinfection has been done in various applications, such as beverage and food industry, aquaculture, and industry water.

2.4.2 Ultrasound Disinfection

As the concern of *Legionella* emerges along with the energy transition in supplying heat in the household, an approach should be applied to provide safe hot water. A study suggested to add local supplementary heating device to boost the hot water or limit the use of hot water (Yang, et al., 2016). Those approaches seem not practical and the potency of the ultrasound cavitation for disinfection is seen as an opportunity to be an alternative in preventing the *Legionella* risk in hot water system with the heating system using heat pump.

Among all types of cavitation that are mentioned in the previous section, only acoustic and hydrodynamic cavitation supposedly give the suitable intensity for water disinfection (Gogate, 2007). The inactivation of the bacteria by cavitation bubbles occurs through several mechanisms (Gogate, 2007; Lambert, et al., 2010):

a) Mechanical effects caused by cavitation (turbulence, circulation currents, shear stress)
b) Chemical effects due to generation of active free radicals (H^+ and OH^-)
c) Heat effects, the cavitation creates local hot spots (locally high temperature and pressure)
d) Disagglomeration of bacteria, thus the disinfectants are able to pass through the microbial cell membranes

The main mechanism that cause the biocide effect from ultrasound is the mechanical effects (Gogate, 2007; Lambert, et al., 2010) since the pressure forces, created by the collapse of gas bubbles, enter the bacterial solution or near the bacterial cell membrane. This pressure leads to mechanical fatigue that can damage the cell wall (Joyce, Phull, Lorimer, & Mason, 2003). Whilst, chemical effect and heat effects act as supporting mechanisms in disinfection (Gogate, 2007). In the chemical mechanism, the radicals attack the cell wall and weaken it, hence the cell wall disintegrates (Joyce, Phull, Lorimer, & Mason, 2003), while the heat effect creates local temperature within 1000-5000 K range (Lambert, et al., 2010). Furthermore, H^+ and OH^- radicals are generated from homolysis of water due to extreme temperature and pressure condition (Mahvi, 2009). Therefore, the chemical and the heat effect are dependent on each other.

Different from the other methods of disinfection, ultrasound disinfection in water does not require chemical additive in the treatment. Because of that, this disinfection method does not generate

hazardous disinfection by-product (Hulsmans, et al., 2010), hence there is no environmental concerns associated with the disinfection by-product that are needed to be anticipated (Mahvi, 2009). However, high ultrasonic intensity is required to have 100% killing rates by ultrasound disinfection alone, and the costs of it is therefore high (Joyce, et al., 2003). In practice, combinations between ultrasound and other disinfection method are used. The combination between disinfection methods yield synergetic effect by declumping and breaking the bacteria agglomerates (Arrojo, et al., 2008) which makes it easier for the disinfectant to diffuse to the cell wall.

2.4.3 Affecting Factors of Ultrasound Disinfection

a) Frequency

As the disinfection method uses acoustic cavitation in the range of ultrasound frequency, acoustic frequency is certainly an important factor in the disinfection process. The frequency affects the size and the number of generated bubbles. As the frequency lowers, the cavitation is easier to occur, and the influence gets more aggressive (Vasilyak, 2010). As mentioned before, cavitation has a declumpling effect. A study by Joyce, et al. (2003) found that a high frequency is able to distinguish between the declumping and deactivation phases, while in low frequency, the actual deactivation is disguised by the declumpling effect.

b) Contact time and water flow

Previous studies have proven that the disinfection efficacy increases as the contact time increases. Jyoti & Pandit (2001) found that 85% removal is reached after 20 minutes of treatment. Also, in circulating systems, a bacterial reduction of more than 99% has been achieved when the water passing through the ultrasonic disinfection is more often due to a high flow rate. However, in a once-through experiment, a low flow rate yields higher reduction (Hulsmans, et al., 2010). Therefore, the more contact with the ultrasound wave, the higher the reduction efficacy.

c) Water volume

Mahvi (2009) has discovered that a smaller volume of water gives a higher removal efficacy and produces a more rapid disinfection at the same treatment duration. Similar results are also obtained in another study, where rapid disinfection occured in a smaller treated water volume (Joyce, et al., 2003). Another finding from a circulating pilot experiment setup showed that within the same flow rate and residence time, smaller a water volume has a higher bacterial reduction (Hulsmans, et al., 2010).

d) Electrical power

Ultrasound wave emission depends on electrical power provided due to its power dissipation and better cavitation. A previous study shows that higher electrical power input, a higher intensity, and a higher power dissipation gave a higher bacterial reduction (Hammes, et al., 2010). A higher power dissipation corresponds with the formation of hydrogen peroxide from radicals (Gogate, 2007), which certainly increases the biocidal effect.

3 | Experimental Setup

This research aims to make a design of and build a pilot experimental setup intended to investigate the biofilm growth in household water supply systems under ultrasound disinfection process. The experimental set-up is made from copper pipes with internal diameter of 19 mm and comprises of some number of smaller pipe segments called pipe-coupons for biofilm sampling. The flow of the water in this set-up will be upflow. This experimental set-up is shown in Figure 2.

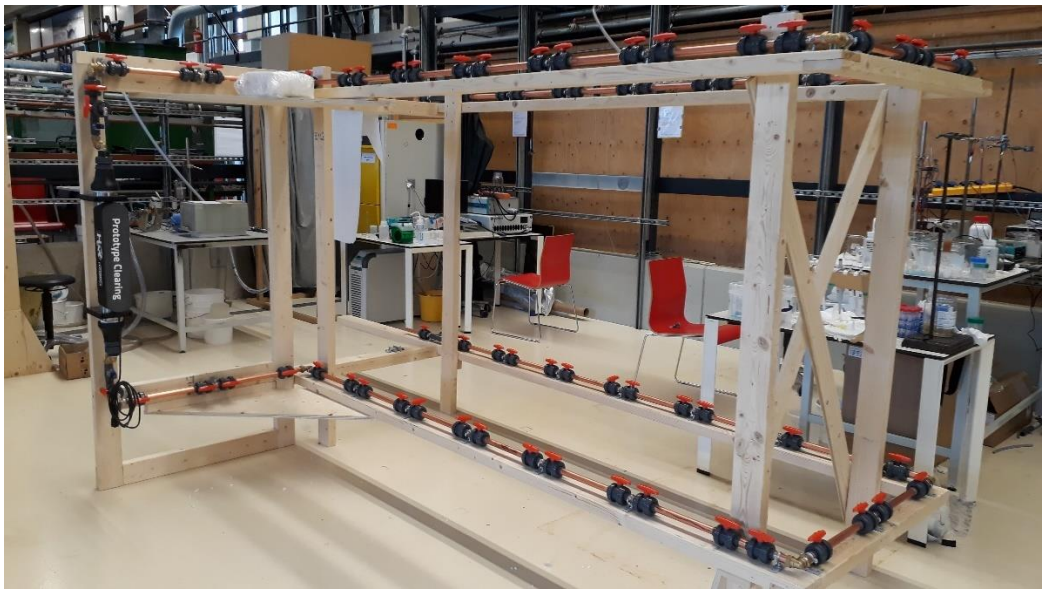


Figure 2. Experimental set-up design

Copper pipe is chosen because the pipe material is the most common material used for the installation of domestic drinking water connections in the Netherlands (Slaats, et al., 2001). This material has been used for years to deliver drinking water in house and other buildings. This is because copper has a good resistance to corrosion (Buijs, 2011), however, there is still a possibility that it can enter into the water, under certain conditions, through uniform corrosion on the surface pipe (Critchley, et al., 2001).

3.1 Installation

Since the biofilm formation is greatly affected by the hydraulic condition inside the pipe, the experimental set-up is equipped with devices that can indicate and control the hydraulic conditions. The experimental set-up comprises of valves at the beginning and the end of the system, a pressure meter and a flow meter were installed in the beginning of the set-up, water tap and festo connection, ultrasound disinfection device, and flow cytometer. Below are the explanations of the components for the experimental set-up

3.1.1 Sampling taps and festo connections

In monitoring the water quality, sampling taps are installed and followed by a festo connection (shown in Figure 3). Water taps are intended for water sampling for the observation of the water quality under ultrasound disinfection. While, the festo connections are used for the connection to the flow cytometer (will be further discussed in the next chapter).

Figure 3. Water tap and festo connection

The locations for these taps and connection are at the beginning of the set-up network (after flow meter), before and after the ultrasound device and at the end of the set-up, and at the end of the setup. These locations are chosen on purpose to see the extent of the ultrasound effect in the pipe network. This is based on the conflicts between the study and claim from the company. Previous studies report that the effect of ultrasound occurs in very short distance, around 2-5 cm (Gogate, 2007; Vasilyak, 2010). While the company states that the effect will be in the whole system as the effect can reach up to 40 meters in fresh water (H2O Technics, 2019). This disagreement might be caused by the different of treatment operation. Previous studies used ultrasound bath operation, which the water is treated in a batch system, while the ultrasound device from H2O Technics works in a continuous flowing water system. Therefore, these taps are important to see whether the operation different gives different results in terms of bacteria level reduction.

3.1.2 Pipe-coupons design

The goal of the research is to investigate the biofilm growth under ultrasound disinfection. In the real distribution network, biofilm sampling can only be done by pipe extraction from the system (Prest, 2016). There are two approaches available in investigating the biofilm in DWDS, which are cut-out pipes and application of inserted device into the pipe, known as coupons (Douterelo, et al., 2014) that are placed for long-term biofilm formation (Prest, et al., 2016). However, the use of inserted-coupons can distort the hydraulic condition inside the pipe and commonly, it changes the shear stress and turbulence regimes that might influence the development of biofilm (Douterelo, et al., 2014). Hence, the cut-out pipe approach is chosen with some modification as depicted in Figure 4.

Figure 4. Coupon design

The modification of the approach is at the application of additional valves at both of the pipe segment ends are intended for sampling easiness. Previous studies demonstrated the cut-out pipes for the biofilm analysis were closed using pre-disinfected caps to inhibit contamination (Liu, et al., 2017). With the valves, the inside sampled segment will be avoided from contact with the outside environment, hence minimizing the risk of contamination although the experimental set-up will be placed in the laboratory. Besides, the biofilm will be isolated before the pipe segment is removed from the system. This makes the biofilm is taken with the initial condition (i.e. water chemistry and bacteria composition), therefore, the result is expected to represent the actual condition of the system at the sampling time.

In this research, the segmented pipe of 20 cm is considered as a coupon because the pipe segment serves the similar purpose to the commercial coupons. Therefore, this pipe segment is named as pipe-coupon in this research. This kind of coupon is preferable because biofilms are not uniformly distributed in drinking water distribution (Liu, et al., 2016). Besides, the pipe connections are new, hence, the biofilm formation might not be abundant in short time. Based on the study of Zlatanović, et al. (2017), also using tap water as the water source, the stabilization of biofilm in a copper pipe network was performed for 210 days before the first analysis. Therefore, longer pipes will provide more area for biofilm to attach and grow, so that the probability to have biofilms during the sampling period (will be explain in the next chapter) is higher. The inserted type of coupon might limit the possibility of getting the biofilm in the early stage of biofilm sampling.

3.1.3 Pressure meter and flow meter

This experimental set-up is expected to be connected directly to the drinking water connection, therefore, it is very likely that the pressure will fluctuate which can affect the amount of water passing through the set-up. Pressure changes can result in changes in the hydraulic conditions, therefore, it helps to indicate the hydraulic condition during the experiment and whether it affects the biofilm formation. Whilst, the flow meter is required to have a minimum capacity of 8 L/min due to the range of intended water velocities of the experimental set-up. The chosen capacity of the flow meter is based on the required velocity of water in DWDS based on the self-cleaning velocity. A maximum water velocity of 0.4 m/s is decided to be the limit of the velocity in the system. This constraint is considered, so that the velocity is safe and feasible to avoid resuspension of particles in the pipe network, regardless the pipe diameter. This maximum velocity has been implemented in the Netherlands since 1999 (Vreeburg, et al., 2009). As the lower limit, 0.15 m/s is already considered as the velocity for a self-cleaning networks (van der Hoek, et al., 2018).

3.1.4 Ultrasound device

The ultrasound device that is used in this research is prototype device of the Clearing type provided by H2O Technics as depicted in Figure 5. This type of ultrasound device is made to be applied in water supply system to fight *Legionella* (KVK, 2018).

Figure 5. Clearing ultrasound device

Ultrasound disinfection takes place in the metal tube with the source of the energy comes from the acoustic wave generated in the resonator. The declumping effect together with microjet and shock wave effect are expected to be able to detach the biofilm from the pipe surface as explained in the previous chapter regarding ultrasound application on surface cleaning. The ultrasound device is mounted to the pipe connection using transducer on both sides of the tube. In operation, the device works at a frequency of 40 Hz with the power up to 110 dB (H2O Technics, 2019). As the request from the company, the device should be installed vertically.

As shown in Figure 6, there are additional valves before and after the ultrasound device, also at the end of the system. The valves before and after the ultrasound device are intended for the easiness of the mounting of the device. Besides, it is used to maintain some amount of water when the ultrasound device is not in used, as requested by the company. This is because the water inside the device will cool down the device.

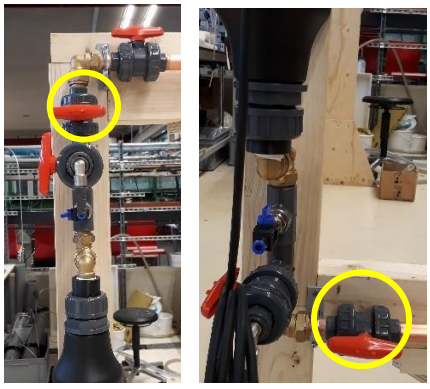


Figure 6. Additional valves

3.1.5 Flow cytometer

Flow cytometer is used to obtain the information of TCC in the water before and after the treatment using ultrasound disinfection. Flow cytometer from Sigrist BactoSense is installed and connected to the experimental set-up through the festo connections. The method of the analysis is online measurement, which allows to measure a constant flow of water from the piping system. The flow cytometer is connected to the system with a PFA tube with diameter of 1/4" (6.35 mm) and the outlet of this device is delivered through another PFA tube with similar diameter. Water flow coming to the flow cytometer should be in the range of 200-400 ml/min.

Until several years ago, heterotrophic plate count (HPC) was used for monitoring biological stability in drinking water (van Nevel, et al., 2017). This method represents the microbes that are isolated under certain conditions of media composition, incubation time, incubation temperature, and means of medium inoculation (Allen, et al., 2004). However, it is found that this method does not represent hygienic relevance in the measurement because neither microbial abundance nor composition are generated from the result (van Nevel, et al., 2017). The reason is because HPC only enumerate a fraction or subpopulation of microorganisms in water (Allen, et al., 2004). Compared to cultivation independent methods, HPC gives several orders of magnitude lower than the cultivation independent measurement (Vital, et al., 2012). The inability of HPC in producing the actual number of the bacteria is because of the carbon concentration in HPC media is excessive, up to 800 times, but the cultivation environment is restricted. Therefore, it is expected that some cells are not cultivated under this situation (Hammes, et al., 2008). This causes the "unseen majority", which major part of bacteria is not detected in HPC that is assumed to be in "viable but not cultivable" (VBNC) state or simply not cultivable in the HPC method (van Nevel, et al., 2017). In addition, *Legionella*, the main target for this research, is doubted to grow on HPC media (Allen, et al., 2004). It is clear that the HPC method is not viable for aiming the goal of the research. Besides, the result of FCM can be obtained in around 30 minutes, which is an advantage of FCM over HPC (HPC gives result in 2-10 days) (van Nevel, et al., 2017).

Flow cytometry (FCM) is believed to be able to overcome the shortcomings of the HPC method. This method allows enumeration of bacteria through staining of specific cellular features or specifically targeted cells with antibodies (Hammes, et al., 2008). These stained cellular will pass through a light source, usually a laser beam and the cells excite and emit light with higher wavelengths. Moreover, FCM can distinguish the living and non-living cells through two steps staining, usually SYBR Green I and Propidium iodide (van Nevel, et al., 2017). Measurement with FCM allows to obtain a broad information of bacteria concentration in water. Total cell concentration is indicated with total cell count (TCC) which represents the total of living and abiotic particles, while, the living cells only are counted as intact cell count (ICC). In principle, SYBR Green I will stain the intact cells, and propidium iodide will stain the damaged cells (van Nevel, et al., 2017). Furthermore, the result from FCM can differentiate between the active and inactive part of the microbial community through the nucleic acid in bacteria cells. High nucleic acid (HNA) bacteria is regarded as the active part, and the inactive part is indicated by the low nucleic acid (LNA) bacteria (SIGRIST, 2018). The FCM from Sigrist works with both SYBR Green I and Propidium iodide stains, therefore, it is able to detect TCC and active cells, which will result clearer and more representative result for the water samples than using HPC method.

Nevertheless, FCM method has some limitations and additional measures should be done to confirm the results of FCM. It is unable to separate the aggregated bacteria from the single cell bacteria. In one example, sonication is used to break the bacteria clumps (van Nevel, et al., 2017). According to that, clumping bacteria will not be a major problem in this research using ultrasound as the disinfection means, since it has a deagglomerating effect (Arrojo, et al., 2008; Joyce, et al., 2003). Another limitation is FCM that only detects intact and damaged cells, but not intact-but-dormant cells. ATP is a common way to conform the result of FCM since ATP is the "energy currency" (Vital, et al., 2012) that indicate whether the bacteria are active. The ATP measurement is usually done for cell-bound ATP and free ATP in the water, which the cell-bound ATP represents the active bacteria.

3.1.6 Water heater

However, in the current set-up the water heater is not applied yet. An additional water heater should be installed to perform the experiment with water temperature of 40°C. Typical tankless washroom water heater is suggested to be used to heat the water. This type of water heater is preferable because there is no need of having a water storage, which means that less space required and minimize the possibility of creating a perfect environment for the bacteria to grow. Once the hot water gets cooled, there will be some period where the temperature of the water is in the perfect range for the bacteria to grow. Therefore, the tankless type of water heater is an option to avoid further bacteria proliferation.

4 | Research Plan

The experiment is planned to be divided into two stages. First stage is investigating the efficacy of ultrasound disinfection in reducing bacteria level in water. Second stage is biofilm sampling that is planned to be conducted around 7 months after the experiment of the first stage starts. In the first stage, the experiment is intended to be conducted in two different hydraulic conditions, which when water is flowing through the set-up and when water is stagnant in the water network. The stagnant condition is considered based on previous studies by Zlatanović, et al. (2017) that found the quality of water in pipe network after stagnation changed, such as, copper concentration and bacteria abundance increased. Another study by Lautenschlager, et al. (2010), conforms the results. Therefore, the disinfection method using ultrasound in stagnant water might be interesting to be investigated.

Some parameters that indicate the bacteria activities will be observed in order to investigate the efficacy of the ultrasound disinfection in reducing the bacteria level and mitigating the biofilm growth. However, since not all the parameters are feasible to be examined in TU Delft Water Lab, some of the parameters will be sent to Haarlem laboratory. Below is the explanation regarding the chosen observed parameters.

- **Bacteria abundance**. The bacteria abundance will be examined using FCM. However, it is not possible to measure more than one point at the same time. Therefore, water samples are taken from the different sampling points and the concentration of bacteria will be analysed later in the Haarlem laboratory together with the other parameters.
- **Bacteria community**. The composition of the bacteria will be analysed using qPCR sequencing in Haarlem laboratory. This parameter is monitored in the water samples and the biofilm samples. For the water samples, one sample from flowing water experiment and one sample from stagnant water experiments will be taken for the community analysis. For analysis of microbial community in biofilm, the pipe-coupon is taken out from the setup and brought to the Harlem laboratory.
- **TOC.** TOC is measured instead of DOC because it will include the organic bounded to particle which also can be the organic source for the bacteria. Measurement of this parameter will indicate the potency of the microorganisms to grow in the water. An example from previous study, showed the indication that bacteria consumed organic carbon during stagnantion to proliferate (Lautenschlager, et al., 2010). TOC will be measured with the available test kit by Hach in Water lab.
- **ATP.** As a complementary data, ATP is a good indicator for viability of microorganisms since it represents the active biomass (Lautenschlager, et al., 2013). Besides, ATP measurement is a culture independent analysis method, hence, the result can be used for accurate and rapid assessment for microbiological activity in water (Vital, et al., 2012). Most of the time it is measure altogether with flow cytometer measurement to conform the result. ATP test kit is available in Water Lab to be used for the analysis.
- **Temperature.** When the water samples are taken, the temperature is also measured directly using the multimeter probe. The temperature is measured in every sampling point to monitor whether the ultrasound disinfection will increase the water temperature during the operation.

Based on previous study, ultrasound sonication can cause temperature increase that might also cause the disinfection (Arrojo, et al., 2008).

- **Copper concentration.** This kind of metal concentration becomes important to be measured since it leaches at higher temperature (Zlatanović, et al., 2017) and it is relevant to the condition in which the experiment will be conducted (under water temperature of 40°C). In addition, microorganism activities can cause corrosion (Wagner & Chamberlain, 1997) and the copper can be released to the water, which is toxic for human health after reaching a certain concentration (Critchley & Fallowfield, 2001). Whilst, bacteria still be able to survive when the copper concentration is at toxic level (Liu, et al., 2016). Since the concentration of the copper is likely very small compared to the sensitivity of the test kit, it can be measured using ICP-MS provided in the laboratory.

Copper concentration becomes one of the focus because microbial activity and copper pipes has a mutual relationship, as reported from the previous study by Wagner and Chamberlain (1997). Microbiologically induced corrosion (MIC) was found to cause unexpected pitting corrosion of copper pipework. Another study also found that microbial activity in copper pipe network is able to initiate pitting corrosion in both cold and hot water systems (Fischer, et al., 1992). The site of the corrosions are the place where the bacteria colonize forming biofilm because of the availability of the loose copper as their nutrient in the form of trace metals (Liu, et al., 2016). On the other hand, biofilm activity can also affect the copper concentration in the water. Copper concentration might increase in water due to the production of acidic metabolic products which promote metal ionisation. Another way by the binding of copper ions within the bacteria cell walls, which will increase the concentration of the copper in the water once the biofilm detach from the pipe (Critchley & Fallowfield, 2001).

4.1 Experiment Stages

4.1.1 Ultrasound Disinfection Efficacy

As previously explained, this stage of the experiment is about observing the water quality under ultrasound disinfection. The experiment is planned to be conducted in 4 variations of temperature (room temperature and up to 50°C) and 4 variations of flow rate (1.7-6.8 L/min). In this research, room temperature indicates the temperature of water that goes out from the tap. It is assumed that water that comes out from the tap has approximately the same temperature as the room due to the temperature equilibrium. The flow rate is determined based on the velocity of the water (0.1-0.4 m/s). For each variation, the experiment takes 90 minutes after flushing and stabilization of the water temperature. The flushing and stabilization are estimated to be 30-60 minutes. After the temperature are stable at all sampling points, ultrasound device can be turned on and it marks the time of 0 minute for the experiment. Water samples for measuring the parameters previously mentioned are taken in every 30 minutes. Table 1 shows the planning of water sampling during the experiment.

The interval of the sampling is 30 minutes is because of the FCM is able to analyse one sample in 30 minutes. Moreover, each sampling point will be analyse once in each variation due to the constraint

from the FCM. Since FCM is only able to analyse one point at the time, there is an order for each sampling point is anlysed using FCM. Table 2 indicates the order of the sampling point for FCM analysis, while the number order of the sampling point in the set-up is depicted in Figure 7.

Table 1. Water sampling planning

Parameters	Time (mins)			
	0	30	60	90
Temperature				
FCM				
ATP				
TOC				
Cu				

Samples are taken Sample are not taken

Table 2. FCM sampling point order for bacteria abundance analysis

Min	Flow rate			
	1.7 L/min	3.4 m/s	5.1 m/s	6.8 m/s
0	1	4	3	2
30	2	1	4	3
60	3	2	1	4
90	4	3	2	1

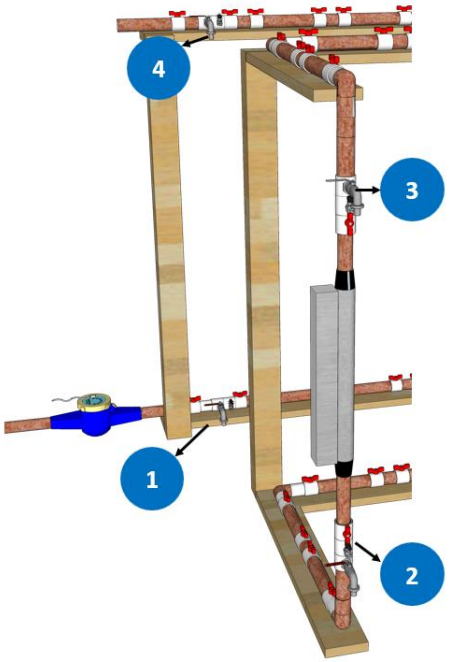


Figure 7. FCM sampling point order

The purpose of switching the FCM connection at the set-up is to get a quick overview whether there is any change in the level of the bacteria concentration in different sampling points to indicate the effect of ultrasound disinfection. This is possible because the operation of FCM is intended to be online, which the device will take the sample directly from the experimental set-up through the festo connection for the direct analysis. Besides, it is a way to double check the result with the result from laboratory analysis.

The same measuring arrangement is also planned for analysing the water quality in the stagnant water experiment. There will be 4 variations of stagnation duration, which are 2, 4, 6, and 8 hours, while the temperature of the water will have 2 variations (room temperature and 40°C). Based on the previous study, the duration of stagnation is linearly connected to the quality change of the water, for example the high bacteria concentration is higher after longer stagnation time (Zlatanović, et al., 2017). Moreover, this experiment will show whether the treatment in stagnant water will have different efficacy.

Both experiment in flowing water and stagnant water are planned to be conducted within 3 weeks, as shown in Table 3. The experiment period only takes in to account the weekdays, therefore, the experiments last for 15 days in total. However, some changes are certainly possible to occur due to some adjustments that need to be taken in the experiment.

Table 3. Planning for first stage experiments

Sampling time		**Variation**		
		Temperature	**Flow rate (L/min)**	**Stagnation duration**
Week 1	Day 1	Room temperature	1.7 and 3.4	
	Day 2	Room temperature	5.1 and 6.8	
	Day 3	25°C	1.7 and 3.4	
	Day 4	25°C	5.1 and 6.8	
	Day 5	40°C	1.7 and 3.4	
Week 2	Day 6	40°C	5.1 and 6.8	
	Day 7	50°C	1.7 and 3.4	
	Day 8	50°C	5.1 and 6.8	
	Day 9	Room temperature and 40°C		2 hours
	Day 10	Room temperature		4 hours
Week 3	Day 11	Room temperature		6 hours
	Day 12	Room temperature		8 hours
	Day 13	40°C		4 hours
	Day 14	40°C		6 hours
	Day 15	40°C		8 hours

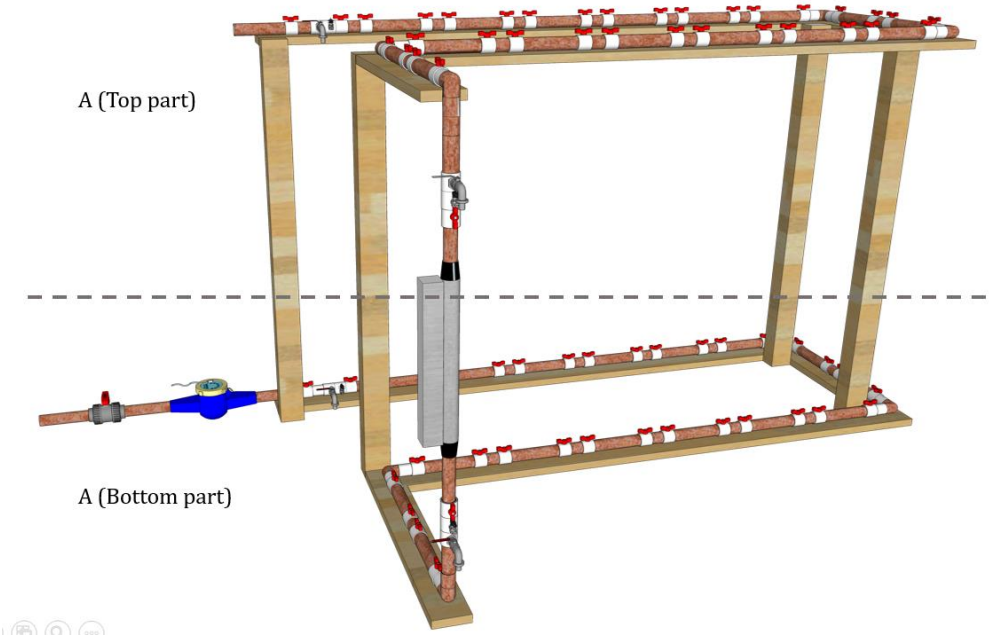
4.1.2 Biofilm Sampling

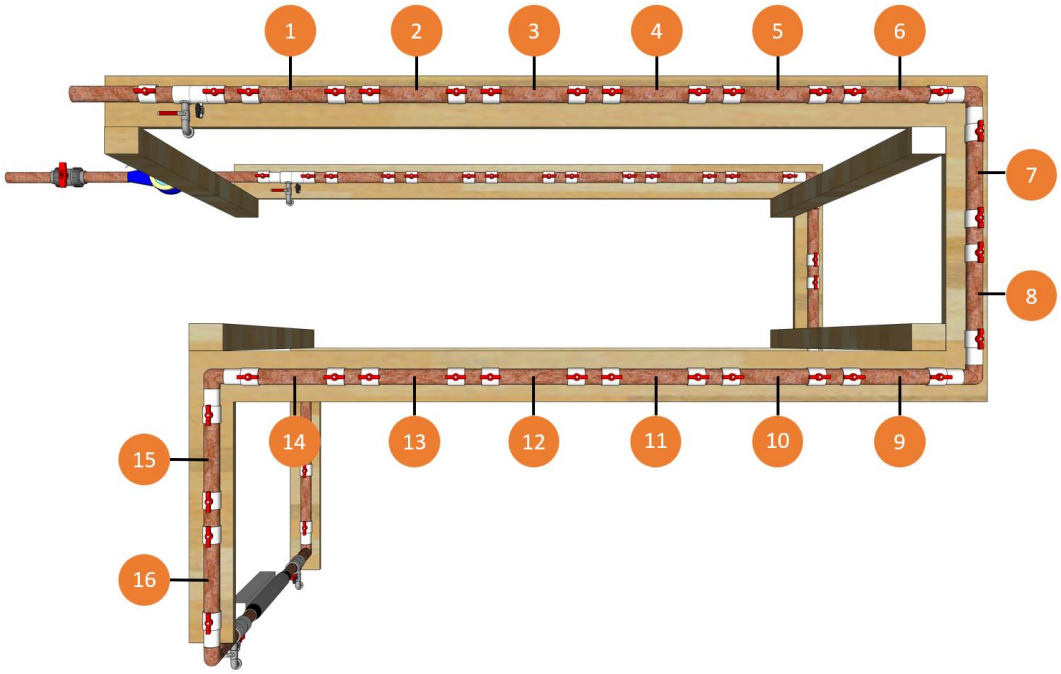
This stage of experiment will be conducted around 210 days (around 7 months) after the start of the first stage of experiment. This is because of the result from the previous study that the stabilization of biofilm will occur around 200-300 days (Lehtola, et al., 2004) and another study consider 210 days for stabilization is sufficient (Zlatanović, et al., 2017).

The sampling method for this experiment is to take out pipe-coupons by closing the valve and unscrew them from the setup. These pipe-coupons will be replaced with the new one. The sampling is planned to be performed monthly and the samples will be duplicate. The order of the pipe-coupons to be taken out is shown in Table 4 and the location order of the pipe-coupons is depicted in Figure 8. In between the end of first stage experiment and the first biofilm sampling, the set-up needs to be flushed. Referring to the previous study of Zlatanović, et al. (2017), the flushing will be in 5 minutes, twice a day and at least 4 times a week.

Table 4. Pipe-coupons sampling order

Sampling month	Pipe-coupons
1	1A, 2A, 15A, 16A, 1B, 2B, 15B, 16B
2	7A, 8A, 9A, 10A, 7B, 8B, 9B, 10B
3	5A, 6A, 11A, 12A, 5B, 6B, 11B, 12B
4	3A, 4A, 13A, 14A, 3B, 4B, 13B, 14B





*Top and bottom are symmetric, therefore, the number code for top and bottom part are the same

Figure 8. Sketch of location for pipe-coupons order

5 | Conclusion

In the future, the usage of gas will be strictly limited in order to cut CO_2 emission, especially in the Netherlands. Renewable energy sources will soon replace the gas in the form of heat pump for heating system in household, both for space heating and hot water system. This new mechanism of heating allows the household to have a hot water with the comfortable temperature (40°C) directly, which the system no longer mix one part of water that is heated up to 60°C and one part of cold water. However, this increases the risk of bacteria proliferation in the household connection because it offers a suitable environment for microorganisms to grow, especially *Legionella*.

Ultrasound disinfection is a new technology that is believed to be able to physically eliminate microorganisms in drinking water network, which no additional chemical is needed. This method appears to be an option to enhance the safety of drinking water in the household due to the possibility of recontamination during the distribution process. Disinfection process under this condition is expected to produce chemically and biologically safe drinking water. This technology is challenged to be effective in removing bacteria, specifically *Legionella*, under lower temperature of hot water supply as the heating energy source will be replaced in the coming years.

A lab-scale experimental set-up was designed and built to be able to investigate the effect of ultrasound disinfection in the household connection. The decisions for the design are based on the factors that are considered to affect the microorganism stability in the household connection. Also, results from previous studies were taken into account in the design. In addition, the devices (pipe-coupons and FCM), included in the design, were chosen due to the advantages in order to produce good quality analysis, therefore, the results would be representative. The designated experimental set-up is expected to generate a rather broad view of effect from ultrasound disinfection, as it provides to monitor different parameters in different distance.

6 | Recommendation

The produced design of the experimental set-up has considered the operation that can be applied for the research, which are recommended in conducting the research using the experimental set-up:

- Adjust the sampling time according to the ability of the FCM due to the analysis time for each sample takes 30 minutes. Moreover, FCM is only able to take one sample at one time, therefore, if the result between FCM and laboratory analysis is not satisfying, the analysis of FCM can be switched to manual analysis, instead of online analysis, so that every sample can be analysed using FCM.
- Operate the system under stable conditions since the biofilm growth is greatly dependent on hydraulic condition in the pipe network.
- A control system that can monitor the quality of water without ultrasound disinfection might be still required to ensure the results of the experiment.

Beside the recommendation for operating the experimental set-up, some recommendations are needed for the future research that might be able to complete the knowledge of ultrasound disinfection in DWDS:

- Intermittent operation of ultrasound disinfection, for instance 5 minutes treatment with 15 minutes interval between one treatment to another one.
- Investigating the disinfection process in different pipe material, such as PVC, as the application of different material of pipe increases.
- Comparison between application of ultrasound disinfection in the new pipe networks and old pipe works. In real practice, the device is possible to be installed in an old pipe system since replacing the pipe network is costly. Therefore, it is interesting to observe the capability of ultrasound disinfection in eliminating microorganisms in an old pipe system.
- Scale up the set-up from the lab-scale experimental set-up to pilot scale set-up to have a closer outlook of the ultrasound disinfection in domestic drinking water system.

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