MASTER THESIS

Dynamics of Water Quality and Biofilm Formation in a Pilot Drinking Water Distribution System with and without Residual Disinfectants

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Dynamics of Water Quality and Biofilm Formation in a Pilot Drinking Water Distribution System with and without Residual Disinfectants

By

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PREFACE

This thesis is focusing on the dynamic of water quality and biofilm formation within the drinking water distribution system, especially considering the effects of residual disinfectants. Since drinking water in the Netherlands is treated without disinfectants, the post-addition of free chlorine and monochloramine and the comparisons among these three cases are intriguing. Hence, in collaboration with the Dutch water company Oasen, a new pilot drinking water distribution system was built in Kamerik. Computer models were also implemented in this study for a comprehensive understanding.

While, when I started this research from January, it was a slightly different plan, with a focus on analyzing the system at the genetic level. After a few months of preparation, the pilot system was all set up and ready to start. However, all of a sudden, the outbreak of COVID-19 made everything difficult. The pause of experiments made me think a lot and found it quite interesting to apply computer models in this study. That is how I have the structure of my thesis. The modelling part was like a new chapter to me. I started from the very beginning and gradually found my way.

This research was not easy, and it has been a long journey for me. Frankly, I cannot achieve what I have now without the help of my committee. Gertjan, Hans, Gang and Lihua, all of them were always supporting me, and offered me lots of inspiring thoughts. I truly appreciate their efforts and thank them for their excellent guidance during this journey. I want to thank Oasen as well, providing me with such an opportunity to conduct my study. Besides, I want to thank my parents for always supporting me no matter what and thank all of my friends for accompanying me through the most difficult time.

Lastly, thank you for reading my thesis, and I hope you will find something interesting.

时浩然

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ABSTRACT

Drinking water safety is of great concern all around the world. Two strategies can be pursued to maintain biological stable drinking water during distribution in the network: (i) without a residual disinfectant, in which bacterial growth is controlled by very low biodegradable nutrients in the water by extensive pre-treatment and a well-maintained network and (ii) with a residual disinfectant in the water during distribution. Independent of the strategy, maintaining water quality during the transportation processes has always been a challenge, and there is a need for a better understanding of the impact of biological stability strategies using systematic studies. In this study, the impact of no residual disinfectant, residual chlorine and residual monochloramine on chemical and microbial water quality were investigated. The research questions were:

1. How to describe concentration of free chlorine and monochloramine in the pilot DWDS?
2. What is the impact of chlorine and monochloramine on chemical water quality in the pilot DWDS, particularly disinfection by-products (DBPs) formation?
3. What is the impact of chlorine and monochloramine on microbial water quality and biofilm formation in the pilot DWDS?

Both modelling and experimental studies were performed involving three identical, pilot-scale distribution systems of the same material (PVC) operated at the same time with identical conditions (e.g., temperature, flow rates, chlorine concentration). Bulk water, suspended solids and biofilm samples were periodically sampled and analysed for various parameters (e.g., residual disinfectant concentration, elements, ATP, cell count, DBPs). Numerical modelling was applied to describe the decay of chlorine, the formation of THMs and formation of biofilm.

<u>Description of the concentration of free chlorine and monochloramine:</u>

The stability of residual disinfectants was firstly investigated by both experiments and modelling. The prepared stock solutions of high concentration free chlorine and monochloramine in ultrapure water have been proved to be stable for a week. Thereafter, studies with the pilot DWDS were done fed with drinking water from the Kamerik water treatment plant. Once chlorine added into the feed water, the decay happened relatively fast at the very beginning. A parallel second order model was hence implemented and can adequately describe the decay behaviour of free chlorine and monochloramine. When chlorine was dosed into the system, a large chlorine demand was noticed in the loops with free chlorine, which is mainly due to its reaction with ammonia and organic matter present in the feed water.

Impact on chemical water quality:

THMs formation (i.e., TCM, DBCM, BDCM) was only observed in free chlorine applied loops, with an average of 4 μg/L THMs at the end. The THMs predictive model gave a linear relationship between free chlorine demand and THMs concentration, with the simulated value in the same order of magnitude as the measured THMs concentration. As for the monochloramine applied loops, no THMs were detected, and it is most direct impact on physiochemical water quality was the increase of ammonia.

Impact on microbial water quality:

Compared to the measurements in the loop without disinfection, a residual free chlorine caused a clear reduction of both bulk water bacteria concentration (37%, judged by flow cytometric intact cell counts) and suspended solids-related bacteria concentrations (25%, measured by ATP). While monochloramine was less effective in reducing the bacteria concentrations, but it strongly delayed and reduced sediment and biofilm formation in the network pipes at the very early stage. Residual free chlorine also caused an 8 times higher biofilm growth rate compared to monochloramine during the observation period. The numerical model predicted a stable biofilm thickness after about 300 days network operation of about 3 μm for no disinfectant, 2 μm for free chlorine, and 7 μm for monochloramine, which corresponded to the trend for thickness and growth rates as observed in this initial three weeks in the pilot networks under the different disinfection regimes. Data obtained from pipes in drinking water distribution systems in the field suggested that the model has predictive value, but this needs to be further developed and validated.

Conclusions:

The combination of experimental research in the pilot distribution network and modelling of disinfectant decay, THMs formation and biofilm formation was found to be suitable for investigating the dynamics of chemical and microbial water quality and biofilm formation under different disinfection regimes. Even though this study was able to investigate only the initial period after start-up of the pilot networks, differences were observed between the disinfection regimes: chlorine reduced concentrations of bacteria in bulk water and suspended solids, delayed biofilm formation and produced THMs. Monochloramine also reduced bacterial concentrations, but to a lesser extent, and increased the ammonia concentration.

It is anticipated that studies with pilot distribution networks will lead to better control of drinking water safety, in terms of biological stability and DBPs formation. Meanwhile, computer models applied in this study fitted disinfectant decay kinetics, and appeared to correspond with the experimental data for THMs and biofilm formation, and hence provided more insights in terms of predicting the behaviour of the system in the future, while their accuracy can also be calibrated and verified by the future experimental data.

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LIST OF ABBREVIATIONS

AOB	Ammonia-oxidizing bacteria
AOC	Assimilable organic carbon
ATP	Adenosine triphosphate
BDCM	Bromodichloromethane
BF	Pipe wall biofilm
BW	Bulk water
CLSM	Confocal laser scanning microscopy
DBCM	Dibromochloromethane
DBPs	Disinfection by-products
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DWDSs	Drinking water distribution systems
DWTPs	Drinking water treatment plants
EC	Electrical conductivity
EPS	Extracellular polymeric matrix
ESEM	Environmental scanning electron microscopy
FCA	Free chlorine added
HAAs	Haloacetic acids
ICC	Intact cell counts
MCA	Monochloramine added
N-DBPs	Nitrogenous disinfection by-products
NOB	Nitrite-oxidizing bacteria
NOM	Natural organic matter
NRD	No residual disinfectant added
OCT	Optical coherence tomography
SEM	Scanning electron microscopy
SS	Suspended solids
T	Temperature
TBM	Tribromomethane
TCM	Trichloromethane
THMs	Trihalomethanes
TOC	Total organic carbon

Chapter 1. Introduction

1.1 Background

Treated water leaving from drinking water treatment plants (DWTPs) is safe and stable. While during the distribution, various physicochemical and biological processes (e.g., the accumulation/ resuspension of loose deposits and the attachment/detachment of biofilm) might occur in drinking water distribution systems (DWDSs) (Isabel et al., 2019; Fish et al., 2016). This might cause hygienic and aesthetic issues, such as the increase of taste and odour in tap water, proliferation of opportunistic pathogens (Feazel et al., 2009; Pryor et al., 2004) and bio-corrosion of pipes (Beech & Sunner, 2004) in DWDSs. It should be addressed that most issues in DWDSs are related to microbial regrowth, which has received extensive attention worldwide. Nonetheless, the microbial ecology and bacteriology in real DWDSs are rather complicated, and have not been fully understood (Berry et al., 2006).

As emerging environmental pollutants, the presence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in drinking water has received many attention (Schwartz et al., 2003). Drinking water can be viewed as a reservoir for the ARB and ARGs, and also a crucial medium for their spread between environment and human beings. Increasing evidence has shown that water chlorination can actually contribute to the enrichment of certain type ARGs ($ampC$, $aphA2$, $blaTEM{-}1$,$tetA$,$tetG$, $ermA$ and $ermB$), and water transportation is effective to decrease the levels of ARGs to a lower content (Shi et al., 2013; Xi et al., 2009; Xu et al., 2016). Besides, chlorination, especially chloramination, can lead to reversible shifts of microbial communities (Hwang et al., 2012), and it has also been found a strong correlation between bacterial community shift and antibiotic resistome alteration (Jia et al., 2015).

Among the four phases identified in DWDSs, namely: bulk water (BW), suspended solids (SS), pipe wall biofilm (BF) and loose deposits (LS) (Liu et al., 2013), it is traditionally believed that more than 98% of the total bacteria from DWDSs is found in the pipe wall biofilm (Mackay et al., 1999). Indicated as the greatest contributors to biomass in DWDSs, biofilms formed along the inner pipe walls and attached with each other, embedding in the extracellular polymeric matrix (EPS) (Fish et al., 2016). Biofilms can act as the reservoir of pathogens, such as *Legionella*, *Cryptosporidium* and *Aeromonas* (Walker et al., 1995). By forming biofilms, most of microbes present in the DWDSs are able to survive (Flemming et al., 2002). The existence of biofilms poses a potential risk to the drinking water safety. Hence, researchers are commonly focus on the study of biofilm bacteria and bulk water bacteria.

Only few studies have assessed different phases in DWDSs to compare their contribution in the overall microbial regrowth (Liu et al., 2014), especially limited researches on loose deposits. But few reported studies suggested that they can act as reservoirs for bacteria as well as organic carbon (Gauthier et al., 1999; Zacheus et al., 2001). When sudden hydraulic change happens, the re-suspended loose deposits will be counted as suspended solids, and might cause water quality deterioration (Gauthier et al., 2001; Lehtola et al., 2004). However, pilot systems are mostly used to study pipe wall biofilm. The time length requirement and complex factor dependency to form LS make it of greater difficulty to sample them in the pilot DWDSs. Besides, to sample all phases together in field DWDSs is also of great difficulty.

Generally, there are two main approaches which are commonly used in practice to control microbial growth in DWDSs: (1) producing biologically stable water by limiting growth supporting nutrients and (2) distributing water with disinfectant residuals. For instance, few countries like the Netherlands supply safe drinking water to customers by producing biological stable water. Instead of using residual disinfectants, the drinking water is distributed with extremely low assimilable organic carbon (AOC) concentration (Hammes et al., 2010; Wielen & Kooij, 2010). In this case, a systematic approach was established and applied in the Netherlands, including applying the best sources water, preventing entrance of microbes, using bio-stable materials and so on (Smeets et al., 2009). The major concern brought by the residual disinfectants is that the side effects might outweigh its benefits.

In contrast, in most countries, a measurable residual disinfectant is commonly remained in the distribution system to inhibit microbial regrowth, control biofilms and prevent recontamination. The precise mechanism behind it is still uncertain. It is normally believed that chlorine compounds are able to inactive bacteria to a lower level and restrain the growth of algal (Hrudey, 2009). But the inactivated bacteria might regrow when the living condition is optimal (Zhang et al., 2015). The regrowth of bacteria and biofilm accumulation still happens (Chu et al., 2003), plus the main drawback of forming disinfection by-products (DBPs). Besides, there are also many factors have shown their influences on the disinfection effects, especially the type and dosage of disinfectants.

1.2 Research Objectives

Both strategies to pursue distribution of biological stable water (applying residual disinfectants or producing and distributing water limiting in growth promoting nutrients) is normally sufficient to maintain a good water quality. They have shown enough power to inhibit the microbial growth in bulk water. Especially, free chlorine is globally applied as a residual disinfectant due to its obvious disinfection effects and low prices. While considering its major drawback of forming DBPs, the switch from free chlorine to monochloramine is becoming popular among many places. Although the drinking water quality meets the standards, biofilms are still universally detected in DWDSs. Typically, it is indicated that residual disinfectants are not quite effective to penetrate into biofilms and limit regrowth. Furthermore, the decay of the disinfectants, which is universal in practice, dramatically lowered the disinfection effectiveness. This might contribute to the regrowth or even biofilm formation in practical DWDSs. While the secrets behind the resistance of biofilm microorganisms to disinfection remain unclear. Moreover, suspended solids, indicated to act as an indirect indicator for the assessment of the biofilm detachment and loose deposits resuspension, are overlooked generally (Liu et al., 2016).

Besides, distribution system modelling aims to help the designers, operators and managers of the water distribution systems deliver safe and stable drinking water to customers. It is mainly used to predict the behaviours of the system, thus provide more insights in solving various problems. For example, computer models can be used for hydraulic modelling, predicting the water flow and pressure; or water quality modelling, simulating the kinetics of residual disinfectants, DBPs, and so on. Meanwhile, the numerous biological reactions can also be simulated by computer models. The investigation in biofilm modelling has been gradually enhanced with time.

To systematically understand the dynamics of the water quality in DWDSs, a pilot DWDS, which was developed without and with disinfectants (free chlorine & monochloramine), was designed. The variations of three phases in the pilot DWDS, including bulk water, suspended solids, and biofilm, were investigated in this study. Besides, mathematical models were ubiquitously implemented to simulate the microbial regrowth in bulk water as well as pipe wall biofilm. The disinfectants decay and corresponding DBPs formation was also simulated in this study to further evaluate the water quality. The computer models applied and calibrated in this study were expected to act as a powerful tool for managers to optimize the design, operation and the ultimate water quality in DWDSs.

Overall research objective

How to describe the development of biofilm over time and concentration of disinfectants and disinfectant by-products in (a pilot) DWDS using modelling and monitoring?

Detailed research questions

1. How to describe concentration of free chlorine and monochloramine in the pilot DWDS?
    - *How fast will chlorine decay in ultrapure water and bulk water?*
    - *How to simulate the chlorine decay behaviour by computer model?*
2. What is the impact of chlorine and monochloramine on chemical water quality in the pilot DWDS?

  - *What types and concentrations of DBPs (mainly THMs) will be formed after applying residual disinfectant? And how to predict their formation using a DBP formation model?*
  - *What will be the impacts of residual disinfectants on selected conventional water quality parameters?*

3. What is the impact of chlorine and monochloramine on microbial water quality and biofilm formation in the pilot DWDS?
   - *How to simulate the formation of drinking water biofilm? What are the key influencing parameters under different conditions?*
   - *What will be the differences of biofilm growth rate and thickness under three conditions of no residual disinfectant, free chlorine and monochloramine addition?*

1.3 Thesis outline

The thesis is structured as it follows:

Chapter 2: Literature review. As presented in chapter 2, a literature review was firstly conducted for the understanding of the research objectives, including the basic concepts such as physical, chemical, biological and water quality integrity in drinking water distribution systems. Extra attentions were paid on chemical disinfection and biofilms. The development of researches on chlorine decay, DBPs formation and biofilms were illustrated, as well as the relationship among them. Moreover, the investigation into computer modelling evolution also offered more insights for this study. The followed experimental design and model establishment were based on the literature.

Chapter 3: Methodology. In this study, a pilot system was initially built to sample three phases of DWDSs, specifically, bulk water phase, suspended solid phase and biofilm phase, followed by the measurements of physiochemical and microbiological parameters. In addition to experimental part, computer models also played an important role in predicting chlorine decay and DBPs formation, as well as simulating the biofilm formation.

Chapter 4: Results & Discussion. This chapter aims to answer all the research questions. Starting with the stability of stock solution, then implementing models to fit the chlorine decay bottle test results and obtain the decay constants. The real situation of chlorine dosing into the pilot system was analysed, considering the pump regime and chlorine demand. Besides, the analyzation of various water quality parameters was conducted, among different sampling time and locations. The formation of THMs was detected in the pilot system, as well as modelled by theoretical equations. As for the microbiological characterization of the pilot system, the bulk water phase, suspended solids phase and biofilm phase were separately described. While a biofilm model was built on a longer time scale, providing more insights for the following studies.

Chapter 5: Conclusions & Recommendations. The main finding of this thesis is summarized in this chapter, and presented general conclusions related to the research objectives of this study. Besides, a few recommendations were also proposed for the further investigation.

Chapter 2. Literature Review

The ultimate purpose of water treatment and supply is to provide safe drinking water at the customers' tap. But it is commonly detected a lower water quality after distribution (Liu et al., 2013), indicating water deterioration happened within the DWDSs. On the contrary, DWDSs should not only distribute drinking water, but also act as a barrier to the contamination and microbial regrowth. According to the World Health Organization drinking water guideline, it must be microbiologically safe and ideally biologically stable water to enter the distribution system (WHO, 2017). However, the term of 'biological stability' is rather less defined, regarding to its meaning and how to achieve. It normally implies no change of microbial community during distributing. Except few countries like the Netherlands, Switzerland, Austria and Germany, they producing the bio-stable water by the limitation of microbial regrowth related nutrients (Hammes et al., 2010). For most of the other countries, a residual disinfectant is applied to enforce the bio-stability by inhibiting microbial regrowth.

2.1 Chemical Disinfection

There has been many researches connecting the distribution system conditions with the microbial activity (Lehtola et al., 2004; Liu et al., 2017; Simões et al., 2010). Mostly reported chemical changes include chlorine decay and DBPs formation (He et al., 2017; Hwang et al., 2012). While the complex relationship between chemical change and microbial change has mainly be indicated by the impacts of residual disinfectant on nitrification processes (Liu et al., 2019; Regan et al., 2003). As for the stability of disinfectants, both chemical and microbial changes share the similar influencing factors, including the water quality and operation condition. While the changes of microbial activities could further lead to chemical changes of disinfectant stability. It is essential to comprehensively understand the overall changes of water quality that would happen from DWTP to the taps of customers.

Free chlorine and monochloramine are the two types of secondary disinfectants that have been most widely used in DWDSs to control the microbial growth, with significantly influence on microbial structure (Berry et al., 2006). Especially, free chlorine is more popular due to its relatively low price and easy availability. It has also been found that the free chlorine residual is more effective to provide the final barrier from pathogen intrusion than chloramine in DWDSs (Propato & Uber, 2004). To achieve a comparable disinfection efficiency, applying monochloramine requires longer contact time and higher concentration. Nevertheless, the major concern of chlorination is the formation of DBPs (Richardson, 2009; Sharma et al., 2014).

As an emerging concern in the drinking water safety, DBPs has received many attentions. Different sorts of DBPs are formed when disinfectants and the natural organic matter (NOM) as precursors (e.g., humic substances, carboxylic acids, and carbohydrates) react unintentionally (Bond et al., 2011). The DBPs are widely believed to be related to the increase of health risks, since it was indicated that some DBPs are mutagens, carcinogens, teratogens, or developmental toxicants (McGeehin et al., 1993; Zierler et al., 1988). According to US Environmental Protection Agency (2006), trihalomethanes (THMs) and haloacetic acids (HAAs) are the two regulated classes of hundreds DBPs (Karanfil et al., 2008). It was reported that the formation of THMs is affected by the chlorine dosage and residence time, the types and concentrations of NOM, as well as pH, water temperature, and so on (Amy et al., 1987). In general, higher levels of the above parameters lead to higher concentrations of THMs (Sadiq & Rodriguez, 2004).

As chloramination forms less DBPs and has a lower taste threshold, the application of monochloramine is increasing worldwide (Hua & Reckhow, 2007; Krasner et al., 2013). Remarkably, the shift from chlorination to chloramination has been proved to be efficient to reduce the concentrations of regulated THMs and HAAs (Seidel et al., 2005). However, chloramination tends to form certain nitrogenous disinfection by-products (N-DBPs) of high genotoxicity and cytotoxicity (Bond et al., 2011; Mitch et al., 2003).

2.1.1 Effects of secondary disinfection

The quality of distributed drinking water can be influenced by various factors, like the presence of residual chlorine, the material of pipes, hydraulic conditions, as well as particles and biofilms (Liu et al., 2016; Masters et al., 2015; Wang et al., 2014). Among all the factors, it is indicated that the presence of residual disinfectant has the major impacts on microbiological water quality (Gillespie et al., 2014). Chlorine and chloramine can be used to limit microbial regrowth, but it also has the potential to select certain species with resistance to oxidants (Proctor & Hammes, 2015). During disinfection, some of the bacteria will transform into a viable, but non-culturable (VBNC) state. These VBNC bacteria is capable of surviving in the harsh environment and regrowing when the conditions are optimal (Mustapha et al., 2015; Zhang et al., 2015).

Besides, the type and concentration of disinfectants can impact the microbial growth, and also the relationship between AOC and bacterial growth to a large extent (Zhang et al., 2016). But the mechanisms behind it remain unknown. It is commonly believed that the added chlorine is capable of destroying the bacteria membranes and therefore inhibiting the activities of certain important enzymes. As a strong oxidizing agent, chlorine can break up organic matter. In the chemical disinfection process, the reaction between chlorine and organic matter can also increase AOC concentration (Fass et al., 2003; Liu et al., 2002; Wilson et al., 2017). Serving as the carbon source to microorganisms, AOC can subsequently affect the bio-stability of drinking water.

When using monochloramine as the secondary disinfectant, ammonia is introduced into drinking water by the formation and decay of chloramine. At is known that the nitrifying biofilm is part of the distribution system nitrification, it can bring some undesirable issues. For example, the growth of ammonia-oxidizing bacteria (AOB) will be promoted, and produce nitrites as well as other soluble organic compounds (Bruce et al., 1994). It may further sustain nitrite-oxidizing bacteria (NOB) growing (Regan et al., 2003). Overall, it will increase the concentration of nitrite and nitrate, accelerate monochloramine decay, decrease pH and dissolved oxygen, and eventually lower water quality (Skadsen, 1993; Zhang et al., 2010). The figure below shows detailed the effects of nitrification processes within chloraminated DWDS, considering different disinfectant strategy and hydraulic conditions.

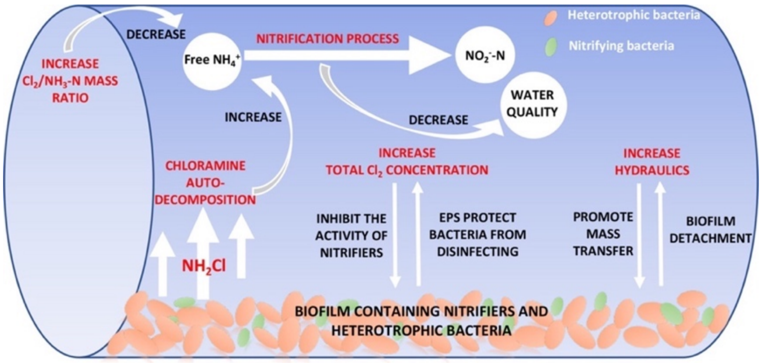


Figure 2.1 Chloramine applied DWDS under different conditions (Shi et al., 2020)

On the other hand, the selection caused by chemical disinfectants also happens within drinking water biofilms, but have yet been fully understand (LeChevallier et al., 1988a; Zhang & DiGiano, 2002). In respect of biofilm bacteria inactivation, it is shown that free chlorine is impeded in biofilm penetration (De Beer et al., 1994). The increase of free chlorine concentration will not necessarily lead to the increase of disinfection efficiency in biofilm. While monochloramine performs in penetrating into biofilms, due to its decreased reactivity and increased persistence (LeChevallier et al., 1988b). There is one hypothesis indicating that the chlorine is neutralized by organic matters from water and biofilm, before diffusing into the biofilm (Chen & Stewart, 1996)

Applying monochloramine as residual disinfectant is increasing globally, concerning the stricter drinking water regulations. The application of free chlorine could contribute to an excessive formation of DBPs (Vikesland et al., 2001), while monochloramine is normally considered to be less reactive in bulk water than free chlorine. Although monochloramine performs better with respect to the new regulations, there are still certain unavoidable shortcomings. For example, the higher CTs (concentration times contact time) required for the inactivation of pathogen (Chiao et al., 2014). The decomposition and releasement of ammonia into bulk water might lead to the instability of system and nitrification (Wilczak et al., 1996).

Once nitrification happens, the decrease of monochloramine is very fast. Therefore the inactivation and regrowth of AOB must be seriously considered to maintain the water quality (Odell et al., 1996). There have been some reports focusing on the survival of AOB under monochloramine applied systems. A study from California (Wolfe et al., 1990) showed that the resistance of nitrifiers were 13 times higher to monochloramine than to free chlorine. Another study in Finland (Cunliffe, 1991) compared the nitrification occurrence and the number of nitrifying bacteria in different DWDSs. It was found that the amount and oxidation potentials of AOB and NOB were higher in the chloramine applied systems, comparing with the application of free chlorine. The pipe wall detachment with high concentration of nitrifiers was found to contribute to an increase in turbidity. Moreover, there has been found a positive correlation between nitrifying bacteria and heterotrophic bacteria as well as turbidity. Hence, the measurements of pH, DO, ammonia, nitrite, nitrate, chloramines dosage and residual concentrations can be used to indicate the activity of nitrifying bacteria within DWDSs (Odell et al., 1996; Wilczak et al., 1996)

2.1.2 *Disinfectant residual stability*

The concentrations of chlorine and chloramine always decrease with time. Maintaining an appropriate concentration of residual disinfectant during the distribution processes, requires deeper understanding of the decay behaviours. When disinfectant is added into the system, it initially reacts with NOM and other inorganic components in the bulk water. This is also called 'bulk decay', compositing the major part of chlorine decay. As for the rest, disinfectants can also react with the pipe and biofilm, called 'wall decay' (Hallam et al., 2002). It was demonstrated that chlorine decay is highly dependent on the water quality, the pipe material that has direct contact with the water flow. Besides, there are also lots of other factors affecting the disinfectant stability by influencing the microbial activity. As shown in figure 2.2, all the parameters have impacts on disinfectant residual stability, with only a few been introduced in detail here.

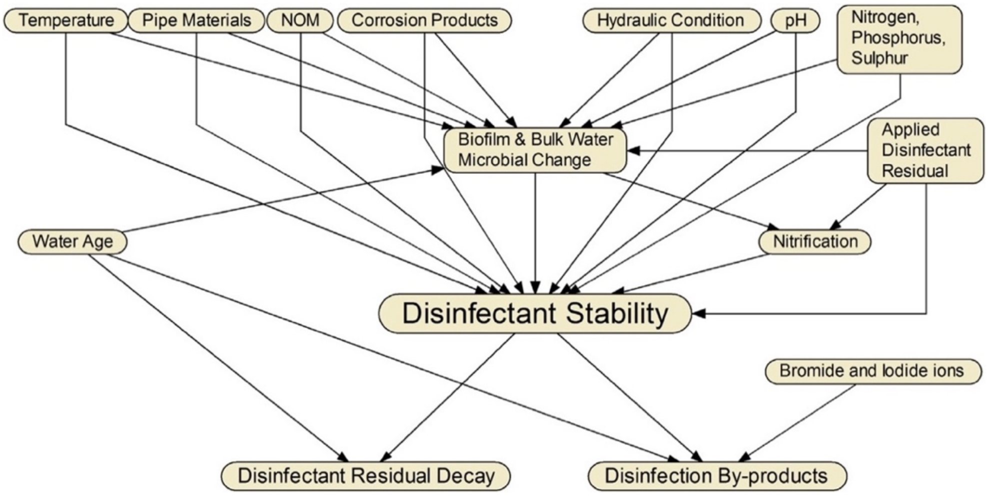


Figure 2.2 Factors influencing the stability of residual disinfectants (Li et al., 2019)

a. Temperature

It is noticeable that the disinfectant decay normally increases with increased temperature (Monteiro et al., 2017). Part of the reason behind it is probably the influences of temperature on microbial activities. Meanwhile, even with no microorganism existing, chlorine and chloramine also show a temperature-dependent self-decay reaction (Ndiongue et al., 2005). Besides, an increase in the temperature is also able to accelerate the formation of DBPs and promote the degradation of some DBPs (Abusallout et al., 2017; Ye et al., 2009; X. Zhang et al., 2013).

b. Pipe materials

The rate of wall decay varies largely with the materials of pipe (Hallam et al., 2002; Kiéné et al., 1998). Comparing with plastic pipes, metal pipes show a higher potential of wall decay, especially of the long-serving metal pipes (Clark, 2011). The selection and regrowth of chlorine-resistant bacteria was also found to be more obvious, when copper pipe was applied instead of polyethylene (PE) pipe (Lehtola et al., 2005). However, PE pipes were related with forming higher concentrations of THMs, than ductile iron pipes and stainless-steel pipes (Zhang et al., 2017). As for the new pipes, it is demonstrated that flushing of the new systems before in use would reduce the chlorine loss (Al-Jasser, 2007).

c. Residual disinfectants

The stability of disinfectant residual varies with its type. Chloramine performs better stability in DWDSs, since it would hydrolysis slower than free chlorine in liquid condition (Rice & Gomez-Taylor, 1986). However, it will decay much faster when nitrification happens (Sathasivan et al., 2008). The dominant species of DBPs are also highly depended on the types of disinfectants (Joo & Mitch, 2007). Take N-DBPs as an example, *N*-nitrosamines and haloacetamides are the dominant species in the chloramine applied system, while haloacetonitriles are more commonly found in chlorinated system (Liew et al., 2016).

d. NOM and nutrients

NOM and other available nutrients can impact the disinfectant stability directly and indirectly. There has been found a positive relationship between the availability of AOC and the microbial growth in both bulk water phase and biofilm phase (Chu & Lu, 2004; Fang et al., 2010; Keinänen et al., 2002). On the contrary, under poor nutrient conditions, an enhanced biofilm formation has also been detected (Elhariry et al., 2012). For example, with a low AOC level, bacteria grow faster in biofilm than in bulk water (Graham A Gagnon et al., 2000; Tsai et al., 2004). Hence, a contradictory result can be drawn that the growth rate of heterotrophic biofilm increases with a decrease of available carbon source (Ellis et al., 1999).

2.1.3 *Disinfectant decay mechanisms and modelling*

By reacting with organic and inorganic matters present in water, the concentrations of free chlorine and chloramine in water are reduced over time. Free chlorine is able to break the double bonds between the carbon elements of a long-chain NOM molecule. Hence, the complexity of DBPs is inversely proportional to the number of organic fragments. In other words, the more fragments, the simpler the structure of DBPs. As for monochloramine, it is more stable than free chlorine, but still decays in water. Besides reacting with NOM (Duirk et al., 2005; Vikesland et al., 1998), it also undergoes an auto-decomposition (Duirk et al., 2002; Valentine & Jafvert, 1988). The auto-decomposition begins with a hydrolysis reaction that produces free chlorine and ammonia. The monochloramine reacts with the released free chlorine and forms dichloramine, but it will then rapidly decompose into chlorides and ammonia.

Besides, chlorine also reacts with other inorganic compound, for example, metals (Fe, Mn), nitrites and sulphates. Normally, iron exists in water in the reduced form, Fe^{2+}, which will react with both free chlorine and monochloramine as shown in Eq.1 and 2 (Vikesland & Valentine, 2002). It was also found that the reaction between monochloramine and soluble ferrous iron is first order, and pH dependent. To be specific, the increased pH will lead to a higher reaction rate. Another field investigation indicated that such reactions are the major reason of free chlorine decay on the metal pipe surface.

$$2Fe^{2+} + HOCl + H^+ \rightarrow 2Fe^{3+} + Cl^- + H_2O \qquad \text{(Eq.1)}$$

$$2Fe^{2+} + NH_2Cl + H^+ \rightarrow 2Fe^{3+} + Cl^- + NH_3 \qquad \text{(Eq.2)}$$

Meanwhile, both free chlorine and monochloramine can react with nitrites and form nitrates, as Eq.3 and 4 showed (Vikesland et al., 2001). The reaction between nitrite and chloramines is more important, considering nitrite as an intermediate product of nitrification. Besides, the newly formed ammonia also are nutrients for nitrifying bacteria. and the free ammonia produced by this reaction is used as a food source by nitrifying bacteria.

$$NO_2^- + HOCl + H_2O \rightarrow NO_3^- + Cl^- + H_3O^+ \qquad \text{(Eq.3)}$$

$$NO_2^- + NH_2Cl + H_2O \rightarrow NO_3^- + Cl^- + NH_4^+ \qquad \text{(Eq.4)}$$

a. <u>Bulk decay</u>

A first-order kinetic model (Eq.5) has been proved sufficient in predicting the decay behaviour of chlorine in bulk water phase, related with the initial chlorine concentration. However, this approach is normally site specific, and cannot be used to explain the dissimilitude between different waters (Powell et al., 2000). There have been some studies found that the water quality parameters would highly impact the kinetic constants for decay reactions in the bulk water, like temperature, organic content and so on (Hua et al., 1999). A second-order kinetic model has been investigated by Powell et al. (2000), shown in Eq.6, which can be considered as first order with respect to chlorine concentration (C) as well as the concentration of other reacting compounds (R).

$$\left(\frac{dC}{dt}\right)_{bulk} = -K_b \cdot C \qquad \text{(Eq.5)}$$

$$\left(\frac{dC}{dt}\right)_{bulk} = -K_b \cdot C \cdot R \qquad \text{(Eq.6)}$$

The decay of monochloramine happens no matter humic materials exist or not. The kinetics behind its dissipation have also been studied, and adapting the existing free chlorine decay model to simulating the decay of chloramine (Maier et al. 2000). The first-order model was firstly applied as shown in Eq.5. K_b was considered as a constant, which expressed the single decay coefficient under different conditions with different values. While another model developed by Duirk et al. (2002) predicted monochloramine decay without the presence of NOM, but mainly the auto-decomposition of monochloramine.

b. Wall decay

Chemical and biological characteristics of different pipe materials vary significantly, so do the potential reactions. Previous studies have proved a lower chlorine demand within synthetic material systems, such as PVC and PE (Hallam et al., 2002). While metallic pipes show a higher chlorine demand, due to the reactions between chlorine and elemental metal and/or corrosion products (Frateur et al., 1999). The rate of reactions, the limitations of mass transfer, and the ratio of wetted surface to volume, all these factors have major influence on wall decay and must be considered (Hallam et al., 2002; Vikesland et al., 2001). Hence, Eq.7 showed the expression for the wall decay rate.

$$\left(\frac{dC}{dt}\right)_{wall} = -\frac{A}{V} \cdot K_W \cdot C_W \qquad \text{(Eq.7)}$$

It is difficult to measure the concentration of chlorine at the pipe wall (C_W). Instead, the chlorine concentration within bulk water phase can be used (C). Depending on flow regime of the pipe, K_W should be adjustable to suit the mass transfer limitations. As developed by Rossman et al. (1994), a film resistance model was shown in Eq.8. It indicated that the mass transfer of chlorine to the pipe wall is proportional to the difference in chlorine concentration between the bulk water phase and the pipe wall. K_F, as a mass transfer coefficient, is depended on the flow conditions. If it is assumed that chlorine reacts as it reaches the pipe wall and there is no accumulation, the rate of mass transfer of the disinfectant would be equal to the rate of chlorine decay at the pipe wall as shown in Eq.9. Assuming that the chlorine does not accumulate in the pipe wall, all the reactions happen within the bulk water. Then the mass transfer rate of the disinfectant is equal to the rate of its decay at the pipe wall. By solving Eq.8 and 9, the new obtained model is shown in Eq.10.

$$\left(\frac{dC}{dt}\right)_{\text{mass transfer}} = -\frac{4}{D_p} \cdot K_F \cdot (C - C_W) \quad \text{(Eq.8)}$$

$$\left(\frac{dC}{dt}\right)_{\text{wall}} = \left(\frac{dC}{dt}\right)_{\text{mass transfer}} \quad \text{(Eq.9)}$$

$$K_W \cdot C_W = K_F \cdot (C - C_W) \quad \text{(Eq.10)}$$

Where K_F = coefficient of mass transfer

K_W = coefficient of wall reaction rate

K_{tW} = coefficient of overall wall decay

D_p = pipe diameter

C = chlorine concentration

c. Temperature effects

The influence of temperature variations can be described using the van't Hoff – Arrhenius equation, as shown in Eq.11. Given the equation, Eq.12 can be further obtained by considering the rate constant at operation temperature and set as a base temperature.

$$K_T = A \cdot \exp\left(-\frac{E_0}{RT}\right) \quad \text{(Eq.11)}$$

$$K_T = K_{20} \cdot \theta^{(T-20)} \quad \text{(Eq.12)}$$

Where E_0 = activation energy

R = ideal gas law constant

K_T = dissipation constant at temperature T

θ = temperature correction factor

A = constant

2.1.4 DBPs occurrence and predictive models

However, the reactions between chlorine and NOM as well as other inorganic components lead to the production of DBPs. To predict the formation of DBPs in drinking water, numerous models have been developed. Some studied on the correlation of DBPs and NOM in raw water (White et al., 2003), and some investigated different water qualities and operational parameters with different species of DBPs (Gallard & von Gunten, 2002; Gang et al., 2003). However, most of these models applied empirical relationships, with only a few studies based on kinetic relationships (Adin et al., 1991; Clark & Sivaganesan, 1998b; Clark et al., 2001; Golfinopoulos & Arhonditsis, 2002).

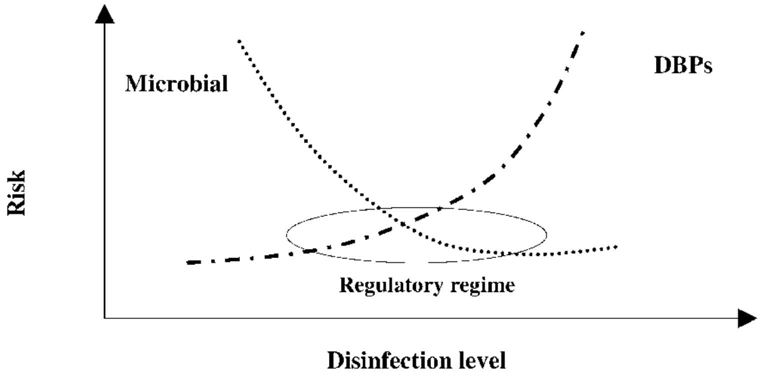


Figure 2.3 Trade-off between disinfection level and risks (Sadiq & Rodriguez, 2004)

The DBP models are being developed for multiply purposes. Not only serving as an alternative of field monitoring, it can also be used to study the kinetics and mechanisms behind the DBPs formation, and thus control it from happening. Predictive models of DBPs formation involves the establishment of empirical and/or mechanical relationships between water quality and other operational parameters. The current models are based primarily on experimental data obtained from field and/or laboratory studies.

Various sampling points are needed for sample collection on site, including raw water, finished water (with residual disinfectants), and distributed water (within the distribution systems). While laboratory-scale studies are usually based on batch sampling of raw water and treated water. It has been proven to be more reliable than field studies in developing empirical models, because it is easier to control conditions in laboratory than on site. But the main drawback of laboratory studies is that the effects of distribution system on residual disinfectant concentrations are overlooked.

In contrast, the field-scale experiments allow human exposure. But there are certain parameters that would influence DBPs formation being very difficult to estimate by field research. For example, to estimate the real contact time of residual disinfectants within distribution system required deep research on tracers as well as hydraulic simulation models, but it is time-consuming, and the accuracy cannot be assured. Another major drawback of field-scale model is that they are generally very site-specific (Sadiq & Rodriguez, 2004).

2.2 Biofilms in DWDSs

Biofilms are universally detected, harboured the majority of microorganisms in the DWDSs. The presence and activity of bacteria within in biofilms shows potential impacts on the infrastructure, the operation management, and even water quality. It is worth noting that biofilms are practically impossible to be eliminated. For instance, little effects of the residual disinfectants on the reduction of biofilm bacteria were observed in a full-scale DWDS, although it was found that planktonic bacteria can be reduced effectively (Gagnon et al., 2005). This might attribute to the protection of the microorganisms in biofilms from disinfectants and fluctuations by EPS (Fish et al., 2017). Nonetheless, the mechanisms of the influence of disinfectants, especially chlorine and chloramine, on the biofilm formation processes is still poorly understood, which deserves special attention.

2.2.1 *Role of biofilm in DWDSs*

The attached microorganisms in the biofilms have certain advantages over planktonic cells. Biofilm can protect the microbes from hostile environment and provide accommodation and nutrients. As a diverse microbial ecosystem, different microorganisms are able to coexist, and even contribute to each other's nutritional acquisition (Stoodley et al., 2002). Biofilms pose a greatly threat than the planktonic cells in terms of water quality. Except a great microbial abundance, the biofilm bacteria show more resistance on disinfectants than planktonic cells (Zhang et al., 2019). Although various microbial groups coexist in the biofilms, studies commonly focus solely on bacteria with respect to water quality.

Many factors have been proved to influence the development of biofilm in DWDSs, including the characteristics of pipes (e.g., material, roughness, diameter), hydraulic conditions, temperature, the concentration of biodegradable organic matter, the disinfectant residual (Douterelo et al., 2016; Niquette et al., 2000, Sharpe et al., 2017). Amongst, the disinfectant residual and biodegradable organic matter have been indicated to be the key controlling factors of the biofilm development (Momba et al., 2000). The addition of residual disinfectants could alter the physical structures of biofilm. For example, it is shown that biofilm became thinner and more compact in the chloramine-applied system, after the increase of chloramine concentration or prolong the contact time (Ling & Liu, 2013). Generally, it is accepted that EPS, as a final barrier, prevents the disinfectants penetrating into biofilm. A few stated mechanisms include the disinfectant being degraded by the enzymes in EPS or being bonded to the matrix. Thus, the cohesive forces of EPS may also be reduced. It is found with the long-serving system, the aged biofilms are more likely to detach under sudden hydraulic changes.

The recent studies have connected biofilm with DBPs. It is found that the chlorination of microbial carbon related with biofilm makes contribution to the formation of total DBPs, but the mechanisms behind it can be distinctly separate from NOM as precursors (Abokifa et al., 2016). Due to the chlorination of pure bacteria and/or EPS, the released microbial carbon is mainly composed of dissolved organic compounds, for example, proteins, nucleic acids and polysaccharide (Wang et al., 2013, 2012). Therefore, the active diffusion of plankton cells (Xue & Seo, 2013) or passive diffusion caused by hydraulic changes (Kaplan, 2010), can all be added into the total DBP precursors from the biofilm matrix.

2.2.2 *The formation of biofilms*

As shown in Figure 2.3, the formation of biofilm is a sequential process, including five stages of microorganisms transportation to pipe wall surfaces, the initial attachment, the formation of micro colonies, the production of EPS, and the final maturation (Morvay et al., 2011; Sauer & Camper, 2001). During this process, the biofilm growth rate of attached bacteria reaches highest at the early stage of its formation, with the highest flux of biodegradable organic matter (Block et al., 1993).

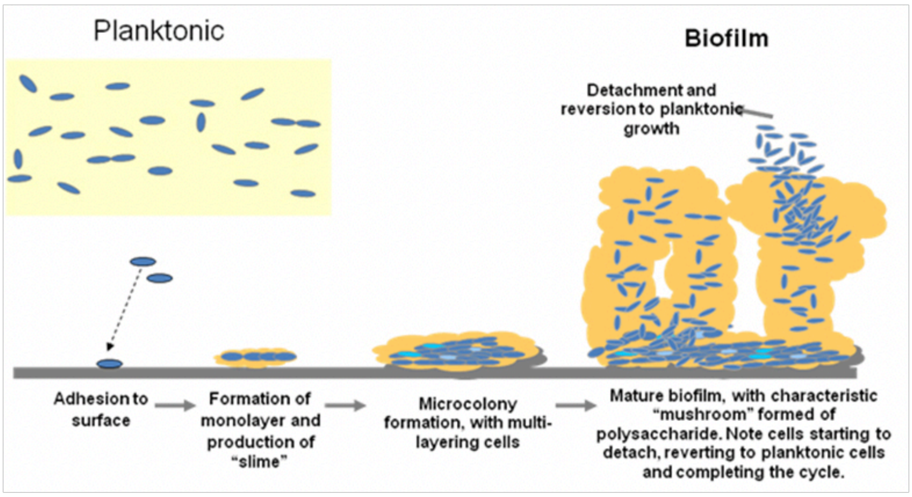


Figure 2.4 Biofilm formation processes (Vasudevan, 2014)

Biofilm formation process starts with the formation of a monolayer on the surface by the random attachment of many planktonic cells in bulk water (Martiny et al., 2003). Given the low selection pressure for attachment of bacteria, it is likely to emerge a highly diverse community. The secondary colonization of bacteria is followed, because it can provide bacteria with a relatively safe and nutritious environment. Then the competition shows up in the secondary community, and the less competitive bacteria is vanished. As the system is dominated by some superior competitors, the community diversity decreases. While during maturation, the richness and evenness of biofilm increase again. This final increase of species reflects the complex spatial structure with many bacterial functional groups (Jackson et al., 2001).

Organic polymers produced and excreted by biofilm consist the polysaccharide matrix, also referred to as EPS. While not only the different types of micro-organisms influence the chemical structure of EPS, but also the environmental conditions. It has been reported that glycocalyx formation is of great importance for the surface-attached cells to survive the shear forces (Ridgway & Olson, 1981). Besides, considering the boundary layer effects and the secretion of extracellular coatings, bacteria closely attached to the inner pipe wall of water flow are less vulnerable to chemical disinfectants (Momba et al., 2000). Hence, the viability and regrowth of microorganisms can be greatly promoted when bonded to suspended particles on the interior side of the pipeline (Ridgway & Olson, 1981)

Numerous studies have been conducted to characterize the biofilms in DWDSs (Douterelo et al., 2014, 2016; Revetta et al., 2013), facilitating the understanding of the biofilm structure and further controlling its development. Microscopy techniques, such as scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) and optical coherence tomography (OCT), are useful tools for studying the formation of biofilms and monitoring the morphological changes (Alhede et al., 2012; Priester et al., 2007; Wagner & Horn, 2017). However, SEM samples are required to be processed (fixed, dehydrated, and coated with a conductive material) before being visualized of the biofilm structure (Bergmans et al., 2005), which might destroy the biofilm structure. While using Environmental SEM (ESEM) is able to address this problem, because there is no preparation needed for this technique (Alhede et al., 2012). Moreover, with the development of the technologies, next generation sequencing (e.g., Illumina Miseq, Nanopore sequencing) is progressively utilized to depict the microbial community structure in drinking water biofilms (Douterelo et al., 2016; Liu et al., 2018), providing new insights with high resolution.

2.2.3 *Biofilm modelling*

It has always been a major challenge to control biofilm formation in DWDSs. Thus, the purpose of a biofilm model is to predict its behaviours, and ultimately control the growth properly. Notably, during the biofilm formation processes, the dominant processes are microbiological reactions and mass transport (Rittmann et al., 2018). An assembly of microbiological processes is no longer sufficient in practice. Physical processes, including hydraulic changes, fluid mechanisms, and mechanical strength have also been successfully connected with microbiological aspects (Edel et al., 2019). As the increase of knowledge in this field and also computer modelling technology, biofilm models have also become more and more complicated. However, by comparing with experimental data, the increased complexity and computation intensity do not assure its accuracy (Morgenroth et al., 2000; Picioreanu et al., 2000; Van Loosdrecht et al., 2002).

When choosing an appropriate biofilm model, the first thing is to look at the modelling objectives and the available data. To achieve the required complexity of computer model, certain simplifications are necessary. Thus, the consequences of these simplifications also need to be taken into consideration. Besides, a good biofilm model should match as much goals as possible. According to the IWA Task Group on Biofilm Modelling, the goals for biofilm modelling include the understanding of fundamental mechanisms, the integration of different mechanisms, the evaluation of novel processes, the pre-modelling for experiments, and also the improvement of process performances (Picioreanu et al., 2006).

a. Major types of biofilm models

Nowadays, the major biofilm models include analytical model, pseudo-analytical model, one-dimensional (1D) numerical model and multi-dimensional numerical model (Boltz et al., 2010). Analytical model is only build for single species and single substrate, and its applied simplification makes the flux of dissolved substrates can be easily calculated (Pérez et al., 2005). Pseudo-analytical model also does not require computational intensity. It was originally developed for one species and one limiting substrate, based on 1D numerical treatment. But the output of the numerical calculation is transformed into algebraic equations to avoid numerical solutions (Saez & Rittmann, 1992). Generally, the use of analytical model and pseudo-analytical model is very limited.

As for the 1D numerical model, it is sufficient to not only describe the bacteria growth, but also help to better understand the complex interactions within multispecies (Rittmann et al., 2018). But 1D model also has its limitations. Given the nature of one dimension, all the quantities are calculated parallel to the substratum and all the gradients are considered perpendicular to the substratum (Wanner & Morgenroth, 2004; Wanner & Reichert, 1996). The bulk water phase can only be simplified as a completely mixed compartment. Hence, 1D models are not able to characteristic the biofilm morphology of multi-dimensions. It can only give a uniform biofilm thickness.

The advanced biofilm models are built on two-dimensional (2D) or three-dimensional (3D) structure, to capture the heterogeneity of biofilm structures. With more power added into the models, more exceptional insights occur, but the requirements for operators' computational skills and computers' capability also increase largely. 2D or 3D models can treat biomass as a continuum or discrete elements (Boltz et al., 2010). It can be discrete on girds (e.g., cellular automata), or as particles (e.g., individual-based models) (Lardon et al., 2011; Tierra et al., 2015). Hence, there are more than one direction for the modelled biofilm to grow. Fluid motion and the advective mass transport can also be considered. The corresponding problems include that the definition between the biofilm matrix and the liquid phase become less clear, and the complexity becomes much higher than 1D model (Boltz et al., 2010).

b. Model definition

As an aggregation of microorganisms and other particles, biofilm has a gel-like structure embedded in EPS. Different sections of biofilm are defined as bulk liquid, boundary layer, biofilm phase and substratum. The four major compartments are shown in figure 2.4. There is a water phase within a defined biofilm system, but the main characteristic is the solid phase. Above the biofilm is a mass-transfer boundary layer between the water phase and solid phase. At the bottom part, there is connecting the biofilm with the pipe surface. The components of each compartment include various important parts of the model, such as the biomass, substrates, products and other materials. Then, particulate and dissolved components can be separated in biofilm models. The main particulate components include cells and EPS, which compose the solid phase of biofilms. These components are normally connected with one another or the substratum physically. But they still hold the potential of detachment and suspending in the bulk liquid phase. On the other hand, the substances, metabolites and other species swimming in the liquid phase of biofilm are defined as dissolved components.

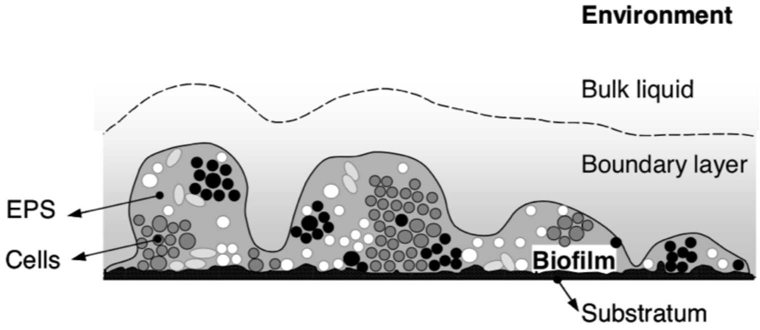


Figure 2.5 Major compartments defined in biofilm system (Picioreanu et al., 2006)

Transformation, transportation, and transfer processes happen among different components. For example, the synthesis of new biomass requires the consumption of certain substrate. The two important processes are the utilization of substrate and diffusion, and they are closely related. The use of biofilm internal matrices creates gradients and facilitates the mass transport of diffusible substances, and the transport of diffusible substances allows further utilization. Both of them create a board gradient. In the end, all the processes within one compartment are linked in one mass balance equation, including parameters and reaction rates (Picioreanu et al., 2006).

The parameters are commonly intrinsic, and their values affect the results highly, as well as the selection of the structure, components, and processes. Parameters can be categorized as system specific and universal (Rittmann et al., 2018). The system specific parameters, like the biofilm thickness and density, are decided based on the considered biofilm system. While the universal parameters can be obtained from literature, for example the diffusion coefficient of dissolved components, the yield of biomass and so on. In the meanwhile, the changes of selected parameters do not always lead to a clear impact on the modelling output. But in some cases, a minor change would also cause a profound influence. Hence, sensitivity analysis can perfectly solve this problem and provide more information on selecting the highly accurate values for model developers.

Chapter 3. Methodology

The methodology of this study is composed of two main parts. The first part is a new pilot-scale DWDS. Field sampling was conducted for different phases within it. However, due to the time constrain, the results from field could only indicate the very initial stage. For further understanding, computer models were introduced to simulate the behaviours of the system. And lab-scale experiments were also designed and performed to calibrate the model results.

3.1 Pilot System

3.1.1 System design

A pilot-scale drinking water distribution network was built in the DWTP in Kamerik, the Netherlands. The new system contains 9 parallel loops in total, in order to compare three cases of no residual disinfection, free chlorine addition and monochloramine addition in triplicate. Each loop is 12m long and made of 40 PVC coupons (D = 32 mm). These coupons can be removed temporarily from the loop for biofilm sampling. Water velocity in all loops is kept at 0.05 m/s, and the residence time is 4 min. Continuous flow is applied in this case, with outflow of the system being discharged directly. The concentrations of total chlorine (for loop 4, 5 and 6) and free chlorine (for loop 7, 8 and 9) are kept at about 0.4 mg/L and 0.1 mg/L, respectively. For the convenience of sampling, there is one sampling taps located at the communal feed water pipe. And for each loop, two sampling taps are installed at the water inlet and outlet as well. The detailed design of the pilot system is illustrated in the figures below.

Figure 3.1 Pilot system on site

Preparing stock solution

To prepare the free chlorine stock solution, sodium hypochlorite solution ('bleach solution') purchased from BOOM (with 60 – 185 g/L active chlorine content). Diluting the bleach solution to a concentration inside the spectrophotometric detection range, the diluted concentration can be measured and then multiply it by the dilution factor to get the original solution concentration. When chlorine reacts with ammonia, monochloramine (NH_2Cl) is firstly formed, followed by dichloramine ($NHCl_2$) and trichloramine (NCl_3). The secret of preparing monochloramine is to control the mass ratio of Cl_2 and NH_4^+-N precisely at 4, and the pH value above 8. The same volume of NH_4Cl solution and diluted bleach solution are prepared. The pH value of NH_4Cl solution is adjusted to about 8, and then adding diluted bleach solution dropwise into the NH_4Cl solution with vigorous stirring. The calculation equations are shown as follow:

$$V_{Cl} = \frac{C_{stock}(g/L)}{71 g/mol} \div C_{bleach}(g/L) \times 71 g/mol \times V_{total}(L) \qquad \text{(Eq.13)}$$

$$M_N = \frac{V_{Cl}(L) \times C_{bleach}(g/L)}{4} \div 14 g/mol \times 53.5 g/mol \qquad \text{(Eq.14)}$$

3.1.2 Field sampling and measurements

Bulk water samples and suspended solids samples were collected from the inflow and outflow of this system. The disinfectant was quenched immediately by adding 5% (w/v) sodium thiosulfate. Microorganisms in the bulk water and suspended solids were collected onto the membranes of different pore sizes. A continuous water filtration setup was developed and shown in figure 3.2 below. As water flowing through membrane filters, suspended solids-related microorganisms can be intercepted by 1.2 μm membrane, and the microorganisms in bulk water can be obtained by filtering through 0.22 μm membrane. Biofilm samples can be obtained by using a swab to scrap certain area from the inner surfaces of the pipe. The detailed sampling program is listed in Appendix A. Generally speaking, the sampling frequency is once per month.

Figure 3.2 Continuous water filtration setup

Table 3.1 Sample collections for different measurements

Measurements	BW	SS	BF
T, EC, pH, DO	Feed[1] & In[2] & Out[3], 1L[4]	\	\
HCO_3^-, NO_3^-, NO_2^-, NH_4^+	Feed & In & Out, 200mL	\	\
TOC	Feed & In & Out, 300mL	\	\
DBPs	Feed & Out, 50mL	\	\
Elements (Fe, Al, Ca)	Feed & Out, 200mL	Feed & Out, 1.2µm 2L	Swab, 2cm×2cm
ATP	Feed & In & Out, 150mL	Feed & In & Out, 1.2µm 2L	Swab, 2cm×2cm
Cell number	Feed & In & Out, 15mL	Feed & In & Out, 1.2µm 2L	Swab, 2cm×2cm
DNA extraction	Feed & In & Out, 0.22µm 20L[5]	Feed & In & Out, 1.2µm 30L	Swab, 4cm×4cm

[1] Communal water source of the pilot system
[2] Inflow of each loop
[3] Outflow of each loop
[4] Collected water volume
[5] Filtrated water volume

The major tasks of field sampling include water collection and water filtration. Most of the samples were sent and analysed by Vitens, a Dutch drinking water company with a professional lab for water quality measurements. Besides, physiochemical parameters of bulk water, such as temperature (T), pH, electrical conductivity (EC), dissolved oxygen (DO) were measured on site by WTW™ MultiLine™ 3420 Portable Digital Multiparameter. Concentrations of free chlorine and total chlorine at the inflow, middle point and outflow of each loop, were also frequently measured by Hach DR300 Pocket Colorimeter on site. The method behind the portable meter is DPD method, with detection range of 0.02 - 2 mg/L Cl_2. Flow cytometer (FCM) and DNA extraction were conducted at Water Lab in TU Delft. FCM was performed by BactoSense Automated Flow Cytometer from SIGRIST. DNA was extracted by Fast DNA SPIN Kit for Soil from MP Biomedicals and saved for the future sequencing. For each sample, details of sampling locations and measurements are listed in table 3.1.

For those parameters measured at Vitens, the concentrations of bicarbonate, ammonia and nitrate can be measured by Ion chromatography (IC), TOC by TOC analyser. ATP can be easily assessed by BacTiter-Glo reagent and a luminometer. For DBPs, most of the halogenated DBPs can be analysed and quantified by using a liquid/liquid extraction (LLE) – GC - ECD method (Krasner et al., 2012). By the analysis of 'screening VAK/VGK', many organic matters can be detected. DBPs were only measured in free chlorine applied loops and monochloramine loops are measured. The targeted DBPs in this study were mainly THMs, including Dichloromethane (DCM), Bromochloromethane (BCM), Trichloromethane (TCM), Tribromomethane (TBM), Bromodichloromethane (BDCM), Dibromochloromethane (DBCM), and Sum Trihalomethane (TTHMs)).

Besides, a systemically method of performing FCM for BW, SS and BF was established in this study, as shown in the figure below. The BW samples can be directly measured by collecting water sample and upload in the sampling device of FCM. To obtain bacteria from SS and BF, membrane and swab are used, respectively. The first step of preparing SS sample is to filtrate 2 L water through 1.2 μm mixed cellulose esters (MCE) membrane, and the biofilm sample is prepared by using a swab to scrap 2 cm × 2 cm inner surface of pipe wall. Then a water bath ultra-sonification is applied to detach cells from membrane and swab. Three cycles of 2-minute ultra-sonification are implemented, with an interval of 2 minutes between. 5 mL new sterile water is added into the system for each cycle; thus, the final solution contains 15 mL. Since the existing of particles from the used material is inevitable, it is necessary to dilute the samples, for example 100 times, in case of blocking the sampling device.

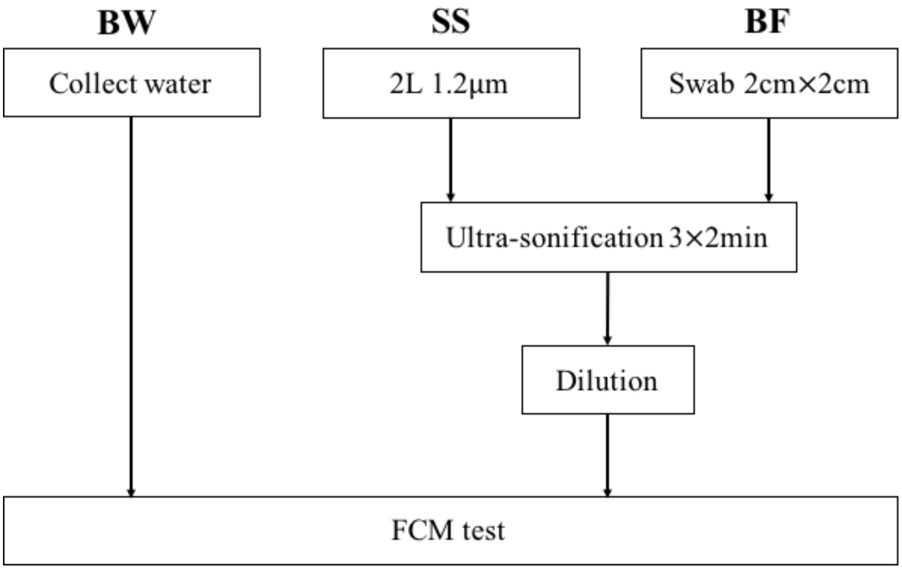


Figure 3.3 Sample preparation for FCM

The applied FCM automatically conducted staining, mixing and incubation. Dyes used are SYBR Green, which is distributed evenly throughout the sample. It binds to the DNA of a microbial cell and fluoresces, emitting green light when illuminated by blue light. The DNA-dye-complex absorbs at 497 nanometres blue light and emits 520 nanometres green light. The following incubation aims to allow the dye to bind to the DNA of each microbial cell, and then heating at 37 °C for 10 minutes. For the detection, fluorescence is measured by 3 detectors, simultaneously, at a wavelength of 525 nm for FL1, at 715 nm for FL2, and at 488 nm for the Side Scatter. The detection limit is 1.0×10^2 cells/mL - 5.0×10^6 cells/mL.

3.2 Modelling Approaches

Computer modelling has become more and more important in the drinking water industry. It is able to provide useful information with relatively few resources. But the accuracy of model output also heavily depends on obtaining reliable input information as well as validation. As mentioned before, the main modelling objectives in this research include biofilm formation, residual disinfectants decay and DBPs formation. The computer models are mainly conducted by Auqasim (Reichert, 1998). As a convenient and often-used software in water systems, it includes data analysis, sensitivity analysis, and fitting for the optimal parameters. Besides, it is also based on robust numerical algorithms, without the need to adjust numerical parameters.

Bacterial growth in the mass and biofilm phase is controlled by the availability of the substrate and the ambient chlorine concentration. Because biofilms are dynamic, bacteria are constantly exchanged between the biofilm and most of the water phase. Bacteria in biofilms also produce EPS, which can make up the majority of the biomass in biofilms. EPS can also separate from the biofilm and enter the mass phase. Chlorine is mainly consumed by reaction with organic substrates in the bulk phase and the consumption of EPS in biofilm and bulk phases. The processes involved in the biofilm model are 1) the attachment of free bacteria to the surface, 2) the separation of bacteria from the biofilm, 3) the growth of bacteria from the biofilm with inhibition of disinfectant, 4) chlorine in large quantities of water, and 5) attenuation of disinfectant by bacteria in the biofilm and the surface of the reactor. Detailed processes are shown in figure 3.4.

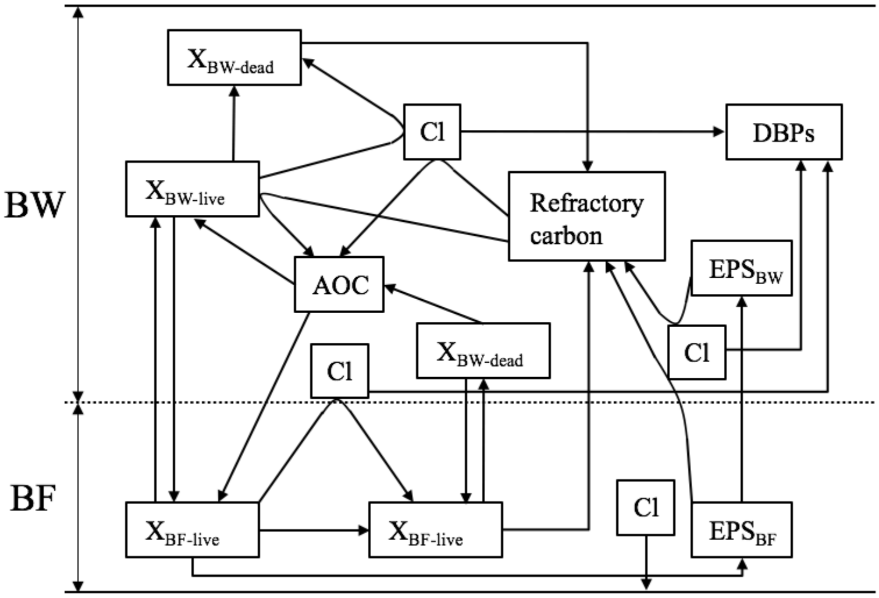


Figure 3.4 Major processes in modelling

3.2.1 Disinfectant decay

a. Chlorine decay bottle test

Before modelling the chlorine decay, the first step was to conduct a bottle test to obtain the decay coefficients. When designing the bottle test, different variables were taken into consideration, such as the changes of temperatures and initial chlorine concentrations. Wall decay was also considered, by cutting the coupon into small pieces and soaked in ultrapure water. The detailed design is shown in table 3.3. However, before conducting chlorine decay tests, the preparation of chlorine demand free (CMD) glassware is of great importance. It is prepared by soaking the freshly cleaned glassware in 20 mg/l free chlorine using ultrapure water and bleach solution overnight. Then, the glassware was emptied, and rinsed with ultrapure water for 4 times. Lastly, all glassware was left to dry or heat at 60 °C for 2 hours (Chiao et al., 2014; Powell, Hallam, et al., 2000).

Another important preparation is to cut the pipe into small pieces (about 2 cm×1cm). Then carefully calculate the water volume required for each piece. It can be derived from the following equations:

$$\frac{V_{water/coupon}}{S_{inside/coupon}} = \frac{\pi R^2 \cdot L}{2\pi R \cdot L} = 2R \tag{Eq.15}$$

$$S_{piece} = 2 \cdot A + S_{edges} \tag{Eq.16}$$

$$V_{water/piece} = 2R \cdot S_{piece} \tag{Eq.17}$$

Table 3.2 Design of the bottle test

Type	**Group**	**Water source**	**Initial concentration (mg/L)**		**T (°C)**		
Bulk decay-free chlorine	Control Group	Ultrapure water	0.1	1.0	4	14	24
	Experimental Group	Feed water from pilot system	0.1	1.0	4	14	24
Bulk decay-monochloramine	Control Group	Ultrapure water	0.4	1.5	4	14	24
	Experimental Group	Feed water from pilot system	0.4	1.5	4	14	24
Wall decay-free chlorine	Experimental Group 1	Pre-treated coupon	0.1		4	14	24
	Experimental Group 2	No pre-treated coupon	0.1		4	14	24

To perform the bottle test, bleach solution and a pre-prepared monochloramine solution were needed. Firstly, the experimental group water samples were collected from Kamerik, feed water of the pilot system. Ultrapure water from Water Lab was applied as the control group. Then, the bleach or prepared monochloramine solution were added into the water sample, in order to obtain the desired chlorine concentrations. After splitting the samples in small bottles and covered with foil, the samples were stored at different temperatures. Lastly, the chlorine content of each bottle under different T was analysed periodically by Hach DR300 Pocket Colorimeter. For free chlorine, the sampling frequency was set as 0, 2min, 4min, 6min, 8min, 10min, 15min, 30min, 60min (1h), 3h, 5h, and 7h. While for monochloramine, the experimental time was prolonged, set as 0, 5min, 10min, 15min, 30min, 60min (1h), 3h, 6h, 16h, 22h, 30h, 42h and 78h.

b. Chlorine decay model

After getting the experimental data, the first-order decay can be easily derived, and a R^2 value from the first order decay model from Excel. Then, Aquasim was applied to estimate the optimal parameter values for the quadratic model and obtain the optimal value for the attenuation dataset. The optimal parameters were those that allowed the model to predict chlorine decay as accurately as possible in each case. As explained by Fisher et al. (2012), the Aquasim software takes an initial set of parameter values and calculates the sum of squares of the differences between each experimental data point and the corresponding prediction model. This sum is derived from the distribution of the data to form the Chi square (χ^2). Then, use the simplex technique to systematically vary the parameter values in order to find the optimal set (minimizing After getting the experimental data, the first-order decay can be easily derived, and a R^2 value from the first order decay model from Excel. During this period, we used

Aquasim software to estimate the optimal parameter values for the quadratic model and obtain the optimal value for the attenuation dataset. The optimal parameters were those that allowed the model to predict chlorine decay as accurately as possible in each case. As explained by Fisher et al. (2012), the Aquasim software takes an initial set of parameter values and calculates the sum of squares of the differences between each experimental data point and the corresponding prediction model. This sum is derived from the distribution of the data to form the Chi square (χ^2). Then, use the simplex technique to systematically vary the parameter values in order to find the optimal set (minimizing χ^2).

3.2.2 *DBPs formation*

The DBPs formation, as chemical changes, have been widely studied by many researchers. However, the relationship between the microbial activity in DWDSs and the chemical changes is much tighter, but with less attention (Li et al., 2019). There are many factors that have impacts on both of the microbial and chemical changes, such as the water quality, type and concentration of disinfectants, etc. They may influence the stability of residual disinfectant and thereby form the by-products. Hence, to model the formation of DBPs is rather complicated, frequently associated with temperature, reaction time, pH, chlorine concentration, bromide concentrations, total organic carbon (TOC), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and so on. In this study, the main focus among all the DBPs is THMs. A total THM (TTHM) formation equation has been developed as follows (Clark & Sivaganesan, 1998b). The formation process can also be simulated by Aquasim. Although there has been some recent paper indicating that certain component of the biofilm can act as the DBPs precursors, there is current few researches connecting them together.

$$\mathrm{TTHM} = \mathrm{D} \cdot [\mathrm{C_{A0}} - \frac{\mathrm{C_{A0}} \cdot (1-\mathrm{K})}{1-\mathrm{K} \cdot e^{-ut}}] \qquad \text{(Eq.18)}$$

where, D = the ratio of TTHM formed to chlorine consumed;

C_{A0} = the initial concentration of disinfectant at time = 0;

K = dimensionless constant;

u = rate constant;

t = time.

3.2.3 Biofilm formation

a. Model definition

Given the dynamic feature of biofilms, bacteria can continually exchange between biofilm phase and bulk water phase, by attachment and detachment. Biofilms include autotrophic and heterotrophic bacteria. The AOC concentration has been reported to have a significant influence on the microbial diversity and growth rate, especially heterotrophs. As the Netherlands has a very low AOC concentration of drinking water, it is hence considered as limiting factor.

As mentioned above, residual disinfectants would not pose big threats to the microbes harboured inside the biofilm. Free chlorine has been proven to be capable on the removal of bulk water bacteria, but monochloramine is reported to be more effective in penetrating deep inside the biofilm. Hence, many reactions and parameters are needed to be taken into consideration. Interestingly, the disinfection by-products can also be the source of AOC. While the main autotrophic organisms of DWDS include nitrifying bacteria and AOB. The introduced ammonia by applying monochloramine is able to promote the regrowth of AOB. But the by-products of AOB might cause water quality problems and potentially sustain NOB and heterotrophic bacteria (Fish et al., 2016).

Before establishing the biofilm model, the following assumptions were made in order to simplify the condition. First, the system was considered closed, with no exchange with the surrounding environments. Second, the newly formed biofilm was assumed as continuous and homogeneous, with a constant thickness. Then, the initial concentration of bacteria within water and biofilm were considered as fixed. Finally, the DWDS was implemented as a complete mix reactor in Aquasim.

b. Model equations

In order to simplify the complexity of the model, the processes listed in table 3.2 were mainly considered. The required parameters include biofilm thickness, boundary layer, biofilm density, influent and effluent substrate concentrations, substrate diffusivity in the liquid phase and in the biofilm, the bulk water flow rate and so on. AQUASIM do not consider any information about units. It means that all the units will be defined by user and keep consistent. As for the selection details about parameter selection were listed in Appendix E. Generally, the universal parameters are found from literature, focusing on drinking water biofilms. There were also some specific parameters decided based on the field sampling results.

Table 3.3 Stoichiometry of the biofilm formation process

Process	**Dissolved components**			**Solid components**		**Process rate**
	S_C	S_N	S_O	X_H	X_A	
Heterotrophic growth	$-\frac{1}{Y\_H}$	-	$-\frac{1-Y\_H}{Y\_H}$	1	-	$\mu_{\max\_H} \cdot (\frac{S_C}{S_C + Kc_H} \cdot \frac{S_O}{S_O + Ko_H}) \cdot X\_H \cdot k_{Cl}$
Heterotrophic inactivation	-	-	-	-1	-	$b_{\text{Ina}\_H} \cdot X\_H \cdot (1 + K_{Cl} \cdot C_{Cl})$
Heterotrophic respiration	-	-	-1	-1	-	$b_{\text{Res}\_H} \cdot (\frac{S_O}{S_O + Ko_H}) \cdot X\_H$
Autotrophic growth	-	$-\frac{1}{Y\_A}$	$-\frac{1-Y\_A}{Y\_A}$	-	1	$\mu_{\max\_A} \cdot (\frac{S_O}{S_O + Ko_A} \cdot \frac{S_N}{S_N + Kn_A}) \cdot X\_A \cdot k_{Cl}$
Autotrophic inactivation	-	-	-	-	-1	$b_{\text{Ina}\_A} \cdot X\_A \cdot (1 + K_{Cl} \cdot C_{Cl})$
Autotrophic respiration	-	-	-1	-	-1	$b_{\text{Res}\_A} \cdot (\frac{S_O}{S_O + Ko_A}) \cdot X\_A$

Chapter 4. Results & Discussion

Three sections were divided in this chapter to answer the corresponding three research questions in order. Before studying the effects of residual disinfectants on DWDS, it is important to firstly understand the essence of chlorine and monochloramine. In this study, the main focus is their stability, in both control group and experimental group. Then, physiochemical and microbiological parameters were used to analyze three phases of the distribution system, specifically bulk water, suspended solids and biofilm. Field sampling and model simulation were both applied. Experimental data can be used for model development as well as calibration, on the other hand, the computer simulation pointed out what to expect in the future. The continuous study is hence necessary to verify the modelling results.

4.1 Concentration Variances of Residual Disinfectants

The most fundamental question of this study is to understand the behaviors of residual disinfectants. The preparation of high concentration stock solution has been introduced before. However, many researches have revealed the decay of chlorine under different conditions (Gang et al., 2003; Laura Monteiro et al., 2017; Vikesland et al., 2001). Generally, free chlorine can maintain stable in ultrapure water, while slow decay can be detected at the higher concentrations of monochloramine. It is mainly due to the auto-decomposition of monochloramine. For further understanding, the decay in source water was tested with lab-scale experiments, and then fitting the experimental data to solve the decay constants. Hence, the last step was dosing stock solution into the pilot system, then practical matters, chlorine demand and the chlorine decay along pipe were addressed.

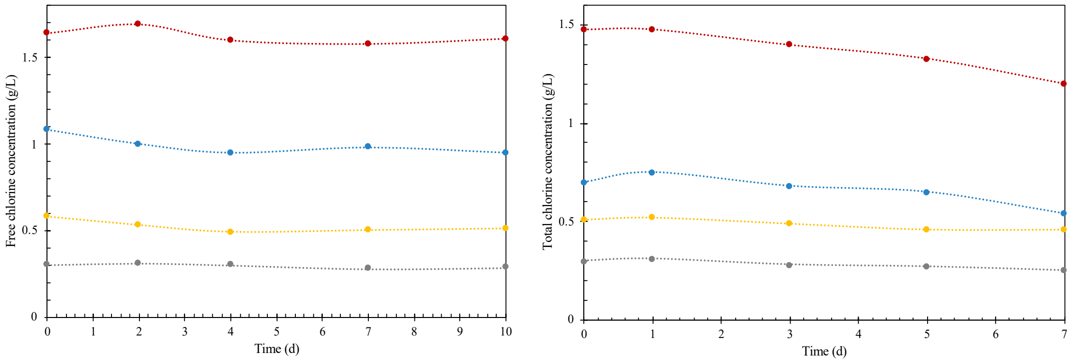
4.1.1 Stability of stock solution over time

In this study, the free chlorine and monochloramine with high concentration was suggested to act as the stock solution for disinfectants dosing into the pilot system. The stability of the stock solutions with different concentrations prepared by using ultrapure water was tested. The stock solution of free chlorine was prepared by directly diluting the commercial bleach solution, the concentration of which varies from 60 to 110 g/L. The results show that there was no difference of the free chlorine concentration within 10 days under different concentrations (e.g., 0.3, 0.6, 1.1 and 1.6 g/L), which indicated the extraordinary stability of the free chlorine at these certain concentrations.

While as the most effective component of the combined chlorine for disinfection, monochloramine is widely used in practice to maintain the biosafety of the drinking water. The secret of preparing monochloramine is to control the mass ratio of Cl_2 and

NH_4^+-N precisely at 4, and the pH value above 8. From figure 4.1-b, it is noticeable the lower the concentration of monochloramine, the better its stability. When the total chlorine is 1.5 or 0.7 g/L, the decay rates were 0.040 and 0.023 g/L·d^{-1}, respectively. Whereas the stock solutions of 0.3 and 0.5 g/L were stabilized over a week with a minor decay rate below 0.01 g/L·d^{-1}. The phenomena that higher decay rates occurred under higher total chlorine concentrations is consistent with the previous study by Zhang et al. (2012). In the study, monochloramine was reported to be able to undergo auto-decomposition within ultrapure water. The auto-decomposition of monochloramine was consisted of a series of reactions, highly depended on various parameters, such as T, pH, the initial chloramine concentration, Cl/N ratio and so on (Vikesland et al., 2001).

Overall, given the stability of the stock solutions under different concentrations and the practical concerns (e.g., the efficiency of the dosing pumps, discussed in Chapter 4.1.3), 0.3 g/L and 0.25 g/L were selected as the concentrations of the free chlorine stock solution and monochloramine stock solution, respectively. In this way, the stability of stock solution can be assured, as well as the frequency of preparing stock solution can be lower to a manageable level, regarding to the practical need of collecting and transporting certain volume water.



a. **b.**

Figure 4.1 Stability of free chlorine stock solutions

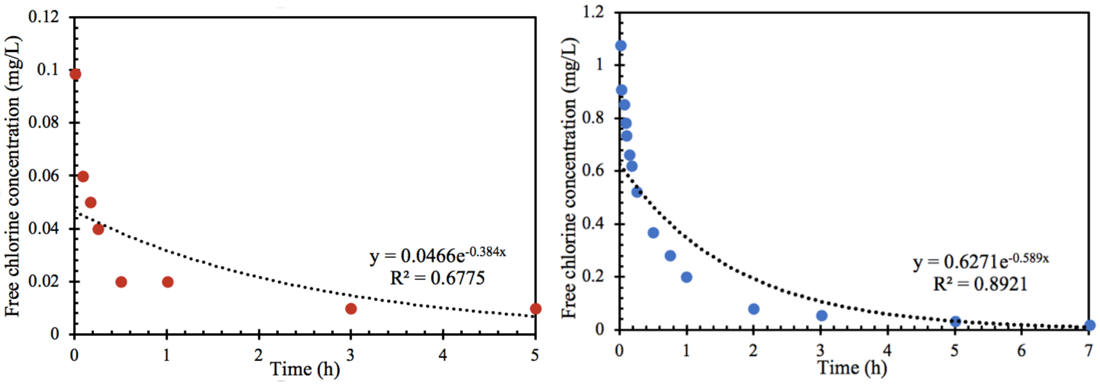
(**a:** Free chlorine decay behaviour; **b:** Monochloramine decay behaviour)

4.1.2 The decay of residual disinfectants concentration over time in bulk water

It was widely reported that the components in bulk water such as the natural organic matters and some metals, could react with residual disinfectants, which results in the consumption of the disinfectants and declining disinfection ability. Therefore, the understanding on the bulk water decay is important. Specifically, bottle tests were carried out to explore the decay behaviour of free chlorine and monochloramine. Notably, it was indicated that temperature could significantly affect the chlorine decay. Considering the potential influence of both temperature and initial concentration, hence, the chlorine decay behaviour was investigated under two initial concentrations (0.1 and 1.0 mg/L for free chlorine, 0.4 and 1.5 mg/L for monochloramine) and three different temperatures (4, 14 and 24 °C). The chlorine decay was determined through the experimental data conjoined with computer models. It aims to determine the kinetic order of chlorine decay reactions and provide the accurate definition of decay constants in such bulk water.

a. Free chlorine decay

As discussed before, initial chlorine concentrations as well as temperature would have impacts on the chlorine decay behaviour. Based on the data obtained from the laboratory bottle tests, different models can be implemented. Free chlorine is rather stable when diluted by ultrapure water. But if bacteria existing, the decay happens in short time. Firstly, a simply first-order model can be used to fit the data points. As the most straightforward method, it is of great simplicity and easiness. The free chlorine decay coefficients for a lower and higher initial concentration were 0.384 and 0.589, which represent the percentage of decayed chlorine versus time. Meanwhile, the decay rates within 5 hours were 0.018 and 0.209 mg/L·h^{-1}, respectively. It can be concluded that the decay of free chlorine happened faster at a higher initial concentration, with a larger decay rate.



a. **b.**

Figure 4.2 First-order decay model of free chlorine
(**a**: Low initial concentration, **b**: High initial concentration)

However, the R^2 of the first-order models were not ideal. Especially within the first hour, the first-order model failed to fit in the faster decay trend, which can be considered as the effects of fast reacting agents. While the rest data points can be viewed as a slow reacting phase. A parallel first-order decay model can be implemented. As is shown below in figure 4.3, the fit curve is composed of two first-order models, linked at about half an hour. Both phases can better fit the experimental data, with the R^2 values higher than 0.90. The result showed that the decay coefficients were 2.265 and 0.493 mg/L·h^{-1}, describing the fast and slow reacting potentials relatively. Due to the limited data points for the lower initial concentration, it is not sufficient to divide and model the decay in two stages. While the major problem with such model is that the theory behind it might not be correct. Since the assumption here is that the first stage is fast reacting agent reacts only, and then the slow reacting agent starts to react in the second stage. But actually, both FRA and SRA react at the same time. There is no evidence indicating the SRA would take longer time before reactions.

Hence, parallel second-order model can be introduced. Regardless of the initial concentrations and temperature, all the chlorine decay data (free and combined chlorine) can be described by two parallel second-order reactions, with both FRA and SRA started reacting at the same time and no division required (G. Kastl et al., 2003). There has been a study compared the efficiency of parallel second-order model with traditional first-order model or second-order model, which proved the parallel second-order model to be most accurate (A. J. Kohpaei et al., 2011).

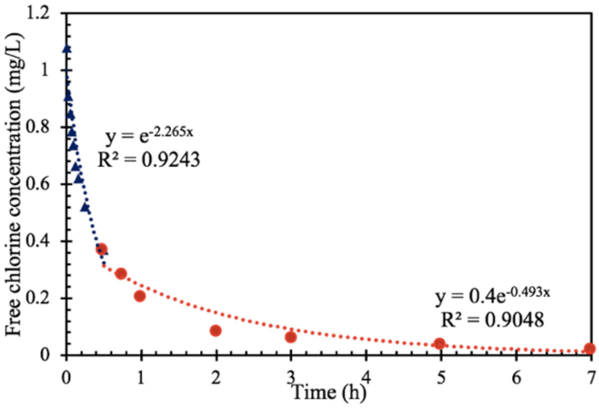
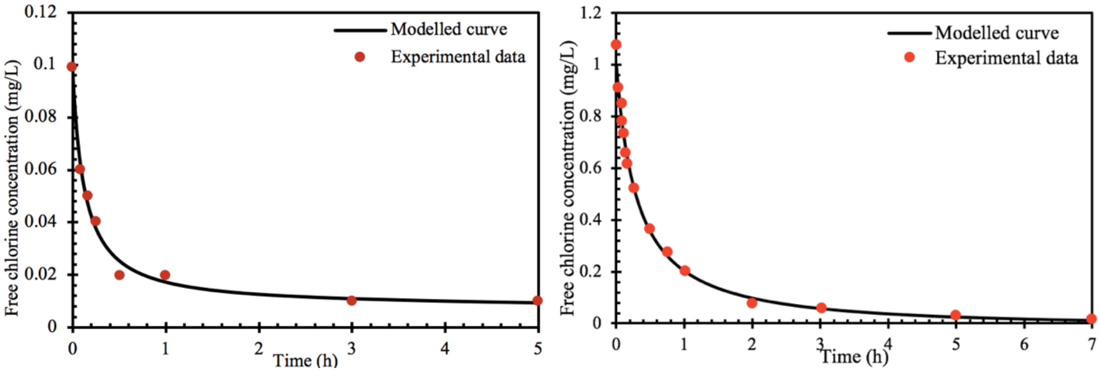


Figure 4.3 Parallel first-order decay model of free chlorine

As figure 4.4 showed, the data was perfectly fitted to the curve. The χ^2 values obtained from Aquasim were also very small, below 0.005. The estimated decay coefficients of FRA and SRA are listed in table 4.1 below, in comparison with several published articles (all used drinking water but involving source water and different steps at a DWTP). Firstly, it can be noticed that the fast decay coefficient was higher when the initial chlorine concentration was slightly higher, from 5.22 to 5.50 $mg/L\cdot h^{-1}$. It also shows that the majority chlorine decay happened due to the fast reacting agents, while the slow reacting phase only contribute a small percentage of decay but lasted for a much longer time. By comparison with literature, the FRA decay constants were in the same range, but the SRA decay constants obtained in this study were much higher than others. However, it is important to notice the time scale. Given the particularity of drinking water quality in the Netherlands, the chlorine decay happened much faster than most of the other studies, which normally describe the decay model on a time scale of about 100 hours (I. Fisher et al., 2017; A. J. Kohpaei et al., 2011; L. Monteiro et al., 2014; Sathasivan et al., 2020). But in this study, the free chlorine disappeared in only 7 hours. It is therefore easy to explain the differences of slow reacting coefficient.



a. **b.**

Figure 4.4 Parallel second-order decay model of free chlorine
(**a**: Low initial concentration, **b**: High initial concentration)

Table 4.1 Comparisons of free chlorine decay coefficient with literature

	This study		(G. J. Kastl et al., 1999)	(A. Kohpaei et al., 2011)	(Fisher et al., 2017)
	a	**b**			
k_{FRA} (mg/L·h^{-1})	5.22	5.50	0.38	3.35-9.80	0.17-9.92
k_{SRA} (mg/L·h^{-1})	1.98	0.82	0.013	0.016-0.37	0.0047-0.071
k_{SRA}/k_{FRA}	0.38	0.15	0.034	0.0005-0.037	0.0007-0.028

Applying the same procedure for the data points obtained at different temperatures, the results showed the effects of temperature on the decay behaviour. Due to the experimental intensity, the samples were split after one hour and then stored at different temperatures. The temperature effects on a lower initial concentration of free chlorine were not studied, considering the free chlorine already decayed below the detection range in one hour. As shown in figure 4.5, the start point was about 0.2 mg/L. The overall decay trends of all three temperatures were identical, but it can still be noticed that the decay of free chlorine was enhanced with elevated temperature. The listed coefficients shown in table 4.2 shows that the SRA coefficient decreased as temperature increase, which also means that the proportion of SRA is higher at lower T. On the other hand, as the major source of decay, FRA was much higher at 24 °C. However, due to the limited data points, the estimated coefficients perform better in describing the curve, instead of understanding the mechanisms. Hence, it is difficult to judge the relationship between temperature and decay coefficients of FRA and SRA.

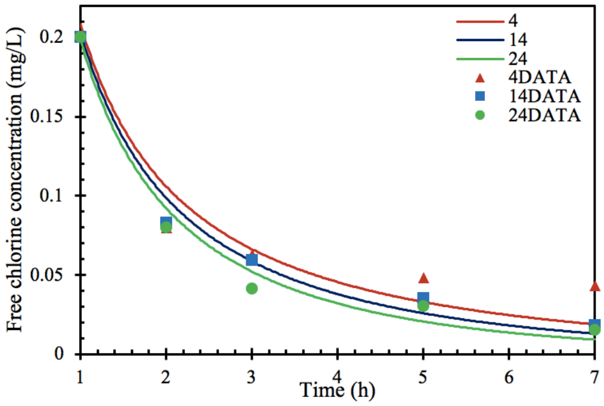


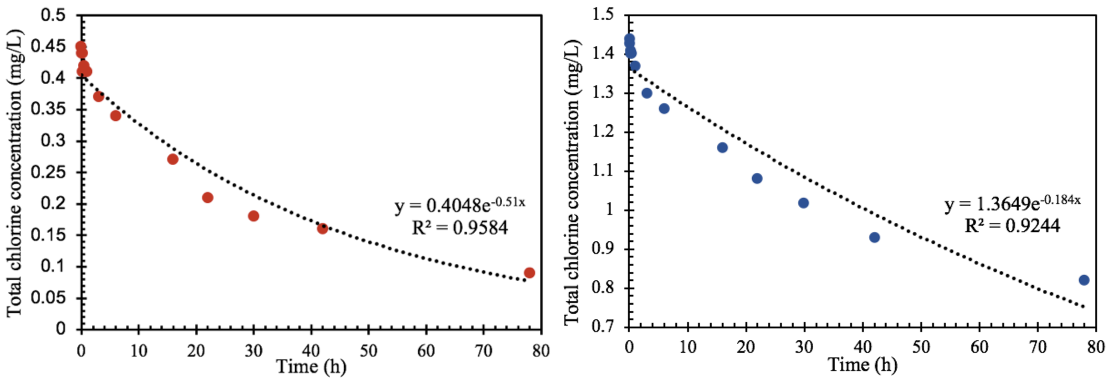
Figure 4.5 Temperature effects on free chlorine decay

Table 4.2 Free chlorine decay coefficients under different temperatures

T (°C)	4	14	24
k_{FRA} (mg/L·h^{-1})	0.003	0.002	2.5
k_{SRA} (mg/L·h^{-1})	0.26	0.09	0.035
χ^2	0.011	0.009	0.010

b. Monochloramine decay

The decay behaviour of chloramine is more complex than free chlorine, due to the auto-decomposition. Even only presented in ultrapure water, the decay of monochloramine would also happen within only few days. But the decay takes a much longer time, compared with free chlorine decay. The figures below showed the simple first-order model. The fit for the lower initial concentration was better with a higher R^2. As for the other case, it is probably due to the time length not enough to reach equilibrium. The fitted exponential line looked more like a straight line, and the R^2 was slightly lower. The decay rates of low and high monochloramine over 80 hours were 0.0044 and 0.0075 mg/L·h^{-1}. It can be found that the decay increased at higher initial concentration, which is the same trend as free chlorine decay.



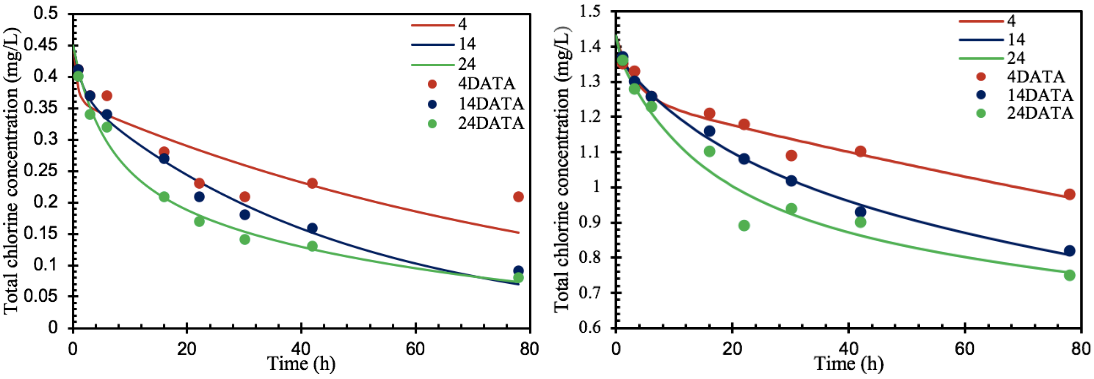
a. **b.**

Figure 4.6 First-order decay model of monochloramine
(**a**: Low initial concentration, **b**: High initial concentration)

The fitted line can be improved by using the parallel second-order model. In this model, not only the two reacting agents were considered, but also the auto-decomposition of monochloramine. As described before, the auto-decomposition of monochloramine also happens in ultrapure water, which normally accelerated by higher temperature and higher initial chloramine concentration (Vikesland et al., 2001; Zhang et al., 2013; Zhang et al., 2012). All of the three processes, contributed to chloramine loss, were implemented in the model, and obtained the estimated value by fitting the model to experimental data with smaller error. Different initial concentrations and different temperatures were all considered.

As shown in figure 4.7, perfect fit curves were made illustrating the monochloramine decay. The results were summarized in table 4.3. Overall, the decay behaviour of all the cases were similarly, and larger decay rates can be observed at higher temperature. It is clearly to see from the table that the auto-decomposition rates of monochloramine increases as the temperature increasing at both initial concentrations. While the higher initial concentration also leads to a higher value comparing with the value obtained under same temperature but smaller initial concentration. This trend is in agreement with the above-mentioned studies.

Considering the figures of all decay coefficients, the auto-composition only contributed a small part of monochloramine loss, while the rest part reacted with FRA or SRA. But the relationship between FRA, SRA and T remined unclear. A higher average value of SRA decay was noticed at higher initial concentration, while the FRA decay coefficients were higher at the lower initial concentration. It showed a shift from fast reacting-dominant to slow reacting-dominant as the initial concentration increases. However, those obtained decay coefficients were highly depended on the experimental data. More experiments are needed to draw a specific conclusion.



a. **b.**

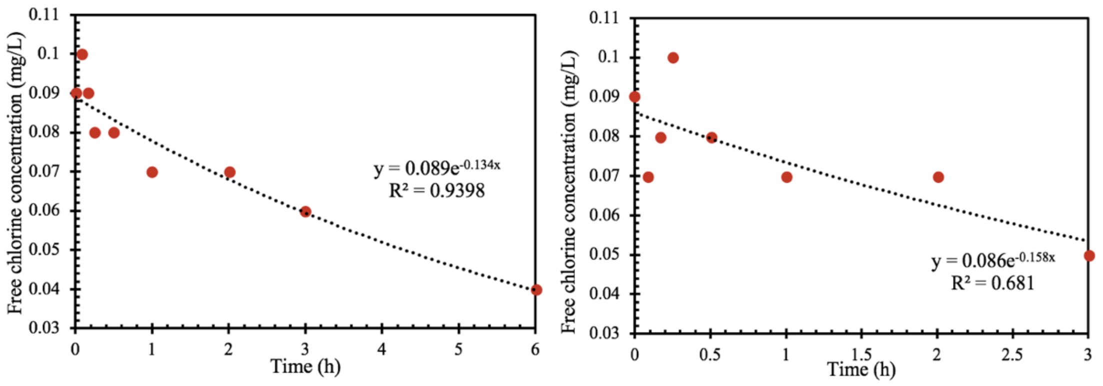
Figure 4.7 Parallel second-order monochloramine decay models
(**a**: Low initial concentration, **b**: High initial concentration)

Table 4.3 Monochloramine decay coefficients under different cases

Initial chlorine concentration (mg/L)	T (°C)	Auto-decomposition rate ($mg/L·h^{-1}$)	k_{FRA} ($mg/L·h^{-1}$)	k_{SRA} ($mg/L·h^{-1}$)	χ^2
0.45	4	0.0013	5.42	0.0004	0.003
	14	0.018	6.37	0.0003	0.012
	24	0.038	2.76	0.0003	0.002
1.46	4	0.0042	1.33	0.08	0.002
	14	0.0044	0.032	1.2	0.02
	24	0.0046	0.099	2	0.01

c. Wall decay

Normally, large wall decay comes from biofilm and reactive pipe material. In this study, new PVC pipe was applied. To investigate the free chlorine decay consumed by pipe wall, it is important to correct calculate the ratio between the area of pipe material and the corresponding water volume. The pre-treatment of soaking pipe in high concentration free chlorine solution aims to eliminate the chlorine demand from the material. While both of the pipe materials with and without pre-treatment gave a similar wall decay coefficient, 0.134 and 0.158 mg/L·h^{-1}, respectively. According to Hallam et al. (2002), the new PVC pipe has little impact on wall decay, with an average coefficient of 0.09 mg/L·h^{-1}. In the meanwhile, comparing the data points within the first hour, both cases showed similar fluctuation, and the wall demand become obvious after one hour. The residence time of the system is only 4 min; hence the wall would cause a maximum of 0.009 mg/L free chlorine decay, which is negligible.

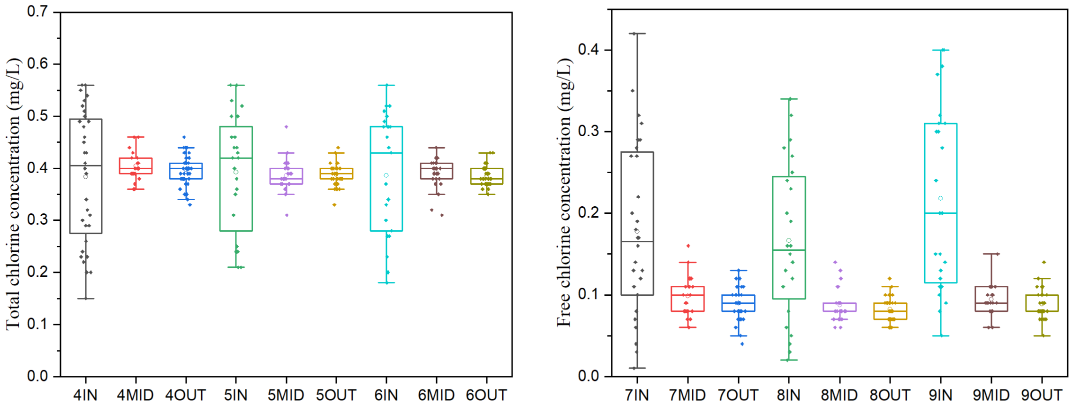


a. **b.**

Figure 4.8 First-order model of wall decay
(**a**: Pre-treated pipe material, **b**: No pre-treatment)

4.1.3 Stability of chlorine concentrations along distance

Given the selected stock solution concentrations, noticeable differences can be observed at the different locations of each loop. Take an example of loop 4, the water samples were collected at three points, when chlorine was just dosed into the system and being mixed through a static mixer (4IN), when the water travelled half way of the loop (4MID), and when water was about to leave the system (4OUT). The chlorine concentration at the inflow shows a larger variance. While as water mixing thoroughly, the concentrations and middle points and end points are relatively stable at the desired level, 0.4 mg/L for monochloramine. Similar trends can be observed for free chlorine. The concentrations were also maintained at around 0.1 mg/L at both middle and end points. The variance and deviation of the start points were also similar. The reason behind it such variance is the inconsistency of dosing pumps on site, which worked on a fixed pattern.

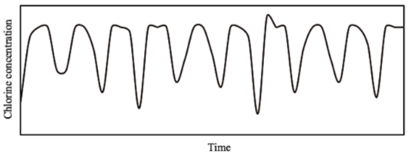


a. **b.**

Figure 4.9 The concentration variances of residual disinfectants
(**a**: Loop 4&5&6 with monochloramine addition, **b**: Loop 7&8&9 with free chlorine addition)

To understand the chlorine variances along the distribution, the range of chlorine concentrations were shown in table 4.4, as well as a modelled dosing pattern. The picture briefly illustrates the concentration variance caused by the dosing pump, with peaks and occurring in cycles. By observing the working pattern of pumps under different dosing velocities, it was found that there is a fixed interval of no pumping, after certain volume had been dosed. As for different dosing velocity, the dosing pattern also varies. For example, when dosing 300 mL/h, the pump would stop dosing for 10 seconds within every 1 minute, and a 7-second interval within 30 seconds at the dosing velocity of 500 mL/h. To minimize the fluctuation, the choice of dosing velocity played the most important role. A higher pumping velocity leads to more frequent intervals, but with shorter time. While a lower pumping velocity takes longer time before the interval happens, but the higher stock solution concentration probably causes more serious influence on the chlorine concentration at the inlet of each loop.

Table 4.4 Chlorine concentrations along the loop



	Monochloramine	Free chlorine
Dosed (mg/L)	0.50	0.65
IN (mg/L)	0.15-0.56	0.01-0.42
MID (mg/L)	0.35-0.46	0.06-0.14
OUT (mg/L)	0.34-0.43	0.05-0.13

Considering the above-mentioned situations, the final pumping velocity is decided at around 300 mL/h based on the experimental results, with small changes allowed to cope with the decay of stock solution. The tested chlorine concentrations at the inlet of loops should fall in the range of [0.1, 0.55] mg/L for loop 4, 5, 6; and [0.06, 0.33] mg/L for loop 7, 8 and 9. Comparing with the data in table 4.4, the inflow concentrations can be well explained by the pump regime, and such large fluctuations are inevitable.

In operation, loop 4, 5, 6 and loop 7, 8, 9 were under identical conditions per group, viewed as triplicate. Based on single factor analysis, no significant difference was detected within both groups. Hence, the average concentration of monochloramine applied loops at the inflow, middle point, and outflow were 0.391, 0.394 and 0.387 mg/L. Similarly, the free chlorine concentrations were 0.187, 0.093 and 0.087 mg/L, respectively. While the amount of actual dosed concentrations can be obtained by the relationship among stock solution concentration, dosing velocity and the flow rate within loops. Through calculation, the dosed stock solutions into the loops were 0.503 mg/L for monochloramine, and 0.646 mg/L for free chlorine.

It is clear to see that an immediate free chlorine decrease happened from 0.646 to 0.187 mg/L within only few seconds. The first reason comes from the dosing pump. Since it is dosing in an intermittent way, the dosed amount within a specific period varies. In this way, the accuracy of the pump cannot be assured. Expect that, part of the reason was the rapid decay in static mixer, due to the larger contact area and unstable hydraulic condition. Meanwhile, the presence of ammonia in bulk water would also consume free chlorine. Ammonia was detected in the feed water within the range of 0.0025 to 0.022 mg/L. To calculate the amount of free chlorine that would react with ammonia, the reaction is assumed to form NCl_3, considering the excess free chlorine in water and the low availability of ammonia. The reaction is listed in Eq.19. After calculation, the residual free chlorine was in the range of 0.369 - 0.614 mg/L, which is still higher than the inflow concentration. While another reason for such rapid decay could be the formation of THMs in bulk water. Since THMs were only detected in the free chlorine applied loops, in another way, neither significant loss nor THMs formation were found in the monochloramine applied loops.

$$3HOCl + NH_3 \rightarrow NCl_3 + 3H_2O \qquad \text{(Eq.19)}$$

4.2 Impacts of Residual Disinfectants on Chemical Water Quality in Pilot DWDS

The purpose of adding residual disinfectant is mainly to control the microbial regrowth, and hence maintain the water quality during distribution. While various chemical and physical processes also happen, which might lead to nonnegligible impacts. In this study, the chemical water quality analyzation was conducted and mainly focused on the bulk water phase. Conventional water quality parameters, organic and inorganic matters were measured, as well as THMs. As mentioned above, the three loops under the same condition were viewed as triplicate, without significant differences.

4.2.1 Conventional water quality parameter

Conventional water quality parameters include pH, EC and DO, were tested on field and listed in Table A.6. No trends can be observed directly for the pH and EC values under different conditions. Only the DO values showed a slightly increase at outlet comparing with inlet among all the cases. However, according to the single factor analysis, no significant variance was detected between week 0 and 3, between water inlet and outlet, or among the NRD, MCA and FCA loops, the *p*-values were all higher than 0.05. The results showed that the addition of residual disinfectants does not necessarily lead to the change of the conventional water quality parameters.

As for the measurements for TOC and other inorganic ions, the results were listed below. No significant variances were detected based on the values of TOC, HCO_3^- and NO_3^-. While the most straightforward change was the increase of ammonia in MCA loops, about 1500 times higher than the average value of the other two cases. This can be directly related to the addition of monochloramine, which would release ammonia into the bulk water. In the meanwhile, the nitrite concentrations of FCA loops were clearly lower than others. This could be related to the oxidation of nitrite by adding free chlorine, which would react fast through the static mixer.

Table 4.5 Organic and inorganic substance concentrations

		TOC (mg/L)		**HCO_3^- (mg/L)**		**NH_4^+ (mg/L)**		**NO_3^- (mg/L)**		**NO_2^- (mg/L)**	
		IN	**OUT**	**IN**	**OUT**	**IN**	**OUT**	**IN**	**OUT**	**IN**	**OUT**
NRD	week0	6.411	6.461	225.54	211.57	0.0030	0.0034	13.97	14.08	0.0041	0.0044
	week3	6.351	6.262	223.00	223.03	0.0065	0.0073	13.95	13.68	0.0080	0.0077
MCA	week0	6.522	6.492	231.15	231.29	0.1502	0.1596	14.14	14.34	0.0051	0.0048
	week3	6.408	6.449	219.67	221.43	0.1491	0.1326	13.96	14.17	0.0070	0.0057
FCA	week0	6.492	6.528	233.41	233.37	0.0133	0.0191	13.74	13.95	0.0019	0.0007
	week3	6.386	6.439	226.20	226.01	0.0039	0.0052	14.14	13.92	0.0019	0.0018

4.2.2 THMs occurrence

The main DBPs to be detected in this study was THMs. Normally, TCM is the most common THM and the principal disinfection by-product in chlorinated drinking-water. Then, Br-THMs (e.g., BDCM, DBCM, TBM) could form in proportions to the decrease in TCM content, when bromides presented (Wahman et al., 2007). According to WHO, the guideline values for TCM, BDCM and DBCM are 300, 100 and 60 μg/L (WHO, 2017). THMs were formed only in FCA loops, and no THMs has been detected yet in MCA loops. By calculating the ratio of different THMs, about 8% was BDCM, less than 1% was DBCM, and more than 90% was TCM. The average values were 0.38, 0.05 and 4.47 μg/L, respectively for week 0, 0.36, 0.04 and 3.85 μg/L for week 3. The averaged total THMs (TTHMs) declined from 4.80 μg/L in week 0 to 4.21 μg/L in week 3. All the values here were much smaller than the WHO guideline values, and also smaller than other studies, which were normally within the range of dozens to almost one hundred μg/L (Zhao et al., 2018).

Another thing to notice is that the detected values for week 3 were all slightly lower than week 0, as the temperatures of week 3 were lower than week 0 (an average of 1 degree). This could be related to the disinfectant stability decrease at elevated temperature, which accelerated the decay of free chlorine and also promoted the formation of THMs. The experimental results verified this theory. A 1-degree decline in temperature led to a 0.06 μg/L lower TTHMs concentration. The relationship between temperature and THMs formation could be proposed when more THMs data at different temperatures obtained.

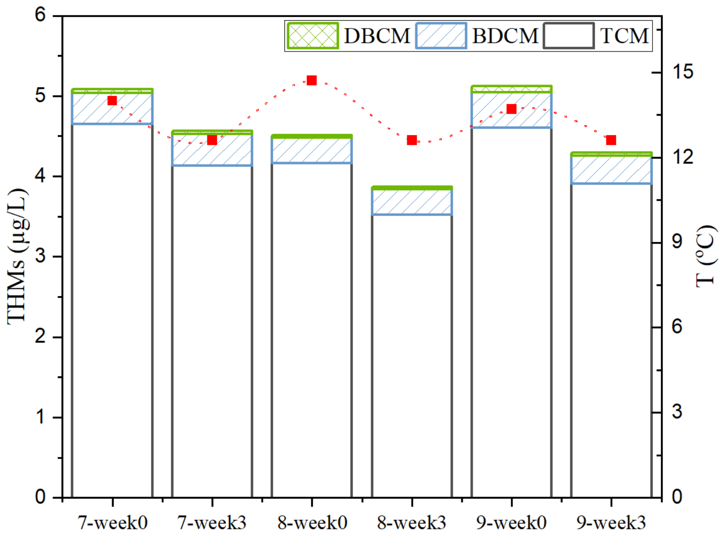


Figure 4.10 The formation of THMs in free chlorine applied loops

4.2.3 THMs predictive model

The researches on THMs have been largely conducted on understanding the complex mechanisms of its occurrence, but the THMs predictive models are mainly empirical or semi-mechanistic, due to the complexity of NOM and variables involved in reactions (Sathasivan et al., 2020). While most of the empirical equations are very site-specific and used to fit the experimental data (Sohn et al., 2004). On the other hand, the semi-mechanistic model normally connects the chlorine decay with THMs formation. The linear relationship between them was firstly proposed by Clark & Sivaganesan (1998a), and gradually confirmed by many other researchers (Boccelli et al., 2003; Gallard & Von Gunten, 2002; G. Kastl et al., 2003). Hence, as discussed in chapter 3.2.3, a theoretical model was considered and conducted in this study. The selected value was also based on literature, with D = 0.77, K = 0.78 and M = 0.42 (Sadiq & Rodriguez, 2004). The figure below illustrated that decreased chlorine concentration and increased THMs concentration, the linear relationship was also showed between the chlorine demand and THMs formation.

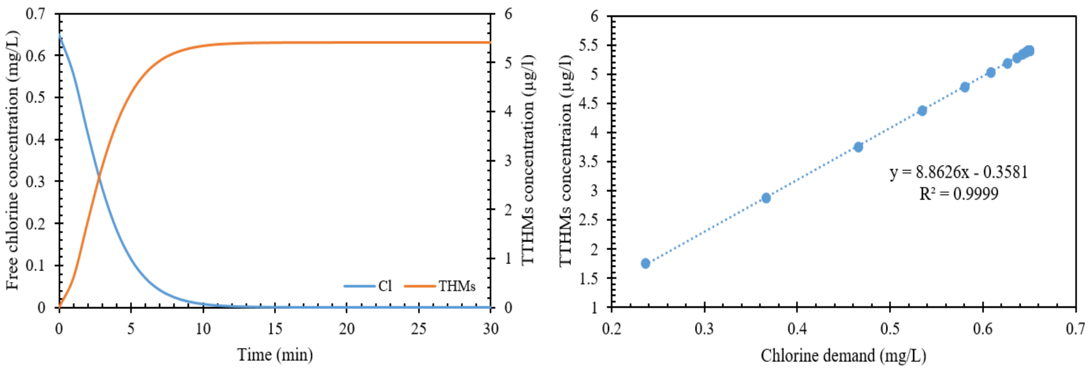


Figure 4.11 THMs predictive model

The model can be validated by the experimental data. However, there is no enough data points in this study. The residence time was only 4 min in the loop, and the reactions were quenched after sample collection. A prolonged time scale is applied in this model in order to have a complete trend. It can be seen that the measured TTHMs value was in agreement with modelled value. It is noticeable that the initial free chlorine concentration was adopted the calculated dosing concentration (0.646 mg/L), but the chlorine decay trend was not identical with the real system. While as in such model, the part of unproductive chlorine demand at the beginning was not considered. Although this model offered a TTHMs value in agreement with the measurement, its accuracy cannot be assured. More experiments are needed for the model calibration.

4.3 Impacts of Residual Disinfectants on Microbial Water Quality and Biofilm Formation in Pilot DWDS

As mentioned above, FCM measures the intact cell counts (ICC) in water samples, and ATP is commonly used to represent viable microorganisms. Both of these two factors can be used to indicate the microbial changes within DWDSs. But they do not often offer the same trend, even in the bulk water phase, which increases the difficulty in analyzing the situation. Farhat et al. (2018) attribute the reasons to the nature of ATP measurements, for example, the potential missing of peak value and the value differences between different growth stages. Similar observation was also found (Nescerecka et al., 2018). Hence, the biomass in bulk water phase was represented by FCM results in this study, which was specifically designed to assess the bacterial water quality by counting the single cells, both stable and reliable.

As for the suspended solids and biofilm phase, ultra-sonification might not be sufficient enough to detach single cells out of the material. While some fiber or particles were inevitable detached from membrane and swab, which could block the sampling device. The last-step 100-times dilution might compromise the actual cell counts of original system. Therefore, as another quick test for representing biomass, ATP was used to analyze the bacteria in suspended solids and biofilm phase. While on the other hand, the combination of both results might offer more insights into the growth potential over time.

4.3.1 Bulk water phase

The figures below show the FCM and ATP results for all three conditions and both the inflow and outflow of each loop. Three identical loops of one case was viewed as triplicate. The average values were listed as columns, and the corresponding error bars indicating standard deviation of triplicate samples. No significant difference is expected between week 0 and week 3. The ICC values for both weeks averaged 4.67×10^5, 4.87×10^5, 4.16×10^5 cells/mL at the inlet of NRD, MCA and FCA loops, respectively. The corresponding outlet values were 4.81×10^5, 4.89×10^5 and 2.61×10^5 cells/mL. Similarly, the average ATP values were 7.99, 5.78 and 4.13 ng/L at inflow, and 7.75, 5.04 and 5.18 ng/L at outflow.

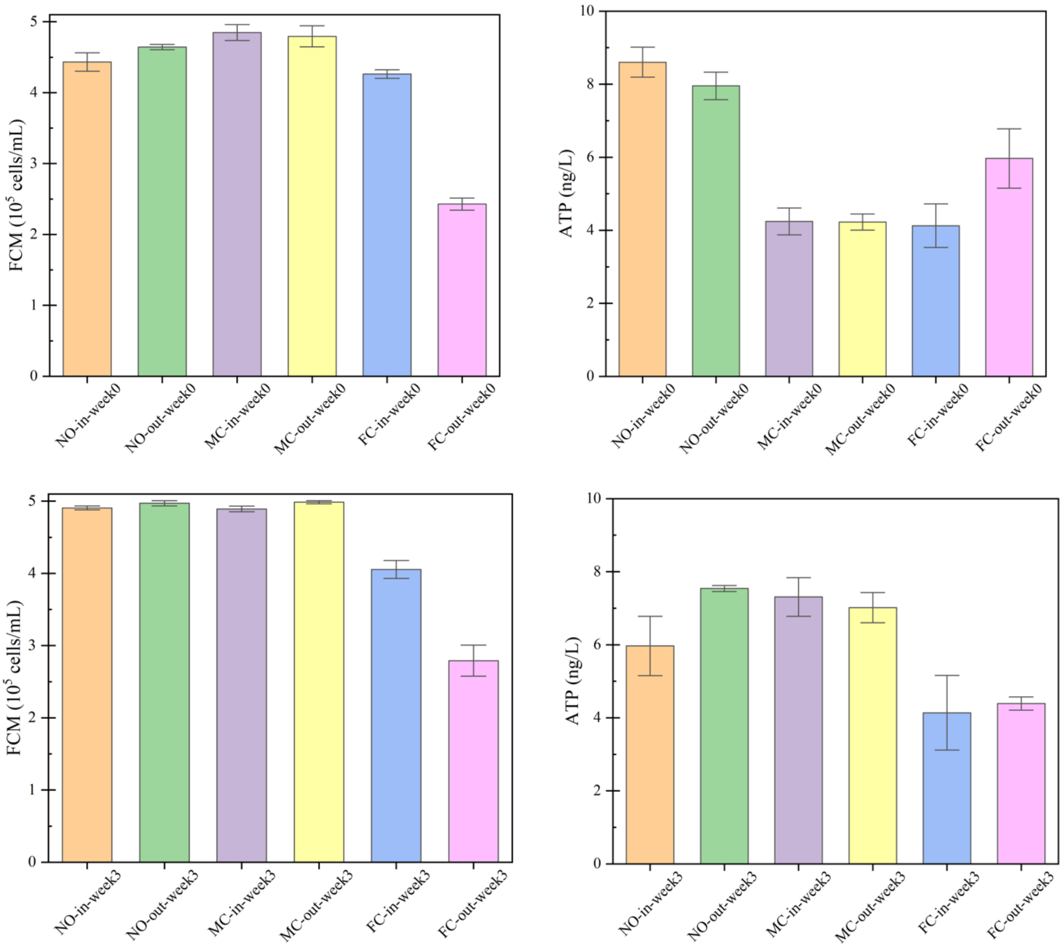


Figure 4.12 Bulk water inlet and outlet samples measured cell counts and ATP

From the FCM result, it can be seen that the effects of free chlorine were very obvious in reducing the cell count from the water inlet to outlet (with an average of 37% reduction). While no major differences between NRD (averaged 3% growth) and MCA (averaged 0.4% growth) loops, only the monochloramine can slightly inhibit the microbial regrowth within the loop. It was expected that the ATP test results would show a similar trend. However, no such relationship could be observed, probably due to the higher fluctuation as well as standard deviation of ATP tests. What can be observed was that the ATP value of the FCA loops was lower than the NRD loops.

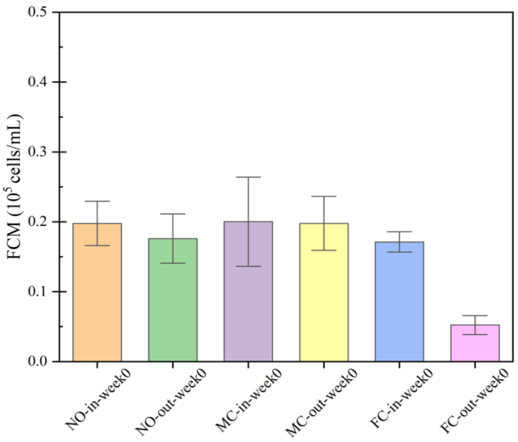
To be specific, the percentages of intact cell counts variances from inflow to outflow were calculated and listed in table 6.1. For the NRD loops, it showed the microbial regrowth ability in bulk water, with an average of 4.81% increase. In the meanwhile, the application of monochloramine did not give an obvious trend of increase or decrease, indicated its ability to inhibit regrowth to some extent. As for free chlorine, it is clearly to see the cell counts decreased largely.

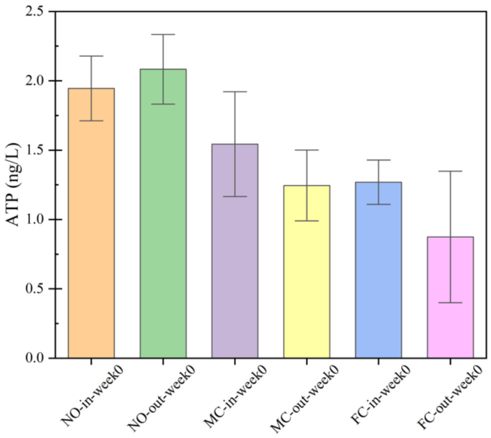
Table 4.6 Comparisons of bulk water cell counts variances

		Cell count variance of three loops			Average
NRD	Week0	6.71%	6.14%	1.58%	**4.81%**
	Week3	1.38%	2.01%	0.46%	**1.29%**
MCA	Week0	-0.19%	-1.05%	-2.13%	**-1.12%**
	Week3	2.07%	0.85%	2.80%	**1.91%**
FCA	Week0	-41.13%	-43.70%	-44.30%	**-43.04%**
	Week3	-31.37%	-34.89%	-27.35%	**-31.20%**

4.3.2 Suspended solids phase

Similar analyzation was conducted for suspended solids samples as well. Cell counts decreases at the outflow were found for all the cases. The ICC values for both weeks averaged 0.29×10^5, 0.23×10^5, 0.21×10^5 cells/mL at the inlet of NRD, MCA and FCA loops, respectively. The corresponding outlet values were 0.24×10^5, 0.20×10^5, 0.09×10^5 cells/mL. Similarly, the average ATP values were 1.65, 1.30 and 1.04 ng/L at inflow, and 1.97, 1.34 and 0.78 ng/L at outflow. Generally, suspended solids-related bacteria only contribute to about 20% of the bulk water bacteria, calculated by ATP measurements. Free chlorine has proven its effectiveness in reducing cell counts in suspended solids to a large extent (with an average of 25%). As for the MCA condition, there is no clearly distinction of ATP variance from inflow to outflow (averaged 3% growth). And a more obvious growth in suspended solid phase can be detected in NRD loops (averaged 20%).





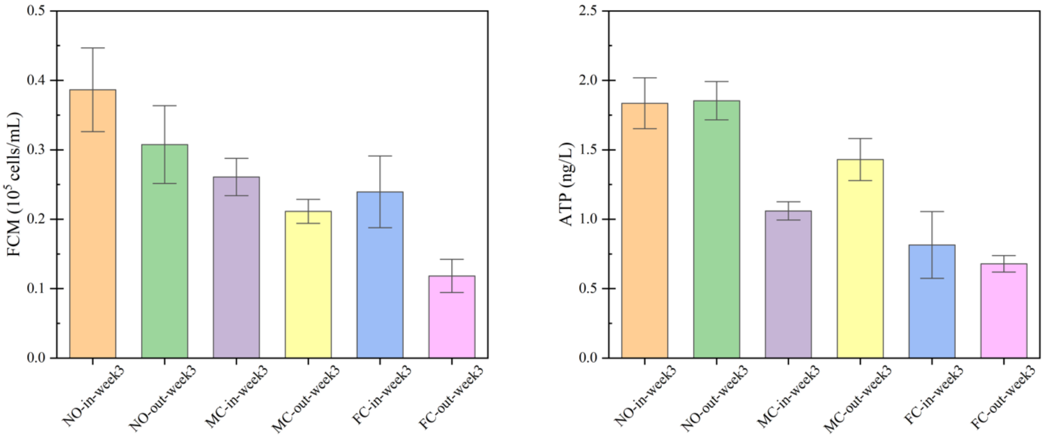


Figure 4.13 Suspended solids inlet and outlet samples measured cell counts and ATP

4.3.3 *Biofilm phase*

Combined both FCM and ATP results, a slightly growth of biofilm can be observed, especially for the NRD loops. However, it is too early to jump into any conclusion, since the values were still relatively very low. However, with the ATP results, it can be roughly concluded that the biofilm growth rates vary from three cases: NRD > MCA > FCA, with average growth ratios of 121%, 1.69% and 13.7%, respectively. While the large variance of biofilm sample measurement mainly comes from the sampling method. It is enviable to make contact with bulk water when scraping the inner surface of pipe wall.

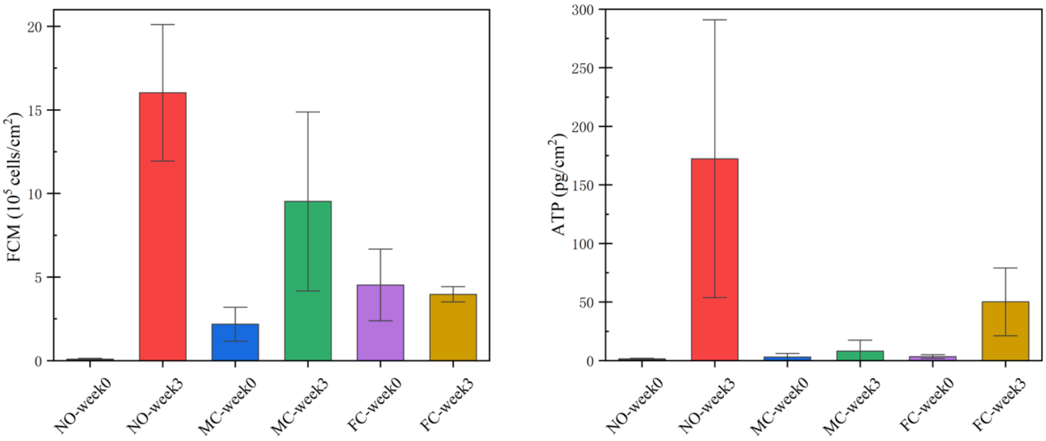


Figure 4.14 Biofilm samples measured cell counts and ATP

4.3.4 *Biofilm formation model*

a. **Parameter selection**

Among all the biofilm models, the increased complexity is necessary when meso-scale structure and microbial diversity are studied. While as only the biofilm thickness and growth rates were focused in this study, such complexity was not needed for the macroscale output. Hence, a few assumptions were made to describe a generic biofilm, as describe in chapter 3.2.3. By considering both heterotrophic and autotrophic bacteria, their growth, respiratory and inactivation processes were stated in table 3.2 as well as the stoichiometries. For the model input parameters, for example, the coefficients of different reactions, the diffusion rates of all substates, the yield and so on, more details can be found in Appendix E.

The definition of parameters was actually the assumption used in modelled processes, which could have significant influence on the numerical value. Since most of the parameters were selected from literature, tracking how those values were originally obtained and keeping consistent in the units would help deepen understanding of the model and select more suitable parameters. For example, the diffusion coefficients were defined by only considering diffusion in the liquid phase. While when the whole biofilm compartment diffusion is considered, a correction factor would normally be applied (Wanner & Reichert, 1996). As for kinetic parameters, the experimental derived rates were always higher than the true value due to the limitation of mass transfer, while only true values should be used in the model (Beccari et al., 1992).

Hence, when selecting the parameters, the definition of their origin needs to be carefully considered. Most of the values applied in this study were selected from similar researches on drinking water biofilms, while sensitivity analysis was hence implemented to investigate the influences of changing those values. The results are shown in Appendix F. Due to the low AOC concentration of water source, AOC acts as the limiting factor for heterotrophs, with most of the bacteria inside NRD and FCA are considered as heterotrophs. While the ammonia increase caused by monochloramine addition can promote the growth of autotrophs and gradually take a large proportion within the biofilm, hence ammonia was also considered as limiting substrate only for MCA biofilms. Overall, the influence of half-saturation concentration of AOC on heterotrophs is very significant. The impacts of variable changes can be fatal, considering the previous very thin thickness. The sensitivity analysis results for the MCA biofilms were more diverse, largely influenced by autotroph-related processes, instead of heterotrophs. This result can also be related with the MCA biofilm growth trend. With a very low growth rate at the beginning controlled by heterotrophs, then autotrophs started to grow when condition was optimal and gradually took the major part of the biofilm biomass.

b. Modelling results

By using AQUASIM to model the growth of biofilm under three conditions, the predicted biofilm growth is shown in figure below. The mature thickness of NRD, MCA and FCA biofilms are around 3.6, 7.6, and 2.4 μm, respectively. Three stages can be defined within 500 days. Stage one is the initial period, at which stage the effects of residual disinfectants heavily influence the formation potential of biofilms. Since free chlorine tends to be more effective in reducing the cells within both BW and SS, less planktonic cells would have the chance to attach on the pipe wall. Hence, the red line at the bottom grew much slower. As for monochloramine, it is not as sufficient as free chlorine in the direct reduction of cells. But it also showed the ability of inhibiting the biofilm growth for about 120 days. Then in the second stage, major changes started to happen. Firstly, the MCA biofilm outgrew NRD biofilm, with a much higher growth rate, and also lasting longer. Eventually, the MCA biofilm was nearly twice the thickness of NRD biofilm. On the contrary, the FCA biofilm maintained its slower growth rate, and the mature thickness was also less than NRD biofilm. Lastly, the maturation of MCA biofilm and FCA biofilm takes about 400 days, while it only takes more than 200 days for the NRD biofilm.

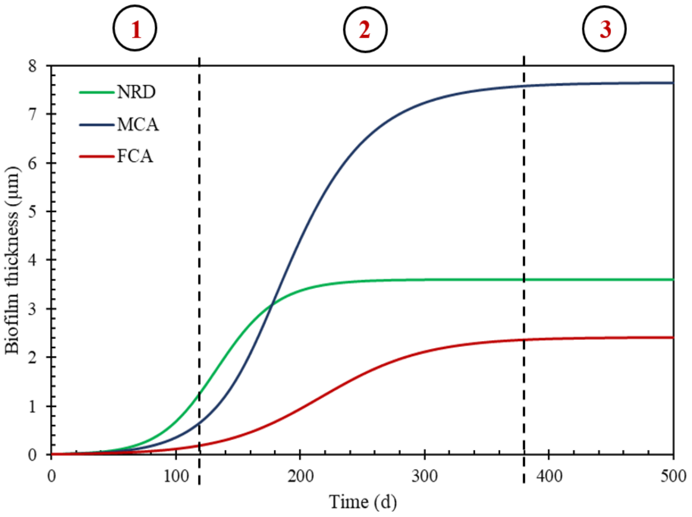


Figure 4.15 Biofilm modelling result

While another parameter to look into is the growth rates of biofilms. The computer model was continuously used to represent a period of 500 days. As shown in figure 4.16, the biofilm showed a higher growth rate when no residual disinfectant existing, up to 0.05 /d. On the other hand, both disinfectants performed well by inhibiting the biofilm growth in this early stage. While the growth rate of free chlorine applied biofilms was higher than monochloramine application within in the first 90 days, and both reached 0.004 /d. The ATP test result also verified this trend. Since the ATP values of FCA loops increased higher than MCA loops within three weeks. While after 90 days, a noticeable turning point of the growth rate between FCA and MCA showed up, the MCA biofilms started to shower a much higher growth potential, which explains the trends of biofilm thickness in stage 1. Among the three cases, NRD biofilms took the shortest time to reach the peak value of growth rate, followed by MCA and FCA biofilms. This trend also illustrated the time required for biofilm maturation.

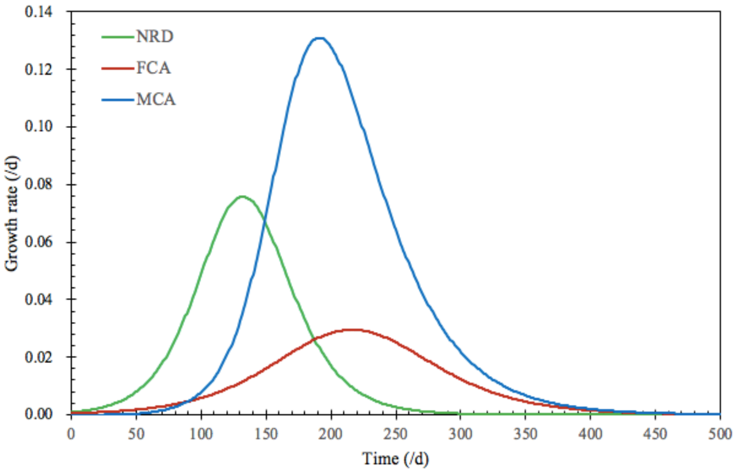


Figure 4.16 Growth rates of predicted biofilms

Due to the time constrain, the current observed biofilm growth was only very limited. No validation of the modelling output can be performed. Hence, it is necessary to continue the field sampling for a longer time, in order to verify the accuracy of the biofilm model. Nevertheless, this model also offers some new insights into the study. It is normally considered monochloramine would perform better in controlling biofilm growth, but the model output in this study gave the opposite. According to Michael (2019), biofilms with and without residual chloramine were compared. It is found that the chloramined biofilm had a relatively lower biomass as well as a lower alpha-diversity. However, the chloraminated DWDS was in the USA with an initial residual total chlorine concentration of 3.8 mg/L. Hence, the difference showed in this study is probably due to the low concentration of monochloramine. Since a lager concentration and longer contact time are commonly required for monochloramine to perform effectively as free chlorine.

While the impacts of free chlorine on biofilm formation is straightforward. The negative correlation of chlorine addition and biofilm formation has been studies, especially on developing biofilms (Chu et al., 2003). As time prolong, chlorine can also lead to selection and accumulation of certain resistance bacteria in both monochloramine and free chlorine applied loops. However, there is a study on the single-species biofilms showing that the chlorine resistance of biofilm basically had no relationship with the potential of biofilm formation (Zhu et al., 2020).

Chapter 5. Conclusions & Recommendations

5.1 Conclusions

The characteristics of the water quality and the potential of DBPs and biofilm formation without and with disinfectants (free chlorine and monochloramine) was systematically investigated in this study through a newly built pilot DWDS, conjoined with mathematical modelling.

Description of the concentration of free chlorine and monochloramine:

The concentration of free chlorine and total chlorine in the pilot system was described by both experimental tests and mathematical modelling. Firstly, the stability of free chlorine and monochloramine with high concentration in ultrapure water which was expected to serve as the stock solution for dosing into the pilot system was tested. The results showed that free chlorine was stable in ultrapure water at all concentrations (i.e., 0.3, 0.6, 1.1 and 1.6 g/L), with no significant variations observed over 10 days. For monochloramine, favourable stability was detected under low concentrations (i.e., 0.3 and 0.5 g/L) within 7 days, while slow decay was observed under higher concentrations (i.e., 0.7 and 1.5 g/L). Given the observed stability and practical concerns (e.g., the inconsistency of the dosing pumps), 0.3 g/L free chlorine and 0.25 g/L monochloramine were selected as the stock solution when dosing into the pilot.

Besides, the variations of the chlorine concentration in feed water over time was measured by bottle tests at two initial concentrations and three different temperatures. It was indicated that free chlorine decay at low (0.018 mg/L·h^{-1}) and high (0.209 mg/L·h^{-1}) initial concentrations was much faster than monochloramine decay (0.0044 and 0.0075 mg/L · h^{-1}, respectively). In addition, the decay rate of free chlorine and monochloramine was higher when the initial concentration was higher. Higher temperature also resulted in more rapid decay. The experimental data was simulated by using the first order, second order and parallel second order decay models in Aquasim, respectively. It was found that the parallel second order model was the optimal one to fit both free chlorine and monochloramine data, which could be applied to accurately describe the chlorine decay in feed water. The wall decay was also simulated, with a coefficient slightly over 0.1 mg/L·h^{-1}). Considering the real system of new PVC pipe material with pre-soaking in high concentration free chlorine, the wall decay was negligible in this study.

Therefore, after dosing free chlorine into the pilot DWDS, rapid decay occurred in the first few seconds as flowing through the static mixer (from 0.646 to 0.187 mg/L), and further chlorine decay was observed in the first part of the pilot system (from 0.187 to 0.093 mg/L). While the chlorine concentration did not reduce further in the second half. The chlorine demand mainly comes from the presence of ammonia and the formation of THMs. For monochloramine, the trend was slightly different, with less decay happened. It can only be noticed an obvious decay from 0.503 to 0.391 mg/L between the calculated dosing volume and the inflow concentration. After that, monochloramine can remain stable within the loop.

Impact on chemical water quality:

The impacts of the residual disinfectants on the water quality in perspective of chemical characteristics in our pilot DWDS were investigated. Unsurprisingly, no significant differences of conventional water quality parameters (i.e., pH, EC, DO, TOC, and HCO_3^-) were observed with and without disinfectants. However, due to the introduction of monochloramine, the context of N-related parameters, such as NH_4^+, were higher in monochloramine applied loops than others. Importantly, THMs (i.e., TCM, DBCM, BDCM) were only detected under free chlorine applied condition, with TCM (around 4.16 μg/L) being the dominant THM. While no THMs were detected in monochloramine applied system. It should be noted that the prediction of THMs formation in this study was mainly based on theoretical equation. A linear relationship between free chlorine demand and THMs concentration was generated. However, it is suggested that more efforts should be made to combine the practical data and theoretical model together to further calibrating the DBPs related model established in the current study.

Impact on microbial water quality:

The impacts of the residual disinfectants on the microbial features within bulk water, suspended solids and biofilm in the pilot system were evaluated simultaneously. Especially, the biomass in bulk water was represented by flow cytometer, while the biomass in suspended solids and biofilm was represented by ATP content only, considering the unreliability of performing FCM on non-liquid phase. Remarkably, free chlorine significantly reduced the intact cell counts in both bulk water (-37%) and suspended solids phase (-25%); while monochloramine maintained biomass at the same level from inlet to outlet (+0.4% in bulk water and +3% in suspended solids), compared to the regrowth within chlorine-free conditions (+3% in bulk water, +20% in suspended solids).

As expected, the measured biofilm formation in the first three weeks of operation was minor, especially with residual disinfectants. Specifically, the calculated average growth of biofilms by ATP measurement were in the order of no disinfectant (121%) > free chlorine (13.7%) > monochloramine (1.69%). To better perform the biofilm development over time under these three conditions, the biofilm model was implemented in Aquasim, with consideration of three main processes of growth, respiratory and inactivation within both heterotroph and autotroph. According to the sensitively analysis, it was indicated that the parameters directly related to the heterotroph growth or death had more impacts on the biofilm formation processes under no-chlorine and free chlorine conditions, with AOC considered as the limiting substrate. Noticeably, when monochloramine added into the system, more parameters needed to be addressed. Autotroph would start to grow and gradually contribute to a larger part of the total biomass. Hence, both heterotroph and autotroph were equally important, with AOC and NH_4^+ considered as limiting substrates.

Regarding to the optimized parameters, the biofilm formation under different conditions was immensely described in Aquasim. The results displayed that the biofilm growth rate was distinctive under different conditions. Especially, the biofilm growth rate under monochloramine condition was highest (maximum growth rate 0.13 /d), followed by the growth rate in chlorine-free condition (0.075 /d), and free chlorine condition (0.03 /d). The chlorine-free system took the shortest time to reach the plateau (120 d), followed by the monochloramine applied system (190 d), and the free chlorine applied system (210 d). Ultimately, a stable biofilm thickness was predicted after about 300 days, 3 μm for no disinfectant condition, 2 μm with free chlorine, and 7 μm with monochloramine.

5.2 Recommendations

For the future study, the following recommendations can be proposed:

Firstly, more ideas can be implemented into the chlorine decay bottle test. For example, the initial concentration can be set at much higher concentrations (i.e., 5 and 10 mg/L), and the time scale can be prolonged. Besides, it is always more convincing to repeat the experiments two or three times. In the meanwhile, the decay of chlorine can be connected with the formation of DBPs directly. Hence, for each bottle, it is advised to not only analyze the chlorine content, but also the concentrations of several selected DBPs. In this way, a more detailed DBPs predictive model can be established.

Secondly, the current focused DBPs was mainly THMs, which has also been widely studied. THMs was only detected in free chlorine added loops in this study, which proved that monochloramine can reduce DBPs formation. However, there are certain N-DBPs, which only formed with the presence of chloramine. The concentrations of N-DBPs are normally very low, but the risks are higher than THMs. As an emerging concern of higher toxicity, it would complete the study by also including the formation of N-DBPs into consideration.

Next, the flow cytometer was specifically designed to count the single cells in water phase. It is possible to use it for the suspended solid samples and biofilm samples. But the pre-treatment is very important. The results in this study were not 100% reliable, mainly due to the last step dilution and the efficiency of ultra-sonification. Hence, the design of flow cytometer preparation methods can be improved with another ultra-sonic method or adding a filtration step before testing the samples instead of diluting.

Lastly, one more recommendation is about the morphology study of biofilms. During the preliminary experiments, both CLSM and OCT have been tested. Different dyes can be used to represent different proportions of biofilm structure and visualized by CLSM. For example, three stains (SYTO 63, Fluorescein-5-isothiocyanate and Concanavalin A tetramethyl-rhodamine) can be applied at the same time, but dye cells, proteins and carbohydrates, separately. As for the OCT measurement, its major advantage is the no pre-treatment needed, in which way the morphology would not be destroyed. But the minimum thickness requirement for OCT is 2 mm, indicating that the use of OCT is only useful when a mature biofilm (> 2 mm) has developed.

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Appendix A. Sampling Program

Table A. 1 Detailed sampling schedule

			0-1	0-2	0-3	1-1	1-2	1-3
Date			01.09	08.09	15.09	22.09	29.09	06.10
Bulk Water	**ATP**	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	HCO_3^-, NO_3^-, NO_2^-, NH_4^+	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	TOC	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	Elements	Feed	-	2	2	-	2	2
		Outflow	3	3	3	3	3	3
	DBPs	Feed	-	2	2	-	2	2
		Outflow	3	3	3	3	3	3
	T, EC, pH, DO	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	Cell number	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	DNA extraction	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
Suspended Solids	**ATP**	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	Elements	Feed	-	2	2	-	2	2
		Outflow	3	3	3	3	3	3
	Cell number	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
	DNA extraction	Feed	-	2	2	-	2	2
		Inflow	3	3	3	3	3	3
		Outflow	3	3	3	3	3	3
Biofilm	**ATP**		6	6	6	6	6	6
	Cell number		6	6	6	6	6	6
	DNA extraction		3	3	3	3	3	3
Measurement places			Vitens		On site		Water Lab	

Appendix B. Bottle test results

Table A. 2 Low concentration free chlorine decay

Time interval (h)	0	0.0833	0.1667	0.25	0.5	1	3	5
Ultrapure water	0.102		0.105			0.101	0.83	0.85
Feed water from pilot system	0.099	0.06	0.05	0.04	0.02	0.02	0.01	0.01

Table A. 3 High concentration free chlorine decay

Water Type	Time interval (min)											T	Time interval (h)			
	0	2	4	5	6	8	10	15	30	45	60		120	180	300	420
Ultrapure water	0.93						0.91				0.91	4		0.81	0.80	0.82
												14		0.89	0.78	0.72
												24		0.85	0.83	0.84
Feed water from pilot system	1.08	0.91	0.85	0.78	0.73	0.66	0.62	0.52	0.37	0.28	0.20	4	0.08	0.06	0.05	0.04
												14	0.08	0.06	0.03	0.02
												24	0.08	0.04	0.03	0.01

Table A. 4 Low concentration monochloramine decay

Water Type	Time interval (min)					T	Time internal (h)							
	0	5	10	15	30		1	3	6	16	22	30	42	78
Ultrapure water	0.42	0.41	0.41	0.41	0.41	4	0.4	0.37	0.38	0.38	0.39	0.34	0.4	0.33
						14	0.41	0.39	0.4	0.36	0.41	0.35	0.34	0.3
						24	0.4	0.41	0.4	0.36	0.34	0.32	0.34	0.31
Feed water from pilot system	0.45	0.44	0.44	0.41	0.42	4	0.41	0.37	0.37	0.28	0.23	0.21	0.23	0.21
						14	0.41	0.37	0.34	0.27	0.21	0.18	0.16	0.09
						24	0.4	0.34	0.32	0.21	0.17	0.14	0.13	0.08

Table A. 5 High concentration monochloramine decay

Water Type	Time interval (min)					T	Time internal (h)							
	0	**5**	**10**	**15**	**30**		**1**	**3**	**6**	**16**	**22**	**30**	**42**	**78**
Ultrapure water	1.5	1.49	1.48	1.48	1.49	4	1.49	1.48	1.48	1.36	1.43	1.38	1.37	1.26
						14	1.48	1.41	1.44	1.38	1.39	1.32	1.24	1.18
						24	1.49	1.48	1.49	1.36	1.38	1.24	1.18	1.1
Feed water from pilot system	1.43	1.44	1.41	1.4	1.4	4	1.35	1.33	1.26	1.21	1.18	1.09	1.1	0.98
						14	1.37	1.3	1.26	1.16	1.08	1.02	0.93	0.82
						24	1.36	1.28	1.23	1.1	0.89	0.94	0.9	0.75

Appendix C. Field sampling data

Table A. 6 Conventional water quality parameters

			pH	EC (µS/cm)	DO (mg/L)
NRD	in	week0	8.28	763.00	9.09
		week3	8.09	756.67	9.16
	out	week0	8.19	762.67	9.34
		week3	8.10	759.00	9.45
MCA	in	week0	8.09	764.00	9.31
		week3	8.08	765.00	8.90
	out	week0	8.08	765.00	9.57
		week3	8.06	764.00	9.05
FCA	in	week0	8.12	751.33	9.26
		week3	8.10	772.00	8.99
	out	week0	8.09	752.33	9.48
		week3	8.13	771.33	9.13

Table A. 7 Experimental data of FCM and ATP tests on bulk water samples

			FCM ($\times 10^5$ cells/mL)	ATP (ng/l)
NRD	in	week0	4.670	7.985
		week3	5.677	5.899
	out	week0	4.807	7.747
		week3	5.665	6.092
MCA	in	week0	4.870	5.777
		week3	5.114	6.079
	out	week0	4.890	5.042
		week3	5.000	5.905
FCA	in	week0	4.159	4.132
		week3	4.074	4.200
	out	week0	2.610	5.178
		week3	3.388	3.409

Table A. 8 Experimental data of FCM and ATP tests on suspended solid samples

			FCM ($\times10^5$ cells/mL)	ATP (ng/l)
NRD	in	week0	0.1977	1.4592
		week3	0.3864	1.8350
	out	week0	0.1760	2.0833
		week3	0.3074	1.8533
MCA	in	week0	0.2002	1.5433
		week3	0.2608	1.0600
	out	week0	0.1978	1.2450
		week3	0.2113	1.4300
FCA	in	week0	0.1712	1.2683
		week3	0.2394	0.8150
	out	week0	0.0522	0.8740
		week3	0.1183	0.6783

Table A. 9 Experimental data of FCM and ATP tests on suspended solid samples

		FCM ($\times10^5$ cells/mL)	ATP (ng/l)
NRD	week0	0.091	1.409
	week3	16.03	172.36
MCA	week0	2.182	3.041
	week3	9.526	8.175
FCA	week0	4.529	3.413
	week3	3.971	50.17

Appendix D. FCM original data

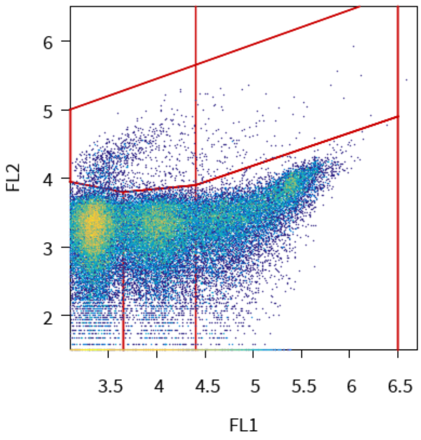
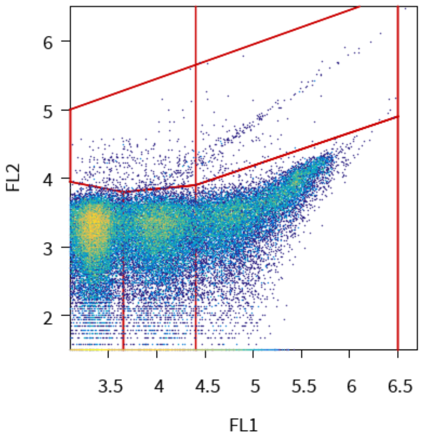
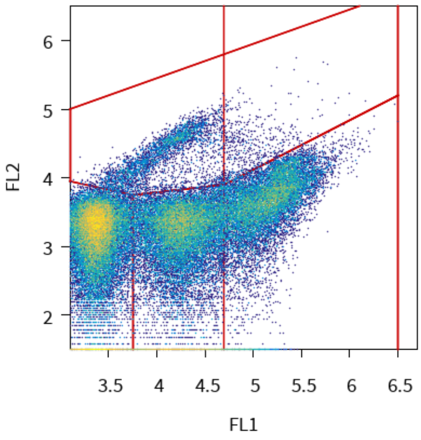
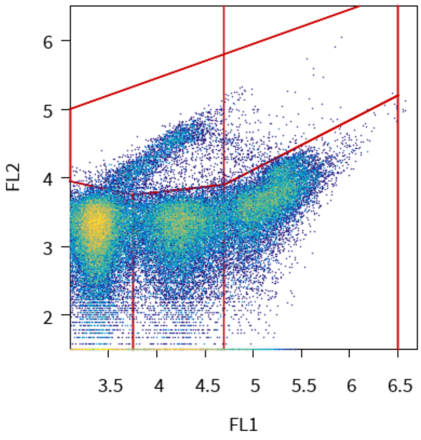
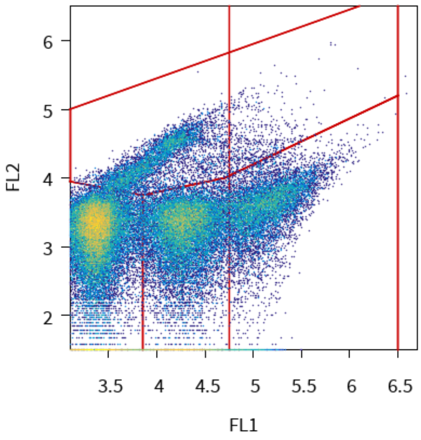
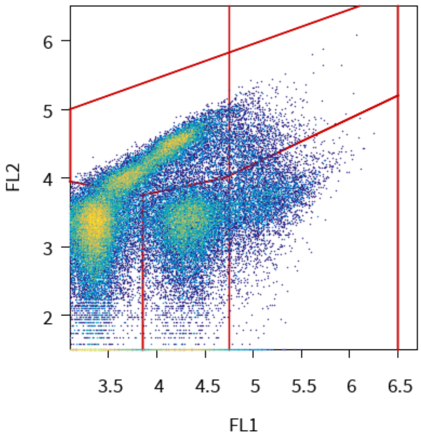
Table A. 10 The original data of FCM tests

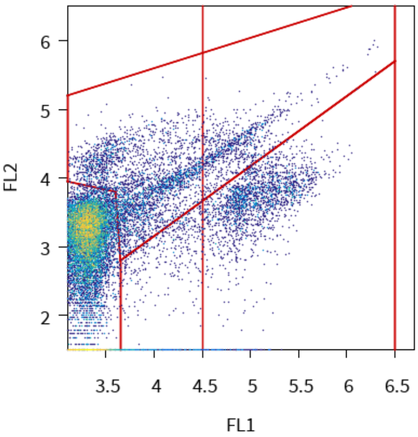
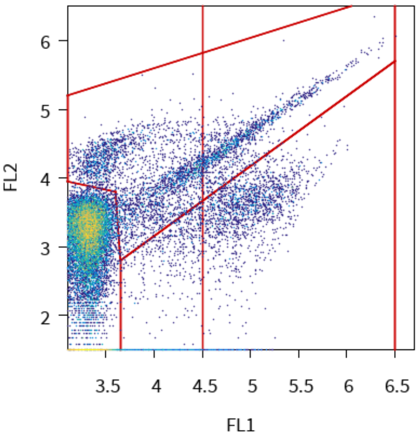
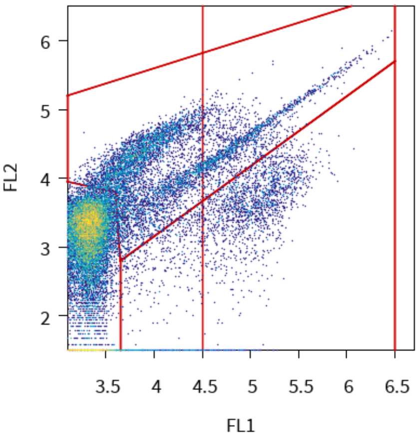
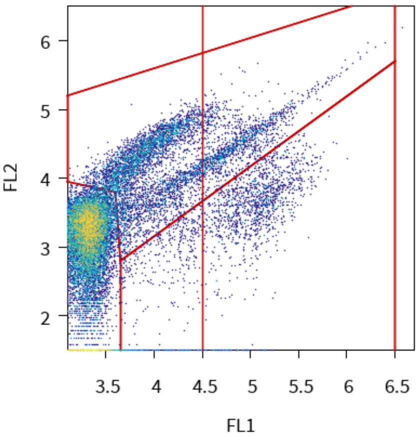
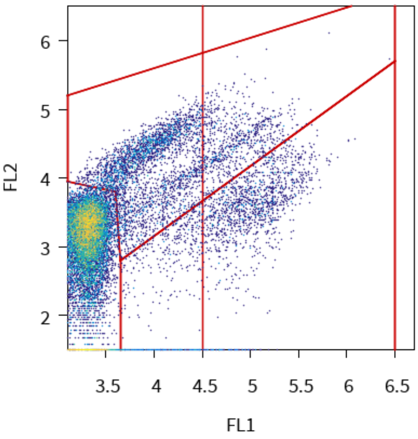
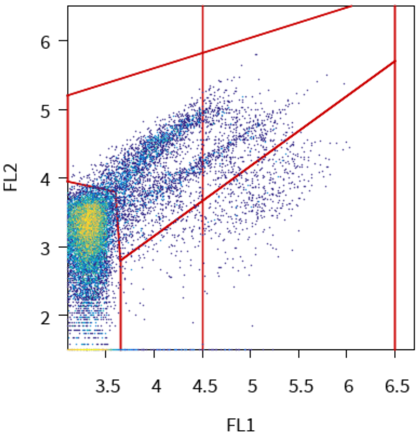
Name	ICC per mL	TCC per mL	HNAP
1-in-0831	431488	445400	40.77%
2-in-0831	441088	446366	41.68%
3-in-0831	457100	464355	42.35%
1-out-0831	460444	468688	42.06%
2-out-0831	468188	490577	41.51%
3-out-0831	464311	474400	42.14%
1-bf-0901	13677	2159866	44.41%
2-bf-0901	3833	1559133	17.08%
3-bf-0901	30344	496677	8.97%
1-in-ss-0901-100	11711	73011	76.53%
2-in-ss-0901-100	21933	55477	75.33%
3-in-ss-0901-100	26811	62855	72.56%
1-out-ss-0901-100	28033	73433	68.89%
2-out-ss-0901-100	18655	51700	67.36%
3-out-ss-0901-100	23711	63177	68.55%
Sterile water -0908	1533	4633	49.25%
Feed-1-0908	454066	484244	36.08%
Feed-2-0908	484844	562955	36.34%
4-out-0908	497455	524980	33.85%
5-out-0908	476066	511278	33.94%
6-out-0908	480888	513744	33.55%
4-in-0908	496507	575188	32.42%
5-in-0908	471057	532755	32.52%
6-in-0908	470633	527366	33.71%
Feed-ss-1-0909-100	22533	73044	71.60%
Feed-ss-2-0909-100	26411	89866	73.45%
4-in-ss-0909-100	17488	60311	71.28%
5-in-ss-0909-100	34288	123066	71.26%
6-in-ss-0909-100	28288	107711	72.07%
4-out-ss-0909-100	32111	116933	71.07%
5-out-ss-0909-100	24900	100111	69.97%
6-out-ss-0909-100	22122	88022	72.53%
4-bf-0909-100	3577	231588	11.18%
5-bf-0909-100	1744	202811	19.09%

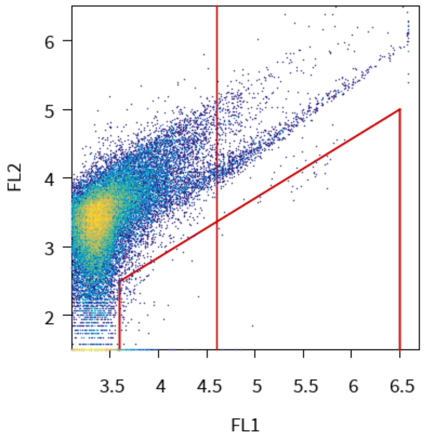
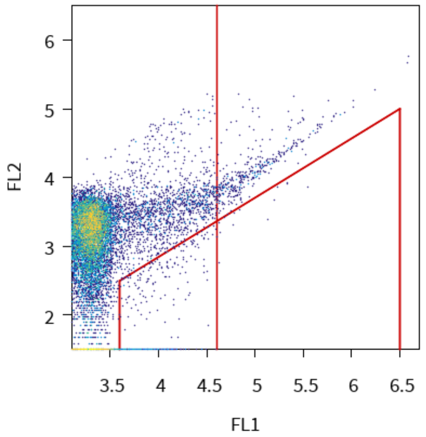
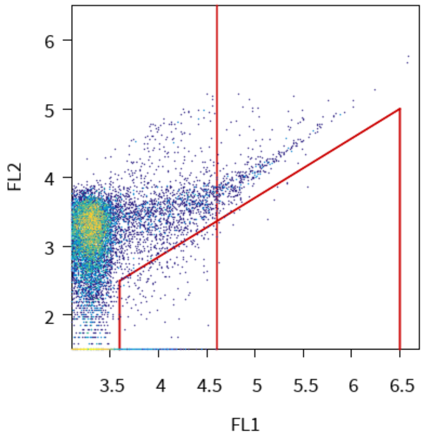
6-bf-0909-100	1660	201170	21.45%
Sterile-0917	3222	6544	94.82%
Feed-1-0917	443344	482955	37.49%
Feed-2-0917	467911	511488	38.06%
7-out-0917	426500	472011	25.47%
8-out-0917	432196	461555	23.26%
9-out-0917	420120	458277	23.03%
7-in-0917	251155	503988	34.94%
8-in-0917	243344	495709	33.37%
9-in-0917	234022	495395	33.25%
9-bf-0917-100	3611	164177	16.28%
8-bf-0917-100	1333	168633	24.98%
7-bf-0917-100	2302	400491	30.10%
Feed-ss-1-0917-100	35500	103233	70.80%
Feed-ss-2-0917-100	37933	117544	72.08%
7-in-ss-0917-100	21911	64600	72.41%
8-in-ss-0917-100	25055	85566	71.66%
9-in-ss-0917-100	21522	73055	73.52%
7-out-ss-0917-100	8163	49838	69.89%
8-out-ss-0917-100	7831	41755	64.53%
9-out-ss-0917-100	4877	27311	65.14%
Sterile	1677	4066	17.17%
Feed-1-0922	452566	495788	36.82%
Feed-2-0922	490788	535055	37.19%
1-out-0922	493266	544122	37.48%
2-out-0922	487955	546011	37.44%
3-out-0922	490888	539766	37.66%
1-in-0922	500066	533322	37.56%
2-in-0922	498200	531066	37.86%
3-in-0922	493155	536422	37.82%
1-bf-0922-100	5000	195766	44.00%
2-bf-0922-100	3022	199011	40.80%
3-bf-0922-100	4800	215288	32.63%
1-in-ss-0922-100	51888	130322	71.82%
2-in-ss-0922-100	59366	158600	74.88%
3-in-ss-0922-100	43322	126400	74.04%
1-out-ss-0922-100	46557	166700	75.37%
2-out-ss-0922-100	43922	132088	75.54%
3-out-ss-0922-100	32500	108055	73.47%
Sterile water	177	4711	24.86%
Feed-1-0929	475433	524033	36.23%

Feed-2-0929	493266	539211	36.09%
4-out-0929	500877	547011	36.92%
5-out-0929	496488	541655	36.84%
6-out-0929	498422	544944	37.01%
4-in-0929	490733	536933	36.44%
5-in-0929	492300	542777	36.27%
6-in-0929	484824	538045	35.46%
4-bf-0929-100	1133	144300	6.80%
5-bf-0929-100	2500	180222	4.00%
6-bf-0929-100	3988	190566	3.89%
Feed-ss-1-0929-100	26722	80422	73.76%
Feed-ss-2-0929-100	32944	101755	71.30%
4-in-ss-0929-100	30977	103855	74.00%
5-in-ss-0929-100	38077	121022	72.75%
6-in-ss-0929-100	35277	125644	72.16%
4-out-ss-0929-100	26815	106740	72.58%
5-out-ss-0929-100	30833	106900	72.00%
6-out-ss-0929-100	26888	94577	70.79%
Sterile-1006	388	2366	39.95%
Feed-1-1006	407344	437933	32.78%
Feed-2-1006	435153	467950	32.30%
7-out-1006	269988	454911	21.89%
8-out-1006	263622	438322	21.60%
9-out-1006	303722	454500	23.16%
7-in-1006	393388	470733	29.90%
9-in-1006	404888	459488	29.39%
8-in-1006	418066	464788	30.63%
7-bf-1006-100	1200	154322	16.67%
8-bf-1006-100	1000	155077	18.80%
9-bf-1006-100	977	162133	11.36%
Feed-1-ss-1006-100	28911	72344	74.83%
7-in-ss-1006-100	31064	112557	70.50%
8-in-ss-1006-100	25509	82313	72.26%
9-in-ss-1006-100	39200	114911	71.40%
7-out-ss-1006-100	16471	88554	59.81%
8-out-ss-1006-100	12311	67666	57.94%
9-out-ss-1006-100	18544	88097	58.77%

Table A. 11 ICC figures under different cases

		IN	OUT
BW	**NRD**		
	MCA		
	FCA		

SS	**NRD**		
	MCA		
	FCA		

BF	**NRD**	
	MCA	
	FCA	

Appendix E. Parameter Selection of Biofilm Model

Table A. 12 Parameters used in the biofilm model

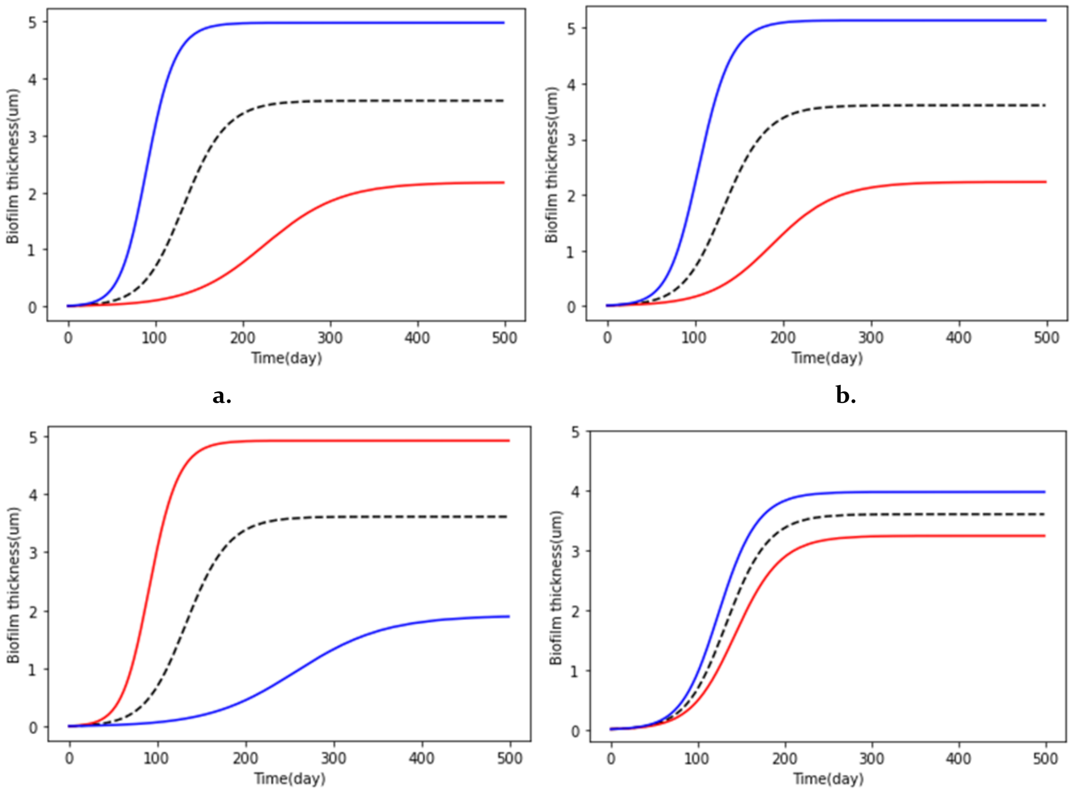
Variables	Description	Units	Value	Reference
b_Aut	Respiratory rate of autotrophic bacteria	d^{-1}	0.03	1
b_Het	Respiratory rate of heterotrophic bacteria	d^{-1}	0.32	1
b_InaAut	Inactivation rate of autotrophic bacteria	d^{-1}	0.03	1
b_InaHet	Inactivation rate of heterotrophic bacteria	d^{-1}	0.08	1
D_Aut	Diffusivity of autotrophic bacteria in bulk water	m^2/d	0.000285	1
D_C	Diffusivity of AOC in bulk water	m^2/d	0.0001	1
D_Het	Diffusivity of heterotrophic bacteria in bulk water	m^2/d	0.000285	1
D_N	Diffusivity of N in bulk water	m^2/d	0.00017	1
D_O	Diffusivity of DO in bulk water	m^2/d	0.0002	1
kax	Bacterial attachment coefficient	d^{-1}	0.1	2
kdx	Bacterial detachment coefficient	d^{-1}	0.4	2
K_Chet	Half-saturation concentration of AOC on heterotrophs	g/m^3	4	1
K_Naut	Half-saturation concentration of N on autotrophs	g/m^3	1	1
K_Oaut	Half-saturation concentration of DO on autotrophs	g/m^3	0.5	1
K_Ohet	Half-saturation concentration of DO on heterotrophs	g/m^3	0.2	1
LL	Thickness of biofilm boundary layer	m	0.00000001	-
mue_Aut	Maximum autotrophic bacteria growth rate	d^{-1}	4.17	1
mue_Het	Maximum heterotrophic bacteria growth rate	d^{-1}	9.52	1
Qin	Water inflow	m^3/d	3.5	-
rho_Aut	Autotrophic bacterial density in bulk water	g/m^3	0.0001	1
rho_Het	Heterotrophic bacterial density in bulk water	g/m^3	0.0001	1
Y_Aut	Yield of autotrophic bacteria	-	0.24	1
Y_Het	Yield of heterotrophic bacteria	-	0.63	1

References: 1. (Pérez et al., 2005) 2. (Jegatheesan et al., 2000)

Appendix F. Sensitivity analysis of biofilm models

Sensitivity analysis for NRD biofilm:

Due to the low AOC concentration of water source, AOC acts as the limiting factor for heterotrophs. For the NRD biofilms, most of the bacteria inside are heterotrophs. Hence the influence of half-saturation concentration of AOC on heterotrophs is very significant, considering the previous very thin thickness. By increasing 10%, the growth rate became slower, and the mature thickness was only about 2 µm. While a 10% decrease promoted the mature thickness to 5 µm, and also shorten the time. Similar situations happen with respiratory rate of heterotrophic bacteria, only the time needed for biofilm maturation was shorter when 10% increase applied in its original value. In the meanwhile, the influence of maximum heterotrophic bacteria growth rate was also very similar but with opposite direction. Lastly, the influence of heterotroph inactivation rate was relatively smaller.



a. b.

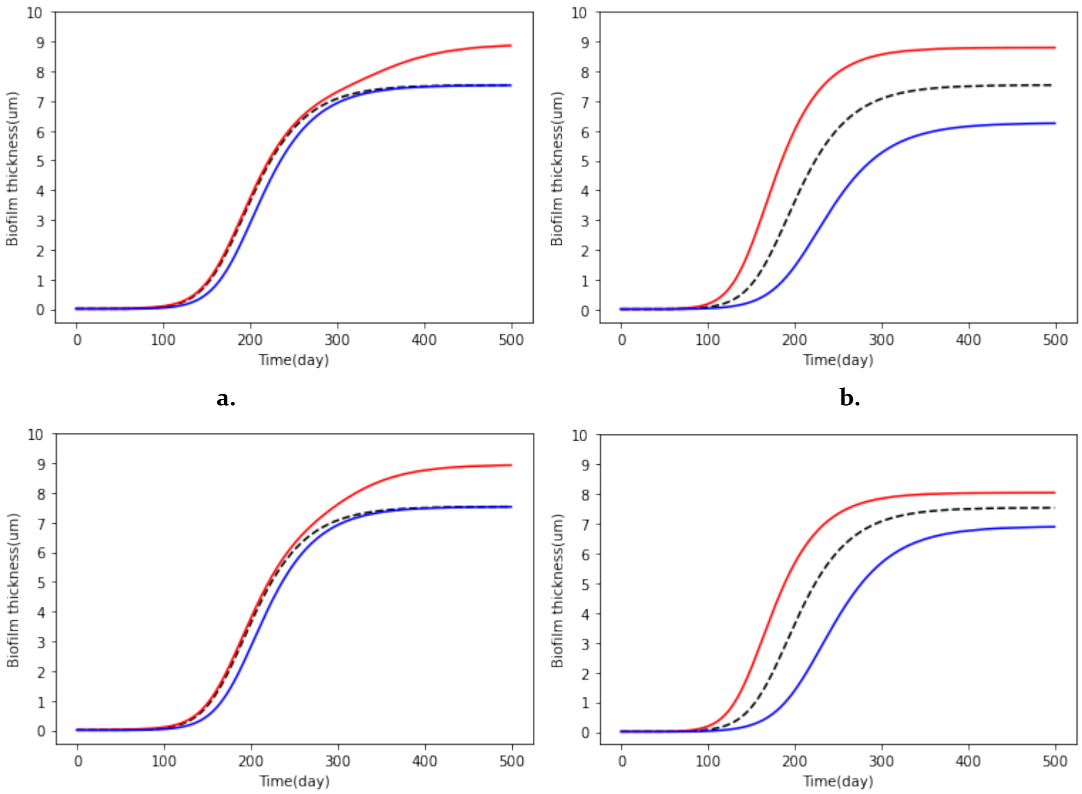
c. d.

Figure A. 1 Sensitivity analysis of no residual disinfectant biofilm

(a. Half-saturation concentration of AOC on heterotrophic bacteria, b. Respiratory rate of heterotrophic bacteria, c. Maximum heterotrophic bacteria growth rate, d. Inactivation rate of heterotrophic bacteria)

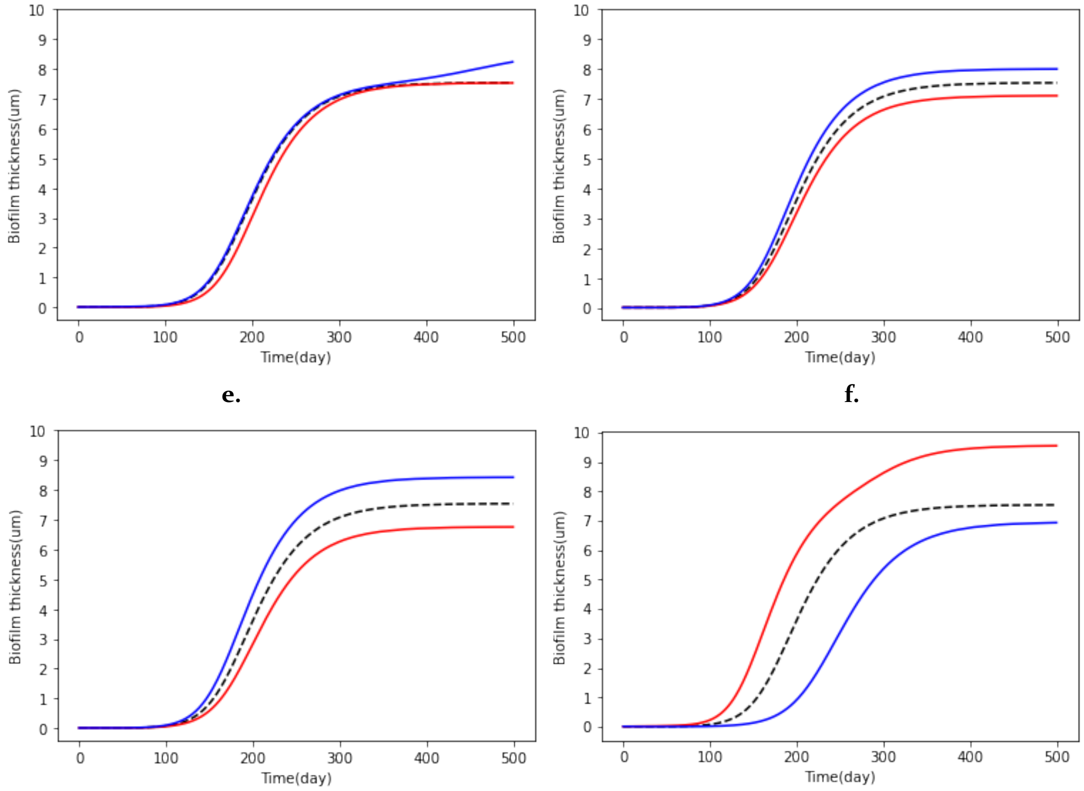
<u>Sensitivity analysis for MCA biofilm:</u>

The sensitivity analysis results for the MCA biofilms were more diverse. Since autotrophs also play an important role in the chloramine applied biofilm. The changes of autotroph-related parameters also presented large influence. From figure A.2-a, c and e, it can be seen that the when the condition became better for heterotroph growth, the new line did not change much at the first 250 days, but it showed the potential of keep growing until 500 days with a smaller growth rate. While when the condition became less favourable, the biofilm would also reach the original mature thickness, but took longer time. As for the other parameters, the influences were more straightforward.



a. **b.**

c. **d.**



e. f.

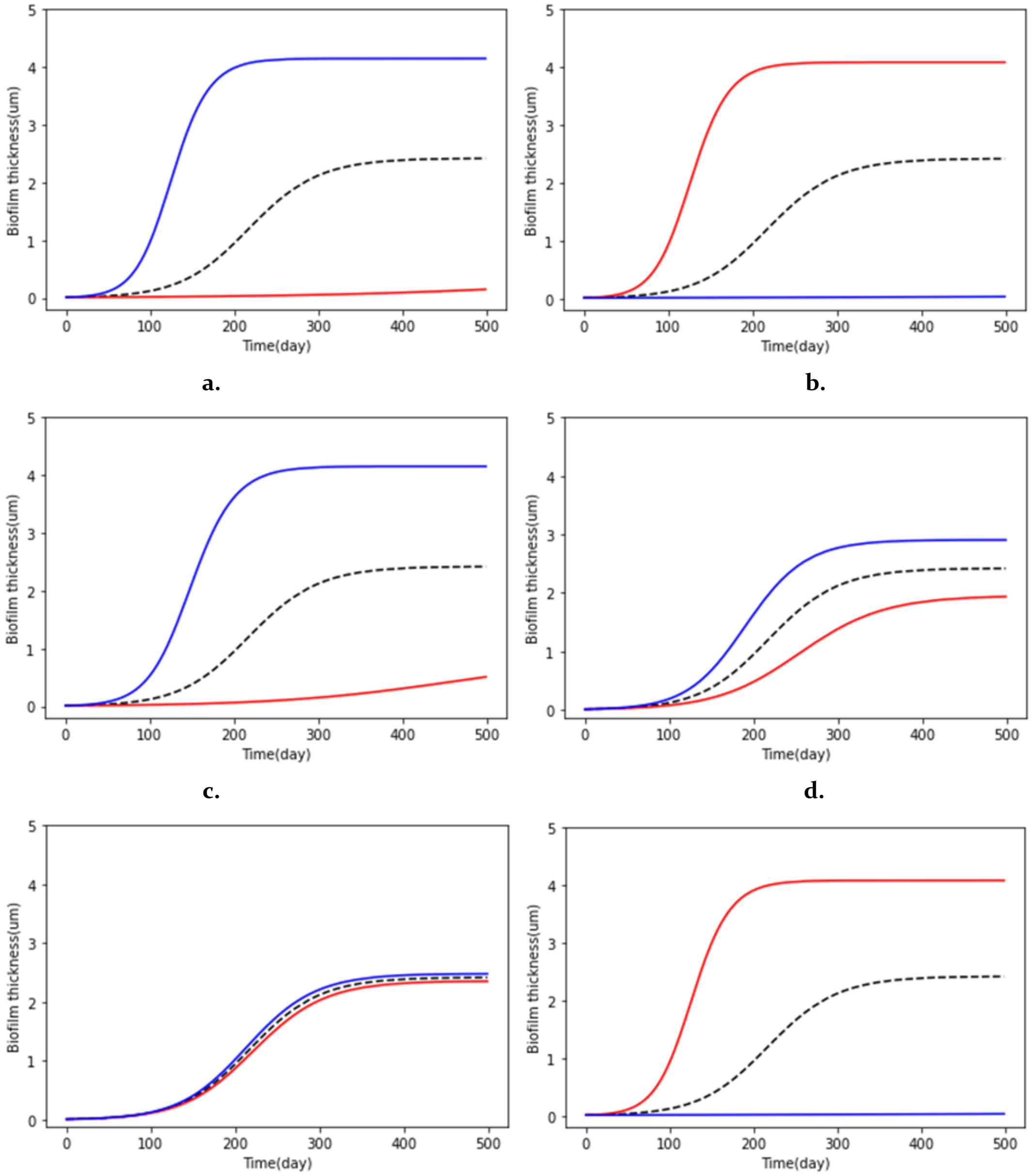
g. h.

Figure A. 2 Sensitivity analysis of monochloramine applied biofilm

(a. Half-saturation concentration of AOC on heterotrophic bacteria, b. Half-saturation concentration of NH_3 on autotrophic bacteria, c. Maximum heterotrophic bacteria growth rate, d. Maximum autotrophic bacteria growth rate, e. Respiratory rate of heterotrophic bacteria, f. Respiratory rate of autotrophic bacteria, g. Inactivation rate of autotrophic bacteria, h. chloramine caused inactivation)

Sensitivity analysis for FCA biofilm:

Considering the existing very low thickness of FCA biofilm, the impacts of variable changes can be fatal. As is shown in figure A.3-a, b, c and f, the biofilm showed little growth, when the cases became worse. Especially in b and f, basically no growth within 500 days. On the other hand, when the condition become optimal, the biofilm maturation took shorter time, and formed double thickness as before. All these four parameters were directly linked with the bacterial growth or death. While for the other two parameters related to bacteria inactivation. The influences became smaller.



a.

b.

c.

d.

e.

f.

Figure A. 3 Sensitivity analysis of free chlorine applied biofilm

(a. Half-saturation concentration of AOC on heterotrophic bacteria, b. Maximum heterotrophic bacteria growth rate, c. Respiratory rate of heterotrophic bacteria, d. Inactivation rate of heterotrophic bacteria, e. chlorine caused inactivation, f. chlorine inhibited growth)

Appendix G. Preliminary Experiments

1. Comparisons of DNA concentration and purity based on different extraction methods

Two methods are used to extract DNA from drinking water. 10 L tap water was directly collected from Water Lab, and then filtered through 0.22 μm membrane. The membrane collected microorganisms from bulk water. From Tab.1, it is clear that the modified DNeasy PowerSoil Kit (MDPK) gave a better DNA concentration than Fast DNA Spin Kit (FDSK). As for the purity, A260/A280 ratio is the premier measure of nucleic acid purity, a ratio of 1.8 is normally regarded as 'pure' DNA. In this case, both methods are not able to give a high A260/A280 ratio, while FDSK performs slightly better than MDPK. Although the concentration is not high enough, this can be compensated by an appropriately increase of water volume. As for a mature commercial kit, FDSK can be easily operated, and also show a better repeatability. Overall, FDSK is selected for the following experiments.

Table A. 13 The results of different DNA extraction methods

DNA extraction method	DNA concentration (ng/μL)	A260/A280 ratio	A260/A230 ratio
Fast DNA Spin Kit	2.03	1.4	0.08
	3.55	1.4	0.33
	2.46	1.45	0.28
	3.35	1.38	0.15
Ameet Modified DNeasy PowerSoil Kit	8.06	1.3	0.89
	23.4	1.23	0.9
	13.9	1	0.91
	18.2	1.41	0.73

2. Effects of chlorine addition

In the pilot system, free chlorine and monochloramine will be added. The residence time of disinfectant is about 5 minutes. Sodium thiosulphate ($Na_2S_2O_3$) is used to quenching chlorine. It is important to investigate how the chlorine addition will influence the DNA extraction results. Apparently, the addition of free chlorine into the same volume of tap water significantly reduced the DNA concentrations, when the water is filtered through 0.22 μm membrane. Table 2 also shows that free chlorine might not be very effective on the removal of suspended solids-related microorganisms, with only a slight decrease of DNA concentration.

Table A. 14 The comparisons of unchlorinated and chlorinated tap water

Membrane pore size (um)	**Water volume (L)**	**Chlorine addition**	**Chlorine quenching**	**DNA concentration (ng/μL)**	**A260/A280 ratio**	**A260/A230 ratio**
0.22	20	/	/	21	1.46	0.22
		/	/	26.6	1.51	0.26
		free chlorine	$Na_2S_2O_3$	4.63	1.58	0.34
		free chlorine	$Na_2S_2O_3$	4.05	1.75	0.26
1.2	30	/	/	23.4	1.49	0.16
		/	/	24	1.54	0.4
		free chlorine	$Na_2S_2O_3$	19.2	1.56	0.12
		free chlorine	$Na_2S_2O_3$	17.5	1.51	0.24

3. Optimizing filtered water volume

After DNA extraction, the following sequencing processes requires a minimum concentration of above 20 ng/μL. More water volume means higher concentration, but also longer filtration time. So, it is rather crucial to determine the least needed water volume. As shown in table 3, when the volume of chlorined water has been increased to 60 L, the DNA concentrations appears to be sufficient.

Table A. 15 The results of different filtered water volume

Membrane pore size (μm)	**Filtered water volume (L)**	**Water filtration time (h)**	**Disinfectant addition**	**Chlorine quenching**	**DNA concentration (ng/μL)**
0.22	20	2.5	free chlorine	$Na_2S_2O_3$	4.63
	20	2.5			4.05
	30	3.5			10.3
	40	4.5			9.41
	40	4.5			12
	60	7			27.7
	60	7			29.6

4. Effects of chlorine quenching

As mentioned above, the residence time of disinfectant is short, while water filtration takes much longer time. If chlorine is not quenched, it means the disinfection time is prolonged, and might keep reducing the DNA concentration. The comparison result is shown below. It proves that chlorine quenching is needed in this case. Otherwise, the residence time will be different.

Table A. 16 The comparisons of chlorinate quenching and no quenching

Membrane pore size (μm)	**Filtered water volume (L)**	**Disinfectant addition**	**Chlorine quenching**	**DNA concentration (ng/μL)**
0.22	60	free chlorine	$Na_2S_2O_3$	27.7
			$Na_2S_2O_3$	29.6
			/	16.9
			/	20.4

5. DNA extraction trials of water from Kamerik

For the above results, all the DNA extractions used tap water from Water Lab. However, water quality of different places is also a major influence of the results. It is more accurate to repeat Step 3, 4 and 5 using water collected from Kamerik. From Tab5, there is no big variations between different scenarios. It is due to the high chlorine consumption of this type water. The same amount of free chlorine stock solution was added, but it could not maintain 0.1 mg/L after 5 min. The results also gave a high DNA concentration than using the tap water from Water Lab, which means less volume will be needed.

Table A. 17 The DNA extraction results of three different scenarios

Membrane pore size (μm)	**Filtered water volume (L)**	**Disinfectant addition**	**Chlorine quenching**	**DNA concentration (ng/μL)**
0.22	30	/	/	49.3
		/	/	53
		free chlorine	/	55
		free chlorine	$Na_2S_2O_3$	44.3
		free chlorine	$Na_2S_2O_3$	53

Then, a similar experiment was carried out, with carefully examination of the residual disinfectant concentrations. The results listed below give several important information. Firstly, 15 L water seems to be enough for all the cases, even with chlorine added. Secondly, chlorine quenching has more effects on free chlorine than monochloramine. Since monochloramine requires a much longer residence time, it would not change a lot within this short time. It also arouses the problem that the addition of monochloramine might not be effective. Finally, the repeatability of all results is rather poor.

Table A. 18 The DNA extraction results of five different scenarios

Membrane pore size (μm)	**Filtered water volume (L)**	**Disinfectant addition**	**Chlorine quenching**	**DNA concentration (ng/μL)**
0.22	15L	/	/	26.9
	15L	/	/	33.1
	15L	0.1mg/L free chlorine	$Na_2S_2O_3$	18.2
	15L	0.1mg/L free chlorine	$Na_2S_2O_3$	24.6
	15L	0.1mg/L free chlorine	/	15.4
	15L	0.1mg/L free chlorine	/	19.7
	15L	0.4 mg/L monochloramine	$Na_2S_2O_3$	22.9
	15L	0.4 mg/L monochloramine	$Na_2S_2O_3$	38.7
	15L	0.4 mg/L monochloramine	/	28.1
	5L	0.4 mg/L monochloramine	/	6.41

The water volume is slightly increase to 20 L, to reduce the error caused by dividing 5 L water from a 10 L bucket. Because there might be more particles settled down at the bottom of the bucket and cause the second round 15 L giving a higher DNA concentration. 1.2 μm membrane was also studied, but the results are rather strange. The reason behind it is still unknown, probably due to operation errors.

Table A. 19 The DNA extraction results of different membrane pore sizes

Membrane pore size (μm)	**Filtered water volume (L)**	**Disinfectant addition**	**Chlorine quenching**	**DNA concentration (ng/μL)**	
0.22	20	/	/	36.4	30.9
		/	/	37.8	28
		0.1mg/L free chlorine	$Na_2S_2O_3$	34.1	29.2
		0.1mg/L free chlorine	$Na_2S_2O_3$	22.6	28.4
		0.4mg/L monochloramine	$Na_2S_2O_3$	37.2	30
1.2	30	/	/	11.8	10
		0.1mg/L free chlorine	$Na_2S_2O_3$	30.2	29.6

6. CLSM photos of biofilm coupon

Sample preparation: The first step is to cut the coupon into small pieces (about 1cm *1 cm). Outside and edges of samples can be cleaned with 70% ethanol, and the inner side is rinsed with 0.8% saline.

Sample staining: By staining different substances with different colours, it will be clear to distinguish from cells to EPS. Three stains (SYTO 63, Fluorescein-5-isothiocyanate (FITC) and Concanavalin A tetramethyl-rhodamine (Con A)) are selected in order to dye cells, proteins and carbohydrates, separately. According to (Katherine E. Fish et al., 2015), when multiple fluorescent stains applied, the application can be conducted in the sequence of cell, protein, and carbohydrate. Between different phases, sterile phosphate buffer saline (PBS) is used to washing the samples for 1 minute, avoiding the staining of lectins.

Table A. 20 Fluorophores for biofilm characterization (McSwain et al., 2005)

Fluorophore	Concentration	Incubation	Excitation	Lambda range
SYTO 63	20 μM	30 min	633 nm	650.7-704.2 nm
FITC	0.1mg/ml	60 min	488 nm	500.9-704.2 nm
Con A	0.1mg/ml	30 min	543 nm	554.4-704.2 nm

Besides, a pre-washing is needed before staining with FITC. 0.1 M sodium bicarbonate will be used in order to keep amines in the non-protonated form (M. Y. Chen et al., 2007). To ensure the staining effects, samples need to be incubated in dark at room temperature.

Observation and analyzation: Samples will be observed by Nikon A1R confocal microscope from Faculty Applied Science of TU Delft, under three channels based on the excitation wavelength and lambda range listed in table1. With all the multispectral data obtained, software, such as DAIME, COMSTANT and IMARIS can be used for the analyzation of confocal images.

Pre-test: To ensure the feasibility of CLSM observation over biofilm, a pre-experiment was carried out using a piece of coupon with mature biofilm. Firstly, the coupon was cut into a small piece. Then, Syto 9 and PI were used to stain live and dead cells. Live cells would be in green, while dead cells in red. A narrow view of figure 3 and a slope in figure 4 show that the sample was not small enough. Light can only go through the nearest part, while the other parts are dark. By a fully scanning, the 3D image shows the whole surface, but with a slope. Fig. 4 also shows that the biofilm is monolayer.

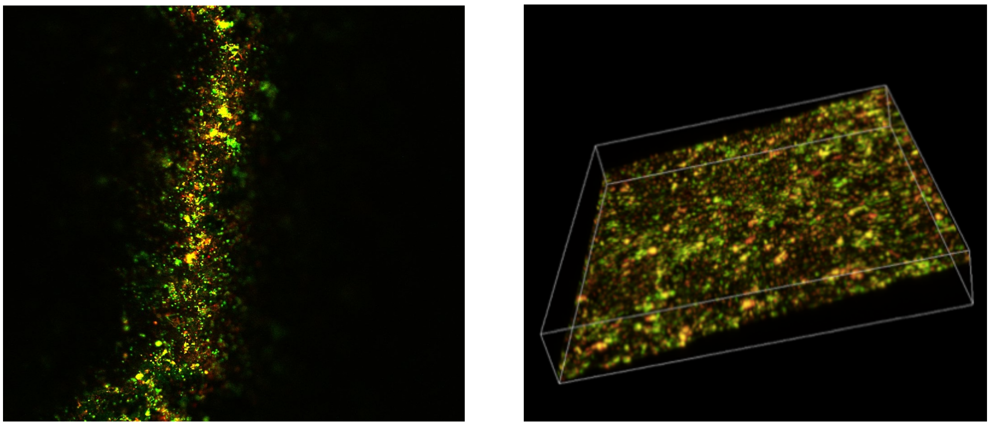


Figure A. 4 2D and 3D CLSM photo