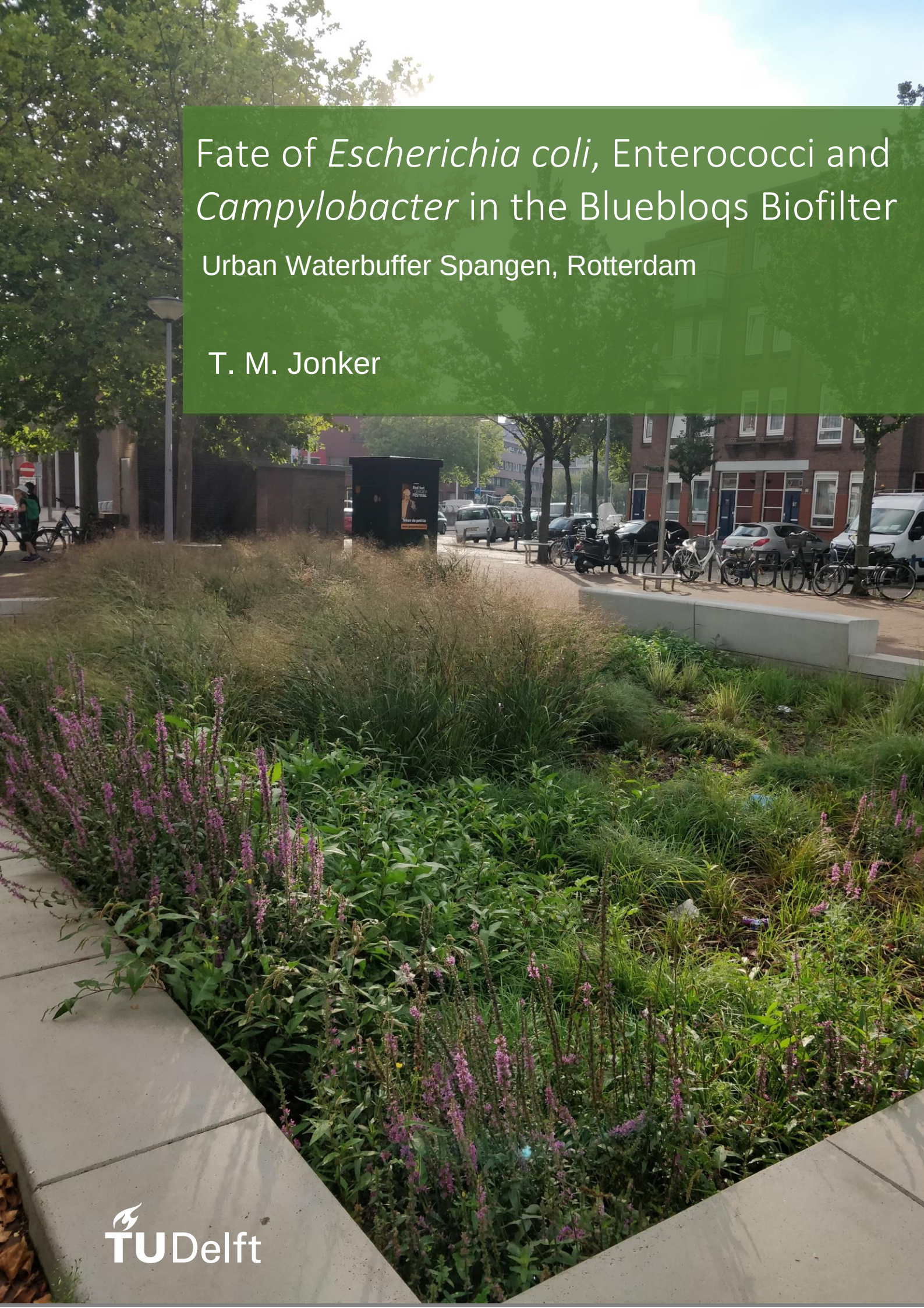

Fate of *Escherichia coli*, Enterococci and *Campylobacter* in the Bluebloqs Biofilter

Urban Waterbuffer Spangen, Rotterdam

T. M. Jonker

TU Delft

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By

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Abstract

Biofilters are implemented in urban environments to treat stormwater but knowledge on their pathogen removal capacity at field scale is limited. Adequate pathogen removal is, however, needed if treated water is reused. This research evaluates the fate of *Escherichia coli*, enterococci and *Campylobacter* in a field scale biofilter in Rotterdam. This biofilter is part of the Urban Waterbuffer Spangen that combines biofiltration with aquifer storage and recovery to provide irrigation water for Sparta's sports field. Microbiological water quality was analysed to assess the removal capacity of the biofilter. To identify factors causing adverse treatment performance, system construction, and operation were investigated, water flows in the biofilter were assessed, and hydraulic conductivity was measured. Lastly, model simulations were used to investigate how short-circuiting and event frequency and duration affect microbiological outflow concentrations.

Results showed leaching of enterococci and *Campylobacter* and minimal retention of *Escherichia coli*. Leaching is likely caused by secondary contamination with bird faeces and multiple design and operational choices lead to low microbial retention. Coarse filter media results in high hydraulic conductivity, and consequently, short hydraulic retention times. Electrical conductivity measurements revealed short-circuiting pathways between the inlet and outlet. Lastly, uneven distribution of feed water presumably reduced the effective reactor volume. It is recommended to upgrade biofilter design by incorporating small particles in the filter media and maximizing the distance between the inlet and outlet. Additionally, reintroduce a ponding zone and rearranging event frequency and duration to lower microbial outflow loads is advised. Model simulations suggest that short, frequent events are beneficial to control microbial loads. However, further work to clarify microbial decay rates is recommended. To conclude, results imply that the biofilter is currently unable to improve the microbiological water quality but can become a treatment barrier if design and operation are upgraded to tackle encountered problems.

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1 Introduction

1.1. Background

Population growth, rapid urbanization, and climate change lead to new challenges in urban water management [59]. Extreme rainfall events will occur more frequently and availability of potable water is decreasing while water demand for non-potable uses is increasing [133]. Consequently, the risk of pluvial flooding and water scarcity is expanding. To tackle these problems, new urban water features are emerging that temporarily store rainwater and treat it for reuse purposes [105]. The Bluebloqs system (Field Factors) is a modular system for rainwater treatment, storage, and reuse [36]. It couples biofiltration with aquifer storage technologies. Biofilters are vertical, often planted, infiltration systems that are increasingly being implemented in urban environments to treat stormwater runoff [93, 98]. Aquifer storage is a technology to increase urban water storage capacity and cover for variability in supply and demand [90]. The Bluebloqs system was implemented in the Urban Waterbuffer Spangen to tackle problems related to heavy rainfall while adding more green to the neighbourhood. [36].

1.1.1. Urban Waterbuffer Spangen, Rotterdam

The Urban Waterbuffer (UWB) Spangen was built at Sparta stadium in Rotterdam in 2018 [132]. Figure 1.1 shows the 5 step concept of the UWB Spangen. Water from various urban areas, defined as stormwater, is harvested and drained by a rainwater sewer to an underground buffer. The buffer retains captured stormwater to ensure a lower infiltration rate for following treatment steps than the precipitation rate during a rainfall event. After storage, water is first treated in the pipeline leaving the buffer with a Sedipoint system (Fraenkische, Germany). This system removes coarse material and light non-aqueous phases [133]. From there, stormwater is pumped to the Bluebloqs biofilter for further treatment. Afterwards, the water is stored in the aquifer using Aquifer Storage and Recovery (ASR) where further purification takes place [102]. Lastly, the recovered water is used for irrigation of the Sparta stadium sports field and as water for a play feature [133].

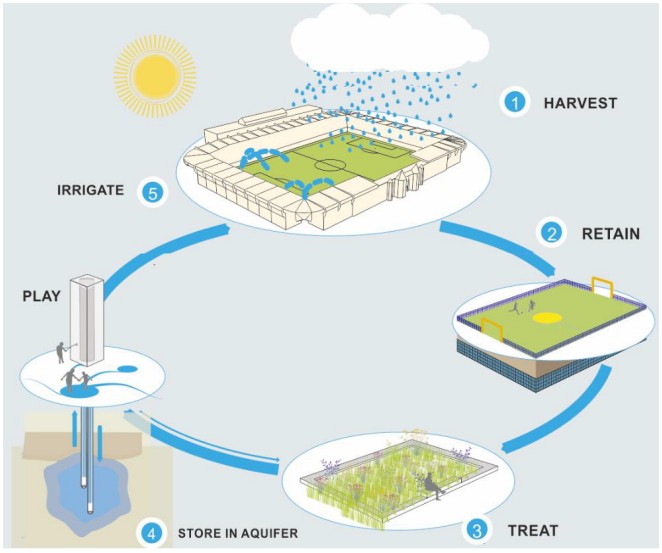


Figure 1.1: Conceptual scheme of the 5 steps of the Urban Waterbuffer. From [132].

1.1.2. Bluebloqs Biofilter 1.0

The Bluebloqs Biofilter in Spangen is Field Factor's first biofilter that is implemented in a full-scale prototype and is the main focus of this research [102]. The filter design, "Bluebloqs 1.0" (Figure 1.2) is based on a constructed wetland and slow sand filtration [102]. Stormwater is delivered on top of the biofilter, where it is distributed over the surface to create a ponding layer. From here, water vertically infiltrates into the vegetated filter layer. In this layer, chemical, physical, and biological treatment processes take place by exploiting plants, microbes, and filter media. The water flows down through a transition layer into the drainage layer, from where it is further transported. Additionally, the system has a raised outlet to create a submerged zone (SZ). The novelty of this biofilter is its flexibility and adaptability in dimension and form for each case [102]. Furthermore, as part of the Urban Waterbuffer, the biofilter is operated via a programmable operation and control system. Therefore, the inflow and outflow of the biofilter can be controlled. This is unique, as most biofilters are designed as passive treatment systems [112].

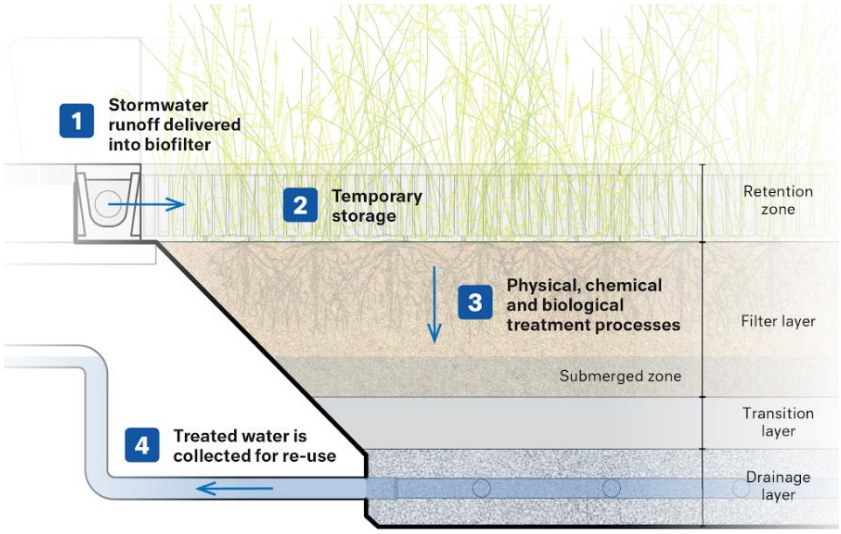


Figure 1.2: Design of the Bluebloqs Biofilter 1.0. From [102].

1.2. Problem Description

Rainwater is initially free of pathogens, such as bacteria, viruses, and protozoa, but quality deteriorates during rainwater harvesting [88, 105]. In this process, rainwater that falls on rooftops and ground surface areas, such as roads, driveways, footpaths, gardens, and laws, is captured and stored. Rainwater that has been in contact with ground surface areas and rooftops is defined as stormwater. Stormwater is contaminated with human enteric pathogens if it receives seepage from combined sewers due to, for example, bad design [59]. Zoonotic pathogens can enter rainwater runoff via animal faeces on surface areas (e.g., roofs or streets). Additionally, pathogens that can naturally occur in water, such as *Legionella pneumophila*, have been found in rainwater runoff and rainwater harvesting systems [101, 109].

When urban water features harvest stormwater for reuse, there is a risk that humans are exposed to pathogens in stormwater. Contaminated droplets can be ingested, inhaled, or come into contact with the skin depending on the reuse application [109]. Waterborne pathogens have the potential to cause large-scale disease outbreaks as they are generally infectious at low doses. Additionally, infectious diseases can be acute of nature and can spread via secondary transmission [59]. Soller et al. (2010) reported that 97% of non-foodborne diseases were caused by 8 common waterborne pathogens, namely protozoa (*Cryptosporidium* spp. and *Giardia lamblia*), bacteria (*Campylobacter jejuni, Salmonella enterica, E. coli* O157:H7), and viruses (norovirus, adenovirus, and rotavirus) [113]. Correspondingly, pathogens are considered a significant human health concern of new urban stormwater concepts [25, 59]. This highlights the importance of adequate treatment of urban stormwater to ensure the microbiological safety of recovered water.

A biofilter is a treatment technology that is increasingly being used in urban environments to improve stormwater quality [92]. To assess the microbial removal efficiency of these features, studies often focus on faecal indicator bacteria (FIB) as a proxy for pathogens. These indicator organisms are easier to measure and current water quality requirements, e.g., the European Bathing Water directive, are often based on FIB [93]. FIB indicate the presence of faecal contamination and, accordingly, the presence of pathogens. Commonly used FIB include *Escherichia coli* (*E. coli*), enterococci, and faecal coliforms. Biofilter removal efficiencies for FIB have been studied at both laboratory scale and field scale. Laboratory scale studies show varying removal efficiencies of 0.45-2.50 $\log_{10}$ [24, 70, 93]. Field scale biofilter studies show *E. coli* removal efficiencies from -0.9 $\log_{10}$ leaching to 1.4 $\log_{10}$ removal [25, 130]. Lower and more variable field scale performance can be explained by more complex operational conditions and maintenance issues, such as short-circuiting and microbial regrowth, compared to controlled operational conditions in a laboratory [26]. Besides, a vast amount of studies show that variations in biofilter removal performance depend on design factors, such as filter media and vegetation, operational conditions (e.g., hydraulic loading rates), and environmental conditions [25, 26].

Previous research on microbial removal performance by biofilters has mainly focused on laboratory scale column experiments [93]. As a result, current knowledge about field scale biofilter applications is limited [93]. Additionally, laboratory experiments are usually of small size, capture limited design and environmental conditions, and use synthetic stormwater. Hence, findings of laboratory scale experiments cannot be translated to field scale performance [93]. Studying field scale biofilters can, therefore, broaden current understanding of the microbial fate in field scale biofilter applications and examine laboratory scale findings. Additionally, most research on microbial treatment in biofilters focused on faecal indicator bacteria, hence, research based on pathogen measurements is sparse. Studies that considered pathogen removal, however, showed that indicator organisms behave differently in biofilters than pathogens [22, 25]. Furthermore, FIB can originate from non-faecal contamination sources [93]. Consequently, their use as a proxy for pathogens is increasingly being questioned. Hence, to improve understanding of pathogen occurrence and removal in biofilter systems, FIB should not only be evaluated but site-specific pathogen information should be collected. Moreover, this contributes to reducing uncertainties in risk management [104].

The microbiological treatment performance of the Urban Waterbuffer was previously evaluated by KWR Watercycle Research [132]. Monthly grab samples were taken from the inflow and outflow of the biofilter and aquifer from January to July 2019 to determine concentrations of faecal indicator bacteria (FIB) *E.coli*, enterococci, and total coliforms. The results showed no removal of FIB in the Bluebloqs biofilter and about 1 $\log_{10}$ removal in the aquifer after one day of residence time [132]. Based on these results, KWR performed an indicative risk analysis in which only the ASR system was considered as a treatment barrier. The highest risk was expected during irrigation of the Sparta sports fields in the break of a football match. *Campylobacter* was considered the main risk driver and its concentrations were assumed based on *E. coli* measurements, as no site-specific data was available for *Campylobacter*. Infection risk of 5.1 % was found, which was considered unacceptable as a maximum risk of infection of 0.01%, similar to drinking water, was aimed for [132]. To mitigate this risk and obtain target microbial concentrations of <1 CFU/100mL in the recovered water, it was proposed to have at least 3 days between the end of infiltration and the onset of extraction [132].

1.2.1. Research Objectives

KWR's research provides initial insight into the system, but much remains unknown. Firstly, inflow and outflow water quality dynamics during feeding events were not studied. Individual samples were taken at variable moments during events, and an effort was made to take samples when the system was running but this was not always possible [132]. As a consequence, the data only provide snapshots of the varying concentrations over the biofilter. More insight into these dynamics can, however, improve understanding of the microbial fate in the system. Moreover, KWR's research focused on the aquifer as the only treatment barrier improving microbiological water quality and, correspondingly, reducing human health risks. Factors that can lead to poor microbial treatment in the biofilter were not investigated. Accordingly, opportunities to enhance the role of the biofilter as a microbial treatment barrier to

reduce human health risks remain unknown. Lastly, no site-specific *Campylobacter* data was collected. Consequently, information on what happens with human pathogens during treatment in the biofilter is missing and the performed human health risk assessment is uncertain [132].

Above mentioned reasons lead to the following aim of this thesis: investigate the fate of *E. coli*, enterococci and *Campylobacter* in the Bluebloqs biofilter and assess if the microbiological treatment performance can be improved to reduce human health risks associated with reusing the water for irrigation of the Sparta sports fields. This leads to the following research objectives:

1. Analyse the capacity of the Bluebloqs biofilter to remove *E. coli*, enterococci, and *Campylobacter*.
2. Identify factors that can lead to poor microbiological removal performance of the biofilter.
3. Investigate how the system can be upgraded to enhance the role of the biofilter as a microbiological treatment barrier.

This study acts as a case study to extend current understanding of microbiological removal performance of field scale biofilter applications for both indicator organisms and human pathogens. Additionally, it can support risk management at the UWB and contribute to optimisation of the system for future implementations.

1.2.2. Approach

To meet the objective of this research, the subsequent methodology is followed:

1. A literature review is performed to obtain insight into microbiological contamination sources of stormwater, microbial removal processes in a biofilter, and the effect of design and environmental conditions on these processes.
2. The system is analysed to obtain a thorough understanding of the set-up and operation. This information provides research context and is necessary to formulate a field measurement plan and interpret water quality data from field measurements.
3. The water quality flowing in and out of the biofilter is systematically monitored over multiple feeding events to capture variability over time and observe trends. This information provides insights into the microbial loads entering and exiting the biofilter.
4. Biofilter hydraulics are investigated with hydraulic conductivity tests and by assessing physico-chemical water quality dynamics over the biofilter.
5. Main microbial transport and removal processes are modelled in PHREEQC. This model is used to investigate the effect of hydraulic conditions and operational choices on microbial treatment performance.
6. Results are analysed to evaluate the fate of microbes in the biofilter and identify causes of low treatment performance. Additionally, possibilities to improve the treatment performances are investigated.

2

Literature Study

This chapter provides a theoretical background on the microbiological quality of stormwater (Section (2.1), the treatment processes that can occur to remove microbes in a biofilter (Section 2.2) and the factors that can affect these treatment processes (Section 2.3).

Figure 2.1 shows a conceptual model for the removal of indicator and pathogenic microorganism in a biofilter. Microbes from various pollution sources enter the biofilter via stormwater. These pathogens can be removed from the water when they are retained in the system due to physical and physicochemical processes. Subsequently, the retained microbes can be permanently removed from the biofilter by biological processes. The effectiveness of both retainment and removal processes determine the overall removal performance of the biofilter. Design and environmental conditions are found to extensively influence these processes [93, 98]. Understanding these factors and their link to removal processes is, therefore, needed to effectively design biofilters as a reliable treatment step [45]. In red, adverse processes are shown that can lead to poor treatment performance.

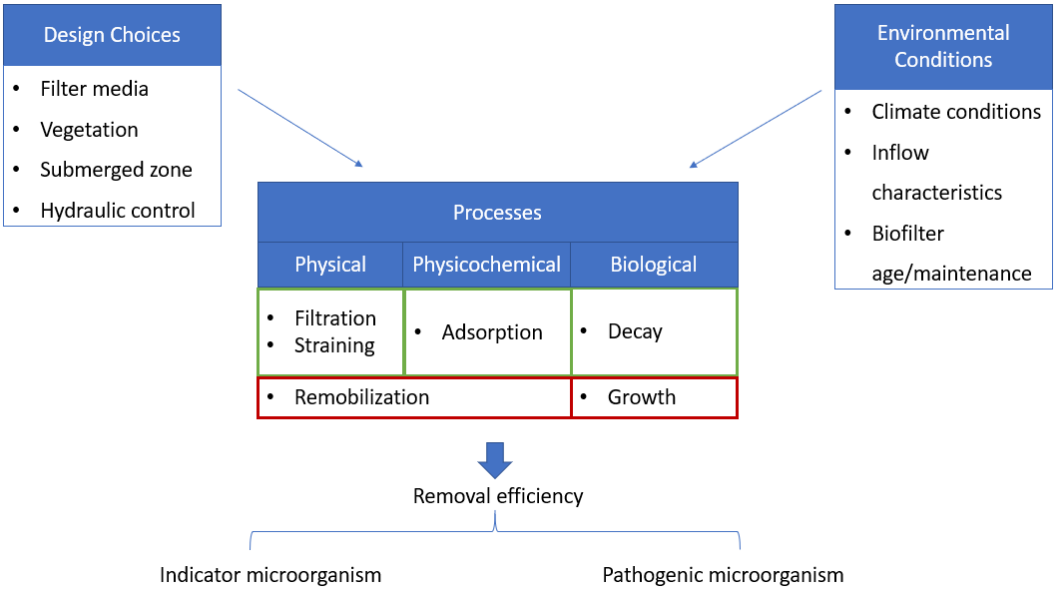


Figure 2.1: Conceptual model for the removal of indicator and pathogenic microorganism by biofilters. Adjusted from [93].

2.1. Microbiological Quality of Stormwater

This section discusses the characteristics of waterborne pathogens (Section 2.1.1), variability in microbial stormwater quality (Section 2.1.2), and the use of indicator organisms to monitor pathogens (Section 2.1.3).

2.1.1. Waterborne Pathogens in Stormwater

Waterborne pathogens are transmitted in water and can cause infectious diseases when humans get in contact with contaminated water. Pathogens of concern include protozoan cyst (2-50 μm), bacteria (0.2-5 μm), and viruses (20-100 nm) [45]. Pathogenic characteristics resulting in great concern are; (1) high presence in the environment or highly infectious at low doses, (2) capability to proliferate outside a host under favourable environmental conditions, (3) ability to remain viable and infectious in the environment for extended periods, and (4) high resistance to water treatment [10]. Pathogens can be present in stormwater as individual particles (free-floating), in aggregates, or attached to other particles.

The source contaminating the stormwater, determine which pathogens enter the system. Important sources for waterborne pathogens in stormwater are human and zootic faeces [10]. Besides these enteric pathogens, pathogens can naturally occur in water systems. As rainwater itself is free of pathogenic contaminants, it is important to determine if rainwater gets in contact with pathogenic sources. Rainwater reuse concepts collect rainwater from various origins, such as pluvial floods, rainwater runoff, rainwater roof harvesting containers and, separate rainwater sewage.

Human pathogens have been found in pluvial floods contaminated with wastewater originating from a combined sewer system [33]. This wastewater can contaminate stormwater due to backflow or cross-contamination with a combined sewer [33]. Rainwater runoff can be contaminated with animal faeces from impervious surfaces, with *Campylobacter, Giardia*, and *Cryptosporidium* frequently reported [33]. faecal contamination from wild animals (e.g., birds, mammals, and reptiles) is a probable pathogen source of rainwater roof harvesting systems, as they can access roof surfaces [4]. Research on rainwater roof harvesting containers show the presence of faecal indicators (*E. coli*, enterococci), various zoonic pathogens (such as, *Campylobacter*, *Giardia*, *Salmonella*) and *Legionella pneumophila)*, which can naturally be present in aquatic environments [3]. *Legionella pneumophila* has also been found in pluvial floods [107] and rainwater runoff [101]. Schets et al. [109] detected *Legionella* in stored rainwater in reservoirs in the Netherlands during a three-year water quality monitoring study. Besides, the reservoirs were frequently contaminated with faeces and occasionally contained human enteric pathogens (*Campylobacter, Cryptosporidium, Giardia, Aeromonas hydrophila)*.

2.1.2. Variability of Microbiological Stormwater Quality

The variability of contamination sources leads to considerable variability in water quality of harvested rainwater [59]. Besides, climate change plays a part in stormwater quality variability. Due to climate change, higher frequency and strength of storms and droughts are expected which affect the concentration of pathogens present in stormwater [105]. Increased faecal indicator and pathogen concentrations are observed after heavy rainfall [109], indicating a relationship between flow magnitude, microorganism wash-off, and transport. Floods can occur more frequently due to increased heavy rainfall, which might lead to an increased health risk because disease outbreaks commonly follow floods [21].

Besides climate change, seasonality contributes to stormwater variability as indicator bacteria concentrations in the input flow typically increase with higher daily temperature [50, 130]. Statistically significant seasonal effect of relatively higher inflow *E. coli* concentrations in warm periods compared to cold periods were found [25]. Besides temperature, sunlight radiation can influence growth/die-off processes of *E.coli* in stormwater [78].

2.1.3. Microbial Indicators and Pathogens

A wide range of pathogenic microorganisms can be present in stormwater in low, but, highly variable concentrations. Consequently, quantification and detection of pathogens are operationally difficult and costly [45]. To overcome this problem, faecal indicator bacteria (FIB) are widely used to characterise the presence of pathogenic microorganisms in water. FIB include non-pathogenic bacteria that are found in faeces and are usually more abundant and easier to measure than pathogens. Therefore, FIB (total coliform, faecal coliform, Enterococcus and *E. Coli*) are used in many regulatory water quality requirements [93]. When using FIB, a larger number of samples can be collected, hence greater stormwater variability and faecal contamination magnitude can be captured [95]. FIB measurements can, therefore, provide insight into site-specific stormwater quality dynamics and potential pathogen concentrations when the sources of faecal contamination are known [95].

The adequacy of using FIB to indicate human pathogens in stormwater is, however, questioned [59, 93]. Therefore, it is important to understand the limitations of this approach. FIB indicate if stormwater is contaminated with faeces, but provide limited insight into which pathogens are present. Besides, FIB not only originate from human faeces as non-human and non-faecal sources have been identified [93]. Lastly, FIB can multiply in environments under a wide range of climatic conditions [41]. Biofilters, for example, can have wet, nutrient rich environmental conditions which can be beneficial for FIB persistence [58]. Additionally, some researchers found that FIB behave differently than pathogens in biofilter systems [22, 25, 93]. As previous studies on microbial removal of biofilters are mostly focussed on FIB, knowledge about actual pathogen removal is sparse.

2.2. Microbial Removal in a Biofilter

This section discusses the main processes governing microbial removal in a biofilter. Physical removal processes are considered in Section 2.2.1. Section 2.2.2 present physicochemical processes and Section 2.2.3 discusses biological processes that can remove microbes from the system.

2.2.1. Physical Removal Processes

Physical removal processes are based on particle size exclusion and are mainly affected by the grain size of the filter bed and the cell size and shape of microbes [115]. During filtration, particles which size exceeds the filter media pore diameter are filtered out. Consequently, filtration will mainly remove particle attached microorganism as free-floating protozoa, bacteria and viruses are often too small [30]. A part of these smaller particles can be removed via straining [30]. Straining involves the trapping of particles at narrow pore throats or grain junctions [98]. Straining is generally found to be important when the average size of the microorganism is > 5% of the grain sizes that compose the biofilter [115]. This process will, therefore, mainly occur in biofilters that contain a percentage of clay and silt. Next to straining related to grains, a particle can be trapped in stagnant water zones between grains or in thin water films [93]. Figure 2.2 presents the main physical processes that can remove microbes in a biofilter.

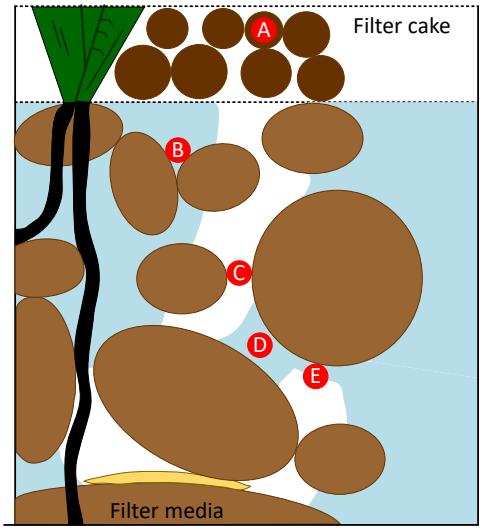


Figure 2.2: Close-up of typical unsaturated biofilter media showing filter cake layer, media grains, pore water, plant roots, and infauna (figure not drawn to scale). Microbes are shown in red and main processes regarding microbial removal in a biofilter are denoted by A-E. (**A**) illustrates microbes attached to large particles that cannot pass through the filter media and are captured in the filter cake. (**B**) shows capture by straining in narrow pore throats and (**C**) and wedging at grain junctions. Specific to unsaturated media are (**D**) straining in a pendular ring of water between grains and (**E**) thin-film straining against a sediment grain. Adjusted from [93, 98]

2.2.2. Physicochemical Removal Processes

Adsorption is a physicochemical process that removes particles from water by attachment to filter media. This process plays an important role in microbial removal as it can capture pollutants of small sizes [93]. The physiochemical properties of the influent (e.g., ionic strength), the adsorbent (e.g., grain diameter), and the adsorbate (e.g., surface properties) determine if adsorption can take place [93]. Adsorption processes contain two stages: (1) transport of particles to biofilter media grains and (2) particle attachment to biofilter media grains surface. Particle attachment to various interfaces can be explained with the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory which states that particles tend to merge due to Van der Waals forces but are simultaneously repulsed by electrostatic forces due to the presence of an electric double layer [54]. This electric double layer is an ionic environment in the vicinity of a charged surface that is formed as the charged surface attracts oppositely charged counterions. Because a double layer represents repulsive forces, the compression of this layer can result in the attachment of a particle to a surface. The attraction of bacteria to filter the media surface depends on the thickness of this double layer, which is a function of ion concentration and valency [115]. If the thickness reduces, pollutants may get close enough to the surface for Van der Waals attraction forces to overcome the electrostatic repulsion barrier [115].

Adsorption takes place in saturated and unsaturated zones of filter media and is particularly important for colloid removal, hence for microbial removal [98]. In saturated sediments, attachment occurs at the grain-water interface, and in unsaturated sediments, additional air-water interfaces are present where binding takes place via strong capillary forces [98]. When biofilters are vegetated, plant root systems form additional attachment sites. Figure 2.3 shows the main microbial physicochemical removal processes.

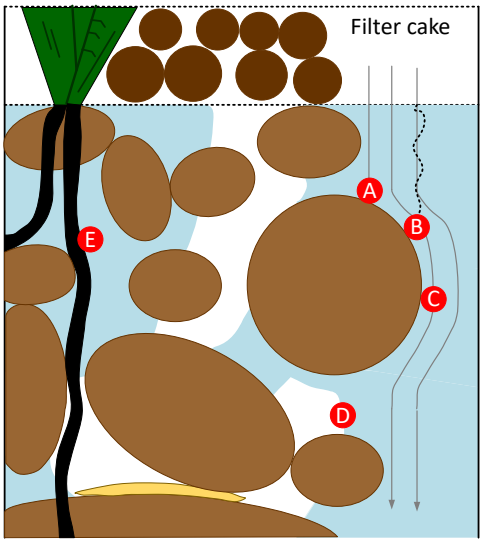


Figure 2.3: Close-up of typical unsaturated biofilter media showing filter cake layer, media grains, pore water, plant roots, and infauna (figure not drawn to scale). Microbes are shown in red and main physicochemical processes regarding microbial removal in a biofilter are denoted by A-E. (**A**) shows capture by sedimentation, (**B**) by diffusion, and (**C**) by interception. (**D**) illustrates trapping of microbes at the air-water interface and **E** adsorption to plant roots. Adjusted from [93, 98]

2.2.3. Biological Removal Processes

Biological removal of microorganisms occurs when there is a net decay compared to the growth of microbes. This largely depends on the persistence of microbes in the biofilter. Microbial decay is induced by abiotic and biotic factors. Abiotic factors include environmental stressors such as sunlight, ultraviolet radiation, temperature, osmotic stress, moisture content, and nutrient availability [93]. Abiotic factors comprise grazing and competition with native microorganisms in soil and on plant roots. Native infauna (protozoa, bacteriophages) graze on microbes and decompose them into compounds for their metabolism [93, 128]. Microbial competition consists of two forms (1) contest competition, whereby native microorganisms excrete a substance that harms nearby competitors, and (2) scramble competition, whereby native microbes obtain nutrients more efficiently than competitors. This harms distant competitors but might help nearby microbes [98]. Figure 2.4 shows main biological removal processes

for microbial removal in a biofilter.

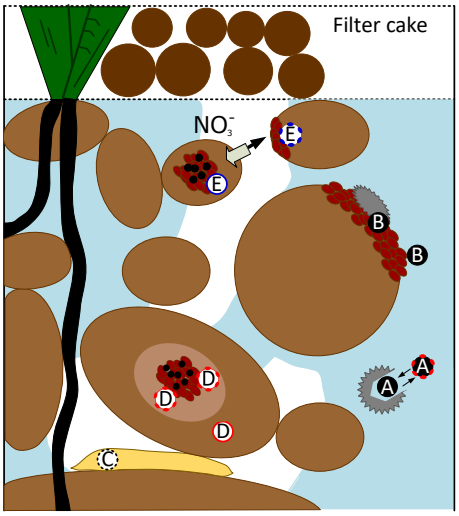


Figure 2.4: Close-up of typical unsaturated biofilter media showing filter cake layer, media grains, pore water, plant roots, and infauna (figure not drawn to scale). The main biological processes affecting bacterial survival in a biofilter are denoted by A-E. (**A**) is ingested (A-solid), digested and excreted as waste (A-dashed) by protozoa (grey particles). (**B**) illustrates protozoa biofilm grazing and (**C**) ingestion by nematodes. **D** shows contest competition whereby native microbial biofilm (brown and black plaque) excrete a substance that harms nearby competitors (D-dashed) but not distant cells (D-solid). **E** illustrates scramble competition in which native biofilm communities obtain nutrients (in this case nitrate) more efficiently than species E. Adjusted from [93, 98]

2.3. Influence Design and Environmental Conditions on Treatment Processes

Design choices and environmental conditions can influence biofilter removal processes. section 2.3.1 and Section 2.3.2 elaborate on the current knowledge-based on previous research findings of the impact of these factors on microbial removal processes. It should be noted that most of these findings are based on laboratory-scale studies and knowledge gaps about the effects still exist.

2.3.1. Impact of Design Choices

Design choices comprise engineered components that can affect microbial treatment. The main design elements that influence biofilter removal processes are filter media, vegetation, submerged zone, and hydraulic control elements.

Filter Media

Filter media is the core of the biofilter as it supports microbial communities, plants, and is involved in most removal processes [26]. It typically consists of a mixture of coarse and fine sand, compost, and an overlying layer of mulch [93]. Listed is current knowledge on how filter media affects the removal of microorganisms by biofilters per filter media aspect:

- The type of filter media grains is an important factor governing all removal processes. The level to which physical removal of microbial contaminants takes place depends on filter media grain size distribution and associated pore sizes. Generally, physical removal increases with decreasing grain size [115]. Grain size distribution also influences physicochemical processes. Smaller particle sizes have larger specific surface areas, hence provide more adhesion sites. Besides, increased surface roughness provides a larger surface area and reduces shear forces that drive desorption. Research on impact of filter media on *E.coli* removal found that fine/coarse sand has limited adsorption capacity compared to loamy sand, which contains a majority of silt and clay particles [19, 26]. The presence of clay particles has a positive effect on microbial adsorption due to their large specific surface area and positively charged edges [115]. On the contrary, clay particles can prolong the survival of FIB as they can protect bacteria from predators, have a high water-holding capacity, and high ability to hold nutrients [37, 115].

- Various laboratory scale column studies looked at the approach of incorporating amendments, such as activated carbon, zeolite, and biochar, to sand based biofilter media. Activated carbon and zeolite have a high specific surface area and porosity and could, therefore, improve attachment and straining. Li et al.[69] found microbial removal capacity of granular activated carbon (GAC) amended filter media in 15 cm columns of 0.58 at a velocity of 720 mm/h. Studies on zeolite amendments show *E. coli* $\log_{10}$ removal of 0.2-0.64 in 15-20 cm layers [71]. Biochar, such as wood pellets and wood chips, can greatly vary in physicochemical properties and therefore in removal efficacy [93]. 0.14 - 1.18 $\log_{10}$ reductions were found depending on biochar type [82, 84, 97]. GAC and biochar are both carbonaceous materials, which can leach dissolved organic matter (DOM). DOM in a solution can compete with FIB for adsorption sites, thus decreasing FIB attachment. Besides, DOM can increase or reduce electrostatic repulsive forces affecting physicochemical processes [98, 115].

- Next to introducing secondary media to biofilters, filter media modifications can affect the removal properties of biofilters. From drinking water systems it is known that the addition of multiple metal oxides, metal hydroxides, and chemicals with antimicrobial properties can increase microbial removal during sand filtration [93]. Chemical modifications may improve microbial removal in the following ways [67, 115]: (a) creating positively charged surface sites that favour microbial immobilization (e.g., metal oxide coating); (b) reduction of the electrostatic barrier for microbial attachment by lowering negative surface charge on filter media grains with, for example, metal hydroxide or polymeric modification; (c) adding biocides that inactivate microbes to the surfaces, for example, nano-metallic coating.

  Iron-oxide coating is the most used surface modifier and studies investigating the efficiency of FIB removal from urban stormwater in iron-oxide coated bioretention media show better results than bioretention media without coating. Zhang et al.[128] found increased removal from 0.52 $_{10}$ to 1.96 log $_{10}$ in 2.5*23 cm column experiments using 0.3-0.5 mm sand with a hydraulic loading of 8 cm/h. Mohanty et al.[83] observed an increase from 0.3 log $_{10}$ to 2.0 log $_{10}$ in 2.5*15 cm columns filled with 0.6-0.8 mm iron coated quartz sand fed and hydraulic loading of 1.2 cm/h. The coating of secondary media was also researched and commonly showed better FIB removal to unmodified amendment media [93]. For example, Li et al.[69] found an increase in log $_{10}$ removal from 0.58 to 1.13 between a GAC amended filter and a GAC-Cu modified amended filter. A second study of these authors found an increase from 0.53 log $_{10}$ to 3.44 log $_{10}$ from a zeolite amended filter to a zeolite-Cu modified amended filter [71].

Figure 2.5 summarises the relationship between pathogen removal processes and filter media design.

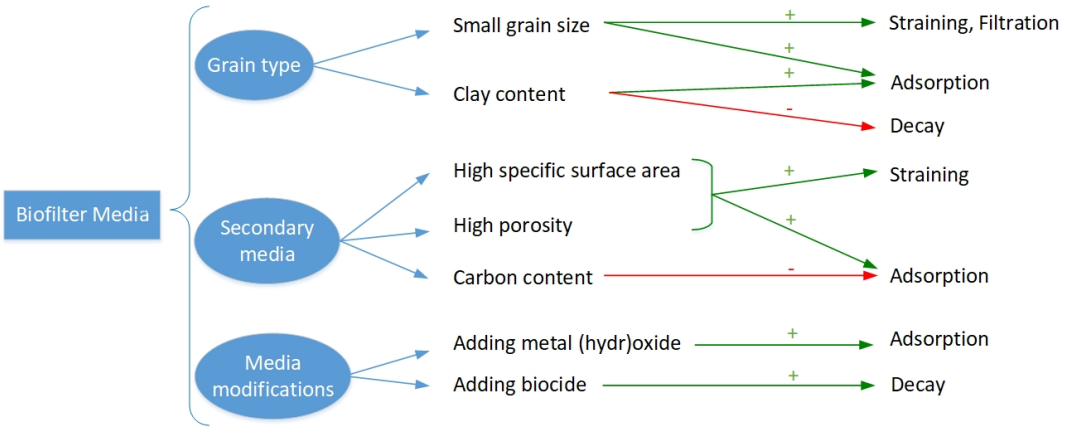


Figure 2.5: Relationship between filter media design components and biofilter removal processes. A green arrow indicates increased pathogen removal and a red arrow decreased pathogen removal.

Vegetation
Research on the effect of vegetation on *E. coli* removal shows different results depending on vegetation type and plant density [24, 26, 27]. This is often explained by distinct characteristics of plant species, such as root growth, rooting depth, nutrient metabolism, and survival capability of plants in a specific environment [63, 93, 102]. Besides, some studies show higher FIB removal in unplanted biofilters [63] while others suggest that plant selection can enhance *E. coli* removal in stormwater biofilters [24, 26]. More heterogeneous and increased soil porosity is seen at some vegetated systems due to, for example, the formation of preferential flow paths by root systems [49, 58, 63]. These studies found shorter HRT which negatively affects physicochemical removal processes and filtration processes. Other studies suggest positive effects on HRT in biofilters due to reduced overall stormwater volume and velocity by evapotranspiration and exfiltration [24, 26]. As a result, lower infiltration rates were found and improved *E. coli* removal.

Lower infiltration rates are suggested to be linked to high plant densities because they lead to an increased reduction in stormwater flow [24]. Besides plant density, plants with extensive root systems positively affect physicochemical processes because they provide more attachment sites [26, 27]. Additionally, plants with extensive root systems provide a high surface area for biological activity such as the growth of rhizosphere microbial communities [29]. These native microbes can compete with microbiological contaminants leading to increased die-off [26, 27]. Moreover, microbes can either be stimulated or inhibited by exudates (oxygen, amino acids, and sugars) from plant roots [24, 27]. Chandrasena et al.[27] found that root exudates adversely impact *E. coli* survival and that leaf and flower/seed extracts might perform antibacterial activity against *E. coli*. Besides die-off, biodegradation is influenced by vegetation as plants can stimulate infauna activity, which can result in increased grazing [93]. Lastly, plants take up nutrients and water. Via nutrient uptake, plants compete directly with microorganisms [98]. Shrubs species with higher nutrient removal efficiency shows higher FIB removal [24]. Besides, water uptake results in lower moisture content which affects microbiological activity [98]. Figure 2.6 summarises the relationship between pathogen removal processes and vegetation.

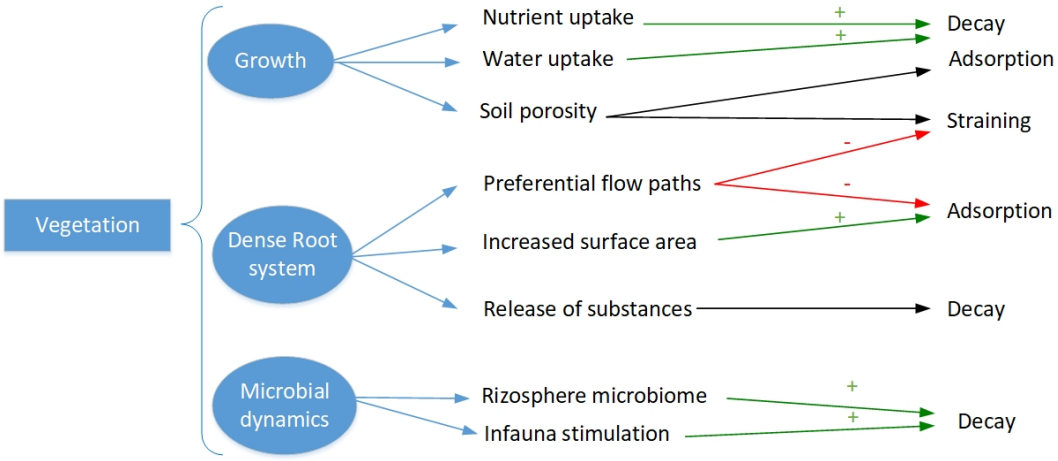


Figure 2.6: Relationship between vegetation and biofilter removal processes. A green arrow indicates increased pathogen removal, a red arrow decreased pathogen removal and a black arrow indicates that both increase and reduced removal can occur.

Hydraulic Control
Important hydraulic factors that influence FIB removal are moisture content, degree of saturation, hydraulic loading rate, and hydraulic retention time. Moisture content, defined as the quantity of water contained in a soil [122], is largely determined by the moisture retention capacity of the filter media. For example, clay has a better water holding capacity than sand. High moisture content can inhibit the development of fine fissures and macropores during extended dry periods [59]. This prevents the formation of cracks and macropores that lead to FIB leaching during subsequent rain events [93, 98]. Besides, a high moisture content promotes vegetation growth, which affects FIB removal in various ways, as discussed under **Vegetation**. In addition, biofilm formation is fueled by increased moisture

content. This can lead to abundant and more active native bacteria and native soil protozoa and, subsequently, increased competition and grazing. On the other hand, moist soil can prolong the survival of FIB [93, 98]. Consequently, longer survival times are seen in loamy soils compared to sandy soils, as loamy soils often contain more moisture [115].

Similarly, saturated soils prolong microbial survival compared to unsaturated soils [115]. Besides biological processes, filter media saturation affects physical processes by influencing the flow through a biofilter. For instance, unsaturated conditions are found more effective for straining than saturated conditions, as unsaturated flow occurs through the smallest pores in filter media [115]. Besides, additional straining mechanisms are found in unsaturated soil compared to saturated soil (e.g., thin-film straining, Section 2.2.2) [98]. Moreover, physicochemical removal in unsaturated biofilters might be higher due to additional water-air interfaces [98]. However, some studies suggest that above mentioned positive effects are less under transient flow (consecutively filling and draining of a biofilter) compared to saturated flow, as propagating wetting fronts can mobilize FIB due to e.g., scouring [83, 98]. Besides, by controlling filter media saturation, redox conditions can be manipulated which can favour, for example, organisms adapted to anoxic or anaerobic conditions [45].

Inflow distribution control also influences redox conditions, as turbulent inflow stimulates oxygenation of inflow water. Additionally, distribution control determines hydraulic loading rates which affect flow rates through filter media. High hydraulic loading rates lead to high flow rates and increased transport through large pores, whereby straining is decreased [115]. Additionally, high flow rates lead to increased hydrodynamic shear stresses that can promote remobilization of microbes [18, 19, 115] and loss of biofilm [26]. High loading rates are also detrimental for adsorption of pollutants, as this results in shorter contact time between adsorbent and adsorbate [17, 18]. A too high loading rate results in a shorter residence time of stormwater in the biofilter, defined as hydraulic retention time, and reduced utilization of attachment sites of a filter media [115].

A submerged zone (SZ) is a common measure to create both unsaturated and saturated conditions in a biofilter, and maintain a high moisture content between storm events. It is a saturated, organic-rich layer near the base of a biofilter that creates a vertical gradient in moisture and redox conditions. It is often amended with an organic carbon source to stimulate anaerobic metabolic processes [93]. Rippy's (2015) meta-analysis shows that a SZ significantly increases the removal of FIB, with average values found to increase from 0.9 log $_{10}$ to 1.9 log. This might be due to the positive effects of decreased flow velocities and the prevention of desiccation of the biofilter. Figure 2.7 summarises the relationship between pathogen removal processes and hydraulic control.

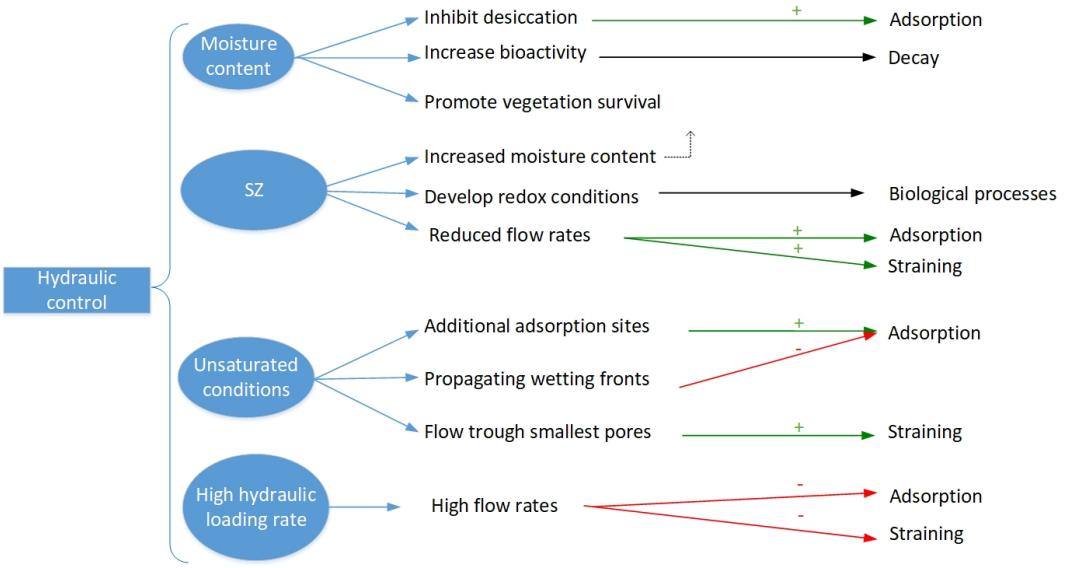


Figure 2.7: Relationship between hydraulic control elements and biofilter removal processes. A green arrow indicates increased pathogen removal, a red arrow decreased pathogen removal and a black arrow indicates that both increase and reduced removal can occur.

2.3.2. Impact of Environmental Conditions

Environmental conditions include factors that cannot be engineered but potentially modify treatment processes. This section describes previous research on these factors.

Climate Conditions

Previous studies speculate that dominant removal processes differ during wet weather and dry weather conditions. Straining, adsorption, and desorption are expected dominant processes in biofilters during wet weather conditions while inactivation and die-off are significant during dry periods between rain events [23, 128]. Besides, it is suggested that the length of antecedent dry periods, i.e., the period between rain events, affects FIB removal. Too short dry periods can result in insufficient time for microbes to decay. As a consequence, filter media will quickly reach its capacity to adsorb microbes and detachment will begin, which can result in increased outflow concentrations [23]. Prolonged dry weather periods and associated desiccation can result in cracks that support flush out of FIB [26]. No published research was found that investigated the effect of antecedent dry periods in field scale biofilters. However, lab-scale studies show contradictory findings. For example, Soberg et al.[118] found improved *E. coli* log reductions with increasing antecedent dry periods for biofilters with and without a SZ: <3 days showed leaching while >17 days showed reduction. Other studies show reduced log removal with increasing dry periods due to drying out of the biofilter [24, 70]. This negative effect was, however, reduced by incorporating a submerged zone.

Generally, the survival of bacteria decreases with increasing temperature [115]. Cold temperatures are reported to enhance FIB survival but this is speculated to be driven by a reduction in competitive and predatory microorganism during cold weather and not temperature itself [28, 130]. This is in line with the observation that FIB growth rates elevate in warm weather. A field scale researched by Zhang et al.[130] found that bacterial removal efficiency was independent of temperature, whereas column experiments show increased bacterial decay with elevated temperatures. This is expected to be linked with increased levels of protozoa and biofilm bacteria whereby die-off of trapped *E.coli* via predation and competition increases [130]. Some research suggests temperature effects on physicochemical processes, stating a reduction in attachment with decreasing temperature due to changes in viscosity [115]. Figure 2.8 summarises the relationship between pathogen removal processes and climate conditions.

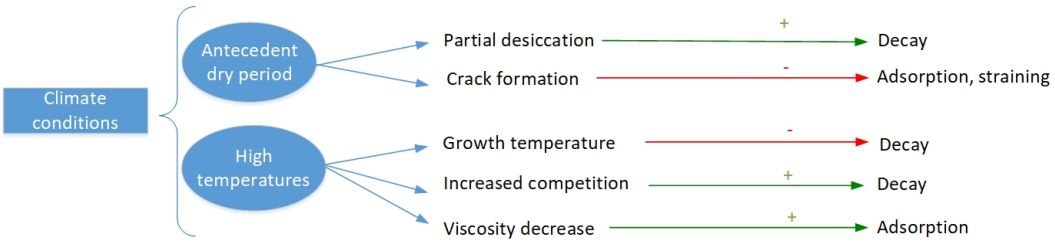


Figure 2.8: Relationship between climate conditions and biofilter removal processes. A green arrow indicates increased pathogen removal and a red arrow decreased pathogen removal.

Inflow Characteristics

Inflow characteristics include factors such as pH, salinity, co-occurring contaminants, and the state of microbial contaminants, i.e., free floating or attached to a suspended solid. Various factors affect physicochemical processes [38] as these depend on the physicochemical properties of the influent, adsorbate, and absorbent (Section 2.2.2). For example, microbes have varying pH at which they are electrically neutral (isoelectric point) and consequently, the optimal pH for adsorption differs per microbe [38]. Besides, pH regulates the ionization of surface groups of soil particles that determine their electrostatic charge (often negative). This is also influenced by the attachment of ions from the influent to these particles. The importance of pH in determining the surface charge increases at low ionic strength [115]. It is expected that the greatest difference between particle surface charge and bacteria charge leads to the greatest adhesion [115].

High salt concentrations are found to result in decreased survival due to the osmotic stresses posed on the microbes [100]. Besides, increased salt concentration (increased ionic strength of a solution) leads to more adsorption as it reduces the thickness of the electric double layer [115]. Increasing effectiveness is found at higher salt valency as this leads to a larger electrostatic double-layer compression [38]. A decrease in pathogen removal is found at increasing inorganic and organic matter content. This is usually present in the form of humic substances, which are negatively charged colloids, that can compete with microbes for attachment sites [102]. Lastly, high pollutant inflow concentration leads to fewer adsorption sites compared to the number of microorganisms, which increases the outflow concentration of microorganisms. This effect was found at a field scale biofilter [25] and validates lab-finding [23].

Besides effects on physicochemical processes, biological processes are influenced by inflow characteristics. Both high and low pH can lead to decreased microbial viability [115]. For example, pH values lower than 3-4 are found to be hostile for bacterial survival and *E. coli* survives better in neutral-to-alkaline soils than in acid soils with optimal values in the range 5-6.4 [115]. Besides, various abiotic factors influence microbial die-off due to variability amongst microorganisms in adaption capacity [93]. For example, FIB are more sensitive to abiotic stresses than native soil flora as they lack adaption capacity, such as downregulating metabolic rates when nutrient availability is low, to survival in sediments [28, 128]. Besides, high nutrient availability in stormwater can increase pathogen survival.

Lastly, the state of the microbe matters as a contaminant attached to a particle behaves differently than in its free-floating state. The microbe-particle connection changes the size, shape, and surface properties of the microbe which impacts filtration and attachment processes. Besides, die-off and predation are affected as the connection affords microorganisms protection against biotic and abiotic factors [13, 56]. Free-floating pathogens are expected to be mostly captured by physicochemical processes than physical processes in porous media due to their small size [23, 128]. Figure 2.9 summarises the relationship between pathogen removal processes and inflow characteristics.

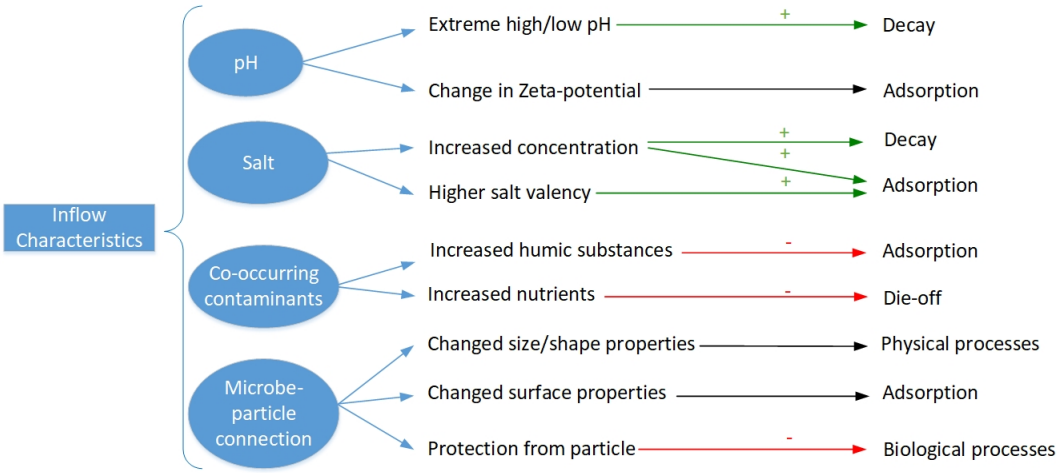


Figure 2.9: Relationship between inflow characteristics and biofilter removal processes. A green arrow indicates increased pathogen removal, a red arrow decreased pathogen removal and a black arrow indicates that both increase and reduced removal can occur.

Native organisms

Native microorganisms comprise indigenous microbial communities that are part of the biofilm and other organisms living in the biofilter. A biofilm is a densely packed community of microbial cells that is likely to grow on filter media grains or within pore spaces and thereby naturally alters filter media and affects microbial removal [93, 98]. Biofilm can influence the removal of microbial contaminants in various ways; (a) altering the porosity and thereby influencing the hydraulic flow through the porous media; (b) modifying surface properties (e.g, altering grain surface roughness and electrostatic charge) and consequently physiochemical interactions [119]; and (c) introducing additional microbial removal mechanism such as straining and predation [70, 75, 128]. Contradictory effects of a biofilm on microbial removal are found; in some cases, they can incorporate FIB and increase their survival [56] and in

other cases enhance FIB removal via predation, microbial competition, and straining due to restricted pore sizes [74, 115]. No consensus has been made on the effect of a biofilm on microbial removal as the biofilm itself is complex. As a result, the magnitude and direction of effect (increase or decrease) of a biofilm are difficult to reliably predict.

Infauna comprises all invertebrates living in a biofilter, such as earthworms, ants, and snails, and their composition shows to vary significantly among biofilter systems [80]. To date, little is known regarding the effect of invertebrates on microbial removal in biofilters [93]. However, plant growth and infiltration can be influenced by infauna [80] which consequently can affect microbial removal by altering biofilter residence time and plant root architecture [93]. For example, bioturbation by earthworms leads to soil oxygenation which is linked to increased aerobic microbial activity. Infiltration rates can be influenced by bioturbation of infauna, which can lead to preferential flow paths that decrease the hydraulic retention time in a biofilter and negatively affect physicochemical removal processes. Micro- and mesofaunal, such as protozoans (2-50 μ m) and nematodes (30 μ -1 mm), can control microbial biomass significantly by grazing [93, 98]. Zhang et al.[128, 129] found enhanced *E. coli* removal in sediments with high protozoan concentrations. Figure 2.10 summarises the relationship between pathogen removal processes and native microorganisms characteristics.

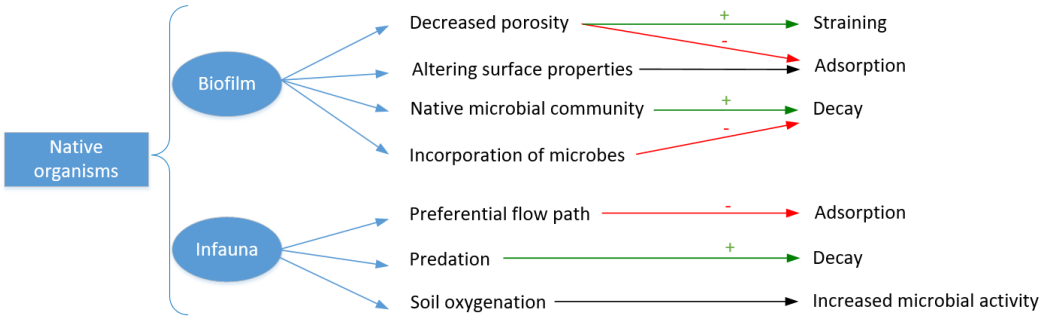


Figure 2.10: Relationship between native organisms and biofilter removal processes. A green arrow indicates increased pathogen removal, a red arrow decreased pathogen removal and a black arrow indicates that both increase and reduced removal can occur.

Biofilter age and maintenance

Biofilter age and maintenance is related to the degree of clogging of a biofilter. Clogging can be a result of biomass growth and the accumulation of solid materials inside a biofilter. It can result in an increase in hydraulic retention time and a change in flow patterns [115]. Experimental evidence shows more efficient bacteria removal in clogged filtration systems compared to unclogged systems [115].

3

System Description

This chapter describes and analyses the system set-up and operational conditions of the Urban Waterbuffer. Section 3.1 describes the project location and set-up of the Urban Waterbuffer and biofilter. Thereafter, Section 3.2 explains the system operation and the water flows resulting from this operation.

3.1. Site Description

The biofilter is part of the Urban Waterbuffer, which is located in the neighbourhood Spangen in the city of Rotterdam, The Netherlands (Figure 3.1). The neighbourhood mainly comprises of residential areas and has 10.200 inhabitants [99]. The UWB is realised next to the Sparta football stadium which has a water demand for irrigation of the sports field of approximately 15.000 $m^3/year$ [132]. In the past, drinking water was used for this but after the realisation of the UWB, part of this drinking water is replaced with stormwater treated in the UWB.

Figure 3.1: Project area (Urban Waterbuffer) and its rainwater catchment area in the neightbourhood Spangen in Rotterdam. Adjusted from [132].

The UWB is connected to the existing rainwater collection system (Figure 3.2) near the Sparta Stadium which collects rainwater from multiple surface areas (Table 3.1). For the design of the UWB, a total connected drainage area of about 46,000 m^2 was estimated. KWR's research, however, showed that less than 50% of the precipitation on the collection area reaches the buffer. Therefore, part of the estimated 46,000m^2 is probably not connected to the collection system, but this was not further investigated [132].

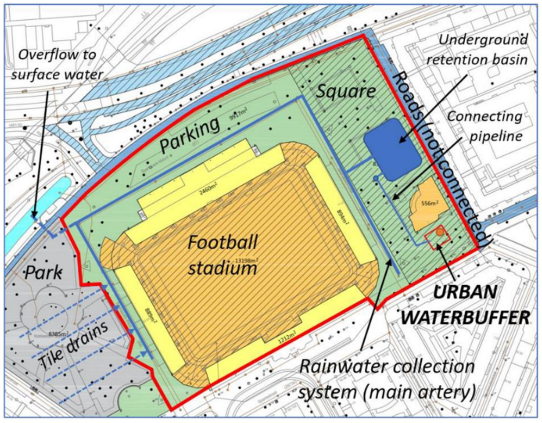


Figure 3.2: Stormwater collection area at the Urban Waterbuffer. Stormwater is collected within the red border. From [133].

Table 3.1: Rainwater receiving areas that discharge towards the Urban Waterbuffer Spangen. From [132, 133].

Type of Rainwater Receiving Area	Surface Area (m2)	Remark
Roof	6,000	Bitumen, Zinc
Parking place / square (paved)	18,300	Bricks
Sport Fields (Pitch+surroundings)	13,200	Artifical gras, pavement
Park	8,400	Green, pavement
Total	***45,900***	***Mixed***

3.1.1. Urban Waterbuffer Set-Up

The UWB consists of a stormwater collection and storage system, a Sedipoint for pretreatment, and a Bluebloqs biofilter and ASR system for main treatment (Figure 3.3).

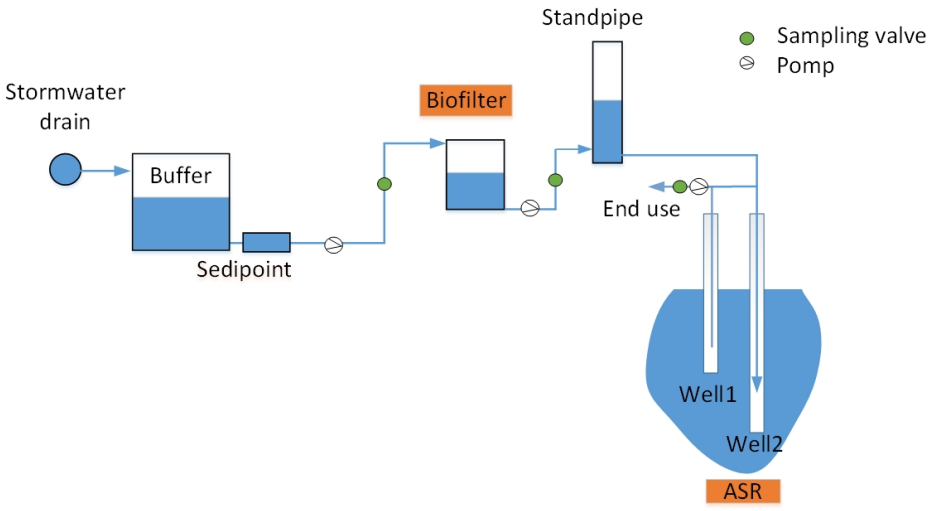


Figure 3.3: Scheme of the Urban Waterbuffer consisting of a stormwater drain from the stormwater collection system, a buffer, a sedipoint system for pretreatment, a biofilter, a standpipe and an ASR system consisting of well 2 for infiltration and well 1 for recovery. Figure not drawn to scale. Adjusted from [132].

Collected water is drained to a 1400 m^3 retention buffer located underneath a public sports field. The function of the buffer is to collect water from precipitation events to enable controlled water flows to the biofilter and aquifer. If the buffer capacity exceeds, rainwater flows into a river via an overflow connection from the rainwater collection system [132]. In the pipeline leaving the buffer, water is treated with a Sedipoint system that removes coarse material (>0.1 mm) and light non-aqueous phases such as

oil [132]. This treatment step aims to decrease physical clogging of the system [102]. After pretreatment in the sedipoint, water is pumped to the Bluebloqs Biofilter where it infiltrates through the biofilter and is collected at the bottom. From there, water is pumped to the standpipe (diameter 400 mm, height 3.3 m) from where it flows into an aquifer via the ASR well (Figure 3.4). This comprises two partially wells (diameter 160 mm) located in a single borehole. The wells are separated with a clay layer (1.0m) to prevent short-circuiting between the wells. Water is injected in the sand aquifer (-16.75-26.25 below surface level, depth: 10.5 m, porosity: 0.3) via the deepest well (W2, depth: 20-26.5 m below surface level), floats upwards due to the lower density of freshwater compared to the surrounding brackish water, and is recovered from the shallow well (W1, depth: 17-19 m below surface level). Monitoring wells (MW1.1 - MW1.5) are installed at 7 meters distance from the ASR well to evaluate the influence of injection and extraction of the water [132].

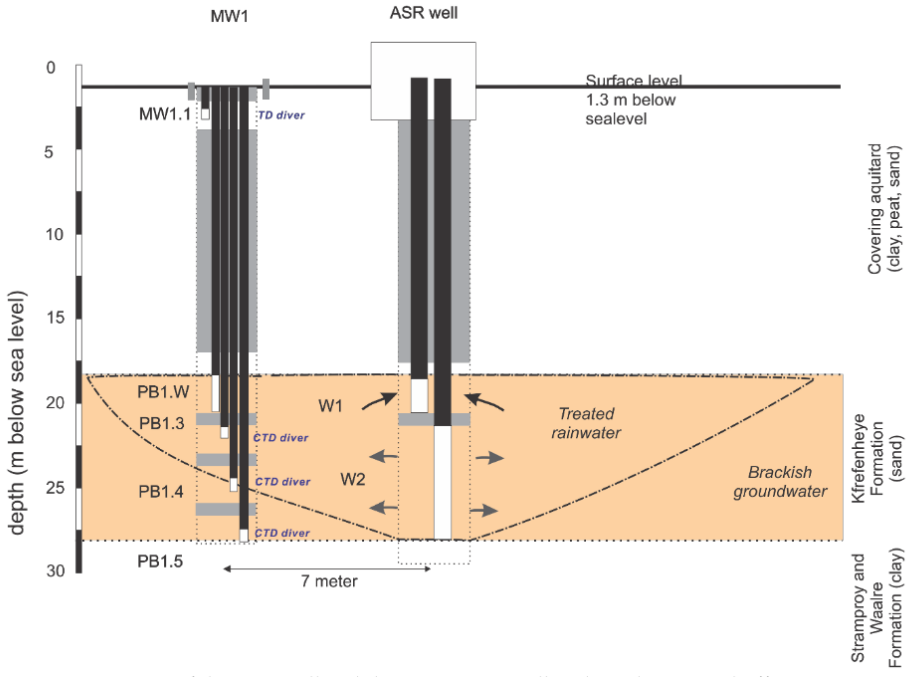


Figure 3.4: Cross-section showing the ASR well, including W1 and W2 and monitoring wells (MW 1.1 - 1.5) at the Urban Waterbuffer, Spangen. From [133].

3.1.2. Bluebloqs Biofilter Set-Up

The biofilter is a frustum with an upper surface area of 94.38 m^2, a lower surface area of 55.44 m^2, and a total volume of 81.46 m^3 (Fig 3.5). It consists of a filter layer, a transition layer, and a drainage layer (Fig 3.6). The filter layer (0.6 m), transition layer (0.2 m) and drainage layer (0.41 m) comprise of quartz sand with grain sizes varying from 0.4-0.8 mm (p=0.38, k=0.12 cm/s), 0.8-1.25 mm (p=0.39, k=0.40 cm/s), and 1.25-2.0 mm (p=0.40, k=0.51 cm/s) respectively. Grain size distribution of the sands are provided in Appendix A.1.

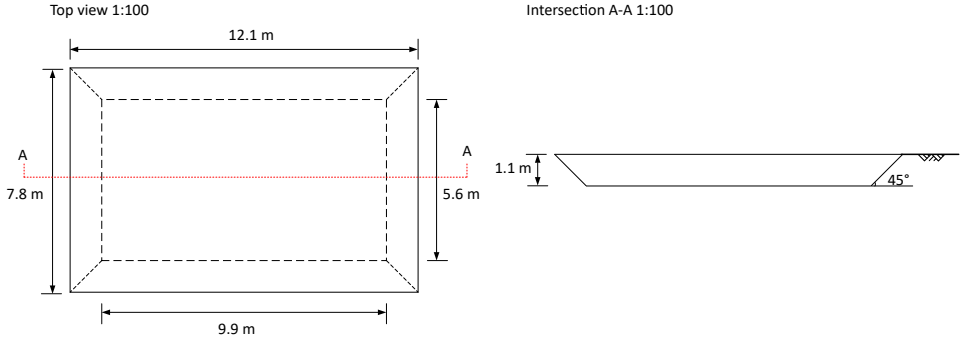


Figure 3.5: Biofilter top view and intersection A-A. The top of the biofilter is located at street level.

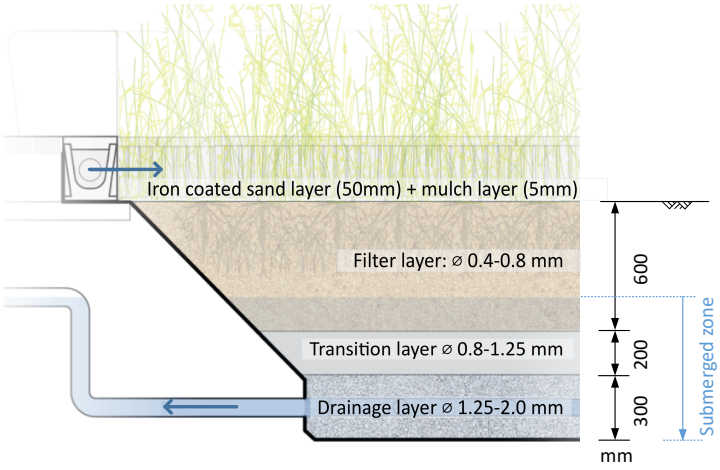


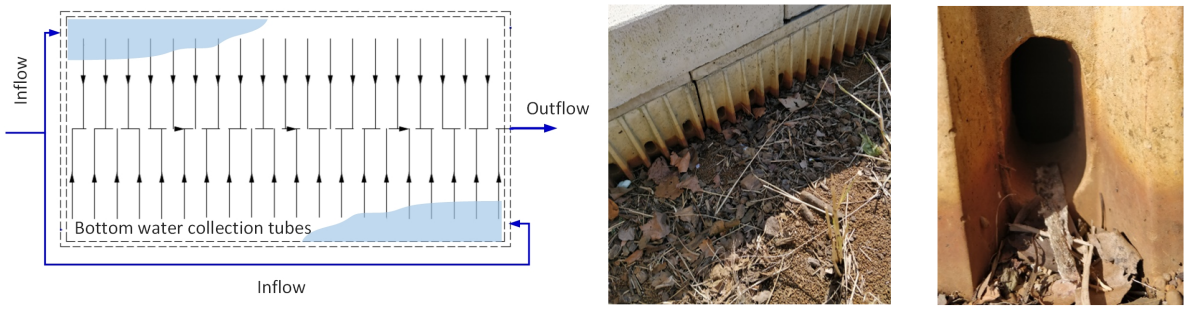
Figure 3.6: Schematic representation of the Bluebloqs biofilter 1.0 showing filter media layering and diameters (⌀) range of the grain sizes used in these layers. Adjusted from [102].

A layer of iron oxide coated sand (5 cm) is added after 5 months of operation to improve the adsorption capacity of the biofilter [132]. Besides, a mulch layer (5 mm) is added on top of the filter layer to retain moisture for vegetation [102]. Figure 3.7 shows the top layer material of the biofilter. The filter layer is planted with multiple species to provide robustness for survival (*Carex acuta, Carex hirta, Lythrum salicaria, Filipendula Ulmaria, and Molinia caerulea*). Moreover, these species are selected because they can grow and survive in the Dutch climate [102].

Figure 3.7: Top layer of the biofilter consisting of iron coated sand and mulch layer. From left to right: iron coated sand with mulch, bare spots in the top layer revealing filter layer sand, and close-up of the iron coated sand.

Hydraulic Control Elements

Water from the buffer is distributed to the biofilter via a concrete channel that surrounds the surface area (Fig 3.8b). Water enters the channel at two locations and in the design this water would spread over the whole surface area and create a ponding layer. However, system operators decided that this led to too high risk and this ponding layer was abolished. Currently, the water spreads over around 20% of the biofilter area (Figure 3.8a). It vertically infiltrates into the system and is collected at the bottom via perforated tubes that lead the water to a central drainage tube. From there, water flows out of the biofilter towards the ASR system. To prevent desiccation of the biofilter between inflow events, the system has a raised outlet which creates a submerged zone (SZ, Figure 3.6).



(a) Water distribution and collection system

(b) Inflow construction

Figure 3.8: Left figure (a): Bluebloqs biofilter 1.0 water distribution and collection system. Inflow at the top at two locations and collection at the bottom via perforated tubes that lead the water to the centered drainage tube, from where the water leaves the biofilter. From [132]. Right figure (b): Inflow Construction Biofilter. Left figure shows part of the concrete channel with holes. Right figure shows one inflow hole.

3.2. System Operation

UWB Spangen is operated with an automatic operation and control system. Multiple parameters (e.g., water levels, a full list is presented in Appendix A.2) are logged every 30 minutes on a Programmable Logic Controller (PLC). This section describes the operation of the system based on an analysis of the PLC data. First, section 3.2.1 explains how water flows are regulated in the UWB. Thereafter, the concept of feeding events is explained. Lastly, other water flows concerning the biofilter are described. A thorough understanding of the biofilter operation and infiltration events are needed to set-up a measurement plan.

3.2.1. Water Flow in the Urban Waterbuffer

Water flow in the UWB is regulated by pumps and valves that activate when critical water levels (determined by pressure sensors) are reached. Fig 3.9 shows a scheme of the water flow and the sensors and pumps that regulate this flow. Frequency regulated pumps sense pressure from a pressure transducer and adjust their volumetric flow rates by adjusting their motor speed to meet system requirements, electric valves open when a specific pressure is reached, and flow regulated pumps activate after a specified volume has passed.

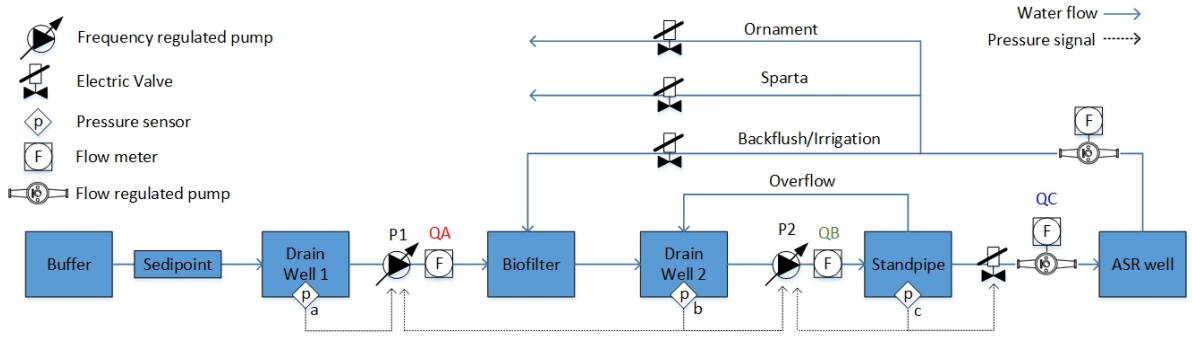


Figure 3.9: Treatment scheme of the UWB including the flow regulating equipment. Pressure signals measured by pressure sensors are transmitted to frequency regulated pumps, electric valves and flow regulated pumps. These control the water flow through the system, which is measured by flow meters. Based on [132].

Biofilter inflow and outflow

During rain events, the stormwater collection system drains water into the buffer. A pressure sensor in drain well 1 (DW1) measures the water level in the buffer. When a critical water level in the buffer is reached, the pressure sensor activates pump 1 (P1) that starts pumping water (QA) from the buffer to the biofilter. Subsequently, pump 2 (P2) activates and starts pumping water (QB) from the biofilter to the ASR system. The inflow stops when the water level in drain well 2 (DW2) reaches a maximum

set point. This water level drops again as water is pumped to the standpipe until a level is reached that cues the inflow pump to start again. Due to this operation, a feed pump cycle consists of multiple inflow intervals. The event stops when the buffer is emptied to a specified level. The outflow will continue until the water level in DW2 has dropped to a set level, which corresponds to the submerged zone level in the biofilter.

Standpipe inflow and outflow
P2 pumps water from the biofilter to the standpipe (diameter 400 mm, height 3.3 m) where water is stored until the pressure has built up that is required for injection in the aquifer. This pressure is reached when the standpipe is filled for 90%, which corresponds to 0.37 m^3 of water [132]. The pressure signal opens an electric valve and water infiltrates by gravity into the aquifer (QC). To prevent overflowing of the standpipe, the water level in the standpipe regulates the frequency of P2, contributing to fluctuations in the outflow rate (QB) from the biofilter to the standpipe [132]. As an additional measure, an overflow to drain well 2 is created, which pressure regulates P1. This, in turn, controls the inflow of the biofilter (QA), and consequently, the outflow (QB) towards the standpipe during an infiltration event.

ASR injection and recovery
From the standpipe, water (QC) flows via the ASR well into the aquifer where it is stored until water is recovered for either the Sparta sports field, the water pillar, a backflush, or irrigation of the biofilter. Water is recovered to Sparta when the water level in the buffer tank at Sparta drops to a specified level and water is pumped to the water pillar when the handle on this pillar is moved. To prevent clogging of the wells, a backflush occurs from well 2 to the biofilter after injection of every 2500 m^3. Irrigation of the biofilter with water from well 1 happens 3 times a day. Before October 2019, both wells were used for injection and recovery. Since October 2019, well 2 is used for injection and well 1 for recovery.

3.2.2. Feeding Events

Section 3.2.1 explains the regulation of water flows in the UWB. The feed of water from the buffer to the biofilter (QA) and the subsequent biofiltration of this water is called a feeding event. In the past, these feed cycles could happen anytime after or during a precipitation event when the critical water level in the buffer was reached due to incoming rainwater. Moreover, since the UWB is in operation the settings of the critical water levels cueing inflow and outflow have been changed multiple times. This resulted in varying event lengths and antecedent dry periods (Figure 3.10).

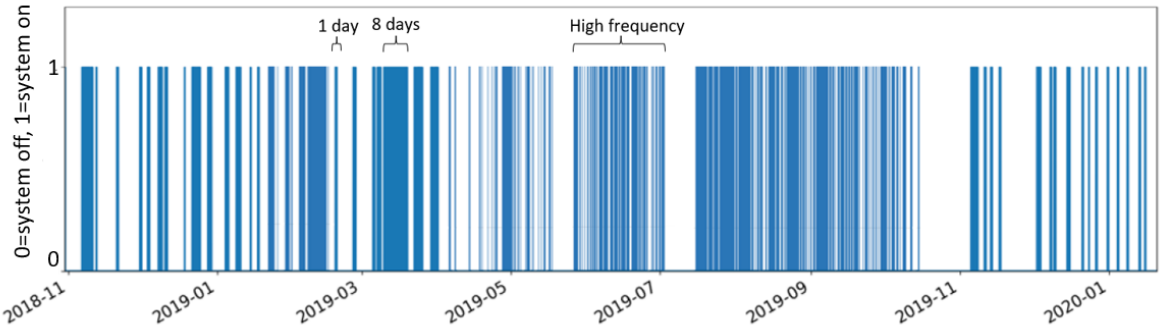


Figure 3.10: Bar plot of infiltration events. Every bar stands for an infiltration event and its width indicates the length of the event. An infiltration event is defined as the dosage of water from the buffer on the biofilter and the subsequent biofiltration of this water. Data from the FTP-server of the Urban Waterbuffer.

A drawback of this unpredictable operation is that it is almost impossible to plan monitoring rounds and enable sampling during an event. As field scale biofilters are variable systems, it is essential to minimize variability between sampling moments to enable meaningful comparison between samples. To facilitate consistent sampling during a feed cycle, the operational settings were changed for this research in the following way:

Activate P1 (Figure 3.9) that pumps water from the buffer to the biofilter when the buffer water level is between >40%, if:

1. It is Tuesday
2. It is 8:00 AM
3. It is between 8:00 - 12:00 AM

To prevent buffer overflow, a second activation level is set between 75% that cues the pumps to start immediately. Other settings of the pump, such as volumetric flow rates are unchanged.

3.2.3. Additional Water Flows to the Biofilter

Besides a weekly infiltration event, water is fed with water from the upper well (well 1) three times a day (Figure 3.11). This was introduced in March 2020 as abstracted water started to present a foul smell. To remove the smell before recovery of the water by Sparta, it was decided to periodically drain the water to the biofilter. On average, 19 m^3 per day is pumped up from the upper well, dosed on the biofilter, and infiltrated back into the lower well (well 2). Due to this recirculation, water in the submerged zone that initially consists of feed water from the buffer (approximately 27 m^3) is replaced with water from the ASR system within 1 day after the feeding event. The water that is dosed on the biofilter from the ASR well is high compared to the feed water from the buffer, approximately 120 m^3 compared to 44 m^3. Accordingly, the water in the SZ is replaced 4 times between events (Figure 3.12). Analysis of water flows is restricted by the data provided by the automatic log system of the Urban Waterbuffer. As data is logged every 30 minutes, the timing of flows cannot accurately be determined, hence the time frame in which the flows occur is presented (Figure 3.12) but actual times can differ. Assuming similar volumetric inflow rates as observed during feeding events (19-20 m^3), recirculating flows are expected to last between 15-30 minutes.

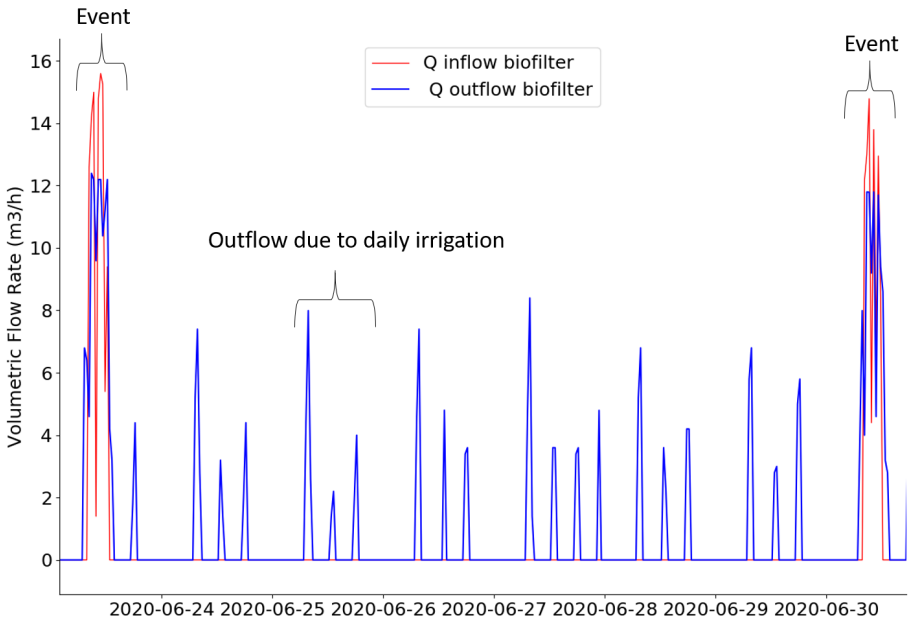


Figure 3.11: Weekly water flows from the buffer to the biofilter (in red) and from the biofilter to the ASR system (in blue). The flows from the biofilter to the ASR indicate the outflow due to daily irrigation of the biofilter with aquifer water.

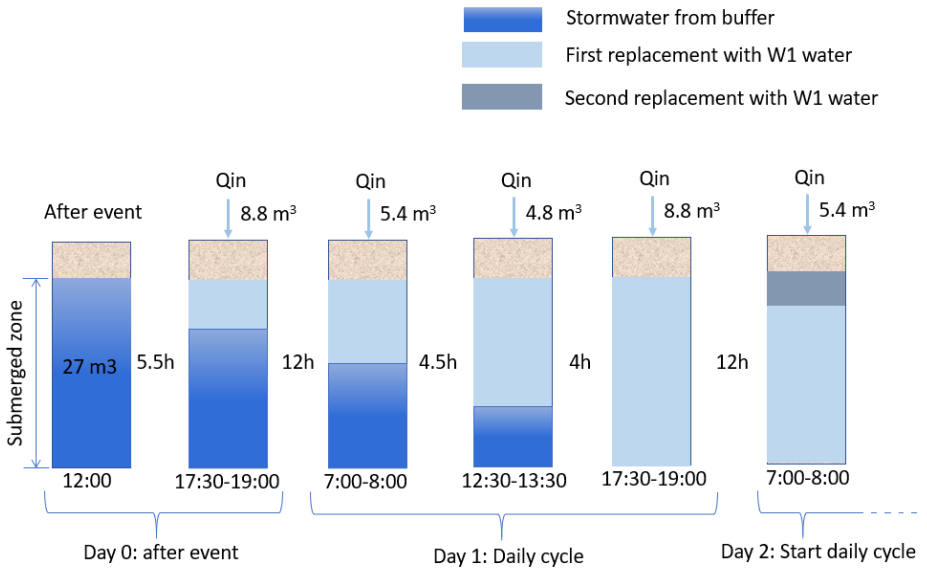


Figure 3.12: Water in the biofilter initially consist of stormwater from the buffer after a feeding event. Every day, this water is partly replaced with water from the ASR system. Water from well 1 is pumped to the biofilter and, consequently, part of the present water flushes out. Due to this operation, water in the biofilter is replaced with aquifer water in less than 2 days. The figure shows the different irrigation stages of the biofilter after the end of an event. Qin represent the average inflow water volume from the aquifer that is dosed on the biofilter per irrigation step.

4

Materials and Methods

This chapter presents the materials and methods carried out to improve understanding of microbial fate in the biofilter. Biofilter hydraulic conductivity was investigated (Section 4.1), water quality measurements were carried out from June 2020 until August 2020 (Section 4.2), and main transport and removal processes were modelled (Section 4.3).

4.1. Hydraulic Conductivity Measurements

The hydraulic conductivity of the biofilter was measured with an in situ hydraulic conductivity test, described in Section 4.1.1. To provide an estimate of the initial hydraulic conductivity, the hydraulic conductivity of the 3 sand types that make up the biofilter media were determined with a permeability test (Section 4.1.2). Porosity was also measured in this test to estimate the biofilter porosity. The actual field porosity, however, is highly variable due to, e.g., compaction, plant roots, and clogging. Consequently, it is hard to accurately measure porosity in the field and this has, to date, not been done at the research site.

4.1.1. In Situ Hydraulic Conductivity Test

A single ring infiltrometer under constant head test was carried out to estimate the saturated hydraulic conductivity of the filter layer. This method is recommended by the Facility for Advancing Water Biofiltration to determine in situ hydraulic conductivity [92]. The goal of this test is to continuously poor water into a ring to keep a constant head and continue doing this until the volume poured per minute has been stable for approximately 30 minutes [92].

For this test, a PVC ring with a bevelled edge and a 50 mm and 150 mm level marked on the inside was used. The ring was pressed 50 mm into the filter layer after removing the mulch and iron coated sand layer. Jerrycans were filled with approximately 60 L before the start of each test to provide sufficient water. The test was conducted at 2 different constant heads: 50 mm and 150 mm. These heads were kept constant by pouring water into the ring with 500 mL, 1 L, and 2 L measuring cylinders. Every minute, the volume poured into the ring was noted down. First, the test was conducted for a 50 mm head and when an approximate steady-state infiltration rate was reached, the test continued for 30 more minutes. Thereafter, the test was repeated for a head of 150 mm. From the calculated volumetric flow water, the hydraulic conductivity was estimated with the following equation for a steady flow [92]:

$$K = \frac{G}{a}\frac{Q_2 - Q_1}{H_2 - H_1} \tag{4.1}$$

Where K is the hydraulic conductivity, H1 (50 mm) and H2 (150 mm) are the pressure heads, Q1 and Q2 are the steady flows obtained per pressure head, and G is a shape factor of the PVC ring estimated as G = 0.316 depth/radius + 0.184 [92].

The test was carried out at 4 locations in the biofilter were different hydraulic behaviour was expected (Figure 4.1). First, the test was conducted at an inflow corner (1) where water flows over the surface during an event. Second, the test was conducted near the outflow of the biofilter. The third location was chosen in the middle of the biofilter, where no water flows over the surface during an event. The same accounts for the corner that was chosen as the last location (4).

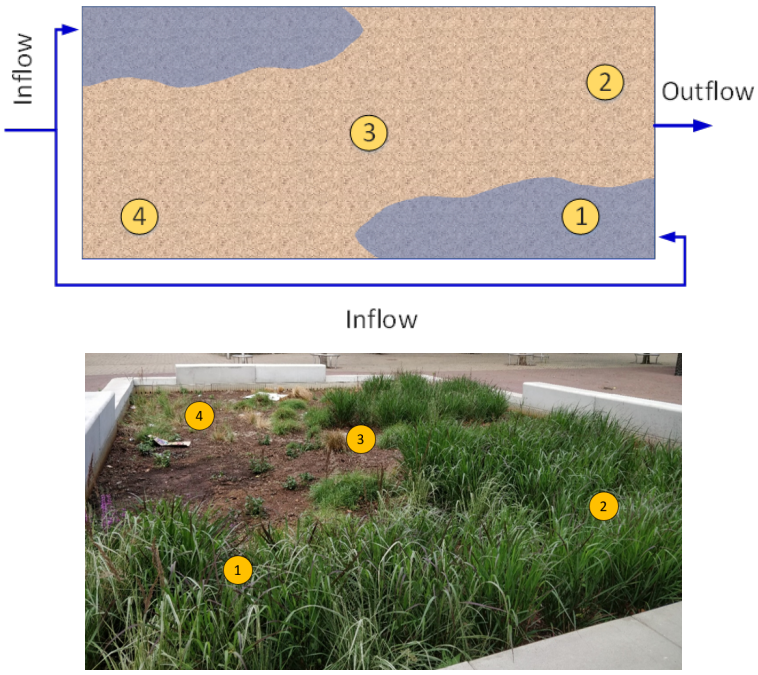


Figure 4.1: Locations where the hydraulic conductivity was measured. 1) wet corner, 2) outflow, 3) middle, 4) dry corner.

4.1.2. Permeability Test

Fig 4.2 shows the permeability test set-up that was used to determine the porosity and hydraulic conductivity of the 3 sand types that make up the biofilter media. The biofilter media comprises 0.6 m quartz sand with grain sizes varying from 0.4-0.8 mm, 0.2 m quartz sand (0.8-1.25 mm), and 0.3 m calibrated quartz sand (1.25-2.0 mm). The sand was supplied by Kremer Zand en Grind and specific details can be found in Appendix A.1. The test was carried out for all sand types separately.

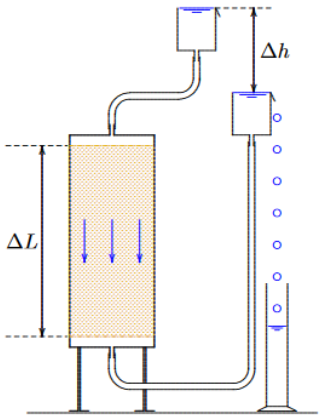


Figure 4.2: Permeability test set-up.

To test the drainage layer sand (0.8-1.25 mm) and transition layer sand (1.25-2.0 mm) the column was filled with a known mass of sand (m_{grains}) to 0.3 m and 0.2 m (ΔL) respectively. This corresponds to the heights of these layers in the biofilter. This was not possible for the filter layer (0.6 m) as the column was too small. Therefore, it was decided to fill the column to 0.3 m. The sand was compacted by hammering on the outside of the column until a stable top level was reached. This was thought to be the most representative compaction for the field situation that could be achieved in this test. A constant head was maintained and water flowed through the sand due to a difference in the head (Δh) between the two reservoirs. Discharge (Q) was calculated 3 times per test by collecting a known volume of water over a measured period. Darcy's law (Equation 4.2) was used to calculate the hydraulic conductivity (k).

$$Q = k * A\frac{\Delta h}{\Delta L} \tag{4.2}$$

The porosity (p) was calculated with Equation 4.3 and Equation4.4. An estimate of the biofilter porosity was made by normalizing the porosity of the sands over their volumetric contribution in the biofilter.

$$p = \frac{V_p}{V_{total}} = \frac{V_{total} - V_{sand}}{V_{total}} \tag{4.3}$$

$$V_{sand} = \frac{m_{grains}}{\gamma_{grain}} \tag{4.4}$$

Where: γ_{grain} = 2650 kg/m^3 [122].

4.2. Water Quality Measurements

Water quality flowing in and out of the biofilter was monitored during 5 feeding events to investigate microbial water quality dynamics in the inflow and outflow, physicochemical characteristics, and water flows in the biofilter. To enable consistent monitoring, the system operation was arranged for one feeding event per week from 8:00-12:00 AM, as explained in Section 3.2.2. Events were monitored during the summer months: 2 in June, 2 in July, and 1 in August. Additional information on these events is provided in Appendix B.

Firstly, Section 4.2.1 explains the choice to investigate *E. coli*, enterococci and *Campylobacter* in this study. Thereafter, Section 4.2.2 elaborates upon the sampling approach. Section 4.2.3 discusses the methods used to process the samples to obtain *E. coli*, enterococci, and *Campylobacter*. Lastly, Section 4.2.4 explains how the data was further processed to evaluate microbiological changes in the biofilter (Section 4.2.4).

4.2.1. Microorganism of Interest

Indicator bacteria *E. coli* and enterococci were studied in this research because they are the operational control for microbiological water quality in the EU drinking water directive and the Dutch ”Waterleidingbesluit”. Both state that *E. coli* and enterococci have to be absent in a 100 mL sample. Similarly, this norm was set at Sparta for the water quality of the recovered water [132]. In addition, both organisms were measured in previous research at the UWB [132], and elaborating this data set can provide insight in e.g., faecal contamination variability.

Detection of different pathogen types, for example, virus, protozoa, and bacteria, that cause different illnesses, e.g. gastrointestinal, respiratory, skin, can provide a total overview of pathogen removal. However, due to time constrain, this research focuses on the detection of the pathogen of the highest risk. Considering the origins of the received rainwater, the rainwater in the UWB system is hypothesised to be free from human faecal contamination. Contamination from birds and domestic animals, for example, cats, dogs, rodents, however, is probable. From a health risk perspective, three reference pathogens are recommended that represent the upper boundary of risk; rotavirus, *Cryptosporidium* and, *Campylobacter* for respectively pathogenic viruses, protozoa, and bacteria [125]. Since human faecal contamination or pig contamination is not expected, viral risk is not considered [10]. Additionally, research on Dutch rainwater found *Campylobacter* to be present, while *Cryptosporidium* was not detected [105, 106]. Therefore, *Campylobacter* is selected as target pathogen.

4.2.2. Sampling Approach

One aim of the fieldwork was to improve understanding of the microbiological loads flowing in and out of the biofilter. For this, samples were collected during feed cycles from the sampling valves in the underground technical room at the Urban Waterbuffer. The location of these valves in the flows is indicated in Figure 3.3 with green circles. To minimise the time in the underground chamber, tubes were connected to the valves and the ends of these tubes were placed outside in measuring cups. The valves were continuously opened to create flow-through cells in these cups: 1 inflow cell and 1 outflow cell. pH, EC, and DO meters were placed in each cell and values were noted down every 5 minutes. EC and DO meters were calibrated 24 hours before the start of sampling, pH meters were calibrated

within 30 minutes before sampling. Samples were collected from the flow-through cells in sterile bottles and stored in a coolbox.

Figure 4.3: Flow-through cells for inflow and outflow water of the biofilter in which EC, PH, DO and temperature are measured and from which samples are taken for the microbiological analyses.

Figure 4.4 presents the number of samples taken per event and the composite samples that were composed of the individual samples to reduce the workload in the laboratory. To illustrate that grab samples were processed together as a composite sample in the laboratory, individual grab samples are connected with a black line (Figure 4.4). Outflow water quality was expected to vary more than inflow water quality as this water is fed from the buffer. Therefore, more outflow samples were taken. In total, 17 inflow samples and 36 outflow samples were analysed per bacteria.

Multiple factors led to a difference in the number of samples taken between events. Firstly, the number of samples per event was increased when time in the laboratory allowed to process more samples. In this way, microbial dynamics could more extensively be investigated. Furthermore, the number of samples differed per event depending on the available time at the site. It was aimed to start sampling from the beginning of the inflow feed and continue as long as possible. However, this was not always achievable. Lastly, stops in the inflow feed were discovered during the events but the timing was not fully understood in the first 2 events. Hence, little volumes in the grab samples made it necessary to merge grab samples from different inflow feeds. When feed cycles were better understood, inflow composite samples were based on the separate inflow periods.

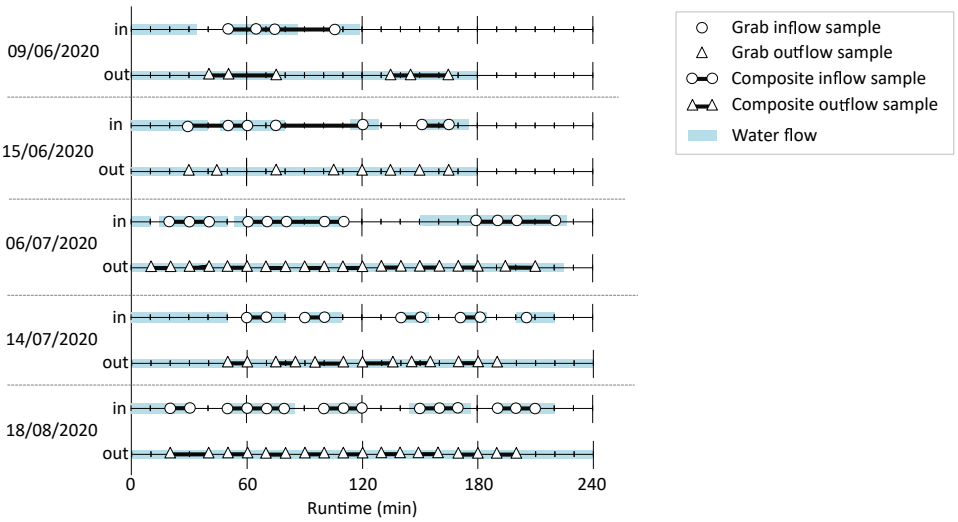


Figure 4.4: Sampling timeline per event. Time 0 represents the start of the feed of water from the buffer to the biofilter and the water flow is presented as blue areas. Circles represent discrete inflow samples and triangles discrete outflow samples. Discrete samples that were combined to create a composite sample are connected with a black line.

4.2.3. Sample Processing

Samples were transported to the laboratory and processed within 24 hours to minimise decay or growth between sampling and processing. Before processing, the samples were stored at 4°C. All samples were analysed for *E. coli*, Enterococci, and *Campylobacter* using culturing methods. Due to the variable nature of the inflow and outflow water quality, multiple volumes were processed in an attempt to obtain 25-100 CFU per plate. Volumes <0.1 μL were processed with the spread plate method, volumes >0.1 μL with the membrane filtration method. For this, volumes were filtered through Whatman 0.2 μm pore size, 47 mm diameter cellulose acetate membranes (Sigma-Aldrich). For the last event, a 0.45 μm pore size was used which could have led to some deviation in bacteria enumeration. This is further discussed in Section C.2.

For all bacteria, agar plates were prepared according to the procedure and dried until visible moisture was gone in the flow cabinet before usage. For *E. coli* detection and enumeration ChromoCult Coliform agar (Merck) was used. After incubation for 24 hours, all blue colonies were counted with a colony counter. To detect enterococci, Slanetz and Bartley enterococcus agar (Thermo Fisher Scientific, CM0377) was used. The agar plates were first incubated for 4 hours at 35°C to aid the stressed organism to grow and, thereafter, for 44 hours at 44°C for the selection of enterococci. All red and maroon colonies were counted as enterococci. The presence of *Campylobacter* was determined using CHROmagar (bioTRADING Benelux B.V). Plates were placed in a candle jar to create a microaerophilic atmosphere (Figure 4.5) and were incubated for 36-48 hours at 42°C. After incubation, all red colonies were counted as *Campylobacter coli, C. jejuni, C. lari*.

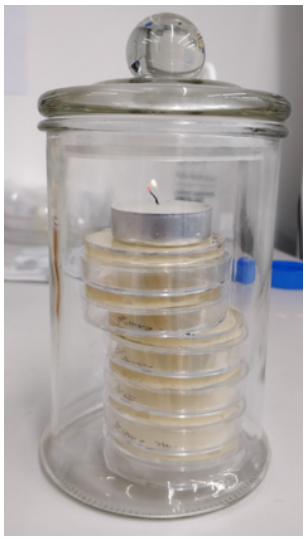


Figure 4.5: The candle jar method used to create oxygen and carbon dioxide conditions favourable for *Campylobacter* growth.

4.2.4. Data Analysis

Microbiological results were calculated in CFU/100 mL according to the United States Environmental Protection Agency method [16]. For this, the most reliable plates from a sample were selected. Plates with atypical colony growth (e.g., indistinct colonies due to swarming) and too numerous colonies to count were discarded. Additionally, plates with counts lower than 5 colonies were thought too unreliable and were discarded, with exception of plate counts performed with a filtration volume of 100 mL. Thereafter, plates with >25 colonies were selected as higher colony counts improve the reliability of the results. When this was not possible, the count closest to 25 CFU was chosen. When multiple volumes resulted in acceptable counts, the counts were first converted to CFU/100 mL, and then averaged for the final reported value.

If colonies on all plates of a sample were too numerous to count (TNTC), the concentration was estimated by taking the upper limit count and the smallest filtration volume that resulted in TNTC. Results were reported as greater-than (>) values. The upper limit count for *E. coli* was chosen at 150 CFU and

for Enterococci and *Campylobacter* at 200 CFU. A lower limit was chosen for *E. coli* due to background bacteria that can grow on the agar, in comparison with the more specific agars used for Enterococci and *Campylobacter*. For all filters without colonies but filtered with 100 mL, a concentration of <1 was reported. If no reliable data was obtained from a sample, No Data was reported and the reason was specified.

Microbial water quality changes over the biofilter
Based on the microbial inflow and outflow concentrations, an indication of the treatment performance of the biofilter was determined with two methods. Firstly, outflow concentrations observed after 1 pore volume had flushed from the biofilter were compared with inflow concentrations that entered the biofilter 1 pore volume before. It was assumed that these outflow samples mainly consisted of filtered feed water. The differences between inflow and outflow concentrations were calculated as the difference between the logarithmic (base 10) inflow concentrations and logarithmic (base 10) outflow concentration and expressed as log removal. A negative value shows that bacteria are added to the system, while a positive value indicates that bacteria are removed.

Secondly, outflow concentrations that would occur if submerged zone water and feed water would solely mix and no reaction over the biofilter would take place were compared with measured outflow concentrations. Based on this, changes in microbial concentrations that take place in the biofilter were estimated. This method was proposed by Appelo and Postma[8] to determine chemical reactions during fresh/saltwater displacement. With Equation 4.5, the fractions of aquifer water in the outflow samples were determined. For $EC_{aquifer}$ the logged concentration of aquifer water was taken and for $EC_{stormwater}$ the average EC value of the inflow. Thereafter, the microbial concentrations in the outflow samples solely based on mixing were obtained with Equation 4.6. For this, $m_{aquifer}$ was assumed the first outflow microbial concentration and $m_{stormwater}$ the average inflow concentration. Lastly, sampled outflow concentrations were compared with calculated concentrations to obtain an estimate of the number of microbes that were added or removed in the biofilter with help of Equation 4.7.

$$f_{aquifer} = \frac{EC_{sample} - EC_{stormwater}}{EC_{aquifer} - EC_{stormwater}} \tag{4.5}$$

$f_{aquifer}$ = fraction of aquifer water in the outflow sample (-)
EC_{sample} = EC outflow measurements (μS/cm)
$EC_{stormwater}$ = average of inflow EC measurements (μS/cm)
$EC_{aquifer}$ = EC value of the aquifer water present in the biofilter (μS/cm)

$$m_{mix} = f_{aquifer} * m_{aquifer} + (1 - f_{aquifer}) * m_{stormwater} \tag{4.6}$$

m_{mix} = concentration from conservative mixing of aquifer water and stormwater (CFU/100mL)
$m_{aquifer}$ = concentration of aquifer water similar to first outflow concentration (CFU/100mL)
$m_{stormwater}$ = average concentration of inflow water (CFU/100mL)

$$m_{react} = m_{sample} - m_{mix} \tag{4.7}$$

m_{react} = concentration change due to reaction in biofilter (CFU/100mL)
m_{sample} = measured concentration outflow water (CFU/100mL)
m_{mix} = concentration from conservative mixing of aquifer water and stormwater (CFU/100mL)

4.3. Modelling Microbial Transport and Removal

Besides field measurements, main microbial transport and removal processes were modelled in PHREEQC to improve understanding of multiple design and operational choices on microbial fate. PHREEQC is a program based on equilibrium chemistry of aqueous solutions interacting with e.g., sorption surfaces which can be used to model kinetic reactions and one-dimensional transport processes [91]. Main processes and equations are explained in Section 4.3.1. The schematization of the model is provided in Section 4.3.2. An attempt was done to obtain a site-specific rate constant to describe main processes by performing two laboratory tests, which are explained in Section 4.3.3. The concepts tested with the model and the sensitivity analysis performed are discussed in Section 4.3.4 and Section 4.3.5.

4.3.1. Processes and Equations

Microbes are transported via advection, diffusion, and dispersion, are retained in the filter media, and subsequently decay or remobilize again [20]. Adsorption is assumed the dominant mechanism removing microbes from the water phase during transport through the biofilter, as coarse filter media minimises straining and microbes are too small for physical filtration (Section 2.2.1). The decay of the retained microbes, subsequently, removes the bacteria from the biofilter.

Equations that were previously applied in other studies to model these processes are used in the study [20, 40, 55, 111]. The transport and fate of microbes in the water phase (C) is described with Equation 4.8 [55, 111]. The first two terms on the right side of the equation account for the dispersive and advective transport, respectively. Thereafter follows the removal of microorganisms from the aqueous phase due to attachment to the filter media and decay. The last term represents an influx of microbes to the aqueous phase from the attached phase.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - v\frac{\partial C}{\partial z} - k_{att}C - \mu C + k_{det}\frac{\rho}{n}M \tag{4.8}$$

Where:

C = Concentration microbes in the aqueous phase (N/L)
t = time (s)
D = hydrodynamic dispersion coefficient (m^2/s)
z = distance in the flow direction (m)
v = pore water velocity (m/s)
k_{att} = attachment rate (s^{-1})
μ = inactivation rate (s^{-1})
k_{det} = detachment rate (s^{-1})
ρ = dry bulk density (kg/m^3)
M = concentration of attached microbes (N/kg)
n = porosity (-)

The concentration of attached microbes (M) is described with Equation 4.9 [20]. The first term on the right side of the equation represents the retention of microbes from the aqueous phase on the filter media. The second term accounts for a decrease in the concentration of attached microbes due to detachment to the aqueous phase and the last term represents microbial decay.

$$\frac{\partial M}{\partial t} = \frac{n}{\rho}k_{att}C - k_{det}M - \mu M \tag{4.9}$$

The die-off rate of microbes in the aqueous phase and attached phase is temperature dependent and described by the often applied Arrhenius-van't Hoff equation, Equation 4.10 [20, 40, 128].

$$\mu = \mu_0\theta^{T-20} \tag{4.10}$$

Where:

μ = inactivation rate (s^{-1})
μ_0 = Standard die-off rate at standard temperature (d^{-1})
θ = Temperature correction coefficient for die-off (-)
T = Temperature in (°C)

4.3.2. Schematization

The biofilter was schematised as a one-dimensional column divided into 20 cells with a total height of 110 cm and Δz = 5.5 cm. Flow direction was set from low to high cell numbers, hence a forward flow in a plug flow regime (Figure 4.6). The boundary conditions for the first and last cells were defined as flux boundary conditions and saturated conditions were assumed. The cells were filled with an initial solution, numbered similarly. During an event, the biofilter was fed with a solution containing a certain concentration of microbes. The advective transport of these microbes was modelled by shifting these solutions from cell to cell [91]. A one-dimensional transport algorithm simulated dispersion and diffusion

for each shift [91]. Additionally, for each shift and mixing step, kinetic reactions were integrated over time while maintaining equilibrium with any phase present in the cell [91]. Between events, stagnant conditions were simulated. During these dry periods, there was no advective flow but only diffusion, and no microbes were fed to the biofilter.

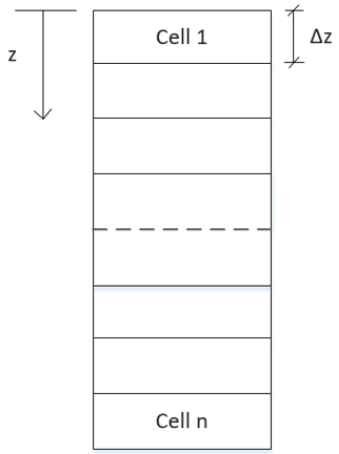


Figure 4.6: Schematization of the PHREEQC model.

4.3.3. First-order Rate Constants: Adsorption, Desorption and Decay Rate

To gain an impression on the adsorption and desorption rates, the depletion of *E. coli* cells from a solution by adsorption onto clean filter layer sand was measured in an adsorption experiment. The decay of *E. coli* and enterococci in a soil sample was monitored to assess die-off rates in the submerged zone. The following paragraphs describe the materials and methods used for these experiments. The observed rates were compared with literature values and the most applicable rates were chosen for the model simulations.

Adsorption and Desorption Rates

10 beakers were filled with 26 g of sterilised filter media sand (quartz sand 0.4-0.8 mm, autoclaved) supplied by Kremer Zand en Grind. A 1 liter solution was prepared with 1000 mL Ringer's buffer and 0.1 mL $1*10^8$ *WR1 E. coli* stock culture to approximately obtain a start concentration of $1*10^4$ CFU/mL. This solution was mixed and 10 mL was added to the beakers to create saturation of the soil samples similar to the saturated zone. The soil to water ratio was estimated from the assumed biofilter porosity of 0.38. An additional beaker with solely the solution and no filter sand acted as a control to assess changes in microbial concentrations other than caused by adsorption to the sand. The experiment started after adding the solution to the beakers by continuously shaking the beakers at 120 rpm. At times t=15,30,45,60,90,120,150,180, and 240 minutes a beaker was removed from the shaker from which 0.1 mL, 0.5 mL and 1 mL samples were taken. The 0.1 mL sample was serial diluted and bacteria concentrations were determined by the spread plate method and membrane filtration method depending on the volume. Based on these values, the concentration attached to the sand grains were estimated with Equation 4.11 [53].

$$X = \frac{(C_0 - C) * V}{W} \tag{4.11}$$

Where:

X = bacteria concentration adsorbed per gram soil at time=t_i (CFU/gram)
C_0 = concentration in aqueous solution at time=0 (CFU/mL)
C_t = concentration in aqueous solution at time=t_i (CFU/mL)
V = volume of water in the soil sample (mL)
W = weight of the soil (grams)

The adsorption rate was estimated using the first-order kinetic sorption equation, Equation 4.12 [126],

assuming that the last measured concentration in the water phase was equal to the equilibrium concentration. The desorption rate was, thereafter, estimated using the Equation 4.8, assuming no transport and no decay during this experiment. Rates were calculated for all measured times and from these values, the mean and standard deviation were determined.

$$C_t - c_{eq} = (C_0 - C_{eq}) * e^{-K_{att} * t} \tag{4.12}$$

Where:

C_t = concentration in aqueous solution at time=t_i (CFU/mL)
C_0 = concentration in aqueous solution at time=0 (CFU/mL)
C_{eq} = concentration in aqueous solution at equilibrium (CFU/mL)
t = time (hours)
K_{att} = first order adsorption rate (hour^{-1})

Decay Rate
A filtration layer soil sample was taken at the end of the sampling round on 14/07/2020. This sample consisted of multiple sub-samples taken over the surface area of the biofilter to a depth of approximately 30 cm after removing the iron-coated sand and mulch layer. The soil sample was saturated with the last taken inflow sample at the field and transported to the laboratory. Thereafter, the sample was mixed and divided into 10 sub-samples. 5 of these samples were stored at room temperature and 5 in a climate chamber of 10°C. All samples were stored in the dark.

On day 1, day 2, day 6, and day 7 the water in the soil samples was analysed for *E. coli*, enterococci and *Campylobacter*. Pore water was drained out of the soil sample by vacuum and analysed with the same methods as described in section 4.2.3.

The die-off rate was estimated using the first-order kinetic equation (Chick's law) [110].

$$N_t = N_0 e^{-\mu t} \tag{4.13}$$

Where:

N_t = Number of bacteria at time t=t_i (CFU/100mL)
N_0 = Number of bacteria at time 0 (CFU/100mL)
t = time (days)
μ = first order die-off rate constant (day^{-1})

Sensitivity Analysis
Obtained rates were compared with literature values to assess the validity of the measurements and choose the most applicable rates. To assess the sensitivity of model outcomes for the chosen first-order rate constants, the rates were adjusted to the lowest and highest values stated in the literature for biofilter systems which were summarised by Shen et al.[111]. First-order adsorption rate constants were varied from 2-5.86 h^{-1}, desorption rate constants from 6*10^{-5}-0.48 h^{-1}, and decay rate constants from [111].

4.3.4. Model Simulation: Short-Circuiting

Short-circuiting is defined as a phenomenon in which a portion of inflow water remains in the biofilter for a much shorter time than the presumed mean residence times due to velocity heterogeneity in the system [72]. The effect of these fast flowing pathways on microbial removal in the biofilter was investigated with the model.

The event of 14/07/2020 in which 44 m^3 was dosed in 4 hours was simulated with a plug flow model and with a multipathway model. In the plug flow model, the inflow volume was evenly distributed over the biofilter, while in the multipathway model different loads were dosed on different parts of the biofilter resulting in multiple flow paths. It must be noted that these flow paths similarly follow a plug flow regime. For the multipathway model, the biofilter was estimated to comprise 3 areas (Figure 4.7). An inlet area (A1) where short-circuiting is expected due to the short distance to the outlet point, an inlet

area (A2) where average to fast flow is expected, and an area (A3) that does not directly receive inflow and, accordingly, is expected to have a slow flow.

A fixed ratio between the different flow paths was estimated: A1 and A2 comprise 10% of the filter area which each represent 3.2m^3 pore volume, and A3 comprises 80% of the filter area representing 25.4m^3 pore volume (total pore volume=31.8 m^3). With optimization methods, the best composition of these areas could be selected to achieve the highest correlation with experimental data. Simulating the experimental data is, however, beyond the aim of this research.

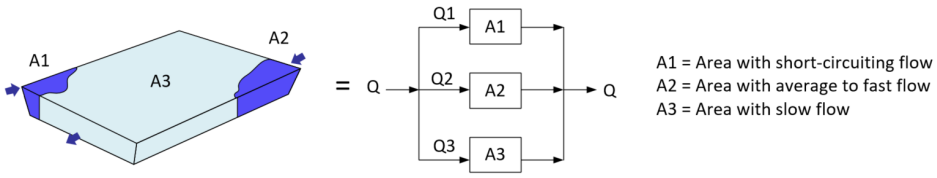


Figure 4.7: Schematization of biofilter in 3 different areas. The total inflow is divided over these areas to simulate different flow velocities: a fast flow through A1, an average to fast flow through A2 and a slow flow through A3.

Flow velocity heterogeneity was simulated by dosing higher inflow volumes on areas A1 and A2 compared to area A3, which resulted in higher flow rates through areas A1 and A2 than through A3 (Table 4.1). Three scenarios were tested in which the amount of inflow volume received differed per area and, accordingly, the times the pore volume of the area was flushed. From scenarios 1-3, the total volume dosed on the inflow corners (A1 and A2) was increased and, correspondingly, the volume dosed on the dry surface area (A3) was decreased. All simulation started with a clean biofilter, i.e., no pathogens were present in the biofilter, and microbial inflow concentrations are similar for all areas.

Table 4.1: Characteristics of the different model runs to investigate the effect of short-circuiting in the biofilter. In the plug flow run 100 % of the inflow volume is dosed on the total surface area, while in the multipathway scenario runs the total amount of inflow volume is distributed over 3 different surface areas resulting in different flow velocities through the biofilter.

	Area	Inflow volume (% of total inflow volume)	Vertical velocity (m/h)	Times PV flushed
Plug flow	Total (100%)	100%	0.12	1.37
Multipathway scenario 1	A1 (10%)	20%	0.23	2.75
	A2 (10%)	10%	0.12	1.37
	A3 (80%)	70%	0.1	1.20
Multipathway scenario 2	A1 (10%)	30%	0.35	4.12
	A2 (10%)	20%	0.23	2.74
	A3 (80%)	50%	0.07	0.86
Multipathway scenario 3	A1 (10%)	40%	0.46	5.49
	A2 (10%)	40%	0.46	5.49
	A3 (80%)	20%	0.03	0.34

4.3.5. Model Simulation: Event Frequency and Duration

A stormwater volume can be processed by multiple frequent short events or less frequent longer events. To assess the effect of this operational choice on microbial outflow concentrations, daily events of 4 hours were compared with 3-daily events of 12 hours (Figure 4.8). A total period of 18 days was considered. Characteristics of the two scenarios are presented in Table 4.2. Simulations were performed assuming that inflow water is distributed over the total surface area. Simulations started with a clean biofilter and a pore volume of 31.8 m^3 was assumed that was flushed in 2.7 hours.

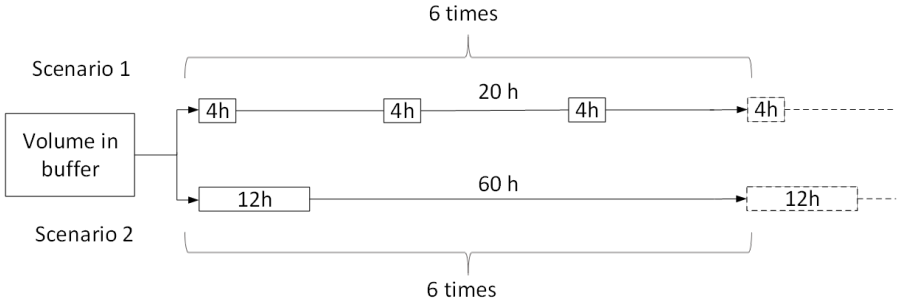


Figure 4.8: Model scenarios to process a volume of water by events with different duration and frequency.

Table 4.2: Characteristics of two scenarios tested in the model to investigate the effect of short frequent events and long less frequent events on microbial outflow concentrations.

	Scenario 1: short events	Scenario 2: long events
Event duration	4 hours	12 hours
Pore volume flushed during event	1.5	4.5
Dry period between events	20 hours	60 hours
Number events	18	6
Number dry periods	18	6
Total time	18 days	18 days

5

Results and Discussion

This chapter presents and discusses the results to examine the microbiological removal capacity of the biofilter, factors that lead to adverse microbial treatment, and how the system can be upgraded. First, the results of the methods are discussed in Section 5.1-5.4. Thereafter, implications of the findings for microbial fate are explored in Section 5.5. Section 5.6 present ways to improve microbial treatment. Lastly, research limitations and uncertainties are discussed in Section 5.7.

5.1. Hydraulic Conductivity

This section presents the clean sand hydraulic conductivity (Section 5.1.1), field hydraulic conductivity (Section 5.1.2), and compares findings with other field scale biofilters (Section 5.1.3). As mentioned in the literature review (Section 2.3.1), hydraulic conductivity affects flow rates through the system and, accordingly, hydraulic retention times. This, in turn, influences the extend of microbial retention in the biofilter.

5.1.1. Clean Biofilter Media Porosity and Hydraulic Conductivity

The biofilter comprises of 3 different types of dried and calibrated quartz sand. Table 5.1 shows the porosity and hydraulic conductivity of these sands that were determined with the permeability test. Porosity between the sand types are comparable, but an increase in hydraulic conductivity is seen with an increase in grain size distribution. Hydraulic conductivity and porosity values (Table 5.1) are in accordance with values found in literature for clean sand [6]. As the sand is relatively coarse and, subsequently, has large pore spaces, hydraulic conductivity values are high. With the results, a biofilter porosity of 0.39 was estimated and a pore volume of 31.77 m^3. The porosity is comparable with field scale porosity estimated in other studies. Chandrasena et al.[7] estimated a porosity of 0.38 for two sand-based biofilters, and Zhang et al.[127] assumed a porosity of 0.40 for a sand biofilter with submerged zone. However, it must be kept in mind that the porosity and the pore volume are rough estimates of the biofilter porosity. Field scale porosity is expected to be heterogeneous and affected by, amongst others, clogging and vegetation and should, therefore, be measured in situ.

Table 5.1: Hydraulic conductivity and porosity of the sands used in the Bluebloqs Biofilter determined with the permeability test.

	Sand Type		
	AcquaSilica® 0,4-0,8mm	AcquaSilica® 0,8-1,25mm	Dried and calibrated sand 1.25-2.0mm
Biofilter application	Filter layer	Transition layer	Drainage layer
ΔL (m)	0.3	0.2	0.3
Porosity (-)	0.38	0.39	0.41
Hydraulic conductivity (mean) (mm/h)	4320	14760	18360
Hydraulic conductivity (std) (cm/s)	68	1404	1620

5.1.2. In Situ Biofilter Hydraulic Conductivity

The hydraulic conductivity of the filtration layer was determined in situ using the single ring infiltrometer test at 4 locations in the biofilter, as described in Section 4.1.1. Figure 5.1 shows the locations and Table 5.2 presents the hydraulic conductivity per location. Hydraulic conductivity in the middle of the biofilter is highest (9740 mm/h), followed by the dry corner (5510 mm/h). These values are larger than the hydraulic conductivity of the clean filter layer sand (4320 mm/h) determined in the laboratory (Table 5.1). In contrast, the hydraulic conductivity near the inflow corner (990 mm/h), and near the outflow (3040 mm/h) are smaller than the clean sand hydraulic conductivity. These differences are likely caused by the uneven distribution of feed water over the biofilter surface that can result in differences in clogging, hydraulic compaction and desiccation.

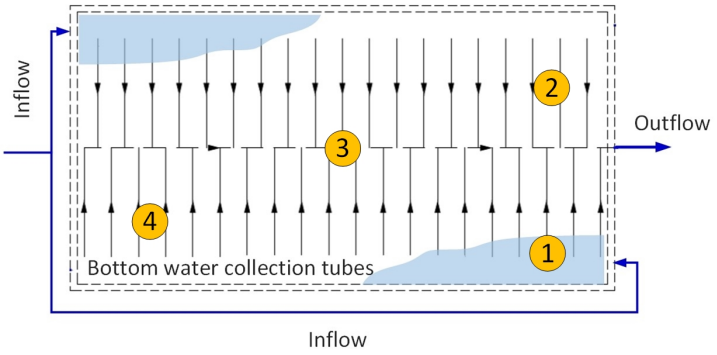


Figure 5.1: Locations of the hydraulic conductivity measurements presented on the water distribution and collection system of the biofilter.

Table 5.2: Results of the hydraulic conductivity measurements per location.

Location	Location description	Hydraulic conductivity (mm/h)
1	Wet corner due to inflow over the surface of the biofilter	990
2	Location nere the outflow of the biofilter. During an event no water flow over the surface	3040
3	Middle of the biofilter, surface stays dry during an event	9740
4	Dry corner, no water flows over the surface of this corner during an event	5510

Location 1, which has the lowest hydraulic conductivity (990 mm/h), is the only location that directly receives inflow water. The decreased hydraulic conductivity compared to the clean sand hydraulic conductivity can, therefore, be explained by clogging and compaction due to hydraulic loading. This corroborates with previous studies that demonstrate that clogging and compaction lead to reduced field scale biofilter hydraulic conductivity [31, 51]. Hydraulic conductivity near the outflow corner (location 2, 3040 mm/h) is also lower than the clean sand hydraulic conductivity. As this area does not directly receive inflow water, an explanation for the lower hydraulic conductivity is that relatively dirty inflow water from the inlet laterally spreads beneath the surface towards the outlet. This implies that short-circuiting paths are present between the inlet and outlet of the biofilter. Considering that one inlet point is located near the outlet point (Figure 5.1) short-circuiting is plausible. This is verified by an analysis of the EC outflow trend, which is discussed in Section 5.2.

The middle of the biofilter (location 3, 9740 mm/h) and the 'dry' corner (location 4, 5510 mm/h) show larger hydraulic conductivity than the clean sand hydraulic conductivity. A possible explanation is that more cracks and fine fissures are present at these locations due to generally dry conditions. Multiple other studies argue that increased infiltration rates after long dry periods can be attributed to the formation of macropores and fine fissures [24, 34, 70]. Furthermore, high hydraulic conductivity suggests little clogging and compaction in these areas, which is expected as these areas do not receive inflow water. Additionally, new vegetation was planted just before the hydraulic conductivity test was performed, which could have loosened the soil structure. The high hydraulic conductivity in the middle of the biofilter is probably caused by digging near this area, which was done to investigate the water depth inside the filter.

Lastly, lowest hydraulic conductivity values were found in the most vegetated areas (Figure 4.1). This seems to contradict finding from other studies that suggest that root growth leads to macropores which increase hydraulic conductivity [51, 66, 73]. On the other hand, it might support the finding of Archer et al.[9] that long and thin roots can reduce the hydraulic conductivity by creating a 'mat' in the soil. A more probable explanation is, however, that the influence of vegetation on the hydraulic conductivity is less dominant than the difference in hydraulic loading on the biofilter that leads to differences in clogging, compaction, and drying of the biofilter.

5.1.3. Comparison Hydraulic Conductivity to other Field Scale Biofilters

The hydraulic conductivity of this biofilter is high compared to other field scale biofilters. For example, Le Coustumer et al.[31] measured the hydraulic performance of 37 field scale biofilters in Australia with the same in situ hydraulic conductivity test (Section 5.1.2). Their study found that 39% of the biofilters had a hydraulic conductivity <50 mm/h, 44% between 50-200 mm/h and 17% above 200 mm/h with a maximum value of 600 mm/h. Clogging was mentioned as primary reason for low hydraulic conductivity values [31]. Specifications on biofilter design were not provided, so a closer comparison with this study cannot be made. However, most biofilters are designed according to the Australian design guidelines [31]. These guidelines argue that the optimal biofilter hydraulic conductivity is 100-300 mm/h, which can be achieved by incorporating a small percentage of fine soil particles (e.g., clay and silt) in a sand based filter media [92]. Fine particles are known to reduce hydraulic conductivity due to their larger water holding capacity than sand [122]. Zinger et al.[131] indeed found lower hydraulic conductivity of two biofilters containing fine constituent in their filter media. The biofilter Monash carpark, which contains 3% silt and 1% clay, showed an initial hydraulic conductivity of 300 mm/h, and the biofilter at Kfar Sava, consisting for 83% of fine to very fine sand, of 140 mm/h. Filter media specifications of these biofilters are given in Appendix E. The high hydraulic conductivity of the Bluebloqs biofilter compared to other field scale biofilter can, thus, be explained by the relatively coarse sand with high hydraulic conductivity (Table 5.1) used in the filter media.

5.2. Physicochemical Water Quality

Physicochemical quality of the water flowing in and out of the biofilter was monitored during 5 feeding events, as explained in Section 4.2. Section 5.2.1 analyses changes in these parameters throughout the events to improve understanding of water flows inside the biofilter. Section 5.2.2 discusses inflow and outflow physicochemical water quality characteristics to obtain insight into environmental conditions that can affect microbial retention and survival.

Figure 5.2 shows inflow and outflow electrical conductivity (EC), pH, dissolved oxygen (DO), and temperature (T) values per event plotted over cumulative volume. The initial volume in the biofilter before the start of an event is shown with a grey dotted line and comprises aquifer water, as discussed in Section 3.2.3. This volume is estimated from the logged water level in the biofilter at the start of an event, further described in Appendix C.1. The pore water volume of the biofilter (31.8 m^3), which is estimated from the porosity of the biofilter media, is presented with a black dashed line. No EC or temperature data were collected on 18/08/2020 due to a broken EC meter. Consequently, temperature data is missing and EC data was taken from the log files of the Urban Waterbuffer.

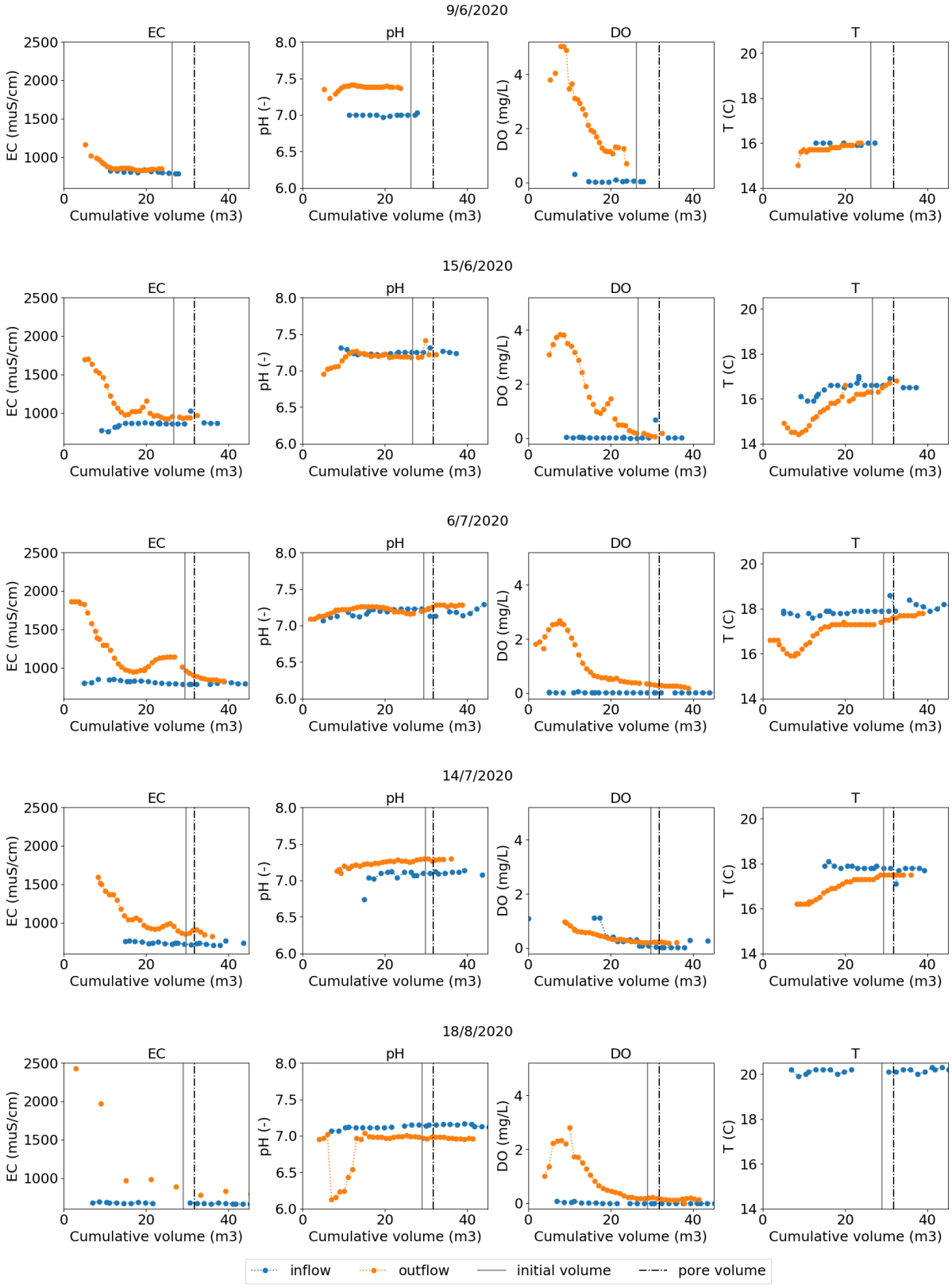


Figure 5.2: Inflow and outflow electrical conductivity (EC), pH, dissolved oxygen (DO) and temperature (T) measurements per event over the cumulative volume (m^3). Black lines represent the pore water volume and grey lines in the outflow graphs show the water volume in the biofilter at the start of an event.

5.2.1. Water Flow in the Biofilter

Outflow physicochemical water quality approaches inflow water quality throughout the event, with exception of some small peaks and drops (Figure 5.2). A decrease in outflow EC is almost immediately observed, while DO and T concentrations first deviate from inflow values. This section analysis these trends to improve understanding of water flows in the biofilter.

Trend outflow EC

Outflow EC decreases towards inflow EC throughout the events (Figure 5.2). This can be explained as follows: at the start of an event the biofilter is filled with aquifer water which has a higher EC than stormwater. During the event, water in the biofilter mixes with feed water from the buffer which results in decreasing EC of the outflow. Values keep decreasing as the portion of feed water in the outflow increases. The observed trend in outflow EC demonstrates that the flow in the biofilter deviates from a plug flow regime (Fig 5.3). Measured values are more comparable to a mixed flow regime but drops are more extensive and appear faster. Short-circuiting flows can explain the quick drop in outflow EC after the start of the events. The extend of these decreases implies that a large portion of feed water flows through these short-circuiting pathways, suggesting overall short retention times in the biofilter. The construction of one inlet point near the outlet location (Figure 5.1) is presumed to be a main factor causing preferential flow between the inlet and outlet [2, 46, 92]. Additionally, the uneven distribution of feed water over the biofilter and, accordingly, the uneven clogging of the biofilter are probable factors contributing to mixing and short-circuiting inside the biofilter [46].

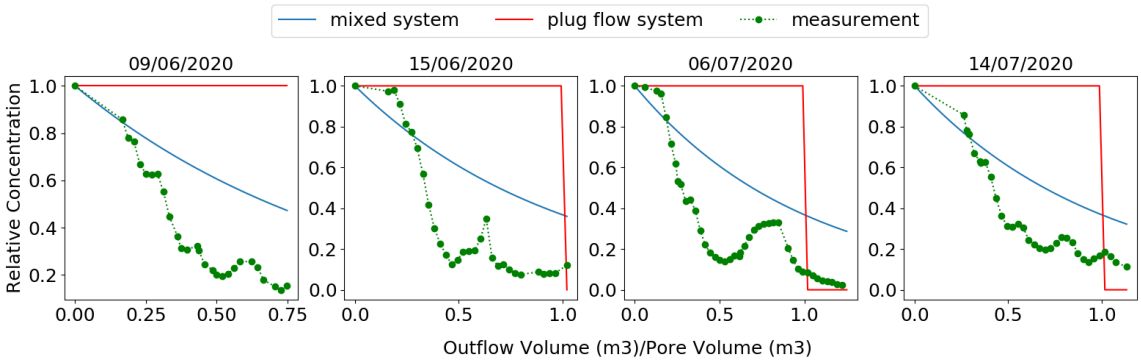


Figure 5.3: Comparing measured electrical conductivity (EC) values with EC values of an ideal mixed flow and plug flow regime.

Trend outflow dissolved oxygen and temperature

At the start of events, a peak in dissolved oxygen and a drop in temperature is observed before values start approaching inflow concentrations (Figure 5.2). This paragraph outlines possible factors that could explain observed trends, however, directly monitoring these mechanisms is recommended to improve understanding.

The initial increase in dissolved oxygen values might be explained by higher oxygen concentrations at the top of the biofilter than at the bottom at the onset of events (Figure 5.4). As water from higher locations reaches the outlet, this would result in increasing DO concentrations in the outflow. Highest dissolved oxygen concentrations are expected at the top of the biofilter as this water is in contact with air in the unsaturated zone. Oxygen from this air can directly diffuse into the water [85] and transport via advection and diffusion to lower parts of the biofilter. In the root zone, additional aeration of the water can occur by oxygen excretion from plant roots [116]. At the bottom, however, no extra oxygen source is expected. Therefore, dissolved oxygen near the bottom could deplete faster by, for example, microbial growth processes than at higher locations. Another factor that can contribute to increasing DO concentrations after the start of an event, is aeration of the inflow by entrapped air in the unsaturated zone. As only a small part of the biofilter surface area receives inflow water (Figure 3.8a), trapped oxygen in these areas might deplete fast which would mainly cause aeration at the start of an event. As more feed water reaches the outflow, DO values start decreasing towards inflow values. This implies that less oxygen is transferred to the water during an event than between events. A possible explanation for this is that aeration of the water by the inlet construction is little and that oxygen transfer rates,

for example, atmospheric diffusion rates and plant oxygen release rates, are too low to aerate the fast flowing feed water through the system, but are sufficient to aerate the submerged zone water between events. To improve understanding of what causes the observed trend, monitoring oxygen dynamics within the biofilter is suggested.

Similar to dissolved oxygen, temperature stratification could explain declining values at the start of an event (Figure 5.4). Monitoring was done in the morning, hence, colder temperatures are presumed at the top of the biofilter due to cooling during the night. In addition, relatively cold aquifer water was dosed on the biofilter in the evenings before feeding events. As cold water from higher locations reaches the outlet, this can result in declining values until the portion of warmer feed water in the outflow increases and results in overall warmer temperatures.

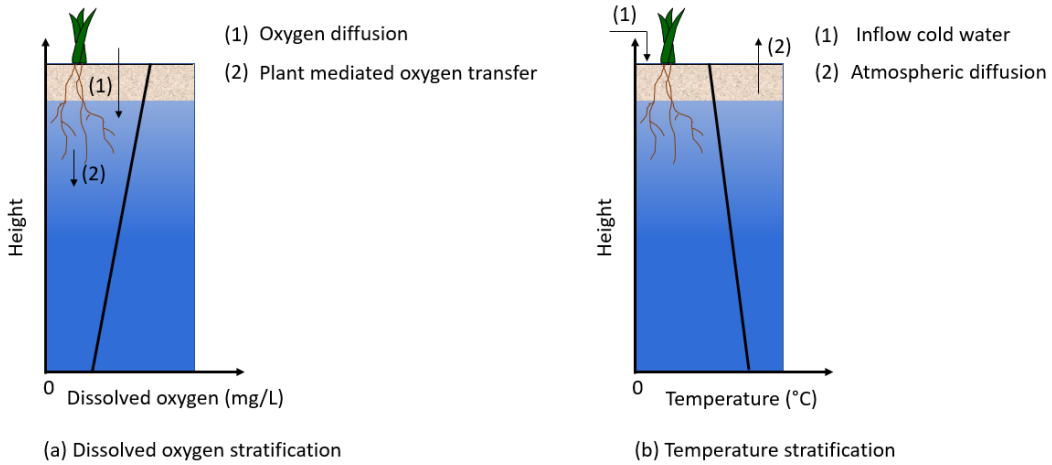


Figure 5.4: Left figure (a): Concept dissolved oxygen (DO) stratification over the height of the biofilter between events. Atmospheric diffusion and plant mediated oxygen transfer are expected to result in increased DO concentrations in higher parts of the biofilter compared to the bottom. Right figure (b): concept temperature stratification over the height of the biofilter before the onset of an event. Cooling during the night and inflow of cold water are expected to lead to lower temperatures at the top than the bottom.

Small peaks during the event

Despite the overall decline in outflow EC, some increases are observed as well. A possible explanation for this is that the portion of aquifer water in the outflow increases during the times that the inflow is off (Figure 4.4), as this lowers the amount of feed water that reaches the outlet. The peaks indeed appear after the inflow stops and are more pronounced when the inflow has been off for a longer time (Figure 5.5). Interestingly, differences are seen in the delay in which the peaks show after the inflow has stopped. Peaks seem to appear faster when feed water has been dosed on the biofilter for a longer timespan, for example on 15/06/2020 and 06/07/2020, compared to shorter inflow runs in which less inflow water is dosed, e.g., on 14/07/2020. A possible explanation for this is that the extend of fast flowing pathways expands with increased feed volumes. This could result in a wider spread of inflow water over the biofilter surfaces which may conduct more water to fast flowing areas. It must, however, be noted that data was manually noted down, hence discrepancies can exist between actual times and recorded times.

If deviations in the EC trend are the results of inflow stops, deviations in DO and temperature measurements are expected at similar moments. Considering DO values (Figure 5.2), a peak comparable to the EC peak is observed on 15/06/2020. The effect on 14/07/2020 is less pronounced, which is probably caused by the small difference between inflow and outflow values. Despite this, a less steep decline is observed. No deviation is, however, seen on 06/07/2020. The presence of a ponding zone during this event is a notable difference from other events. This might have caused expanded mixing of submerged zone water and feed water resulting in overall lower oxygen concentrations in the outflow. However, this seems unlikely as the peak in EC is clearly observed. It, thus, remains unclear what

causes this observation. Considering the temperature measurements (Figure 5.2), feed water stops seem to result in periods of relatively constant instead of increasing temperatures. This is less obvious on 15/06/2020 due to an outlier at the moment of the deviation. On the whole, these deviations underpin the observation that feed water mixes with submerged zone water in the biofilter during events.

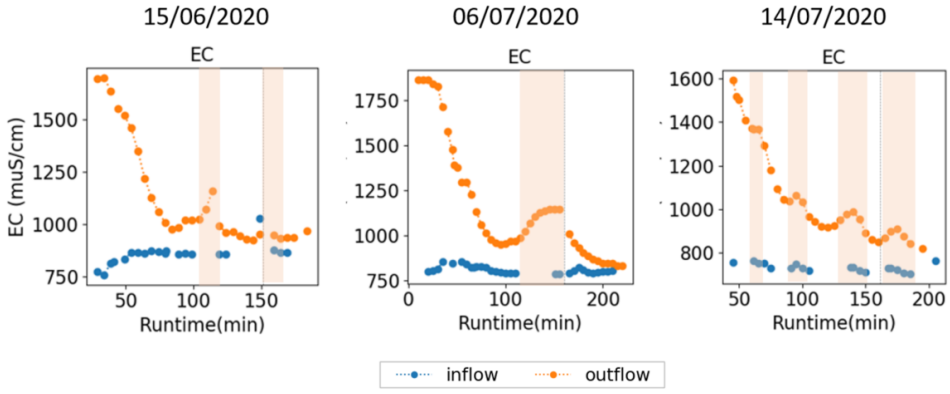


Figure 5.5: From left to right: Electrical conductivity (EC) of 15/06/2020, 6/7/2020 and 14/7/2020 over time. The peaks are indicated with red shaded areas. The blank spaces between the blue inflow measurements indicate that the inflow was off.

Difference inflow and outflow quality after 1 pore volume has flushed
Lastly, results show that after 1 pore volume has flushed, outflow values still differ from inflow values. For example, inflow and outflow EC differ 10-100 μS/cm, DO 0.1-0.2 mg/L and pH about 0.1. This indicates that aquifer water is still present in the biofilter after 1 pore volume is flushed. Reasons could be that part of the water in the biofilter flows out slowly or might even be stagnant. Furthermore, this suggests that feed water flow is dominant over the outflow of water in the submerged zone, again pointing to preferential flow paths. It must, however, be noted that the pore volume is an estimation based on porosity determined in the laboratory. Even though the estimated porosity is comparable with other research and, therefore, assumed an acceptable estimation (Section 5.1.1), biofilter pore volume will differ due to, e.g, clogging and compaction. Therefore, the observed differences between inflow and outflow values after 1 pore volume has flushed have to be interpreted with caution.

5.2.2. Physicochemical Inflow and Outflow Characteristics

This section examines the monitored inflow and outflow physicochemical characteristics.

Inflow characteristics
Inflow water is a mix of stormwater from multiple storm events that is stored in the buffer before fed to the biofilter. Accordingly, the water quality is relatively stable over an event. The following inflow characteristics are observed:

- Inflow EC covers a range of 660-1030 μS/cm with a median value mean of 792 μS/cm. EC of stormwater is generally lower. Wolfgang et al.[44], for example, found a mean EC of 180 μS/cm for 94 urban runoff events in Australia. Research by Zuurbier and Van Dooren [132] revealed that high EC of the inflow water at the studied site is caused by leaching of shallow groundwater into the rainwater sewer that drains to the buffer. This was detected by camera inspections and based on the observed inflow on dry days, Zuurbier and Van Dooren estimated that 10-20 m^3/day leaches into the buffer [132].
- Inflow dissolved oxygen concentrations are below <0.1 mg/L with exception of DO values measured on 14/07/2020 that reach 1.11 mg/L. As no differences were found in the antecedent dry period, weather conditions, or operation to the other events, these higher values are likely caused by a bias in the DO meter due to, for example, erroneous temperature calibration of the meter.

  The observed concentrations are lower than dissolved oxygen concentrations generally found in urban runoff of >5.0 mg/l [11, 35, 47]. Incoming stormwater is expected the only oxygen source

for water in the buffer as, for example, dark storage conditions prevent oxygen input by photosynthesis. Therefore, oxygen depletion during storage in the buffer could explain the low DO concentrations of the inflow water. This can occur if stormwater retention times in the buffer are long and sufficient biological degradable compounds are present in the water.

Biological degradable compounds were not monitored in this research. However, Boogaard et al.[15] found mean biochemical oxygen demand (BOD) values of Dutch stormwater of 5.7 mg BOD5/L, which indicates that in 5 days 5.7 mg DO/L is consumed. Stormwater stays in the buffer for more than 5 days due to the weekly feeding events, and even long residence times are expected as the buffer was not fully replenished during the research period. Specifically, 570 m^3 buffer water was fed to the biofilter, while the buffer was on average filled with 900 m^3. Because of these reasons, oxygen depletion is probable.

- Inflow pH ranged from 6.74-7.31 with a mean value of 7.14. As rainwater is generally slightly acidic [109], the neutral values suggest that the pH increases due to stormwater harvesting and storage. Factors that could play a role are anaerobic respiration processes in the buffer, such as denitrification [1].
- Inflow temperatures did not vary widely during an event (<0.5°C) as the effect of diurnal cycles on the underground buffer is probably little. Inflow temperature, however, increased over the events from 15.8-20.3°C. This indicates that water in the buffer heats up when average daily temperature increases (Appendix B.1).

Outflow characteristics
Biofilter outflow consist of a mix of aquifer water and filtered feed water. At the start of an event, mostly aquifer water flows out and over the event the portion of filtered feed water in the outflow increases. Regarding first outflow concentrations, the following things are noted:

- Outflow EC increases from 1200 μS/cm in the first event (9/06/2020) to 2430 μS/cm in the last event (18/08/2020). Furthermore, values are higher than the mean aquifer EC of 640 μS/cm monitored in previous research at the site [132]. This indicates salinization of the aquifer, which is probably caused by a net-uptake of water. Log files show an increase in aquifer EC around June 2020 (Appendix. C.3) which is presumably the result of little injection of water due to a dry period in which the system only ran a few times. Additional salinization is likely caused by the low treated volume of 44 m^3 per week during the research period compared to the water demand of 24 m^3-80 m^3 per day [132]. Lastly, the frequent recovery of water from the upper well caused by the recirculating water flows could induce mixing of stored water with brackish groundwater, as it might provide little time for the injected water to float upwards and restore the freshwater bubble.
- First outflow dissolved oxygen concentrations declined from 3.9 mg/L in June to 1.0 mg/L in August. In addition, an increase in first outflow temperatures is seen over the monitoring period. This could partly explain the declining dissolved oxygen concentrations, as cold water can contain more oxygen than warm water. However, multiple other factors, such as vegetation differences, could have contributed to DO concentrations in the outflow.
- Outflow pH is neutral but seems to increase in the first 10 m^3 of outflow as seen on 09/06/2020, 15/06/2020, and 06/07/2020. This indicates that aquifer pH is lower than inflow pH. Various processes can contribute to this, such as oxidation of sulphur and nitrogen compounds and mineral dissolution in the aquifer [64]. The pH could also decrease inside the biofilter due to e.g., nitrification[2]. The drop in pH on 18/08/2020 is likely a measurement error.

[1] Denitrification: $6NO_3^-$ + 5 CH_3OH –> $3N_2$ + $5CO_2$ + $7H_2O$ + $6OH^-$
[2] Nitrification: $2NH_3$ + $3O_2$ –> $2NO_2$ + $2H^+$ + $2H_2O$

5.3. Microbiological Water Quality

Inflow and outflow *E. coli*, enterococci and *Campylobacter* concentrations were measured to improve understanding of the microbial fate in the biofilter. Section 5.3.1 and Section 5.3.2 discuss observed inflow and outflow concentrations and compare these with literature. Thereafter, water quality changes over the biofilter are analysed in Section 5.3.3.

Table 5.3 presents the observed *E. coli*, enterococci and *Campylobacter* inflow and outflow concentrations. In Appendix B additional information on the number of processed samples is provided. No outflow *Campylobacter* concentrations were determined on 14/07/2020 and no inflow concentrations on 18/08/2020 due to swarming and contaminated growth. Despite this, *Campylobacter* was detected in all samples. Fig 5.6 shows the inflow and outflow microbial concentrations over the cumulative inflow and outflow volume per event. The initial volume in the biofilter before the start of an event is shown with a grey line and the pore water volume of the biofilter with a black dashed line. Plots of 09/06/2020 are not provided, as only 2 composite outflow samples and 1 composite inflow sample were analysed.

Table 5.3: Inflow and outflow concentrations of *E. coli*, enterococci, and *Campylobacter*. Median values are presented and minimum and maximum values are given between parentheses.

Date	*E. coli*		Enterococci		*Campylobacter*	
	Inflow	Outflow	Inflow	Outflow	Inflow	Outflow
09/06/2020	$2.7x10^4$	$1.6x10^4$ ($9.8x10^3$-$2.3x10^4$)	$7.1x10^2$	$9.9x10^2$ ($3.5x10^2$-$1.6x10^3$)	$1.0x10^2$	$9.6x10^1$ ($8.5x10^1$-$1.1x10^2$)
15/06/2020	$1.0x10^4$ ($9.7x10^3$-$1.3x10^4$)	$2.8x10^3$ ($1.8x10^2$-$4.8x10^3$)	$1.5x10^2$ ($1.4x10^2$-$1.6x10^2$)	$2.7x10^2$ ($7.6x10^1$-$4.8x10^2$)	$9.3x10^1$ (8.0x101-$1.1x10^2$)	$9.8x10^1$ ($7.0x10^0$->$2.9x10^2$)
06/07/2020	$1.8x10^4$ ($8.5x10^3$-$2.1x10^4$)	$5.7x10^3$ (<$3x10^1$-$1.1x10^4$)	$1.3x10^2$ ($1.0x10^2$-$3.2x10^2$)	$4.1x10^2$ ($3.6x10^1$-$1.2x10^3$)	$2.6x10^1$ ($1.4x10^1$–$7.0x10^1$)	5.0 ($1.0x10^0$-$6.1x10^1$)
14/07/2020	$6.1x10^3$ ($5.2x10^3$-$7.7x10^3$)	$7.5x10^2$ ($3.7x10^2$-$1.8x10^3$)	$1.2x10^2$ ($6.8x10^1$-$1.4x10^2$)	$9.87x10^1$ ($3.0x10^1$-$2.0x10^2$)	$1.2x10^1$ ($9.0x10^0$-$2.3x10^1$)	No data
18/08/2020	$6.4x10^2$ ($5.5x10^2$-$7.2x10^2$)	$2.3x10^4$ ($1.6x10^4$ ->$3.0x10^4$)	$4.0x10^1$ ($2.7x10^1$-$7.1x10^1$)	$3.1x10^2$ ($3.2x10^1$->$1.0x10^3$)	No data	$1.0x10^1$ ($2.0x10^0$-$8.5x10^1$)

5.3.1. Microbiological Inflow Concentrations

The presence of *E. coli*, enterococci and *Campylobacter* shows that inflow water is contaminated with faeces. Variations are observed in concentrations between microorganisms (Figure 5.7), during events, and between events (Table 5.3).

Firstly, *E. coli* concentrations were 1-2 orders of magnitude higher than enteroccoci concentrations, and 2-3 orders of magnitude higher than *Campylobacter* concentrations. Differences between enterococci and *Campylobacter* are less, generally 1 order of magnitude. These microbial differences can be explained by the diversity of microbial concentrations in faecal sources contaminating stormwater [10, 59, 132], and show that *E. coli* is more abundant in the faecal sources polluting the water at the Urban Waterbuffer than enteroccoci and *Campylobacter*.

In addition, inflow concentrations varied during events (Table 5.3). This suggest that the microbiological water quality in the buffer is not homogeneous, hence that water is not completely mixed in the buffer. Lastly, variability in concentrations was observed between events. Multiple factors can result in microbial variations observed between events. Firstly, harvested stormwater quality can vary substantially due to, for example, temperature and rainfall intensity [50, 59, 130]. Additionally, microbial changes are expected during storage in the buffer as the retention times in the buffer are likely high, as explained in Section 5.2.2. For example, lowest inflow concentrations were found on 18/08/2020, the event with the highest daily temperature (Appendix B.1). This could be explained by extended microbial decay in the buffer due to increased microbial activity at higher temperatures.

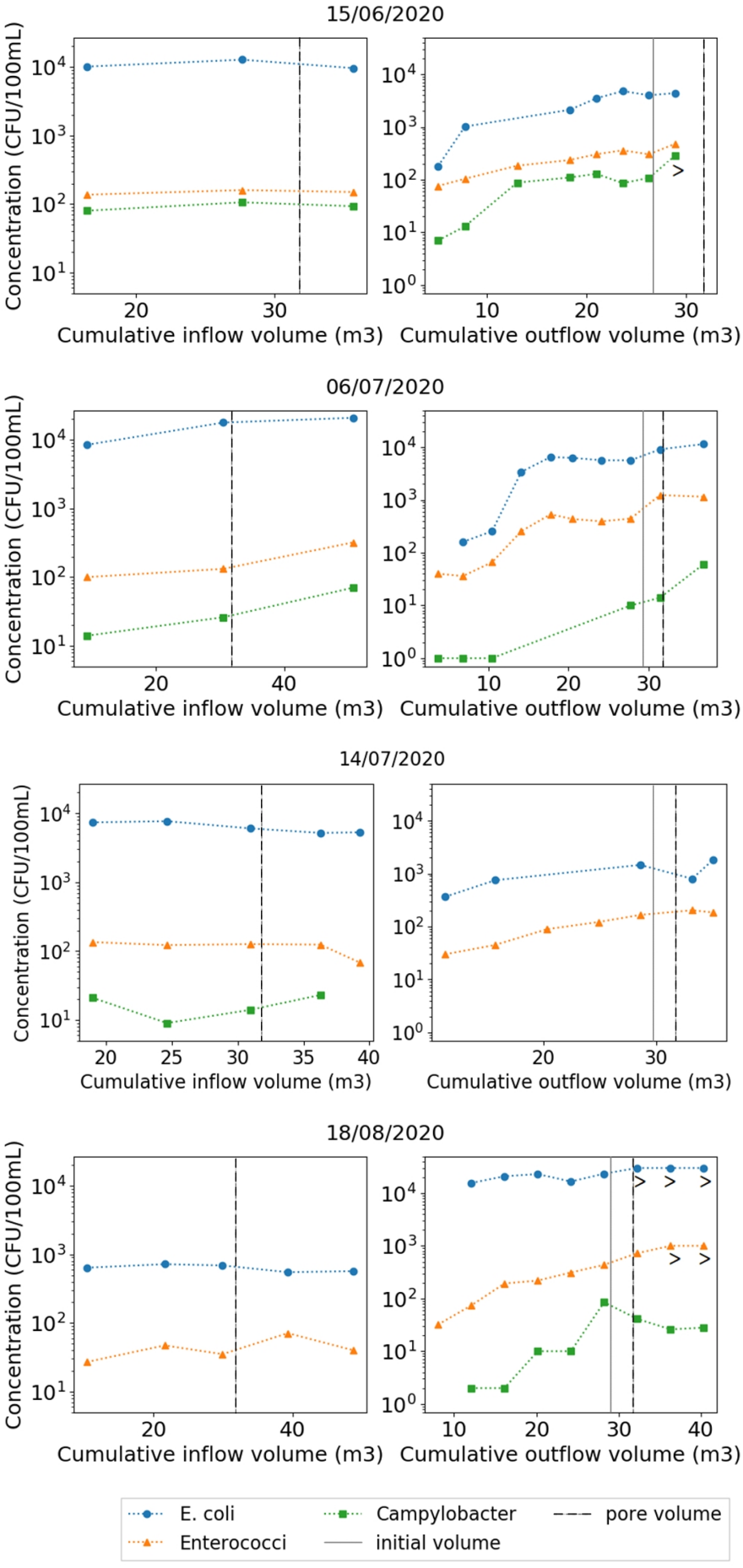


Figure 5.6: Measured *E. coli*, enterococci and *Campylobacter* inflow concentrations over cumulative inflow volume (left) and outflow concentrations over cumulative outflow volume (right). Black lines represent the pore water volume and grey lines in the outflow graphs show the water volume in the biofilter at the start of an event. Right pointing single angle quotation marks indicate that concentrations are higher than the plotted value but were too numerous too count to quantify.

Similarly, low temperatures in the buffer could contribute to the highest inflow concentrations observed on 9/06/2020. This is in line with the study of Schets et al.[109] who investigated temperature-dependent decay of *E. coli* in water from an underground reservoir found increased decay at higher temperatures: <1 log at 15°C compared to >2 log at 35°C.

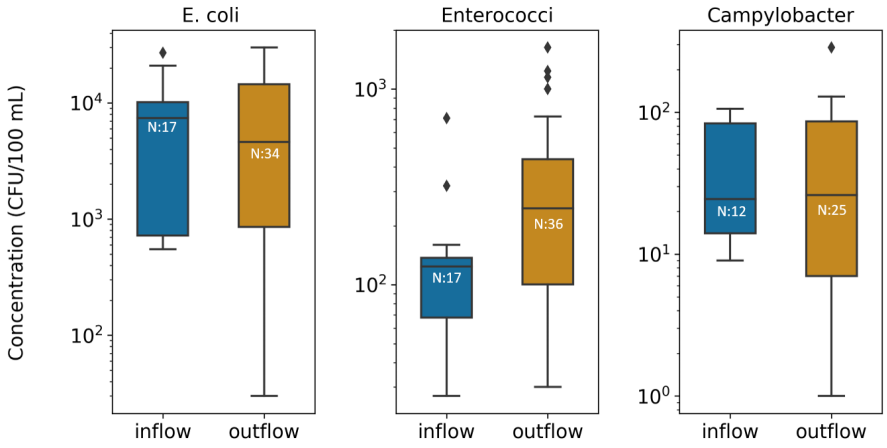


Figure 5.7: Boxplots showing the distribution of inflow and outflow concentrations per bacteria over all events. The median, 25th and 75th percentiles, minimum and maximum values and outliers (diamonds) are shown.

Comparison inflow concentrations to literature

Inflow concentrations reflect well with stormwater quality found in other Dutch residential areas (Table 5.4). Concentrations are, however, lower than ranges found in urban floodwater originating from a combined sewer, as stormwater in current study is not polluted with sewage. In contrast, concentrations are higher than detected in rooftop harvested rainwater, which can be explained by additional pollution from, for example, street runoff. Sales-Ortell and Medema [105], who investigated stormwater runoff in a water plaza in Spangen, also found lower microbial concentrations. However, drainage areas were pre-cleaned the day before sampling, which was not the case in current study. Lastly, and most notably, inflow concentrations are considerably higher than observed in the previous research at the Urban Waterbuffer. Zuurbier and Van Dooren [132] found *E. coli* concentrations ranging from 14-200 CFU/100 mL and enterococci from <1-100 CFU/100 mL in monthly samples taken over the period 12/2018 until 7/2019.

Higher pollutant loads entering the system could explain elevated concentrations in current study. This can be caused by changes in the connected catchment area, such as an increase in the number of infected animals carrying the target bacteria, or expansion of the drainage area connected to the rainwater sewer [5, 79, 132]. In the first operational year of the Urban Waterbuffer it was, for instance, assumed that not all drainage areas (Figure 3.2) were yet connected to the rainwater sewer [132]. The low volume of water infiltrated during this research period (570 m^3), however, implies that still not all areas are connected.

Furthermore, the low treated volume shows that the buffer was not fully replenished during the research period. Another possibility is, therefore, that a one-time high pollutant load influenced concentrations over the whole monitoring period. Additionally, results indicate oxygen depletion in the buffer (Section 5.2.2), which might have led to increased survival rates of the target bacteria compared to previous research period. Studied bacteria can, namely, withstand low oxygen conditions and could, therefore, have an advantage over aerobic bacteria in the buffer.

Differences in monitoring strategy and analytical methods applied between the researches are other factors that can contribute to deviating findings. Samples taken during a feeding event could, for instance, contain higher microbial concentrations than samples taken when the system was off as these samples likely contain water from the pipelines instead of the buffer. Further research that clarifies the drainage areas connected to the UWB and monitors water quality changes in the buffer is needed to improve understanding on the higher inflow concentrations found in current study.

Table 5.4: Comparison of microbial inflow water quality in current study with concentrations found in multiple stormwater research in The Netherlands. Urban stormwater runoff complies stormwater from residential areas, roofs and roads.

Water type	*E. coli* (CFU/100 mL)	Enterococci (CFU/100 mL)	*Campylobacter*	Reference
Stormwater UWB	$5.5*10^2$-$2.7*10^4$	$2.7*10^1$-$7.1*10^2$	$9*10^0$ - $1.0*10^2$ [a]	Current study
Stormwater UWB	14-$2*10^2$	<1-$1*10^2$		[132]
Urban stormwater	$6.7*10^3$			[15]
Urban stormwater	$3.4*10^4$			[14]
Urban stormwater	$1.0*10^4$		$1.0*10^3$ [b]	[103]
Urban floodwater storm sewer			1->10^2 [c]	[33]
Urban floodwater combined sewer	$8.7*10^3$- $1.0*10^5$	$5*10^4$- $2.1*10^5$		[120]
Stormwater from cleaned square	14-$1.5*10^2$		35-$1.1*10^3$ [b]	[105]
Rooftop harvested rainwater	0-53	2-$1*10^2$		[109]

[a] Concentration in CFU/100 mL
[b] Concentration in gencopies/100 mL. Not all genomic *Campylobacter* is culturable, concentrations could be lower in CFU/100 mL [132].
[c] Concentrations in most probable number per 100 mL. This is equivalent to colony forming units

5.3.2. Microbiological Outflow Concentrations

Similar to physicochemical parameters (Section 5.2.1), microbial outflow concentrations approach inflow concentrations during the events (Figure 5.6). As outflow samples comprise a mixture of aquifer water and filtered feed water, outflow concentrations cover a wider range than inflow concentrations (Figure 5.7).

First outflow concentrations, that mainly comprise of aquifer water, were 1-2 orders lower than outflow concentrations at the end of the monitoring rounds (Figure 5.6). This difference indicates that microbes are removed in the aquifer. However, microbes were still observed in these samples which implies that microbes are present in the submerged zone water at the start of events. This could be caused by desorption of microbes from the biofilter into the aquifer water due to concentration differences. Another possible explanation is that aquifer water used to irrigate the biofilter still contained microbes. Based on *E. coli* and enterococci outflow concentrations ranging from 4-200 CFU/100mL and 4->80 CFU/100 mL respectively, Zuurbier and Van Dooren [132] suggest that it takes between 3-10 days to lower microbial concentrations <1 CFU/100 mL in the aquifer. As microbial outflow concentrations in this study are 1-2 orders of magnitude higher, and aquifer water is frequently recovered, irrigation water likely contains microbial concentrations >1 CFU/100mL.

Comparing outflow water quality with different water sources (Table 5.5), concentrations fall in the ranges of water quality found in freshwater, recreational lakes, and canals. Accordingly, outflow is not microbiological safe to reuse and requires additional microbial treatment.

Table 5.5: Comparison of *E. coli*, Enterococci and *Campylobacter* outflow concentration ranges in current study with concentration ranges of different water sources.

Water type	Concentration in CFU/100 mL		
	E. coli	Enterococci	*Campylobacter*
Stormwater Urban Waterbuffer[a]	10^2-10^4	10^1-10^3	10^0-10^2
Raw sewage [b]	10^5 – 10^7	10^4- 10^5	10^0- 10^4
Fresh water [c]	10^2 - 10^3	10^2	10^0 - 10^2
Recreational lake [d]	10^1- 10^4	10^0- 10^2	
Amsterdam canal [e]	10^1- 10^3	10^0-10^2	

[a] Results current study
[b] [12, 52, 132]
[c] [52]
[d] Mean concentration of 5 recreational sites [108]
[e] Mean concentration of 3 canals [108]

5.3.3. Microbiological Water Quality Changes over the Biofilter

This section analyses microbiological water quality changes over the biofilter to assess the capacity of the biofilter to remove microbes. Inflow and outflow quality are compared in two different ways, as explained in Section 4.2.4. Thereafter, possible causes that lead to the observed behaviour are discussed.

Figure 5.8 shows the difference between outflow water quality observed after 1 pore volume has flushed with mean inflow water quality. *E. coli* concentrations measured near the end of events generally stayed below mean inflow concentrations, shown as a positive log removal in Figure 5.8. This suggests that a part of the inflow *E. coli* is retained in the biofilter. In contrast, enterococci and *Campylobacter* concentrations exceeded mean inflow concentrations, implying that these microbes are leached from the biofilter. However, as previously explained (Section 5.2.1), outflow water contains a proportion of aquifer water and filtered feed water. Accordingly, inflow and outflow concentrations cannot directly be compared to evaluate microbial treatment in the biofilter. To overcome this problem, and indicate microbial quality changes over the biofilter throughout events, a second method was applied.

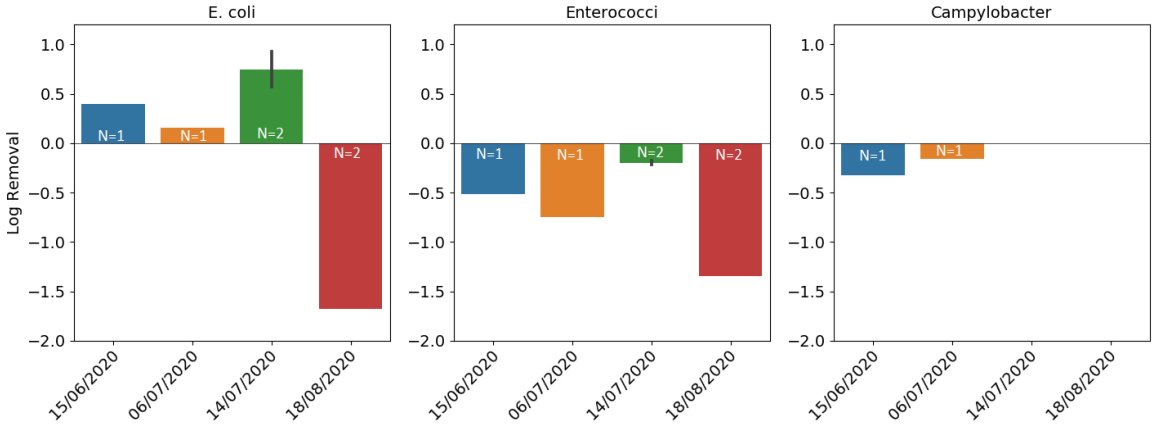


Figure 5.8: Difference *E. coli*, enterococci and *Campylobacter* inflow and outflow concentrations after 1 pore volume has flushed. A log value >0 means that outflow concentrations were lower than inflow concentrations, while <0 means that outflow concentrations were higher. 1 log represents 90% removal.

The microbial outflow concentrations that would be observed from mixing aquifer water with feed water were compared with actual outflow concentrations (Section 4.2.4). The estimated mixing fractions of aquifer water and filtered feed water in the outflow samples are provided in Appendix C.4. Figure 5.9 shows the calculated concentrations in the water if no addition or removal would take place during the event with unfilled markers, and actual measurements with filled markers. If actual measurements are higher, this indicates that microbes were added to the system and lower measurements suggest that microbes were removed. The following things are observed that are further analysed in the subsequent section:

- A part of the inflow *E. coli* is retained in the biofilter in 3 of the 4 analysed event. On 18/8/2020 *E. coli* leached from the system.
- Enterococci and *Campylobacter* were added to the water during the events. Plots suggest higher leaching of enterococci than *Campylobacter*.

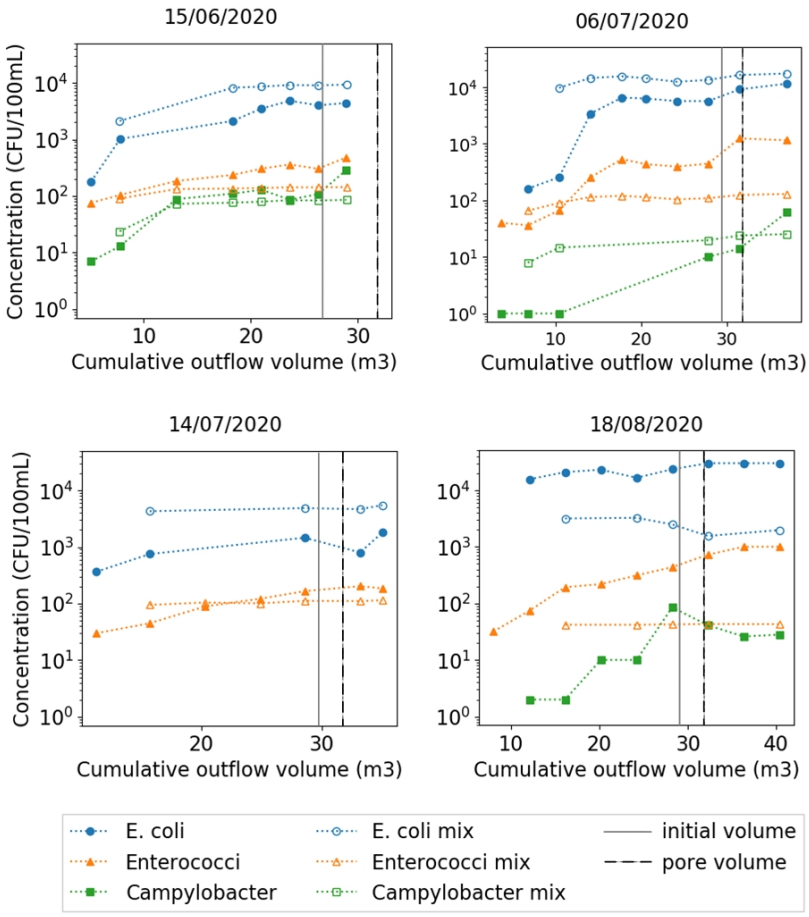


Figure 5.9: Measured outflow concentrations over cumulative outflow volume (closed markers) compared with mixed outflow concentrations if no removal or addition of microbes would take place (open markers).

Possible causes for increasing outflow concentrations

There are several possible explanations for the observed leaching of target bacteria in the biofilter. Bacteria could leach from the system if microbial concentrations in the biofilter are high compared to inflow concentrations, which can result in net-desorption [23, 25]. Chandrasena et al.[23] suggest that this can happen if retained microbes grow in the system or if low loading events follow shortly after high loading events. The latter is not expected as observed inflow concentrations are generally high and there is a 6-day dry period between events. Growth of the target bacteria in the biofilter is also unlikely as indicator bacteria commonly decay in natural systems due to their low persistence to biotic and abiotic factors compared to native microorganisms [28]. Zhang et al. [130] suggest that direct contamination on the biofilter surface can lead to leaching of bacteria. As the prevalence of bacteria in animal faeces can differ [10], this seems a likely factor contributing to the observed bacteria leaching.

Leaching of enterococci and *Campylobacter* can be caused by direct contamination of the biofilter with bird faeces. Both enterococci and *Campylobacter* are often isolated from birds and their prevalence in birds is high compared to *E. coli* [1, 62, 65, 77, 86, 114, 123]. Furthermore, citizens working adjacent to the Urban Waterbuffer confirmed that a pigeon population inhabits the biofilter surroundings and regularly observed birds flying over the biofilter. Accordingly, bird droppings on the biofilter are likely. Despite this, the explanation can only be valid if bird populations in Spangen are less infected with *E. coli* compared to enterococci and *Campylobacter*. To validate the secondary contamination source, microbial source tracking could be applied.

Similarly, direct contamination could explain the high *E. coli* concentrations on 18/08/2020. From the start of this event, *E. coli* outflow concentrations were 2 orders higher than inflow concentrations and concentrations further increased during the event. In contrast, enterococci and *Campylobacter* out-

flow concentrations show similar trends and orders of magnitude as observed in the other events. It could, therefore, be that a faecal contamination source on the biofilter leached *E. coli* into the system. Dog faeces are a probable source for high *E. coli* concentrations [65, 132]. Another explanation of the elevated concentration at the start of the event is that aquifer water is contaminated with high *E. coli* concentrations compared to enterococci and *Campylobacter* concentrations. This seems less likely as aquifer pollution by, for example, a leaking sewer or leaching of manure constituents, would probably result in increased concentrations of all target bacteria.

In addition to faecal contamination sources, microbial characteristics might lead to interspecies differences in outflow concentrations. Results suggest that fewer enterococci are retained than *E. coli* and *Campylobacter*. This could be caused by differences in cell shape and motility. Stevik et al.[115] suggest that long-rod shaped bacteria, e.g., *E. coli*, are better removed than spherical cells, such as enterococci. *Campylobacter* is spiral-shaped but has long dimension (on average 0.5-5 μm [121]) compared to enterococci (diameter of 0.8 μm [39]) which might also lead to better retention. In addition, Soberg et al.[118] proposed that differences in motility can lead to interspecies differences in microbial removal. An explanation for this is that motile bacteria, such as *E. coli* and *Campylobacter*, can move towards biofilms that might take up these bacteria and provide food and protection [118]. This could lead to a difference in microbial retention when motile bacteria are more often incorporated or adsorbed to a biofilm than non-motile bacteria. As multiple enterococci species are non-motile[114, 118], this might be a factor contributing to the lower enterococci retention observed.

5.4. Modelling Microbial Transport and Removal

This section first presents and discusses the results of the laboratory test carried out in an attempt to obtain site-specific adsorption, desorption, and decay rate constants (Section 5.4.1). Thereafter, Section 5.4.2 investigates the effect of the uneven inflow load and preferential flow paths on microbial outflow concentrations and compares simulated tracer results with EC measurements. Lastly, Section 5.4.3 examines what the effect is of event duration and frequency on microbial outflow concentrations.

5.4.1. Adsorption, Desorption and Decay Rate

Adsorption and desorption rate estimates

The depletion of *E. coli* cells from a solution by adsorption onto sterilized filter media sand under saturated conditions were measured in a laboratory experiment, as described in Section 4.3.3. Figure 5.10 shows the measured concentration in the water phase (blue markers) and the subsequent calculated adsorbed concentrations on the sand grains (red markers). Based on these measurements, the first-order adsorption rate constant was estimated with Equation 4.12 to be 4.2 $\pm$ 1.6 h^{-1}. The desorption rate was estimated based on calculated adsorption rates with Equation 4.11 to be 0.08 $\pm$ 0.05 h^{-1}.

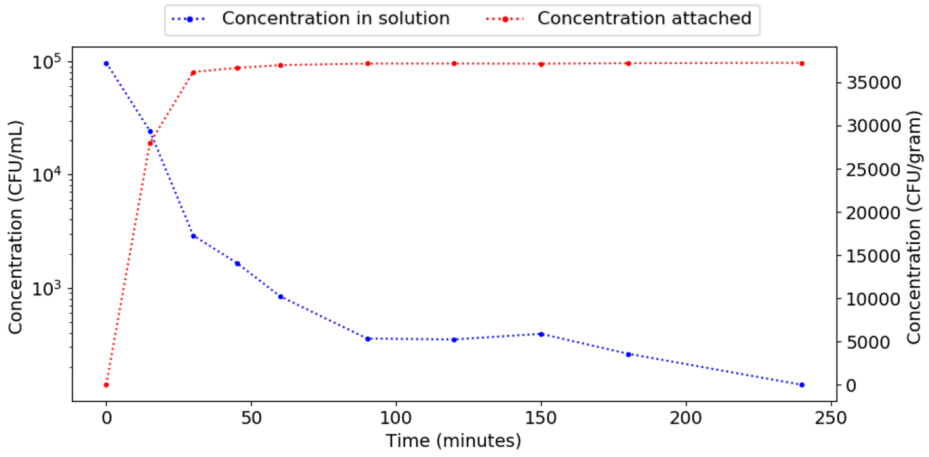


Figure 5.10: Measured *E. coli* concentrations in the solution shown as blue markers, and linked calculated adsorbed concentrations shown as red markers.

The rates are high compared to *E. coli* rates obtained from similar tests [76, 124] but fall within the wide range of rates found for saturated and unsaturated porous, namely adsorption rates ranging from 0.20 - 5.86 h^{-1} and desorption rates from 6*10^{-5} - 0.48 h^{-1} [19, 43, 111]. Variability between rates can be caused by multiple factors that affect the adsorption process, such as water content and soil structure (Section 2.2.2). Shen et al.[111] investigated rate constants in a laboratory study for different biofilter designs and for one field scale biofilter. Their results show average adsorption and desorption rates of 2.00 h^{-1} and 0.09 h^{-1}, respectively [111]. The adsorption rate in this study is thought to be unrealistic high and values are uncertain. Factors that likely contributed to this are the high microbial concentration in the starting solution, additional microbial adsorption to the beaker, loosening of the soil by shaking of the beakers, and estimations in the calculations. Therefore, the average rates found by Shen et al.[111] are used as rate constants to model adsorption and desorption processes.

Decay rate estimate

To investigate the decay rate of target bacteria at room temperature (+/- 25°C) and at 10°C, microbial concentrations in pore water from a field collected soil sample were measured at multiple days, as explained in Section 4.3.3. No *Campylobacter* data was obtained due to overgrowth of the plates. Figure 5.11 shows the measured *E. coli* and enterococci concentrations from the pore water and regression lines that were used to determine the decay rate constants. The regression curves at 10°C show a poor fit with the data. Furthermore, measured concentrations are highly uncertain due to contamination of the water samples. This is caused by the release of biofilm during the extraction of pore water from the soil. An effort was made to prevent plating of the biofilm by taking aliquots from top water after leaving the biofilter to settle. However, multiple samples were presumably polluted with additional microbes from the biofilm, impairing the accuracy of decay observations.

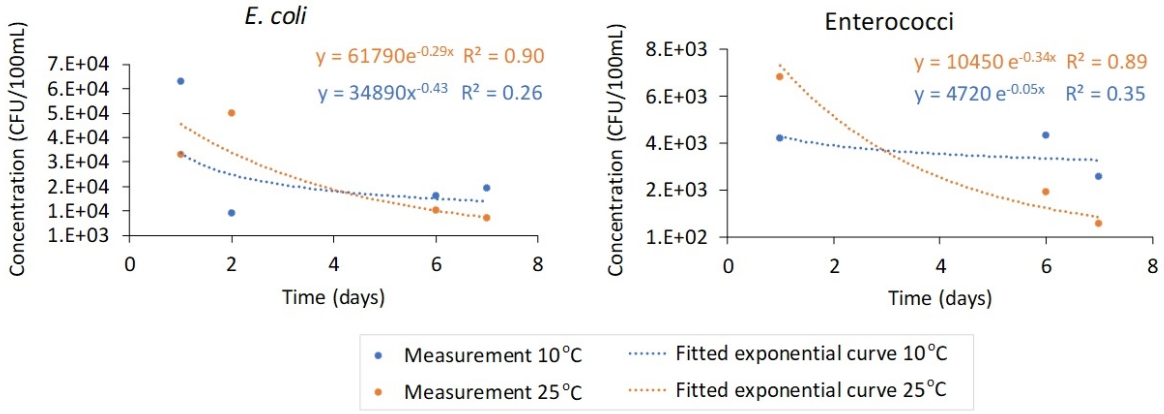


Figure 5.11: Measured *E. coli* and enterococci concentrations in the soil sample of the biofilter at 10°C 25°C with fitted regression lines to determine first order die-off rate constants for *E. coli* and enterococci and r-squared values.

A higher *E. coli* decay rate was observed at 10°C (0.43 d^{-1}) than at 25°C (0.29 d^{-1}). This contradicts with a common finding in literature that increasing temperatures result in increased microbial inactivation rates [40, 117]. Studies on *E. coli* survival in biofilter media at different temperatures show similar results (Table 5.6). *E. coli* rates are, therefore, thought unreliable. Enterococci showed increased decay at 25°C (0.34 d^{-1}) compared to 10°C (0.05 d^{-1}) and rates are comparable with decay rates found by Zhang et al.[130] and Chandrasena et al.[28] (Table 5.6). Even though the rates are highly uncertain and environmental stressors, such as UV radiation and moisture content variability, were not taken into account, observed enterococci rates are thought applicable due to the similarities with literature values. From the observed rates, μ_0=0.22 d^{-1} and θ=1.11 (-) were determined (Equation 4.10). These values are in line with μ_0 and θ parameters obtained in other researches regarding microbial modelling, namely μ_0:0.06-1.23 and θ:1.01-1.19 [111].

Table 5.6: *E. coli* die-off rates in biofilter media from other studies. Conventional bioretention media contains sandy loam soil, sand, and mulch. Adjusted from [27].

Environment	Decay rate (day^{-1})	Temperature (°C)	pH	Reference
Conventional bioretention media	0.90	25 ± 3	5.4	[128]
Conventional bioretention media	0.3	5		[130]
	0.39	15		
	0.35	25		
	0.45	37		
Washed sand filter media	0.05	15	7.0	[28]
	0.10	21	7.0	
Loamy sand filter media	0.07	15	6.8	[28]
	0.11	21	6.8	

5.4.2. Model Scenario: Short-Circuiting

This simulation aimed to improve understanding of the effect of short-circuiting pathways on the microbial fate in the biofilter by comparing a plug flow model with a multipathway model, as explained in Section 4.3.4. For the multipathway model, the biofilter was divided in three areas: A1 and A2 both comprising 10% of the filter area, and A3 comprising 80% of the filter area (Figure 4.7). Three multipathway scenarios were tested in which the inflow volume dosed on the short-circuiting areas increased from scenario 1-3 (see Table 4.1). Figure 5.12 shows the tracer breakthrough curves of the plug flow model and the multipathway scenario runs and compares these with normalized inverted outflow EC measurements.

The breakthrough time in the multipathway scenario runs is lower than in the plugflow model. Furthermore, the time decreases as more volume is dosed on fast flowing areas and, accordingly, a larger volume reaches the outflow sooner. In Scenario 1 the tracer is mostly flushed out, while in the other scenarios part of the tracer remains in the biofilter as the slow flowing area (A3, Table (4.1)) is not completely flushed during the event. Field EC measurements correspond best with the outflow of multipathway scenario 3 in which 80% of the volume is fed to fast flowing areas. This corroborates that a large portion of feed water flows through short-circuiting pathways in the biofilter, as was suggested after analyzing the EC outflow trends (Section 5.2.1).

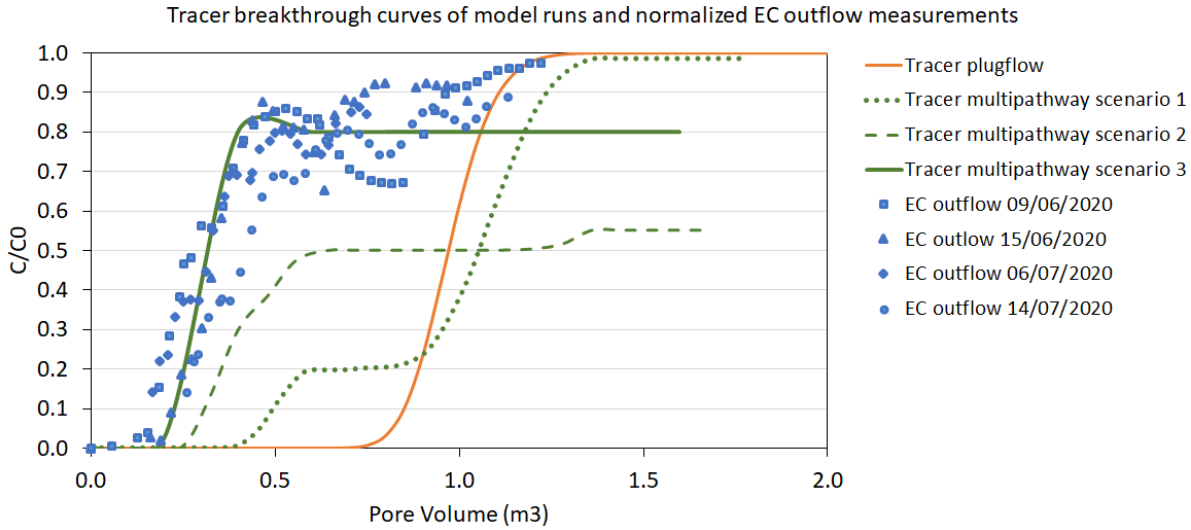


Figure 5.12: Tracer breakthrough curves from a plugflow model and a multipathway model consisting of 3 different flow paths: A1 and A2 comprise 10% of the filter area and A3 80%. In scenario 1, 30% of the inflow volume flows through A1 and A2 pathways, in scenario 2 50% and in scenario 3 80%. Blue markers show normalized inverted EC outflow measurements of the biofilter of multiple events.

Figure 5.13 shows the pathogen breakthrough curves of the different model runs and *E. coli* measurements normalized over mean inflow concentrations. The first event and last event were not considered as only 2 outflow concentrations were measured in the first event, and outflow concentrations were already high from the start of the last event due to direct contamination (Section 5.3.3).

Higher microbial outflow concentrations are observed when the volume dosed on the fast flowing areas increases (Figure 5.13). These areas are flushed more often during the events than the slow flowing area (Table 4.1). Consequently, the fast flowing areas receive a higher total microbial load. This results in higher net-adsorption rates (Section 4.3.1), but the proportion of microbes that adsorb remains similar. Therefore, more microbes will reach the outflow from the high loaded areas. This is in line with other studies that show an increase in microbial outflow concentrations related to increased hydraulic loads and high flow velocities [17, 18, 25, 115]. Higher outflow concentrations from fast flowing areas overrule the lower outflow concentration from area 3, that is not completely flushed during the events (Table 4.1). Hence, this simulation shows that multiple high loaded, fast flowing areas result in increased microbial outflow concentrations. Additionally, no microbial decay was observed during the 4 hour events. This agrees with the finding from other researchers that microbial retention processes are dominant during wet periods and decay during dry periods [23, 128].

As previously mentioned, simulated outflow concentrations are not representative for field measurements and outflow samples contained a mix of aquifer water and filtered feed water. Hence, caution must be taken when comparing the measured values with the model outcomes. However, Figure 5.13 shows that *E. coli* outflow concentrations are comparable to the outflow of a biofilter in which a considerable part of the feed volume flows through short-circuiting pathways.

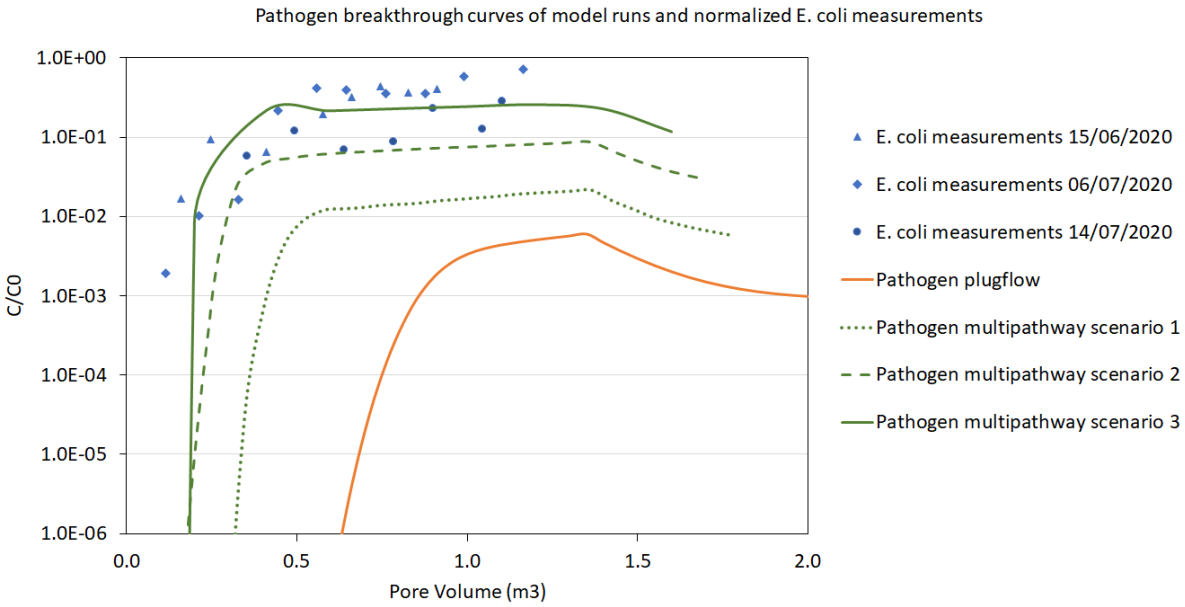


Figure 5.13: Pathogen breakthrough curves from a plugflow model and a multipathway model consisting of 3 different flow paths: A1 and A2 comprise 10% of the filter area and A3 80%. In scenario 1, 30% of the inflow volume flows through A1 and A2 pathways, in scenario 2 50% and in scenario 3 80%. Blue markers show normalized *E. coli* measurements of multiple events.

Sensitivity Analysis

A sensitivity analysis for the assumed adsorption and desorption rates is provided in Appendix D. It shows that the model is most sensitive to the adsorption rate. However, outcomes remain similar: multiple flow paths lead to diminished microbial retention compared to plug flow. An exception is seen when the adsorption rate is low (0.2 h^{-1}) which results in low microbial removal for all scenarios.

5.4.3. Model Scenario: Event Frequency and Duration

The effect of event frequency and duration to process a volume of water on microbial outflow concentrations was investigated with the model, as explained in Section 4.3.5. Microbial outflow concentrations resulting from daily feeding events of 4 hours are compared with 3-daily feeding events of 12 hours. Figure 5.14 shows the pathogen breakthrough curve of the two model scenarios. In these runs, one pore volume represents 31.8 m^3 and 2.7 hours.

Outflow concentrations for both scenarios increase until an approximately steady state is reached after 80 pore volumes, similar to 9 days (Figure 5.14). At this state, microbial outflow concentrations at the start of 12 hour events are lower than at the start of 4 hour events. This is caused by higher microbiological decay between 12 hour events than between 4 hour events due to extended dry periods. The longer feed period at the 12 hour events, however, leads to higher peak loads during the event. This can be explained as more microbes are adsorbed as the feed continues and, consequently, desorption rates increase. This results in a higher influx of microbes from the adsorbed phase back into the water in 12 hours events compared to 4 hour events. Despite the lower outflow concentrations at the start of the 12 hour events, this leads to a higher average outflow concentration.

Results of the model run, thus, imply that microbial outflow loads are higher when a volume is processed in one 3-daily 12 hour event compared to 3 daily 4 hour events. Therefore, operating the system with more frequent short runs seems beneficial if the aim is to lower peak outflow concentrations, e.g., for human health risk control, or to lower loads towards the ASR system, for example, to reduce retention times in the subsurface. However, model simulations do not represent field conditions, therefore, actual effects on system choices should be verified by monitoring outflow concentrations.

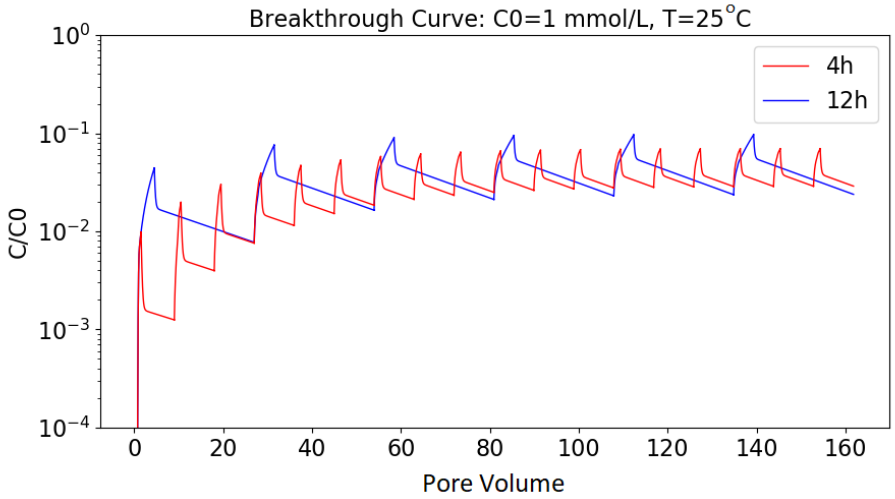


Figure 5.14: Pathogen breakthrough curve of events that last 4h with 20h antecedent dry period in red and events that last 12h with 60h antecedent dry period in blue. 1 pore volume represents 2.7 hours. Model is run at 25 °C

Outcomes model simulation at 10°C

The effect of a temperature of 10°C instead of 25°C on the outcome was evaluated. The result of this model run is provided in Appendix D. Differences between the two operational scenarios are smaller at 10°C than at 25°C. This can be explained by the higher decay rate at 25°C resulting in larger differences in decay between short and long dry periods. Differences in outflow load between 4h and 12h events, however, remains similar.

5.5. Implications of Design, Operation and Environmental Conditions on Microbial Fate

This section evaluates possible implications of research findings on microbial fate, comprising microbial transport, retention, and decay, in the biofilter. Design, operation, and environmental conditions are consecutively discussed in Section 5.5.1- Section 5.5.3. Lastly, Section 5.5.4 compares the microbiological treatment performance of the biofilter with other field scale biofilter studies.

5.5.1. Implications Design on Microbial Fate

Biofilter design choices affect biofilter hydraulics which influences microbiological retention. High hydraulic conductivity of the biofilter was found as solely coarse material with low water retention capacity was used in the filter media (Section 5.1). The location of the inlet near the outlet induces short-circuiting flows in the biofilter (Section 5.2.1) and model simulations imply that a considerable amount of feed water flows through these preferential flow paths (Section 5.4.2). These design choices thus result in high flow velocities through the biofilter and, accordingly, short hydraulic retention times. This can negatively affect microbial adsorption due to short contact times between the microbe and filter media (Section 2.3.1). Moreover, increased flow rates can result in increased shear forces inducing remobilization of previously retained microbes. Both factors can lead to an increased breakthrough of microbes. In addition to the low adsorption capacity, the coarse material presumably minimises the chance of bacteria retention by straining. Stevik et al.[115] argue that straining occurs if the average bacterial cell size is larger than the diameter of the smallest 5% of the particles in the filter media. With cell diameters ranging from 0.5-1.5, 0.5-4, 0.5-5 μm for *E. coli*, enterococci and *Campylobacter* respectively, all bacterial cell sizes exceed the smallest particles size of 0.4 mm [39, 96, 121]. Additionally, Chandrasena et al.[23] argue that bacteria straining is unlikely in filter media with grain sizes >0.02 mm.

5.5.2. Implications Operation on Microbial Fate

Being part of the Urban Waterbuffer, inflow and outflow of the biofilter is controlled as explained in Section 3.2. This section evaluates the effect of current operation on microbial fate.

Uneven distribution of feed water over the biofilter surface

System analysis showed that the ponding zone was abolished leading to uneven distribution of feed water over the biofilter (Section 3.1.2). The varying hydraulic conductivity (Section 5.1.2) caused by irregular clogging, compaction, and desiccation of the biofilter implies that this operation leads to diminished use of the biofilter volume to treat water. A smaller effective reactor volume, and consequently a lower adsorption capacity, can result in faster breakthrough of pollutants and higher microbial outflow concentrations, as was demonstrated in Section 5.4.2

Event frequency and duration

During the research period, the biofilter was once a week fed with stormwater from the buffer for 4 hours. In total 570 m^3 stormwater was treated and the buffer was on average filled with 900 m^3. This implies that the buffer was not fully replenished during the research period. Corresponding residence times were likely high which could have contributed to oxygen depletion in the buffer (Section 5.2.2). Enterococci and *E. coli* are facultative aerobic, i.e., can survive at both aerobic and anaerobic conditions [39, 42]. *Campylobacter* is microaerophilic, hence, requires low oxygen concentrations for growth (2-10%) but cannot withstand atmospheric levels of oxygen [60]. Target bacteria can, thus, have an advantage over aerobic bacteria in the buffer. Decreased competition with these bacteria might lead to higher survival rates of the studied bacteria during storage. This could have contributed to the high inflow concentrations observed. Further salinization of the aquifer is another possible effect of the low volumes that were likely to low to meet water demand. Due to the recirculating water flows, this salinization resulted in subsequent salinization of the biofilter. Possible effects of this salinization on microbiological removal processes are addressed in Section 5.5.1.

Recirculating water flows between the biofilter and aquifer
Analysis of the automatically logged water volumes in the Urban Waterbuffer revealed recirculating water flows between the aquifer and biofilter that occurs 3 times a day (every 4-12h) and replaces submerged zone water with aquifer water between events (Section 3.2.3). On average 120 m^3 is recirculated per week which is approximately 3 times more water than dosed on the biofilter during a feeding event of on average 44 m^3. This operation is expected to affect microbiological water quality in the following ways:

- Higher and more frequent microbial loads on the aquifer are expected compared to an operation in which feed water remains in the biofilter between events. Feed water in the submerged zone is replaced within 4-26 hours providing little time for microbial decay before being injected into the aquifer (Figure 3.11). Increasing microbial loads on the aquifer are further expected as flushing of the biofilter with aquifer water can cause daily leaching of bacteria due to (1) dissolving of microbes from direct pollution source into the recirculating water, (2) net-desorption of attached microbes into the relatively clean aquifer water, and (3) remobilization of previously retained microbes by the propagating wetting fronts in the biofilter [83, 98]. Flushing of the biofilter between events could, however, reduce loads during an event as more adsorption sites might be available at the start of a feeding event.

- Residence times in the aquifer are uncertain which causes concerns whether microbiological loads are sufficiently reduced before water is recovered by Sparta. Due to the frequent recovery, water potentially short-circuits between well 1 and well 2 resulting in little treatment between injection and recovery [132]. In addition, residence times in the aquifer can not be controlled as water is daily injected and recovered. A risk control measure based on a minimal time between injection and abstraction as previously proposed by KWR is, thus, not possible with current operation [132].

5.5.3. Implications Environmental Conditions on Microbial Fate

Results provide insight into some abiotic factors that were previously found to affect microbial removal processes (Section **??**). The following influences of monitored parameters are expected:

- EC conditions (1200-2500 μS/cm) are not expected to result in an osmotic shock and, consequently, increased decay as the measured values are below salt tolerance values found for the target bacteria ([39, 57, 121]). Due to aquifer salinization, the EC conditions could potentially keep increasing towards the maximum salinity of the groundwater (5500 μS/cm) [132] but this value is still expected to be too low to actually promote decay. Decreased growth potential at increasing salt concentrations has, however, been observed [87]. The increased salinity of the biofilter might positively affect adsorption processes as this can result in a decrease in the electrical double layer, as explained in the literature review (Section 2.2.2, Section **??**).

- Neutral pH is observed in both inflow and outflow water which is generally found to support faecal bacteria survival [28, 94]. The biofilter is also expected to have a neutral pH, as this was previously detected by Li and Davis [68] and Hathaway et al.[49] for sand-based bioretention media. Consequently, pH is also not expected to be a stress factor for microbial survival.

- Oxygen concentrations in the system are low ranging from 0 in the buffer to <5 mg/L in the biofilter. As previously explained (Section 5.5.2), the target bacteria are expected to withstand these low oxygen conditions which could potentially lead to increased survival.

- Factors that could promote bacteria decay are low water contents and drying of the biofilter [28, 38]. Therefore, the part of the biofilter above the SZ might be most hostile to bacteria. However, due to the frequent irrigation of the biofilter with aquifer water, this effect is expected to be little.

Generally, the observed physicochemical characteristics are not expected to pose challenging conditions on the target bacteria. The most important factor affecting microbial decay is probably related to biotic factors, which are often stated in literature to have a vast influence on microbial survival [93, 98, 128, 129]. Therefore, it would be interesting to further investigate decay in the biofilter and the effect of biotic factors on this decay.

5.5.4. Comparison Bluebloqs Biofilter to other Field Scale Biofilter Studies

Microbial treatment performance differs per field study depending on environmental conditions, inflow water quality, and differences in design and operation. Information on these factors is often missing which hinders comparison between studies. For example, no information was found on hydraulic retention times and flow conditions of other studied biofilters. Keeping this in mind, some aspects of the current biofilter study can be compared to prior studies. Characteristics of other field scale biofilter studies can be found in Appendix E.

Similar to other field scale biofilter studies (Table E.2), microbial inflow and outflow concentrations cover a wide range, highlighting the variable conditions surrounding stormwater biofilters. Assessment of microbial concentrations flowing in and out of the biofilter (Section 5.3.3) showed variable performance over the events. Similarly, a range of treatment performances was observed over the events monitored in other studies (Table 5.7). However, the current study shows worse microbiological removal efficiencies than most other field scale biofilter studies.

Table 5.7: Removal performance results of other field scale studies on stormwater biofilters. Median or arithmetic mean values are values within parentheses are the reported minimum and maximum values. Negative values indicate addition of microbes and positive values removal. Adjusted from [127].

Field scale biofilter	Log removal			Nr of sampling	Reference
	E. coli	Enterococci	*Campylobacter*	events	
RMGC[a]	1.38 (0.4-1.84)		0.78 (0.35-1.57)	20	[25]
Monash carpark[a]	1.18 (0.82-1.80)		0.9 (-0.28-2.05)	6	[25]
Bioretention Charlotte[b]	1.10 (0.06-4.00)			14	[48]
Bioretention-D[b]	0.52	0.98		20	[49]
Bioretention-S[b]	-0.34	-0.03		20	[49]
Maryland CP[a]	0.02 (-1.48-2)			13	[130]
Maryland SS[a]	-0.9 (-0.96-0.3)			13	[130]
Kfar Sava[a]	2.0 (1.4-3.7)			9	[131]

[a] Median value
[b] Arithmetic mean value

Considering *E. coli*, minimal retention to leaching was observed in the current study (Section 5.3.3). A possible explanation for the higher removal of *E. coli* in other studies could be the incorporation of fine sand, clay, and silt in the filter media (Table E.1). This can increase hydraulic retention times and adsorption capacity of the filter media resulting in increased microbial retention [25, 32, 131]. The systems Monash carpark, Bioretention Charlotte, and Kfar Sava have considerably lower hydraulic conductivity than found in the current study, namely between 30-300 mm/h (Table E.1) compared to >900 mm/h (Table 5.2), which could contribute to increased retention. To the author's knowledge, *Campylobacter* removal was only studied at the RMGC biofilter and Monash carpark (Table 5.7). In contrast to current research, Chandrasena et al.[25] found overall *Campylobacter* removal. Leaching was observed during one event and suggested causes were the release of previously retained *Campylobacter* as inflow loads were low compared to previous high loading events and uncertainty in microbial detection methods. Similar to better *E. coli* removal in these biofilters, the incorporation of fine particles in the filter media likely contribute to better *Campylobacter* removal. Furthermore, direct contamination could have been less or absent during the monitored events.

Similar to this study, leaching of bacteria was found in Bioretention-S and Maryland SS (Table 5.7). Hathaway et al.[49] attributes poor microbial treatment in system SS compared to system CP to a higher soil water flux caused by the smaller filter layer depth, namely 250 mm compared to 600 mm. This resulted in decreased contact times between microbes and filter media. The reason for the high soil water flux is, thus, different than the short-circuiting flows and high hydraulic conductivity in the current study. However, the implication remains similar: a high flow rate can lead to diminished microbial treatment. Corresponding with the current study, Zhang et al.[130] suggest that direct contamination with animal faeces contributed to poor microbial treatment in Bioretention-S.

Results of the current study show minimal retention of *E. coli* and leaching of *Campylobacter* and enterococci, which implies that the fate of these bacteria over the biofilter differs. This is in line with the study of Chandrasena et al.[25] who found higher *E. coli* removal and state that *E. coli* behaves significantly different than *Campylobacter* in the studied biofilters. Similar to this study, the treatment performance of *E. coli* and enterococci differ in Bioretention-D and Bioretention-S. However, Hathaway et al.[118]) observed better enterococci removal than *E. coli* removal. This difference could be caused by differences in direct contamination on both biofilters.

5.6. System Improvement Opportunities

The previous section highlights the impact operation and design can have on microbial fate and shows the need for further measures to enable microbiological safe water for reuse. This section discusses how the system can be upgraded to mitigate encountered problems. Section 5.6.1 focuses on improving microbial retention in the biofilter during an event, Section 5.6.2 considers how system operation can be arranged to improve the microbiological water quality, and Section 5.6.3 proposes a way to reduce the change of short-circuiting in the aquifer by changing aquifer construction. Lastly, Section 5.6.4 provides recommendations for microbial risk management.

Discussed changes to the system will impact the treatment of other constituents. Therefore, adjustments should not be made before investigating possible effects on target pollutants. If no changes are desired or if pathogen removal remains inadequate to provide microbial safe water, an option is to introduce post-disinfection, such as UV radiation, prior to irrigation of the sports fields.

5.6.1. Improve Retention Capacity of the Biofilter

The large grain size, high hydraulic conductivity, short-circuiting pathways, and the uneven inflow distribution diminish pathogen retention during an event (Section 5.5). The following steps can be taken to improve this:

- Distribute feed water over the total surface area by increasing the height of the allowed water level in the biofilter during a feeding event. A more even distribution of inflow water can expand the effective reactor volume and, consequently, the capacity of the biofilter to retain microbes. Furthermore, it can reduce clogging of specific surface areas which lowers the potential of short-circuiting flows past these clogged areas. Lastly, distribution of the water over the total surface area allows for more contact with the iron-coated sand layer on top of the filter which could improve microbial retention due to its greater positive surface charge and surface roughness compared to quartz sand [128]. This was previously demonstrated by laboratory studies, as discussed in Section 2.3.1. However, these layers were approximately 2 to 3 times larger than the layer on top of the biofilter and hydraulic loading rates were roughly 2-10 times lower than hydraulic loading rates that could be achieved by a more even inflow distribution on the studied biofilter, hence positive effects might be little. To minimise safety risks regarding water ponding, the height above the filter media can be minimised.

- Incorporate soil particles with smaller grain sizes and better water holding capacity, such as clay, in the filtration layer. This lowers hydraulic conductivity and thus increases hydraulic retention times, which is beneficial for microbial adsorption. Furthermore, it improves the retention capacity of the biofilter by generating a higher specific surface area for adsorption and enhancing the chance of particle straining. Previous laboratory studies demonstrate better microbial removal by incorporating fine particles [19, 26] and improved microbiological removal performance in other field scale biofilters that contain fine particles suggest the same (Section 5.5.4).

- Minimise short-circuiting by locating inflow points at a maximum distance from outlet points [2, 46, 92]. For example, inlet points could be distributed evenly over the biofilter surface, which is seen in vertical flow wetlands [61]. However, this construction can be less robust and reduces the accessibility of the biofilter area.

5.6.2. Control Water Flows and Residence Times

Water flows and residence times in the biofilter and aquifer can be controlled to improve the microbiological water quality in the system. The concept of controlled operation to improve microbiological water quality consist of three steps:

1. Set an adequate dry period between events to enable sufficient microbial decay of previously retained microbes before the onset of a new event. This prevents fast saturation of the biofilter media in the subsequent event and remobilization of previously retained microbes before they have decayed, as discussed in Section 2.3.2. The length of this period thus determines microbial outflow concentrations at the start of a new event.
2. Control outflow concentrations during an event by controlling the biofilter runtime. As outflow concentrations increase during an event, short runtimes result in low outflow loads but similarly low outflow volumes, while longer runtimes increase outflow concentrations and volumes. Accordingly, this affects the time required in the aquifer to reduce microbial loads and the amount of water available for recovery. For example, shorter runtimes enable frequent recovery of less volume compared to the occasional uptake of large volumes.
3. Set a fixed time between aquifer injection and subsequent recovery of the injected water to provide sufficient reduction of the microbiological load introduced by the biofilter. Accordingly, this depends on the chosen dry period between events and the event duration.

The above steps are connected and should be optimised to obtain sufficient microbial treatment and meet water demands. For this, further research is needed to clarify microbial decay in the biofilter between events and microbial removal in the aquifer over time. As a first indication, model simulations (Section 5.4.3) suggest that shorter, more frequent runs result in lower microbial outflow concentrations compared to longer less frequent runs. Moreover, long residence times and salinization of the aquifer imply that the total water volumes processed per week should be increased (Section 5.5.2).

To enable the discussed operation, recirculating water flows between the biofilter and aquifer should be abolished. The unpleasant odour of the recovered water resulted in the decision to recirculate aquifer water to the biofilter before it is recovered by Sparta (Sec.3.2.3). This odour was not encountered in the first year of operation and should be solved first. Improving overall water quality, such as oxygen conditions and microbial levels, could already be beneficial but further work is required to investigate what causes this odour, e.g. sulfate reducing bacteria in the aquifer, and how to prevent or treat this.

5.6.3. Reduce Chance of Short Residence Times in the Aquifer

A concern of current operation is that water might short-circuit between the lower and upper well when injected water is quickly recovered (Section 5.5.2). It is proposed to set a fixed time between aquifer injection and recovery to ensure sufficient retention time in the aquifer (Sec. 5.6.2). However, this restricts the uptake of water by the end user. If this is undesirable, infiltration and abstraction wells could be separated into two boreholes to increases the distance between these wells and accordingly, extend the travel time between injection and recovery. A type of managed aquifer recharge used to achieve additional water treatment by extending residences times is Aquifer Storage Treatment and Recovery (ASTR) system System (Figure 5.15) [89, 90]. This asks for more drastic changes to the current system but could be directly applied in future applications to improve the robustness of treatment in the aquifer.

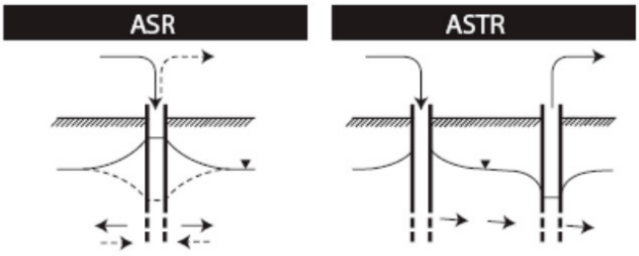


Figure 5.15: Left: Aquifer Storage and Recovery (ASR) system. Right: Aquifer Storage Treatment and Recovery (ASTR) system. From [90].

5.6.4. Recommendations Risk Management

Improved understanding of *E. coli*, enterococci and *Campylobacter* concentrations entering and exiting the biofilter during feeding events lead to the following suggestions concerning risk management:

- Varying inflow and outflow concentrations cannot be captured by a single sample taken during an event. Accordingly, evaluation of the treatment performance of the biofilter based on a single grab sample can be inaccurate. For example, samples taken at the start might imply treatment while samples were taken near the end show leaching. To allow direct comparison between inflow and outflow samples for evaluation of the treatment performance, sampling should take place after all preceding water has flushed out. Lastly, the system should be running when taking samples to prevent sampling of dead water volumes that do not represent actual concentrations.
- In the study conducted by Zuurbier and Van Dooren [132] at the Urban Waterbuffer, *E. coli* measurements were used as a surrogate for *Campylobacter* concentrations. This research, however, found 2-3 orders higher concentrations of *E. coli* than *Campylobacter*. Additionally, observed differences in outflow trends of these bacteria imply that direct contamination sources, that have a main effect on outflow concentrations, are different. This suggests that *E. coli* should not be employed as a proxy for *Campylobacter*. Considering the shared avian pollution source, enterococci seems a better indicator for *Campylobacter* behaviour in the biofilter. Nonetheless, most certainty in risk assessments can be achieved when monitoring *Campylobacter* concentrations. It must, however, be noted that the obtained *Campylobacter* data set is small. To improve understanding of *Campylobacter* dynamics and its relation to *E. coli* and enterococci, these bacteria need to be monitored for a longer period.
- Zuurbier and Van Dooren [132] proposed a minimum time of 3 days between the injection of water into the ASR and subsequent recovery to mitigate human health risks of reusing the water for irrigation of the Sparta sports fields. Higher microbial concentrations in both inflow and outflow water were, however, observed in this study (Section 5.3). Consequently, the minimum time required in the aquifer to attenuate the encountered levels needs to be reconsidered. Hence, research investigating the removal in the aquifer over time is needed.

5.7. Limitations and Uncertainty

This section discusses the main limitations and uncertainty of this study and examines how this affected the outcomes. Three main limitations are related to the methodology applied.

Firstly, hydraulic conductivity measurements are subjected to several sources for possible error: water was manually poured into the cylinder which made it impossible to constantly obtain a stable hydraulic head, installation of the ring in the soil presumably disturbed soil compaction, and preferential flow on the sides of the cylinder might have led to higher flow rates. Additionally, the top layer was assumed to limit hydraulic conductivity. Therefore, hydraulic conductivity deeper in the biofilter was not measured. The limiting hydraulic conductivity of areas that do not directly receive inflow water, could, however, be located deeper in the biofilter. Lastly, measurements were only performed once per location due to time limitations. Because of these reasons, hydraulic conductivity values are uncertain. Nevertheless, the high hydraulic conductivity of the biofilter and variations over the biofilter area are evident. To improve certainty on biofilter hydraulic conductivity, a deep ring infiltration test could be performed and measures can be repeated.

Secondly, problems were encountered with the analytical methods chosen to enumerate *E. coli* and *Campylobacter*. ChromoCult Coliform agar was used as a growth medium for *E. coli* but showed to be inconvenient for the target water as high concentrations of coliform bacteria were present. Background growth of these bacteria on the agar led to difficulties in the enumeration of *E. coli* colonies. Consequently, the amount of data available for analysis was reduced. Despite this, enough data was obtained to provide insight into *E. coli* inflow and outflow concentrations and trends. To prevent encountered problems, a medium only allowing *E. coli* growth is recommended for future studies. In addition, *Campylobacter* showed to be more difficult to culture than *E. coli* and enterococci. Consequently, concentration predictions are less precise and the usable data set for *Campylobacter* is small limiting the

ability to assess trends. Despite this, the presence of *Campylobacter* was detected in all samples, and trends were observed that comply with the other bacteria. To facilitate *Campylobacter* cultivation, it is advisable to use an incubator in which specific oxygen conditions and carbon dioxide conditions can be controlled. Lastly, as water quality at the site is variable, the reliability of the data can be improved by performing sample analysis in triplicates instead of duplicates.

A last methodological limitation is related to the monitoring strategy. Due to the unanticipated hydraulic behaviour of the system, monitoring rounds were too short to drain all initial water from the system. Consequently, outflow samples contained a mixture of filtered feed water and aquifer water. Therefore, a direct comparison between inflow and outflow was not possible. To overcome this problem, water quality changes over the biofilter were estimated based on the mixing fraction of aquifer water and filtered feed water (Section 4.2.4). These values provide an estimation of the treatment performance in the biofilter but are based on assumptions and, therefore, uncertain. In addition, microbial decay in the biofilter could not be determined as submerged zone water was replaced between events. Despite these limitations, trends were observed in the data that improve understanding of microbial fate in the biofilter. In future research, monitoring rounds should be extended to ensure drainage of all initial pore water and recirculating water flows should be stopped to assess microbial decay in the biofilter.

Besides the above-mentioned limitations, generalizibility of the results is limited. Firstly, the data set obtained in this study is small due to the restricted availability of the monitoring site and the short research period. Samples were taken during the summer months (June-August) and only 5 events were monitored. Consequently, this data set does not represent the behaviour of the system over the whole year. Moreover, the biofilter is subjected to instantaneous variations in inflow water quality and direct contamination. Understanding of such variations can be expanded by monitoring over a longer period. Lastly, results cannot simply be extrapolated to other field scale biofilters. For example, inflow water quality can differ substantially per site and biofilters can be subjected to various environmental factors. Despite this, some findings apply to a broader context. Results, for example, highlight the need to prevent short-circuiting and show adverse effects of coarse filter media on pathogen removal. Furthermore, results show that direct contamination can lead to leaching of bacteria, and underline that multiple microorganisms should be monitored to improve understanding of microbiological removal in biofilters.

6

Conclusion & Recommendations

6.1. Conclusion

This research aimed to evaluate the fate of *E. coli*, enterococci and *Campylobacter* in the Bluebloqs biofilter and assess if the microbiological treatment performance can be improved to reduce human health risks regarding reuse of the treated water.

The first objective was to examine the capacity of the biofilter to remove *E. coli*, enterococci and *Campylobacter*. By analyzing microbiological inflow and outflow dynamics over 5 feeding events, leaching of enterococci and *Campylobacter* and minimal retention of *E. coli* was revealed. The results, thus, imply that the biofilter was unable to reduce enterococci and *Campylobacter* inflow concentrations and had limited capacity to reduce *E. coli* concentrations.

The second objective was to identify factors that lead to adverse removal efficiency. *E. coli*, enterococci, and *Campylobacter* trends suggest that direct contamination contributed to poor treatment performance. Leaching of *Campylobacter* and enterococci was likely caused by bird droppings, whereas direct deposition of dog faeces on the biofilter probably caused leaching of *E. coli*. The variability observed between events and microorganisms highlights the complex conditions surrounding field scale biofilters and emphasizes that microorganisms from multiple origins should be assessed to evaluate the microbiological removal performance of field scale biofilters.

In addition, multiple design factors were identified that diminish the microbiological retention capacity of the biofilter. Firstly, the coarse filter media led to high hydraulic conductivity in the biofilter resulting in short hydraulic retention times. Consequently, the contact time between microbes and the filter media is expected to be low which is detrimental for adsorption. Presumably, adsorption is the main removal process during a feeding event as the filter media is likely too coarse to facilitate microbial straining. Another design factor resulting in unfavorable conditions for microbial retention is the construction of one inlet point near the outlet. EC measurements revealed short-circuiting flows between these points. Comparison of EC outflow trends with model simulations implied that a considerable amount of feed water flows through these preferential flow paths. This leads to high flow velocities through some part of the system and, consequently, reduces the chance of adsorption to take place. Furthermore, this can result in high shear forces which promote remobilization of previously retained microbes.

Lastly, factors regarding operation were identified that can result in poor microbiological treatment. Firstly, feed water was only distributed over a small part of the surface of the biofilter. This implies that only a part of the biofilter volume is effectively used to treat water. Additionally, operational settings lead to concerns regarding treatment in the aquifer. Water is frequently recovered and, consequently, residence times in the aquifer are uncertain. Therefore, it is questioned if retention times in the subsurface are sufficient to reduce incoming microbial loads from the biofilter. Further actions are, thus, recommended to ensure the microbiological safety of the water available for reuse.

The last objective was to investigate how the system can be upgraded to enhance the role of the biofilter as a microbiological treatment barrier. Regarding design, incorporating smaller soil particles in the filter media and maximizing the distance between inlet and outlet are recommended to improve the retention capacity of the biofilter and minimise short-circuiting.

Concerning operation, reintroducing an even distribution of inflow over the biofilter is advised. Additionally, it is recommended to rearrange event frequency and duration to provide sufficient microbial decay between events and control microbial outflow loads during events. Model simulations suggest that shorter, more frequent runs lead to lower outflow concentrations than longer, less frequent runs. However, further research is recommended to clarify microbiological decay rates between events. Additionally, it is believed that upgrading the biofilter is not enough to provide microbiologically safe water for reuse. Therefore, improving the reliability of the aquifer as a microbiological treatment step is also recommended. This can, for example, be achieved by setting a minimum time between injection and recovery, or by extending the distance between injection and recovery wells.

To encapsulate, the results of this study show that the biofilter with current design and operation is not a treatment barrier for microorganisms. However, it is believed that the system could improve microbiological water quality entering the aquifer if system design and operation are upgraded to aid microbiological treatment.

6.2. Recommendations for Future Research

Based on the outcomes of this study, the following recommendations are made for future research:

- It would be interesting to investigate how water quality evolves after all aquifer water has flushed out by prolonging monitoring rounds. This can help to verify the extent of leaching or potential retention of *E. coli*. Furthermore, it is advised to monitor microbial decay in the biofilter to assess how much time is needed to reduce microbial loads between events. For this, irrigation of the biofilter with aquifer water should be discontinued.
- Water quality changes in the aquifer should be monitored to assess the treatment performance. For this, the injection and recovery of aquifer water should be paused. Based on this information, the time required in the aquifer to lower microbiological loads can be determined.
- Monitoring the water quality in the buffer is recommended to assess how the quality changes during storage, for example, to what extent and in which time frame. If needed, this information helps to determine measures to prevent water quality deterioration in the buffer.
- Further work regarding the flows in the aquifer is needed to clarify how much water is taken up by Sparta and to assess if the clay layer separating the wells is capable of preventing short-circuiting when water is frequently recovered.
- The actual drainage area connected to the Urban Waterbuffer should be investigated to improve understanding of the sources of microbiological pollution contaminating the water and the water flows entering the buffer.
- In addition to *Campylobacter*, it would be interesting to monitoring *Legionella pneumophila*, a pathogen that can naturally occur in water and is transmitted via aerosols, as the treated water is used for irrigation of the sports fields.
- This research focused on bacteria removal in the biofilter. However, the previous study detected the presence of viral indicators, i.e., somatic coliphages, and F-specific RNA bacteriophages, in the inflow and outflow of the biofilter [132]. These assessments were only performed once. However, it is recommended to further investigate if these microorganisms are consistently present at the Urban Waterbuffer, as this could indicate contamination of the water with human enteric pathogens, hence, potential connections with the sewer.
- Laboratory studies suggest that secondary amendments with a high specific surface area and porosity, such as zeolite, could improve the retention capacity of biofilter media [83, 128]. However, research on the effects of such amendments is still limited to small scale laboratory experiments, hence, it would be interesting to assess the potential of such amendments under field conditions.

A

Technical Specifications Urban Waterbuffer Spangen

This appendix shows technical specifications of the Urban Waterbuffer Spangen. Section A.1 shows the grain size distributions of the used filter media, Section A.2 shows the sensors present at the Urban Waterbuffer, and Section A.3 presents the calculations that were done to estimate the dead water volumes in the pipelines.

A.1. Grain Size Distribution Curves of the Filter Media Sands

Fig A.1 show the grain size distribution graphs of the materials used in the filter layer, transition layer, and drainage layer. All information is from the distributor: Kremer Zand & Grind.

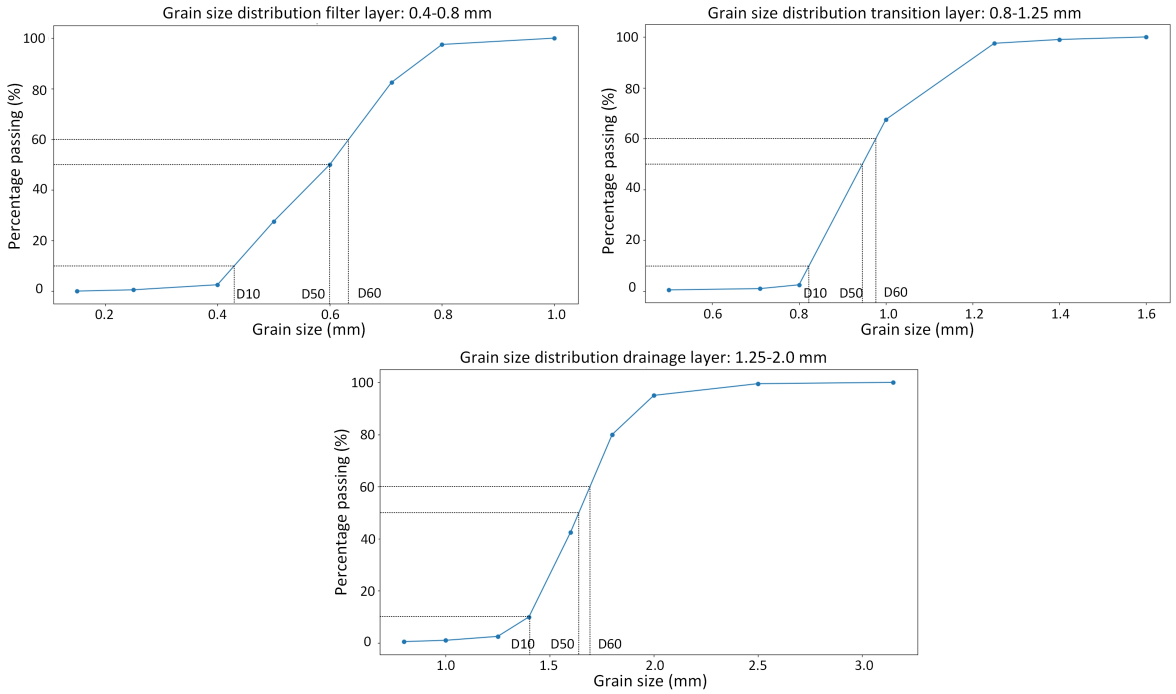


Figure A.1: Grain size distribution of the filtration layer sand, transition layer sand, and drainage layer sand. D10, D50, and D60 represent particle sizes that corresponding to 10%, 50% and 60% finer particles in the particle sizes distribution.

A.2. Monitoring System

To monitor the functioning of the Urban Waterbuffer, various sensors are installed that log their data every 30 minutes on a Programmable Logic Controller (PLC). Table A.1 presents these sensors, their location, and what they measure.

Table A.1: Sensors present at the Urban Waterbuffer that log data every 30 minutes on a Programmable Logic Controller. From [132].

Measurement	Location of Sensor	Unit	Sensor Type
Water level retention buffer	Drainage Well 1	m or Pa	Pressure sensor
Supply Bluebloqs biofilter	Pipeline buffer to biofilter	m^3	EM-Flow sensor
Water level Bluebloqs biofilter	Drainage Well 2	m or Pa	Pressure sensor
Supply standpipe	Pipeline biofilter to standpipe	m^3	EM-Flow sensor
Water level standpipe	Standpipe	M or Pa	Pressure sensor
Infiltration volume ASR well 1 + 2	Pipeline standpipe to ASR well	m^3	EM-Flow sensor
Withdrawal volume ASR well 1 + 2	Pipeline ASR well to watertechnical room	m^3	EM-Flow sensor
EC ASR well infiltration	Pipeline biofilter to standpipe	mS/m	EC-sensor
EC ASR well 1+2 withdrawal	Pipeline ASR well to watertechnical room	mS/m	EC-sensor

A.3. Dead Water Volumes Pipelines

Stagnant water from the pipeline has to be drained before samples are taken. This section shows the calculation of the water volumes present in the pipelines that run from the inlet and outlet of the biofilter to the sampling valves in the water technical room. With help of technical drawings the length of the pipelines was assumed. Based on these lengths and diameters, the volume in the pipes were calculated. Thereafter, the time it takes to drain this volume 1.5 times was calculated.

Using Figure A.2, the volume in the pipeline running from the buffer to the biofilter inflow sampling valve is assumed: $A_{125} * L_{125} + A_{90} * L_{90} = 0.012 * 4.10 + 6.36 * 10^{-3} * 1.61 = 0.061m^3$. Based on Figure A.3 the volume in the pipeline from the biofilter to the outflow sampling valve is assumed: $A_{125} * L_{125} + A_{90} * L_{90} = 0.012 * 12.26 + 6.4 * 10^{-3} * 1.39 = 0.16m^3$.

Table A.2 shows the estimated dead volumes in the pipelines and the time it takes to drain this water when the system is running.

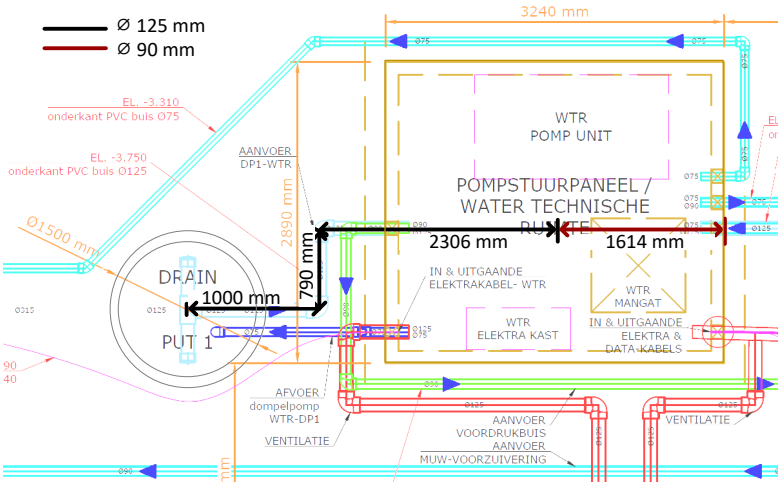


Figure A.2: Pipeline segment that needs to be drained before taking a sample of the biofilter influent. This segment is part of the pipeline running from the buffer towards to biofilter. Adjusted from [132]

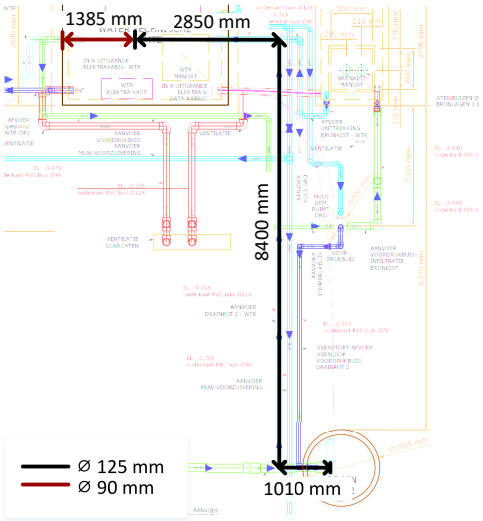


Figure A.3: Pipeline segment that needs to be drained before taking a sample of the biofilter effluent. This segment is part of the pipeline running from the biofilter towards to ASR well. Adjusted from [132]

Table A.2: Dead water volume in the pipelines from target sampling point to sampling valves and the time it takes to drain these volumes for 150% before sampling.

	Target water sample	
	Biofilter inflow	Biofilter outflow
Dead water volume [m3]	0.061	0.16
Volume to be drained [m3] (150%)	0.092	0.24
Qavg [m3/h]	14	11.4
Time [s]	24	76

B

Data Collection

This appendix provides additional information on the samples and data collected during field work at the Urban Waterbuffer. Section B.1 provides additional information per sampling event. Section B.2 presents the amount of physicochemical data and microbiological data collected per event.

B.1. Sampling Event Information

Table B.1 provides additional information on the conditions of the monitored sampling event. Figure B.1 shows photographs taken of the biofilter at the sampling days to show the development of vegetation during the monitoring period.

Table B.1: Information on the sampling events at the Urban Waterbuffer. Vin and Vout represent the total inflow and outflow volume, Qavg in and Qavg out show the average inflow and outflow volumetric flow rate, T shows the average temperature on the sampling date.

Date	Weather	Duration (min)	Vin (m3)	Vout (m3)	Qavg in (m3/h)	Qavg out (m3/h)	T (°C)
09/06/2020	cloudy	180	27.9	23.8	19.9	7.9	12
15/06/2020	cloudy	184	38.8	32.5	19.1	10.6	19
06/07/2020	windy, rain showers	225	55.7	39.7	20.1	11.0	17.0
14/07/2020	drizzly	240	43.6	44.3	20.0	11.1	18
18/08/2020	sunny, rain showers	240	51.5	48.4	20.9	12.1	20

09/06/2020

15/06/2020

06/07/2020

14/07/2020

18/08/2020

Figure B.1: The biofilter at the sampling days.

B.2. Number of Data

Table B.2 shows the number EC, pH, and DO data collected. Table B.3 shows the number of *E. coli*, enterococci and *Campylobacter* data obtained.

Table B.2: Number of electrical conductivity (EC), pH and dissolved oxygen (DO) data measured of the inflow and outflow water of the biofilter per date.

Date	EC in	EC out	pH in	pH out	DO in	DO out
09/06/2020	12	26	12	26	11	26
15/06/2020	21	30	21	30	21	30
06/07/2020	30	43	30	43	30	43
14/07/2020	19	32	19	32	18	31
18/08/2020	21	9	25	38	25	38

Table B.3: Number of *E. coli*, Enterococci and *Campylobacter* data from the inflow water and outflow water of the biofilter per date. Between brackets the number of samples that did not obtain results due to too numerous colonies too count (TNTC) or too little growth (<5 CFU) are given.

Date	*E. coli*		Enterococci		*Campylobacter*	
	Inflow	Outflow	Inflow	Outflow	Inflow	Outflow
09/06/2020	1	2	1	2	1	2
15/06/2020	3	7 (1 <5CFU)	3	8	3	7 (1 TNTC)
06/07/2020	3	9 (1 <5CFU)	3	10	3	6 (4 <5CFU)
14/07/2020	5	7	5	7	5	No data[b]
18/08/2020	5	5 (3 TNTC)	5	7 (2 TNTC)	No data[a]	9

[a] No data due to swarming
[b] No data due to contaminated growth

C

Additional Information Results and Analyses

This appendix contains supplementary information on the experiments and calculations carried out in this research. Section C.1 shows how the volume of water present in the biofilter at the start of an event is calculated. Section C.2 presents results of a test that was performed to assess the effect of pore size on *E. coli* and enterococci detection. Section C.3 shows volumetric flow rates entering and exiting the aquifer and corresponding electrical conductivity values. Lastly, Section C.4 presents the assumed fractions of aquifer water in the outflow samples.

C.1. Volume Water in Biofilter

This section explains how water volume present at the start of an event is calculated. This volume depends on the water level set as submerged zone (SZ) height. The water level in the biofilter is measured in drainage well 2 with a pressure sensor and logged as % of the well filled. These measurements are used in this calculation.

The base of the biofilter is considered as 0%. The volume of the fruster is calculated with Equation C.1. For every water level, the height is determined with $h = 1.1 * (waterlevel/100)$. The lower area (55.44 m^2) is constant, and the upper area is calculated with Equation C.2.

$$V_{frustrum} = \frac{h}{3} * (A_1 + A_2 + \sqrt{A1 * A2}) \tag{C.1}$$

Where:

h = height of frustrum
A_1 = area of upper base
A_2 = area of lower base

$$A_1 = (h * 2 + 9.9) * (h * 2 + 5.6) \tag{C.2}$$

Where:

h = height of the water level: h = 1.1 * (water level/100)
9.9 = length of lower base
5.6 = width of lower base

C.2. Membrane Filtration Technique - Membrane Pore Size

This section shows the results of a tests that was performed to assess the effect of membrane pore size on detection and enumeration of *E. coli* and Enterococci. The water used for this tests was obtained from the Urban Waterbuffer when the system was not running. Consequently, stagnant water from the drains might have ended up in the samples. Therefore, these samples cannot be used to evaluate the treatment performance of the system but were solely used for microbiological analysis technique optimisation. In this test, 2 volumes were filtered through 0.22 μm and 0.45 μm Whatman cellulose acetate membranes to visually assess the differences between the outcomes. It is acknowledged that this test is very uncertain due to the limited samples processed and only provides a first insight in differences that might arise from the use of different pore sizes.

The results for *E. coli* (Figure C.1) showed that more *E. coli* (blue colonies) are retained on a membrane with pore diameter 0.22 μm compared to 0.45 μm when the same amount of water is filtered. For enterococci (Figure C.1) this difference is not found, which might be explained by the larger dimension of enterococci. Consequently, the switch to the 0.45 μm might have led to less retention on *E. coli*. However, as 0.45 μm is common practice in *E. coli* enumeration [81], and 0.22 μm retained more microorganisms, results from analysis with 0.22 μm and 0.45 μm are believed valid. It is, however, uncertain what the actual effect of the switch is so this must be kept in mind when assessing results.

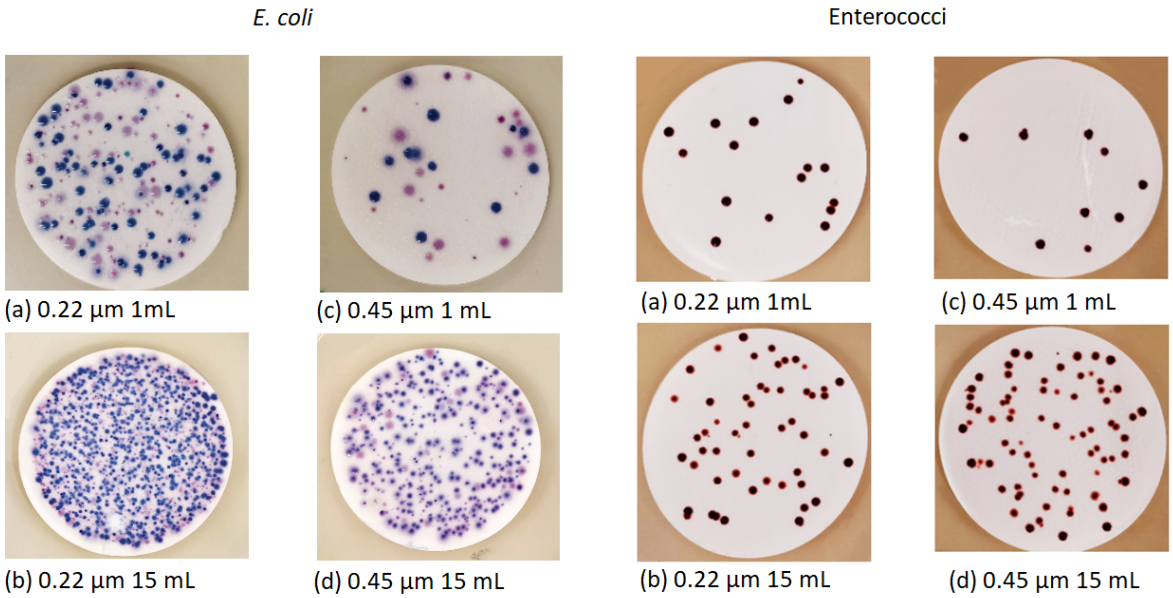


Figure C.1: Results for *E. coli* and enterococi detection using the membrane filtration technique with 0.22 μm (a, b) and 0.45 μm (c,d) Whatman cellulose acetate membranes (incubation 37°C). Top shows 1 mL filtration and bottom 15 mL.

C.3. Salinization of the Aquifer

Figure C.2 presents volumetric flow rates entering and exiting the aquifer that were retrieved from the automatic logged data from the Urban Waterbuffer. It shows that little water was injected into the aquifer from mid January 2020 - June 2020. However, caution must be taken when considering this figure as biases were found in the automatic log files. For example, no data was logged over February 2020. Figure C.3 shows electrical conductivity (EC) values monitored in well 1 and well 2. A peak in EC is seen after June 2020 implying salinization of the aquifer.

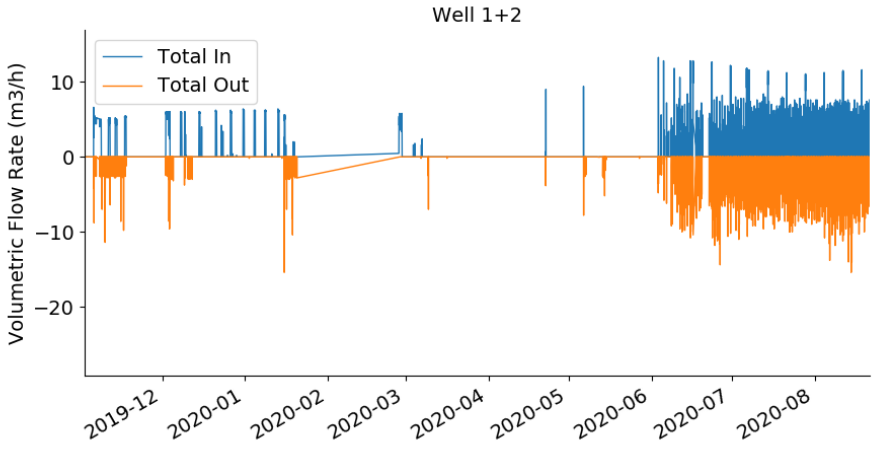


Figure C.2: Total volumetric flow rates (well 1 + well 2) entering and exiting the aquifer in 2020. In the period of January 2020-June 2020 little water flows were detected.

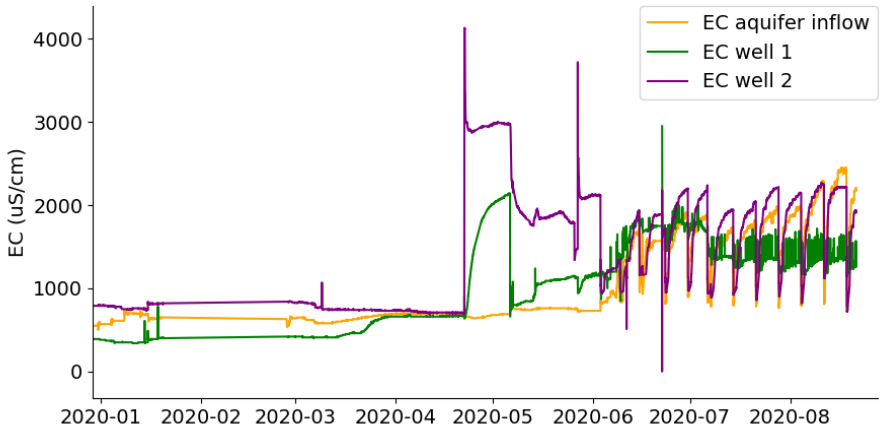


Figure C.3: Electrical conductivity (EC) of the water flowing into the aquifer, at well 1 and well 2. The observed peak indicates salinization of the water in the aquifer.

C.4. Fraction Aquifer Water in Outflow

Figure C.4 shows the fractions of aquifer water in the outflow volume that were estimated from electrical conductivity measurements, as explained in Section 4.2.4.

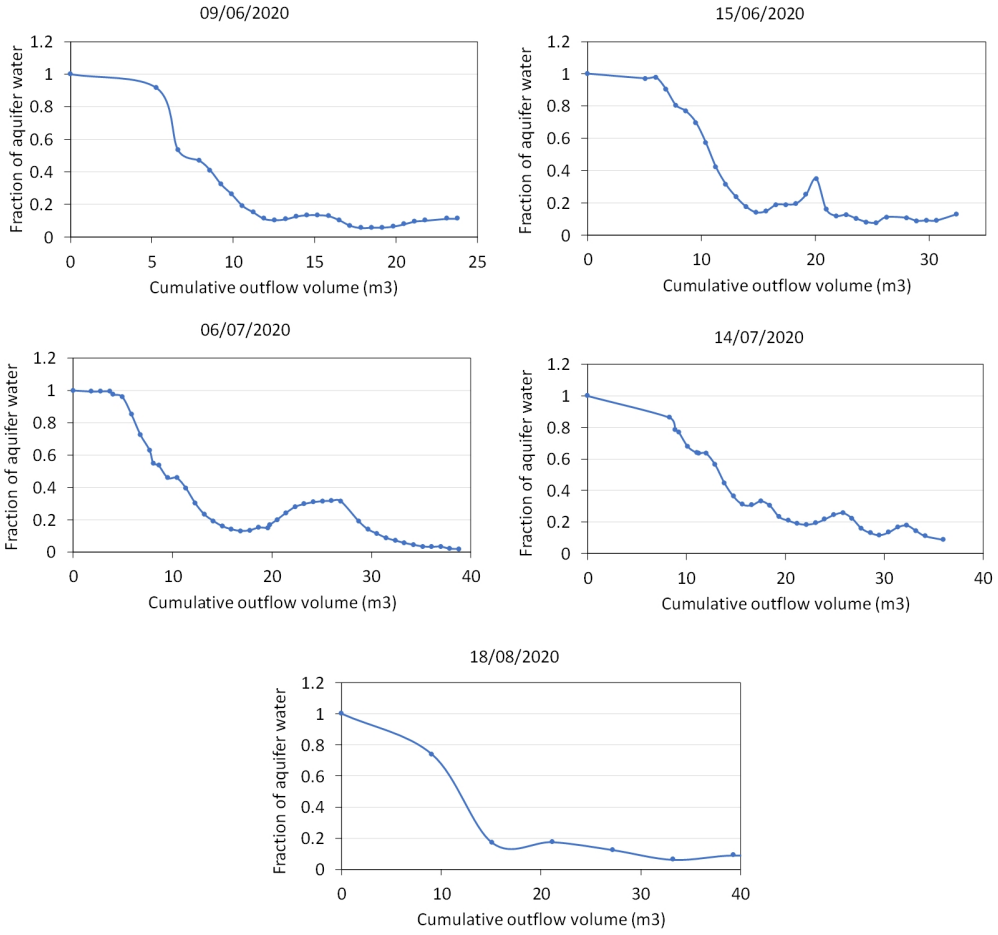


Figure C.4: Fractions of aquifer water in the outflow samples estimated from the electrical conductivity measurements. The markers indicate the fractions that were calculated from an electrical conductivity measurements.

D

Sensitivity Analysis

Adsorption and desorption rate Figure D shows the results of the sensitivity analysis performed for the plugflow and multipathway model. Adsorption and desorption rates are adjusted to the lowest and highest value stated in literature for biofilter systems [111]. For adsorption these are 0.2-5.86 h^{-1} and for desorption $6*10^{-5}$-0.48 h^{-1}. The decay rate was not changed as there was no decay during the simulated runs.

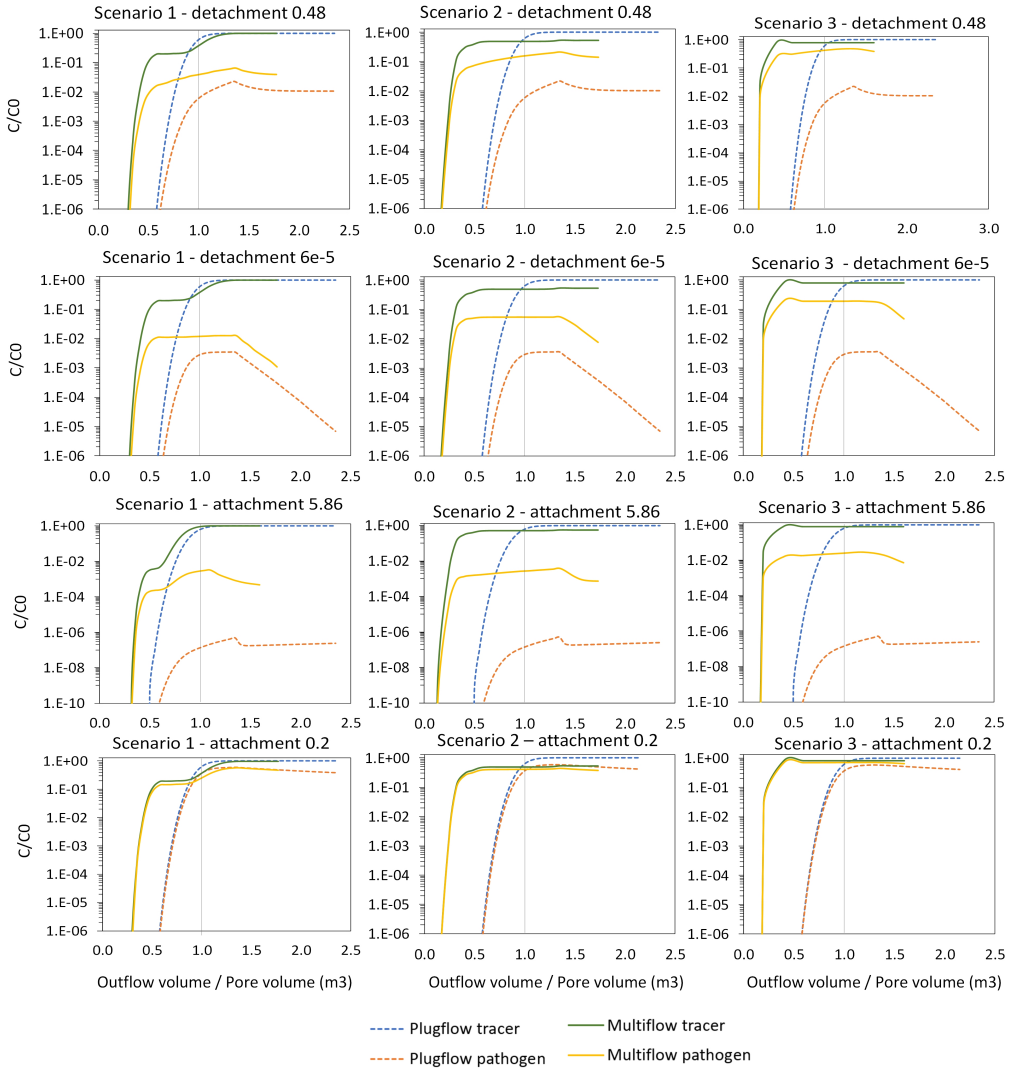


Figure D.1: Sensitivity analysis: testing effect of adsorption and desorption rates on the plug flow model and multipathway scenarios.

Event frequency and duration: model run 10°C

Figure D.2 shows the model outcomes for a temperature of 10°C.

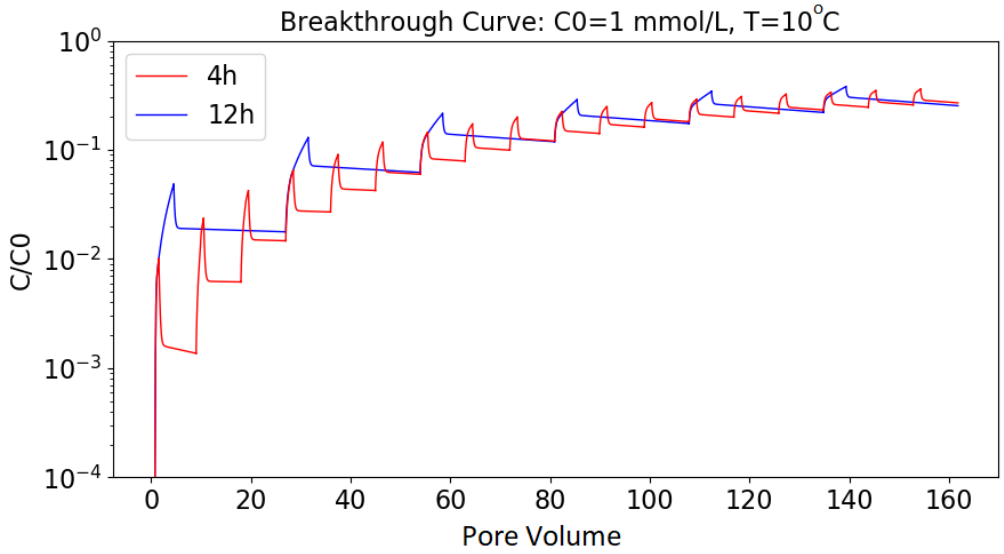


Figure D.2: Sensitivity analysis: testing effect of 10°C on event frequency and duration scenarios

E

Field Scale Biofilter Studies

Table E.2 shows inflow and outflow concentrations measured in other field-scale biofilter studies and Table E.1 shows an overview of the characteristics of field-scale biofilters researched in these studies.

Table E.1: Field-scale biofilter studies with biofilter characteristics.

Field-Scale Study	Hydraulic conductivity [mm/h]	Filter Layer Depth [mm]	Filter Layer Composition [% w/w]		Reference
RMGC	-	600	99.1% 0.6% 0.3%	Sand Silt Clay	[25]
Monash carpark	Day 0: 300 Day 500: 220	400	96% 3% 1%	Sand Silt Clay	[25, 131]
Bioretention Charlotte	-	1200	94.3% 5.7%	Sand Silt and Clay	[58]
North Carolina (D)	-	600	87% 4% 4%	Sand Silt Clay	[49]
North Carolina (S)	-	250	88% 5% 5%	Sand Silt Clay	[68]
Maryland CP	-	900	80% 13% 7%	Sand Silt Clay	[68]
Maryland SS	-	610	54% 26% 20%	Sand Silt Clay	[68]
Kfar Sava	Day 0: 140 Day 110: 30	400	71% 14% 12% 3%	Fine Sand Medium/Coarse sand Very fine sand Clay, Silt	[131]

Table E.2: Inflow and outflow *E. coli*, enterococci and *Campylobacter* concentrations measured in other field-scale biofilter studies. Concentrations are given in MPN or CFU per 100 mL.

Field-Scale Study	Bacteria source	E. coli concentration		Enterococci concentration		Campylobacter concentration		Reference
		Inflow	Outflow	Inflow	Outflow	Inflow	Outflow	
RMGC	Urban stormwater	10^2-10^5	10^1-10^4			10^{-1}-10^1	10^1 – 10^0	[25]
Monash carpark	Semi-natural stormwater	10^3-10^4	10^2-10^3			10^{-1} – 10^1	10^1 – 10^0	[25]
Bioretention Charlotte	Stormwater parking	10^0->10^3	10^0 -10^3					[58]
North Carolina (D)	Stormwater parking	10^2	10^1	10^2	10^1			[68]
North Carolina (S)	Stormwater parking	10^2	10^2	10^2	10^2			[68]
Maryland CP	Urban stormwater	10^0-10^4	10^{-1}-10^4					[68]
Maryland SS	Stormwater parking	10^0-10^4	10^{-1}-10^4					[68]
Kfar Sava	Urban stormwater	10^2-10^4	10^0-10^2					[131]

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