Influence of the schmutzdecke microbial community and varying operational conditions on the performance of biosand filters for domestic drinking water treatment

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Declaration

This work has not been previously accepted in substance for any degree and is not being concurrently submitted in candidature for any degree. This thesis is the result of my own independent work/investigation, except where otherwise stated.

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List of abbreviations

ACFs Activated carbon filters

ANOVA Analysis of variance

ASU Aquatic services unit

AWCD Average well colour development

BAC Biologically active carbon

BAF Biologically active filter

B-BSF Bench-scale biosand filter

BSF Biosand filter

CAWST Centre for Affordable Water and Sanitation Technology

CECs Contaminants of emerging concern

CFU Colony-forming unit

CLPPS Community level physiological profiles

CPBB Coniferous pinus bark biomass

DBP Disinfection by-product

DO Dissolved oxygen

DOC Dissolved organic carbon

DS Distribution system

DWTP Drinking water treatment plant

$\mathbf{d_{10}}$ Effective diameter

EC Electrical conductivity

E. coli *Escherichia coli*

EDCs Endocrine disrupting chemicals

GC Gas chromatography

HAA Haloacetic acid

HPLC High performance liquid chromatography

HWT Household water treatment

HWTS Household water treatment and safe storage

KM Kaplan-Meier

LOI Loss-on-ignition

MCPP Mecoprop

MIB 2-methylisoborneol

MLSB Membrane lauryl sulphate broth

MTBE Methyl tert-butyl ether

NOM Natural organic matter

OD Optical density

PBS Phosphate buffer saline

PCA Principal component analysis

PCR Polymerase chain reaction

POU Point-of-use

PVC Polyvinyl chloride

RCTs Randomized control trials

rpm Revolutions per minute

$\mathbf{r_s}$ Spearman correlation coefficient

RSF Rapid sand filter

RT-PCR Reverse transcription polymerase chain reaction

RWD Resting water depth

SDGs Sustainable Development Goals

SEM Scanning electron microscope

SODIS Solar disinfection

SSF Slow sand filter

SUVA Specific ultra violet absorbency

THM Trihalomethane

THMFP Trihalomethane formation potential

TON Total oxidised nitrogen

TPs Transformation products

TSS Total suspend solids

TTC Thermotolerant coliforms

UC Uniformity coefficient

UNICEF United Nations International Children's Emergency Fund

UV_{254} Spectral absorption coefficient (Ultraviolet absorbency at 254 nm)

VBNC Viable but non-cultivable

WHO World Health Organization

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This PhD has taught me many things but, more than anything, it has taught me to be tenacious, be curious, never stop questioning, build on the knowledge of others and most importantly, believe in myself.

“*In time and with water, everything changes*”

- Leonardo da Vinci

Abstract

Household water treatment technologies offer an interim solution for people who remain without access to a safe drinking water source. Biosand filters (BSFs) are among the most sustainable point-of-use (POU) water treatment techniques in terms of treatment efficiency and user satisfaction. However, the lack of knowledge pertaining to the impact of operating conditions on filter performance and the microbial community structure of the schmutzdecke is potentially limiting the optimisation of BSFs.

This thesis provides insight into the impact of varying operating conditions including filter media characteristics, resting water depth and ambient temperature on the performance of BSFs. In addition it provides the first insight into changes in the schmutzdecke bacterial community structure during the maturation of BSFs.

This thesis has shown that deviations from the recommended sand grain sizes was not detrimental to filter performance, indicating that there is potential to ease the current recommendation for a maximum grain size of ≤ 0.7 mm. It was also found that variations in the resting water depths of between 2 and 11 cm did not negatively impact the removal of microbial indicator organisms or the metabolic activity and functional diversity of the schmutzdecke. However, operating temperature was found to impact the performance of BSFs, with filters operated at a lower temperature (10 ± 1 ˚C) having a higher *E. coli* removal when compared with filters operated at 26 ± 1˚C. This suggests that householders may experience variations in filter performance due to seasonal temperature changes. A further interesting observation in this thesis was that the rate of bacterial family colonization and succession differed in three, full-scale BSFs. However as the filters aged the same bacterial families eventually dominated the schmutzdecke in all three filters. It was also found that bacterial family dominance in the feed water was not a driver for the subsequent dominance of the family (*Planctomycetaceae* and *Acidobacteria)* in the schmutzdecke.

The findings of this thesis, along with further studies on the effect of operating conditions on filter performance and schmutzdecke development, will enable the design and operation of BSFs to be optimised.

Chapter 1

Sand filter technologies for drinking water treatment

1.1 Abstract

Access to safe drinking water is a basic necessity for human life. However, as the world population continues to grow, the demand on the world's water supply increases, but the quality of the supplies is deteriorating. The deterioration in source water quality has led to a renewed interest in biologically active filters (BAFs) to treat drinking water. Conventional drinking water treatment processes have been found ineffective in removing a new group of contaminants which are currently unregulated and referred to as "emerging", "contaminants of emerging concern", "new" or "micropollutants". This review examines the three different gravity-fed sand filter systems (slow sand filters (SSFs), rapid sand filters (RSFs) and biosand filters (BSFs)) and their effectiveness in treating drinking water, including water containing micropollutants. The current literature suggests that biological and mechanical processes that occur within sand filters offer an effective method to treat water contaminated with an array of contaminants. Nevertheless there is still a need for further research in particular areas.

1.2 Introduction

The provision of drinking water in adequate amounts, and of acceptable quality, is a basic necessity for human life; however securing long term supplies of drinking water of acceptable quality remains a major global challenge (Reid *et al*., 2003). Benner *et al*. (2013) highlight some of the challenges facing the world's drinking water resource as the world population continues to grow. A key issue is that the quantity of available drinking water is decreasing due to over withdrawal for human and agricultural activities. The quality of available supplies is also deteriorating due to the continued input of nutrients and anthropogenic chemicals from point (e.g. wastewater discharges) and diffuse (e.g. agricultural runoff) sources.

The major proportion of drinking water consumed globally is from ground water, comprising 40 to 50% of municipal water use in the USA (Bouwer and Crowe, 1988) and 70% in Europe (Navarrete *et al*., 2008). Such supplies undergo minimum treatment prior to human consumption. When surface water is used as a source of drinking water, it often requires extensive treatment to meet drinking water guidelines due to eutrophication and the direct discharges of pollutants to such water supplies. Water treatment is not a new concept as it dates back over 6000 years when the Greeks used a number of techniques to improve aesthetic quality of drinking water including charcoal filters, boiling, straining and exposure to sunlight (WHO, 2003). In the 21st century conventional drinking water treatment generally employs physicochemical processes which aim to eliminate pathogenic organisms, reduce turbidity and control the organoleptic properties (Benner *et al*., 2013; Bouwer and Crowe, 1988). The typical stages in conventional treatment are screening, coagulation, flocculation, sedimentation, rapid sand filtration and disinfection (Kameya *et al*., 1997; Sakoda *et al*., 1996). Some treatment systems may also require a pH adjustment stage prior to the disinfection stage, and some countries also have a fluoridation step, including Ireland.

The drinking water treatment industry faces an increasing challenge in treating drinking water in a sustainable and cost effective manner because water supplies are becoming increasingly contaminated with an array of potentially toxic organic and inorganic compounds, and with pathogens such as *Cryptosporidium* and viruses (Schwarzenbach *et al*., 2006; Bouwer and Crowe, 1988). Recent studies have

indicated the potential of biological treatment to aid with some of the treatment challenges facing the water treatment industry. The use of biological treatment in drinking water is not new, but dates back to 1804 when John Gibb designed and built the first slow sand filter (SSF) in Paisley, Scotland and sold the surplus treated water to the public (Baker and Taras, 1948). However a renewed interest in biological treatment has occurred in recent times with biological treatment offering a number of advantages over the conventional treatment system. Advantages associated with biological treatment include (i) reducing the cost to treat a litre of water because biological treatment has a lower chemical requirement and (ii) a higher efficiency for removal of natural organic matter and inorganic compounds, resulting in lower regrowth potential in the distribution system (DS) and decreased chlorine demand (Camper *et al*., 2003; LeChevallier *et al*., 1992; Rittmann and Vernon, 1984). SSFs are widely used in Europe and Asia, however the United States of America have been slow to accept biological treatment, as water authorities strife to impair biological activity in the treatment by pre-chlorination, scouring and frequent backwashing of filter media (Bouwer and Crowe, 1988). Since the development of the original SSF, a number of other sand filter systems have been developed, including rapid sand filters (RSFs) and, more recently domestic-scale slow sand filters referred to as biosand filters (BSFs). This review summarizes the sand filter technologies adopted in drinking water treatment, along with the appropriateness of such technologies in treating drinking water supplies.

1.3 Biological treatment

All biological treatment processes adopted in drinking water treatment rely on the activity of biofilms (Bouwer and Crowe, 1988), where biologically active microorganisms are retained within the biological system and are typically attached to a granular media. There are a number of different biological treatment methods adopted in drinking water treatment plants (DWTPs) including SSFs, RSFs and activated carbon filters (ACFs). While the operating cycle of biological treatment methods are similar (Figure 1.1), the duration of each stage of the cycle varies. For example, in SSFs, filter maintenance only takes place when filtration rate falls below the recommended filtration rate of between 0.1 to 0.4 $m^3/m^2/hr$ (Huisman and Wood, 1974), whereas in RSFs, backwashing is completed at set time intervals, regardless of the rate of head loss development. The following section briefly describes the

basic characteristics of different sand filter technologies. All the filters described in the following sections are gravity fed systems.

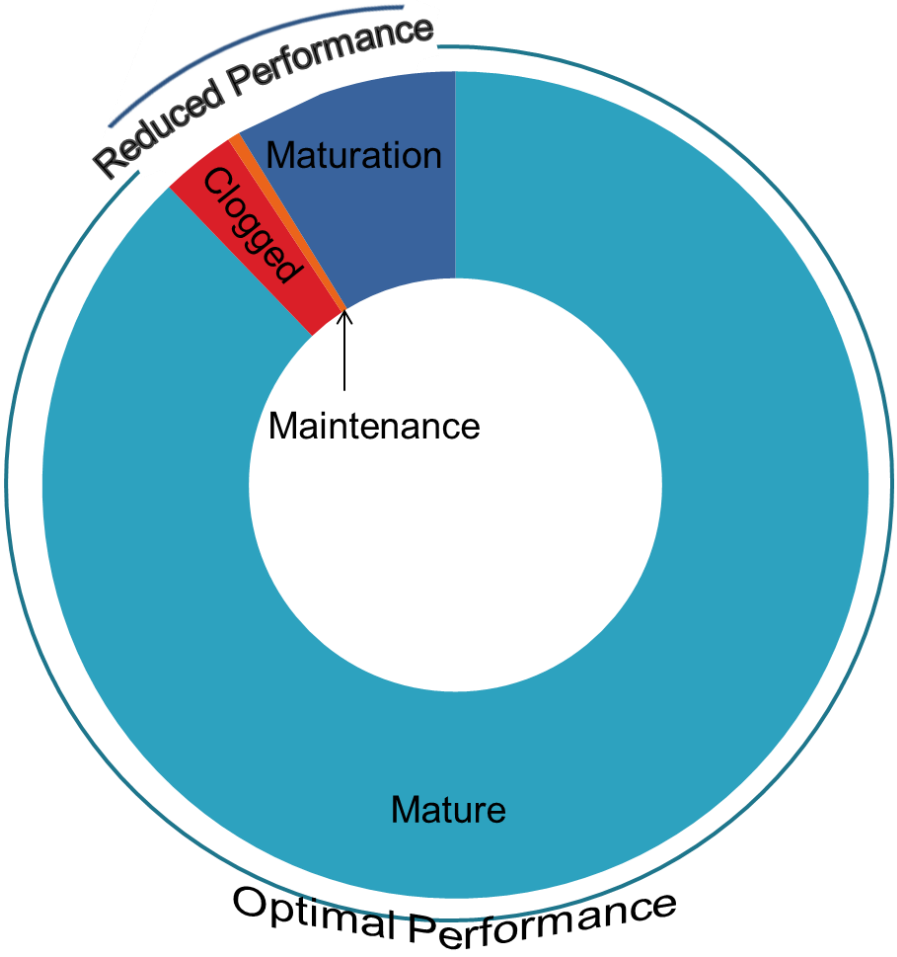

Figure 1.1: Schematic of the operational cycle of biologically active filters (Adapted from Haig (2014)).

1.3.1 Slow sand filtration

Slow sand filters (SSFs) date back to 1804 when John Gibb designed and built an experimental slow sand filter (Baker and Taras, 1948). SSFs were further developed by Robert Thorn, and subsequently James Simpson implemented the first public supply at the Chelsea Water Company, London in 1829 (Baker and Taras, 1948). Fine sand (effective diameter 0.15 – 0.30 mm and uniformity coefficient 1.5 – 3.0) is used in SSFs, allowing for a filtration rate of between 0.1 and 0.4 $m^3/m^2/hr$ (Huisman and Wood, 1974). As feed water percolates through the sand, suspended solids and colloid particles are deposited at the top of the sand column. Deposition of suspended solids and the development of the schmutzdecke, a biological community

at the sand water interface (Bouwer and Crowe, 1988) (see section 1.4.2), results in filter clogging and the need for filter maintenance, which involves scraping the top 1 – 2 cm of the sand column. This may be required at weekly or month intervals to restore filtration rates to a satisfactory level (Huisman and Wood, 1974). SSFs are effective at improving the physical, chemical and bacteriological quality of the treated water, but also the low cost, ease of construction and the ease of operation adds to the advantages associated with the technology (Huisman and Wood, 1974). In addition unlike other water treatment methods SSFs have a low energy requirement (Haig *et al*., 2011).

Slow sand filters combining multiple treatment mechanisms including physical, chemical and biological within a single system (WeberShirk and Dick, 1997). Extensive research has been undertaken to understand the mechanical processes (Huisman and Wood, 1974; Bellamy et al., 1985; WeberShirk and Dick, 1997), but the biological processes remain poorly understood, which is contributing to SSFs remaining somewhat of a "black box" system. Recent advances in molecular techniques have allowed for studies to assess the microbial ecology of full-scale and laboratory-scale SSFs (Haig *et al*., 2014) and also to gain insight into the mechanisms contributing to pathogen removal (Haig *et al*., 2015).

1.3.2 Rapid sand filtration

Rapid sand filters (RSFs) gained popularity in the early 20th century over the SSFs, due to their smaller footprint and, when combined with chemical coagulation, their greater tolerance to changes in the water quality of the influent supply to the filter (Haig *et al*., 2011). It is the difference in the properties of the medium that distinguishes RSFs and SSFs: the filter medium in a RSF is typically coarser with an effective grain size (d_{10}) of 0.6 – 2.0 mm. With the larger grain size medium, the pore spaces are larger, which reduces the resistance of the downward percolation of water through the medium, resulting in higher filtration rates compared to SSF, typically 5 – 15 $m^3/m^2/hr$ (Huisman and Wood, 1974). Generally, water in RSFs has a residence time of 7.5 to 12 mins, although this is dependent on filter loading rate. With larger pore spaces and the associated increased flow rate, contaminants are deposited at deeper depths within the medium. As a result backwashing is required at

regular intervals to restore both filtration rates and the quality of the treated water (Huisman and Wood, 1974).

Rapid sand filtration as a sole treatment method would provide little effective microbial purification and chemical improvement of the treated water; even the physical straining action is reduced in RSFs due to the large pore spaces which result in high filtration rates. Thereby, RSFs are typically used as one of final stages of treatment having been preceded by chemical treatment, flocculation and sedimentation which remove the majority of the contaminants in the feed water before it reaches the RSF. The remaining contaminants that reach the RSF are easily removed in their coagulated state (Huisman and Wood, 1974).

Although schmutzdecke development occurs in RSFs and is believed to contribute to filter performance and a degree of bacterial purification, the overall contribution of the biofilm to the treatment efficiency of the filter is believed to be low because of the frequency of backwashing (Huisman and Wood, 1974; Bouwer and Crowe, 1988).

1.3.3 Biosand filtration

The biosand filter was developed by David Manz at the University of Calgary in the late 1980s, and now more than 650,000 BSFs are used by more than 4 million people globally (Ngai *et al*., 2014). The most significant difference between a BSF and a SSF is that a BSF is charged intermittently rather than continuously, typically once daily (Elliott *et al*., 2011). In BSFs water is poured into the top of the filter and passed through the diffuser plate before blending with the resting water above the filter media. Two unique features of the BSF are the diffuser plate and the elevated outlet pipe. The diffuser plate is a plastic or metal sheet with 2 mm diameter holes which promotes uniform flow of the feed water over the sand column, avoiding any disturbance to the filter media (Elliott *et al*., 2008). The outlet tube of a BSF rises up the external wall of the filter to a height about the filter media of 5 cm, as recommended by the Centre for Affordable Water and Sanitation (CAWST) (CAWST, 2012).

A key advantage of the intermittent operating of BSFs is that it allows for the technology to be scaled down for domestic use (Buzunis, 1995; Sobsey *et al*., 2008).

However, earlier studies had discouraged the intermittent operation of commercial-scale SSFs due to the development of anoxic conditions in the schmutzdecke (Buzunis, 1995). In the design of BSFs it was hypothesised that reducing the level of water over the filter media (resting water depth) during the filter resting period would maintain aerobic conditions in the schmutzdecke (Buzunis, 1995). The current guideline recommends a resting water depth (RWD) of 5 cm but not greater than 6 cm (CAWST, 2012).

Similar to other biological treatment technologies, a schmutzdecke develops at the sand water interface in BSFs. The schmutzdecke is a biological community that develops in biologically active filters (BAFs) at the sand water interface and is also often referred to as the biolayer or biofilm (Bouwer and Crowe, 1988). The most efficient removal of microbial indicator organisms has been reported with an intact schmutzdecke (Earwaker, 2006; Elliott *et al*., 2008; Stauber *et al*., 2006). However, there is no direct evidence that the biological community in the schmutzdecke is solely responsible for bacterial removal and other factors, including physical straining, may be involved (Elliott *et al*., 2011; Elliott *et al*., 2015). BSFs have been identified as a highly effective technology when measured in terms of filter performance, user satisfaction and sustained rates of use (Aiken *et al*., 2011; Duke *et al*., 2006; Fiore *et al*., 2010; Liang *et al*., 2010; Stauber *et al*., 2009; Tiwari *et al*., 2009).

1.4 Influential factors on sand filter performance

There are three components to the sand filter technology which are important in determining filter performance: the supernatant water reservoir, the schmutzdecke and the filter media.

1.4.1 Supernatant water reservoir

The supernatant is the water above the filter media. The supernatant provides a pressure head to overcome the resistance of the filter media bed, allowing for water to flow through the filter media. The supernatant also allows for some pre-treatment of the feed water to occur prior to the water flowing through the filter, including particle settlement, particle agglomeration and oxidation (Hendricks, 1991). The difference in design and operation of SSFs and RSFs compared to BSFs is that the

supernatant depth is maintained in SSFs and RSFs. To allow for its intermittent operation, a BSF has a resting water depth of 5 cm and, during filter charging, the water level above the filter media increases to a maximum of 21 cm. As water percolates through the BSF the level of water above the filter media returns to 5 cm until the next charge event (CAWST, 2012).

1.4.2 Schmutzdecke

The colonization and succession of the microbial community in the schmutzdecke is poorly understood in sand filters. However, recently Haig *et al*. (2014) provided the first insight into the microbial ecology of full-scale SSFs and found that the schmutzdecke microbial community structure of the laboratory-scale filters replicated that of full-scale filters with filter age. A study of the microbial community in RSFs and BSFs has not been completed to date and such a study may identify different microbial community structures resulting from different operating conditions between the different filter systems (SSF, RSF and BSF).

The schmutzdecke is credited with the majority of *E. coli* removal in SSFs, with studies having reported greater than 1 – 2 log reduction of *E. coli* in filters with a schmutzdecke, compared with filters without (Bellamy *et al*., 1985; Hijnen *et al*., 2004). However, it remains to be explored whether the schmutzdecke contributes to *E. coli* removal by mechanical or biological mechanisms. The schmutzdecke is not believed to contribute to virus removal, but rather the removal of viruses occurs within the filter media (Hijnen *et al*., 2004).

In BAFs, maintenance depends on filter type but the main two methods include filter backwashing (which is used in RSFs), and filter scraping (typically performed for SSF). Reducing the biomass of the schmutzdecke increases the filtration rate of BAFs, but the removal of excessive amounts of biomass results in the loss of microbial activity, thereby reducing the treatment efficiency of the filter (Urfer, 1998). Simpson (2008) provided a detailed review of the approaches to optimizing biofilm activity and control of biofilm thickness. The review discussed the various methods that can be adopted to control microbial biofilm growth on BAF media, including control influent nutrient loading, dissolved oxygen and pH, together with other methods adopted including use of chemical (oxidative) disinfection and the more commonly used method of backwashing (Simpson, 2008).

1.4.3 Filter media

The filter media used in sand filter systems is a fundamental component of the technology and it is the differences in sand size which differentiate the sand filter systems. In both SSFs and BSFs a fine sand is used: Huisman and Wood (1974) recommended an effective diameter (d_{10}) of between 0.15 – 0.30 mm and uniformity coefficient (UC) (d_{60}/d_{10}) 1.5 – 3.0 for SSFs. For BSFs, Lukacs (2002) recommended a d_{10} range of 0.15 – 0.4 mm and a UC of 1.5 – 3.0 whereas Manz *et al*. (1993) recommended UC < 5. Elliott *et al*. (2008) recommended a narrower d_{10} range of 0.19 – 0.22 mm and a higher UC of 3.5 – 4.0 for BSFs. In comparison, the sand used in RSF is coarser with recommended d_{10} of 0.6 – 2.0 mm (Huisman and Wood, 1974), which allows for the higher filtration rate that occurs in RSFs.

1.5 Performance of sand filters

Sand filter systems combine both biological and mechanical processes within single stage treatment units and have been shown to be highly effective for drinking water treatment. Slow sand filters have higher removal efficiency for pathogenic microorganisms compared with RSFs, with the reported removal of *Cryptosporidium* of > 99.9% (Hijnen *et al*., 2007) and 99 to 99.99% of Gardia cysts (Bellamy *et al*., 1985). Given the similarity in filter media characteristics between in SSFs and BSFs, it is not surprising that studies have found similar removal of enteric bacteria with removals to range from to 90 to 99.9% in SSFs (Hijnen *et al*., 2007) and from 96 to 99% in BSFs (Duke *et al*., 2006; Mahmood *et al*., 2011; Vanderzwaag *et al*., 2009).

The removal of ammonium from drinking water is of significant importance because ammonium can cause nitrification to occur in the distribution network which can lead to a number of issues with the water supply, including decreases in pH resulting in the supply becoming corrosive, aesthetic problems (colour and taste) and biological instability (Rittmann *et al*., 2012). Although nitrification in BAFs is used to remove ammonium in drinking water, the process can experience problems associated with temperature (Andersson *et al*., 2001; Kors *et al*., 1998; Van der Aa *et al*., 2002), insufficient oxygen availability (Lytle *et al*., 2013) and nutrient limitation (phosphate) (de Vet *et al*., 2012). Hence, the performance of sand filter systems in removing contaminants is affected by variations in operating conditions (temperature) and feed water characteristics.

1.6 Challenges relating to drinking water treatment

Today, a new group of contaminants (so-called "emerging", "new", "contaminants of emerging concern (CECs)" or "micropollutants") pose a threat to human health and some of these contaminants are unregulated. This new group of contaminants comprise products used in everyday life such as endocrine disrupting compounds (EDCs), pharmaceuticals, veterinary growth stimulators and personal care products (Caliman and Gavrilescu, 2009; Benotti *et al*., 2008; Kolpin *et al*., 2002). As most emerging contaminants are from human use, their emission is an issue for wastewater treatment and determining the fate of CECs in wastewater treatment plants is important (La Farre *et al*., 2008). Once released into the environment CECs are subject to degradation (e.g. biodegradation, chemical degradation and photochemical degradation) which contributes to their elimination. However, different transformation of CECs can occur depending on environmental position (groundwater, surface water and sediments) or whether the compounds are present in wastewater treatments plants or DWTPs, resulting in the formation of transformation products (TPs) that differ in their environmental behaviour and ecotoxicological profile (Boxall *et al*., 2004).

Determining the fate of emerging contaminants in the environment is further complicated by their low environmental concentrations (sub-µg/l) therefore, preconcentration prior to analysis is required most often by solid phase extraction for water samples (Batt *et al*., 2008; Gracia-Lor *et al*., 2011; Gros *et al*., 2012; López-Serna *et al*., 2011; Petrović *et al*., 2003). Analyitical techniques including high performance liquid chromatography (HPLC) (Batt *et al*., 2008; Gracia-Lor *et al*., 2011; Gros *et al*., 2012; Bayen *et al*., 2014; Berset *et al*., 2010; López-Serna *et al*., 2011; Yu *et al*., 2012) and gas chromatography (GC) (Steven *et al*., 2002) have been used as the primary separation techniques in the analysis of complex mixtures of CECs. Mass spectrometry is most commonly used as the detection systems (Batt *et al*., 2008; Bayen *et al*., 2014; Berset *et al*., 2010; Gracia-Lor *et al*., 2011; Gros *et al*., 2012; López-Serna *et al*., 2011; Yu *et al*., 2012; Steven *et al*., 2002).

During the last decade, CECs have been reported in the influent and effluent of wastewater treatment plants (Batt *et al*., 2008; Glassmeyer *et al*., 2005; Vanderford and Snyder, 2006), surface waters (Bartelt-Hunt *et al*., 2009; Conley *et al*., 2008;

Ferrell and Grimes, 2014; Tabe *et al*., 2009) and, of greater concern, in drinking water treatment and distribution systems (Benotti *et al*., 2008; Focazio *et al*., 2008; Huerta-Fontela *et al*., 2011). Although conventional drinking water treatment methods can reduce the concentration of micropollutants, the removal of such micropollutants is often incomplete (Benner *et al*., 2013). More advanced treatment technologies, such as advanced oxidation, microfiltration, ultrafiltration, nanofiltration, reverse osmosis and activated carbon filtration have all been explored as treatment methods for the removal of pathogens, disinfection by-products, inorganic and synthetic organic chemicals (Jacangelo *et al*., 1997; Van Der Bruggen *et al*., 2003; Ikehata *et al*., 2008). As the cost benefit of such advanced technologies remains unclear there is a renewed interested in role of biological active filters (BAFs) for drinking water treatment.

1.6.1 Micropollutants

The conventional drinking water treatment process was not designed to remove micropollutants (Westerhoff *et al*., 2005; Snyder, 2007) and it is therefore possible that consumers may be exposed to CECs through drinking water abstraction from contaminated water sources (Houtman, 2010).

Therefore the renewed interest in BAFs has occurred as recent laboratory studies have shown that autochthonous bacterial communities in SSFs are capable of transforming various pesticides and wastewater-derived micropollutants (Meffe *et al*., 2010; Richter *et al*., 2008; Zearley and Summers, 2012; Zuehlke *et al*., 2007). Zearley and Summers (2012) assessed the fate of 34 micropollutants in SSFs (including pesticides, pharmaceuticals, personal care products and some EDCs and reported a wide range of removal performances for different micropollutants ranging from no measurable removal for 13 compounds (e.g. carbamazepine) to removal below the detection limit (e.g. ibuprofen and triclosan)) Other studies in full-scale DWTPs have shown the effectiveness of sand filters in removing compounds, including methyl tert-butyl ether (MTBE) (Arvin *et al*., 2005) and 2-methylisoborneol (MIB) and geosmin (Ho *et al*., 2007). It has also been indicated that enhanced removal of micropollutants can occurred in SSFs with enhanced contact times achieved by reducing the hydraulic velocities (Zearley and Summers, 2012; Zuehlke *et al*., 2007). Rapid sand filters have also been shown to be effective

at removing micropollutants, with Hedegaard *et al*. (2014) reporting removal of the herbicide mecoprop (MCPP) with a concentration of 0.08 µg/L in the feed water to a concentration below the detection limit when the feed water was aerated followed by primary and secondary rapid sand filtration. Although recent studies have demonstrated the effectiveness of sand filters in removing micropollutants, they have focused on changes in the concentration of the primary micropollutants from the feed water to the filtered water. However, the concentrations of the persistent TPs formed as result of the biotransformation process have been ignored and the biological mechanisms involved remain unidentified (Benner *et al*., 2013). Recent studies suggest that sand filters can remove micropollutants, based on observations of changes in the parent compound concentration in the filtrate (Arvin *et al*., 2005; Ho *et al*., 2007). However, significant research questions still remain regarding the concentrations of persistent TPs and potential adaptation to the operation of sand filters to enhance the removal of micropollutants.

1.6.2 Disinfection and disinfection by-products

Chemical disinfection is widely used in drinking water treatment, typically involving the use of chlorine in order to inactivate microorganisms. While chlorine is a highly effective disinfectant it reacts with natural organic matter (NOM) and bromide ions resulting in the formation of disinfection by-products which have significant implications as human mutagens, carcinogens and teratogens (Hamidin *et al*., 2008). Disinfection by-products (DBPs) are a group of over 600 different species, the most common include trihalomethanes (THMs), haloacetic acids (HAAs) and nitrosamines (Richardson *et al*., 2007). As DBPs are emerging as a major challenge in commercial drinking water treatment, a renewed interest in BAFs has occurred because the microorganisms that colonize the filter media in BAFs use the natural organic matter (NOM) as a substrate, thereby reducing the formation of DBPs but also reducing the microbial regrowth potential in the distribution system (Langlais *et al*., 1991).

Little information is available that directly shows the capacity of SSFs to remove THM precursors including NOM and humic substances (Collins *et al*., 1992). However, Fox *et al*. (1984) indicated the potential that SSFs offer in reducing the organic loading by reporting 15 – 19% reductions of TOC and THM formation

potential (THMFP). Further studies are needed to determine the entire effectiveness of sand filters in reducing the organic loading in drinking water and hence reducing the risk of DBP formation.

1.7 Bioaugmentation

Bioaugmentation of sand filters using single strains or microbial consortia that have the capacity to degrade or mineralise target micropollutants may offer the potential to enhance the removal and mineralization of micropollutants (Benner *et al*., 2013). Although results are encouraging, bioaugmentation has only been explored in a limited capacity for the purpose of drinking water treatment. For example, McDowall *et al*. (2009) found a higher reduction (75%) of geosmin (associated with adverse taste and odour) following the augmentation of SSFs with a consortium of geosmin-degrading bacteria previously isolated from the schmutzdecke of a sand filter column in the Hoefel *et al*. (2006) study, when compared with the non-inoculated filters where the geosmin removal was as low as 25%. Similarly, Haig *et al*. (2016) also found that the augmentation of SSFs with estrogen-degrading bacteria resulted in significantly higher removals of estrone and estradiol compared with non-augmented filters. However, the adoption of bioaugmentation to enhance filter performance relies first of all on the isolation and identification of individual strains or microbial consortia with the metabolic capacity to biodegrade or bio-transform the target micropollutants (Benner *et al*., 2013).

1.8 Summary

The main role of DWTPs is to remove pathogenic microorganisms, reduce turbidity, reduce the nutrient loading and improve the sensory properties of the final drinking water. However, a renewed interest in how drinking water is treated has emerged, primarily due to changes in the quality of the water being treated for drinking water purposes. Such an emerging issue is the concentration of emerging contaminants which may have adverse health effects, because conventional treatment process is ineffective in the removal of such contaminants. Recent research has indicated the potential of sand filtration in tackling some of the major challenges in drinking water treatment, such as pollutants in the emerging contaminant category but, there is the need for further research to support the effectiveness of sand filtration in degrading emerging contaminants.

1.9 Thesis statement

In order to improve the operation and performance of BSFs, a greater understanding of the impact of different operational conditions and the schmutzdecke microbial community structure on filter performance is required. Specifically this thesis will address the following questions:

1. Does sand properties including grain size distribution, shape, roughness and inorgranic composition impact schmutzdecke development and filter performance?
2. What is the impact of resting water depth on the schmutzdecke and filter performance?
3. Does operating temperature affect the microbial community and filter performance?
4. What bacterial families are present in the feed water, schmutzdecke and filter water for full-scale Hydraid® filters?
5. How does the schmutzdecke community change with filter maturation?

1.10 Thesis outline

This thesis is structured as follows:

Chapter 2 presents a detailed literature review, summarising the various aspects of BSFs including the fundamental theory, design, operation, performance and sustainability of the technology.

Chapter 3 uses full-scale Hydraid® filters to examine the effect of sand characteristics including grain size and chemical composition on BSF performance and development of the schmutzdecke microbial community; from an operation perspective, this is to determine if there are differences in filter performance asiring from the sourcing of sand locally.

Chapter 4 presents the use of bench-scale BSFs to examine the impact of different resting water depths on filter performance and the development of the schmutdecke.

Chapter 5 uses the bench-scale filters as used in chapter 4 to explore the effect of different ambient temperatures on filter performance and schmutzdecke development. Furthermore, this chapter explores the retention of *E. coli* over severals days after its intial introduction by spiking the feed water with an antibiotic resistant (kanamycin) *E. coli* (pGFPGUSPlus was gifted from Claudia Vickers (Addgene plasmid # 64401)).

Chapter 6 presents molecular (next generating sequencing (MiSeq)) and water quality analysis for three replicate, full-scale, Hydraid® filters which were sampled periodically for six months. The analysis provides the first insight into the schmutzdecke microbial community structure in BSF.

Chapter 7 provides a sythesis of the contribitions and finds of this thesis and explores avenues for future work.

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Chapter 2

Are biosand filters an effective point-of-use technology for developing nations or is their distribution premature?

2.1 Abstract

Access to safe drinking water is a significant global challenge with a sizable portion of the world's population remaining without access to safe drinking water source. The majority of people without access to safe drinking water live in developing countries in rural isolated locations where reality of a centralized, piped, drinking water treatment system will not be achievable for many people for decades to come. While communities wait for the development of centralized pipe water supplies, point-of-use water treatment technologies offer a real intern solution for people to treat water domestically. Biosand filters, which are domestic scale, intermittently operated slow sand filters, have been identified amongst the most effect point-of-use water treatment technology when measured in terms of user satisfaction, sustained rates of use and reduction of diarrheal disease. This paper reviews the challenges and advantages of using biosand filters, and evaluates whether the distribution of biosand filters is premature. Further research needs are also identified.

2.2 Introduction

Access to an improved drinking water source remains a major global challenge with 784 million people globally continuing to consume drinking water from unimproved sources, of which 173 million people rely on the direct use of surface water (WHO and UNICEF, 2014). The majority of those using unsafe drinking water supplies are living in developing nations including sub-Saharan Africa, South and East Asia (Dungumaro, 2007; UNICEF, 2009). Target 6.1 of the recently agreed Sustainable Development Goals (SDGs) addresses the lack of access to safe drinking water and sanitation and aims to '*achieve universal and equitable access to safe and affordable drinking water for all'* by 2030 (UN, 2015).

Exposure to waterborne pathogens can occur when using contaminated water for drinking or cooking, washing or bathing, and even exposure to aerosols could potentially result in infection (Gadgil, 1998). Hence, the consumption of untreated drinking water has the potential to cause illness due to the presence of viruses, bacteria or protozoa. Illness is not severe in most cases, but it has sometimes been the source of major epidemics (Arnal *et al*., 2001).

Reliance on expensive, resource-intensive, centralised water treatment systems will leave people in rural, isolated locations in developing nations without safe potable water far into the future (Mintz *et al*., 2001). It is therefore widely accepted that household water treatment and safe storage (HWTS) practices, which include boiling, filtration and chlorination, are necessary for developing nations and regions devastated by natural disasters (Sobsey, 2002). Studies completed by Clasen *et al*. (2006a) and Fewtrell *et al*. (2005) have shown that household water treatment (HWT) techniques, often referred to as point-of-use (POU) treatment, can reduce the average risk of diarrheal disease by 35%. Single interventions, such as POU treatment systems, have been shown to be as effective as multiple interventions that include water, sanitation and hygiene measures (Fewtrell *et al*., 2005). A number of different POU water treatment technologies are available including liquid or tablet chlorination, solar disinfection (SODIS), ceramic filters and biosand filters (BSFs) (Clasen, 2009). Many HWT require consumables (chlorine) or replacement components (e.g. ceramic filters), which necessitate the need for a supply chain which is critical to the sustainability of some HWT technologies (Ojomo *et al*.,

2015). Other domestic treatment methods such as solar disinfection, chlorination and boiling are only effective for microorganism removal, but the efficiency of such technologies are diminished in high turbidity water (Ahammed and Davra, 2011).

A review completed by Sobsey *et al*. (2008) identified BSFs, also known as Manz filters: as the most sustainable treatment technique in terms of removal efficiency of turbidity and microbial pathogens. However, some studies suggest that research conducted on POU technologies such as BSFs are biased and lack hard scientific evidence, and that the current global distribution of such technologies is premature (Schmidt and Cairncross, 2009; Hunter, 2009). Vanderzwaag *et al*. (2009) highlighted that a BSF may not be a suitable technology if an appropriate implementation strategy is not adopted because it could affect the receptiveness of the population to future aid. However, a more recent study by Kennedy *et al*. (2013) supports the use of a BSF paired with a disinfection method (solar disinfection, chlorination) and Divelbiss *et al*. (2013) also recognized that no single factor or invention in isolation can improve the health of a population; rather a combination of factors is needed to improve health. Nevertheless, Divelbiss *et al*. (2013) still indicated that a BSF deserves to be recognized as an effective tool in reducing the outbreaks of diarrhoeal disease in developing nations. It is estimated that since the development of the BSF in the late 1980s by David Manz more than 650,000 BSFs are now in use by more than 4 million people globally (Ngai *et al*., 2014).

In the last decade reviews (Loo *et al*., 2012; Sobsey *et al*., 2008; Lantagne and Clasen, 2009; Peter-Varbanets *et al*., 2009) have discussed the role of HWT technologies in improving drinking water quality in the absence of an improved drinking water source and as part of an emergency response. This paper focuses on assessing challenges faced by BSF users in the operation of the filter, while also providing an assessment of user satisfaction and sustained rates of BSF use from field studies. Moreover, the relevance of modifying the filter media to overcome some of challenges relating to BSFs is discussed. This paper will further evaluate whether the current state of research supports the distribution of BSFs, as a viable domestic water treatment method to reduce the disease burden associated with the consumption of unsafe drinking water supplies.

2.3 Filter operation

The most significant difference between a BSF and a slow sand filter (SSF) is that a BSF is charged intermittently rather than continuously, typically once daily (Elliott *et al*., 2011). In BSFs, water is poured into the top of the filter and passes through a diffuser plate before entering the resting water above the filter media. The diffuser plate is a unique feature of the BSF design, which avoids schmutzdecke disturbance during filter charging by promoting uniform flow over the sand surface. The diffuser plate is a plastic or metal sheet with 2 mm diameter holes (Elliott *et al*., 2008). Water percolates through the sand column due to gravitational force. Similar to a conventional SSF, a BSF combines multiple functions within a single unit including settlement, straining and filtration, all aiding in the removal of chemicals and microorganisms to produce potable water (Earwaker, 2006). A key advantage is that there are no recurring costs once installed, because maintenance of the filter can be done by the householder (Jenkins *et al*., 2011; Tiwari *et al*. 2009; Sobsey *et al*., 2008). A BSF is a low cost, affordable, domestic water treatment method (costing US $20-30/unit) that can be constructed at a community level by locally skilled people using local materials (Duke *et al*., 2006).

The biologically active layer commonly referred to as the schmutzdecke, but also referred to as the biolayer or biofilm, develops at the sand water interface. The most efficient bacterial removal from source waters by BSFs has been associated with an intact schmutzdecke (Earwaker, 2006; Elliott *et al*., 2008; Stauber *et al*., 2006). However, there is not sufficient evidence that the biological community of the schmutzdecke is solely responsible for the bacterial removal and other factors, including physical straining, may be involved (Elliott *et al*., 2011; Elliott *et al*., 2015). Early research on BSFs focused on the mechanical processes that occur within the filter (Huisman and Wood, 1974; Ellis and Wood, 1985; Fogel *et al*., 1993); and hence the biological processes remain under investigated, and hence poorly understood. Schmutzdecke colonization over the operation cycle of a SSF has been assessed (Haig *et al*., 2014), but such a study has not been completed for BSFs. However Hwang *et al*. (2014) have assessed the schmutzdecke for opportunistic pathogens using a culture-dependant method at specific time intervals. Elliott *et al*. (2015) suggested that the increased removal of viruses with filter age is more likely

attributed to slower pore velocities which occur with head loss development, allowing for enhanced filtration at depth. Wang *et al*. (2014) and Elliott *et al*. (2011) attributed deep-bed biological maturation to enhanced virus removal during the filter resting period.

Recent studies on BSFs have begun to focus on the impact of varying operating conditions on filter efficiency (Baumgartner *et al*., 2007; Jenkins *et al*., 2011; Young-Rojanschi and Madramootoo, 2014). A number of studies have reported the impact of many operational and design parameters on the reduction of microbial indicators (e.g. total coliforms, *E. coli*) including factors such as premature clogging as a result of feed water with high turbidity (Lee, 2001), daily charge volume and resting period (Elliott *et al*., 2008; Jenkins *et al*., 2011; Lynn *et al*., 2013), filtration rate (Jenkins *et al*., 2011; Kennedy *et al*., 2012), physical disturbance to the filter (Mahaffy *et al*., 2015; Napotnik and Jellison, 2014), filter media size/type (Ghebremichael *et al*., 2012; Jenkins *et al*., 2011; Ngai *et al*., 2007; Tellen *et al*., 2010; Elliott *et al*., 2015), intermittent versus continuous operation (Young-Rojanschi and Madramootoo, 2014) and multi-day residence periods (Young-Rojanschi and Madramootoo, 2015). Given the vast array of design and operational parameters that have been identified to impact filter performance, it is evident that a filter could underperform due to poor construction and management. This is a challenge which can only be corrected through greater education of BSF users and continued support from the implementing organizations over the lifetime of the filters.

Recent studies have shown the importance of filter charge volume and residence time on filtered water quality. Results from Elliott *et al*. (2009) indicated significant viral removal within the displaced pore water, compared with the water that passed directly through the filter. Jenkins *et al*. (2009) reported improved removal of faecal coliforms and MS2 bacteriophage, but their study only compared resting periods of 5 and 16 hours. However an earlier study using a longer residence time of 36 hours, reported reduced removal of total coliforms compared with a 12 hour residence time, indicating that a longer residence time negatively impacts filter performance (Baumgartner *et al*., 2007). A recent study by Young-Rojanschi and Madramootoo (2014) compared intermittent and continuously operated BSFs on filter performance

and, while the intermittent operation of the BSF significantly improved water quality, a higher removal for *E. coli*, MS2 bacteriophage and turbidity occurred under continuous operation. Furthermore the Young-Rojanschi and Madramootoo (2015) study looked at the multi-day residence periods of 1, 2 and 3 days. Their study found no significant difference in *E. coli* removal between the three residence periods, but there were significant differences for other quality parameters, including DO, NO_3^--N and NO_2^--N, with longer residence times resulting in increased nitrite concentration in filtered water. Despite numerous studies having examined the efficiency of BSFs in the removal of contaminants, such as bacteria, viruses and heavy metals, Young-Rojanschi and Madramootoo (2014) were of the opinion that the workings of a BSF are poorly understood.

2.4 Filter media

While some operation conditions employed in a BSF are similar to a commercial-scale, SSF, including no backwashing or pre-treatment of influent water. However, the depth of the sand column in BSFs is 0.4 m, compared with a recommended starting depth of > 0.8 m for SSF with a minimum sand depth of 0.5 – 0.7 m (Elliott *et al*., 2008). The Centre of Affordable Water and Sanitation (CAWST), Calgary recommended an effective sand size (d_{10}) of 0.15 mm – 0.20 mm, a uniformity coefficient (UC) (d_{60}/d_{10}) of 1.5 – 2.5 and a maximum grain size of ≤ 0.7 mm for BSFs (CAWST, 2012). While the physical properties (grain shape, size and packing) of the filter media are important in filter functioning, in particular in relation to filtration rates, Ngwenya *et al*. (2015) suggested that the transport of bacteria through the filter is controlled by the magnitude of the surface charge of the bacteria cells relative to that of the filter media.

A recent study by Elliott *et al*. (2015) assessed the effect of varying filter media characteristics on the filter performance, in terms of bacterial and virus removal. While the two types of sand (Accusand and crushed Granite) used in the study had differing inorganic composition, grain angularity and size distribution, a significant difference in *E. coli* removal was not observed. High virus removal was observed in the columns containing crushed granite, which was attributed to the higher surface Al and Fe concentration on the granite media, and which is believed to produce a higher positive surface charge, hence increasing the media electrostatic interaction

with viruses (Elliott *et al.*, 2015). Although grain size is important in terms of maintaining appropriate flow rates, these recent studies suggest that other filter media characteristics such as grain surface properties may play important roles in enhancing the treatment performance of BSFs.

2.5 Filter performance

The efficiency of the BSF in reducing diarrhoeal disease has been well documented with a number of field (Table 2.1 and 2.2) and laboratory studies reporting the efficiency of BSFs in relation to the removal of faecal indicator organisms, including faecal coliforms and *E. coli*. While field trials have demonstrated the effectiveness of BSFs at improving water quality, greater variability in *E. coli* reductions have been reported in field trials in comparison to laboratory trials (Fiore *et al*., 2010; Vanderzwaag *et al*., 2009). BSF field trials have reported bacterial removal to range from negative to 100% (Duke *et al*., 2006; Earwaker, 2006; Kaiser *et al*., 2002; Stauber *et al*., 2006), while laboratory studies have reported virus removal to range from 0 to 0.75 $\log_{10}$ measured using MS2 and PRD-1 bacteriophage surrogates and 1.14 $\log_{10}$ of echovirus type 12 (Elliott *et al*., 2008).

Variation in turbidity removal has also been reported between field trials (Table 2.1) (Aiken *et al*., 2011; Earwaker, 2006; Kaiser *et al*., 2002; Lee, 2001; McKenzie *et al*., 2013; Vanderzwaag *et al*., 2009) and de Aceituno *et al*. (2012) suggested that removal for different sources of turbidity (anthropogenic and natural sources) needs to be further studied. Recent studies have also shown that nitrification/denitrification in BSFs leads to a higher concentration of nitrate and nitrite in the treated water (Chiew *et al*., 2009; Murphy *et al*., 2010a; Murphy *et al*., 2010b). This raises concerns about the health risk associated with using BSFs in regions which have a high nitrogen concentration in the feed water.

Field studies indicate that there is variation in the removal efficiency of faecal coliforms and *E. coli*. A possible explanation for the reported variation in filter performance may be the concentration of indicator microorganisms in the feed supply as Vanderzwaag *et al*. (2009) showed that a higher concentration of bacteria in the feed supply resulted in higher $\log_{10}$ reduction rates, which is consistent with the earlier finding of Baumgartner *et al*. (2007). Furthermore Stauber *et al*. (2011)

and Elliott *et al*. (2008) indicated that flow rate and BSF ripening were contributing factors in the removal of microbial indicator organisms. Baumgartner *et al*. (2007) observed an inverse relationship between the volume of water charged to a BSF and the removal of faecal coliforms, with higher volumes of water charged per day resulting in a reduced removal of faecal coliforms, which is consistent with the finding of Elliott *et al*. (2006). de Aceituno *et al*. (2012) suggested that more research is needed to understand the influence of water dosing frequency, flow rate, ripening and influent water characteristics on the efficiency of BSFs. The diarrhoeal disease reduction achieved by concrete BSFs and plastic housing BSFs are comparable, with similar removal efficiency of indicator organisms (de Aceituno *et al*., 2012; Stauber *et al*., 2011; Stauber *et al*., 2012; Stauber *et al*., 2009; Liang *et al*., 2010; Tiwari *et al*., 2009).

2.6 Altering filter performance by modifying filter media

Recent laboratory studies have focused on enhancing the removal of heavy metals (Chiew *et al*., 2009; Avilés *et al*., 2013) and microbiological contaminants by modifying the filter media with iron oxides (Ahammed and Davra, 2011; Murphy *et al*., 2010a) or silver nanoparticles (Mahmood *et al*., 1993). Such modifications have the potential to enhance the efficiency of BSFs during the maturation period when reduced treatment efficiency is typically observed.

2.6.1 Enhancing bacterial and virus removal

An example of an approach to modify the surface properties of the filter media is to coat the sand grains with an electropositive material (Scott *et al*., 2002). Lukasik *et al*. (1999) found that coating the sand grains of the filter media with ferric and aluminium hydroxides maintained a consistent removal efficiency after passing 192 litre of water through the filters, with *E. coli*, bacteriophage MS2, and poliovirus 1 removal efficiencies of 80%, 99.9% and 90% respectively. Lukasik *et al*. (1999) further found that even with feed water with varying pH and temperature, microorganism removal efficiencies were higher in the modified filters in comparison to the unmodified filters. The observed increases in the removal of microorganisms in the modified media are attributed to the

Table 2.1: Summary of field studies completed on BSFs

Location of Study		Number of filters in study	Time since installation	Parameter measured	Removal efficiency	Problem	Positive perception of users	Continued use rate	Reference
Cameroon		89	3 years	*E. coli* (12 h residence time) E. coli (No residence time)	90.2% - 92.4% 70% - 82%	6, 9	4,5	*	(Klopfenstein *et al*., 2011)
Dominican Republic		55	1 year	*E. coli*	93%	*	*	*	(Stauber *et al*., 2006)
Dominican Republic (Bonao)		328	1 year	Faecal coliform Turbidity	84–88% 29.5%	3, 10-13	5	90%	(Aiken *et al*., 2011)
Ethiopia		57	5-6 years	*E. coli* Turbidity	87.9% 69%	6, 10	5	75.5%	(Earwaker, 2006)
Ghana	Batamyili Village	25	< 6 months	*E. coli* Faecal coliform	65% 55%	8	*	*	(Collin, 2009)
	Savelugu	4	3 months	*E. coli* Faecal coliform	89% 72%				

Table 2.1 – (continued)

Location of Study	Number of filters in study	Time since installation	Parameter measured	Removal efficiency	Problem	Positive perception of users	Continued use rate	Reference
Nepal	39	2 years	Total coliform E. coli Turbidity	99.5% (After been in operation for 45 days) 53% 75%	10	*	*	(Lee, 2001)
Pakistan	42	*	*E. coli*	97%	7, 9	1	*	(Mahmood *et al.*, 2011)
Haiti (Artibonite Valley)	55	< 1 year to 12 years	*	*	2, 10-12, 14, 15	*	53%	(Sisson *et al.*, 2013b)
Haiti (Artibonite Valley)	107	1 to 5 years, with average 2.5 years	*E. coli*	98.5%	8, 9	1, 4	*	(Duke *et al.*, 2006)
Haiti	187	80 filters < 3 months	*E. coli*	73% initially to 85% after 3 months and 98.5% after 2.5 years on average	8, 9	1 - 4	*	Baker (2006)

Table 2.1 – (continued)

Location of Study	Number of filters in study	Time since installation	Parameter measured	Removal efficiency	Problem	Positive perception of users	Continued use rate	Reference
Kenya, Cambodia, Mozambique, Vietnam, Honduras, Nicaragua	600	≥ 3 months	Faecal coliform Turbidity	93% 32.5%	6-10	5	98.4%	(Kaiser *et al*., 2002)
Kenya	30	< 6 months	Faecal coliform Turbidity	95.6% 32.4	8, 9	*	*	(McKenzie *et al*., 2013)
Nepal	12	Recently	*	*	6, 9	*	*	(Lukacs, 2002)
Nicaragua	199	12 months (average)	*E. coli*	80%	2, 8, 9, 10, 11	5	77%	(Fiore *et al*., 2010)
Nicaragua	234	3 and 8 years	*E. coli* Total coliforms Turbidity	96% 98% 88%	9, 10, 16, 17	*	10.3%	(Vanderzwaag *et al*., 2009)

[1]Ease of Use
[2]Poorer tasting water
[3]Smell
[4]Appearance of filtered water
[5]Improved health within the household
[6]Filter maintenance knowledge varied between household

Table 2.1 – (continued)

[7]Poor understanding of the link between water, sanitation, hygiene and illness
[8]Post filtration recontamination
[9]Insufficient education/training of BSF users
[1O]Damage to BSF unit/Loss of Sand/Filter clogging
[11]BSF infested with ants
[12]Currently not living in household/Using neighbours BSF
[13]Poor perception/didn't like using BSF
[14]Filter Clogging
[15]Households non confident in the effectiveness of the systems (e.g. against cholera)
[16]Filter water was accessible to animal
[17]Poor follow up by technician
*Not assessed/relevant to the study

Table 2.2: Summary of randomized control trials (RCTs) completed on biosand filters (BSF)

Intervention group number of households		Control number of households		Removal efficiency (intervention group)	Reduction in diarrheal disease in BSF households compared to control	Location of study	BSF type	Reference
Starting number	Number that finished the study	Starting number	Number that finished the study					
30	30	30	29	Faecal coliform – 94.4%	54% in children < 5 years	Kenya	Concrete	(Tiwari *et al*., 2009)
81	75	86	79	*E. coli* – 83%	47% in children < 5 years	Bonao, Dominican Republic	Concrete	(Stauber *et al*., 2009)
107	105	102	102	*E. coli* – 95%	47%	Cambodia	Concrete	(Liang *et al*., 2010)
90	89	86	82	*E. coli* – 61% Total coliforms – 38%	39% for all age groups	Copan Honduras	Plastic - Hydraid	(de Aceituno et al., 2012)
90	90	99	99	*E. coli* – 93.3 – 99.3%	59% for all age groups	Cambodia	Plastic	(Stauber *et al*., 2011)
117	117	143	143	*E. coli* – 97%	60% for all age groups	Rural Tamale, Ghana	Plastic	(Stauber *et al*., 2012)

increased electrostatic interactions (Lukasik *et al*., 1999). Ahammed and Davra (2011) also found that a 10 cm thick layer of iron oxide coated sand in the middle of the BSF sand layer resulted in higher bacterial removal efficiency during the maturation period, when compared with a conventional, unmodified BSF. In common with Lukasik *et al*. (1999), Ahammed and Davra (2011) did not observe significant chemical changes in the filtered water quality between the unmodified and modified filter. However, further research is required to investigate the duration for which the iron oxide coated sand grains would remain effective in removing bacteria, in addition to the duration for which the modified filters would continue to produce acceptable water quality (Ahammed and Davra, 2011).

Bradley *et al*. (2011) attempted to increase the efficiency of the BSF to remove viruses by amending the filter media with iron nails and steel wool. They observed a 99.9% removal of viruses through adsorption to positively charged iron oxides, a phenomenon which had previously been identified by Gutierrez *et al*. (2009), Moore *et al*. (1981) and Rao *et al*. (1968). The period of efficient virus removal is dependent on the composition and quality of iron material added, and the source water characteristics (Bradley *et al*., 2011). However, the efficiency of the BSF containing steel wool decreased significantly after 170 days, indicating that the absorption sites had been exhausted. The use of steel nails was not suitable because they allowed non-uniform flow which lowered filter efficiency.

Baig *et al*. (2011) studied the use of coniferous pinus bark biomass (CPBB) at different depths within the sand layer of BSFs. The purpose of the CPBB was to increase the efficiency of the BSFs through absorption, due to the strong positive surface charge on the CPBB. A CPBB layer of 5 cm thickness within the sand achieved 100% removal of *E. coli* from day 30 to day 45 after filter set-up compared to 90% for the control filters. In comparison, the *E. coli* removal by conventional, unmodified BSFs, recorded in studies by Stauber *et al*. (2006), Duke *et al*. (2006), Elliott *et al*. (2006), Ngai *et al*. (2007) and Devi *et al*. (2008) only ranged from 93% to 99.9%.

2.6.2 Heavy metal removal

The presence of arsenic in drinking water has been identified as a major challenge for the treatment of drinking water in developing countries. High concentrations of arsenic have been detected in drinking water supplies in, Mongolia, Vietnam, Taiwan and Nepal (Smedley and Kinniburgy, 2002). A number of recent studies have explained the possibility of enhancing BSFs to improve removal efficiency for arsenic. Roberts *et al*. (2004) modified a BSF with steel nails; arsenic was removed through the formation of iron (III) oxides from the rusting nails. As the filter was recharged, the nails reacted with the oxygen bearing water forming insoluble hydroxides which removed soluble arsenic through the mechanism of adsorption and co-precipitation. The precipitate formed was then trapped by the sand resulting in the removal of arsenic (Roberts *et al*., 2004). Chiew *et al*. (2009) took a different approach and suspended iron nails on the diffuser plate. They found similar arsenic removal efficiency with or without iron nails, suggesting that the addition of nails to the filter design had no benefit in terms of arsenic removal. A possible explanation for this was the limited contact time between the nails as the water passed through the diffuser plate (Chiew *et al*., 2009). The encrustation of iron oxides on the nails further limited the ability of the nails to oxidise, especially while exposed to air during the resting period of the filters. During such periods the Fe (III) oxides produced were coating the surface area of the nails and reduced the generation of Fe (II), which is needed to generate sufficient reactive Fe (III) oxide to scavenge aqueous arsenic. Chiew *et al*. (2009) also found the removal of arsenic through BSFs to be reliant on the iron and phosphate levels in the feed water to the filter.

2.7 Sustainable use of biosand filters

Aiken *et al*. (2011) observed that 90% of BSFs in the Dominican Republic were still in use approximately one year after installation which is similar to observations from Duke *et al*. (2006). However, studies completed by Earwaker (2006), Sisson *et al*. (2013b), Fiore *et al*. (2010) and Vanderzwaag *et al*. (2009) found lower continued use rates, ranging from 10.3% to 77%. Sisson *et al*. (2013a) used the Kaplan-Meier (KM) estimator (statistical time to event analysis) in their study on 55 concrete BSFs in the Artibonite Valley near Deschapelles, Haiti. The KM analysis showed the probability of BSFs still being in use exceeded 80% up to 5 years after installation,

and was nearly 40% at 12 years. While the rate of continued use of BSFs varied between field studies, overall, the average rate of continued use remains higher than that of other POU technologies such as ceramic filtration, solar disinfection and chlorination. The continued use rate for POU water treatment techniques has been shown to be as low as 5%, and ranging to as high as 80% (Hunter, 2009; Rose *et al*., 2006; Rainey and Harding, 2005; Arnold and Colford, 2007; Clasen *et al*., 2006b; Crump *et al*., 2005; Luby *et al*., 2008; Colindres *et al*., 2008).

The same reasons were given by householders for abandonment of their BSF in many field studies. Issues recently identified by Sisson *et al*. (2013a) were similar to previous studies, which had identified poor understanding amongst BSF users of the link between hygiene, water quality and sanitation (Mahmood *et al*., 2011; Hurd *et al*., 2001) and insufficient education and training in the use of BSFs (Fiore *et al*., 2010; Duke *et al*., 2006; Baker, 2006; Kaiser *et al*., 2002; Klopfenstein *et al*., 2011). Recontamination of filtered water during storage due to animal or human contact (Collin, 2009; Duke *et al*., 2006; Fiore *et al*., 2010) and damage to the BSF unit (e.g. loss of sand and filter clogging) (Aiken *et al*., 2011; Earwaker, 2006; Fiore *et al*., 2010; Kaiser *et al*., 2002) have all been identified as issues resulting in potential abandonment of the BSF (Table 2.1). Infestation of the BSF with ants was another reason given by some households for discontinued use of the filter (Aiken *et al*., 2011; Fiore *et al*., 2010) (Table 2.1). The Sisson *et al*. (2013a) study observed that chlorination of the feed supply to the BSFs was a common practise amongst BSF users, which may have adverse effects on the development of the schmutzdecke.

A number of studies have indicated operational problems associated with BSFs that could be minimized through greater education of BSF users and the provision of long-term technical and maintenance support, which would potentially increase the rate of continued use of BSFs (Momba and Kaleni, 2002; Sisson *et al*., 2013b; Earwaker, 2006). Divelbiss *et al*. (2013) found that households with lower education levels were more likely to maintain their BSF properly. Divelbiss *et al*. (2013) found that households with properly operated BSFs acknowledged their health benefits, and had experienced reductions in diarrheal disease since the installation of the BSF.

Recent studies have begun to focus on the role of the implementation strategy on the receptiveness of the household to the technology and ultimately to the sustained rates

of use achieved. Ojomo *et al*., (2015) identified the importance that HWTS technologies are perceived as aspiration to ensure those without access to a safe drinking water supply are inclined to adopt a HWTS technology. A number of factors have been found to enhance the uptake and continued use of HWTS technologies including the visual appeal of the HWTS product and the treated water, as well as the cultural and religious beliefs, and the social status achieved through ownership of a HWTS technology (Ojomo *et al*., 2015). The importance of having community leaders, religious leaders, or other well respected individuals within communities, to both promote HWST technologies and support users of the technology has been shown to increases the continued use of the technology in the community (Ojomo *et al*., 2015; Figueroa and Kincaid, 2010).

Studies have also indicated that the sustainability of HWTS technology is influenced by how the household receives the product. When the product is provided free of charge, it often remains unused. For communities who cannot afford the product, users could make a contribution to the manufacturing, distribution and installation, which reduces or eliminates the cash contribution required (Clasen, 2008). As a result, the users make an investment in the product, which has been shown to reduce the number of households that abandon their filters (Clasen, 2008; Ojomo *et al*., 2015).

2.8 Concluding remarks and recommendations

A huge effort is needed to reduce the portion of the world's population that remain without access to an improved drinking water source. The high cost associated with the infrastructure and necessary maintence of a safe, piped, water supply means that many people in developing countries that typically live in rural, isolated locations will remain without access to a safe piped water supply for decades to come. Hence BSFs offer an interim solution to treat drinking water at the domestic level. The literature discussed in this review highlights the challenges and advantages of BSFs with a significant proportion of the literature supporting the distribution of BSFs as a system which offers people without access to a safe drinking water, a low cost, sustainable domestic water treatment method. However, field studies of the current users of BSFs indicates the importance of sufficient education and a support system for BSFs users, in order to empower the users to maximise filter performance,

ultimately achieving user satisfaction and the continued use of the filter. Appropriate support systems within communities could be as community leaders and/or technicians from the implementing organization, to ensure that households continue to use their BSFs into the future. Such a support network would increase the likelihood that BSFs are maintained at their optimum treatment performance and reduce the negative impacts associated with consumption of unsafe drinking water and loss of confidence in the filters.

It remains imperative that future research focuses on understanding the processes that occur within a BSF, because currently the BSF remains somewhat of a "black box" system. The identification of processes that lead to the effective removal of physical, chemical and biological agents in water that passes through the filter remain poorly understood. Research that gains insight into the processes that contribute to the removal of contaminants, through trophic interactions or physical mechanisms will lead to greater insight into the removal mechanisms contributing to filter performance. This will lead to the identification of how the operation of the filter could potentially be enhanced to optimise filter performance.

While field and laboratory studies have indicated some variation in the treatment performance of BSFs, there is evidence that BSFs are effective at reducing the risk of individuals contracting water related diseases. A number of recent laboratory studies have focused on the effect of varying operational parameters on filter performance. However, the current poor understanding of the influence of operational parameters, such as sand characteristics (e.g. grain chemical composition, grain shape/texture), and household operation on schmutzdecke colonization and development, suggest that higher treatment efficiency could potentially be achieved.

The current research has indicated the effectiveness of BSFs at treating drinking water and some insights have been gained into the effect of different operational parameters on filter efficiency. However, further advances in understanding the full functioning of BSF will bring significant benefit to BSFs users by allowing for optimization of the operation of a BSF to achieve water treatment efficiencies that consistently meet World Health Organization (WHO) Drinking-Water Guidelines (WHO, 2011).

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Chapter 3

Effect of sand characteristics, grain size and chemical composition on flow rate, schmutzdecke development and treatment performance in biosand filters

3.1 Abstract

Biosand filters (BSFs) are domestic-scale, intermittently operated, slow sand filters (SSFs), used primarily in developing regions of the world where centralized piped water supplies are unavailable. Sand used in BSFs is often sourced locally and, given the geographical distribution of BSFs, it is likely to have varying inorganic compositions and grain properties (shape and roughness). Six full-scale Hydraid® BSFs were used in the study and were operated for 142 days. Triplicate filters were loaded with well sorted sand or poorly sorted sand to compare thermotolerant coliform reduction performance and the development of the schmutzdecke. The larger maximum grain size of the poorly sorted sand resulted in higher flow rates compared with the well sorted sand, yet the differing flow rates between the sand types was not found to affect the removal of thermotolerant coliforms. The microbial community in the schmutzdecke of the well sorted sand was found to have higher metabolic activity and diversity indices during the early and middle phases. Overall the different sand properties (grain size, shape, roughness and inorganic composition) were not found to affect the removal performance of thermotolerant coliforms, whereas they were found to affect the development of the schmutzdecke. As the filters matured the metabolic activity, richness and diversity of the schmutzdecke microbial community became similar for the sand types.

3.2 Introduction

Access to safe drinking water remains a major global challenge with 663 million people remaining without access to an improved drinking water source (UNICEF and WHO, 2015). Progress towards universal access to a safe drinking water source has gained renewed focus when world leaders pledged their support for the Sustainable Development Goals (SDGs). Target 6.1 of the SDGs aims to '*achieve universal and equitable access to safe and affordable drinking water for all'* by 2030 (UN, 2015). The World Health Organization (WHO) recommends the use of point-of-use (POU) water treatment techniques for households which remain without access to a continuously piped, treated water supply (WHO and UNICEF, 2012). A number of different POU water treatment techniques are available, of which biosand filters have been identified amongst the promising in terms of treatment efficiency for turbidity and microbial pathogens (Sobsey *et al*., 2008). While more than 500,000 people use BSFs (Clasen, 2008), the operational parameters that influence their performance remains poorly understood.

The sand used in BSFs is fundamental in the treatment performance of the filter and is often obtained locally by BSF users from readily available sources. Given the geographical distribution of BSFs, the sourcing of sand locally is expected to result in sand with different characteristics, such as varying chemical composition, and different grain sizes, shape and roughness. Recent studies have indicated the importance of the physical properties of the filter media, particularly effective grain size with Lukacs (2002) and CAWST (2007) recommending an effective grain size (d_{10}) range of 0.15 – 0.4 mm and a uniformity coefficient (UC) of 1.5 – 3.0. Elliott *et al*. (2008) recommended a narrower d_{10} range of 0.19 – 0.22 mm and a higher UC of 3.5 – 4.0 whereas Manz *et al*. (1993) recommended a UC < 5.

A recent study by Ngai *et al*., (2014) identified that a common problem with BSFs is that "*the sand for the BSF was not properly selected and prepared, resulting in too high of a flow rate, which compromises the filter's performance*". Of the BSF users surveyed, "*only 52% of the BSFs had correctly installed sand*" (Ngai *et al*., 2014). A number of studies have indicated that sand size and type impacts bacterial reductions (Jenkins *et al*., 2011; Ngai *et al*., 2007; Tellen *et al*., 2010; Ghebremichael *et al*., 2012). However, the influence of sand characteristics including chemical

composition, grain shape and roughness on the colonization of the schmutzdecke and the treatment performance of the filter remain poorly understood. Elliott *et al*. (2015) conducted the first study to assess the effect of chemical properties and grain size distribution on filter performance and found no difference in removal of *E. coli* between the sand types. However the removal of viruses was affected by sand properties (Elliott *et al*, 2015).

The objectives of this study were to assess (i) the impact of flow rate and filter age on the removal of thermotolerant coliforms and the physicochemical properties of the filtered water, (ii) the effect of sand properties (grain size, shape, chemical properties) on filtered water quality and (iii) the impact of different sand properties on schmutzdecke development.

3.3 Materials and Methods

3.3.1 Filter and media preparation

Six full-scale plastic housing, BSF units were obtained from Hydraid® (Grand Rapids, Michigan) and were operated as two sets of three replicate filters. The Hyraid® filters used in the study had a maximum loading head of 17 cm. Since this study has been completed, Hyraid® have increased the sand column depth and the maximum loading head has been reduced to 12 cm. Filters were filled with 5 cm of underdrain gravel (10 mm diameter), 5 cm of separating gravel (3.15 – 5.6 mm) and 45 cm of sand (Figure 3.1). Three filters were loaded with well sorted sand which had a maximum grain size of 0.7 mm, an effective diameter (d_{10}) of 0.172 mm and uniformity coefficient (UC) of 1.77. The second sets of filters were loaded with poorly sorted sand which was purchased from a local quarry. This poorly sorted sand had a maximum grain size of 1 mm, a d_{10} of 0.175 mm, and a UC of 2.75. All filter media was washed with tap water and the sand was autoclaved at 121˚C for 20 minutes prior to been used in the study. Filters were charged once daily with 20 l of water. The daily dose volume represented the lower limit of a typical water requirement for a household in a developing nation of 20 – 40 l (Elliott *et al*., 2008). However, the 20 l daily dose volume resulted in a higher charge-to-volume ratio than the current recommended charge-to-pore volume ratio of 1:1 or less (CAWST, 2012).

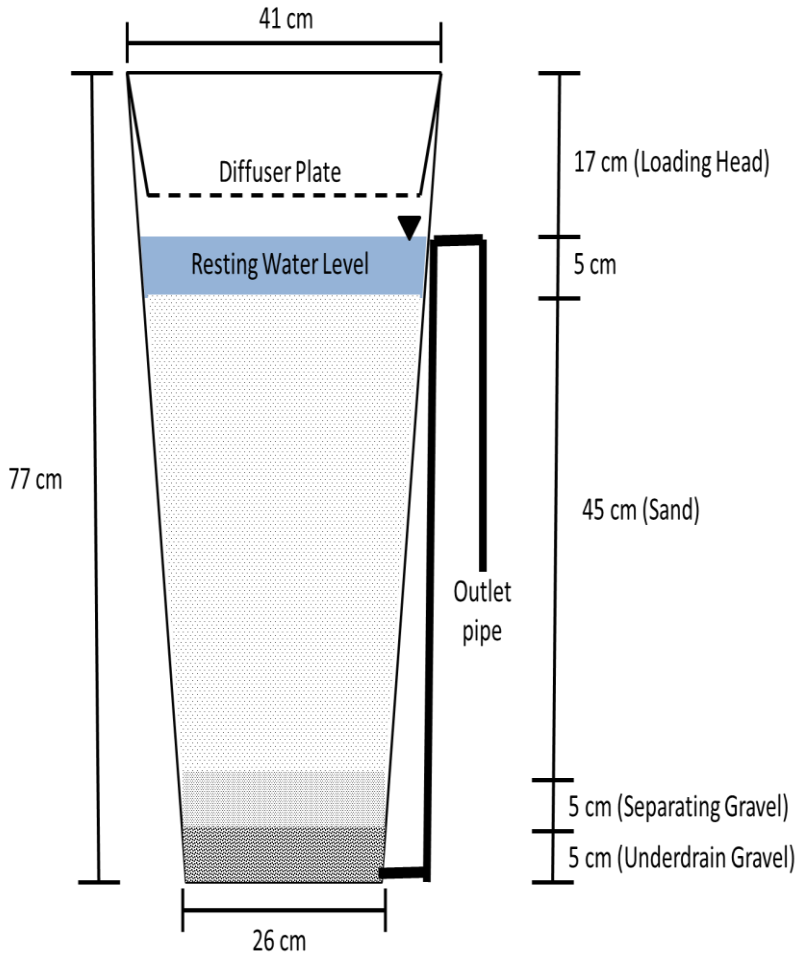


Figure 3.1: Hydraid® biosand filter used in the study.

3.3.2 Sand analysis

Standard sieve analysis was used to determine the effective grain size (d_{10}) (sieve mesh diameter by which 10% of the sand weight passes) and the uniformity coefficient (UC) (degree of sand uniformity, calculated by dividing d_{60} by the d_{10}). To determine these values, approximately 200 g (dry weight) was sieved and the cumulative weights of sand against the sieve mesh diameter were plotted. Microscopic imaging of both sand types was completed using Leica VZ700 C light microscope fitted with Leica DVM2500 camera (Leica Microsystems, Meath, Ireland) and scanning electron microscope (SEM) using a TM-1000 Tabletop Microscope (Hitachi, Tokyo, Japan).

Elemental analysis of the sand media was completed by the Aquatic Services Unit (ASU) in order to determine the concentrations of calcium, magnesium, manganese,

iron and aluminium. For this, 2.0 g subsamples of sand were placed in a 100 ml glass digestion tubes, to which was added, 9 ml of concentrated analytical grade nitric acid and 3 ml of concentrated analytical grade hydrochloric acid. The samples were digested for 12 hours at 100˚C in a Tecator 2040 digester (Foss, Hillereod, Denmark), cooled and then filtered through Whatman GF/C filter paper and made up to 100 ml with deionised water. To determine the iron, magnesium and calcium concentrations samples were analysed using a fame atomic absorption spectrometer, while aluminium and manganese concentrations were determined using the graphite furnace method with a SpectrAA 300 atomic absorption spectrometer (Varian Inc., Palo Alto, USA). The acid digestion procedure does not dissolve silicate-based minerals. Therefore the elements found in the analysis were present in acid soluble-components on the surface of the sand grains and could therefore potentially affect the sorption characteristics of the sand.

3.3.3 Feed water

Feed water was obtained from the Curraheen River, Co. Cork. Sufficient water was collected every week to feed the six filters, and was stored in an aerated container at the ambient temperature of the filters until use.

3.3.4 Water sampling and analysis

Feed water was sampled prior to dosing the filters and was analysed immediately. Grab water samples of the filtered water were collected in 250 ml sterile bottles one hour after charging the filters. Filtered water samples were collected directly from the outlet tube, which had been cleaned with a 70% ethanol solution prior to collecting the sample.

Daily measurements were taken of the feed and filtered water for dissolved oxygen (DO), pH, electrical conductivity (EC) and temperature. DO was measured using a portable meter (Handy Polaris, OxyGuard®), while the pH, EC and temperature were measured using a WTW multimeter 320i (WTW, Weilheim, Germany). Weekly samples were collected to determine total oxidised nitrogen (TON), nitrite (NO_2^- - N), orthophosphate (PO_4^{3-}), dissolved organic carbon (DOC), spectral absorption coefficient (UV_{254}) and total suspended solids (TSS). The ASU completed the total oxidised nitrogen (TON) and nitrite analyses using automated flow injection analysis

(Lachat Quickchem 8000, Zellweger Analytics Inc., Milwaukee, USA). Dissolved organic carbon analysis was also completed by the ASU using a total organic carbon analyser (TOC-VCPH/CPN, SHIMADZU, Kyoto, Japan). Analysis of the feed water to determine the total alkalinity was completed by the ASU using the titration method with 0.01 M EDTA. Orthophosphate concentration were determined using the ascorbic acid method (reference number: 4500-PE) and the UV_{254} was measured using the method (reference number: 5910 B) outlined in APHA *et al*. (2012) using the Thermo Scientific™ GENESYS 10 UV scanning spectrophotometer. Specific ultraviolet absorbance (SUVA) was calculated from the ratio between DOC and UV_{254}. Changes in the suspended organic and inorganic matter loading in the feed and filtered water were determined by measuring the TSS with method 2540 D (APHA *et al*., 2012). Thermotolerant coliforms were enumerated using the membrane filtration technique (reference number: 9222 D) (APHA *et al*., 2012), using membrane lauryl sulphate broth (Oxoid Limited, Hampshire, United Kingdom).

3.3.5 Schmutzdecke analysis

In order to characterise changes in the schmutzdecke, the filters were sampled at biweekly intervals. At each sampling event three cores (2 cm depth by 1.5 diameter) were randomly taken across the top of each sand column. These samples where then pooled for each filter. A proportion of the sample was analysed to determine the organic matter and carbon content using the loss-on-ignition (LOI) method (Dean Jr, 1974). Samples were dried over night at 105˚C and the organic matter content was then determined using an ignition temperature of 550˚C (Dean Jr, 1974). A further proportion of the sample was used for community level physiological profiles (CLPPS) using Biolog Ecoplates™ (Biolog Inc., Hayward, CA). A Biolog Ecoplate™ consists of 31 carbon substrates replicated three times per plate. Samples (3 g wet weight) were shaken at 270 rpm for 2 hrs in 27 mL of phosphate buffer saline (PBS) and were then allowed to settle for 30 minutes. The prepared supernatants were diluted with PBS to adjust the optical density (OD) at 420 nm to approximately 0.06, after which 150 µL of the diluted solutions were inoculated into each well of the Biolog Ecoplate™. The bacteria in solution attempted to metabolize the carbon sources, if successful, this resulted in the release of a purple-coloured

formazan in the wells (Preston-Mafham *et al*., 2002). The plates were subsequently incubated at 25˚C. The OD at 590 nm was measured at 96 hrs using a multi-well plate reader (Bio-rad 680 XR Microplate Reader). The average well colour development (AWCD) was calculated by dividing the total well colour development (minus the control well OD) by number of wells for each sample (31).

3.3.6 Schmutzdecke diversity and richness calculations

All functional diversity and richness indices were calculated using the 96 hr OD readings for each Biolog Ecoplate™. The species richness was calculated by counting the number of positive wells with an OD of greater than or equal to 0.06, after subtracting the OD of the control well. The functional diversity of the microbial community of the schmutzdecke was determined using the Shannon diversity index (H'). The Shannon diversity index was calculated as:

$$H' = - \sum p \, (\ln(p))$$

Where, p is the ratio of the relative absorbance value of each well on the Biolog Ecoplate™ plate to the sum of the absorbance value of the 31 wells.

Principal component analysis (PCA) of the Biolog Ecoplate™ data was conducted using the Multi-Variate Statistical Package (Version 3.1, Kovach Computing Services) using the absorbance values at 96 hrs.

3.3.7 Statistical analysis

Arithmetic means and standard deviations were calculated for all data and are present unless otherwise stated. The removal performance for each water quality parameter was calculated as a percentage. All statistical analysis was completed using the software package IBM SPSS Version 22 (SPSS Inc., Chicago, USA). Outliers were assessed by inspection of a boxplot, Shapiro-Wilk's test was used to test for normality, and Levee's test for equal variance was used to assess the homogeneity of variances. The non-parametric Mann-Whitney U test was applied to data that failed to meet the assumption of the independent sample t-test. Correlations between water quality parameters and filter age were explored using the non-parametric Kendall tau procedure.

3.4 Results

3.4.1 Filter media

Light microscopy and SEM imaging of the sand grains showed clear differences in grain properties including shape, texture and uniformity between the well sorted sand and the poorly sorted sand. The well sorted sand grains were generally well rounded (Figure 3.2), but also the SEM image showed the surface to be well fractured (Figure 3.3). In comparison, the poorly sorted sand grains were angular (Figure 3.2), with a high degree of fracturing (Figure 3.3). Chemical analysis of the sand revealed higher concentrations of Fe, Mg, Ca, Al and Mn on the surface of the poorly sorted sand (Table 3.1). Concentrations of Fe were several orders of magnitude higher for the poorly sorted sand (29.950 mg/g) in comparison to the well sorted sand (0.158 mg/g). Similarly the Mg concentration for the poorly sorted sand was higher than the well sorted sand at 4.600 mg/g and 0.017 mg/g respectively (Table 3.1).

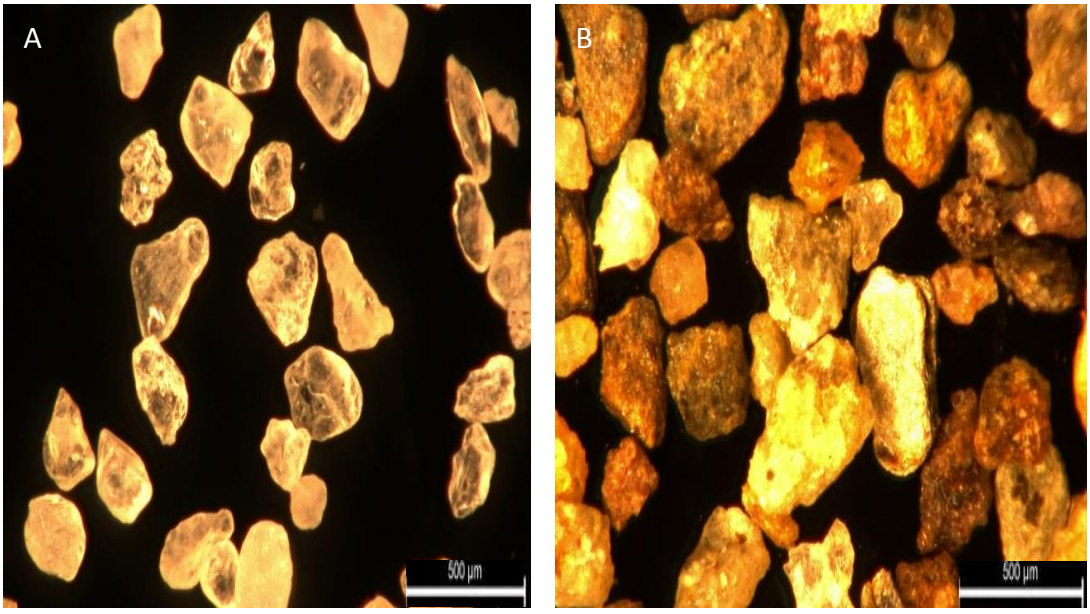
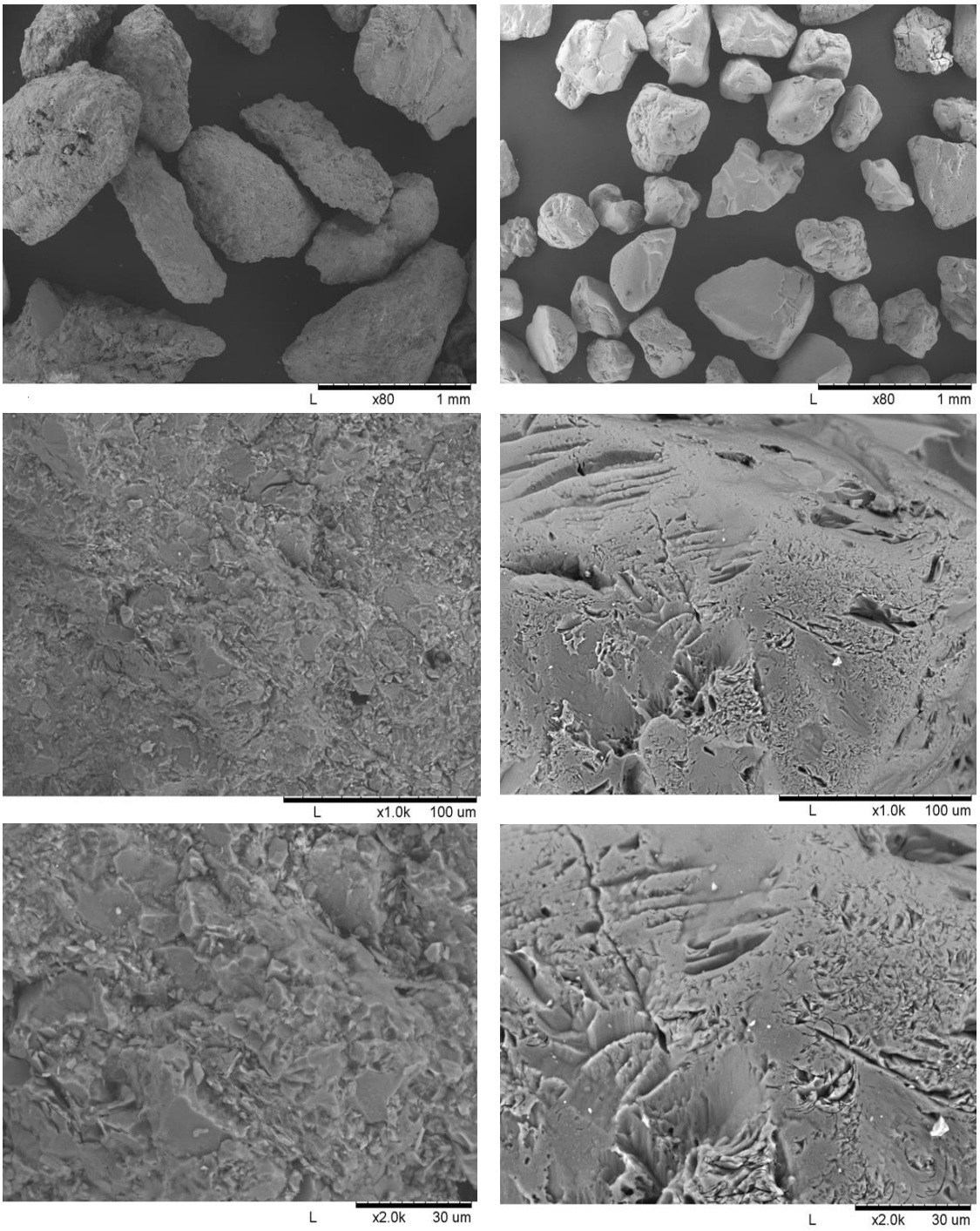


Figure 3.2: Light microscopic image of the two different sands used in the study, **A**: Well sorted sand, **B**: Poorly sorted sand.



Poorly sorted sand Well sorted sand

Figure 3.3: SEM images of well sorted sand and poorly sorted sand with increasing magnification.

Table 3.1: Characteristics of the two different sand types.

Parameter	Well sorted Sand	Poorly sorted Sand
Fe (mg/g)	0.158	29.950
Mg (mg/g)	0.017	4.600
Ca (mg/g)	0.037	0.132
Al (mg/g)	0.397	15.080
Mn (mg/g)	0.001	0.818
Maximum grain size (mm)	0.70	1.0
Effective diameter (mm)	0.172	0.175
Uniformity coefficient	1.77	2.75
Porosity	0.42	0.40

3.4.2 Filtration rate

Filtration rate was measured immediately after charging the filters, when head water pressure was at its maximum and filtration was at its highest. The peak filtration rate at the start of the study (day 0) was higher in the filters containing the poorly sorted sand (0.106 ± 0.01 $m^3/m^2/hr$) in comparison to the well sorted sand (0.183 ± 0.009 $m^3/m^2/hr$) (Figure: 3. 4). Over the duration of the study a greater decline in filtration rate occurred for the poorly sorted sand in comparison with the well sorted sand. The peak filtration rate on day 141 of the study was 0.098 ± 0.005 $m^3/m^2/hr$ for the well sorted sand and 0.145 ± 0.006 $m^3/m^2/hr$ for the poorly sorted sand. The difference in filtration rate between the sand types was found to be statistically significant (U = 0.000, Z = -25.272, p-value < 0.001).

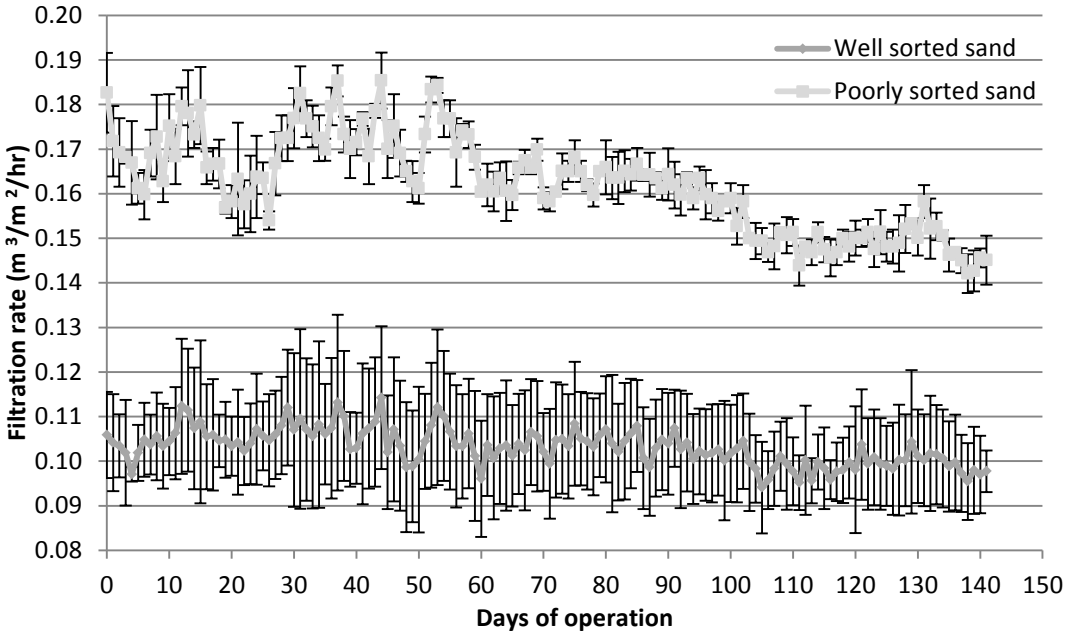


Figure 3.4: The peak daily filtration rate for the filters containing well sorted sand poorly sorted sand (mean ± standard deviation, n = 3).

3.4.3 Water quality

The median removals of thermotolerant coliforms (TTCs) were not statistically different between the well sorted sand (88.07%) and the poorly sorted sand (88.31%) (U = 5620.5, Z = -1.129, p-value: 0.259). The mean concentration of TTCs in the influent water to both sets of filters was 547 ± 757 CFU/100 ml, placing the influent water in the high risk category according to the WHO (1997) Risk Classification Scheme (Table 3.2). The mean concentration in the filtered water from the well sorted sand filters (74 ± 126 (37)) and the poorly sorted sand filters (77 ± 135 (37)) reduced the risk to the intermediate category based on the WHO Risk Classification Scheme (WHO, 1997) (Table 3.2). The highest removal of TTCs occurred at the late phase (day 95 – 142) of filter operation for both sets of filters (Figure 3.5). During the middle phase (day 48 – 94), a slight reduction in percentage removal of TTCs occurred compared with the early phase. A significant positive correlation was found between percentage removal of thermotolerant coliforms and filter age for both sets of filters (Table 3.3).

Table 3.2: Risk classification scheme for thermotolerant coliforms (TTCs) or *E. coli* in drinking water according to WHO (1997).

TTC counts per 100 ml	**Remark**
0	Meets WHO Guidelines
1 – 10	Low Risk
10 – 100	Intermediate Risk
100 – 1000	High Risk
> 1000	Very High Risk

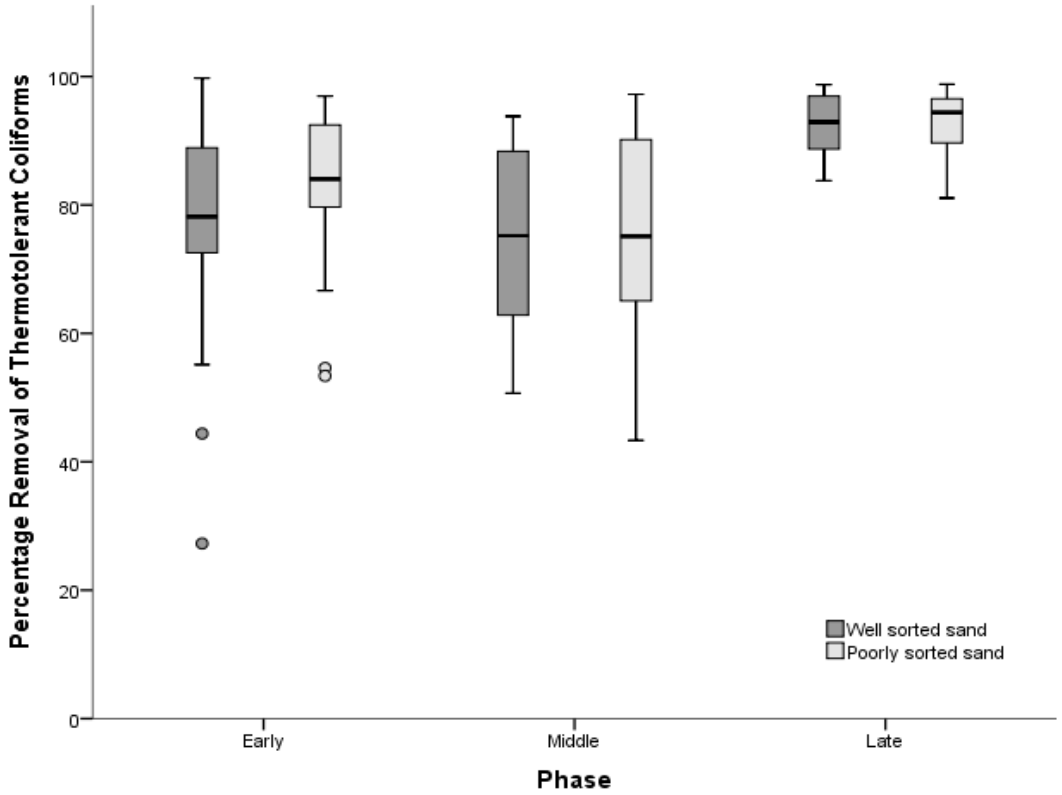


Figure 3.5: Percentage removal of thermotolerant coliforms for each sand type (well sorted sand and poorly sorted sand) in the different age phases. Early: 0 – 47 day, middle: 48 – 94 days and late: 95 – 141 days. The top and bottom boundaries of the boxes show the 75th and 25th percentile. The bold lines within the boxes represent the median values (50th percentile) and the end of the whiskers show the maximum and minimum values.

A strong positive correlation was found between percentage reductions of DO and filter age for both the well sorted sand (R = 0.525 (p-value < 0.001)) and poorly sorted sand (R = 0.422 (p-value: < 0.001) filters (Table 3.3). The difference between the median DO concentration over the duration of the study between the well sorted sand (9.00 mg/l) and poorly sorted (8.90 mg/l) sand was not statistically significant (U = 87565.5, Z = - 0.884, p-value: 0.377) (Table 3.4). Significant differences in the median EC concentration of the filtered water was found between the well sorted sand (306 µs/cm) and poorly sorted sand (304 µs/cm) (U = 43518, Z = -13.166, p-value < 0.001). Greater reductions in pH occurred in the filtered water from the poorly sorted sand filters in comparison with the well sorted sand filters, and this difference was significant (U = 7601, Z = -23.147, p-value: < 0.001) (Table 3.4). The alkalinity of the feed water was determined from three grab samples and was found to be 116 ± 3.61 mg/l as $CaCo_{3.}$

Higher nitrite concentrations occurred in the filtrate from both sand types in comparison with the feed water during the initial filter maturation period. However, after 30 days of filter operation the nitrite concentration was lower in the filtrate in comparison with the feed water concentration for both sand types. This observation is supported by a strong positive correlation between the percentage removal of nitrite and filter age for both the well sorted sand (R = 0.077 (p-value: < 0.001) and poorly sorted sand (R = 0.674 (p-value: < 0.001) (Table 3.3). However no significant difference was found for the nitrite concentration between the sand types (U = 1972.5, z = - 0.060, p-value: 0.952). The median nitrate concentration of the filtered water from both sand types was not found to be statistically different (U = 1970, z = -0.071, p-value: 0.944) (Table 3.4).

A reduction in UV_{254} values occurred in both sets of filters, but greater reductions occurred in the sand columns with poorly sorted sand. There was a statistically significant difference in the median UV_{254} absorbance between the filtered water from the poorly sorted sand filters (0.052) and the well sorted sand filters (0.062) (U = 1188, Z = -3.887, p-value < 0.001) (Table 3.4). Similarly, the filtered water from the poorly sorted sand had a lower SUVA in comparison with the filter water from the well sorted sand filters and this difference was found to be statistically significant (U = 511.5, Z = - 8.370, p-value; < 0.001) (Figure 3.6).

Table 3.3: Kendall tau correlations of filter age and the percentage removal of water quality parameters.

Parameter	Well sorted sand	Poorly sorted sand
TTC	R = 0.402** (p-value < 0.001)	R = 0.302** (p-value < 0.001)
DO	R = 0.525** (p-value < 0.001)	R = 0.422** (p-value < 0.001)
TON	R = - 0.089 (p-value: 0.313)	R = 0.092 (p-value: 0.296)
Nitrite	R = 0.777** (p-value < 0.001)	R = 0.674** (p-value: 0.001)
Nitrate	R = - 0.181* (p-value: 0.039)	R = - 0.023 (p-value: 0.794)
Orthophosphate	R = - 0.303** (p-value: 0.001)	R = - 0.445** (p-value: < 0.001)
DOC	R = 0.298** (p-value: 0.001)	R = 0.187* (p-value: 0.033)
UV_{254}	R = 0.132 (p-value: 0.131)	R = - 0.136 (p-value: 0.120)
SUVA	R = - 0.164 (p-value: 0.061)	R = - 0.409** (p-value: < 0.001)
TSS	R = 0.008 (p-value: 0.932)	R = 0.138 (p-value: 0.156)

Blue denotes positive correlations
Red denotes negative correlations
*correlation is significant at the 0.05 level (2-tailed)
** correlation is significant at the 0.01 level (2-tailed)
DO: dissolved oxygen, DOC: dissolved organic carbon, SUVA: specific ultraviolet absorbance, TTC: thermotolerant coliforms, TON: total oxidised nitrogen, TSS: total suspended solids, UV_{254}: spectral absorption coefficient.

Table 3.4: Median concentrations for each physicochemical parameter measured during the study for the feed and filtered water from BSFs with different sand types.

Parameter	Feed Water	Well sorted sand filtered water	Poorly sorted sand filtered water	p-value
DO (mg/l)	10.00 (9.48 – 10.60)	9.20 (8.50 – 9.83)	9.15 (8.50 – 9.80)	0.377
pH	8.07 (8.01 – 8.13)	7.90 (7.83 – 7.94)	7.67 (7.59 – 7.73)	< 0.001
EC (µs/cm)	302 (298 – 304)	301 (299 – 304)	296 (291 – 300)	< 0.001
TON (mg/l)	6.22 (5.95 – 6.76)	6.196 (5.99 – 6.63)	6.17 (5.99 – 6.69)	0.979
Nitrate (mg N/l)	6.207 (5.931 – 6.731)	6.173 (5.986 – 6.600)	6.172 (5.984 – 6.659)	0.944
Nitrite (mg N/l)	0.023 (0.016 – 0.034)	0.007 (0.001 – 0.032)	0.006 (0.001 – 0.028)	0.952
Orthophosphate (mg/l)	0.023 (0.020 – 0.026)	0.024 (0.021-0.027)	0.017 (0.012 - 0.020)	< 0.001
DOC (mg C/l)	2.170 (1.905 – 2.745)	2.090 (1.800 – 2.770)	2.100 (1.850 – 2.460)	0.766
UV_{254}	0.065 (0.056 – 0.093)	0.062 (0.054 – 0.083)	0.053 (0.043 – 0.067)	< 0.001
SUVA (l/mg-M)	3.23 (2.80 – 3.54)	3.10 (2.87 – 3.41)	2.54 (2.30 – 2.71)	< 0.001
TSS (g/l)	0.096 (0.093 – 0.104)	0.096 (0.094 – 0.105)	0.096 (0.094 – 0.103)	0.342

.Interquartile range in parenthesis, p-values test for a significant difference between the filtered water from the well sorted sand and poorly sorted sand. DO: dissolved oxygen, DOC: dissolved organic carbon, EC: electrical conductivity, SUVA: specific ultraviolet absorbance, TON: total oxidised nitrogen, TSS: total suspended solids, UV_{254}: spectral absorption coefficient.

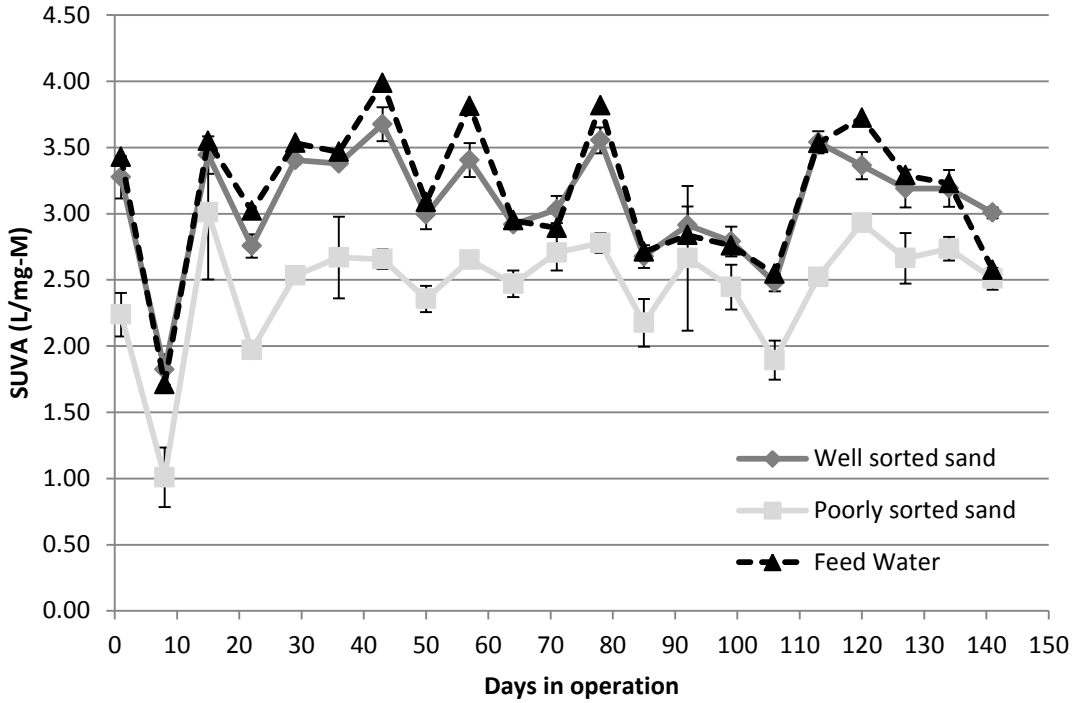


Figure 3.6: Specific ultraviolet absorbency (SUVA) concentration of the feed and filtrate water from BSFs with different sand types. Error bars represent the standard deviation, n = 3.

Both sets of filters performed well in terms of total suspended solid removal, with Mann-Whitney U test showing no significant difference in median TSS concentration between the filters with well sorted sand and poorly sorted sand (U = 1808.0, Z = - 0.950, p-value: 0.342). A positive correlation was found between the removal of TSS and filter age for the well sorted sand (R = 0.008) and poorly sorted sand (R = 0.138) but these correlations were not significant (Table 3.3).

3.4.4 Metabolic function of the schmutzdecke

The average well colour development (AWCD), which is a measure of the metabolic activity of the microbial community in the schmutzdecke, showed an increased metabolic activity for both sand types with filter age (Figure 3.7). Although the microbial community for the well sorted sand displayed a higher metabolic activity in comparison to the filters containing the poorly sorted sand, there was no significant difference in AWCD between the two sets of filters (U = 25.0, Z = -1.890

p-value: 0.063). The metabolic activity of the schmutzdecke microbial community was similar for both sand types at the end of the study (Figure 3.7).

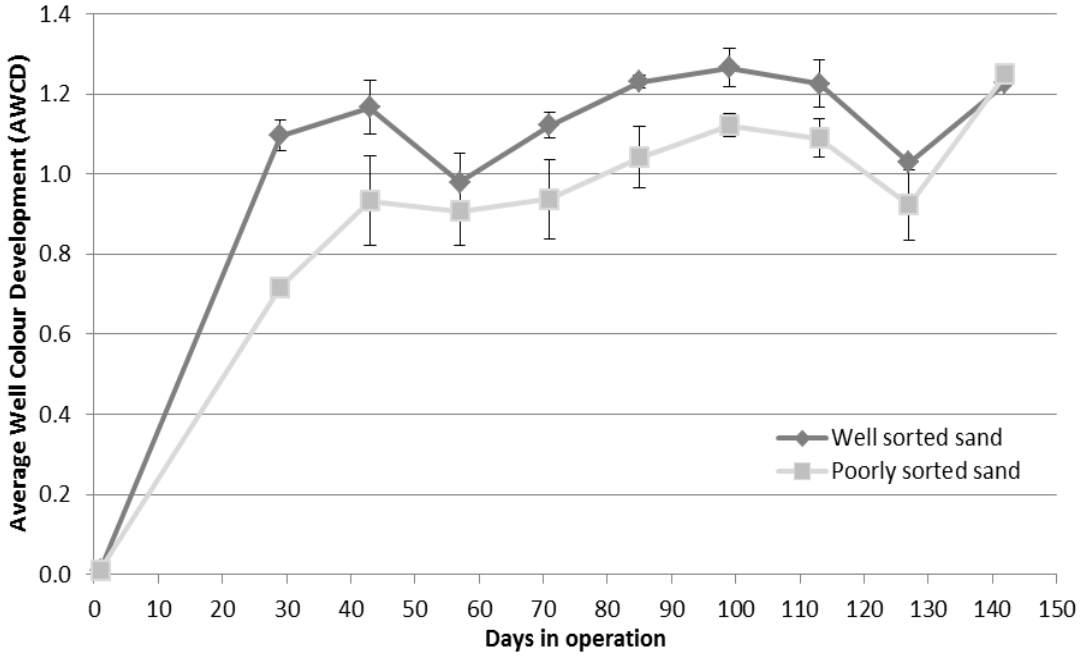


Figure 3.7: Metabolic activity of the microbial communities in the schmutzdecke over the duration of the study in BSFs with different sand types. Error bars represent standard deviation, n = 3.

The functional diversity increased gradually with filter age; the well sorted sand had a slightly greater richness index up to day 71 in comparison with the filters containing the poorly sorted sand. After 71 days of filter operation, the richness index reached a relatively stable level for both sets of filters. Similarly, a higher Shannon diversity index occurred for the well sorted sand over the duration of the study (Table 3.5). The median richness for well sorted sand (31) and poorly sorted sand (31) was effectively the same and not statistically different (U = 420.5, Z = -0.507, p-value: 0.612). However, the median Shannon diversity index for the well sorted sand (3.29) and poorly sorted sand (3.26) was found to be statistically different (U = 258, Z = -2.839, p-value: 0.005).

Table 3.5: Functional diversity of the schmutzdecke microbial communities during different days of filter operation for the different sand types.

Days of operation	Well sorted sand		Poorly sorted Sand	
	Richness	**Shannon Diversity**	**Richness**	**Shannon Diversity**
0	0.67 ± 0.58	2.23 ± 0.180	0.67 ± 0.58	2.28 ± 0.586
29	30.33 ± 1.15	3.28 ± 0.020	29.33 ± 1.15	3.17 ± 0.014
43	31.00 ± 0.00	3.32 0.023	30.33 ± 1.15	3.25 ± 0.041
57	31.00 ± 0.00	3.27 ± 0.015	30.33 ± 0.58	3.23 ± 0.037
71	30.67 ± 0.58	3.29 ± 0.020	30.67 ± 0.58	3.24 ± 0.039
85	30.67 ± 0.58	3.31 ± 0.031	30.67 ± 0.58	3.26 ± 0.013
99	31.00 ± 0.00	3.32 ± 0.005	31.00 ± 0.00	3.30 ± 0.028
113	30.67 ± 0.58	3.31 ± 0.024	31.00 ± 0.00	3.28 ± 0.007
127	30.00 ± 1.00	3.24 ± 0.008	30.33 ± 1.15	3.21 ± 0.067
142	30.67 ± 0.58	3.32 ± 0.021	31.00 ± 0.00	3.31 ± 0.002

The principal component analysis (PCA) of the carbon utilization on the Biolog Ecoplates^{TM} (Figure 3.8) illustrates that the metabolic functions of the microbial communities at the different time points over the duration of the study were distinct, and the metabolic functions of the microbial community changed over the duration of the study. For the well sorted sand, the PCA plots show that metabolic function for day 29 of the study are clustered on the positive side of axis one, with only the metabolic functions on day 0 clustering on the negative side of axis one and two (Figure 3.8). Similarly, clear shifts in the metabolic activities of the microbial community in the schmutzdecke of the poorly sand filters occurred with filter age. Also replicate filters tend to cluster indicating a similar metabolic function (Figure 3.8).

The organic matter content, determined using the loss-on-ignition method, was also found to differ between the two sand types. The initial organic matter content of the sand on day 0 in top 2 cm of the sand columns was higher for the poorly sorted sand (0.149 ± 0.013%) in comparison with the well sorted sand (0.005 ± 0.002%) (Figure 3.9). The percentage loss-on-ignition (LOI) peaked for the poorly sorted sand on day 112 (0.229 ± 0.056%) and for the well sorted sand on day 70 (0.076 ± 0.002%) (Figure 3.9). The difference in organic matter content between the two sand types was found to be statistically significant with the poorly sorted sand having a higher

mean rank (40.89) in comparison to the well sorted sand (14.11) (U = 3.00, Z = -6.254, p-value < 0.001)

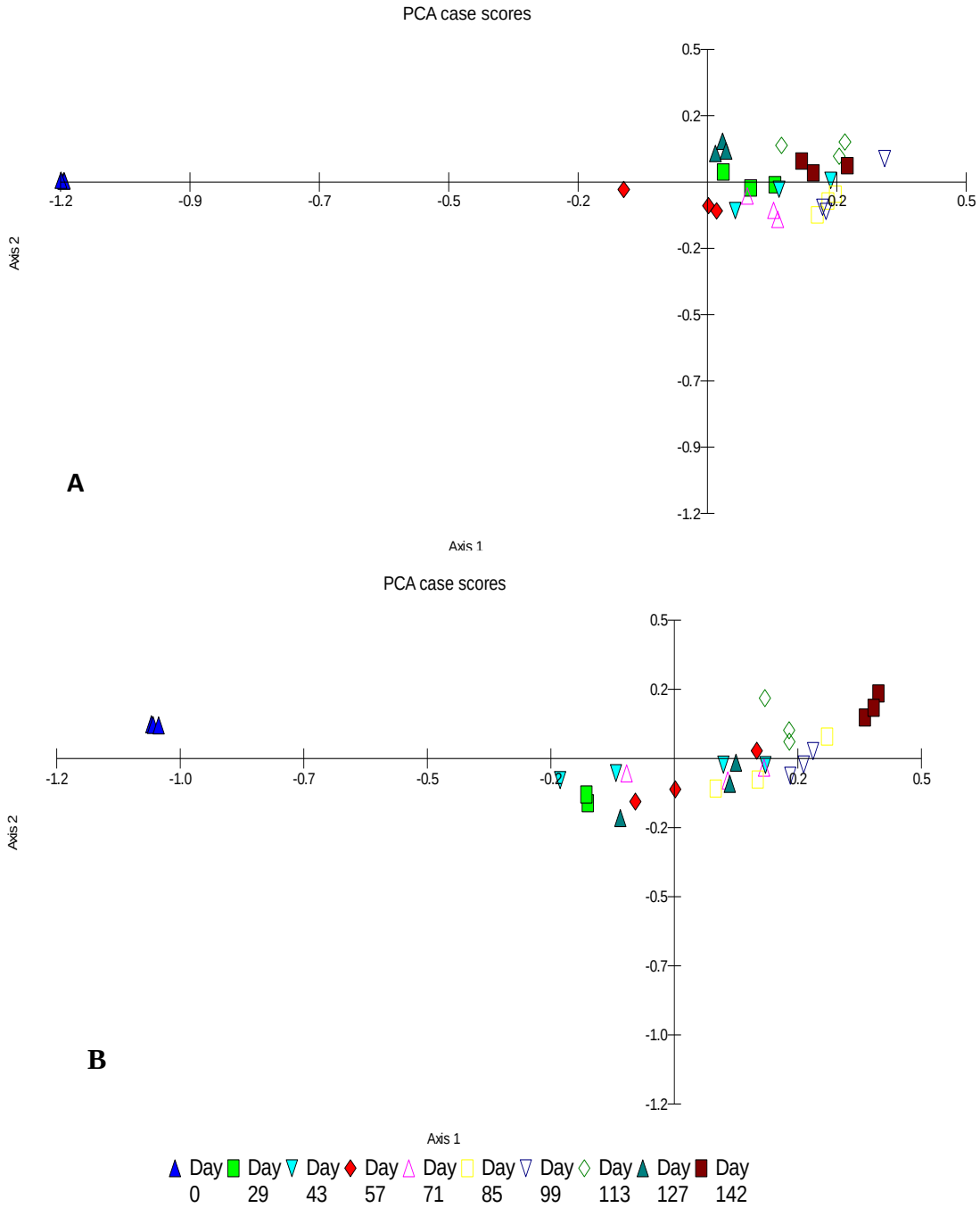


Figure 3.8: Principle component analysis (PCA) of carbon utilization on Biolog Ecoplates™ by the schmutzdecke microbial community at different time points during the study. **A:** filters containing well sorted sand **B:** filters containing poorly sorted sand.

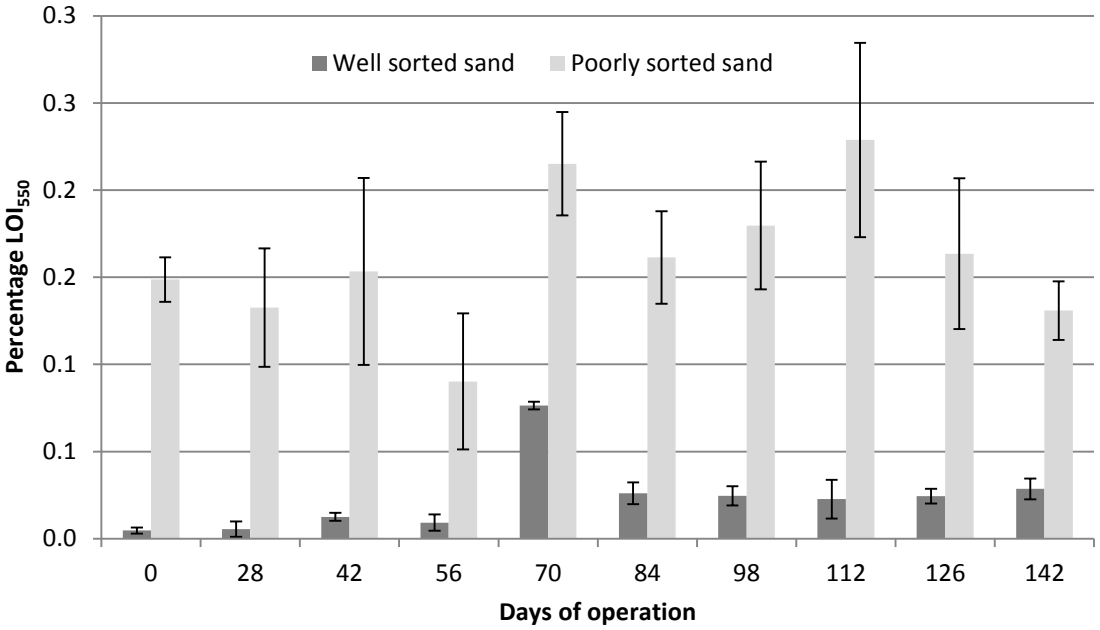


Figure 3.9: Loss-on-ignition, percentage accumulation of organic matter in the schmutzdecke of BSFs with different sand types.

3.5 Discussion

3.5.1 Media properties

Given the geographical distribution of BSFs, the sourcing of sand locally results in varying sand characteristics including varying grain shape, grain texture and inorganic composition. Currently the guidelines regarding sand selection for use in BSFs only relates to grain size (CAWST, 2007; Elliott *et al*., 2008; Lukacs, 2002, Manz *et al*., 1993).

Earlier studies have indicated the importance of a lower flow rate in optimising particle settlement and increasing the contact between bacteria, filter media and the schmutzdecke (Jenkins *et al*., 2011; Kennedy *et al*., 2012). This study found that the increased filtration rate in the filters containing poorly sorted sand did not affect the removal of thermotolerant coliforms in comparison with well sorted sand. This observation is supported by Chan *et al*. (2015) who reported that lower filtration rates did not enhance filter performance. The decline in the filtration rate and hence head loss development, was faster in the poorly sorted sand filters compared with the filters containing well sorted sand. The difference in grain size, shape, roughness and

inorganic properties may be a contributing factor to the differences in head loss development. Saiers and Ryan (2005) suggested that grain sphericity and shape is important contributor to contaminant retention. Microscopic imaging of the sand grains for both sand types in this study revealed the poorly sorted sand was angular in comparison with the well sorted sand were the grains were more rounded. Furthermore Knappett *et al*. (2008) attributed increased grain angularity to increased straining and trapping of colloids in saturated media, which potentially explains the greater decline in filtration rate that occurred for the poorly sorted sand.

Imaging of the sand grains by SEM showed that the poorly sorted sand had rougher surface properties compared with the well sorted sand. This rough sand surface could have implications for filter performance as a study completed by Shellenberger and Logan (2002) on glass beads found that there was a large protrusion of microspheres on rough glass beads compared with smooth beads, indicating that sand grain surface roughness could potentially influence microbial colonization of schmutzdecke, but also removal of microbial organisms through adhesion. Although there were differences in the physical properties of the sand grains between the sand types, the performance of the filters did not differ significantly, despite the differences in flow rate. This would indicate that the higher degree of grain angularity, the roughness, organic matter content and the surface properties of the poorly sorted sand may have enhanced filter performance. Elliott *et al*. (2015) also assessed the effect of different sand types on BSF performance and found granite sand had much higher concentrations of Calcium, Magnesium, Manganese, Iron and Aluminium. This study also found large differences in the concentrations of Fe, Mg, Ca, Al and Mn between the sand types, although the chemical form of the metals on the surface of the sand were unknown. Oxide forms of metals, including iron and aluminium oxide as well as hydroxide coatings have been shown to enhance the reductions of bacteria in sand filters, by enhancing the attachment efficiencies of cells (Ahammed and Davra, 2011; Lukasik *et al*., 1999; Truesdail *et al*., 1998). However Foppen *et al*. (2008) found that feed water quality, in particular the presence of dissolved organic matter can block metal oxide and hydroxide as absorption sites for *E. coli*. Therefore, while metal oxide and hydroxide absorption sites can enhance the removal of bacteria, it likely that their effectiveness is short lived and sensitive to feed water quality (Elliott *et al*., 2015).

3.5.2 Filter Performance

While both sand types failed to achieve the standards set by the WHO guidelines for thermotolerant coliforms (WHO, 2011), both sand grades did reduce the risk to the consumer. The average thermotolerant coliform loading of the feed water over the duration of the study places the water into the high risk category, however passing the water through the sand filters reduced the water to the intermediate risk category based on the WHO Risk classification scheme (WHO, 1997). Elliott *et al*. (2015) compared two different sand types and found no significant difference in *E. coli* reductions between different sands despite their different inorganic composition, grain angularity and grain size distribution. This study supports this earlier finding and indicates that there is potential to ease the current guidelines regarding sand size to be used in BSFs. Elliott *et al*. (2015) did, however, find a difference for viral removal between the sand types and attributed the increased removal of viruses in the sand with the higher concentrations of Al and Fe, which are believed to produce a positive surface charge aiding better electrostatic attachment of viruses.

Sand type was not found to affect the performance of the filters in terms of TON, nitrite and nitrate concentration in the filtrate. For both sand types, the nitrite concentration in the filtered water decreased significantly with filter age in comparison with the feed water, indicating that some nitrification/denitrification was occurring. Murphy *et al*. (2010) also suggested that nitrification/denitrification was occurring in BSFs in a study in Cambodia. To determine the true extent of nitrification/denitrification future studies would benefit from the inclusion of ammonium measurements. The nitrate and nitrite concentration of the feed and filter water from both sand types in this study remained well below the WHO Guidelines for Drinking-Water Quality of 11 mg/L NO_3^- – N and 0.9 mg/L NO_2^- – N (WHO, 2011).

Some filtered water physicochemical properties were found to differ between the sand types including pH, EC, orthophosphate, UV_{254} and SUVA. While these differences indicate a difference in the performance of BSFs with different sand types, the observed differences in these parameters would not directly impact the health of consumers. Filtered water from the poorly sorted sand filters showed a greater decrease in pH and EC compared with the well sorted sand. This difference

could be attributed to the different chemical properties of the filter media, because Young-Rojanschi and Madramootoo (2015) attributed increases in filtered water pH and EC to the leaching of calcium carbonate from the filter media in their study. The difference in orthophosphate and UV_{254} reductions between the sand types could possibly be attributed to different schmutzdecke microbial community structures given the differences in the metabolic activities between the sand types however, this requires further investigation. The observed differences in filtrate SUVA is an important observation because SUVA provides an estimation of the dissolved aromatic carbon content. Water with a high DOC and SUVA levels are considered to be problematic from a disinfection by-product (DBP) formation aspect (Ates *et al*., 2009). Based on the lower median SUVA concentration, the filtered water from the poorly sorted sand columns would be more suitable for subsequent chlorination, whereas filtered water from the well sorted sand would be more suitable for solar disinfection (SODIS), which would avoid the risk associated with the formation of DBPs.

3.5.3 Schmutzdecke development

The similarity of the AWCD in the Biolog Ecoplates^{TM} between the sand types at the end of the study indicates that the microbial communities in the schmutzdecke in both sand types had similar metabolic activity. However, during the early stages of filter operation there were clear differences in the metabolic activity between the microbial communities in the schmutzdecke. The initial difference in metabolic activity of the schmutzdecke microbial community between the sand types is most likely due to differences in filtration rate. Differences in filtration rate and sand properties, including grain shape (angularity), surface texture and inorganic composition, are likely to influence particle trapping and microbial attachment, potentially contributing to the differences in the rate of schmutzdecke development. Elliott *et al*. (2015) suggested that differences in the inorganic composition of the filter media would affect cell attachment. However, despite higher concentrations of metals in the poorly sorted sand, this difference was not found to enhance the metabolic diversity and richness during the start-up stage of the filters. Even though a higher filtration rate decline occurred for the filters containing the poorly sorted sand, indicating a higher rate of filter clogging which could be attributed to

biological growth and particle trapping, the metabolic activity of the biological community in the schmutzdecke was higher in the well sorted sand. However, as the filters age the metabolic activity of the microbial community in the schmutzdecke converge despite the differences in sand properties and filtration rates between the sand types.

3.6 Conclusions and recommendations

The rate of schmutzdecke development, as measured by the decline in filtration rate differed between the sand types with the poorly sorted sand filters having a greater filtration rate decline over the 142 days of the study. Slight reductions in the removal of TTCs occurred during the middle phase (48 – 94 days) but the removal of thermotolerant coliforms increased again in the late phase, which coincided with a decline in filtration rate for both sand types. Although both sand types reduced the concentration of thermotolerant coliforms, they both failed to reduce the thermotolerant coliforms to a level that met the WHO Guidelines Drinking-Water Quality (WHO, 2011). The filtered water from both sand types did, however, provide a lower risk to the household if consumed in comparison to the feed water. As discussed in the method section, the charge volume to both sand types was greater than the recommend charge-to-pore volume ratio of 1:1. Therefore the absolute reductions of thermotolerant coliforms reported in this study may not be representative of those in BSFs that meet the current BSF design guidelines (CAWST, 2012). Nevertheless these results provide a useful comparison of the effect of sand type on the filtered water quality and schmutzdecke development.

Thermotolerant coliform reductions did not differ significantly between the sand types even though they had different inorganic compositions (higher concentrations of Fe, Mg, Ca, Al and Mn), angularity (poorly sorted sand was more angular), maximum grain size (≤ 1.0 for the poorly sorted sand and ≤ 0.7 for the well sorted sand) and grain size distribution (much wider distribution for poorly sorted sand). Further research is needed to understand the effect of sand grain size on filter performance, but also to determine the direct effect of sand inorganic properties on the development of the schmutzdecke and filter performance.

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Chapter 4

Influence of resting water depth on schmutzdecke development and filter performance in domestic scale biosand filters

4.1 Abstract

Biosand filters are a point-of-use water treatment technology, often referred to as intermittently operated, slow sand filters, developed for domestic use. A fundamental design feature of biosand filters is the resting water depth (RWD) which is the level of water maintained over the sand column during the filter resting period. Sixteen bench-scale biosand filters (B-BSFs) were constructed to represent full-scale biosand filters. Four different resting water depth conditions of 2 cm, 5 cm, 8 cm and 11 cm were assessed. All resting water depth conditions had the same maximum loading heads (17 cm) and daily dose volumes. Feed water, filtered water and control samples were analysed for a number of parameters including total coliforms, *E. coli*, dissolved oxygen (DO), electrical conductivity (EC), pH, nitrate, nitrite, orthophosphate and total suspended solids. All four different RWD conditions achieved greater than 90% removal of total coliforms and *E. coli* from the feed water after 20 days of filter operation. No significant differences in physicochemical characteristics of the filtered water were observed between the different RWD conditions. The study also found that the microbial community in the biolayer under the different RWD conditions had similar metabolic activity and functional diversity. This study found that the performance of B-BSFs was not related to RWD.

4.2 Introduction

Access to an improved drinking water source remains a major global challenge with 784 million people globally continuing to consume drinking water from unimproved sources, of which 173 million people rely on the direct use of surface water (WHO and UNICEF 2014). A promising point-of-use water treatment technology for people without access to an improved drinking water source is a biosand filter (BSF): a domestic scale, intermittently operated, slow sand filter (Elliott *et al*. 2008). Biosand filters are among the most promising point-of-use water treatment technologies available, in terms of treatment performance (Sobsey *et al*. 2008). Biosand filters have a number of key advantages, which include no reoccurring costs once installed because filter maintenance can be completed by the owners of the filter. Another advantage is that filters can be constructed locally, using locally available material and locally skilled people (Jenkins *et al*. 2011; Sobsey *et al*. 2008; Tiwari *et al*. 2009).

With over 500,000 people worldwide using BSFs, a number of field studies have assessed implementation, user satisfaction and filter performance in terms of removal efficiency of thermotolerant coliforms and *E. coli* (Duke *et al*. 2006; Earwaker 2006; Kaiser *et al*. 2002; Lukacs 2002; Stauber *et al*. 2006; Vanderzwaag *et al*. 2009). Fewer studies have assessed the impact of the filter design, on the microbial removal efficiency and associated schmutzdecke development although a number of recent studies have assessed the impact of optional parameters including intermittent versus continuous operation, multi-day residence periods and daily charge volume on the microbial removal efficiency under well-controlled laboratory conditions (Young-Rojanschi and Madramootoo 2015; Young-Rojanschi and Madramootoo 2014; Elliott *et al.* 2008; Jenkins *et al.* 2011; Lynn *et al*. 2013).

A design feature of BSFs, which allows for the filter to be operated intermittently, is the outlet pipe, which rises up the wall of the filter to discharge at a point which is higher than that of the sand column, thereby maintaining a level of water over the sand column during the filter resting period, (i.e. the period during which there is no discharge from the filter). The RWD must be of sufficient depth to dissipate the initial force of water during filter recharge, thus avoiding disturbance of the schmutzdecke (Kubare and Haarhoff 2010). However, Buzunis (1995) suggested that

RWD should be minimized, in order to maximise the diffusion of oxygen through the RWD to the schmutzdecke during the filter resting period, to support the biological metabolism of organic contaminants in the schmutzdecke.

The Centre for Affordable Water and Sanitation (CAWST) recently recommended a RWD of 5 cm but, not to be greater than 6 cm (CAWST 2012). Field studies have also observed variation in RWDs in households. Earwaker (2006) field study in Ethiopia observed RWDs to range from 0 – 20 cm, and attributed such low and high RWDs to undesirable conditions for the survival of the microbial community in the schmutzdecke. The schmutzdecke community became starved of oxygen at higher RWDs, but at low RWDs the schmutzdecke was vulnerable to damage, potentially affecting the efficiency of the BSF in the removal of harmful pathogens (Earwaker 2006). Contrary to observations by Buzunis (1995), a study by Vanderzwaag *et al*. (2009) in Nicaragua observed an increased log reduction for *E. coli* and total coliforms with increased RWD.

It remains unclear from field studies whether a variation in RWD of 0 to 20 cm influences the development of the schmutzdecke and ultimately the treatment performance of the filter. Therefore the main objectives of this study under controlled laboratory conditions were to (i) assess the impact of varying RWD on schmutzdecke development, (ii) determine whether a reduced RWD resulted in schmutzdecke disturbance during filter recharge, and (iii) examine if schmutzdecke disturbance did occur, whether it impacted schmutzdecke development and the treatment performance of the filter in terms of the removal of microbial indicator organisms.

4.3 Materials and Methods

4.3.1 Filter design

The bench-scale sand columns used for this study were of a similar design to those used by Young-Rojanschi and Madramootoo (2014), which replicated the design of the CAWST V10 filter (CAWST 2012). The bench-scale column filters were constructed using 10 cm diameter polyvinyl chloride (PVC). The diffuser plate, with pore size of 2 mm, was located 2 cm above the resting water level. The maximum loading head for all treatments was 17 cm. The study consisted of sixteen filters: four

replicate bench-scale BSF columns (Figure 4.1 and 4.2) each with resting water depths of 2, 5, 8 and 11 cm.

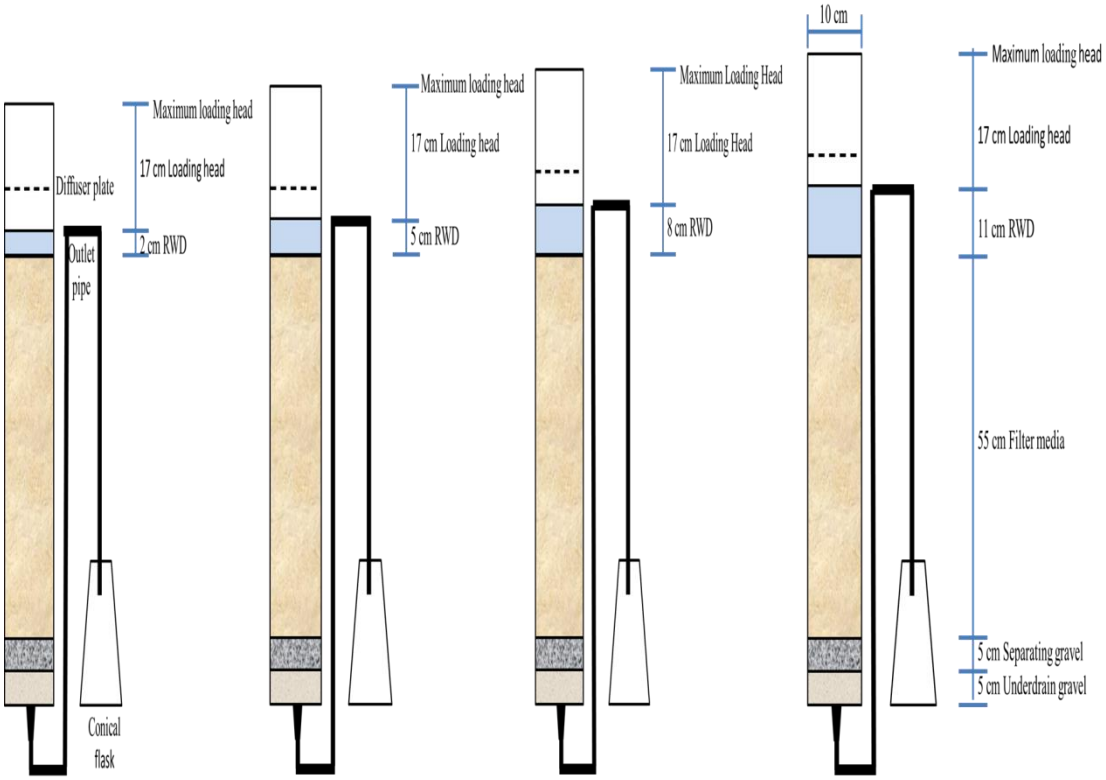

Figure 4.1: Schematic of bench-scale biosand filters with different resting water depths.

Figure 4.2: Bench-scale biosand filters used in the study with different resting water depths.

All filter media was washed with tap water and the sand was autoclaved at 121˚C for 20 minutes prior to use. The filter media was locally purchased silica sand, chosen for its high purity, low organic matter concentration and low uniformity coefficient. The sand comprising the filter beds was analysed using standard sieve analysis to determine the effective grain diameter and uniformity coefficient. To determine the effective grain diameter, 464 g (dry weight) was sieved and the cumulative weights of sand against the sieve mesh diameter were plotted. The media had a maximum diameter of 0.71 mm, an effective diameter (d_{10}) of 0.21 mm, a uniformity coefficient (UC) of 1.73 and a porosity of 0.38. The separating gravel had a diameter range of 3.15 – 5.6 mm and the underdrain gravel had a diameter of 10 mm. Filters were partially filled with water prior to loading the filter with underdrain gravel and sand to avoid trapping of air.

The dose volume of 1.6 l was approximately equivalent to the pore volume of the sand. All treatment groups were dosed once daily with 1.6 l or a total of 156.8 l over the 98 day duration of the study.

4.3.2 Feed water

Feed water for the filters was collected from the River Lee, Co. Cork at weekly intervals and stored in aerated containers at the ambient temperature of the filters until use.

4.3.3 Water quality measurements

Feed water was sampled prior to dosing the filters and were analysed immediately for dissolved oxygen (DO) using a Handy Polaris, OxyGuard® meter, while the temperature, pH and electrical conductivity (EC) were measured using a WTW multimeter 320i (WTW, Weilheim, Germany). Control samples were taken by collecting approximately 1 l of feed water in a sterile, loosely-capped, glass bottle, which was covered in black plastic and placed alongside the filters. The control sample was then analysed with the corresponding filtered water samples at each sampling event.

Filtered water samples were well-mixed composite samples that were collected in 3 l sterile glass flasks. The outlet tube of each filter was cleaned with a 70% ethanol solution prior to being placed in the sample flask, immediately after measuring the flow rate. Sampling flasks where left in place for two hours after charging the filters, by which time 85 – 100% of the water had filtered through. Flow rate was measured immediately after charging the filters, when the head water pressure was at its greatest, and the flow rate was therefore at its highest.

Feed and filtered water samples were analysed daily for pH, DO and EC. Weekly samples were collected to determine total oxidised nitrogen (TON), nitrite (NO_2^--N), orthophosphate, dissolved organic carbon (DOC) and spectral absorption coefficient (UV_{254}). Total oxidised nitrogen and nitrite were determined using automated flow injection analysis (Lachat Quickchem 8000, Zellweger Analytics, Inc. Milwaukee, USA), by the Aquatic Services Unit (ASU) at University College Cork. The nitrate (NO_2^--N) was determined by deducting the nitrite (NO_3^--N) concentration from the TON concentration. Dissolved organic carbon (DOC) analysis was also completed by the ASU using a total organic carbon analyser (TOC-VCPH/CPN SHIMADZU, Kyoto, Japan). Orthophosphate concentrations were determined using the ascorbic acid method (reference number: 4500-P E) (APHA *et al*., 2012), while UV_{254} was

measured using the method (reference number: 5910 B) outlined in APHA *et al*., (2012) using the Genesy 10 UV-Vis spectrophotometer (Thermo Electon Scientific Instrument LLC, Madison, WI, USA). Additionally, the total alkalinity of the feed water was determined from three grab samples of the feed water taken over the duration of the study. The total alkalinity was determined by the ASU using the titration method with 0.01 M EDTA. Weekly sampling was completed to determine total coliforms and *E. coli* loadings using the membrane filtration technique (reference number: 9222 D) as described in APHA *et al*., (2012), using CHROMagar™ Liquid ECC as the broth (CHROMagar, Paris, France). Plates were incubated at 37˚C for 18-24 hrs. The total suspended solids (TSS) concentration of the feed and filtered water was determined using the method (2540 D) outlined in APHA *et al*. (2012).

4.3.4 Schmutzdecke analysis

The metabolic function of the microbial community in the schmutzdecke of the filters was assessed using Biolog Ecoplates™ (Biolog Inc., Hayward, CA) at biweekly intervals. Cores with a 1 cm diameter were taken of the top 2 cm of the sand column. Subsamples (3 g wet weight) were taken from each core and were shaken at 270 rpm for 2 hours in 27 mL of phosphate buffer saline (PBS). Samples were then allowed to settle for 30 minutes. The supernatant was diluted with PBS until the optical density (OD) was approximately 0.06 measured at 420 nm using a Genesy 10 UV-Vis spectrophotometer (Thermo Electon Scientific Instrument LLC, Madison, WI, USA). Each well on the Biolog EcoPlates™ was inoculated with 150 µL per well of the diluted supernatant, and incubated at 25˚C. The OD at 590 nm was then measured using a multi-well plate reader (Model 680 XR, Microplate Reader, Bio-Rad laboratories, Inc) at 96 hrs.

At the end of the study further samples of the top 2 cm of the sand column were taken and dried overnight at 105˚C. A portion of the dried sample was taken to analyse the organic matter content using the loss-on-ignition (LOI) method (Dean Jr, 1974).

4.3.5 Schmutzdecke diversity and richness calculations

All functional diversity and richness indices were calculated using the 96 hr OD readings for each Biolog Ecoplate™. The species richness was calculated by counting the number of positive wells with an OD of greater than or equal to 0.06, after subtracting the OD of the control well. The functional diversity of the microbial community of the schmutzdecke was determined using the Shannon diversity index (H'). The Shannon diversity index was calculated as:

$$H' = - \sum p (\ln(p))$$

Where, p is the ratio of the relative absorbance value of each well on the Biolog Ecoplate™ plate to the sum of the absorbance value of the 31 wells.

Principle component Analysis (PCA) of the Biolog Ecoplate™ data was conducted using the Multi-Variate Statisitcal Package (Version 3.1, Kovach Computing Services) using the absorbance values at 96 hrs.

4.3.6 Statistical analysis

All statistical analysis was completed using IBM SPSS Version 22 (SPSS Inc., Chicago, USA). Normality was tested using the Shapiro-Wilk test, equal variance was tested using the Levees test for equal variance and outliers were determined by visual inspection of boxplots. One-way ANOVA was used to test for differences between sets of filters, when data conformed to the assumptions of the test. Non-normal data were analysed using the non-parametric Kruskal-Wallis test, and when appropriate pairwise comparisons were performed using the Dunn (1964) procedures with a Bonferroni correction for multiple comparisons. Correlations between water quality parameters (percentage removal) and filter age were examined using the Kendall's tau correlation coefficient. A paired sample t-test was used to compare control samples with the corresponding feed water samples. Control and feed water parameters that failed to meet the assumptions of the paired sample t-test were analysed using the non-parametric Signed Rank test.

4.4 Results

4.4.1 Filtration rate

The mean initial filtration rate at the start of the study, day 0, for the 2 cm, 5 cm, 8 cm and 11 cm RWD conditions were 0.51 ± 0.06 $m^3/m^2/hr$., 0.49 ± 0.06 $m^3/m^2/hr$, 0.47 ± 0.05 $m^3/m^2/hr$ and 0.46 ± 0.03 $m^3/m^2/hr$ respectively. A one-way ANOVA was conducted to determine whether the difference in mean filtration rate between the treatment groups was statistically different at day 0 of the study, but no significant difference was found ($F(3, 12) = 0.591$, p-value: 0.633). Similar declines in filtration rate occurred in the filters with different RWDs over the 98 days of operation (Figure 4.3). A Kruskal-Wallis test was run to determine whether the median filtration rate differed between the RWD conditions and a statistically significant difference was found (χ^2 (3) = 89.816, p-value < 0.001). The poc hoc analysis indicated a statically significant difference in the median filtration rate between the 11 cm RWD (0.45 $m^3/m^2/hr$) and the other three RWD conditions, 2 cm (0.48 m^3/m^2hr) (p-value < 0.001), 5 cm (0.48 $m^3/m^2/hr$) (p-value < 0.001) and 8 cm (0.47 $m^3/m^2/hr$) (p-value < 0.001).

Some schmutzdecke disturbance was observed when charging the 2 cm RWD filters, with sand particles becoming suspended during filter recharging. To a lesser extent some disturbance to the sand column was also observed at the 5 cm RWD, while no disturbance was observed at the higher RWD conditions.

4.4.2 Filtered water quality

A paired sample t-test was used to compare control samples with the corresponding feed water. Control samples had a lower DO (9.3 ± 0.3 mg/l) compared to the feed water (9.5 ± 0.3 mg/l) (p-value < 0.001). Also, the control had a lower pH (7.62 ± 0.29) compared with the feed water (7.68 ± 0.27) (p-value: 0.001) (Table 4.1). The paired sample t-test was also used to compare the nitrite concentration between the control (0.017 ± 0.011) and feed water (0.016 ± 0.010) (p-value 0.031). No statistically significant differences were found between the feed and control water for nitrite and TON. Nitrate, orthophosphate, UV_{254}, TSS and EC failed to meet the assumption of the paired sample t-test and hence were analysed using the non-parametric sign rank test, but no statistical differences were found for these parameters between the control samples and the corresponding feed water with the

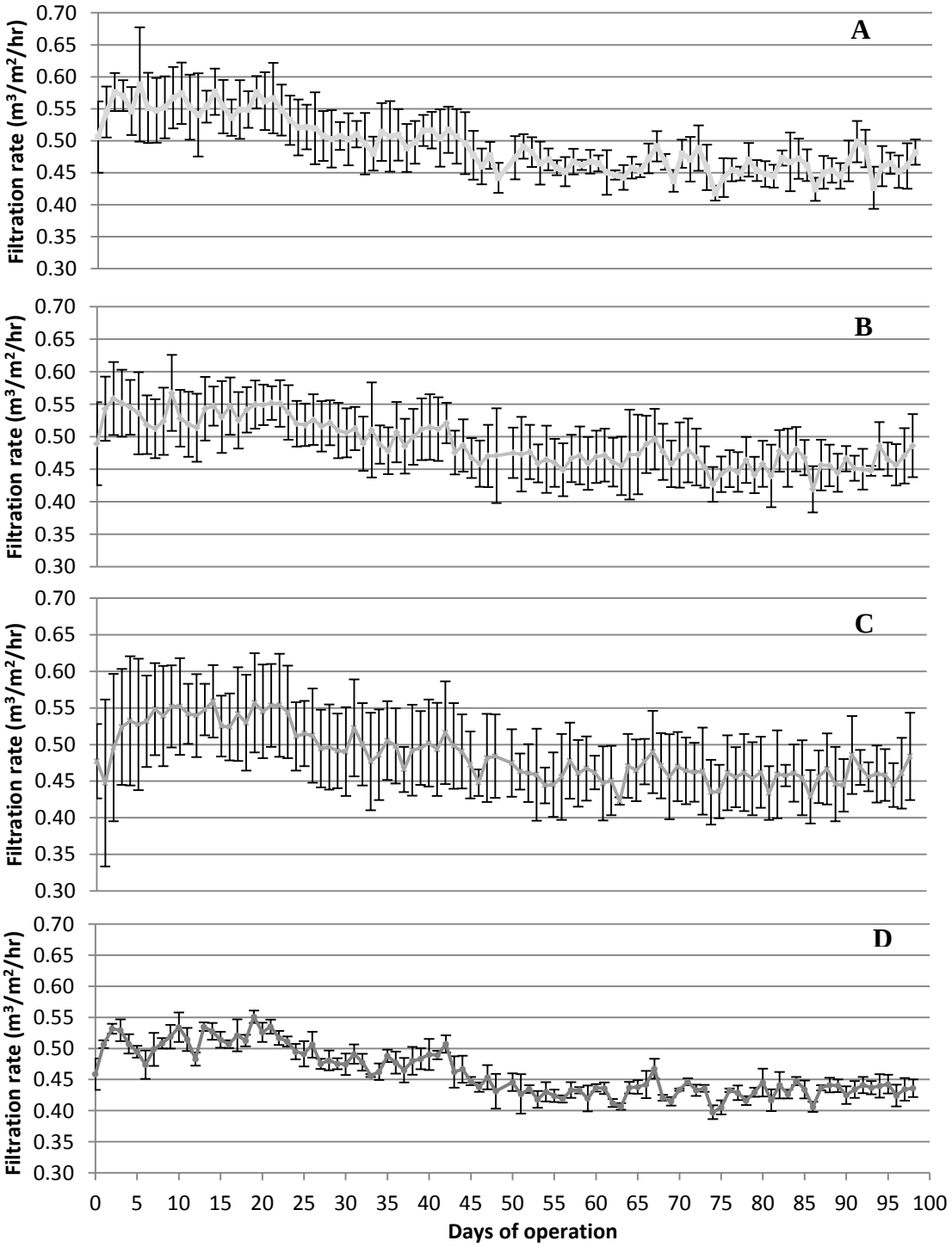

Figure 4.3: Changes in mean filtration rate at four resting water depth (RWD) conditions (A = 2 cm RWD, B = 5 cm RWD, C = 8 cm RWD, and D = 11 cm RWD) in bench-scale biosand filters. Error bars represent standard deviation (n = 4).

exception of electrical conductivity (p-value: 0.001). The *E. coli* and total coliform concentration (CFU/100 ml) also failed to meet the assumptions of the paired sample t-test and were analysed using the non-parametric sign test. A statistically significant difference was found between the median total coliform concentration in the feed water (3317 CFU/100 ml) and the control (3188 CFU/100 ml) (p-value: 0.030), while no statistical difference was found between the median *E. coli* concentration in the feed water (195 CFU/100 ml) and the control (175 CFU/100 ml) (p-value: 0.115). The DOC of the feed water was determined from thirteen samples taken over the duration of the study and was 5.1 ± 0.7 mg C/l. The alkalinity was determined from three grab samples taken from the feed water during the study and was found to be 56.7 ± 13.7 mg/l as $CaCo_3$.

The filtered water for all four RWD conditions (2 cm, 5 cm, 8 cm and 11 cm) met all drinking water quality parameters set out in the WHO Guidelines for Drinking-Water Quality, 4th Edition (WHO 2011), with the exception of total coliform and *E. coli* concentration of 0 CFUs/100mls (Table 4.1). Some variability in total coliform removal was observed in the early stages of filter operation, but after day 20 all treatment groups consistently achieved greater than 90% removal (Figure 4.4). There was no significant difference in total coliform and *E. coli* removal between the four RWDs (χ^2 (3) = 2.410, p-value: 0.493; χ^2 (3) = 0.753, p-value: 0.861, respectively). A positive correlation was found between the total coliform removal and filter age, however a significant positive correlation was only found at the higher RWDs of 8 cm and 11 cm (Table 4.2). A negative correlation was found between *E. coli* removal and filter age for all four RWDs (Table 4.2).

There was no significant difference in filtered water quality between the four RWDs for pH, TON, NO_2^--N, NO_3^--N, PO_4^{3-} and UV_{254}. Dissolved oxygen (DO) consumption occurred across all treatment groups, with the highest average DO consumption occurring in the filters with the lower RWD (2 cm and 5 cm) (Table 4.1). The Krushal-Wallis tested show the median filtered water DO concentration was statistically different between the 2 cm RWD (8.6 mg/l) and the 11 cm RWD (8.7 mg/l) (p-value: 0.020). The median EC of the filtered water from the 2 cm RWD filter (181.4 µS/cm) was statistically different from the 8 cm RWD filter (179.5 µS/cm) (p-value: 0.033). Across all treatment groups, the filter water had a lower pH compared to the feed water (Table 4.1). A significant positive correlation was found

between filter age and percentage removal of total oxidized nitrogen (TON), nitrate and nitrite for all treatment groups (Table 4.2).

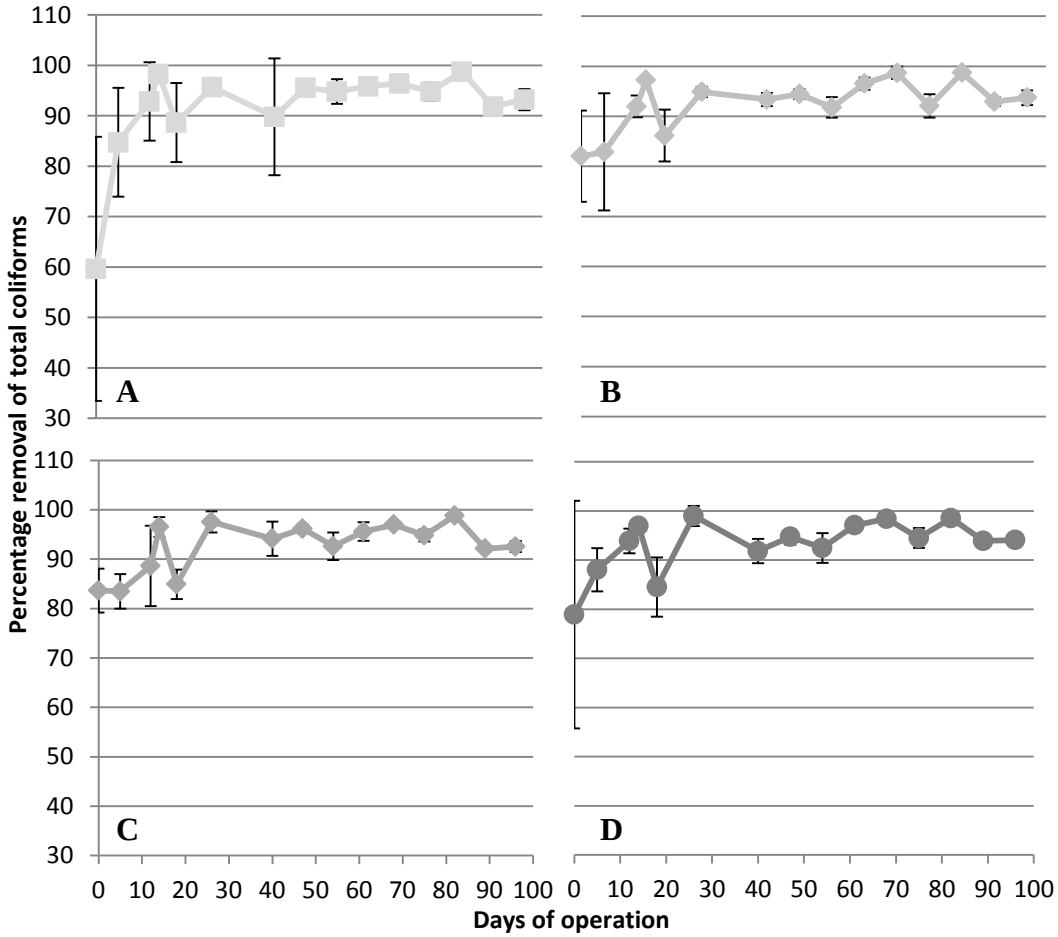

Figure 4.4: Mean percentage removal of total coliforms with filter age for different four resting water depth (RWD) conditions (A = 2 cm RWD, B = 5 cm RWD, C = 8 cm RWD, and D = 11 cm RWD) in bench-scale biosand filters. Error bars represent standard deviation, n = 4.

Table 4.1: Summary of the physical, chemical and biological properties of the feed water, control and filtered water from biosand filters with different RWDs (mean ± standard deviation, n = 4)

Parameter	Number of samples	Feed water	Control	2 cm RWD	5 cm RWD	8 cm RWD	11 cm RWD
Total coliform removal (%)	14 – 15		9.9 ± 17.2	93.6 ± 5.3	93.8 ± 4.1	92.5 ± 5.7	93.9 ± 4.4
***E. coli* removal (%)**	14 – 15		7.4 ± 33.4	94.2 ± 7.8	94.1 ± 7.3	93.8 ± 7.6	94.4 ± 7.7
Electrical conductivity (µS/cm)	98	167.7 ± 13.2	168.8 ± 12.6	179.1 ± 9.8	178.4 ± 10.5	177.4 ± 9.9	177.9 ± 11.7
pH	98	7.68 ± 0.27	7.62 ± 0.29	7.51 ± 0.27	7.52 ± 0.26	7.49 ± 0.27	7.52 ± 0.25
Dissolved oxygen (mg/L)	98	9.5 ± 0.3	9.3 ± 0.3	8.6 ± 0.5	8.6 ± 0.5	8.7 ± 0.4	8.7 ± 0.4
Nitrate – N (mg/L)	13	1.622 ± 0.356	1.623 ± 0.350	1.635 ± 0.341	1.632 ± 0.339	1.654 ± 0.325	1.653 ± 0.327
Nitrite – N (mg/L)	13	0.016 ± 0.010	0.017 ± 0.011	0.010 ± 0.014	0.012 ± 0.015	0.011 ± 0.018	0.008 ± 0.012
Orthophosphate (mg/L)	14	0.016 ± 0.027	0.016 ± 0.026	0.012 ± 0.003	0.012 ± 0.005	0.012 ± 0.003	0.011 ± 0.002
UV_{254}	14	0.200 ± 0.042	0.194 ± 0.044	0.154 ± 0.030	0.157 ± 0.032	0.156 ± 0.031	0.158 ± 0.033

Table 4.2: Kendall's tau correlation of filter age and percentage removal for water quality parameters at different resting water depths in biosand filters

Parameter	2 cm RWD	5 cm RWD	8 cm RWD	11 cm RWD
Coliforms	0.053 (0.0.567)	0.167 (0.076)	0.265** (0.003)	0.270** (0.003)
E. coli	-0.310** (0.001)	-0.085 (0.368)	-0.168 (0.070)	-0.020 (0.830)
TON	0.325** (< 0.001)	0.420** (< 0.001)	0.451** (< 0.001)	0.346** (0.001)
Nitrate - N	0.197* (0.047)	0.340** (0.001)	0.374** (< 0.001)	0.333** (0.001)
Nitrite – N	0.608** (< 0.001)	0.634** (< 0.001)	0.604** (< 0.001)	0.599** (< 0.001)
PO_4^{3-}	0.059 (0.539)	0.044 (0.640)	0.122 (0.197)	0.004 (0.966)
UV_{254}	-0.207* (0.028)	-0.211* (0.025)	-0.156 (0.097)	-0.179 (0.057)
TSS	-0.249* (0.011)	-0.207* (0.037)	-0.129 (0.186)	-0.196 (0.053)
DO	-0.202** (< 0.001)	-0.202** (< 0.001)	-0.278** (< 0.001)	-0.205** (< 0.001)

Correlation coefficient and p-value in parentheses
Blue denotes positive correlations
Red denotes negative correlations
*correlation is significant at the 0.05 level (2-tailed)
** correlation is significant at the 0.01 level (2-tailed)
DO: dissolved oxygen, TON: total oxidised nitrogen, TSS: total suspended solids, UV_{254}: spectral absorption coefficient.

4.4.3 Schmutzdecke changes with filter maturation

Changes in the microbial community of the schmutzdecke were assessed at biweekly intervals using Biolog Ecoplates™. The average well colour development (AWCD), which indicates the metabolic activity of the microbial community, increased rapidly for the schmutzdecke community during the first 10 days of filter operation and reached a relatively stable level after day 30 for all four RWDs (Figure 4.5). All RWDs displayed similar metabolic microbial activities on all days, with no significant difference between RWD conditions (F = 2.605, p-value: 0.056).

The diversity indexes (Table 4.3), based on the diversity of the carbon sources utilised on the Biolog Ecoplates™ by the schmutzdecke forming microorganisms, indicate changes did occur with filter age for all RWDs. The Shannon Diversity Index (H') increased rapidly at the initial stages of the filter maturation, for all RWDs, up until day 28 when the H' index reached a relatively stable level for the

remaining period of filter operation. A similar diversity index was recorded for all four RWD conditions (λ^2 = 2.014, p-value: 0.569). The Richness index also reached a relatively stable level by day 28 and no statistical difference was found between the metabolic functional richness of the microbial community in the schmutzdecke of the four different RWD conditions (λ^2 = 3.904, p-value: 0.272).

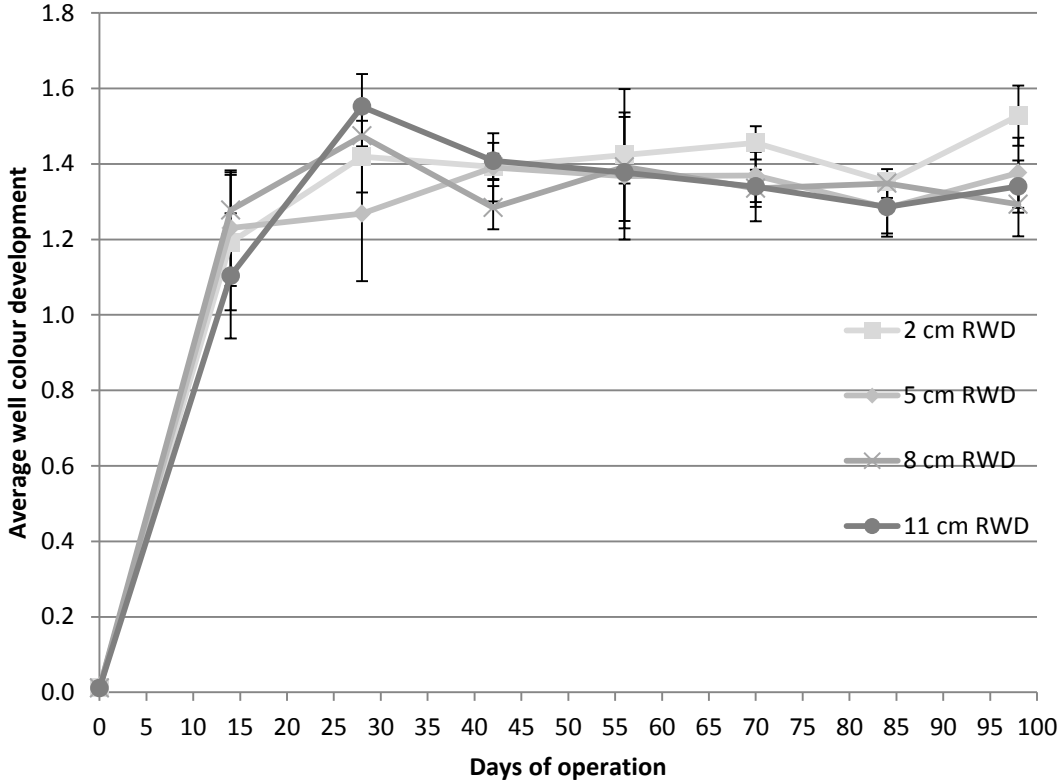

Figure 4.5: Metabolic activity of the microbial community in the schmutzdecke represented by the average well colour development (AWCD) of the different resting water depth (RWD) conditions. Error bars represent the standard deviation of replicate samples (n = 4).

Table 4.3: Mean functional diversity of microbial communities in the schmutzdecke of biosand filters under different resting water depth (RWD) conditions (mean ± standard deviation, n = 4).

Days of operation	Shannon Diversity Index (H')				Richness Index			
	2 cm	5 cm	8 cm	11 cm	2 cm	5 cm	8 cm	11 cm
14	3.24 ± 0.06	3.27 ± 0.04	3.23 ± 0.08	3.23 ± 0.03	29.25 ± 1.50	30.00 ± 0.82	29.75 ± 1.20	29.75 ± 1.89
28	3.15 ± 0.03	3.13 ± 0.02	3.17 ± 0.02	3.14 ± 0.02	30.00 ± 0.00	28.75 ± 2.22	30.75 ± 0.50	31.00 ± 0.00
42	3.14 ± 0.02	3.14 ± 0.02	3.13 ± 0.06	3.14 ± 0.03	29.75 ± 0.96	30.75 ± 0.50	29.75 ± 0.50	30.50 ± 0.58
56	3.14 ± 0.03	3.12 ± 0.03	3.15 ± 0.02	3.16 ± 0.02	30.25 ± 0.96	29.75 ± 1.26	30.00 ± 0.00	30.25 ± 0.50
70	3.16 ± 0.01	3.15 ± 0.03	3.13 ± 0.04	3.10 ± 0.07	30.25 ± 0.50	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00
84	3.14 ± 0.01	3.10 ± 0.03	3.12 ± 0.02	3.08 ± 0.06	29.75 ± 0.50	29.25 ± 0.96	29.50 ± 0.58	29.00 ± 0.82
98	3.15 ± 0.05	3.11 ± 0.04	3.12 ± 0.04	3.10 ± 0.07	30.50 ± 0.58	29.50 ± 0.58	28.75 ± 1.26	30.50 ± 1.00

As seen in Figure 4.6 the time profiles for the metabolic functions of the microbial communities of the schmutzdecke for the four different RWDs display clear shifts at different stages in filter maturation. The PCA reveals that the metabolic function of the microbial communities under the different RWDs show some similarities at the initial stages of schmutzdecke development, and move towards a more distinct metabolic function diversity between the different RWD conditions. There is evidence of some variation between replicates, as replicates tend not to cluster.

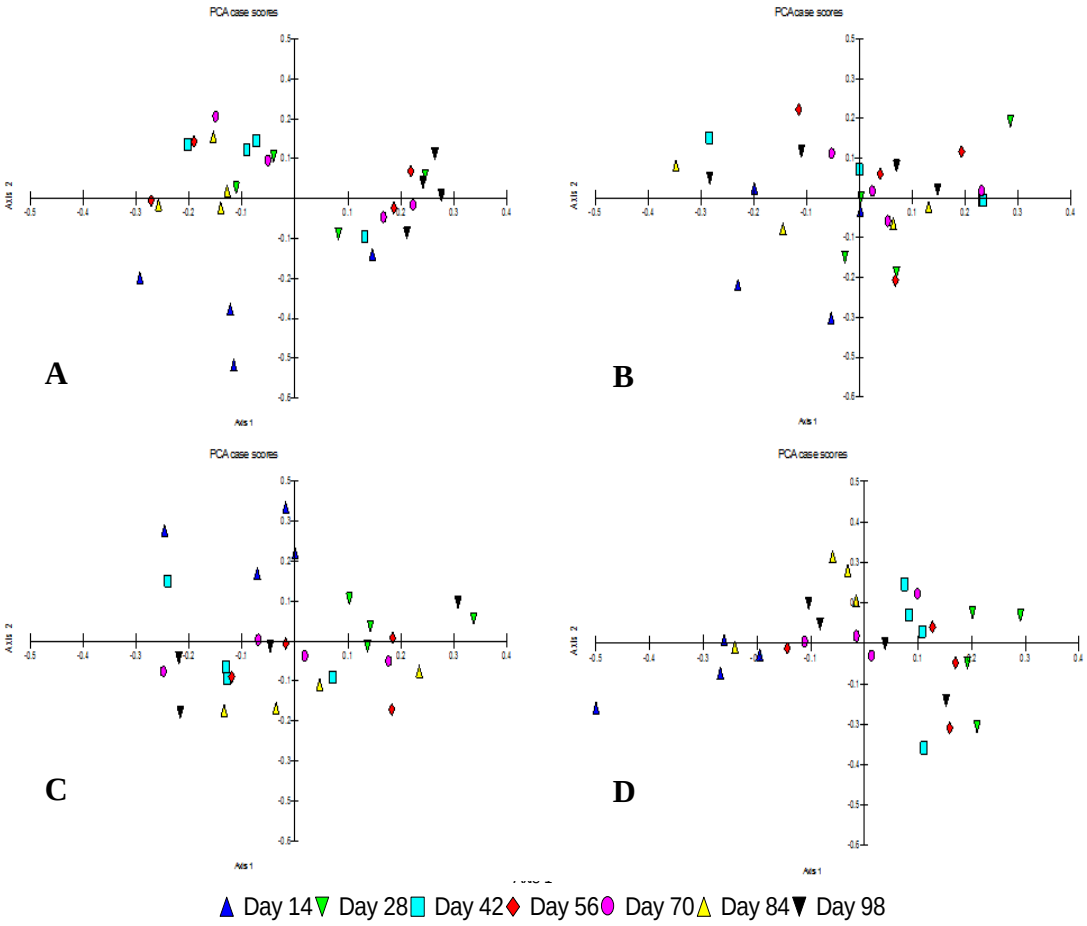

Figure 4.6: Principle component analysis (PCA) of the Biolog Ecoplate^{TM} optical density readings for the four different resting water depth conditions (A = 2 cm RWD, B = 5 cm RWD, C = 8 cm RWD, and D = 11 cm) in bench-scale biosand filters

After 98 days of filter operation, sand samples were taken to compare the accumulation of organic matter in the schmutzdecke of the four different RWD conditions. A gradual increase in organic matter occurred with increased resting water depth. The percentage organic matter in the top 2 cm of the sand column in the 2 cm RWD (0.0110% ± 0.0019), 5 cm RWD (0.0111% ± 0.0009), 8 cm RWD (0.0160% ± 0.0095) and 11 cm RWD (0.0194 ± 0.0068) was higher at the higher RWD conditions. A one-way ANOVA was conducted, but the difference in mean percentage LOI for the four different resting water depth conditions was not statistically significant ($F(3, 12) = 1.89$ p-value: 0.185).

4.5 Discussion

It is evident from this study that RWD is not an influential factor on the performance of the bench-scale BSFs with no significant difference in total coliform and *E. coli* removal at RWD conditions of between 2 and 11 cm. However, all filters were operated with resting periods of 24 ± 1 hr and therefore it is not possible to predict whether a multi-day resting period would influence the performance of the filter under different RWD conditions. Young-Rojanschi and Madramootoo (2015) assessed the impact of multi-day resting periods on filter performance with RWDs of 5 cm and concluded that resting periods of up to three days was not deterimental to filter performance. As the resting period in this study was 24 ± 1 hr it is likely that the microbial community in the schmutzdecke had not yet exhausted the dissolved oxygen and nutrients in the feed water between charge events. A study of the influence of multi-day resting periods at higher RWDs on filter performance would further improve the understanding and optimisation of BSFs.

All RWD conditions achieved an average removal of > 90% for total coliforms and *E. coli* over the duration of the study. These reductions are comparable with ealier laboratory studies which have reported microbial (faecal coliform, *E. coli*) reductions of between 93% and 99% (Buzunis 199; Elliott *et al*. 2008; Palmateer *et al*. 1999; Stauber *et al*. 2006). In field studies, a reduced removal performance of microbial indicator organisms (faecal coliforms, *E. coli*) of between 60% and 99.9% has been reported (Duke *et al*. 2006; Earwaker 2006; Lee 2001; Stauber *et al*. 2006; Vanderzwaag *et al*. 2009). A positive correlation was found between total coliform removal and filter age, with the removal performance of total coliforms increasing

with filter age. However, a negative correlation was found between *E. coli* percentage removal and filter age for all four RWDs. This inverse relationship was unexpected because previously Stauber *et al*. (2006) had reported increased *E. coli* reduction with filter age.

Young-Rojanschi and Madramootoo (2014) observed some disturbance to the sand column during filter recharging with a RWD of 5 cm and suggested increasing the RWD to avoid disturbance to the schmutzdecke during filter recharging. This study supports this recommendation as an increased RWD was found to provide greater protection to the schmutzdecke, with no disturbance to the sand column being observed during filter recharging at 8 cm and 11 cm RWDs. This avoided disturbance to the sand column might be a contributing factor to the higher percentage organic matter in the top 2 cm of the sand column at the 11 cm RWD. This higher organic matter content at the highier RWD is likely to have contributed to the significantly higher decline in filtration rate observed with 11 cm RWD in comparison with the lower RWDs tested. The higher accumulation of organic matter would reduce pore space, impeding the flow of water, hence reducing the filtration rate.

The schmutzdecke is the zone in the slow sand filter with the highest biological activity (Unger and Collins 2008) and therefore expected to have the highest dissolved oxygen consumption, which was confirmed by Young-Rojanschi and Madramootoo (2015). CAWST (2012) suggested that RWDs should be maintained at a moderate level of 5 cm, but not greater than 6 cm. However, Young-Rojanschi and Madramootoo (2014) found that anoxic conditions occured in the upper sand column layer at the end of a 24 hr residence period with only a 5 cm RWD. Further studies are required to examine the oxygen profile at the sand water interface over the daily filtration cycle and during extended (multi-day) filtration cycles. This would help to determine whether the biological community derives sufficient oxygen from the feed water and the resting water during the filter resting period, or whether the biological community relies on the transfer of oxygen across the RWD to the schmutzdecke during a multi-day residence period.

The maturation period of BSFs is poorly understood, with a number of researchers suggesting the duration for the maturation period to range from two weeks (Jenkins *et al*. 2011) to 30 days (Baumgartner *et al*. 2007). This study observed marked changes in filter performance for the removal of microbial indicator organisms, as well as changes in physicochemical properties of the filter water, in comparison to the feed water, after 20 days of filter operation. While this current study observed slight increases in filtered water nitrate concentration, the nitrite concentration decreases significantly compared with the feed water, indicating that some nitrification/denitrification was occuring. Murphy *et al*. (2010) study in Cambodia suggested that nitrification/denitrification was occurring in BSFs. To determine the true extent of nitrification/denitrification future studies would benefit from the inclusion of ammonium measurements. However, the nitrate and nitrite concentration of both the feed water and the filtered water for all treatment groups remained well below the WHO Drinking-Water Guideline Values of 11 mg/L NO_3^--N and 0.9 mg/L NO_2^--N (WHO, 2011).

For the purpose of this study, the sand column depth was maintained the same (55 cm) for all RWD conditions. In full-scale filters, adjusting the RWD could only be achieved by adjusting the sand column depth or by adjusting the height of the outlet pipe, both of which would affect the maximum loading head of the filter. A reduced sand column depth would affect both the pore volume and filtration rate. With a reduced pore volume, a reduced volume of water would have to be charged to the filter per filteration cycle in order to maintain the recommended charge-to-pore volume ratio of 1:1 or less (CAWST 2012). If the sand column depth is maintained in full-scale BSFs but the RWD is changed by adjusting the height of the outlet pipe, the loading head of the filter would be reduced which would affect the filtration rate.

4.6 Conclusions and recommendations

The performance of bench-scale BSFs was not found to be related to the RWD. All RWDs tested experienced a decline in filtration rate with filter age, with the highest filtration rate decline occuring at the highest RWD. The greater protection provided to the schmutzdecke by a higher RWD is likely to contribute to greater accumulation of organic matter and reduced filtration rate. The similarity in metabolic functional

diversity indicated that the higher RWD did not affect the community diversity of the schmutzdecke. Further studies required to determine the oxygen profile through the RWD to determine whether an increased RWD leads to increasing oxygen stress at the sand surface or whether the increased volume of water provides sufficent oxygen and nutrients to the schmutzdecke. This might help to determine the maxiumum resting period that would not result in premature die off (residence period > 24 hrs) of the microbial community of the schmuzdecke.

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Chapter 5

The impact of operating temperature on the retention and removal of faecal bacteria by biosand filters

5.1 Abstract

Biosand filters are used in many different countries under very different climatic conditions. The aim of this study was to determine the impact of ambient temperature on the performance of the filter in removing a microbial indicator organism and on the development of the microbial community in the filter. Bench-scale filter columns were operated at constant temperatures of 10 ± 1˚C and 26 ± 1˚C. Feed and filtered water was tested for a number of parameters including, pH, dissolved oxygen (DO), electrical conductivity (EC), total oxidised nitrogen (TON), nitrite, orthophosphate, dissolved organic carbon (DOC), UV_{254} and *E. coli* colony forming units. Changes in the metabolic activity and diversity of the schmutzdecke were determined using Biolog Ecoplates™. Filters operated at the lower temperature achieved a higher *E. coli* $\log_{10}$ reduction when compared to the filters operated at 26˚C (3.39 $\log_{10}$ versus 2.73 $\log_{10}$). Filtered water from the 26˚C filters had a lower nitrate and nitrite concentration and higher DO consumption when compared with filters operated at the lower temperature. The filters operated at the lower temperature also had a higher retention potential, i.e. *E. coli* were retained in the filter for a longer period before being released.

5.2 Introduction

Access to safe drinking water is an important public health and development issue for both developed and developing nations. Currently, 663 million people remain without access to an improved drinking water source (UNICEF and WHO, 2015), and the consumption of contaminated drinking water results in approximately 502,000 diarrheal deaths each year (WHO, 2015). Target 6.1 of the Sustainable Development Goals (SDGs), which were agreed in 2015 as the global agenda for 2030, builds on the success of the Millennium Development Goals and provides a renewed focus on safe drinking water by aiming to '*achieve universal and equitable access to safe and affordable drinking water for all'* by 2030 (UN, 2015). To help achieve this target, point-of-use (POU) drinking water treatment and safe storage technology may offer a real interim solution, giving people without access to a safe, piped drinking water source a method to treat drinking water domestically (Sobsey, 2002). There is evidence in recent studies that household interventions are linked to a reduced risk of diarrheal disease outbreaks (Clasen *et al*., 2007; Fewtrell *et al*., 2005). Biosand filters (BSFs) are a POU technology which is affordable (Loo *et al*., 2012) and provided the difficulties in the distribution of BSFs to rural isolated locations are overcome they can be made accessible to a large proportion of the world population who remain without access to safe drinking water (Mahmood *et al*., 2011).

A number of field studies have assessed the performance of BSFs in relation to the removal of pathogenic microorganisms. Such studies have reported removal performances to range from 87% - 98.5% (Mahmood *et al*., 2011; Klopfenstein *et al*., 2011; Fiore *et al*., 2010; Duke *et al*., 2006; Earwaker, 2006, Stauber *et al*., 2006). Similarly, laboratory-based studies have assessed the performance of BSFs in the removal of *E. coli* and found removal rates to range from 90 – 99% (Elliott *et al*., 2008; Stauber *et al*., 2006). The reported variations in removal efficiencies for *E. coli* suggest that further studies are needed to understand the mechanisms contributing to the removal of pathogenic microorganisms in BSFs.

Determining the fate of potential pathogenic microorganisms in BSFs is fundamental in understanding how the design and/or operation of the filter could potentially be adjusted to optimise filter performance. The mechanisms contributing to the removal

of pathogenic microorganisms by slow sand filters have been attributed to mechanical processes (WeberShirk and Dick, 1997; Huisman and Wood, 1974; Bellamy *et al*., 1985) and to naturally occurring biological processes in the filter, including predation and bio-oxidation (Ellis and Wood, 1985; Huisman and Wood, 1974; Bahgat, 1999; Fogel *et al*., 1993). However, the biological processes that occur in a BSF have not been verified or comprehensively studied to date. Marine and terrestrial studies have shown that microbial reduction is influenced by top-down mechanisms (predation by protozoa and viral lysis) and bottom-up mechanisms (nutrient availability) (Hunter and Price, 1992; Lloyd, 1973; Pace and Cole, 1994; Rosemond *et al*., 2001; Weber-Shirk and Dick, 1999). A study by Haig *et al*. (2015) showed that the removal of *E. coli* in slow sand filters (SSFs) was primarily by top-down trophic interactions with protozoa being the main *E. coli* grazers, but viral lysis was also found to be contributing factor to *E. coli* removal. Gaining insight into the fate of pathogens in BSFs at different operating temperatures will enable the filter distributors to provide training to BSF users on how to optimise filters during varying seasonal temperatures. It will also allow filter distributors to provide advice on the suitability of secondary treatment such as solar disinfection (SODIS) or chlorination during periods of low or high ambient temperature when filter performance may be affected.

The aim of this current study on BSFs was to determine (i) the removal and the delayed breakthrough (retention potential) of *E. coli* under two different ambient temperatures and (ii) the effect of ambient temperature on the functional activity and diversity of the microbial community in the schmutzdecke and deep within the sand column.

5.3 Materials and Methods

5.3.1 Bench-scale filters

The bench-scale filters used for this study were of a similar design to that used by Young-Rojanschi and Madramootoo (2014). The filters were constructed using 10 cm diameter polyvinyl chloride (PVC) piping (Figure 5.1 and 5.2). Three filters were operated in a constant temperature room at 10 ± 1 ˚C and a further three filters were in a different constant temperature room at 26 ± 1 ˚C. Sampling ports were installed at four different locations on the filter; 1 cm above the sand surface and 5 cm, 10 cm

and 50 cm below the sand surface (Figure 5.1). All bench-scale filters were maintained in dark conditions throughout the study, except for brief periods during filter charging and sampling.

The filter medium used in the study was locally purchased silica sand, which was selected for its high purity and low organic matter content. All sand was washed and autoclaved at 121 ˚C for 20 mins before use. The sand comprising the filters beds was analysed using standard sieve analysis to determine the effective grain diameter and uniformity coefficient. To determine the effective grain size, 732 g (dry weight) was sieved and cumulative weight of the sand against the sieve mesh diameter were plotted. The sand had a porosity of 0.40, and maximum grain size of < 0.7 mm, effective diameter (d_{10}) of 0.23 mm and a uniformity coefficient (UC) (calculated by dividing d_{60} by d_{10}) of 1.70. The two underdrain layers were also locally purchased. The separating gravel had a grain size range of 3.15 – 5.6 mm and the underdrain gravel had a grain size of 10 mm. All underdrain gravel was washed prior to use in the study. Filters were partially filled with water prior to loading the underdrain gravel and sand to avoid trapping of air.

The daily charge volume for all filters was equivalent to approximately that of the media pore volume. Both sets of filters were dosed once daily with 1.4 l which was equivalent of 92.4 l over the 66 day duration of the study. The filters were charged by pouring the water into the filters to a maximum loading head of 17 cm. The initial force of water was dissipated by the diffusor plate (which had 2 mm diameter holes) which was located 2 cm above the resting water level, hence minimizing disturbance to the sand column during filter charging.

5.3.2 Feed water

Feed water was collected from the River Lee, in County Cork at weekly intervals and stored in aerated containers until use. Daily charge volumes for both temperature treatments were placed in each constant temperature room overnight to ensure that feed water temperature equilibrated with that of the filters, hence avoiding any temperature shock to the filters. Feed water was spiked daily with *E. coli* (ATTC # 11303) to increase the feed water *E. coli* concentration. Overnight cultures of *E. coli* were grown at 37˚C in tryptic soy broth and the stock was diluted using autoclaved feed water into daily aliquots for 7 days and stored at 4˚C until use. The *E. coli*

(ATTC # 11303) was used to spike the feed water to both temperature treatments on all days of the study, with the exception of day 46. On day 46 of the study the feed water was not spiked with the *E. coli* (ATTC # 11303) strain, but instead the feed water was spiked with antibiotic resistant (Kanamycin) *E. coli* (pGFPGUSPlus was a gift from Claudia Vickers (Addgene plasmid # 64401)) (Vickers *et al*., 2007), to the same concentration as the *E. coli* (ATTC # 11303) strain in order to determine the retention of *E. coli* in BSFs.

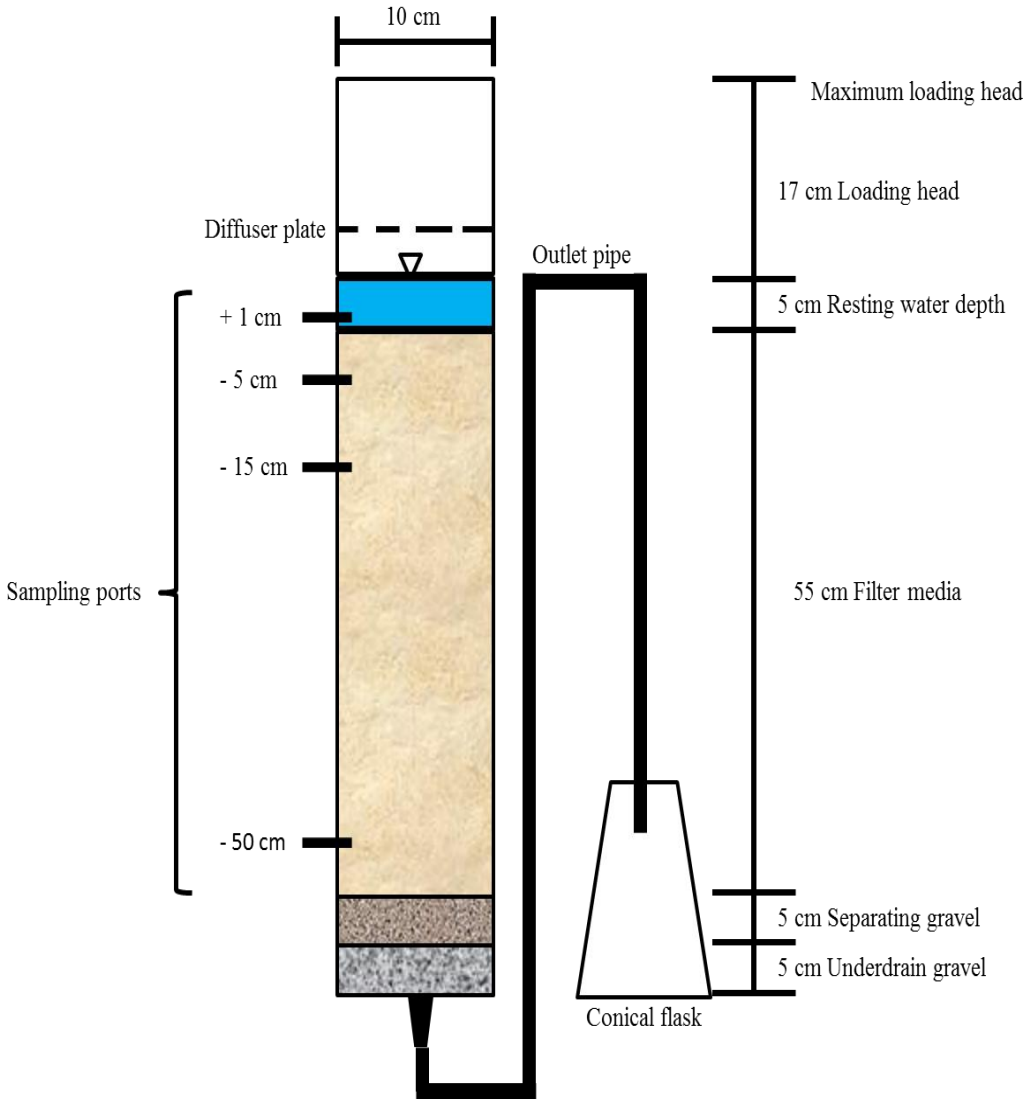

Figure 5.1: Schematic of bench-scale biosand filters used in the study.

Figure 5.2: Photograph of the bench-scale filters using in the study.

5.3.3 Water sampling and analysis

Water samples from each filter were collected daily in sterile conical flasks. The outlet tube of the filter was cleaned with a 70% ethanol solution prior to placing the tube into the flask. The conical flask was left in place for 2 hours after charging the filter, at which point 90 – 100% of the charge volume had filter through.

As the residence period for the filter was 24 ± 1 hrs, the feed water was analysed the day before the filtered water samples. The feed water samples were analysed immediately for pH, temperature, dissolved oxygen (DO), electrical conductivity (EC) and *E. coli*. Feed samples for dissolved organic carbon (DOC), Nitrite (NO_3^--N), total oxidised nitrogen (TON), UV_{254}, total suspended solids (TSS) and Orthophosphate (PO_4^{3-}) analyses were stored at 4˚C and analysed the next day with the corresponding filtered water samples. Total oxidised nitrogen and nitrite analyses were completed by the Aquatic Services Unit (ASU) at University College Cork using automated flow injection analysis (Lachat Quickchem 8000, Zellweger Analytics, Inc. Milwaukee, USA). The nitrate concentration was determined by

deduction of the nitrite concentration from the TON concentration. The ASU also carried out the DOC analyses using a total organic carbon analyser (TOC-VCPH/CPN, SHIMADZU, Kyoto, Japan). Analysis of the feed water to determine the total alkalinity was completed by the ASU using the titration method with 0.01 M EDTA. Orthophosphate concentration was determined using the ascorbic acid method (reference number: 4500-P E) and the UV_{254} was measured using the method (reference number: 5910 B) outlined in APHA *et al*., (2012) using the Genesys 10 UV-Vis spectrophotometer (Thermo Electon Scientific Instrument LLC, Madison, WI, USA). The specific ultraviolet absorbance (SUVA) was calculated from the ratio between DOC and $UV_{254.}$ Total suspended solids for the feed and filtered water were analysed using method 2540 D given in APHA *et al*. (2012). The *E. coli* concentration of the feed and filtered water were analysed using the membrane filtration technique (reference number: 9222 D) (APHA *et al*., 2012) using CHROMagar^{TM} Liquid ECC as the broth (CHROMagar, Paris, France) and plates were incubated at 37˚C for 18-24 hrs.

On day 46, after the filters had been charged with feed water spiked with antibiotic resistant (Kanamycin) *E. coli* (pGFPGUSPlus was a gift from Claudia Vickers (Addgene plasmid # 64401) (Vickers *et al*., 2007), water samples were taken from each sampling ports (-1 cm, 5 cm, 15 cm and 50 cm) to develop a profile of *E. coli* concentrations with depth, and also to determine changes in *E. coli* concentration during the filter residence period. Samples were taken from each sample port at 10 hrs and 24 hrs after charging the filters. The concentration of the antibiotic resistant (Kanamycin) *E. coli* in the filtered water was determined for twenty days after the initial introduction on day 46. Water samples were analysed for *E. coli* using the membrane filtration technique (reference method: 9222 D) (APHA *et al*., 2012), using CHROMagar^{TM} Liquid ECC as the broth (CHROMagar, Paris, France). In order to get direct counts of the Addgene plasmid (# 64401), kanamycin (50 ug/ml) was added to the CHROMagar^{TM} liquid ECC. Plates were incubated at 37˚C for 18-24 hrs. *E. coli* reductions at the sample depths were determined by subtraction of the $\log_{10}$ *E. coli* per 100 ml of the filtered water concentration from the $\log_{10}$ *E. coli* per 100 ml of the feed water concentration (Elliott *et al*., 2008).

5.3.4 Schmutzdecke development

Changes in the schmutzdecke microbial community were determined using community level physiological profiling (CLPP) using Biolog Ecoplate™ (Biolog Inc., Hayward, CA). Cores with 1 cm diameter were taken from the top 2 cm of the sand column. Subsamples (3 g wet weight) were shaken at 270 rpm in 27 ml of phosphate buffer saline for 2 hrs. Samples were then allowed to settle for 30 mins and the supernatant was gradually diluted with phosphate buffer to achieve an optical density (OD) of approximately 0.06 at 420 nm measured using a Genesy 10 UV-Vis spectrophotometer (Thermo Electon Scientific Instrument LLC, Madison, WI, USA). Each well of the Biolog Ecoplate™ was subsequently inoculated with 150 µl of the diluted supernatant and the plates were incubated at 25˚C and OD at 590 nm was measured after 96 hours using a multi-well plate reader (Model 680 XR, Microplate Reader, Bio-Rad laboratories, Inc).

At the end of the study sand samples were taken from three samples ports (5 cm, 15 cm and 50 cm) to assess changes in the functional diversity of the microbial community with sand column depth. The sand samples taken at depth were subject to the same processing techniques as taken from the top 2 cm of the sand column at biweekly intervals using Biolog Ecoplates™.

At the end of the study samples were also taken of the top 2 cm of the sand column to determine the organic matter concentration using the loss-on-ignition (LOI) method (Dean Jr, 1974). Samples were dried over night at 105˚C and the organic matter content was then determined using an ignition temperature of 550˚C (Dean Jr, 1974).

5.3.5 Schmutzdecke diversity and richness calculations

Functional diversity and richness indexes were calculated using the 96 hr OD readings for each Biolog Ecoplate™. The species richness was calculated by counting the number of positive wells with an OD of greater than or equal to 0.06, after subtracting the OD of the control well. The functional diversity of the microbial community of the schmutzdecke was determined using the Shannon diversity index (H'). The Shannon diversity index was calculated as:

$$H' = - \sum p (\ln(p))$$

Where, p is the ratio of the relative absorbance value of each well on the Biolog Ecoplate™ plate to the sum of the absorbance value of the 31 wells.

Principle component Analysis (PCA) of the Biolog Ecoplate™ data was carried out using the Multi-Variate Statisitcal Package (Version 3.1, Kovach Computing Services) using the absorbance values at 96 hrs.

5.3.6 Statistical analysis

All statistical analysis was performed using IBM SPSS Version 22 (SPSS Inc., Chicago, USA). Normality was assessed using the Shapiro-Wilk test, while outliers were visually determined from boxplots. The assumption of equal variance was tested using Levene's test for equal variance. An independent sample t-test was used to compare the *E. coli (ATTC* # 11303) $\log_{10}$ reduction between the two treatment treatments. A non-parametric Mann-Whitney U test was used to compare the differences in filtration rate, physicochemical properties, *E. coli* (Addgene plasmid # 64401) retention potential, percentage LOI and schmutzdecke average well colour development (AWCD) between the two treatment treatments as the data did not conform to the assumptions of the parametric independent sample t-test. A pair sample t-test was used to test for differences in the *E. coli* reduction between the sample ports (+1 cm, - 5 cm, - 15 cm, - 50 cm) and between the 10 hr and 24 hr sample points. Correlations between Log_{10} reduction of *E. coli* and filter age were examined using the Spearman Rank Order correlation coefficient.

A two-way analysis of variance (ANOVA) was used to compare the metabolic activity (AWCD) at the various sand column depths between the two temperature treatments. A one-way ANOVA and Tukey post hoc test were used to test whether the Shannon index and Richness index (section 5.2.5) for sand samples taken at different depths in each temperature treatment were statistically different. Where data failed to meet the assumptions of the parametric test, the non-parametric Kruskal-Wallis test was used and for pairwise comparisons a Bonferroni correction was applied using the procedure described by Dunn (1964).

5.4 Results

5.4.1 Filtration rate

Filtration rate was measured immediately after charging the filters when the head water pressure was at its highest, and the filtration rate was at its maximum. The mean filtration rate at the start of the study (day 0) for the 10˚C and 26˚C filters was 0.74 ± 0.00 $m^3/m^2/hr$ and 0.83 ± 0.05 $m^3/m^2/hr$ respectively. A decline in filtration rate occurred under both operating temperatures over the duration of filter operation. However, decline in filtration rate was more rapid for the filters operated at higher temperature (26˚C) (Figure 5.3). The median filtration rate for the 10˚C filters (0.58 $m^3/m^2/hr$.) and the 26˚C filters (0.51 $m^3/m^2/hr$.) were statistically different (U = 11,986 Z = 6.692, p-value < 0.001).

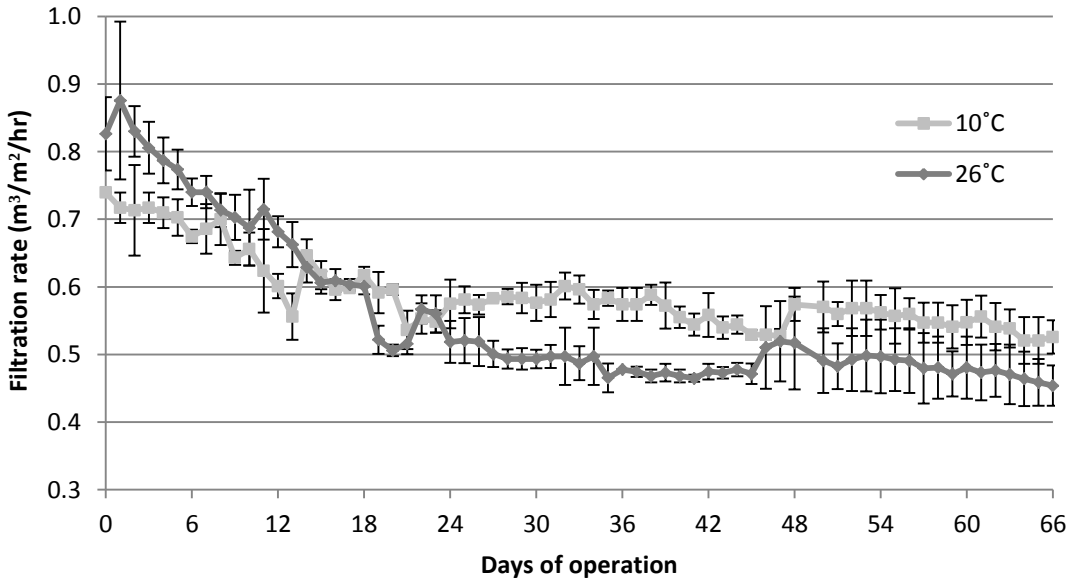

Figure 5.3: Mean filtration rates for biosand filters operated at two different operating temperatures. Error bars represent the standard deviation, n = 3.

5.4.2 Water quality

Filtered water quality from the BSFs under both operating temperatures met the World Health Organization (WHO) Guidelines for Drinking-Water Quality (WHO, 2011) for the parameters measured but, not for *E. coli*, which failed to achieve 0 CFU/100 ml. The feed water to filters at both temperatures treatments was from the same source; however adjusting the feed water temperature prior to charging the

filters resulted in statistically significant difference in some physicochemical properties including DO, EC, orthophosphate, TSS, DOC and SUVA between the feed water at 10˚C and 26˚C (Table 5.1). The total alkalinity of the feed water was determined from 5 samples taken over the duration of the study and was found to be 73 ± 4 mg as $CaCO_3$/l and 72 ± 4 mg as $CaCO_3$/l for the 26˚C and 10˚C filters respectively. The filtered water from the two temperature treatments was statistically different for pH, DO, EC, temperature, TON, nitrate, orthophosphate, TSS and SUVA. No significant difference was found between the filtered water from the two treatments for nitrite and DOC (Table 5.1). Exceptionally high DOC concentrations where found for the filtered water from the filters operated at 10˚C, on days 2, 39 and 44. However, over the duration of the study, the DOC in the filtered water from the filters operated at 10˚C was always higher than the corresponding feed water.

Dissolved oxygen consumption increased for both temperature treatments with filter age. Even though the feed water supply to the filters at 26˚C had a lower median DO concentration (8.3 mg/l) compared with the feed supply to the 10˚C filters (10.3), higher DO consumptions occurred in the filters operated at 26˚C (Table 5.1). For both temperature treatments the filtered water had a higher EC and a lower pH compared with the feed water to each treatment. The median SUVA concentration was lower in the filtered water from the 10˚C filters (0.86 l/mg-M) compared the 26˚C filters (1.27 l/mg-M), despite the feed water to the 10˚C filters having a higher median SUVA concentration (2.18 l/mg-M) compared with the filters operated at 26˚C (1.01 l/mg-M) (Table 5.1).

5.4.3 *E. coli* reduction

The mean *E. coli* concentration of the feed water to the filters operated at 10˚C was 249,273 ± 144,953 CFU/100 ml, with a maximum and minimum range of 511,200 to 13,400 CFU/100 ml. Similarly, the feed water to the filters operated at 26˚C had a mean of 246,254 ± 146,244 CFU/100 ml and a maximum and minimum concentration of 519,200 to 21,550 CFU/100 ml. Both sets of filters under different operating temperatures achieved significant reductions in *E. coli* with filter age. However, none of the filters achieved the limit set for *E. coli* in drinking water of 0 CFU/ 100 ml by WHO, (2011). Figure 5.4 suggests that a higher mean *E. coli* $\log_{10}$ reduction occurred in the filters operated at 10˚C (1.48 ± 0.64), and this was found to

be statistically different from the 26˚C filters (1.18 ± 0.60) (t (76) = 2.119, p-value: 0.037). Furthermore an increased $\log_{10}$ reduction occurred with filter age for both temperature treatments (Figure 5.4). This was confirmed with a significant positive correlation between the *E. coli* $\log_{10}$ reduction and filter age for the filters operated at 10˚C (r_s = 0.919, p-value < 0.001) and 26˚C (r_s = 0.839, p-value < 0.001).

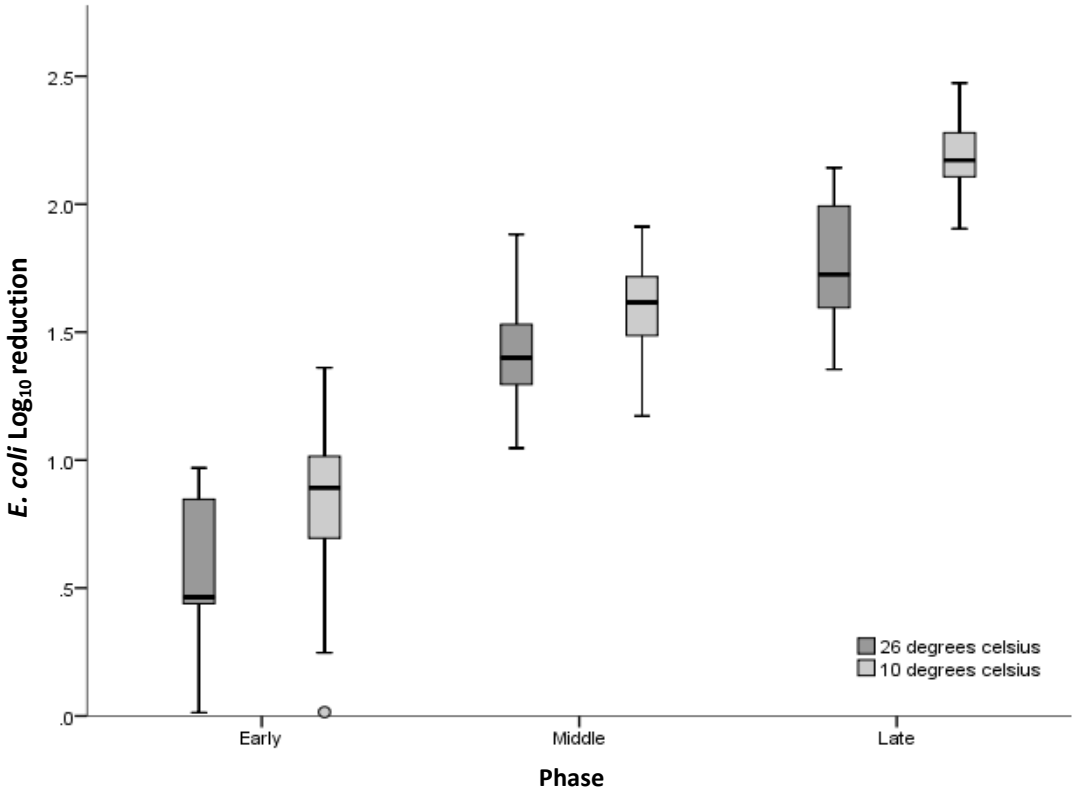

Figure 5.4: Boxplot depicts the mean $\log_{10}$ reduction of *E. coli* with filter age for filters operated at 10˚C and 26˚C. The top and bottom boundaries of the boxes show the 75th and 25th percentile and the ends of the whiskers show the maximum and minimum values. Bold lines within the boxes represent median values (50th percentile). Early age phase: 0 – 22 days, middle: 23 – 44 days, late: 45 – 66 days.

Table 5.1: Summary of the physicochemical properties of the feed supply and filtered water from biosand filters operated at two different ambient temperatures.

Parameter	Number of samples	10˚C Ambient Temperature		26˚C Ambient Temperature		p-value[1]	p-value[2]
		Feed	**Filtered**	**Feed**	**Filtered**		
pH	67	8.08 (7.93 - 8.19)	7.94 (7.87 – 8.01)	8.09 (7.92 – 8.20)	7.98 (7.92 – 8.02)	0.019*	0.598
DO, mg/l	67	10.3 (10.1 – 10.5)	9.3 (9.0 – 9.6)	8.3 (8.0 – 8.5)	6.9 (6.6 – 7.2)	< 0.001*	< 0.001*
EC, µs/cm	67	198.6 (188.3 – 204.0)	206.0 (202.0 – 213.0)	207.0 (196.2 – 212.0)	225.0 (220.6 – 228.0)	< 0.001*	< 0.001*
Temperature, ˚C	67	12.8 (12.3 – 13.6)	12.9 (12.6 – 14.1)	21.6 (21.0 -22.2)	23.2 (22.7 – 23.7)	< 0.001*	< 0.001*
TON, mg/l	10	1.431 (1.119 – 1.643)	1.399 (1.088 – 1.573)	1.256 (1.057 – 1.501)	0.199 (0.010 – 0.857)	< 0.001*	0.442
Nitrite, mg N/l	10	0.008 (0.006 – 0.023)	0.041 (0.010 – 0.087)	0.011 (0.007 – 0.028)	0.029 (0.001 – 0.111)	0.317	0.684
Nitrate, mg N/l	10	1.425 (1.110 – 1.591)	1.347 (1.057 – 1.475)	1.249 (1.038 – 1.462)	0.163 (0.009 – 0.692)	< 0.001*	0.408
Orthophosphate, mg/l	10	0.006 (0.004 – 0.009)	0.014 (0.011 – 0.015)	0.004 (0.004 – 0.005)	0.016 (0.012 – 0.020)	0.016*	0.053
DOC, mg C/l	10	4.09 (3.04 – 5.01)	9.425 (6.630 – 17.903)	9.02 (6.93 – 9.71)	7.255 (5.998 – 12.525)	0.204	0.001*
SUVA, l/mg-M	10	2.18 (1.47 – 2.79)	0.86 (0.44 – 1.38)	1.01 (0.78 – 1.32)	1.27 (0.76 – 1.47)	0.049*	0.004*
TSS, g/l	9	1.612 (0.284 – 5.028)	0.036 (0.000 – 0.124)	0.252 (0.102 – 0.652)	0.108 (0.000 – 0.234)	0.034*	0.040

Median values with interquartile range in parentheses, p-value[1] represents comparison between filtered water from the filters operated at 10˚C and 26˚C and p-value[2] represents comparison between feed water for the filters operated at 10˚C and 26˚C. Asterisk denotes statistical significance. DO: dissolved Oxygen, DOC: dissolved organic carbon, EC: electrical conductivity, SUVA: specific ultraviolet absorbance, TON: total oxidised nitrogen, TSS: total suspended solids.

5.4.4 *E. coli* depth profile

On day 46 the feed water to both sets of filters was spiked with the Addgene plasmid (# 64401), with a concentration of 165,750 CFU/100 ml and 159,500 CFU/100 ml to the filters operated at 10˚C and 26˚C respectively. The filters operated at 10˚C achieved higher $\log_{10}$ reduction rates with depth when compared with the filters operated at 26˚C (Figure 5.5). In both temperature treatments the most effective zone for the removal of *E. coli* was in the top 15 cm of the sand column. The resting water level was also a region of effective *E. coli* removal in both operating temperatures. In addition, *E. coli* removal also occurred deep within the sand column in the filters operated at 10˚C. The filters operated at 26˚C showed further *E. coli* reductions with depth for the samples taken 24 hrs after charging the filters, while for the 10 hr sample point a lower *E. coli* $\log_{10}$ reduction occurred at the 50 cm sample port in comparison to the 15 cm sample port (Figure 5.5). There was no statistically significant difference in *E. coli* $\log_{10}$ reduction during the residence period for samples taken at -1 cm ($t(2) = 3.844$, p-value: 0.062), 5 cm ($t(2) = 2.382$, p-value: 0.140), 50 cm ($t(2) = 0.566$, p-value: 0.628) in the filters with 10˚C ambient temperature. There was however a statistically significant difference between the 10 hr and 24 hr samples from the 15 cm sample port for the filters operated at 10˚C ($t(2) = 6.971$, p-value: 0.020). For the 26˚C treatment there was no significant difference in *E. coli* concentration between samples taken at 10 hrs and 24 hrs at 5 cm ($t(2) = -1.646$, p-value: 0.242) and 15 cm ($t(2) = -0.893$, p-value: 0.466) samples ports with a reduced *E. coli* $\log_{10}$ reduction occurring with increased residence periods for both depths. A statistically significant difference occurred between the 10 hr and 24 hr samples taken at – 1 cm ($t(2) = 16.191$, p-value: 0.004) and 50 cm ($t(2) = 34.623$, p-value: 0.001). Interestingly the *E. coli* (Addgene plasmid # 64401) concentration was found to be higher in the filtered water on day 47 compared to the concentration at the 50 cm depth sample port at the 24 hr sample point (day 47) for both operating temperatures.

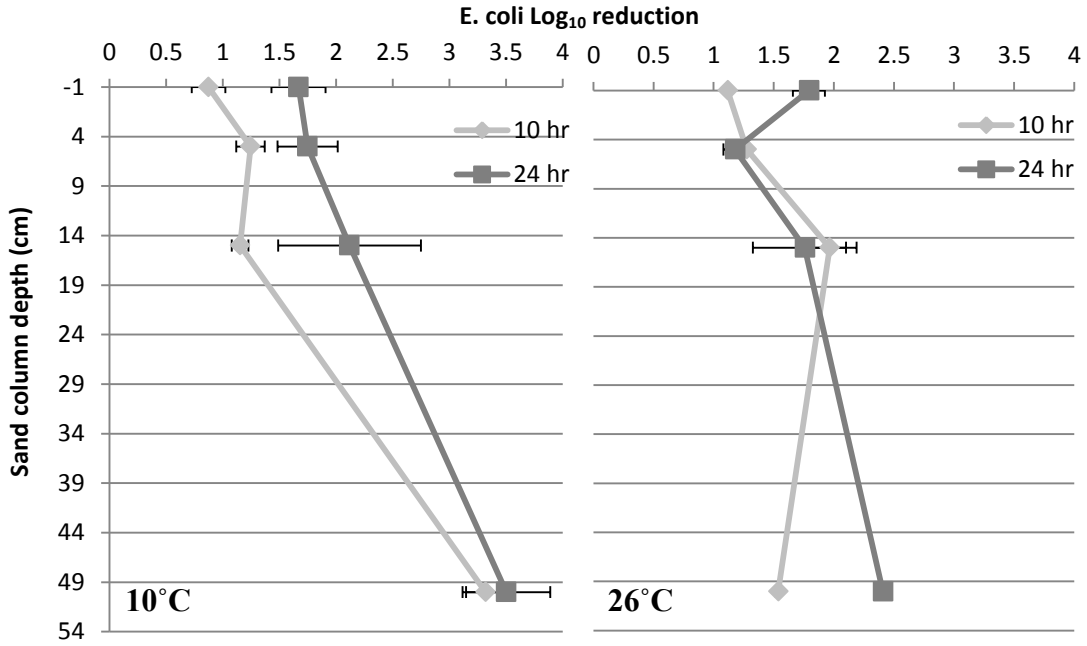

Figure 5.5: *E. coli* removal with sand column depth (mean ± standard deviation, n = 3).

5.4.5 Retention of *E. coli*

E. coli concentration in the filtered water on day 47, 24 hrs after charging the filters with feed water spiked with the Addgene plasmid, was higher for the filters operated at 26˚C (1433 ± 257 CFU/100 ml) compared with the filters operated at 10˚C (1067 ± 351), but this difference was not statistically significant (t (4) = -1.460, p-value: 0.218). However, from day 49 the *E. coli* (Addgene plasmid # 64401) concentration in the filtered water from the 26˚C filters was lower than the filtered water from the 10˚C filters (Figure 5.6). While from day 53 onwards filters at both temperatures had ≤ 3 CFU/100 ml in the filtered water (Figure 5.6). It is therefore apparent that while the filtered water from the 26˚C filters have a higher *E. coli* (Addgene plasmid # 64401) concentration on day 47, the filter operated at 10˚C had a longer retention potential (i.e. released a higher concentrations of *E. coli* over a longer period following the initial introduction of the *E. coli*). The median *E. coli* concentration for the 10˚C filter (6.17 CFU/100 ml) and the 26˚C filters (0.67 CFU/100 ml) over the 20 days following the charging event with spiked *E. coli* (Addgene plasmid # 64401) was statistically different (U = 1,109, z = -3.655, p-value < 0.001).

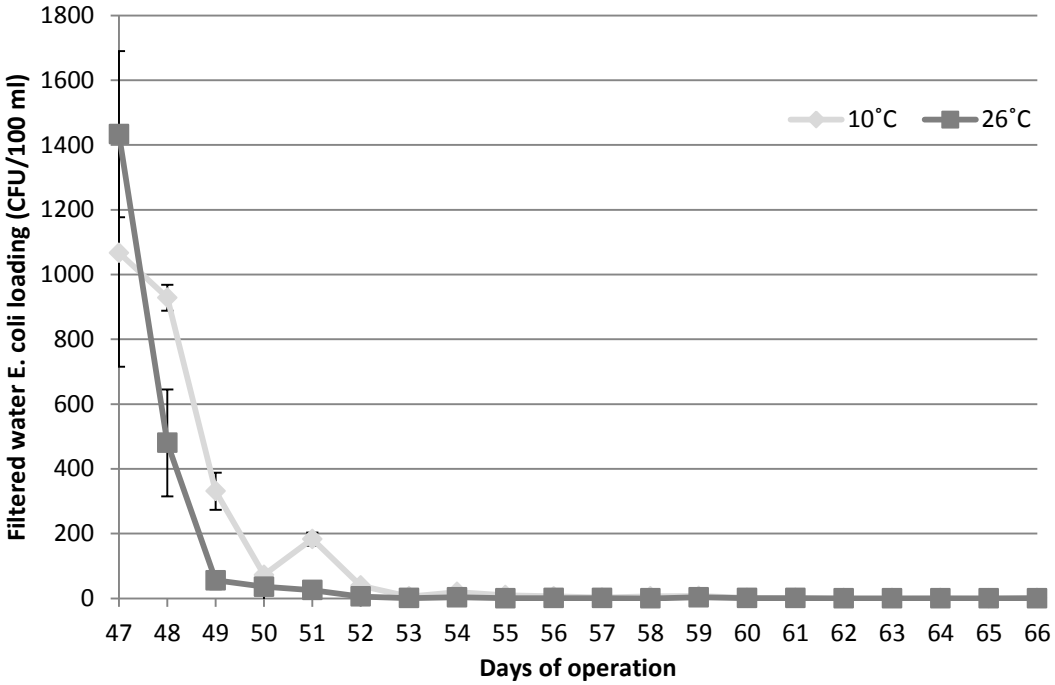

Figure 5.6: Mean *E. coli* (Addgene plasmid # 64401) concentration in the filtered water from the filters at different operating temperatures (mean ± standard deviation, n = 3).

5.4.6 Functional activity, diversity and richness of the schmutzdecke

The average well colour development (AWCD), which is a measure of the metabolic activity of the microbial community, indicated that the metabolic activity increased rapidly in the schmutzdecke from day 0 to day 13 under both temperature conditions. A lower AWCD (Figure 5.7) occurred from day 28 onwards for both temperature treatments in comparison to the metabolic functional activity recorded on day 13. Operating temperature did not appear to influence the functional metabolic activity of the microbial community in the schmutzdecke, with similar AWCDs recorded for both temperature treatments (U = 156, Z = - 0.190, p-value: 0.864).

The functional diversity of the schmutzdecke community increased gradually with filter maturation for both temperature treatments (Table 5.2). A higher Shannon diversity index occurred in the schmutzdecke of the 10˚C filters in comparison with the 26˚C filters, yet the median Shannon diversity index was not statistically different for the filters operated at 10˚C and 26˚C (U = 145, Z = - 0.538, p-value: 0.606). The Shannon diversity index for the schmutzdecke was statistically different at different sample time points over the duration of the study for the filters operated

at 10˚C (χ^2 (5) = 11.952, p-value: 0.035). Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons which revealed statistically significant differences in the Shannon diversity index between day 0 and day 13 (p-value: 0.016) at 10˚C. No statistically significant difference was found for the Shannon diversity index for the schmutzdecke at the different sample time points for the filters operated at 26˚C (χ^2 (5) = 10.810, p-value: 0.055). Similarly, a statistically significant difference was not found for the biolayer Richness index between the filters operated at the two different temperature treatments (U = 135.5, Z = - 0.898, p-value: 0.406). At the different sample time points, the Richness index was found to be statistically different for the filters operated at 10˚C (χ^2 (5) = 13.824, p-value: 0.017) and 26˚C (χ^2 (5) = 11.304, p-value: 0.046).

On day 66, the median percentage organic matter in the top two centimetres of the filters operated at 10˚C (0.027% (0.026% – 0.028%)) and 26˚C (0.017% (0.016%-0.026%)) was not statistically different (U = 3, Z = - 0.655, p-value: 0.513).

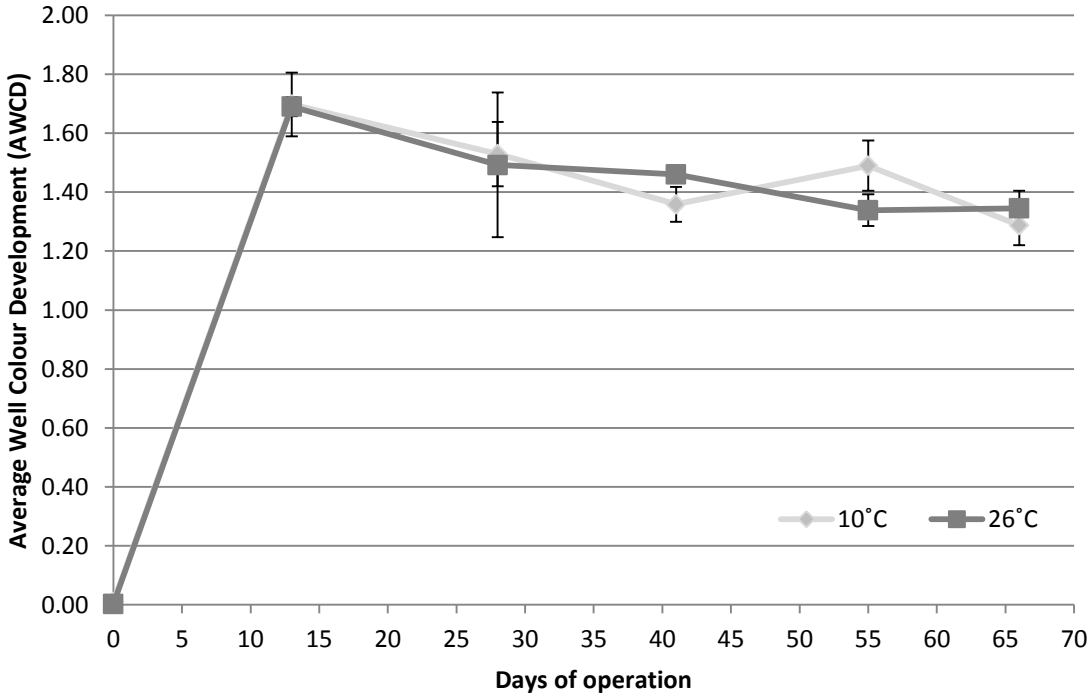

Figure 5.7: Metabolic activity of the microbial community in the schmutzdecke of biosand filters operated at the two different ambient temperatures. (mean ± standard deviation, n = 3).

Table 5.2: Functional diversity analysis of the schmutzdecke microbial community in biosand filters operated at two different ambient temperatures (mean ± standard deviation, n = 3).

Day	Richness Index		Shannon Diversity (H') Index	
	10˚C	**26˚C**	**10˚C**	**26˚C**
0	0.67 ± 0.58	0.00 ± 0.00	2.007 ± 0.757	1.202 ± 0.574
13	31.00 ± 0.00	30.67 ± 0.58	3.373 ± 0.014	3.362 ± 0.014
28	29.67 ± 0.58	31.00 ± 0.00	3.309 ± 0.034	3.314 ± 0.080
41	31.00 ± 0.00	29.67 ± 0.58	3.289 ± 0.002	3.282 ± 0.040
55	31.00 ± 0.00	30.33 ± 0.58	3.337 ± 0.011	3.287 ± 0.018
66	30.33 ± 0.58	30.00 ± 1.00	3.312 ± 0.024	3.323 ± 0.033

The PCA plots of the carbon utilization on the Biolog Ecoplates^{TM} (Figure 5.8) show the schmutzdecke had similar metabolic functions between the two treatments on day 0. The metabolic functions show high similarity for both temperature treatments on day 13 and day 28 located on the positive side of axis two. There are clear shifts in the metabolic function of the schmutzdecke community with filter age as the metabolic functions remain on the positive side of axis two, but shift closer to axis one.

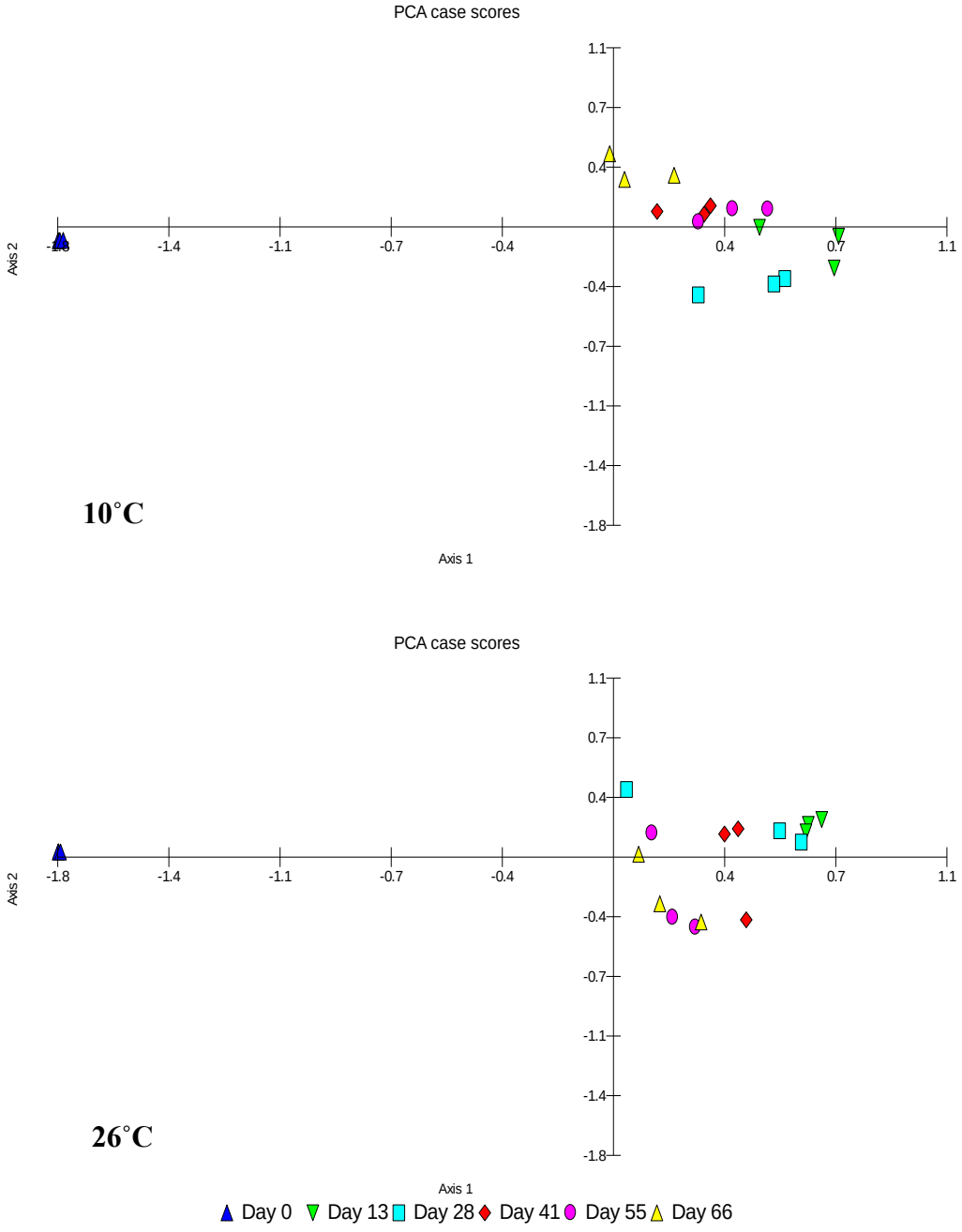

Figure 5.8: Principle component analysis (PCA) of metabolic activity and functional diversity of the schmutzdecke community in biosand filters operated at two different temperatures.

5.4.7 Functional activity, diversity and richness with sand column depth

The microbial communities showed declining metabolic activity with sand column depth at both temperatures (Figure 5.9). The filters operated at 26˚C had a higher AWCD at the sand surface (0 cm) compared with the filters at 10˚C. However, a greater decline in AWCD occurred with sand column depth in filters operated at 26˚C compared with the 10˚C treatment. A two-way ANOVA showed no statistically significant interaction in metabolic activity (AWCD) between filter operating temperature and sand column depth ($F(2, 12) = 0.173$, p-value: 0.843).

The Shannon index and the Richness index, calculated based on the carbon source utilization rate declined with sand column depth (Table 5.3). A one-way ANOVA showed there was a significant difference for the Richness index at different depths in the sand column at 10˚C ($F(2,6) = 9.023$, p-value: 0.016). The Tukey post hoc analyses revealed that the Richness index was statistically different between sand samples taken at 5 cm and 50 cm, with a higher Richness index at 5 cm depth (7.6667, 95% CI (2.127 to 13.206), p-value: 0.013). The Richness index profile over sand column depth at 26˚C failed to meet the assumptions of the one-way ANOVA and hence were analysed using the non-parametric Kruskal-Wallis test, which identified a statistically significant difference for the calculated median Richness index with sand column depth ($\chi^2(2) = 6.330$, p-value: 0.042). The post hoc analysis revealed a statistically significant difference between the median Richness index at the 5 cm depth (29) and the 50 cm depth (24) (p-value: 0.036). There was no statistically significant difference in the Shannon diversity index depth profiles between the two temperature treatments ($t(15) = 0.135$, p-value: 0.895). The Shannon diversity index for the microbial community at different depths in the sand columns for the filters operated at 10˚C was found to be statistically different ($F(2, 5) = 8.245$, p – value: 0.026). The Tukey post hoc analysis revealed that the increase in the Shannon diversity index at 50 cm depth compared with the 5 cm depth was statistically different (0.4446, 95 % CI 0.0871 to 0.8020, p – value: 0.022). As the Shannon diversity depth profile for the 26˚C filter violated the assumptions of homogeneity of variance, the differences were tested using the Kruskal Wallis test ($\chi^2(2) = 6.489$, p-value: 0.039). Subsequent post hoc analysis revealed statistically significant differences in the diversity of microbial communities at 50 cm and 5 cm depth, using the Shannon diversity index (p-value: 0.034)

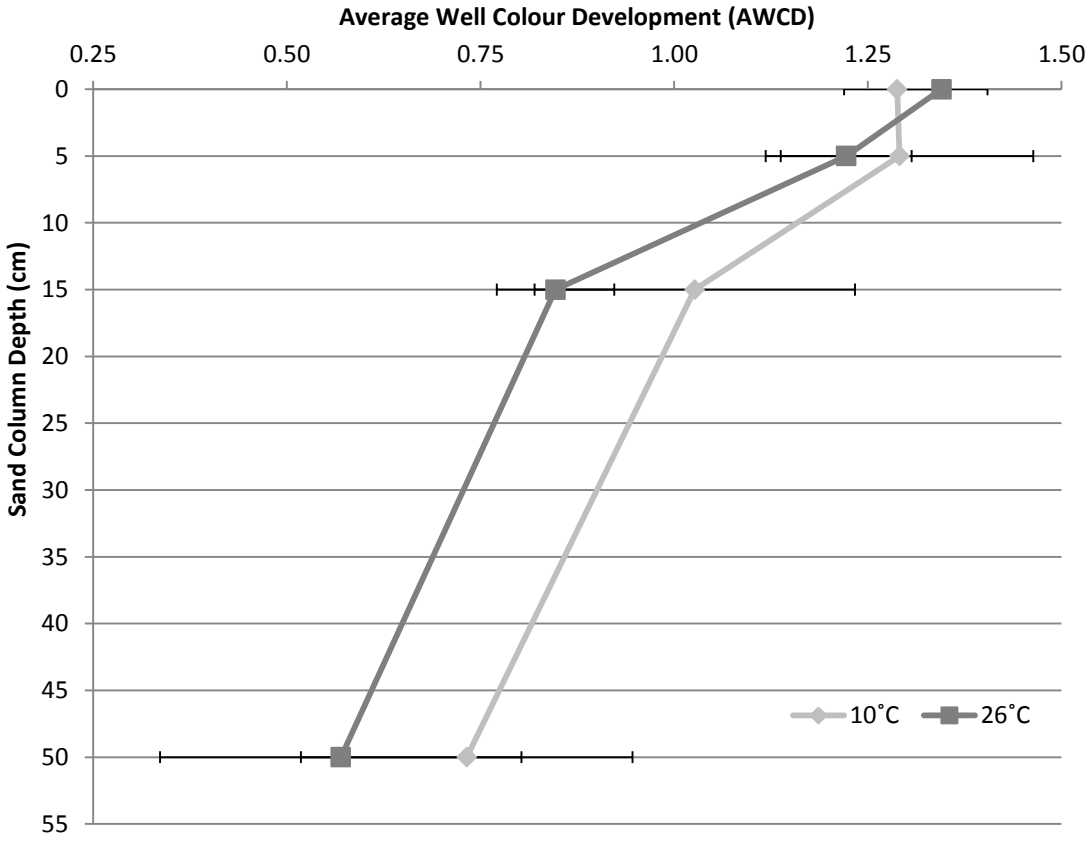

Figure 5.9: Average well colour development (AWCD) of the microbial community at different depths in the sand column (0 cm sand surface) of biosand filters operated at two different temperatures (mean ± standard deviation, n = 3).

Table 5.3: Average species richness and diversity at different sand column depths in biosand filters operated at two different ambient temperatures. (mean ± standard deviation, n = 3).

Depth (cm)	**Richness Index**		**Shannon Diversity (H') Index**	
	10˚C	**26˚C**	**10˚C**	**26˚C**
5	31 ± 0	29.33 ± 0.58	3.30 ± 0.07	3.30 ± 0.02
15	27.33 ± 2.31	28.00 ± 1.00	3.09 ± 0.16	3.15 ± 0.09
50	23.33 ± 3.06	22.67 ± 5.13	2.91 ± 0.13	2.92 ± 0.17

5.5 Discussion

5.5.1 Filtration rate

Slower filtration rates have been reported to enhance filter performance, by enhancing particle settlement and allowing for increased contact time between

bacteria, sand grains and the schmutzdecke (Jenkins *et al*., 2011; Kennedy *et al*., 2012). However in this current the filters operated at 26˚C had a lower median filtration rate, yet a higher removal of *E. coli* occurred in the filters operated at 10˚C. This suggests that the slower filtration rate at the higher temperature did not enhance filter performance. This observation is supported by Chan *et al*. (2015), who also found that slower filtration rates did not enhance the bacterial removal performance.

5.5.2 Filter performance

This study showed that the operating temperature of the BSFs was an influential factor on filter performance. Despite the feed water supply to both sets of filters being from the same source, the physicochemical properties, DO, EC, DOC and SUVA differed considerably between the feed water to the two different temperature treatments (10˚C and 26˚C). The difference can be explained by the increased water temperature leading to a decreased concentration of DO and an increased concentration of dissolved substances (Delpla *et al*., 2009). Higher DOC and DO consumption occurred in the filters operated at 26˚C indicating a higher biological activity occurred in the filters operated at the higher temperature.

The median EC and pH of the filtered water decreased slightly compared with the feed water for both temperature treatments. Young-Rojanschi and Madramootoo (2015) attributed increases in filtered water pH and EC to the leaching of calcium carbonate from the filter media. Decreased nitrate and increased nitrite concentrations occurred in the filtered water compared with the feed water in both temperature treatments suggesting that denitrification was occurring in the filters, as had previously been observed by Murphy *et al*., (2010). Higher nitrate reductions occurred in the filters operated at the higher temperature. However, the nitrate and nitrite concentration of the feed and filtered water from both temperature conditions did not exceed the WHO (2011) Drinking-Water Guideline concentration for nitrate and nitrite of 11 mg/L and 0.9 mg/L respectively.

The SUVA is an important drinking water quality parameter as SUVA provides an estimation of the dissolved aromatic carbon content in the water. Water with a high DOC and SUVA levels are considered to problematic from a disinfection by-product (DBP) formation aspect (Ates *et al*., 2009). Based on the lower median SUVA concentration in the filtered water, the filters operated at the lower temperature

would be more suitable for the application of a secondary treatment using chlorination. If a secondary treatment method was to be used on the filtered water from the filters operated at the higher ambient temperature, then an alternative secondary treatment method, such a solar disinfection (SODIS), would reduce the risk associated with the formation of DBPs.

5.5.3 *E. coli* removal

E. coli is widely used as an indicator organism because it is easily detected, it does not significantly multiple in the environment outside the host, and it can survive for sufficient periods of between 4 and 12 weeks outside the host depending on environmental conditions (Edberg *et al*., 2000). These properties make it the best single biological indicator for monitoring the potential presence of pathogens in drinking water (Edberg *et al*., 2000). The *E. coli* reductions reported in this study are in line with other studies (Elliott *et al*., 2008; Stauber *et al*., 2006); however in this study the mean *E. coli* reductions were significantly higher in the filters operated at 10˚C (1.48 $\log_{10}$) compared with the filters operated at 26˚C (1.18 $\log_{10}$). This indicates that households may experience differences in the performance of their filters due to seasonal temperature variations.

Increased reduction of *E. coli* occurred with filter age has also been reported in other studies (Elliott *et al*., 2006; Elliott *et al*., 2008; Stauber *et al*., 2006) and this is often attributed to the development of the schmutzdecke. However, there is no direct evident that the increased reduction of the indicator organism with filter age is linked to the development of the biological community in the filter, and other factors may be contributing to the enhanced performance with age, such as physical straining (Elliott *et al*., 2011). Therefore, further research is needed to assess the mechanisms directly contributing to the removal of bacteria in BSFs and also to determine the effect of varying the physicochemical properties of feed water on filter performance.

5.5.4 Retention of faecal bacteria

The current study also assessed the fate of *E. coli* within the filter column. The objective was to determine whether the *E. coli* in the filtered water was the same *E. coli* that was charged to the filter 24 hrs earlier, or whether short term retention of *E. coli* occurred in the filter, followed by release several days later. Filters operated at

the lower temperature had higher *E. coli* $\log_{10}$ reduction, but these filters also had a higher retention potential, as *E. coli* which was charge on day 46 was released from the filters over several days. During the filter residence period, the period during which there is no discharge from the filter, a number of mechanisms are believed to be contributing to the removal and retention of bacteria, including natural die-off, adsorption and predation (Kennedy *et al*., 2013). Nutrient loading is also believed to be a contributing factor (Kennedy *et al*., 2013). Ambient temperature is likely to be the controlling factor for the different retention potentials observed between the two temperature treatments, because Edberg *et al.* (2000) observed that microbes survive longer in colder water.

Baumgartner *et al*. (2007) and Jenkins *et al*. (2011) both reported that the duration of the residence period of a BSF has a significant effect on its reduction of *E. coli*. However, this study found the *E. coli* concentration was not significantly different at the different sand column depths with increased residence time (from 10 hrs to 24 hrs). Similarly Young-Rojanschi and Madramootoo (2014) found no statistically significant reduction in *E. coli* concentration during the residence period. The *E. coli* depth profiling also showed that filters operated at a higher temperature did not have a consistent decline in the *E. coli* concentration with depth. The higher concentration at the 50 cm depth may be attributed to the higher filtration rate that occurred, immediately after charging the filters, resulting in the water at 50 cm having passes through the sand column faster than the water in the upper portion of the column. However, a similar high initial filtration rate would also have occurred in the filters operated at 10˚C and yet an increased *E. coli* reduction occurred with depth. Another possible explanation is that the higher *E. coli* concentrations with depth in the filters operated at the higher temperature was a result of the *E. coli* being transported through the sand column and settling near the sample port. The filtered water from the both temperate treatments had a higher *E. coli* concentration in comparison to the *E. coli* concentration recorded for the 50 cm depth at 24 hrs, indicate that *E. coli* may have been resuspended when flow rate resumes after charging the filters.

5.5.5. Metabolic activity and diversity of the microbial community

As the feed water to both sets of filters was from the same source, it was assumed that the same microbial communities were available for the development of the

schmutzdecke under both operating temperatures. The duration of the maturation period is poorly understood for BSFs with time periods of two weeks (Jenkins *et al*., 2011) to 30 days (Baumgartner *et al*., 2007) being suggested. However, the results from this study show that that the metabolic functional activity of the microbial community in the schmutzdecke of the filters increases rapidly up to day 13 and reaches a relatively stable level after day 13 regardless of temperature. The richness and diversity indexes were also relatively stable after day 13 indicating that the maturation period was reached after 13 days of operation for both temperature treatments. Future studies should assess the microbial ecology under different operating temperatures, because although the metabolic activity and diversity of the microbial community of the schmutzdecke were similar, the community structures may differ.

Depth profiling of the filters from both temperature treatments showed that the activity, diversity and richness of the microbial community declined with filter depth. This decline in metabolic activity could possibly be due to reduced oxygen availability with filter depth, although Young-Rojanschi and Madramootoo (2014) did not find a consistent decrease in DO with depth for intermittently operated BSFs. Therefore the decline in functional activity, diversity and richness of the microbial with depth might be linked to factors including nutrient availably, however this requires further investigation.

5.6 Conclusions and recommendations

This study provides the first insight into the effects of ambient temperature on BSF performance and the development of the schmutzdecke. The study has shown that temperature is a confounding factor for filter performance, with a lower temperature resulting in a higher filter performance. Filters operated at a lower temperature (10˚C) performed better in removing *E. coli* and produced water with a lower SUVA concentration. At the higher operating temperature the filtered water had a lower concentration of nitrogen forming compounds in comparison to filters operated at 10˚C. However different operating temperatures did not affect the metabolic activity or diversity of the microbial community in the filters.

The difference in operating temperatures resulted in a difference in the influent water characteristics, which may have been a confounding factor to the enhanced *E. coli*

removal observed at the lower temperature. Differing physicochemical properties of feed water resulting from changes in water temperature could also be experienced by household users of BSFs due to seasonal temperature changes.

However, a limitation of this study was that viable *E. coli* were characterised by the *E. coli* that were cultivable. There may have been *E. coli* released from the filter that were in a viable but nonculturable (VBNC) state, resulting in a potential under-estimation of viable *E. coli* concentration determined in this study. Therefore, it would be beneficial if future studies determined the VBNC *E. coli* concentration. It would also be useful to assess BSF performance over an entire annual cycle in order to expose the same filters to differences in temperature, between day and night, and also between seasons.

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Chapter 6

Schmutzdecke community structure in replicate, full-scale, biosand filters

6.1 Abstract

Previous studies on biosand filters (BSFs) have focused on the effect of different operating conditions on the removal of pathogenic microorganism, while the biological community structure of the filter remains unstudied. This study provides the first insight into the colonization and succession of bacteria in the biolayer, or schmutzdecke, of BSFs, while also determining the bacterial community structure of the influent and effluent. Three full-scale BSFs were operated for 171 days and the performances, in terms of effluent (filtrate) water quality, were found to be reproducible. The bacterial communities of the influent (feed water), schmutzdecke and effluent samples were characterised by sequencing 16S rRNA genes in samples taken periodically during the trial. The colonisation of the sand, and the succession of the bacterial families in the schmutzdecke, differed during the early (0 – 35 days) and middle (36 – 114 days) phases of filter maturation for the three filters. In the late phase (115 – 171 days) the dominant bacterial families in the three BSFs were *Planctomycetaceae* and *Acidobacteria* Gp6. Interestingly, bacterial family dominance in the schmutzdecke was not found to be driven by the bacterial families that dominated the influent. A likely contributing factor to the different bacterial dominance in the early and middle phases is the differing declines in filtration rates between the filters.

6.2 Introduction

Access to safe drinking water is a particularly acute problem in regions of the world lacking large-scale water treatment infrastructure (Grabow 1996) and regions devastated by natural disasters where the drinking water supply becomes contaminated or disturbed due to infrastructure damage (Faruque *et al*. 2005; Roig *et al*. 2011). In these cases, point-of-use (POU) water treatment techniques have become the technology of choice to provide access to clean drinking water (Loo *et al*. 2012; WHO and UNICEF 2012). Several different point-of-use (POU) water treatment techniques are available of which biosand filters (BSFs) are amongst the most effective when measured in terms of user satisfaction, sustained rates of use, and reduction of diarrheal disease (Aiken *et al*. 2011; Duke *et al*. 2006; Fiore *et al*. 2010; Liang *et al*. 2010; Stauber *et al*. 2009; Tiwari *et al*. 2009). Thus, BSFs offer a realistic and proven interim technical solution allowing households without access to a safe, piped water supply a method to treat drinking water domestically.

Recent studies have focused on the influence of operational parameters including: resting periods (Lynn *et al*. 2013; Young-Rojanschi and Madramootoo 2015; Young-Rojanschi and Madramootoo 2014), hydraulic flow rate (Elliott *et al*. 2008; Kennedy *et al*. 2012), filter media characteristics (Elliott *et al*. 2015; Ghebremichael *et al*. 2012; Jenkins *et al*. 2011; Ngai *et al*. 2007; Tellen *et al*. 2010) and physical disturbance (Mahaffy *et al*. 2015; Napotnik and Jellison 2014) on the performance of the filter with respect to pathogen removal as assessed by the concentration of indicator organisms. Earlier studies have also reported that lower flow rates enhance filter performance by increasing the contact time between bacteria, filter media and the schmutzdecke (Jenkins *et al*., 2011; Kennedy *et al*., 2012). However, recently Chan *et al*., (2015) found that lower filtration rates did not enhance filter performance. While these recent studies are important in providing guidance on how householders should operate their filters to optimise filter performance, there is however the need for a deeper understanding of the colonization and succession of the microbial community that forms the schmutzdecke in BSFs.

BSFs remain somewhat of a "black box" system and while the name "biosand filter" suggests that the biological community is contributing to filter performance direct evidence is lacking (Elliott *et al*. 2011). This study assessed the reproducibility of

three BSFs operated in parallel with the same influent source, daily charge volume and environmental conditions. The study aimed to determine whether BSFs operated in parallel would have similar declines in filtration rate (head loss development), filter performance (effluent water quality) and bacterial colonization of the sand, together with the rate of bacterial family succession in the schmutzdecke over a six month period.

Biosand filters are often described as small scale intermittently operated slow sand filters (SSFs) (Jenkins *et al*. 2011; Kubare and Haarhoff 2010; Ngai *et al*. 2007) and, while a recent study completed by Haig *et al*. (2014) assessed the microbial ecology of SSFs, a molecular assessment of the schmutzdecke in BSFs as conducted here has not previously been completed. Such a study could identify differing community structures in BSFs and SSFs, given their differences in design and operation, in particular their feed flow patterns (Elliott *et al*. 2011). One of the first studies to provide some indication of community composition of the schmutzdecke of BSFs was Hwang *et al*. (2014), whose study adopted a culture-dependent approach to evaluate the opportunistic pathogens in both the influent and schmutzdecke. Wang *et a*l. (2014) assessed the microbial diversity by 16S rRNA gene pyrosequencing of samples taken from the schmutzdecke and at five different sand column depths in full-scale biosand filters at a single time point. However, this current study provides the first assessment of microbial 16S rRNA community in the influent, schmutzdecke and effluent of BSFs, to determine the bacterial families involved in the maturation of the schmutzdecke, ultimately leading to the identification of the community structure of the effluent.

6.3 Materials and Methods

6.3.1 Filter design and operation

Three full-scale Hydraid® (Grand Rapids, MI 49503) BSFs were used in the study. Distinguishing features of the BSF are the elevated outlet tube and the diffuser plate (Figure 6.1). The outlet tube was located 5 cm over the sand column allowing the filter to remain saturated during the filter resting period. The diffuser plate, with 2 mm diameter holes, was located 2 cm above the resting water level, allowing for the even distribution of water over the resting water and thus avoiding disturbance to the sand column during filter recharging. The maximum loading head for all filters was

14 cm. Each filter contained 5 cm of underdrain gravel (10 mm diameter), 5 cm of separating gravel (3.15 – 5.6 mm) and 48 cm of sand (Figure 6.1). The sand used in the study was pure silica sand, selected for its high purity and low organic matter content. The sand had a maximum grain size of 0.7 mm, an effective size (d_{10}) of 0.21 mm and a uniformity coefficient (UC) of 1.59. All filter media was autoclaved at 121˚C for 20 minutes before use. The ambient temperature over the duration of the study ranged from 8 ˚C to 23˚C.

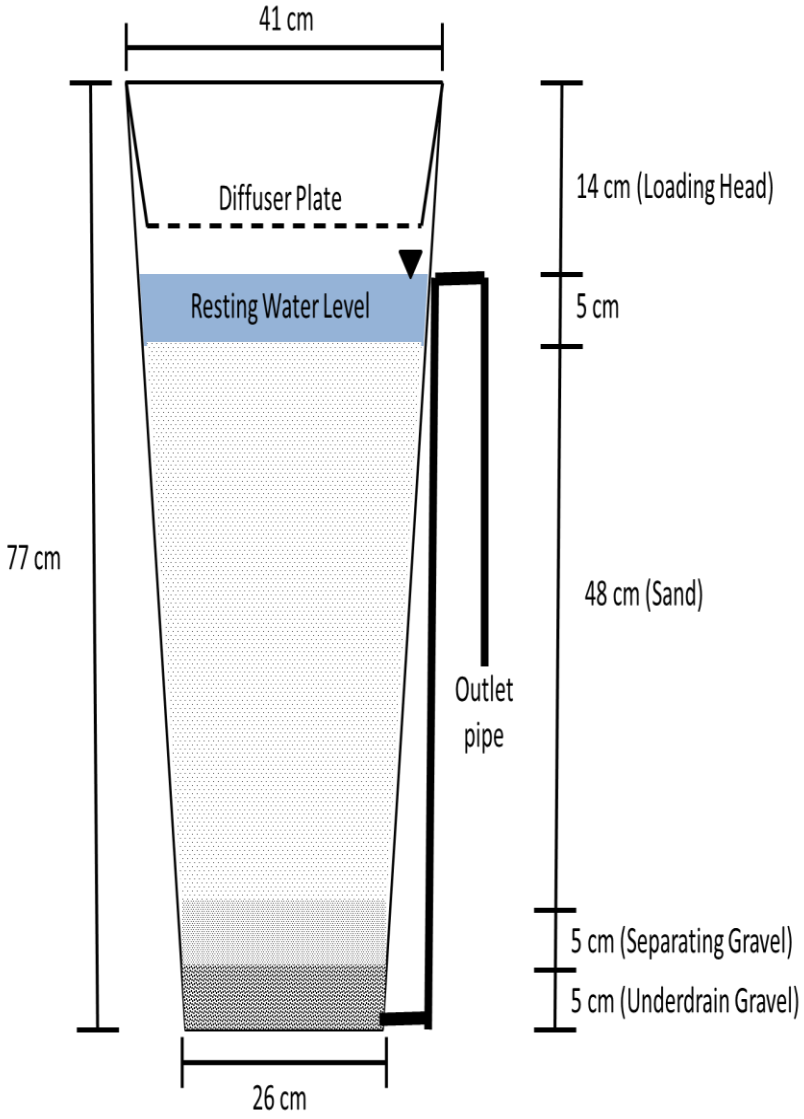

Figure 6.1: Schematic of full-scale Hydraid® biosand filters used in study.

6.3.2 Influent supply

Influent water was collected from the River Lee, County Cork, Ireland at weekly intervals and stored a in a tank with air stones until use. Filters (F1, F2 and F3) were charged daily with 7 l, i.e. a total of 1,197 l over the 171 days of the study. Daily charge volume to the filters was approximately that of media pore volume. The alkalinity was determined from three grab samples taken from the river at the time of water collection and was found to range from 42 – 75 mg/l as $CaCO_3$.

6.3.3 Water quality analysis

The peak filtration rate was determined immediately after charging the filter, when head water pressure was at its greatest and filtration rate was at its maximum. Once flow rate was measured, the outlet tube was cleaned with a 70% ethanol solution prior to being inserted into a sterile glass flask, which was left in place for 3 hours allowing for > 90% of the charge volume to filter. Influent samples were collected the day before effluent water samples and were analysed immediately for pH, temperature, electrical conductivity (EC) with a WTW mutlimeter 320i (WTW, Weilheim, Germany), and dissolved oxygen (DO) was measured with a Handy Polaris OxyGuard® meter.

Weekly samples were taken for total coliforms and *E. coli* and were enumerated using the membrane filtration technique (reference number: 9222 D) (APHA *et al*., 2012) in CHROMagar™ Liquid ECC broth (CHROMagar, Paris, France). Plates were incubated at 37˚C for 18-24 h. Weekly analysis also included total oxidized nitrogen (TON), nitrite, orthophosphate, dissolved organic carbon (DOC) and the spectral absorption coefficient (UV_{254}). TON and nitrite were measured by the Aquatic Services Unit (ASU), University College Cork using an automated flow injection analysis (Lachat Quickchem 8000, Zellweger Analytics, Inc. Milwaukee, USA). The nitrate concentration was determined by deducting the nitrite concentration from the TON concentration. Dissolved organic carbon was also measured by the ASU using a total organic carbon analyser (TOC-VCPH/CPN, SHIMADZU Kyoto, Japan). Analysis of the influent water to determine the total alkalinity was also completed by the ASU by titration with 0.01 M EDTA. Orthophosphate measurements were completed using the ascorbic acid method (reference number: 4500-P E) (APHA *et al*., 2012) and the spectral absorption

coefficient (UV_{254}) was measured using the method (5910 B) outlined in APHA et al. (2012), using the Thermo Scientific™ GENESYS 10 UV scanning spectrophotometer. The specific ultraviolet absorbance (SUVA) was calculated from the ratio between DOC and UV_{254}. The total suspended solids (TSS) concentration of the influent and effluent was determined using the method (2540 D) outline in APHA *et al*. (2012).

6.3.4 Sample collection for 16S rRNA extractions

At each sampling event three cores (2 cm depth by 1.5 cm diameter) were randomly taken across from the top of each sand column. These samples were then pooled and stored. Samples (500 ml) of influent and effluent water were filtered using 0.22 µm polycarbonate filters (Whatman, Piscataway, New Jersey), which were also stored, along with the sand samples at -20˚C for subsequent DNA extraction.

6.3.5 DNA extraction and 16S rRNA gene sequencing

DNA was extracted using the Maxwell® 16 tissue DNA purification kit and subsequent purification of the DNA was completed using the automated Maxwell® 16 instrument. A spectrophotometer was used to quantify purified DNA (Qubit system, and the Broad-Range Qubit Assay; Life Technologies). The V4 region of 16S rRNA genes was polymerase chain reaction (PCR)-amplified using Golay barcoded primers (Caporaso *et al*., 2012) and the KAPA HiFi HotStart PCR Kit. The 515F and 806R primers were used with the following conditions: initial denaturation at 95°C for 5 min; with 25 cycles of 98°C for 20 s, 60°C for 15 s and 72°C for 40 s; followed by final extension at 72°C for 1 min (Caporaso *et al*., 2012). The PCR products were gel-purified and the quantity of the PCR product was determined using the High-Sensitivity Qubit Assay (Life Technologies). The pooled multiplexed library normalized to 5 ng/µl DNA was sequenced on an Illumina MiSeq platform. Raw sequences data were analysed using a modified version of the Illumina MiSeq SOP pipeline (Kozich *et al*., 2013) in Mothur (http://www.mothur.org/wiki/MiSeq _SOP). Filtering of ambiguous base calls (maxambig = 0), amplicon size (maxlength= 300, minlength = 200), barcode mismatches (bdiffs = 0), primer mismatches (pdiffs = 2) and homopolymers (maxhomop = 8) was done following Weigel and Erwin (2016). Silva database was used to align the sequences and the sequences were then trimmed to the V4 region. The precluster (Huse *et al*., 2010)

and UChime algorithms were run, and chimeras sequences were removed. Each dataset was subsampled to the lowest read count (n = 2,372) and all analyses were based on the final subsampled data sets. A threshold of 1% was employed to define rare or abundant taxa.

6.3.6 Statistical analysis

All statistical analysis was completed using software package IBM SPSS Version 22 (SPSS Inc., Chicago, USA). Shapiro-Wilk's test was used to test for normality, and Levene's test for equal variance was used to assess the homogeneity of variance. One-way analysis of variance (ANOVA) and a Tukey post hoc was used to test for differences between phases (Early (0 – 25 days), Middle (48 – 94 days) and Late (95 – 141 days)) for each filter and also differences within phases between filters. Data which failed to conform to the assumptions of the one-way ANOVA were analysed using the non-parametric Kruskall-Wallis test and pairwise comparisons were completed using Dunn (1964) procedure with Bonferroni correction for multiple comparisons. Kendall's tau correlation coefficient was used to assess the relationship between filter performance (percentage removal) and filter age, and also between bacteria family relative abundance and filter age. Shannon diversity indices, evenness and rarefaction curves were calculated for all samples.

6.4 Results

6.4.1 Filtration rate

The decline in peak filtration rate and the associated head loss development differed between the replicate filters (Figure 6.2). In the early phase there was a statistically significant difference in median filtration between F3 (0.060 $m^3/m^2/hr$) and F1 (0.072 $m^3/m^2/hr$) (p-value: < 0.001), F3 and F2 (0.072 $m^3/m^2/hr$) (p-value: < 0.001). There was no significant difference in the median filtration rate between F1 and F2 (p-value: 0.250).

In the middle phase there were also significant differences in median filtration rate between all three filters, with significant differences between F3 (0.055 $m^3/m^2/hr$) and F2 (0.064 $m^3/m^2/hr$) (p-value: < 0.001), F3 and F1 (0.065 $m^3/m^2/hr$) (p-value: < 0.001) and F1 and F2 (p-value: 0.020). In the late phase statistical differences in the median flow rate occurred between F3 (0.048 $m^3/m^2/hr$) and F2 (0.051 $m^3/m^2/hr$) (p-

value: 0.012) and F3 and F1 (0.052 $m^3/m^2/hr$) (p-value: < 0.001), no statistical difference in filtration rate occurred between filters F1 and F2.

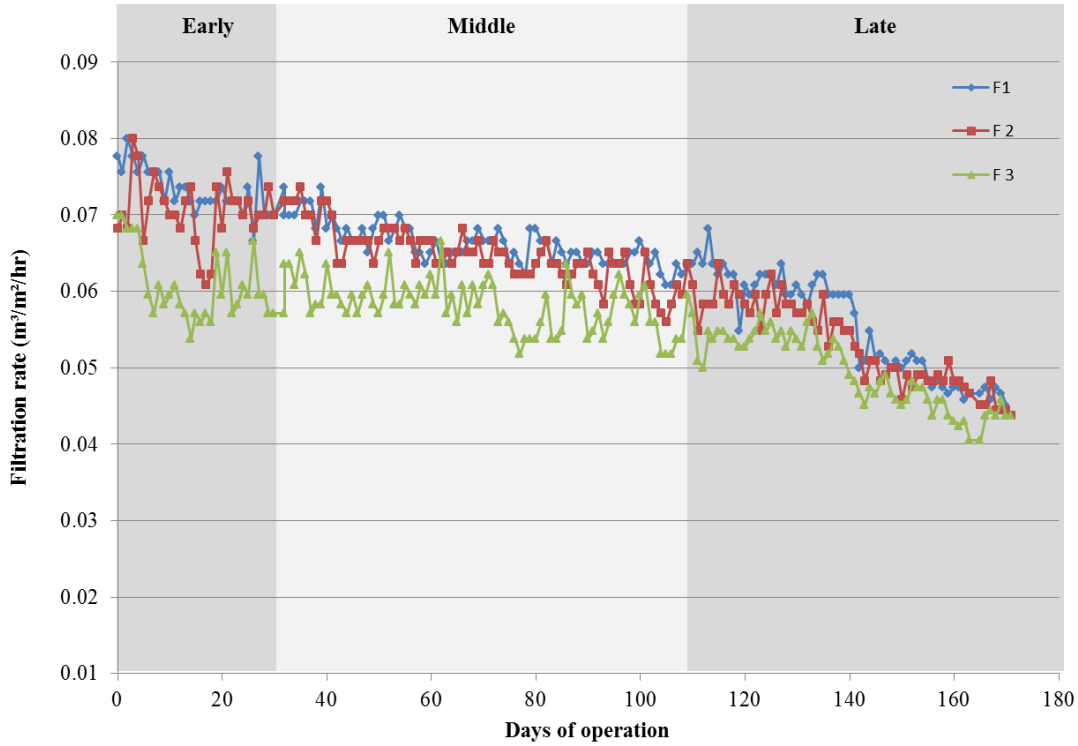

Figure 6.2: Filtration rate for three replicate, full-scale, biosand filters. Early age phase: 0 – 35 days, middle: 36 – 114 days and late: 115 – 171 days.

6.4.2 Water quality

Increased removal of total coliforms occurred with filter age for all filters (Figure 6.3). There was also no significant difference in the removal of total coliform between the three filters over the duration of the study (p-value: 0.754). Similarly, there was also no significant difference in the *E. coli* removal performance between the filters operated in parallel (p-value: 0.450) (Table 6.1).

The influent and effluent physicochemical characteristics are summarized in table 6.1. Overall there was no significant difference in the effluent physicochemical properties between three replicate filters operated over the duration of the study. The pH, dissolved oxygen, nitrite, UV_{254}, SUVA and TSS concentration in the effluent were lower compared to the influent for all three filters. The effluent from all filters exhibited increased EC and nitrate concentrations compared to the influent (Table 6.1).

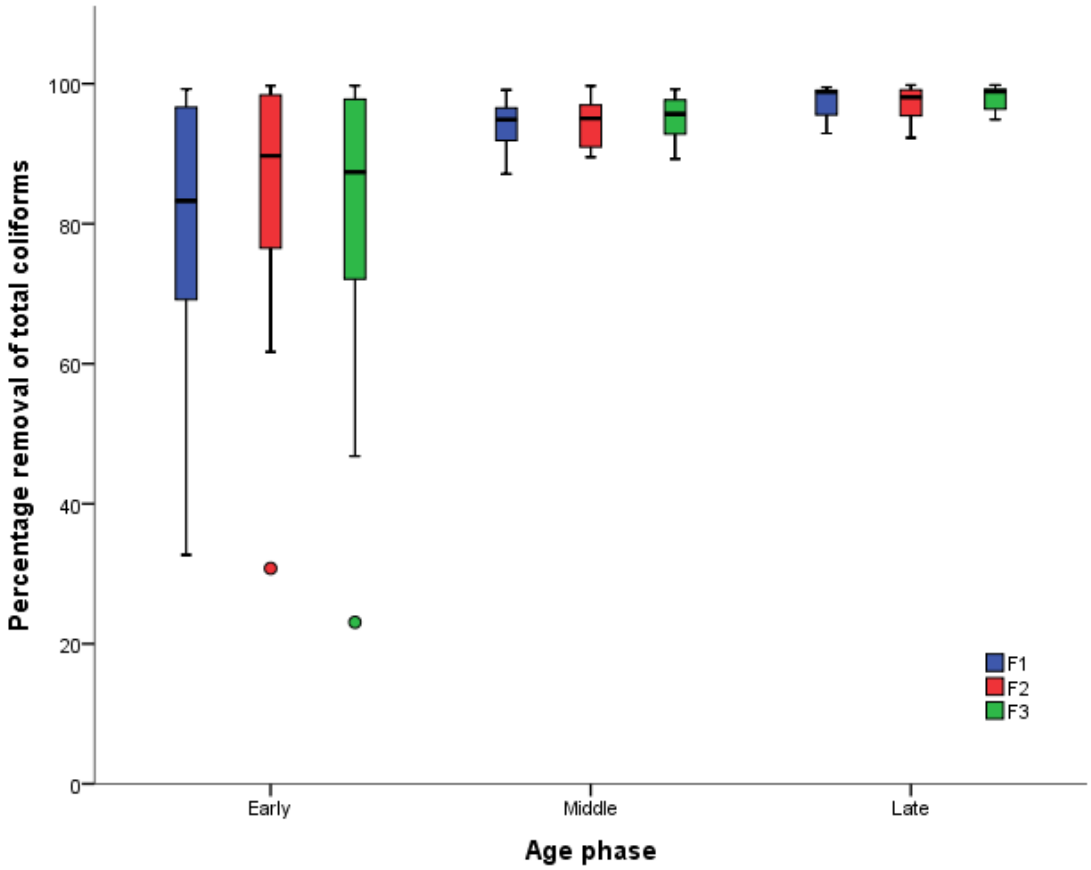

Figure 6.3: Box-whisker plots of the removal efficiency of total coliforms in the different age phases for each filter (F1-F3). Early age phase: 0 – 35 days, middle: 36 – 114 days and late: 115 – 171 days. The top and bottom boundaries of the boxes show the 75th and 25th percentile. The bold lines within the boxes represent the median values (50th percentile) and the end of the whiskers show the maximum and minimum values.

6.4.3 Bacterial community structure

Rarefaction curves indicate that the true diversity of the bacterial community in schmutzdecke, influent and effluent water is likely to be underestimated, indicated by the non flattening of the rarefraction curves (Wooley *et al.*, 2010) (Figure 6.4). The read cut off was 2,372 and hence samples which fell before this cut off were removed from the analysis (Schmu: F2 day 15 and F1 day 43 and effuent: F3 day 85). Species evenness was found to decrease in the influent, schmutzdecke and effluent samples with filter age. During each phase (Early, Middle and Late) a lower species evenness was consistently found in the effluent compared to the influent and schmutzdecke (Figure 6.5 (A)).

Table 6.1: Summary of the phyical, chemical and biological characteristics of the influent and effluent from three replicate, full-sclae, biosand filters (mean ± standard deviation).

Parameter	Number of samples	Influent	F1	F2	F3	p-value
Total coliforms removal (%)	32		88.9 ± 16.1	90.9 ± 13.9	90.2 ± 16.4	0.754
***E. coli* removal (%)**	32		85.3 ± 18.2	83.9 ± 27.2	89.1 ± 17.1	0.450
pH	172	7.77 ± 0.25	7.56 ± 0.23	7.58 ± 0.22	7.59 ± 0.21	0.607
Dissolved oxygen (mg/L)	172	9.67 ± 0.48	8.63 ± 0.76	8.63 ± 0.81	8.57 ± 0.81	0.687
Electrical conductivity (µS/cm)	172	170.9 ± 16.5	180.3 ± 14.4	180.0 ± 13.5	181.4 ± 14.3	0.526
Nitrate – N (mg/L)	24	1.68 ± 0.38	1.78 ± 0.37	1.78 ± 0.37	1.78 ± 0.37	0.999
Nitrite – N (mg/L)	24	4.44 ± 2.55	0.47 ± 1.56	0.75 ± 1.76	1.34 ± 2.66	0.942
Orthophosphate (mg/L)	25	0.011 ± 0.004	0.016 ± 0.004	0.016 ± 0.003	0.016 ± 0.003	0.957
DOC (mg C/L)	24	5.24 0.94	4.70 ± 0.82	4.64 ± 0.76	4.73 ± 0.80	0.920
UV_{254}	25	0.202 ± 0.042	0.150 ± 0.030	0.155 ± 0.032	0.157 ± 0.031	0.490
SUVA (L/mg-M)	24	3.86 ± 0.36	3.21 ± 0.43	3.36 ± 0.42	3.34 ± 0.45	0.427
TSS (mg/L)	25	0.352 ± 0.293	0.145 ± 0.204	0.120 ± 0.279	0.258 ± 0.322	0.339

p-value represents comparison between the filtered water from the three replicate, full-scale, biosand filters. DOC: dissolved organic carbon, SUVA: specific ultraviolet absorbance, TSS: total suspended solids, UV_{254}: spectral absorption coefficient.

The Shannon diversity index ranged from 1.93 to 2.47 for the influent, 2.73 to 2.12 for the schmutzdecke samples (F1 - F3), and 1.90 to 2.22 for the effluent samples (F1 - F3). Comparison of the Shannon index for the influent, schmutzdecke and effluent samples revealed that the highest diversity occurred in the early phase, with a progressive decline in diversity occuring in the middle and late phases for the influent, schmutzdecke and effluent samples (Figure 6.5 (B)). The Shannon diversity index converged for the influent and effluent, together with the diversity of bacterial community in the schmutzdecke of the three filters (F1 - F3) during the late phase (Figure 6.5 (B)).

6.4.4 Schmutzdecke colonization and succession

Shifts in the relative abundance of the dominant bacterial families in the schmutzdecke occurs with filter age in all three filters (Figure 6.6). In the early phase, F1 and F2 were the most similar, with the most abundant bacterial families in the schmutzdecke of F1 being *Moraxellaceae* (10.05%), *Planctomycetaceae* (8.28%), *Shingomonadaceae* (5.63%), *Xanthomonadaceae* (5.58%) and *Oxalobacteraceae* (4.27%). In F2 *Moraxellaceae* (14.94) and *Planctomycetaceae* (10.74%) were the most dominant, followed by *Oxalobacteraceae* (6.54%), *Shingomonadaceae* (5.63%) and *Xanthomonadaceae* (4.44%). The ordering of the dominant bacteria in F3 differed as *Oxalobacteraceae* (12.12%) had the highest relative abundance, followed by *Planctomycetaceae* (9.26%), *Moraxellaceae* (7.11%), *Comamonadaceae* (5.04%) and *Pseudomonadaceae* (3.72%).

As the filters continued to mature, changes in bacterial family dominance occured in the middle phase. The most abundant bacterial families in F1 were *Comamonadaceae* (22.15%), *Planctomycetaceae* (6.83%), *Shingomonadaceae* (4.73%), *Moraxellaceae* (3.63%) and *Bradyrhizobiaceae* (3.39%). *Planctomycetaceae* (F2: 18.57%, F3: 16.95%) and *Oxalobacteraceae* (F2: 6.68%, F3: 5.47%) were the families with the highest relative abundance in F2 and F3. *Acidobacteria* Gp6 family (3.87%), *Moraxellaceae* (3.87%) and *Pasteuriaceae (*3.48%) subsequently had the highest relative abundance in F2. The next most

abundant families in F3 were *Moraxellaceae* (4.80%), *Shingomonadaceae* (4.42%) and *Acidobacteria* Gp6 (3.32%).

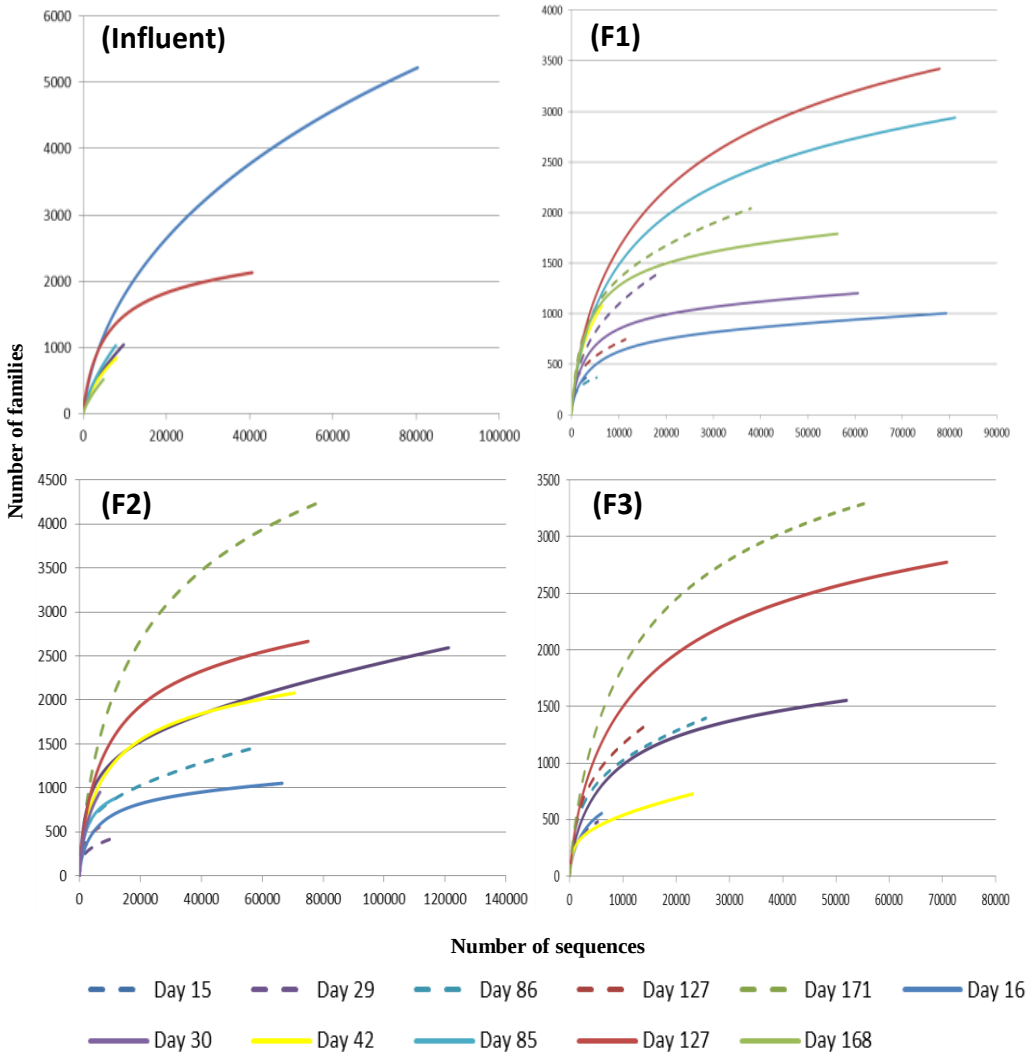

Figure 6.4: Rarefaction curves. Dash line: schmutzdecke samples, solid line: water samples.

In the late phase there was a convergence as the most abundant families in the schmutzdecke in all three were *Planctomycetaceae* (F1: 18.47%, F2: 19.86%, F3: 14.61%) and *Acidobacteria* Gp6 family (F1: 3.99%, F2: 4.34% and F3: 5.73%). *Rhodobacteraceae* (2.53%), *Shingomonadaceae* (2.32%), and *Hyphomicrobiaceae* (2.00%) had the next highest relative abundance in F1, whereas in F2 *Rhodobacteraceae* (2.63%), *Acidobacteria* Gp4 family (2.55%), *Moraxellaceae* (2.46%), and *Sphingomonadaceae* (2.39%) were the next most abundant families.

Acidobacteria Gp4 (2.96%), *Rhodobacteraceae* (2.89%), *Hyphomicrobiaceae* (1.86%) and *Sphingomonadaceae* (1.84%) had the next highest relative abundance in F3. In all three phases for each of the filters there was a high abundance of unclassified organisms in the schmutzdecke ranging from 23.10% - 27.92% in the early phase, 25.44% - 35.44% in the middle phase and 25.11% - 41.60% in the late phase.

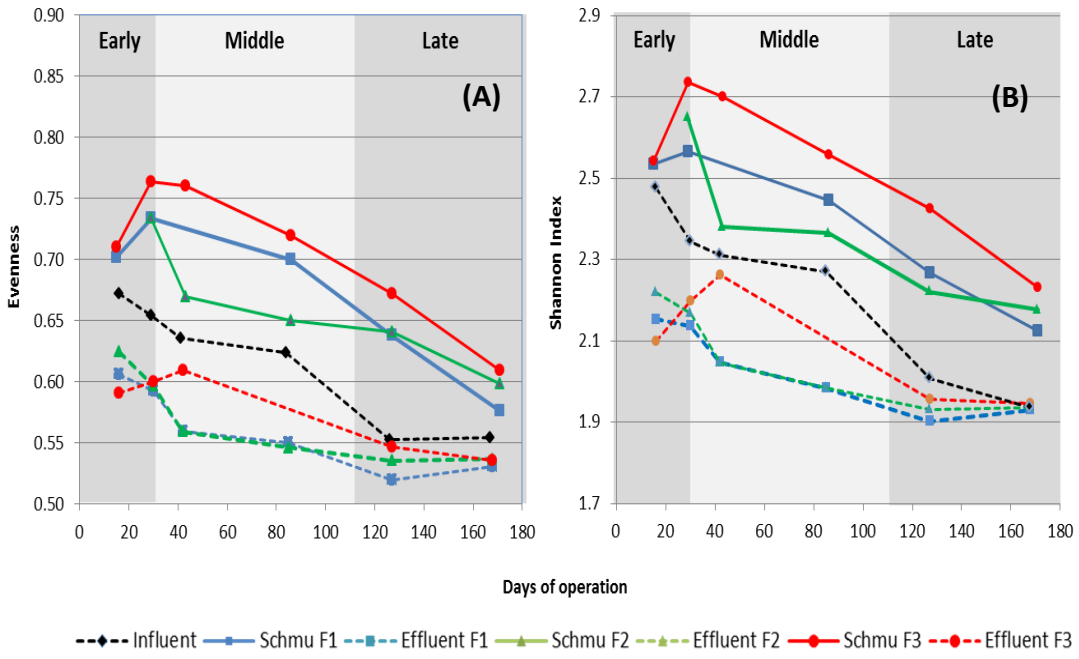

Figure 6.5: Scatter plots show how the evenness (A) and Shannon index (B) change for the influent, schmutzdecke and effluent samples with filter age. Early age phase: 0 -35 days, middle: 36 – 114 days, and late: 115 -171 days.

When the relative abundance of the dominant bacterial families in the different phases were correlated with filter age, a negative correlation was found for the bacterial families *Moraxellaceae* (F1: R = -0.40, p-value: 0.327; F2: R = -0.60, p-value: 0.142; F3: R = -0.60, p-value: 0.091; Influent: R = -0.50, p-value: 0.173) and *Oxalobacteraceae* (F1: R = -0.80, p-value: 0.050; F2: R = -0.80, p-value: 0.050; F3: R = -0.73, p-value: 0.039; Influent: R = -0.07, p-value: 0.851). These were the dominant families in the schmutzdecke in the early phase. However, a positive correlation was found for the dominant bacteria *Planctomycetaceae* and filter age in the schmutzdecke in the late phase, despite a negative correlation being found between the relative abundance of the family in the influent and filter age (F1: R =

0.40, p-value: 0.327; F2: R = 0.60, p-value: 0.142; F3: R = 0.47, p-value: 0.188; Influent: R = -0.47, p-value: 0.188).

6.4.5 Influent and effluent bacterial community structure

Similar bacterial community structures were evident in the influent and the effluent, with *Comamonadaceae*, *Burkholderiaceae*, *Methylophilaceae* and *Oxalobacteraceae* being the four major bacterial families. Across the three different phases, the most abundant bacterial families in the influent continued, although the ordering of the most abundant families changed (Figure 6.6). In the early phase the most abundant families in the influent were *Comamonadaceae* (15.23%), *Burkholderiaceae* (12.58%), *Methylophilaceae* (8.35%), *Oxalobacteraceae* (4.65%) and *Flavobacteriaceae* (4.18%). Likewise in the middle phase the most abundant families were *Comamonadaceae* (18.83%), followed by *Oxalobacteraceae* (12.36%), *Burkholderiaceae* (11.89%), *Methylophilaceae* (4.46%) and *Flavobacteriaceae* (2.51%). Similarly *Comamonadaceae* (31.29%) was the most abundant family in the late phase, followed by *Methylophilaceae* (6.80%), *Burkholderiaceae* (4.30%), *Oxalobacteraceae* (2.91%) and *Cytophagaceae* (1.86%).

Analysis of the effluent water from the filters at the different phases showed that the most abundant bacterial families in the influent water were also typically the most abundant in the effluent, although the ordering based on relative abundance also differed. *Comamonadaceae* was the most abundant bacterial family across all three phases in the effluent for each of the filters, with the exception of F1 in the early phase where the most abundant family was *Burkerholderiaceae* (9.47%), followed by *Comamonadaceae* (8.9%), *Oxalobacteraceae* (6.5%) and *Subdivision2 family incertae sedis* (2.98%). In the early phase, the next most abundant bacterial families, after *Comamonadaceae,* were *Oxalobacteraceae* (11.4%), *Burkerholderiaceae* (5.7%) and Subdivision3 family (3.53%) in F2. Similarly, in F3 the next abundant families were *Burkerholderiaceae* (8.89%), *Oxalobacteraceae* (5.74%), and *Planctomycetaceae* (2.9%). In the middle phase, *Comamonadaceae* was the most abundant in all three filters: 13.6%, 15.5% and 9.4% in F1, F2 and F3 respectively. In the late phase the most abundant bacterial families were *Comamonadaceae* (F1: 12.4%, F2: 14.67%, F3: 15.79%), *Burkerholderiaceae* (F1: 4.1%, F2: 4.37%, F3: 4.29%), *Oxalobacteraceae* (F1: 3.2%, F2: 3.74%) and *Methylophilaceae* (F1: 2.98%,

F2: 3.19%). The effluent from F3 had a higher relative abundance of *Methylophilaceae* (3.65%) compared with the *Oxalobacteraceae* (3.5%) family. It was also found that there was high similarity between the bacterial structure of the influent and effluent in the different age phases and in the filters operated in parallel (Figure 6.7). Also, there was high similarity between the bacterial communities in the schmutzdecke at all age phases and filters (Figure 6.7).

Furthermore, when the relative abundance of bacterial families in the influent was compared with the relative abundance of the same family in the effluent, the relative abundance was generally lower. However, there are cases were the relative abundance of some bacterial families were higher in the effluent compared to the influent. In the early phase, a higher relative abundance of *Oxalobacteraceae* occurred in the effluent compared with the influent for all three filters. Similarly, in the middle phase, increases in the relative abundance of some of the bacterial families occurred in the effluent water compared with the influent, including the family *Planctomycetaceae* where the relative abundances were 2.05%, 2.17% and 4.96% in the effluent in F1, F2 and F3 respectively, compared to 1.10% in the influent to all filters. Likewise, in the late phase there were also increases in the family *Planctomycetaceae* where the relative abundances were 2.05%, 2.17% and 4.96% in the effluent in F1, F2 and F3 respectively, compared to 1.10% in the influent to all filters. Likewise, in the late phase there were also increases in the relative abundance of some bacterial families in the effluent for the three filters in comparison to influent including the *Oxalobacteraceae* and *Acidobacteria* Gp6 family. Similar to the schmutzdecke samples, rare species and unclassified organisms contributed a significant portion of the diversity in influent and effluent samples analysed.

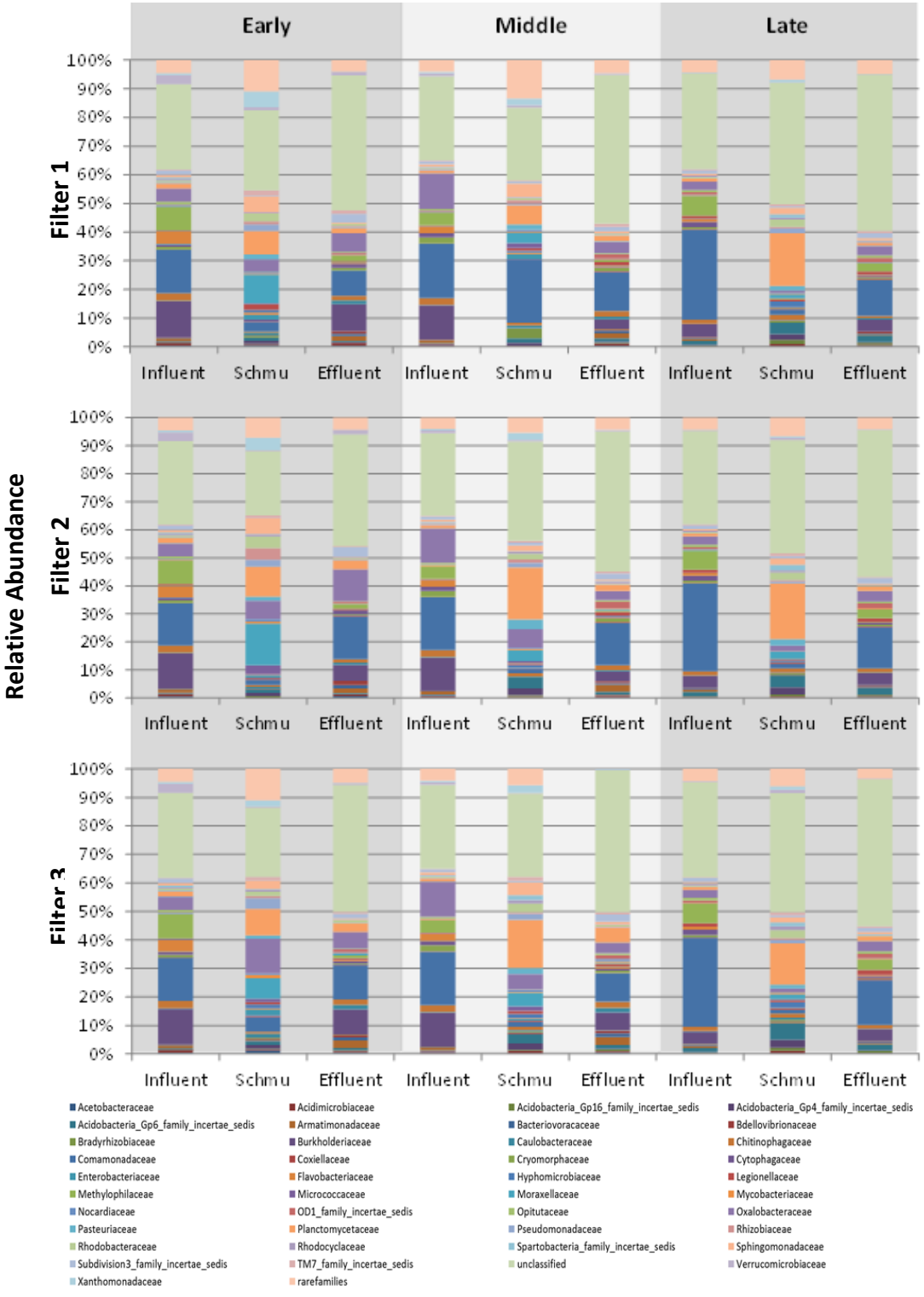

Figure 6.6: Stacked bar graph showing the average relative abundance of each of the families for the influent, schmutzdecke and effluent samples for each of the three phases. Early phase (0-35 days), middle phase (36-114 days) and late phase (115-171 days).

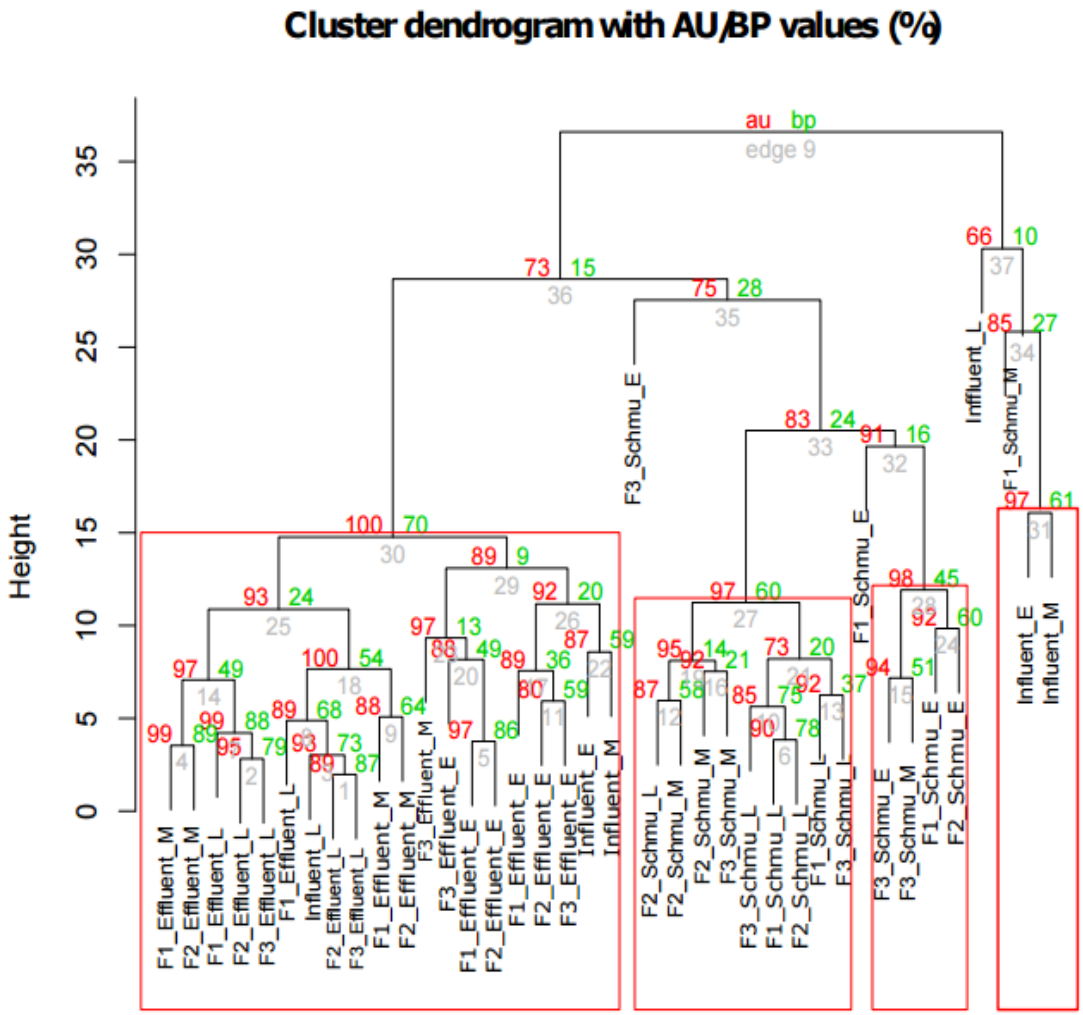

Figure 6.7: Hierarchical cluster dendrogram of the microbial community at the family level for influent, schmutzdecke and effluent in the three BSFs (F1, F2 and F3) in the different age phases (E: Early, M: Middle, L: Late). Values in red are the AU (Approximately Unbiased) p-values, values in green are the Bootstrap probability values and values in grey are the standard error. Clusters with AU ≥ 95% are indicated by the red rectangles.

6.5 Discussion

The microbial ecology of SSFs has previously be assessed (Haig *et al.*, 2014), however this current study is the first to assess the development of the bacterial community in the schmutzdekce of BSFs, while also simultaneously assessing the bacterial community structure of the influent and effluent. This study indicated that

the relative abundance of bacterial families in the influent were not found to be a driver for family dominance in the schmutzdecke; i.e. bacterial families with the highest relative abundance in the influent did not subsequently have the highest relative abundance in the schmutzdecke. The establishment of *Planctomycetaceae* as the dominant bacteria family in the schmutcdecke of all three filters in the late phase was not found to be result of an increase in the abundance of the bacterial family *Planctomycetaceae* in the influent water. Interestly the Wang *et al*., (2014) study which assessed the community structure of the schmutzdecke at a single time in full-scale BSF found the *Alphaproteobacteria* and *Gammaproteobacteria* classes had the highest relative abundance. The difference in the schmutzdecke community structure between the Wang *et al*., (2014) study and the current study would suggested that the schmutzdecke community is distinct and not reproducible for biosand filters with different feed water supplies.

The different rates of bacterial family succession and dominance in the schmutzdecke, between the filters operated in parallel during the early and middle phases, could possibly be attributed to the higher flow rate resulting in a reduced contact time and increased shear stress, in turn affecting bacterial colinization and succession. However, by the late phase the same bacterial families (*Planctomycetaceae* and *Acidobacteria* Gp6) were dominant in the schmutzdecke of all three filters, despite differences in flow rate, suggesting that BSFs operated under the same conditions with the same influent water are reproducible with filter age.

The duration of the maturation period is poorly understood for BSFs, with research suggesting different time periods of two weeks (Jenkins *et al*., 2011) to 30 days (Baumgartner *et al*., 2007). Young-Rojanschi and Madramootoo (2014) suggested that filters operated for 60 days might not have reached maturity and that the performance may continue to improve with filter age. However, the characteristics of "filter maturity" remain undefined. If the efficency of the filter in terms of the removal of indicator organisms is to be used as the defining characteristic of a filter reaching maturation, based on the finding of this current study and the removal of the microbial indicator total coliforms, the filters had reached maturation by day 35 (end of the early phase) because the removal of total coliforms had reached a high, stable level. However, if the defining chacteristic of a BSF reaching maturation is the

stablization of the schmutzdecke, then this current study would suggest that it could take as long as 114 days before the schmutzdecke bacterial community stabilizes.

It was also found that the bacterial community evenness and diversity in the influent, schmutzdecke and effluent decreased with filter age. This decline was unexpected as Haig *et al.* (2015) found greater microbial community evenness led to better filter performance. Also, greater microbial community evenness has been attributed to providing greater robustness and functional stability, enabling the microbial community to be adaptable to changes in influent water characteristic (Wittebolle *et al*., 2009). The decline in the evenness and diversity of the schmutzdecke microbial community in this study may be explained by the different feed flow pattern of a BSF compared to a SSF. The intermittent operation of a BSF compared with the continuous operation of a SSF leads to varied environmental conditions in the schmutzdecke during the filter resting period, i.e. the period during which there is no discharge from the filter. Young-Rojanschi and Madramootoo (2014) reported sharp declines in DO in the top portion of the BSF during the filter resting period which further demonstrates the competitive environment of the schmutzdecke for not only space but available resources. The establishment of *Planctomycetaceae* as the dominant bacterial family in the schmutzdecke as the filters matured is likely aided by the high tolerance the family has shown to different environmental conditions (Kulichevskaya *et al*., 2008; Kulichevskaya *et al*., 2007; Fuerst and Sagulenko, 2011).

A further important observation of this study is that BSFs operated in parallel, under the same environmental conditions and receiving the same influent, are reproducible in terms of the effluent water physicochemical and biological characteristics. By day 35, the performance of the filters, with respect to the reduction of microbial indicators, were in line with filter performance in other laboratory studies which reported removal of indicator bacteria to range from 90 – 99% (Elliott *et al*., 2008; Stauber *et al*., 2006). Effluent from the filters met the WHO Guidelines for Drinking-Water Quality (WHO, 2011), with the exception of the guideline value for microbial indicator organisms of 0 CFU/100 ml.

This current study indicates that the stabilization of the bacterial community structure may be contributing to the enchanced peformance. As the filters matured and the bacterial community structure stabilized, the removal of microbial indicator organisms also continued to increase. Young-Rojanschi and Madramootoo (2014) found that the highest removal of indicator microorganisms occurred in the first 5 cm of the sand column, indicating the importance of the schmutzdecke to filter performance. Similarly Wang *et al*., (2014) attribed the inceased removal of the MS2 bacteriophage specifically in the schmutzdecke (2.1 ± 0.2-$\log_{10}$ reduction in filters operated between 22 and 28 days to 3.2 ± 0.2-$\log_{10}$ reduction between 152 and 201 days) to the highest diversity of the microbial community in the BSFs. Earlier studies also suggest the importance of an intact schmutzdecke, with sharp decreases in the reduction of bacteria occuring when the schmutzdecke is removed (Hijen *et al*., 2004; Unger and Collins, 2008). The convergence of a similar bacterial community stucture in all filters in the late phase of this study is intriguing and suggests, as found by Massol-Deya *et al*. (1997), that the microbial community converge to one that supports optimum performance.

Despite the three BSFs being replicates in terms of sand grain characterisitics and depth, loading head and dose water volumes, differences in filtration rate occured between the replicate filters. A decline in filtration is expected as the filter matures, with particulate accumulation and biological growth (Elliott *et al*., 2008). Earlier studies have reported that lower filtration rates result in enhanced filter performance, by allowing increased particle settlement and also increased contact time between bacteria, filter media and schmutzdecke (Jenkins *et al*., 2011; Kennedy *et al*., 2012). An interesting observation in this study was that, while the filtrations rates differed, the performance of the filters in terms of the removal of microbial indicator organisms (Total coliforms and *E. coli*) was not affected. Chan *et al*. (2015) also recently reported that a lower filtration rate did not enhance filter performance.

6.6 Conclusions and recommendations

This study has shown that the relative abundance of bacteria in the influent water was not a driver for bacterial dominance in the schmutzdecke. Further research is now required to determine how the dominant bacterial families in the schmutzdecke

directly contribute to filter performance. It remains unclear what is driving the differences in the dominant bacterial families in the schmutzdecke between the filters during the early and middle phases. It is likely that the difference in flow rate between the filters is impacting the bacterial colonization of the schmutzdecke and rate of succession. However, further experimental work is required to confirm this.

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Chapter 7

Synthesis and recommendations

7.1 Synthesis

This chapter discusses the main findings of the thesis while also outlining some limitations of the work and recommends some aspects that would benefit from further research. The focus of this thesis was to build on earlier studies that have assess the effect of mode of operation on filter performance (Lynn *et al*., 2013; Young-Rojanschi and Madramootoo, 2015; Young-Rojanschi and Madramootoo, 2014; Elliott *et al*., 2008; Kennedy *et al*., 2012). This thesis added to the limited existing knowledge on the community structure of the schmutzdecke of BSFs, by using next generation sequencing (MiSeq) of the 16S rRNA gene to determine the bacterial community of the feed water, schmutzdecke and filtered water. This enabled the contribution of the available bacteria in the influent to the development and maturation of the schmutzdecke to be determined. Understanding the impact of different operational conditions together with the biological community structure will enable optimisation of the BSF design, operation and maintenance. This will ultimately ensure user satisfaction which will promote the long term use of these filters.

Specifically this thesis aimed to address the following questions:

1. Does sand properties including grain size distribution, shape, roughness and inorganic composition impact schmutzdecke development and filter performance?
2. What is the impact of resting water depth on the schmutzdecke and filter performance?
3. Does operating temperature affect the microbial community and filter performance?
4. What bacterial families are present in the feed water, schmutzdecke and filtered water for full-scale Hydraid® filters?
5. How does the schmutzdecke community change with filter maturation?

To address these questions, a two-pronged approach was adopted to assess the impact of different operating conditions. The development of the schmutzdecke was assessed by determining changes in the metabolic function of the microbial community (Chapters 3 - 5) and community structure (MiSeq) (Chapter 6). The impact of different operational conditions on filter performance was determined

using both biological and physicochemical techniques. Overall this thesis has shown that BSFs are highly effective and capable of greatly improving drinking water quality.

7.2 Filter media characteristics

Filter media is a fundamental component of the BSF technology and stringent guidelines exist regarding sand size selection (CAWST, 2007; Elliott *et al*., 2008; Lukacs, 2002). However, given the geographical distribution of BSFs, it is assumed that sand is sourced from readily available sources locally (e.g. crushed rock, sand quarry, river sand, beach sand and desert sand) resulting in the sand having different grain properties (i.e. shape, surface texture and chemical composition). The data presented in Chapter 3 are similar in some aspects to the study of Elliott *et al*. (2015), but contribute further knowledge by determining the influence of sand properties on the development of the schmutzdecke. The rate of change in metabolic function of the microbial community in the schmutzdecke was found to differ between the sand types; although as the filters matured their metabolic activity, diversity and richness became similar despite the differences in sand properties. The results in Chapter 3 also showed that the greater grain size distribution of the poorly sorted sand resulted in an increased flow rate which was not found to affect filter performance. This indicates the potential to ease the current sand size guidelines of ≤ 0.7 mm.

7.3 Implications of resting water depth (RWD)

Earwaker (2006) found RWDs to range from 0 to 20 cm in a field study in Ethiopia and proposed that low and high RWDs lead to undesirable conditions for the survival of the microbial community in the schmutzdecke. In the current literature there are conflicting reports of the impact of RWD depth on filter performance. The results of Chapter 4 show that filters operated under the same conditions but with different RWDs (2 cm, 5 cm, 8 cm and 11 cm) were equally efficient at removing total coliforms. Furthermore, the development of the schmutzdecke was not found to be affected by different RWDs and similar metabolic functions were found for the different schmutzdecke communities.

Chapter 4 did not identify a direct relationship between a high or low RWD depth and filter performance. However the resting period was always 24 ± 1 hr and did not determine whether a multi-day resting period would influence the performance of the filter under different RWD conditions. It could be anticipated, that under multi-day resting periods, anoxic conditions would gradually develop in the schmutzdecke due to the distance that oxygen would have to diffuse from the atmosphere to the schmutzdecke under high RWD conditions. Therefore, the effect of different RWDs combined with multi-day resting periods requires further investigation.

7.4 Performance under different ambient temperatures

Chapter 5 explored the impact of ambient temperature on filter performance. Higher mean *E. coli* reductions occurred in BSFs operated at a lower temperature. Interestingly, the metabolic function of the schmutzdecke community was not found to be affected by ambient temperature. This is the first study to show that BSF performance differs under different temperature conditions, indicating that householders may experience differences in filter performance under different seasonal temperature variations. Along with this, it is interesting that despite no difference in filter media characteristics an increased retention of *E. coli* occurred in filters operated at lower temperatures. Therefore, the fate of pathogenic organisms in BSFs and their removal requires further investigation.

7.5 Characterization of the schmutzdecke of full-scale filters

Chapter 6 describes the periodic sampling of the feed water, schmutzdecke and filtered water of three full-scale BSFs, in order to determine the influence of the feed water bacterial community on the development of the schmutzdecke, and to determine how the schmutzdecke bacterial community structure changed as the filters aged. The results demonstrated that the relative abundance of bacteria in the feed water was not a driver for the subsequent dominance of particular bacterial families in the schmutzdecke. It was also found that during the early (0 – 35 days) and middle (36 – 114 days) phases of filter maturation the bacterial families differed between the filters, but by the late phase (115 – 171 days) the same families dominated the schmutzdecke in all filters (*Planctomycetaceae* and *Acidobacteria Gp6*). As the filters aged, the evenness, diversity and richness of the bacterial communities decreased in the schmutzdecke. This is the first study on BSFs to

examine the 16S rRNA community in the feed water, schmutzdecke and filtered water of BSFs.

7.6 Filtration rate

Chapter 3, 5 and 6 all support the observation of Chan *et al*. (2015) that lower filtrations do not enhance filter performance. In chapter 6, the rate of bacterial family succession in the schmutzdecke of filters operated under the same conditions differed during the early (0 – 35 days) and middle (36 – 114 days) phases of filter maturation, and this difference could possibly be attributed to the differences in filtration rate.

7.7 Recommendations for further research

The research conducted for this thesis has demonstrated that the performance of BSFs is influenced by various operational parameters (temperature and sand properties). Furthermore, the results have shown that the bacteria families which dominate the schmutzdecke of biosand filters is not driven by the relative abundance of bacteria families in the feed water. Nevertheless, the results presented in the preceding chapters indicate several opportunities for further research.

Further research should focus on the mechanisms contributing to the removal of pathogenic organisms as little is known about the directly contribution of the biological community in the filter to the removal of pathogenic organisms. Future studies should adopt approaches such as stable isotope labelling to track the movement of the microbes through the BSF column, and determine whether the biological community in the filter are the most important mechanism in pathogen removal/retention or whether physical mechanisms are the primary mechanism in the removal of pathogen organisms.

Future studies would benefit to examine the dissolved oxygen profile through the RWD and at the sand water interface, under different RWD conditions. This would help determine whether an increased RWD results in an increased oxygen stressed environment at the sand water interface or whether the increased volume of water associated with the increased RWD provides a sufficient supply of DO to the schmutzdecke during the filter resting period. This would allow for the identification

of the optimum RWD to avoid oxygen stressing the schmutzdecke during the filter resting period.

This thesis provides the first insight into the bacterial community colonization and succession in the schmutzdecke of full-scale BSFs. However, future studies would benefit to also complete 18S rRNA profiling. While understanding the structure and composition of microorganism in the schmutzdecke is important, understanding the activity and function is also important in order to get a full picture of the microbial ecosystem. Hence, future studies of the functional gene using metatranscriptomic (reverse transcription polymerase chain reaction (RT–PCR) or Microarray) would be beneficial. Also in this thesis, the development of the schmutzdecke was assessed over a 6 month period however, future work would be benefit to study the entire life cycle in order to determine if the same microbial community structure re-established post the completion of filter maintenance (wet harrowing).

The effect of source water quality and the impact of changing source water on the performance of BSFs in removing pathogenic organisms need to be assessed. It is likely that the majority of users of BSFs will use different water sources (i.e. river water, rain water) to feed their BSFs over a yearly cycle depending on availability. Future studies should assess the impact of varying feed water characteristics (turbidity and nutrient loading) on filter performance. In this thesis the coliform group were used as the indicator organisms to determine filter performance however, future studies would benefit to determine the fate of viruses in BSFs, including human enteric viruses, to determine the impact of operating conditions on virus removal.

7.8 Conclusion

The work presented in thesis has provided greater understanding of the effects of different operating conditions on the development of the schmutzdecke and BSF performance. This thesis further provides the first insight into the schmutzdecke microbial community structure in full-scale BSFs. Using the finding reported in this study along with some future studies will allow for the advancement of operational guidelines, enabling households to operate their BSF to achieve optimum filter performance. It is the belief of the author that the correct operation of a BSF offers the most effective, sustainable and cost-effective technology to treat drinking water

for the poor and marginalized in the developing regions that remained without a safe piped water supply.

7.9 References

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