TU Delft

Environmental Engineering

Master Thesis

Bacteria and viruses removal in slow sand filters

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November 2021

Acknowledgments

Time flies, the snowflakes of the cold winter of Delft in 2020 are still flying in my memory, but right now, I'm writing this paragraph far away in Sichuan, China. I can always feel the peculiarities of my precious study career at TU Delft. It's a journey across space and time, a process of self-exploration, denial, and affirmation. If I have to choose two of the most important points in this period, I would say the day I met my fiancée in the Netherlands and the day I joined the big family of Prof. Dr. Ir. Jan Peter van der Hoek, Dr. Ir. Doris van Halem and Ir. Shreya Trikannad.

To start with, I want to give a big shout-out to my mentors Jan Peter van der Hoek, Doris van Halem for their guidance, insight, and support. I'm very grateful for their kindness, patience, and encouragement, which helped me out whenever I was struggling with my thesis. At the same time, I feel really happy and warm to have both of them enlighten me in the right direction and keep me on track. Big respect to my mentor Shreya Trikannad! I appreciate that she treats me like her own little brother and offers me meticulously help not only in the whole master thesis but in daily life. I've truly learned a lot from them, from every small detail in the thesis to the big direction of life. I thank Thom Bogaard for providing invaluable feedback and guiding me to think critically. I thank Ahmed Mahmoud for providing precious advice on the experimental setup design and a great help for difficulties that I met during the lab. I am very grateful for the help from the laboratory as well. Thank you, Armand Middeldorp, for being a solid backup to help and assist me whenever needed. Your quick response and valueble information help me find the most powerful and efficient way to solve my problem and advance my process.

Next, I would like to show my respect to my friends who always hear me out and offer me help. Thanks to Lucas for being a 'different' person with me in the white lab. His positive attitude toward life and superb rap skills really helped me through many depressive days. Thanks to Connie Au for her precious idea and help, which really helped me have an easier life in the lab. Thanks to Sadhna for her every big smile and daily chat. It's really good to have a friend to hear you out every day, which helps me get over my fear of expressing myself in English.

Finally, I have to thank my fiancée Jianyi Tang for her accompany, understanding and help. I'm so lucky that I could get the chance to meet her there. She changed my thoughts about the world and my recognition of myself. She makes me feel much stronger and more confident than ever before. She cheered me up whenever I got depressed. Without the support from whom, this would have been an impossible job for me.

Abstract

Though slow sand filtration is one of the oldest and effective means of drinking water treatment, the mechanisms contributing to bacteria and viruses removal are not well understood. The lack of understanding of actual removal potential and different mechanisms occurring in the filter bed has limited the development of new filter design and operation. This research aims at assessing the bacteria and viruses removal capability of filter material in different depths from the top 40 cm of full-scale slow sand filter (SSF) operated for 436 days. In addition, the focus is to identify the key removal mechanisms that aid bacteria and viruses removal in the schmutzdecke.

The results show that three depths: 0-5, 5-20 and 20-35 cm contribute to E. coli removal of 0.55, 1.3 and 1.04 logs, PhiX174 removal of 0, 0.30 and 0.14 logs. The log reduction value of E. coli and PhiX174 is rather similar in different layers, even though the schmutzdecke is considered to be the critical component for E. coli removal. It indicated that the deeper layers are also important in a well-established SSF. No removal of PhiX174 was observed in 0-5 cm with a thick biofilm, which indicates that the thickness of a certain level would impact the performance of virus removal.

To determine mechanisms, filter material from 0-5 cm was operated under three conditions: active, inactive, and ignited condition. The results show E. coli removal of 0.68, 0.74, and 0.43 logs, PhiX174 removal of 0, 0, and 0.28 logs for active, inhibited, and ignited sand, respectively. Contrary to previous studies, no function of microbial mechanisms is observed for E. coli removal. That key mechanism might change with the different maturity levels of SSFs might be a possible reason. In addition, this may ascribe to incomplete microbial active inhibition. On the other hand, despite evidence that virus removal enhances with filter maturation, schmutzdecke did not improve PhiX174 removal. Poor virus removal may be attributed to higher interstitial velocity along with higher shearing forces caused by abundant biofilm within the schmutzdecke.

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Symbols and Abbreviations

AOC	Assimilable organic carbon
BDOC	Biodegradable dissolved organic carbon
dATP	dissolved ATP
EPS	Extracellular polymetric substance
EC	Electrical conductivity
HRT	Hydraulic loading rate
LRV	Log reduction value
PVC	Polyvinyl chloride
SSF	Slow sand filter
tATP	total ATP

Chapter 1: Introduction

Global drivers associated with population growth, demographic shifts, and climate change place increasing stress on fresh and sustainable water supplies. In this context, the development and application of efficient drinking water treatment technologies are needed for the continuous improvement of water quality and access. High water quality demands may be fulfilled using a variety of methods such as reverse osmosis, UV, and ozonation. The employment of these technologies, on the other hand, has a relatively high operational cost. Nowadays, rather than technological limitations, economic constraints are the limiting issue, especially for developing countries. Conventional filtration, especially slow sand filtration, is known for its economic benefits among these innovations and has been widely used in the development of safe and biological-stable drinking water (Urfer et al., 2016). These filters gained popularity due to their simple design and construction, ease of operation, and capacity to improve physical-chemical and biological water quality in a single process (Huisman & Wood, 1974).

Slow sand filters (SSF) date back to 1804 when John Gibb built and constructed the slow sand filter system in Paisley, Scotland (Huisman & Wood, 1974). After that, in 1829, the method modified in practical details was first applied for public use when James Simpson made an installation to treat the water from Chelsea Water Company in London (Huisman & Wood, 1974). By 1852, for its evident advantages, the Metropolis Water Act required all water derived from the River Thames within 5 miles of St Paul's Cathedral to be treated before being supplied to the public (Logsdon et al., 2006).

Due to specific treatment requirements such as low filtration rate, large area, and influent turbidity limitation, SSF was substituted by rapid sand filtration (Lauderdale et al., 2012). However, SSFs have gained increased interest in the last three decades (Zhao et al., 2019) owing to the advantages such as chemical-free treatment depending on water quality, energy efficiency, and ease of design and operation (Lauderdale et al., 2012; Li, 2016). Most importantly, SSFs exhibit great capability of removing bacteria, viruses, cysts, and various chemical contaminants. Slow sand filtration showed a wide range of removal efficiencies for total coliforms (0.3-3.5 log units), fecal coliforms (2-2.4 log units), E. coli (1.9-4.1 log units), and enterococci (0.7 – 3.7) (Bauer et al., 2011b; Farooq & Al-Yousef, 1993; Keraita et al., 2008a; Langenbach et al., 2009, 2010; Sadiq et al., 2003).

In addition, SSFs are widely applied for drinking water and wastewater treatment (Verma et al., 2017). For its' effectiveness in removing particulate suspended matter, it is applied for the treatment of groundwater. Because of its capacity to remove organic substances and pathogenic organisms, it is also appropriate for surface water with moderate turbidity (Huisman & Wood, 1974; Keraita et al., 2008b; Moreira Neto et al., 2012). In wastewater treatment, SSF is one of the most promising post-treatment

methods for upflow ananrobic sludge blanket effluents and has been extensively employed for tertiary treatment (Verma et al., 2017).

Chlorine is not preferred in the Netherlands, either as a primary or residual disinfectant. While it is generally recognized that chemical disinfection improves the quality of drinking water, the Dutch believed that the drawbacks of disinfection by-products outweigh the benefits (Smeets et al., 2009). In the Netherlands, slow sand filtration is applied to ensure the safety and biostability of water for its' capability of the pathogen, assimilable organic carbon (AOC), and biodegradable dissolved organic carbon (BDOC) removal. Combined with enhanced pretreatment including soil passage, ozone, and activated carbon filtration (Guchi, 2015a), the regrowth of microbes could be greatly avoided in the distribution system. SSFs have gained wide attention from countries like the Netherlands, Switzerland, and England, due to their simple operation and cost-effectiveness.

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Chapter 2: Literature review

2.1 Elements of slow sand filter

As shown in Figure 1, SSF is made up of five basic elements: Supernatant, schmutzdecke, filtration sand layer, under-drainage system, and control systems.

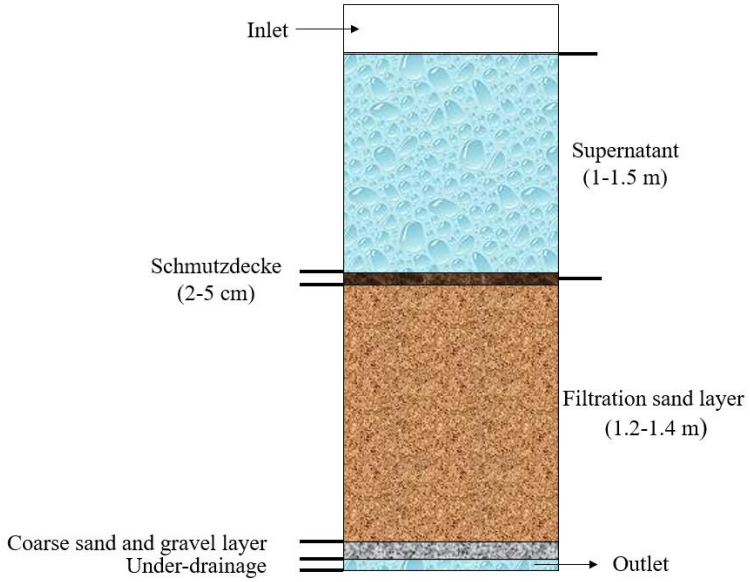

Fig. 1. Scheme of the elements of the slow sand filter

Supernatant: The function of supernatant water is to provide a constant head of water above the filter media, which maintains the pressure that can make the water pass through the filter (Huisman & Wood, 1974). The depth of supernatant water is designed according to the expected maximum resistance. Normally the water depth above the sand bed is maintained at about 1–1.5 m with a maximum of 2 m (Ratnayaka et al., 2009). There would be a freeboard higher 20-30 cm than the supernatant level.

Schmutzdecke: It is a biological layer formed on top of the filter as raw water passes through the filter. Schmutzdecke is richly populated with protozoa, bacteria, algae and other forms of life (Dizer et al., 2004). In the SSF system, the Schmutzdecke is considered to be the most effective removal element (Barrett et al., 1991; Unger & Collins, 2008a).

Sand bed: The sand bed harbors various processes that contribute to contaminant removal. The recommended uniformity of filter sand is 1.5 – 2. It should be made up of hard and durable grains, which should not contain more than 2% of calcium and magnesium. The generally effective diameter of the filter medium is between 0.15 mm – 0.35 mm (Huisman & Wood, 1974). The height of the filter bed usually lies at 1.2 m

– 1.4 m (Huisman & Wood, 1974).

Under-drainage system: This system provides a mutable function that not only supports the filter medium but also reduces the possibility of obstruction. It includes coarse sand and gravel layer and an under-drainage layer. With the under-drainage system, treated water can flow through the underside of the sand layer (Huisman & Wood, 1974).

Control system: Generally, valve control is used in the control system to adjust the velocity of flow to make sure the raw water level won't drop below an expected level during operation (Huisman & Wood, 1974).

2.2 Schmutzdecke

2.2.1 Definition and development of Schmutzdecke

When a sand bed is submerged in the nutrient-rich water for an extended length of time, biologically active mats containing photosynthetic microorganisms (if the light is accessible) and heterotrophic bacteria would develop. ‘Schmutzdecke’ is a German word. 'Schmutz' means dirt, and 'Decke’ stands for covering. It’s a general term (cake layer or slime layer is used as well for some researchers) applied to describe these biologically active mats (Adin, 2003). The composition of schmutzdecke can vary widely with seasons. Generally, plankton, algae, diatoms, heterotrophic protists, rotifers, and bacteria are found. Schmutzdecke is the place where inert suspended particles may be mechanically strained, organic material and nitrogenous substances decomposed, and microorganisms entrapped (Huisman & Wood, 1974). The character of the schmutzdecke also changes with the properties of the source water. Overall, the growth of schmutzdecke requires the presence of sufficient food, oxygen, and a proper temperature. The quantity of organic material provided by influent raw water limits the proliferation of microorganisms inside the schmutzdecke. With hydraulic loading rate (HLR) of 0.1 m/h – 0.3 m/h and sand size of 0.15 cm -0.35 cm, schmutzdecke primarily develops in the upper 0.5-2 cm of the sand bed (Ratnayaka et al., 2009).

Huisman & Wood proposed that at the initial stage of the development of schmutzdecke, the filter should be operating continuously and without interruption for at least several weeks depending on the climate and characters of the raw water. The cleaner raw water needs a longer maturity time (Huisman & Wood, 1974). Bellaymy et al. (1985) reported that the ripening period could be shortened by adding synthetic polymers to agglomerate particles in the raw water to speed up their removal at the filter surface, allowing the filter cake to form more rapidly (Bellamy et al., 1985b). The concept was endorsed by the experiment of Jellison et al. In just 24 hours, a modified filter with continuous polymer feed matured effectively and generated water with turbidity below 1NTU. Haig et al. pointed that inoculation with pre-conditioned microbial biomass can lead to the fast development of SSF. The results of laboratory experiments showed that no-sterile columns removed pathogens after 4-6 weeks, while clean sand columns

needed 7-10 weeks to achieve the same level of performance (Haig et al., 2014). Microbial reductions improved with ripening, according to Elliott et al. (2008), although a more considerable decrease was seen after 30 days. In the study of Elliott et al., microbial reductions improved for up to 53 days, which suggested that enough ripening time was needed for better removal performance.

2.2.2 Role of schmutzdecke

The improvement of water quality is associated with the formation of schmutzdecke on top of the filter (Yao et al., 1971). Barrett et al. (1991) observed bacteria removal was higher in the schmutzdecke by 1-3 logs compared to the deeper depths. By measuring green fluorescent protein, Unger & Collins (2008a) reported that the schmutzdecke entrapped significantly more challenge bacteria than other depths, while a large number of E. coli was also detected in the deeper layers. The result indicates that the entire filter bed also plays an important role in E. coli removal.

Virus removal capacity is thought to be independent of the existence of schmutzdecke. 2-2.8 $\log_{10}$ MS2 elimination was acheived after the scraping of schmutzdecke, which is similar to the general performance of viruses' removal found by other researchers (Hijnen et al., 2004b). (Mcconnell et al., 1984) found that reovirus was spread across the whole bed, while most parts of reovirus were found in the first 42 cm. (Bauer et al., 2011a) suggested that virus removal was enhanced in model systems with the development of schmutzdecke and deeper sand biofilm. (Schijven et al., 2013) also pointed out that the reduction of MS2 would increase with the ageing of schmutzdecke and temperature.

Ranjan indicated that schmutzdecke had not only a filtration function but also acted as a bypass to connect the penetration zone (top 0.5-2 cm) to the deeper zone. The deeper zone thus can serve as a continuation area of biological action (Ranjan & Prem, 2018). (Verma et al., 2017) raised the same point that schmutzdecke are contributors to microbes attached to sand particles in deeper layers.

2.3 Bacteria and viruses removal in SSF

Slow sand filtration can enhance the bacteriological quality of water by removing bacteria, viruses, protozoa, cysts, and various contaminants (Lauderdale et al., 2012; Li, 2016). The performance of SSF could be influenced by multiple parameters like filtration rate, sand type, temperature, and design of columns (Huisman & Wood, 1974).

2.3.1 Bacteria removal

Bacteria is typically in the range of 0.5 to 5 μm (Blackett et al., 1948). Researchers found that nearly 2-3 log removal of pathogenic bacteria is removed during slow sand filtration (Dijk et al., 1978). Schuler et al. (1991) indicated that 1 log of the Total

Coliform could be eliminated in slow sand filtration. Huisman et al. (1974) pointed out a factor between 2-3 log can be achieved in the total bacteria removal.

Studies have indicated the growth of schmutzdecke on top of the slow sand filter had a significant impact on bacteria removal. (Unger, 2006) indicated that removal in schmutzdecke could reach 1-1.7 log removal. (Hijnen et al., 2004b) suggested that SSFs with a schmutzdecke had 1-2 $\log_{10}$ greater reduction than filters without schmutzdecke. Weber-Shirk and Dick (1997) pointed out biological mechanisms were primarily responsible for particles smaller than 2 μm like E. coli. On top of that, the author indicated that bacteria removal by sticky biofilm was of little effect on bacteria removal proposed. Whereas, some researchers didn't agree with this point and proposed the function of sticky biofilm on removal (Bellamy et al., 1985b; Huisman & Wood, 1974). In addition, the significant role of protozoan grazing in bacteria removal was stated by showing that the diversity and size of flagellate and ciliate populations in the filter were inversely associated with E. coli (Guchi, 2015a).

2.3.2 Virus removal

Viruses are one orders of magnitude smaller (0.01 to 0.1 μm) compared to bacteria (Montgomery, 1985). Due to the size difference between bacteria and viruses, the removal mechanisms responsible for primary elimination are distinct. Viruses are thought to be removed via adsorptive mechanisms since the straining mechanism is hard to impact particles with such tiny size (Bitton, 1975; Cliver & Herrmann, 1972; Drewry & Eliassen, 1968; Gerba & Lance, 1978). Hammes et al. (2011) suggested that extracellular polymeric substance (EPS) and organic matrix of schmutzdecke could enhance the adsorption of the virus. Researchers also emphasized the significant function of the biological community in virus removal (Gordon & Toze, 2003). When the microbial activity was suppressed with sodium azide, Elliott et al. (2011) found a great decrease in virus reduction, which indicated the importance of the function of microbial activity.

Wheeler et al. (1988) indicated that the scraping of the schmutzdecke won't significantly affect the removal of MS2. On top of that, they also emphasized the vital function of deep sand bed in virus removal through micropredation and raised the possible function of adsorption process. This point was endorsed by Hijnen et al. (2004), who found that only 0.2 log removal can achieve after filtration in columns packed with top 20 cm ripened sand. McConnell et al. presented that whether schmutzdecke exists or not, the sand bed is clean or conditioned, the virus removal would appear. Gerba (1984) suggested that virus sorption is strain-dependent and can be influenced by several parameters like ionic strength, pH, and temperature. Troyan and Hansen (1989) raised that the removal of viruses increases with increasing bed depth and decreasing filtration rate and increasing water temperature (Troyan & Hansen,1989).

2.4 Removal mechanisms in slow sand filtration

Slow sand filtration (SSF) can remove microorganisms (bacteria, viruses, protozoa, which may be pathogenic), organic compounds, and particles (Graham, 1999). The superior performance of the filters is due to the integrative action of various physical-chemical and biological mechanisms that occur simultaneously throughout the sand bed.

The difference of definition between physical-chemical and biological mechanisms is that if the process needs the presence of microbial activity within the filter bed. The physical-chemical mechanism is achieved without the presence of microbial activity. Thus, if a nonliving organic matter in the filter bed aids in particle removal, it would be classed as physical-chemical, despite the fact that life was required to create the organic matter. The attachment process caused by biofilm within the filter bed was credited to biological mechanism even though the attachment process itself would be a physical-chemical process. If sand grains cause to attachment process, then this process will be classified as a physical process, because microbial activity is not required here (Weber-Shirk & Dick, 1997).

2.4.1 Physical-chemical mechanism

The physical-chemical processes of filtration are classified into two categories: transport mechanism and attachment mechanism (Guchi, 2015b; Huisman & Wood, 1974).

a) Transport: The transport mechanism is the primary process through which particles are brought into contact with the media. It is a general term including straining (screening), sedimentation, diffusion, interception, inertial and centrifugal forces (Guchi, 2015b; Huisman & Wood, 1974).

i. Straining: Straining or screening process is one of the main kinds of transport mechanism. It happens when particles in raw water are larger than the grain pore size(Huisman & Wood, 1974). Generally, when the average cell size exceeds 5% of the grain size that makes up the porous medium, straining would become an essential removal mechanism. Due to non-uniform grain size distribution, part of heterogeneous sands is also small enough to interfere with the transport of matter (Stevik et al., 2004).

ii. Sedimentation: Sedimentation takes place when the density of the particle is greater than water. Particles would deviate from the flow direction and settle on the sand surface for the function of settling velocity (Ellis, 1985). Different from traditional settling tanks in which only the bottom can be the place of deposition, theoretically,

sedimentation in the filter could happen on all the total upward-facing surface area of the sands (Huisman & Wood, 1974).

iii. Interception: Interception is caused by the accumulation of particles settled on the media surface. The deposited particles can decrease the pore size and function as additional collectors for subsequent particles (Montgomery, 1985).

iv. Inertial: The particle will deviate from the flow path when the inertial of the particle is greater than the hydrodynamic force that leads the water to pass the sand filter (Montgomery, 1985).

b) Attachment: The primary forces that keep particles in place after they've contacted the sand grain surface are called attachment mechanisms. Electrostatic attraction, Van der Waals force, and adherence are the main categories of attachment. Generally, the combination of those forces is also called adsorption (Guchi, 2015b; Huisman & Wood, 1974). Ellis (1985) suggested that adsorption is more critical for smaller particles. The detachment of particles could happen when the interstitial velocity increases along with hydrodynamic shear force due to accumulation of particles and development of biofilm.

i. Electrostatic attraction: The attraction between opposite electrical charges. Due to the structure of clean quartz sand, the sand bed is usually negative charge. Organic particles like bacteria and viruses typically have a negative charge and thus are repelled by the clean sand bed, which partly explain the bad performance when the filter bed is completely clean. However, some positive charge particles are prone to be attracted to the sand bed during the initial commissioning and operation of filters. Researchers have found that standard brass sieves could lead to zinc and copper contamination of the sand grains (Brown et al., 2002; Chu et al., 2000). Cations of iron, manganese, aluminum, and other metals from the raw water can be accumulated on the filter media during the initial ripening process and help to remove negatively charged particles.

ii. Van der Waals force: Mass attraction between atoms, molecules, and surfaces. Although compared to mechanisms previously described, the effect of Van der Waals force is minor, the force is ubiquitous and plays a role in both transport and attachment processes.

iii. Adherence: Slimy substance called extra-cellular polymeric substances (EPS) generated by bacteria, and other microbes would be developed due to the accumulation of organic particles. The sticky layers on the surface of the schmutzdecke and sand grains, which could aid in the attachment of particles from the raw water.

2.4.2 Biological mechanism

Bacteria, viruses, and protozoans can be effectively removed by SSF (Barrett et al., 1991; Ellis, 1985; Mcconnell et al., 1984; Schijven et al., 2006; Wheeler et al., 1988). Physical-chemical mechanisms are only part of the reasons that contribute to pathogen removal. The contribution of biological mechanisms in the biofilm within the upper layer of sand bed can't be neglected (Huisman & Wood, 1974).

a) Predation: A food chain could be developed with the maturity of schmutzdecke. Small organisms could feed on particulate matters. Higher-order eukaryotes could be predators to bacteria. Pathogens and organic compounds are consumed along with the food chain (Collins et al., 1992; Ellis, 1985). Predatory activities are considered the primary reason for microbial pathogens reduction (Stevik et al., 2004).

b) Biodegradation: Bacteria coming from the raw water could multiply using the organic matter accumulated in the filter media. Part of the foods are oxidized by the bacteria and are transferred to energy to meet their need for metabolism. Another part of foods would be converted to cell materials for their growth. Dead bacteria could be the liberation of organic matter that could be used again for the bacteria at greater depth. The quantity of organic material provided by the inflowing raw water limits the bacterial population. As a result, the growth is followed by a corresponding death. (Huisman & Wood, 1974).

c) Enhancement of straining and attachment: The EPS excreted by bacteria could contribute to the form of biofilm. The sticky biofilm on the sand surface will increase the attachment mechanism. When microorganisms proliferate in the filter, the pore size shrinks. As a result, the straining mechanism may be improved (Bellamy et al., 1985a; Guchi, 2015b; Stevik et al., 2004; Weber-Shirk & Dick, 1997).

Chapter 3: Problem statement and research questions

The slow sand filtration is an effective way to produce safe and biological-stable water. The efficiency of removing pathogens, organic compounds, and particles is seen to be impacted by the existence of the schmutzdecke and the maturity of the sand bed (Calixto et al., 2020).

Researchers suggested that 90% of pathogen indicator bacteria and coliphages are reduced in the schmutzdecke layer (Adin, 2003; Wotton, 2002). As a result, schmutzdecke has received a great deal of attention, while the knowledge of underlying layers is rather poor. The coexistence of biologically active biofilms and particulate matters accumulated led to complex removal mechanisms contributed by the function of microbial community and the media surface (Bellamy et al., 1985b; Huisman & Wood, 1974). As such, despite the slow sand filter being one of the oldest water treatment techniques, the role of physical-chemical and biological mechanisms on bacteria and viruses removal remains unclear (Pfannes et al., 2015).

Slow sand filtration is regarded as an all-around treatment technique as it incorporates many purifying features. It combines settling, straining, filtering, organism removal, organism inactivation, chemical change, and storage into a single unit (Huisman & Wood, 1974). For that reason, SSF used to be heavily loaded with only sedimentation, rapid filtration, or coagulation as pretreatment (Huisman & Wood, 1974). Much of the information about SSF we applied today comes from the time when the SSF was heavily loaded. However, the situation of slow sand filtration is different from the past, which might cause deviation in the understanding of the function of SSF. Extensive pretreatments before slow sand filtration are applied nowadays. As indicated in Figure 2, before surface water enters SSF, micro sieves, rapid sand filter (3.5 m/h), dune filtration, softening, powdered active carbon dosing, aeration, and rapid sand filter (4.5 m/h) are performed. Various indicators like ammonia, suspended solids, organic matters, and pathogens have already been reduced to some extent. As a result, SSF receives a lower load compared to the past. The function of schmutzdecke, the deeper layer, and the whole SSF could change.

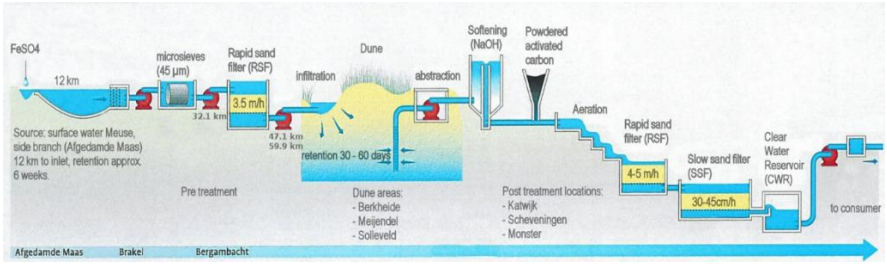

Fig. 2. Drinking water treatment processes in Dunea water company, Netherland

In order to develop new design and operational conditions of slow sand filtration, it is crucial to have a detailed understanding of pathogen, AOC, and particles removal processes occurring in the schmutzdecke and deeper layers of the filter (bacteria and viruses are focused in this thesis). Researchers indicated the function of active microbial community in pathogen removal in schmutzdecke. However, it is still unclear whether the removal is mediated by the microbial community or by modification of the media surface. In addition, there is a need to investigate the pathogen removal potential of different depths in order to improve our knowledge on the stratification of processes throughout the sand bed. Besides, it is important to renew our knowledge on SSF operated in low-loaded influent nowadays.

In this regard, the focus of this research is to investigate the bacteria and viruses removal of sand from different depths of full-scale SSF from a drinking water treatment plant in the Netherlands. Furthermore, the aim is to identify the key mechanisms in the schmutzdecke that contribute to bacteria and viruses removal. In order to achieve the main aim, the following questions are addressed:

a) What is the contribution of different depths of slow sand filter to bacteria and viruses removal?
b) Which are the key mechanisms that contribute to bacteria and viruses removal in the schmutzdecke?

Based on the research questions and the knowledge we got in the literature review, the hypothesizes are:

a) Schmutzdecke was the most important part in pathogen removal and the place had most significant microbial activity in previous studies. Thus, compared to other deeper layers, the schmutzdecke would have the highest removal capacity, and the removal efficiency would decrease with the deeper depth due to the reduction of biofilm.
b) Due to the relatively smaller size and negative charge of virus and bacteria, physical processes might not be a decisive mechanism in pathogen removal. With the most prominent biomass within the top of the column, the microbial community could play a significant role in virus and bacteria removal.

Chapter 4: Materials and methods

4.1 Experimental design

Two experimental setups were constructed to investigate both research questions. The sand material for the experiments was collected from the full-scale SSF at the Dunea water treatment plant from the Monster site. The filter was running for 436 days at the time of sample collection. The scraping event provided the opportunity to sample the sand the filter material up to a depth of 40 cm. The feed water applied for filtration columns was the influent of the full-scale SSFs at Monster. Water was collected every week, and the water quality is shown in Table 1.

Setup 1 was aimed at investigating the contribution of different depths. Six columns packed with sands from three different depths of the full-scale filters were applied. From the collected 40 cm of sand material, three depth layers were separated: 0-5, 5-20, 20-35 cm sand. Each of depth was filled in duplicate columns. Three layers were chosen since they are representative of different conditions. The microbial activity is most pronounced in the upper layer (0-5 cm), which was evident by black sticky slime film. While for 20-35 cm, little sign of biofilm could be observed. 5-20 cm was only with a small part of the slimy film on the upper part of the depth. The contribution of different depths can be evaluated by comparing the removal efficiency in spiking experiments of bacteria and viruses indicators. Though, in the realistic operation of SSF, the concentration would get lower when the water goes deeper. Separate operation on sand from different depths could help us have a better understanding on the potential capacity of different depths without the impaction of concentration effect. Besides, it could also give us insights on how deeper layers would work after scrapping.

Setup 2 focused on the key mechanisms in schmutzdecke. Since generally top 5 cm is removed in the cleaning process of SSFs in the Netherland to restore the treatment efficiency, 0-5 cm layer was considered to be representative of the schmutzdecke layer in this study. Two columns were filled with sands from 0-5 cm depth of the full-scale filter. To determine removal mechanisms, sand under three conditions was applied: active sand, inhibited sand and ignited sand. Active sand is the sand with active biomass grown on its surface. Inhibited sand refers to active sand where biological activity is inhibited by 400 mg/L of sodium azide. For ignited sand, inhibited sand was dried at 105 ℃ for one hour to remove residual water content and was heated in a muffle furnace under 550 ℃ for four hours.

Comparison among removal capability of bacteria and viruses indicators of columns in different sand conditions can indicate the role of the microbiological community. Getting insights into key mechanisms in schmutzdecke would help with the understanding of the role of schmutzdecke in the whole SSF and aid in the adjustment

of ripening/re-ripening period and enhancement of removal performance.

Table 1. Feedwater quality of slow sand filters in Dunea water treatment plant

Parameters	Value
pH	8.55
ATP (ng/L)	6.9
DOC (mg/L C)	3.21
AOC (µg/L C)	5.71
E. coli (CFU/100L)	<1
Ammonia (mg/L N)	0.01
Nitrate (mg/L N)	0.70
Nitrite (mg/L N)	0.05
Phosphate (mg/L p)	0.092

4.2 Sodium chloride tracer test

The conservative tracer test is widely applied in column experiments to measure porous media properties (e.g., residence time, pore volume, and confirmation of plug flow). In the experiments, sodium chloride tracer tests were conducted before indicator organisms spike tests to ensure the flow pattern is approximately plug flow. Electrical conductivity (EC) was chosen to be a proxy of the concentration of NaCl. The stock solution was prepared by adding 2.5 g NaCl into 1 L deionized water (2500mg/L). Effluent samples were collected before the tracer test to get the background. The NaCl stock was injected into columns for several minutes, and effluent samples were collected for 2 minutes according to regular intervals.

The samples were 100 times diluted by deionized water to get a suitable measurement volume for the EC meter. The details of sample collection are indicated in Table 2.

Table 2. Sample collection frequency for tracer test

Setup	Dosing time (min)	Collecting frequency	Duration (min)
1 (different depths)	30	0-50 min: every 5 min 50-120 min: every 10 min	120
2 (schmutzdecke mechanism)	20	0-20 min: every 4 min, 20-40 min: every 5 min 40-80 min: every 10 min	80

4.3 Indicator organisms spike test

Indicator organisms E. coli and PhiX174 were applied as microbial surrogates to study the performances of bacteria and viruses, respectively. PhiX174 was chosen for its more conservative than MS2 due to its higher isoelectric point and lower contact angle (Attinti et al., 2010; van der Wielen et al., 2008). To assess the contribution of different layers to bacteria and viruses removal, the spike tests were performed on 0-5, 5-20, 20-35 cm duplicate columns successively. Columns with active, inhibited and ignited sand were tested phase by phase. The comparison among the removal efficacy obtained from the spike tests could indicate the role of different mechanisms.

E. coli and PhiX174 were co-injected into the column. The strains were inoculated in demi water to an initial concentration of 10^6 CFU/PFU/ml. To prevent the dead volume and dilution effect, the supernatant level was decreased as low as possible during the spike tests. The samples were collected with the same frequency as the tracer test. Table 3 shows the details of the indicator organisms spike test.

Table 3. Sample collection frequency and analysis for spike test

Setup	Spike items	Dosing time (min)	Effluent sample collection	Duration (min)	Analysis
1 (different depths)	E. coli and PhiX	30	0-50 min: every 5 min 50-120 min: every 10 min	120	The spread plate technique & Plaque assay
2 (schmutzdecke mechanism)	E. coli and PhiX	20	0-20 min: every 4 min 20-40 min: every 5 min 40-80 min: every 10 min	80	The spread plate technique & Plaque assay

4.4 Column design and operation

4.4.1 Packing of columns

Before filling the columns with sand, the valves at the effluent site were closed. After one-fifth of water was filled into columns to avoid air trapped in the sand bed, the sand was transferred into columns carefully with continuous shaking. The column was backfilled from the effluent site with a HLR of 0.03 m/h once the packing was finished. The filters were backfilled slowly until the water level rose to 2 cm higher. The backfill velocity was increased with an increment of 0.01 m/h for every 20 min. The same

backfill measures were used when the columns were accidentally drained during the daily operation.

4.4.2 Setup 1: Contribution of different depths

As shown in Figure 3, the filtration columns were made of acrylic pipes with a diameter of 1.5 cm and a height of 10 cm (for 0-5 cm bed depth) and 28 cm (for 5-20 cm, 20-35 cm bed depth). Coarse gravel (1.4 mm) was applied underneath as a support layer. Columns were divided into three groups (two duplicates in one group) according to sands from the different depths. Each column was built with one influent port, one effluent port, and one side pipe. The supernatant level and the height of the support layer were varied according to the bed depth. Table 4 indicates the detailed design of six columns.

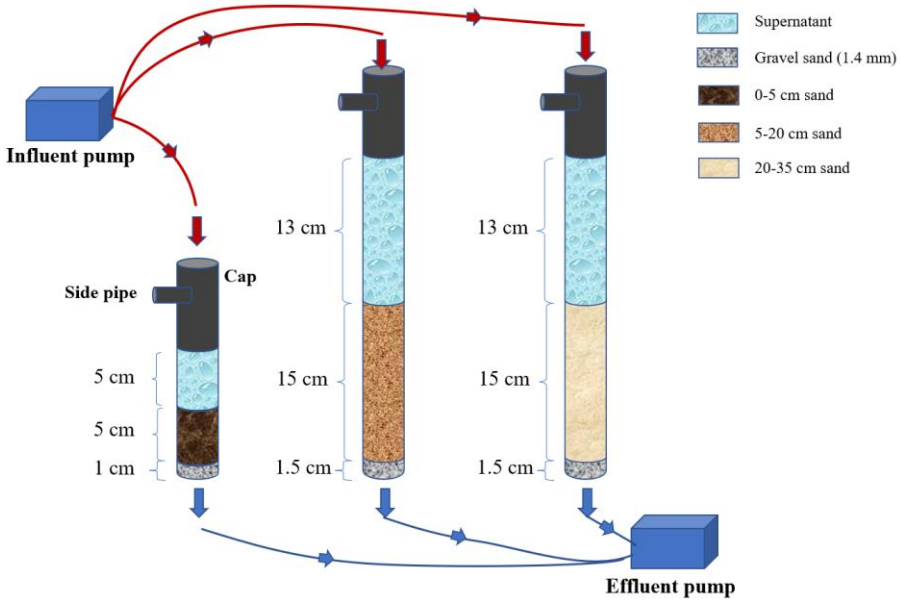

Fig. 3. Scheme of setup 1 (contribution of different depths)

Table 4. Column's design of setup 1

Bed depth of sand from the full-scale SSF (cm)	Bed depth (cm)	Supernatant (cm)	Support layer (cm)	Number of columns
0-5	5	5	1 cm	2
5-20	15	13	1.5 cm	2
20-35	15	13	1.5 cm	2

A suction pump was applied at the effluent site to control the effluent velocity. The influent velocity was set to be slightly higher than the effluent to avoid drainage of the columns. The excess water was drained through the side pipe to keep a stable supernatant level. Columns were covered with aluminum foils to avoid algae growth, as shown in Figure 4.

Fig. 4. Picture of column setup 1 (contribution of different depths)

4.4.3 Setup 2: Key mechanism in schmutzdecke

Experiment setup 2 (Figure 5) only includes columns packed with sands from 0-5 cm of full-scale sand filter. The columns were made by polyvinyl chloride (PVC) soft tubing with an inner diameter of 1.5 cm. Columns in setup 2 have only one influent and one effluent port. The support layer was filled with coarse gravel (1.4 mm) up to 1cm and the supernatant was sustained at 2 cm.

Columns were operated under a pressure system to overcome the relatively higher head loss of the schmutzdecke layer. The influent site and effluent site were sealed, and the pressure was provided by the peristaltic pump from the influent site. The filtration rate was set at 0.3 m/h. Columns were covered with aluminum foils to mimic the dark situation of the full-scale slow sand filtration. The picture of columns setup 2 is attached

below (Figure 6).

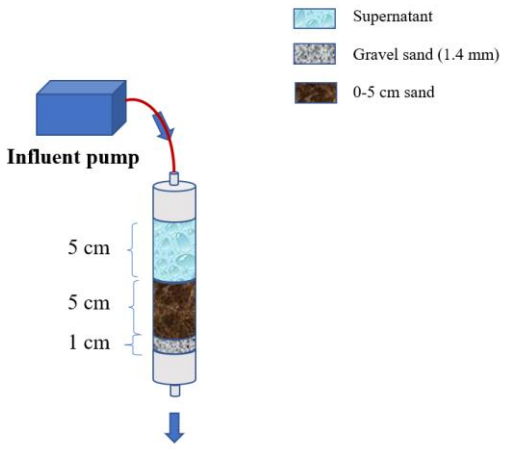

Fig. 5. Scheme of setup 2 (key mechanisms in schmutzdecke)

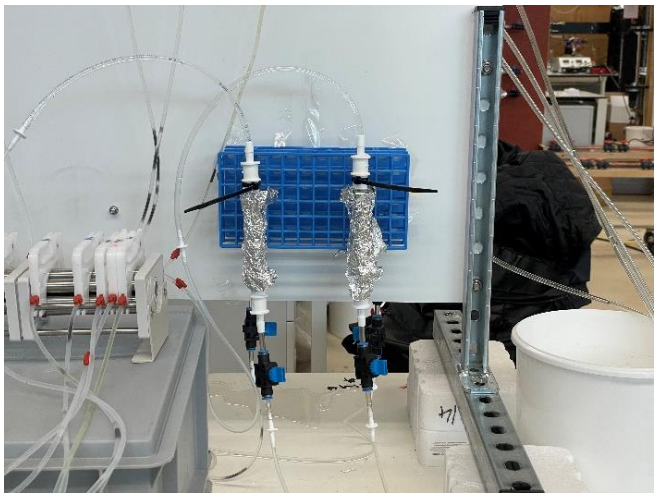

Fig. 6. Picture of columns setup 2 (key mechanisms in schmutzdecke)

4.5 Sodium azide and ATP measurement

Biological activity in the column was inhibited by dosing sodium azide at a concentration of 400 mg/L for 5 days. Inhibition was confirmed by measuring cellular activity on the sand before and after azide addition. ATP was measured as a direct indicator of effectiveness biological activity inhibition. Quench Gone 21 wastewater test kit was used in ATP measurement.

4.5.1 The total ATP (tATP) analysis

The total ATP indicates the ATP from both living and dead cells. 1 ml of well-mixed sample was collected by small specula and added to a 2ml UltraLyse 30[21] (Extraction) Tube. The mixture was inverted several times and incubated for at least 1 min in the tube. The UltraLyse 30[21] (Extraction) Tube was poured into a new 8ml Ultralute/Resin Tube. The mixture was transferred back and forth between UltraLyse 30[21] (Extraction) Tube and Ultralute/Resin Tube to mix well. 100µL of mixture contents in Ultralute/Resin Tube and 300µL of Luminase[w] to 12*55mm test tube. After being swirled several times, the test tube was inserted into the luminometer measure. The value was recorded as RLU_{tATP}.

4.5.2 The dissolved ATP (dATP) analysis

Only ATP from dead cells was measured in dATP analysis. 100µL of the well-mixed sample was added to a 10ml LumiSolve (Stabilizer) Tube. The tube was capped and inverted three times to mix. The mixture was incubated for at least 1min. 100µL of the LumiSolve (Stabilizer) Tube contents and 300µL of Luminase[w] were added into a new 12*55mm test tube. The test tube was swirled gently five times and inserted into the luminometer and measured. The value was recorded as RLU_{dATP}.

4.5.3 ATP Calculations

To convert RLU value to ATP concentration, the calculation below was followed. Cellular ATP (cATP) was the final parameter that serves as a direct indicator of total living biomass quantity.

$tATP\ (ng\ ATP/ml) = RLU_{tATP} / RLU_{ATP1} * 11\ (ngATP / ml)$

$dATP\ (ng\ ATP/ml) = RLU_{dATP} / RLU_{ATP1} * 101\ (ngATP / ml)$

$cATP\ (ng\ ATP/ml) = tATP\ (ng\ ATP/ml) - dATP\ (ng\ ATP/ml)$

4.6 Microbiological cultures preparation

E. coli WR1 strain (NCTC13167) and PhiX174 somatic coliphage (ATCC1370 6-B1) are applied as pathogen indicators due to similar characteristics to vital pathogens and low risk to people. The removal efficiency of microbial indicators through columns was monitored during column experiments. The culture preparations refer to ISO 10705-2:2000 for somatic coliphages and ISO 9308-1:2014 for *E. coli*.

4.6.1 *WR1 E. coli* stock culture

A vial of *WR1 E. coli* was taken out from a -80 ℃ freezer and waited until it was

completely thawed. 0.1 ml culture was spread on the M-Lauryl Sulphate agar and incubated in the incubator maintained at 35 ± 3 ℃ for 20 ± 4 hours. 50 ml TYGB and 500 µl of Ca-Glucose were added to an autoclaved Nephelometric conical flak.

3-5 yellow colonies taken from the incubated M-Lauryl Sulphate agar were suspended into a culture flask and incubated at 37 ℃ for 18 h while continuously shaking at a speed of 100 ± 10 rpm. 10 ml sterile glycerol was pipetted into the flask and thoroughly mixed after compliment of incubation. At last, each of 2.5 ml cryo-vials was filled with 1-1.5 ml aliquots and stored at -80 ℃ freezer.

The stock culture's concentration could be determined by making serial dilution with phosphate buffer saline. The suitable dilution should be around 10^{-5} – 10^{-6} times. 0.1 ml of diluted sample was injected and distributed uniformly on Chromocult coliform agar plates using a sterile spreader. All measurements were conducted in duplicate. The plates were put upside down in the incubator with a temperature of 36 ± 2 ℃ for 24 h. The concentration obtained was around 5 x 10^{8} CFU/ml.

4.6.2 PhiX174 host E. coli WG5 stock culture (ATCC70078)

Around 3ml of Modified Scholten's' Broth (MSB) was applied to hydrate a lyophilized ampoule of the reference host culture. 50 ± 5 ml MSB stored in the fridge was added into a 300ml conical flask and waited until it went back to room temperature. The rehydrate culture was transferred to a flask and shaken in the incubator for 20 ± 4 hours at 36 ± 2 ℃. In the culture flask, 10 ml of sterile glycerol was added. Every 1 ml solution was distributed into 2 ml cryo-vails and stored at the freezer maintained at -80 ℃.

4.6.3 Coliphage PhiX174 stock culture

A high concentration of PhiX174 stock culture was obtained from the Civil Engineering department at TU Delft. The stock was found to have a concentration of 10^{12} pfu/ml. To achieve an initial concentration of 10^{5} pfu/ml, this stock was further diluted using demineralized water.

4.7 Microbiological assays

Samples at different time points were serially diluted before assays to get a suitable number of colonies on plates. The dilution factor was varied according to time, bed depth of columns, and the spike items. 4.5 ml phosphate-buffered saline (PBS) was added to a 10 ml tube. 0.5 ml sample was added to the tube to get 5 ml total volume. Mixed it well, and 10 times dilution was reached. Further dilution could be reached according to the same procedures.

4.7.1 E. coli assays

The E. coli assays were performed by the spread plate method. 0.1 ml diluted or undiluted sample was added to the chromocult coliform agar. It was spread in the same direction by a sterile spreader. The spread was continued until the sample was entirely absorbed by coliform agar. Each sample was done by duplicate. Inoculated plates were put into the incubator with a constant temperature of 36 ± 2℃ and incubated for over 8 h.

4.7.2 PhiX174 assays

100 ml of MSB was added in a conical flask and placed on a shaker in the incubator to warm up. One vial of WG5 culture was removed from the freezer and being thawed at room temperature. Until the WG5 culture turned from solid to liquid state, 1 ml of WG5 was added into pre-warmed MSB, and the flask was put back to the incubator on the shaker. The absorbance was measured by spectrophotometer with a single wavelength at 550 nm every 30 min. The culture was continued until the absorbance was 0.5-0.6 (typically 5-6 hours). At this point, the estimated WG5 concentration was 10^8 CFU/ml, and the inoculum culture was taken out and placed in melting ice. Within the same working day, the inoculum culture was utilized.

For PhiX174 assays, semi-solid Modified Scholtens'Agar (ssMSA) was melted in a water bath at 99 ± 2℃. The volume needed for semi-solid depended on the samples that needed to be measured (Usually 200 ml). After the ssMSA was melted, it was transferred to another water bath at 45 ± 2 ℃ to cool down. Once the ssMSA was cool down to 45 ± 2 ℃, each 25 ml ssMSA was added with 150 µL calcium chloride and mixed well. 2.5 ml ssMSA was distributed to small sterile glass tubes and maintained at 45 ± 2 ℃.

1ml of inoculum culture (E. coli WG5) and 1 ml of diluted or undiluted sample were added to 2.5 ml of ssMSA in the glass tube. The tube was rolled with palms carefully to ensure good mixing. The mixture was then poured onto the Modified Scholtens'Agar (MSA). After the mixture was completely set, the plates were incubated in the incubator for over 4 h. Each sample was examined in duplicate.

Chapter 5: Results

5.1 Conservative tracer test

For setup 1, the duplicate columns of each depth interval showed similar breakthrough curves. C/C_0 (effluent to influent concentration) started from 0.1 as background value. The time needed to reach the peak for 0-5 cm (35 min) is less than 5-20 & 20-35 cm (40 min, 45 min, respectively). The results of the tracer test indicated approximated plug flow characteristics of columns. The detailed results of NaCl tracer tests of setup 1 are shown in Appendix A.

For setup 2, the peak of C/C_0 setup 2 was near 0.8 instead of 1. The downward curves of in inhibited and ignited sand showed slightly time shift like an earlier decrease for one of the duplicate columns. The time required to reach the peak for active sands was 25 min, while for inhibited sand and ignited sand, 16 min and 25 min were needed respectively. Plug flow characteristic were shown in tracer tests for setup 2 as well. The detailed results of NaCl tracer tests of setup 2 are attached in Appendix B.

5.2 Role of different depths in pathogen removal

Setup 1 focused on exploring the contribution of different depths of SSF to E. coli and PhiX174 removal. The concentration of PhiX174 and E. coli was determined during the spike tests as shown in Figures 7-12. The relative residual concentration given by the ratio of effluent and influent concentration (C/C_0) was applied as Y-axis for compensating the difference in initial spike content. The spiking started at the beginning of the experiments and the time at which dosing was stopped was presented with the dashed line. Log reduction values (LRVs) were calculated [$LRV = \log_{10} (C_0/C)$] for the comparison of E. coli removal by different layers as shown in Table 5. The average value of relative residual concentration was applied for duplicate columns. Error bars were presented in the figures according to two duplicate data.

5.2.1 E. coli removal by different depths

The columns filled with sand from 0-5, 5-20, 20-35 cm showed 0.55, 1.30, 1.04 log removal of E. coli, respectively, as shown in Figures 7-9. The highest removal was observed in 5-20 cm depth with 1.30 log removal. 1.04 log removal was observed within 20-35 cm sand. The cumulative LRV for these three depths reached 2.89 log removal. The increase of breakthroughs was delayed with the increase of depth. For 0-5 cm (Figure 7), an increase was shown at the first 5 mins and it reached to peak at 20 mins. The increase of the breakthrough curves can only be seen after 30 mins for 5-20 and 20-35 cm columns. The peaks were shown at 35 mins and 45 mins for 5-20 cm depth and 20-35 cm depth respectively.

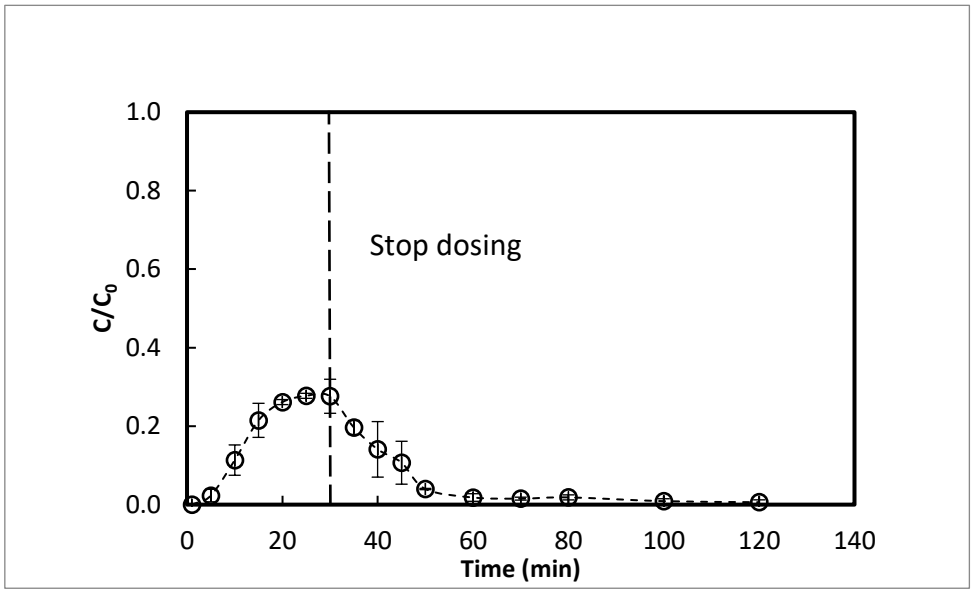

Fig. 7. E. coli breakthrough curve in 0-5 cm layer

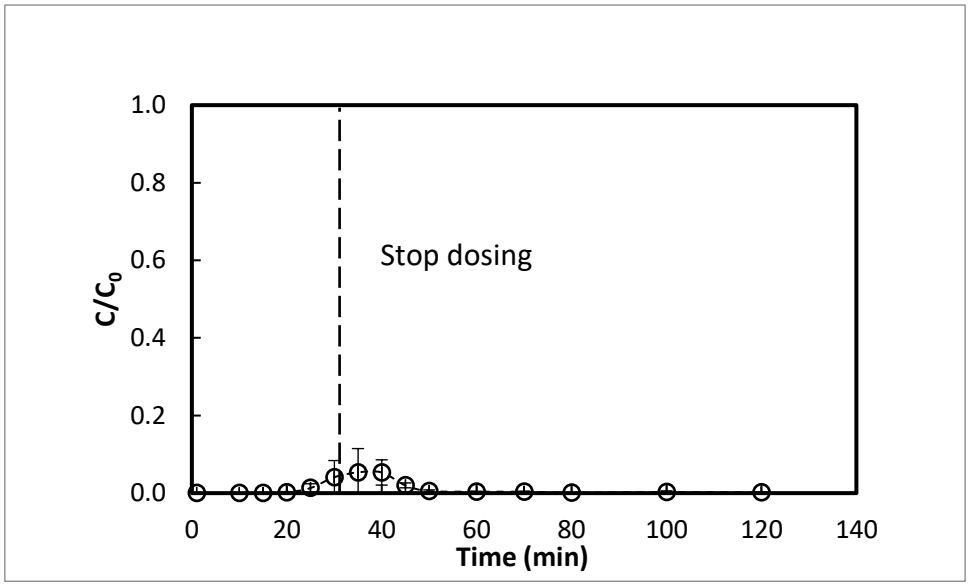

Fig. 8. E. coli breakthrough curve in 5-20 cm layer

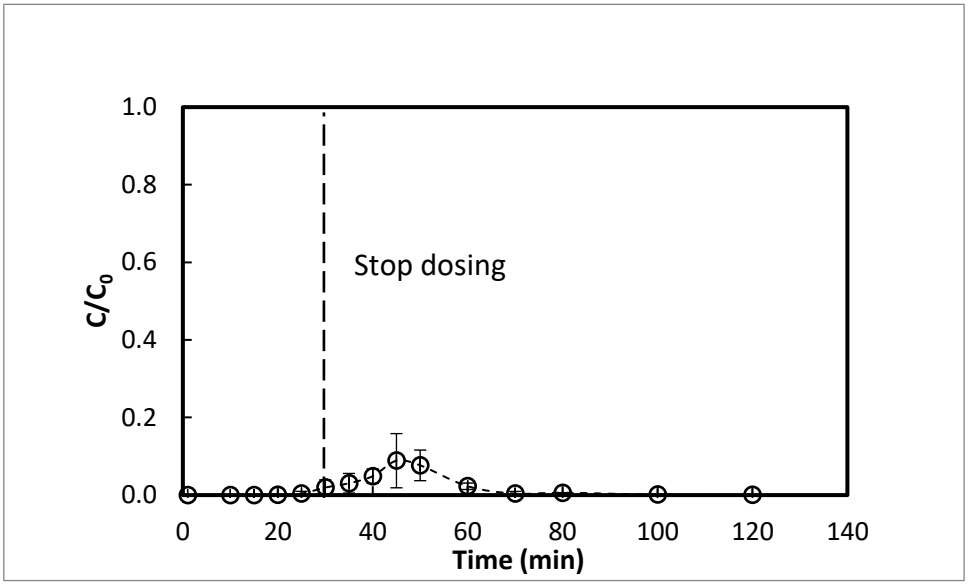

Fig. 9. E. coli breakthrough curve in 20-35 cm layer

5.2.2 PhiX174 removal by different depths

A log removal of 0, 0.30 and 0.14 was observed by 0-5, 5-20, 20-35 cm columns accordingly, as shown in Figures 10-12. The relative residual concentration went up to 1.25 after filtration by 0-5 cm sand. 5-20 cm exhibited the highest removal capacity with 0.30 log. The LRV was 0.14 log when it comes to 20-35 cm. The cumulative removal could reach 0.44 log in PhiX174 removal.

The existence of PhiX174 was observed at effluent port for the first 5 mins in 0-5 cm depth. For deeper layers (5-20 & 20-35 cm), an increase was noticed at 20 mins, at which a peak was already shown in 0-5 cm depth. A longer time was needed to reach the peak effluent concentration for 15 cm length columns. 30 mins and 40 mins were required for 5-20 cm and 20-35 cm depth respectively.

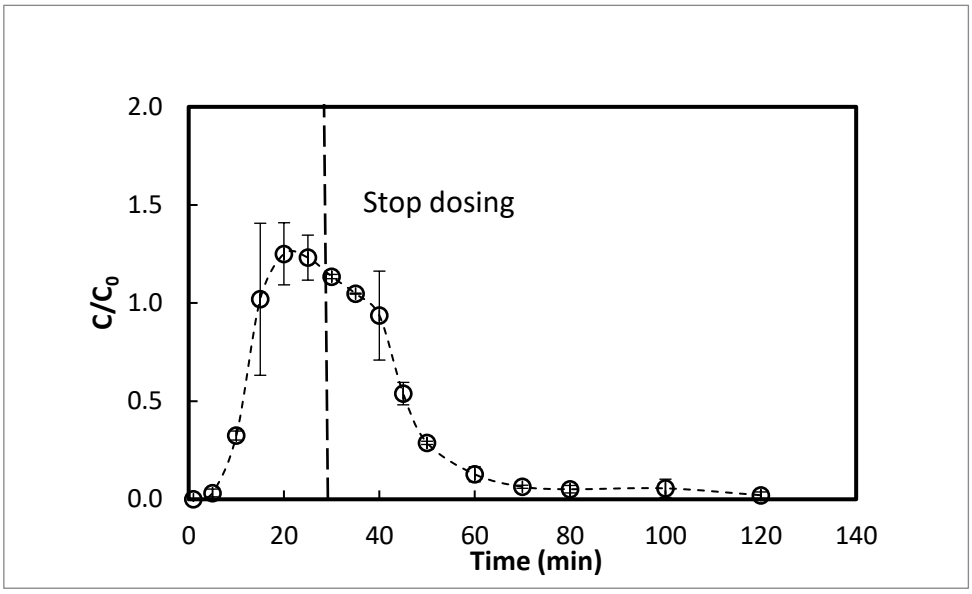

Fig. 10. PhiX174 breakthrough curve in 0-5 cm layer

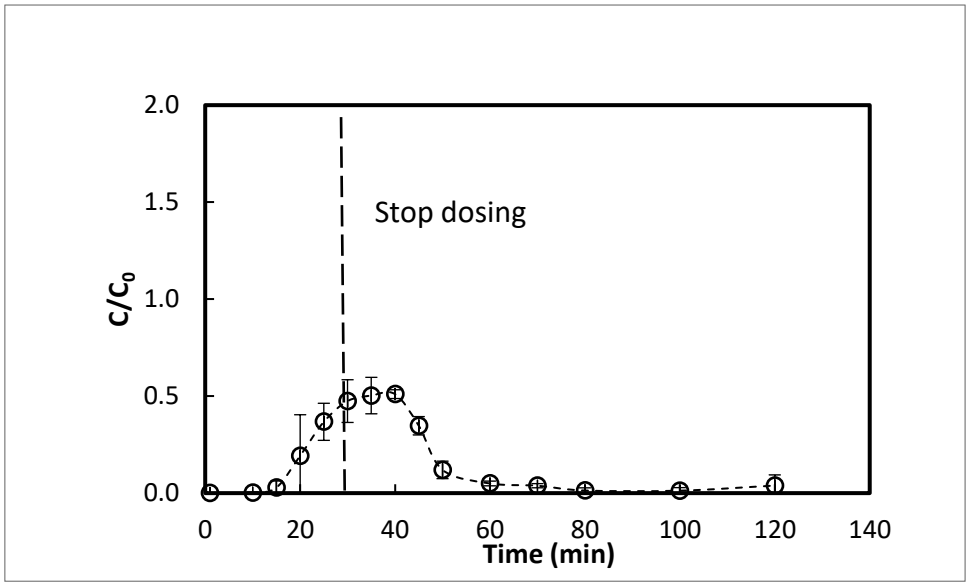

Fig. 11. PhiX174 breakthrough curve in 5-20 cm layer

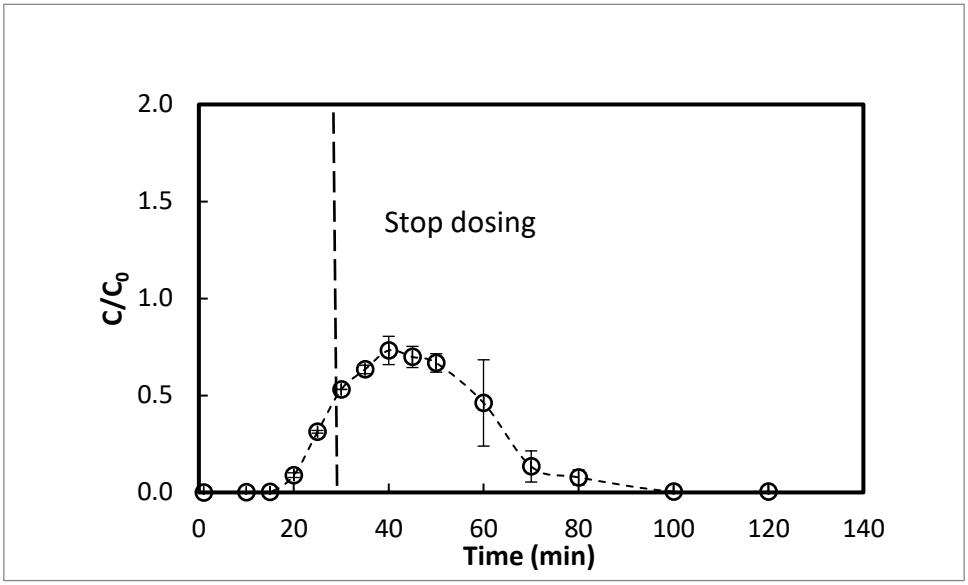

Fig. 12. PhiX174 breakthrough curve in 20-35 cm layer

5.2.3 Performance of different layers on bacteria and viruses removal

The E. coli and PhiX174 removal efficiency are summarized in Table 5. All layers exhibited varying degrees of E. coli removal, while only 5-20 and 20-35 cm depth showed the ability of virus removal. For 0-5 cm, the virus concentration could reach even higher than influent. Overall, more efficient removal of E. coli was observed for the top 35 cm sand. The cumulative removal through 35 cm depth could reach 2.89 $\log_{10}$ for E. coli and 0.44 $\log_{10}$ for PhiX174.

Table 5. Removal of E. coli and PhiX174 of different depths

Bed depth (cm)	E. coli removal efficiency ($\log_{10}$)	PhiX174 removal efficiency ($\log_{10}$)
0-5 (schmutzdecke)	0.55	0
5-20	1.30	0.30
20-35	1.04	0.14
Cumulative removal ($\log_{10}$)	2.89	0.44

5.3 Removal mechanisms in schmutzdecke

Setup 2 was aimed at determining the key mechanisms contributing to bacteria and viruses removal in the schmutzdecke. Indicator organisms, including E. coli and PhiX174, were measured in experiments of filtration columns under three different conditions (active, inhibited and ignited). The relative residual concentration given by the ratio of effluent and influent concentration (C/C_0) was applied as Y-axis for compensating the difference in initial spike content. LRV was calculated for columns under different conditions. The average value of relative residual concentration was applied for duplicate columns and error bars were presented in the figures. The vertical dashed line marked the time at which the dosing was stopped.

5.3.1 Inhibition performance evaluation

To inactivate the biological activity, the sodium azide (400 mg/l) was inducted for consecutive 5 days in columns of setup 2. The tATP and dATP were measured on day 1 and day 5 to calculate ATP concentration in one column of setup 2 to verify the efficacy of inhibition. As indicated in Table 6, the ATP concentration decreased from 117.68 ngATP/ml on day 1 to 29 ngATP/ml on day 5, which proved the effectiveness of inhibition.

Table 6. ATP concentration on day 1 and day 5

	Day 1	**Day 5**
tATP (ng/ml)	120.56	41.90
dATP (ng/ml)	12.76	12.92
ATP (ng/ml)	117.67	28.98

5.3.2 E. coli removal in active, inhibited and ignited sand

The columns operating under different conditions showed E. coli removal of 0.68, 0.74 and 0.43 log for active, inhibited and ignited sand, respectively, as shown in Figure 13. The active sand and inhibited sand showed a similar value of removal efficiency. As such, no significant role of the microbial community can be observed through the E. coli breakthrough between active sand and inhibited sand. Counts of E. coli were detected from the first 4 min and reached to peak at 20 min when the dosing was stopped.

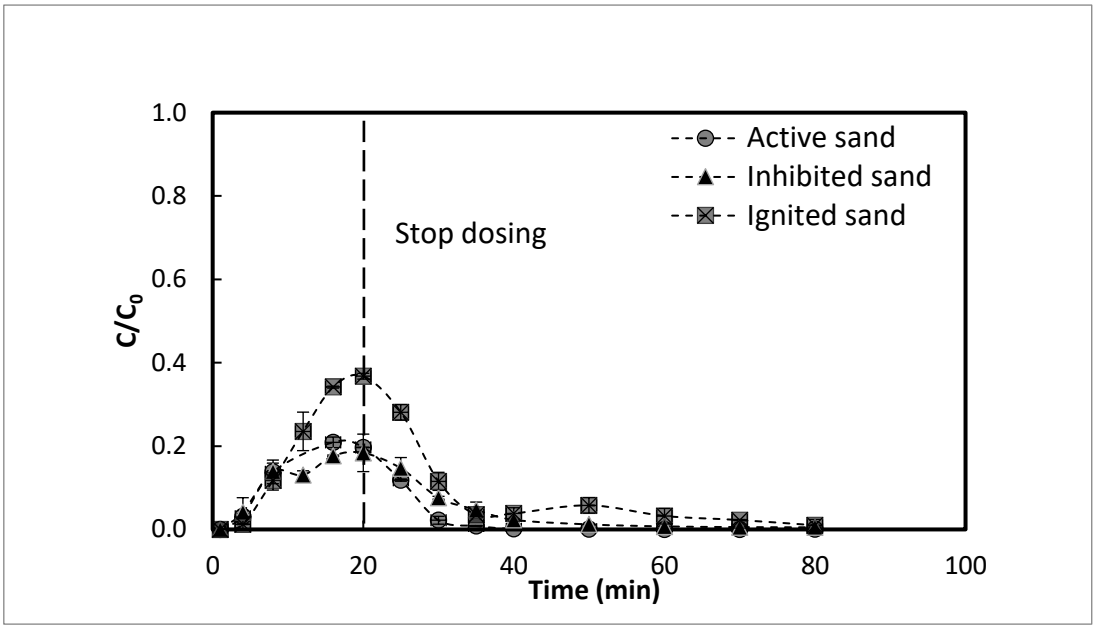

Fig. 13. E. coli breakthrough curves in active, inhibited and ignited sand

5.3.3 PhiX174 removal in active, inhibited and ignited sand

The results showed PhiX174 removal of 0, 0 and 0.28 log for active, inhibited and ignited sand (Figure 14). Only ignited sand exhibited capability of PhiX174 removal. The C/C_0 was over 1 after passing through the schmutzdecke under active and inhibited sand conditions. Similarly, the microbial community didn't show the effect on PhiX174 removal. Active sand showed a slightly earlier increase at 4 min. Inhibited and ignited sand showed a similar shape to the curve. The C/C_0 peaked at 20 minutes for all sand conditions when dosing was stopped.

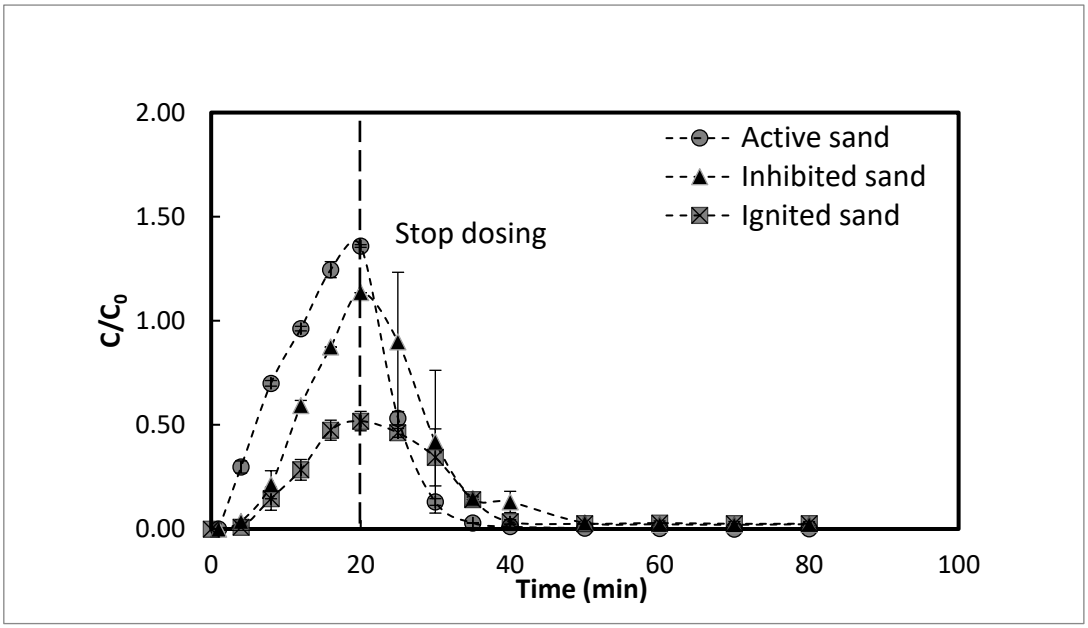

Fig. 14. PhiX174 breakthrough curves in active, inhibited, ignited sand

The schmutzdecke showed much better performance in E. coli removal than PhiX174 removal. For the active or inhibited layer, 0.68, 0.74 $\log_{10}$ of E. coli could be reduced, while no removal of PhiX174 was observed. As such, only bacteria can be removed in active and inhibited sand. The ignited sand exhibited capability of removal as well, with 0.43 $\log_{10}$ removal for E. coli and 0.28 $\log_{10}$ for PhiX174. The removal efficiency of E. coli and PhiX174 was summed up in the Table 7.

Table 7. E. coli and PhiX174 removal in active, inhibited and ignited sand

Indicator organisms	Removal in active sand ($\log_{10}$)	Removal in inhibited sand ($\log_{10}$)	Removal in ignited sand ($\log_{10}$)
E. coli	0.68	0.74	0.43
PhiX174	0	0	0.28

Chapter 6: Discussion

6.1 Role of different depths in bacteria and viruses removal

In the investigating of removal of different depths, two different lengths (5 cm and 15 cm) were applied for depth interval 0-5 cm and 5-20, 20-35 cm. For that reason, longer time was required for longer columns to reach the peak of C/C_0. At the same time, the earlier increase was observed as well for shorter columns due to the lower pore volume.

The removal of different depths on E. coli and PhiX174 removal was tested using sand from different depths of a full-scale filter in a continuous flow column. The LRVs of E. coli were 0.55, 1.3, 1.04 logs at depths 0-5, 5-20, 20-35 cm respectively. While, LRVs of PhiX were 0, 0.30, 0.14 logs at 0-5, 5-20, 20-35cm, respectively. These results show that the deeper layers had a similar removal capacity as the schmutzdecke, particularly for E. coli. In addition, PhiX174 removal was not affected by the schmutzdecke and underlying layers seem to play a role. This aligns with the previous studies of Hijnen et al., Schijven et al., Wheeler et al., where virus removal was unaffected by schmutzdecke.

Schmutzdecke is considered to be a critical component, especially for bacteria removal. Experiments have shown that the development of schmutzdecke was primarily responsible for the enhanced E. coli removal and scraping of schmutzdecke would greatly impact filter performance. (Elliott et al., 2015; Hijnen et al., 2004a; Unger & Collins, 2008a). However, other researchers pointed that the scraping of schmutzdecke did not alter the effluent water quality from the established SSF that has been operating for 20 years (Chan et al., 2018). Chan et al. compared a well-established SSF (with 20 years operation time) with the other two newly packed SSFs (with 1 year operation time) in the same production line packed from previous scraping events. Results showed that only newly-packed SSFs would be hugely impacted by the scraping event. No change of bacteria profiles or breakthrough of microbial indicators or TOC was found in the effluent of 20-year-operation SSF. Chan et al. indicated that scraping could greatly affect newly-packed columns since a well-functioning microbial community didn't establish in the deeper layer. The effect of schmutzdecke on filter functioning may be more apparent in filters when the deep sand bed biofilm cannot substantially purify the influent water for a variety of reasons (Chan et al., 2018). Haig et al. proved this point indirectly by presenting the results that communities between sand samples from different depths were found to be highly similar in two well-established full-scale SSFs. It indicated that the deeper sand bed had the potential to have the same removal capability as the upper part with enough operational time given.

Studies with respect to the evaluation of schmutzdecke were mostly performed by ripening clean sand in-lab or pilot scale columns instead of investigating mature sand directly from full-scale filters. The ripening time for the microbial community in deeper

sand bed to establish is limited for lab-scale columns (Hijnen et al., 2004b; Pfannes et al., 2015; Unger & Collins, 2008b). Unger & Collins used a laboratory-scale column ripened for 15 days and pointed out the significant influence of scraping on the performance of the column. But it is possible that in such a short period, schmutzdecke might be the only part that has relative higher microbial activity in the column, and removing it off could influence the effluent water quality. Although Hijnen et al. and Phannes et al. operated the pilot-scale column for several weeks, it is still limited compared to 1-year operated filter materials applied in this study. The different maturity level might exist and lead to different results of the role of schmutzdecke.

5-20 and 20-35 cm depth with little and no biofilm respectively exhibited higher capability in PhiX174 removal than 0-5 cm. However, with thick biofilm in schmutzdecke (0-5 cm), we noticed a higher effluent concentration than influent, indicating no removal of PhiX174, which throws doubt to the statement that EPS could enhance the adsorption of the virus (Hammes et al., 2011). And by running a T-test of PhiX174 log removal in 5-20 and 20-35 cm ($p = 0.11$), no statistically significant difference was found, which suggested the biofilm might not greatly aid in PhiX removal, but the thickness of biofilm could be a factor that would influence the removal since no PhiX174 was removed in 0-5 cm.

6.2 Cumulative removal efficiency in bacteria and viruses removal

Though individual layers were evaluated independently in setup 1, the cumulative LRVs was considered to represent the true performance of slow sand filters because all layers are operated in a SSF as a whole in real situations. For E. coli, 2.89 log removal was achieved, which is in the range of 1.6-4.5 log removal for pilot-scale SSFs conducted by researchers in the Netherlands (Schijven et al., 2013). As indicated in Figure 15, the E. coli removal along with depth clearly shows a nearly linear relationship.

Similar experiments were performed by Pfannes et al. (2015) with laboratory-scale SSFs packed with sands of different sizes and ripened for 12 weeks. In the experiment, columns C1 (d10 = 0.25mm), C2 (d10 = 0.4mm), and C3 (d10 = 0.63 mm) packed with sand in different effective diameter were tested on E. coli removal capacity. When compared to the finding of the C1 column (d10 = 0.25mm) that has a similar effective diameter to us (d10 = 0.3mm), a comparable removal efficiency between 2.5 and 3 $\log_{10}$ removals was found (Fig. 12) (Pfannes et al., 2015). However, different from this study, the top 5 cm layer exhibited much higher removal than the rest of the columns in Pfannes' experiments, indicating the stratification of removal processes in the filter bed. After the highest removal shown in the top 5 cm, the efficiency decreased immediately in 5-10 cm depth. And a further slight reduction was observed between 5-10 and 10-20 cm (Pfannes et al., 2015). While, in our cases, an approximately linear shape was observed for the first 35 cm. The removal efficiency slightly decreases with depth. It

indicated the same importance of deeper layers of SSF as schmutzdecke in E. coli removal.

In addition, most of the studies that reported the critical role of schmutzdecke in bacteria removal were either performed by columns with different sampling points at various depths or just with a single outlet and inlet (Elliott et al., 2015; Hijnen et al., 2004a; Unger & Collins, 2008a), which could, to some extent, be impacted by the concentration effect. With the highest spiking concentration contacting the top layer first and decreasing with the increase of depth, the deeper layer could only contact with lower-load concentration, which might hinder the exhibition of its real capability. Different depths operating in different columns in this study could help to have a clearer comparison among different depth intervals and provide insight on the capability of deeper depths when the schmutzdecke is removed in the cleaning process, which can help us have better ideas in the adjustment of re-ripening period of SSFs.

The LRV of E. coli in schmutzdecke was lower compared to the previously reported value (more than 1 log) (Adin, 2003; Haarhoff & Cleasby, 1991; Pfannes et al., 2015; Wotton, 2002). The possible reason could be improper column operation. Accidentally drained-out could happen when the influent tank wasn't refilled in time during night or connectors were disconnected due to some human factors. Besides, that the interval of sand packing and spike test was too short is another possible reason. The capability of columns may have not restored to normal level. The primary goal of this study is to compare the removal capacity of various depths. For all depths of columns, the experiment steps and column running were almost identical. Even if the findings were influenced by the improper operations, the results of the studies still provide informative insight into the function of various depths. In the future experiment, the sand should be packed more carefully and the complete drained-out should be avoided in the daily operation. Longer stabilization should be ensured before the columns are tested.

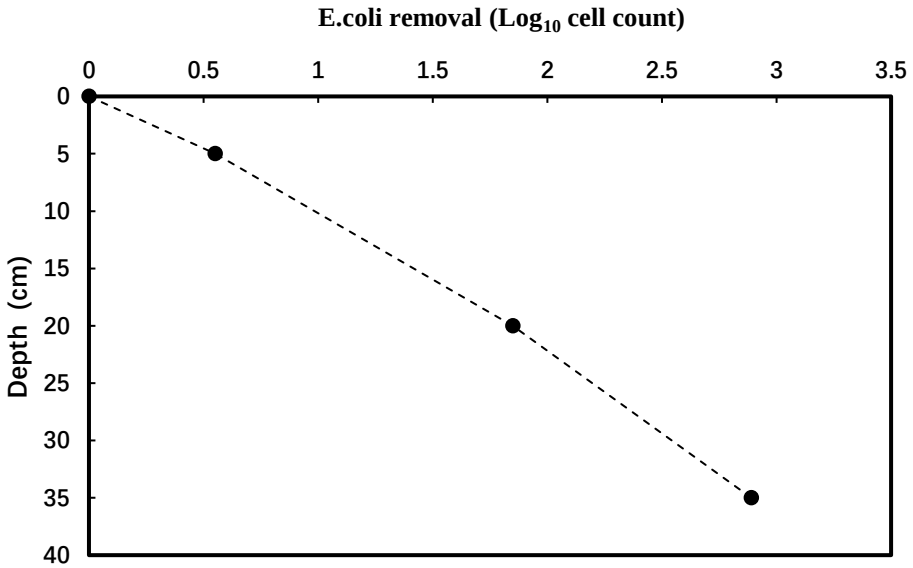

Fig. 15. Cumulative E. coli removal with the depth of the sand bed

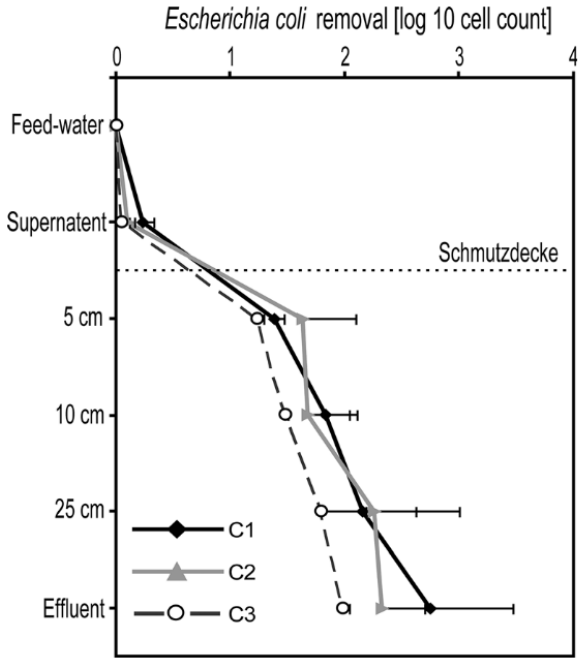

Fig. 16. E. coli removal through SSFs (Pfannes et al., 2015)

The cumulative PhiX174 removal could reach 0.44 $\log_{10}$, which is in line with results of 0.2 - 0.5 $\log_{10}$ found by other researchers (Aronino et al., 2009; Elliott et al., 2008; Hijnen et al., 2004b). The stratification of PhiX174 removal was much more evident than the E. coli in this experiment, as shown in Figure 17: no removal of PhiX174 in the first 5 cm, 0.32 log removal in 5-20 cm and 0.13 log removal in 20-35 cm. With biofilm in 0-5 cm and 5-20 cm, no significant improvement of PhiX174 removal was

noticed in the experiments.

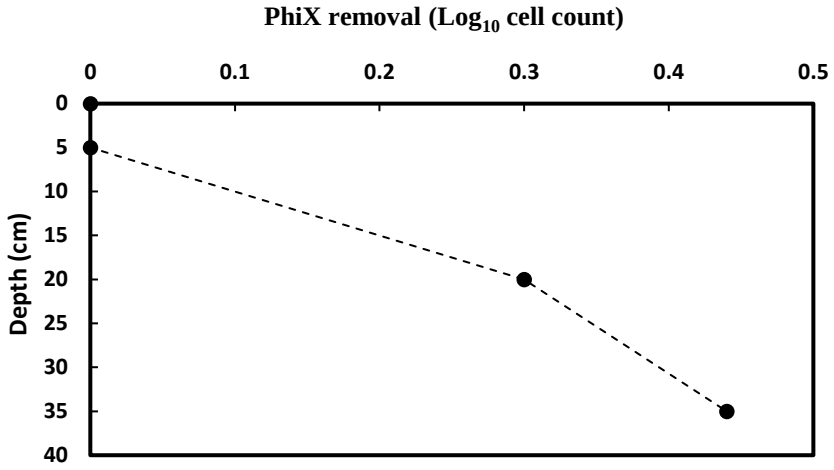

Fig. 17. Cumulative PhiX174 removal with the depth of the sand bed

6.3 Bacteria and viruses removal mechanisms in the schmutzdecke

6.3.1 E. coli removal

The columns under different conditions removed 0.68, 0.74 and 0.43 log of E. coli for active, inhibited, and ignited sand, respectively (Table 7). Schmutzdecke, considered as the most efficient removal compartment in an SSF system, was demonstrated to retain more than 1 log (90%) of bacteria indicators (Adin, 2003; Hendricks, D. W., & Barrett, 1991; Wotton, 2002). Around 0.7 log (80%) were attained in active and inhibited sand in the present study. The possible reasons for its' underperformance might credit to what we've discussed before (chapter 6.2).

It was indicated that straining becomes an important removal process when the average cell size surpasses the size of 5% of the grains that make up the porous medium (Stevik et al., 2004). Due to the small size of E. coli, the screening process cannot be a decisive mechanism in ignited sand. With the exception of the biological mechanisms after ignition, a possible reason responsible for elimination in ignited sand was the adsorption caused by the positive charge metal trapped in the sand bed. Since the sand was sampled from a full-scale sand filter, cations like iron, manganese, aluminum from influent raw water can deposit on negative charge sand bed, which render the positive charge of sand bed and aid to the E. coli removal. This was endorsed by the findings of unpublished results that iron, and manganese were found in ignited sand. Weber-Shirk et al. also pointed out that the presence of metal (aluminum) can be an enhancement of slow sand filters (Weber-Shirk & Chan, 2007). Measurements conducted by Atomic Force Microscopy showed that the adsorption forces between virus and coated sand from high to low was: aluminum oxide-coated sand, goethite-coated sand, and oxide-removed

sand, which aligned with the zeta potential of each kind of sand: 11.52 ± 0.6 mv, 4.1 ± 0.2 mv, -39.5 ± 0.7 mv, respectively (Attinti et al., 2010).

Whereas a study has shown that in the presence of humic acid or dissolved organic matter on the sand, the impact of these metal-based adsorption sites was substantially decreased (Chen et al., 1998; Foppen et al., 2006). As such, this metal-based adsorption might not be the primary reason for E. coli removal in the active and inactive sand bed. As shown in Figure 13, the function of microbial activity is not evident in the schmutzdecke as the similar performance on both active and inhibited sand. In the research conducted by Weber-Shirk and Dick (1997), the impulse of azide for 3 h did not impact the pre-existing biofilm (the performance was reversible, claimed by the author), and the significant decrease observed on E. coli removal proved that the adsorption to biofilm was not the primary reason responsible for elimination. Instead, the function of microbial community was thought to be the main reason (Weber-Shirk & Dick, 1997).

It is worth noting that Weber-Shirk and Dick's tests were performed over a short amount of time before filters were required to be completely matured, while sand that had been running for 483 days was applied in our study. Different maturity levels may also affect the key mechanisms in schmutzdecke. On top of that, we only focus on 0-5 cm, whereas much longer columns (18 cm for Weber-Shirk et al.) were applied. As such, it is possible that the addition of sodium azide makes great impaction on the removal capacity of the deeper layer (more than 5 cm) rather than schmutzdecke. As a result, Weber-Shirk et al. (1997) noticed the changes in the whole column after injection of azide.

No evident sign of microbial community in this study might also be attributed to the incomplete inhibition of sodium azide, possibly due to limited dosing time. Even the same concentration of sodium (6mM) was applied in the previous study, the addition of 5 consecutive days in this study was limited compared to daily addition for 50 days (Elliott et al., 2011). 195 mg/L (3 mM) was chosen in the experiment of Weber-Shirk et al. (1997), while no direct validation of complete inhibition was shown. A longer dosing time of sodium azide should be performed until the ATP concentration reaches minimal concentration and be stable.

Straining could be one of the possible key mechanism in schmutzdecke. Studies showed that SSFs that had a schmutzdecke on top exhibited an elimination of bacteria 1-2 logs better than columns without a schmutzdecke, whereas virus removal was independent of the existence of schmutzdecke (Hijnen et al., 2004b; Mcconnell et al., 1984; Wotton, 2002). This was attributed to the size difference of bacteria and viruses. On top of that, Weber-Shirk and Dick (1997) suggested that the Physical-chemical mechanisms may be responsible for partial removal after the azide pulse. Unger & Collins (2008) found that the mass of EPS (the main components of biofilms) was not significantly correlated to E. coli removal. All these studies point to the critical roles of straining in the

schmutzdecke layer for E. coli removal after excluding the possibility of metal-based adsorption, biofilm adsorption, the function of microbial activity.

Meanwhile, the function of straining could be seen by the comparison of E. coli removal and PhiX174 removal in this study as well. Within active sand, no PhiX174 removal could be observed, while nearly 0.7 log of E. coli can be eliminated. This different performance may attribute to the size difference between these two items (Average diameter:1 μm E. coli and 26 nm for PhiX174) (Aronino et al., 2009). Thus, screening could be the key removal mechanism in schmutzdecke for a well-development SSF.

However, the role of microbial mechanism should not be overlooked, though it isn't obvious in our results. It might be a decisive mechanism for E. coli removal if the operation time of SSF is limited, as indicated by experiments performed by lab-scale columns (Hijnen et al., 2004b; Pfannes et al., 2015; Unger & Collins, 2008b). Besides, for the possible absence of complete inhibition, the function of the biofilm may not be demonstrated in this study.

6.3.2 PhiX174 removal

The columns under different conditions removed 0, 0 and 0.28 log of PhiX174 for active, inhibited, and ignited sand, respectively (Table 7). The virus was considered as the most critical microorganism for the elimination of SSF since only 1.5-2.2 $\log_{10}$ can be reached (Hijnen et al., 2004b; Schijven et al., 2006; Wheeler et al., 1988). Studies pointed out that phages and viruses are barely influenced by the existence of schmutzdecke due to the size (Hijnen et al., 2004b; Schijven et al., 2006; Wheeler et al., 1988). The findings obtained in this study were basically consistent with previous ones: the virus was difficult to remove within schmutzdecke.

As indicated in Figure 14, no removal of PhiX174 can be observed in both active and inhibited sand, indicating that there was no sign of microbial community. However, the function of the microbial community exhibited a decreased removal in viruses after the addition of sodium azide in the research where longer (40 cm) columns were performed (Elliott et al., 2011). In addition, Elliott et al. (2011) also indicated that modified media surface might not be the primary reason for virus reduction. From the results, we could speculate the possibility of better performance of active microbial community in deeper depth (below 5 cm) since in the comparison of active and inhibited 0-5 cm sand, either of each aid in removal in this study, while the influence of azide was indeed observed in Elliott et al. experiments. The impaction of higher interstitial velocity caused by microorganism proliferation and particle accumulation in 0-5 cm could be a possible reason for the division (Aronino et al., 2009). The higher velocity could lead to less retention time, which could greatly impact the function of microbial activity. In addition, with the higher interstitial velocity, the hydrodynamic shear force is accordingly expected to rise at the same time (Ranjan & Prem, 2018). It is conceivable that particles

attached to the layer were flushed out again, resulting in a higher concentration of effluent than influent, as we observed in PhiX174 removal (Figure 14). Furthermore, this explanation is also in line with the inference made before that screening might be the key mechanism in E. coli removal in schmutzdecke as less retention time and higher shear force won't hugely impact the efficacy of the screening process. At last, the incomplete inhibition due to improper operation, as mentioned before, might also be a possible reason.

6.4 Improvements of tracer test

The tracer tests of setup 1 showed that the peak of C/C_0 is nearly 1, while for setup 2, the peak could only reach 0.8 for active, inhibited and ignited sand. NaCl, as a conservative tracer, was supposed to be completely recovered. Only 0.8 got in setup 2 might attribute to the improper design of setup 2. A pressure system was applied to ensure the suitable effluent velocity. The connectors used to connect the filter media and the influent tube had some empty spaces. To operate as a pressure system, this space was filled full with water, which could, to some extent, dilute the concentration of influent water and lead to a lower ratio of C/C_0. For setup 1, the supernatant was reduced as low as possible when tracer tests were applied and a more negligible dilution effect existed in the column. As a result, the recovery of NaCl was closer to 1.

On top of that, for the relatively slow velocity of slow sand filters (0.3 m/h), the effluent sample volume collected in the tracer test according to effluent Table 2 was not enough for EC measurements. For that reason, 100 times dilution was applied to get a higher sample volume. During this process, errors could be easily caused by the inaccurate dosage of water, which can partly explain the incomplete recovery and slight time shift in setup 2.

The inhibited sand and active sand were expected to have similar hydraulic characteristics since the introduction of azide only inactivated the microbial activity. However, inhibited sand showed an earlier increase breakthrough and reach to the peak value at only 16min. Accident drained-out of experiment columns in inhibited sand leading to decrease of biofilm might be a possible reason. On top of that, the connectors at the effluent site of both setup 1 and 2 inevitably could have some empty space before effluent coming out, which could influence the accuracy of the experiment as well. The process happened in effluent tubes could also impact the accuracy of results.

In future experiments, better design and operation of columns are needed. The supernatant should be reduced as low as possible when spike/tracer tests are needed and the dead volume within the structure of columns should be avoided as much as possible. At the same time, the diameter and length of influent and effluent tubing should be decreased.

Chapter 7: Conclusion

This research aimed at investigating the contribution of different depths of SSF to bacteria and viruses removal and the key purification mechanisms in schmutzdecke. Columns packed with sand from three different depths showed removal of 0.55, 1.30, 1.04 logs for E. coli at 0-5, 5-20, 20-35 cm respectively. 0, 0.30, 0.14 logs for PhiX174 was achieved at 0-5, 5-20, 20-35 cm respectively. 0-5 cm (schmutzdecke) was hypothesized to have the greatest removal capacity due to the existence of prominent biofilm. However, the role of deeper depths in E. coli removal was shown as important as schmutzdecke. For viruses, 0-5 cm and deeper layers showed poor removal capacity. The biofilm didn't significantly aid in PhiX174 removal, but the thickness of biofilm could be a possible reason to impact the removal of PhiX174. Overall, schmutzdecke might not have a decisive effect on the removal performance of a well-developed SSF. The underperformance of schmutzdecke compared to the previous study might be due to improper column operations and short intervals between spike test and sand pack. Accidental drained-out of columns might cause decrease of microbial activity and further impact the removal efficiency. Enough intervals needed to stabilize the column and restore the capacity of the filter material.

The schmutzdecke under three conditions showed 0.68, 0.74, 0.43 logs E. coli removal and 0, 0, 0.28 logs PhiX174 removal in active, inhibited and ignited sand respectively. Despite the function of the microbial community being hypothesized to be the key mechanism in bacteria and viruses removal, no sign of the effect of the microbial community was observed in schmutzdecke. The effect of the microbial community may change over time as the biofilm in schmutzdecke ages. Schmutzdecke functions more like a physical filter mat in a well-established SSF as the higher interstitial velocity and shearing forces caused by abundant biofilm might greatly decrease the efficacy of microbial community. Besides, possible incomplete inhibition due to limited time could also be a reason for the little effect of the microbial community. Thus, the role of the microbial community should not be overlooked.

Since schmutzdecke in a well-developed SSF is less important than SSF in the early stages, the re-ripening time might be minimized. The SSF can quickly resume regular operations since deeper layers have the same pathogen-removal capability as schmutzdecke. In addition further studies are still needed to verify the capability of schmutzdecke in mature full-scale SSF.

Chapter 8: Recommendations

a) Filter material and similar influent from full-scale SSF should be applied in lab-scale experiments concerning the impaction of scraping and the role of schmutzdecke. Different experimental conditions may draw different conclusions, as shown in this research, that the effect of the microbial community might be different under different operation time of the sand bed .

b) Further studies on the mechanisms in the deeper layer of SSF should be investigated to get insights on the stratification removal process in SSF and enhancement of efficacy of SSF.

c) Studies investigating the capacity of schmutzdecke should have a clear and reasonable definition of the depth of schmutzdecke. A clear distinction between schmutzdecke and deeper depth should be built to avoid attributing the function of the deeper layer to schmutzdecke layer. Different removal processes and key mechanisms might exist in different depths.

d) In order to fully comprehend the function of biofilm in the SSF, studies on the influence of biofilm thickness on pathogen removal should be conducted since the findings of this study revealed that no PhiX174 was eliminated with the thick biofilm generated in schmutzdecke.

e) Enough stabilization time should be guaranteed between the sand filling and tests in column experiments. On top of that, appropriate restore intervals should be ensured between improper operations and experiments. Environmental variations may influence the microbial community to variable degrees, and the removal ability that SSF should have will take some time to recover.

f) Enough time of azide introduction should be performed until the ATP concentration reaches minimal value and become stable to ensure the effectiveness of the complete inhibition and increase the credibility of the results.

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Appendix A: Tracer results in setup 1 (different depths)

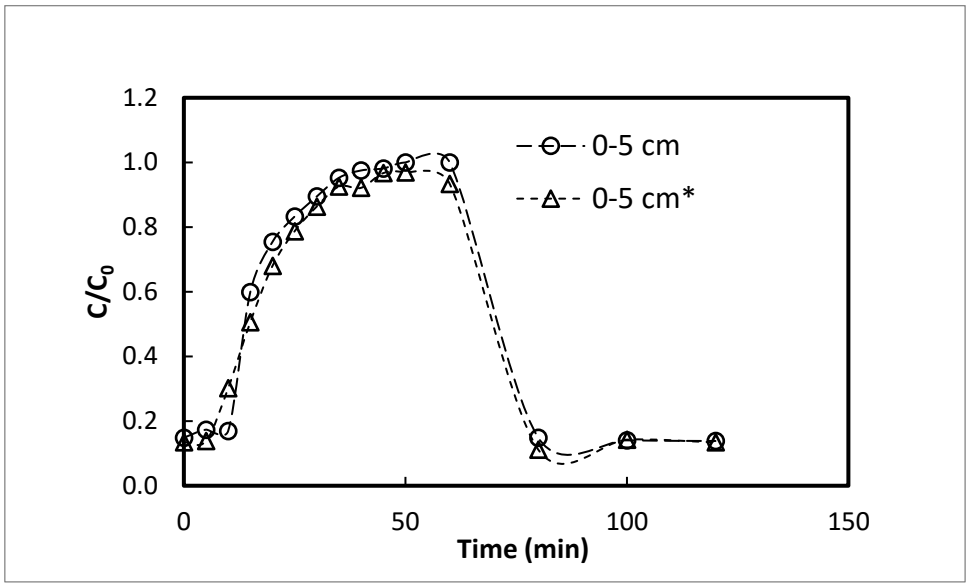

Fig. 18. NaCl breakthrough curves for 0-5 cm (* stands for duplicate column)

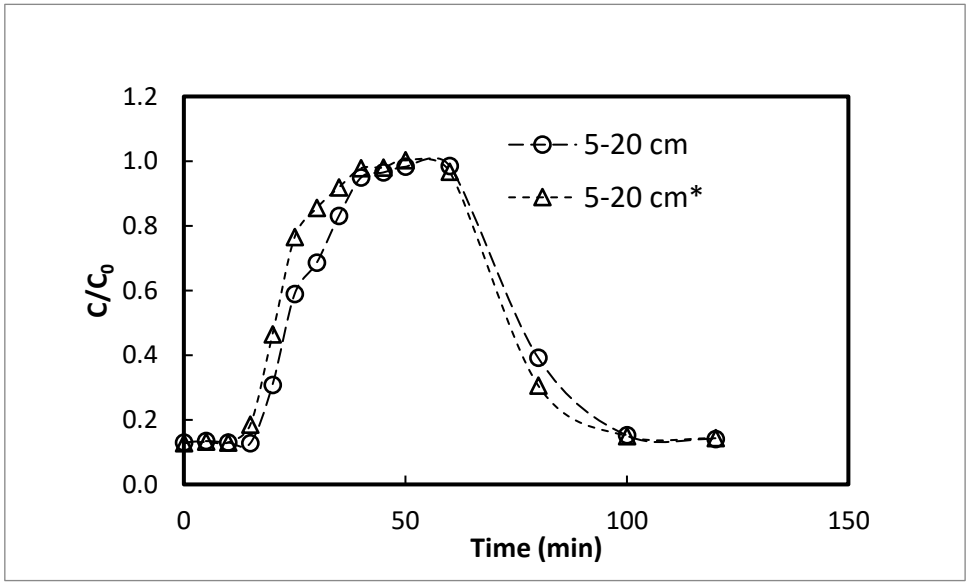

Fig. 19. NaCl breakthrough curves for 5-20 cm (* stands for duplicate column)

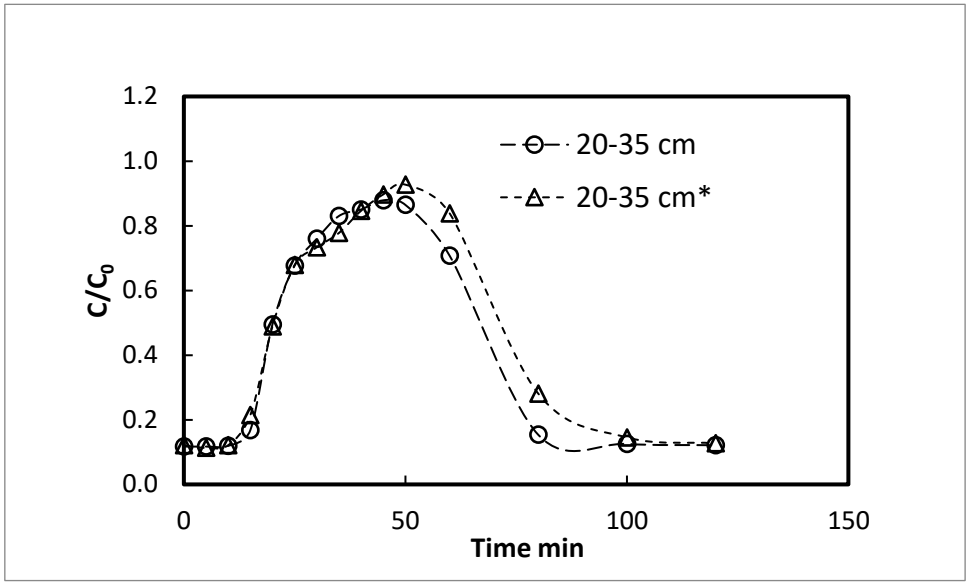

Fig. 20. NaCl breakthrough curves for 20-35 cm (* stands for duplicate column)

Appendix B: Tracer results in setup 2 (key mechanisms)

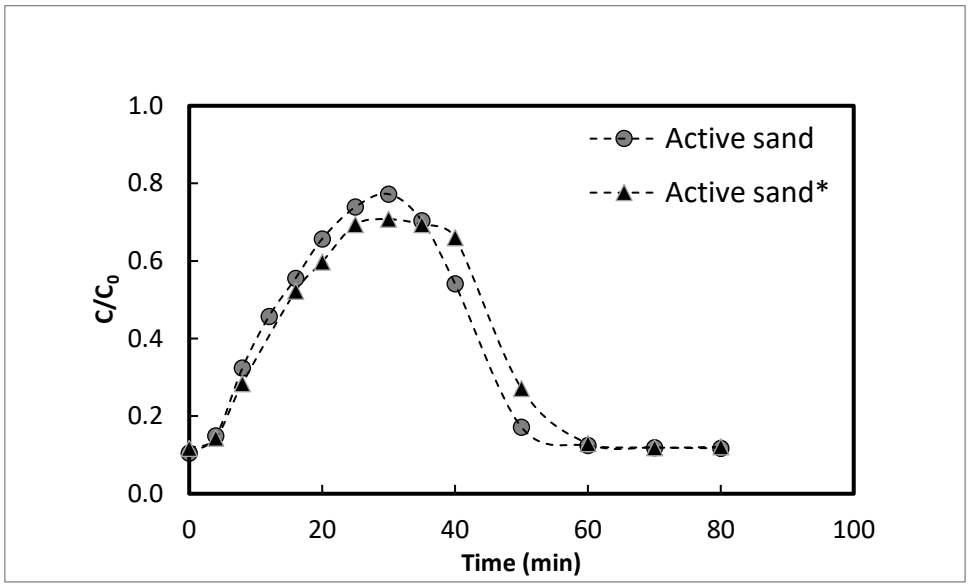

Fig. 21. Nacl breakthrough curves in active sand (* stands for duplicate column)

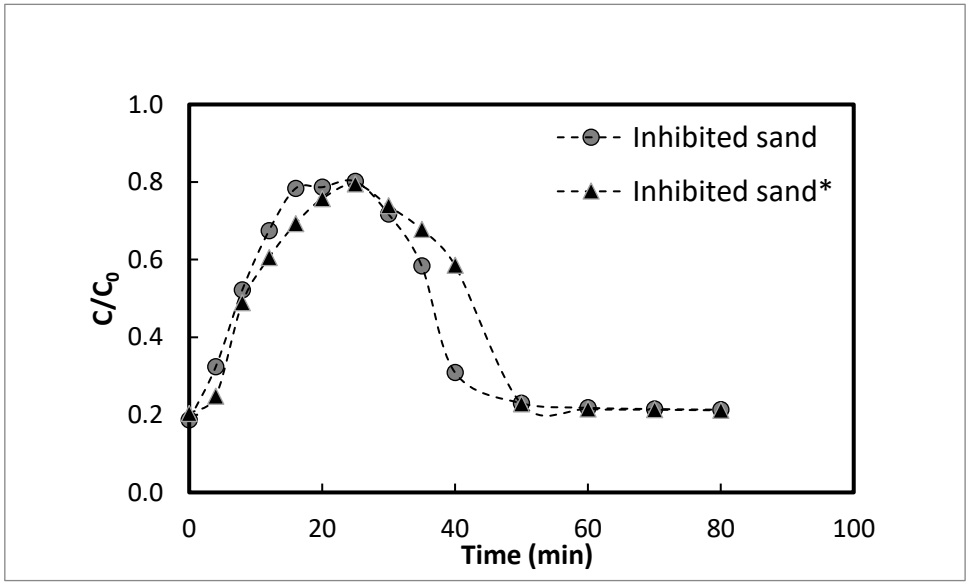

Fig. 22. Nacl breakthrough curves in inhibited sand (* stands for duplicate column)

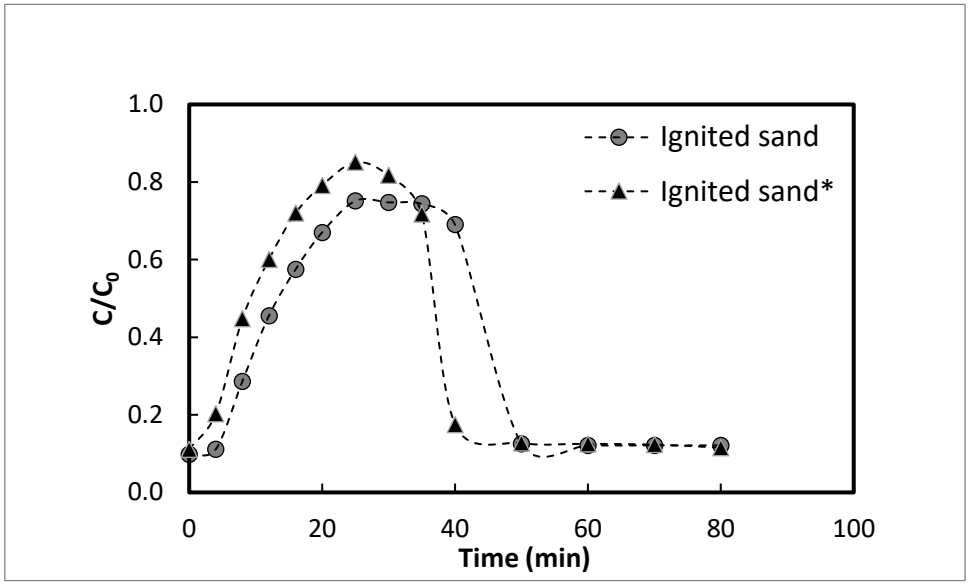

Fig. 23. Nacl breakthrough curves in ignited sand (* stands for duplicate column)