Multi-parametric assessment of a scraped slow sand filter during the ripening period

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Abstract

Being one of the oldest water treatment systems in the world, slow sand filters (SSFs) have played a big role in the water treatment sector due to their reliability and their effective physico-chemical and biological purification capabilities. They are still widely used as a disinfection step in many countries, especially those that do not use chlorine for disinfection. SSFs are gaining popularity in recent times, especially due to their ability to deactivate pathogens like *Giardia lamblia* and *Cryptosordidium parvum*, which have been posing significant challenges to the public drinking water systems for years.

Over time, researchers have mainly focused on understanding the importance of the schmutzdecke layer of the SSFs, as it is understood that the schmutzdecke is essential for their functioning. This has limited the research done in the deeper layers of the sand bed, resulting in a lack of understanding of the activities here.

The aim of this study is to investigate the physico-chemical and biological functions happening in the deeper layers of the SSF, and to determine whether parameters like nitrate, turbidity, DOC, FEEM, ATP and particle cell counts can evaluate the ripening of a scraped bed. Furthermore, the ability of these parameters to sufficiently indicate biological stability of the sand bed is to be assessed.

In order to determine this, two full scale filters were sampled and analysed. One was a scraped bed and the other was a mature bed. They were also compared to judge how differently they behaved throughout the ripening phase.

The results of the tests showed that nitrate, turbidity and ATP are good indicators of ripening. ATP and particle cell counts, when used in parallel, are good assessors of biological stability. FEEM also serves as a useful method to determine why the DOC concentrations in the water samples were similar for both the filter beds. ATP tests conducted on the schmutzdecke sand samples provide evidence of ripening over time for the scraped bed. Further research using parameters like iron, manganese, ammonia and oxygen is required to strengthen the understanding of the workings of the deeper layers of the sand bed and the various physico-chemical and biological mechanisms happening within.

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Delft University of Technology — Thanisha Kannan
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Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

1-1 Motivation behind research

Slow sand filters (SSFs) are one of the oldest treatment processes that are in use till date. SSFs are popular till date due to their efficiency, low cost of installation, operation and maintenance and ease of operation. They have various physico-chemical and biological processes taking place at the top layer and within the sand bed. Furthermore, SSFs are an efficient method of producing water of good bacteriological, physical and organic quality. They are capable of removing various pathogenic organisms like Giradia, Cryptosporidium, which are highly resistant to chlorine disinfection. They can also remove viruses, oxidize ammonia and also biodegradable natural organic matter from the water. They can also reduce the turbidity of water. Their removal efficiencies is given in Table 1.

Table 1: Slow sand filters parameters removal

Parameter	Possible removal	Source
Assimilable Organic Carbon	14 – 40 %	Haig et al., 2011
Dissolved Organic Carbon	5 – 40 %	Haig et al., 2011
Biodegradable natural organic matter	$>$ 80 %	Pike, 1987
E. Coli and Coliforms	2 – 4 log10	Pike, 1987
Turbidity	$<$ 1 NTU	Haig et al., 2011; Pike, 1987

Even various industrial countries like the Netherlands, United Kingdom, France, Belgium use it as a treatment step in a water treatment unit (Huisman and Wood, 1974). The Netherlands, for example, prefer to not use chemicals like chlorine for disinfection. Significant research has been done on the potential harmful effects of chlorine disinfection. Various toxicological studies have suggested that they could cause significant health concerns. In raw water, chlorine reacts with natural organic matter (NOM) and produces disinfection by-products (DBPs) like Trihalomethanes (THMs), Haloacetic acids (HAAs) etc. Chronic exposure to DBPs has been

linked to cause cancer (Mazhar et al., 2020). Hence, some water utilities in the Netherlands use SSFs as a last disinfection step to produce micro biologically safe and bio stable drinking water to avoid the use of a residual disinfectant during distribution. SSFs have proven to be beneficial for disinfection as they are capable of removing or inactivating various pathogens, in the water, which precludes the necessity of using a residual disinfectant during transportation (Smeets et al., 2009).

There is a biologically active layer, called "the schmutzdecke" which grows on top of the sand bed. It is composed of various micro-organisms, such as bacteria, protozoa, planktons, diatoms, rotifers, that also aid in the treatment of water. These microorganisms are responsible for various physical, chemical and biological processes taking place on and within the filter bed. They mainly help to break down organic matter, which is passed on to the deeper layers and may serve as the food source for the biomass in those layers, they help with oxidation of nitrogen, removal of inert suspended particles and various other processes (Huisman and Wood, 1974). A lot of research has been done on the efficacy of the schmutzdecke. However, the deeper sand also contains biomass which contribute to the treatment of water, which is a topic that is not extensively researched (Haig et al., 2011).

The schmutzdecke layer, as advantageous as it is in the treatment of water, can also lead to difficulties in maintenance long term. Due to the growth of the micro-organisms in the sand layer, it can lead to clogging. This clogging prevents the influent water from being able to pass sufficiently through the filter bed and thus leads to head loss in the filter bed. In order to manage the head loss, the filters need to be scraped, and the schmutzdecke is allowed to form again on the filter bed, this process is called "ripening". Ripening is an essential part of the water treatment process, as most research (Campos et al., 2002; Haig et al., 2014; Haig et al., 2015; Huisman and Wood, 1974) suggests. Ripening period is considered crucial from a disinfection point of view. Removal efficiency improves with schmutzdecke development or filter maturation M. Elliott et al., 2015; Unger and Collins, 2008 However, most of these studies were done in a pilot scale study or used newer filter beds. These studies have various limitations in terms of replicating a full-scale sand bed because of the following reasons:

- A pilot-scale study is limited and cannot replicate the complex microbial growth environment that a full-scale sand bed contains. Most of the laboratory-scale studies have been performed on lab-scale microcosms, with carefully controlled parameters, which may not fully represent the complex and diverse microbial communities that full-scale SSFs contain (Chan et al., 2018)
- A new slow sand filter bed is not fully developed and is not representative of the functioning of a mature full scale sand bed (Haig et al., 2015).

One such example is the research conducted by Unger and Collins, 2008 which compared the functioning between 3 kinds of lab-scale filter beds - an unripened bed, a ripened bed and a scraped bed, which were monitored over a period of 3 weeks. The research concluded that the removal of schmutzdecke affected the function of the filter bed and lead to a decrease in the filter-ability of the sand bed. This result is expected in a lab scale study, especially with a new filter bed. The schmutzdecke plays an important role in the filtration process in new SSFs because the substrate concentrations and the biomass acting on the substrates are highest at the surface of the sand bed Bai et al., 2013 and also because a new filter bed

would not have a developed sand biomass in the deeper layers. Absence of a fully developed schmutzdecke in a new sand bed, therefore, inherently reduces the efficiency of the sand bed.

However, on the contrary, research done by Campos et al., 2002 suggested that the deeper sand biomass is sufficiently able to treat the water to meet the water quality requirements. Chan et al., 2018 also concluded from his research, using bacterial profiling, that established SSFs showed consistent performance, and that it was not affected by the removal of the schmutzdecke. This result suggests that a mature biofilm in the deep sand bed is essential for consistent microbial water quality from SSFs. Hence, it can be concluded that the results obtained from lab-scale experiments might be overestimating the impact of the schmutzdecke, which further emphasizes the need for studies conducted at full scale SSF plants for a complete and better assessment of drinking water treatment by SSFs.

Additionally, due to the lack of research done to corroborate this study, it is often believed that during the ripening process, the slow sand filters are practically ineffective and the water that is treated is usually discarded. Dunea, for example, usually discards water for a period of nearly 40 days, when the filter is under the ripening phase. Many water treatment companies also follow this procedure, which could lead to excessive amounts of water wastage. Hence, it is important to have a better understanding of the functioning of the deeper layers of the sand bed biomass. And the ability to follow SSF function in real time, using monitoring parameters, would minimise the time that the filters are kept offline to both ensure maximum supply of treated water and reduced costs Chan et al., 2018.

The scraped bed considered in the study is a slow sand filter that had been in use for about > 500 days before it was scraped. It is important to know that, during the cleaning, only the top 1-2 cm of the schmutzdecke was scraped and the deeper sand biomass was left untouched, thus the scraped bed has biomass in the deeper layer that is fully developed.

1-2 Problem statement and knowledge gaps

It is a well-known fact that the active schmutzdecke layer plays a big role in ensuring the biological stability of water and also helps in maintaining the effluent water quality standards. However, there is not enough research done using the water samples in the deeper layers of the sand bed to understand that they contribute majorly in maintaining the water quality in the absence of an active schmutzdecke.

Furthermore, it is important to conduct water quality tests to determine if a filter has ripened. Several physico-chemical parameters like assimilable organic carbon (AOC), turbidity, nitrate, ammonia, phosphate, dissolved oxygen (DO) and biological tests like Adenosine triphosphate (ATP), Total and faecal coliform, Total cell counts (TCC) could be done to assess the effluent water quality, but doing these tests on a regular basis could be time consuming and also expensive.

Currently, drinking water utilities applying SSF use total and faecal coliform analysis to determine whether a filter has ripened. This could be a misleading analysis if the SSFs are used as a final treatment step, mainly because the influent entering the slow sand filter could already have very low coliform content and this will reflect in the effluent as well. Usually, lower coliform content in the effluent is considered as an indicator for a ripened bed

(Huisman and Wood, 1974). This might work for wastewater but might not be representative for drinking water. This is mainly because of 2 reasons:

- The slow sand filter is the last stage of treatment and so, the water coming into the SSF itself will have very low coliform content.
- The water that Dunea treats (and groundwater in general), is mainly infiltrated through the dunes, before being sent to the treatment unit. The water abstracted from the dunes is usually biologically stable already (Smeets et al., 2009).

Hence, it would be useful to have some reliable parameters to assess the ripening of the filters that are easy to measure and inexpensive so that companies can implement them in order to investigate the ripening process of the filter bed. Furthermore, there is less understanding of how the deeper sand bed of a full-scale SSF behaves during the ripening phase, which serves as a knowledge gap that needs further research.

Therefore, due to the reasons cited above, it can be understood that the ambiguity in the understanding of SSFs with respect to its treatment effectiveness without the schmutzdecke still persists and it needs further investigation.

1-3 Objectives and approach

The main objective of the study is to investigate what parameters can be used to indicate ripening in a scraped SSF. Under this objective, the scraped SSF will be analysed, using temporal and spatial variation, how the function changes over time and how the biological performance varies.

The approach that will be taken in-order to fullfil the objectives are as follows:

- monitoring the scraped bed using physico-chemical parameters to determine if the filter has ripened, and using biological parameters to assess the biological performance of the filter bed
- comparing the scraped bed with a mature bed to analyse how differently they behave. If the scraped bed begins to act like the mature bed over time, it would indicate that the scraped bed is maturing

A schematic diagram showing the approach taken to fulfil the objectives is given in Figure 1.

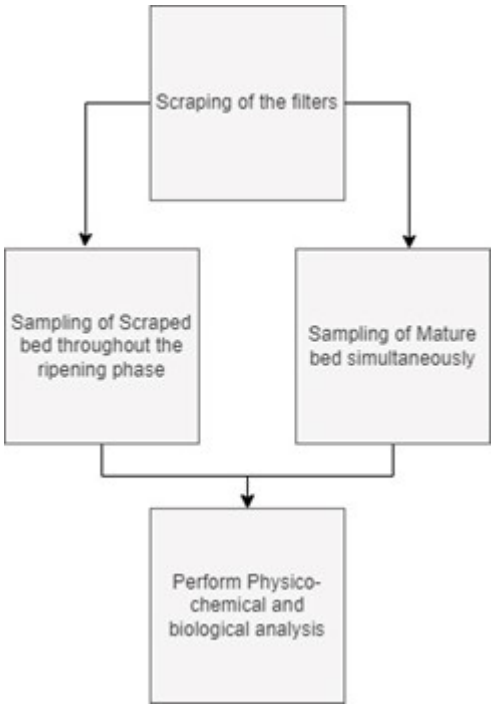

Figure 1: Approach and objectives

1-4 Research questions and hypothesis

The main research questions are as follows:

- *What physico-chemical parameters can assess the ripening of the bed?*
- *Do the scraped and mature beds show similar biological performance over time?*
- *Can the physico-chemical changes through depth be attributed to biologically stable performance within the depths of the sand bed?*

The specific research questions are as follows:

- *Is the change in Nitrate, turbidity, DOC indicative of filter ripening?*
- *Do the physico-chemical parameters stabilize over time for the scraped bed?*
- *If the sand biomass is active in the deep sand bed, would it be possible to indicate that using ATP and TCC?*
- *How do the nitrate, turbidity, DOC parameters vary in the deeper sand bed for a scraped bed versus the mature bed?*

The hypothesis is that the scraped bed does not differ greatly from the mature bed in terms of the variation in physio-chemical parameters because the deeper sand biomass in the scraped bed is fully developed and is able to function like the mature bed. Hence, despite the absence of an active schmutzdecke layer, the scraped bed is able to replicate the mature sand bed in regard to the filter characteristics and performance.

1-5 Outline of the report

- Chapter 2 deals with the past research done on the slow sand filters and it will shed light on the research available so far and how it influences the current research and expected outcomes. It talks about the mechanism of slow sand filtration, role of the schmutzdecke and sand biomass in water treatment and the physical, chemical and biological parameters that are useful to determine ripening of the filter. The previous literature is important to understand why this project was undertaken and what are the research gaps that are intended to be investigated.
- Chapter 3 enlists the materials and methodology used during the study. It contains the information on the experimental setup of the slow sand filters and also the method of sampling, transportation and analyses of the samples. The methods used to determine DOC, turbdity, Nitrate, ATP and TCC are explained.
- Chapter 4 focuses on reporting the results obtained from the experimental study.
- Chapter 5 discusses the obtained results in detail, and sheds light into the reasoning behind the results obtained.
- Chapter 6 presents the conclusions with suggestions for further research and takeaways from the current study.

Chapter 2

Literature Research

2-1 Introduction to slow sand filters

John Gibbs was the very first known person to use elements of the SSF to treat the water from his bleachery in Scotland in 1804. His idea was used and after some improvements and additions were made, the first ever SSF was constructed by James Simpson in 1829 at Chelsea water company in London. This was constructed to treat water that was to be supplied to the general public (Baker, 1949). In its initial stages, SSFs were mainly used as a straining unit to remove suspended solids and turbidity. The breakthrough point that boosted the popularity of the SSFs was after Jon Snow linked the outbreak of the cholera epidemic to the contaminated water sources. This lead to the SSFs becoming a legal necessity to treat all the portable water extracted in Thames, London from 1854 onwards (Huisman and Wood, 1974).

Under the right circumstances, SSFs might still be one of the simplest and most effective methods of treating water. The World Health Organization (WHO) had even stated that SSFs are simple to use, inexpensive, reliable and is still a sought out method for purifying water for some of the major cities in the world. One example is the United Kingdom, where, 20% of the treatment plants still use slow sand filters. And in London alone, about 80% of treatment plants consist of SSFs. Thames Water Authority in London still uses SSFs to provide potable water to almost 8 million people (Kem, 1996).

Comparative studies conducted to compare combined costs of construction and operation of water filtration plants in New Hampshire concluded that the SSFs could provide treated water at a lower cost and also that the SSFs were very effective in treating water where simple operation was desired (Cleasby et al., 1984; Kem, 1996; Mann, 1995).

The main characteristics of SSFs that make it distinct are its slow filtration rate, lack of chemical pre-treatment requirement, no requirement of skilled labour or excessive maintenance and low costs of investment. Other distinguishing characteristics are the small effective size of the sand media, accumulation of source water bacteria and other materials on the top layer (schmutzdecke), uniform sand size at all depths, no requirement of filter backwashing

and long filter run times. These various advantages can make this system very desirable for developing countries or a water treatment unit with a low budget.

Overall, SSFs have been proved to be a very effective method in producing drinking water of good physical, bacteriological and organic quality, and also requires marginal to no chemical disinfection during post-treatment. They are able to achieve 2-4 log removal of coliforms and other pathogenic organisms (Pike, 1987).

There has been a new emerging issue in the field of disinfection which has been a cause of concern for various scientists and water researchers worldwide. Two protozoan pathogens, namely, Giardia lamblia and Cryptosordidium parvum have been posing significant challenges to the public water systems and the delivery of clean, safe drinking water to the public has been a hurdle that many regulators have been dealing with (Askenaizer, 2003). The renewed interest in the SSFs in the recent decades is also caused by its ability to sufficiently purify the water and its efficiency in removing the cysts of Giardia and Cryptosporidium enteroparasites, and additionally, it can also remove the the dissolved organic matter sufficiently, ensuring that no regrowth can occur post treatment (Campos et al., 2002) and thus, biologically stable water is produced. Further research done by Dullemont et al., 2006 also emphasises on the ability of the SSFs to remove viruses and biodegradable organic matter, by almost 80%, making it all the more appealing to the water treatment sectors.

2-2 Components of a slow sand filter

The main components of the slow sand filters are the supernatant, the filter media, the underdrainage and the control valves. A schematic representation of the different parts of the slow sand filter are shown in Figure 2. These different components together result in the proper functioning of the slow sand filter. Different components play different roles in the filter. They are explained as follows:

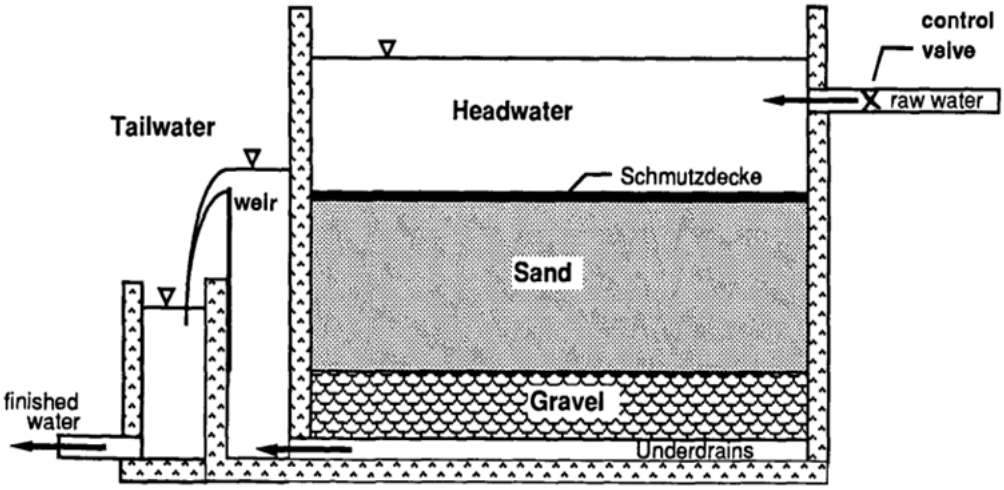

Figure 2: Elements of a slow sand filter (Barrett et al., 1991)

- **Supernatant**
  The main function of the supernatant is make sure there is a sufficient head above the filter media. This supernatant provides enough pressure on the filter enough to carry the water through the sand filter. The supernatant water is usually about 1-1.5 m in depth. This head maintained also ensures that the filter run time is also substantial. The retention period of water in the supernatant layer normally ranges from 5-15 hrs or more. During the retention period, before the water undergoes filtration,a certain amount of purification happens in the supernatant water itself. The suspended solids start to coalesce and settle down and also the breakdown of biodegradable organic matter occurs by the heterotrophic bacteria under the aerobic conditions, in the presence of the dissolved oxygen content.

- **Filter media**
  The filter media is undoubtedly the most important part of the SSF. Most of the purification happens on the sand bed and within the depth of the sand bed. The sand bed usually has varying depth, based on the raw water quality and the desired effluent quality. Normally, it is suggested that, for effective filtration, a depth of 1.2-1.4m. During cleaning and resanding, about 2-5cm of sand is skimmed out from this depth. The depth plays a big role in the treatment of the water. Studies have noted that about 30-40cm is sufficient for the removal of turbidity, whereas, about >60cm is required for the removal of viruses and oxidation of ammonia (Ellis and Wood, 1985). Hence, the sand depth will have to be decided based on the influent water quality to ensure maximum efficiency of treatment. Basically, it is important to know that the poorer the quality of the influent water being treated is, deeper the sand bed must be (Frankel, 1981).

  Two other parameters that influence the filter media are the effective size (ES) and uniformity coefficient (EC). The ES is basically the mesh diameter, expressed in millimeters of a sieve, which retains 90% (by weight) of the material and permits 10% to pass through. The recommended ES range is 0.15-0.4mm (Huisman and Wood, 1974). The UC defines the uniformity of the sand grains in the filter media. It is basically given by mesh diameter of a sieve which retains 60% of the material (by weight) of the material, divided by its ES. It is recommended that the UC always be <3 and preferably less than 2 (Kirkhoven, 1979).

  The sand used in the filter media also must be of specific characteristics. Sand is the main component of the filter media due to its inexpensiveness, easy availability, durability etc. Other materials, like diatomaceous sand can also be used (Campos et al., 2002). It should be rounded and should be free of clay, organic matter and other impurities. The ES of the sand must be selected carefully, in order to make sure the sand grains are not too fine. Sand that is too fine would surely increase the quality of purification but will add to reaching head loss much quicker. Schmutzdecke is also an important part of the filter media which develops at the top, but that will be discussed in the upcoming section.

- **Gravel media**
  Below the filter media made of sand, there is a gravel layer. The gravel layer lies between the sand filter media and the underdrainage. The main purpose of this layer is to ensure that the sand from the filter bed does not seep through and block the

underdrain. Additionally, it also ensures that the filtered water is abstracted uniformly as it passes on to the underdrainage. The gravel media consists of gravels of various sizes, with the coarsest gravel at the bottom and the finest at the top. (Huisman and Wood, 1974)

- **Underdrain**
  The underdrain is a network of pipelines that act as the drain. There are various drains that connect to the main drain line. This underdrain mainly acts as a drainage which collects the treated water that passes through the filter media. Furthermore, underdrain is also designed such that it provides additional support to the filter media. Normally, these pipelines are made of perforated cement or PVC (Ellis and Wood, 1985).

- **Flow control**
  Various control valves and weirs also play an important role in the filter. The flow control system is used for the regulation of the filtration rate through the sand bed. This is mainly necessary to avoid the raw water levels dropping too fast or to levels more that is required. The control valves are also important because they prevent any disturbances occurring to the sand media.

2-3 Scraping and ripening process

The scraping of the filter bed is an essential process that is required when the slow sand filter bed starts to undergo head loss. The filter surface gets clogged due to the accumulation of various particles, micro-organisms and the development of biomass, which gradually leads to a build-up of head loss. Scraping of the filter bed is when the schmutzdecke and the subsequent few centimetres (normally 10-40cm, depending on the filter bed) of sand below is scraped off.

When the filter is operational and it reaches resistance where the regulating valve has to be kept fully open, that is indication that the filter media needs to be cleaned (Huisman and Wood, 1974). The resistance tends to affect the filtration rate as well and causes it to decline. If not scraped off on time, the resistance can keep increasing on the filter bed. Another indicator of potential head loss is the deterioration of the effluent water quality (For example, increase in turbidity levels, coliform content).

Normally, the length of the filter run time depends on factors, namely:

- the rate of filtration
- and uniformity of the sand in the filter bed
- quality of the influent water

Post the scraping process, the filter has to undergo a ripening period, where the microbial community needs to re-develop for the filter to be fully functional again and for optimal treatment performance. During this ripening period, the filters are normally kept offline until the micro-organisms have somewhat developed. The ripening is considered one of the drawbacks of using a slow sand filter, because of the significant downtime required. Letterman and Cullen Jr., 1985 defined a ripening period as "occurring when a filter which had just been

put in operation removes particulates at a lower efficiency than removed by an identical filter which had been operating for a significant time." They conducted experiments and observed that for a scraped bed filter, the turbidity of the water was higher than for a filter that had been online for a significant period of time.

The ripening of filter bed impacts the quality of effluent water. These effects are reduced by disposing the filtered water until the desired water quality conditions are reached (Logsdon, 1991). The development of the schmutzdecke is necessary to form an effective filtering medium in the slow sand filter. In a study conducted by Cleasby et al., 1984, it was the observed that in a slow sand filter, the filtrate quality improved after the first two days of operation and the filter performance improved gradually. They also suggest a period of filtering to be wasted at the beginning of the run, in cases where the Giardia cysts are of concern.

2-4 The schmutzdecke layer and deep sand bed

After scraping, when a slow sand filter is returned to operation, the filter performance is significantly lower. At the beginning of a fresh run a layer of alluvial mud, organic waste, bacterial matter, algae etc gets accumulated on top of the sand bed. This happens due to the settlement of the water and the straining at the sand surface. This layer is known either as the filter skin or more commonly as the schmutzdecke layer (which means “dirt layer” in German). The consistency of this layer is different for each slow sand filter depending on the time of the year and the materials of which it is composed of. The schmutzdecke layer is biologically active consisting of different types of microorganisms such as algae, protozoa, bacteria, fungi, actinomycetes, planktons, diatoms, bacteriophages etc. This layer is usually known to enhance the effectiveness of the straining process in the slow sand filter (Ranjan and Prem, 2018). The thickness of schmutzdecke layer, which varies from 0.5 to 2.0 cm, starts to develop after 2 weeks of filter operation and to be physically separable, more than a month is required.

2-4-1 Biological activity in the schmutzdecke

The schmutzdecke layer is a sticky layer which contains decomposing organic matter, iron, manganese, silica etc. It mainly acts as a fine filter media and contributes to the removal of particles causing turbidity in the influent. It is an important component of the filter bed because it also doubles up as an initial zone for biological activity and aids in the degradation of some soluble organics in the influent, which are responsible for imparting taste, odour and colour to the raw water (Ranjan and Prem, 2018).

The schmutzdecke gets formed in the initial weeks after a sand bed is put into operation as a result of the establishment of a microbial community at the top-most layers of the sand bed. This community is formed as a result of slow filtration rate, the organic matter and nutrient availability in the influent required for biomass growth. It is mainly composed of organic matter, bacteria, zooplanktons, diatoms and various other microorganisms. As the influent water passes through the schmutzdecke, the foreign particles are trapped and consumed by the microbes in the layer. As the water slowly passes down through the layers of sand, impurities are left behind, leaving the water between 90% and 99% free from bacteria (M. A. Elliott

et al., 2008). The majority of the microbial community are essentially predatory bacteria which feed on the water-borne microbes passing through the filter. The microbial population is limited by the amount of organic material available or supplied by the inflowing raw water; the growth is therefore followed by an equivalent dying off (Ranjan and Prem, 2018).

The schmutzdecke is mainly responsible for the amount of organic matter that is passed onto the deeper sand layers. And eventually, all of the degradable organic matter is converted to carbon-dioxide, water and other harmless inorganic salts like sulphates, nitrates and phosphates. The bacteria oxidize some of the food to derive energy required for their metabolism and some of it goes into producing cell material for their growth (Ranjan and Prem, 2018).

2-4-2 Biological activity within the deeper sand bed

However, the biological activity within the filter bed is also very essential to the satisfactory performance of the filter bed, as shown by several studies (Collins et al., 1992; Collins, 1989; Wang et al., 1995; Collins, 1988, 1990; Weber-Shirk, 1992). Studies like Chan et al., 2018; Collins et al., 1992 have also shown that higher accumulation of biomass in the deeper sand bed has been correlated with higher removals of DOC. Studies like Collins et al., 1989; Huisman and Wood, 1974; Wang et al., 1995 have also reported that, although the upper layer of the bed contains the most concentration of the bacterial population, the bacterial activity is very active beyond the surface layer. Huisman and Wood, 1974 reported that the biologically avtive zone exists until a depth of 30-40cm, but it would also depend on the filtration rate.

A study conducted by Duncan, 1988 conducted an extensive research on the organisms present throughout the depth of the filter bed. It was concluded that the species were very dynamic and varied through the depth of the sand bed. The species reportedly changed, depending on the raw water characteristics, the filter conditions, sudden changes in filtration rate and during the cleaning process (Kem, 1996).

2-4-3 Biological mechanisms in SSFs

Although the SSFs are an essential part of the water treatment process, the biological composition and the various mechanisms happening in the filter bed are poorly understood (Haig et al., 2011). The difficulty in the collection of the representative samples of the schmutzdecke and sand material during the filter operation, along with the lack of a simple routine method to measure the microbial biomass add to the limitations of field scale investigations of the biological mechanisms in the SSFs (Graham and Collins, 2014).

A study conducted by (Campos et al., 2002) highlighted the development of microbial biomass in the sand and in the schmutzdecke layer in pilot and slow sand filters, where one of the filters was covered and the other was left uncovered. It was observed in this study that the schmutzdecke layer was highly spatially variable, whereas the biomass in the deeper sand layer was more uniform. It was speculated that, the microbial activity happening in the schmutzdecke is largely responsible for the spatial distribution of the carbon substrates within the deeper sand bed. It was also observed that the TOC and DOC removal in both the covered and uncovered beds were very similar, concluding that a small population of biomass is sufficient to remove the labile carbon in the influent water (Graham and Collins, 2014).

Ranjan and Prem, 2018 also said that the bacterial activity is most pronounced in the upper layer of the bed and decreases gradually with an increase in depth, as the food becomes more scarce. Although the bacterial activity is small over a depth of 30-40cm, various important biochemical reactions, like, conversion of amino acids into ammonia and nitrates, do take place.

In the SSF, there are certain filtration processes that occur within the schmutzdecke and the deeper sand layers or the biological zone. Huisman and Wood, 1974 reported that four processes take place that are mainly responsible for the purification of the raw water. They are as follows:

- Hostile environment: The conditions found in the SSF are unsuited for the growth of the intestinal bacteria, as they are used to the temperature of the human body, which is around 37°C and they do not thrive under temperatures below 30°C.
- Competition for food: The microbial community require food to carry out their metabolism and generation of new cells. The organisms survive on the substrates that is present in the influent water, and most often, the influent water does not contain enough organic mater to meet their nutritional needs and hence, within the upper layers, there is high competition for food. At deeper layers, food becomes scarcer so most organisms are starved.
- Predation: the predator organisms mostly thrive in the surface layer of the filter bed as they fight amongst one another and feed on other cells.
- Excretion of poisons or toxins: microorganisms in the SSF produce certain chemical or biological substances, which are harmful for the intestinal bacteria, which cannot survive under these conditions.

These combined effects create a hostile environment which is what leads to effective filtration in the sand bed. Most pathogens, like E.coli, entering the sand bed are either dead or inactivated by the time they pass through the sand bed (Ranjan and Prem, 2018).

2-5 Physio-chemical mechanisms in SSFs

Slow sand filters utilize physical, chemical, and biological processes to purify water. The biological filtration process, works in parallel with physical filtration within the same filter bed. Physico-chemical mechanisms can be divided into straining and transport (physical) and adsorption (chemical).

Straining is the main removal mechanism for particles larger than grain pore size and takes place at the sand surface and independent of the filtration rate (Huisman and Wood, 1974). The development of the schmutzdecke layer is due to straining mechanism in SSFs (Campos et al., 2002). The transport mechanism governs the transport of particulate matter to the filter media. These include sedimentation, diffusion, interception, inertial and centrifugal forces. Transport mechanism varies depending on flow rate, particle size, grain size and temperature (IVES, 1960). Adsorption mechanism governs the attachment of particles to the media.

Sedimentation occurs when the mass density of a particle is much greater than that of water and its settling velocity causes the particle to deviate from the flow path and settle on to the media surface. Interception occurs when deposited particles accumulate on the media surface, gradually reducing the pore size, and act as additional collectors for subsequently passing particles.

Adsorption of particles to the media is an important attachment mechanism. Adsorption is a process which favours removal of dissolved substances and colloidal suspensions. The success of adsorption is determined by surface forces (e.g., Van der Waals forces and electrostatic interactions) between the substance to be removed and the sand grains (Huisman and Wood, 1974). Van der Waals forces are always attractive in nature but electrostatic forces can be attractive or repulsive depending on the physicochemical conditions of the suspension. Clean quartz sand which is mostly used as the filter bed material in SSF is negatively charged, and so are the bacteria. Whereas, the metallic ions and organic matter are positively charged. Because of this a ripening period is required so that the SSFs allow for the charges in the filters to accommodate attachment of biological life (Huisman and Wood, 1974). Microorganisms such as algae and bacteria will colonize the filter bed and form a sticky zoogleal biofilm on the sand grains to which particles can become attached to.

2-6 Significance of different parameters in SSFs

The importance of various parameters chosen for analyses are enlisted below

Dissolved organic carbon (DOC)

Since full scale SSF are used as a last step of a series of treatment steps treatment steps and no chlorination is done in the drinking water supply system, it is very important to measure the DOC concentration in the effluent water to maintain the biological stability of water. DOC in finished water can increase the potential for bacterial regrowth in the distribution system. Regulations for DOC are specific to each country, with aesthetic objective in drinking water being approximately 5 mg/L (Arora, 2017).

Turbidity

Turbidity is the degree of reduction of light transmittance. More specifically, it is the measure of how light reflects from suspended particles in water - especially particles such as proteins, minerals, bacteria, algae, dirt, and oil. The cloudier the water, the higher its turbidity value, logically. However, it is not a measure of the actual particles in the water. Turbidity is caused by particles in water that may not be visible on their own but can be seen by the naked eye when they clump together. Many of these particles come from matter commonly found in the environment, such as sediment, algae, or small organisms such as phytoplankton. There are many reasons to measure the turbidity of water, but the primary reason is to measure the cleanliness of water. Turbidity was originally used in the early 1900s as a qualitative measurement to classify the aesthetic quality of drinking water. The current process is similar in that it is based on qualitative observations but includes instruments that use light scattering technology for more specific readings. Quality and aesthetics, health and compliance are just a few reasons why measuring turbidity is important.

Nitrate (NO_3)

Nitrogen is an essential nutrient for all living organisms and is found in proteins, nucleic acids, adenosine phosphates, pyridine nucleotides, and pigments. Nitrate and phosphate are the most important nutrients that are required by the micro-organisms to grow and metabolise. In this study, nitrate was performed to determine if it can determine ripening. Since nitrate is considered an essential nutrient, the consumption of nitrate is expected to have a direct influence on the biomass in the sand bed. A decrease in nitrate over time, with an increased ATP and/or ICC and HNA might be able to indicate biological activity. Various literature (Oh et al., 2018; Poghosyan et al., 2020) suggest that Comammox Nitrospira are one of the most abundant heterotrophic bacteria present in the drinking water treatment plants, especially in oligotrophic waters. Thus, it would be interesting to see how nitrate varies with depth in the filters.

Fluorescence excitation emission matrix analysis (FEEM)

FEEM is a new technique that has been gaining traction over the last decade for its ability to characterise Natural organic matter (NOM) and identify and distinguish between different types of Dissolved organic matter (DOM) in the water samples (Moradi et al., 2018). FEEM data is helpful in water samples where DOC might be problem, and it is necessary to find out which components dominate in DOC so that better maesures can be taken inorder to meet the effluent water quality.

Due to FEEM method's higher sensitivity and selectivity compared, it is increasingly used as a proxy indicator for DOC and other water quality indicators.

By measuring the excitation and emission wavelengths at which a family of components fluoresce, it is possible to represent 3D FEEMs in the form of level curves or contour maps. In this way, complex DOM can be characterized by its fluorescent components. In previous studies conducted by Dong et al., 2021 and Kulikova and Perminova, 2021, the excitation and emission wavelengths for different DOC components were found through experimentation. The wavelengths for different DOC components are mentioned in Tables 2 and 3. In this thesis work, FEEM analysis will be done to determine the composition of DOC present in the drinking water of the SSF and using the excitation and emission wavelength data given in Tables 2 and 3, the specific organic components will be identified.

Table 2: Excitation and emission wavelengths for lower molecular weight compounds (Dong et al., 2021)

Component	Excitation wavelength (nm)	Emission wavelength (nm)	Comment
Tyrosine	280	320	Protein-like compounds that are easily assimilable by the microorganisms
Tryptophan	280	350	Protein-like compounds. Microbial and algal metabolites in rivers and lakes
Amino acids	290	360	Spectrally similar to tryptophan
Phenolic substances	275	306	Benzyl compounds which contain relatively small molecular weight and are easily biodegradable

Table 3: Excitation and emission wavelengths for higher molecular weight compounds (Kulikova and Perminova, 2021)

Component	Excitation wavelength (nm)	Emission wavelength (nm)	Description
Humic acid	320-360	420 -470	They represent the dominant form of organic matter (OM) in the environment and are ubiquitous in marine, aquatic, soil, and sedimentary ecosystems, incorporating up to 94% of the total organic carbon
Fulvic acid	230-260	400-480	They come under the broad category of “Humics”. They are high molecular weight compounds, like the humic acid

Adenosine triphosphate (ATP)

The cellular ATP is a biological analysis which gives a count of the active biomass concentration present in the sand and water samples. ATP plays an important role in cell metabolism. It is present in high concentrations compared to other metabolites and is uniformly distributed in the protoplasm of microorganisms from where it may be readily extracted. Due to its high rate of turnover, ATP could be a good index of cell viability (Ochromowicz and Hoekstra, 2005). Thus it is established that cellular ATP is a good indicator of biological activity. ATP is essential in determining the cellular activity, as it gives an idea about how the biological activity varies within different depths of the sand bed. In sand samples, ATP can serve as a parameter that could assess ripening and can evaluate the biological performance over time.

Total cell counts (TCC)

The total cell counts were measured by a method called Flow cytometry (FCM). FCM refers to the analysis of suspended particles based on how they scatter light and/or fluoresce when passing through a laser beam. These particles may include bacteria, viruses, protozoa, cell fragments, inorganic debris etc (Safford and Bischel, 2019). The principle of FCM allows for individual qualitative and quantitative characterization of cells suspended in a liquid medium. It is also a quick, reliable and cost effective method, which makes it an attractive measurement for water quality analysis. It provides information related to the physiological state of the cells through the use of adapted fluorochromes. It can enable detection of contamination of drinking water in about 15 minutes (Hammes et al., 2010; Helmi et al., 2018). The HNA cells are considered to be the active cells whereas the LNA cells are considered to be inactive or dead cells. These cells are differentiated based on how they contribute to the biological activity in the water samples. The TCC quantifies the cells that are active and contribute to the production of biomass, the inactive cells that are alive but do not contribute to the production of biomass, intact cells which might be dormant at the time of sampling but might contain potential activity and the dead cells which are only considered to be organic particles (Lebaron et al., 2001).

Chapter 3

Materials and methodology

The drinking water treatment process of Dunea consists of a number of successive treatment steps in order to produce safe drinking water that is in accordance with the Dutch drinking water quality standards. A schematic outline of the treatment steps is shown in Figure 3.

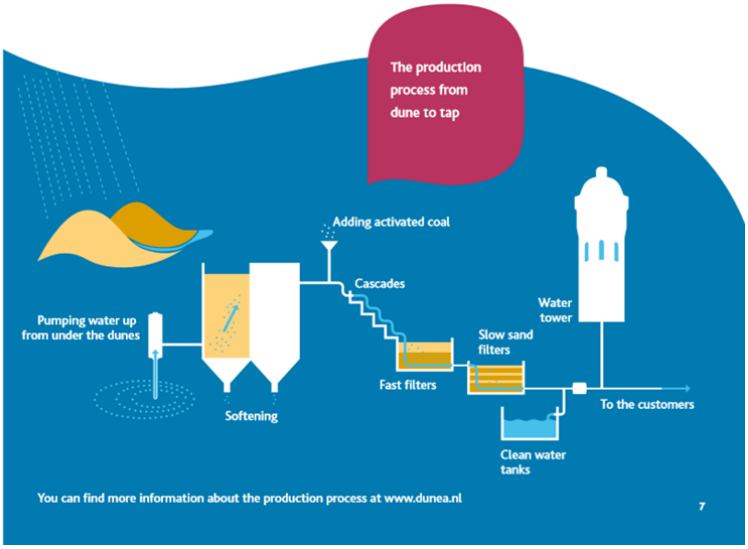

Figure 3: Dunea water treatment plant

The water is withdrawn from the river of Afgedamde Maas, a side branch of the River Meuse. After abstraction, the water is made to undergo some pre-treatment steps, which include sieving and Rapid sand filtration. For sieving, micro sieves of 45μm are used. After sieving, the water is made to go through the RSF, where the filtration rate is maintained at 3.5m/h. It is then sent through two large pipelines to undergo dune filtration. The dune filtration is mainly used for storage of water, and is also very efficient in eliminating micro-organisms and pathogens from the water. There are 3 main dune locations - Berkheide, Meijendel and Solleveld.

This water is pumped into the dunes using a system of open infiltration ponds. One part of the water is used for infiltration of the deeper aquifer using deep wells so as to replenish them. An average of 30-60 days retention time is allowed in the soil, after which it is extracted out to undergo post-treatment steps.

After dune filtration, the water is treated in a series of post-treatment steps. There are 3 locations where post-treatment happens, namely, Katwijk, Monster and Scheveningen. These steps include softening with dosing of sodium hydroxide (NaOH), treatment by dosing Powdered activated carbon (PAC), aeration using cascades, dual media rapid sand filtration where the filtration is maintained at 4-5m/h and slow sand filtration where the filtration rate is maintained at 30-45cm/h as the last treatment step. These filter steps are essential to make sure the water is safe for consumption. The slow sand filters, used as the last treatment unit serves as a good disinfection step, which produces microbiologically safe and biologically stable water with minimum re-growth potential and also eliminates the need to dose chlorine and other chemicals.

Subsequently, the water is usually stored in clean water reservoir tanks, after which it is then delivered to customers through the distribution network.

3-1 Water quality of the influent (after Dune extraction), post RSF treatment and post SSF treatment

Table 4: Water quality standards after abstraction from the dunes, the water quality after rapid sand filtration and the final water quality after treatment with slow sand filtration

Parameter	Unit	Post Dune Abstraction	Post RSF	Post SSF
Iron	µg/l	429.8	9.4	2.2
Manganese	µg/l	94.8	0.3	0.0
DO	mg/l	-	-	9.8
TCC (Total)	cells/mL	4.1E+05	3.3E+05	2.0E+05
TCC HNA	cells/mL	9.5E+04	5.2E+04	1.8E+04
TCC LNA	cells/mL	3.2E+05	2.8E+05	1.8E+05
TCC (Alive)	cells/mL	3.2E+05	2.7E+05	1.6E+05
TCC HNA	cells/mL	6.4E+04	3.9E+04	1.3E+04
TCC LNA	cells/mL	2.6E+05	2.3E+05	1.5E+05
ATP	ng/L	61.8	6.2	1.5
Ammonium	mg/L N	0.3	0.0	0.0
Nitrate	mg/L N	0.8	1.1	1.1
Nitrite	mg/L N	0.0	0.0	0.0
Calcium	mg/L Ca	68.4	43.2	43.1
Magnesium	mg/L Mg	8.3	N/A	8.2
HCO_3	mg/L HCO_3	206.9	177.9	177.9
pH	pH	7.7	8.5	8.5
DOC	mg/L C	2.4	#N/A	2.1
AOC	µg/L C	#N/A	5.5	3.7

Table 4 is the average value that is expected for the water quality during the various treatment steps. All the slow sand filters in Dunea are expected to have similar values in the effluent.

3-2 Filter characteristics

For sampling and analysis, two SSFs were used, one was a scraped sand bed and the other was a mature sand bed. The filter specifications are as given in Table 5.

Table 5: Filtering specifications of the scraped bed and mature bed

Characteristics		Scraped bed	Mature bed
Filter run time (months)*		68	56
Filtration rate (m/h)		0.26	0.26
Effective sand size (mm)	d10	0.4	0.43
	d50	0.63	0.65
	d90	1.32	1.35
Supernatant water level (m)		1	1
Area of the filter (m^2)		2383	2359

**Note: The filter run time for the scraped bed is the number of months the filter was in operation before it was scraped. For the mature bed, it shows the number of months the bed has been in operation since it was last scraped.*

3-2-1 Scraping of the filter bed

The sand bed usually undergoes cleaning or "scraping" wherein, the filter is cleaned by removing the schmutzdecke layer. At Dunea, the filters are cleaned once the sand beds show head loss. They are normally cleaned by removing the top 10 cm of the sand bed, along with the Schmutzdecke. In literature, usually 2 cm of the top sand layer is reported sufficient for removal during scraping (Campos et al., 2002).

The efficiency of the filter is determined by measuring turbidity and E.Coli in the effluent water. E.Coli should be 0 CFU/100 ml and turbidity levels less than 0.1NTU. Current SSF's are expected to have DEC of 3 log for bacteria and 1 log for viruses.

Figure 4 was taken at Dunea when the sand bed was being scraped, Figure 5 is the mature bed that was also sampled simultaneously, Figure 6 shows the scraped and mature beds that were sampled during the study in parallel.

Figure 4: Scraping of a slow sand filter (scraped bed)

Figure 5: Slow sand filter still in production (mature bed)

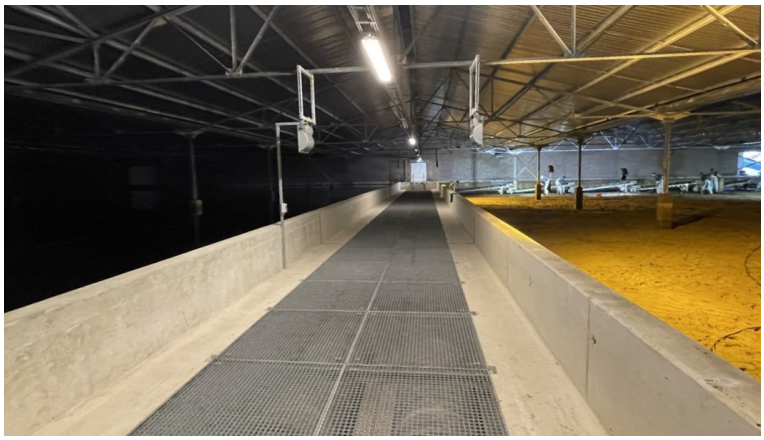

Figure 6: Mature bed and scraped bed in parallel

3-2-2 Sampling and transportation

The sampling was initially done weekly for the scraped bed and once in 2-3 weeks for the mature bed. Water and sand samples were taken from both the filter beds.

The water samples were taken from the sampling points that have been installed by Dunea. Sampling ports were installed near the side wall of the filter, these pipes go into the filter bed, and are about 50-60cm long. They were used to sample water from different depths. The sampling ports were installed at different depths, starting from the supernatant and were consecutively installed 10cm apart from each other, at 8cm, 18cm and so on, until 78cm. The sampling ports at Dunea is shown in Figure 7.

Figure 7: Sampling points at Dunea

The various samples taken and the parameters measured are shown in Figure 8. For the spatial distribution, the water samples were collected in duplicates using 180mL sampling vials from both the scraped and the mature beds. The sampling points (mentioned above)

were used to collect water samples from the supernatant, 8cm, 18cm, 28cm, 38cm and 48cm. Figure 9 shows a rough outline of the bed along with the different sampling points.

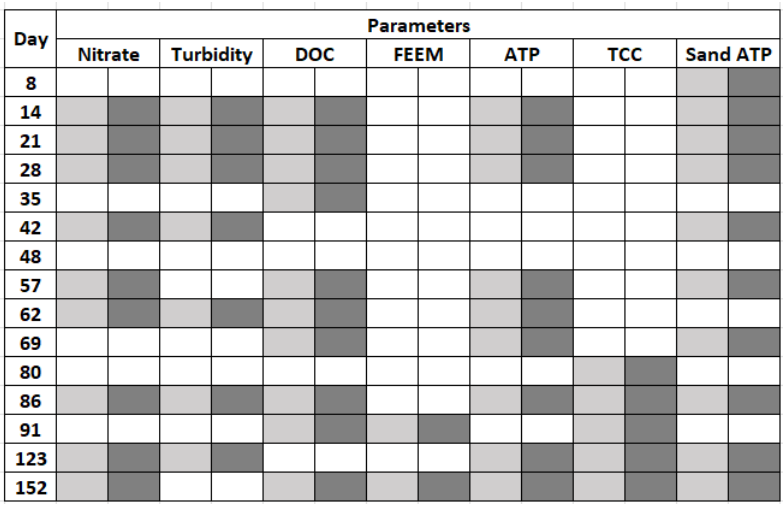

Figure 8: The various parameters and the dates of sample collection for the scraped and mature beds. The scraped bed has been represented by light grey and the mature bed has been represented by dark grey

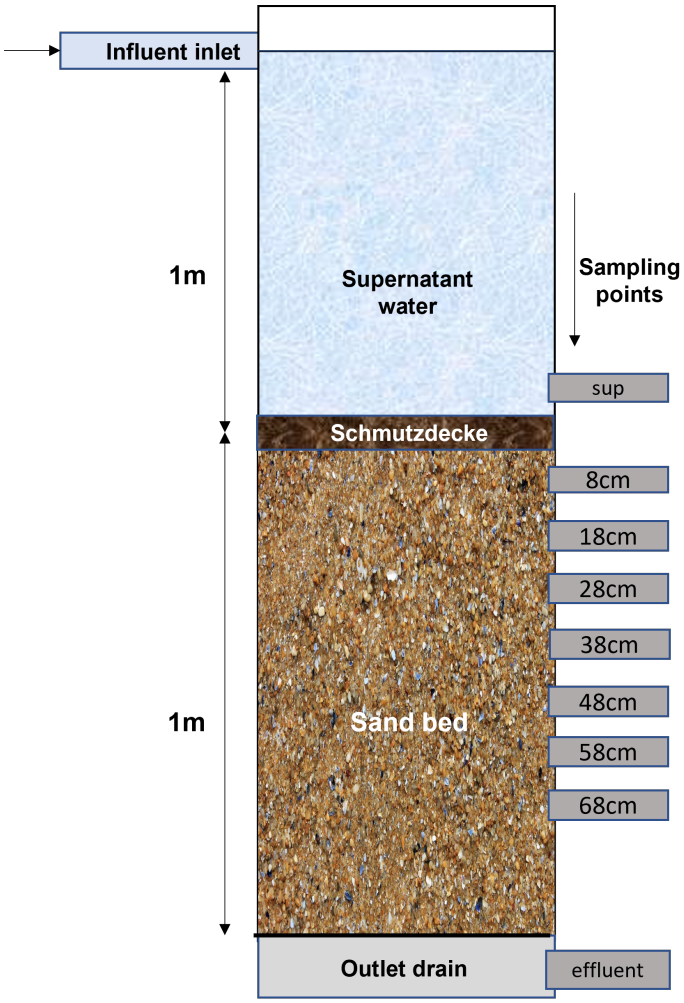

Figure 9: Schematic diagram of the different sampling points in the slow sand filter

The sampling points had taps which could be opened out to sample the water. The taps were normally left open 10-15 minutes before samples were taken to avoid collecting stagnated water. The samples to be measured for Total cell counts (TCC) were measured in separate sampling vials and sent to the Het Waterlab for analysis.

The sand samples were scraped from the top of the sand bed (mostly the schmutzdecke) using a sterilised sand corer. They were collected also in duplicates in 15mL sampling vials.

The samples were transported to the TUDelft Waterlab in bags containing icepacks and with as minimal interaction with the outer surroundings and sunlight as possible. The ATP, TCC and DOC tests were performed within 24 hours. The Nitrate and turbidity tests were done usually within 48 hours. Samples were also collected for Fluorescence Emission Excitation Matrix method (FEEM), which was done at IHE institute. FEEM samples were measured within 1-2 weeks after sampling.

3-3 Analyses

The physico-chemical analyses done include nitrate, turbidity, DOC and FEEM, and biological analyses include ATP and total cell counts.

3-3-1 Physio-chemical analyses

Dissolved organic carbon (DOC)

The full scale SSFs are used as a last treatment step and thus, it is very important to measure the DOC concentration in the effluent water in order to maintain the biological stability of water. DOC presence in treated water can increase the bacterial regrowth potential in the distribution system. Regulations for DOC are specific to each country. For aesthetic purposes, the drinking water standard for DOC is approximately <5 mg/L.

The DOC analysis was done using TOC-V CPH Shimadzu Analyser. The instrument mainly measures the total carbon (TC), inorganic carbon (IC) and calculates total organic carbon (TOC) in the water samples. Oxidative combustion-infrared analysis is the method used for the TOC measurement.

The samples for the DOC were prepared in the following manner. They were made to first pass through the 0.45um Whatman Spartan syringe filters (30/0.45RC rinse filter). And then, 1.6mL of 2M Hydrochloric acid is added to the samples. The sample vials are then sealed tightly using an aluminium foil and cap.

Standards and blanks were also prepared for the measurement. The number of blanks depends on the number of samples to be measured. For the weekly analyses, one blank was prepared for every 6-7 samples. The blanks were prepared by adding 30mL ultrapure water into the vials and adding 1.6mL of 2M HCl. The standard was prepared by using the pre-made standard stored in the refrigerator in a similar manner as the rest of the samples. The standard and blanks were also sealed using aluminium foil and cap.

After performing routine checks of the analyser, the samples, standard and blanks were places in the analyser. The analyser normally takes about over 5-6 hours for the measurement of about 13 samples. The standard value should also be checked to ensure that it falls close to 10mg/L and the blanks should be around 0.2mg/L.

Turbidity

In this study, turbdity was measured as a possible indicator to define whether the filter is ripening (in comparison with the mature bed). Turbidity is also considered an important water quality parameter. High levels of turbidity in water samples can protect microorganisms from the effects of disinfection and stimulate the growth of bacteria. No health-based guideline value for turbidity has been proposed: however, according to World Health Organization, 1970, turbidity should be mainted below 10 NTU for effective disinfection.

The turbidity meter was first calibrated using stabilised formazin standards of 0.01 NTU, 1 NTU, 20NTU and 100NTU every time before measuring the sample. The turbidity measurements were done in duplicates to avoid the errors in measurement (Hach, 2022b).

Nitrate

Nitrate was also measured as a possible indicator for ripening. Nitrate is an essential nutrient that is required by micro-organisms to grow and metabolise. Consumption of nitrate in the scraped bed over time could indicate ripening. Nitrate is measured in the water samples using the cuvette test (LCK339), using 2,6-dimethylphenol. This kit has a measuring range of 1-60mg/L (Hach, 2022a).

Fluorescence emission excitation matrix (FEEM)

FEEM analysis was done in supplement to the DOC analysis to determine the DOC composition in the water samples at different depths. FEEM results would give a better qualitative and quantitative characterisation of DOC, which would help in analysing how the DOC is being consumed at different depths.

For the preparation of the samples, the water samples were made to pass through a filter of 0.45um to filter in the dissolved organic carbon and they were collected in 15 mL sampling vials and filled up to the brim to avoid the formation of air bubbles. The samples were then stored in a refrigerator at 3 degrees Celsius until they were measured. The samples were usually measured within 2 weeks post refrigeration. The FEEM analysis was done at the IHE Institute for Water Education.

3-3-2 Biological analyses

Adenosine triphosphate

ATP is an important biological analysis which can be used as a potential indicator of ripening. Cellular ATP (or cATP) counts are capable of determine quantitatively how much active biomass is present in the water and sand samples. The ATP test, both for the water and sand samples was performed using the Lumina Ultra test kits. Both the measurement of sand and water samples are similar but with some minor differences which will be elucidated below. The ATP kit used was to measure the cellular ATP (cATP).

The ATP measurement occurs with the reaction between the substrate Luciferin and the enzyme Luciferase, which occurs naturally in the tails of fireflies. The reaction between these 2 substances produces light which is detected in a luminometer as Relative Light units (RLU). The reaction is as given below:

$$\text{Luciferin} + \text{Luciferase} \xrightarrow[\text{ATP}]{\text{Mg}^{2+}} \text{Luciferin}-\text{Luciferase}-\text{AMP} + \text{Pyrophospahte}$$

$$\text{Luciferin}-\text{Luciferase}-\text{AMP} \longrightarrow \text{Oxyluciferin} + \text{Luciferase} + \text{CO}_2 + \text{AMP} + \text{Light}$$

AMP=Adenosinemonophosphate

Under optimum conditions, 1 light photon per molecule of ATP is produced. The measurement is made in duplicates and the average value is used in calculation. The RLU value is then converted into biomass carbon concentration and represented in pg/mLwater or ng/g sand.

ATP standard calibration

In a 12*55mm test tube, two reagents, 100μL of Ultracheck and 100μL of Luminase are added. The test tube is swirled gently 5 times and immediately inserted in a luminometer to measure the value, given in relative light units (RLU). If the RLUstd is greater than or equal to 5000, a new luminase reagent needs to be rehydrated and the calibration needs to be repeated to obtain an appropriate value.

ATP water and sand measurement

Usually for the ATP measurement of drinking and sanitary water, 50-100mL of sample is recommended. 60mL of water sample was used for every measurement for the study. The samples are measured in duplicates to avoid errors in measurement. The water sample is filtered through a 0.45um filter at a rate of 3-5mL/s. The sample water is then discarded. Post this, the filter and the plunger of the syringe are removed. The filter is re-attached and 1mL of ultralyse solution is added into the syringe barrel and this solution is pushed through the syringe and collected in a 9mL Ultralute tube. After this the solution is mixed thoroughly. For the assay, 100μL of Ultralute solution is transferred into a 12*55mm test tube. To this, 100μL of Luminase enzyme is added and the mixture is gently swirled 5 times and then immediately inserted into the luminometer to measure the value in RLU. The calculation to convert the RLU value to the actual ATP concentration (pgATP/mL) is given as:

$$\text{cATP (pgATP/mL)} = \text{RLU}_{\text{sam}}/\text{RLU}_{\text{std}} * (10,000)/\text{V}_{\text{sam}} \text{ (mL)}$$

cATP stands for cellular ATP, RLU_{sam} is the RLU value of the sample, RLU_{std} is the calibration value, V_{sam} is the volume of sample taken (60mL in this case) and the factor 10,000 is the dilution factor.

For measuring sand samples, the collected sample is first centrifuged at 10,000rpm for 5 mins to separate out the sand and schmutzdecke particles from the supernatant water. After this, the supernatant water is carefully discarded and the solids are properly mixed. For the sample preparation, 1g of the solids is weighed in an aluminium dish and then added to a 15mL sampling vial. 5mL ultralyse 7 reagent is then added to it. The mixture is then mixed thoroughly. 5 minutes of incubation time is allowed. Post this, 1mL of the ultralyse solution is transferred to a 9mL ultralute tube and the solution is mixed vigorously. For the assay, 100μL of solution from the Ultralute tube and 100μL of luminase is transferred to a 12*55mm test tube and swirled gently 5 times. The solution is then immediately measured in the luminometer to obtain the RLU value. The calculation to convert the RLU values to total ATP (tATP) is given below:

$$\text{tATP (pgATP/mL)} = \text{RLU}_{\text{sam}}/\text{RLU}_{\text{std}} * (50,000)/\text{M}_{\text{sam}} \text{ (g)}$$

tATP is the total ATP which measures the ATP of both the living and the dead cells within the sand deposit. RLUsam is the RLU value of the sample, RLUstd is the calibration value and Msam is the mass of sample taken (1g in this case) and 50,000 is the dilution factor. To convert the value to ng, the value is to be divided by 1000.

Total cell count (TCC)

Total cell count is done using the method called Flow Cytometry (FCM). FCM method is a good measure to use to determine the quantitative relationship between cells with low and high nucleic acid (LNA and HNA) content in the water samples. The HNA cells are regarded as the more active while LNA are considered to be inactive, dead or dormant cells (Antonel et al., 2007). The total cell count is an important parameter because it can give a measure of the actively growing cells that contribute to the production of biomass, the living but inactive cells which do not participate in bacterial production at the time of sampling but have potential activity (often called dormant cells), and the dead and inactive cells that should be considered only organic particles (Lebaron et al., 2001).

The cell components are fluorescently labelled and excited by the laser to emit light at different wavelengths. The total and living cells are identified by straining the cells with fluorochromes. SYBR Green 1 and Propidium iodide are used to distinguish between the living and the dead cells.

- SYBR Green 1: This fluorochrome is capable of penetrating through the cell wall and binds to the DNA of the cell. And when irradiated (excitation 488nm), this fluorochrome emits light and this emission would fall within the spectrum of wavelength 475-700nm.
- Propidium Iodide: This fluorochrome can penetrate through ruptured cell walls and can bind to its DNA. When irradiated (excitation 488nm), it emits light, which falls within the spectral range of 550-700nm.

For TCC measurements, the water samples were collected in airtight and sterilised 100mL vials and sent to Het Water Laboratory in Haarlem for analyses.

Chapter 4

Results

Sand sampling

4-1 tATP analysis of Schmutzdecke - sand samples

The sand samples were analysed for ATP and the results obtained are as shown in Figure 10. It can be seen that the scraped bed shows an increase in the ATP concentration over time. On day 8, which is a week after the scraping was done, the ATP concentration in the sand sample for the scraped bed was 53.3 ngATP/g sand, whereas for the mature bed, the ATP concentration was 190 ngATP/g sand. Over a period of 144 days, the ATP in the scraped bed increased to 189 ngATP/g sand, which is a 72% increment.

It is interesting to observe that, the highest increase observed was 40% from day 21 to 42. After day 42, the ATP increases further until day 152.

The mature bed, on an average, had an ATP concentration of 236 ngATP/g sand (throughout the sampling period). There are slight variations in the initial samples because the location of sampling was changed.

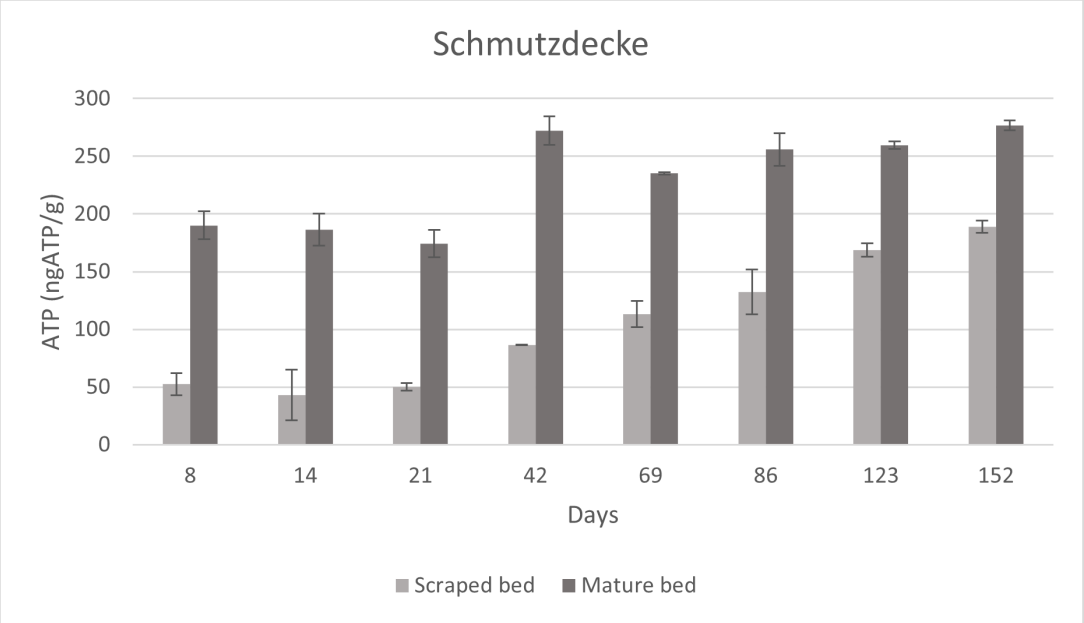

Figure 10: ATP in Schmutzdecke

The schmutzdecke development can be observed on the sand bed as the reddish-brown layer starts to develop on the sand bed. The scraped bed, without the schmutzdecke contains very low biomass and subsequently lower biological activity. From Figures 11 and 12, which were sampled 55 days apart, it can be observed that over time, the scraped bed's sand sample starts to resemble the mature bed aesthetically.

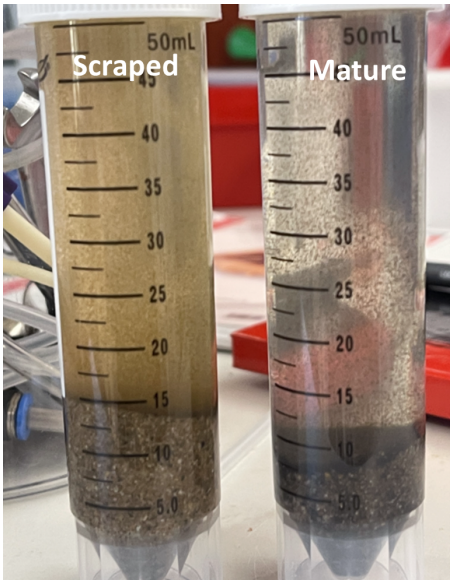

Figure 11: Sand samples taken on day 14

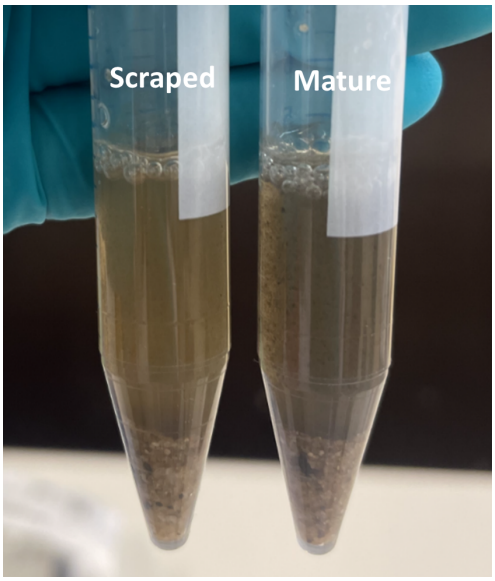

Figure 12: Sand samples taken on day 69

Water sampling

The results for the water samples have been segregated into 2 categories - temporal variation and depth variation. The temporal variation shows how the scraped bed samples vary from the time of scraping until five months later. For comparison, the mature bed samples were also analysed simultaneously. The depth variation shows how the parameters behave through the depth of the filter beds over time. Within these 2 categories, they are further divided into physico-chemical parameters and biological parameters.

4-2 Part 1: Temporal variation

4-2-1 Physico-chemical parameters

Nitrate

Figure 13 shows the nitrate concentration in the influent versus the effluent for the scraped and mature beds. For the scraped bed, nitrate concentration in the influent and the effluent were close until day 60. Post this, there is a clear decrease in the effluent concentration compared to the influent concentration. From day 0 until day 57, the removal in the scraped bed ranged from 0.7-2.6%. After this period, the average removal was about 8.3%, with the highest removal being 11% on day 152.

The mature bed (Figure 14) showed a consistent nitrate removal throughout the days. The effluent concentration was always lower than the influent for all days. The removal percentage was in the range of 12-18%, with the highest removal being 18.4% on day 86.

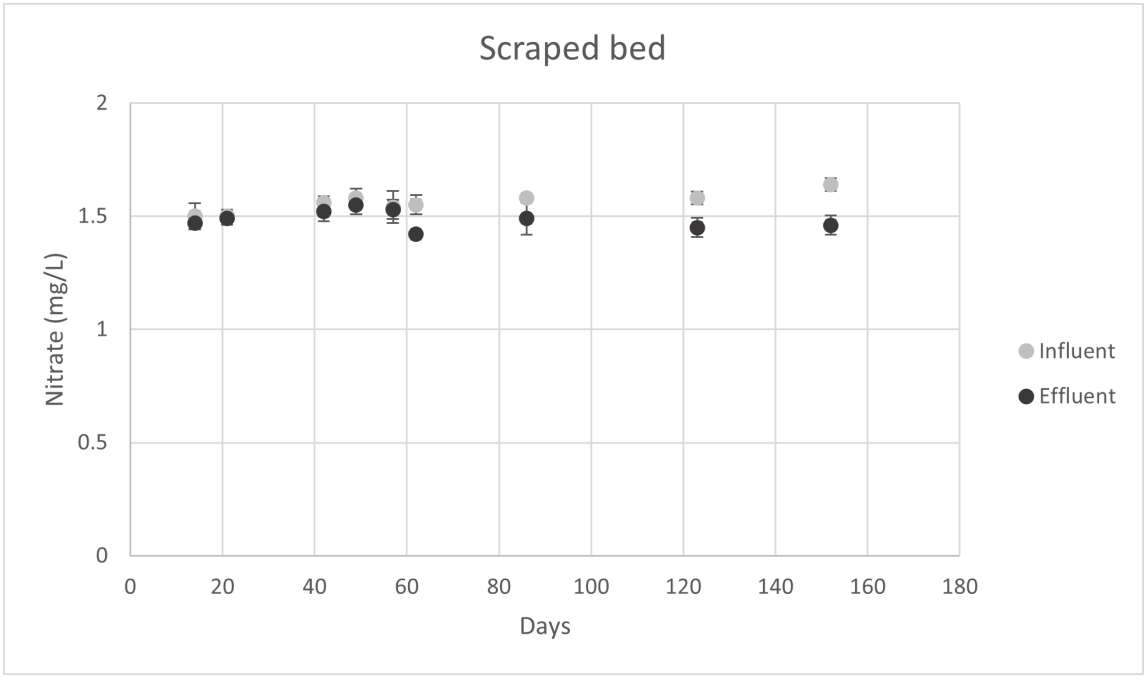

Figure 13: Nitrate concentration for the scraped bed

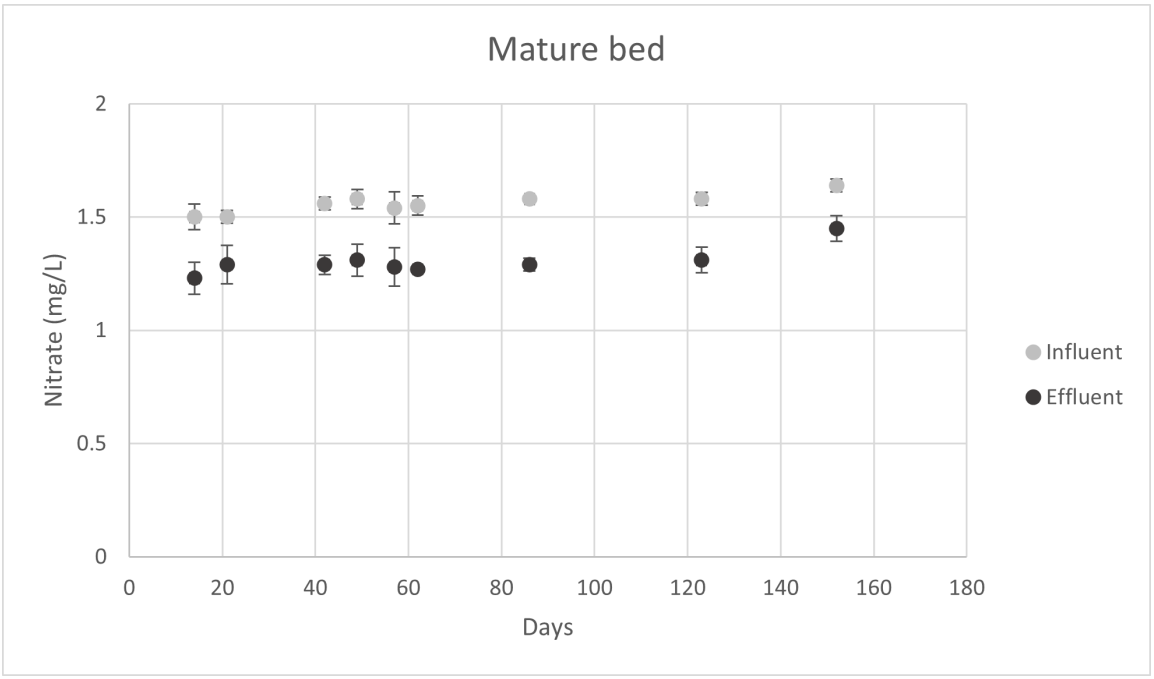

Figure 14: Nitrate concentration for the mature bed

Turbidity

Turbidity trend for the scraped bed is very similar to that of nitrate. The turbidity in the effluent starts to decrease for the scraped bed after day 42. Until day 42, negative percentage removal was observed in the effluent. But on day 49 onwards, there was turbidity removal in the effluent. On day 42, there was -1.8% removal and it increases to 5.3% on day 49. After day 49, positive removal was observed till the last sampling day. The highest removal being on day 123 at 20%, which is a significant increase from the first day of sampling, on day 14, where the removal was -2.4%. This can be observed in Figure 15.

The mature bed, on the other hand, shows nearly consistent removal. The removal percentage ranging from 9.6% on day 21 to 20% on day 152. This can be observed in Figure 16. The average turbidity removal throughout the sampling period was around 13%.

It can be observed that on day 123, the scraped bed's turbidity removal was 19.9% which is higher than the mature bed as well, which was 14.4%.

The effluent turbidity varies based on the influent. A correlation study was conducted, where the R^2 values obtained were 0.76 for the scraped bed and 0.87 for the mature bed respectively.

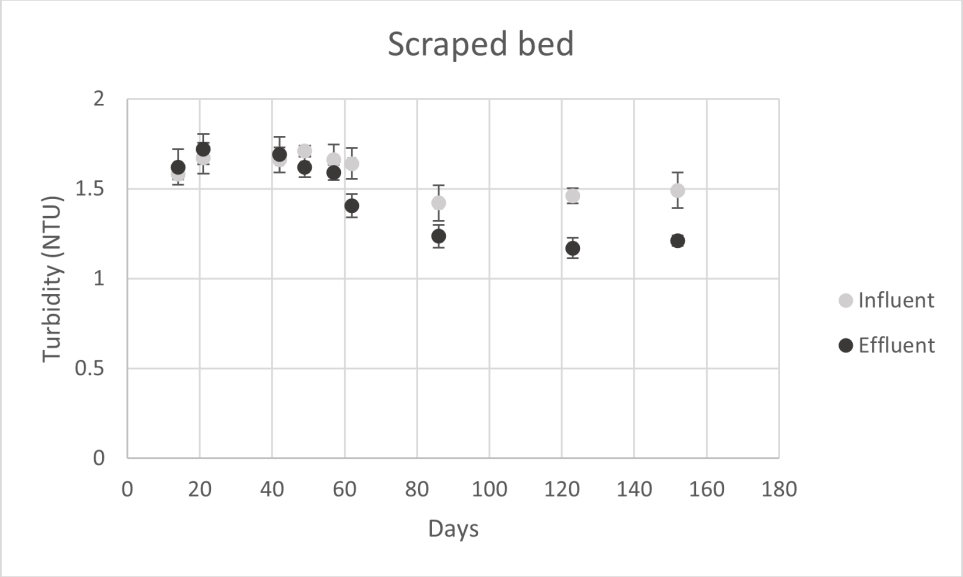

Figure 15: Turbidity for the scraped bed

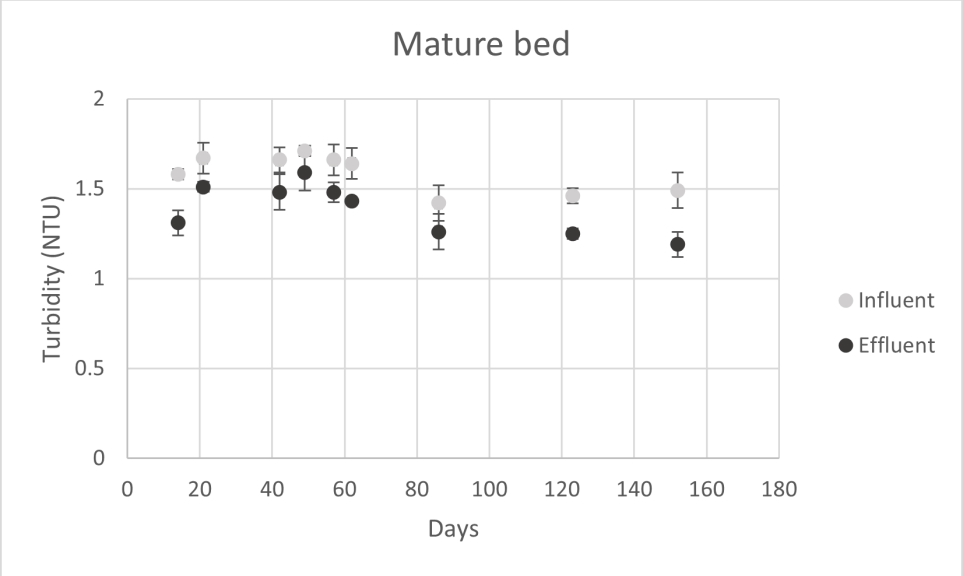

Figure 16: Turbidity for the mature bed

Dissolved organic carbon (DOC)

The DOC concentration of the effluent for both beds are very similar as seen in Figures 17 and 18. The DOC concentration in the influent shows fluctuations through time and the effluent concentrations vary with respect to it.

For the scraped bed, on day 14, the DOC concentration of the effluent was much lower, with the removal percentage being 26.9% as compared to the mature bed, which had a 25.5% removal. The DOC concentration of the effluent for the scraped bed starts to resemble that of the mature bed from day 49 onwards. The average DOC removal percentage reported for the scraped bed was 10% and 12% for the mature bed.

A t-test was conducted to analyse the similarity of the effluent concentrations for the scraped vs mature bed and the p value was determined as 0.84, which further indicates that both the beds have similar removal capacity.

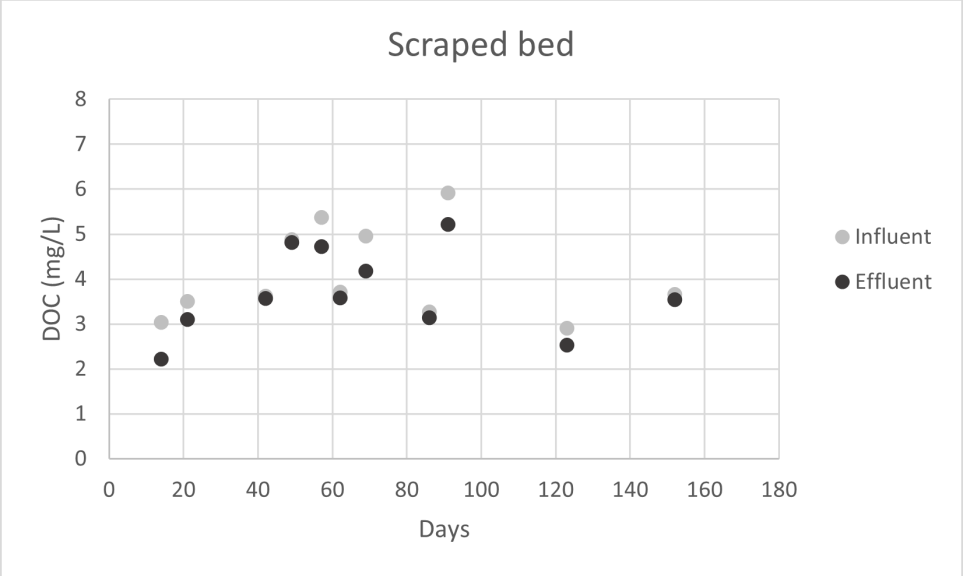

Figure 17: DOC for the scraped bed

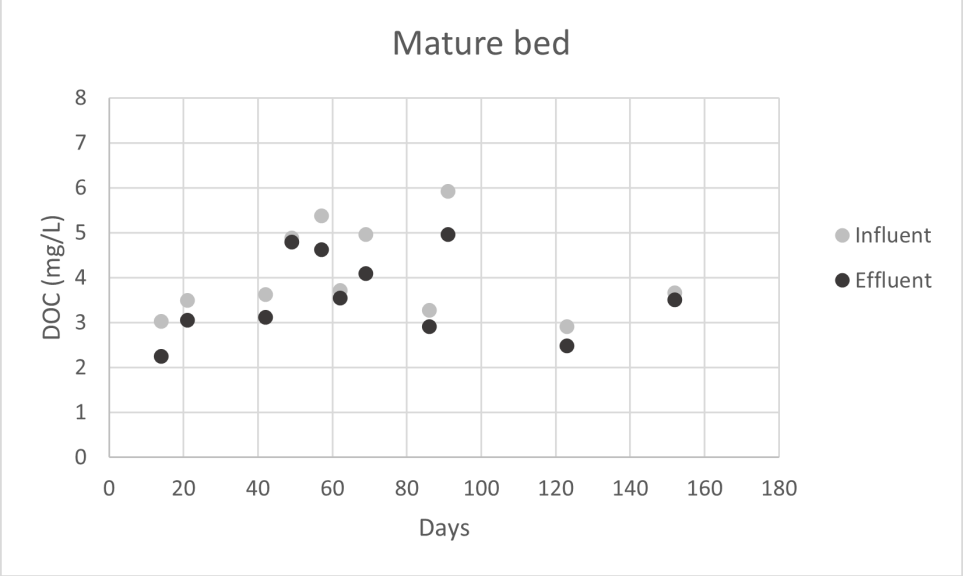

Figure 18: DOC for the mature bed

Adenosine triphosphate (ATP)

The scraped and the mature beds both show similar trends for ATP. It is evident from Figures 19 and 20 that both beds have ATP removal in the effluents. For the scraped bed, the lowest ATP removal was reported at 42% on day 86 and the highest removal was reported at 80% on day 14. The average ATP removal for the scraped bed was reported to be 61%.

For the mature bed, the lowest ATP reported was 58% on day 123 and the highest was 81% on day 21. The average removal was 74%.

The ATP in the effluent varies greatly with the influent ATP. When the ATP in the influent is high, the effluent ATP also is high, for both the beds. Both beds had very high influent-effluent ATP correlation. The scraped and mature beds had correlation factors of 0.74 and 0.65 respectively. However, when the ATP in the influent exceeds a certain limit, the bed might be unable to meet the consistent removal capacity. It can be seen in Figure 19 for the scraped bed, where, on day 86, the influent had the highest ATP of 7.8pg/L and the removal was only 42%, whereas, on day 14, the ATP concentration in the influent was 4.64 and the removal was the highest (80%).

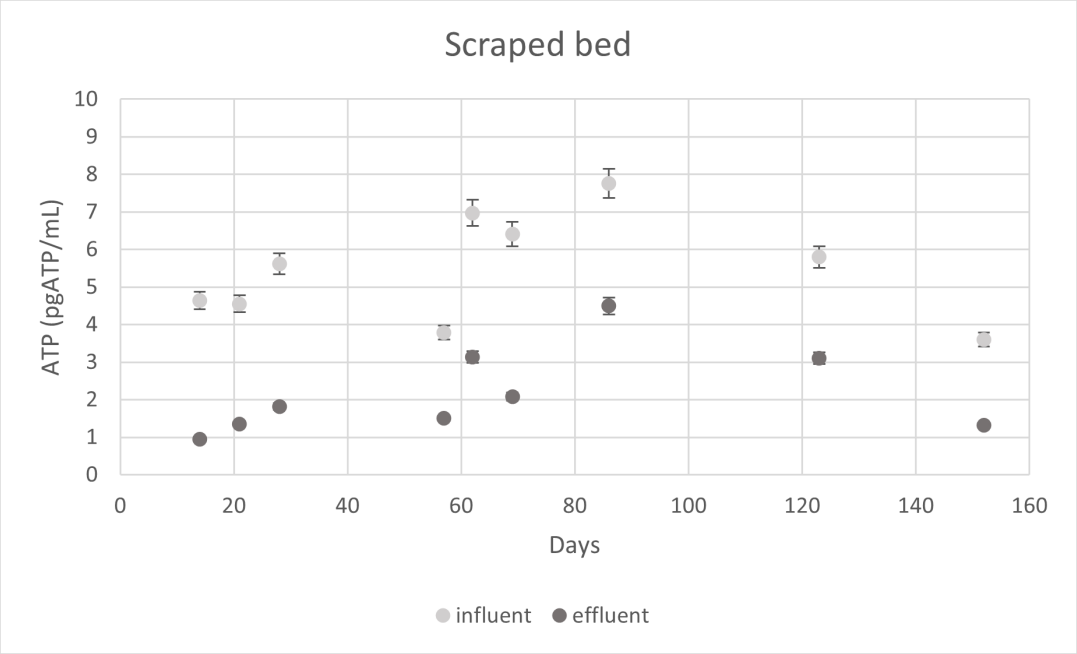

Figure 19: ATP for the scraped bed

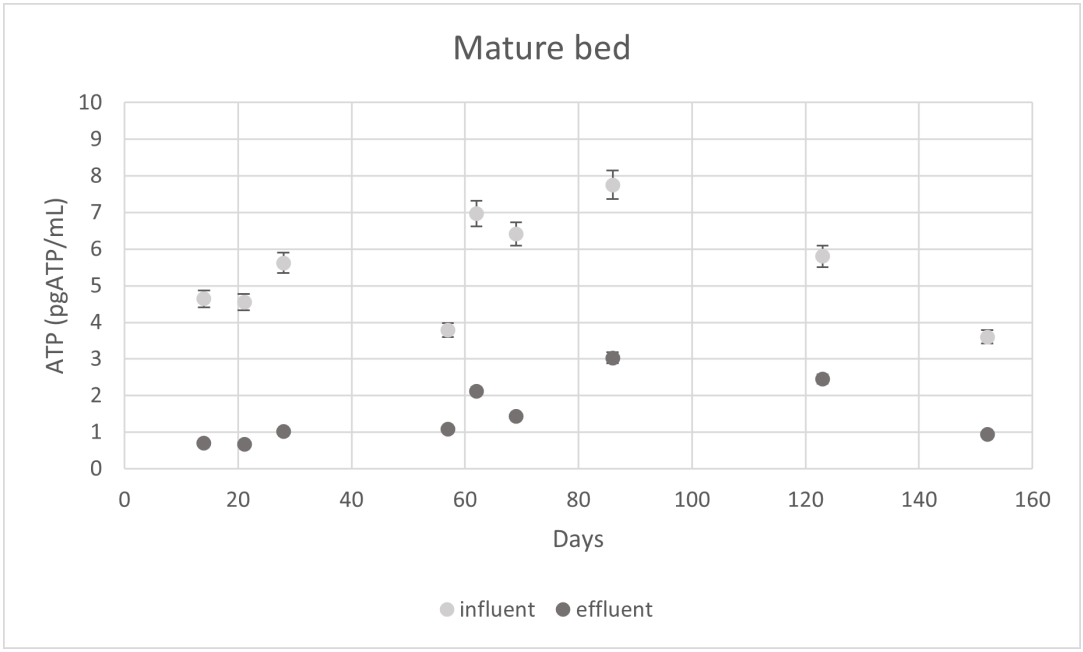

Figure 20: ATP for the mature bed

4-3 Part 2: Depth variation

4-3-1 Nitrate trends in deeper sand bed

The nitrate trend for the scraped bed is similar throughout the days for both beds. The scraped bed, shown in Figure 21, has a decreasing trend until 18cm. At 48cm, an increasing trend can be observed, and it decreases again at the effluent. For all days, from the influent to the supernatant, on an average, a decrease of 13% was observed, and it falls to an average of 3.5% and 1.9% at 8cm and 18cm respectively. At 48 cm, however, the nitrate concentration increases, at an average of 20% before decreasing to 3% in the effluent.

The mature bed also shows a similar trend as the scraped bed, with a decrease in nitrate until 18cm, and then an increase at 48cm. The decrease until 18cm is more prominent than that of the scraped bed, which can be observed in Figure 22. There is an average increase of about 23% at 48cm, followed by a drop at the effluent. A t-test was performed for different depths for different days to determine whether the beds behaved similarly, shown in Appendix -4 in the Appendix. The result indicated that the beds were dissimilar on day 14 but behaved similarly for days 42 - 123.

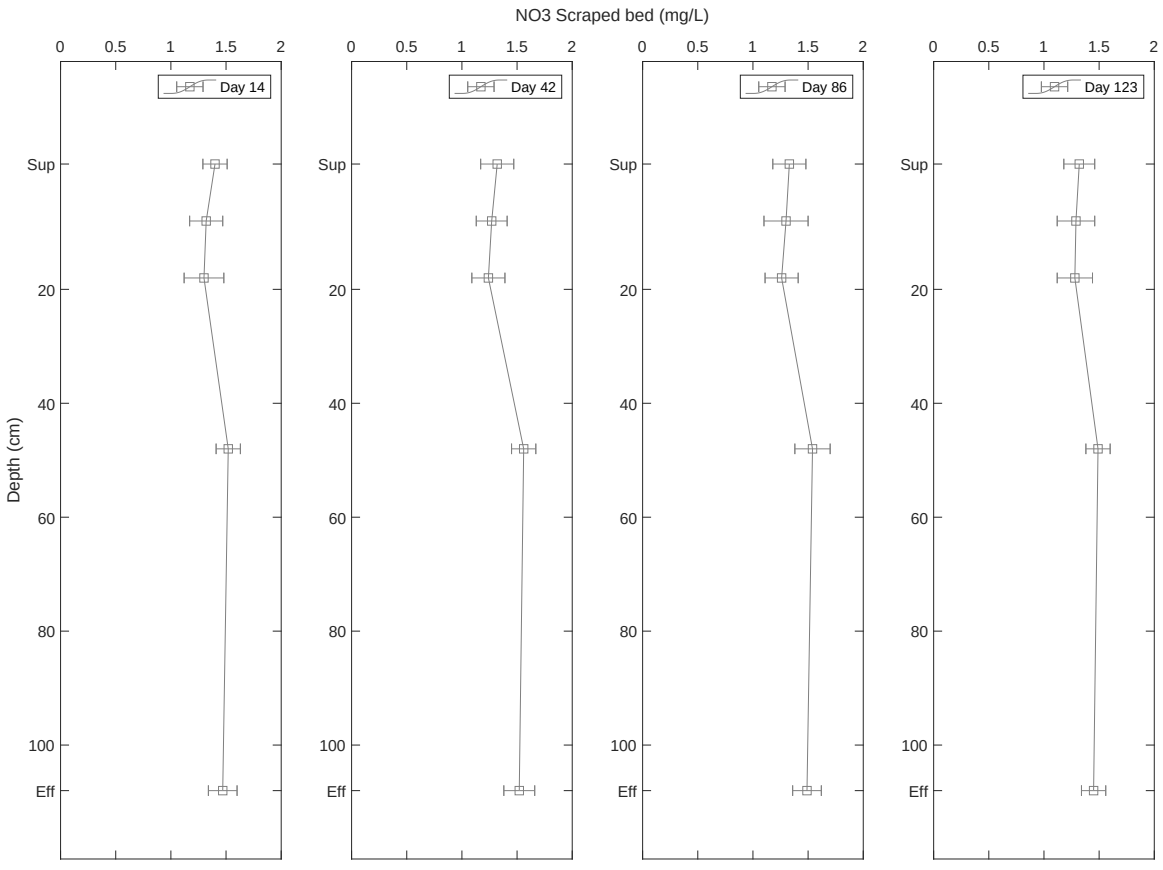

Figure 21: Nitrate depth trend for the scraped bed

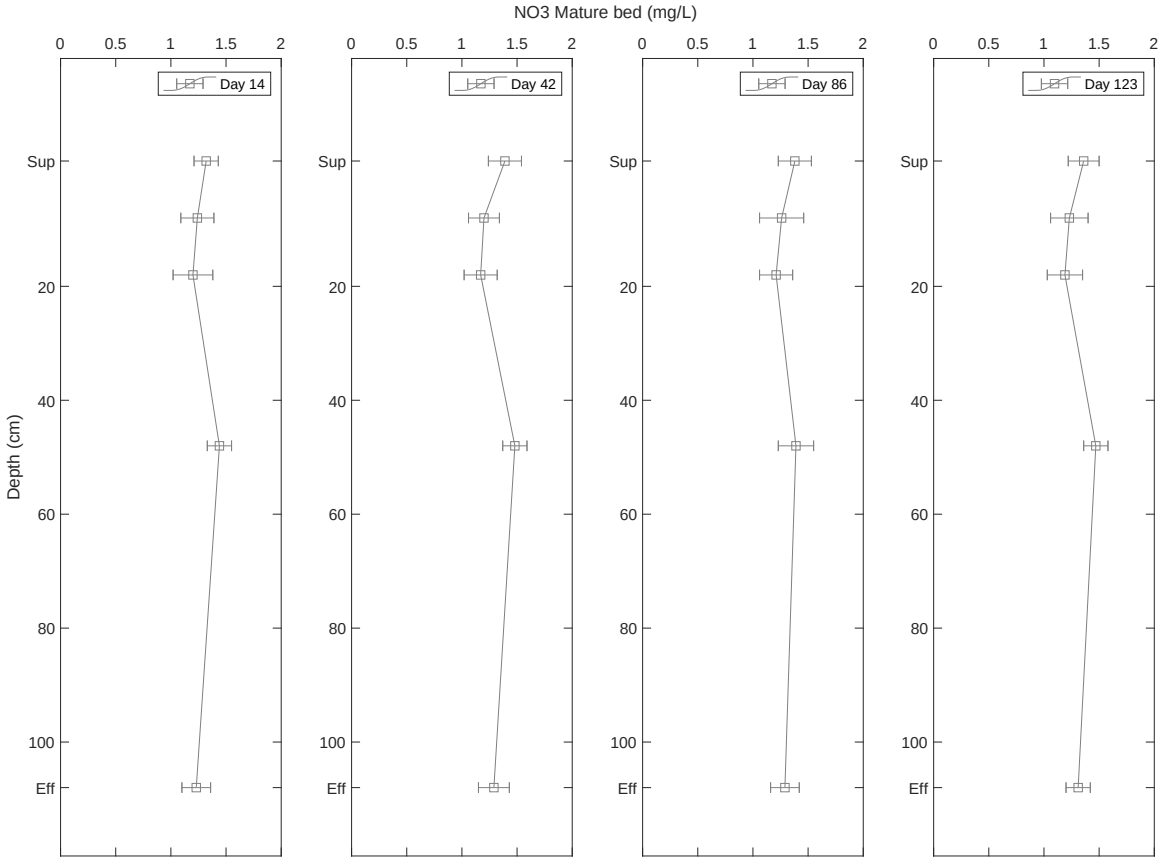

Figure 22: Nitrate depth trend for the mature bed

4-3-2 Turbidity removal in different depths

The mature bed has a more stable turbidity removal compared to the scraped bed, whereas the turbidity in the scraped bed fluctuated throughout the depths for different days.

It can be observed in Figure 23 that the turbidity concentration in the scraped bed reduces at 8 cm and then slightly increases at 18 cm. A further decrease is seen at 48 cm and in the effluent. The lowest removal, on average, was observed at 18cm, where there was an increase in the turbidity concentration by an average of 4% from 8-18 cm. The highest removal observed was 7% at the effluent.

The Figure 24 shows the Turbidity trend in the mature bed. The removal in the mature bed was more consistent throughout the depths for different days. The lowest turbidity removal observed was 1.63% on average, at the supernatant, whereas the highest turbidity removal observed was 5% on average in the effluent.

According to the correlation graph, Figure 49, in the Appendix, the turbdity removal varies with the influent concentration for both beds, which show a very high influent-effluent correlation. Thus, when the influent turbidity is very high, it will be reflected in the effluent.

A t-test was conducted, shown in Appendix -4 in the Appendix which showed that the scraped bed had a turbidity trend similar to the mature bed on day 14. But on day 42, it was dissimilar.

It was similar as the days went by, showing a p value > 0.05 on days 86 and 123.

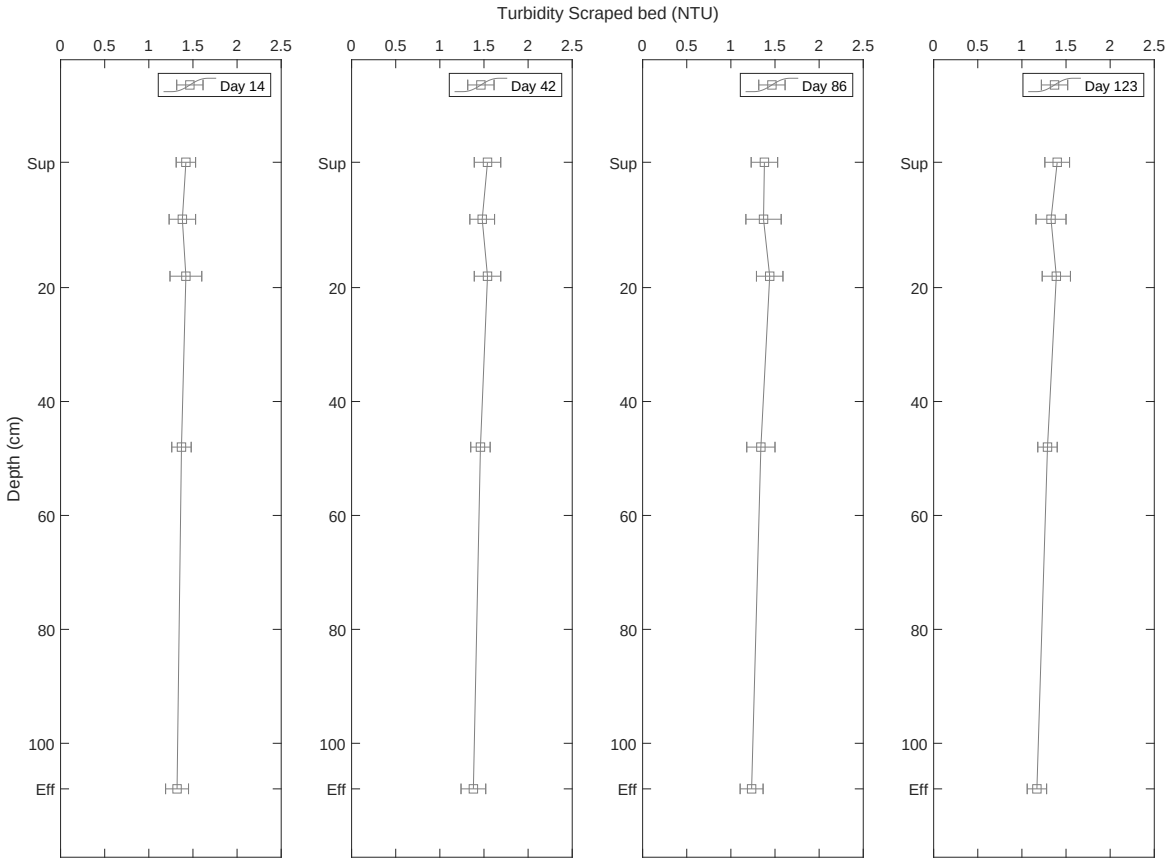

Figure 23: Turbidity depth trend for the scraped bed

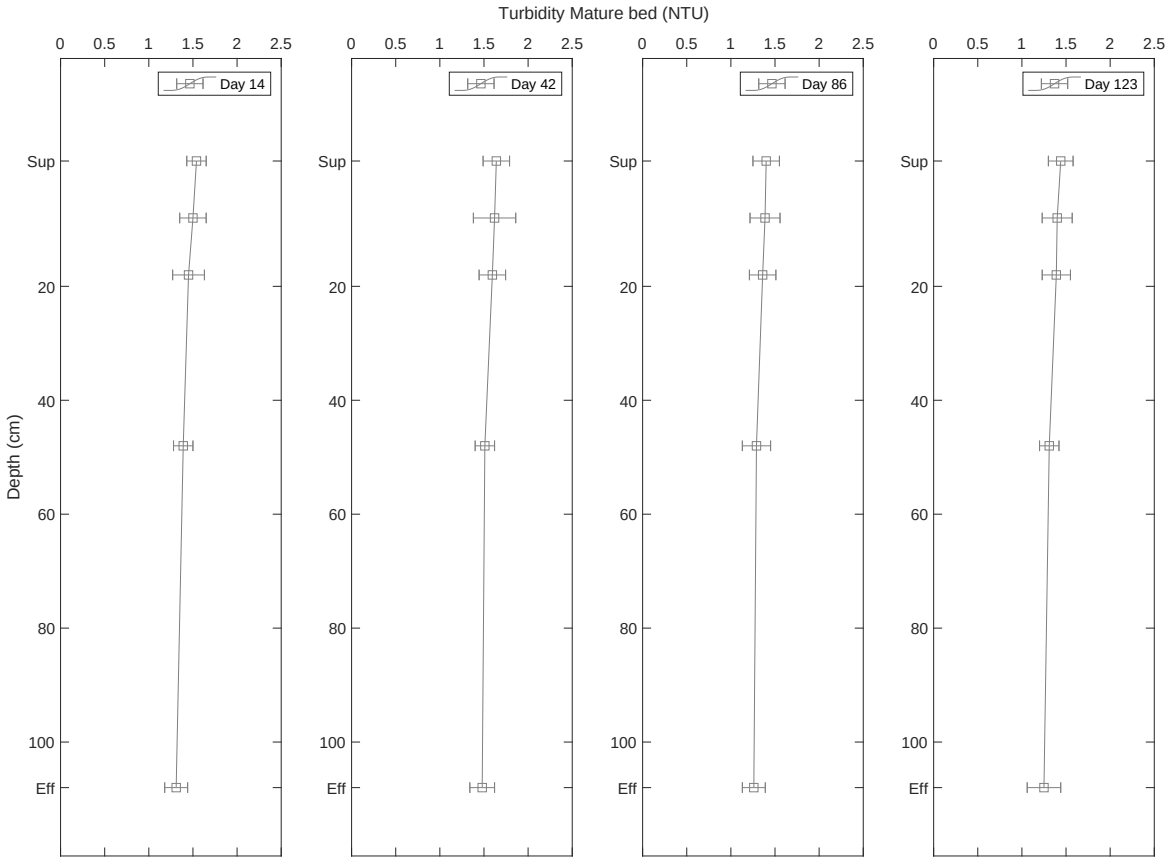

Figure 24: Turbidity depth trend for the mature bed

4-3-3 DOC trends along the depths for scraped vs mature bed

Figures 25 and 26 show the DOC removal throughout the depths. This parameter is limited for both beds, and appears to be very inconsistent. It is interesting to observe that even the mature bed has fluctuating removal percentages for different days and throughout the depths.

For the scraped bed, the highest removal on average was 8.4% in the supernatant samples , and the lowest removal on average was observed at 18cm where the removal was -7.7%, which indicates that the DOC increased from 8–18cm. For the mature bed, the highest removal observed on average was 9.3% at the supernatant, and the lowest removals were observed at 8 and 18cm. At both 8 and 18cm, there is an increase in the DOC concentration, leading to negative removal percentages of -2.2% and -2.3% respectively. However, from Figures 25 and 26, it can be seen that at day 14 and day 123, both beds have similar trends, which is also reflected in their total average removal percentages throughout the depths as well. (5.4% for scraped bed and 5.5% for mature bed for day 14 ; 2.6% for scraped and 3% for mature bed for day 123). For the rest of the sampling days, both beds showed inconsistent removal throughout the depths.

A t-test was also done to analyse the similarity of the DOC concentration within depths for different days for both beds. The results are presented in Appendix -4. The p values obtained for all 4 days were > 0.05, which shows that both beds behave similarly for all days.

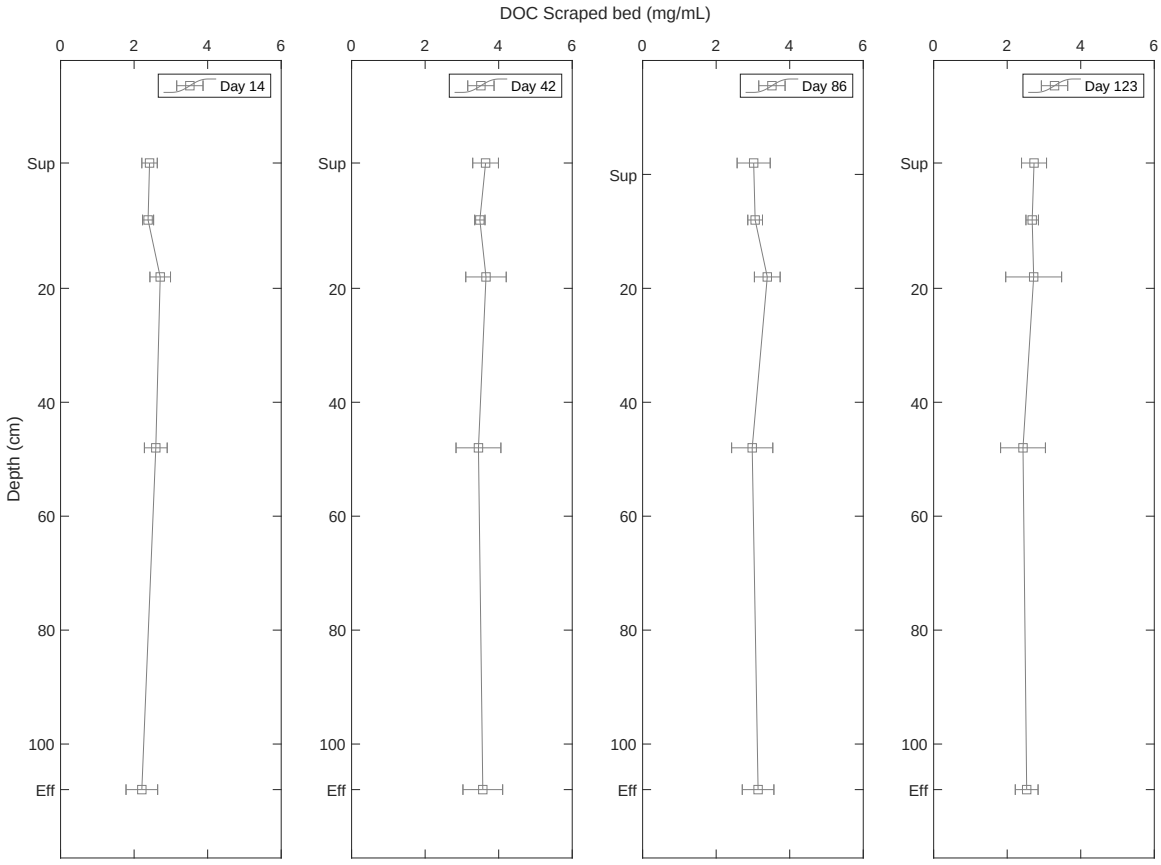

Figure 25: DOC depth trend for the scraped bed

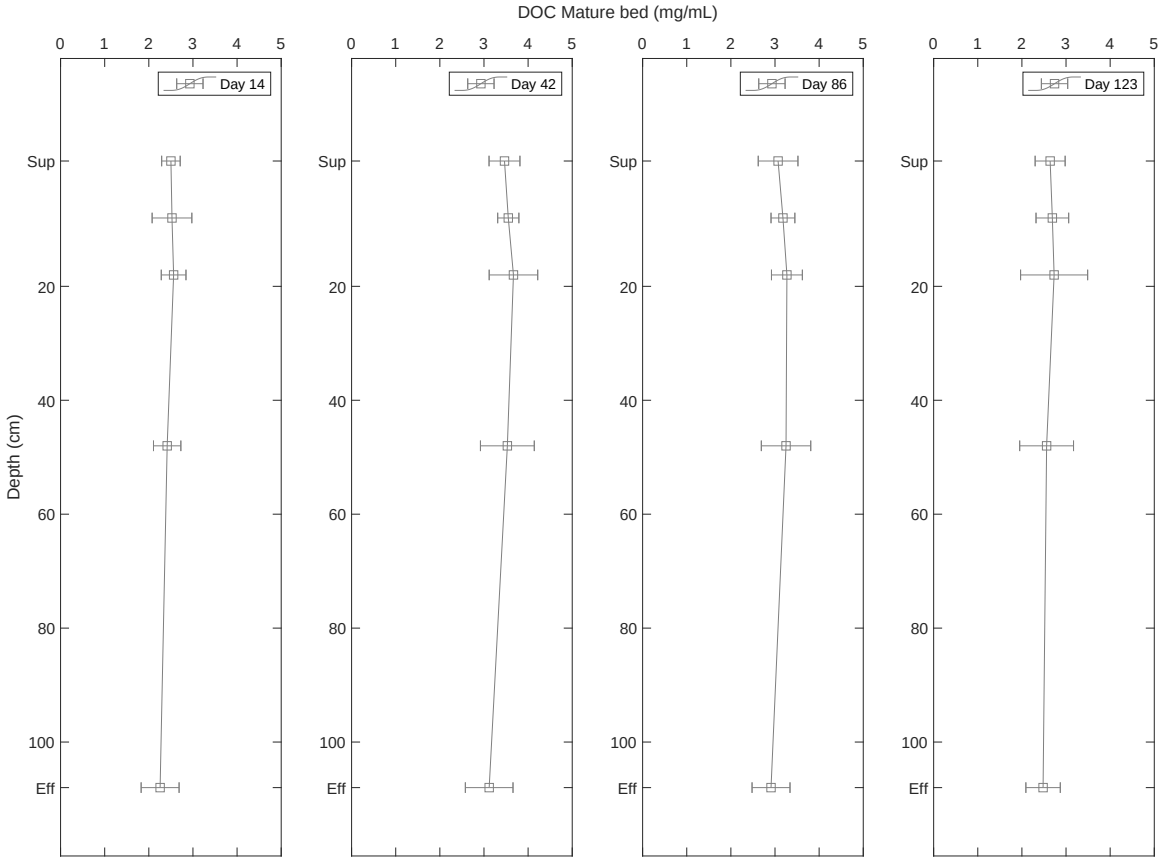

Figure 26: DOC depth trend for the mature bed

4-3-4 Fluorescence Emission Excitation Matrix (FEEM) method for categorisation of DOC

FEEM method was performed on the samples obtained on day 86 (6 May 2022) to analyse the composition of the DOC present in the samples. A numerical value or factor is assigned to the different components, which is a quantitative measure of the components present. The different DOC compounds considered were tyrosine, tryptophan, amino acids and phenolic substances which are of lower molecular weights. The higher molecular weight substances like humic and fulvic acids were also checked. Tables 6 and 7 show the different values for both the scraped and the mature bed. Figures 40 and 41 of the appendix shows the different relative percentages of the compounds present.

The heat maps obtained from the FEEM analyses are shown in Figure 42 and Figure 43 in the Appendix for the scraped and mature beds respectively. These maps were divided into 4 quadrants for ease of interpretation. The different wavelengths for the different compounds used for the FEEM analyses is mentioned in Tables 2 and 3.

For the scraped bed, from Table 6, it can be observed that, although the fulvic acid dominates in the influent, the deeper layers contain higher amounts of phenolic substances, followed by fulvic acid. This trend was different for the mature bed, where the fulvic acid dominated in almost all the depths, followed by humic acid. Tyrosine was the highest compound present

amongst the lower molecular weight compounds both in the scraped and the mature filter. The DOC trends can also be explained by the FEEM table. For DOC, shown in Figure 25, for day 86 in the scraped bed, it can be observed that, at 18cm, there is an increase in the DOC concentration. The other depths do not show any peaks. The FEEM table (Table 6) given for the scraped bed also follows this trend. At 18cm, very high values of phenolic substances, humics and fulvics were present. The mature bed has very stable DOC concentrations throughout the depths for day 86, which is also the case with the FEEM data. The highest value was seen for the fulvic acid, the other compounds had similar values.

Table 6: FEEM intensity values for different compounds present in the scraped bed at different depths

Scraped bed	**Lower molecular weight compounds**				**Higher molecular weight compounds**	
Depth	**Tyrosine**	**Tryptophan**	**Amino acids**	**Phenolic substances**	**Humic acid**	**Fulvic acid**
Influent	1.01	0.89	0.66	0.53	1.5	2.02
Supernatant	2.1	0.44	0.65	2.86	0.98	1.37
8cm	2.22	0.22	0.44	2.6	0.59	1.03
18cm	1.89	0.45	1.52	8.5	3.91	2.44
48cm	0.69	0.67	0.64	3.18	1.67	2.33
Effluent	0.81	0.87	0.91	0.27	0.78	0.6

Table 7: FEEM intensity values for different compounds present in the mature bed at different depths

Mature bed	**Lower molecular weight compounds**				**Higher molecular weight compounds**	
Depth	**Tyrosine**	**Tryptophan**	**Amino acids**	**Phenolic substances**	**Humic acid**	**Fulvic acid**
Influent	1.01	0.89	0.66	0.53	1.5	2.02
Supernatant	1.85	1.11	0.67	0.88	1.33	2.14
8cm	1.31	0.9	0.42	0.6	1.36	2.29
18cm	1.51	0.92	0.66	0.51	1.16	2.65
48cm	1.21	1.08	0.67	0.64	1.3	1.95
Effluent	0.97	0.69	0.43	0.6	1.36	1.66

4-3-5 ATP in water samples

The trend of ATP in the scraped bed and the mature bed are shown in Figures 27 and 28. It can be observed that during the initial period of sampling, until day 42, the ATP concentration in the scraped bed (days 14 and 42) fluctuate for most of the depths, and shows an inconsistent trend. From day 86 onwards, the ATP trends look more consistent. The highest removal for the scraped bed was 50% on average, which was mostly observed at the effluent, followed by an average of 20% which was observed at 8cm. At 18cm and the supernatant, there was an increase in the concentration of ATP (15% and 9% respectively) from the previous depths. The mature bed showed a consistent trend throughout the depths for different days. The highest removal observed for the mature bed was also at the effluent, at an average of 51% followed by 19% at 8cm. The mature bed mostly has a an average % decrease in the ATP concentration for all depths except for 18cm, where there was an increase in ATP concentration by 4%. A t-test was performed to analyse the similarity of ATP concentration between both beds for different days. The results are shown in appendix -4. It is seen that the ATP values were similar in both beds for days 42 and 152 (p values of 0.370 and 0.106 respectively) and the values were dissimilar for all the other days (p value > 0.05).

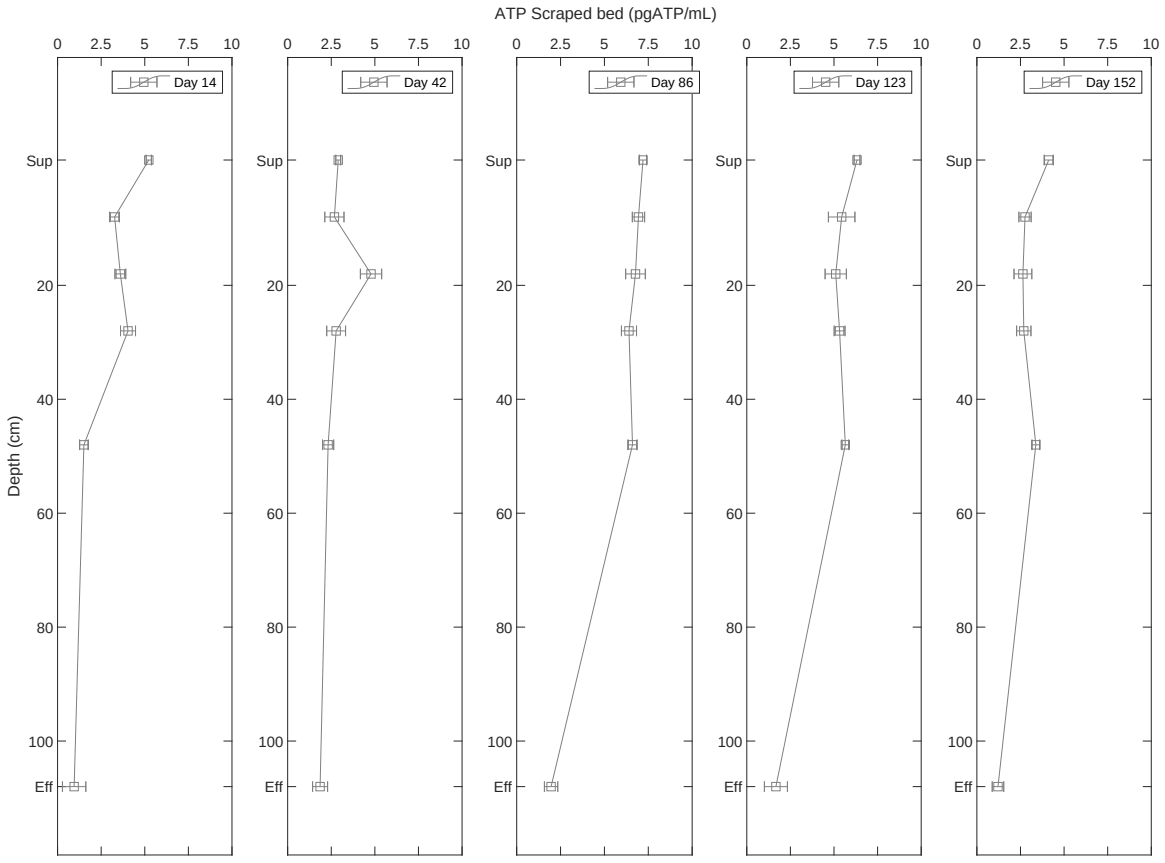

Figure 27: ATP depth trend for the scraped bed

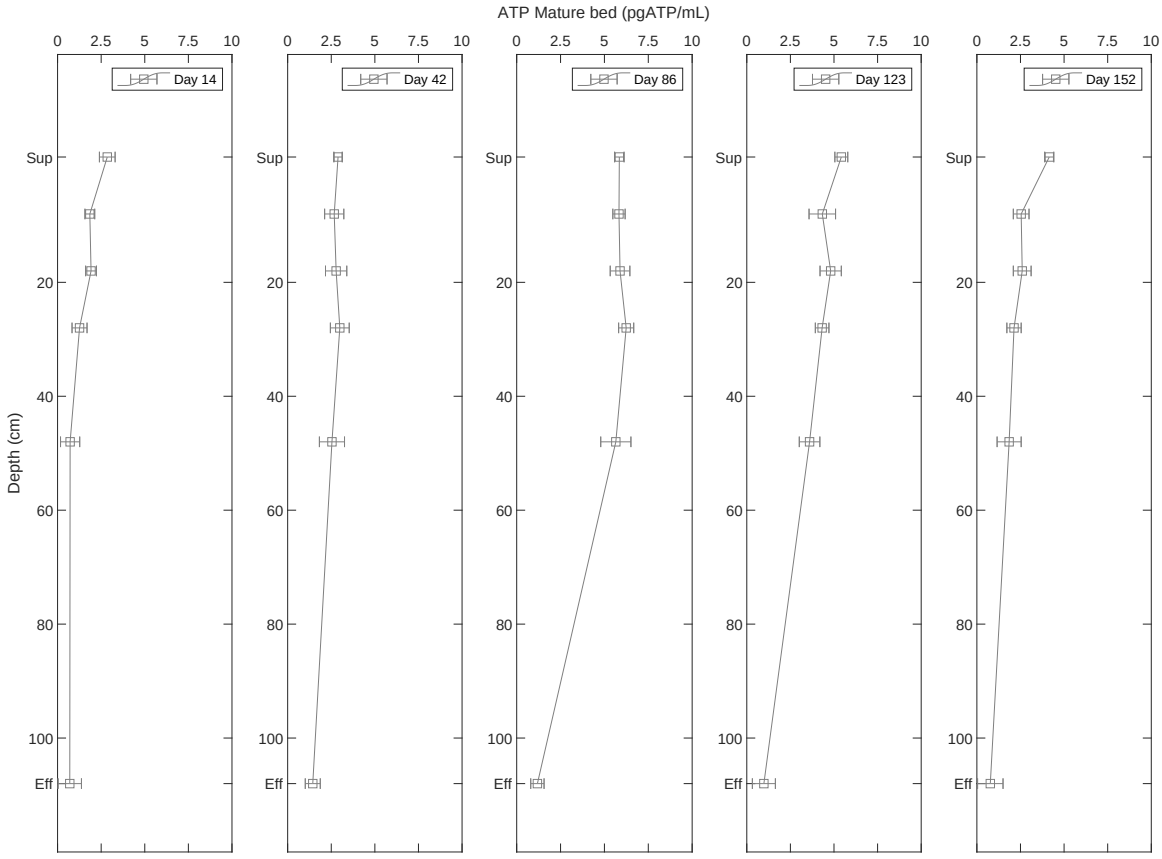

Figure 28: ATP depth trend for the mature bed

4-3-6 Particle cell counts

Using the ATP tests, it is possible to determine only how much of living organisms are present in the samples. To add more depth to the ATP value and attain additional information of biological activity in the sand bed, cell counts were performed, which give information about the total and live cell counts present in the samples, and also how much of these cells are active using HNA and LNA counts.

For the scraped bed, it can be observed from Figure 29 that both the total and live cell counts are highest in the influent and the supernatant. As the depth increases, the total and the live cell counts decrease. The influent shows high concentrations of total and live cells mostly for all days, as expected. But excluding the influent, it can be observed that the highest concentration of total cells in the scraped bed on average was observed to be $2.82 \times 10^5 \pm 0.5 \times 10^5$ cells/mL at the supernatant, whereas the highest concentration of live cells obtained was observed to be an average of $1.74 \times 10^5 \pm 0.48 \times 10^5$ cells/mL. The lowest total and live cells were observed in the effluent (average concentration of $1.72 \times 10^5 \pm 0.16 \times 10^5$ cells/ mL and $1.23 \times 10^5 \pm 0.38 \times 10^5$ cells/mL respectively).

For the mature bed, it can be observed from Figure 30 that the highest total and live cell concentrations (after the influent) is at the supernatant (an average of $2.24 \times 10^5 \pm 0.88 \times 10^5$ and $1.72 \times 10^5 \pm 0.41 \times 10^5$ respectively). The concentration of both the total and live cells

reduces with an increase in depth. At 48 cm, the concentration of both the total and live cells are very similar to the effluent concentration, with a difference of, on an average, 1.7×10^4 cells/mL and 400 cells/mL respectively.

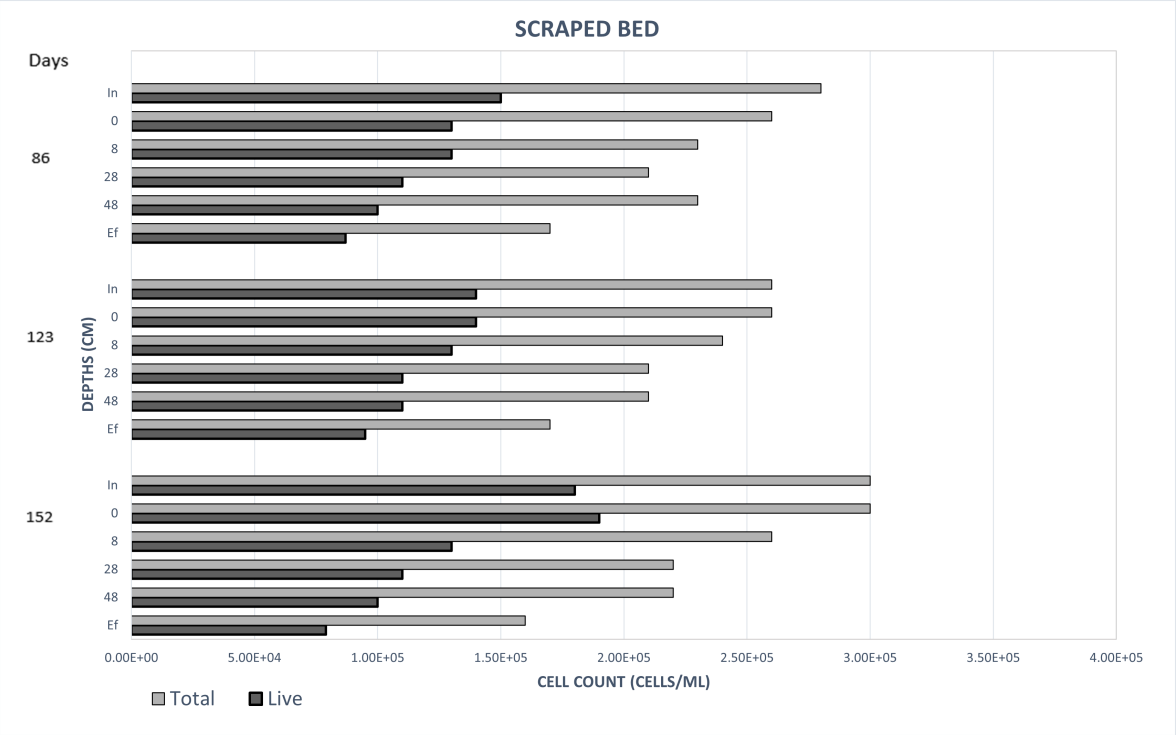

Figure 29: Cell counts for the scraped bed

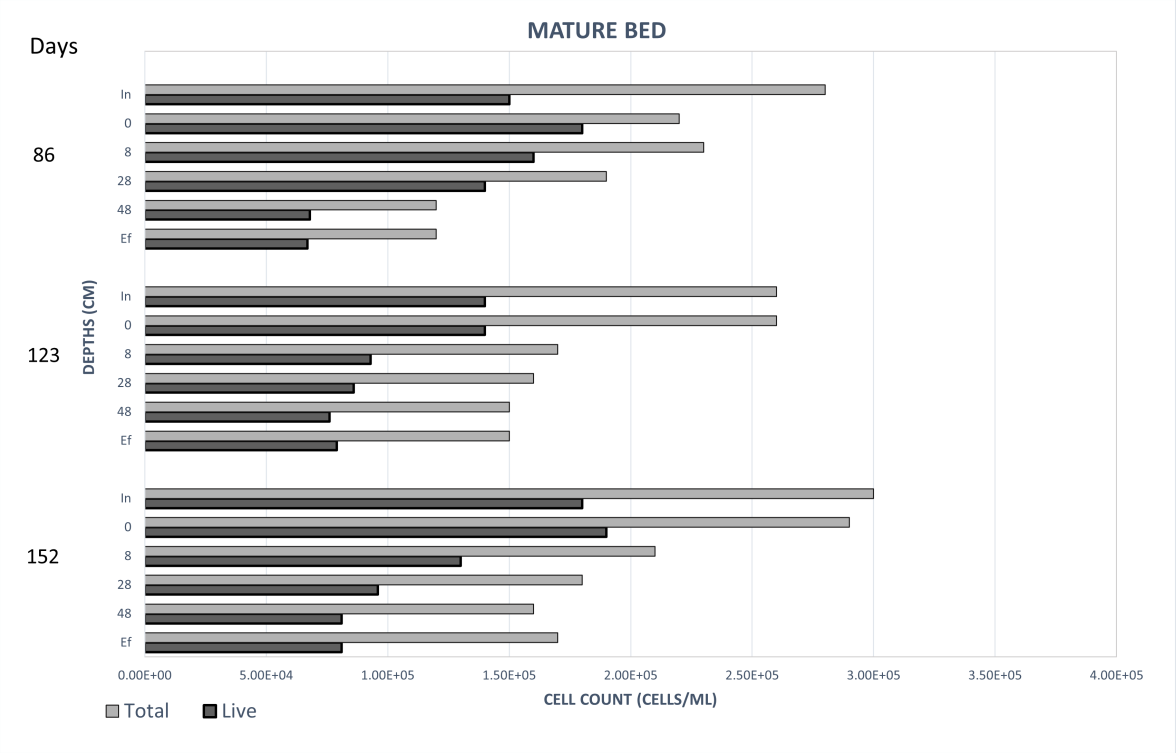

Figure 30: Cell counts for the mature bed

4-3-7 LNA and HNA

The LNA and HNA cells analysed were part of the live cell counts. From figures 31 and 32 it can be observed that both the beds clearly contain much higher LNA concentrations. The HNA concentrations for both beds seem to be lower for almost all depths. The scraped bed shows an inconsistent HNA trend for different depths throughout the days but overall, it can be said that the concentration decreases with an increase in depth. For some days, a slight peak is observed at 48cm for the scraped bed.

The percentage of LNA and HNA in the live cells of the influent and effluent are shown in Figures 33 and 34. It can be observed that the LNA in both figures are similar but there is a decrease in HNA concentration for all days in the effluent.

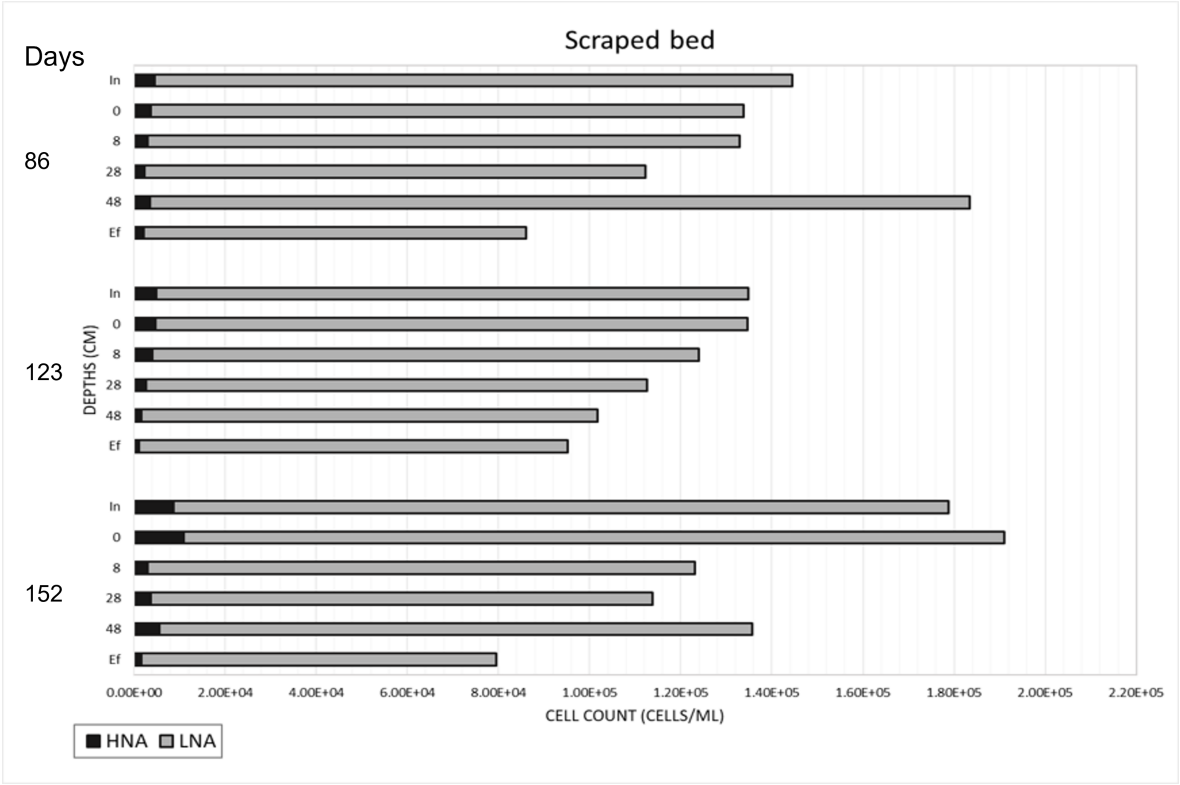

Figure 31: LNA and HNA for the scraped bed

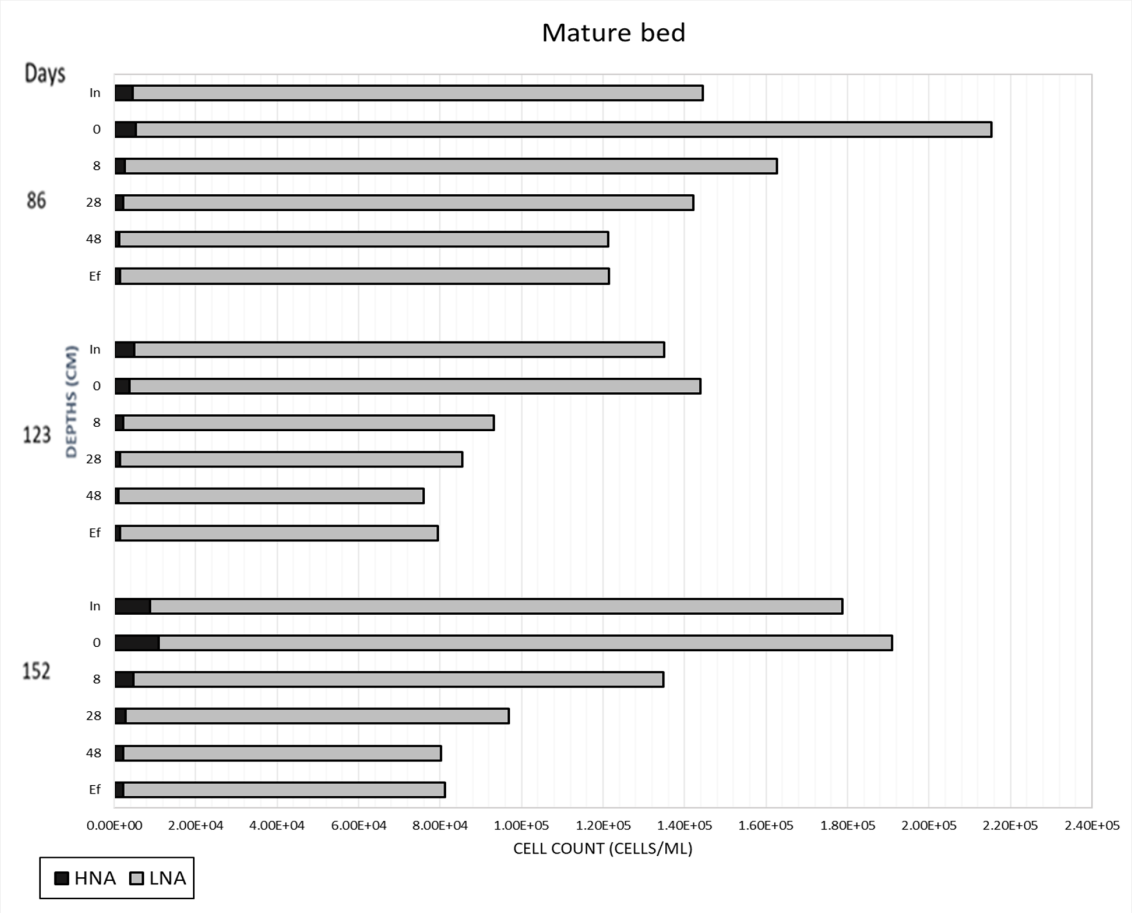

Figure 32: LNA and HNA for the mature bed

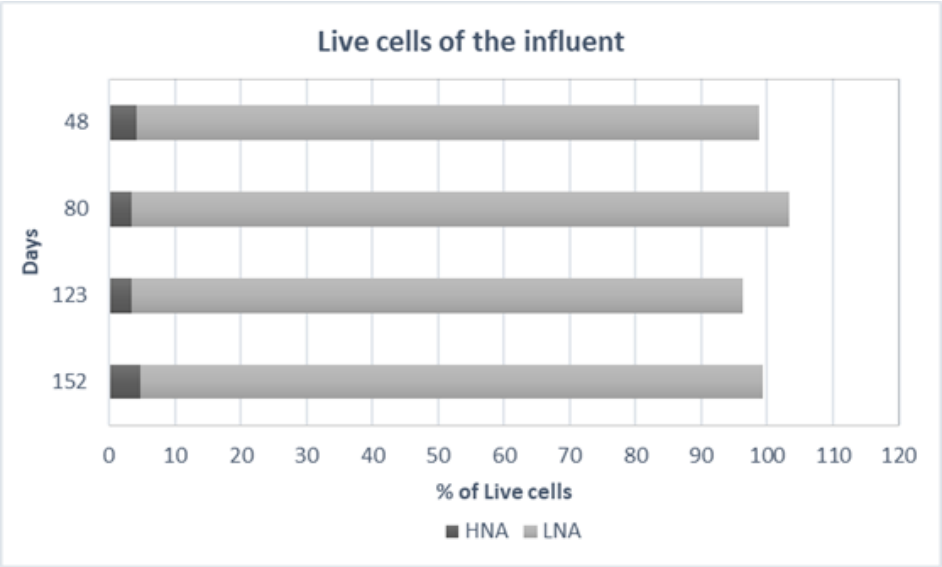

Figure 33: Live cells for the influent

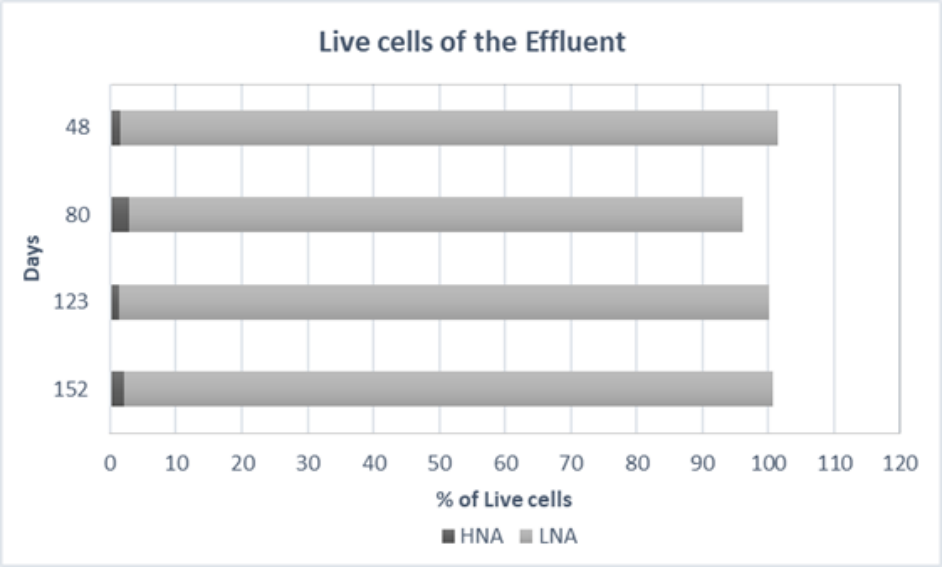

Figure 34: Live cells for the effluent

Chapter 5

Discussion

5-1 Ripening indicators

The reason for measuring various parameters for the scraped bed was to analyse whether they could be used as a means for indicating stability over time. From the results obtained, it is seen that certain parameters are capable of indicating ripening over time.

From Figure 10, we can see a clear increasing trend for ATP over time for the scraped bed. It can be observed that the ATP in the scraped bed sand samples start to rapidly increase and resemble the mature bed after day 42 onwards. The sand samples collected are a good indicator of ripening over time. The ATP concentration showed an overall increasing trend, which is indicative of an actively developing schmutzdecke layer. This is, however, not the case for the ATP water samples. The ATP trends for the scraped and the mature bed showed a p value > 0.05 for 2 out of 5 sampling days. Thus, the ATP water samples are inconsistent for the scraped bed during the ripening period and cannot be used independently to indicate ripening.

Turbidity in the scraped bed is also indicative of ripening. It can be observed in Figure 15 that the turbidity concentration of the effluent in the scraped bed started to resemble that of the mature bed after day 49 onwards. There was a drastic change in the removal % from day 14 to day 152.

The figure 35 shows the nitrate correlation between the scraped and the mature bed for several sampling days throughout the depths. It can be observed that the correlation between both the days were very low initially, with the R^2 value ranging between 0.1-0.2. However, this increases as the days increase. By the end of the sampling period, the value of R^2 was ≤ 0.8, indicating that the scraped bed was starting to behave very similar to the mature bed. Thus, nitrate could be used as a parameter to assess ripening of the sand bed.

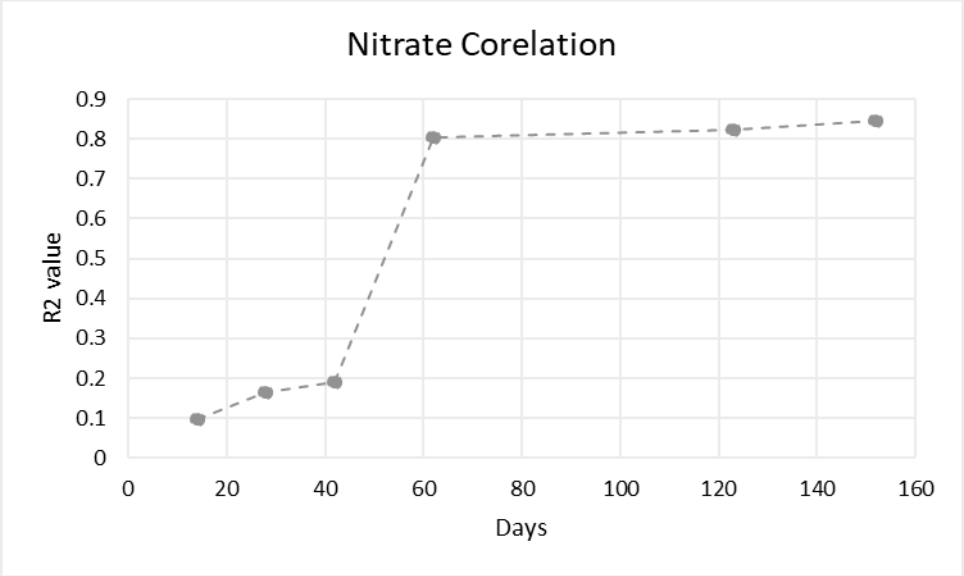

Figure 35: Nitrate correlation between scraped and mature bed

According to certain studies, like Chen et al., 2021; Oh et al., 2018, a good correlation can be expected of ATP with live cells and HNA, which can indicate potential biomass growth over time. However, from results obtained in the current study, it was difficult to determine whether ATP and Particle cell counts can be used in parallel to determine biologically stable performance (and thus ripening) over time. This is because, from the correlation matrix Table 15 in the appendix, for different days, the correlation between the parameters vary considerably. Additionally, there is no data for particle cell counts before day 48 for the scraped bed, and hence, it was not possible to determine how the corelation for ATP and cell counts vary over time.

ATP and total cells show a good correlation but live cells, LNA and HNA do not. This could be a result of overestimation in the cell count as it takes into account the actively growing cells which contribute to production of biomass and the dead and dormant cells. Additionally, ATP is sensitive to temperature and the exposure to sunlight could lead to an an increased ATP concentration (Ochromowicz and Hoekstra, 2005). This could also cause an overestimation of ATP values. Thus, total cells and ATP together can be indicative of biomass activity over time, if the samples are transported carefully. Nonetheless, more data is required to be certain.

5-2 Relationship between physico-chemical and biological parameters

From the correlation matrix shown in Table 8, it can be seen that ATP and turbidity show the highest correlation for all days for the scraped bed. The ATP values vary based on turbidity. According to literature (Azhdarpoor et al., 2019), turbidity increase can indeed cause an increase in ATP. A decrease in the turbidity in deeper sand layers can decrease the pathogenic organisms that can pass through the filter bed and into the effluent.Ferguson

et al., 1996, who investigated the relationship between pathogens and river water quality indices, reported that following increasing turbidity, water coliform is increased, as well. By performing total coliform tests along with ATP and turbidity, the stability of the filter bed can be determined over time.

Turbidity also shows a good correlation with total cells, live cells, LNA and HNA for most days for both the scraped and the mature bed, as seen in Table 15 and Table 16 in the Appendix. A study conducted by Azhdarpoor et al., 2019 also said that turbidity can provide nutrients for microbial population growth in downstream treatment plants. Additionally, the organic and inorganic particles provide good bedding for germs by providing food.

The ATP trends are expected to vary depending on the availability of the carbon substrates and nutrients available for the micro-organisms to grow. The DOC and ATP trends were analysed for the scraped bed to check the trends. Ideally, it is expected that when the DOC concentration increases, the ATP concentration would also increase at that depth. However, since DOC analysis performed in the influent using FEEM was determined to have more higher molecular weight compounds (for almost all the days), we can expect that the trend might not be good, as the microorganisms do not consume high molecular weight compounds because of their inability to synthesize these nonbiodegradable compounds easily. The correlation analysis performed shows that the R^2 values for all days between the ATP and DOC were $\leq$ 0.5 which indicates that the DOC and ATP do not have a very good correlation as expected for both beds.

The NO_3 and ATP concentrations were also analysed to check if there was any correlation between them. It was hypothesized that with an increase in NO_3, the ATP would increase, as nitrate is an essential nutrient required by the microorganisms for growth and metabolism (Aslan, 2008). The correlation between ATP and NO_3 observed, however, did not reflect that. The R^2 value obtained for all days were also ≤ 0.5. The sudden increase in nitrate at 48cm for the scraped bed (in Figure 21) does not seem to have any effect on the ATP concentration observed at the same depth. The total and live cell counts also show a slight decrease in the cell counts at 48cm, further proving that nitrate might not be a parameter that can be relied upon to determine biological activity in the sand for a ripening bed.

It is also interesting to observe that the schmutzdecke sand samples for the scraped bed show a significant increase from day 42 onwards, which is mentioned in Section 4-1. A similar trend is also seen in the water samples for nitrate and turbidity as well (Figures 13 and 15).

The turbidity removal until day 49 was negative, indicating that the turbidity was higher in the effluent than in the influent (discussed in Section 4-2-1). After day 42, the turbidity removal increased, showing a positive removal. Similarly, nitrate also showed lower turbidity removals until day 49 and it increases significantly after (discussed in Section 4-2-1.

The close similarity between these trends could indicate that the ATP increase in the schmutzdecke can cause a decrease in the effluent turbidity and nitrate water samples. However, the water samples showed that an increase in turbidity can cause an increase in ATP. The water samples for nitrate do not show any correlation with the ATP values, hence it is difficult to come to a conclusion, due to the conflicting nature of the results.

Table 8: Correlations between parameters for the scraped bed

Day	Parameter	ATP	DOC	Nitrate	Turbidity
14	**ATP**		0.2296	0.1101	0.7106
	DOC		-	0.0379	0.8118
	Nitrate			-	0.0264
	Turbidity				-
42	**ATP**	-	0.3632	0.3814	0.7819
	DOC		-	0.1004	0.2719
	Nitrate			-	0.0017
	Turbidity				-
86	**ATP**	-	0.0032	0.1479	0.7015
	DOC		-	0.0248	0.2335
	Nitrate			-	0.1214
	Turbidity				-
123	**ATP**	-	0.1756	0.0158	0.6865
	DOC			0.0007	0.681
	Nitrate				0.0003
	Turbidity				-

Table 9: Correlations between parameters for the mature bed

Day	Parameter	ATP	DOC	Nitrate	Turbidity
14	**ATP**	-	0.2844	0.2538	0.8039
	DOC		-	0.3706	0.6672
	Nitrate			-	0.1614
	Turbidity				-
42	**ATP**	-	0.3345	0.0792	0.7336
	DOC		-	0.0069	0.4555
	Nitrate			-	0.0172
	Turbidity				-
86	**ATP**	-	0.1089	0.0815	0.7797
	DOC		-	0.0699	0.2363
	Nitrate			-	0.1411
	Turbidity				-
123	**ATP**	-	0.2651	0.0643	0.889
	DOC		-	0.116	0.7238
	Nitrate			-	0.0412
	Turbidity				-

5-3 Influence of different parameters over depth

A carbon substrate is essential for most of the microorganisms' survival. Organic carbon acts as a substrate for the microorganisms to grow. Although multiple factors affect the microbial growth, organic matter, mainly the biodegradable fraction, has a determining effect since it provides carbon and acts as an energy source essential to the growth of heterotrophic bacteria (Bouteleux et al., 2005). The DOC analysis done for both beds (shown in Figures 25 and 26) indicated that the DOC concentration in the influent was already very low, at an average of 4mg/L. The DOC distribution through the sand bed was also very similar for both the sand beds, which could be due to the low concentration. The removal percentage of the beds at the effluent was on an average 11% and 15% for the scraped and mature bed respectively.

FEEM method performed to analyse the composition of low and high molecular weight compounds revealed that the water samples contained high amounts of high molecular weight compounds. High molecular weight compounds like humics and fulvics are not easily assimilable by the micro-organisms, which could lead to the DOC passing through the entirety of the sand bed unaffected, if they are not consumed by the micro-organisms. A t-test was conducted to determine if both beds behaved similarly throughout the depths. The results, seen in Appendix -4, showed that, for all days, the p values were > 0.05. This indicates that the difference between both beds were insignificant, which goes on to show that both beds behaved similarly.

The t-test conducted for nitrate (shown in Appendix -4 in the Appendix) indicates that nitrate values were dissimilar initially after scraping but started to resemble the mature bed after day 42. Literature suggests that nitrate is removed effectively until 50cm (Nakhla and Farooq, 2003). And also, studies like Oh et al., 2018 have suggested that nitrifiers like nitrospira are present in abundance in oligotrophic water. They have a high yield propensity and can survive in water treatment plants with low oxygen accessibility (Maddela et al., 2021). The scraped bed has an established sand biomass in the deeper layers, which could mean that it behaves similar to the mature bed, with an active nitrifier population, even in the absence of the schmutzdecke.

The microbial activity is an important part of the SSFs, not just at the schmutzdecke but also at the deeper sand layers. This sand biomass is very important, especially in the deeper sand layers as they are responsible to perform the filtration and purification when the schmutzdecke is scraped, to maintain the water quality standards in the acceptable range. However, performing only one type of biological test like ATP or plate counts do not give specific details about how much of the cells are active. This can be overcome by using ATP and cell counts as complimentary methods of analysis. The study published by Vital et al., 2012 also suggested that using a combination of both methods provided a more detailed insight into the specific treatment processes than using only one technique alone.

With respect to the Cell count data, the increase in ICC and HNA values over time can also indicate increased biological activity. This can show that the sand biomass is active in the deeper sand bed. This can prove that the deeper sand biomass is capable of treating the influent water, even in the absence of the schmutzdceke. Research done by Campos et al., 2002 also suggested that the sand biomass in the deeper sand bed is capable of treating the water effectively, even in the absence of the schmutzdecke. By analysing parameters like total coliforms, faecal coliform, turbidity, AOC and other parameters that can indicate whether the

sand bed meets the effluent water quality standards along with the TCC measurements, a sand bed could be put into operation without the need to wait for months for the schmutzdecke to fully develop.

Chapter 6

Conclusions and Recommendations

6-1 Conclusions

- The schmutzdecke ATP samples were able to determine ripening over time. Turbidity and nitrate were also able to indicate ripening over time.
- The ATP, ICC and HNA parameters, when done in parallel are capable of analysing biologically stable performance of the scraped bed over time.
- Turbidity is a parameter that can directly influence the biological stability of the filter bed. Turbidity has a good correlation with ATP and particle cell counts. Nitrate and DOC values did not show good correlations with ATP.
- FEEM measurements done in parallel with DOC can determine the composition of DOC compounds. Abundance of higher molecular weight compounds in the influent can lead to high DOC concentrations in the effluent, which can result in the effluent standards not being met.
- DOC concentrations vary similarly for both beds over depth as the days increase.
- Nitrifiers in the deeper sand bed could be responsible for the nitrate depth variation acting similar to the mature bed over time.

6-2 Recommendations for Dunea

- Turbidity and nitrate are the physico-chemical can be measured to indicate ripening over time. The schmutzdecke ATP and HNA water samples are the biological parameters that can be used to determine ripening.
- Along with regular ATP measurements, the ICC and HNA measurements could be performed intermittently after a filter is scraped to analyse whether there is biological

activity in the deeper sand biomass. If the ICC, HNA and ATP sampled in the deeper layers show similar values over the weeks after scraping is performed then it can be indicative of an established sand biomass. According to literature, an established biomass in the deeper bed is capable of treating the water sufficiently, even with the absence of a schmutzdecke. Additionally, regular analysis of turbidity, ammonia, nitrate, DOC (along with the ICC and HNA) can determine whether the effluent water quality falls within the required standards.

If these water standards are being met and the deeper sand biomass show a biologically stable cell activity (using ATP, ICC and HNA measurements), then it can be concluded that the sand bed is safe to be put back into operation, even in the absence of a fully functional schmutzdecke. This could minimize the period of time for which the filter needs to be kept offline.

6-3 Recommendations for further research

- One of the main problems encountered during the study was transportation of the ATP and cell count samples as they are sensitive to ambient temperature. It would be ideal to measure ATP and cell counts in-situ to avoid contamination of the samples. If in-situ measurements are not possible, then the samples need to be transported with utmost care, especially during summer, as higher temperatures can affect ATP and cell count values, even if exposed for short periods of time.
- The cell count samples were analysed by an external laboratory and the values were highly variable, due to which the data were inconsistent. It is advisable to measure all the samples in the same laboratory to avoid discrepancies.
- Parameters like phosphate, ammonia, oxygen, temperature and pH can give better insight into the overall functioning of a scraped bed over time. And also, iron and manganese can be analysed in schmutzdecke, as the biomass accumulate these minerals over time, which can also aid in an improved water quality. Due to the constraints in time and access to necessary equipment to perform the analyses like phosphate, ammonia, iron and manganese, these parameters could not be analysed. However, they could be interesting parameters to consider for further research.

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Appendix

-1 Nitrate

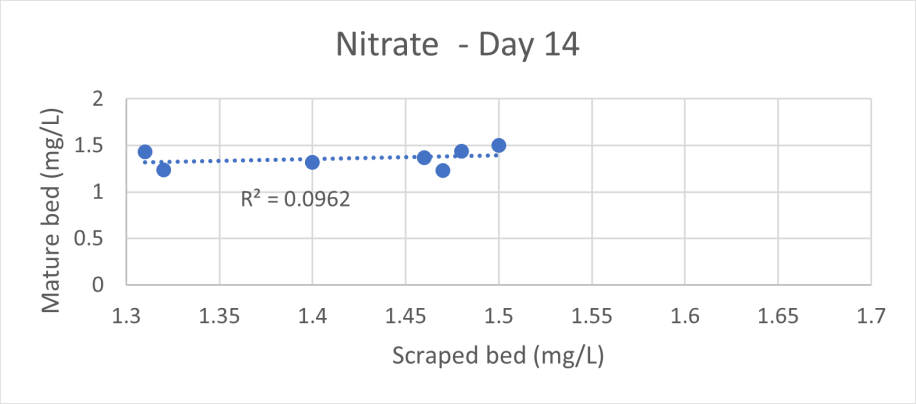


Figure 36: Nitrate correlation - Day 14

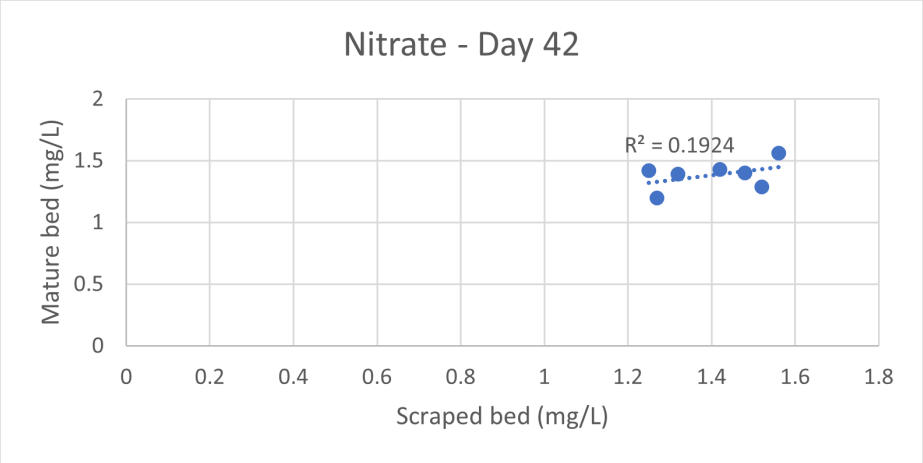


Figure 37: Nitrate correlation - Day 42

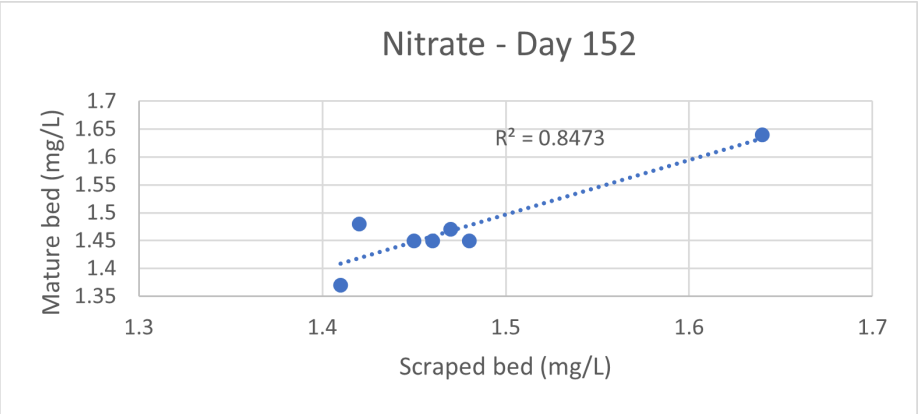


Figure 38: Nitrate correlation - Day 152

-2 DOC and FEEM

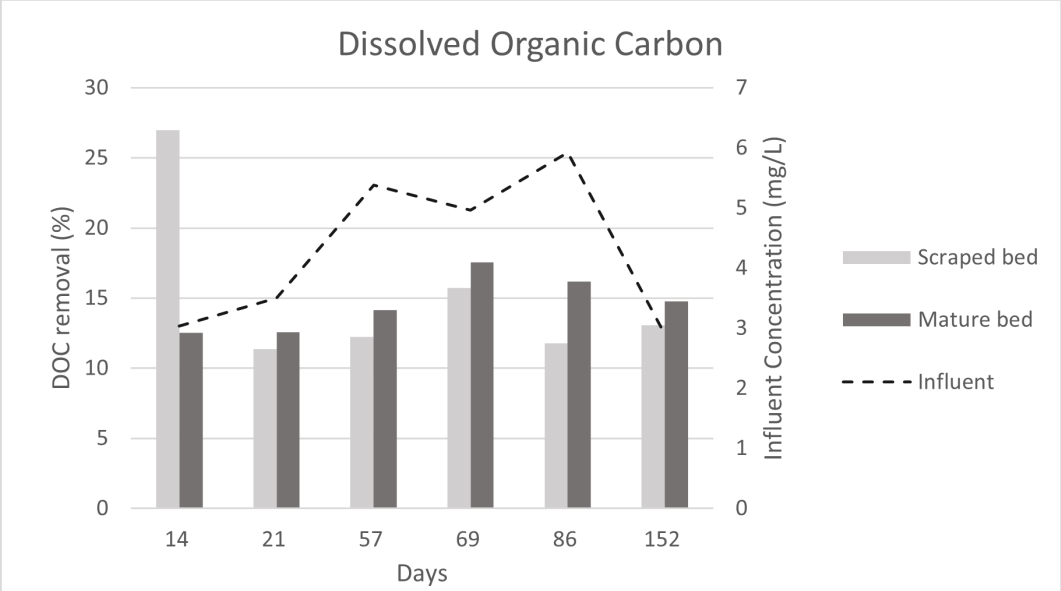


Figure 39: DOC removal over time

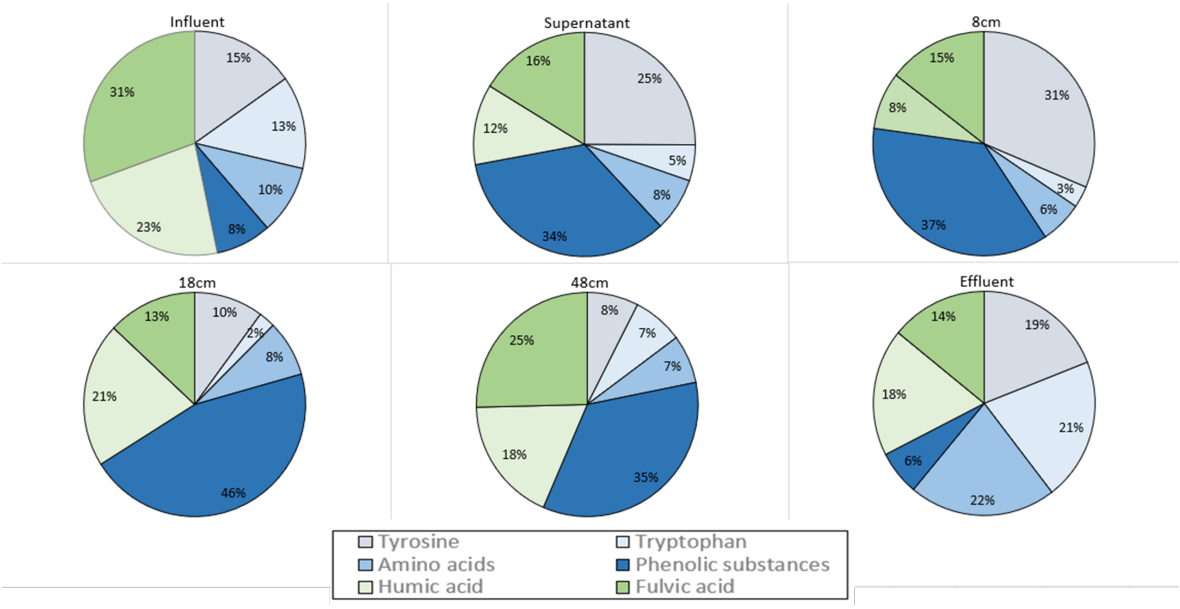


Figure 40: Percentage composition of DOC compounds in the scraped bed on day 86 using FEEM data

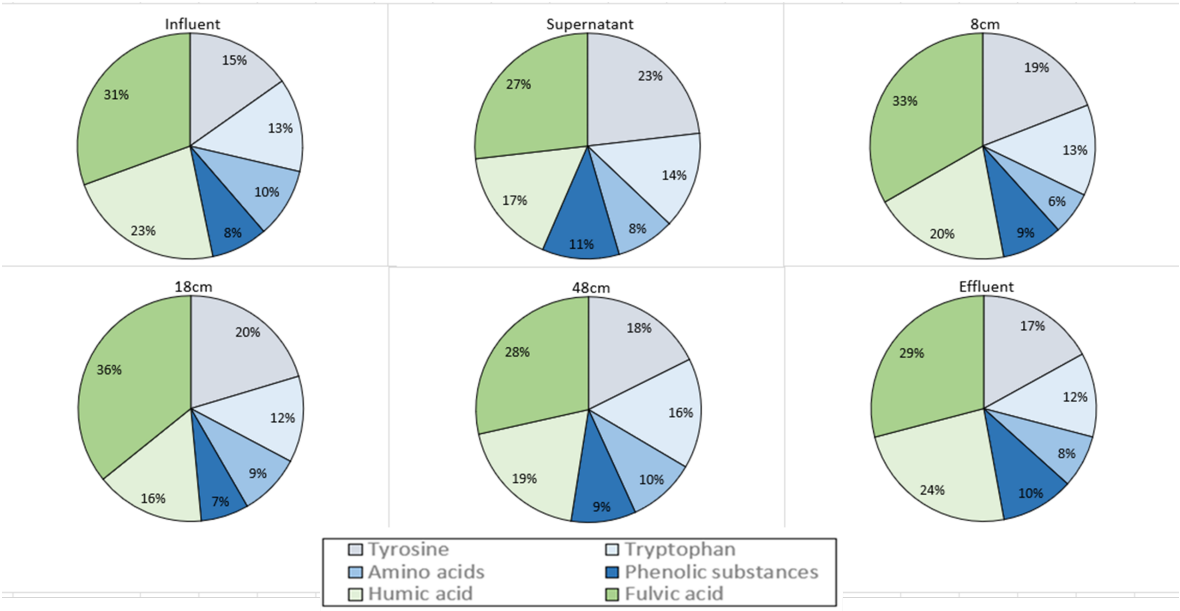
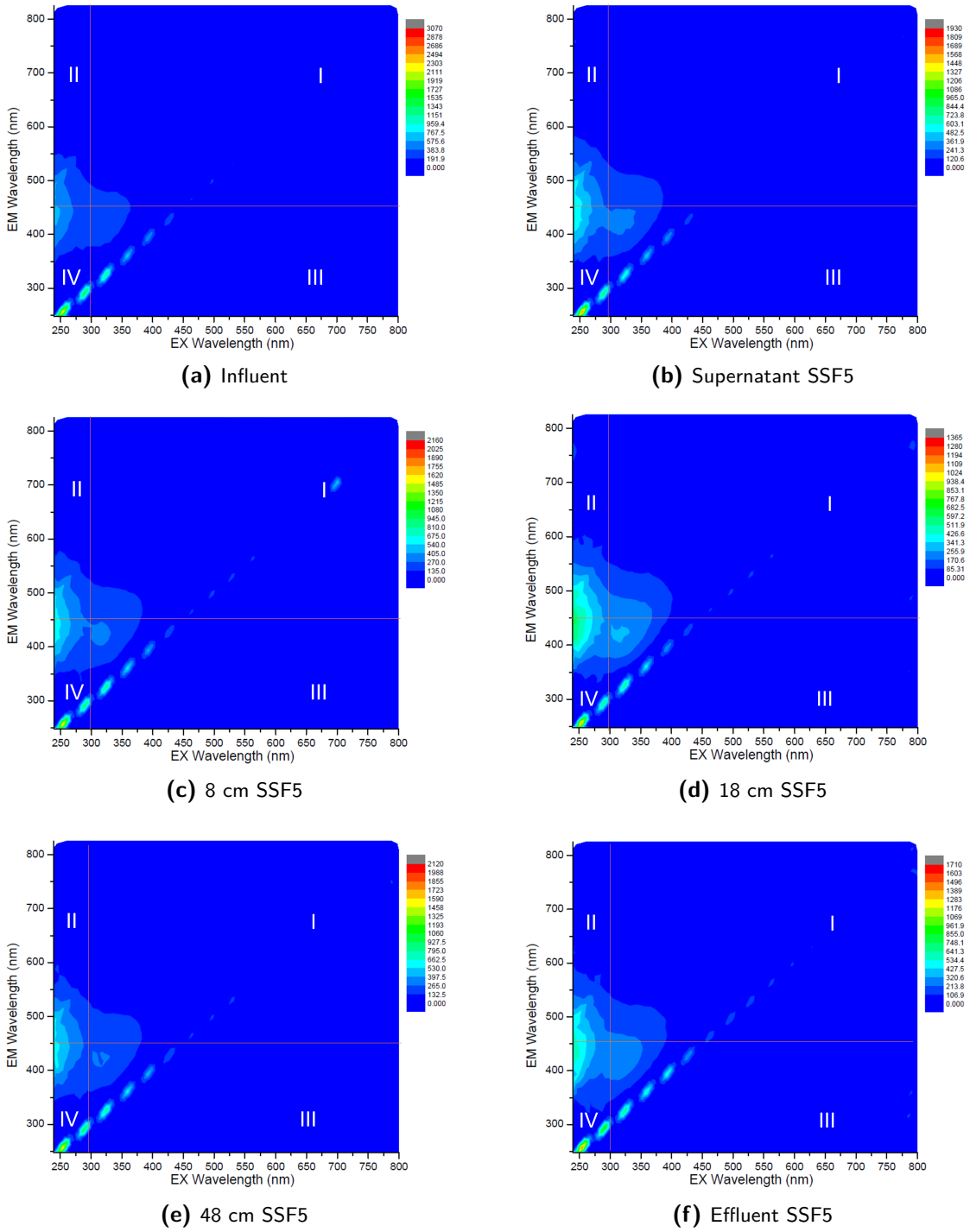


Figure 41: Percentage composition of DOC compounds in the mature bed on day 86 using FEEM data

-2-1 FEEM Heatmaps

Scraped bed



(a) Influent

(b) Supernatant SSF5

(c) 8 cm SSF5

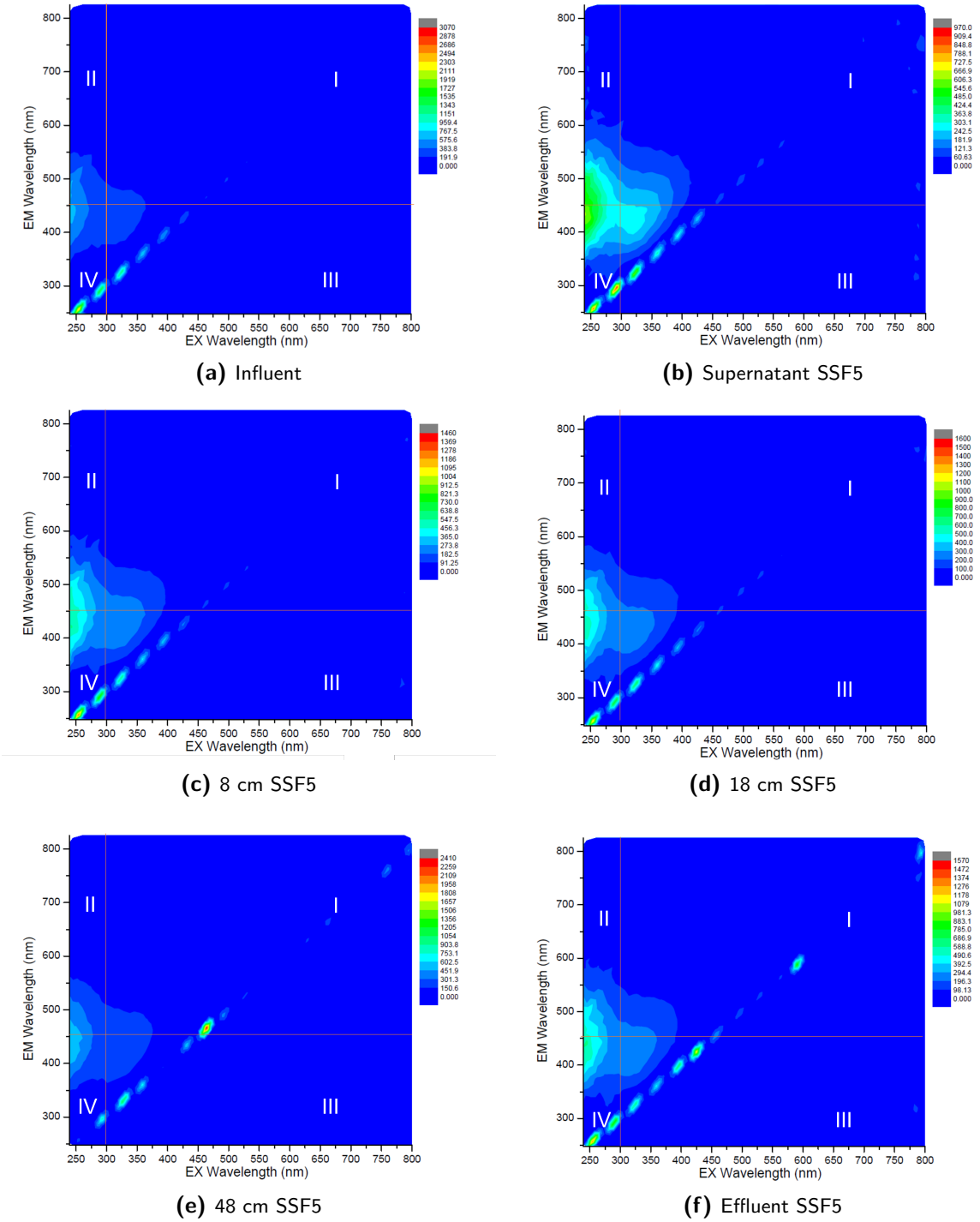
(d) 18 cm SSF5

(e) 48 cm SSF5

(f) Effluent SSF5

Figure 42: Heatmaps for different depths of the scraped bed on day 86

Mature bed



(a) Influent

(b) Supernatant SSF5

(c) 8 cm SSF5

(d) 18 cm SSF5

(e) 48 cm SSF5

(f) Effluent SSF5

Figure 43: Heatmaps for different depths of the mature bed on day 86

-3 Particle Cell Counts

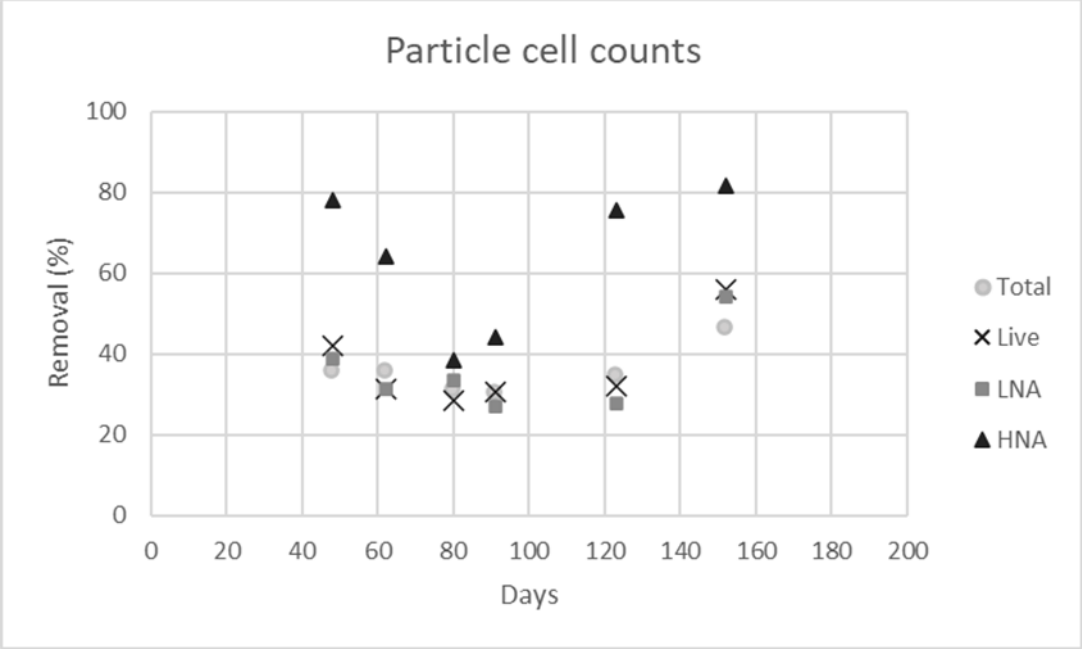


Figure 44: Percentage removal of different cells in the scraped bed on different days

-4 T-test values

Day	p value
14	0.0206
42	0.1848
86	0.1508
123	0.1523

Table 10: t-test values between nitrate concentration of both scraped and mature bed for different days.

Day	p value
14	0.1062
42	0.0059
86	0.5623
123	0.0585

Table 11: t-test values between turbidity concentration of both scraped and mature bed for different days.

Day	p value
14	0.0206
42	0.1848
86	0.1508
123	0.1523

Table 12: t-test values between nitrate concentration of both scraped and mature bed for different days.

Day	p value
14	0.015
42	0.370
86	0.007
123	0.013
152	0.106

Table 13: ATP t-test values between scraped bed and mature bed for different days

Day	p value
14	0.875
42	0.734
86	0.843
123	0.958

Table 14: t-test values between DOC concentration of both scraped and mature bed for different days.

-5 Correlation values

Table 15: PCC correlations between parameters for the scraped bed

Day	Parameter	Total cells	Live cells	LNA	HNA
42	**ATP**	0.8786	0.964	0.9408	0.8192
	DOC	0.2549	0.304	0.3828	0.1154
	Nitrate	0.0784	0.2847	0.2468	0.0005
	Turbidity	0.5097	0.7637	0.7849	0.5148
86	**ATP**	0.5924	0.76	0.7119	0.4086
	DOC	0.0046	0.145	0.142	0.0422
	Nitrate	0.0565	0.1291	0.1134	0.1823
	Turbidity	0.9032	0.088	0.3671	0.8013
123	**ATP**	0.7131	0.5918	0.5232	0.4878
	DOC	0.6174	0.6878	0.7854	0.8115
	Nitrate	0.0013	0.0006	0.0102	0.0086
	Turbidity	0.9362	0.9095	0.8651	0.8518

Table 16: PCC correlations between parameters for the mature bed

Day	Parameter	Total cells	Live cells	LNA	HNA
86	**ATP**	0.3846	0.1667	0.1571	0.3846
	DOC	0.8116	0.0055	0.0012	0.043
	Nitrate	0.249	0.0055	0.0008	0.2706
	Turbidity	0.0579	0.5073	0.4515	0.7302
123	**ATP**	0.5671	0.5773	0.5538	0.5671
	DOC	0.5279	0.5447	0.4454	0.7095
	Nitrate	0.0419	0.0334	0.0154	0.0407
	Turbidity	0.6944	0.7262	0.6985	0.6871

Day 48	**ATP**
Total	0.8786
Live	0.964
LNA	0.9408
HNA	0.8192
Day 62	**ATP**
Total	0.5706
Live	0.7603
LNA	0.7119
HNA	0.4086
Day 123	**ATP**
Total	0.7131
Live	0.591
LNA	0.5232
HNA	0.4878
Day 152	**ATP**
Total	0.7716
Live	0.8942
LNA	0.8893
HNA	0.8074

Table 17: Correlation values between ATP, Total cell count, Live cell count, LNA and HNA cells for different days of the scraped bed

Day 86	**ATP**
Total	0.3846
Live	0.1667
LNA	0.1571
HNA	0.1848
Day 123	**ATP**
Total	0.5671
Live	0.5773
LNA	0.5538
HNA	0.5066
Day 152	**ATP**
Total	0.826
Live	0.8961
LNA	0.8922
HNA	0.8783

Table 18: Correlation values between ATP, Total cell count, Live cell count, LNA and HNA cells for different days of the mature bed

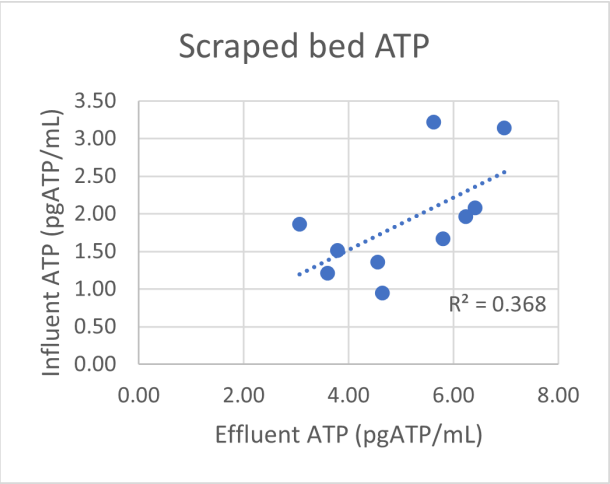


Figure 45: Influent-effluent correlation for ATP of scraped bed

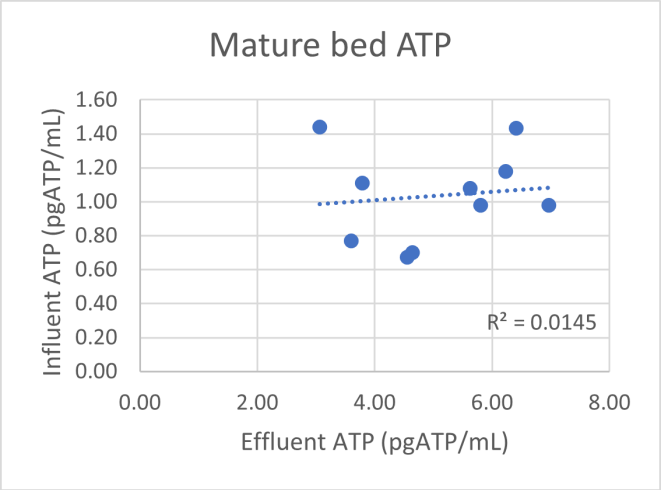


Figure 46: Influent-effluent correlation for ATP of mature bed

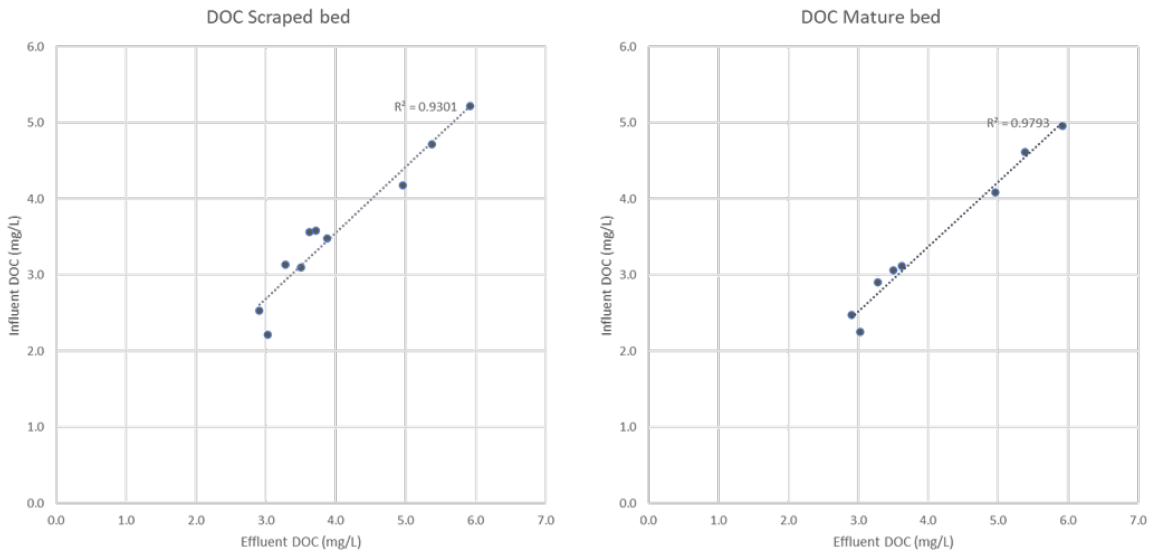


Figure 47: Influent-effluent correlation for DOC of both scraped bed mature bed

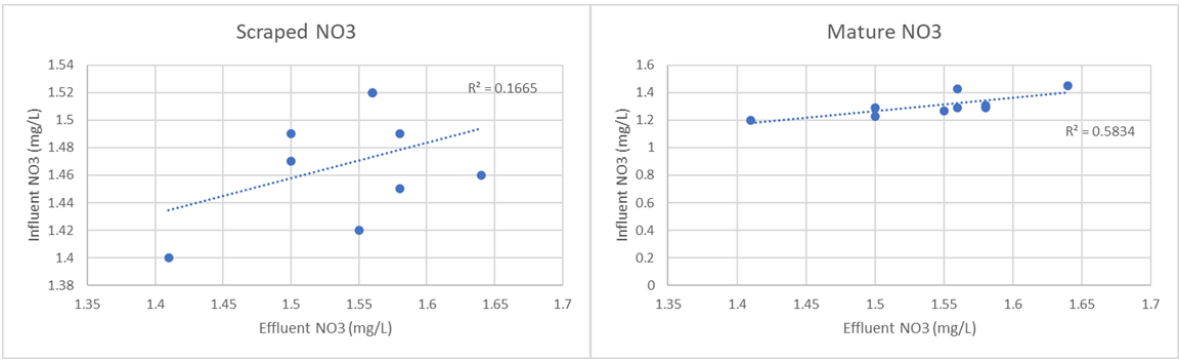


Figure 48: Influent-effluent correlation for nitrate values of both scraped bed mature bed

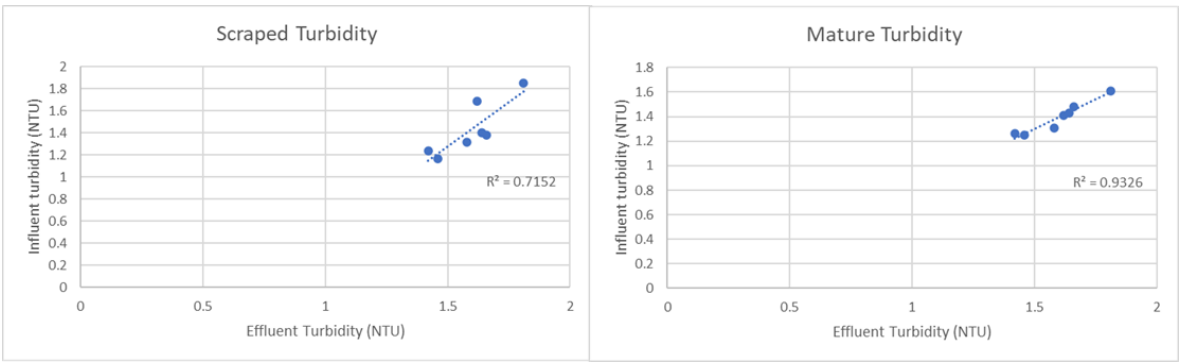


Figure 49: Influent-effluent correlation for turbidity values of both scraped bed mature bed

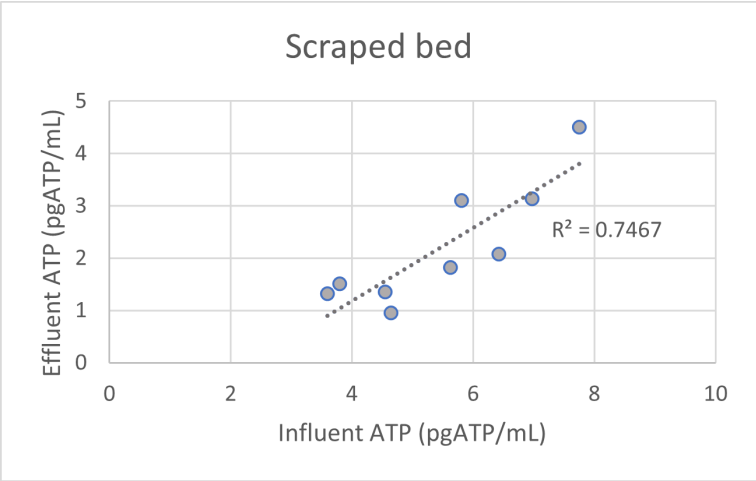


Figure 50: ATP correlation for the scraped bed

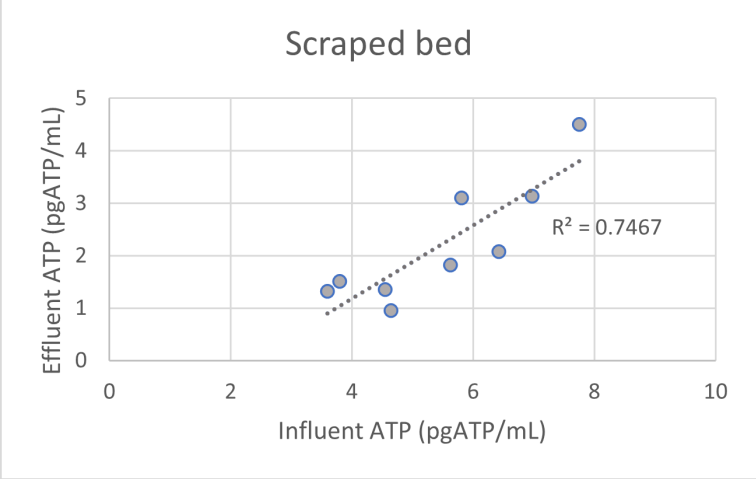


Figure 51: ATP correlation for the mature bed

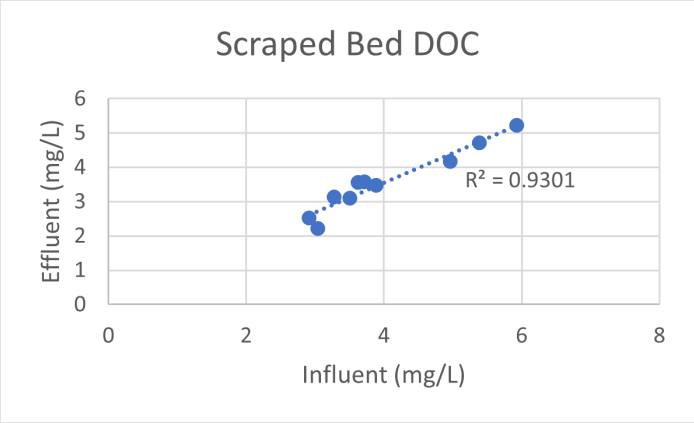


Figure 52: DOC correlation for the scraped bed

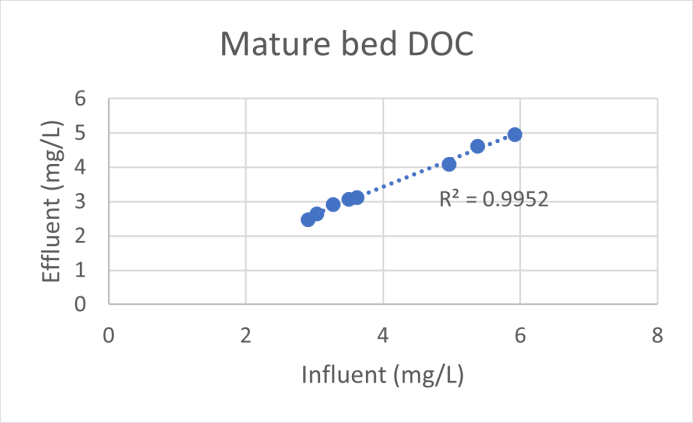


Figure 53: DOC correlation for the mature bed