Optimising the Ripening Period of Slow Sand Filters

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Optimising the Ripening Period of Slow Sand Filters

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Summary:

This work is carried out in collaboration with Dunea and TU Delft. The aim of this study was to optimize the ripening period of slow sand filters and to identify the indicators of ripening. Combination of full scale and column scale slow sand filters were used to achieve the goals.

Ripening period is required for the formation of biological community (schmutzdecke layer) over the sand layer and within the top layers of the sand bed during which filter performance is sub-optimal. The ripening period depends on factors such as influent water quality (nutrient loading), temperature and filtration rate. In order to optimise the ripening period of slow sand filter, two possible approaches were investigated. First, how can we retain the maximum biological activity within the filter bed at the time of scraping (optimise scraping) and second how to accelerate the growth of microorganism when a filter is put into operation by changing operational parameters

In order to ensure maximum biological activity is retained within the filters at the time of scraping, biomass concentration in different layers of sand bed which is responsible for head loss was quantified followed by quantifying the inactivation potential and biological activity in different layers of sand bed. This was done by measuring Adenosine Triphosphate (ATP) and Cell count of the sand samples in column and full scale SSF and carrying of spiking experiments after removal of different layer of sand bed. Spiking experiments were done only in columns SSF. Spatial Distribution of biomass on the filter bed was also investigated.

In order to accelerate the growth of microorganism's, three possible solutions were investigated in column SSF. First was the use of additional nutrients, second was to increase the filtration rate and third was the use of microbial inoculum (schmutzdecke) from a matured filter. The effect of different operational parameters on the efficacy of column SSF was determined by measuring particle counts, turbidity, dissolved organic carbon and total nitrogen in influent and effluent. Along with this spiking experiment of *E.Coli WR1* and *MS2 bacteriophage* were carried out during stages of filter operation. Physical, chemical & microbial parameters that were used to assess the efficacy of SSF were correlated to each other and most suitable indicators of ripening were identified.

In total there were 8 columns, running in duplicates with six of them running at filtration rate of 0.1 m/hr and two at 0.5m/hr. Two columns with 0.1 m/hr were used a reference for comparison. Two columns running at 0.1 m/hr were seeded with inoculum from one of the full scale filters and other two at 0.1 m/hr were seeded with additional nutrients.

The Biomass concentration decreased with depth in both full scale and column slow sand filters. More than 80% of biomass accumulation takes place in schmutzdecke and top 2 cm of sand bed. Position of the inlet valve source affects the spatial distribution of biomass on the filter surface due to lateral gradients and leads to uneven biomass growth. Decimal Elimination Capacity of column SSF decreased after the removal of sand layers with most significant reduction in DEC was observed after the removal of schmutzdecke (>1 log).

Column with added microbial inoculum were able to mimic the full scale filters. Removing of schmutzdecke and top 2 cm of sand bed where most of the biomass accumulation takes place, columns were still able to achieve more than 3 log removals for bacteria and 1 log for virus. This is higher than the values required by current full scale SSF's in their operation. Effluent turbidity and particle counts were less than 0.1 NTU and 200/ml even after the removal of schmutzdecke in columns with inoculum.

Combining the results of the biomass distribution and spiking experiments carried out in the columns with microbial inoculum, it can be concluded that ripening period of the SSF will be drastically reduced if the scraping of only 4 cm of sand bed takes place including schmutzdecke.

To reduce the start up time of a new filter, addition of microbial inoculum (schmutzdecke from a matured filter) is the better solution in comparison to addition of nutrients or increasing the filtration rate. Although the purpose of adding inoculum or nutrients or increasing the filtration rate was same: that is to increase the biological activity in the sand bed. Columns with inoculum reached more than 2.5 log removals in first 30 days of operation as compared to others and took only 24 and 27 days to reach median levels of turbidity of 0.1 NTU and particle count less than 200/ml respectively.

Reductions of bacteria, viruses, turbidity and particle counts increase substantially with time as filters ripens. No such pattern was observed in the DOC and TN removal, they were more a function of the influent water quality and independent of the ripening period. Particle count was a better surrogate than turbidity as an indicator of ripening of the filter. Using them together would provide a better insight regarding the ripening as the correlation between them increases as the filter ripens. DOC and TN cannot be used as indicators of ripening.

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Chapter 1

Introduction

This Chapter includes motivation behind the research, problem statement, research approach and objectives and the outline of the thesis.

1.1 Motivation behind Research

Slow Sand Filtration is one of the most ancient water treatment techniques used worldwide to treat microbially impacted surface waters by biological and physico-chemical removal mechanisms *(Anderson et al., 2009)*. With technological advancements, SSF has often been replaced by high-rate filtration methods such as rapid sand filtration, many countries (eg Netherlands, United Kingdom, Brazil & India) still uses SSF because it does not require chemical pretreatment, functions over a wide range of influent water quality, requires minimal maintenance, and is cost-effective to build and operate *(Logsdon et al, 2002)*.The efficient removal of pathogenic microorganisms such as bacteria, viruses *(Graham, 1999)*, giardia cysts *(Bellamy et al, 1985; Hendricks, 1991)* and cryptosporidium *(Fogel et al, 1993)* gives it an edge over the other filtration techniques. Indeed, *Wegelin (1988)* stated "no other single water treatment process can improve the physical, chemical, and bacteriological water quality of surface water better than slow sand filtration."

The high efficiency of water treatment achieved by slow sand filtration is partly explained by the process of slow filtration rate (0.1-0.5 m/h) and fine effective size of the sand but also because of the accumulation of biologically active schmutzdecke layer on the surface and within the upper layers of sand bed *(Huisman & Wood, 1974)*. These layers contain the greatest biological activity and highest levels of bacterial colonization in the sand layer *(Calvo-Bado et al., 2003a).*

A major limiting factor to the application of slow sand filters is the ripening period which is required at the beginning of each run when a filter is put in operation *(Letterman, 1985)*.

As the filtration progresses, the biomass concentration increases in the schmutzdecke layer and sand bed and build-up the head loss. Ultimately, the filter has to be cleaned by scraping of the schmutzdecke layer and few centimeters of sand bed.*Ellis (1985)* reported that draining of SSF during the cleaning process causes significant death of microorganisms living in the filters and a major reduction in biological activity. In addition, an exposed sand surface creates highly aerobic conditions with no nutrients for microorganisms to consume, which leads to the consumption of extracellular materials as a food source *(Ellis, 1985)*. The loss of this extracellular material leads to reduced biomass in the filter bed and subsequent washout of microorganisms when the filter is put back in operation, due to which the filter requires a ripening period.

Filter ripening is a complex process that involves both biological and physiological mechanisms *(Joubert et al., 2008)*. Ripening period is required for the formation of biological

community (schmutzdecke layer) over the sand layer and within the top layers of the sand bed during which filter performance is sub-optimal *(Joubert et al., 2008)*. As filtration progresses, the schmutzdecke layer builds up with diverse organisms such as protozoa, bacteria, algae and other forms of life and contributes to the removal of pollutants from water *(Dizer et al., 2004).*

The ripening period depends on factors such as influent water quality (nutrient loading and temperature) and filtration rate. These variables results in uncertainty in the operation of slow sand filters and variable ripening period.

1.2 Problem Statement & Research Gaps

It has been widely acknowledged that the development of the microbial community (bacteria, viruses and eukaryotes) is integral to the elimination of pathogens *(Bauer et al., 2011; Weber-Shirk and Dick, 1997a, 1999)*, breakdown of organic matter (*Eighmy et al., 1992)* and the oxidation of ammonia to nitrate *(Aslan, 2008)*. Since the importance of biological activity in the schmutzdecke layer and sand bed in the efficacy of SSF is accepted, a frequently asked question is whether this activity can be enhanced and can its establishment or development be speeded up *(Hunter et al., 2013)*?

The biological activity occurs because of the microorganisms, the growth of which depends on the availability of nutrients *(Vital et al, 2010)*. Organic carbon, especially assimilable organic carbon (AOC) has been considered to be the main nutrient controlling microbial growth *(Van der Kooij et al., 1982)*. However, recent studies have shown that if water contains high amounts of organic matter, microbial growth in water can be limited by phosphorus instead of organic carbon *(Lethola et al., 2000)*. The growth of the microorganisms is essential for the development of the biological (schmutzdecke) layer and the ripening of the filter.

As ripening proceeds, schmutzdecke layer will be gradually visible. There will be a slight increase in the head loss in the sand bed due to build up of the schmutzdecke layer *(Visscher et al., 1987)*. These are the indicators that ripening is producing satisfactorily but bacteriological and chemical analysis of the effluent is required to ensure the filter has been ripened. Coliform bacteria and turbidity are commonly used as indicators of ripening.

No health-based guideline value for turbidity has been proposed; ideally, median turbidity should be below 0.1 NTU for effective disinfection *(WHO guidelines, 2008)*. Coliforms level should be 0 CFU/100 ml according to Dutch drinking water guidelines. Recently particle

counts and ATP measurements are also used as the indicators of ripening of the Slow Sand Filters. Researchers have shown that there may be a high probability of Cryptosporidium and Giardia in drinking water when the concentration of particles larger than 2 μm is more than~200 particles/mL *(Hargesheimer et al. 1998)*, suggesting that the particles in water treatment effluent can be used as a substitute index of the Protozoa *(Cook 1995)* and consequently a surrogate for water quality monitoring against pathogens.

The complex tests (chemical and bacteriological) are generally applied as part of validation and verification activities rather than as part of operational monitoring. These complex tests are costly and time consuming. Therefore if a correlation between these simple and rapid observations such as turbidity and particle count can be established with chemical and microbial test, they can be substituted as indicators of ripening, thus saving time and money.

1.3 Objective & Approach

In order to optimise the ripening period of slow sand filter, two possible approaches were suggested as shown in figure 1. First, is to retain the maximum biological activity within the filter bed at the time of scraping (optimise scraping) and second to accelerate the growth of microorganism when a filter is put into operation by changing operational parameters.

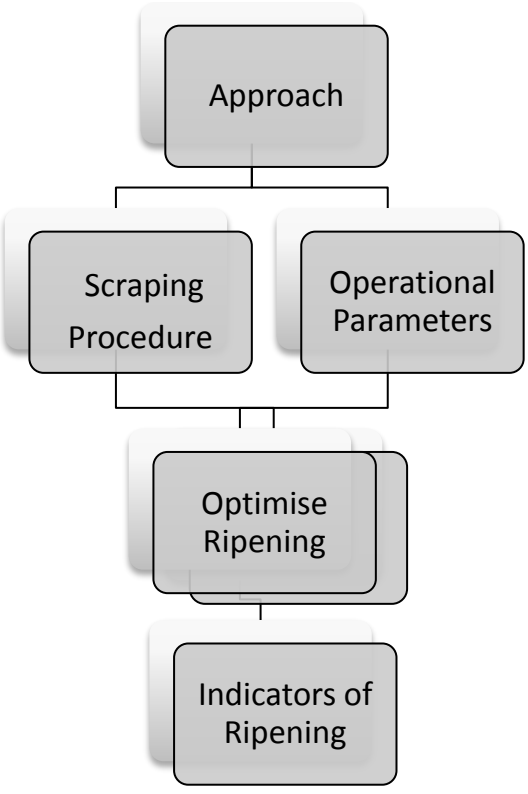

Figure 1: Approach used to optimise and identify the indicators of Ripening

In order to optimise the scraping procedure, the biomass distribution, the inactivation potential and the biological activity of different layers should be known. As explained earlier the growth of microorganism depends on the availability of nutrients, therefore after a thorough literature review, three possible approaches were investigated. First, increasing the filtration rate to increase the nutrient loading second is the use of additional nutrients and third is the use of scraped schmutzdecke from a matured SSF as an inoculum for a new filter. The purpose of all the three approaches is to accelerate the growth of microorganisms. Based on the above approach, the current study had multiple objectives which were answered by using a combination of full scale and column scale Slow Sand Filters.

The following objectives (sub) were defined for the current study:

1. To optimise the scraping procedure

1.1 Determine the vertical distribution of biomass growth in slow Sand Filter.
1.2 Determine the effect of scraping (removal of schmutzdecke and sand bed) on the efficacy of slow sand filter.
1.3 Determine the spatial variability of biomass growth in slow sand filter.
1.4 Determine the biological activity in different layers of sand bed.

2. To accelerate the biological activity in the filters

2.1 Determine the effect of operational parameters: filtration rate (0 .1 m/hr vs. 0.5 m/hr), additional nutrients and addition of microbial inoculum on water quality parameters such as turbidity, particle count, dissolved organic carbon (DOC) & total nitrogen (TN) removal during the ripening period.
2.2 To determine the effect of operational parameters: filtration rate (0 .1 m/hr vs. 0.5 m/hr), nutrient loading and addition of microbial inoculum on bacteria *(E.Coli WR1)* and virus *(MS2 bacteriophage)* removal during different stages of ripening.

3. To identify the indicator of Ripening

1.4 Outline of the thesis

Chapter **2** presents a literature review of the Slow Sand filter relevant to our study that includes the physical and biological mechanism of filtration, role of schmutzdecke and sand bed in bacteria and virus removal, quantification of biomass, operational parameters affecting the ripening of slow sand filter.

Chapter **3** outlines the materials and methodology used that includes experimental description of the full scale and column SSF. The methodology used to measure biomass concentration and biological activity, inactivation potential of sand bed by carrying out spiking experiments of *E.Coli WR1* and *MS2 bacteriophage*, methods to measure turbidity, particle counts, dissolved organic carbon (DOC), total nitrogen (TN) and details of spiking experiments during stages of ripening.

Chapter **4** contains the results and the discussion part of objective 1. Chapter **5** contains the results and the discussion part of the objective 2. Chapter **6** contains results and the discussion part of objective 3. Chapter **7** contains the conclusion and recommendation for further work.

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Chapter 2

Literature Review

This Chapter includes the overview of SSF, mechanism of filtration, role of schmutzdecke and sand bed in bacteria & virus removal, biomass distribution and operational parameters affecting the ripening period of SSF.

2.1 Overview of SSF process

The first known water treatment system to use components of slow sand filtration was made by John Gibbs for his bleachery in 1804 in Paisley, Scotland. After some improvements in his design James Simpson constructed first slow sand filter in 1829 to be used in public water supply at Chelsea Water Company in London *(Baker, 1949)*. Initially slow sand filters were regarded as a mechanical means of straining out of suspended solids and turbidity and the original benefits were seen as good aesthetic quality of water *(Huisman & Wood, 1974)*. But after Jon Snow linked the outbreak of diseases cholera and typhoid to waterborne contamination, SSF become a legal requirement for all portable water extracted from river Thames from 1852 *(Huisman & Wood, 1974)*. The effectiveness of the SSF was further convinced during a cholera epidemic in two cities of Germany, Altona & Hamburg. Both cities withdraw their water from the River Elbe, Hamburg delivered their drinking water untreated while the Altona filtered whole of its supply, avoiding epidemic when the river water become infected with cholera organisms.

2.1.1 Element of Slow Sand Filter

In slow sand filters, the water purification is achieved by a combination of physiochemical and biological processes. The basic elements of a slow sand filter are: supernatant water layer, sand bed, underdrain system and a flow control system as shown in figure 2.

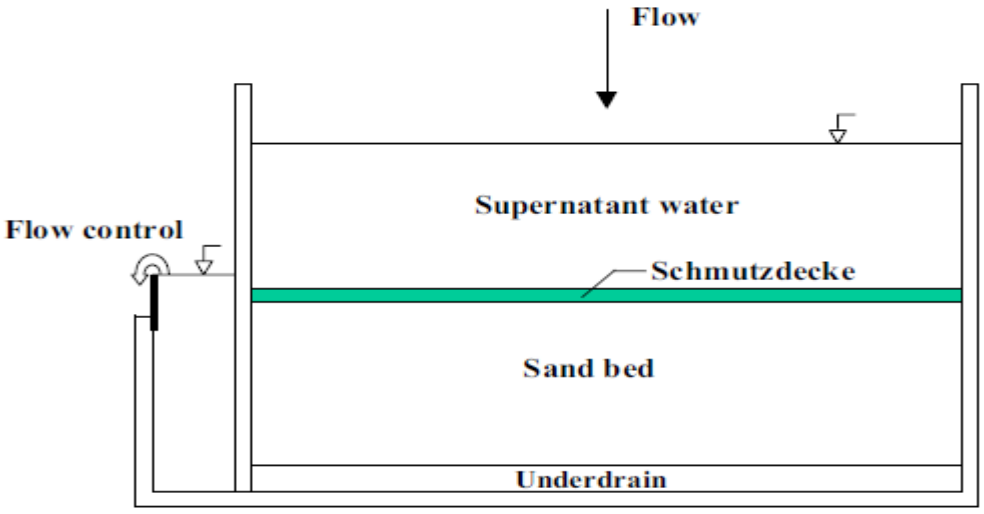


Figure 2: Schematic representation of a Slow Sand Filter

1. Supernatant Water: The supernatant water provides a constant head above the filter medium (Sand Bed) that pushes the water downwards.

2. Filter medium: Sand is the most commonly used filter bed medium because of its low cost, durability and availability, although other granular materials such as diatomaceous earth can also be used *(Campos, 2002).*
3. Underdrain: The underdrain serves the dual purpose of supporting the filter bed and providing passage to the water from the filter bed *(Visscher et al., 1987)*.
4. Flow Control: A flow control system regulates the filtration rate through the sand bed in order to prevent the raw water level dropping below a predetermined level during operation.

2.1.2 Design parameters

SSF is regarded as an effective treatment technology for good quality surface water with turbidity less than 10 NTU and colour less than 5 CU *(Campos, 2002, Sharpe et al, 1994)*. Variation in the ability of slow sand filters to reduce turbidity below 1 NTU has been observed under different influent condition *(Logsdon, 1987)*. The different degrees of turbidity reduction in some cases have been attributed to the nutrient condition of the filters which helps in the growth of bio-population *(Logsdon, 1987)*. Bellamy observed that raw water from the Rocky Mountains with initial turbidity of 6 – 8 NTU with low nutrient concentration when passed through SSF's was not able to lower the turbidity below 1 NTU as compared to an open water source of turbidity 0.4-4.6 NTU with higher nutrient concentration *(Bellamy et al, 1985a, 1985 b)*. Slow sand system varies considerably in their design aspects depending on the influent water quality. Table 1 summarise the main characteristics (Design Parameters) of the SSF system reported by various authors.

Table 1: Characteristics of Slow sand Filter (Adapted from Pyper and Logsdon 1991, Galvis et al, 1998)

Design Criteria	Ten States Standards (1997)	Huisman and Wood (1974)	Visscher et al. (1987)
Filtration Rate ($m^3/m^2/hr$)	0.08-0.24	0.10-0.40	0.10-0.20
Effective Sand Size (mm)	0.30-0.45	0.15-0.35	0.15-0.30
Uniform Coefficient of Sand	<2.5	<3	<5
Initial Depth of Sand (m)	0.8	1.2	0.8-0.9
Depth of Supernatant Water (m)	≥0.9	1-1.5	1
Depth of Support Media Including Underdrains (m)	0.4-0.6	Not stated	0.3-0.5

Raw water quality limits the used of slow sand filters because suspended and particulate matter tends to be removed at the top of the filter and slow sand filters have limited capability to remove inorganic contaminants and synthetic organic chemicals *(Logsdon,*

1987). An important parameter in the raw water is the algae content, almost all the surface waters contains algae *(Cleasby et al, 1984a)*. The amount and nature of algae depends on the temperature, turbidity, concentration of nutrients and the amount of sunlight *(Huisman, 1974)*.

Raw water quality limitations of slow sand filters have been overcome by using pretreatment techniques, such as micro straining, roughing filters and pre ozonation. Further modifications were made in the design of SSF to overcome the limitations such as use of filter mats and surface amendments *(Rollins, 1991).* Filter mats such as non woven fabric were placed on the top of sand bed which increased the porosity and surface area, thus more efficient filtration medium than sand resulting in longer filter runs. Another important advantage of using filter mats is limiting the passage of impurities through the fabric into the sand and a simple cleaning arrangement *(Graham & Mbwette, 1988; Vochten et al., 1988; Pulin et al., 2007).*

Bauer et al., (1996) used the combination of granular activated carbon and slow sand filtration to achieve higher organic removal, reduction of disinfection by products (THM) and micropollutants. The combination resulted in capital cost savings and potential for rapid implementation. Another major modification made to improve the operational aspect of SSF was covering of the filter bed. Covering of a slow sand filter reduces algal biomass growth in the supernatant water and lower level of organics (AOC & DOC) *(Campos, 2002; Schellart, 1996)*. It prevents the deterioration in the quality of water during periods of low temperature, thus no frozen filter surfaces and higher capacity all over the year. No faecal contamination of birds and thus no introduction of coliforms, pathogenic microorganism, fertilizing nitrogen and phosphorus compounds in the influent water over the filter *(Schellart, 1996)*.

2.1.3 Cleaning & Resanding

The long hydraulic detention time of water above the sand bed leads to the development of substantial biological community. The particles that deposit on the sand bed include organic material and microorganisms that contribute to the development of schmutzdecke layer. Depending upon the influent water quality (turbidity and suspended solids concentration), the duration for which a slow sand filter can be operated varies greatly (60 days to more than fifteen years). The filter surface becomes clogged due to the gradual accumulation of inert particles, microorganisms and the development of biological community. This results an increase in hydraulic resistance to flow and increase in headloss *(Huisman, 1974)*.

SSF's are cleaned by scraping the schmutzdecke layer and the top few centimeters of sand bed (varies depending on the filter) as shown in figure 3. Filter cleaning is done when with maximum water head above the sand and the outlet valve fully open, it is no longer possible to achieve the designed flow rate *(Camps et al, 2002).* The headloss needs to be measured regularly in order to determine the time of cleaning. Without any measurement of head loss the only true indicator of build up resistance is the degree of opening of the regulating valve. Cleaned filters require a ripening period during which the microbial communities re-establish before becoming fully efficient again.

Figure 3: Cleaning of a full scale filter at Scheveningen, Dunea NV

In order to remove the air from interstitial spaces which causes flow blocking and initial head loss, the filter is refilled with water in slow upflow procedure untill there is at least 30 cm of water at the surface *(Letterman, 1991; Kors, 1996).* The scraped sand can be reused after washing it in a shaker/hydrocyclone process reducing most of the suspended solids and washed sand is then left to dry in the sunlight and is ready for reuse as shown in figure 4. The sand bed needs to be refilled after consecutive scrapings when it reaches its minimum level (usually 0.5-0.6m). The resanding frequency and depth of sand bed that needs to be refilled depends on the scraping frequency which can vary from 8 to 10 years *(Collins, 1991).*

(a) *(b)*

Figure 4: (a) Shaker (b) Sand cleaning machine at Scheveningen Dunea NV

The bed can be restored back to its original depth by using several methods as suggested by *Toms & Bayley* which includes resanding, trenching and deep skimming. In dry resanding method, the bed is double skimmed and then clean sand is put evenly onto the top untill full bed depth is restored. In trenching the clean sand replaces the older sand at the bottom and the older sand is placed on the top. Deep skimming is done by completely replacing the entire sand upto the gravel support *(Toms & Bayley, 1996). Kors et al., (1996)* studied the effect of dry resanding in comparison to wet slurry method in which sand is mixed with water and sand slurry is pumped onto the filter bed. Dry technique resulted in serious head loss development and additional head loss at the interface of old sand and the refilled sand in comparison to wet slurry method.

2.2 Removal Mechanisms in SSF

A number of complex forces contribute to the removal mechanism in slow sand filters. SSF's are capable of removing pathogenic microorganisms such as bacteria, cysts, viruses and parasites *(Poynter and Slade, 1978; Graham, 1999)*. Along with that SSF are able to remove variety of contaminants with different removal capacity as mentioned in Table 2. There are two main removal mechanisms that operate in slow sand filters: Physico-chemical mechanism and biological mechanism. Although these processes have been given separate names, physico-chemical process acts simultaneously along with the biological mechanism

of removal which makes them very difficult to separate. As said by E. B White, there's no limit to how complicated things can get, on account of one thing leading to another, SSF present a perfect example justifying this quote.

Table 2: Performance Summary of SSF [Adapted from Gimbel & Collins (2006), Haig S. (PhD, 2011)]

Water Quality Parameter	% Removal Capacity	Reference
Assimilable Organic Carbon	14-40	*Lambert & Graham (1995)*
Biological Dissolved Organic Carbon	46-75	*Lambert & Graham (1995)*
Chemical Oxygen Demand	15-25	*Haaroff & Cleasby (1991)*
Cryptosporidium	99.99	*Hijnen et al., (2007)*
Giardia Cysts	90-99.99	*Bellamy et al., (1985a,b); Pyper, (1985)*
Coliform removal	97 -100	*Cleasby et al., (1984a)*
Dissolved Organic Matter	25-75	*Graham (1991)*
Enteric Viruses	99-99.99	*Polynter & Slade (1977)*
Dissolved organic Carbon	5-40	*Lambert & Graham (1995)*
Enteric Bacteria	90-99.9	*Hijnen et al., (2007)*
Iron	30-90	*Ellis (1985)*
Manganese	30-90	*Ellis (1985)*
Pesticides	0-100	*Lambert & Graham (1995)*
Total Organic Carbon	15-25	*Haaroff & Cleasby (1991)*
Colour	25-40	*Ellis (1985)*
Turbidity	90-98	*Smet & Vissher (1989)*
Zoospores	99-100	*Calvo- Bado et al., (2003)*

2.2.1 Physico-chemical mechanism

Physico-chemical mechanism can be divided into straining & 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, 1974).* Building up of the schmutzdecke layer is in part a consequence of the straining mechanism operating in SSF *(Campos, 2002)*. Transport mechanisms are responsible for removing particles out of their flow streamlines into the proximity of the grain surface. These include sedimentation, diffusion, interception, inertial and centrifugal forces. The significance of the various transport mechanism depends principally upon the flow rate, particle size, grain size and temperature *(Ives, 1970)*. Sedimentation occurs within the pore space (spaces between grains) of the SSFs and removes particles which are smaller than the pore space by settling on the sand grains *(Haig, 2011)*. Figure 5 shows how inertial and centrifugal forces act upon the particles with specific gravity higher than that of the surrounding water causing them to move out of the flow line and deposit in the crevices between the sand grains.

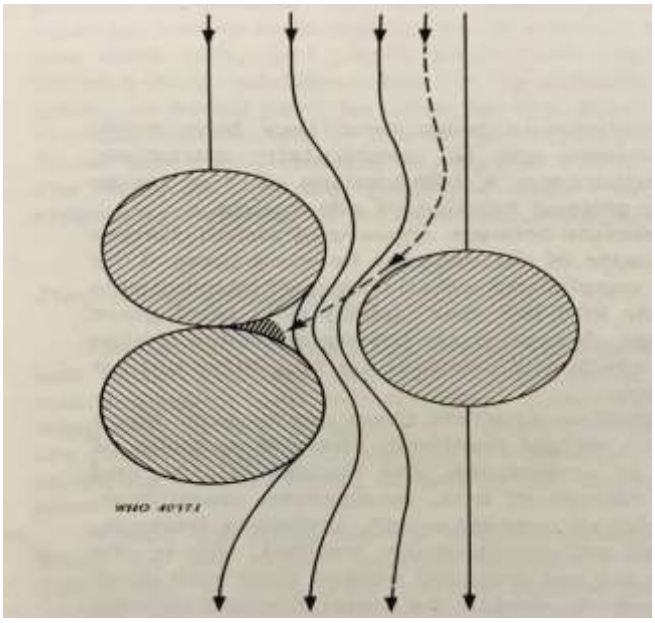


Figure 5: Transport of discrete particles in Water (Source: Huisman, 1974)

Adsorption is a physicochemical removal process which favours removal of dissolved substances and colloidal suspensions. The success of absorption 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 & 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, along with bacteria while the metallic ions and organic matter is positively charged. Because of this a ripening period is required for slow sand filters to allow for the charges in the filters to accommodate attachment of biological life *(Huisman, 1974).*

2.2.2 Biological Mechanism

The major modes of biological action in SSF are believed to be the microbial competition and biodegradation in the schmutzdecke layer and sand bed and bactivory by protozoa *(Hunter et al., 2013).* The microbial community of the slow sand filters is diverse with variety of organisms performing different functions *(Duncan, 1988). Nakamoto, 1999* defines slow sand filters as an ecosystem where various organisms work under aerobic condition. The most predominant organisms are gram-negative pigmented bacteria such as Pseudomonas and Aeromonas along with algae, protozoa, and higher order eukaryotes (*Eighmy et al., 1993)*. Other predacious fauna include meiofaunal species (0.1 to 1mm in size), which feed on individual bacterial or algal cells, suspended particles, or other species *(Duncan, 1988).* Small organisms like Protozoa catch and trap any particulate matter

including germ cell. In this way there is a food chain from small protozoa to insect larvae, oligocheata, etc which is key for water purification.

The importance of biological activity in the SSF was investigated by *Webber-Shrik & Dick, (1997a)* by investigating the physicochemical and biological removal mechanism of E.Coli and particles in the presence and absence of sodium azide. Sodium Azide is used an inhibitor for biological activity. Presence of sodium azide resulted a decrease in removal of both E.Coli and particles smaller than 2µm indicating the importance of biological activity in performance of Slow Sand Filter *(Weber-Shrik & Dick, 1997b).*

The biological activity in the slow sand filters increases with increased residence time in the filters. *Elliott et al,. (2006)* demonstrated that removals of E. coli, bacteriophage *MS2* and PRD1, and a human enteric virus (echovirus type 12) by intermittent household scale biosand slow sand filters increases when water retained in the filter beds was allowed to sit idle overnight, thus providing longer residence times for the seeded microorganisms to be eliminated by predation or attachment to biofilms and media grains. Removals increased as the filters became biologically mature, further suggesting biological mechanisms of removal *(Elliott et al., 2006).*

A new biological mechanism of removal has been proposed by several authors where by incoming pathogenic bacteria is either out competed or inactivated by naturally occurring bacteria known as autochthonous bacteria in the sand bed *(Guchi, 2015)*. *Sattar et al., (1999)* found that presence of autochthonous microorganism in the natural environment resulted a decline in the concentration cryptosporidium, and the phenomenon was termed as "bioantagonism".

The actual mechanism of bioantagonism is yet to be understood clearly and no specific microorganism have been identified which are responsible for this. *Uhl et al., (2000)* also observed that the presence of autochthonous bacteria resulted a sharp decrease in the concentration of pathogens in biofilters. Similar kind of autochthonous bacteria could be responsible for oocyst decay in slow sand filters *(Scleary, 2005)*. The reason for this could be the fact the all bacteria requires organic matter to thrive and grow but the growth rate of autochthonous bacteria is high even in low concentration of organic matter (less than 1mg/l) thus outcompeting the pathogens which require high concentration *(Uhl et al., 2000)*.

2.3 Mechanism of Viruses & Bacterial removal by Slow Sand filters

Slow sand filtration employs both physico-chemical processes (adsorption/attachment to sand grains and biofilms) and biological processes (predation and inactivation by microbial enzymes) in the removal and inactivation of bacteria & viruses. Published literature reviews have credited slow sand filtration with E. Coli removal ranging from 2-4 log, *MS2*removal 1.5-2 log *(Hijnen et al., 2004)*, 0.2-2.2 log removal *(DeLoyde et al., 2005)*, enteric virus removals of 2 to 4 logs *(Amy et al., 2006)*.

The major modes of virus removal/ inactivation (table 3) are attachment to sand grains and biofilms. Some authors have reported that elimination of viruses by biological processes may be an equally important process during slow sand filtration *(McConnell et al., 1984; Poynter and Slade, 1977)*. The mechanism of removal of bacteria (table 4) is similar to viruses but since bacteria is much bigger in size as compared to virus, adsorption/ attachment to granular media is less pronounced in bacterial removal and more due to biological processes such as predation and microbial activity in the schmutzdecke and sand bed. Some eukaryotes are known to be predators to bacteria, while some microorganisms simply produce substances that are toxic to enteric bacteria *(Lloyd, 1973).*

Table 3: Mechanism that contribute to the Virus removal / inactivation during slow sand filtration

Removal Mechanism	Influence
Biological Activity	Increased biological activity and longer residence time in non-sterile water leads to increased virus inactivation*(Poynter and Slade, 1977; Elliott et al., 2006)*
Attachment to Biofilms	Viruses can be entrapped in or adsorbed onto biofilms *(Wheeler et al., 1988; Storey and Ashbolt, 2001 and 2003)*
Predation	Filter feeding protozoa and bacteria can ingest viruses *(Kim and Unno, 1996; Cliver and Herrman, 1972)*
Adsorption/attachment to granular media	Viruses undergo reversible adsorption/attachment; long term detachment has been observed after seeding stops *(Schijven et al., 2003; Hijnen et al., 2004; Dullemont et al., 2006)*

Table 4: Mechanism that contribute to the Bacterial removal / inactivation during slow sand filtration

Removal Mechanism	Influence
Role of Schmutzdecke	The removal of bacteria increases as the ripening proceeds, schmutzdecke builds up *(Dullemont et al., 2006)*
Predation	Filter feeding protozoa can ingest bacteria from both the suspension and schmutzdecke surface *(Lloyd , 1973)*
Biological Activity	Increased filter bed maturity and longer residence time in the filter bed increases the bacterial removal *(Bellamy et al, 1985 a,b)*

2.3.1 Adsorption

Adsorption plays a significant role for the removal of virus. The adsorption of viruses to sand grains is typically poor due to the negative surface charges that both exhibit at natural water pH *(Schijven et al., 2000)*. The most significant force preventing virus adsorption to a grain surface is electrostatic repulsion. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory predicts that as like-charged particles get closer together, repulsive energy increases and creates an energy potential barrier that must be overcome in order for adsorption to occur *(Schijven et al., 2000).* If the repulsive barrier is overcome, then the proximity of the two particles allows van der Waals attraction to dominate. The result is that rapid, strong and irreversible adsorption will occur in the primary minimum *(Schijven et al., 2000).*

The DLVO theory also states that a weakly attractive secondary minimum exists at further separation distances, but adsorption in the secondary minimum is rapid and reversible *(Schijven et al., 2000).* Therefore, viruses would be removed permanently during filtration if irreversible adsorption in the primary minimum occurred, while viruses adsorbed in the secondary minimum would desorbs and be washed out of the filter over time *(Schijven et al., 2002)*.

It has also been demonstrated that increased ionic strength leads to increased virus adsorption to granular media *(Bales et al., 1991; Lance et al., 1982*). Multivalent cations have been shown to improve the adsorption rate of more negatively charged *viruses (Harvey et al., 2004)*. If ionic strength is decreased, previously adsorbed viruses can detach due to double layer expansion and increased repulsion between virus and media *(Bales et al., 1993; Lance et al., 1982; Funderburg et al., 1981)*.

2.3.2 Attachment to Biofilms

Biofilms contain many sorption/attachment sites particularly extracellular polymeric substances (EPS), which are a web-like matrix of polysaccharides, proteins, and lipids which are capable of accumulating inorganic and organic particles, including bio colloidal enteric viruses *(Storey and Ashbolt, 2003; Flemming, 2010).* Bacteria such as Pseudomonas aeruginosa are known to produce extra-cellular polymeric substances (EPS), polysaccharides and proteins, which serve to anchor bacteria to surfaces *(Dai et al., 2002)*. *Wheeler et al. (1988)* suggested that these extra- cellular polymers could also provide binding sites for viruses. Since biofilms generally have a porous, low-density structure when hydraulic shear is low and a smooth, patchy, and dense structure when shear is high, low flow rates will promote the development of porous biofilms that offer more attachment sites for particles *(van Loosdrecht et al., 1995)*. This study was carried out in waste water

where the thickness of the biofilm is much higher (due to higher organic concentration) as compared to biofilms in drinking water.

2.3.3 Predation

The most commonly proposed mechanism for predation is that predators graze on bacteria and detritus attached to sand grains *(Huisman & Wood, 1974: Poynter & Slade, 1977)*. The second proposed mechanism is that suspension feeding predators remove suspended particles as the particles flow through the filter *(Lloyd, 1973)*. Predators that graze on attached bacteria potentially free up sites for future bacteria attachment while suspension feeding predators directly remove particles from the mobile phase. *Campos et al., (2002)* propose that grazing activities by protozoa might be a result of the saturability of the entire adsorption site and the ability of bacteria to grow in the sand bed and detach again.

Lloyd (1973) & Richards (1974) suggested a relationship between bacterial removal and the number of Vorticella or flagellates and ciliates in the slow sand filter. In a series of laboratory scale experiments carried out by Lloyd it was observed that in the absence of ciliate protozoa, non predatory mechanism of bacteria removal did not exceed 96% while using 1000 V. Convallaria resulted in more than 99.8 % removal. It was also observed that these ciliate protozoa did not penetrate deep into the sand bed which was in agreement with the field surveys carried out by *Richards, (1974)* and *Evins & Greaves, (1979)*. Protozoa derive their nutrition by grazing on algae and bacteria, in some cases, on smaller protozoa and by ingesting particulate organic matter *(Tebbutt, 1988)*. Since most of the algae and bacteria are present in the supernatant water layer and top few centimeters of the sand bed, the protozoan population decreases with depth in the sand bed.

Galal et al., (1989) investigated the vertical distribution of the ciliated protozoa and the bacterial populations at the Ashford common water treatment along with the particulate organic carbon and chlorophyll-a. The bacterial population was largest in the top 0-10 cm of the sand bed and sharply declined with depth. Similar trends were observed in the densities of the ciliate organisms, concentration of POC and chlorophyll- a.

2.3.4 Role of Schmutzdecke and Sand bed

It has been reported by many authors that sand bed plays a significant role in the removal of viruses as compared to the schmutzdecke, whereas schmutzdecke plays a major role in the removal of Bacteria *(Dullemont et al., 2006; Hijnen et al., 2004; McConnell et al., 1984; Poynter and Slade, 1977)*.

A study by *Dizer et al., (2004)* found that coliphages removal by a SSF increased as the filter matured. Removal of Coliphages 138 was 0.4 logs in the first experiment when a schmutzdecke layer was absent and 2 logs in the second experiment after two months of ripening when a Schmutzdecke developed.

Similarly, *Windle-Taylor (1969)* found that while poliovirus-1 was effectively removed by a mature SSF as compared to the clean sterile sand where there was no removal. A study by *Wheeler et al., (1988)* demonstrated that the schmutzdecke layer and biological maturity of SSFs were responsible for virus removal. In one experiment, *Wheeler et al., (1988)* showed that a biologically mature SSF with a schmutzdecke layer removed simian rotavirus SA11 concentrations by 1 log, compared to no removal in an acid washed sand filter or a clean (sterile) sand filter for the same detention time. This implies an important role of microorganisms in the schmutzdecke and filter bed for virus removal. In addition, a mature SSF would be expected to have improved physical filtration capacity due to accumulation of material in the sand bed, including extracellular polymeric substances produced by the resident microorganisms and other organic and inorganic matter.

A series of spiking experiments were carried out by *Hijnen et al., (2004)* to assess the Decimal Elimination Capacity (DEC) of slow sand filter for virus, bacteria and oocysts of cryptosporidium in full scale, pilot plant and columns experiments. *Hijnen et al.* reported that schmutzdecke scraping had a marginal effect on *MS2*removal but DEC for bacteria was reduced by 2 $\log_{10}$ after scraping.

Based on the results, *Hijnen et al. (2004)* concluded that, in addition to biological activity, both straining and adsorption are significant removal mechanisms in SSFs controlling the elimination of microorganisms larger than viruses. Therefore, they argue that schmutzdecke scraping did not affect phage removal because they are too small to be strained by the schmutzdecke *(Hijnen et al., 2004)*.

Bellamy et al., (1985 b) reported a reduction by a factor of 10 - 100 in E. coli removal after the scraping of schmutzdecke layer and replacement of sand respectively implying most of the bacteriological removal in SSF occurs within the schmutzdecke layer where as virus removal is affected by the maturity of the sand bed. *Bellamy et al., (1985a)* also reported that a new SSF with virgin sand and virgin support gravel seeded with Giardia cysts achieved 2.1 logs removal, compared to >4.6 logs removal in an 80-week-old SSF with mature sand and gravel beds. *Bellamy et al., (1985 a)* also found that cyst removal did not deteriorate after filter scraping implying the efficacy of the maturity of sand bed.

Poynter and Slade (1977) found that scraping of the top 2.5 cm of schmutzdecke from pilot SSFs had a negligible effect on poliovirus-1 removals for short-duration cleaning. The short duration filter cleanings lasting only a few hours caused minor decreases (0 to 0.3 logs) in poliovirus-1 removal. However, after the filters were drained for 24 hours (without schmutzdecke scraping), poliovirus-1 removals dropped by ~1.1 logs. Poynter and Slade (1977) concluded that SSF performance for microorganism removal was impacted more negatively by the duration of SSF draining than by the scraping of the schmutzdecke.

2.4 Biological Aspects of SSF

The maximum treatment efficiency or the optimal performance of slow sand filters is achieved only when the filter is fully matured and acclimatized with a steady biomass population. A steady state is achieved when there is equilibrium between the microorganism's population and the availability of substrate (nutrients) under the ambient water quality conditions.

The biomass population changes dynamically responding promptly to the changes in temperature, influent organic concentration and dissolved oxygen and tries to re-establish a steady state population *(Duncan, 1989)*. The effect of temperature is more prominent as compared to other factors as observed by *Seger and Rothman, (1996)* in the biomass population at the top of the sand bed which is much more affected by changes as compared to deeper levels.

2.4.1 Characteristics of the Schmutzdecke:

As mentioned earlier, as the filtration progresses the schmutzdecke layer builds up and contributes to the removal of water pollutants and microorganisms. There are various descriptions available for schmutzdecke development in slow sand filter filters suggesting that the characteristics of the schmutzdecke vary significantly depending on location and season. The term schmutzdecke has been described by *Huisman & Wood (1974)* as a thin slimy matting of material organic in origin, consisting of filamentous algae and other forms of life including diatoms, protozoa, rotifers and bacteria. *Nakamoto (1993)* defines schmutzdecke as a sticky algal mat formed on the sand surface of a slow sand filter.

The development of a Schmutzdecke layer on a sand surface occurs through a sequence of specific processes *(Cooksey and Wigglesworth-Cooksey, 1995)*. Bacteria are the primary colonisers of the sand surface and attached via electrochemical interactions e.g., Van der Waal. The surface colonisation by bacteria proceeds through an ordered series of recruitment processes; first, pioneer species of bacteria (primary colonisers) interact with

the conditioning film and form the initial assemblage of surface biota and biopolymers *(Marshall, 1992)* followed by the diatoms, insect larvae and invertebrates *(Wolfaardt et al., 1994)*. Attached bacteria play crucial role in the biodegradation of contaminants and clogging of the porous media *(Rittman, 1993)*. The net accumulation of the bacteria in porous media is controlled by four processes: growth, deposition, decay and detachment.

Joubert at al., (2008) studied the formation of microbial community using Environmental Scanning Electron Microscopy (ESEM) during an 8 week ripening period. Bacteria appear to be the primary colonisers of the sand surface with extracellular matrix covering the bacteria *(figure 6.2)* followed by the colonisation of the sand particles with diatoms *(figure 6.3)*. After 3rd week, the physical structure of the sand surface began to alter due to the accumulation of microorganisms and their extracellular and breakdown products. Diatoms grow in number and became more apparent in week 4 *(figure 6.4)*. The accumulation in the biofilm and an increase in microbial biodiversity, continued in Weeks 5 and 6 *(figure 6.5)* with diatom becoming the dominant species in number and variety. The trend continued in week 7 *(figure 6.6)* and diatoms also become embedded in the biofilm. The microorganism community continue to grow and the sand particles were covered to such an extent that no part of the sand surface was visible after week 8 and only the biofilm covering the sand grain could be seen *(Joubert et al., 2008)*.

2.4.2 Characteristics of the Sand Bed

In addition to the development of schmutzdecke layer on the top of the sand, maturity of the sand bed is a critical factor influencing the removal properties of SSF. Knowledge of biomass in the sand bed is important to understand the headloss development in SSF system, as too much biomass accumulation can clog filters *(Campos et al., 2002)*. Concept of maturation is both microbiological and physicochemical. The former requires the establishment of vigorous population of protozoa, metazoan, fungi, bacteria and algae. The latter requires the establishment of appropriate physicochemical properties throughout the filter medium in order to facilitate adsorption *(Wheeler, 1988).* The biological maturity of the SSF bed, which increases over a finite time period but is not directly measurable, has a major influence on virus, oocyst and cyst removals. It has been consistently shown that removal increases as filter maturity increases. Each layer of the sand bed has its own inactivation potential depending on the vertical distribution of microorganisms and biomass.

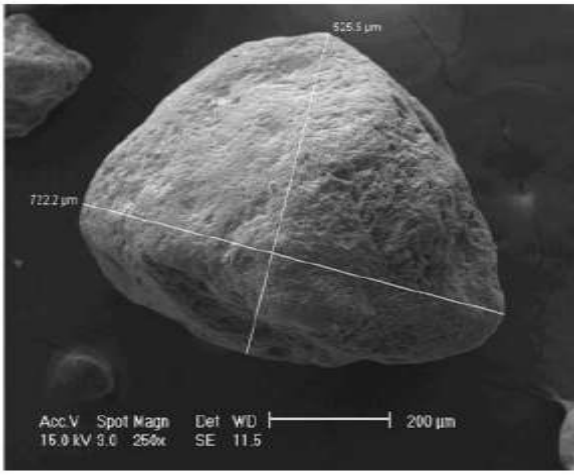


Fig 6.1 Micrograph of Control Sample

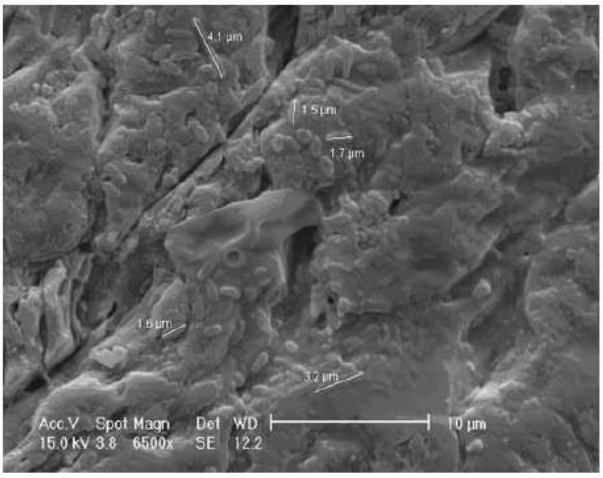


Fig 6.2 Micrograph of Sampled removed at Week 1 proliferation of bacteria

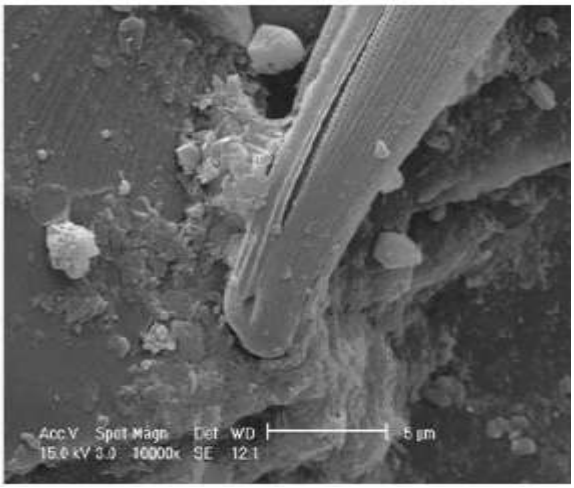


Fig 6.3 Micrograph of Sampled removed at Week 3 demonstrating the first sign of diatoms

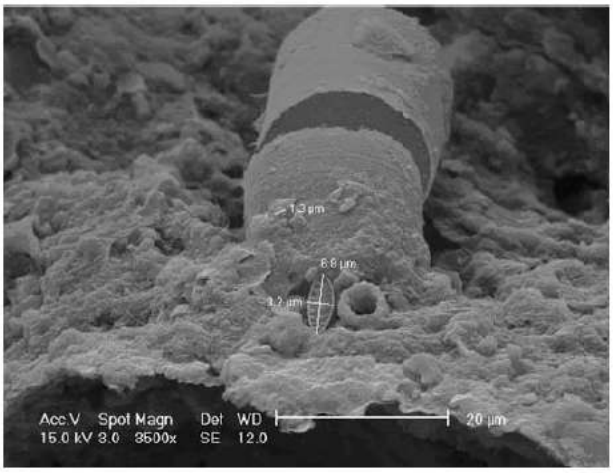


Fig 6.4 Micrograph of sample removed at week 4 exhibiting a variety of diatoms

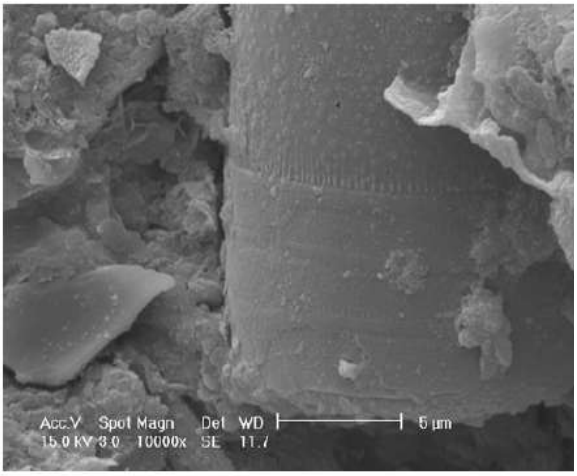


Fig 6.5 Micrograph of sample removed at week 5 showing Melosira Varians

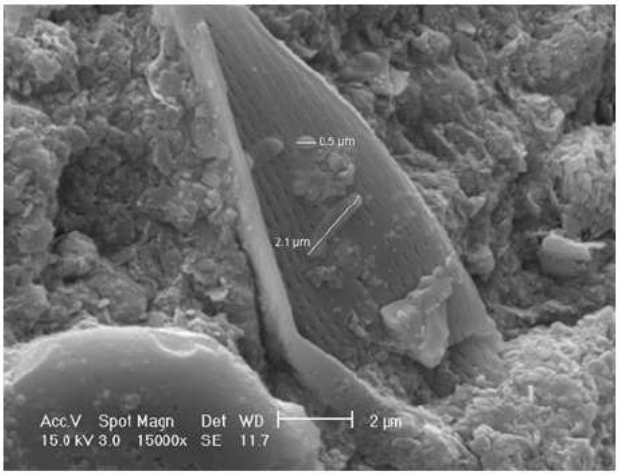


Fig 6 .6 Micrograph of sample removed at week 7 showing a combination of bacteria and diatoms.

Figure 6: Visual Representation of the Microbial Community during an 8 week ripening period (Source: Joubert et al., 2008)

2.4.3 Quantification of Biomass

As the filtration progresses, the biomass accumulates in the schmutzdecke and contributes to the headloss development. Therefore quantification of biomass growth in schmutzdecke and sand bed and the factors affecting its growth would improve understanding of the complex interactions between the different (biological and physico-chemical) processes operating in the SSF *(Campos et al., 2002)*.

The vertical distribution of biomass in sand bed depends on the food availability which in turn depends on the influent concentration of the organic matter and filtration rate. At higher filtration rate the substrate/food is penetrated deep into sand bed allowing the algae and detritus particles to penetrate deeper and thus motivating protozoa to penetrate deeper into sand bed. Since higher filtration rate allows microorganism to grow deeper into sand bed, it can also result in the breakthrough of bacteria into the effluent *(Huisman, 1974)*. The size of sand media can also effect the vertical distribution of biomass; bigger pore size allows deeper penetration of organic matter into san bed and thus more substrate availability in deeper layers *(Scleary, 2005)*.

There are various methods to quantify microbial biomass in the schmutzdecke layer and sand bed of SSF *(Duncan, 1988)*. The different approaches (as shown in table 5) used to measure biomass concentrations confound inter-study comparisons of biomass development and behaviour in SSF because of the inconsistent units and sampling intervals *(Campos et al., 2002; Yordanov, 1999).*

The distribution and activity of the microbial populations within the media of covered slow sand filters were investigated by *Eighmy et al., (1992, 1994)* and *Collin et al., (1994)* Quantification of the bacterial populations in the schmutzdecke layer was done by measuring biomass and bacteria counts. The bacterial population decreased with the depth and the filter biomass was significantly correlated to bacteria counts. In the schmutzdecke the bacterial population counts were $1*10^{9}$ /g dry weight of media and the biomass content of the filter bed was 2.5-4.5 mg/g dry weight.

Biological activity in the uncovered slow sand filters (ozonated and non ozonated) was characterised by *Seger & Rothman, (1996)* by measuring ATP using luciferine-luciferase method and total cell count by epifluorosence microscopy. The biological activity decreased with depth and was not observed below 10 cm. The maximum amount of the ATP was in the top 5 cm of the ozonated filter was approximately $7.5x10^{-8}$ g/g dry weight which was higher than the reference filter receiving non ozonated water at 17 ^{0}C. Similar to Eighmy and

Collins, a significant correlation was observed in the bacterial counts and the biomass development in both the reference and the ozonated filter.

Table 5: Different Method to quantify Biomass

Biomass Determination	Technique	Reference
Bacterial Population	Acriflavine Direct Cell Counts[1]	Eighmy et al, 1992
ATP content	Luciferene- Luciferase Method[2]	Seger & Rothman, 1996
Total Cell Count	Epifluorosence Microscopy	Seger & Rothman, 1996
Cell Protein	Follin Reactive Material[3]	Yordanov et al,1996
Chlorophyll a	Direct Extraction from the sand sample using boiling 90% ethanol*	Yordanov et al,196
Total Biofilm Carbohydrate	Phenol Sulphuric Acid Method**	Yordanov et al,1996
Viable Bacteria Numbers	Heterotrophic Spread Plate Counts on R2A medium	Yordanov et al,1996
Biomass Carbon	Chloroform-Fumigation[4]	Campos et al, 2002
ATP Content	Luciferene- Luciferase Method	Knezev et al, 2004

* As described by Nusch, 1980,

* * as described by Dubois et al., 1956

Yordanov, (1966) also quantified the biomass in covered slow sand filter using viable bacterial numbers, proteins, total biofilm carbohydrate and chlorophyll-a. Similar trends in biomass distribution with depth were observed in all the techniques as done by Eighmy and Collins. It was also observed that head loss development is associated with the biomass growth and consequently clogging of the filters. The net biomass accumulation was observed to be higher in the winter season due to the lower activities of protozoan's as they are associated with the removal of biofilm from the surface.

Campos et al., (2002) investigated the microbial biomass development in the sand and schmutzdecke layer in full-scale slow sand filters, operated with and without a light excluding cover. A simple logistic positive growth rate function of biomass concentration was observed as compared to the linear growth rate which was observed by the *Nakamoto,*

[1] The Acriflavine Direct Cell Count (ADCF): This method utilises acriflavine, a DNA specific Strain for easy enumeration of Bacteria in clear and humic waters *(Bergstrom et al, 1986)*.

[2] Luciferene- Luciferase Method is a rapid method for the determination of ATP. The ATP measurement is based on the reaction between two Proteins derived from flies luciferin (substrate) and luciferase (enzyme).

[3] The Folin Reactive Material (FRM): This method applies a standard solution for example a bovine albumin and folin reagants to measure cell protein through spectrometric analysis.

[4] In Chloroform Fumigation extraction method samples are exposed to chloroform vapour for 24 h or longer to lyse the microbial cells. Then the fumigated and non-fumigated controls are extracted with 0.5 M K_2SO_4. The difference between fumigated and non-fumigated carbon (C) is a measure of the chloroform labile C which is then multiplied by a factor to give microbial biomass C *(Setia et al., 2012)*.

(1999). Despite large differences in biomass growth in covered and uncovered filters, no differences were observed in the water quality of effluents from either filter and generally consistent removals of TOC and DOC were observed throughout.

Usually the major contribution to headloss in uncovered SSF occurs in the schmutzdecke and in the immediate underlying sand (top 2 to 4 cm) as most of the biomass accumulation take place in these regions but it is difficult experimentally to differentiate the contributions between these. *Campos et al, 2002* built a model to predict the headloss development at different depths of the sand bed and the schmutzdecke using the influent water quality concentrations (inert material, chlorophyll-a, bacteria, protozoa, non-living POC, dissolved organic carbon (DOC), phosphorus, nitrate-nitrogen, ammonium-nitrogen), temperature, flow rate, filter surface area, supernatant water level, filter bed depth, duration of filtration runs, and several other input parameters *(Campos, 2002).* Figure 7 provides an example for one particular SSF run. In this case, the schmutzdecke contributed very little headloss during the first 50 days of the run, but became increasing important with further time and was the major component of overall headloss at the end of the run.

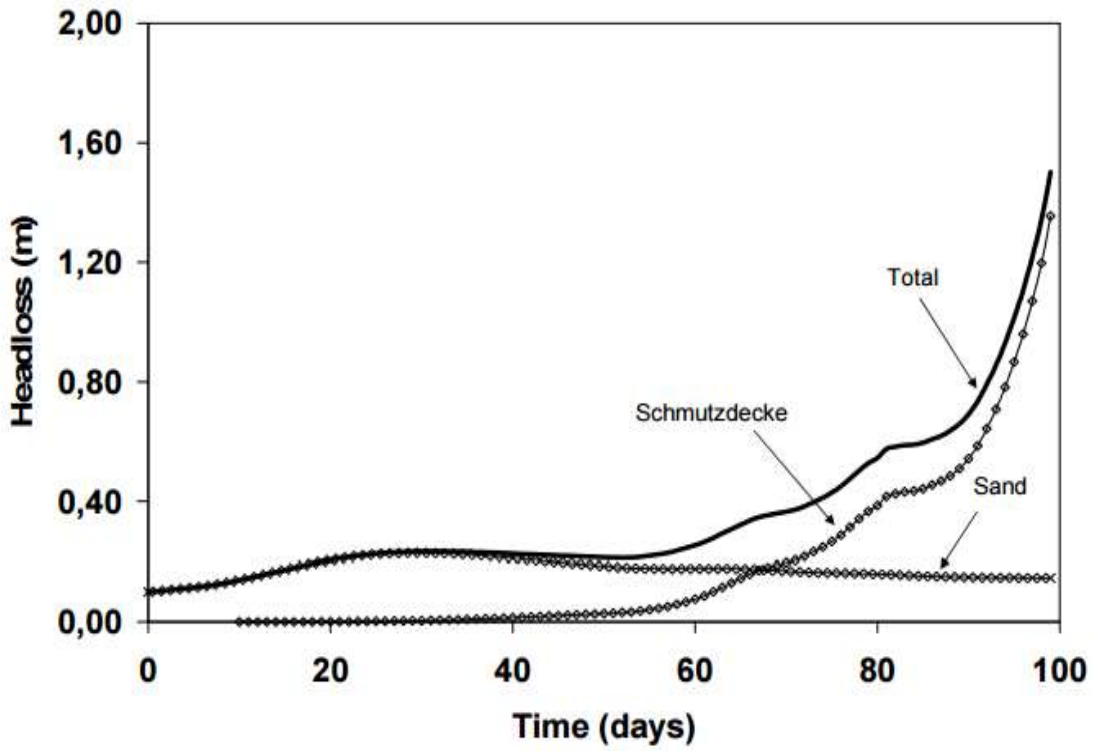


Figure 7: Example of model prediction of sand and schmutzdecke headloss development with time (Source: Campos et al., 2006b).

2.5 Factors affecting the Ripening Period

At the start of the filter the effluent is discharged into the waste depending on the influent water characteristics (several weeks in tropical climates and much longer in colder regions) *(Huisman et al., 1974)*.This allows the schmutzdecke and microbial community on the sand grains to grow. This is period is termed as ripening. During the ripening period the filter performance is suboptimal. Ripening period depends on the nature of raw water quality, temperature and filtration velocity which can take upto 30- 60 days to develop *(Duncan, 1988)* but can be speeded up by increasing the temperature *(Huisman et al, 1974).*

The ripening of the slow sand filter depends on the biological activity of the microorganism that grow in the schmutzdecke and sand bed. The organisms may be characterized by the compounds they use as sources of energy, as source of carbon, and as the hydrogen acceptor *(Van der Kooij et al, 1982)*. The growth of the microorganism required for the biological activity is bound by the availability of nutrients. Assimilable organic carbon (AOC) is the portion of total organic carbon (TOC) which is utilized by organisms for growth and has been suggested to be the main nutrient for microbial growth *(Miettinen et al, 1977; Le Chevallier et al, 1991)*.

Miettinen et al, (1997) found that inorganic nutrients also play a significant role in the microbial growth in water among which phosphorus and nitrogen are the two main inorganic nutrients. Microbes can readily utilize orthophosphate which are present in organic and inorganic colloids *(Jones et al., 1988; Baldwin, 1998)* which reduces their biological availability. *Sathasivan et al, (1997)* demonstrated the significance importance of inorganic phosphorus in controlling the regrowth of microbial community using different combination of acetate, phosphorus, and other inorganic nutrients. The results indicated the bacteria cannot simply grow on simple organic carbon source and tap water, bacterial growth suppressed when phosphorus was limiting.

In order to enhance the start phase of the SSF, *Van der Hoek et al.,(1996)* used the water of the of rapid sand filtrate instead of water after rapid sand filtration-ozonation-biologically activated carbon filtration. This was done to allow the filters to mature at a relatively high DOC load (2-2.5 mg C/l). The water after ozonation- biologically activated carbon filtration probably would contain a too low level of nutrients (DOC) to develop a schmutzdecke.

Bellamy et al. (1985a) compares the efficiency of the filters by lowering and enhancing biological activity. For lowering the biological activity, growth was prevented by using chlorine at concentration of 5 mg chlorine residual /L and biological activity was augmented by adding sterile synthetic sewage to the filter. The results of the experiments are shown in

the table 6 demonstrating the activity of the biological community increased from minimal biological community for the chlorinated filter to the augmented activity for the nutrients added filter, the percent removals of coliform, standard plate count bacteria, and turbidity increased significantly.

Table 6: Effect of Biological activity on operational performance of SSF, (Bellamy et al, 1985)

Parameter	No Biological Community , Filter 3* (Percent Removal)	Ambient Activity, (Control Filter) Filter 1	Augmented Biological filter (Percent Removal) Filter 4+
Total Coliform	60.1	97.5	99.9
Standard Plate Count	-89	-41	58
Turbidity	5	15	52

* Filter 3 was chlorinated between runs, + Nutrients were added continuously to filter 4

The major modes of biological action in SSF are believed to be the microbial competition and biodegradation in the schmutzdecke layer and sand bed and bactivory by protozoa *(Hunter et al., 2013).* The growth rate of protozoa is severely affected by the temperature. Ciliate protozoa cannot recolonize when the temperature is less than 3^0C. *Lloyd (1996)* also found when filter beds were drained for cleaning, rapid desiccation of these ciliate protozoa can occur and lead to reduced predation and impaired filter performance (microorganism breakthrough) upon start-up. *Sanchez et al. (2006)* found that scraping of pilot SSFs reduced protozoa populations on the surface of the sand bed from approximately 32,000 to 0 protozoa/cm^2, from 66,000 to 0 protozoa/cm^2, and from 30,000 to 3,700 protozoa/cm^2 in three different experiments. The impact of SSF cleaning on protozoa was less severe when less sand was scraped off. Protozoa populations returned to pre-scraping levels after 68 to 320 hours of operation *(Sanchez et al., 2006)*.

2.6 Operational parameters affecting the efficacy of SSF

Factors such as hydraulic loading rate, water temperature, sand depth, and biological maturity plays a significant role in the ripening of the filter and correlated to the removal of bacteria and viruses as shown below in table 7. The principal concern with increasing the loading rate is the possible reduction in the removal efficiency and the economic drawbacks of the shorter filter run. But it has been observed that removal efficiency of the slow sand filters is more a function of the filter bed maturity and bed depth rather than hydraulic loading *(Rollins et al., 1991; Wheeler et al., 1988; Duncan et al., 1988)*. The effect of the flow rate and sand depth on SSF efficiency suggests that to a certain extent, a reduction in

efficiency caused by higher flow rate might be mitigated by increasing sand depth. *(Wheeler et al., 1988)* but on increasing the flow rate a reduction in the filter run time is observed. All these factors add uncertainty in the working of the slow sand filters.

Table 7: Factors Affecting Bacteria & Virus Removal in SSF

Factor	Influence
Hydraulic Loading Rate	Major importance; lower HLRs promote greater virus and bacteria removals due to increased residence time in the SSF Range tested: 0.05-0.5 m/h *(Lance et al., 1982; Wang et al., 1981;Poynter and Slade, 1977)*
Water Temperature	Major importance; warmer temperatures increases virus and bacterial removals due to increased biological activity *(Dullemont et al., 2006; Poynter and Slade, 1977)*
Sand Depth	Greater virus removals observed for SSFs with deeper beds, likely due to increased residence time in the SSF and increased opportunities for attachment *(Graham et al., 1996; Slade, 1978)*
Biological Maturity	Virus and Bacterial removal increases with increased biological maturity. Maturity of sand bed plays a more significant role in the removal of virus as compared to bacteria. *(Dizer et al., 2004; Wheeler et al., 1988; Poynter and Slade, 1977)*
Schmutzdecke Scraping	No major effect on virus removals; SSF draining >24 h for cleaning may desiccate microbes in the bed. Significant difference in the removal of E. Coli on removal of schmutzdecke. *(Hijnen et al., 2004; Dullemont et al., 2006; McConnell et al., 1984; Ellis, 1985)*

2.6.1 Filtration rate:

Controlling the rate of filtration is the key to adequate functioning of a slow sand filter. Typically the filters are operated at a rate between 0.1 m/hr to 0.4 m/hr in case of surface waters *(Rachwal et al., 1988: Ellis et al., 1985)*. Higher filtration rate tends to clog the filter within a shorter period of time, thus minimising run period and increasing cleaning frequencies which are time consuming as well as costly. It has also been observed that in case of slow filter rate, the DO concentration becomes severely low during the night and the microorganisms near the surface sand layer escapes to the deeper sand layer resulting in the clogging of the filter. These microorganisms may also be leaked into the filtrate *(Nakamoto, 2014).* An indication of the filter being operated at a very slow rate for too long will be the presence of iron bacteria in the filtered water *(Toms & Bayley, 1996)*. In cases where water is of very good quality higher filtration rate can be applied. In Amsterdam slow sand filters are operated at a rate of 0.6 m/hr as it is one of the last step in of a series of treatment steps involved *(Visscher, 1996)*. *Muhammed et al. (2014)* observed that turbidity

and colour removal efficiency decline considerably with higher filtration rates, although the filtrate quality remains reasonably good.

Rachwal et al., (1996) carried out studies in Thames Water's Kempton Park showed that the filtration rate can be increased from 0.1 m/hr to 0.5 m/hr with an increment of 0.1 m/hr over 3 to 7 days without any deterioration in the SSF water quality but on further increasing the rate to 0.8 m/hr with the use of traditional fine (0.3mm ES) sand, a significant bed headloss was observed. It was also observed that in comparison to uncover SSF, covered filters achieved upto 4 times higher filter productivity at the same filtration rate. *Van der Hoek et al., (1996)* studied the effect of filtration rate (0.3 m/h & 0.6 m/hr) on two different sand types (silver sand d_{10}-d_{90}= 0.19-0.35mm & river sand d_{10}-d_{90}=0.25-0.84 mm) but no significant effect on the filtrate quality was observed.

Poynter and Slade (1977) demonstrated that greater virus removal was achieved at an HLR of 0.2 m/h compared to 0.5 m/h over a one year study period (Table 8*). Poynter and Slade (1977)* concluded that even though higher HLRs led to less efficient virus and bacteria removal, satisfactory removal of viruses and bacteria can be obtained at high HLRs and water temperatures as low as 5°C.

Table 8: Effect of HLR and Temperature on Virus Removal (Polynter & Slade, 1977)

Season	Water Temp.	SSF Number	HLR	Polivirus-1 Log Removal	Difference in Removal
Winter	5-8	1 2	0.2 0.5	2.5 1.8	0.7
Spring	9-18	1 2	0.2 0.5	3.7 2.9	0.8
Summer	16-18	1 2	0.2 0.4	4.5 2.9	1.6
Fall	9-16	1 2	0.2 0.4	3.3 2.4	0.9

The supernatant water level in the slow sand filters can be kept constant or variable during the filter run time. *Bernardo et al, (1996)* studies the performance of two filters at various filtrate rates but one with a constant supernatant water level and the other with variable supernatant water level. No significant effect was observed in the ripening period for both the filter and he behaviour of both the filters were same for the different filtration rate in terms of turbidity removal, total iron, apparent colour, total coliform counting and CFU. Similar study was carried out by *Anggraini et al., (2014)* and no significant difference was observed in regards to turbidity under both conditions.

2.6.2 Temperature

Numerous studies have demonstrated that higher water temperatures can lead to higher inactivation of viruses such as *MS2*, poliovirus-1, echovirus, bacteriophage PRD1, and hepatitis A (*Sobsey et al., 1995; Yahya et al., 1993b; Yates et al., 1990*). *Nasser and Oman* (*1999*) seeded viruses into various water samples and found that inactivation of hepatitis A virus and poliovirus-1 was greater at high (20-30°C) vs. low temperatures (4-10°C). They hypothesized that this was due to greater microbial activity at the higher temperatures (*Nasser and Oman, 1999*).

Poynter and Slade (1977) found that poliovirus removal by SSF consistently increased with increasing water temperature over a four year study period. They attributed the increased virus removals at higher temperatures to increased biological activity. Increased activity of microorganisms living in SSFs that prey on or inactivate viruses was likely the cause of the higher virus removals at higher temperatures.

An approximate 2 log increase in *MS2* and *E. coli* removals were observed in warm water SSF experiments (13 to 16°C) by *Dullemont et al., (2006)* compared to the experiments at cooler temperatures (10°C). These enhanced microorganism removals were attributed to increased biological activity in the SSFs at warmer temperatures *(Dullemont et al., 2006*). Taylor et al., studied the influence of filtrate rate and temperature on virus removal. At 0.20 m/hr and 11° to 12°C, removal was 99.9999% in comparison to 99.8% for 0.40 m/hr at 6°C. In another set of experiments there was 99.8% removal at 0.20 m/hr but only 91% at 0.40 m/hr *(Logsdon, 1987*).

Toms & Bayley, (1996) studied the relationship between the filtration rate and temperature at Thames Water Utility, London and observed there is a low temperature limitation on the capacity of filter to remove faecal indictor organisms. At water temperature below 4°C, at filtration rate of 0.3m /hr it was not possible to achieve average concentration of less than 50 E.coli/100 ml but at the same temperature with filtration rate of 0.2 m/hr, the average concentration was within the criteria of 10 E.coli/100 ml. These observation were similar to the one reported by *(Bellamy et al, 1985a).* On decreasing the temperature from 17°C to 5°C or 2°C, there was deterioration in coliform removal from the 99% level to about 90% for the colder waters. Also, total coliform removal was found to be adversely influenced by increases in filtration rate from 0.04 to 0.4 m/hr *(Bellamy et al., 1985 a)*.

2.6.3 Sand Depth

There is no fixed value of the sand bed depth and there is considerable uncertainty to an acceptable figure of bed depth. *Graham, (1996)* carried out studies to investigate the performance of the filters using reduced sand bed depth in the range of 0.2-0.5 m by introducing a non woven synthetic fabric layers over the filter bed (Table 9). The advantage of using reduced bed depth is the reduction in capital cost, also using fabric layer results in longer filter run time because of a lower rate of pressure headloss development. But there was a decrease in the treatment performance as the bed depth decreased. In a study by *Graham et al. (1996)*, virus removal was found to increase with greater SSF sand depth as shown below.

Table 9: Effect of Sand Depth on Virus Removal

	Sand Depth (cm)	Polivirus-1 (Log Removal)	Bacteriophage (log Removal)
SSF A	20	3.2	1.9
SSF B	30	4.0	2.9
SSF C	50	>4.0	3.5

*effective sand diameter=0.3 mm, UC =2.1, hydraulic loading rate 0.15 m/hr

Study carried out by *Poynter and Slade, (1974)* determined that SSF performance was enhanced by greater sand depths and that greater sand depth could be used to offset the negative impact of high HLRs. In the study, Poynter and Slade found that average poliovirus removals were 0.5 to 1.0 logs greater in a 60 cm deep SSF compared to a 30 cm deep SSF. *Slade (1978)* found less poliovirus-1 removals in full-scale SSFs with bed depths of 30 and 45 cm compared to those reported by *Poynter and Slade, (1974)* for 60 cm deep pilot-scale SSFs. The lower removals were attributed to the shallower sand depths *(Slade, 1978)*.

Sand bed depth plays a major role in the oxidation of nitrogenous compounds but not so in the bacteriological treatment efficiency as it mostly occurs in the top 400 mm of sand bed *(Muhammad et al, 1996)*. *Muhammed et al, 1996* studied the effect of the different sand depth on the percent removals of faecal coliform, total coliform, turbidity and colour for different effective sand size as shown in the table 10. The percentage reduction decreased with decreasing sand bed depth and increase in the effective sand size. Turbidity and colour removal efficiencies are affected more by reducing depth, which shows the importance of adsorption throughout the filter column in purifying water by SSF. A decrease in sand bed

depth causes a reduction in total surface area of the sand grains and ultimately reducing the adsorption capacity of the sand bed.

Table 10: Effect of Sand Bed depth and Effective sand size on the performance of SSF

Effective Size Sand	Sand Bed Depth (m)	Fecal Coliform (% Removal)	Total Coliform (% Removal)	Turbidity (% Removal)	Colour (% Removal)
0.2 mm	0.73	99.6	99.7	96.5	95.10
	0.40	98.4	99.0	87.5	72.0
0.35 mm	0.73	99.30	99.30	96.50	95.10
	0.40	97.40	98.70	86.50	72.0
0.45 mm	0.73	99.00	98.60	96.20	92.0
	0.40	95.90	98.10	85.00	66.00

2.7 Summary of relevant findings and knowledge gaps in the literature

- SSF supports a substantial biological community due to long hydraulic retention time of water above and across the filter bed.
- The major modes of virus removal/ inactivation are attachment to sand grains and biofilms. Some authors have reported that elimination of viruses by biological processes may be equally important during slow sand filtration.
- The major modes of removal of bacteria are predation and inactivation by microbial enzymes in the schmutzdecke layer.
- Biomass concentration increases with time and has a major contribution to the headloss development which leads to the cleaning of the filter bed and scraping of the schmutzdecke layer *(Campos et al., 2002).*
- The vertical distribution of biomass depends on the influent water quality, temperature and filtration rate.
- The depth of the sand bed that needs to be scraped depends not only on the biomass distribution, but also on the inactivation potential of different layer of sand bed.
- Most of the studies that are carried out only quantify the vertical distribution of biomass; very little information is available regarding the spatial distribution of biomass on a SSF bed, presence of lateral substrate gradients and the effect of position of inlet water source on biomass growth.
- Variety of methods has been used to quantify the biomass growth (table 7). In most of the studies a single method has been used to assess the biomass growth. It is generally agreed that a combination of methods that focuses on different indicators of viability is superior to any individual method, especially when natural microbial

communities are assessed *(Hammes, Berney et al. 2008)*. Moreover, no single method can determine all relevant aspects, so it is important to use combination of different methods.

- At the start of the filters, the effluent is discharged into waste untill filter reaches optimal performance. This period is termed as Ripening.
- Ripening of the filters depends on the biological activity of the microorganisms which in turn primarily depends on the operational parameters influent water quality (nutrient loading), temperature and the biological activity remaining after scarping of schmutzdecke and sand bed.
- In order to shorten the ripening period, the growth of the microorganisms which are responsible for biological activity needs to be accelerated.
- Coliform bacteria and turbidity are the commonly used indicators of ripening rather than complex microbial or chemical tests. The complex tests are generally applied as part of validation and verification activities rather than as part of operational monitoring.
- No relationship exists between this simple and rapid observation (turbidity and particle counts) to the ripening period of slow sand filter which would help in minimising time and cost.

2017

Chapter 3

Materials & Methodology

This Chapter includes the description of full scale and column SSF filters and methodology used. Two full scale slow sand filters operated by Dunea at treatment locations Monster & Katwijk were studied, along with 8 SSF columns (running in duplicates) installed in the Water Lab at TU Delft.

3.1 Full Scale Slow Sand filters

The current drinking water treatment process of Dunea consists of a number of successive treatment steps as a multiple barrier approach, which is in accordance with the Dutch standard guidelines for surface water treatment.

The water is withdrawn from the river near Brakel and purified through sand filters, before carrying it through two large pipes to the dunes between Monster and Katwijk. The purified river water is pumped into the dunes in so-called infiltration ponds. At the dunes, the water is infiltrated via a system of open infiltration ponds. A minor part of the water is infiltrated with deep-wells to replenish the deeper aquifer. The water is pumped up, after an average residence time of two months. The important function of the dunes is the storage of water and elimination of micro-organisms and pathogens, in order to obtain microbiological safe water.

After abstraction from the dunes, the water is post-treated in a series of treatment steps, consisting of softening, aeration with preliminary dosing of powdered activated carbon (PAC) for the removal of pesticides, dual media rapid filtration and slow sand filtration. Finally the water is stored in clear water reservoirs, from where it is distributed to the customers. In Figure 8 schematic overview of the treatment step is shown.

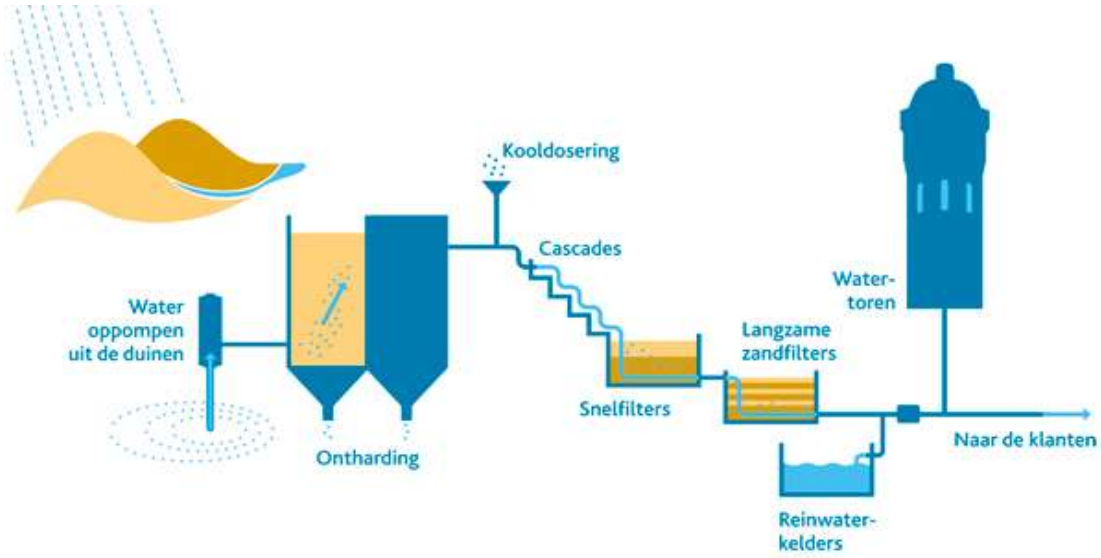


Figure 8: Schematic of Drinking Water Treatment at Dunea

With an extensive and controlled infrastructure of multiple treatment steps, different barriers against pollutants and pathogens are created. The last step of the treatment process is the Slow Sand filter which is used as a post disinfectant treatment step. As a result; primary and secondary disinfection by chlorination can be eliminated, while still

microbiological stable and safe drinking water is obtained, which causes minimal re-growth in the distribution network.

3.1.1 Characteristic of the Filters

The characteristics of the filters under study are mentioned in the table 11. Regarding the operational mode of the filters, both the filters were covered. Total production capacity of the treatment plant at Katwijk is 25 million m^3/year and at Monster 9 million m^3/year. The supernatant water level is variable in both the filters but is always greater than 1 meter. At the treatment plant in Monster, the influent source is a single source situated at the middle of one side whereas in Katwijk, the influent supply is uniformly distributed on one of the sides.

Table 11: Characteristics of the Filters

Filter Details	Monster (5B)	Katwijk (8.4)
Filter Age	2006	1990
Depth of sand Bed	90 cm	70 cm
Effective Sand size	0.35 mm	0.31 mm
Supernatant Water level	>1 meter	>1 meter
Filter Run Time (Scraping of sand)	243 days	450 days
Area of the filters	375 m^2	850 m^2
Filtration rate	35 to 40 cm/hr	25 to 40 cm/hr

3.1.1.1 Temperature

The influent water temperature to SSF ranges from 8 to 16 ^{0}C at both the treatment locations. Regular measurement of temperature was done at Monster treatment plant as shown below in fig 9. The highest temperature occurred in the month of August & September while lowest around January and February.

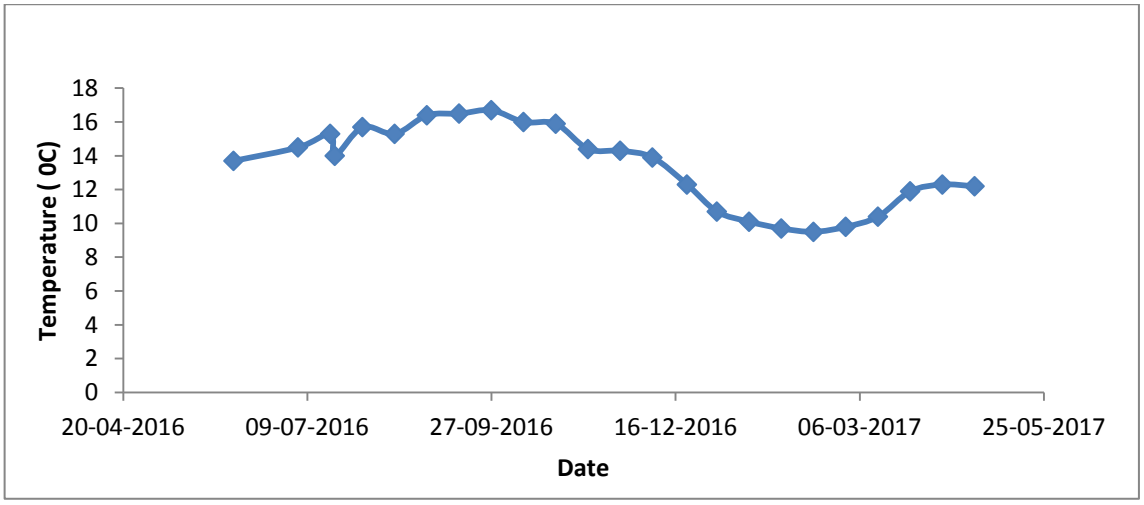


Figure 9: Influent Water Temperature to SSF at Monster Treatment plant

3.1.1.2 Turbidity

Because of extensive pretreatment, the quality of the influent water to the SSF is very good. The influent turbidity levels were less than 0.1 NTU at Monster treatment works as compared to Katwijk, where it was slightly higher but still below 1NTU as shown in figure 10. Turbidity at Katwijk occasionally exceed upper limit it might be because of the passage of flocs through the rapid filters (Chipps, 2002).

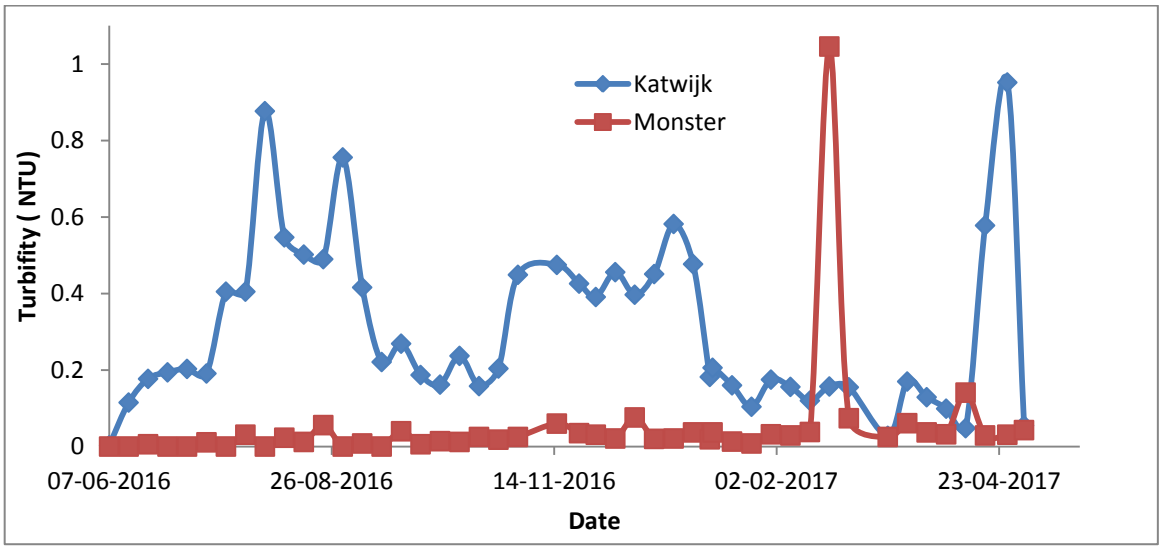


Figure 10: Influent turbidity to SSF

3.1.1.3 Nitrate (NO^-_3)

Nitrate (NO^-_3) concentration was measured regularly for around 1 year, but on average the influent raw water concentration was approximately 6.0 mg/L at Katwijk and 4.8 mg/L in Monster during the period of June/2016 and April/2017 as shown in figure 11.

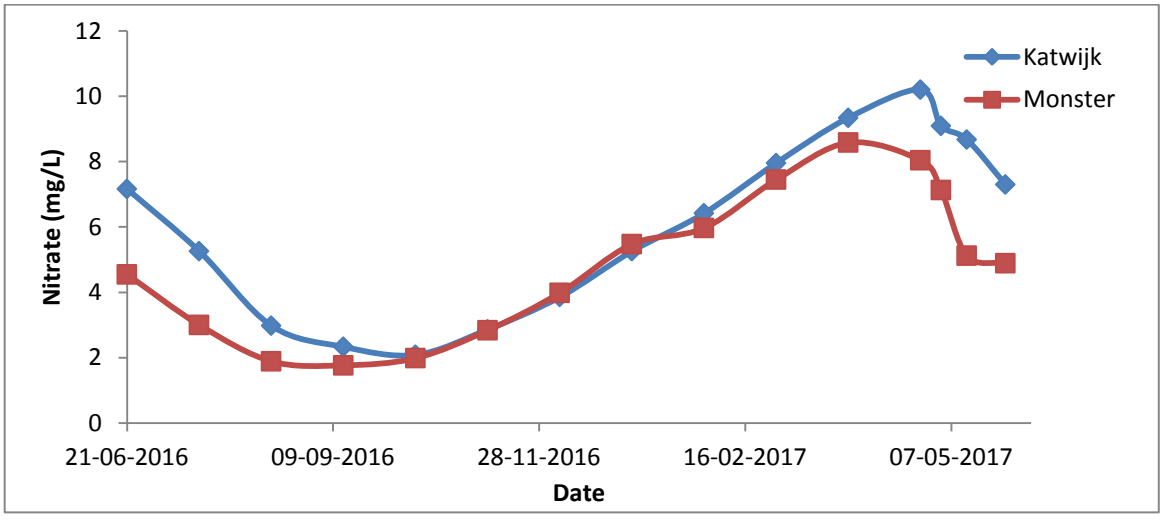


Figure 11: Influent Nitrate Concentration to SSF

There was no significant removal of nitrate (p=0.18 for 5B, p = 0.13 for 8.4) during this period, in both the filters. Moreover in some cases the effluent concentration of nitrate is higher than the influent concentration. The increase in the nitrate concentration in the effluent might be because of nitrification. Nitrification is the biological oxidation of ammonia or ammonium to nitrite followed by the oxidation of the nitrite to nitrate as shown in equation 1 and 2. On average, the influent and effluent ammonia concentration in SSF 5B were .012 mg/l and .009 mg/L, while in SSF 8.4 the average influent and the effluent concentrations were .012 and .01 mg/L respectively as shown in figure 12.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad \text{.....(1)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad \text{.....(2)}$$

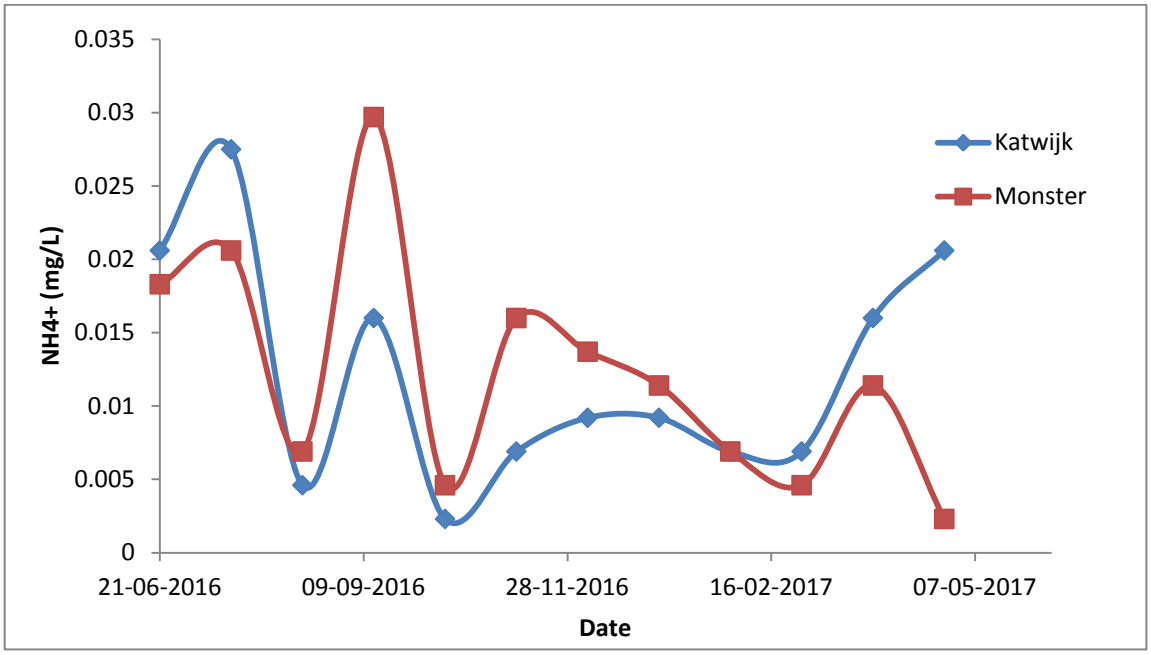


Figure 12: Influent Ammonia Concentration

The average influent and effluent ATP values were 6.55 ng/L and 1.23 ng/ L respectively. The average influent and effluent AOC concentration were 4.6 ug/L C and 3.8 ug/L C respectively.

3.1.1.4 Cleaning & Start up Procedure

The filters are cleaned when the outlet valve is fully opened and it is not possible to achieve the desired flow rate. The filters are cleaned by removing the top 10 cm of sand bed including schmutzdecke which is much higher than the reported value in literature of 2-3

cm by *Campos et al., (2002); Visscher et al., (1996) and Huisman, (1974).* The scrapped sand is washed, dried and used again.

Due to very low level of nutrients and microbial activity, the development of the schmutzdecke (biological layer) takes very long time, around 53 days. During the ripening period, the filter is non operational and the water passing through the filters is recircualted in the ratio 2:1 to fresh water, untill the filter reaches it optimal removal efficiency. 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.

3.1.2 Methodology used

In order to ensure maximum biological activity is retained within the filters at the time of scraping, first step is to determine the biomass concentration in different layers of sand bed which is responsible for head loss and secondly by carrying out spiking experiments of bacteria and virus the contribution of different layers of sand bed in the efficacy of the filters can be assessed. By combining biomass and cell count the biological activity in different layers of sand bed can also be quantified. Since carrying out spiking experiments in full scale filter is not feasible, it will be done in column scale filters. The biomass distribution in these filters will also be quantified and compare with the full scale filter.

This will help to optimise the depth of sand bed that needs to be cleaned and ultimately helps to retain maximum biological activity during the scraping process.

The effect of scraping can be further understood by studying the spatial variation of biomass growth in the filter. This would lead to a better understanding of the lateral substrate gradients that exists on the filter bed and the effect of the direction of influent water source on the biomass growth which results in uneven scraping of the filter bed and might results in longer ripening period.

The Full scale filters were analysed for the biomass concentration by measuring ATP content using Luciferene –Luciferase method and cell counts by using Flow Cytometry. Two different methods were using to quantify the biomass activity. As explained by *Goldschmidt et al, 2010* that by combining cell count and cell activity together, not only the number of the cells and the activity of the cell can be studied, but the physiological state of the cell can also be assessed *(Vital et al., 2008; Goldschmidt et al., 2010).*

3.1.2.1 Sample Collection

The samples from the full scale slow sand filter at Katwijk and Monster were collected as shown in figure 13. The filters at Katwijk & Monster were running for 450 and 243 days respectively at the time of sample collection. Before the day of sample collection the filters were drained out.

Figure 13: Sample Collection at Monster Treatment Plant

In order to study the spatial distribution of biomass, sand and schmutzdecke samples were collected from three different locations as shown in figure 14. Schmutzdecke was easily distinguishable by the sand bed by colour. The schmutzdecke was dark black in colour where as samples from sand bed were dark brown in colour.

To study the vertical distribution of biomass, samples were collected from the schmutzdecke and 3 different depths from the sand bed (0-2 cm, 4-6 cm, 8-10 cm) from all three locations. A hole was dug adjacent to the point of sample collection. This was done to avoid the mixing of different layers of sand bed so that undisturbed sand samples can be collected and to divide the sand bed into three different layers below the schmutzdecke. Schmutzdecke samples were first collected, followed by the collection of sand samples from 0-2 cm of sand bed, after that 2- 4 cm layer of sand bed was scraped and next sample from the depth of 4-6 cm was collected. Similarly sand samples from 8-10 cm of sand bed were collected.

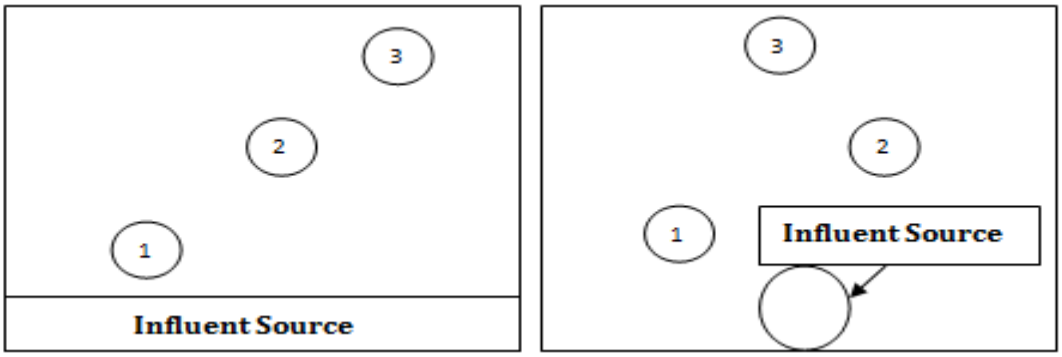


Figure 14: Point of Sample Collection (a) Katwijk (b) Monster

Samples were collected using a sterilized spatula and stored in plastic containers before transferring them to HWL laboratory in a cool box. Sand and schmutzdecke samples were analysed within 24 hours for biomass and cell counts using Luciferene Luciferase and flow cytometry method respectively.

3.1.2.2 Analysis Description

A. Luciferene Luciferase method

The biomass concentration was determined by measuring the ATP values in the sand suspension and then converting them into organic carbon concentration using a conversion factor. Conversion factor was determined in calibration measurements. The calibration curve was obtained by measuring standard ATP solutions of known concentration in the same assay conditions *(KIWA, 1997)*. The ATP measurement is based on the reaction between two Proteins derived from flies luciferin (substrate) and luciferase (enzyme) that occurs in presence of ATP as shown below. Light is produced, which is measured and displayed in Relative Light Units (RLU).

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

$$\text{Luciferine-Luciferase-AMP} \longrightarrow \text{Oxyluciferene} + \text{Luciferase} + CO_2 + \text{AMP} + \text{Light}\ (h\vartheta)$$

AMP=Adenosinemonophosphate

The light produced is measured with a sensitive photometer and expressed in Relative Light Units (RLU). Under optimum conditions, 1 light photon per molecule of ATP is produced. The determination is made in duplicates and the average value is used in calculation. The RLU value converted into biomass carbon concentration value using the below mentioned formula.

Biomass Carbon Concentration (ng/L) = {(RLU_{sample} - RLU_{Blanc})/Conversion Factor}* Dilution Factor

Conversion factor =4.63

Dilution factor = 1000

The biomass values obtained by this method were in ng/L which was converted to ng/g of dry weight of sand using the formula:

Biomass Organic Carbon (ng/g) = {Biomass Organic Carbon (ng/L)/Wt. of dry sand (g)} * Volume in which sand was immersed (L)

B. Flow Cytometry

The cell count was done by using Flow cytometry method which is able to distinguish between the High Nucleic Acid content (HNA) and Low Nucleic Acid Content (LNA) cell. The HNA cells are considered to be more active while LNA are regarded as inactive, dead or dormant cells *(Andrade et al., 2007)*. The total cell count includes the actively growing cells which contribute to 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)*.

Flow cytometry uses principle of light scattering and fluorescence. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths. Total Cell counts and living cells are determined after staining of the cells with fluorochromes. Fluorochromes SYBR Green I and Propidium Iodide can make a distinction between living (undamaged) and dead (damaged) cells.

SYBR Green l: Fluorochromium penetrates through all cell walls and binds to the DNA material of the cell, as this fluorochromes is irradiated (excitation, 488 nm), it will emit light and the emission falls into the spectrum with a wavelength of 475-700 nm (max. 525 nm).

Propidium Iodide: Fluorochromium penetrates through damaged cell walls and binds to the DNA material of the cell. When this fluorochromes is irradiated (excitation, 488 nm), the light emits. This emission is in the spectrum with a wavelength of 550-700 nm (max. 617 nm).

The fluorescence and light scattering signals are detected by scattering and fluorescence detectors and were converted into counts. The values reported were in ng/L which was converted to ng/g of dry weight of sand using the formula:

Cell Count (ng/g) = {Cell Count (ng/L)/Wt. of dry sand (g)} * Volume in which sand was immersed (L)

3.2 Columns Slow Sand Filters

3.2.1 Column Design

A schematic of the experimental set up of SSF columns is shown in figure 15. There were 8 columns which were running in duplicates. The columns were 1m long and 4 cm in diameter made from polyvinyl chloride (PVC). The columns were cleaned with ethanol, chlorine and sodium thiosulphate and rinse with demi water to avoid any microbial activity. The columns were mounted on a big board and were clamped on to it as shown in figure 17. Columns were packed with a layer of coarse gravel (3–6 mm) followed by fine sand of effective size of 0.3 mm similar to what is used in the full scale filter to an overall depth of approximately 0.6 m.

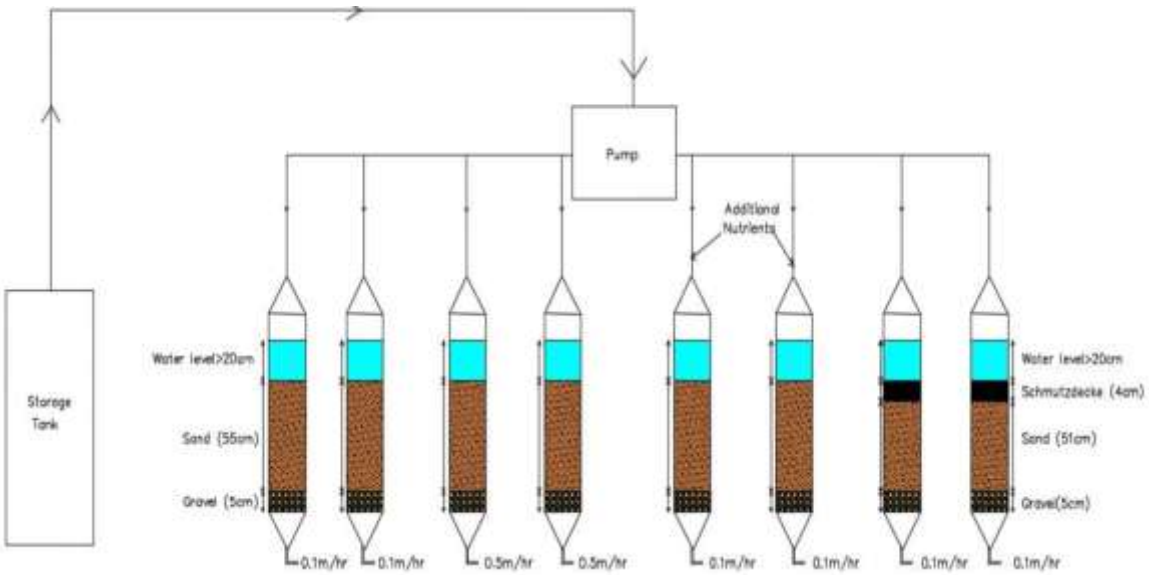


Figure 15: Schematic of the Columns Setup

Columns were filled one fourth of the water and sand was slowly added to it, continuous shaking of the columns was done to make the sand bed compact and at the same time filters were backfilled slowly at the rate of 0.1 m/hr. The filter were backfilled slowly untill the water level reaches 0.1 m above the sand surface. This was done to ensure that there is no accumulation of air. After this the filter was filled upto its working level. The initial rate of

filling was low (.02 m/hr) to prevent scouring of the sand, but as the layer of supernatant rises, the rate was gradually increased with increment of .02 m/hr every hour untill the desired supernatant water level was reached. The ratios of the column diameter (D) to sand diameter (d) was 115 (Table 14), which were above the minimum D/d ratio of 50 recommended by *Lang et al. (1993)* to ensure that pilot filters accurately represent the filtration performance of full-scale filters (headloss build-up, effluent turbidity, effluent particle counts).

3.2.2 Column Operation

The influent water to the columns was the influent to the rapid sand filter from the full scale filter instead of the effluent of rapid sand filter similar to the full scale filter. This was done to provide higher DOC loading. The influent was brought from the treatment plant once every week and supplied to the columns by the use of the peristaltic pump. The pump was calibrated to ensure constant rate of supply. The calibration curve is shown in appendix A. The start date of the columns was different as shown below in table 12.

Table 12: Start Date of Experiment

	Column 1 & 2	Column 3 & 4	Column 5 & 6	Columns 7 & 8
Start Date	10 May'2017	14May'2017	24 May'2017	17 May'2017

The columns were running in duplicates under different operating conditions as shown in figure 16. In order to accelerate the biological growth in the filters, three possible approaches were investigated in the column scale slow sand filter. The Columns 1 & 2 were used as a reference with filtration rate of 0.1 m/hr. The columns 3 & 4 were running with filtration rate of 0.5 m/hr which will result in higher nutrient loading and thus more substrate available to the microbial community to grow. The columns 5 & 6 were seeded with additional nutrients with concentration 5 times the influent. The concentration added was 5 times to maintain the same mass balance of the nutrient loading with respect to the column operating at the filtration rate of 0.5 m/hr. In this way both the columns are subjected to same nutrient loading and the effect of filtration rate and nutrient loading on the ripening of the columns can be assessed.

Based on the analysis of the influent water quality, phosphate P (0.059mg/L) was the main source of total phosphorus (.062 mg/L). Nitrite N and Ammonia N concentration was below 0.1 mg/L, Nitrate N (1.12 mg/L) was the main source of Nitrogen in the influent. Total N concentration was 1.2 mg/L which was slightly higher than the combined values of nitrite, nitrate and ammonia (1.15mg/L) indicating the presence of organic nitrogen. Organic

nitrogen may be in the form of a living organism, humus or in the intermediate products of organic matter decomposition. Analysis of different forms of nitrogen and phosphorous were carried out by using HACH cell kits. The DOC concentration in the influent was 1.78 mg/L. The DOC concentration was determined by TOC-V CPH Shimadzu Analyser. The detailed description of which is provided in the next section.

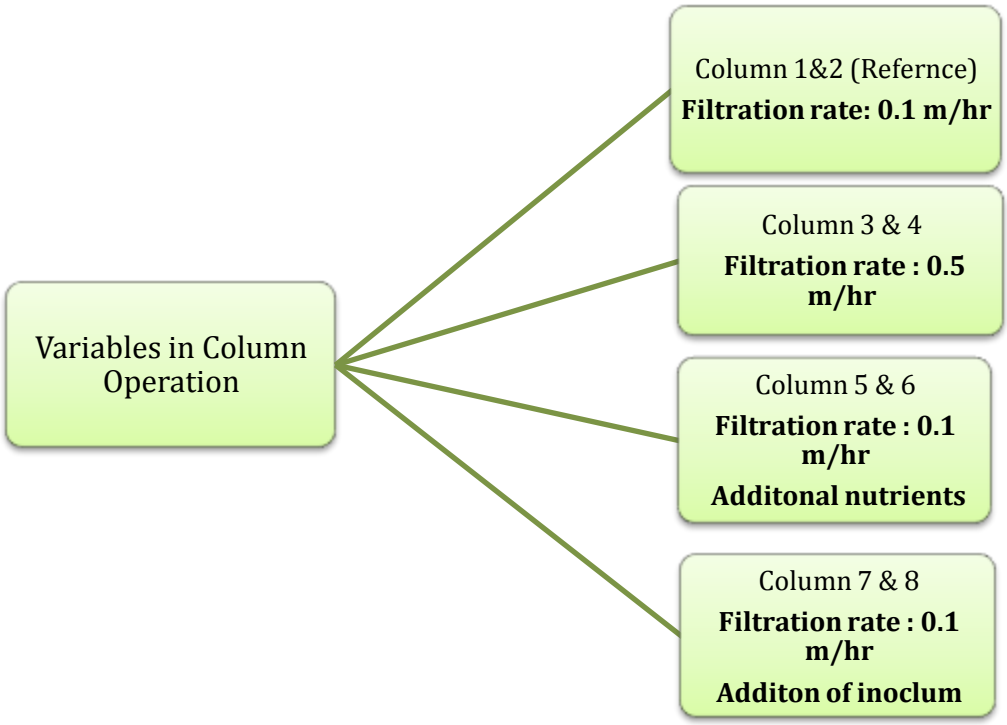


Figure 16 : Variable in column operations

Since our objective was to observe the behaviour of column when additional nutrients were added and not to observe the effects of different sources of nutrients, easily available sources were used. Sodium dihydrogen phosphate was used as a phosphorus source, Ammonium Chloride as nitrogen source (since Nitrate N was already present in the influent) and Sodium Acetate as a carbon source. The nutrient addition was 5 times the influent concentration, Therefore Total N= 6 mg/L, Total C= 8.9 mg/L, Total P = 0.31 mg/l were used. The influent nutrient concentration to columns 5 & 6 were adjusted weekly based on the influent concentration to the other columns.

The columns 7 & 8 were added with microbial inoculum in the top 3-4 cm over the sand bed. The inoculum used was the schmutzdecke layer from the Katwijk filter. The microbial inoculum was added to the columns within 3 hours to preserve the biological activity of the microorganisms. The percentage of the inoculum added to the sand bed was 4/56 *100 = 7 % (56 cm is the depth of sand bed) which is slightly higher than the actual filters, where the

thickness of the schmutzdecke was around 3 cm and depth of sand bed was 70 cm making it 3/70 *100 = 4.29%. The operating characteristics of the column are presented in table 13.

Table 13: Operating Characteristics of the Columns

Characteristics	Column 1& 2	Column 3 & 4	Column 5 & 6	Column 7 & 8
Sand Effective Size (mm)	0.3	0.3	0.3	0.3
Bed Depth (cm)	60	60	60	56
Filtration rate (m/hr)	0.1	0.5	0.1	0.1
Nutrient Loading	-	-	5 * influent	-
D/d Ratio	~115	~115	~115	~115
Microbial Inoculum	-	-	-	✓ (4cm)

D= columns diameter d= Sand diameter

The flow rate in the columns was controlled by the valve at the end of the columns. The effluent flow rate was maintained daily by adjusting the outlet valve. There was no measurement of head loss but during the entire duration of experiment the outlet valve was never fully opened in any of the columns. The supernatant level was controlled by the inflow rate maintaining a head of atleast 20 cm at every point of time. Study carried out by *Anggraini et al, 2014* suggested that there is no significant difference due to the different type of supernatant layer in regard to the turbidity removal. The influent to the columns was recircualted in the ratio 2:1 to fresh water similar to the actual treatment plant. A secondary pump was used for the purpose of recirculation. The calibration curve of the pump is shown in appendix A. The description of the column setup is shown in figure 17.

Figure 17: Set up of Column Experiments

3.2.3 Methodology used

The growth of microorganism responsible for biological activity is bound by the availability of substrates (nutrients). In order to accelerate the biological growth in the filters, three possible approaches were proposed in the column scale slow sand filter. First is the use of additional nutrients; second is to increase the filtration rate which will result in higher nutrient loading and thus more substrate available to the microbial community to grow. The third option is the use of scraped schmutzdecke from a matured SSF as a microbial inoculum. The efficacy of filter under various operating condition will be quantified by carrying out the spiking experiments during stages of ripening and continuous monitoring of water quality parameters such as turbidity, particle count, dissolve organic carbon and total nitrogen

In most cases, operational monitoring is based on simple and rapid observations or tests, such as turbidity or structural integrity, rather than complex microbial or chemical tests. The complex tests are generally applied as part of validation and verification activities rather than as part of operational monitoring. No relationship exists between these simple and rapid observation with chemical and microbial test. Therefore in these study theses simple test such as turbidity and particle counts will be monitored along with the chemical parameters such as DOC & TN and microbial test will be used a part of validation process, the microbial test will be carried out during different stages of filter operation. Chloride tracer test will be carried out to calculate the HDT of the SSF.

3.2.4 Water Quality Analysis

3.2.4.1 Turbidity

Turbidity monitoring was done to assess the ability of the columns to meet the regulatory effluent requirements and in order to maintain filter performance at typical full-scale efficiencies for pathogen spiking tests. High levels of turbidity can protect microorganisms from the effects of disinfection, stimulate the growth of bacteria. No health-based guideline value for turbidity has been proposed: however, median turbidity should be below 0.1 NTU for effective disinfection *(WHO, 2008)*. Therefore, in this study one indicator to define whether filter is ripened or to define the ripening period, the median turbidity would be used and its value should be less that less than or equal to 0.1 NTU.

Turbidity of the influent and effluent was measured daily by using HACH turbidmeters. The turbidmeters were calibrated using stabilised formazin standards of 0.01 NTU, 1 NTU, 20

NTU and 100 NTU every time before measuring the sample. The turbidity measurements were done in duplicates to avoid the errors in measurement.

3.2.4.2 Particle Counts

The rationale for the use of turbidity as an indicator for evaluation of filtration efficiency has been based on the assumption that microorganisms, many of which contribute little to turbidity are removed with efficiency equal to or better than that of inorganic, light-scattering, and light absorbing particles such as silts and clays *(O'Connor et al, 2001)*.It was also assumed that most microorganisms were embedded in or attached to the surface of larger particles in suspension. From this, it was followed that the removal of those larger particles will ensured the removal of virtually all the pathogenic microorganisms from the source water. Later on this rational was discarded after the events surrounding the waterborne outbreak of cryptosporidium Carrollton, Georgia where water turbidity was used as a surrogate for protection against pathogens.

Therefore in our current study, particle counts were also measured in the influent and effluent concentrations. Researchers have shown that there may be a high probability of Cryptosporidium and Giardia in drinking water when the concentration of particles larger than 2 μm is more than~200 particles/mL *(Hargesheimer et al. 1998)*, suggesting that the particles in water treatment effluent can be used as a substitute index of the Protozoa *(Cook 1995)* and consequently a surrogate for water quality monitoring against pathogens. Therefore one way to define the ripening of the filter is the effluent particle concentration which should be less than 200 counts/ml.

The particle count in the influent and the effluent was measured twice a week by using liquid particle counting system model-9703 and HIAC particle distribution Analysis Software. Before each measurement system was rinsed with demi water and measurement was done in triplicates. At the start of measurement particle count of Demi water was measured to ensure the accuracy of the system. Average Particle Count of demi water was around (15$\pm$3 /ml).

3.2.4.3 Temperature

Temperature measurement of the influent was done regularly. The figure in appendix D presents the temperature of the influent water during the entire study period.

3.2.4.4 Dissolved Organic Carbon (DOC) & Total Nitrogen (TN)

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, therefore 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. Along with DOC, TN measurements were also done.

DOC and TN measurements were done using TOC-V CPH Shimadzu Analyser. The analysis was done weekly. For the measurement of DOC and TN, blanks and standard solutions were prepared to check the accuracy of the measurement. Since the measurement is done for DOC, the samples were first passed through the 0.45 um syringe filters (Whatman Spartan 30/0.45RC Rinse filter).

Blanks were prepared by adding 30 ml of ultra-pure water into a TOC free vial with 1.6 ml of 2 M Hydrochloric acid and closing it by using the aluminum dish and cap. The amount of blank depends on the number of the samples for example after every 5 samples one blank was placed.

Two types of standard solution were prepared for both DOC and TN for every analysis. New standard solution were prepared by adding 1 ml of the stock standard solution (1000PPM) using the 1000 µl pipet to the 100ml volumetric flask by adding ultra-pure water. The flask was closed with a stopper and mixed vigorously for homogenization. 30ml of this solution was put into a vial and the vial was closed using the aluminum dish and caps. Old standard solutions were already prepared in refrigerator. 1.6 ml of 2 M Hydrochloric acid was added to 30ml of the old standard solution in the vial and the vial was closed using aluminum dish and cup.

The samples, standards and the blanks were placed in the analyzer. The completed run was valid only when the old and new standard solutions have a value of approximately 10 mg/L and the blanks a value of 0.2 mg/L.

3.2.5 Chloride Tracer Test

Chloride tracer tests were performed to determine hydraulic detention times of the SSFs. Chloride was selected as a tracer because it is conservative, non-reactive, and short-term exposures should not negatively impact the filters biological community. The stock solution for each chloride tracer test was prepared by mixing 3.5 g NaCl into 1 L of deionized water. The chloride tracer stock solution had a chloride concentration of 55,800 mg/L and conductivity of 9.29 milli Siemens per centimeter (mS/cm). The chloride stock was applied

as a continuous feed on the top of the column filter for 2.5 hours using a peristaltic pump with Teflon® tubing.

To determine if conductivity measurements could be used as an indicator to monitor the tracer, a calibration curve was generated using the influent water to the columns and a series of increasing standards of chloride solutions. The resulting calibration curve is shown in Appendix B figure B.1 displaying a higher linear correlation (R^2 = 0.9919). Thus conductivity measurements were appropriate to measure the chloride trace during tracer test.

Each filter was tested independently to avoid any confounding effects of testing multiple filters in series. The flow rate was the same as the flow rate during the for the filters during normal operation i.e for columns 1,2,3,4,5,6 it was 0.1 m/hr and for column 7&8 it was 0.5 Conductivity measurements were taken before chloride injection, during injection, and after the injection pump was stopped. Therefore, background and steady state conductivities were recorded. The steady state values were recorded at the time the injection pump stopped (210 min). From the results in Figure B.2 to B.5 (Appendix B), hydraulic detention times (HDTs) were determined as the time it took for conductivity to drop to half way between the steady state and background values after the chloride injection pump was stopped. Results of the tracer test are shown in table 14.

Table 14: Chloride tracer Results

	0.1 m/hr		0.5 m.hr		0.1 m/hr N		0.1 m/hr S	
Column / Conductivity (ms/cm)	1	2	3	4	5	6	7	8
Background	0.54	0.57	0.54	0.54	1.36	1.34	0.50	0.53
Steady	6.52	7.13	8.73	8.96	7.81	7.78	6.89	7.11
Target	2.98	3.28	4.09	4.21	3.20	3.20	3.17	3.27
HDT (t50) min	102	100	60	54	137	132	104	111

**Target Conductivity is the 50% value between background and steady state conductivity*

The t50 time represents the time it takes for 50% of the chloride tracer to pass through the filter. The t50 time was selected as an estimate of HDT because it is more conservative than the t10 time (i.e. time for 10% of the tracer to pass through the filter, which is typically used for microbial inactivation estimates). Theoretical HDT was calculated as shown in table 16. By combining the theoretical HDT (table 15) and t_{50} values calculated from the tracer

experiments (table 14), the porosity of the filter beds was determined. The value of the porosity is provided in table 16.

Table 15: Theoretical HDT

Parameter	0.1 m/hr	0.5 m/hr
Area (m^2)	.001256	.001256
Flow(m^3/min)	.0001256	.000628
Depth (m)	0.55	0.55
Theoretical HDT of media	330p	66p

Assuming porosity = p

At that time of carrying out tracer test the supernatant water level over the columns with 0.1 m/hr was maintained at a minimum level of 2-3 cm to avoid the dilution effect where as it was 25 cm in the columns with higher filtration rate (it was not possible to maintain the low supernatant level with higher filtration rate as it results in scouring of the top layer). Therefore, the theoretical value used for the calculation of porosity in columns running at 0.5m/hr was 66p + HDT above water bed {(.25m)/(0.5m/hr)= 36 min}. The porosity values calculated using the table 15 & 16 is a rough estimation of the filter bed porosity as the effect of dilution was not used in the calculation and t_{50} value calculated using the tracer test was done only once after first spike which can change as the filter bed matures and schmutzdecke develops.

Table 16: Calculation of porosity using tracer test and theoretical HDT

Columns	HDT (t_{50}) min	Theoretical t_{90} min	Porosity (p)
1	102	330p	0.30
2	100	330p	0.30
3	60	66p + 36	0.36
4	54	66p + 36	0.27
5	137	330p	0.41
6	132	330p	0.40
7	104	330p	0.31
8	111	330p	0.33

3.2.6 Spiking Experiments

The spiking experiments were carried out two columns at a time i.e for the duplicates. In total there were 8 E. Coli spikes per column resulting into 64 spikes for all the columns. For *MS2* there were 3 spikes per column resulting into 24 spikes. The timeline of the spiking event is shown in the table 17.

Table 17: Timeline of the spiking Event

Days after the start of the experiment	E. Coli Spike	*MS2*spike
30 days	✓	✓
44 days	✓	
58 days	✓	
80 days	✓	✓
Removal of Schmutzdecke*	✓	✓
Removal of 0-2 cm sand bed	✓	
Removal of 4-6 cm sand bed	✓	
Removal of 8-10 cm sand bed	✓	

* Schmutzdecke was removed after 90 days; sand bed of 0-2 cm was removed next day and so on till 8-10 cm of sand bed was removed. After the removal of schmutzdecke, the samples were stored in a sterile plastic container and sent to the HWL lab the same day for the analysis of microbial biomass and Cell Count using the same method as describes earlier in section 3.1.2.2. Samples were analysed within 24 hours. Similarly the sand samples from 0-2 cm sand bed, 4-6 cm and 8-10 cm were sent to the lab the following days and were analysed within 24 hours.

A number of materials were needed for the E. Coli & *MS2* challenge tests, including TYGB agar, TYGB broth, phosphate buffered water and Ca-glucose solution.

Tryptone-Yeast-Extract-Glucose Agar (ssTYGA) was made by combining 1 L Demi Water, 10 g of Trypticase peptone, 9 g of granulated agar, 1 g of yeast, and 8 g of NaCl. The ingredient was mixed completely in hot water and the pH was adjusted to 7.2± 0.1. The medium was distributed into the bottles which were autoclaved at 121^{0}C. After that it was cooled down and stored in the refrigerator at 4^{0}C.

Tryptone-Yeast-Extract-Glucose (TYGB) broth was prepared by combining 1 L Demi Water, 10 g of Trypticase peptone, 1 g of yeast, and 8 g of NaCl. The ingredient was mixed completely in hot water and the pH was adjusted to 7.2± 0.1. The medium was distributed into the bottles which were autoclaved at 121^{0}C. After that it was cooled down and stored in the refrigerator at 4^{0}C.

Phosphate buffered water (PBW) was needed for serial dilutions of *E. Coli* and *MS2*samples. Phosphate buffered was prepared by using 0.58 g Monosodium phosphate (NaH_2PO_4), 2.5 gm Disodium phosphate ($Na_2H_2PO_4$) and 8.5 g sodium chloride (NaCl) in 1 L of demi Water. After the preparation, the solution was autoclaved at 121^{0}C and check for the pH which needs to be around 7.2± 0.1. After that it was cooled down and stored in the refrigerator at

4^0C. Before the experiment PBW was dispensed into sterile test tubes with volume of 4.5 ml in each.

Ca- Glucose solution was prepared using 3 g of $CaCl_2.2H_2O$, 10 g of Glucose in 100 ml of demi water. The ingredients were dissolved in the water while heating gently. After that the mixture was cooled down and passed through a 0.22µm membrane filter and stored in refrigerator at 4^0C. The beakers and the glass bottles used in the spiking experiments were autoclaved at 121^0C before use to avoid any background contamination.

E. coli *WR1* is widely used as a reference strain for enumeration of E. coli and is used here as reference for bacterial removal *(Mooijman et al., 1991)*. The details of the E.coli spiking are provided in Appendix C.1. MS2 analysis done using double agar layer (DAL) method according to international standards ISO 10705-1:1995 (See appendix C.2).

2017

Chapter 4

Results : Objective 1

The objective of this chapter was to optimise the scraping procedure.

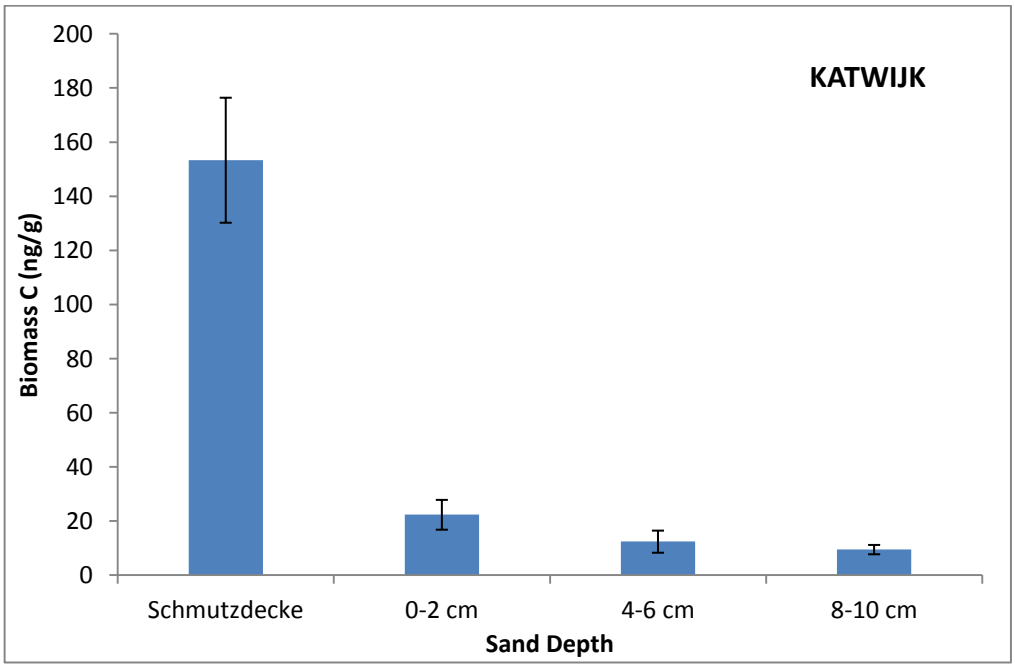
4.1 Vertical Distribution of Biomass

The biomass distribution was calculated in organic carbon concentration which is reported as per gram of dry weight of sand. This made it possible to directly compare the amount of sand biomass to the amount of Schmutzdecke biomass.

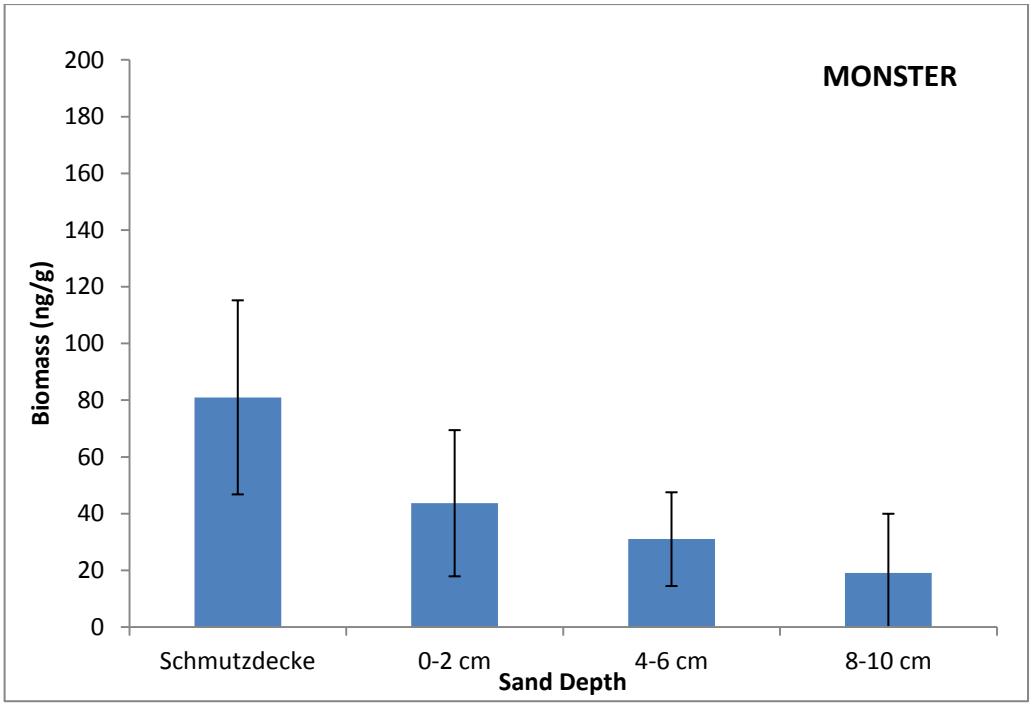
4.1.1 Full Scale SSF

The patterns in the interstitial microbial biomass concentration in the schmutzdecke layer and sand bed are shown in figure 18. The values are the average of the biomass concentration from three different locations at a particular depth in a SSF. The error bars represents the standard deviation between the measurements at different locations.

The most notable difference between the filters was their age. The biomass concentration in the schmutzdecke layer is much higher in Katwijk as compared to Monster. The filter run time at Katwijk & Monster was 450 and 243 days respectively at the time of sample collection, suggesting that the biomass concentration in the schmutzdecke layer increases with time. Another reason for higher biomass concentration in the schmutzdecke layer in Katwijk as compared to Monster is the influent water turbidity, which is higher in Katwijk (0.3± 0.2, N=46) as compared to Monster (0.045± 0.026, N=45) leading to the accumulation of inert and organic particles and thus higher biomass. The filter run time is much higher as compared to the values reported in the literature, because the SSF under investigation is a last step of series of treatment step where the concentration of organic substrate which is essential for biomass growth is very low.



(a)



(b)

Figure 18 : Average value of Biomass growth (a) Katwijk (b) Monster

In general the biomass concentration decreased with depth in both the filters, suggesting substrate limitation to microbial growth with depth. The reduction in biomass growth is much sharp in filters at Katwijk as compared to Monster. The percentage reduction at Katwijk is 85% from schmutzdecke to 0-2 cm, 45 % from 0-2cm to 4-6 cm and 23% from 4-6 cm to 8-10 cm where as it is 46%, 29% and 38 % from schmutzdecke to 0-2 cm, 0-2 to 4-6 cm and 4-6 cm to 8-10 cm respectively in Monster. The accumulation of organic matter, microorganisms and suspended and inert particles from the influent water in the exopolymeric matrix lead to the faster biomass development in the Schmutzdecke as compared to the sand. The biomass concentration at different depth and location is show in the table 18. The values reported in the table are in ng/g dry wt. of sand.

Table 18: Biomass Distribution

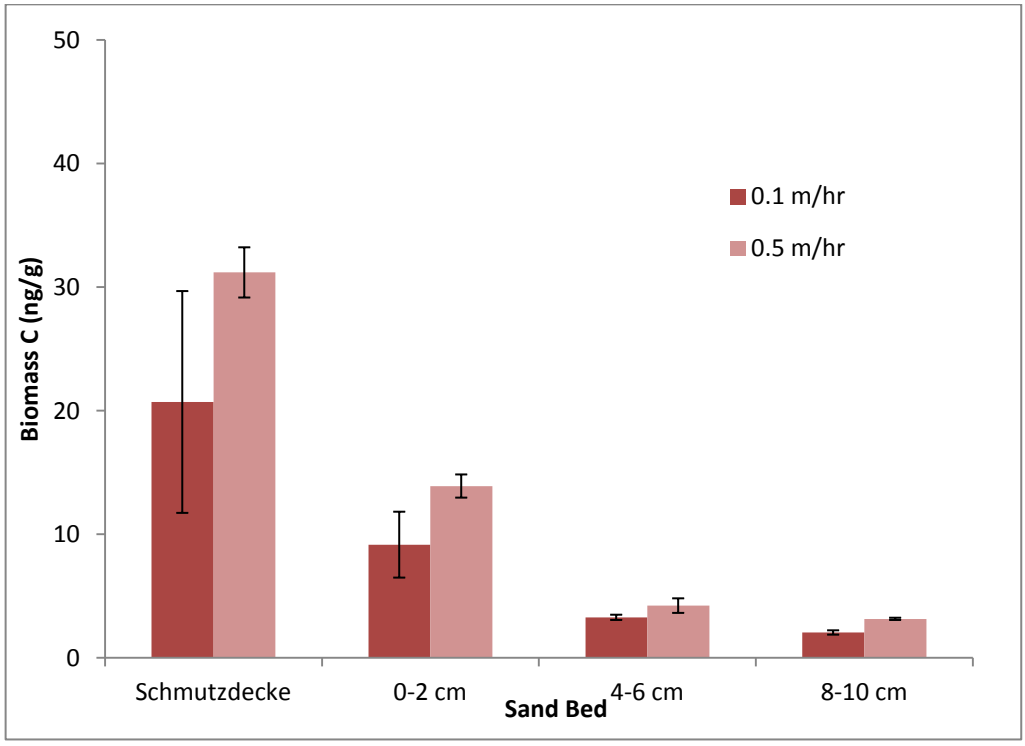
Filter	Location	Schmutzdecke	0-2 cm	4-6 cm	8-10 cm
Katwijk(ng/g)	Location 1	140	25	17	9.8
	Location 2	140	16	9.2	7.6
	Location 3	180	26	11	11
	Average	153±23	22.33±5.50	12.4±4.1	9.46±1.72
Monster(ng/g)	Location 1	56	16	12	4.5
	Location 2	67	48	42	9.7
	Location 3	120	67	39	43
	Average	81± 34	43.6± 25.7	31± 16	19± 20

Higher biomass in the deeper layers at Monster suggests that higher filtration rate pushes substrate into deeper layers. The influent water quality of both the filters is same in terms of concentration of organics and nitrates, although there were some difference in the influent turbidity, filters at Katwijk were able to run for much longer time, despite having higher biomass concentration in the schmutzdecke layer suggesting the importance of maintaining low filtration rate in the working of the sow sand filters.

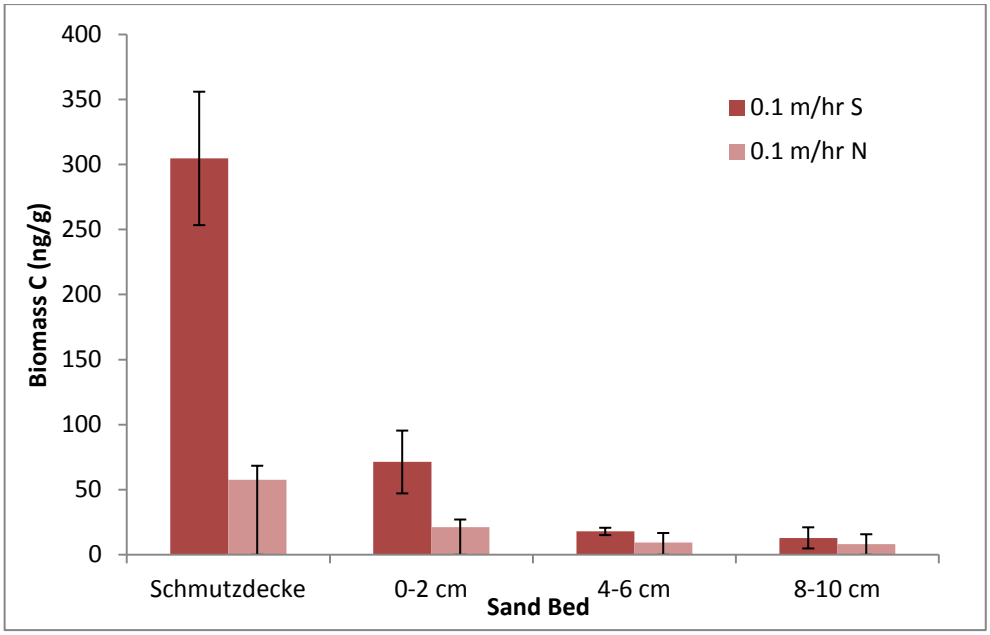
Similar patterns in biomass distribution with depth in slow sand filters have also been reported by *Campos et al. (2006).Duncan, (1988) and Yordanov et al., (1996)*. The values reported by them is higher as compared to these, this is because the filters under the study are covered where the growth is supported only by the substrate present in the influent water as compared to the uncovered ones where photosynthetic inputs of carbon substrates from schmutzdecke to the sand layer occurs *(Campos et al, 2002)*.

4.1.2 Column SSF

Schmutzdecke was distinguished by the sand biomass by colour; schmutzdecke was dark brown in colour where as sand bed was brown. In order to ensure homogeneity in all the columns, schmutzdecke here refers to the top 2 cm of sand. The biomass concentration pattern is presented in figure 19. The value is the average of columns running in duplicates with error bar representing the standard deviation.



(a)



(b)

Figure 19: Biomass distribution in Column SSF (a) 0.1 & 0.5 m/hr (b) 0.1 m/hr with inoculum (S) & Nutrients (N)

The biomass distribution pattern follows the similar pattern as in full scale filter. The biomass concentration decrease with depth and most of the biomass accumulation is in the schmutzdecke and top 2 cm of sand bed.

The columns with added microbial inoculum were able to mimic the full scale filters in terms of biomass concentration growth as seen from the figure 19b. Though, the biomass concentration was much higher in the schmutzdecke layer as compared to the full scale filter, it is because the inoculum used from the filters at Katwijk had a biomass concentration of 153.33 ng/g at the time of addition which further increased as the filtration progressed. As the filtration progressed the biomass in the schmutzdecke layer went into deeper layers of sand bed and thus enhancing the biological activity. The concentration in subsequent depth of sand bed resembles the full scale filter.

Similar effect of higher filtration rate was observed in column SSF. Column with higher filtration rate pushes the substrate into deeper layers of sand bed, thus higher biomass concentration in deeper layers of sand bed as seen in figure 19a. Colum with additional nutrients have higher biomass concentration in comparison to the columns with filtration rate 0.1 m/hr and 0.5 m/hr.

The percentage distribution of biomass concentration in each layer is shown in table 19. The values reported are in ng/g of dry weight of sand. In all the columns, more than 80% of the

biomass is accumulated in the schmutzdecke and top 0-2 cm of the sand bed similar to full scale filters.

Table 19: Percentage Distribution of Biomass Concentration

% distribution	0.1 m/hr	0.5 m/hr	0.1 m/hr N*	0.1 m/hr S**
Schmutzdecke (ng/g)	20.07	31.18	57.60	304.65
% of total	**58.8**	**59.4**	**74.8**	**59.8**
0-2 cm (ng/g)	9.16	13.90	21.27	71.33
% of total	**26.2**	**26.4**	**17.5**	**22.0**
4-6 cm (ng/g)	3.28	4.42	9.33	17.96
% of total	**9.3**	**8.1**	**4.4**	**9.6**
8-10 cm (ng/g)	2.05	3.15	8.13	12.99
% of total	**5.8**	**6.0**	**3.1**	**8.4**
Total	35.20	52.48	93.36	406.94

* N=Additional Nutrients ** S=Microbial Inoculum

4.2 Effect of Scraping on the Efficacy of Slow Sand filter

The effect of scarping on the efficacy of filter surface was studied by carrying out spiking experiments in the columns. In full scale filters, it was not possible to carry out spiking experiments due to safety concern as filters cannot be put out of operation.

The columns were running for 90 days before scraping of layers was done. The schmutzdecke layer was scraped off at 90th day which is in this case was top 2 cm of sand in every column, followed by the scarping of 0-2 cm on 91st day, 4-6 cm on 92nd day and 8-10 cm on 93rd day of sand bed. Spiking experiments of E. Coli WR1 were carried out after scraping of each layer while *MS2* spike was carried out only once after the removal of schmutzdecke.

4.2.1 Role of Schmutzdecke & Sand bed in E.coli WR1 Removal

Schmutzdecke scraping had a significant impact on E. Coli removal because of the reduction in physical straining removal mechanism, which is important for larger organisms such as bacteria and protozoan (oo) cysts, but not for small particles such as viruses. Similar effect was observed in the subsequent removal of the sand. The result of scraping was different for different columns as shown in table 20. In general there was more than 1 log reduction in each of the column immediately after the removal of schmutzdecke scraping. The results of the finding were similar to the value reported in the literature by *Dullemont et al. (2006), McConnell et al. (1984). Hijnen et al. (2004)* reported a reduction of 1 log after the removal of schmutzdecke.

Table 20: Effect of Scraping on E. Coli Removal

	Days	0.1 m/hr	0.5 m/hr	0.1 m/hr S	0.1 m/hr N
Spike 4 (DEC)	**80**	**4.90**	**3.57**	**5.30**	**5.10**
	Removal of				
Spike 5 (DEC)	**Schmutzdecke**	**3.38**	**2.14**	**4.14**	**3.90**
	% Reduction	31.02	40.05	21.88	23.52
Spike 6 (DEC)	**sand 0-2 cm**	**2.53**	**1.76**	**3.56**	**3.23**
	% Reduction	25.14	17.75	14.01	17.18
Spike 7 (DEC)	**sand 4-6 cm**	**1.90**	**1.48**	**2.96**	**2.79**
	% Reduction	24.90	15.90	16.85	13.62
Spike 8 (DEC)	**sand 8- 10 cm**	**1.35**	**0.78**	**2.25**	**2**
	% Reduction	28.94	47.29	23.98	28.31
	Total Reduction*	**72.44**	**78.15**	**57.54**	**60.78**

**Total reduction is based on the difference between 4th spike and 8th spike.*

Columns with microbial inoculum was least affected by removal of schmutzdecke, this is because inoculum was added to a depth of 3-4 cm and during scraping only 2 cm of sand was removed thus still leaving a cm of active layer.

The columns with additional nutrient showed a decrease of 1.2 log removal from 5.1 to 3.9 resulting in 23% reduction in the log removal efficiency. Columns ruuning at 0.1m/hr showed a decrease of 31 % from 4.9 to 3.8. Additional nutrients mean providing more substrate to the microorganisms in the deeper layers of sand bed and hence higher biomass growth in the sand layers below schmutzdecke. Therefore even after the removal of schmutzdecke, the columns with additional nutrient have still sufficient biological activity to achieved higher DEC which can be substantiated from the results of the biomass distribution in figure 21b. Columns with higher filtration rate were found to be most susceptible to the removal of schmutzdecke layer resulting in decrease of 40% from 3.57 to 2.14.

The DEC further reduced on removing the 0-2 cm of sand bed. The percentage reduction was maximum for the columns running at 0.1 m/hr which was 25% as compared to 17.7, 14, and 17.17 in columns running at 0.5 m/hr, 0.1 m/hr with inoculum and 0.1 m/hr with nutrients respectively.

After the removal of 10 cm of sand bed, the DEC was reduced to 1.35, 0.78, 2.25 and 2 for columns running at 0.1 m/hr, 0.5 m/hr, 0.1 m/hr with inoculum and 0.1 m/hr with nutrients respectively. This is because of the loss in biological activity after scraping 10 cm of sand bed. The other reason is the reduction in the depth of sand bed, which plays a significant role in the removal of microorganism from SSF. Since the depth of bed has been

reduced to 50 cm, there is less contact opportunities for bacteria to attach to sand grains and biofilms. Greater detention time in the SSF would allow more time for bacteria removal by predation. Studies in the literature have shown that bacteria removal increases with increased SSF bed depth *(Slade, 1978; Poynter and Slade, 1977)*.

4.2.2 Role of Schmutzdecke in *MS2* removal

In general schmutzdecke scarping had no major impact on the *MS2* removal. The reduction in DEC is shown in table 21.

Table 21: Effect of Scraping on MS2 Removal

	Days	0.1 m/hr	0.5 m/hr	0.1 S m/hr	0.1 N m/hr
Spike 2 (DEC)	**80**	**1.50**	**0.89**	**1.74**	**1.99**
Spike 3 (DEC)	**Removal of Schmutzdecke**	**1.23**	**0.6**	**1.59**	**1.80**
	% decrease	18	32.58	8.62	9.62

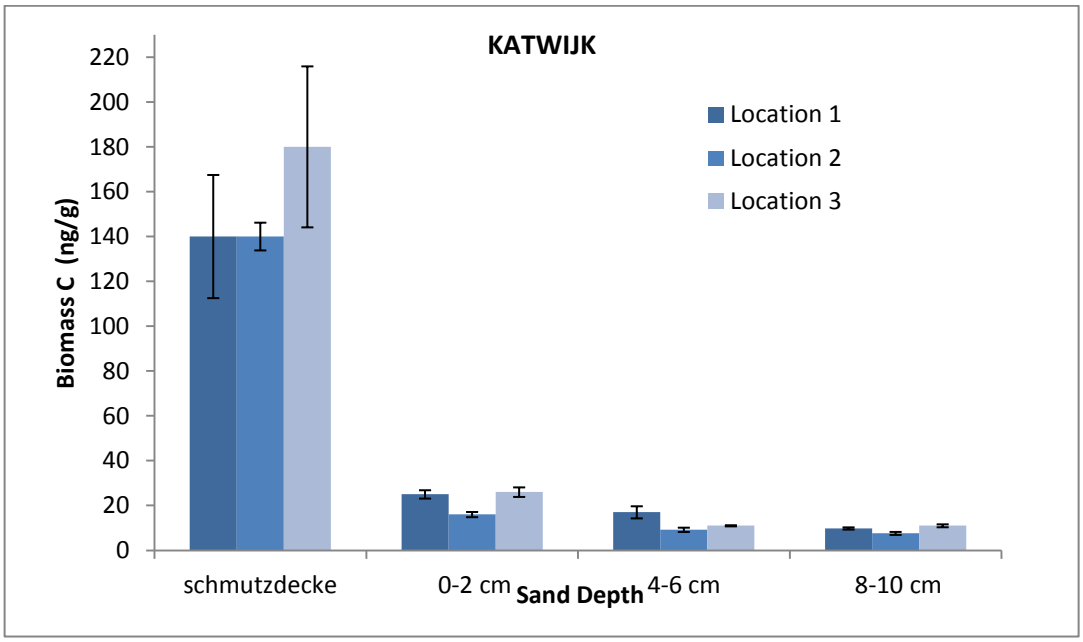
The minor decrease in average *MS2* removal following schmutzdecke scraping observed in the study may be attributed to the loss of predatory microorganisms. Protozoan ciliate suspension feeders primarily inhabit the top of SSFs and can be rapidly desiccated when filters are drained during scraping, which leads to reduced predation of viruses *(Lloyd, 1996)*. *Ellis (1985)* and *Sanchez et al. (2006)* reported that schmutzdecke scraping vastly reduces populations of bacteria and protozoa, both of which are known to prey on viruses *(Kim and Unno, 1996; Cliver and Herrmann, 1972).*

Ellis (1985) also reported that prolonged SSF draining causes microorganisms to utilize some of the extracellular polymeric substances in biofilms as substrate when other substrates are not available which lead to loss of biomass and subsequent washout of bacteria and other microorganisms after filter operation is restarted. One possible reason why filter cleaning had no major impact on *MS2* removals in the current study is because SSF columns were not completely drained when the schmutzdecke was removed. Since full scale SSFs can be drained for days during filter cleaning, the loss of biomass/biofilms and predatory microorganisms at full scale may be more important than observed in this study.

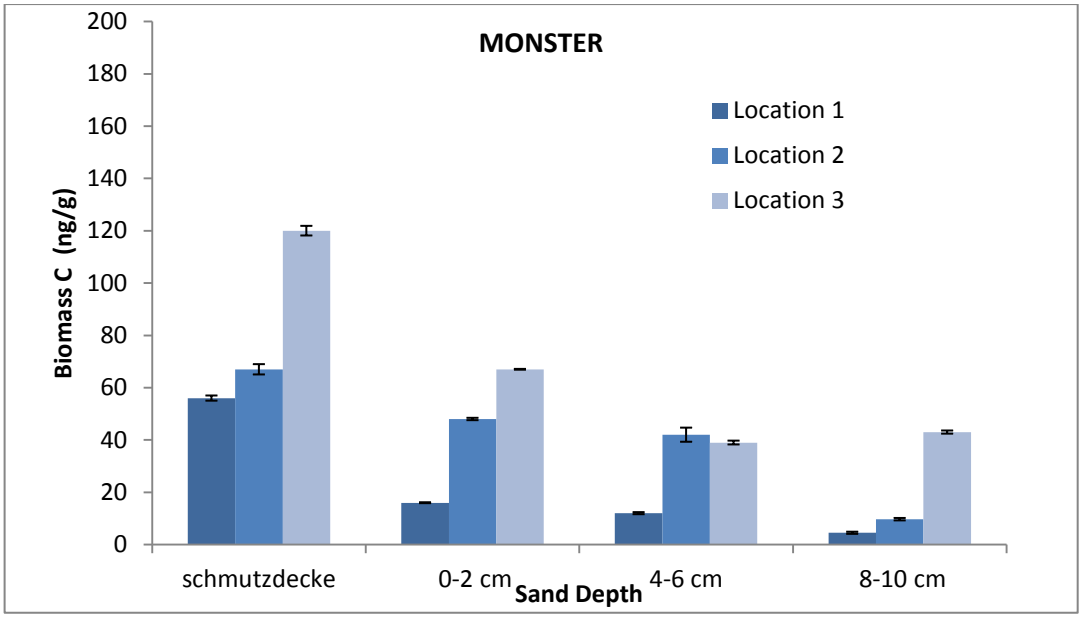
4.3 Spatial Distribution of Biomass

The effect of spatial distribution of biomass was studied in full scale filters. Since the column scale slow sand filters under study were only 4 cm in diameter therefore spatial

distribution of biomass could not be studied in them. The full scale filters were running for 450 and 243 days at Katwijk and Monster respectively at the time of scraping. The spatial variability of biomass growth on sand filter is shown in figure 20. The error graph represents the standard deviation in the duplicate measurements. Location 1 is nearest to the point of entrance of the influent and location 3 is farthest as shown in the figure 14.



(a)



(b)

Figure 20: Spatial distribution of microbial biomass in SSF (a) Katwijk (b) Monster

The spatial variability of the biomass growth on the sand bed is much higher in Monster as compared to Katwijk implying existence of lateral substrate gradients resulting from higher filtration rate and position of the inlet valve of the water to the filter bed. There is a uniform distribution of the influent water at Katwijk from one side whereas there is only a single source situated in the middle of a side at monster (figure 14).

The highest biomass concentration in both the filters is at the point farthest away from the source. At Katwijk, the effect of lateral substrate gradient is significant only in the schmutzdecke layer with standard deviation between three different location to be 23% where as it is 5.5 %, 4% and 1% in sand bed depth 0-2 cm, 4-6 cm and 8-10 cm respectively. At Monster, it is 34%, 25%, 16%, 20% in schmutzdecke, 0-2 cm, 4-6 cm and 8-10 cm respectively.

The spatial differences in the biomass concentration might be because of the habitat heterogeneities caused by differences in the physiochemical characteristics as explained by *Deschesne et al., 2007* such as partially filled or unfilled voids between sand grains that would disperse nutrients and microbes or the dilution of components away from the influent pipe, creating nutritional gradients. Such dispersal of nutrients occurs faster and easier along the surface of SSFs rather than vertically and thus may account for the higher lateral variations as compared to vertical.

High standard deviation in biomass concentration was observed particularly the in the schmutzdecke layer ranging from 48 to 180 ng ATP/g in filters. This might be because of uneven scraping. There is no direct correlation between biomass growth and species (microbial community) evenness but the importance of species evenness in relation to better performance of SSF have been well established by *Wittebolle et al., (2009)*. *Wittebolle et al. (2009)* explained that higher species evenness implies greater robustness and functional stability and therefore a greater ability to adapt to new and fluctuating parameters and a longer run time.

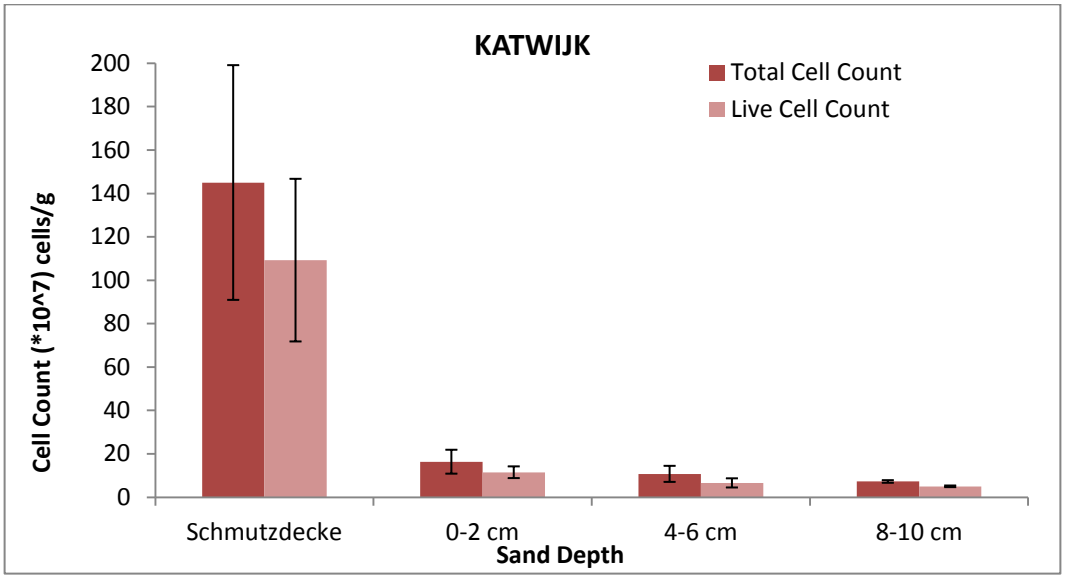
Further in depth studies are required to establish the correlation between the biomass concentration and the species evenness. DNA extraction was done from the current samples from which biomass concentration were determined, which will be analysed later on for microbial community using the Next Generation Sequencing (NGS) technology.

4.4 Biological Activity

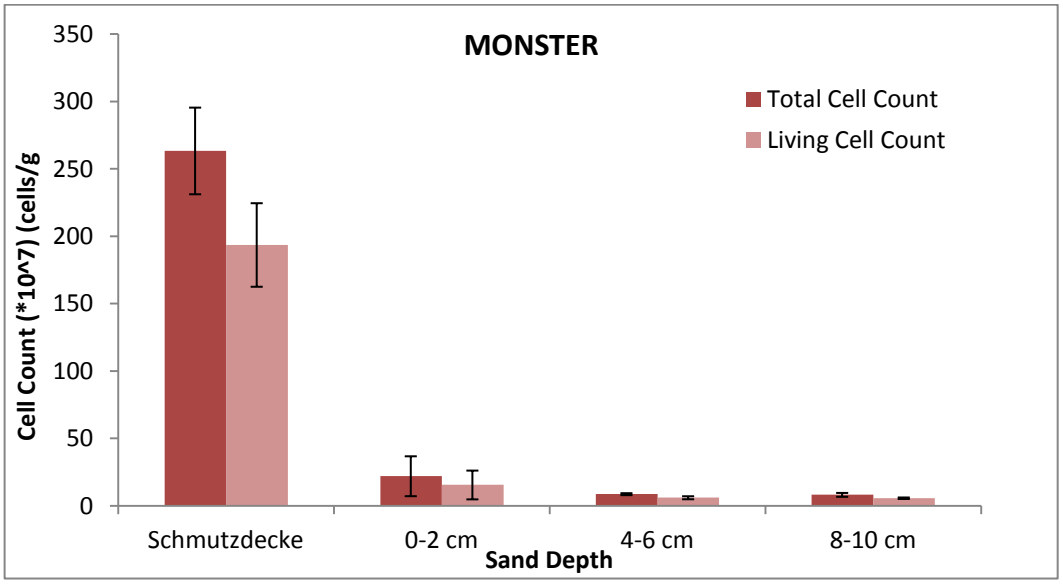
The biological activity in the filters was quantified by using a combination of cell count and ATP content.

4.4.1 Full Scale SSF

The average value of vertical distribution of cell count (Total & Living) in sand bed for filters at Katwijk & Monster is shown in figure 21. The error bar represents the standard deviation between the measurements from 3 different locations at a particular depth on the filter bed. The difference between total and living represents the inactive cell that includes organic particles and suspended particles.



(a)



(b)

Figure 21: Average value of Cell Count from three different locations in SFF (a) Katwijk (b) Monster

The TCC concentration ranged from $6.6*10^7$ to $190*10^7$ cells/g and $6.5*10^7$ to $300.1*10^7$ cells/g in Katwijk and Monster respectively. The distribution of total cell count from both the filter on all the three locations is presented in the table 22.

Table 22: Total Cell count Distribution

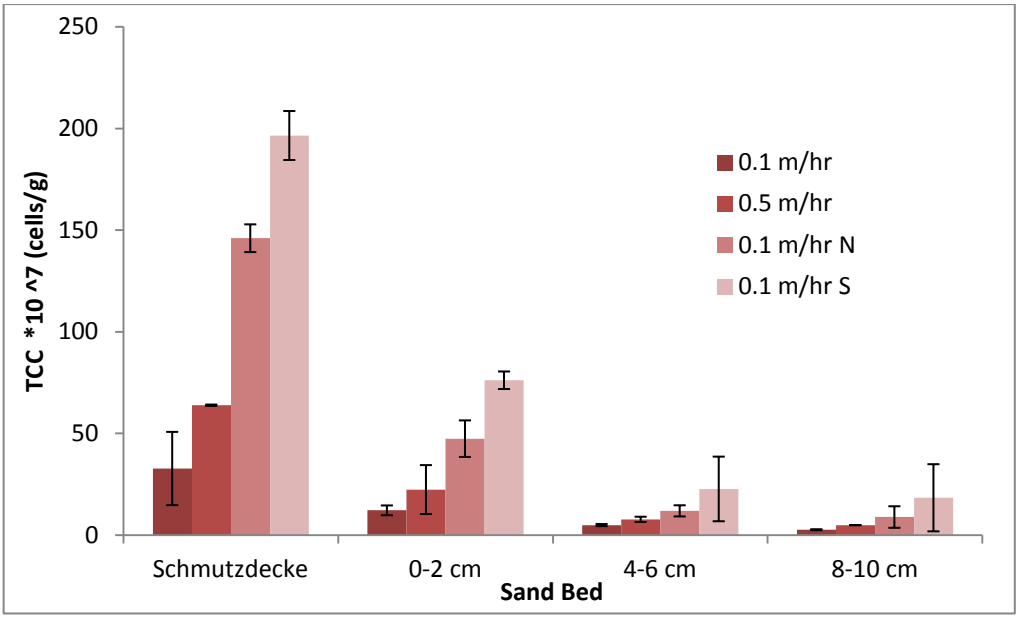
Filter	Location	Schmutzdecke	0-2 cm	4-6 cm	8-10 cm
Katwijk (10^7 cells/g)	Location 1	190	22	15	7.8
	Location 2	85	11	8.6	7.3
	Location 3	160	16	8.6	6.6
	Average	145±54	16.3±5.5	10.7±3.6	7.2±0.6
Monster (10^7cells/g)	Location 1	240	39	9.5	9
	Location 2	300	15	8.5	9
	Location 3	250	12	8.1	6.5
	Average	263± 32	22± 14	8.7± 0.7	8.1± 1.4

Similar to biomass concentration the total cell count decreased with depth and highest cell count was observed in the schmutzdecke layer followed by the top 0-2 cm of the sand bed. Infact more than 90% of the cell count was in top 2 cm and schmutzdecke. The average TCC in the schmutzdecke was higher in Monster (263± 32.24*10^7 cells/g) as compared to Katwijk (145± 54.08*10^7 cells/g) despite having low biomass concentration. This could be because of the effect of inorganics and difficulty in separating cells in the biofilm *(Seger et al, 1996)*. The concentration of inorganics (Ca. Mn, Fe) was determined at Katwijk. Unfortunately the samples from the monster were lost during the transportation and hence cannot be analysed for the inorganic. The distribution of inorganics with depth is provided in the appendix D.

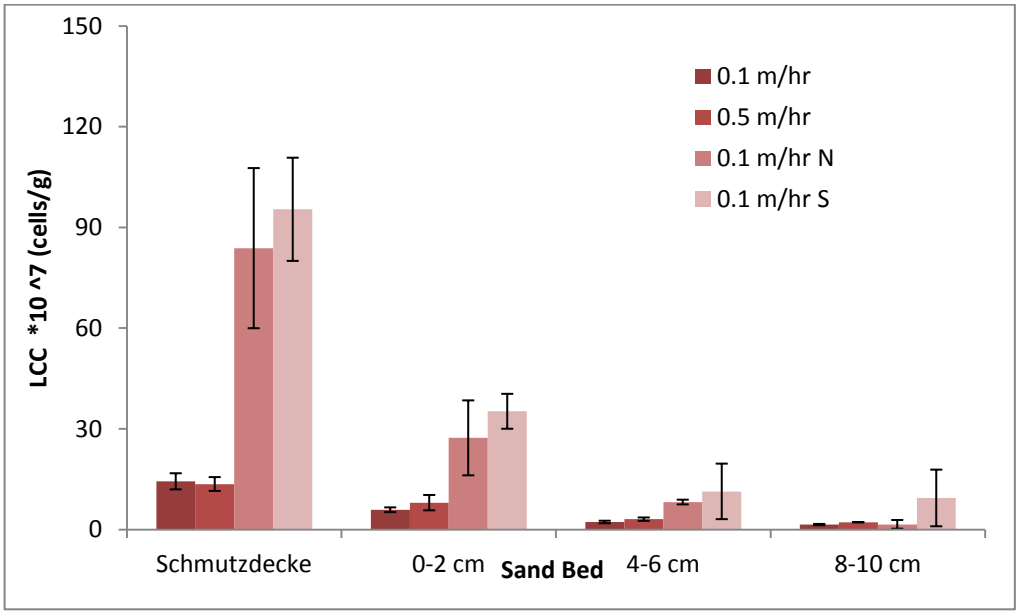
The value of biomass concentration was higher in Katwijk as compared to Monster, though it was opposite in case of cell count. Therefore biomass concentration measurement done using ATP or cell counts determined by using FCM might give an over estimation. Hence it is better to use a combination of one or more technique to determine the cell activity.

4.4.2 Column SSF

The cell count distribution in the column filters is presented in figure 22. The value is the mean of column running in duplicates and error bar represents the standard deviation in average values. Cell Count in the columns follows the similar pattern as in full scale filter that is decrease with depth. Higher cell count was observed in filters with higher filtration rate, microbial inoculum and nutrients as compared to the one without any addition at the filtration rate of 0.1 m/hr.



(a)



(b)

Figure 22: Cell count in SSF (a) Total (b) Living

A comparison between the cell count in column and full scale filter is presented in table 23. It can be seen that columns with microbial inoculum and nutrients have cell counts in the range of full scale filters. The values presented in the table are in 10^7 cell/g. The values for full scale filters are the average from three locations.

Table 23: Total Cell count Comparison in Column and Full scale filter (10^7 cell/g)

SSF	Schmutzdecke	0-2 cm	4-6 cm	8-10 cm
Katwijk	145±54	16.3±5.5	10.7±3.6	7.2±0.6
Monster	263± 32	22± 14	8.7± 0.7	8.1± 1.4
Column (0.1m/hr)	32.7± 18.1	12.17± 2.41	4.92± 0.51	2.63± 0.23
Column (0.5 m/hr)	63.87± 0.34	22.38± 12.03	7.73± 1.28	4.48± 0.03
Column (0.1 m/hr N*)	146.04± 6.82	47.42± 8.99	5.68± 6.07	3.20± 2.74
Column (0.1m/hr S)**	196.56± 12.04	76.17± 4.31	22.7± 15.90	18.35± 16.51

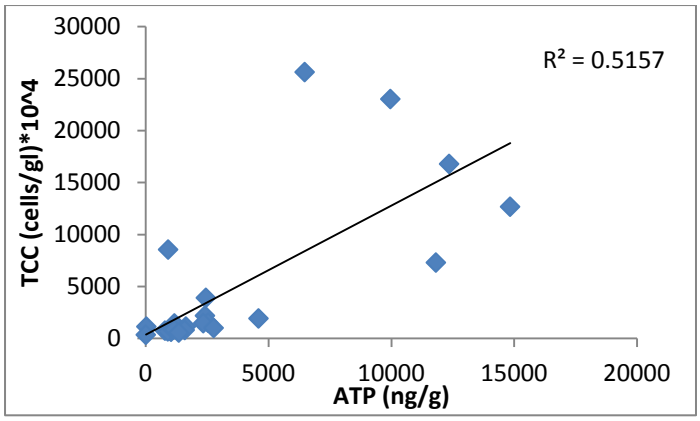
* N= Columns with additional Nutrients ** S=Columns with microbial inoculum

4.4.3 Relation between TCC and ATP

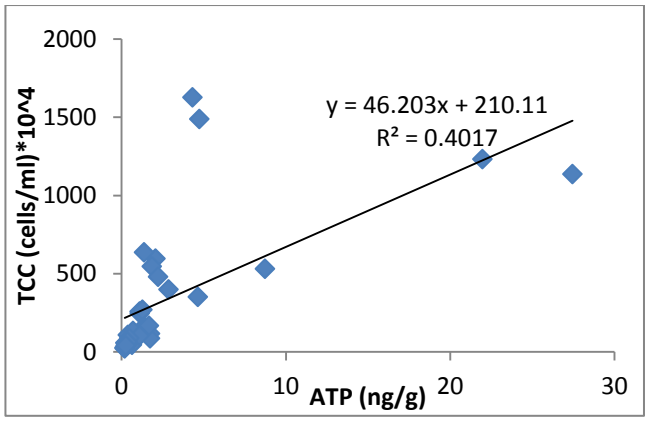
A correlation analysis was performed between TCC and ATP of samples (figure 23). A good relation between TCC and ATP has been reported in literature for drinking water samples, However, a good correlation between TCC and ATP was not found in the current study with R^2 =0.51 and 0.41 in full scale and column SSF respectively. This might be because of the overestimation in the cell count as it includes the actively growing cells which contribute to production of biomass and the dead and inactive cells. The microbial biomass in the SSF might be overestimated with the ATP measurements due to a higher biomass concentration at the top of the filter *(Eighmy et al., 1992)*.

Gasol et al. 1999 suggested that the percentage of HNA cells could be used as a reference for the percentage of actively growing bacteria in marine environments. Therefore, a correlation between the number of active bacteria and the ATP concentrations can be expected since LNA bacteria might be inactive, therefore, may have no contribution to ATP. A correlation between cell counts of HNA bacteria and ATP concentrations was done (figure 24). The correlation coefficient (R^2) was found to be 0.62 in both the full scale and column SSF.

The average ATP content per cell, which is a measure for metabolic activity, was calculated for both the full scale and column filters as show in the Appendix C. The ATP content ranged from $7.3*10^{-8}$ to $1.66*10^{-7}$ ± $0.285*10^{-7}$ ng ATcell^{-1} in filters at Katwijk where as it ranges from 2.3 $*10^{-8}$ to $6.61*10^{-7}$ ± $2.422*10^{-7}$ ng ATcell^{-1} in filters at Monster. The ATP content ranged from $6.6*10^{-8}$ to $8.5*10^{-8}$ ± $0.785*10^{-8}$ ng ATcell^{-1} in columns running at 0.1 m/hr, $4.8*10^{-8}$ to $7.3*10^{-8}$ ± $0.11*10^{-8}$ ng ATcell^{-1} in columns running at 0.5 m/hr, $2.9*10^{-8}$ to $4.0*10^{-8}$ ± $0.73*10^{-8}$ ng ATcell^{-1} in columns running at 0.1 m/hr with nutrients and $1.44*10^{-7}$ to $2.9*10^{-7}$ ± $0.3*10^{-7}$ ng ATcell^{-1} in columns running at 0.1 m/hr with added inoculum.

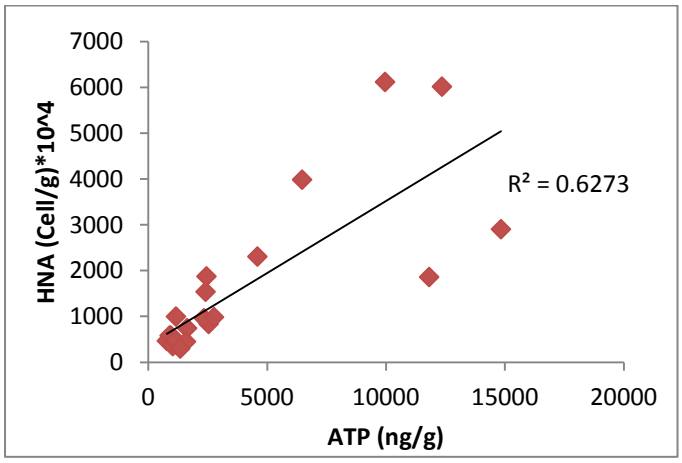


(a)

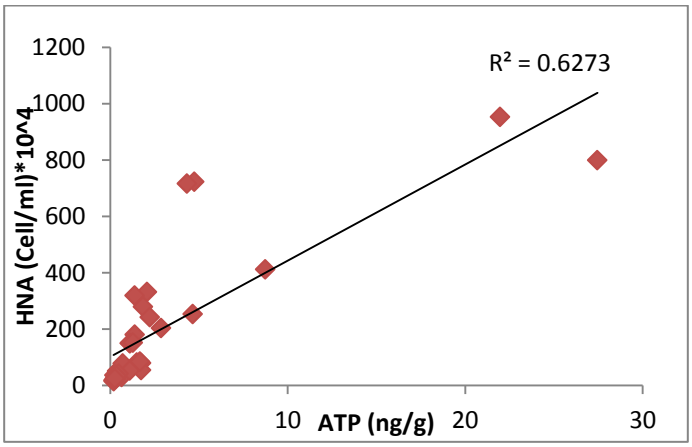


(b)

Figure 23: Correlation between ATP &TCC (a) Full Scale SSF (b) Column SSF



(a)



(b)

Figure 24: Correlation between ATP &HNA (a) Full Scale SSF (b) Column SSF

The median ATP content of cells on SSF was in the range of 10^{-8} to 10^{-7} ng cell^{-1} which is the median value reported for starving cells *(Webster et al., 1985)* and bacteria on membranes used in water treatment ($2*10^{-8}$ –$7*10^{-7}$ ng cell^{-1}) indicating low metabolic activities of the cells on SSF *(Vrouwenvelder et al., 1998)*. Because of the low metabolic activity in the slow sand filters, the depth of sand bed that is removed should be minimised, to ensure maximum activity is retained during scraping procedure and thus reduce the ripening time.

The median ATP content per cell was also calculated using HNA cell which was found to be higher than tha median values calculated using total cell count in both the column scale and full scale filters (Appendix E). Since a better correlation was obtained between ATP and HNA cell, it can be inferred that the activity calculated using HNA cell count is a better representative of the metabolic activities rather than using Total Cell Count.

Objective 1: Summary and Concluding Remarks

- The largest accumulation of biomass was measured in the schmutzdecke followed by the top 0–2 cm of sand bed in both the full scale and column SSF.
- The observed reduction in biomass concentration with depth suggested there is substrate limitation to microbial growth in the deeper layers of the covered filter.
- Higher filtration rate pushes the substrate into deeper layers of sand bed, thus higher biomass concentration in deeper layers was observed both in full scale filter and column SSF.
- Lower filtration results in longer filter run time and most of the deposition of biomass takes place in the schmutzdecke and top 2 cm of sand bed and thus less depth of the sand bed has to be cleaned.
- Different layer of sand bed have different inactivation potential. More than 1 log reduction in DEC of E.coli was observed after the removal of schmutzdecke in all the columns. After scraping of 10 cm of sand bed the percentage reduction in the DEC was 72%, 78%, 57% and 60% in columns running at the filtration rate of 0.1m/hr, 0.5 m/hr, 0.1 m/hr with inoculum and 0.1 m/hr with nutrients respectively. Schmutzdecke scraping had only a minor negative effect on *MS2* removals.
- Column with added microbial inoculum were able to mimic full scale filter in terms of biomass growth and cell count. As the filtration progresses, the biologically active inoculum penetrated into deeper layers of sand bed and were able to achieve more than 2.2 log removal even after the scraping of 10 cm of sand bed.
- Current full scale SSF is expected to have DEC of 3 logs for bacteria and 1 log for virus. After the removal of schmutzdecke and 0-2 cm sand bed where most of the biomass accumulation take place, the DEC of the E.Coli was still above 3 and DEC of the *MS2* was still above 1 in columns with added inoculum.
- Combining the results of the biomass distribution and spiking experiments carried out in the columns with microbial inoculum, it can be concluded that ripening period of the SSF will be drastically reduced if the scraping of only 4 cm of sand bed takes place including schmutzdecke as seen from the spiking results of the column with added microbial inoculum.

- Position of the inlet source also effects the biomass growth on the sand surface and generates lateral gradients. The biomass concentration was highest at location 3 in both the filters (point farthest away from source) implying the existence of lateral substrate gradients on the surface of the bed.

- Substrate gradient results in uneven biomass growth. This is particularly important from the cleaning point of view, uneven scraping of the filter might result in longer time for filter to get ripened due to species unevenness.

- The cell counts followed the similar pattern as the biomass concentration in both the filters. Cell counts were done using flow cytrometry which was able to distinguish between HNA and LNA cell. Correlation coefficient between ATP and TCC was found to be 0.51 and 0.40 in full scale and column filters , where as a better correlation was obtained between ATP & HNA (R^2= 0.62) in full scale as well as column filters, HNA represents the active bacteria and thus a better correlation was obtained.

- Even though most of the biomass concentration (~ 80%) is present in the schmutzdecke and top 0-2 cm of the sand bed in both the full scale and column SSF, the metabolic activity is present throughout the sand bed (0-10 cm)

2017

Chapter 5

Results: Objective 2

The objective of this chapter is to accelerate the ripening period by changing operational parameters and observe the effect of different operating conditions on the efficacy of column slow sand filters during stages of ripening.

5.1Water Quality Measurements

The Columns were run for approximately 100 days, during which continuous measurements of turbidity, particle counts and dissolved organic carbon were done. Turbidity measurements were done daily, particle counts were done twice a week and DOC measurements were done once a week. Along with that temperature measurement were done twice a week and on the day of carrying out spiking experiments. The current study includes data from 10 May to 25 August'2017.

5.1.1 Turbidity

The influent turbidity ranged from 3.43-6 NTU, with average value of 4.55±0.6. Frequency analysis of the influent water turbidity is presented in table 24. The influent and effluent concentration of turbidity to the columns is shown in figure 25 & 26. The y axis in the graph is on logarithmic scale. The effluent turbidity is the average values of columns running in duplicates.

Table 24: Frequency analysis of the influent water turbidity

Occurrence of turbidity in the Range (NTU)	Frequency
3-4	17
4-5	71
5-6	17
5-6	2

The average effluent turbidity and percentage removal in the columns is shown in the table 25. The value represented in the table is for the entire duration of the columns operation.

Table 25: Summary of Effluent Turbidity

Columns	Average Effluent Turbidity (NTU) (Standard Deviation)	% Removal	Median value (N=100)
0.1 m/hr	0.39±0.39	91.43	0.19
0.5 m/hr	0.62±0.54	86.20	0.34
0.1 m/hr S*	0.59± 1.02	87.02	0.155
0.1 m/hr N**	0.33±0.40	92.56	0.15

*S represents the columns in which microbial inoculum were added.

**N represents the columns in which nutrients were added.

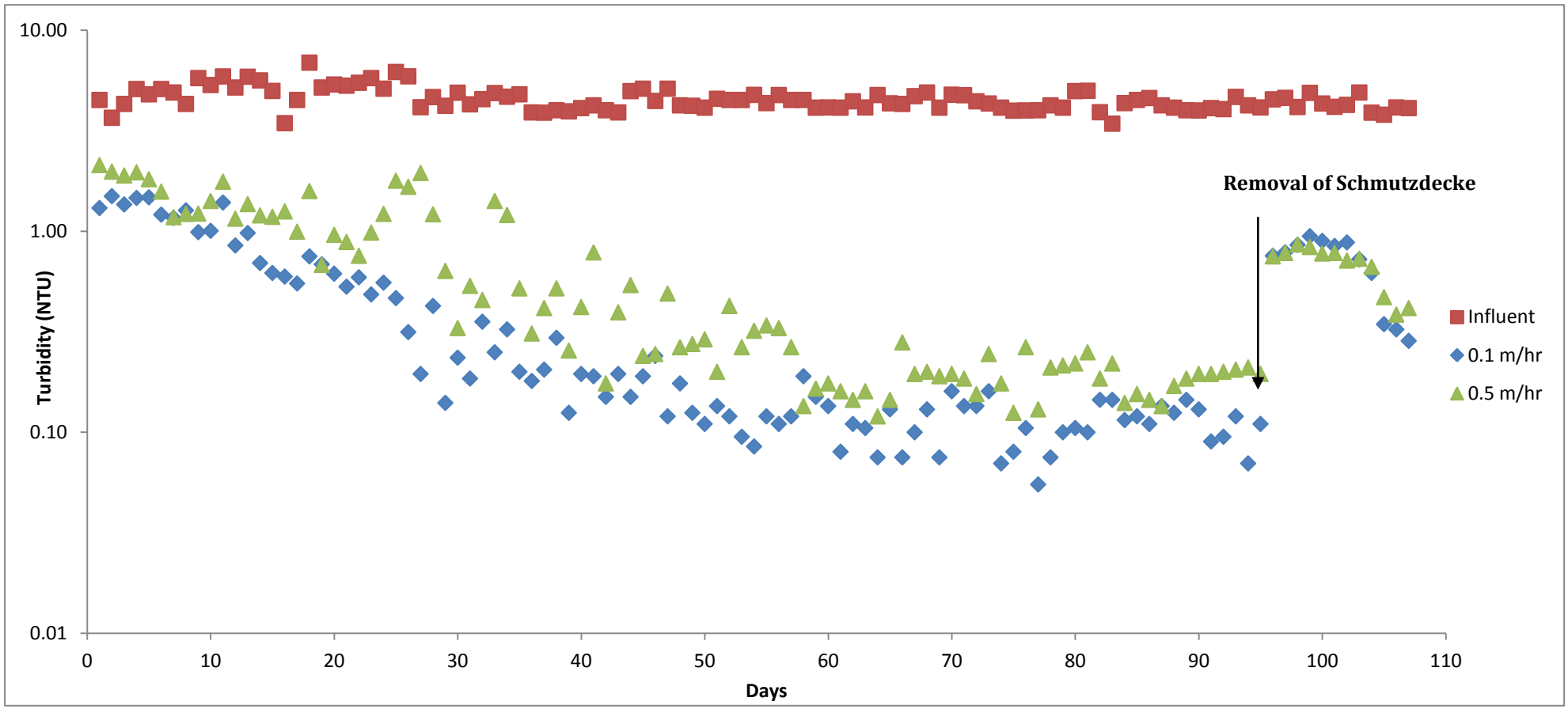


Figure 25: Influent and effluent turbidity to columns running at the filtration rate of 0.1 m/hr and 0.5 m/hr

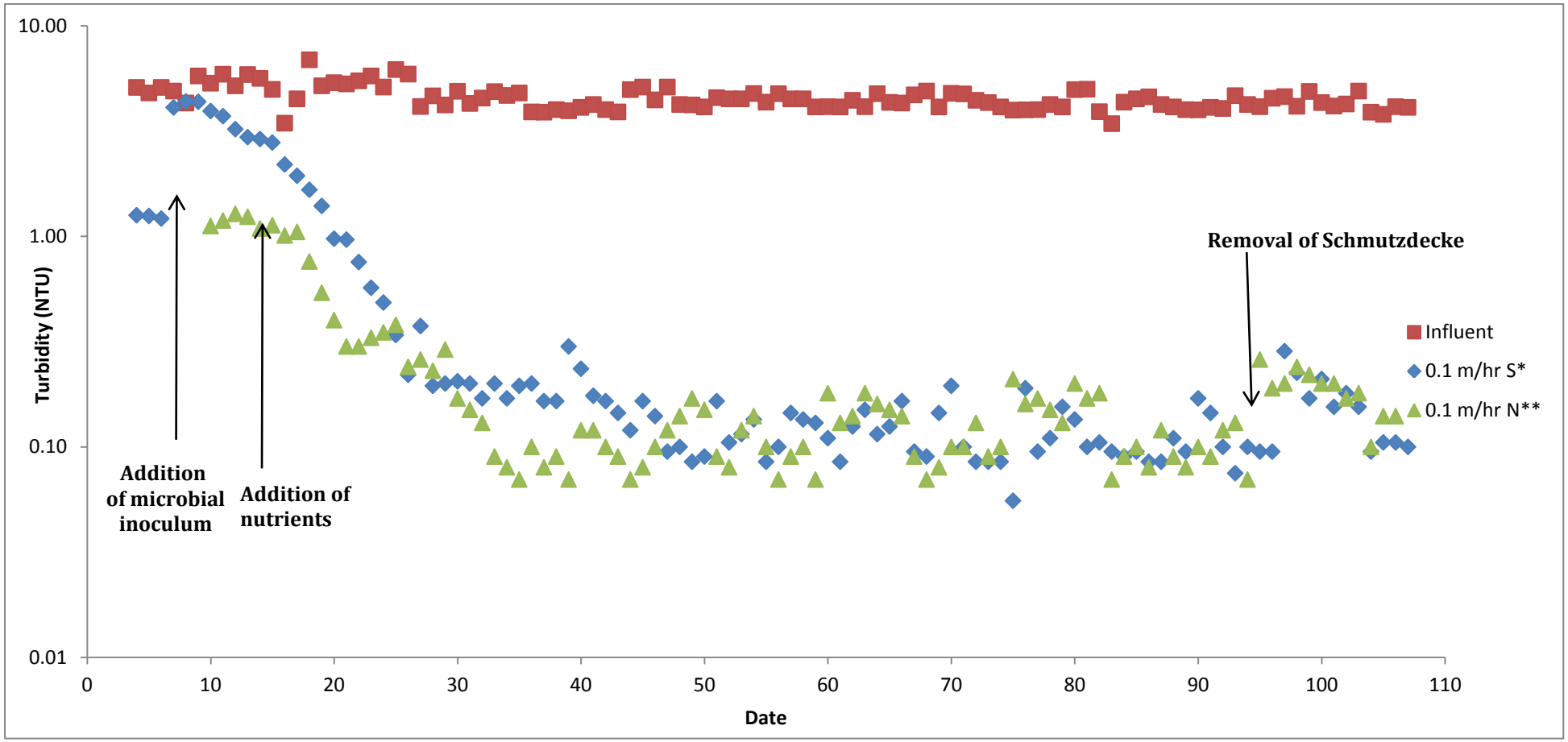


*Figure 26: Influent and Effluent turbidity: Columns running at the filtration rate of 0.1 m/hr with addition of *microbial inoculum(S) and **nutrients (N)*

Column running at 0.1 m/hr: Correlation analysis was performed between the duplicate columns to check the validity of the mean values. The correlation coefficient (R^2) value was found to be 0.92 as shown in the graph F.1 in appendix F.

The average percentage removal is 91.43% which is in the range of values reported in the literature. The median value is 0.19 which is higher than the acceptable limits this is because of the fact that during the initial stages of the SSF, the filters are at sub optimal performance. This is also the rationale behind higher value of standard deviation. The turbidity value was less than 1 NTU for 90% of the time and 70% of times it is less than 0.5 NTU.

During the first 38 days of operation the average turbidity is 0.7 ± 0.43 and for the next 58 days that is before the removal of schmutzdecke (39-90 days), the average turbidity was 0.12 ± 0.039. The median turbidity during these 58 days was 0.1 NTU and never exceeded 0.2 NTU, unaffected by the changes in the influent water turbidity. The average percentage removal during this period was $98\pm0.89\%$ which is higher than the average value for the whole run time.

The increase in the percentage removal and lower value of turbidity after certain point of time in filter operation suggest the importance of ripening. This in turn was confirmed by the removal of schmutzdecke after which the percentage removal decreased to 83% from 97.5% (14.5%) and keep on decreasing as subsequent removal of sand layer. After removal of the last sand bed layer (8-10 cm), the improvement in the turbidity removal was seen after 3-4 days. Even after the removal of schmutzdecke, in no case the effluent turbidity exceeded 1NTU, this is because the influent turbidity is not very high and filters are operating at slow filtration rate of 0.1 m/hr and the temperature is in the range of 20-25^{0}C which promotes biological activity and faster recovery of schmutzdecke. In case of high raw water turbidity and colder temperature the effect may be more pronounced. Therefore, scraping of the schmutzdecke layer should be avoided in challenging operational conditions to minimize the negative impacts on treatment performance.

Column running at 0.5 m/hr: Correlation analysis was performed between the duplicate columns to check the validity of the mean values. The correlation coefficient (R^2) value was 0.97 as shown in the graph F.2 in appendix F.

The average percentage removal was 86.43% which was less than as compared to the columns running at 0.1 m/hr. The median value is also on the higher side which was 0.34. Only 70% of the times the effluent turbidity was less than 1NTU and 58 % times less than 0.5 NTU.

Turbidity removals was much more susceptible to the higher filtration rate (0.5 m/hr vs. 0.1 m/hr), even after 50 days of operation slight changes in the influent turbidity impacted the effluent turbidity. After 57 days of operation that is for the period of 57 -90 days, it was able to reach the median value below 0.1 NTU. The average percentage removal during this period of time was 96.36± 1.006.

After the removal of schmutzdecke, the percentage reduction in turbidity removal was 11% which is less than from the filter running at 0.1 m/hr. This because higher filtration rate might have pushes the substrate into deeper sand levels. Effluent turbidity was still under 1NTU although it took much longer time to recover (7-8 days) and to reach turbidity levels below 0.5 NTU because of the lesser residence time over the filter bed.

Column running at 0.1 m/hr with microbial inoculum (0.1m/hr S): Correlation analysis was performed between the duplicate columns to check the validity of the mean values. The correlation coefficient (R^2) value was 0.987 as shown in the graph C.3 in appendix C.

The effluent turbidity was less than 1 NTU 82.24 % of time and less than 0.5 NTU 78. 5% of time. The average percentage removal is 87% which is less than the filter running at 0.1 m/hr. The lower average percentage removal was observed because a significant increase in the effluent turbidity was observed after the addition of schmutzdecke as shown in figure 26. The schmutzdecke consist of the suspended and inert particles which take time to settle down on the sand surface and were suspended in the supernatant water (black colour) as shown in the fig 29.

Figure 27: Sudden effect of addition of schmutzdecke

The effluent turbidity reached below 1 NTU after 2 weeks of operation. The median value was 0.15 which justified the fact the lower percentage removal was because of the few values of higher turbidity during initial stages resulting from the sudden effect of addition of schmutzdecke. The average percentage removal increased to 96.5±1.02% after that.

After 21 days of operation the average effluent turbidity was 0.13± .049 and the median value was below 0.1 NTU. No significant difference in the percentage reduction was observed because of the removal of schmutzdecke and sand bed. This is because of the two reasons; first the microbial inoculum with high biological activity was added on to top 3-4 cm of sand bed, so after removing schmutzdecke and top 0-2 cm it was still there. The other reason could be; as the filtration progresses the inoculum penetrated deeper and hence improved activity in the deeper layers of sand bed.

Column running at 0.1 m/hr with Nutrients (0.1 m/hr N): Correlation analysis was performed between the duplicate columns to check the validity of the mean values. The correlation coefficient (R^2) value was 0.979 as shown in the graph F.4 in appendix F.

The average percentage removal was 92.56 with median value of 0.15 NTU. The effluent turbidity was less tha 1 NTU 83.81 % of time and less than 0.5 NTU 81.31% of time. After only two weeks of addition of nutrient the effluent turbidity was below 0.2 NTU and remained below that for the entire duration of column experiment. After that the average percentage removal was increased to 96.5± 1.23 %. The median value was below 0.1 NTU after 17 days of operation that is for the duration after 17 days to the time of schmutzdecke removal (17-90 days). Similar to the addition of microbial inoculum filters, no significant difference was observed in the turbidity percentage removal after the removal of schmutzdecke and sand bed from the columns. Higher turbidity reductions after the addition of nutrients might because of high the growth rate of biopopulaion. Similar results were obtained by *Logsdon, 1987* where the influent water with high nutrient concentration resulted in higher turbidity removal in SSF. The average effluent turbidity after median value was equal to or less than 0.1 NTU are presented in table 26.

Table 26 : Summary of Effluent turbidity (Median= 0.1 NTU)

Columns	Effluent Turbidity (NTU) (Standard Deviation)	% Removal	Median value (N=100)
0.1 m/hr (39-90)	0.12±0.039	98.09	0.10
0.5 m/hr (57-90)	0.18±0.540	96.20	0.11
0.1 m/hr S* (21-90)	0.13± .049	97.98	0.10
0.1 m/hr N (17-90)**	0.13± .026	98.06	0.10

Higher filtration rate pushes the substrate deep into the sand bed, similarly additional nutrients and addition of microbial inoculum provides substrate into deeper layers of sand bed as seen from figure 21 (biomass distribution in SSF column). This was evident from the fact the removal of schmutzdecke and different layers of sand bed had comparatively less significant impact on the turbidity removal efficiency as compared to the columns that were running at 0.1 m/hr.

5.1.2 Summary & Concluding Remarks

1. Filtration rate and microbial activity plays a significant role in the turbidity removal during stages of ripening.
2. Columns operating at the rate of 0.1 m/hr took 38 days to reach median turbidity levels of 0.1 NTU, where as columns with microbial inoculum took 21 days and columns with additional nutrients took only 17 days.
3. Column operating at the filtration rate of 0.5 m/ took 57 days to achieve the median turbidity below 0.1 NTU and were more susceptible to changes in the influent turbidity.
4. Even though same nutrient loading was applied on the columns running at 0.5 m/hr and 0.1 m/hr with additional nutrients (N), columns with 0.1 m/hr with N performed much better with lower effluent turbidity values and take much less time to reach median turbidity levels below 0.1 NTU, suggesting the importance of maintaining low filtration rate during initial stages of ripening.
5. During the experimental period, the influent turbidity was not very high (<6 NTU) therefore no filter clogging was observed during the entire period of study. Also the variation in the influent turbidity was not significant and thus there were no peaks or sudden changes in the behaviour.
6. There was no direct measurement of headloss. The effluent flow rate was adjusted by opening the effluent valve which was not fully opened during the entire period of study.

5.1.3 Particle Counts

The average influent concentration was 9971±1580 counts/ ml. The percentage distribution of particles is shown in table 27. The influent and effluent particle counts/ml for the columns is show in Appendix G.

Table 27: Percentage distribution of the particles

Particle Count/ml (Range)	Frequency Percentage
6000-7000	2.5
7000-8000	5
8000-9000	17.5
9000-10000	55
10000-11000	20

Columns running at 0.1 m/hr: The average effluent concentration of the particle was 402 ± 291 particles/ml during the entire column run which is higher than the desired range of 100-200 counts/ml (*Hargesheimer et al. 1998; Cook, 1995)*. The concentration was higher during the initial filtration process. The particle count was below 200/ml after 40 days of operation, after which the average concentration was 122± 20 count/ml and was in the range of 101-189counts/ml. During the period 40-90 days of column operation the average percentage removal was 98.6±0.9. After the removal of schmutzdecke, the particle count increased to 375 counts/ml from 122counts/ml and similar effect was observed after the subsequent removal of sand bed. The average percentage removal after 90 days that is for the period of 90-105 days was 95.4± 0.91 which was still higher than the initial percentage (0-40 days) removal of 93.9 ± 2.1.

Column running at 0.5 m/hr: The average effluent concentration was 774 ±520 /ml which is almost 6 times higher than the recommended values of 100-200 counts/ml (*Hargesheimer et al. 1998)*. The count was below 200/ml only after 58 days of operation. The average effluent concentration for 58-90 days of operation was 185± 58 count/ml with removal efficiency of 98.1± 0.6. Significant increase in the particle count was observed after the removal of schmutzdecke and sand bed. The average particle count increased to 1005± 231 counts/ml for the duration of 90-105 days.

Column running at 0.1 m/hr with microbial inoculum: The average effluent concentration was 351 ±465 /ml. The high value of standard deviation was because of a spike observed in the effluent particle concentration after the addition of schmutzdecke. After 7 days of addition of inoculum, the average concentration was reduced 203±34/ ml. After 27 days of operation, the effluent concentration was below 200 counts/ml and remains that for the entire duration of the study. No significant effect was observed after the removal of schmutzdecke and sand bed in the effluent concentrations. The percentage reduction for the duration of 27-90 days was 98.6± .4 and fro 90-105 days was 98.8± .1.

Column running at 0.1 m/hr with additional nutrients: The average effluent concentration was 260 ±169 /ml. After 7 days of addition of nutrients the average

concentration was reduced 203±34/ ml. After 26 days of operation, the effluent concentration was below 200 counts/ml and remains that for the entire duration of the study. The average effluent concentration was 128± 27 counts/ml after that. No significant effect was observed after the removal of schmutzdecke and sand bed in the effluent concentrations. Table 28 presents the average particle counts during different stages of filter operations.

Table 28: Effluent particle count

Column	0.1m/hr			0.5 m/hr			0.1m/hr S			0.1 m/hr N		
Days of operation	**0-40**	**40-90**	**90-106**	**0-58**	**58-90**	**90-106**	**0-27**	**27-90**	**90-106**	**0-26**	**27-90**	**90-106**
Particle Count/ml	605± 200	138 ± 60	454± 90	999± 481	185 ± 58	1005± 231	875± 581	133 ± 37	112 ± 4	606± 234	130± 30	118 ± 7
% Removal	93.9 ± 2.1	98.6 ±0.9	95.4± 0.91	89.8± 4.5	98.1 ± 0.6	89.92 ± 2.3	91.225 ± 5.8	98.6 ± .4	98.8 ± .1	93.9± 2.4	98.69 ± 0.3	99.8 ± .1

From table 28, it can be concluded that the effluent particle counts is different during stages of column operation and the time taken to reach the optimal value of 100-200 counts/ ml is also different suggesting the effect of operational parameters on the ripening of the columns.

Columns with added inoculum and nutrients performed better than the columns with higher filtration rate and took relatively less time to reach the particle count within acceptable limit of 100-200 counts/ml. It was also observed that there was no significant decrease in the percentage removal even after the scraping of schmutzdecke and different layers of sand bed in columns with added inoculum and nutrients.

This suggests the fact that the filtration rate and biological activity play a significant role in the particle removal from slow sand filters. *Webber and Shrik, (1999a)* highlighted the importance of biological activity in the removal of particles less than 2 µm , though in this case particle count was done for particle greater than 2 µm, suggesting the importance of biological activity in the reduction of particles irrespective of the size.

5.1.4 Summary & Concluding Remarks

1. Similar to turbidity, the effluent particle count is affected by the operational parameters.
2. The reduction in particle count as the ripening proceeds indicates enhanced particle straining due to bio layer formation and maturity of the filter bed.

3. Filter operating at 0.1 m/hr took 40 days to reach the effluent concentration below 200 count/ml where as columns with microbial inoculum and nutrient took 27 and 26 days respectively. Columns running at 0.5 m/hr took 58 days to reach the concentration below 200 counts/ml.
4. No significant difference was observed in the effluent particle counts after the removal of schmutzdecke in columns added with microbial inoculum and nutrients as seen from the values in table 28.
5. Effluent concentration in columns with higher filtration rate was most susceptible to removal of schmutzdecke and sand bed. A 10% decrease was observed in the percentage removal efficiency after the removal of schmutzdecke in column running at 0.5 m/hr as compared to a decrease of 3% in columns running at 0.1 m/hr.

5.1.5 DOC & TN

In the current study, the average DOC removal by SSF columns was in the range of 16.23 to 28.82 % which is in accordance with value reported in the literature (table 2). Summary of the average influent and effluent DOC and TN is presented in the table 29.

Table 29: Summary of the average influent and effluent DOC and TN

	Avg. Influent (mg/L)	**Average Effluent (mg/L)**			
		0.1m/hr	0.5 m/hr	0.1 m/hr S	0.1m/hr N
DOC	1.78 ±.08	1.32 ±.15	1.42 ±.13	1.49 ±.14	6.34 ±.64*
TN	1.20 ±.079	1.05 ±.07	1.07 ±.08	1.12 ±.06	5.31 ±.28**

* The influent DOC values was 8.91±.43

** The influent TN values was 6.03±.39

The percentage removal increased slightly after few days of operation, but there was no significant increase in the percentage reduction with the filter ripening. The influent and effluent DOC value is shown in the graph in appendix H. The effluent DOC concentration is more a function of the influent concentration rather than the maturity of the filter bed or growth of schmutzdecke layer. The average percentage reduction was 25.77±8.33%, 20.05±6.51%, 16.23±8.29% and 28.82±6.24% for columns running at 0.1m/hr, 0.5 m/hr, 0.1 m/hr with microbial inoculum and nutrients respectively. The percentage reduction at different stages of column operation is shown in figure 28.

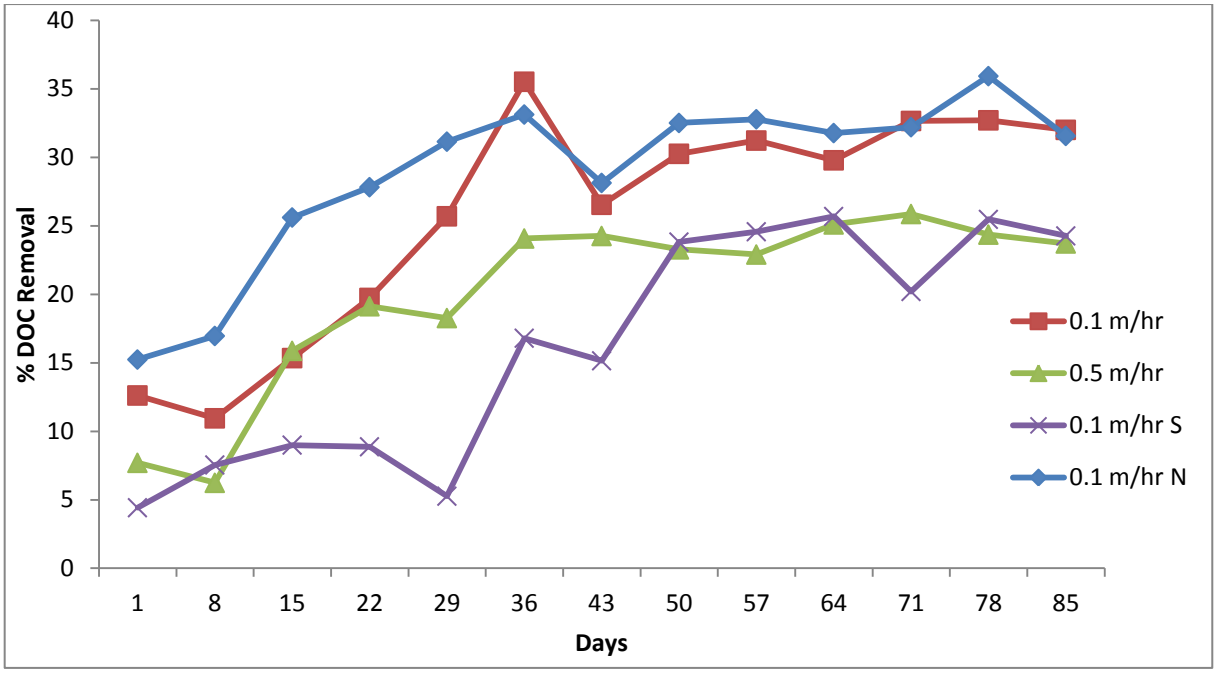


Figure 28: Percentage DOC removal

Higher percentage reduction in the columns running at 0.1 m/hr and 0.1 m/hr with added nutrients implies higher substrate utilization for the growth of microorganisms. 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 a carbon and energy source essential to the growth of heterotrophic bacteria *(Bouteleux et al, 2005)*.

Total Nitrogen was also measured in the effluent and influent. *Miettinen et al, 1996* found that inorganic nutrients also effect the microbial growth among which phosphorus and nitrogen are the two main inorganic nutrients. The influent and effluent DOC value is shown in the graph in appendix H. The average percentage reduction was 12.53±2.55%, 10.77±3.2%, 6.82±2.05% and 11.79±1.98% for columns running at 0.1m/hr, 0.5 m/hr, 0.1 m/hr with microbial inoculum and nutrients respectively. The percentage reduction at different stages of column operation is shown in figure 29. Similarly to DOC removal, total nitrogen removal increased slightly in the beginning as the filter matures but after that no significant increase in the percentage reduction was observed.

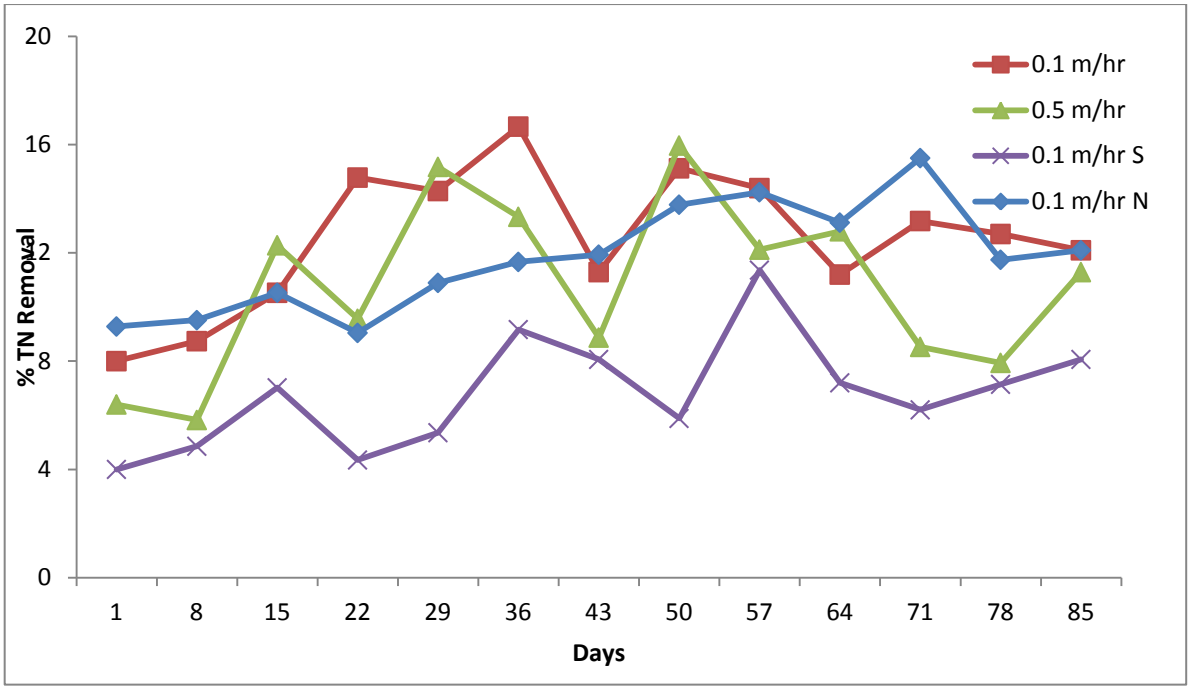


Figure 29: Percentage TN removal

5.1.6 Summary & Concluding Remarks

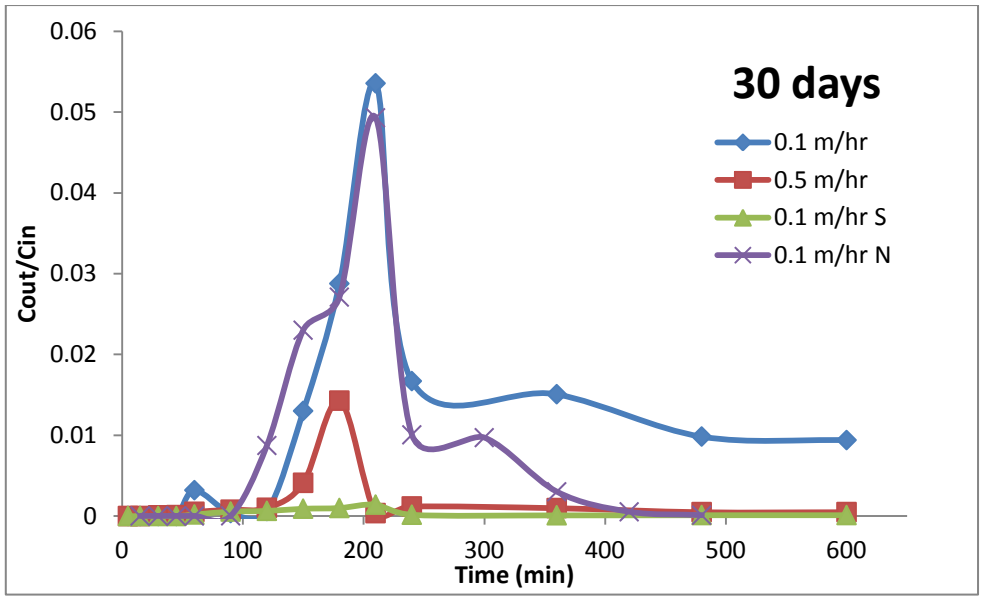
1. The DOC removal by SSF ranged from 16.23 to 28.82 % and TN removal ranged from 6.82 to 12.53 %. Organic carbon and nitrogen is required by the microorganism for their growth.
2. The percentage reduction was least in the column with added inoculum suggesting the presence of organic carbon and nitrogen source in the inoculum which is being utilized by the microorganisms to grow and thus relatively less utilization of the organic carbon and nitrogen from the influent source
3. It also suggests that there is limit to the substrate consumption by the microorganism and DOC and TN removal efficiency of SSF.
4. In case of the columns with added nutrients, the effluent DOC & TN value was 6.34 $\pm$.64 and 5.31 $\pm$.28 respectively which is higher than the recommended values in literature for water to be biologically stable. Since SSF is used a post disinfectant step and no chlorination is done in the supply network, therefore the amount of additional nutrients have to be decided carefully, such that effluent concentration is within the limits.
5. The effluent DOC and TN values were more a function of the influent values rather than the ripening of SSF.

5.2 E. Coli Spike Challenge

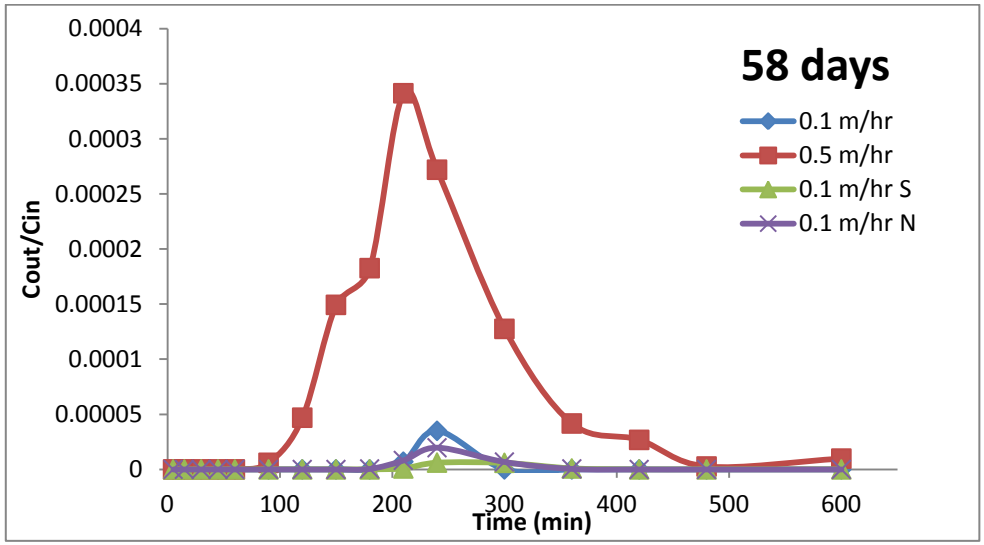
Several *E. Coli WR1* spike challenge test were carried out between 10 May'2017 to 17 August'2017, details of which have already been provided in the table 17. The purpose of these challenge tests was to quantify bacteria removal by slow sand filtration during stage of ripening under different operating condition (filtration rate, nutrient loading, and addition of microbial inoculum). The first spike experiment was carried out at 30 days from the start of the filter; this is because in general a new slow sand filter with fresh sand usually takes 30- 60 days for ripening after the start.

The influent E. Coli *WR1* concentration was around ~ 10^6 /ml. The Decimal Elimination Capacity (DEC) of the filter was calculated using $Log_{10}C_{in} - Log_{10} C_{out}$. The difference between the influent concentration and maximum effluent concentration was used to calculate the DEC. Figure 30 presents the breakthrough cures of spiking experiments carried out during the column operation at different stages of ripening. The first spiking experiment was carried out at 30 days followed by 44, 58 and 80 days. Cout/Cin value used is the mean of the columns running in duplicates.

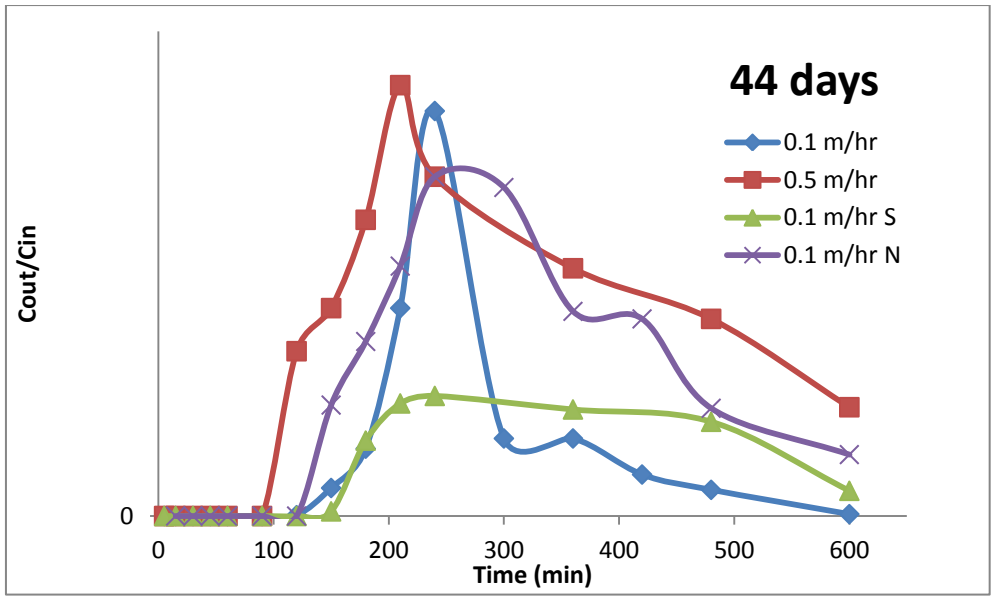
The Cout/Cin was calculated for 10 hours, even though the feeding was done only for 2.5 hours, this was done to check whether there would be of detachment of the bacteria from the sand particles or schmutzdecke after the feeding stops. The maximum Cout/Cin value was around 210 minutes for columns running at 0.1 m/hr as compared to 180 min for columns running at 0.5 m/hr. Even though the filtration rate was 5 times higher, the difference in the time take to reach peak values was for around 30 minutes. Accumulation and retarded transport seemed the most plausible explanations for this phenomenon. After the first spike, the peak shifted further due to the accumulation of particles and build up of the schmutzdecke layer (retardation phenomenon) causing a delay in the breakthrough values.



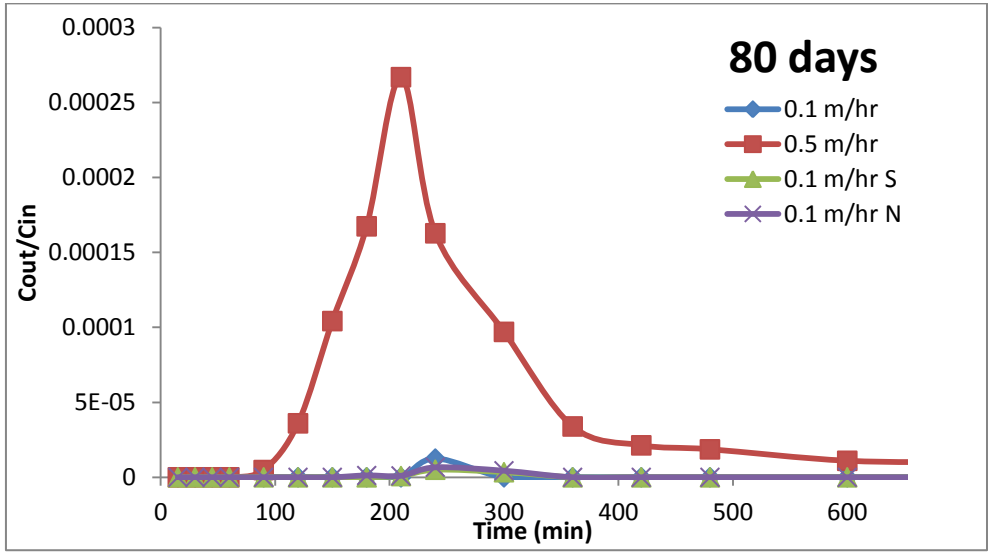
(a) 30 days



(b) 58 days



(c) 44 days



(d) 80 days

Figure 30: Breakthrough Curve of E.Coli WR1 spike

The DEC increased as the filters became more mature with the development of the schmutzdecke layer and biological community in the filter bed but the increase was different for filters operating under different conditions as shown in the table 30.

Table 30: Summary of average removal of E.Coli WR1

	Days	0.1 m/hr	0.5 m/hr	0.1 m/hr S	0.1 m/hr N
Spike 1 (DEC)	**30**	**1.27**	**1.84**	**2.84**	**1.31**
Spike 2 (DEC)	**44**	**3.42**	**2.80**	**3.95**	**3.50**
Spike 3 (DEC)	**58**	**4.45**	**3.17**	**5.20**	**4.71**
Spike 4 (DEC)	**80**	**4.90**	**3.57**	**5.30**	**5.10**

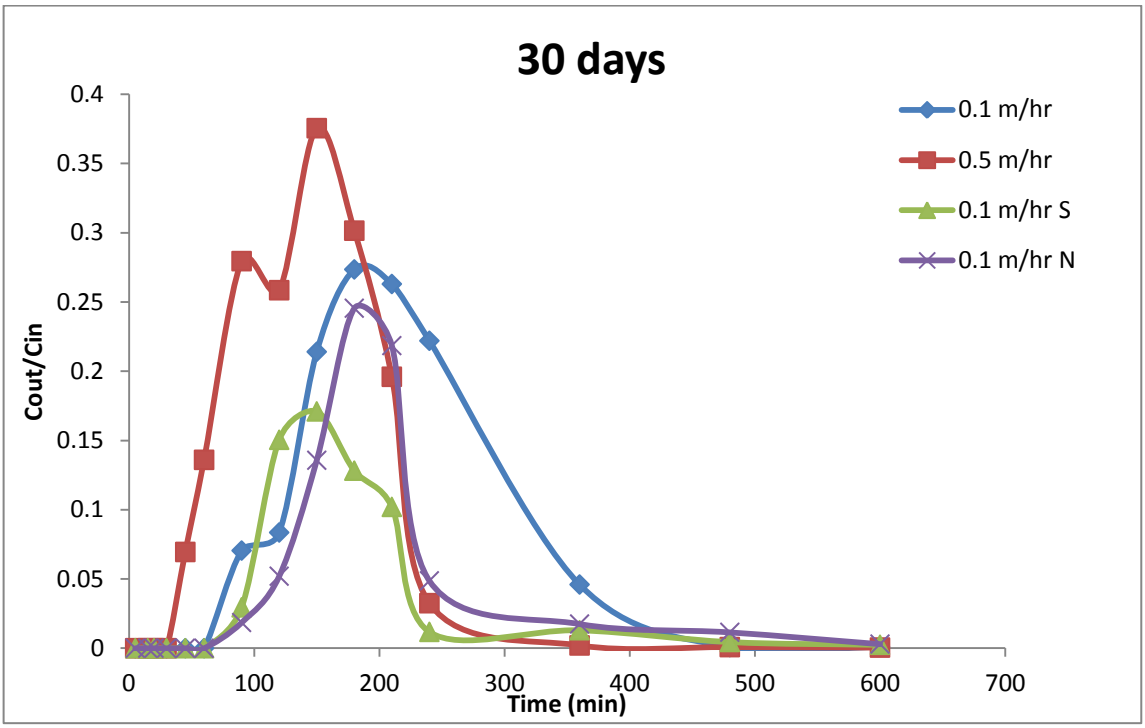
5.3 *MS2* Challenge Test

Similar to *E. Coli WR1*, *MS2* spike challenge were also carried out but only 3 times as mentioned in table 17. The purpose of these challenge tests was to quantify virus removal by slow sand filtration during stage of ripening under different operating condition (filtration rate, nutrient loading, and addition of microbial inoculum).

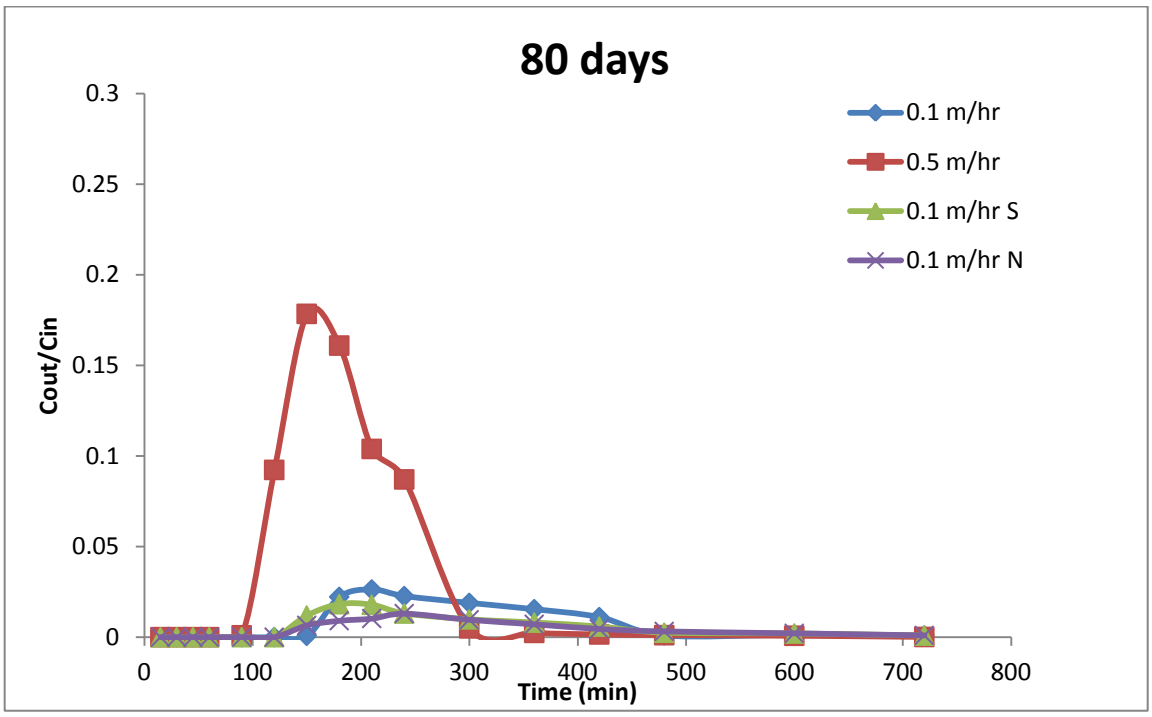
Figure 31 highlights the breakthrough curve of the *MS2* spike experiments. Cout/Cin value used is the mean of the columns running in duplicates. Similarly to E.coli, peak of Cout/Cin for *MS2* was earlier for the columns running at filtration rate of 0.5 m/hr as compared to 0.1 m/hr. Retardation phenomenon was also observed in the *MS2* peak though it was less pronounced as *MS2* is smaller in size as compared to *E. Coli WR1* and thus the maximum Cout/Cin was observed earlier in *MS2* spike as compared to *E.Coli WR1*.

The DEC was calculated the same way as done for the E. Coli as mentioned above. The influent concentration was in the range of 10^6/ml. *MS2* was measured in the effluent concentration for 12 hours after the start of the spiking experiment even though the influent feed with *MS2* was done only for 2.5 hours. As described earlier in the section 2.2.3.1 the adsorption of virus onto sand bed also can be reversible and they can detach afterwards. If ionic strength is decreased, previously adsorbed viruses can detach due to double layer expansion and increased repulsion between virus and media. Therefore measurements were done for a period of 12 hours to observe the effects of detachment.

The Decimal Elimination Capacity (DEC) of the filter was calculated using $Log_{10}C_{in}$ – Log_{10} C_{out}. The difference between the influent concentration and maximum effluent concentration was used to calculate the DEC.



(a) 30 days



(b) 80 days

Figure 31: Breakthrough Curves of MS2spiking Experiments

Table 31 provides the summary of the DEC of *MS2* and percentage decrease with the removal of schmutzdecke.

Table 31: Summary of Average removal of MS2Bacteriophage

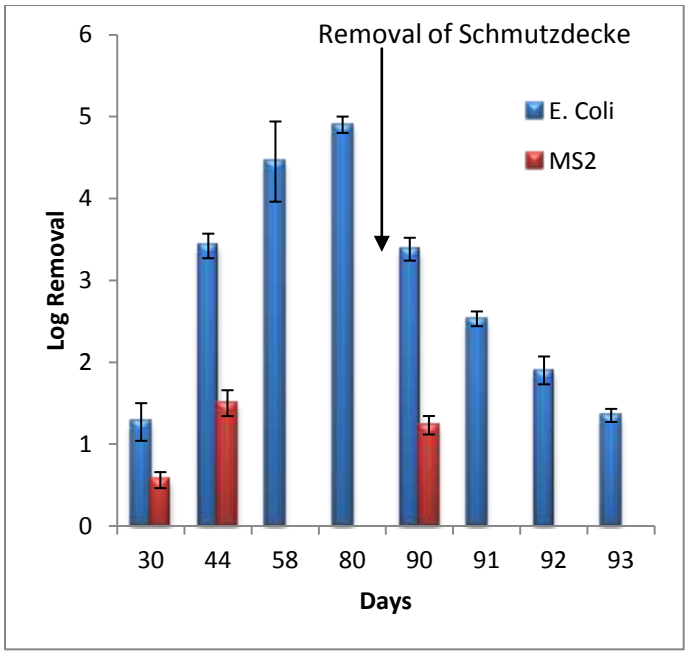
	Days	0.1 m/hr	0.5 m/hr	0.1 S m/hr	0.1 N m/hr
Spike 1 (DEC)	**30**	**0.56**	**0.41**	**0.76**	**0.69**
Spike 2 (DEC)	**80**	**1.50**	**0.89**	**1.74**	**1.99**

The average *MS2* removals are not as high as compared to the values reported in the literature (upto 2.2 logs as reported by *DeLoyde, 2005*). There could be multiple reason of this: first the temperature plays a significant role in the removal of viruses, higher temperature favours more removal due to enhance biological activity (Nasser & Oman, 1999). During the study period the averaged temperature ranged from 18 to 22^0C. Second the age of the filter bed, as the filter becomes more mature; the removal increases, since in this case it was only running for 80 days so this could be one of the reasons.

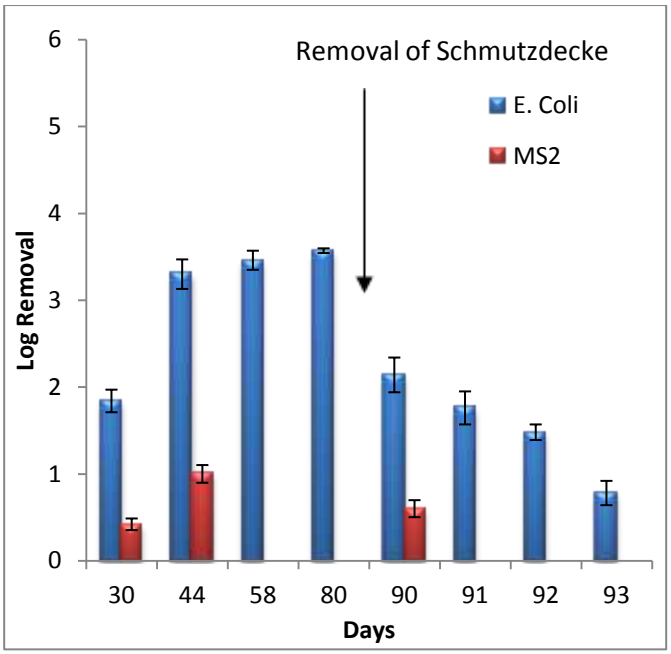
5.4 Analysis of results

A summary of average bacteria and virus removal at different stages of ripening is presented in figure 32. The error bar in the graph represents the standard deviation between measurements of duplicate columns. The removal efficiency of SSF is subject to variable operational conditions, such as temperature, filtration rate, thickness and biological activity of a Schmutzdecke.

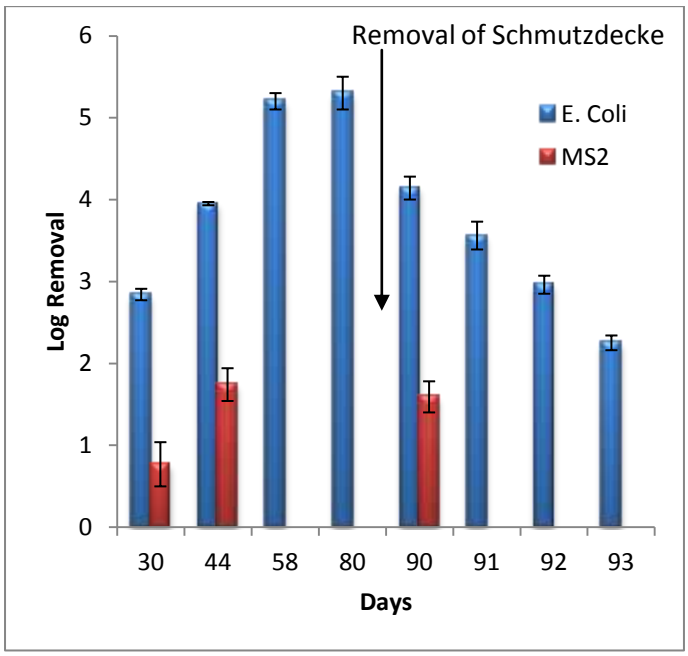
In general, the removal efficiency of both bacteria and virus removal increase as the sand bed matures. The DEC of *E.Coli WR1* was 2-3 Log_{10} higher than the *MS2* removal at the end of 80 days before the schmutzdecke was scraped off. The less removal of bacteriophage *MS2* than of *E. Coli WR1* by SSF implies that the sticking efficiency of *E.Coli WR1* is much higher than that of *MS2 (Schijven et al, 2002)*. Schmutzdecke and top layers of sand bed plays a significant role in the removal of *E.Coli WR1* as compared to *MS2*removal. Factors affecting the removal of *E.Coli WR1* and *MS2* are discussed in details in the subsequent sections.



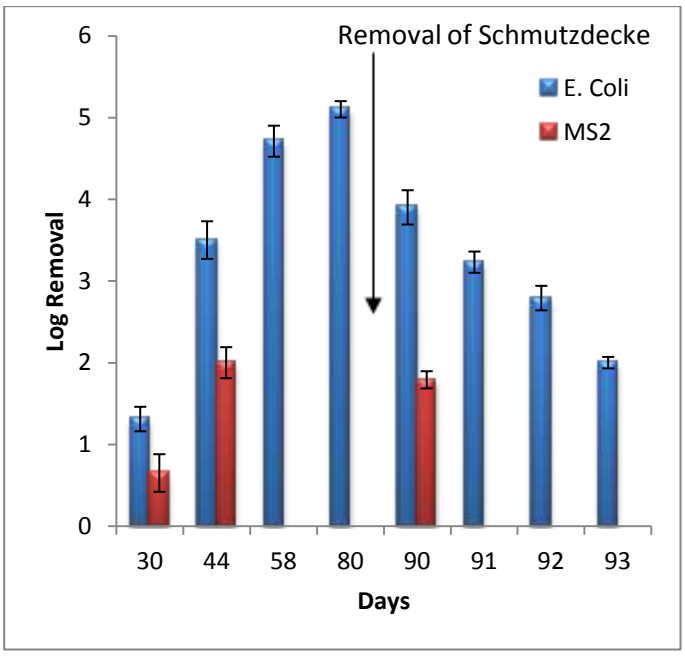
(a)0.1 m/hr



(c) 0.5 m/hr



(c) 0.1 m/hr with microbial inoculum



(d) 0.1 m/hr with Nutrients

Figure 32: DEC for Bacteria and Virus removal at different stage of ripening

*90th day is the first day after the removal of Schmutzdecke

**91st day is the first day after the removal of sand bed 0-2 cm

***92nd day is the first day after the removal of sand bed 4- 6cm

****93rd day is the first day after the removal of sand 8-10 cm.

5.4.1 Factors affecting E. Coli *WR1* Removal

In this study the E. Coli removal by SSF was studied during stages of ripening under different operational condition such as the ffiltration rate, microbial inoculum, nutrient addition and presence and absence of schmutzdecke layer. In general the E. Coli Removal increased as the filtration progress with the development of the schmutzdecke layer and maturity of the sand bed, though the increase was different in different operating conditions.

(a) Filtration Rate

For the first 30 days of operation, the average *E.Coli WR1* removal was 0.47 log higher in columns operating at 0.5 m/hr in comparison to 0.1 m/hr. This could be because of the fact that the higher filtration rate provided higher nutrients for the growth of microorganism and resulting in faster build of the schmutzdecke layer.

After 44 days of operation the columns ruuning at 0.1 m/hr had 0.6 log higher removal than 0.5 m/hr. The DEC of columns operating at 0.5 m/hr becomes stagnant and only showed improvements of 0.37 and 0.4 log after 58 and 80 days of operation respectively. On the other hand columns running at 0.1 m/hr showed improvement of 1 and 0.55 log after 58 and 80 days of operation respectively.

The difference in the improvement between 1st and 2nd spike between the columns suggests that even though higher filtration rate increases the log removal capacity in the beginning of the run (due to high nutrient content) after a certain point of time (in this case it is between 30-44 days), higher filtration rate lead to washout of the microorganism, pushing them deeper inside the sand bed and ultimately causing breakthrough from the columns.

Lower filtration rate will provide more time for attachment to biofilm and there is less shear force with lower possibility of detachment from the sand bed. The long hydraulic retention above the filter bed may provide the substantial growth of biological community and thus higher DEC by inactivation by microbial enzymes and predation. That is why after certain period of time DEC of columns running at higher filtration rate become stagnant. At the end of 80 days columns running at 0.1 m/hr showed 4.9 log removals while it was only 3.57 in columns running at 0.5 m/hr.

(b) Addition of Microbial Inoculum

Significant improvements in the average log removal were shown by the addition of microbial inoculum. After 30 days of operation, columns with additional inoculum showed 1.55 log higher removals as compared to the one without any inoculum. This suggests the

importance of the schmutzdecke layer in the removal of bacteria. Since *E.Coli WR1* is bigger in size as compared to *MS2*, the major removal mechanism in the beginning of the filter operation might be physical straining in the added inoculum.

Although the difference in the DEC between the columns (with & without inoculum at 0.1 m/hr) decreased in the subsequent spiking, it was still 0.53, 0.75 and 0.4 higher in column with inoculum after 44, 58 and 80 days respectively. Though this value can vary depending on the biomass concentration of the added inoculum and the amount of biomass added.

The microbial inoculum added from the matured filter might have (in) organic components accumulated in the biomass that may have a significant effect on the removal efficiency of organisms removed by adsorption. That is why a significant difference was observed in the DEC after 1st spike. The average DEC after 80 days of operation was 5.3 and 4.9 in columns with and without microbial inoculum implying addition of inoculum helps SSF to get ripened faster but once the filter has been ripened, it is expected to achieve same log removal capacity.

(c) Addition of Nutrients

Use of additional nutrients did not result a significant difference in the DEC during the first two spikes (.04 log and .08 log after 30 & 44 days respectively) with respect to the columns running at 0.1 m/hr. After the 3rd and 4th spike difference increased to 0.25 and 0.23.

In comparison to the columns running at 0.5 m/hr which has the same nutrient loading, the columns with additional nutrient showed a significant difference in the DEC as the filter ripens. Though after the 1st spike (30 days) columns with 0.5 m/hr showed 0.53 more log removal but after that in the subsequent spikes columns with 0.1m/hr showed 0.7, 1.54 and 1.53 more log removal than 0.5 m/hr at 44, 58 and 80 days respectively. The reason for this is the same as explained above higher filtration rate causes washout of the microorganism in the effluent.

5.4.2 Summary & Concluding Remarks

1. Average *E. Coli WR1* ranged from 1.27 to 5.3 in column slow sand filters depending on filtration rate, schmutzdecke age and biological maturity of filter bed
2. E. Coli removals increased as SSF biological maturity increased over time, likely because of improved straining and entrapment due to greater biofilm coverage and accumulation of solids in the pore spaces of the filter media.
3. Higher filtration rate provide more substrate at the beginning of the filter leading to higher log removal (1.84) during first 30 days of operation as compare to columns at

0.1 m/hr (1.37) but after 44 days of operation the log removal capacity was almost same and subsequently after that higher in the columns with low filtration rate.

4. Columns with added nutrients and microbial inoculum (0.1m/hr) were able to achieve more than 5 log removal of bacteria after 80 days of operation.
5. Columns with added inoculum were able to achieve greater than 2.5 log removals within 30 days of the start of the filter.
6. From the results of objective 1, it was shown the columns with added inoculum were able to mimic the full scale filter. Hence addition of microbial inoculum presents a possible solution for minimising the ripening period.

5.4.3 Factors affecting *MS2* removal

Both biological and physiochemical mechanisms are responsible for the removal and inactivation of viruses in slow sand filters. Although viruses are too small to be removed by physical straining, physiochemical adsorption/attachment to filter media is an important removal mechanism under favourable conditions. The primary biological removal mechanisms include predation by microorganism, such as bacteria and filter feeding protozoa, and attachment to biofilms and biomass.

In this study the effect of filtration rate, microbial inoculum, nutrient addition and presence and absence of schmutzdecke layer were investigated on removal or inactivation of *MS2 bacteriophage.*

(a) Filtration Rate

The average *MS2* removal was 0.15 logs greater at the filtration rate of 0.1 m/hr as compared to 0.5 m/hr after 30 days of operation which increased to 0.7 after 80 days of operation.

The greater removal efficiency at lower filtration rate were likely because of long hydraulic retention time over the filter bed which enhance the removal by biological mechanism such as predation and inactivation by microbial enzymes. Lower filtration rate will provide more time for the *MS2* phages to attach to the biofilm.

As explained earlier the removal of virus can be reversible, lower filtration rate provide low shear stress which would cause less detachment of attached phages and provide greater opportunity for detached viruses to re-attach lower in the filter. The difference in the average removal was lower at the beginning and increase as the filtration progresses. This could be because in the beginning of the filter operation the major removal mechanism

could be adsorption onto sand grains and as the filtration progress attachment to biofilms became more dominant.

As filtration rate also affects the formation of the biofilm, lower filtration rate promoted the growth of porous and low-density biofilms, which could be expected to provide numerous sites for entrapment of particles such as viruses (*Van Loosdrecht et al, 1995*). The results of the findings were similar to the reported values in literature by (*DeLoyde, 2005; Wang et al., 1981*). Therefore it can be concluded that on increasing the filtration rate the removal efficiency of SSF for virus removal gets decreased. To optimise the working of slow sand filter during initial stages of ripening it should be operated a lower filtration rate preferably with 0.1-0.2 m/hr.

(b) Addition of Microbial Inoculum

The addition of microbial inoculum which was the scraped schmutzdecke layer from one of the matured filter improved the removal efficiency of *MS2* removal, although the difference in the removal was not as significant compared to the columns operating without inoculum.

The average log removal of *MS2* virus was 0.24 and 0.36 higher than the columns operating without inoculum after 30 and 80 days of operation respectively. Increased microbial activity expected to increase virus attachment on the porous and sticky nature of the biofilm *(Storey and Ashbolt, 2003).* The microbial inoculum added from the matured filter might have (in) organic components accumulated in the biomass and thus unavaibilty of the adsorption sites for viruses in the biofilm.

(c) Addition of Nutrients

The addition of nutrients was done to increase the biological activity by providing more substrates to the microorganism to grow. The average *MS2* log removal was 0.13 and 0.77 higher as compared to the columns operating without any addition of nutrients at 30 and 80 days respectively.

The growth of the microorganism is bound by the availability of nutrients. Sodium acetate was added as the organic carbon source which was utilised by the bacteria for growth and energy production. *Kim and Unno, (1996)* and *Cliver and Herrmann, (1972)* reported that certain bacteria that grow in the biofilm prey on viruses and thus enhance the virus removal.

The nutrient addition was done five times the influent water, maintaining the same balance nutrient loading as the columns operating at the filtration rate of 0.5 m/hr. The average *MS2*

log removal was 0.275 and 1.4 times higher with addition of nutrients for columns running at 0.1 m/hr as compared to columns running at 0.5 m/hr at 30 and 80 days respectively. From this it can be inferred that, even though the amount of nutrient loading applied was same, filtration rate plays a major role in the removal of viruses as compared to nutrient loading. Increasing the filtration rate provides more substrate but at the same time promotes the growth of thick non-porous biofilms which decreases the adsorption site and thus lower effective removal of viruses.

5.4.4 Summary & Concluding Remarks

- Average *MS2*log removal ranged from 0.415 to 1.99 in column scale slow sand filters depending on filtration rate, schmutzdecke age and biological maturity of the filter bed.
- It is possible that better *MS2* removals would have been observed if experiments had been carried out at warmer temperatures.
- As the ripening progress, the DEC of the *MS2* virus by SSF increased in all the operating conditions. Although surface charges were not measured in this research, it is likely that *MS2* coliphage had a strong net-negative charge at the near-neutral pH of the influent water, causing repulsion by the negatively charged sand *(Schijven and Hassanizadeh, 2000)* and thus lower removal in the early stages and an increase as the filter bed matures with deposition of other charges enhancing attraction on to the sand surface.
- The DEC was better in the columns operating at low filtration rate. To maximize virus removal from SSF, filtration rate should be kept in the lower range of the values reported (0.1- 0.4 m/hr) in the literature during the ripening.
- In case with columns running at 0.5 m/hr and columns with 0.1 m/hr with nutrients, even though the amount of nutrient loading applied was same, columns with low filtration rate have higher DEC capacity than columns at 0.5 m/hr, thus highlighting the importance of long hydraulic retention time over the filter bed.
- No significant difference was observed in the *MS2* removals in the columns with or without inoculum as observed in the E.Coli removal.
- Columns with additional nutrients showed higher MS2 removal (0.25 logs) in comparison to columns with inoculum (the difference was not the significant) but it was opposite in case of E.Coli removal where it was higher in columns with inoculum (0.2 logs).

Objective 2: Summary and Concluding Remarks

- Addition of microbial inoculum (schmutzdecke from a matured filter) presents a better solution in comparison to addition of nutrients or increasing the filtration rate to fasten the ripening period. Though the purpose of adding inoculum or nutrients or increasing the filtration rate was same: that is to increase the biological activity in the sand bed.
- Higher filtration rate pushes the microorganisms into deeper layers of sand and causes the breakthrough for E. Coli & MS2.
- Higher filtration also adversely affects the turbidity and particle counts removal.
- Lower filtration rate promotes the growth of porous and low-density biofilms, which could be expected to provide numerous sites for entrapment of particles such as viruses.
- Column with added inoculum and nutrient were able to perform better than other columns in terms of turbidity, particle counts removal and DEC of bacteria and viruses.
- Use of addition of nutrients to reduce the ripening time has a major drawback as compared to addition of inoculum. SSF's have a limited capability to remove the organic carbon and nitrogen and since SSF are used as a post disinfectant treatment step and no chlorination is done in the drinking water supply, the biological stability of the water cannot be ensured and thus there is a possibility of microbial regrowth in the drinking water distribution system.

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Chapter 6

Results: Objective 3

The objective of this chapter is to identify the indicators of ripening.

6.1 Correlation between different indicators

Correlation Analysis was performed between different parameters that were measured during the operation of columns SSF to identify the indicators of ripening. The results of the correlation analysis are provided in the appendix I. The summary of correlation coefficient (R^2) between different parameters is given in table 32. The correlation analysis performed in table 34 was performed on the entire data set that is the columns running at the filtration rate of 0.1 m/hr with and without any substrate addition and columns running at 0.5m/hr.

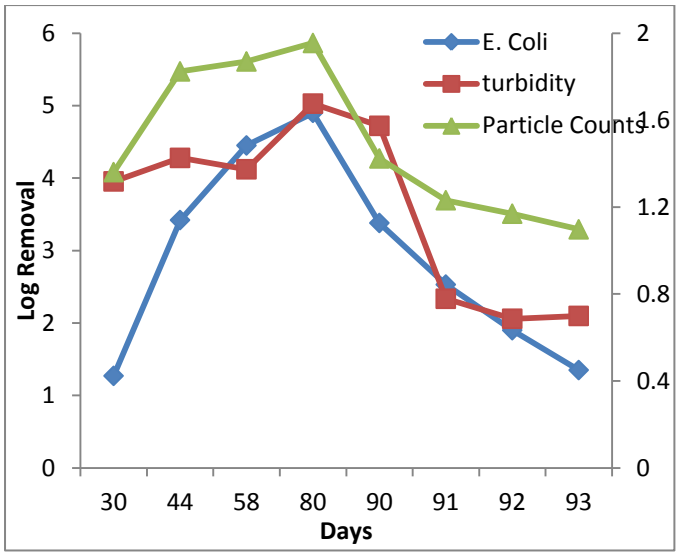
Table 32: Summary of Correlation Coefficient (R^2)

	DEC*	Turbidity	Particle Counts	DOC	TN
DEC*	1				
Turbidity	0.68	1			
Particle Counts	0.70	0.75	1		
DOC	0.29	0.27	0.28	1	
TN	-0.11	.058	-0.18	0.61	1

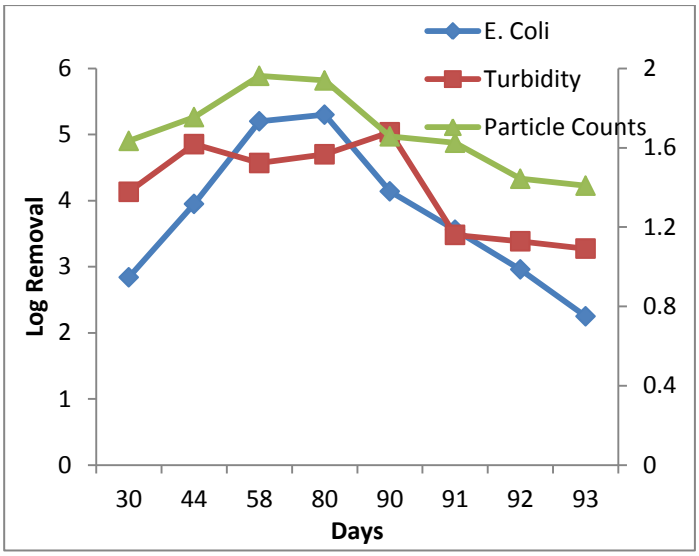
*DEC represents the Decimal elimination capacity of E.Coli; DEC of MS2 was not used to correlate as it was only done after 30 days and 80 days and once after scraping of schmutzdecke.

The DEC of E. Coli, turbidity and particle count is presented in figure 33. The primary vertical axis represents the DEC of E.coli and secondary vertical axis represent the DEC of turbidity and particle counts. Both the axis represents the log removal values. The three of them follows more or less the same pattern that is increased as the filtration progresses, followed by a decrease after the removal of schmutzdecke and sand bed. The R^2 value was higher for DEC with particle counts as compared to turbidity. The trend towards reduction in filtered water turbidity and particle counts (increase in log removal) with time as ripening progressed indicates that filter ripening has enhanced particle straining due to bio layer formation, improved depth filtration by slowing the filtration rate and altering the surface properties of the filtration media.

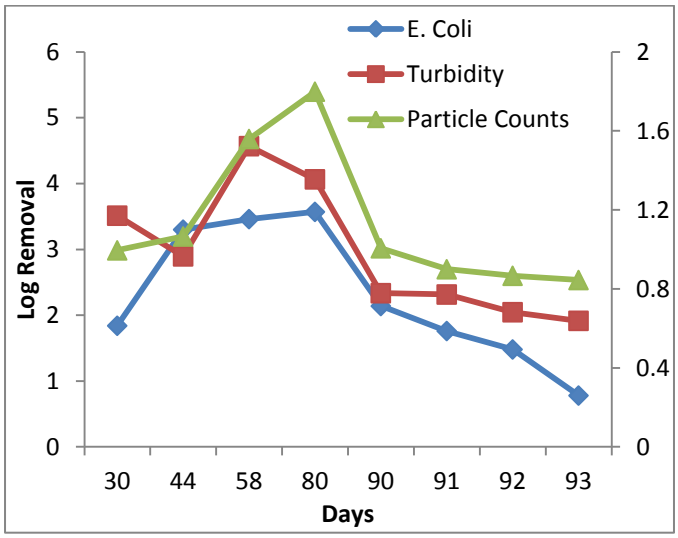
No such trend was observed in the effluent value of DOC & TN and the percentage reduction. The correlation coefficient between the microbial and chemical parameters (DOC & TN) was not significant (<0.30); similarly the correlation between turbidity and particle counts with DOC and TN was not significant (table 32). Effluent DOC & TN is a function of the influent values rather than the ripening of the filter or maturity of the filter bed. The reduction in DEC of E.Coli after the removal of schmutzdecke was much more significant as compared to particle counts and turbidity as shown in figure 33.



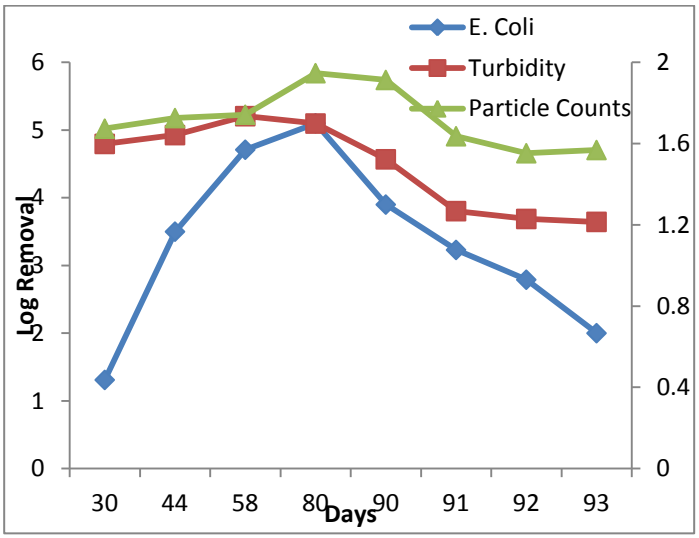
a)0.1 m/hr



(c)0.1 m/hr S (Microbial inoculum)



(b)0.5 m/hr



(d) 0.1 m/hr with nutrients

Figure 33: DEC of E.oli, turbidity and Particle Counts

6.2 Correlation between Turbidity & Particle Counts

Correlation analysis was performed between particle counts and turbidity for columns SSF. The graphs are presented in appendix J. The correlation coefficient (R^2) was 0.75, 0.65, 0.73, 0.79 for the columns running at 0.1 m/hr, 0.5 m/hr, and 0.1 m/hr with nutrients and 0.1 m/hr with microbial inoculum respectively. The correlation values were much higher as compared to the values reported in the literature. Leilei et al, found R^2 <0.1 between turbidity and particle counts in drinking water after sand filtration. It might be because the correlation analysis was performed for data set of only 70 hours during which the filters

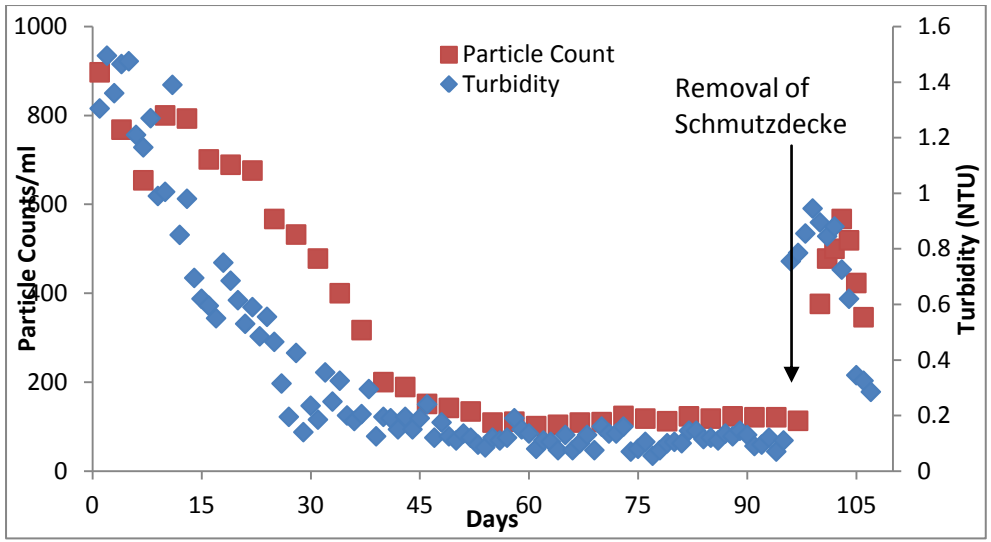
might not be acclimatized to microbial biomass and huge variations were observed in the turbidity and particle count due to the changes in the influent water characteristics.

From the figure 34, it can be observed that during initial stages of filter run when the effluent concentration was higher than 200 counts/ml, the correlation is not that significant. The R^2 value was 0.56, 0.6, 0.65 and 0.45 for the columns running at 0.1 m/hr, 0.5 m/hr, 0.1 m/hr with nutrients and 0.1 m/hr with microbial inoculum respectively before the particle counts was greater than 200/ml and increase to 0.80, 0.72, 0.78, 0.83 after the particle counts was less than 200/ml. Once the filter reaches optimum performance level, a better correlation was observed between particle counts and turbidity. It can also be observed that, even when the effluent turbidity is less than 0.5NTU, the particle count is much higher than the limit of 100-200 counts/ml implying that particle count is thus a more effective control parameter than turbidity for assessing the water quality of sand filter effluent.

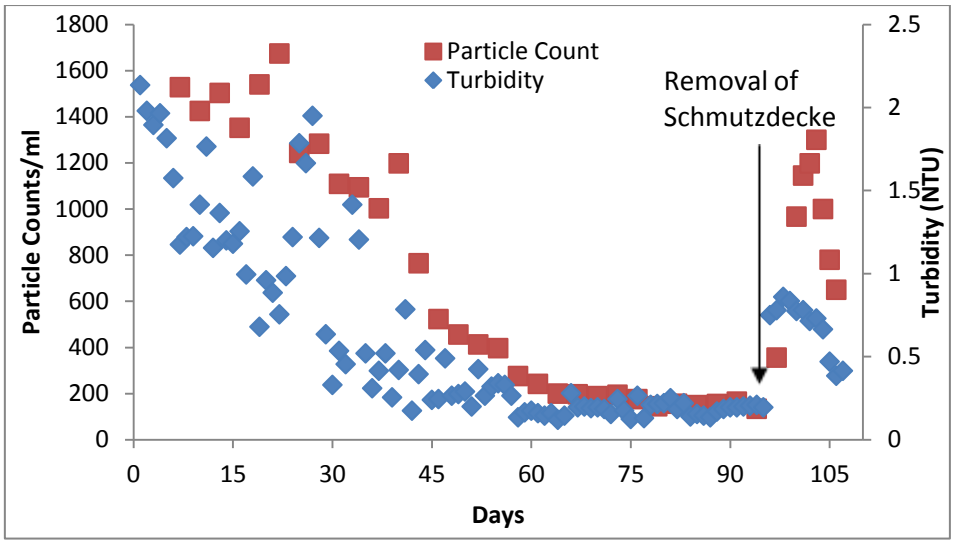
6.3 Objective 3: Conclusion

- The time taken by turbidity to reach median values of less than 0.1 NTU and particle counts to reach levels of 100-200 counts/ ml was variable under different operating conditions which indicate the turbidity and particle count can be used as a surrogate for the ripening of the indicator.
- This was further confirmed by carrying out spiking experiments at different stages of ripening and by measuring DEC of bacteria. Turbidity and particle count follows similar pattern as DEC of bacteria and can be used as a surrogate for the indicators of ripening.
- Reductions of bacteria, viruses, turbidity and particle counts increase substantially with time as filters ripens. No such pattern was observed in the DOC and TN removal, they were more a function of the influent water quality and independent of the biological activity in the schmutzdecke and maturity of the filter bed.
- Particle count is a more effective control parameter than turbidity for assessing the water quality of sand filter effluent as it was observed even when the influent turbidity was less than 1 NTU; the particle count was still higher than 500 counts/ml and much higher in the column running at 0.5 m/hr as compared to 0.1 m/hr.
- A good correlation was observed between turbidity and particle counts once the filter has been ripened. Hence a combination of two should be used to define the ripening of the filter.

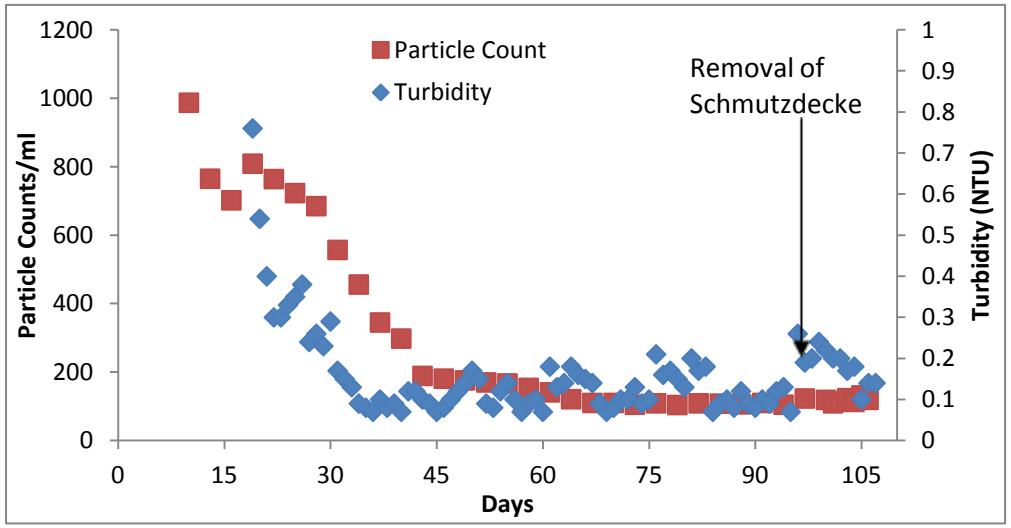
Figure 34: Effluent Particle counts and Turbidity



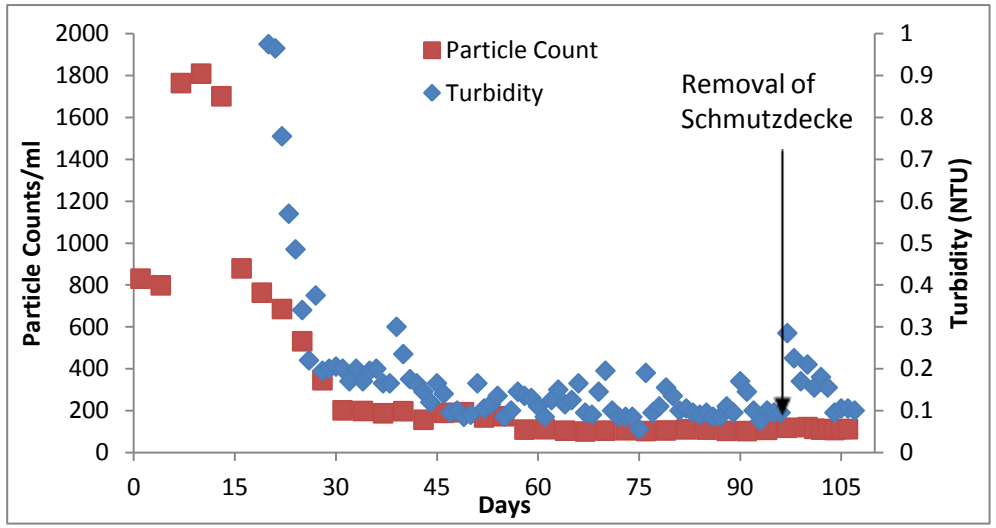
(a)0.1m/hr



(c)0.5 m/hr



(b)0.1 m/hr N



(d)0.1 m/hr S

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Chapter 7

Conclusion and Recommendations

7.1 Conclusion

Though there were multiple objectives in this study, the main aim was to optimise the working of slow sand filters by fastening the ripening period and to identify the indicators of ripening. Two possible approaches were investigated; first was to optimise the scraping procedure and second was to accelerate the biological activity when the new filter is put into operation. Combination of full scale and column scale slow sand filters were used to achieve those goals.

- In order to optimise the scraping procedure, biomass concentration and DEC (bacteria and viruses) for different layers of sand bed has to be quantified.
- Biomass concentration decreases with depth in both full scale and column slow sand filters. More than 80% of biomass accumulation takes place in schmutzdecke and top 2 cm of sand bed.
- DEC of column SSF decreased after the removal of sand layers with most significant reduction in DEC was observed after the removal of schmutzdecke (>1 log).
- Column with added microbial inoculum were able to mimic the full scale filters. Removing of schmutzdecke and top 2 cm of sand bed where most of the biomass accumulation takes place, columns were still able to achieve more than 3 log removals for bacteria and 1 log for virus. This is higher than the values required by current full scale SSF's in their operation.
- Effluent turbidity and particle counts were less than 0.1 NTU and 200/ml after the removal of schmutzdecke in columns with inoculum.
- Thus scarping of schmutzdecke and only top 2 cm of sand bed in SSF can significantly reduce the time taken to put the filter back into operation after scraping.
- Position of the inlet valve source affects the spatial distribution of biomass on the filter surface due to lateral gradients and leads to uneven biomass growth.
- To reduce the start up time of a new filter, addition of microbial inoculum (schmutzdecke from a matured filter) is the better solution in comparison to addition of nutrients or increasing the filtration rate.
- Columns with inoculum reached more than 2.5 log removals in first 30 days of operation as compared to others and took only 24 and 27 days to reach median levels of turbidity of 0.1 NTU and particle count less than 200/ml respectively.
- Particle count is a better surrogate than turbidity as an indicator of ripening of the filter. Using them together would provide a better insight regarding the ripening as the correlation between them increases as the filter ripens.

- DOC and TN cannot be used as indicators of ripening.

7.2 Recommendations for Further Research

- The current experimental study was done on the laboratory scale column filters, which were only 4cm in diameter and 1 meter in length, further research work on pilot scale system would provide us with better insights regarding the use of microbial inoculum and addition of nutrient to enhance the ripening of the filter bed.
- In the current study, the biomass carbon concentration of the microbial inoculum added was around 150 ng/gm of dry sand and it was added on the top 3-4 cm of sand bed. There was no significant difference observed in the removal of *MS2* virus by the use of inoculum in the columns that might be because of the unavailability of the adsorption site in the added inoculum layer. Hence further experimenting with the biomass concentration would improve the efficiency of SSF for virus removal.
- The study is carried out in relatively warm temperature ranges 16-20^{0}C where the microbial activity is high, therefore a further study in cold water temperature must be carried out.
- The nutrients added were five time the influent concentration. Further experimenting with nutrient concentration and the source of nutrients might provide us with better solutions to fasten the ripening of the filter.
- At neutral pH sand bed is usually negatively charged therefore experimenting with the use of positively charged ions or inclusion of positively charged media on SSF Bed as a possible means to enhance the ripening of the filter bed should be investigated. Positively charged media options include iron-oxide coated sand, zero-valent iron beads or filings, and naturally occurring zeolites (alumino-silicate minerals containing cations).

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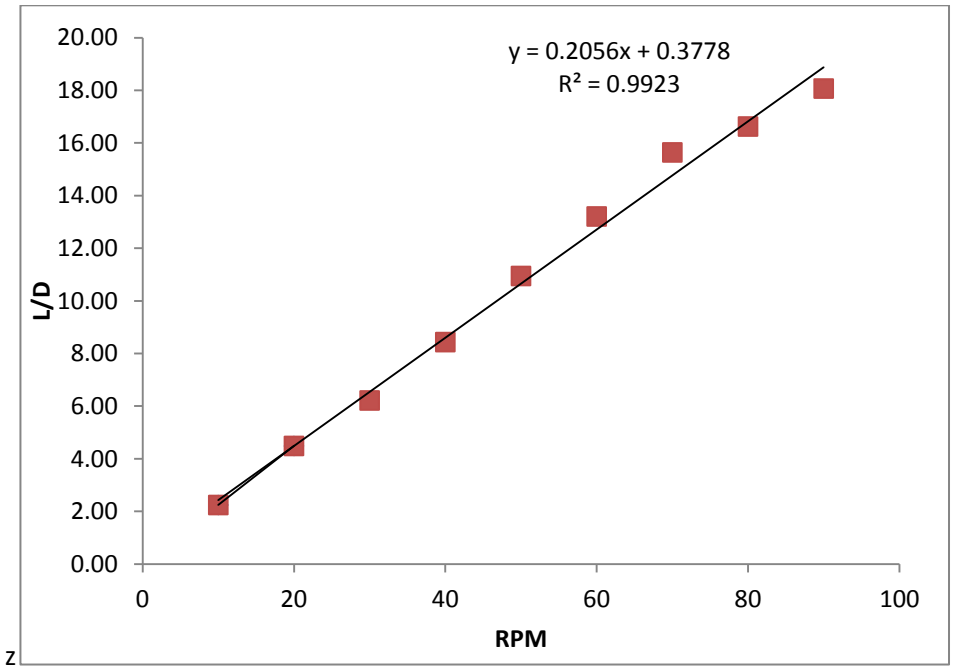
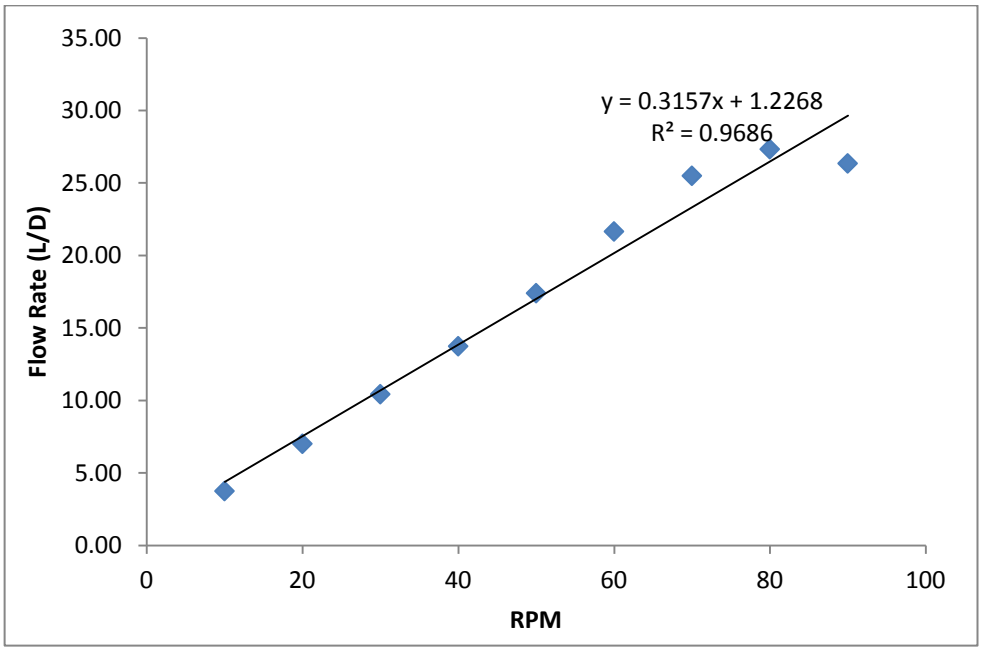
Appendix A: Pump Calibration Curve



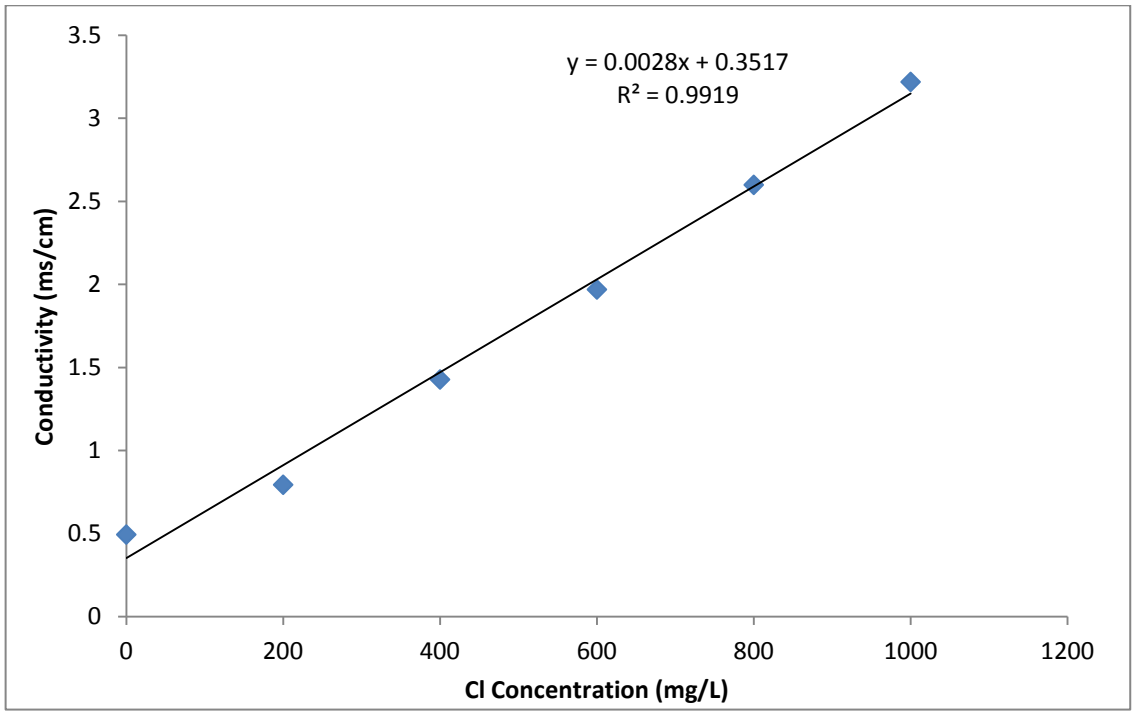
z

A.1 Calibration Curve Pump 1

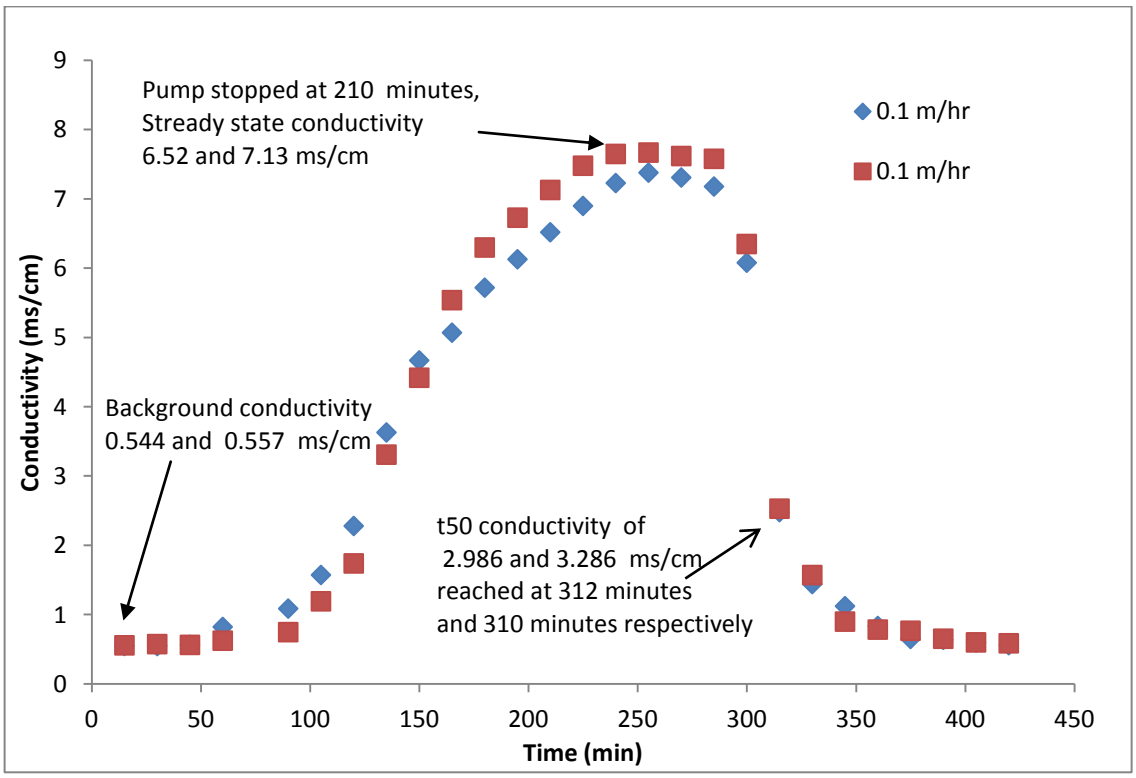


A2. Calibration Curve Pump 2

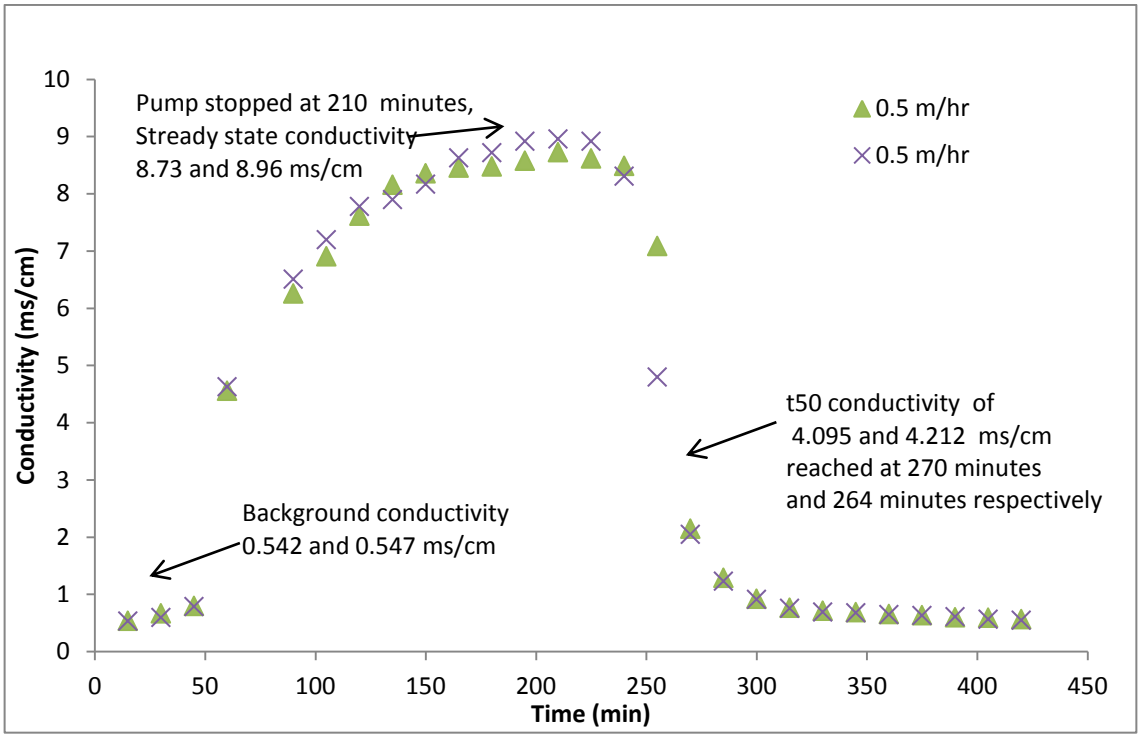
Appendix B: Chloride Tracer Results



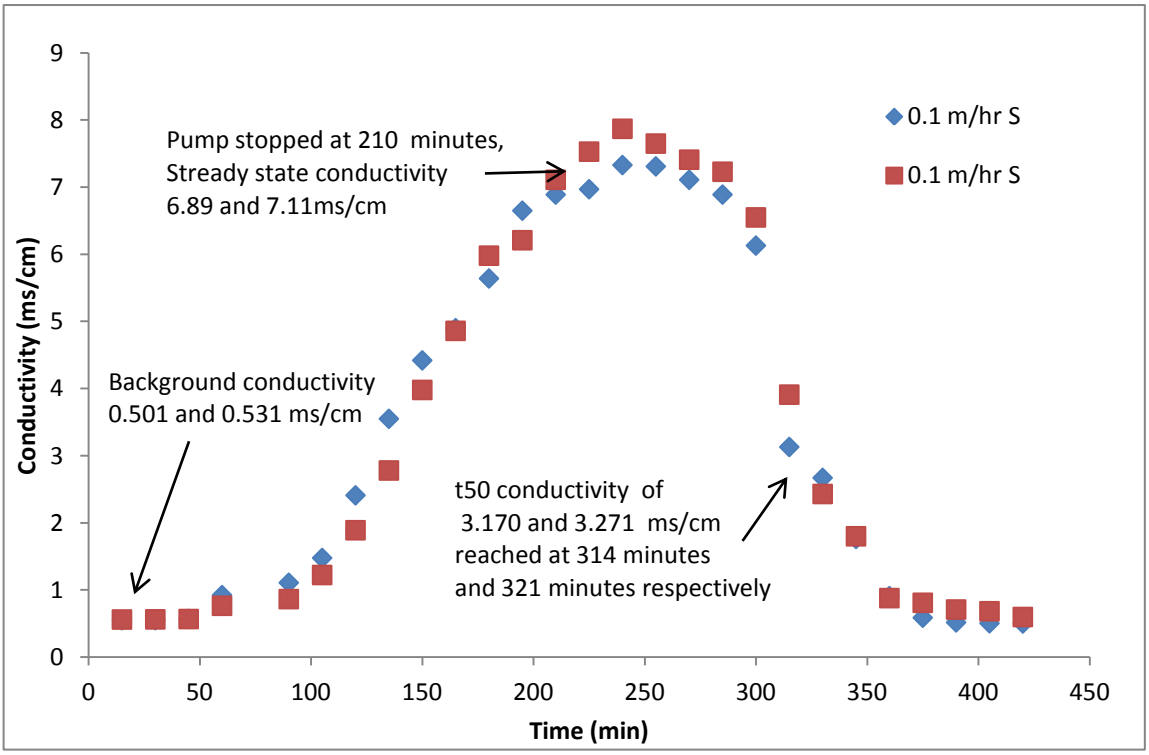
B.1 Chloride Calibration Curve



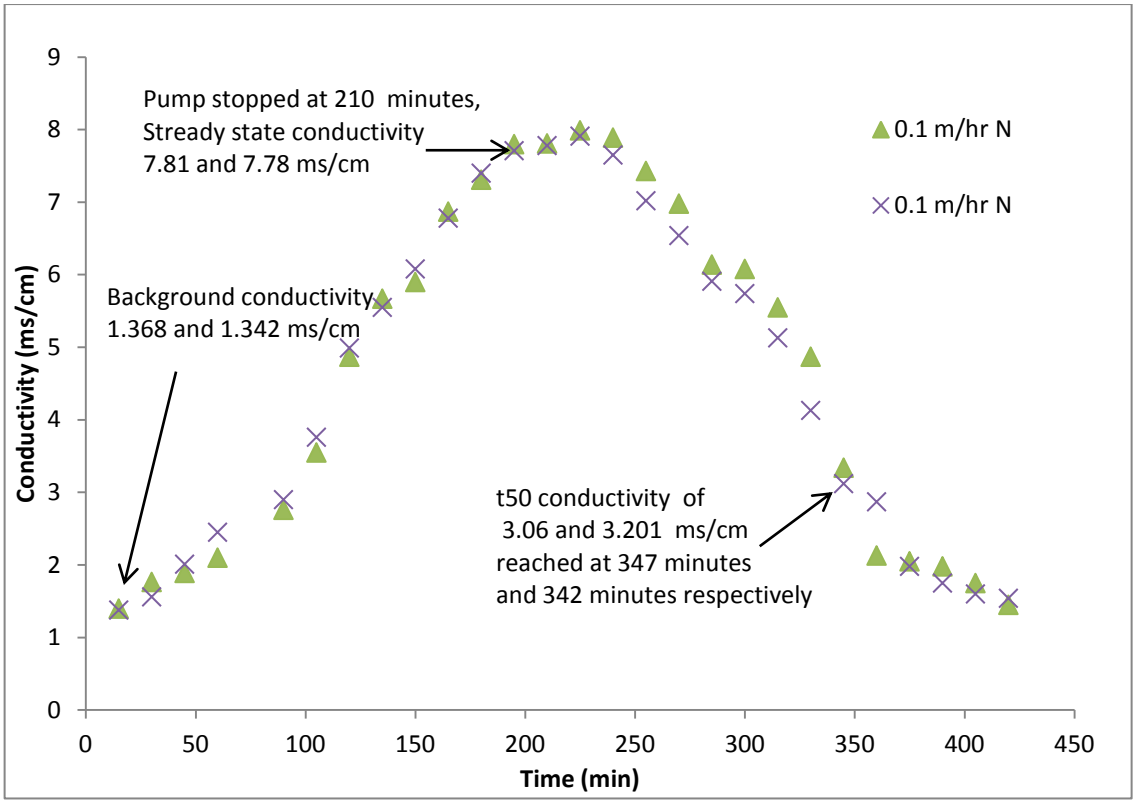
B.2 Chloride Tracer Result: Column at 0.1 m/hr



B.3 Chloride Tracer Result: Column at 0.5 m/hr



B.4 Chloride Tracer Results: Column with microbial inoculum at 0.1 m/hr



B.5 Chloride Tracer Results: Column with additional nutrients at 0.1 m/hr

Appendix C: Spiking Procedure of E.Coli and MS2

C1. E.Coli WR1

(a) Preparation of E. coli

The night before the experiment a vial of *WR1* E. coli was thawed from the -80^0 C freezer which is already prepared. An overnight culture was prepared by adding 50 ml of TYGB and 500 µL of Ca-Glucose to a sterile plastic flask, and then 100 µL of thawed *WR1* was added to it which is inoculated on shaker inside 37°C incubator at 90 rpm and left overnight (usually for 15-17 hours).

At the day of experiment the culture was retrieved from the incubator at appropriate time. The culture was distribute equally into two sterile 50 ml centrifuge tubes each with ~25ml which is then centrifuged at 3000 rcf for 15 minutes at 15°C. After that the supernatant was removed from the centrifuge tubes without disturbing the pellet. The pellets were resuspended into 5 ml of the test water and contents of both vials were combined into one centrifuge tube which resulted in 10 ml of concentrated *WR1* with a concentration of ~10^9.

(b) Filter Seeding

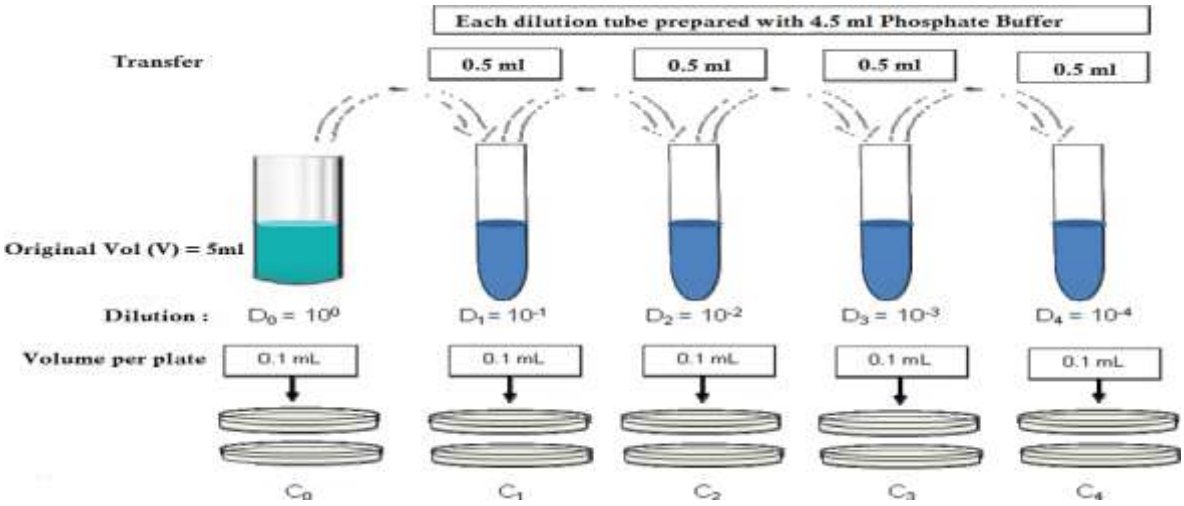
Test Water which is the same as the influent water to the columns was added in to the glass beakers which were sterilised before. For every litre of water, 1ml of the culture was used resulting into concentration of around ~10^6/ ml in the feed water. During seeding, spike bottles were continuously stirred using a magnetic stirrer. Before the start of the spiking experiments calibration was done to ensure the filtration rate. The seeding was done at the same rate as the influent is supplied to the columns. Seeding was done for duration of 2.5 hours with a concentration of ~ 10^6/ml.

(c) Sampling protocol

Effluent samples were collected in sterilised 10 ml tubes after the start of the feeding. The start time of the spiking experiment was when the first drop enters in to the supernatant water making it time 0. The volume of the sample collected was 5 ml. Samples were collected at the following duration after that: 5 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, 300 min, 360 min, 420 min, 480 min, 600 min and 720 min.

(d) Sample Processing

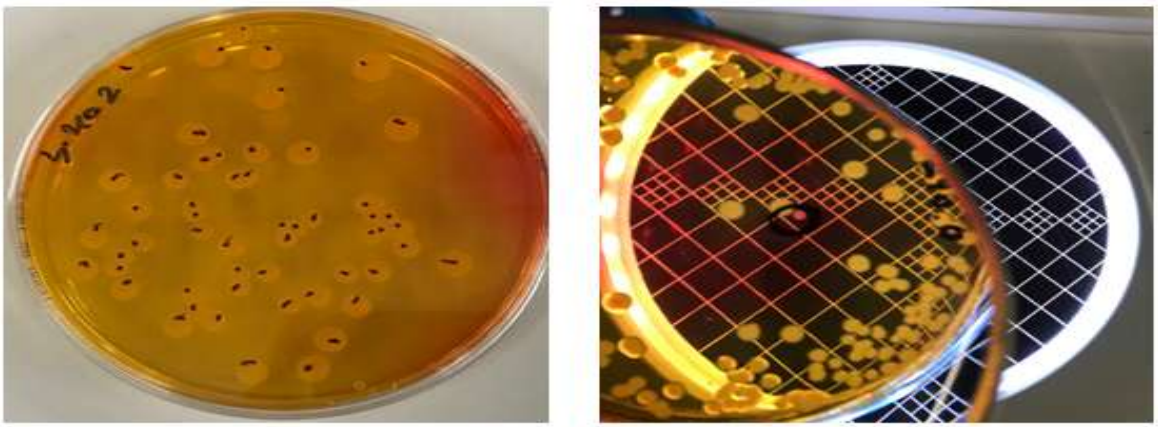
The samples were collected and serial dilution were performed using phosphate buffered solution as shown in fig 18. 0.1 ml of the sample was spread on the on the Membrane Enriched Lauryl Sulphate (*M500.02*) and were spread on the plate surface using sterilised inoculation spreader. Plating was done in duplicates. After that the plates were left to dry out for 15- 20 minutes and then closed and placed in the incubator at 37^{0}C for 24 hours.



Serial Dilution of the samples

(e) Plaque Counting and converting into concentration

Enumeration was performed using a colony counter and plaques were visible as clear spots in the bacterial lawn. Only yellow plaques were counted as they were the indicator of the E. Coli *WR1*. There were some red and pink plaques on the plates too that was because of the phenol red in the plates- it is an indicator of pH and a red colony means it did not ferment lactose which makes the colony acidic A black permanent marker was used to label each plaque that had been counted. In figure 19, the red and yellow colonies on the plates are shown.



(a) *(b)*

Plaques on the plates (a) Yellow colonies (b) Red colonies

The counts were converted into concentration using the formula.

$$T_m = \left[C_m / (D_m * u) \right] \quad \text{..... (1)}$$

Where T_m is the concentration per ml, C_m is the plaque count which is the average of two plates that were used for each dilution, D_m is the dilution factor and u is the amount of volume added on the plate which is 0.1 ml in every case. Since we are using multiple dilutions, therefore the final concentration was calculated using weighted mean formula. The weighted average is more precise than either the estimator from a single dilution or the unweighted arithmetic mean across all of the dilutions.

$$T = (T_m * D_m + T_{m+1} * D_{m+1} + ... + T_n * D_n) / (D_m + D_{m+1} + ... + D_n) \quad \text{..... (2)}$$

Where T_m is the corresponding value to a particular Dilution D_m

The weighted average in equation (2) is based on least squares theory, according to which least square gives unbiased estimates that are linear combinations of the T_m values.

C2. MS2 Bacteriophage

MS2 is used as a surrogate for the human viruses because of their similar shape, size, transport in the environment, ease of measurement, and lack of health risks *(Schijven et al., 2000)*. Bacteriophage (phage) is harmless to humans as they cannot infect human cells because only host bacteria contain the receptor sites needed for virus attachment and infection.

*MS2*is an icosahedral phage 26 nm in diameter and has an isoelectric pH (IEP) of 3.9, meaning that the surface charge is positive at a water pH below 3.9 and negative above pH 3.9 *(Schijven et al., 2000)*. Therefore, *MS2* has a strong negative charge at a natural water pH of 7. The low IEP and negative surface charge causes *MS2* to be repelled from granular media surfaces, which commonly have a negative charge at pH 7 *(Gerba, 1984)*. This results in poor attachment of *MS2* during drinking water filtration or flow through groundwater aquifers and hence making them a good surrogate for viruses.

(a) Preparation of *MS2* Bacteriophage

For the preparation of the culture, WG49 vial thawed from the -80^{0}C refrigerator was added to 50 ml of TYGB solution and 500 µL of calcium glucose solution in a flask. The flask was placed on a stir plate at 100 rpm inside the 37^{0}C incubator for around 3.5 hours. After taking out the flask from the incubator it was placed in a bowl of ice and water.

(b) Filter Seeding

MS2 Bacteriophage and E. Coli *WR1* spiking was done together. Test Water which is the same as the influent water to the columns was added in to the glass beakers which were sterilised before. For every litre of water, 10 µL of the culture were used resulting into concentration of around ~10^6/ ml in the feed water. During seeding, spike bottles were continuously stirred using a magnetic stirrer and feeding was done for 2.5 hours.

(c) Sampling protocol

Effluent sampling was done in the same way as done for E. Coli *WR1* as mentioned above.

(d) Sample Processing

Serial dilutions were done for the *MS2* samples similar to E. Coli WR1.

ssTYGA was prepared depending on the number of samples. Each plate requires 2.5 ml of ssTYGA. After taking out the ssTYGA from the autoclave it was placed in a water bath which is at 45^0C. For each 100 ml of ssTYGA, 1ml of calcium glucose was added into it. 2.5 ml of ssTYGA agar was pipetted into glass tubes and the glass tubes were covered with cap to keep them sterile. All the glass tubes were placed in the water bath.

1 ml of host bacteria (WG49) was pipetted into each tube and this was done only prior 10 minutes before using that tube to avoid the bacteria to sit in the heated water bath. After this, 1ml of the sample was added from each dilution tube to the glass tubes with agar and host bacteria. The plating was done in duplicate therefore two 1 ml samples were added into two tubes.

The glass tubes were taken out of the water, tapped slightly in order to dry water drips and then pour onto the Trypton-Gistextract-Glucose Agar (*T442.02*) plates. The plate was tilted slightly to ensure even distribution. The plates were left to solidify for 20 minutes and then were closed and put in an incubator at 37^0C for 16-18 hours.

(e) Plaque Counting and Calculation of Removals

Plaque counting was done in similar way as done for E. Coli. *MS2*concentrations were calculated by multiplying the plaque counts by the dilution factor. For example, if 75 plaques were counted for a 10^3 diluted sample, then the *MS2*concentration of that sample would be 75x10^3 CFU/mL because 1 mL of sample was processed. The plaque value used for the

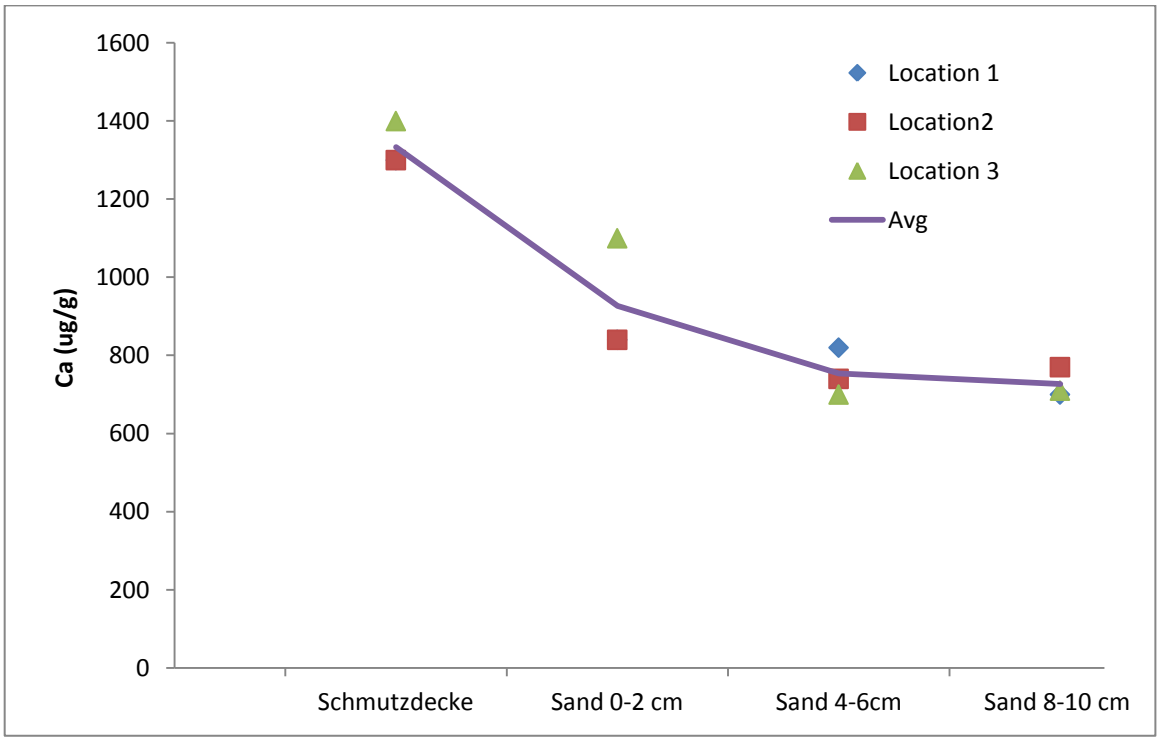
calculation was the average of the duplicates. Similar to the E. Coli, the final concentration was calculated using weighted average formula as shown in equation 2.

Appendix D: Inorganics Distribution

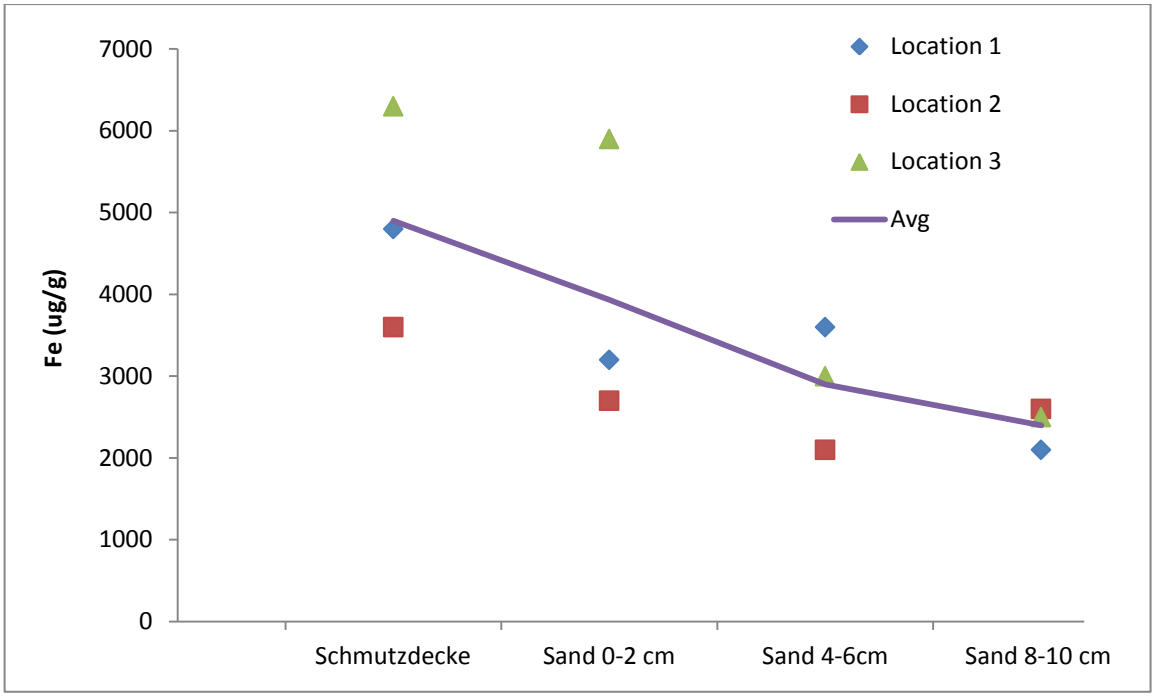
To study the effect of deposition of inorganics, the distribution of Ca, Mn and Fe were also quantified with depth at Monster filter treatment works. The distribution follows the same pattern as the biomass that is decreased with depth as shown in table1. The highest concentration among the inorganics was of iron followed by calcium and manganese.

Table1: Inorganics Distribution

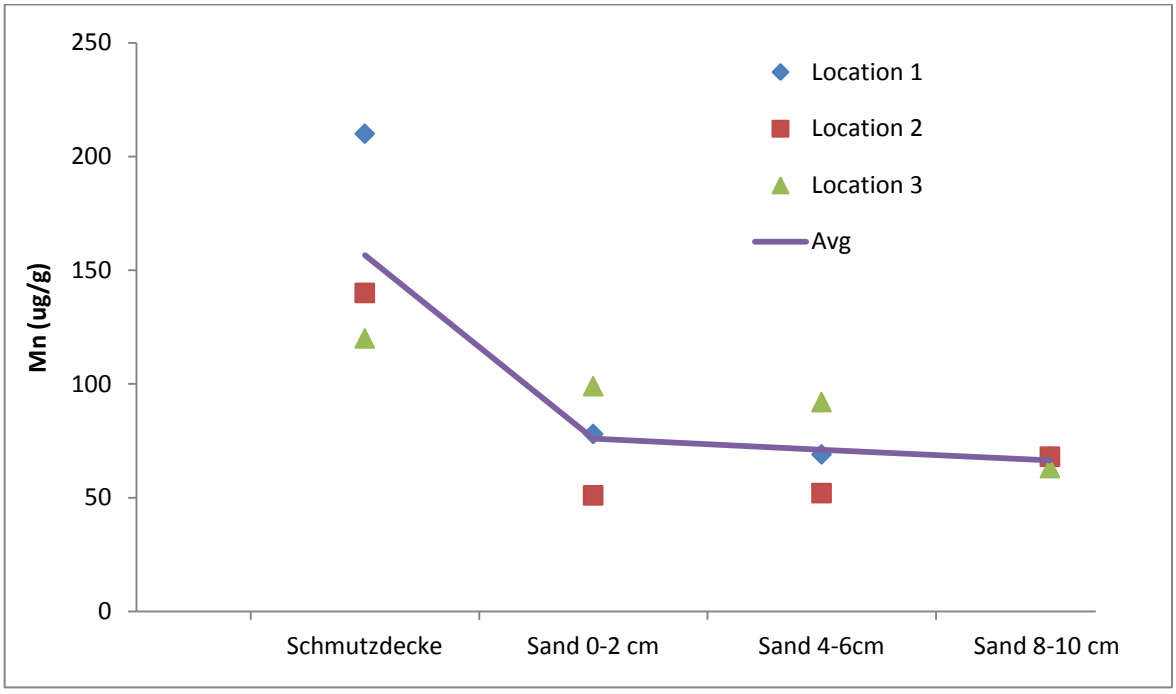
	Schmutzdecke	0-2 cm	4-6 cm	8-10 cm
Iron (µg/g)	4900±1352	3934±1721	2900±754	2400±264
Calcium (µg/g)	1333±57	926±150	753±61	726±38
Manganese (µg/g)	156±47	76±24	71±20	66±3



(a) Calcium Distribution with Depth



(b) Iron distribution with Depth



(c) Manganese distribution with depth

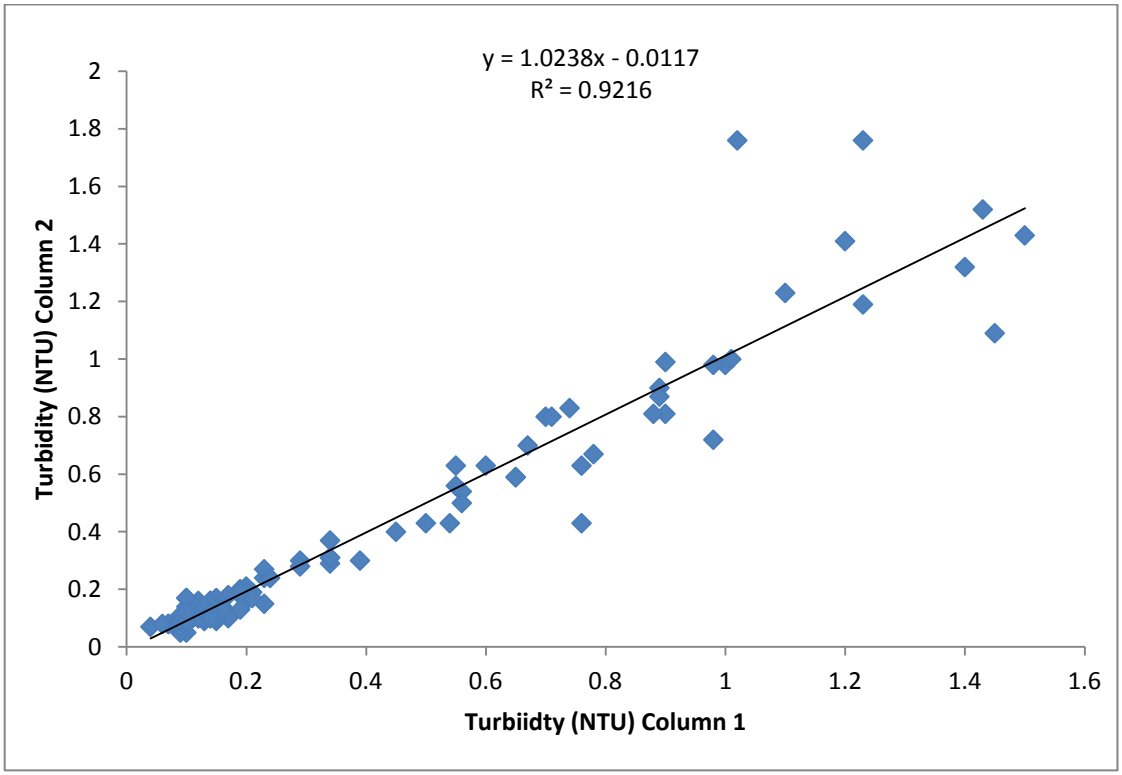
Appendix E: Average ATP content per cell

The values used for the full scale filters were the average of three locations. The value used in columns is the average of column running in duplicates. The concentrations are expressed in10^{-7} ng ATPcell^{-1}.

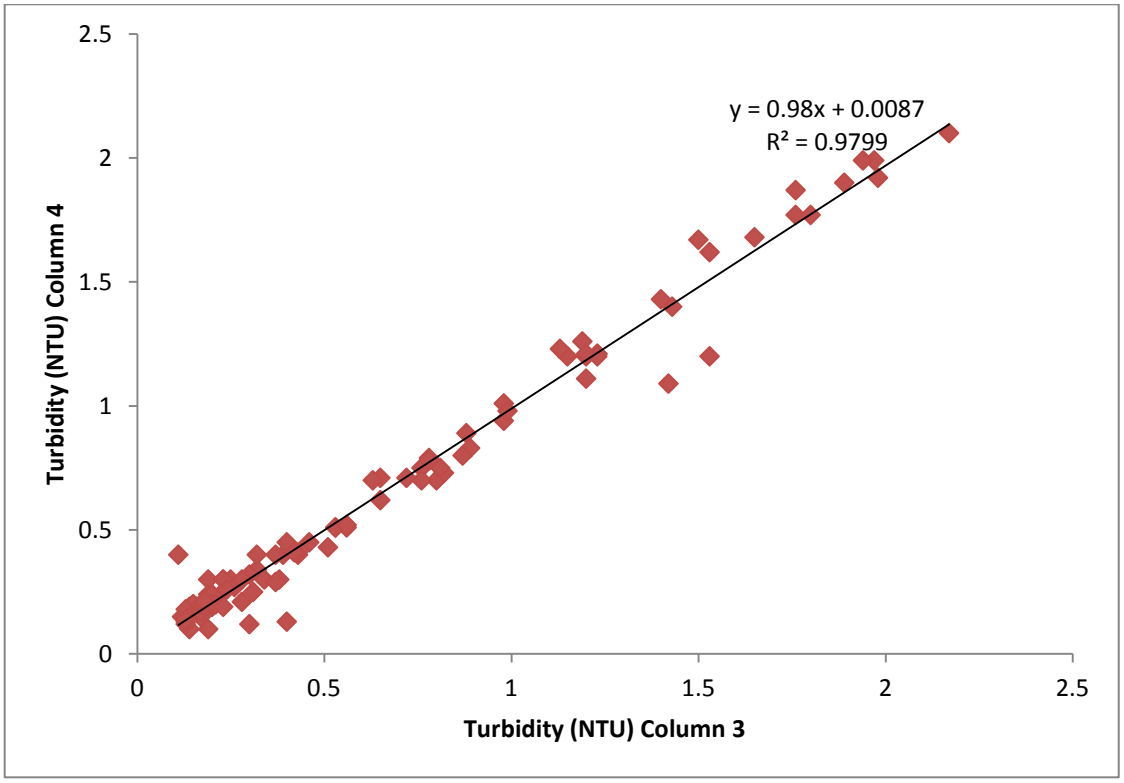
Filter		Schmutzdecke	0-2 cm	4-6 cm	8-10 cm
Monster	ATP/Cell (TCC)	1.169±0.49	1.40±0.24	1.16±0.10	1.32±0.31
	ATP/Cell (HNA)	1.52±0.52	1.95±0.49	1.88±0.15	1.90±0.40
Katwijk	ATP/Cell (TCC)	0.31±0.14	3.06±2.58	3.67±2.08	2.73±3.37
	ATP/Cell (HNA)	0.43±0.22	4.39±3.70	5.57±3.41	3.56±4.06
Columns (0.1 m/hr)	ATP/Cell (TCC)	0.85±0.19	0.78±0.37	0.66±0.02	0.78±0.13
	ATP/Cell (HNA)	1.52±0.52	1.95±0.49	1.88±0.15	1.90±0.40
Columns (0.5 m/hr)	ATP/Cell (TCC)	0.48±0.03	0.73±0.43	0.54±.03	1.45±0.04
	ATP/Cell (HNA)	0.43±0.22	4.39±3.70	5.57±0.01	0.64±.01
Columns N* (0.1 m/hr)	ATP/Cell (TCC)	0.3±0.04	0.28±0.09	0.39±0.05	.43±0.02
	ATP/Cell (HNA)	1.52±0.52	1.95±0.49	1.88±0.15	1.90±0.40
Columns S (0.1 m/hr)**	ATP/Cell (TCC)	2.09±0.44	1.48±0.22	1.57±0.59	1.44±.05
	ATP/Cell (HNA)	0.43±0.22	4.39±3.70	5.57±3.41	3.56±4.06

***N= Nutrients ** S=Schmutzdecke**

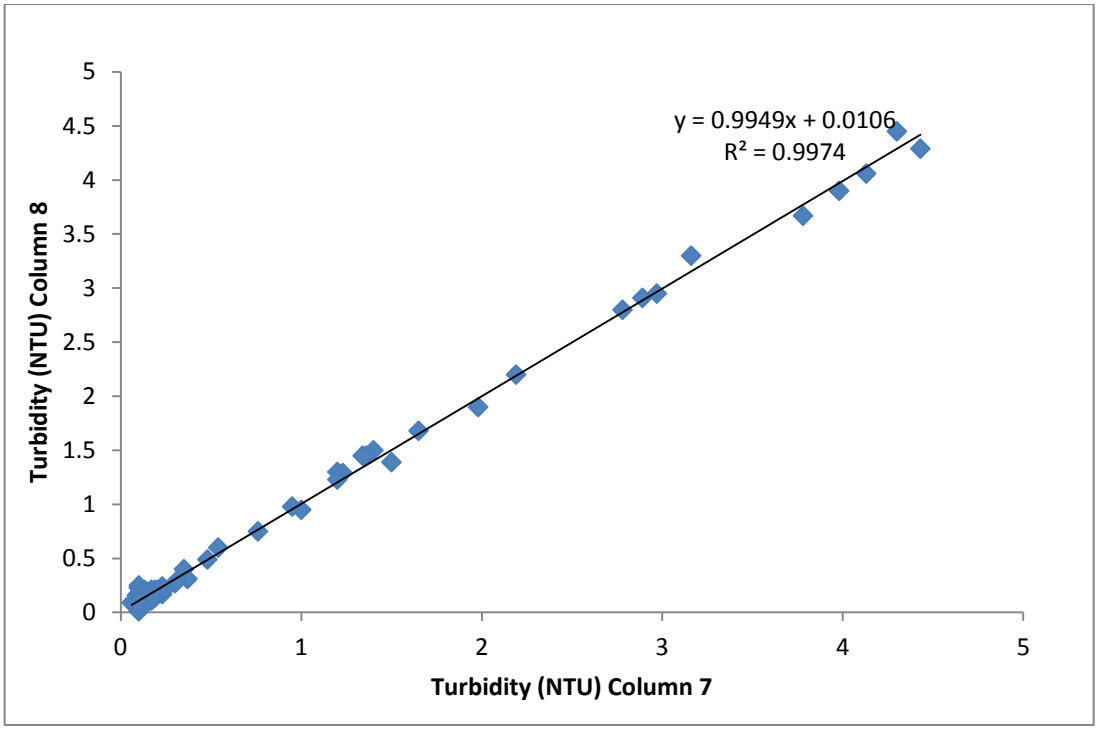
Appendix F: Turbidity Measurements



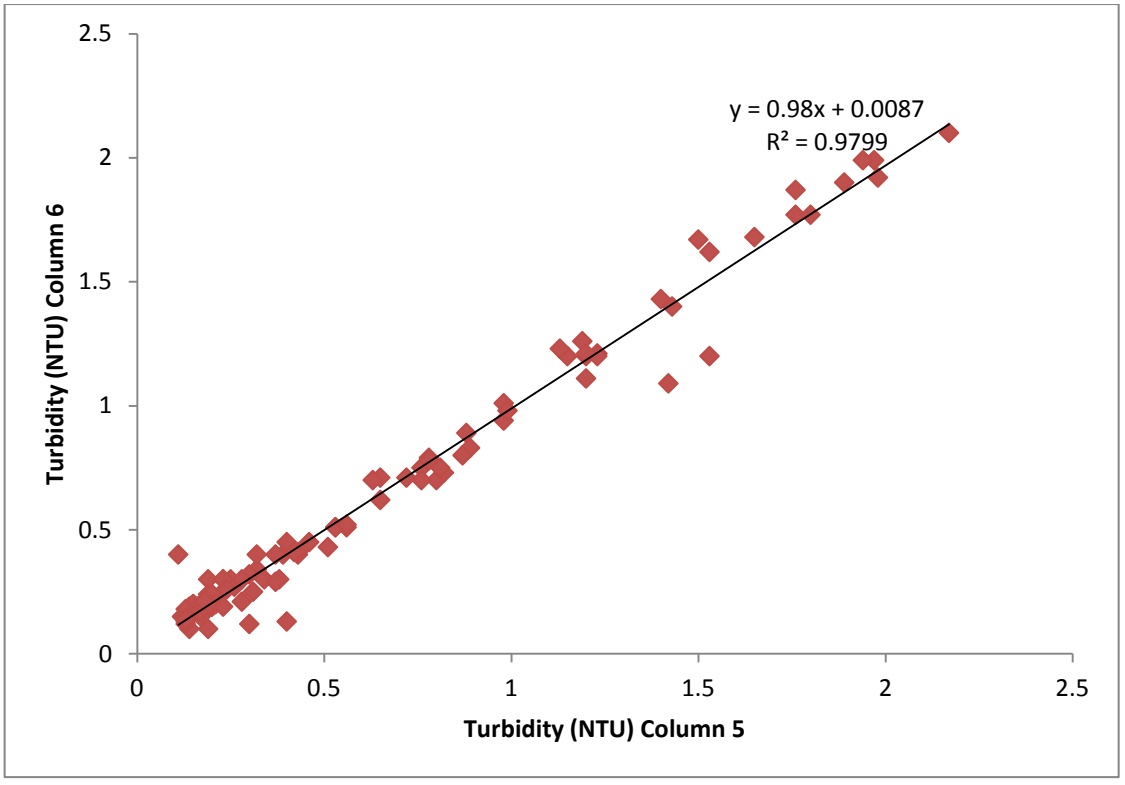
C.1 Correlation between Turbidity measurements for duplicate columns running at 0.1m/h



C.2 Correlation between Turbidity measurements for duplicate columns running at 0.5m/h



C3. Correlation between Turbidity measurements for duplicate columns running at 0.1m/h with inoculum



C4. Correlation between Turbidity measurements for duplicate columns running at 0.1m/h with nutrients

Appendix G: Influent and Effluent Particle Count

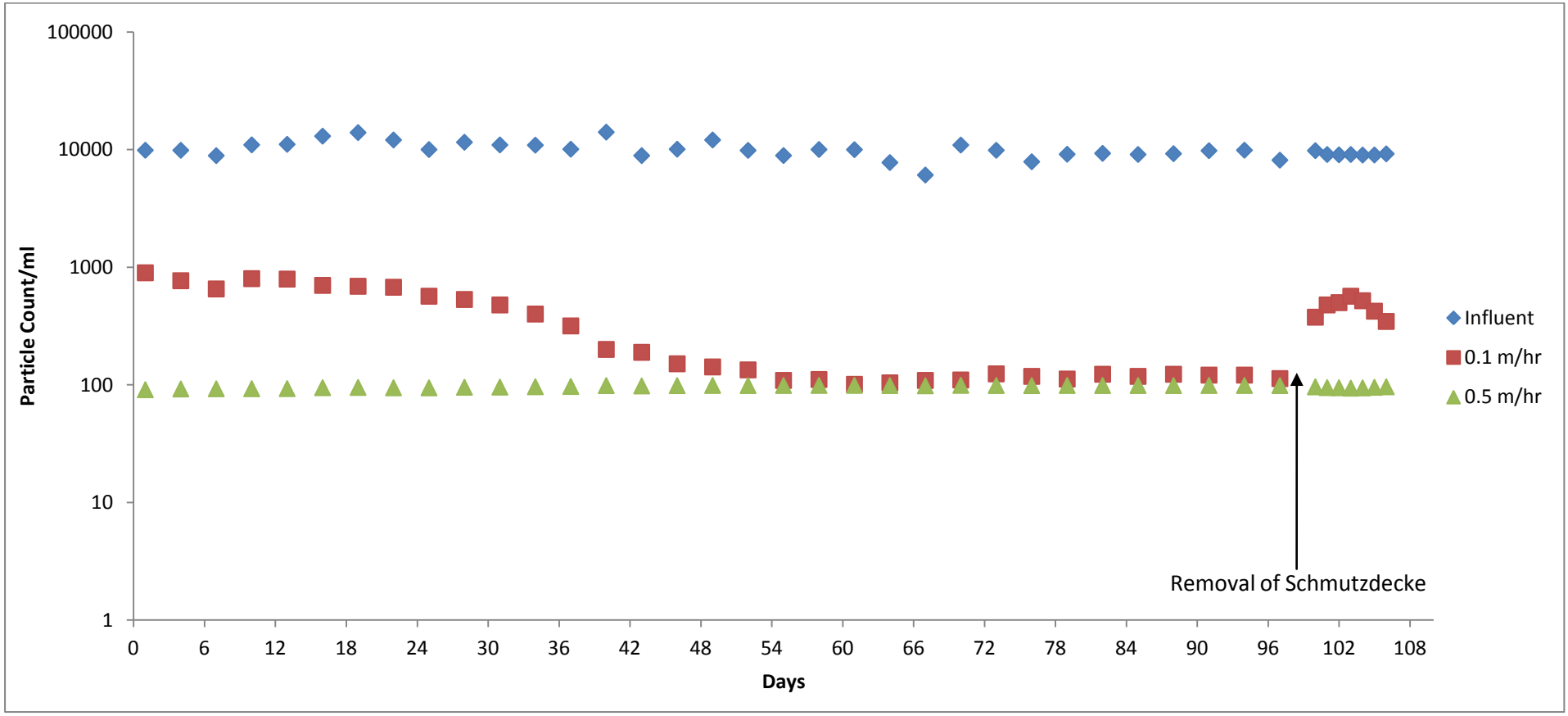


Figure E.1: Influent and effluent particle counts to columns running at the filtration rate of 0.1 m/hr and 0.5 m/hr

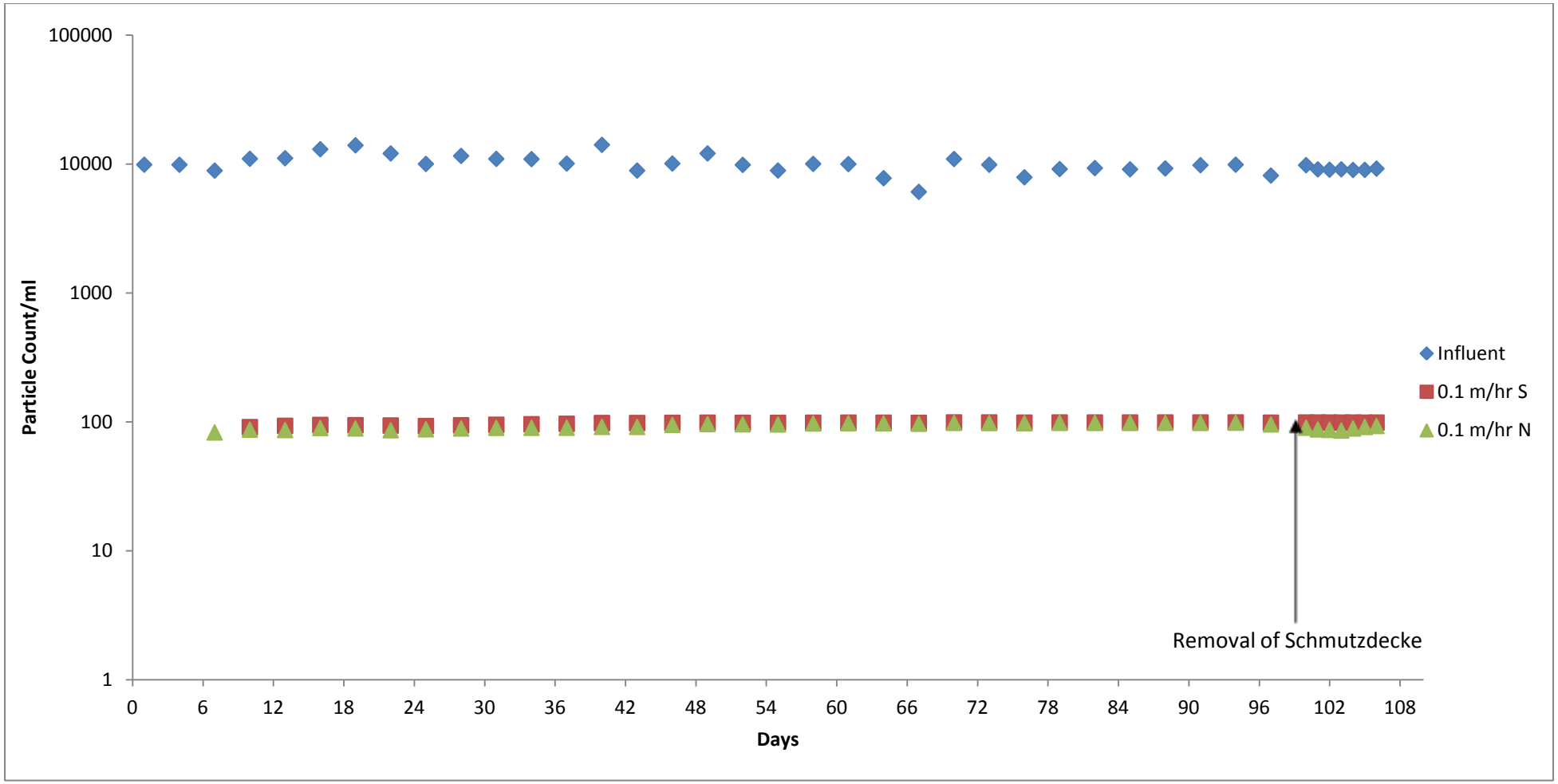
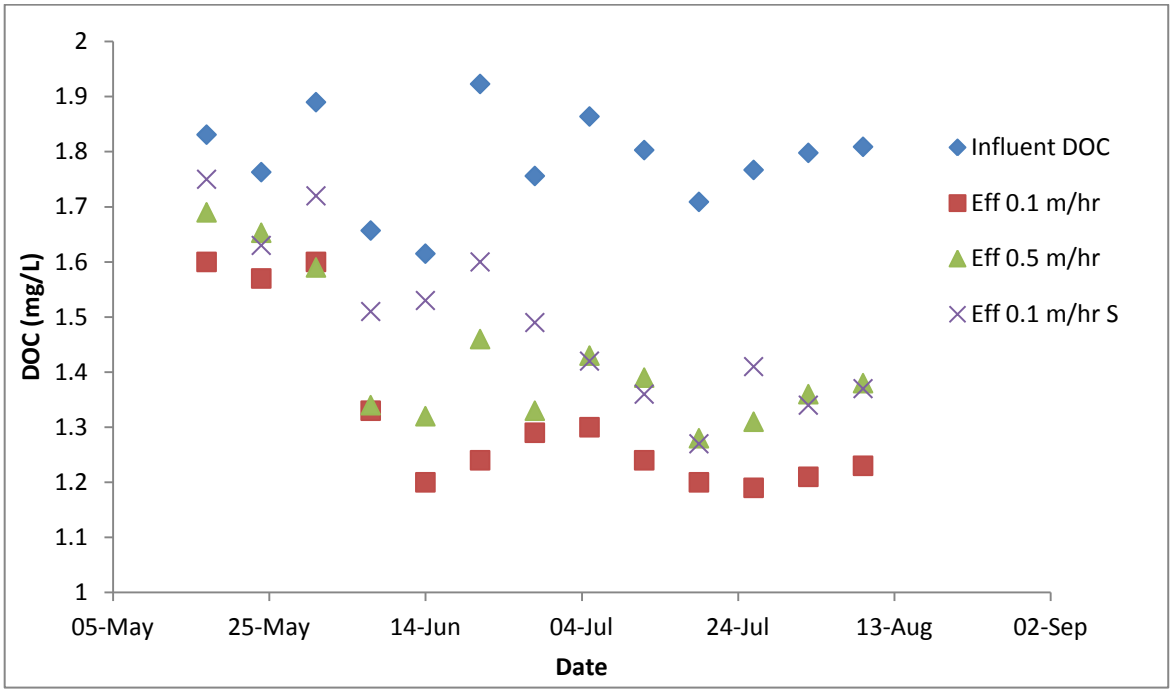
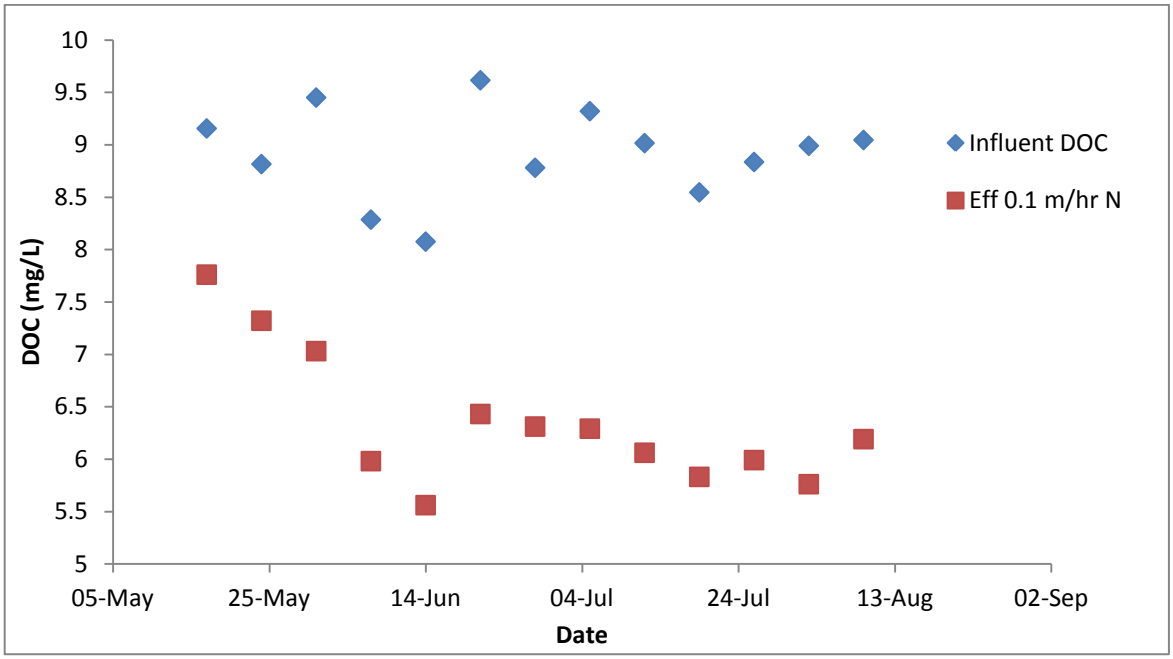


Figure E.235: Influent and effluent particle counts to columns running at the filtration rate of 0.1 m/hr with inoculum (S) and nutrients (N)

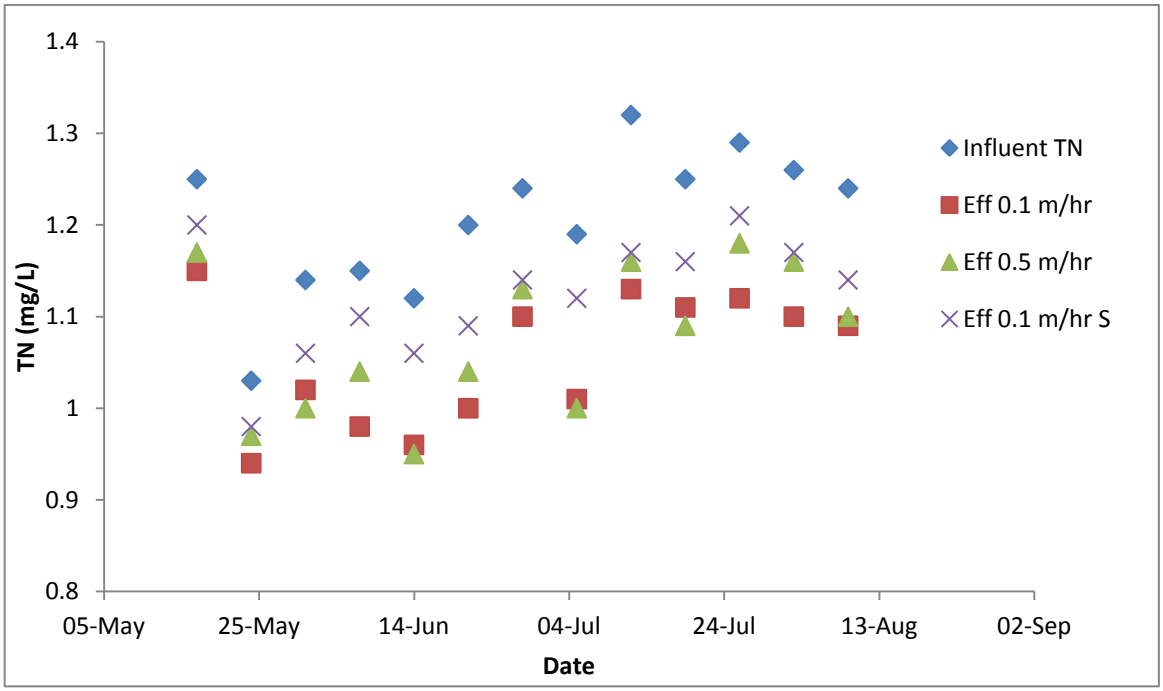
Appendix H: Influent and Effluent DOC & TN



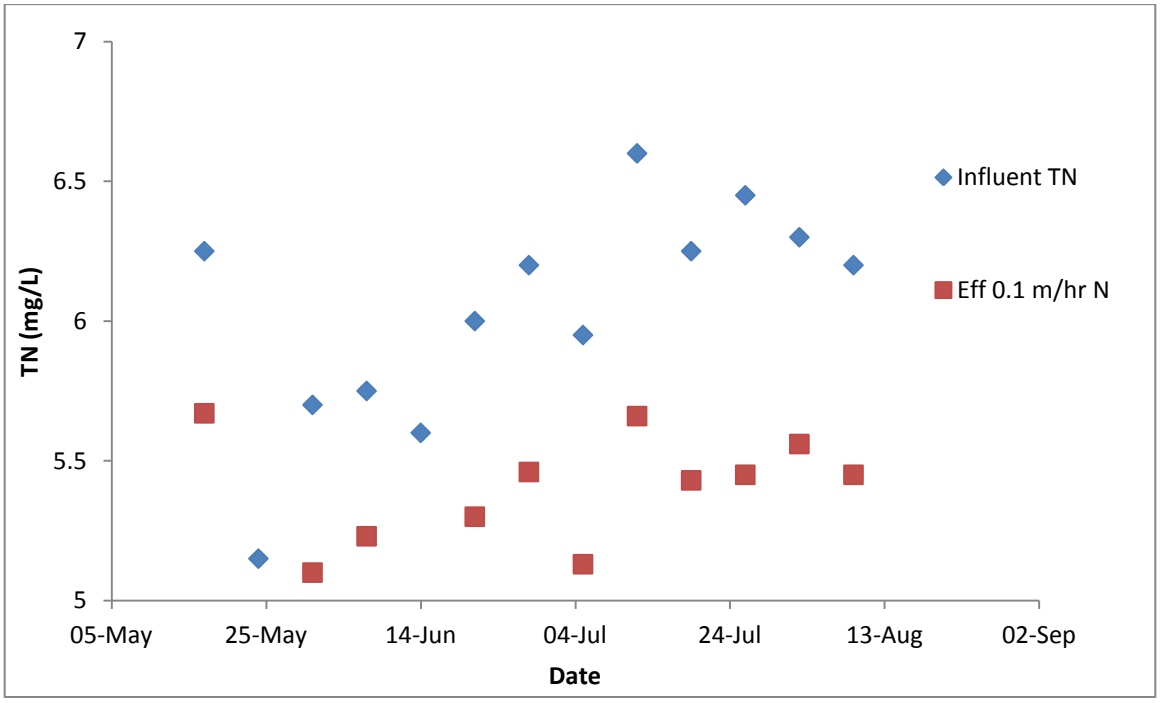
E.1 Influent and Effluent DOC



E.2 Influent and Effluent DOC



E.3 Influent and Effluent TN



E.4 Influent and Effluent TN

Appendix I: Correlation between Indicators of Ripening

F.1 Correlation between DEC, Turbidity and Particle Counts

	DEC	*Turbidity*	*Particle Counts*
DEC	1		
Turbidity	0.68	1	
Particle Counts	0.70	0.86	1

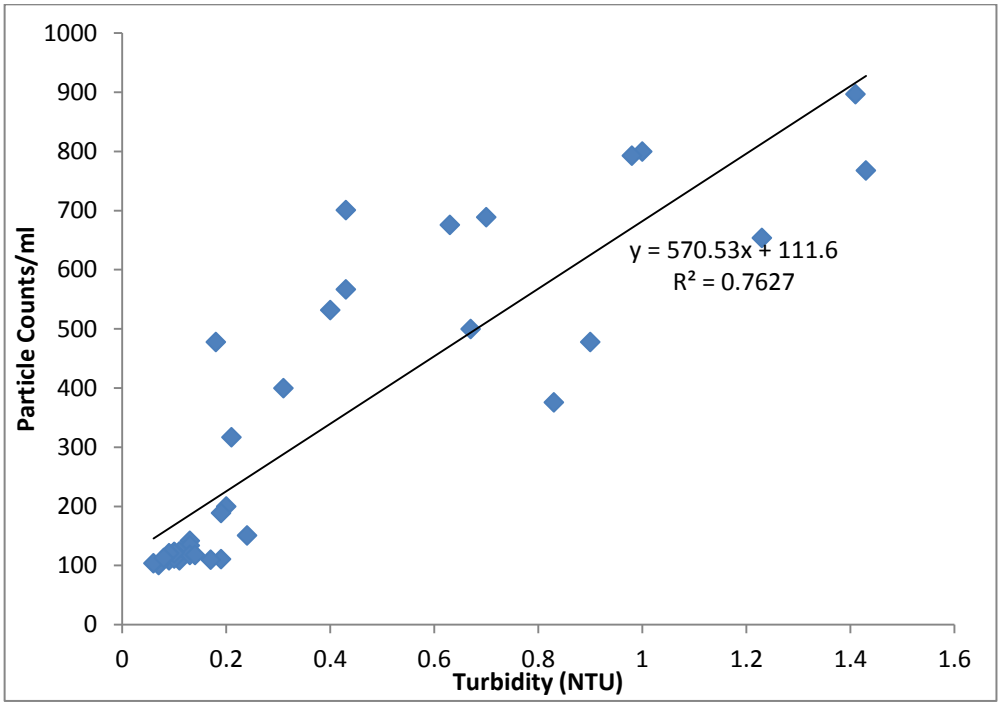
F.2 Correlation between DEC, DOC and TN

	DOC	*TN*	*DEC*
DOC	1		
TN	0.61	1	
DEC	0.30	-0.11	1

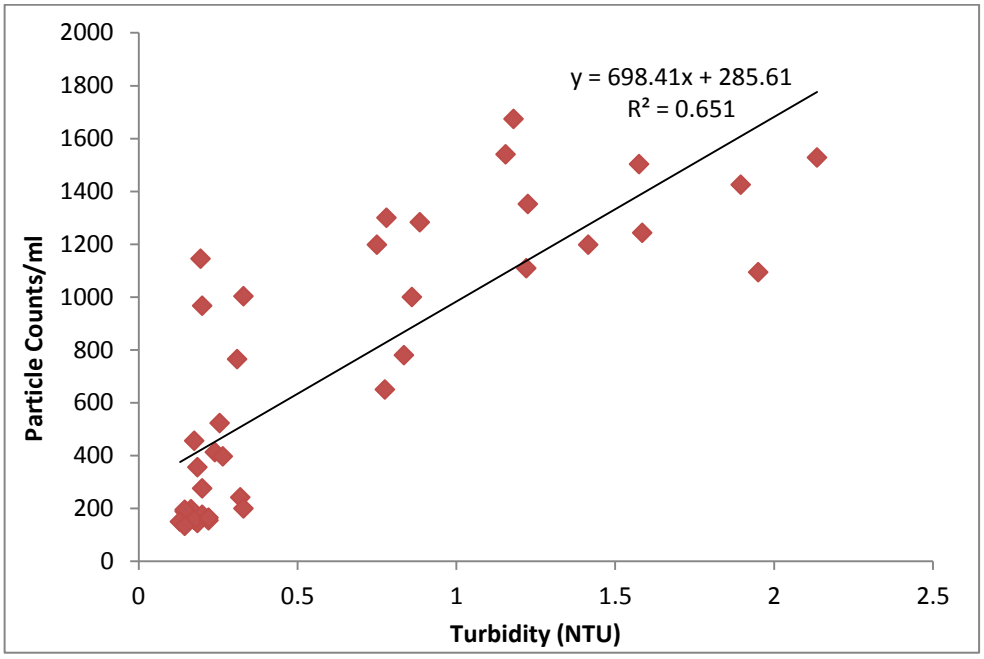
F.3 Correlation between Turbidity and Particle Counts, DOC and TN

	Turbidity	*Particle Counts*	*DOC*	*TN*
Turbidity	1			
Particle Counts	0.83	1		
DOC	0.27	0.28	1	
TN	0.058	-0.18	0.61	1

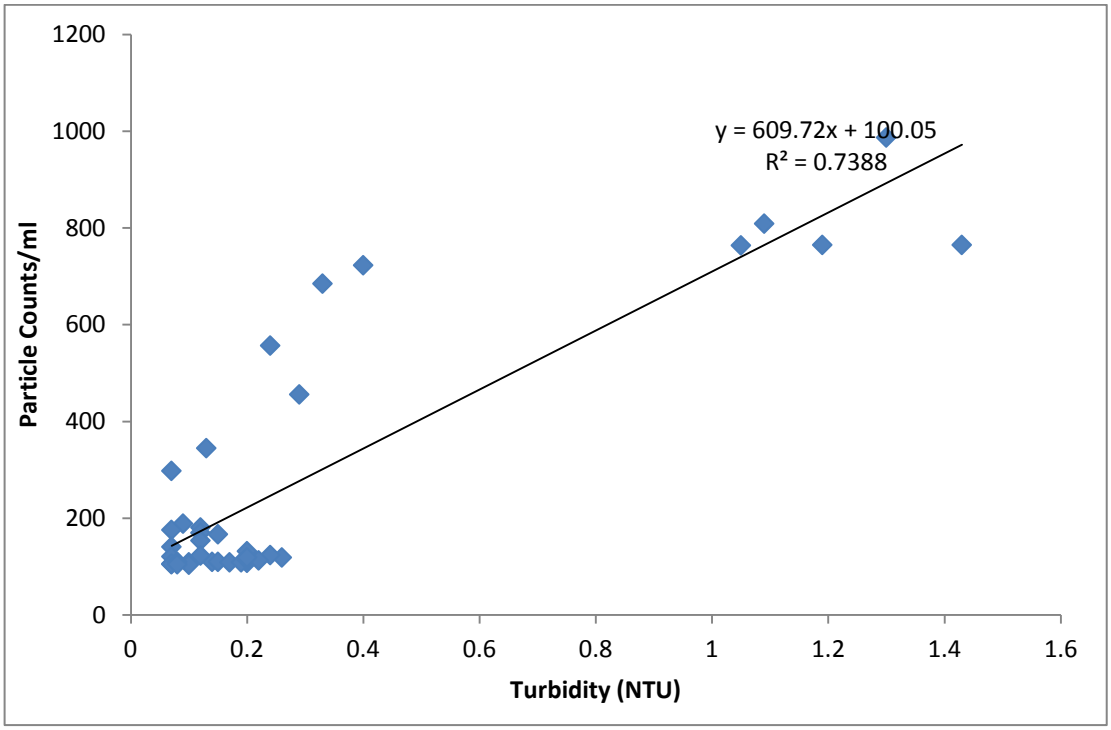
Appendix J: Correlation between Turbidity and Particle Counts



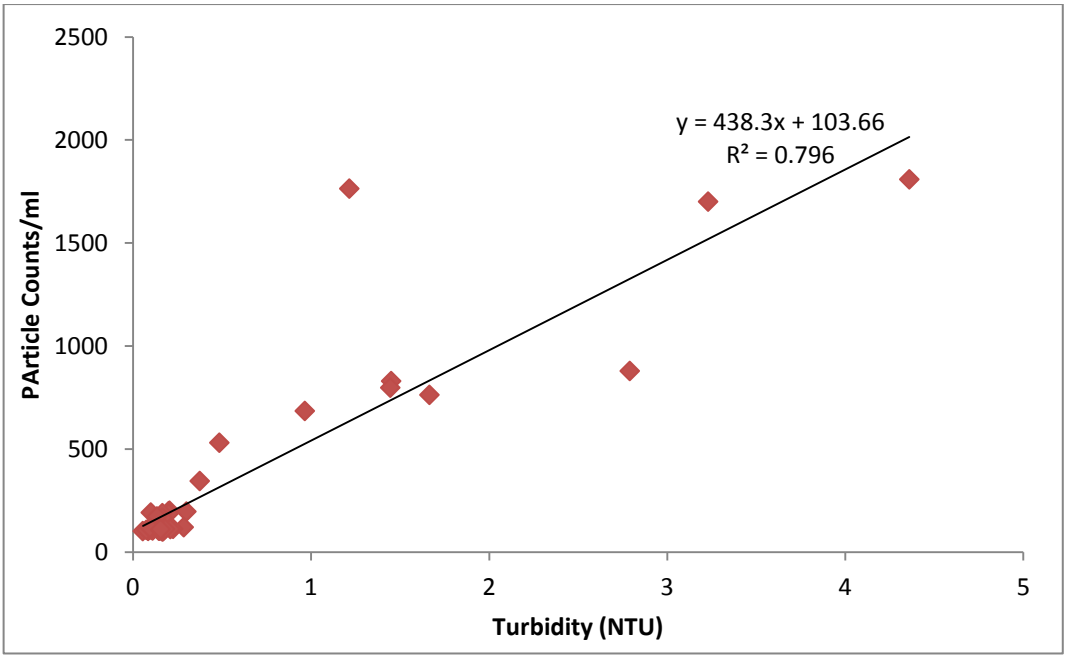
D2. Correlation between Particle Counts & Turbidity for columns running at 0.1m/h



D.3 Correlation between Particle Counts & Turbidity for columns running at 0.5m/h



D.4 Correlation between Particle Counts & Turbidity for columns running at 0.1m/h with Nutrients



D.5 Correlation between Particle Counts & Turbidity for columns running at 0.1m/h with microbial inoculum