INTERMITTENT SLOW SAND FILTERS:

IMPROVING THEIR DESIGN FOR DEVELOPING

WORLD APPLICATIONS

by

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For my Dad

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ABSTRACT

As many as 500,000 people in developing countries rely on an intermittent slow sand filter, called the BioSand filter to provide safe drinking water. However, it has been shown that BioSand filters do not remove all of the pathogens. The mechanisms of water purification in slow sand filtration have not been clearly understood until this day. In order to successfully influence intermittent slow sand filters performance we need to have a full understanding of removal processes that govern their efficiency. In the first part of this study pathogen removal mechanisms in intermittent slow sand filtration were investigated. In the second part of this study the effect on pathogen removal of a zero-valent iron – sand mixed layer, placed at the bottom of an intermittent slow sand filter was investigated.

A series of experiments was performed on sand columns ripened with creek water. It was shown that the majority of *E.coli* was removed during filtration through the schmutzdecke, while the residence time in the biologically active layer did not have a significant effect on its removal. Quite to the contrary, the majority of MS-2 virus was removed with the residence time in the biologically active layer while the filtration through the schmutzdecke did not have significant affect on its removal. Clearly, two different mechanisms govern bacteria and virus removal in intermittent slow sand filtration. The EDX analysis of the schmutzdecke material revealed that it was primarily composed of O, C, Si, Al, Fe, Ca, and Mn. The zeta potential analysis showed that the schmutzdecke was neutral in charge and was composed of positively, neutrally and negatively charged particles. It was determined that a flocculation

process was not a likely mechanism of either *E.coli* or MS-2 removal in intermittent slow sand filtration. The disturbance of the schmutzdecke had a significant impact on *E.coli* removal but only a slight effect on MS-2 removal. The intricate internal structure of the schmutzdecke might affect bacteria removal by an increase in rate of collisions. The disturbance of the schmutzdecke may cause the water to bypass the treatment in the schmutzdecke "network" of polymeric strands.

It was concluded that an additional treatment step is needed in order to efficiently remove viruses; that the residence time in the schmutzdecke affects the quality of the effluent.; that the disturbance of the schmutzdecke might lead to a significant compromise in water quality and thus should be avoided; and that the addition of Al or Fe into the filter influent might benefit the pathogen removal efficiency.

In the second part of the study an intermittent slow sand filter was amended with a zero-valent iron – sand mixture at the bottom of the column in order to improve its performance, especially virus removal. In the first challenge experiment the ZVI amended filter removed 100% of both *E.coli* and MS-2. Although, in the second challenge experiment the zero-valent iron – sand layer caused a decrease in *E.coli*/MS-2 concentration for unknown reasons, both *E.coli* and MS-2, exhibited breakthrough in the effluent.

The ZVI powder can be obtained by sieving iron filings which are a waste byproduct of iron industry. Therefore, it is readily available to communities in developing countries. It was shown that use of ZVI in an intermittent slow sand filter can greatly improve its safety. However, the mechanisms responsible for this benefit and limitations of ZVI are not yet explained and deserve to be investigated.

Chapter 1

INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Currently as many as 500,000 people worldwide rely on a household filter, known as the BioSand filter, to provide their drinking water (Elliott et al., 2008).

This filter's ability to remove *Giardia* cysts and *Cryptosporidum* oocysts is unsurpassed, as it has been shown capable of achieving at least 4-log removal (>99.99%) of those parasites (Palmateer et al., 1999). However, several laboratory and field studies show that the BioSand filter does not remove all pathogens to this extent. *E.coli* reduction in BioSand filters ranged from 90% to 99% for the filters tested in laboratory settings (Buzunis, 1995; Lee, 2001; Elliott et al., 2008; Donison, 2004; Stauber, 2007). In the same studies viruses were removed to even lesser extents ranging from 70% to 87% (Elliott et al., 2008; Stauber, 2007).

Performance of BioSand filters in removing *E.coli* tends to be even lower in field studies. A few field studies have been conducted in the Dominican Republic, Ethiopia, and in Nepal. Average *E.coli* reductions in those studies were 80% (Stauber, 2007), 87.9% (Earwaker, 2007), and 93% (Stauber et al., 2006). However, it is important to note that the performance of the investigated filters varied largely, and while some filters provided 100% removal, others provided no or even negative removals of *E.coli* (Donison, 2004; Earwaker, 2007; Lee , 2001; Stauber, 2007; Stauber et al., 2006).

Since there cannot be any guarantee that water produced by the BioSand filter will be pathogen free, disinfection is recommended (CAWST, 2008). However, disinfection will most likely lead to the formation of carcinogenic disinfection byproducts (DBSs) as slow sand filters are not capable of removing all dissolved organic matter (Amy et al., 2006; CAWST, 2008; Logsdon, 1991; Logsdon et al., 2002).

Due to the high popularity of BioSand filters in developing countries, their inconsistent performance in the field, and the risk associated with post-disinfection, it becomes increasingly important to determine factors that affect pathogen removal in those filters. Although some progress has been made in recent years, the removal processes in slow sand filters are still not clearly understood. The understanding of the mechanisms governing slow sand filter efficiency is essential to successfully influence its performance. The implementation of additional treatment steps that would improve effluent water quality should be also considered.

1.2 OBJECTIVES AND OVERVIEW OF THE STUDY

The purpose of the first part of the study was to gain further insight into removal mechanism in intermittent slow sand filters. The contribution of biological versus physical removal processes was studied. The schmutzdecke material from intermittent SSF was analyzed in order to determine its components and properties. The importance of residence time and intact structure of the schmutzdecke on effluent quality was also investigated.

The purpose of the second part of the study was to investigate the influence of zero-valent iron amendments on the quality of intermittent slow sand filter effluent.

Chapter 2

BACKGROUND

2.1 CONTINUOUSLY OPERATED SLOW SAND FILTER

2.1.1 History

The first slow sand filter was installed by James Simpson in 1829. It was owned by Chelsea Water Company and supplied water in London (Huisman & Wood, 1974). Its advantages became so evident that by 1852 the Metropolis Water Act was passed which required that all water derived from The Thames river within 5 miles of St Paul's Cathedral be treated by slow sand filtration before being delivered to the public (Huisman & Wood, 1974). The cholera outbreak of 1892 had established slow sand filtration as a very effective means of public health protection (Logsdon, 1991).

As described by Hazen (1913), the two German cities, Hamburg and Altona, both drew their water supplies from the Elba River. Altona, however, was located below the Hamburg sewage discharge. As Hazen (1913) stated:

> "Both cities take their entire water-supplies from the Elbe – Altona from a point about 7 miles below the discharge of the sewage of both cities, Hamburg from about 7 miles above. The raw water at Altona is thus polluted by the sewage from the population of both cities, having now together over 700,000 inhabitants, and contains in general 20,000 to 40,000 or more bacteria per cubic centimeter. The raw water of Hamburg has, however, according to the time of year and tide, form 200 to 5000…"

Yet still, it was Altona that suffered less from the cholera outbreak. While Hamburg had 13.39 deaths per 1000 inhabitants, Altona had only 2.13 deaths per 1000 inhabitants (Hazen, 1913). The reason for this enormous difference in the Hamburg and Altona death rate was the fact that Altona had slow sand filters while Hamburg had no water treatment (Hazen, 1913). Other interesting epidemiological data comes from 1890, when the Massachusetts State Board of Health performed a study of the removal of *Salmonella typhi* by slow sand filtration. Based on the results which indicated complete removal of the bacteria (Bellamy et al., 1985c), the decision was made to install a slow sand filtration plant in the city of Lawrence MA. Following that, a great reduction was reported in the occurrence of typhoid in that city (Bellamy et al., 1985c).

The slow sand filter's ability to produce high quality water was unsurpassed for many years. Huisman & Woods in WHO publication "Slow sand filtration" (1974) stated:

> "No other single process can effect such an improvement in the physical, chemical, and bacteriological quality of normal surface waters as that accomplished by biological filtration…. Under suitable circumstances, slow sand filtration may be not only the cheapest and simplest but also the most efficient method of water treatment."

Full scale slow sand filters were still being installed four decades ago (Huisman & Wood, 1974) and they continue to be in use in many European cities (Graham & Collins, 1996). Recently, there has been a renewed interest in potential use of slow sand filtration, especially for small and rural communities (Graham & Collins, 1996).

2.1.2 Design and operation

A slow sand filter is a type of a gravity filter. It typically consists of a rectangular shaped filter box, from 2.5 to 4 meter in depth, which is made of stone, brick or concrete (Huisman & Wood, 1974; Logsdon, 1991). At the bottom of the box is an underdrain made of porous concrete or a system of porous pipes (Huisman & Wood, 1974). The underdrain is covered with gravel that supports the sand-bed and prevents fine grains from escaping into the underdrain system (Huisman & Wood, 1974). The recommended depth of the supporting media is 0.3 – 0.5 meter (Visscher et al., 1987). The recommended depth of the sand bed is 0-8 - 1.2 meter (Huisman & Wood, 1974; Visscher et al., 1987). It is required that there is always a pool of supernatant water above the sand bed which is very important for the proper functioning of a filter (Huisman & Wood, 1974). It is recommended that the depth of supernatant water is 1 – 2 meters (Amy et al., 2006; Huisman & Wood, 1974; Visscher et al., 1987). Very important also is a properly functioning system of valves to regulate the hydraulic loading rate, maintain the desired level of supernatant water above the sand bed, and permit the filter bed to be drained for cleaning and then to be back filled when it is put back in operation (Huisman & Wood, 1974; Visscher et al., 1987).

The recommended filtration rate for a slow sand filter is 0.1 – 0.4 m/h; the recommended effective grain size is 0.15 – 0.35 mm with a preferred uniformity coefficient of less than 2 or 3 (Huisman & Wood, 1974; Visscher et al., 1987). The estimated empty bed contact time is 3 to 10 hours (Amy et al., 2006).

During the filter operation suspended matter like detritus is strained out near the surface of the sand. Over time a slime layer develops on the surface of the sand which consists of organic matter, numerous forms of life such as algae,

plankton, diatoms, protozoa, rotifers, bacteria, and their products (Huisman & Wood, 1974; Logsdon, 1991). Eventually, this layer causes the development of terminal head loss and the filter needs to be cleaned. The typical filter operation cycle is > 30 days (30 -300+ days) (Amy et al., 2006). The filter is cleaned by scraping to remove the top 1 to 2 cm of sand (Logsdon, 1991) or by harrowing and subsequent draining of the supernatant water that contains suspended "dirt" (Collins et al., 1991).

The biggest asset of slow sand filtration is that it can function as a stand alone treatment system (Amy et al., 2006). Contrary to rapid sand filtration, it does not require coagulant chemical feeders, rapid mixers, flocculators, sedimentation basins or filter washing equipment (Logsdon, 1991). However, due to a low filtration rate, slow sand filters normally require much a bigger area than rapid sand filters. According to Huisman & Wood (1974) a rapid sand filtration plant usually requires only 20% of the area needed for a slow sand filtration plant.

For comparison purposes, the sand used in a rapid sand filter is much coarser with an effective size of 0.6 – 2.0 mm (Huisman & Wood, 1974). The pores between the sand grains are larger. A rapid slow sand filter usually operates 20 – 50 times faster than a slow sand filter; the loading rate is 5 – 15 m/h (Huisman & Wood, 1974). Therefore, the deposition of impurities is more rapid, and they are carried deeper into the filter medium (Huisman & Wood, 1974). As a result rapid sand filters need to be cleaned more frequently, as often as every one or two days, and throughout their whole depth (Huisman & Wood, 1974). This is done by backwashing, a process which forces air and water up through the filter in order to re-suspend accumulated in the filter media matter.

The other advantages of slow sand filters are the low cost, and the ease of construction and operation (Amy et al., 2006; Huisman & Wood, 1974). The maintenance involves only occasional cleaning of the filter bed. No coagulants or any chemicals other than chlorine, used as a precaution for disinfecting treated water, are needed. Since no additional equipment is used the cost of fuel and energy is low. Additionally, no water is needed for backwashing, and sludge storage, dewatering, and disposal are less troublesome since chemical coagulants are not used (Huisman & Wood, 1974).

2.1.3 Water purification efficiency

Although slow sand filtration is such an old water treatment technology, the purification processes that make it efficient in contaminants removal are still not fully understood. It is believed that the development of the slime layer called "the schmutzdecke" is primarily responsible for this benefit (Huisman & Wood, 1974; Logsdon, 1991; Unger & Collins, 2008; Weber-Shirk & Dick, 1997b). The word "schmutzdecke" comes from German and means "dirty layer" (Logsdon, 1991).

The schmutzdecke is a biologically active zone at, and directly underneath, the sand/water interface. It develops over time, as a "biofilm" adhering to the sand and capturing suspended matter as it enters the filter. The most successful algae and bacteria produce adhesive gelatinous matter to maximize capture (Rideal, 1997; Huisman & Wood, 1974), growing to produce a visible slime layer in the filter. This becomes a breeding ground for additional trophic levels of both microorganism and macrofauna, within the schmutzdecke or in lower layers (Logsdon, 1991; Huisman & Wood, 1974).

Viruses and bacteria are believed to be removed by both biological and physical removal processes, in the schmutzdecke or below it. In slow sand filtration physical removal processes are far more efficient than in rapid sand filtration (Logsdon, 1991). The detailed description of the biological and the physical removal processes in slow sand filtration can be found in the last section of this chapter.

Previous studies show that slow sand filtration can be very efficient in pathogen removal. The percent removals of coliforms, standard plate count bacteria, viruses and turbidity increase with the increasing activity of the biological community in a slow sand filter (Bellamy et al., 1985b; Poynter & Slade, 1977). The mature filter is capable of reducing coliform bacteria concentrations by 2.5 – 4 logs (Bellamy et al., 1985b; Bellamy et al., 1985c). The schmutzdecke removal (cleaning) results in a 1-2 log decrease in coliform removal efficiency (Bellamy et al. 1985c; Dullemont et al., 2006; Bellamy et al., 1985b). In the research performed by Bellamy et al. (1985b) the additional filter bed disturbance created by mixing the top 10 cm of sand, and pounding on the sand surface, caused a further decrease in coliform removal efficiency by 0.5 – 1 log. *Giardia* cysts can be reduced by virtually 100% for mature bed sand conditions (Bellamy et al., 1985b; Dullemont et al., 2006). Even for a new filter, cysts can be reduced by 99% (Bellamy et al., 1985c). The virus removal efficiency in slow sand filtration appears to be somewhat lower. In the study performed by Poynter and Slade (1977) virus removal efficiency for a mature filter bed conditions ranged from approximately 2.3-log to 4-log. Dullemont et al. (2006), however, reported that viruses were the most critical microorganisms because they were removed the least (removal ranged from 1.7 to 2.2 log) and were affected the least by the presence of the schmutzdecke. It was shown that removal of the schmutzdecke had a negligible effect

on virus removal efficiency (Dullemont et al., 2006; Poynter & Slade, 1977). Drainage of the bed, however, had a strong effect on virus removal efficiency (Poynter & Slade, 1977).

The removal of *Giardia* cysts, coliform bacteria, standard plate count, viruses, and turbidity declines as the hydraulic loading rate increases (Bellamy et al., 1985b; Dullemont et al., 2006; Poynter & Slade, 1977). Doubling the hydraulic loading rate might cause a ten-fold decrease in virus removal efficiency (Poynter & Slade, 1977). However, even at a high loading rate the pathogen removal efficiency can be still relatively high. Bellamy et al. (1985c) reported that at 0.4 m/h removals were 99.98% and 99.01% for *Giardia* cysts and coliform bacteria, respectively. It was shown that a decrease in temperature affects the removal efficiencies of bacteria and viruses but not of *Giardia* cysts (Bellamy et al., 1985a; Dullemont et al., 2006; Poynter & Slade, 1977). Bellamy et al. (1985a) reported that after a temperature drop from 17°C to 5°C *Giardia* removal was still 100% but bacterial removal decreased from 97% to 87%. It was determined that the bacterial removal efficiency is also sensitive to sand size (Bellamy et al., 1985a). Bellamy et al. (1985a) reported 99.4% and 96% of total coliform bacteria reduction for 0.128 mm and 0.615 mm sand, respectively.

Slow sand filters are not capable of removing all dissolved organic matter (Amy et al., 2006; Logsdon, 1991; Logsdon et al., 2002). Various studies have reported from 75% to 10% reduction in COD (Amy et al., 2006; CAWST, 2008; Logsdon, 1991; Logsdon et al., 2002). Such variable results are most likely caused by differences in the organic compounds composition (Logsdon, 1991).

The reduction in influent water turbidity will depend on the type of the suspended matter in the raw water (Bellamy et al., 1985c). If the influent water contains a large number of fine clay particles, the turbidity removal might be as low as 10% (Collins et al., 1991). Therefore, the slow sand filters are appropriate for treating water with moderate turbidity. Purification is the most efficient when the treated water turbidity is no more than 10 mg/l (Huisman & Wood, 1974). It is not recommended that treated water turbidity exceed 50 mg/l for a period longer than a few days (Huisman & Wood, 1974).

2.2 INTERMITTENTLY OPERATED SLOW SAND FILTER

2.2.1 Design

The intermittent slow sand filter, called the BioSand filter, was developed by Dr. David Manz, a professor from the University of Calgary, in the early 1990's (CAWST, 2008). While it is a type of slow sand filter, the design was modified in such a way that the filter can be built as a small household unit that can be operated not continuously, but based on the water demand (CAWST, 2008). The differences between the traditional and the intermittent slow sand filtration are substantial. While the traditional slow sand filter is operated continuously at a constant head and flow rate, the BioSand filter is operated intermittently at a head and a flow rate that vary during the filter run (Stauber et al., 2006). This modification was made possible by ensuring that the design changes allow for filtration to be stopped and then resumed without causing the schmutzdecke disturbance or negatively affecting its biological activity (Manz, 2007).

The BioSand filter consist of a concrete rectangular box, that is 1 meter in height and 40 cm in width (Manz, 2007). The filtering media is approximately 45 cm deep; the separating media is 3 to 5 cm deep (Manz, 2007). The recommended effective sand size is 0.15 to 0.25 mm with a uniformity coefficient of less than 2 (CAWST, 2008). The recommended filtration rate is 0.6 L/minute which is double that recommended for traditional slow sand filtration (measured when the box is full of water) (CAWST, 2008; Manz, 2007). The filter is designed in such a way that the outlet of the spout is located 5 cm above the sand surface. This ensures that there is always an approximately 5 cm deep layer of paused water above the filter bed. The depth of 5 cm was determined to be the best in order to ensure that the schmutzdecke is protected from falling water and that oxygen can freely diffuse (Buzunis, 1995; CAWST, 2008; Manz, 2007). Additionally, as a means of protecting the biofilm from falling water, the filter is equipped with a diffuser. A diffuser is a container with holes on its bottom that sits on the ledge 5 cm above the paused water (Manz, 2007).

River, stream, lake, rainfall or well water can be used, however, the source should be the cleanest possible since there cannot be a guarantee that the BioSand filter will remove all contaminants (CAWST, 2008). The same source of water should be used each time since the biofilm cannot adapt quickly to changes in water quality (CAWST, 2008)..

2.2.2 Operation

The operation of the BioSand filter is simple. A bucket of water is poured into the diffuser and water starts trickling through the small holes in its bottom. While the water flows through the filter it is purified due to the same physical and biological

processes that take place in traditional slow sand filtration. As the water level above the filter bed goes down the flow slows down and eventually stops. However, the level of water above the filter bed remains at the same height as the outlet of the spout (approximately 5 cm above the sand). It is recommended that the next portion of water is poured after at least 1 hour but no more than 6 -12 hours. During the pause time the pathogen and contaminants are “consumed” by the biolayer. However, if the pause time is too long, the nutrients and oxygen supply for the microbial community in the biolayer might become limited which might lead to a compromise in water quality (CAWST, 2008).

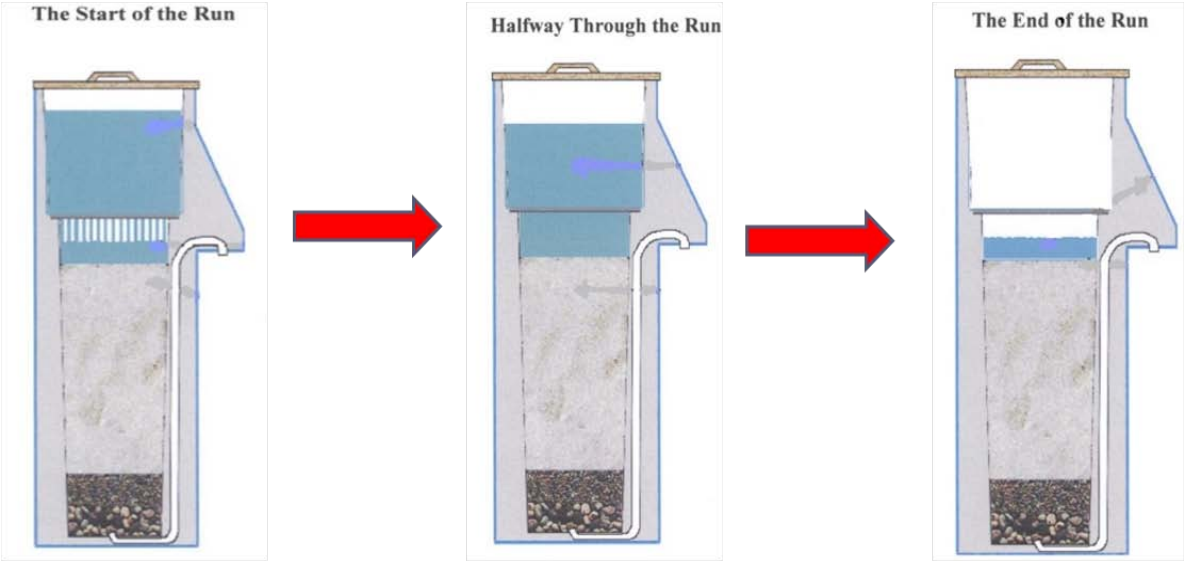


Figure 2.1 The Biosand filter operation (Adapted from CAWST, 2008)

Over time as more and more particles are deposited and the biofilm develops, the headloss becomes significant. When the flow becomes too slow, the filter should be cleaned by agitation of the sand on the filter’s surface. This causes

the re-suspension of the particles that were previously deposited. The dirty water is then poured out. The procedure can be performed as many times as needed in order to restore the desired flow rate (CAWST, 2008). During the first few weeks of a filter operation when the biofilm is not yet developed the effluent from the filter should be disinfected before consuming (CAWST, 2008).

2.2.4 Water treatment for developing countries

The BioSand filter which allows people to treat water by themselves in their homes is believed to be a technology that is especially suitable for developing countries where public water treatment facilities are very rarely available. Additionally, this technology is very affordable. The BioSand filters consist only of concrete, gravel and sand thus they can be built with locally available materials (CAWST, 2008). Due to the ease of construction, slow sand filters can be constructed by local communities/household owners (CAWST, 2008; Huisman & Wood, 1974; Logsdon et al., 2002). Since the maintenance of the filters involves only periodic cleaning, it does not require the support of highly trained outside technicians and can be simply performed by a filter owner (CAWST, 2008).

2.2.3 Water purification efficiency

The Biosand filter can not only significantly improve the look and taste of water but also greatly reduce the amount of pathogens and toxins (CAWST, 2008). The filter's ability to remove *Giardia* cysts and *Cryptosporidum* oocysts is unsurpassed as the filter has been shown to be capable of achieving 4-log or higher removal rates of those parasites (Palmateer et al., 1999). *E.coli* removal in laboratory experiments ranged from 90% to 99% (Buzunis, 1995; Lee , 2001; Elliott et al., 2008;

Donison, 2004; Stauber, 2007). In the same studies viruses were removed to a lesser extent and ranged from 70% to 87% (Elliott et al., 2008; Stauber, 2007). Average *E.coli* reductions in field studies were 80% (Stauber, 2007), 87.9% (Earwaker, 2007), and 93% (Stauber et al., 2006). However, it is important to note that performance of the investigated filters varied largely, and while some filters provided 100% removal, others provided no or even negative removals of *E.coli* (Donison, 2004; Earwaker, 2007; Lee , 2001; Stauber, 2007; Stauber et al., 2006).Since there cannot be any guarantee that water produced by a BioSand filter will be pathogen free, disinfection is recommended (CAWST, 2008).

While the BioSand filter will reduce 50% - 90% of inorganic and organic toxicants (Palmateer et al., 1999) it cannot remove some organic chemicals, e.g., pesticides and fertilizers (CAWST, 2008).

2.3 MECHANISMS OF REMOVAL IN SLOW SAND FILTRATION

A number of different physical and biological removal processes that operate in tandem have been suggested to take place in slow sand filtration.

2.3.1 Biological removal mechanisms

Predation

The results of many studies suggest that predation of bacteria is a highly probable mechanism of pathogen removal in slow sand filtration.

Lloyd (1973) showed that clean sand in a slow sand filter was rapidly colonized by *Vorticella sp.* population and that the number of bacteria in the effluent was inversely proportional to the number of *Vorticella sp*. Unger & Collins (2008) found a strong correlation between *E.coli* removal and protista abundance, with both

E.coli removal and protista abundance increasing with ripening time. The slow sand filtration plants with more numerous protozoa and algae populations have higher turbidity and fecal coliforms removals (Sanchez et al., 2006).

The inoculation of filters with bacterivory organisms i.e., *Vorticella*, and chrysophyte that have been previously isolated from slow sand filters caused an immediate increase in the removal of bacteria (Lloyd, 1996; Weber-Shirk & Dick, 1999). In Lloyd's experiment (1996) non-predatory removal mechanisms in the control cells did not exceed 96%, however, the presence of *Vorticella convallaria* in the inoculated cells enhanced the removal by almost 1000-fold (99.9%).

Weber-Shirk introduced sodium azide into the filter feed water (Weber-Shirk & Dick, 1997a) in order to inhibit biological activity in the filter. Sodium azide temporarily inhibits the biological activity of aerobic organisms by reversibly blocking oxidative phosphorylaton (Weber-Shirk & Dick, 1997a). The rapid decrease in the removal efficiency of *E.coli* after the introduction of sodium azide and the prompt recovery of the initial removal efficiency after the azide was washed out from the column suggested the bacteria removal was by predation (Weber-Shirk & Dick, 1997a).

Natural death and inactivation

Pathogens will die as they enter a hostile environment. As water filters further into the sand bed, food in the form of organic matter becomes limited and competition between organisms fierce (Huisman & Wood, 1974). Furthermore, it was found that *E.coli* is reduced shortly after introduction into the filter's supernatant water (Logsdon et al., 1991). In the Burman & Lewin (1962) study *E.coli* reduction was noticeable 30 cm above the sand surface.

Bactericidal effect of algae

It was shown that extracellular algal products increased the death rate of bacteria over long periods of time (Logsdon et al., 1991).

Increased stickiness of sand surface

Early researchers have suggested that the development of the zoogloea layer on the sand surface increases "stickiness of sand grains" thus causing better adherence of the particles that come in contact with them (Bellamy et al., 1985a; Huisman & Wood, 1974; Rideal, 1997). This would explain why turbidity is reduced more efficiently after the filter ripens (Logsdon et al., 1991).

2.3.2 Physical removal mechanisms

Straining

The particles that can be removed by straining will have a diameter of no less than 15% of the sand grain diameter (Logsdon et al., 1991). That is, it can be expected that for a sand with an effective size of 0.2 mm, particles with a diameter of 30 μm or more will be strained out (Logsdon et al., 1991). Therefore, it can be expected that in slow sand filtration, particles like algae and plant debris are removed by straining, while bacteria and viruses are removed by other physical or biological processes (Logsdon et al., 1991). As more particles are strained out and an increased head loss results in filter cake compression, the pore openings become smaller thus smaller particles can be removed by this mechanism (Logsdon et al., 1991; Weber-Shirk & Dick, 1997b).

Adsorption

The surface area available for adsorption in a slow sand filter is extremely high. Huisman & Wood (1974) estimated that one cubic meter of filter sand has one

and a half hectares of surface. The removal of viruses was found to follow an adsorption isotherm (Logsdon et al., 1991). Results of one study suggest that DLVO or sorption process was responsible for *Cryptosporidium* oocyst removal in intermittent sand filters (Logan et al., 2001).

Interception, sedimentation, and diffusion

The particles that escape straining might still be removed within the filter bed by physical removal processes that involve transport mechanisms bringing particles in contact with sand grains surfaces and the subsequent attachment mechanisms that hold the particles in place (Logsdon et al., 1991). The transport mechanisms include interception, sedimentation and diffusion. With a low filtration rate, a long detention time and fine filter media those mechanisms are expected to be far more efficient in slow sand filtration than in rapid sand filtration (Logsdon et al., 1991).

The model for estimating particle deposition by physical removal processes has been proposed by Yao et al. (1971). In this model a filter bed is seen as an assembly of collectors (sand grains) with which particles come in contact by interception, sedimentation, or diffusion (Yao et al., 1971). The equations for single collector efficiencies, that is a fraction of particles that will strike the collector by a given transport mechanism, are presented below:

$$\eta_I = \frac{3}{2}\left(\frac{d_p}{d_c}\right)^2 \qquad \eta_S = \frac{(\rho_p - \rho_w) g d_p^2}{18\mu V} \qquad \eta_D = 0.9\left(\frac{KT}{\mu d_p d_c V}\right)^{2/3}$$

Where

η_I – the single collector efficiency for interception

d_p - the particle diameter

d_c – the collector diameter

η_s – the single collector efficiency for sedimentation

ρ_p – particle mass density

ρ_w – water mass density

g- gravity acceleration constant

μ – absolute viscosity

V- approach velocity

η_D – single collector efficiency for diffusion

T- absolute temperature

K – Boltzmann's constant

Logsdon (1991) has noticed that in a slow sand filter, transport mechanisms will be much more efficient than in a rapid sand filter. This is because for each transport process the collector efficiency is higher in slow sand filtration than in rapid sand filtration. The sand grain size in a slow sand filter is ½ of the sand grain size in a rapid sand filter; the approach velocity is 100 times smaller for a slow sand filter; the number of collectors in a slow sand filter is roughly double that of a rapid sand filter (Logsdon et al, 1991). Based on those observations Logsdon (1991) had estimated that the single collector efficiency in slow sand filtration is higher than in rapid sand filtration by approximately 200, 34, and 4 times for sedimentation, diffusion and interception, respectively. The sedimentation appears to be the most important transport mechanism. Huisman & Wood (1974) compared pores between sand grains in a slow sand filter to millions of minute sedimentation basins where water suddenly slows down and particles settle onto the nearest sand grain surface.

2.3.3 Attachment mechanisms

It still has not been clearly explained why negatively charged particles become attached to negatively charged sand grains in slow sand filters. We know that for rapid sand filtration to function properly coagulants needs to be added in order to reverse the negative charge of the particles. Kemna said in 1899 (cited by Logsdon (1991)):

> "Why does the sand take the dirt out of water? It seems to me more normal that the water should take dirt away from the sand!"

A couple of different attachment mechanisms have been hypothesized by researchers.

Charge reversal

Huisman & Wood (1974) suggested that due to a negative charge, sand can attract flocculi of iron, iron and aluminum hydroxide, crystal of carbonates, as well as cations of iron, manganese, aluminum and other metals. They further hypothesized that there is "a reversal of the sand surface charge by the attachment of positive particles and the subsequent re-reversal by the attachment of negative particles, with a series of reversals throughout the filter cycle" (Logsdon et al., 1991).

Development of positively charged sections in the schmutzdecke

Other researchers have suggested that the accumulation of microorganisms/their metabolic products (Edwards & Monke, 1967) or "detritus of dead microorganisms in various stages of decomposition" cause development of positively charged sections near the surface of the sand (Jorden, 1963).

Increased collision efficiency

Weber-Shirk (1997) indicated that the previously removed particles modify the filter medium surfaces and thus increase attachment efficiency. Increase in the rate of collisions due to particle deposition within a filter bed was suggested by O'Melia et al. (1978).

Cations in the solution may act as bridges

Jorden (1963) suggested that polyvalent cations in the solution may act as "bondings" between negatively charged particles and negatively charged sand grains.

Flocculation

Bacteria are capable of producing adhesive extracellular polymers that form links between the cell and the solid surface (Stevik et al., 2004; Weber-Shirk & Dick, 1997a). Some researchers have indicated that those polymers are capable of flocculating both organic and inorganic stable colloidal dispersions (Pavoni et al., 1972) and organisms in activated sludge (Tchobanoglous et al., 1991). Therefore, it was hypothesized that extracellular polymers produced by microorganism in slow sand filters might cause destabilization of particles and their further enhanced attachment to the filter media (Logsdon et al., 1991). Flocculation might be enhanced by turbulent mixing that is produced while water flows downward through the filter column (Wotton, 2002) and/or by influent particles in the suspension (Weber-Shirk & Dick, 1997b).

Van der Waals forces

Van der Waals forces also should be mentioned. However, these are short range forces that will only be effective in holding the particle after it overcomes the electrostatic repulsion barrier and comes in contact with the surface of a sand grain (Huisman & Wood, 1974; Logsdon et al., 1991).

Chapter 3

MECHANISMS OF PATHOGEN REMOVAL IN INTERMITTENT SLOW SAND FILTRATION

3.1 INTRODUCTION

It has been almost two centuries since the first continuously operated slow sand filters were put to work. They have greatly improved the quality of life of people, as the number and severity of waterborne diseases has declined significantly ((Hazen, 1913).

Although still not fully understood, the water purification processes in slow sand filters are believed to be dependent on a biologically active layer that develops on the surface of the sand, called "the schmutzdecke". This a German word meaning "dirty layer", because it consists of a living biological zone growing at, and near, the sand/water interface. Various forms of microorganism and macrofauna live in this zone in order to capture and use the organic matter entering the filter. Bacteria and algae in the schmutzdecke produce sticky gelatinous material known as zoogloea (Huisman & Wood, 1974; Logsdon et al., 1991; Rideal, 1997). Slow sand filtration technology is capable of achieving a 2-log to 4-log removal of pathogens (Logsdon et al., 1991) without use of any coagulants or chemicals. Huisman & Wood in WHO publication "Slow sand filtration" (1974) stated:

> "No other single process can effect such an improvement in the physical, chemical, and bacteriological quality of normal surface waters as that accomplished by biological filtration…. Under suitable circumstances, slow sand filtration may be not only the cheapest and simplest but also the most efficient method of water treatment."

Therefore, it is not surprising that the interest in slow sand filtration was recently renewed. In early 1990s the traditional slow sand filter was redesigned, by Dr. Manz of University of Calgary, into a filtration unit that can be operated intermittently and is small enough to be used at home (CAWST, 2008). This technology is believed to be especially appropriate for developing countries due to the low cost and local availability of materials (sand, gravel and concrete) as well as the ease of construction and maintenance (CAWST, 2008). In many developing countries due to the lack of community water systems, this kind of technology might be the only alternative to drinking unsafe, contaminated water. Currently as many as 500,000 people worldwide rely on this filter, called the BioSand filter, to provide drinking water (Elliott et al., 2008).

The Biosand filter can not only significantly improve the look and taste of water but also greatly reduces the amount of pathogens and toxins (CAWST, 2008). The filter's ability to remove *Giardia* cysts and *Cryptosporidum* oocysts is unsurpassed as the filter was shown to be capable of achieving at least 4-log removal (>99.99%) of those parasites (Palmateer et al., 1999). However, it's been shown that intermittent operation of slow sand filters can lead to deterioration in water quality (Huisman & Wood, 1974; Logsdon et al., 2002; Martensson & Jabur, 2006; Petry-Hansen et al., 2006). Several laboratory and field studies show that the BioSand filter is not removing all pathogens. *E.coli* reduction in BioSand filters ranged from 90% to 99% for the filters tested in laboratory settings (Buzunis, 1995; Lee, 2001; Elliott et al., 2008; Donison, 2004; Stauber, 2007). In the same studies viruses were removed to a lesser extent which ranged from 70% to 87% (Elliott et al., 2008; Stauber, 2007). The performance of BioSand filters in removing *E.coli* tends to be even lower in field

studies. A few field studies have been conducted in the Dominican Republic, Ethiopia, and in Nepal. Average *E.coli* reductions in those studies were 80% (Stauber, 2007), 87.9% (Earwaker, 2007), and 93% (Stauber et al., 2006). However, it is important to note that the performance of the investigated filters varied largely, and while some filters provided 100% removal, others provided no or even negative removals of *E.coli* (Donison, 2004; Earwaker, 2007; Lee , 2001; Stauber, 2007; Stauber et al., 2006).

Therefore, since there cannot be any guarantee that water produced by a BioSand filter will be pathogen free, disinfection is recommended (CAWST, 2008). However, disinfection adds to an ongoing treatment cost, and will most likely lead to the formation of carcinogenic disinfection byproducts (DBSs) as slow sand filters are not capable of removing all dissolved organic matter (Amy et al., 2006; CAWST, 2008; Logsdon, 1991; Logsdon et al., 2002).

Due to the high popularity of BioSand filters in developing countries, their inconsistent performance in the field, and the risk associated with post-disinfection it becomes increasingly important to determine factors that affect pathogen removal in those filters. The mechanisms of microorganism removal in the schmutzdecke are still not fully explained. Unger & Collins (2008) stated:

> "Unfortunately, the exact role this interface layer, or schmutzdecke, exerts in microbial removals in both slow sand filtration and river bank filtration systems has not been well defined. The development of a schmutzdecke has been shown to alter conditions within a filter such that the applicability of conventional filtration theory is questionable."

Additionally, the removal mechanisms in an intermittent slow sand filter, while believed to be similar to those in a traditional slow sand filter, might not be exactly the same. This is due to the fact that while a traditional slow sand filter is

operated continuously at a constant head and flow rate, the BioSand filter is operated intermittently at a head and a flow rate that vary during the filter run. Up to date, there has been very little research on the factors that affect the efficiency of intermittent slow sand filters:

> "…there has been almost no systematic, process engineering research to substantiate the effectiveness of the BioSand filter or to optimize its design and operation."(Elliott et al., 2008)

> "Further research is definitely needed to widen the knowledge base concerning slow sand filtration. This especially applies to the relatively recently developed intermittent household slow sand filter. Although the research done to date on the intermittent filter is very encouraging, there are still gaps in the knowledge base and more research needs to be carried out on both its technical and social aspects." (*Biosandfilter.org.*)

The understanding of mechanisms governing an intermittent slow sand filter is essential to successfully influence its performance.

The aim of this study was "filling the gaps" in the basic understanding of removal processes in an intermittent slow sand filter and studying the factors that might affect its performance.

It was suggested before that biological and physical processes work in tandem in slow sand filtration (Huisman & Wood, 1974). No research, however, was done to assess the importance of biological versus physical removal processes. In the present study an attempt was made to separate those two groups of processes. The hypothesis was made that most pathogen removal will occur in the "biological" part of filtration. Other researchers indicated the importance of residence time in the reduction of pathogen numbers (Elliott et al., 2008; Stauber, 2007; Unger & Collins, 2008). The importance of filtration, i.e., interception versus pause period,

i.e., predation, sedimentation, and diffusion in removal of different types of microorganisms was also investigated.

It was hypothesized by other researchers that due to a negative charge sand can attract flocculi of iron, iron and aluminum hydroxide, crystals of carbonates, as well as cations of iron, manganese, aluminum and other metals (Huisman & Wood, 1974), and that microorganisms/their metabolic products (Edwards & Monke, 1967) or "detritus of dead microorganisms in various stages of decomposition" cause development of positively charged sections near the surface of the sand (Jorden, 1963). Although it seems inevitable to determine the composition and charge of the schmutzdecke in order to understand its performance, up to date no research was done on the former and only limited research was done on the latter. The composition and charge of the schmutzdecke were investigated in the present study utilizing EDX analysis and zeta – potential measurements.

It was hypothesized that bacteria living in a slow sand filter may produce extracellular polymer capable of particles destabilization and their further enhanced attachment to the filter media (Logsdon et al., 1991). This hypothesis was evaluated in the current study by performing flocculation experiment with the material derived from the schmutzdecke.

Increase in the rate of collisions due to deposition of particles within a filter bed was previously suggested by O'Melia et al. (1978). The last hypothesis that was evaluated in this study was increased rate of collisions due to intricate schmutzdecke "architecture" formed by previously removed particles and biological growth. In order to evaluate this hypothesis the schmutzdecke structure was disturbed and the effect that this action had on microbial reduction evaluated.

3.2 MATERIALS AND METHODS

3.2.1 Sand and gravel

The sand that was used in the experiments had an effective size of 0.18 mm and a uniformity coefficient of 1.61. The sand was delivered by Unimin Corporation from their plant in Pevely, MO. The chemical analysis of the sand, mean percentage by weight, was as follows:

Silicon Dioxide	99.83%
Iron oxide	0.02%
Aluminium Oxide	0.04%
Calcium Oxide	0.03%
Titanium Oxide	0.01%
Magnesium Oxide	0.01%
Potassium Oxide	0.01%
Loss on ignition	0.05%

Some white play sand was also bought at a Home Depot supply store. Particle size and chemical analysis information were not provided. The sand was sieved to obtain sand grains larger than the sand grains used as filter media. A small amount of this sand was used below the actual sand media and above the gravel, to prevent fine sand from escaping the filter.

Gravel, in two different sizes, was obtained from Ricci Sand company. Particle size and chemical analysis information were not provided. The gravel was used as an underdrain.

3.2.3 Water quality

White Clay Creek water amended with 2.5%-0% of autoclaved for 20 minutes at 121°C primary effluent was used as feed water for the filters. Originally, the primary effluent from a wastewater treatment plant was added in order "to simulate the presence of wastewater" in typical source water in developing countries and to speed up the ripening of the filter (Elliott et al., 2008). Chemical parameters in the feed water were measured on a weekly basis over the time of the study. It was determined that there was significant variability in the level of each of the studied parameters. The addition of primary effluent did not affect any of the parameters to a significant extent, or at least no pattern could be discerned. It was shown that there was no correlation between the amount of primary effluent and any of the parameter concentrations (Figure A.2 – A.5 in Appendix A). Therefore, further addition of primary effluent to filter feed water was ceased. The average, minimum and maximum values for each studied parameter are presented in Table 3.1. This analysis includes all feed water data, ranging from 2.5% to 0% of primary effluent content.

Table 3.1 Chemical quality of feed water

	Average	Minimum	Maximum
Fe (total) (mg/L)	0.18	0.05	0.39
TSS (mg/L)	5.5	3	17.5
pH	7.2	6.7	7.9
COD (mg/l)	11.72	6	21.5

The creek water was collected from the raw water tap at the Newark Water Treatment Plant at weekly intervals. The water was stored in plastic containers at room temperature until used. In cases of an increase in water turbidity, caused by a rain event, the plant operators switch to water from the Newark Reservoir, which is also taken at the same tap. The Newark Reservoir stores water that has previously been drawn from White Clay Creek. The primary effluent was collected at Wilmington Wastewater Treatment Plant biweekly and stored at 4°C until used.

The total iron was measured using FerroVer reagent obtained from the Hach company; the COD was determined using low range COD digestion vials obtained from the Hach company; the total suspended solids were measured with a spectrophotometer DR 5000; the pH was measured with a AR25 Dual Channel pH/Ion meter.

3.2.3 Experimental challenge microorganisms and their enumeration

Two surrogates of human pathogens were used: MS-2 (ATCC 15597B1) and *E.coli* (ATCC 25922). MS-2 was used as a surrogate for human enteric viruses due to its similarity to many enteric viruses, low attachment and survival rate (Dullemont et al., 2006; Hijnen et al., 2004; You, et al., 2005). *E. coli* is a good indicator of fecal contamination in drinking water (Tallon et al., 2005). Tests were performed to ensure that *E.coli* (ATCC 25922) cannot serve as a host for MS-2.

Prior to enumeration all samples were serially diluted ten-fold in buffered dilution water containing magnesium chloride and potassium dihydrogen phosphate. The dilution water concentrate was obtained from the Hach company.

MS-2 was grown in a 4-hour host culture overnight. Next the stock was centrifuged at 3,5000 x g for 10 min causing the host cells to settle. The supernatant

was decanted and filtered through a 0.22 μm syringe driven filter unit to remove the remaining bacterial cells. The supernatant was then subjected to ultrafiltration using Millipore's Amicon Ultra-15 centrifugal devices as described by the company manual. The sample was placed in the Amicon tube and centrifuged at 5,000 x g for 30 minutes in order to remove the soy broth and concentrate the virus stock. The concentrated stock was rediluted in buffered dilution water. Since the ultrafiltration devices are not sterile, the sample was once again filtered through 0.22 μm fitler unit. The stock was stored at 4°C. The virus stock was taken out from 4°C temperature and allowed to reach room temperature before being used in an experiment.

MS-2 was enumerated with the single agar layer method using its *E.coli* host (ATCC 15597) according to EPA Method 1602 (EPA, 2001). The host was grown in 25 ml of Tryptic Soy Broth for 18 hours. A 0.5 ml volume of this overnight culture was transferred to 40 ml of Tryptic Soy Broth and grown for 4 hours to log phase (OD 0.36-0.44) (EPA, 2001). MS-2 samples were enumerated by pouring 0.1 ml of the sample and 0.5 ml of the host culture into a glass tube containing 0.7% Tryptic Soy Agar. This mixture was then poured onto 1.5% Tryptic Soy Agar plates. When the plates solidified, after approximately 15 minutes, they were incubated at 37°C overnight. Plaques that formed overnight were enumerated and results reported as PFU/ml. One sample was assayed multiple times and the number of repeats depended on an experiment. Based on the plaques from all the plates, mean and confidence intervals were calculated.

A new culture of *E.coli* was grown before each experiment. One colony was picked from a streak plate with a sterile loop and transferred to a tube containing 40 ml of Typtic Soy Broth. The tube was incubated at 37°C for 18 hours. The culture

was centrifuged at 3,5000 x g for 10 min causing *E.coli* cells to settle. The supernatant was discarded. The cells were rediluted with buffered dilution water, enumerated and stored at 4°C until the experiment. The culture was taken out from 4°C temperature and allowed to reach room temperature before being used in an experiment.

Samples containing *E.coli* were analyzed by either the spread plate method using Violet Red Bile Agar with MUG media (the last experiment only) or by the multiple-tube fermentation method using A-1 media. For both techniques, procedures described in "Standard Methods For the Examination of Water and Wastewater" and Difco Manual were followed (Difco, 1998; Greenberg et al., 1985). When using the spread plate method colonies were counted after overnight incubation and expressed as CFU/ml. Based on the number of colonies from all of the plates, mean and confidence intervals were calculated. The multiple-tube fermentation method was used to determine the Most Probable Number (MPN) with 95% confidence intervals. MPNs were calculated in an Excel spreadsheet that uses algorithms described by Haas et al. (1999). The method assumes a Poisson distribution (random dispersion) of bacteria in a sample and employs the principle of maximum likelihood in the estimation of the mean (Haas et al., 1999):

> "The best estimate of a set of parameters from a set of data is obtained by maximizing the probability that the particular sample would have been obtained."

In this case the sample is a number of positive versus negative tubes in each dilution.

The estimation of the mean is based on certain probability formulas (trial and error by minimizing negative log-likelihood). The goodness of the fit to Poisson distribution is determined by the index of dispersion test D^2. The test for significant

difference between samples can also be performed, based on a set of *p* values (the proportion of area under the chi-squared distribution less than or equal to the compounded D^2 values). An example spreadsheet output is presented in Figure A.1 (Appendix A).

All statistical tests performed in this study were two-sided in nature.

3.2.4 Experimental setup

Sand was washed with deionized DI water until it was no longer visibly dirty, then it was dried in an oven at 80°C for 3 – 4 days. The column was then packed under saturated conditions, by pouring the sand in increments while stirring with a plexiglass rod to remove any attached air bubbles. As pathogen removal in the slow sand filtration is attributed to biological activity in the schmutzdecke the filters were made shallow, except for the first experiment.

The filter porosity was determined either by a tracer test (the first experiment only) or based on calculations taking into account the empty column weight, the dry media weight, the packed column weight and the volume of the media filling the column. The tracer test was run using sodium chloride. The conductivity of the effluent water was measured with a pocket conductivity meter and the concentration of sodium chloride was calculated based on the previously prepared calibration curve.

Filters were ripened by dosing creek water intermittently, on a daily basis. The peristaltic pump was turned on by timer three times daily, for one hour each time, to provide water from the raw water reservoir onto the filter diffuser. Two pore volumes of the BioSand filter is typical of a higher range of a daily family water demand in developing countries (Elliott et al., 2008). In those experiments more than

two pore volumes were run through the filter daily in order to speed up the ripening process. The challenge experiments were conducted by feeding the water to the filters continuously at a constant rate using a peristaltic pump. The flow rate was kept constant by keeping the head at a constant level.

3.2.4.1 Contribution of biological versus physical processes in pathogen removal

The purpose of this experiment was to estimate the contribution of biological versus physical processes in pathogen removal. In order to do that, an attempt to separate biological and physical removal processes was made. Two columns were packed. The first one was shallow and consisted of approximately 4.5 cm of gravel and separation media, followed by 6 cm of sand. The area of the column was 638 cm^2. The pore volume of the column was estimated at 4,090 ml (2,500 ml in the media and 1590 ml in the supernatant water). This column was ripened with creek water spiked with 2.5% primary effluent for 14 days at a velocity of 0.15-0.12 m/h. The second column was packed with 10 cm of gravel and separation media, followed by 47 cm of sand. The area of the column was 123 cm^2. The pore volume of the column was estimated at 3,414 ml (2,800 ml in the media and 614 ml in the supernatant water). The column had seven sampling ports at different heights. The first five sampling ports were 5 cm apart from each other, the next two 10 cm apart from each other. The sampling ports were designed in such a way that allowed collection of samples through syringe driven needles. This column was not ripened with creek water and was only flushed with DI water daily. The whole experimental setup, with the sampling points' locations, is presented in Figure 3.1.

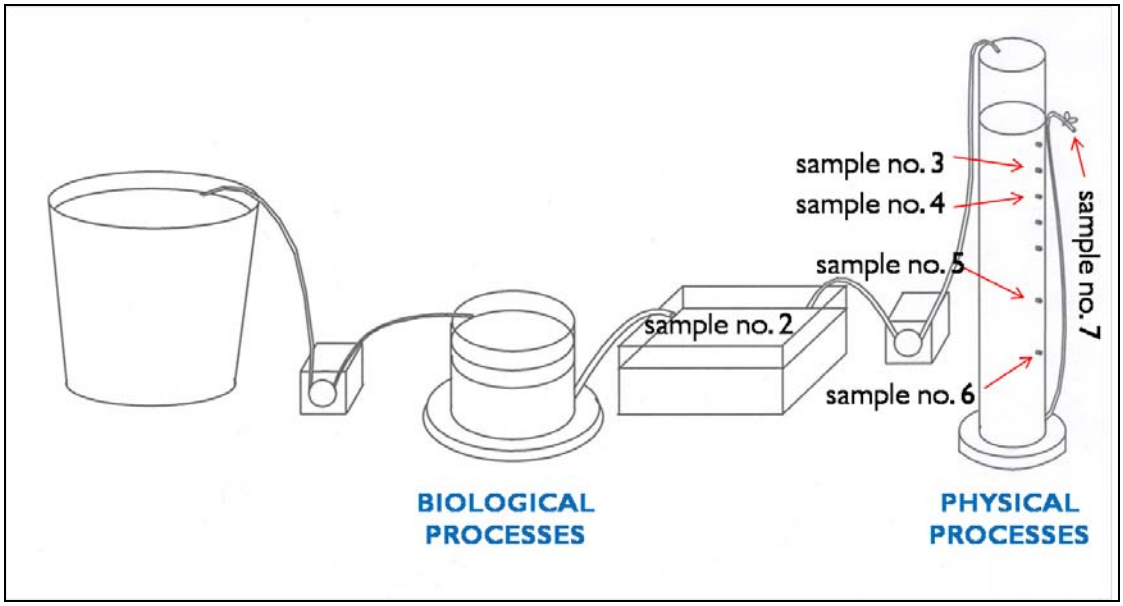


Figure 3.1 Contribution of biological versus physical processes to pathogen removal in intermittent slow sand filtration (experimental setup).

For the challenge experiment the creek water was spiked with 2% of primary effluent and with *E. coli* and MS-2 to an expected concentration of 10^6/ml and 10^4/ml, respectively.

At the beginning 4.15 liters of the spiked creek water were charged into the first ("biological") column in order to increase hydraulic head. Next, 10.5 liters of the spiked creek water were run through the column at a velocity of 0.37 m/h. The first 3.1 liters of the effluent were not captured; the next 7.4 liters of the effluent were captured for analysis and to be used as the feed water to the second column. Following that, 6 liters of effluent from the first column were run through the second column at a velocity of 0.37 m/h. The first 3.5 liters of the effluent were not captured; the next 2.5 liters were captured and a sample for microbial analysis was collected from that

volume. Right after that, samples from four different heights in the sand column were collected (12 cm, 17 cm, 37cm, 47 cm below the sand surface).

There was a control sample to take into account natural die off/increase of *E. coli* and MS-2. Some volume of the schmutzdecke influent was left in the open to the atmosphere beaker. Two samples from that beaker were collected, the first one after filtering 10.5 liters through the first column and the second one after filtering 6 liters through the second column.

For MS-2 enumeration one sample was assayed two times.

For *E.coli* enumeration the multiple tube fermentation method was used utilizing 6 dilutions, 3 tubes per dilution.

It is important to note that only 0.76 of pore volume (1.24 of media pore volume) was run through the first column before collecting the composite sample that was used as an influent to the second column. Since it was suspected that not enough pore volumes could have been run and that the collected composite sample could have been diluted, before estimating the removal efficiency, the *E.coli* /MS-2 concentration in sample no. 1 was recalculated, taking into account the dilution. Before the experiment 4.15 liters of the spiked creek water were charged to the filter that already contained 1590 ml of the supernatant water above the sand surface. The initial concentration was therefore recalculated according to the equation:

(concentration in sample no. 1 x 4150 ml)/(4150 ml + 1590 ml)

This value was an estimation of the *E.coli*/MS-2 concentration in the supernatant water after charging the filter. Although water with the higher concentration was run through the filter afterward and this approach underestimated the removal efficiency it was considered conservative and therefore applied.

3.2.4.2 Effect of residence time on *E.coli* and MS-2 removal

The purpose of the experiments described in this section was to study the effect of residence time in the biologically active layer of an intermittent slow sand filter on *E.coli* and MS-2 removal. A column was packed with approximately 4.5 cm of gravel and separation media, and 2.0 cm of fine sand. The column had a sampling port, located 2 cm below the sand surface, which allowed the collection of the sample through a syringe driven needle. The porosity was estimated at 0.35. The total pore volume was estimated at 520 ml (235 ml in media and 285 in supernatant water). The area of the column was 100 cm^2. The column was ripened with creek water spiked with 1.5% primary effluent. Three experiments were run on this column. In all of the experiments the creek water was spiked with 1% of primary effluent; the concentrations of *E.coli* and MS-2 varied. It was 10^5/ml of *E.coli* and 10^3/ml of MS-2, 10^5/ml of *E.coli* and 10^3/ml of MS-2, 10^3/ml of *E.coli* and 10^2/ml of MS-2 for the first, the second and the third experiment, respectively.

The experimental procedure was to run a certain volume of spiked creek water through the column and then collect a series of effluent samples through a sampling port at different time intervals. The first sample was collected immediately after the spiked creek water was filtered through the column; the following samples were collected after 1 hour and 3.5 hours. Before collecting the second and the third sample approximately 50 ml was drained from the column, for consistency purposes and to make sure that the collected effluent is in fact from the bioactive sand layer. In the first (unripened column) and the second experiments, 3 pore volumes of spiked creek water were run through the filter at a velocity of 0.44 m/h before collecting the samples. In the third experiment 1.5 pore volumes (here 1 pore volume is a pore volume of media only) of spiked creek water were run through the column at

a velocity of 0.29 m/h before collecting the samples. In the third experiment supernatant water in the filter was spiked with *E.coli* and MS-2 to approximately the same concentration as in the spiked creek water.

Following the first experiment the column continued to be ripened for 12 days at 0.44-0.39 m/h. The second challenge experiment was run and after that the filter was cleaned by repeatedly raking and swirling the top 0.5 cm of sand by hand. This caused detachment of pieces of the schmutzdecke that started floating in the supernatant water. The supernatant water was gradually being poured out and replaced with fresh creek water. The procedure continued until no visible schmutzdecke was left on the sand surface. The filter was ripened again at 0.44-0.3 m/h velocity for 16 days and the third experiment was run.

For MS-2 enumeration one sample was assayed three times in the first and the second experiment and seven times in the third experiment.

For *E.coli* enumeration the multiple tube fermentation method was used utilizing 6 dilutions and 3 tubes per dilution in the first and second experiment, and 5 dilutions and 6 tubes per dilution in the third experiment.

3.2.4.3 Components and properties of the schmutzdecke material

The schmutzdecke was grown on a sand media (the filter packed in the same way as in the effect of residence time experiments) with creek water (or creek water spiked with primary effluent) until the time when the headloss was significant and visual examination indicated abundant macrofauna. The filter was then cleaned by raking and swirling the top 0.5 cm of sand by hand. This caused a detachment of pieces of the schmutzdecke which started floating in the supernatant water. The supernatant water was collected into a glass beaker and pieces of

the schmutzdecke flocculated and settled. The supernatant water was decanted and the flocculated material was used in the experiments.

3.2.4.3.1 Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis

The material from the schmutzdecke was analyzed using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDX). The first method provided high resolution pictures of the schmutzdecke material. The second method provided information on the elemental composition of the material.

SEM and EDX analyses were performed on two different samples. The first sample was from the schmutzdecke that was grown for 23 days with creek water spiked with 1.5% of primary effluent. The second sample was from the schmutzdecke that was grown for 20 days with creek water. The creek water was spiked with 1.5% of primary effluent only for the first five days.

Samples were mounted with Silver Conductive Adhesive 18DB70X to aluminum stubs and allowed to air dry. They were then coated with a thin layer of gold palladium in a Denton Vacuum Bench Top Turbo III. Imaging and analysis was done on a Field-Emission Scanning Electron Microscope, Hitachi S-4700. Samples were imaged at 20.0 kV, with a 12 mm working distance using the medium electron detector. For the elemental analysis an Oxford x-Act detector was used.

3.2.4.3.2 Zeta potential analysis

The schmutzdecke material was introduced into creek water that was previously filtered through a non-sterile General Filtration 0.2 μm Membrane MCE. The solution was hand shaken to disperse the schmutzdecke material. As a result the material broke into fine particles of which the zeta-potential was estimated by

particle electrophoresis using a Zeta-Meter 80. In this procedure, the applied voltage causes electrically charged particles to move toward the electrode that has an opposite charge. The velocity with which particles move is proportional to their charge. The velocity of individual particles was measured with a timer as the particles moved along a microscope grid from the start to finish lines. This procedure is not automated, and the instrument operator can select specific particle types to characterize microscopically. Four categories of particles were distinguished in dispersed schmutzdecke material: floc particles >160 μm in diameter, floc particles 160 – 80 μm in diameter, small floc particles 80 – 40 μm in diameter, elongated particles with different lengths, believed to be diatoms which were very numerous in the material characterized by SEM. The velocities of twenty particles from each category were measured. The average velocity for each category was determined and the zeta potential calculated based on the Helmholtz-Smoluchowski equation. Since the particles flocculated during the measurement, the electrophoresis cell containing the analyzed sample was shaken approximately every one minute.

The zeta potential measurements were performed on two different samples. The first sample was from the schmutzdecke that was grown for 21 days with creek water. The second sample was the schmutzdecke that was grown for 16 days with creek water.

3.2.4.3.3 Assessing flocculating properties of the schmutzdecke material

It was observed that after the filter cleaning when the “dirty” supernatant water was poured out, the particles that were suspended in it flocculated and dropped out of the solution very fast. It looked like flocs were “sweeping out” all

the impunities from the water since the visual examination implied that the solution was very clean after all the flocs settled (Figure 3.2).

Therefore, the material from schmutzdecke was tested to determine if it is capable of flocculating either *E.coli* or MS-2. The schmutzdecke was grown for 22 days on a sand media and the material for analysis was harvested as described above. The suspension with an expected concentration - 10^3/ml of *E. coli* and 10^2/ml of MS-2 was prepared by diluting *E.coli* culture and MS-2 stock in creek water. A 20 ml volume of this solution was poured into four identical autoclaved glass vials, each containing a magnetic stirrer. A 1 ml aliquot of the floc was introduced into two of those vials; a 1 ml aliquot of the supernatant was introduced into the remaining two vials (Figure 3.3). Here "supernatant" refers to the water recovered above the floc that settled from the water poured out after a filter cleaning. The vials were shaken vigorously for approximately 30 seconds. Next the contents of the vials were mixed gently with magnetic stirrers for 28 minutes. When the mixing was stopped, 40 minutes were allowed to let the flocs settle. The samples were then collected from all four vials, slightly below the water surface and with care taken to collect all at the same depth. *E.coli* and MS-2 concentrations in the samples were analyzed directly.

For MS-2 enumeration one sample was assayed five times.

For *E.coli* enumeration the multiple tube fermentation method was used utilizing 5 dilutions and 4 tubes per dilution.

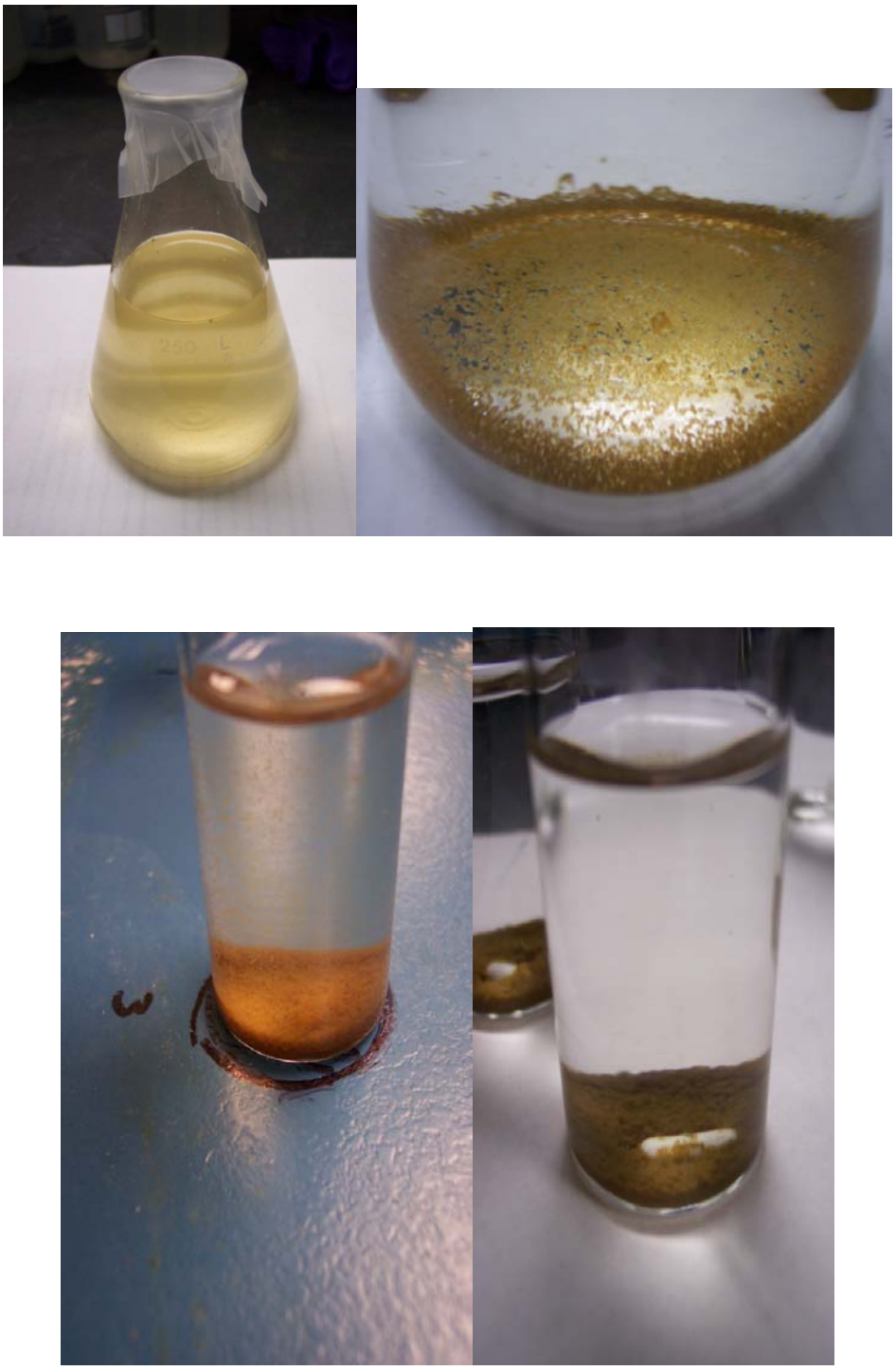
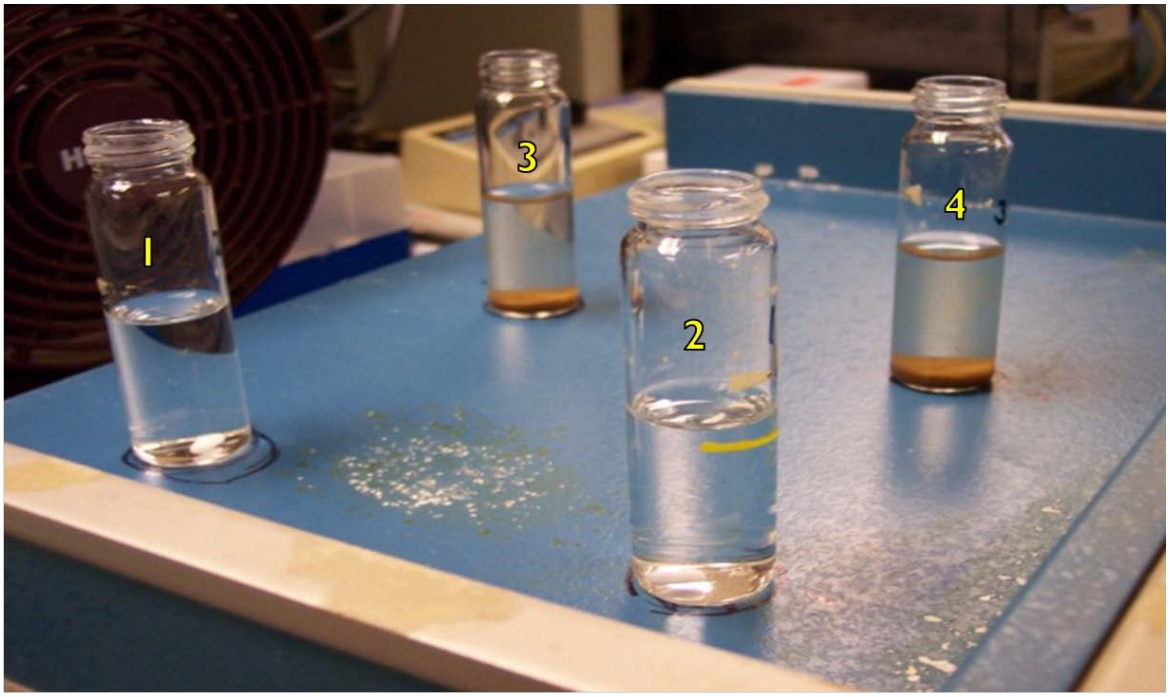


Figure 3.2 Particles flocculating in the supernatant water poured out from the filter that was just cleaned



Number 1 and 2 - 1 ml of the supernatant in 20 ml of creek water containing *E. coli* and MS-2

Number 3 and 4 -1 ml of the floc in 20 ml of creek water containing *E. coli* and MS-2

Figure 3.3 Assessing flocculating properties of the schmutzdecke material (experimental setup)

3.2.4.4 Importance of increased rate of collisions in the schmutzdecke

The purpose of this experiment was to determine if the intact structure of the schmutzdecke might be of importance in pathogen removal. The schmutzdecke was grown on a sand media for 14 days with creek water as described above. The filter was challenged with creek water spiked to an expected concentration of 10^2/ml of *E.coli* and 10^2/ml of MS-2. The experiment was run at a velocity of 0.13 m/h. The sample of effluent was collected after 1.1 pore volumes were filtered through the column. At the same time the first sample of the influent was collected. After collecting the samples the schmutzdecke (first 2 cm of sand) was disturbed by mixing it at 180 rpm for 1 minute using a Phipps & Bird Stirrer (model 7790-400).

A small sample of the schmutzdecke material was collected and examined under a microscope to determine the effect of mixing on the biological life. The protozoa, the main organisms of concern here, seemed to be unaffected as they were numerous and motile as usual.

Following the mixing couple of hours were allowed for the schmutzdecke particles suspended in the supernatant water to flocculate and settle. Then approximately 10 pore volumes of creek water were run through the filter. This was done in order to flush out residual MS-2 and *E.coli* and facilitate settling of dispersed schmutzdecke particles.

The filter was challenged with creek water spiked to an expected concentration of 10^2/ml of *E. coli* and 10^2/ml of MS-2 for a second time. The experiment was run at a velocity of 0.13 m/h (the same velocity as in the first part of the experiment). The sample of effluent was collected after 1.1 pore volumes were filtered through the column. At the same time the second sample of the influent was collected. The removal efficiencies prior and after the disturbance were compared in

order to determine if the intact structure of schmutzdecke is of importance in pathogen removal.

For MS-2 enumeration one sample was assayed five times.

For *E.coli* enumeration the multiple tube fermentation method was used utilizing four dilutions and four tubes per dilution.

3.3 RESULTS AND DISSCUSION

3.3.1 Contribution of biological versus physical processes to pathogen removal

The purpose of this experiment was to estimate the contribution of biological versus physical processes in pathogen removal. In order to do that, an attempt to separate biological and physical removal processes was made. Two columns were packed. The first one was shallow. This column was ripened with creek water spiked with 2.5% of primary effluent for 14 days. The second column had regular depth and sampling ports at different heights. This column was not ripened with creek water and was only flushed with DI water daily. After 14 days of ripening the first column was challenged with creek water spiked with *E.coli* and MS-2. The effluent collected from the first column contained remaining *E.coli* and MS-2 and was used to challenge the second column. The samples were collected from different heights in the column. Based on results removal due to biological versus physical processes was estimated.

3.3.1.1 Results

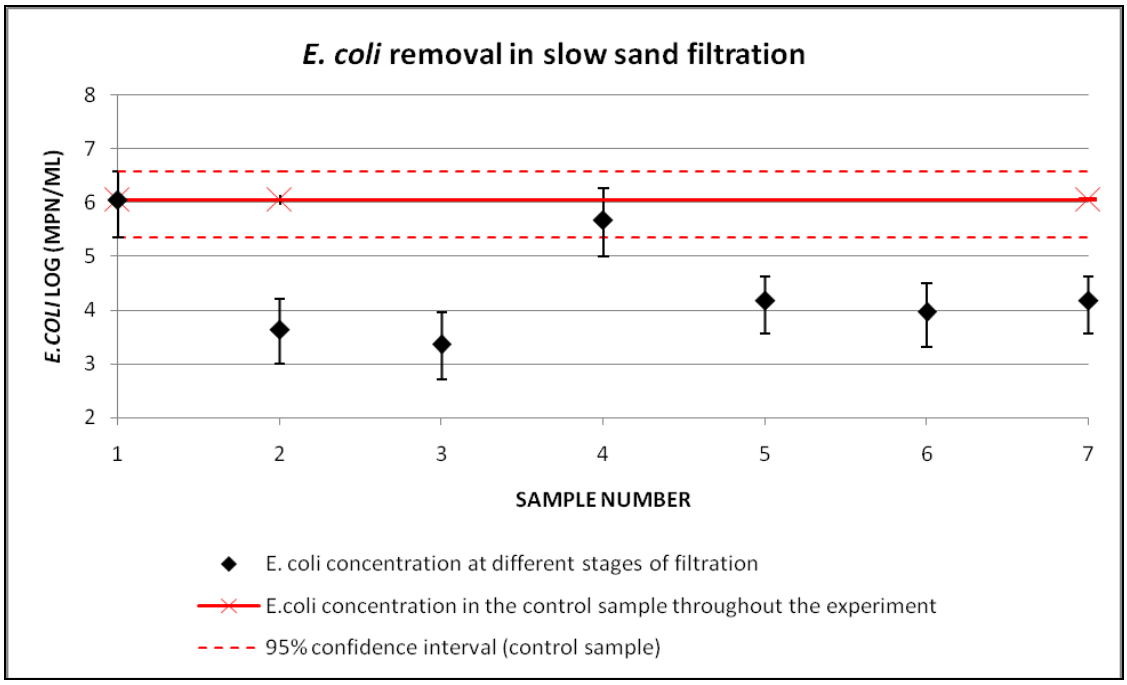


Figure 3.4 ***E.coli*** **removal due to biological versus physical processes in intermittent slow sand filtration**

In this experiment *E.coli* was removed in the first column only. The difference of means test on the sample no.1 and the sample no.2 showed that the samples were statistically different from each other ($p = 1.59*10^{-12}$). There was no removal in the second (unripened) column. The *p* values for the difference of means tests on sample pairs: 2 and 3, 2 and 4, 2 and 5, 2 and 6, 2 and 7, were 0.54, $6.54*10^{-10}$, 0.021, 0.17, and 0.021. *E.coli* concentration in sample no. 4 was much higher than the concentration in the other samples collected from that column. This sample clearly does not match the data set. This raises the question whether this

sample was contaminated during enumeration. The removal in the first column was estimated to be 99.5%. There was no removal of *E.coli* in the second column.

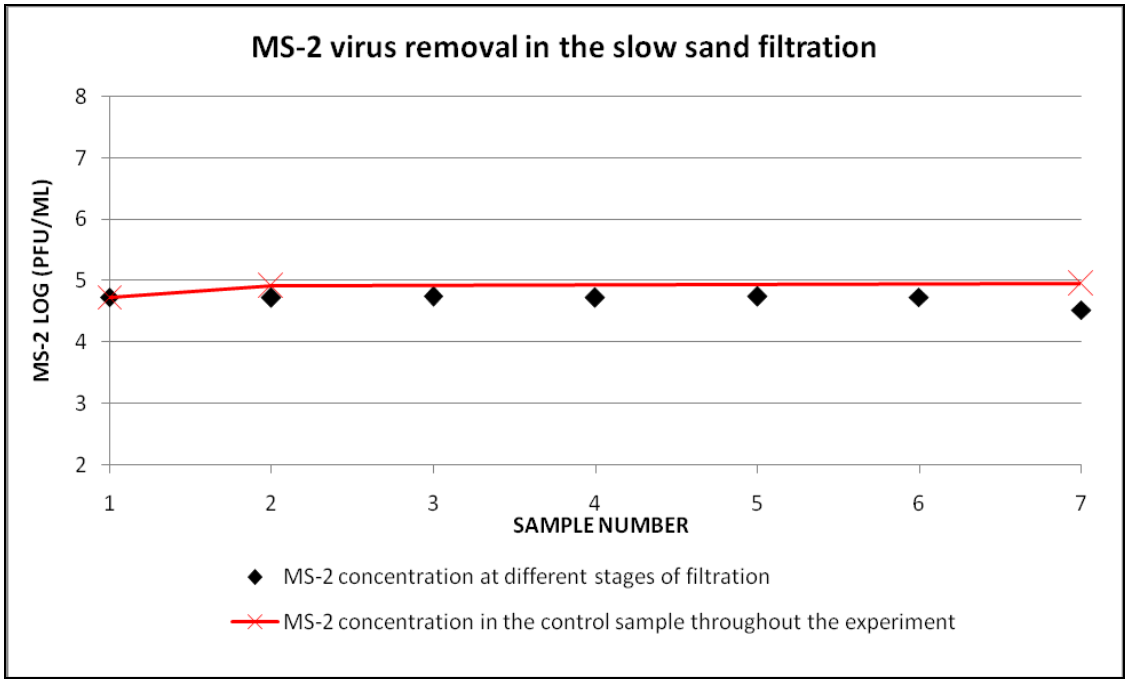


Figure 3.5 MS-2 removal due to biological versus physical processes in intermittent slow sand filtration

MS-2, on the other hand, was not removed to any significant extent in either of the columns. The removal in the first column was estimated to be up to 29% and in the second column 37%.

The conclusion was drawn that *E.coli* removal was due to biological processes attributable to the schmutzdecke development. MS-2 removal was not efficiently removed by either biological or physical processes.

3.3.1.2 Discussion

The results of this experiment are consistent with previous findings concerning bacteria and virus removal in the slow sand filtration. Research has shown that while bacteria are almost completely removed within the schmutzdecke, virus removal in slow sand filters is problematic. The first study on movement and fate of bacteria in slow sand filters was done by Burman & Lewin (1962) in England. The researchers observed that there was marked reduction in bacterial numbers at the first sampling point below the sand surface (13 mm – 76 mm depending on the sand level) with very little further improvement below the second sampling point (89 mm – 76 mm depending on the sand level) (Burman & Lewin, 1962). A pilot study in South Africa investigated fecal coliform removal with filter depth (Williams, 1987). It was shown that at 50 mm below the sand surface, the removal was 90% and at 200 mm it was 99.5% (Williams, 1987). More recent studies done by Unger & Collins (2008) confirmed the results of previous researchers. The results of this study showed that the majority of *E.coli* were entrapped in the schmutzdecke. However, authors have noticed that although the accumulation was much greater in the schmutzdecke a large number of *E.coli* reached the lower sections of the column or even escaped the column and appeared in the effluent (Unger & Collins, 2008).

Hijnen et al. (2004) have shown that filters with the schmutzdecke achieved 1-2 log greater removal of bacteria than the filters without the schmutzdecke, whereas the removal of schmutzdecke did not affect virus removal. Similar results were obtained by Dullemont et.al. (2006) who had shown that viruses are the most critical microorganism for the removal efficiency of slow sand filters. In their experiment viruses were removed the least (1.7 – 2.2 log) and were affected the least by the presence of the schmutzdecke (Dullemont et al., 2006).

An additional treatment needs to be implemented in order to obviate the problem of virus breaking through in slow sand filtration. Such a treatment should also prevent any occasional breakthrough of bacteria or other pathogen and make the filter safe during the initial period of filter ripening when the schmutzdecke is not yet developed. An additional treatment could be some sort of an amendment that would improve filter performance. GAC and zeolite amendments have been proposed before (Bauer et al., 1996; Collins et al., 1991; Manz, 2007; Manz, 2007). Mantz recommended the amendments be added as separate pieces of equipment that are used after filtration so that their maintenance does not interfere with normal operation of the BioSand filter (Manz, 2007).

3.3.2 Effect of residence time on *E.coli* and MS-2 removal

The purpose of the experiments described in this section was to study the effect of residence time in the biologically active layer of an intermittent slow sand filter on pathogen removal. A column was packed with a shallow layer of sand; it was equipped with sampling port located 2 cm below the sand surface. Three challenge experiments with creek water spiked with *E.coli* and MS-2 were run on the column. The first experiment was run on the unripened column. Following the first experiment the column was ripened for 12 days. The second challenge experiment was run and then the filter was cleaned. The filter was ripened again for 16 days and the third experiment was run.

3.3.1.1 Results

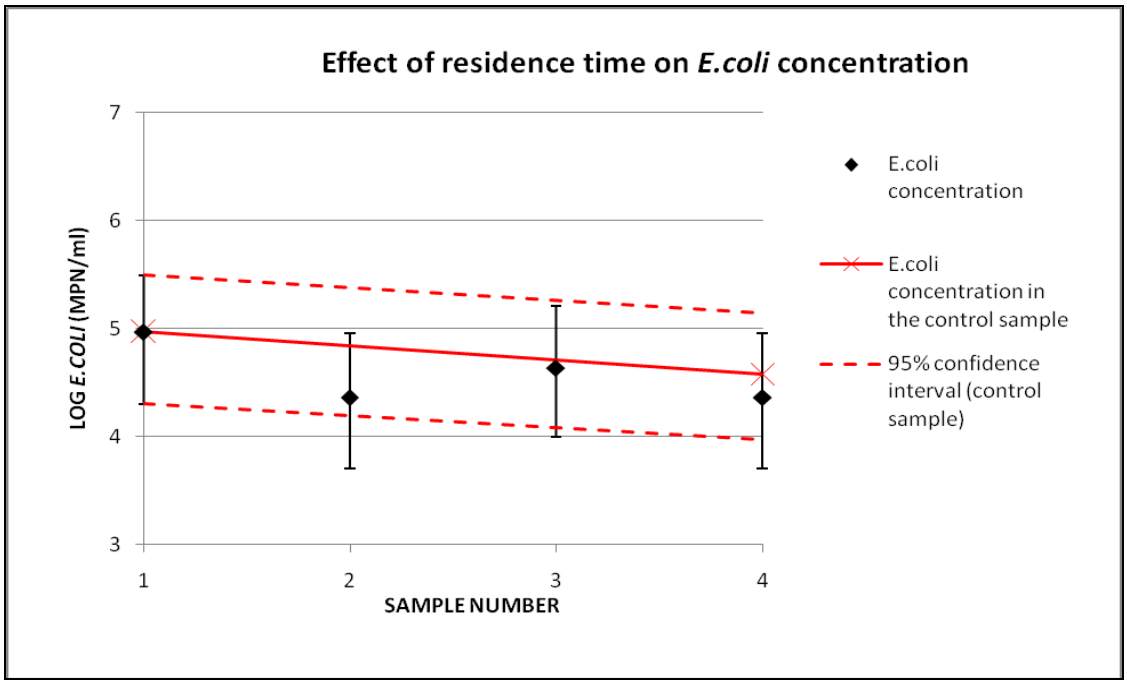


Figure 3.6 Effect of residence time on *E.coli* removal (first experiment)

On the graphs, sample no. 1 – influent to the column, sample no. 2 – effluent from the column at t = 0, sample no. 3 – effluent collected after 1 hour of residence time, sample no. 4 – effluent collected after 3.5 hours of residence time.

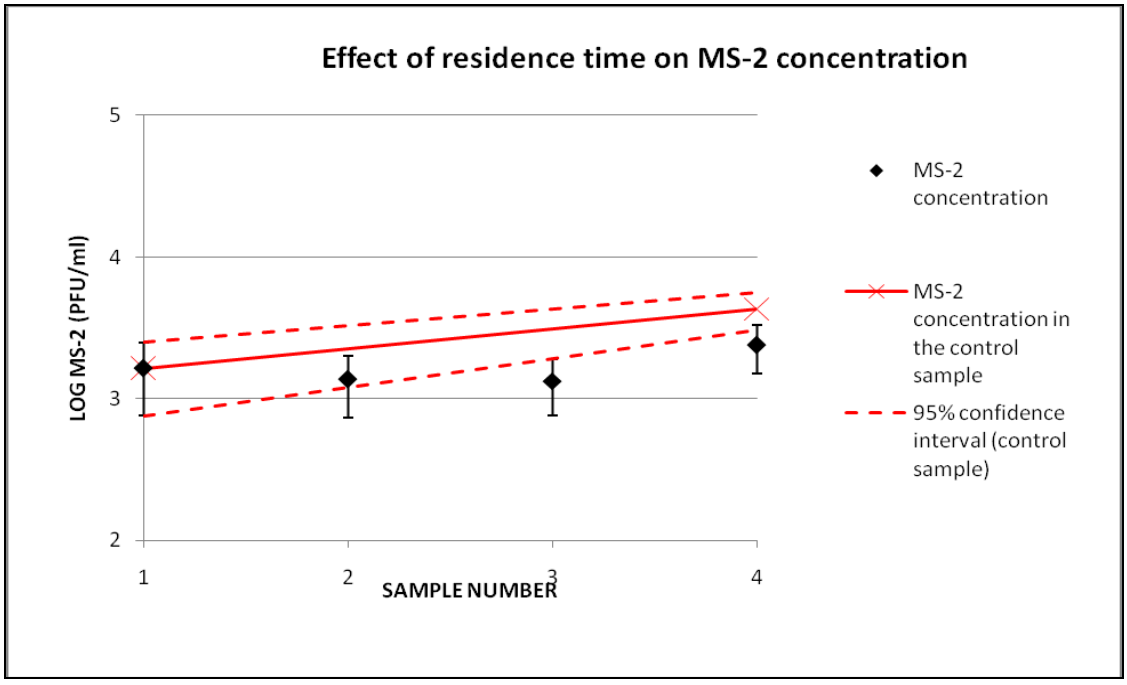


Figure 3.7 Effect of residence time on MS-2 removal (first experiment)

In the first experiment (unripened column) both *E.coli* and MS-2 were not significantly removed either during filtration or residence time.

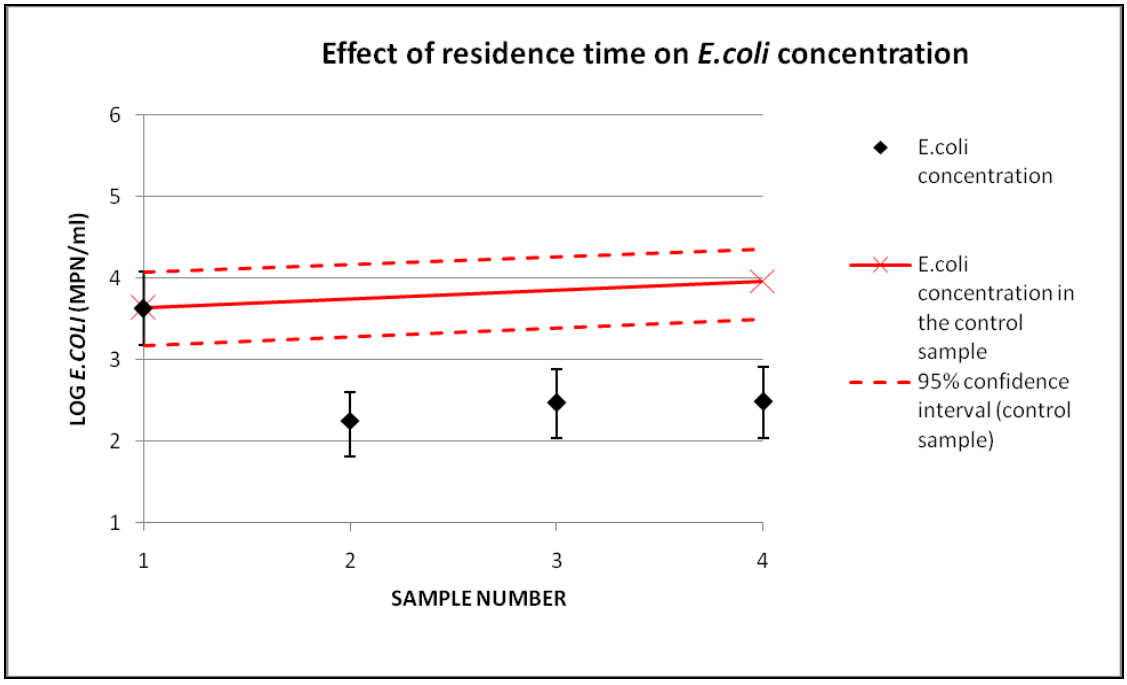


Figure 3.8 Effect of residence time on *E.coli* removal (third experiment)

In the third experiment (column ripened for 16 days) *E.coli* removal was only significant during filtration. The *p* value for a difference of means test between sample no. 1 and sample no. 2 was $4.48*10^{-19}$. The removal was estimated at 96%. The concentration of *E.coli* in sample no. 3 and sample no. 4 was higher than the concentration of *E.coli* in sample no. 2. Therefore, there was no removal of *E.coli* as a function of the residence time.

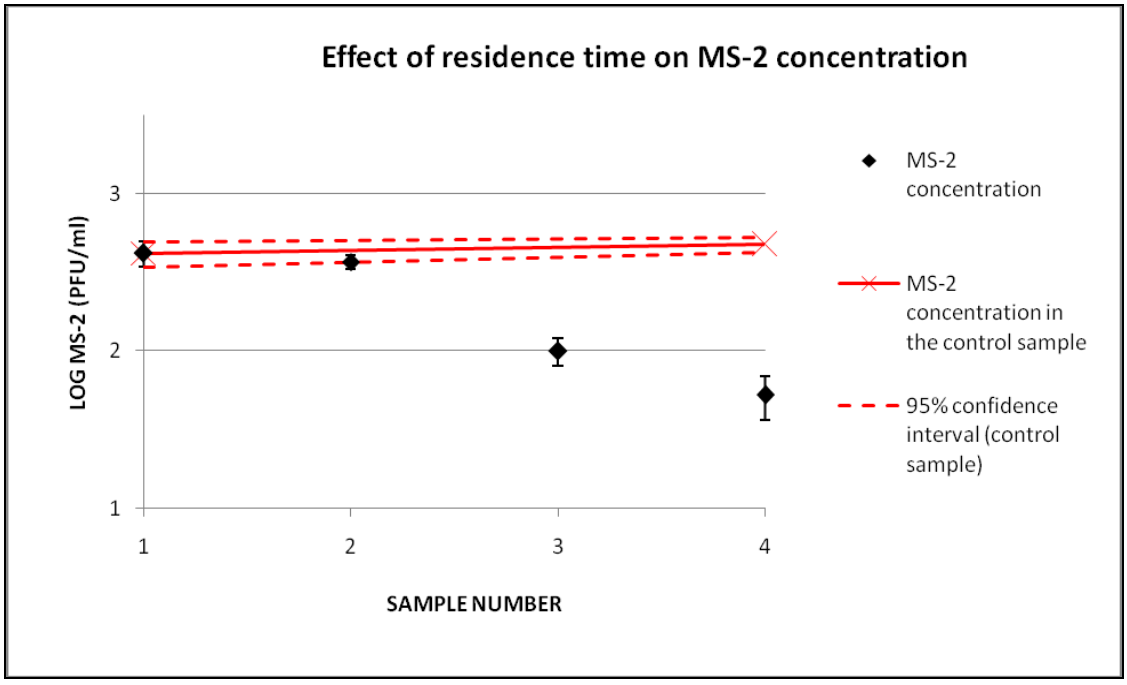


Figure 3.9 Effect of residence time on MS-2 removal (third experiment)

On the contrary, MS-2 removal was only significant during the residence time. The difference of means test between sample no. 1 and sample no. 2 showed that the samples were not significantly different from each other (p = 0.19). The difference of means test between sample no. 2 and sample no. 4 showed that the samples were statistically different from each other ($p = 8*10^{-8}$). The removal with the residence time was estimated at 86%.

Similar results were obtained in the second experiment, with *E.coli* being removed during filtration and MS-2 with residence time. *E.coli* removal during filtration was estimated at 94% (Figure A.6 in Appendix A). MS-2 removal during residence time was estimated at 75% (Figure A.7 in Appendix A).

3.3.2.2 Discussion

In this study the residence time contributed only to removal of MS-2 and did not affect the removal of *E.coli.* In fact, *E.coli* concentration seemed to slightly increase with residence time. These results are in contradiction to Elliot et al. (2008) and Stauber (2007) results. The cited studies showed that the residence time affected both bacteria (*E.coli*) and viruses (MS-2, PRD-1, echo 12). The importance of empty bed contact time on *E.coli* removal was also shown by Unger & Collins (2008). However, on the other hand, the Baumgartner et al. (2007) study of the effect of residence time on coliform removals was inconclusive. They found that while total coliform removal decreased with an increase in the sample collection volume, it was actually higher when the filter pause period was 12 hours versus 36 hours.

The reason for the discrepancies between the results of this study and the results of the studies mentioned above might be because the experiments described here were performed on a very shallow column, and thus only the biologically active section of an intermittent slow sand filter was studied. In the above mentioned studies the removal of bacteria with residence time could have been due to processes in the unripened portion of the column. It was shown that coliform bacteria can survive and even multiply in slow sand filters (Burman & Lewin, 1962; Martensson & Jabur, 2006; Petry-Hansen et al., 2006) The reason for their survival/multiplication is probably because the organic matter, that is a source of energy for bacteria, is abundant in the schmutzdecke/biologically active layer of the filter. On the other hand, in the lower sections of the filter, where food becomes scarce, the numbers of bacteria are likely to decrease.

In the light of recent studies results showing that one of the bacterial removal mechanisms in slow sand filters is predation by protozoa (Lloyd, 1996; Unger

& Collins, 2008; Weber-Shirk & Dick, 1997a; Weber-Shirk & Dick, 1999) the results of this study are somewhat surprising. If predation by protozoa were an important bacterial removal mechanism in this experiment *E.coli* would be removed with residence time. It might be hypothesized that the protozoa species that feed on *E.coli* were not present in the schmutzdecke of the filters used in this study. The creek water used for ripening the filters only occasionally (after a heavy rain event) contained *E.coli*; the concentration of *E.coli* rarely exceeded 20/ml. Such a low *E.coli* concentration could not be sufficient to sustain a population of protozoa grazing on *E.coli.*

It is interesting to note that *E.coli* was removed exclusively during filtration while MS-2 was removed almost exclusively with residence time. Clearly, two different mechanisms governed bacteria and virus removal in those experiments. The mechanism responsible for the bacterial removal appear to be dependant on water movement and mixing, while the mechanism responsible for virus removal, quite to the contrary, lacked those dependencies.

When James Simpson installed the first slow sand filter, he believed that water is purified because the filter acts as a very efficient strainer (Huisman & Wood, 1974). In those times the size of many colloidal particles was not known. Today we know that straining cannot be the only mechanism in slow sand filtration since the bacterial cells are much smaller than the pore sizes of the finest sand used in those filters (Huisman & Wood, 1974). However, even nowadays some researchers suggest that straining might be an important mechanism in bacteria removal (Stauber et al., 2006; Unger & Collins, 2008). Others state that this does not seem probable:

> "…filter cake void size is likely not significantly decreased by additional particle deposition... (Weber-Shirk & Dick, 1997b)"

However, it might be hypothesized that a mechanism that may *look* like straining is in fact an increased rate of collisions by interception during water filtration through the schmutzdecke. Polymer strands, fungal hyphae, particle deposition and biological growth in the schmutzdecke, could contribute to the increased rate of collisions. Rideal (1902) stated:

> "… diatoms and bacteria are normally surrounded by a gelatinous envelope…, so that in the short time the sand of a new filter becomes covered with a living slimy layer which entangles suspended matter and effects the main part of purification.."

The strands of slime were observed in SEM pictures of the schmutzdecke (Figure A.11, Figure A.15 in Appendix A). A "net" of fungal hyphae stretching between sand grains could be observed with a conventional microscope (Figure A.16 in Appendix A). Increase in the rate of collisions due to filter ripening was previously suggested by O'Melia et al. (1978).

On the other hand, for particles with a size of MS-2, sedimentation and interception do not play a significant role. It is believed that MS-2 removal with residence time was due to collisions caused by Brownian motions and subsequent attachment to sites with opposite or neutral charge. The development of the schmutzdecke could cause neutralization of negatively charged sand grains or even a development of positively charged sections (Edwards & Monke, 1967; Jorden, 1963). If this was the case viruses could be removed due adsorption caused by the electrostatic attraction (Logsdon et al., 1991).

3.3.3 Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis

The schmutzdecke was grown on a sand media with creek water (or creek water spiked with primary effluent) for 23 (sample no. 1) and 20 days (sample no. 2) and then harrowed. The harrowing caused a detachment of pieces of the schmutzdecke which started floating in the supernatant water. The supernatant water was then poured out and schmutzdecke particles flocculated and settled shortly thereafter. The floc was analyzed using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDX). The first method provided high resolution pictures of the schmutzdecke material. The second method provided information on the elemental composition of the material.

3.3.3.1 Results

Table 3.2 Elemental analysis of material from the schmutzdecke

	sample no. 1 (% weight)	sample no. 2 (% weight)
O	45.0	39.4
C	20.5	33.1
Si	13.7	11.4
Al	6.5	4.9
Fe	6.2	5.0
Ca	1.9	1.5
Mn	1.7	1.1
K	1.0	0.9
Mg	0.9	0.8
Cu	0.7	-
P	0.6	0.7
S	0.4	0.5
Cl	0.3	0.3
Ti	0.5	0.4
Na	0.3	-

The most abundant elements in the material from the schmutzdecke are oxygen and carbon. This is not surprising taking into account the deposition of organic matter, significant biological life and the accumulation of bacterial products and wastes. The third most abundant element was silica. This is not surprising either since very fine sand and clay particles could have been present in the material. Another reason for high silica content could have been diatoms which were very abundant in the material from the schmutzdecke (Figure 3.14 and Figures A.8 – A.15 in Appendix A). Also present, in addition to silica, were aluminum and iron, which

are clay constituents probably originating in creek water turbidity. They are some of the most abundant elements in the Earth's crust (*Iron oxides, Wikipedia (b)*). The other elements that contributed more than 1% to the material building up the schmutzdecke were calcium and manganese.

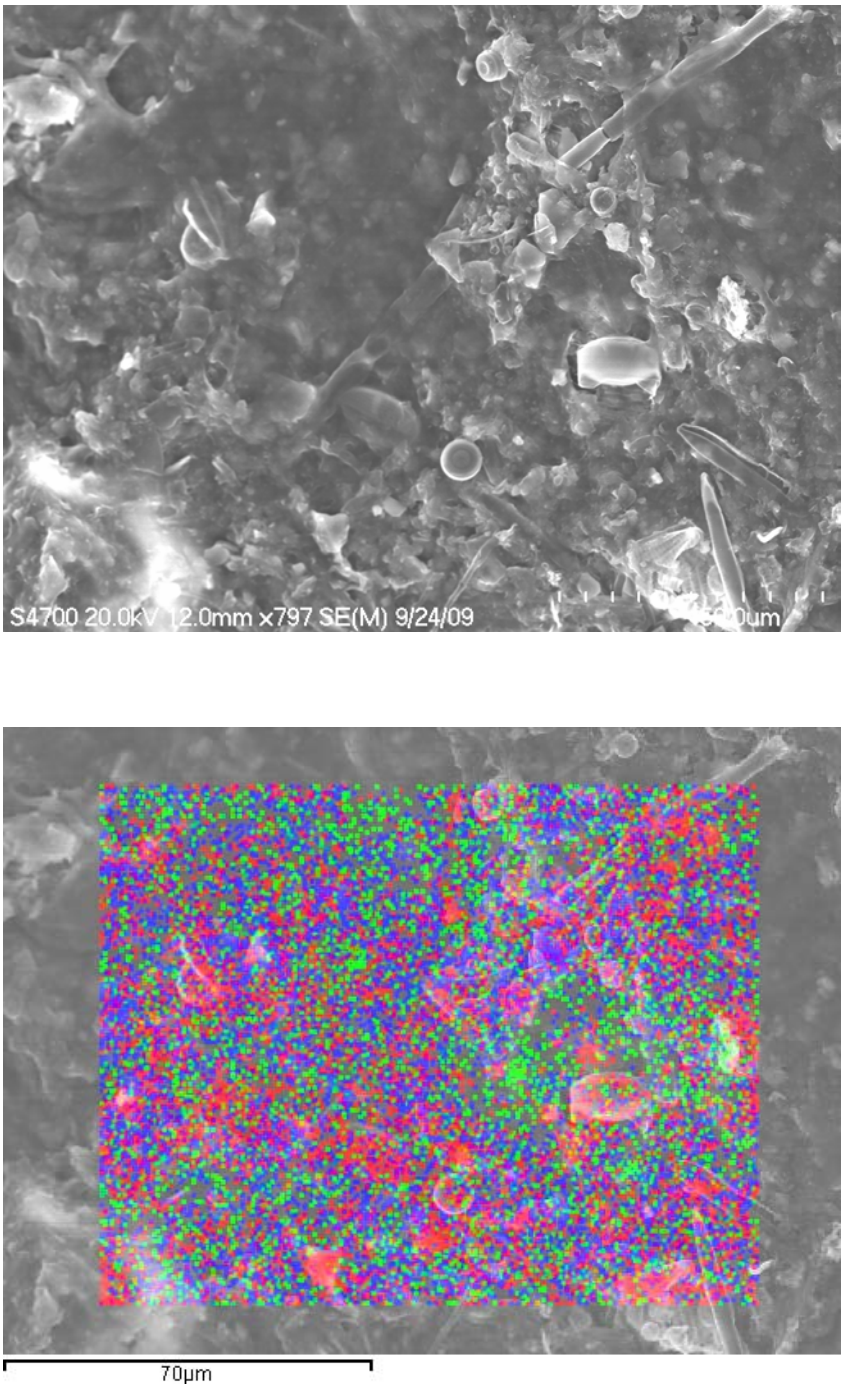


Figure 3.14 Distribution of silica, iron, and aluminum in the schmutzdecke material (sample no. 2); Si – red, Al – blue, Fe - green

In the Figure 3.14 silica is represented by red, aluminum by blue and iron by green. The distribution of all three elements was fairly uniform, except for silica which was somewhat more concentrated in the areas where diatoms were present.

3.3.3.2 Discussion

Liss et al. (1996) studied freshwater and activated sludge flocs. EDX analysis of those flocs showed the inorganic portion to be composed mainly of Fe, Al, Si. The inorganic floc constituents were concluded to be iron oxyhydroxides, clay minerals and silicates (Liss et al., 1996). It is important to note that the present study similarly indicated the primarily inorganic elements in the schmutzdecke to be Si, Al, and Fe.

The fact that aluminum and iron were abundant in the schmutzdecke material is an important discovery taking into account that those two compounds are used in the everyday practice of water/wastewater treatment. These results raise the question whether accumulation of those elements in the schmutzdecke could be of importance in its water purification processes.

Weber-Shirk & Chan (2007) have suggested that aluminum might be responsible for particle removal in slow sand filters. These researchers have achieved 6-log *E.coli* removal in a filter ripened with Cayuga Lake seston extract (Weber-Shirk, 2002). Analysis of the extract with an inductively coupled plasma technique (ICP) revealed that aluminum was 17% of the total metals by mass (Weber-Shirk & Chan, 2007; Weber-Shirk, 2002). They later showed that a relatively small amount of aluminum added to the feed water during the filter ripening cause a substantial improvement in *E.coli* reduction (Weber-Shirk & Chan, 2007).

Researchers suggested long ago that cations present in water may cause charge reversal of negatively charged sand grains (Huisman & Wood, 1974). The results presented here are consistent with this hypothesis since it was observed that a significant amount of cations accumulated in the schmutzdecke. Since bacteria are negatively charged if the surface of sand grains became neutral or positively charged during the ripening period, the removal of pathogen would be more effective due to an opposite charge attraction. The overall charge of the schmutzdecke material was tested in further experiments.

3.3.4 Zeta potential analysis

The schmutzdecke was grown on a sand media with creek water for 21 (sample no. 1) and 16 days (sample no.2) and then harrowed. The harrowing caused a detachment of pieces of the schmutzdecke which started floating in the supernatant water. The supernatant water was then poured out and schmutzdecke particles flocculated and settled shortly thereafter. The floc was dispersed in filtered creek water. As a result the floc broke into fine particles. The solution was then poured into the electrophoresis cell and applied voltage caused electrically charged particles in the solution to move toward the electrode with an opposite charge. Four categories of particles with different sizes were distinguished. The velocities of twenty particles from each category were measured. The average velocity for each category was determined and the zeta potential calculated based on the Helmholtz-Smoluchowski equation.

3.3.4.1 Results

Table 3.3 Zeta-potential of the schmutzdecke (sample no. 1)

	Big floc particles (> 160 µm)	Medium floc particles (80 - 160 µm)	Small floc particles (40-80 µm)	Elongated particles with various sizes (most < 40 µm)
Mean	0.0 mV	+1.9 mV	+2.3 mV	-24.5 mV
Standard error	0.0	1.1	1.5	1.5
Mode	0.0 mV	0.0 mV	0.0 mV	-23.8
Variance	0.0	22.5	63.4	51.7
Min	0.0 mV	0.0 mV	-24.0 mV	-35.1 mV
Max	0.0 mV	+13.3 mV	+13.8 mV	-6.2 mV
Confidence level (95%)	0.0	2.2	2.2	3.3

Table 3.4 Zeta-potential of the schmutzdecke (sample no. 2)

	Big floc particles (> 160 µm)	Medium floc particles (160-80 µm)	Small floc particles (80-40 µm)	Elongated particles with various sizes (most < 40 µm)
Mean	0.0 mV	+0.5 mV	+2.2 mV	-22.5 mV
Standard error	0.0	0.5	1.5	1.9
Mode	0.0 mV	0.0 mV	0.0 mV	-24.6
Variance	0.0	5.5	42.5	74.5
Min	0.0 mV	0.0 mV	-10.9 mV	-35.8 mV
Max	0.0 mV	+10.5 mV	+18.5 mV	-9.2 mV
Confidence level (95%)	0.0	1.1	3	4

Similar to the EDX analysis, the zeta-potential analysis revealed that the two different samples, derived from the two different schmutzdecke showed very

similar characteristics. Based on particles' sizes, four categories of particles were distinguished. Between the two samples, the corresponding categories of particles had similar particle charge mean and variance. In the first category (> 160 μm) the mean particle charge was 0.0 mV and the variance was 0 for both samples. All of the particles in this category were neutrally charged for both samples. In the second category (160 – 80 μm) the mean was +1.9 mV for the first sample and +0.5 mV for the second sample. The variance was 22.5 and 5.5 for the first and the second sample, respectively. The majority of particles in this category were neutrally charged; the mode was 0.0 mV for both samples. The second category differed, however, from the first category since it also contained some particles that were positively charged. The maximum particle charge was +13.5 mV and +10.5 mV for the first and the second sample, respectively. There were no negatively charged particles in this category. Note the increase in charge variance as the size of particles decreased. In the third category (80 – 40 μm) all kinds of particles were observed: neutrally, positively, and negatively charged. The variance was 63.4 and 42.5 for the first and the second sample, respectively. The mean particle charge was +2.3 mV and +2.2 mV for the first and the second sample, respectively. In the last category (mostly < 40 μm) the variance was still significant; however, all the particles were negatively charged. The mean particle charge was -24.5mV and -22.5mV for the first and the second sample, respectively. It is believed that the majority of particles in this category were diatoms which were recognized as such under the microscope. SEM pictures also revealed that diatoms were very abundant in the schmutzdecke. Diatoms are encased by amorphous silica frustules (shells) which under neutral conditions bear significant negative charge (Toster et al., 2009).

3.3.4.2 Discussion

It is interesting to note that as particle size decreased they were more likely to bear either a negative or positive charge. This effect was seen for particles which were < 80 μm. The majority of particles larger than > 80 μm were neutral and only a few were positive. The particles bigger than > 160 μm, however, were almost exclusively neutral. It was also observed that particles were flocculating quickly and thus in a short time all of the particles in the zeta-meter field of view were neutral.

Based on the zeta-potential results it can be concluded that the schmutzdecke had an overall neutral charge. However, negative or positive micro sites might have been present on its surface since the schmutzdecke was composed of particles bearing positive and negative charges. In light of these results Huisman's & Wood's (1974) hypothesis that particle attachment in slow sand filters is due to "a charge reversal by the attachment of positive particles and a subsequent re-reversal by the attachment of negative particles" (Logsdon et al., 1991) becomes plausible. However, because the overall surface of the schmutzdecke was neutral, the charge reversal processes must occur continuously during the slow sand filter run. This suggests that the influent water composition might be of importance and might have an effect on removal efficiencies during slow sand filtration. It is well known that the ionic concentration in solution affects the thickness of the double layer which in turn affects bacterial and viral adsorption on surfaces (Abbott et al., 1983; Jewett et al., 1995; Logsdon, 1991). Jorden (1963) suggested that polyvalent cations may act as "bondings" between negatively charged particles and negatively charged media in the filter. It was shown that higher valence cations such as Fe^{3+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}

enhance bacterial and viral adsorption (Huysman & Verstraete, 1993; Stevik et al., 2004; Logsdon et al., 1991).

It is important to note that the enhanced removal of negatively charged clay particles due to the development of positively charged sections in the schmutzdecke was first suggested by Jorden (1963) and Edwards and Monke (1967). They utilized electrophoresis and streaming potential measurements to understand the mechanisms of colloidal particles removal in filtration columns (Edwards & Monke, 1967; Jorden, 1963).

3.3.5 Assessing flocculating properties of the schmutzdecke material

The schmutzdecke was grown on a sand media with creek water for 22 days and then harrowed. The harrowing caused a detachment of pieces of the schmutzdecke which started floating in the supernatant water. The supernatant water was then poured out and schmutzdecke particles flocculated and settled shortly thereafter. The floc was tested to determine if it is capable of flocculating either *E.coli* or MS-2. Four vials with creek water spiked with *E.coli* and MS-2 were prepared. Two of these vials received a 1 ml aliquot of floc and the other two a 1 ml aliquot of the "supernatant". The contents of vials were mixed vigorously and then gently stirred for 28 min. When the floc settled, the samples were collected from all four vials slightly below the water surface and analyzed directly for remaining *E.coli* and MS-2.

3.3.5.1 Results

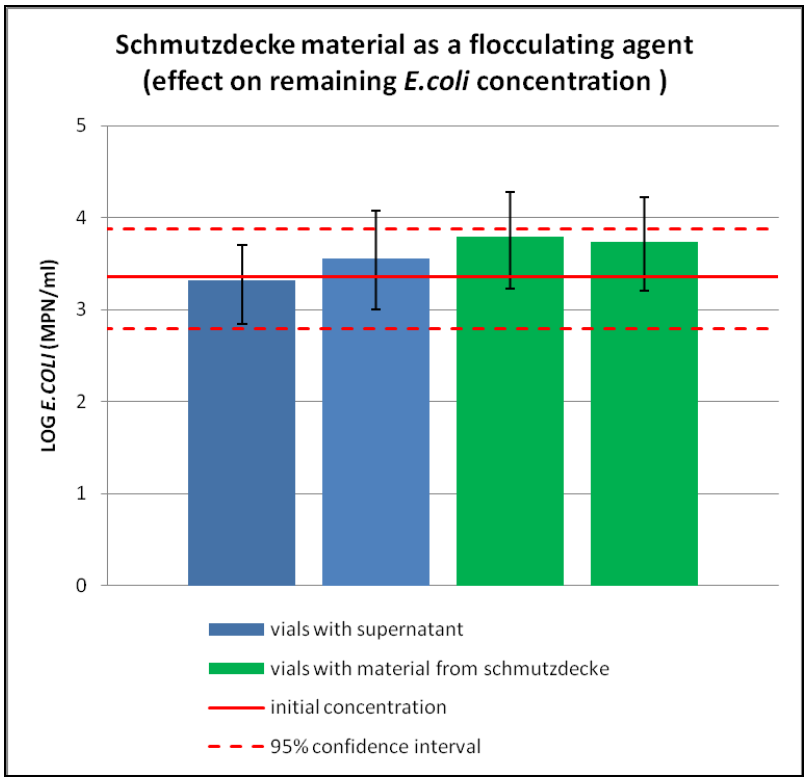


Figure 3.15 The schmutzdecke material as a flocculating agent (effect on *E.coli* concentration)

The material derived from cleaning an intermittent slow sand filter did not have flocculating properties on *E.coli* in this experiment. The initial sample concentration was not statistically different from any of the other sample concentrations, whether containing the supernatant or the floc. The difference of means test between the initial concentration and the concentration in each other vial yielded *p* values of 0.45 and 0.61 for samples containing the supernatant and 0.23 and 0.28 for samples containing the floc.

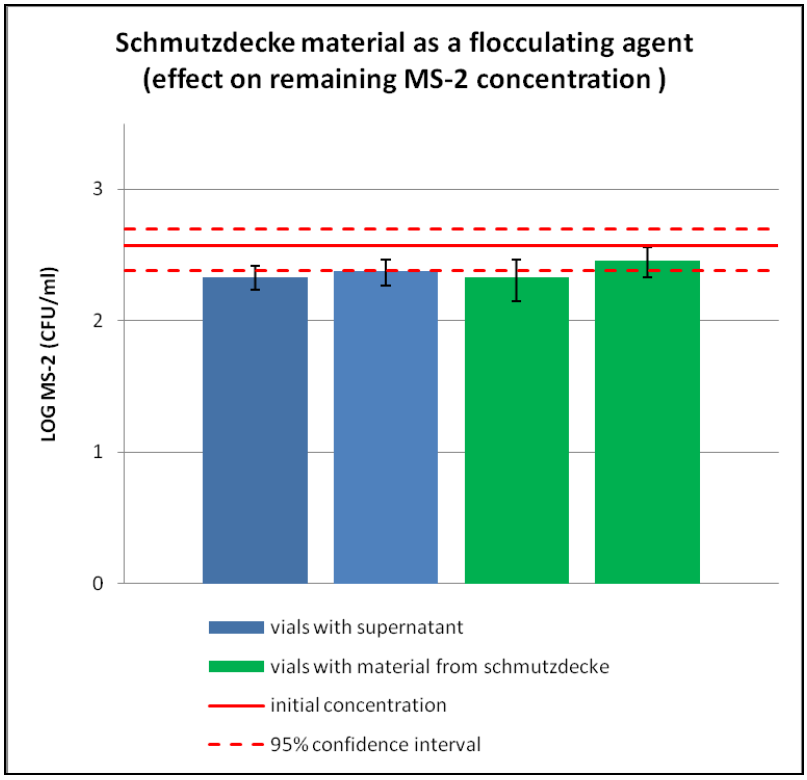


Figure 3.16 The schmutzdecke material as a flocculating agent (effect on MS-2 concentration)

The material derived from cleaning an intermittent slow sand filter did not have flocculating properties on MS-2 in this experiment. Although the initial sample concentration was statistically different from all of the other sample concentrations except for one (one of the samples containing the floc), the samples containing the supernatant and the samples containing the floc were not statistically different from each other. The difference of means tests between them yielded *p* values of 1, 0.049, 0.53, and 0.16.

3.3.5.2 Discussion

Early researchers have suggested that the development of a zoogloea layer on the sand surface increases the "stickiness of sand grains" thus causing better adherence of particles that come in contact with them (Bellamy et al., 1985a; Huisman & Wood, 1974).

It is also known that bacteria are capable of producing adhesive extracellular polymers that form links between the cell and the solid surface (Stevik et al., 2004; Weber-Shirk & Dick, 1997a). Some researchers have indicated that those polymers are capable of flocculating both organic and inorganic stable colloidal dispersions (Pavoni et al., 1972) and organisms in activated sludge (Tchobanoglous et al., 1991). In this study it was observed that particles from the schmutzdecke dispersed in the supernatant water as it was poured out after filter cleaning and that they rapidly flocculated and settled thereafter. The results of this experiment showed, however, that neither *E.coli* nor MS-2 was flocculated by the material from the schmutzdecke. The possible explanation could be that floc was too loose and amorphous to retain the particles. It was hypothesized that polymer strands and fungal hyphae, together with particle deposition and biological growth in the filter, could contribute to the increased rate of collisions. It was hypothesized that the increase would only occur if the schmutzdecke structure was undisturbed. It was believed the schmutzdecke material that was removed from the filter, though it still had the same elemental composition, did not have the same properties anymore. These properties are evidently more complex than a single charge – charge interaction.

3.3.6 Importance of increased rate of collisions in the schmutzdecke

The purpose of this experiment was to determine if the intact structure of the schmutzdecke might be of importance in pathogen removal. The schmutzdecke was grown on a sand media for 14 days with creek water. The filter was then challenged with creek water spiked with *E. coli* and MS-2. The sample of effluent was collected and then the schmutzdecke (approximately 2 cm of sand) was disturbed by mixing it at 180 rpm for 1 min using a Phipps & Bird Stirrer (model 7790-400). Following the mixing 10 pore volumes of creek water were run through the filter. The filter was challenged with creek water spiked with *E. coli* and MS-2 for a second time. The sample of effluent was collected. The removal efficiencies prior and after the disturbance were compared in order to determine if the intact structure of the schmutzdecke is of importance in pathogen removal.

3.3.6.1 Results

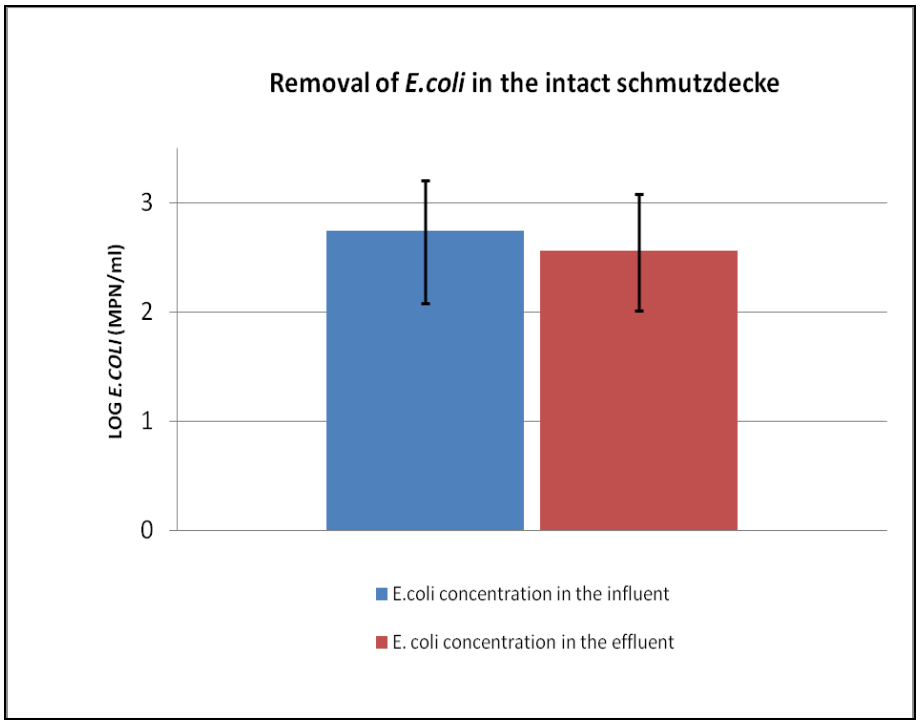


Figure 3.10 Removal of *E.coli* in the intact schmutzdecke

The experiment was run in the winter time and thus *E.coli* removal in the schmutzdecke was not very impressive in the first experiment. Still, the influent and the effluent samples were statistically different from each other ($p = 5.12*10^{-7}$).

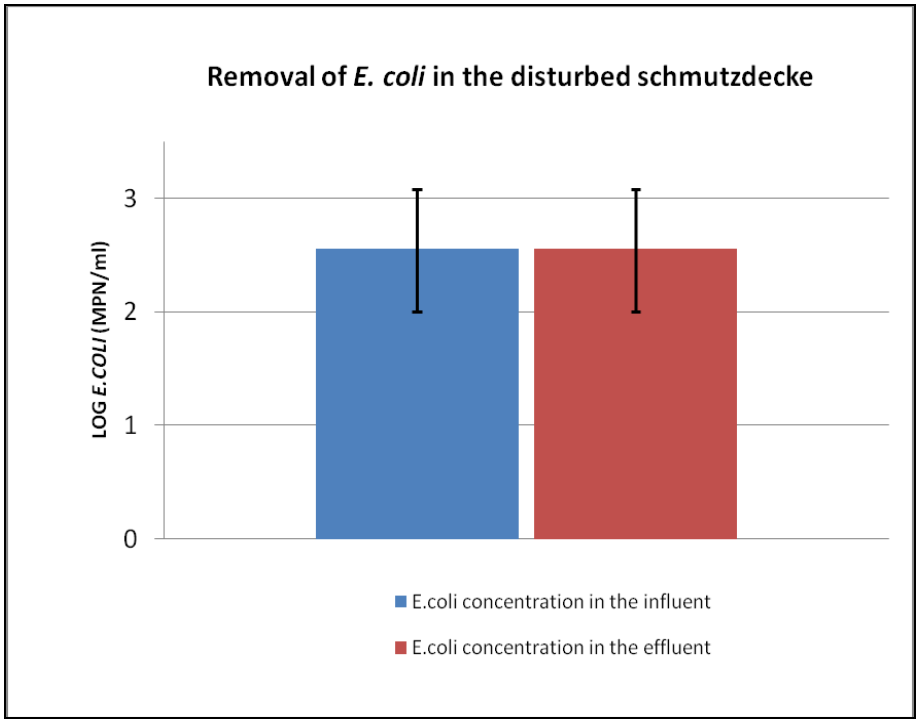


Figure 3.11 Removal of *E.coli* in the disturbed schmutzdecke

The removal of *E.coli* was estimated at 53% in the first experiment and went down to 0% in the second experiment. Therefore, it can be concluded that in this experiment the disturbance of the schmutzdecke structure had a significantly negative effect on *E.coli* removal. It is hypothesized that such a dramatic effect was caused by the decreased rate of collisions due to the destruction of the schmutzdecke internal structure.

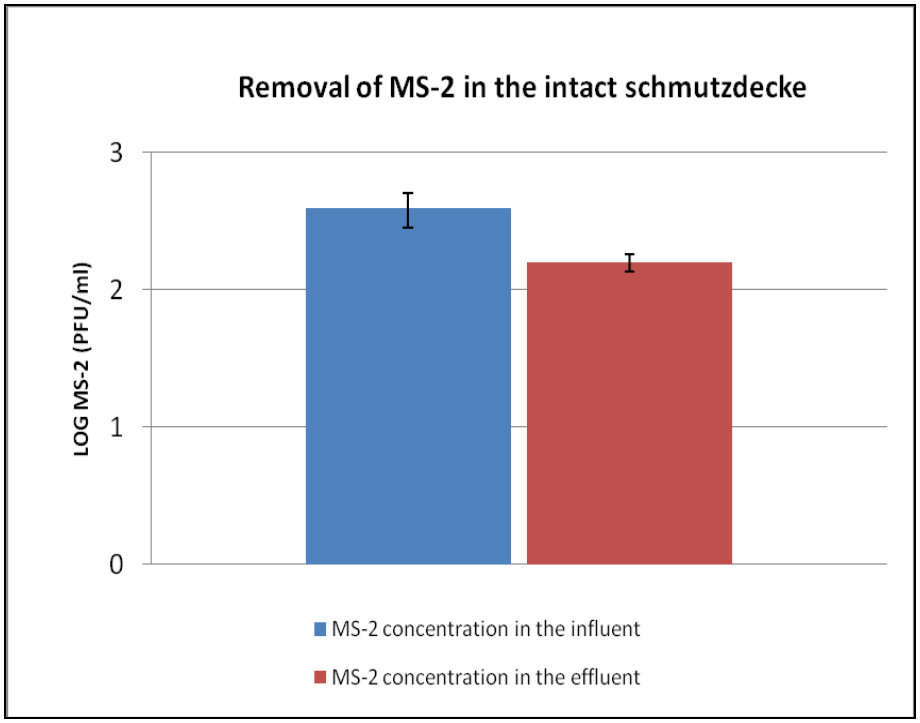


Figure 3.12 Removal of MS-2 in the intact schmutzdecke

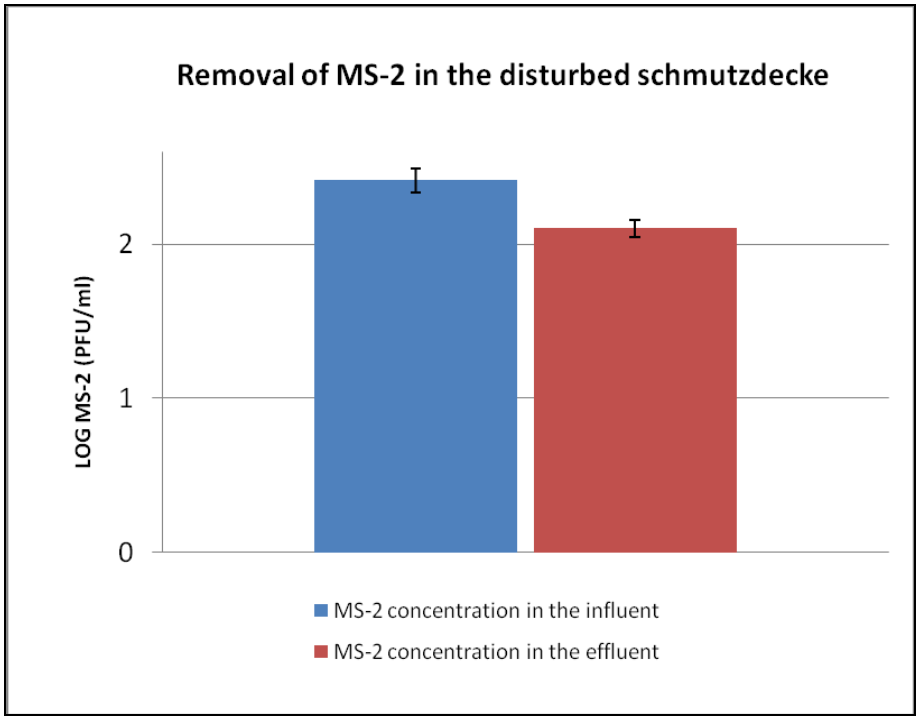


Figure 3.13 Removal of MS-2 in the disturbed schmutzdecke

It is interesting to note that disturbance of the schmutzdecke had only a slight effect on MS-2 removal. The removal of MS-2 in the first experiment was 59% and went down by only 8% in the second experiment. These results are consistent with previous results showing that MS-2 is removed to only a very minimal extent during the filtration through the schmutzdecke. Therefore, disturbance of the schmutzdecke was not expected to have any major effect on MS-2 removal.

3.3.6.2 Discussion

In this study it was hypothesized that the intricate internal structure of the schmutzdecke is the reason for the increased bacteria removal in the first few centimeters of sand in a slow sand filter. It is believed that schmutzdecke is composed

of a "net" of polymer strands, fungal hyphae, deposited particles and biological growth which together contribute to the increased rate of collisions. The strands of slime were observed in SEM pictures of the schmutzdecke (Figure A.11, Figure A.15 in Appendix A). A "net" of fungal hyphae stretching between the sand grains could be observed with a conventional microscope (Figure A.16 in Appendix A).

Liss et al. (1996) had studied freshwater and activated sludge flocs architecture using correlative microscopy (COM, SCLM and TEM) and a novel 4-fold multimethod preparatory technique. The result revealed that:

> "…pores which appeared to be devoid of physical structures under the optical microscopic techniques (SCLM and COM) were observed to be composed of complex matrices of polymeric fibrils (4-6 nm diameter) when viewed by high-resolution TEM…"

It was observed that those fibrils are major components of the flocs' void spaces and that they are associated with surfaces of clay particle and bacteria (Liss et al., 1996). The author noted that a dense fibril network inside the void space of flocs on the one hand provides structural support that promotes pseudoplastic characteristics and on the other significantly increases the surface area available for sorption (Liss et al., 1996).

It is hypothesized that void spaces within the schmutzdecke might have a similar "architecture". The "slime strands" observed in the SEM pictures of the schmutzdecke material in this study (Figure A.11 and Figure A.15 in Appendix A) had a much bigger diameter, approximately 1 μm. It is possible that higher resolution pictures would reveal other important nanoscale structural elements of the schmutzdecke.

The compromise in water quality caused by the disturbance of the schmutzdecke was suggested as early as 1917 by Folwell who stated:

> "Another reason for fixing the low rate is that if by force a high velocity is obtained, the schmutzdecke is apt to be broken and unpurified water pass through the filter."

Recently, Stauber (2007) reported that during her study on the BioSand filter performance the schmutzdecke was accidentally punctured one day. She had noticed that, immediately following this incident, *E.coli* reduction was lower. Stauber (2007) suggested that the mechanism of *E.coli* reduction was perhaps straining. The author believes that it was rather a decreased rate of collisions due to water bypassing the treatment in the schmutzdecke "network".

Increase in the rate of collisions due to filter ripening was previously suggested by O'Melia et al. (1978).

3.4 CONCLUSIONS

Currently, as many as 500,000 people in developing countries rely on the BioSand filter to provide safe drinking water. However, it was shown that the BioSand filter will not remove all pathogens; thus the produced water is not safe to drink without disinfection. Disinfection will most likely lead to the formation of DBPs as slow sand filters are not very efficient in DOM removal. The mechanisms of water purification in slow sand filtration have not been clearly understood so far. We will not be able to successfully influence intermittent slow sand filters performance until we fully understand the removal processes that govern their efficiency. In this study the removal mechanisms in an intermittent slow sand filter were investigated.

It was shown that *E.coli* was removed almost exclusively in the biologically active stage of filtration. MS-2, on the other hand, was not efficiently

removed either by the biologically active or the physical processes governing stages of filtration. In order to efficiently remove viruses an additional treatment step, i.e., some kind of an amendment at the bottom of an intermittent slow sand filter is recommended.

It was further shown that *E.coli* concentrations were only reduced during filtration through the schmutzdecke. The residence time in the biologically active layer did not have any affect on *E.coli* reduction. Quite contrary, while MS-2 concentration was not significantly reduced during filtration through the schmutzdecke, it was dramatically reduced with the residence time in the biologically active layer. Clearly two different mechanisms govern bacteria and virus removal in an intermittent slow sand filtration. It was hypothesized that bacteria are removed due to an increased rate of collisions by interception processes while viruses are removed by Brownian motion and subsequent attachment due to opposite charge attraction.

The elemental analysis of the material from the schmutzdecke revealed that the most abundant elements in its composition were: O, C, Si, Al, Fe, Ca, Mn. The majority of inorganic elements in the schmutzdecke were cations. The zeta potential measurement performed on the particles of dispersed schmutzdecke material revealed that the overall schmutzdecke charge was neutral. However, the schmutzdecke was composed of positively, neutrally and negatively charged particles. This observation lends credence to the hypothesis that the attachment of particles in the schmutzdecke is caused by “a charge reversal due to the attachment of positively charged particles, and a subsequent re-reversal due to the attachment of negatively charges particles and so on” (Logsdon et al, 1991).

Although the schmutzdecke particles flocculated and dropped out of solution relatively quickly *E.coli* and MS-2 were not affected by the flocculation process. The disturbance of the schmutzdecke greatly affected *E.coli* removal while it only slightly affected MS-2 removal. It was hypothesized that "network" of polymer strands and fungal hyphae together with particle deposition and biological growth in the schmutzdecke led to an increase in collisions rate and thus affected bacterial removals.

Based on the results it can be concluded that residence time in the schmutzdecke affects the quality of the effluent. The disturbance of the schmutzdecke might lead to a significant compromise in water quality and thus should be avoided. The addition of Al or Fe into the filter influent might benefit the pathogen removal efficiency.

Chapter 4

IMPROVING PERFORMANCE OF INTERMITTENT SLOW SAND FILTER WITH ZERO-VALENT IRON

4.1 INTRODUCTION

In September of 2000, world leaders came together to strengthen their partnership in fighting extreme poverty around the globe. They adopted the United Nations Millennium Declaration, which defined a series of goals to be achieved by 2015. The focus of Goal 7 Target 3 is to halve, by 2015, the proportion of population lacking access to safe drinking water and basic sanitation (UN, 2010).

Although progress has been made toward achieving Millennium Development Goals and the access to safe drinking has improved for many, the situation did not improve for 884 million people worldwide (WHO/UNICEF, 2010). Waterborne diseases continue to be a major health threat for people in developing countries. In 2006 WHO reported that of 1.8 million deaths caused by diarrheal diseases every year, 88% are due to unsafe water supplies (WHO, 2004). 90% of those deaths are children less than 5 years old (WHO, 2004). It is estimated that 5.7% of all diseases and 4% of all deaths worldwide are caused by inadequate water, sanitation and hygiene (Pruss et al., 2002). Kofi Annan, former United Nations Secretary-General, stated (WHO, 2004):

> "We shall not finally defeat AIDS, tuberculosis, malaria, or any other infectious disease that plagues the developing world until we have also won the battle for safe drinking water, sanitation and better health care."

Point-Of-Use water treatment is a technology that allows people to treat water by themselves, at their homes. It is a good solution for developing countries where centralized water treatment facilities are very rarely available. Often, for people living in those countries, this kind of technology is the only alternative to drinking unsafe, contaminated water. An intermittent slow sand filter, called the BioSand filter is a type of Point-Of-Use technology that is widely implemented in developing countries. Currently as many as 500,000 people worldwide rely on BioSand filters to provide drinking water (Elliott et al., 2008).

The efficiency of a traditional slow sand filter is well-documented; however, the ability of BioSand filters to remove infectious microorganisms is questionable, since intermittent slow sand filters have different design and operational conditions. It is well known that intermittent operation of slow sand filters can lead to deterioration in water quality (Huisman & Wood, 1974; Logsdon et al., 2002; Martensson & Jabur, 2006; Petry-Hansen et al., 2006). Manz, who in early 1990s developed the BioSand filter, stated that (CAWST, 2008):

> "The BioSand filter …cannot guarantee that the water is pathogen free. The BioSand filter should be used as part of multi-barrier approach for providing safe water. …It is recommended to disinfect the water after it has passed through the BioSand filter."

Several laboratory and field studies investigated the efficiency of BioSand filters in removing pathogens.

Labolatory studies prove that BioSand filters are superior in removing *Giardia cysts* and *Cryptosporidum occysts*, achieving 4-log or higher removal efficiency (Palmateer et al., 1999). However, the removal of indicator bacteria, *E.coli*, rarely exceeds 2-log removal efficiency in the laboratory studies. Buzinis, who was the first researcher to study BioSand filter performance, achieved an average of 96%

fecal coliform reduction (Buzunis, 1995) in the laboratory experiment. Other researches have achieved an average *E.coli* removal of 99.5% (Lee , 2001), 99% (Elliott et al., 2008), 90% (Donison, 2004), 97% and 91% (Stauber, 2007), for the filters tested in laboratory settings. In the same studies viruses were removed to a lesser extent. Stauber (2007) has reported the mean reductions of 78% and 87% for MS-2 and PRD-1, respectively. Elliott (2008) has achieved an average of 70% reduction of bacteriophages. It is worth noting also that in traditional slow sand filters viruses are removed the least and therefore are the most critical microorganisms in determining SSF efficiency (Dullemont et al., 2006; Hijnen et al., 2004).

The performance of BioSand filters in removing *E.coli* tends to be even lower in the field studies. Field studies have been conducted in Dominican Republic, Etiopia, and Nepal. Average *E.coli* reductions in those studies were 80% (Stauber, 2007), 87.9% (Earwaker, 2007), and 93% (Stauber et al., 2006). However, it is important to note that performance of the investigated filters varied largely, and while some filters provided 100% removal, others provided no or even negative removals of *E.coli* (Donison, 2004; Earwaker, 2007; Lee , 2001; Stauber, 2007; Stauber et al., 2006)

In the light of those results, and following WHO recommendations that fecal coliform concentration in drinking water ought to be less than 1 CFU/100 ml (WHO, 2006), the water treated by BioSand filter should be definitely disinfected before drinking.

However, the BioSand filter has one more drawback. It does not remove all of the organic substances, especially those that are not easily degradable (Amy et al., 2006; CAWST, 2008; Logsdon, 1991; Logsdon et al., 2002). Therefore

the addition of chlorine as a disinfectant will most likely lead to the formation of carcinogenic disinfection byproducts (DBPs).

Iron and aluminum salts are widely used in water and wastewater treatment. Recently, You et al. achieved 4-log removal of both MS-2 and φ174 shortly after packing the rapid filtration columns with zero-valent iron sand mixture (You et al., 2005; Donison, 2004; Earwaker, 2007). The removal has increased to 5-log for both bacteriophages after a couple days of operation. The suggested mechanism of removal was adsorption to ion oxides and oxihydroxides that formed during elemental iron corrosion (You et al., 2005). The potential advantage of using ZVI is also the removal of organic matter that might be a precursor to DBPs formation (You et al., 2005). It has been shown that iron oxides can adsorb organic substances (Dries et al., 2004; Gu et al., 1994).

The same principle of iron oxides formation was used in the Kanchan Arsenic Filter design, which was first developed to remove arsenic from contaminated ground water. KAF is a modification of BioSand filter. The difference is incorporation of non-galvanized iron nails covered with brick chips in the filter diffuser (CAWST, 2008). The brick chips help to disperse water over iron nails. Nails become rusty quickly after contact with water. Rust indicates formation of iron oxides that is an excellent adsorbent of arsenic. Two studies suggest that KAF may be also capable of removing bacteria. A study in Nepal showed that KAF was capable of removing 85-99% of total coliform bacteria (Ngai et al., 2007). A study in Cambodia showed that all filters had no *E.coli* in the filtered water (Chea et al., 2008). No more studies of bacteria removal in KAF are available.

In recent studies conducted by Tellen (2009) a BioSand filter amended with zero-valent iron (iron filings) outperformed a traditional BioSand filter. The former achieved 2 log removal and the latter achieved 1.5 log removal after 65 days of ripening.

In the previous experiments, conducted by the author, it was shown that the material derived from the schmutzdecke contained a significant amount of iron (6% by weight). It was not clear if iron can play any role in pathogen removal. However, in the experiments performed by Weber-Shirk (2007) the addition of a relatively small amount of aluminum to the slow sand filter feed water caused substantial improvement in filter performance.

The purpose of this study was to investigate the influence of zero-valent iron amendments on the quality of intermittent slow sand filter effluent. The zero-valent iron used in those experiments was in the form of iron filings. Iron filings are a waste byproduct of grinding, filing, or milling of finished iron products. Therefore it is expected that iron filings will be available free of charge to local communities in developing countries.

4.2 MATERIALS AND METHODS

4.2.1 Sand and gravel

The sand that was used in the experiments had an effective size of 0.18 mm and a uniformity coefficient of 1.61. The sand was delivered by Unimin Corporation from their plant in Pevely, MO. The chemical analysis of the sand, mean percentage by weight, is as follows:

Silicon Dioxide	99.83%

Iron oxide	0.02%
Aluminium Oxide	0.04%
Calcium Oxide	0.03%
Titanium Oxide	0.01%
Magnesium Oxide	0.01%
Potassium Oxide	0.01%
Loss on ignition	0.05%

Some white play sand was also bought at Home Depot supply store. Particle size and chemical analysis information were not provided. The sand was sieved to obtain sand grains larger than the sand grains used as filter media. A small amount of this sand was used below the actual sand media and above the gravel, to prevent fine sand from escaping the filter.

Gravel, in two different sizes, was obtained from Ricci Sand company. Particle size and chemical analysis information were not provided. The gravel was used as an underdrain.

4.2.2 Iron

The zero-valent iron used in this study was in a form of iron filings and was obtained from Peerless Metal Powers & Abrasives (Detroit, MI). For the preliminary experiment the iron filings were sieved and particles of size 0.5mm – 0.83 mm were obtained. For the final experiment the iron filings were sieved and particles of size 0.07 – 0.25 mm were obtained and mixed with fine sand in proportion 15:100.

4.2.3 Water quality

White Clay Creek water was used as feed for the filters. The water was collected from the raw water tap at Newark Treatment Plant at weekly intervals. The water was stored in plastic containers at room temperature until used. The turbidity of raw water was observed by the treatment plant's staff daily. Its average was 2-3 NTU. In cases of an increase in water turbidity, caused by a rain event, the plant operators switched to water from Newark Reservoir, which is also taken at the same tap. The Newark Reservoir stores water that has previously been drawn from White Clay Creek. Its turbidity was on average 1.5 NTU.

Some chemical parameters in the feed were measured by the author a few times during this study. The averages for those parameters were:

Total iron	(based on 6 measurements)	0.11 mg/l
Conductivity	(based on 4 measurements)	313 μS/cm
pH	(based on 2 measurements)	7.1
COD	(based on 2 measurements)	1.5 mg/l
DO	(based on 2 measurements)	5.42 mg/l

The total iron was measured using FerroVer reagent obtained from the Hach company; the COD was determined using ultra low COD digestion vials obtained from the Hach company; the conductivity was measured using a pocket conductivity tester (ECTestr11+); the pH was measured using a AR25 Dual Channel pH/Ion meter; the DO was measured using a YSI Model 55 Handheld Dissolved Oxygen system.

4.2.3 Experimental challenge microorganisms and their enumeration

Two surrogates of human pathogens were used: MS-2 (ATCC 15597B1) and *E.coli* (ATCC 25922). MS-2 was used as a surrogate for human enteric viruses due to its similarity to many enteric viruses, low attachment and survival rate (Dullemont et al., 2006; Hijnen et al., 2004; You et al., 2005). *E. coli* is a good indicator of fecal contamination in drinking water (Tallon et al., 2005). Tests were performed to ensure that *E.coli* (ATCC 25922) cannot serve as a host for MS-2.

Prior to enumeration all samples were serially diluted ten-fold in buffered dilution water containing magnesium chloride and potassium dihydrogen phosphate. The dilution water concentrate was obtained from the Hach company.

MS-2 was grown in a 4-hour host culture overnight. Next the stock was centrifuged at 3,5000 x g for 10 min causing the host cells to settle. The supernatant was decanted and filtered through 0.22 μm syringe driven filter unit to remove the remaining bacterial cells. Following that the supernatant was subject to ultrafiltration using Millipore's Amicon Ultra-15 centrifugal devices as described by the company manual. The sample was placed in the Amicon tube and centrifuged at 5,000 g for 30 minutes in order to remove the soy broth and concentrate the virus stock. The concentrated stock was rediluted in buffered dilution water. Since the ultrafiltration devices are not sterile, the sample was once again filtered through 0.22 μm fitler unit. The stock was stored at 4°C. The virus stock was taken out from 4°C temperature and allowed to reach room temperature before being used in an experiment.

MS-2 was enumerated with the single agar layer method using its *E.coli* host (ATCC 15597) according to EPA Method 1602 (EPA, 2001). The host was grown in 25 ml of Tryptic Soy Broth for 18 hours. A 0.5 ml volume of this overnight

culture was transferred to 40 ml of Tryptic Soy Broth and grown for 4 hours to log phase (OD 0.36-0.44) (EPA, 2001)). MS-2 samples were enumerated by pouring 0.1 ml of the sample and 0.5 ml of the host culture into a glass tube containing 0.7% Tryptic Soy Agar. This mixture was then poured onto 1.5% Tryptic Soy Agar plates. When the plates solidified, after approximately 15 minutes, they were incubated at 37°C overnight. Plaques that formed overnight were enumerated and the results reported as PFU/ml. For the final experiment each sample was assayed five times. However, depending on a sample, three to five plates could be used for determining a mean and confidence intervals.

For the preliminary experiment samples were only "screened" on the day of the experiment to determine, based on the number of plaques produced, which dilution is appropriate to be analyzed. Therefore, each sample was assayed at least once on the day of the experiment and then five times the day after the experiment.

MS-2 is difficult to enumerate due to the fact that plaques are not equal in size. Therefore, for the same sample, the numbers from different plates might vary significantly, especially if the plates are overcrowded. The other reason for high sample variance is possible losses of specimen due to retention on the glass tube while the soft agar is poured onto a media plate. Some of the numbers obtained in the enumeration of MS-2 were discarded as outliers (this concerns three samples in the second part of the final experiment, and one sample in the first part of the preliminary experiment). A value was considered an outlier if it was more than 1.5 IQR below Q1 or above Q3 where IQR = Q3 - Q1 (Ott & Longnecker, 2001).

A new culture of *E.coli* was grown before each experiment. One colony was picked from a streak plate with a sterile loop and transferred to a tube containing

40 ml of Typtic Soy Broth. The tube was incubated at 37°C for 18 hours. The culture was centrifuged at 3,5000 x g for 10 min causing *E.coli* cells to settle. The supernatant was discarded. The cells were rediluted with buffered dilution water, enumerated and stored at 4°C until the experiment. The culture was taken out from 4°C temperature and allowed to reach room temperature before being used in an experiment.

Samples containing *E.coli* were analyzed using Violet Red Bile Agar with MUG media by a spread plate method following procedures described in "Standard Methods For the Examination of Water and Wastewater" and the Difco Manual (Difco, 1998; Greenberg et al., 1985). Plates were incubated at 37°C overnight. Colonies were counted after overnight incubation and expressed as CFU/ml. For the final experiment each sample was assayed four times. However, three or four plates could be used for determining a mean and confidence interval. Some of the numbers obtained in the enumeration of *E.coli* were discarded as outliers (this concerns one sample in the second part of the final experiment). In the first part of the preliminary experiment each sample was assayed at least two times; in the second part of the preliminary experiment each sample was assayed at least three times.

Lake water spiked with an expected concentration of 10^3 CFU/ml of *E.coli* and 10^3 PFU/ml of MS-2 was used in the experiments. This concentration was considered to be similar to the actual pathogen concentration that may be encountered in fecally contaminated water (Stauber, 2007).

All statistical tests performed in this study were two-sided in nature.

4.2.4 Experimental setup

Sand was washed with deionized (DI) water until it was no longer visibly dirty, then it was dried in an oven at 80°C for 3 – 4 days. The column was then packed

under saturated conditions, by pouring the sand in increments while stirring with a plexiglass rod to remove any attached air bubbles.

The filter porosity was determined based on calculations taking into account the empty column weight, the dry media weight, the packed column weight and the volume of the media filling the column. The tracer test was run using sodium chloride. The conductivity of effluent water was measured with a pocket conductivity meter and the concentration of sodium chloride was calculated based on the previously prepared calibration curve.

Filters were ripened with creek water intermittently. The peristaltic pump was turned on by timer three times daily to provide water from the raw water reservoir onto the filter diffuser. The challenge experiments were conducted by feeding water to the filters continuously at a constant rate using peristaltic pump. The flow rate was kept constant by keeping the head at a constant level.

4.2.4.1 Preliminary experiments

Two columns were packed with the same amount of gravel, separation media (coarse sand) and fine sand (d_{10}=0.18 mm). The total media depth was 6.5 cm in each column with 2.5 cm deep fine sand layer. Porosity of the media in column no.1 was estimated at 0.38 (246 ml) and in the column no.2 at 0.37 (242 ml). A tracer test was run to determine how many pore volumes have to be run until steady state concentration in the effluent is reached (Figure B.1 in Appendix B).

14.2 grams of iron filing with size 0.5mm – 0.83 mm were distributed evenly over 100 cm^2 cross sectional area of one of the columns. The sand was not covered with iron completely in order to leave some room for habitats that would be present if no iron was applied (Figure 4.1).

Figure 4.1 Uneven distribution of iron over the surface of sand

On the 1st day of filters operation about 5 pore volumes of creek water were run through both columns at a velocity of 0.26 m/h and samples for chemical analysis were collected (Table 4.1) (the total iron in the effluent was measured on the 5th day). On the 2nd day of filters operation the challenge experiment was run at a velocity of 0.26 m/h, using creek water spiked to an expected concentration of 10^3/ml of *E. coli* and 10^3/ml of MS-2. Six effluent samples, collected at different time intervals, were collected and analyzed.

Both columns continued to been ripened with approximately 3.5 pore volumes of creek water daily. The headloss in the column with iron was increasing rapidly over the period of filter ripening and by the 9th day of the operation the flow

rate had to be decreased to 0.1m/h to prevent overflowing. On the same day the second set of samples for chemical analysis was collected (Table 4.1). On the 10^{th} day of the filter operation the second challenge experiment was run at a velocity of 0.096m/h. The creek water was spiked to an expected concentration of 10^3/ml of *E. coli* and 10^3/ml of MS-2. Five effluent samples were collected and analyzed.

Two other small preliminary experiments were run. One column was packed in the same way as described above except that iron filings were placed in the filter diffuser. The second column was packed with 2 cm of gravel, 1 cm of separation media, 1 cm of fine sand, 1.5 cm of 20% iron-sand mixture (iron size was in range 0.07 – 0.25 mm), and 0.8 cm of fine sand. There were no microbial challenge experiments run on those columns.

4.2.4.2 Final experiment

The column was packed with 5.5 cm of gravel, 2 cm of underdrain media (coarse sand), 2.5 cm of fine sand (d_{10}=0.18mm), 3.2 cm of sand-iron mixture (15% iron) and 41 cm of fine sand (d_{10}=0.18mm). The area of the column was 123 cm^2. Total porosity in the media was estimated at 0.39, pore volume at 2620 ml.

On the 1^{st} day of the filter operation approximately 2.5 pore volumes of creek water was run through the column and samples for chemical analysis were collected (Table 4.2). On the 2^{nd} day of the filter operation the challenge experiment was run at a velocity of 0.26 m/h, using creek water spiked to an expected concentration of 10^3/ml of E. coli and 10^3/ml of MS-2. The samples of effluent were collected at two different heights. Location of those two sampling points is presented in the Figure 4.2. Three effluent samples, at different time intervals, were collected from each height.

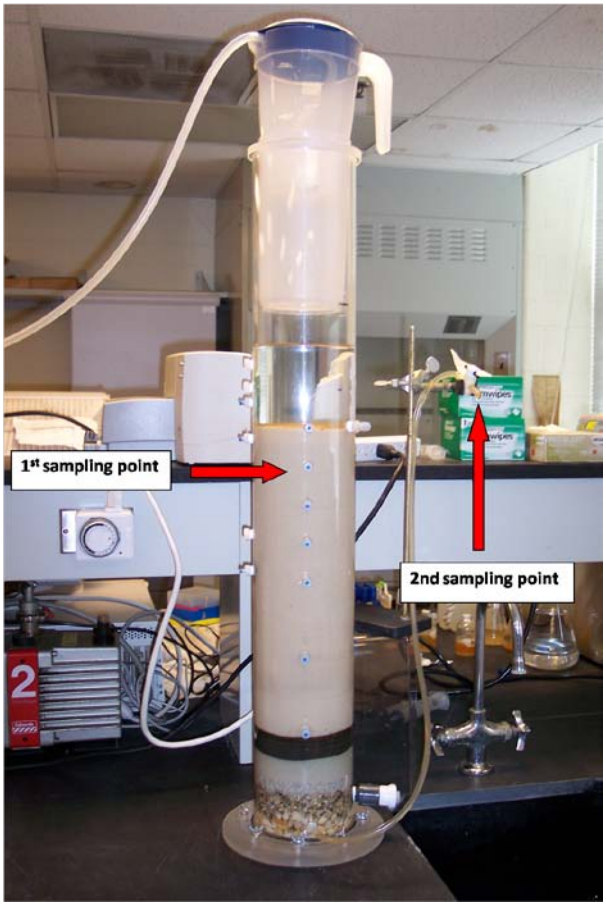


Figure 4.2 Location of sampling points in the first challenge experiment

The column continued to be ripened with 1.2 pore volumes daily. One pore volume of BioSand filter is typical of a lower range of a daily family water demand in developing countries (Elliott et al., 2008). On the 18th day of the filter operation approximately 2.5 pore volumes of creek water was run through the column and samples for chemical analysis were collected (Table 4.2). Additionally on the 4th and the 13th days the effluent grab samples were collected in order to determine

the iron content. The second challenge experiment was run on the 19th day of the filter operation at a velocity of 0.27m/h (the same velocity as in the first experiment), using creek water spiked to an expected concentration of 10^3/ml of E. coli and 10^3/ml of MS-2. The samples of effluent were collected at three different heights. Location of those three sampling points is presented in the Figure 4.3. Three effluent samples, at different time intervals, were collected from each height.

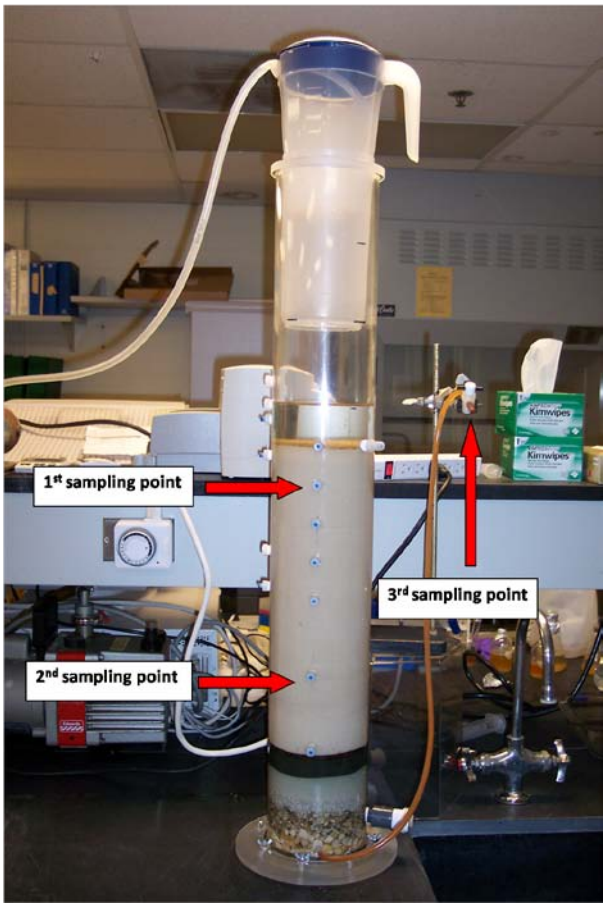


Figure 4.3 Location of sampling points in the second challenge experiment

An additional sampling location was added in order to prove that sand below the schmutzdecke is not affecting the removal much and that the majority of the removal is due to the iron-sand layer that is at the bottom of the column.

4.3 PRELIMINARY RESULTS AND DISCUSSION

The purpose of the experiments described in this section was to determine the effect of iron fillings distributed on the surface of an intermittent slow sand filter on pathogen removal. Two columns were packed with the same amount of gravel, separation media and fine sand. 14.2 grams of iron filings with size 0.5 mm – 0.83 mm were distributed evenly over the sand surface of one of the columns. The challenge experiment was run using creek water spiked with *E.coli* and MS-2. The filters were then ripened for 9 days and the second challenge experiment was run using creek water spiked with the same microorganisms.

Two other small preliminary experiments were run. The first column had iron filings placed in the filter diffuser, the second column had 1.5 cm zero-valent iron – sand layer placed 1 cm below the sand surface.

4.3.1. Microbial challenges (results and discussion)

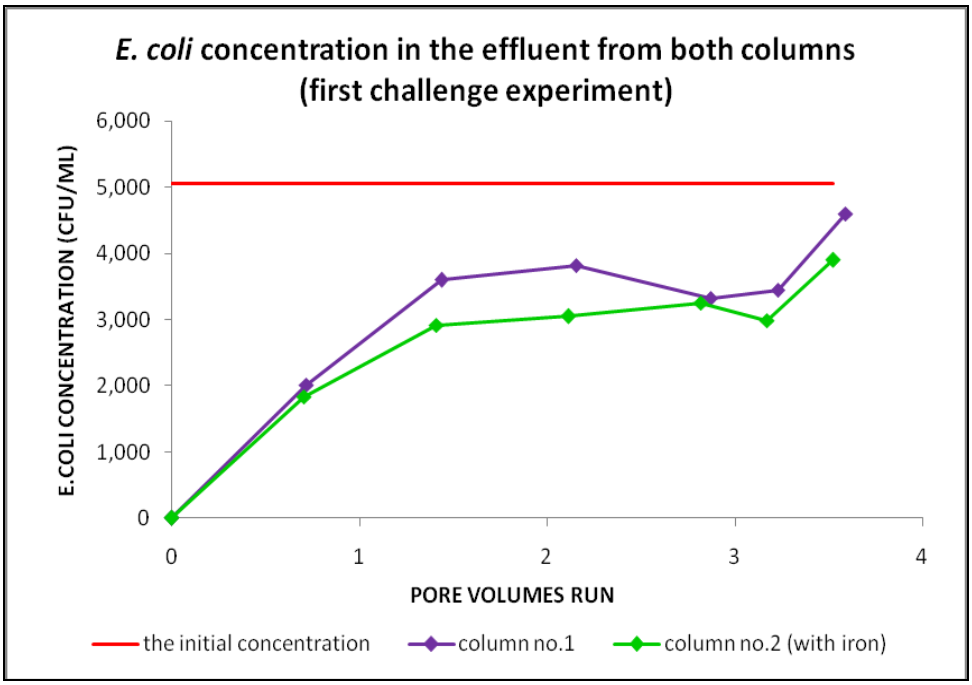


Figure 4.4 ***E.coli*** **removal in both columns on the 2nd day of filters operation (preliminary experiment)**

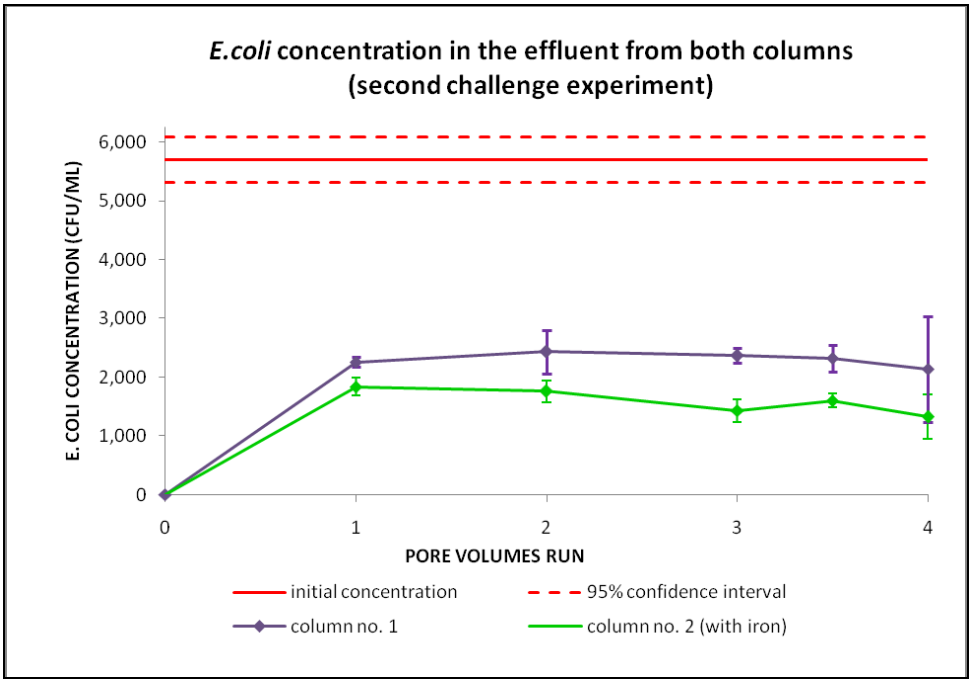


Figure 4.5 ***E.coli*** **removal in both columns on the 10th day of filters operation (preliminary experiment)**

The removal of *E. coli* was higher in the column no.2 (with iron), at least in the second challenge experiment, as proved by a t-test. The p-values of the difference of means tests, that were performed on pairs of samples collected at different time intervals ranged from 0.0004 to 0.037, thus proving that the difference in performances of the two columns was statistically significant. Based on the averages of the last three effluent samples, *E.coli* removal efficiency in the column with iron was 75% and in the column with sand only was 60%.

Although not proven statistically, it was estimated (based on averages of the last three effluent samples), that in the first challenge experiment the removal of *E.coli* in the column with iron was 33% and 25% in the column with sand only.

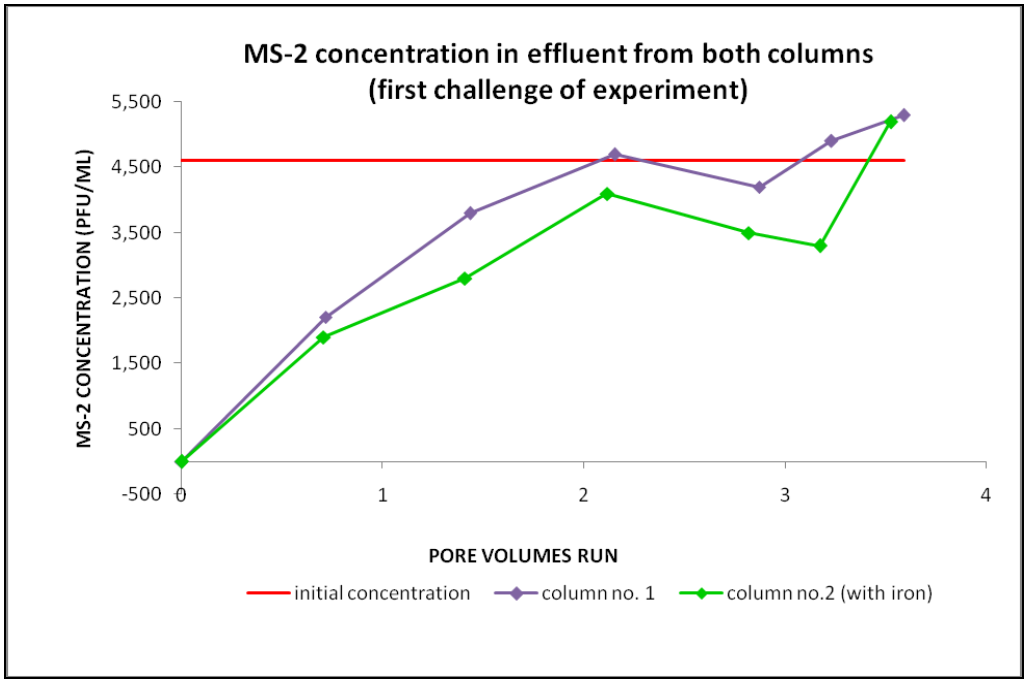


Figure 4.6 MS-2 removal in both columns on the 2nd day of filters operation (preliminary experiment)

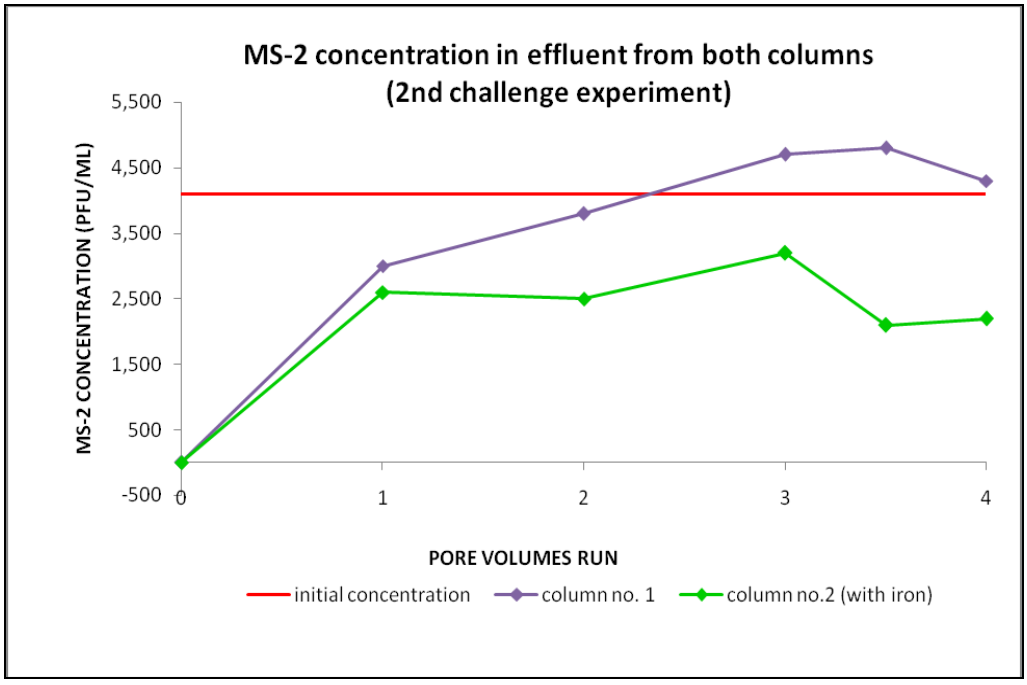


Figure 4.7 MS-2 removal in both columns on the 10th day of filters operation (preliminary experiment)

Although the samples were stored in a refrigerator, the numbers of MS-2 decreased significantly overnight. Therefore the results obtained from assaying the samples one day after the experiment cannot be considered as representative (Figure B.2, and Figure B.3 in Appendix B). (*E.coli* numbers did not change significantly during the overnight storage).

Although this could not be proved by statistical analysis, the removal of MS-2 also seemed to be higher in the column with iron. It was estimated that in the first challenge experiment the removal of MS-2 was 13% in the column with iron and 0% in the column with sand only; in the second challenge experiment it was 39% in the column with iron and 0% in the column with sand only.

It appears that both *E.coli* and MS-2 removals were slightly higher in the second experiment (run after 9 days of ripening). It is unclear if this was due to the formation of iron oxide or due to the lower velocity at which the experiment was run. Although iron caused "ripening" of the sand below (Figure B.4, and Figure B.5 in Appendix B), this ripening was not uniform and it is hypothesized that *E.coli* and MS-2 could get through the sand sections that were not affected by iron corrosions/iron oxides precipitation.

Additionally, two samples of the sand from the surface of both filters were collected and investigated under the microscope. The sand from the column no.1 was rich in various organisms. Protozoa, macrofauna, diatoms and fungi hyphae were observed. The sand from the column no.2 (with iron), however, had very scarce, if any, biological life. Some diatoms were observed, but it was not clear if they lived in the column or were just filtered out from the feed water. No other macrofauna was observed, except for one protozoa. The observation suggested that iron is toxic to the organisms living in the biofilter.

The column that contained 20% iron-sand mixture underneath a layer of sand developed very significant headloss in just 3 days of operation. Therefore, the filter run was terminated. The upper portion of iron-sand mix developed a rusty color, suggesting corrosion. The bottom portion of this layer was black which suggested that the iron present was the ferrous, soluble form. On the 3rd day of the filter operation the total iron in the influent was 0.19 mg/l, and 1.17 mg/l in the effluent. The microscopic examination of the sand sample from the surface revealed very scare biological life. Only a few protozoa, one nematode and no other

macrofauna were observed, suggesting that the iron had diffused upward through the sand layer and negatively affected biological life on the surface.

The column that contained iron filings in the diffuser had some headloss in just 1 hour of continuous filter operation. The headloss was probably due to iron oxides formation and precipitation on the sand grain since the first 3 mm of sand became rusty in color. The filter run was terminated on the first day.

The preliminary results suggested that the best solution might be to place the iron-sand layer at the bottom. It was expected that if the iron is at the bottom it will not affect the biological life near the surface of the sand which is important for the proper performance of the filter. It was also expected that, since less oxygen is available at the bottom, there will be less rapid corrosion and headloss. However, on the other hand, some iron was to be expected in the effluent. To circumvent this disadvantage, in the final experiment, iron particles were spread out more within the iron-sand layer (15% instead of 20%). However, the layer was thicker (3.2 cm) as it was determined that a significant amount of iron is needed in order to achieve high efficiency in pathogen removal. Use of smaller iron particles was also expected to increase removal efficiency since smaller particles have a higher effective surface area.

The summary of pros and cons of different iron configurations in the column are presented in Appendix B (Figure B.6).

4.3.2 Chemical water quality (results and discussion)

Table 4.1 Comparison of effluent water quality from both columns (preliminary experiment)

	Influent			Effluent (column no.1)			Effluent (column no.2 – with iron)		
	1st day	**5th day**	**9th day**	**1st day**	**5th day**	**9th day**	**1st day**	**5th day**	**9th day**
Fe (total) (mg/l)	-	0.18	0.09	-	0.06	0.07	-	0.07	0.09
DO (% saturation)	62.9	-	61.4	58.3	-	49.7	49.1	-	29
pH	6.7	-	7.4	6.8	-	7.4	6.8	-	7.4
Conductivity (uS/cm)	316	-	312	318	-	314	317	-	314

Conductivity and pH were not affected by water filtration through either of the columns. Iron filings did not cause an increase in total iron concentration in the effluent. The total iron concentration in the effluent from column no.2 (with iron) and column no.1 was the same. For both columns the dissolved oxygen content in the effluent was lower than the oxygen content in the influent. For both columns the difference in oxygen content between input and output had increased with ripening. However this difference was much greater for column no.2

4.4 FINAL EXPERIMENT RESULTS AND DISSCUSION

4.3.1 Final experiment results

The purpose of the experiments described in this section was to determine the effect of a zero-valent iron – sand layer placed at the bottom of an intermittent slow sand filter on pathogen removal. Iron particles with sizes ranging from 0.07 mm to 0.25 mm were used. The zero-valent – sand layer contained 15% of iron by volume. Shortly after packing the column the challenge experiment using creek water spiked with *E.coli* and MS-2 was run. The samples of effluent were collected at two different heights in the column. Three effluent samples, at different time intervals, were collected from each height. The column was then ripened with creek water for 18 days and the second challenge experiment was run using creek water spiked with *E.coli* and MS-2. The samples of effluent were collected at three different heights in the column. Three effluent samples, at different time intervals, were collected from each height.

4.3.1.1 Microbial challenges results

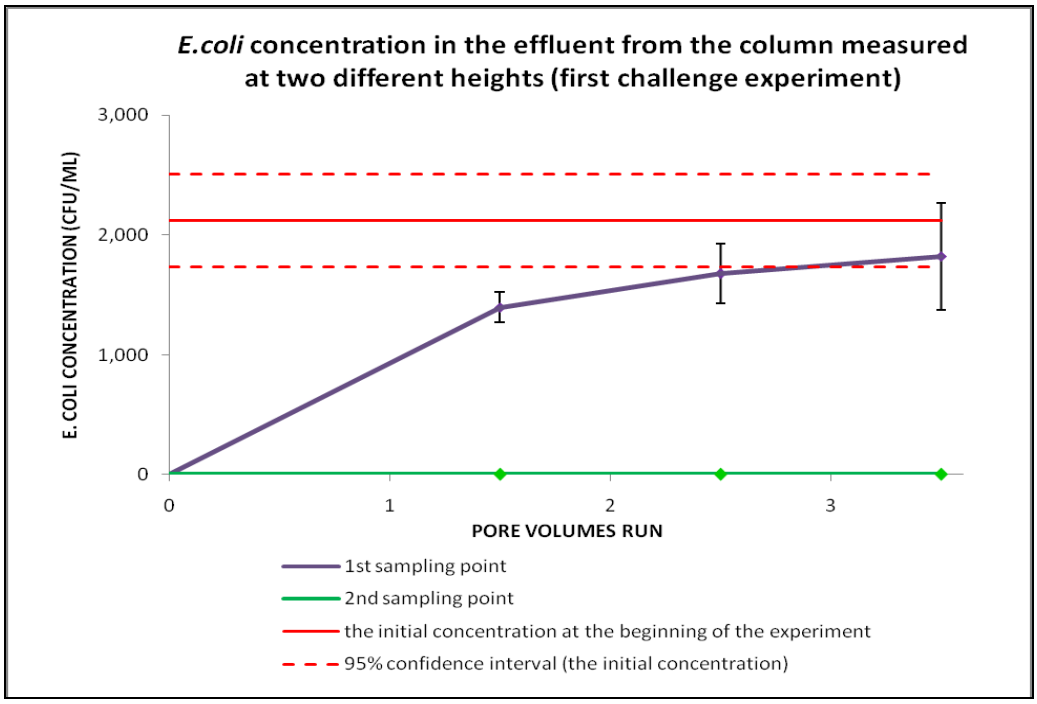


Figure 4.8 ***E.coli*** **removal by the schmutzdecke and by the ZVI layer on the 2nd day of filters operation**

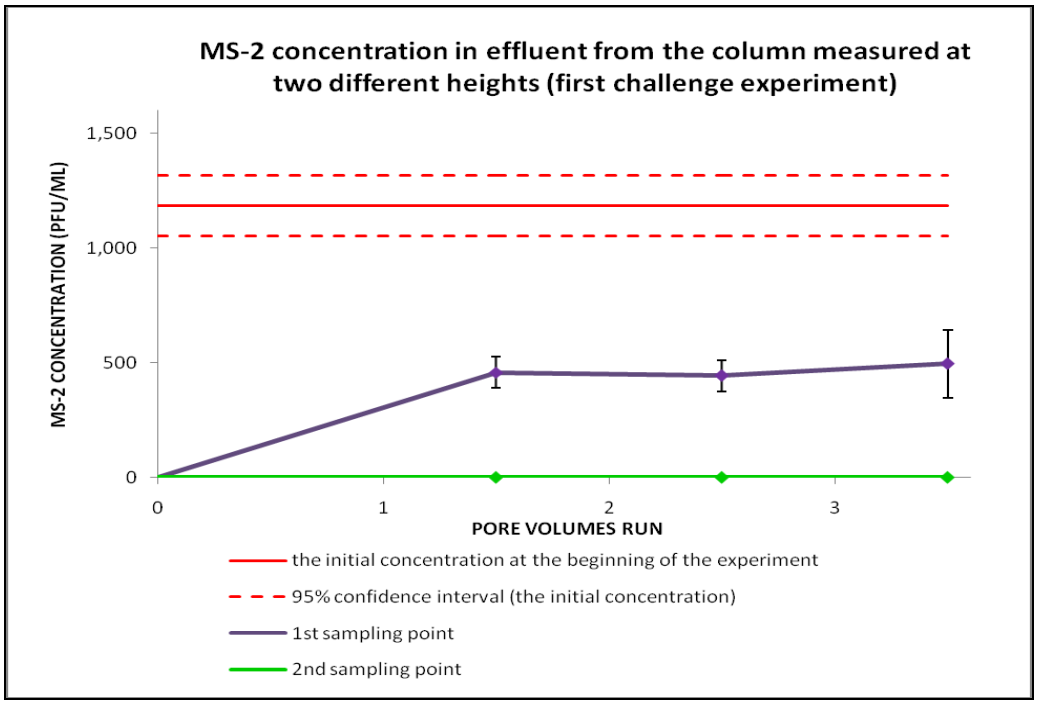


Figure 4.9 MS-2 removal by the schmutzdecke and by the ZVI layer on the 2nd day of filters operation

In the first challenge experiment the iron- sand layer was very efficient in both MS-2 and *E. coli* removal. There was no breakthrough of either of those in the effluent from the column.

The first 5 cm of sand ripened with creek water did not remove any *E.coli*. The initial *E.coli* concentration and *E.coli* concentration in the last effluent sample were compared by a t-test. The *p* value was 0.12 therefore the null hypothesis that there is no difference between samples could not be rejected. However, it was proved by a t-test that the initial MS-2 concentration and MS-2 concentration in the last effluent sample were statistically different (p value of 0.0002). This result suggests that there was 58% removal of MS-2 just by the first 5 cm of unripened sand. It does

not seem very probable. The number of MS-2 in the influent could have changed during the experiment due to natural die off or growth, this kind of phenomena was observed in the previous experiments. In this experiment there was no control sample to account for natural die off/growth effects. The sample from the bucket with feed water (the initial concentration) was collected sometime in the middle of the experiment. In the second challenge experiment two samples of feed water were analyzed – the first sample was collected at the beginning and the second one at the end of the experiment.

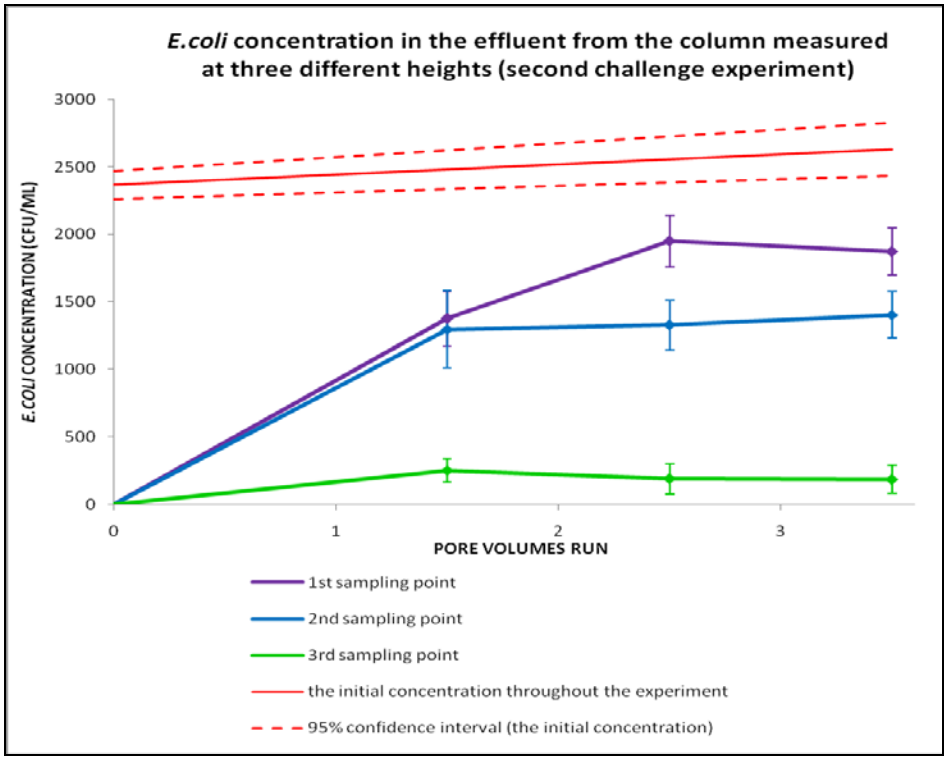


Figure 4.10 ***E.coli*** **removal by the schmutzdecke, by the sand underneath the schmutzdecke, and by the ZVI layer on the 19th day of filters operation**

The data sets from different sampling points were compared by a t-test. Effluent samples collected from the 1st and 2nd sampling points were statistically different, except for the first set of effluent samples. The *p* values were 0.38, 0.0003 and 0.008 for the first, the second and the third set of effluent samples, respectively. These results suggest that there was some removal of *E.coli* by the sand underneath the schmutzdecke.

There was a statistically significant difference between the intial concentration (the influent sample collected at the end of the experiment) and the last effluent sample from the 1st sampling point ($p = 0.0002$). This suggests that there was some removal of *E.coli* by the schmutzdecke.

The effluent samples collected from the 2nd and 3rd sampling points were also statistically different. The *p* values were 0.004, 5.8×10^{-5} and 2.61×10^{-5} for the the first, the second and the third set of effluent samples, respectively. Therefore, the iron-sand layer caused some reduction in *E.coli* concentration.

The *E.coli* concentration in the last effluent sample from each sampling point was compared to the initial *E. coli* concentration (the influent sample collected the end of experiment). The removal was estimated to be: 29% in the schmutzdecke, 18% in the sand underneath the schmutzdecke and 46% in the iron-sand layer. The total *E.coli* reduction was 93%. The majority of *E.coli* removal was due to the iron-sand layer. There was a breakthrough of *E.coli* in the effluent; however, it was not as pronounced as the breakthrough of MS-2.

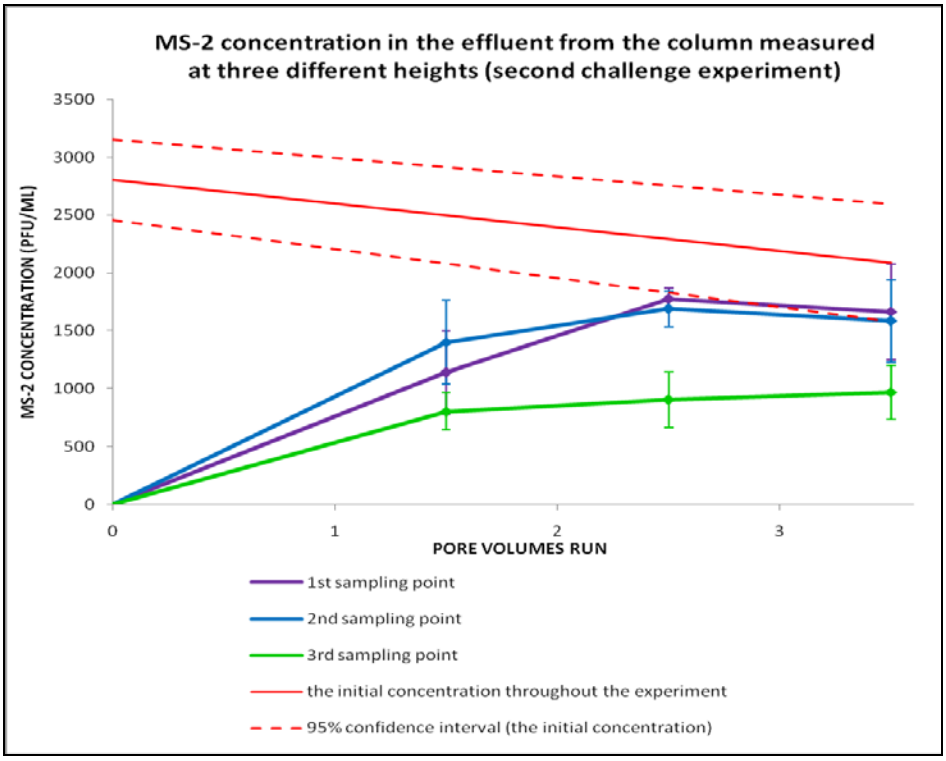


Figure 4.11 MS-2 removal by the schmutzdecke, by the sand underneath the schmutzdecke, and by the ZVI layer on the 19th day of filters operation

The data sets from different sampling points were compared by a t-test. Effluent samples collected from the 1st and the 2nd sampling points were not statistically different. The *p* values were 0.21, 0.14 and 0.69 for the first, the second and the third set of effluent samples, respectively. These results suggest that there was no removal of MS-2 by the sand underneath the schmutzdecke.

On the other hand, the effluent samples from the 2nd and the 3rd sampling points were statistically different. The *p* values were 0.005, 0.0004 and 0.005 for the first, the second and the third set of effluent samples, respectively. There was a statistically significant difference between the intial concentration (the influent

sample collected at the end of experiment) and the last effluent sample from the 3rd sampling point (p = 0.005). Therefore, the iron-sand layer caused some reduction in the number of MS-2 in the effluent. This removal was estimated at 54%.

There was no statistically significant difference between the intial concentration (the influent sample collected at the end of experiment) and the last effluent sample from the 1st sampling point (p = 0.06). This suggests that the removal of MS-2 by the schmutzdecke was neglegible. The result suggests that all removal of MS-2 in the column was only due to the iron-sand layer. The breakthrough of MS-2 in the effluent from the column was very surprising after the previous excellent results.

4.3.1.2 Chemical water quality

Table 4.2 The influence of iron-sand layer on water quality

	Influent				Effluent			
	1st day	**4th day**	**13th day**	**18th day**	**1st day**	**4th day**	**13th day**	**18th day**
Fe (total) (mg/l)	0.09	0.08	0.13	0.08	0.01	0.14	0.91	1.84
DO (mg/l)	5.57	-	-	5.27	0.86	-	-	1.32
Conductivity (uS/cm)	306	-	-	319	305	-	-	319
COD (mg/l)	1.2	-	-	1.8	0.15	-	-	UMR*

*UMR – under measuring range

Apparently, initially the column was removing iron from the water since the iron content in the effluent was lower than the iron content in the influent. However, gradually the iron content in the effluent started increasing and at the end of

the filter run it was 23x higher in the effluent than in the influent. DO was significantly reduced in the column, 88% at the beginning of filter run and 73% at the end of filter run. Conductivity was not affected by water filtration through the column. The COD removal increased with the filter operation. It was reduced by 83% at the beginning of the filter run and by 100% at the end.

4.3.2 Discussion

The breakthrough of MS-2 and *E.coli* in the second challenge experiment was very unexpected. It was observed that, over time, the upper portion of the iron-sand layer became rusty and the bottom portion became green-black. The rusty color suggests the formation of iron oxides. It was shown by many researchers that viruses adsorb on metal oxide surfaces (Chu et al., 2003; Murray & Park, 1980; Schijven et al., 2000; You et al., 2005). Therefore the filter performance was expected to improve as iron corrosion proceeded. In experiment performed by You et al. (2005) the removal of both MS-2 and φ 174 improved from 4-log to 5-log after 10 days of zero-valent iron filter operation. The authors suggested that it was due to the formation of new iron oxides. However in their experiment artificial groundwater was used which did not contained any organics. Oxide coated surfaces of soils, while providing favorable attachment sites for viruses, may be often blocked or competed for by DOM (Blanford et al., 2005; Foppen et al., 2006; Gerba, 1984; Pieper et al., 1997), or biomass growth (Chen et al., 1998). In a worst case scenario, when anionic surfactants are a major component of DOM, the alteration of virus-surface interactions and the elution of previously retained viruses might even take place (Pieper et al., 1997). Therefore, it is probable that the organic substances in the creek water affected the virus removal in the filter. The amount of iron oxides in the column was increasing

gradually, as indicated by the rusty color development, and was the highest at the end of the experiment. This is consistent with COD removal in the column which increased over time from 83% to 100%. On the other hand, over time, there was a decrease in the amount of DO reduced in the column, from 88% to 73%. This suggests that the zero-valent iron might have been almost completely oxidized at this point, especially, since only some of the oxygen depletion may be attributed to the zero-valent iron oxidation and the rest to the biological activity in the column. If nearly all of the zero-valent iron was oxidized and there was no or very little of the new iron oxides formed, the competition for existing adsorption sites would be very high and the organic compounds could have outcompeted viruses.

This poses a new very important question. You et al. (2005) suggested that virus removal in the column with zero-valent iron was due to adsorption on iron oxides. The authors achieved excellent 4-log removal shortly after packing the column. Is it possible that enough iron oxides were formed in such a short time? In the presented experiment 100% removal of the microorganism was also achieved shortly after packing the column (2^{nd} day of filter operation). This removal was not nearly as good in the second experiment, while visible rust left no doubt that a large amount of iron oxides were formed. Is it possible that the removal of microorganisms at the beginning of the experiment was due to a completely different mechanism than the adsorption to iron oxides?

Lee et al. (2008) performed experiments with nano-scale zero-valent iron. They have found that the highest *E.coli* inactivation was achieved with zero-valent iron that was under unsaturated conditions. In the presence of oxygen the bactericidal activity of particles was impaired. The author suggested that this reduction in activity

was due to iron oxidation. The hypothesis was confirmed by an experiment in which oxalate ion, a soluble Fe (III) complex forming agent, was added in order to prevent the buildup of an iron oxide layer on nanoparticles. The addition of oxalate ion has restored bactericidal activity. The authors hypothesized that since iron is a strong reductant it may be capable of inducing "reductive decomposition of functional groups in the proteins and lipopolysacharides of the outer membranes", may cause "oxidative damages via Fenton reactions", or "may disturb the enzymatic function of external membranes proteins by direct electron transfer". The authors suggested that the bactericidal effect of zero-valent particles is caused by "both size-related physical properties and chemical interactions of elemental iron". It is still worthwhile, however, to verify if the microscale zero-valent iron does not have bactericidal properties. If microscale zero-valent iron is proven to have such properties and the inhibition of iron oxidation in the filter can be achieved (e.g., cathodic protection), even if much higher doses need to be used (Lee et al., 2008), it still might be more beneficial to use microscale than nanoscale iron particles. Safety of nanoscale iron particles is still uncertain and their cost will make them unavailable for application in developing countries.

Another hypothesis explaining the observed MS-2 and *E.coli* breakthrough in the 2nd challenge experiment is the increased pressure head resulting in increased pore velocity and preferential flow problems. Iron oxides are insoluble in water and like many other metal oxides can cause clogging of filters (Weber-Shirk & Chan, 2007). Due to iron corrosion the velocity of the flow through the filter had to be decreased from 0.27m/h to only 0.034 m/h in just 18 days. In the 2nd experiment the pressure head was increased in order to achieve the same velocity as in

the previous experiment- 0.27 m/h (Figures B.7 and B.8 in the Appendix B). Blockage of sand pores and adjusting the pressure head to keep the same loading rate might have caused an increased interstitial velocity and preferential flow problems. This might have caused an impairment in physical removal processes because the number of microbes exposed to a filter media and the rate of successful collisions with sand grains were likely to decrease.

The last question that requires attention is why *E.coli* was removed to a much greater extent than MS-2. *E.coli* is much bigger than MS-2. While MS-2 is only 26 nm in diameter (You et al., 2005), *E.coli* is 500 nm in the diameter and up to 3000 nm in length (Bergey et al., 2001). Because of its size, *E. coli* will be more efficiently removed by physical removal processes, like sedimentation and interception. Additionally, bacteria will attach to surfaces more easily because they produce adhesive polymers that "form links between the cell and the solid surface" (Weber-Shirk & Dick, 1997a). This is an important first step in bacterial attachment to the surfaces. Furthermore, flagella that *E.coli* produce will make it more prone to removal (Stevik et al., 2004). Surface appendages may facilitate adsorption process by altering the effective diameter, by increasing adhesive properties (appendages have hydrophobic character), and by their motility which may play a role in overcoming electrostatic repulsion (Stevik et al., 2004). Therefore, *E.coli* had a much greater chance than MS-2 to come in contact with iron and sand grains and be a subject to subsequent attachment or inactivation processes.

4.5 CONCLUSIONS

Currently, as many as 500,000 people in developing countries rely on BioSand filters to provide safe drinking water. However, it was shown that BioSand

filters will not remove all pathogens; thus the produced water is not safe to drink without disinfection. Disinfection will most likely cause the formation of DBPs as slow sand filters are not very efficient in DOM removal.

In the described experiments it was shown that zero-valent iron amendments can greatly increase intermittent slow sand filter performance and safety. In the first challenge experiment the ZVI amended filter removed 100% of both, *E.coli* and MS-2. For unknown reasons, in the second challenge experiment, both *E.coli* and MS-2 broke hrough in the effluent. However, still the majority of *E.coli* and all MS-2 removal was solely due to the iron-sand layer.

The mechanism of *E.coli*/MS-2 reduction by microscale ZVI powder in the filtration columns is still not fully understood and deserves further investigation.

Chapter 5

FUTURE DIRECTIONS

The results of this and previous laboratory and field studies show that intermittent slow sand filters are not capable of removing all pathogens. Disinfection of the effluent poses a risk of DBPs formation due to poor removal of DOM by slow sand filters.

In this study an intermittent slow sand filter was amended with a layer of zero-valent iron – sand mixture. It was shown that this filter was capable of removing both *E.coli* and MS-2 by 100%. However, the mechanism of removal was not explained. It is uncertain if the removal was due to bactericidal effects of microscale zero-valent iron, due to iron oxide formation, or both. The mechanisms of pathogen removal by microscale zero-valent iron should be determined.

The right combination of zero-valent iron grain size and the amount that would ensure complete pathogen removal but on the other hand limit the problems with filter clogging should be determined. The potential problems due to headloss development should be evaluated in long term experiments.

It is recommended that the factors that affect zero-valent iron transformation are studied. Models can be developed that would predict iron performance, i.e. changes in pathogen removal efficiency and development of headloss, and a period of time after which the iron should be replaced. In cases when iron is placed at the bottom of the column, an easy method of iron-layer replacement without causing disturbance to the schmutzdecke should be developed. The alternative

could be to use zero-valent iron as a second step treatment, but, only in cases when it does not significantly raise the cost of filter construction.

Mechanisms of pathogen removal are still not fully understood and it is recommended study of them continues. Although it seems inevitable to include some kind of an amendment in an intermittent slow sand filter in order to ensure that the effluent water is safe to drink, such studies could contribute to improved filter design and operation. Furthermore, the understanding of processes like pathogen removal in slow sand filtration might serve as an inspiration for the development of engineering solutions for complex real life problems. Such solutions are developed by engineers working is the area of biomimetics, a science that studies and applies solutions found in nature for designing of modern technology.

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Appendix A

ADDITIONAL TABLES AND FIGURES FOR CHAPTER 3

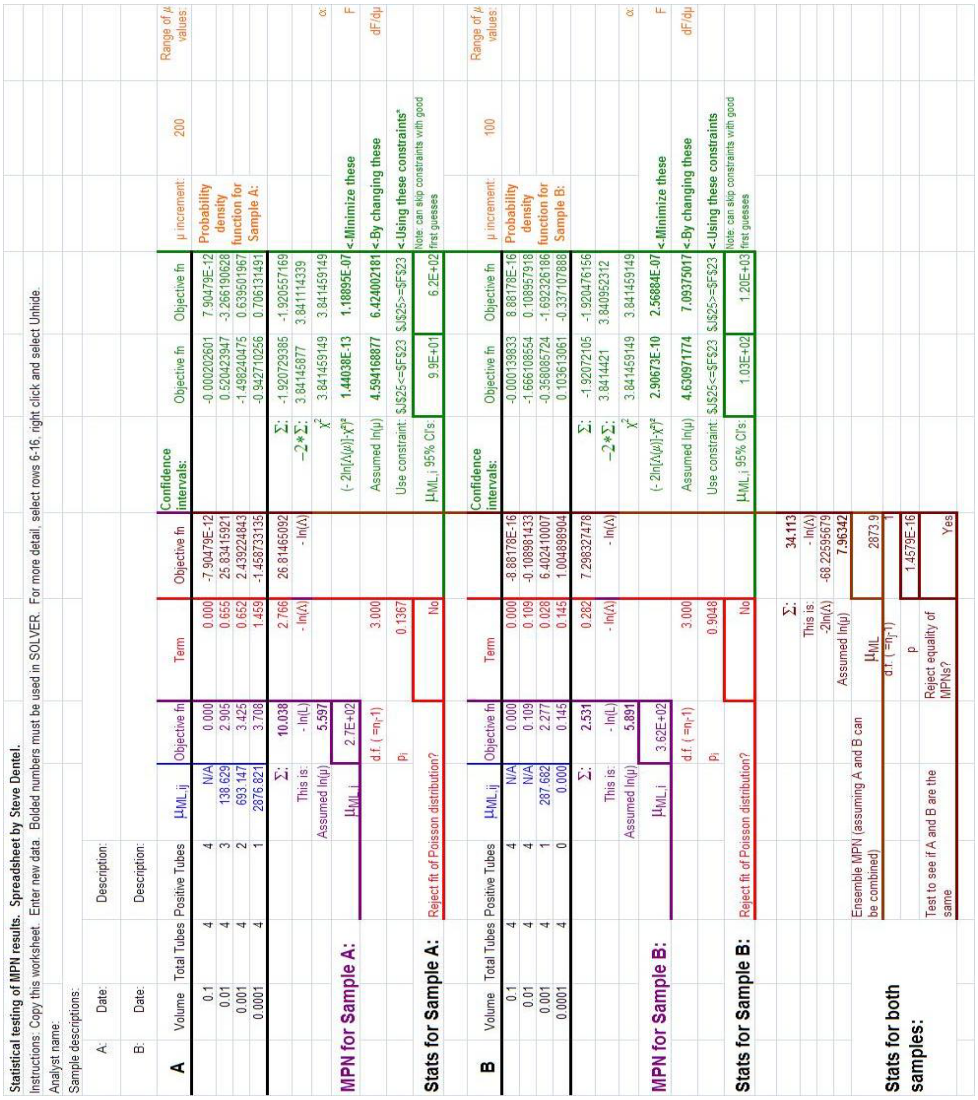

Figure A.1 The example spreadsheet output for MPN calculations

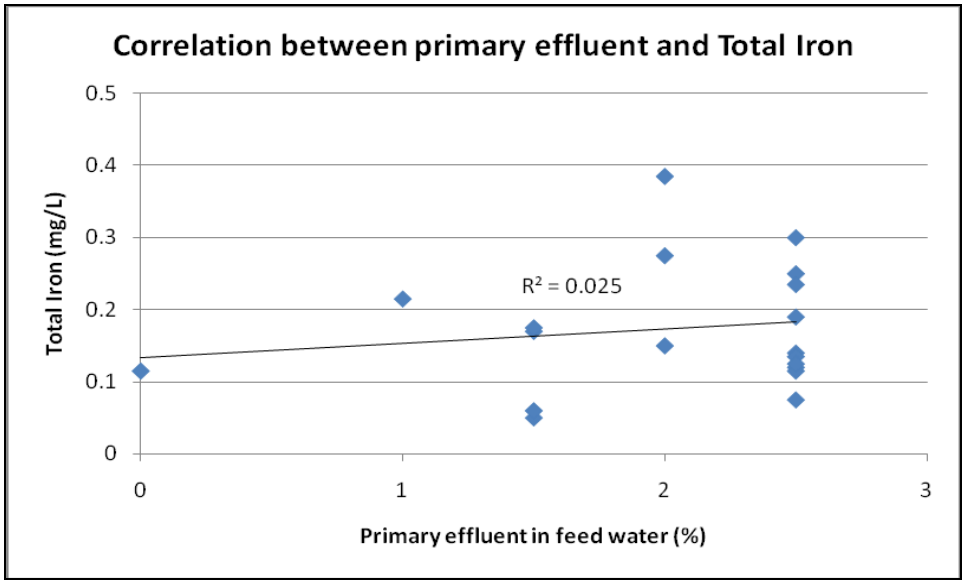

Figure A.2 Correlation between primary effluent content and the total iron concentration in the feed water

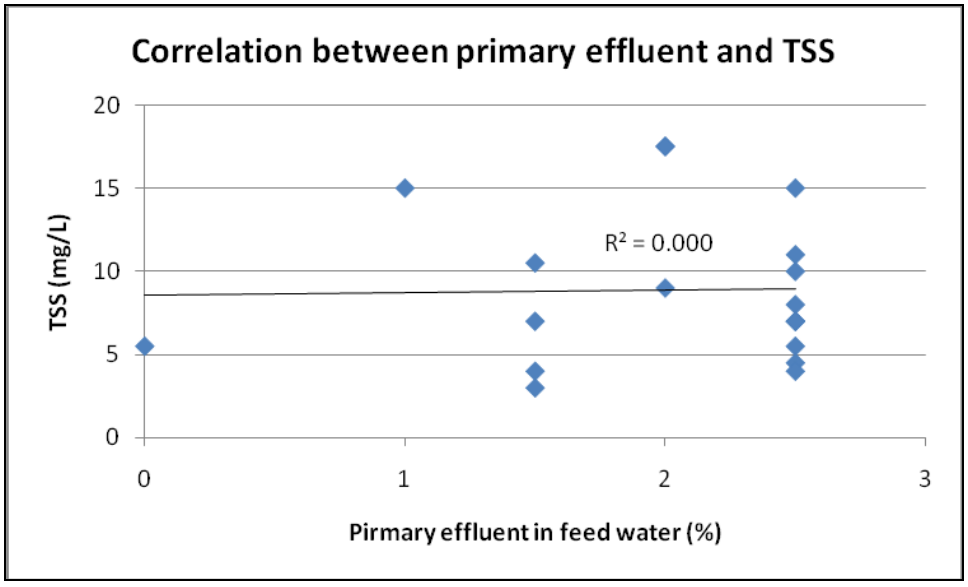

Figure A.3 Correlation between primary effluent content and the TSS in the feed water

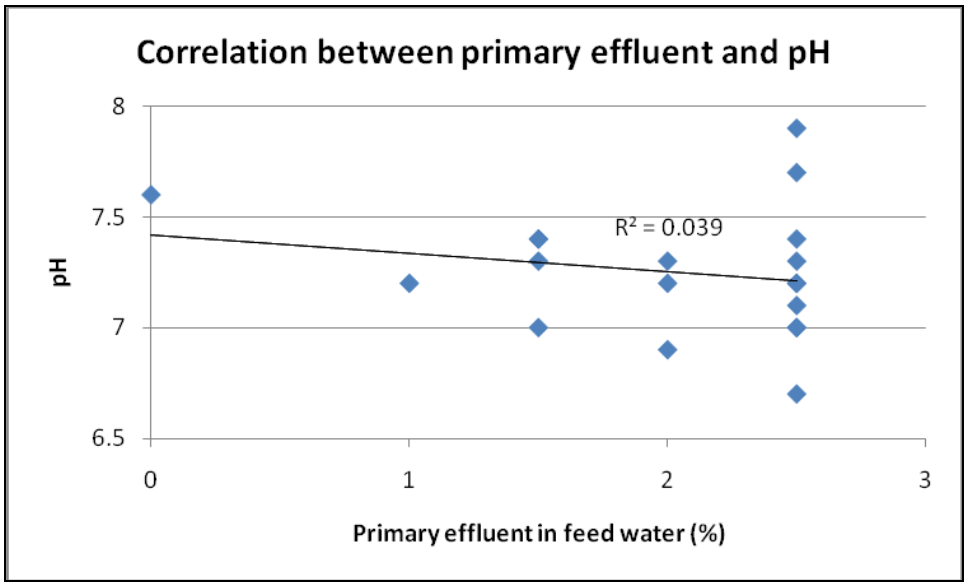

Figure A.4 Correlation between primary effluent content and the pH in the feed water

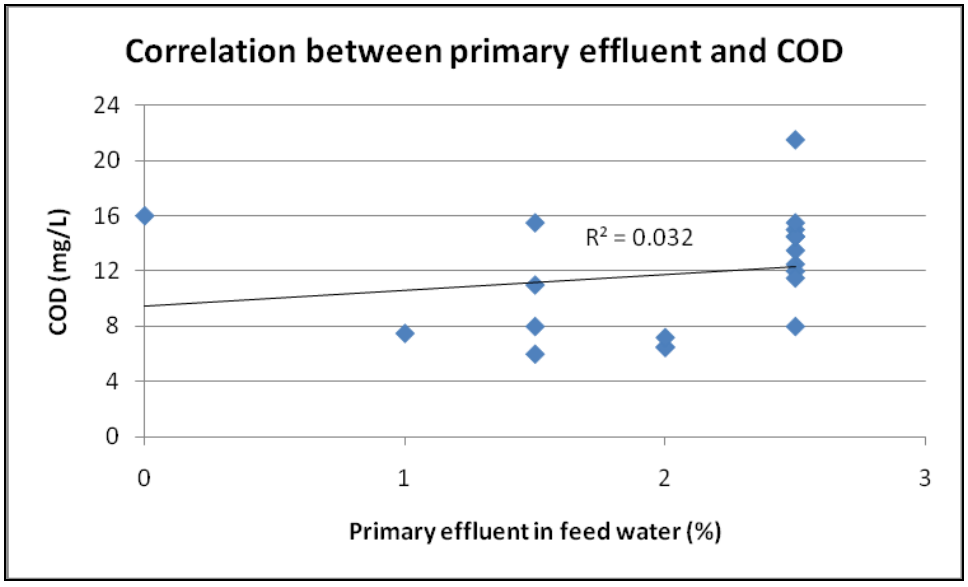

Figure A.5 Correlation between primary effluent and the COD in the feed water

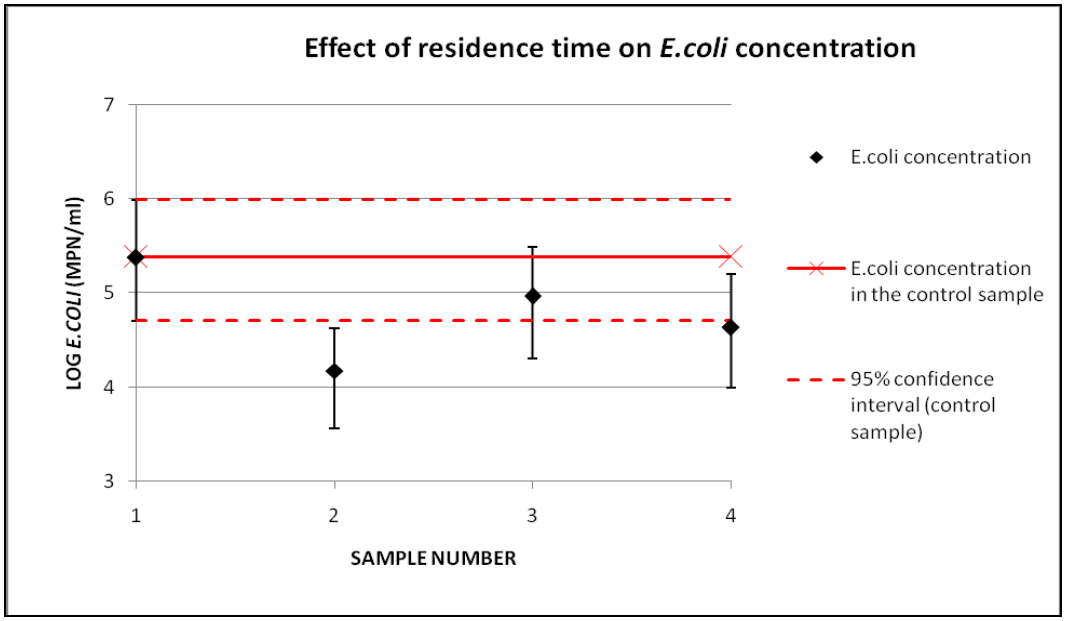

Figure A.6 Effect of residence time on *E.coli* removal (second experiment)

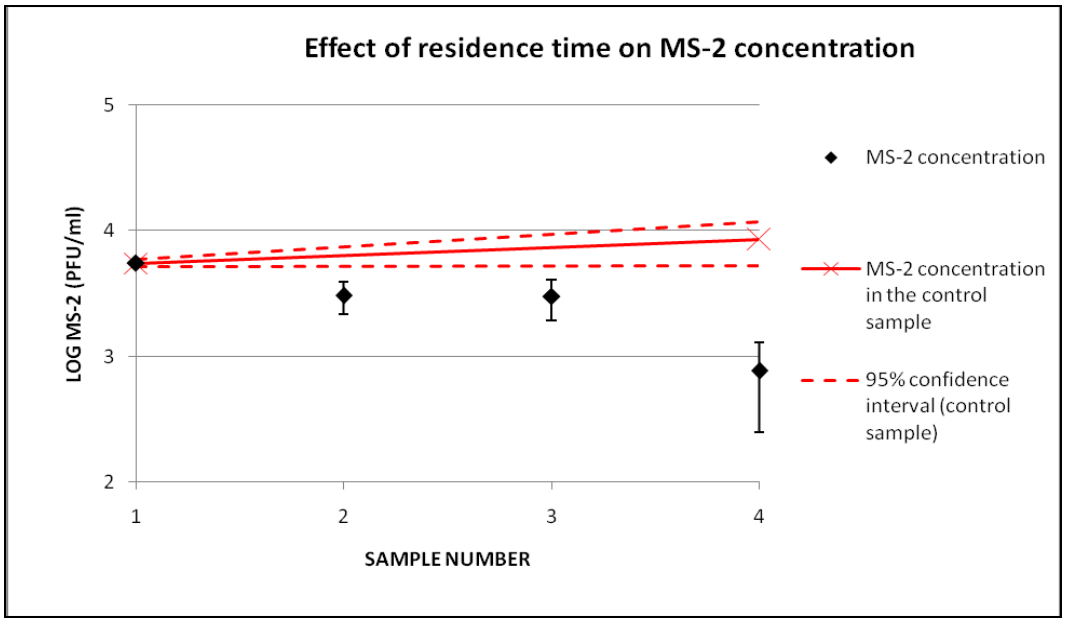

Figure A.7 Effect of residence time on MS-2 removal (second experiment)

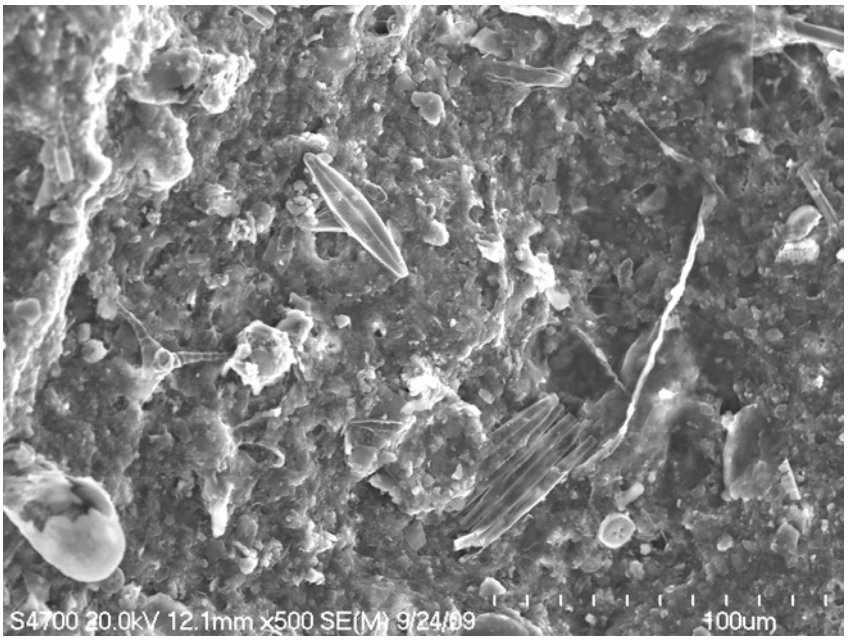

Figure A.8 SEM picture of the schmutzdecke material (1)

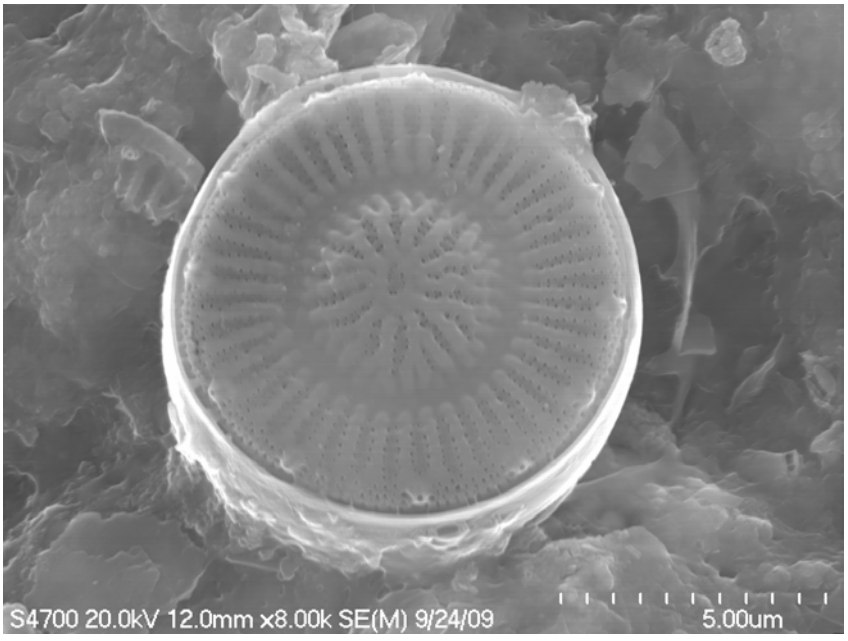

Figure A.9 SEM picture of the schmutzdecke material (2)

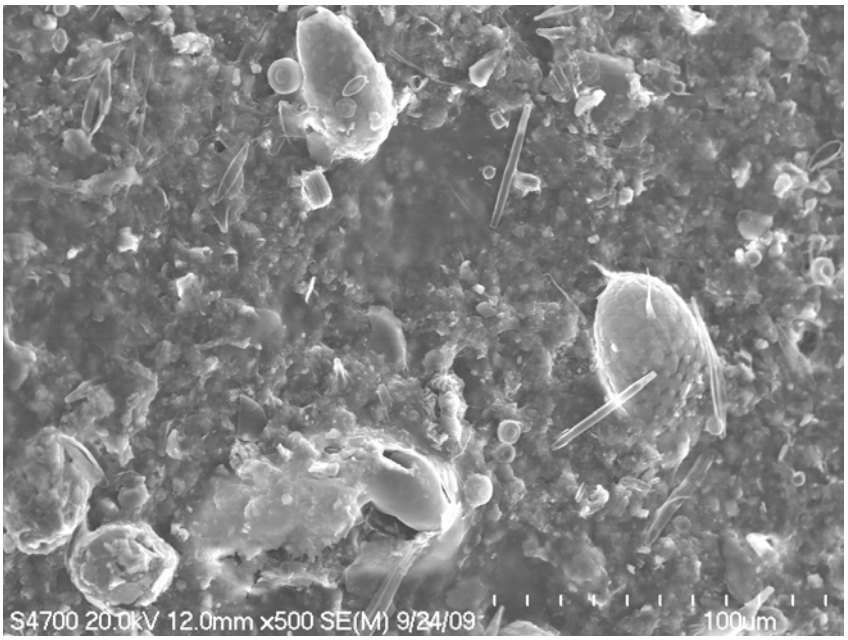

Figure A.10 SEM picture of the schmutzdecke material (3)

Figure A.11 SEM picture of the schmutzdecke material (4)

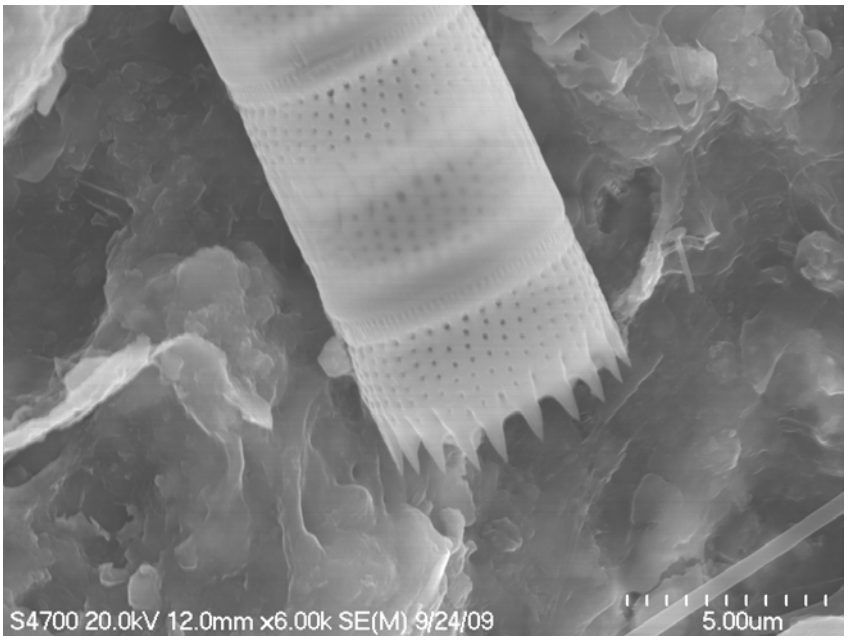

Figure A.12 SEM picture of the schmutzdecke material (5)

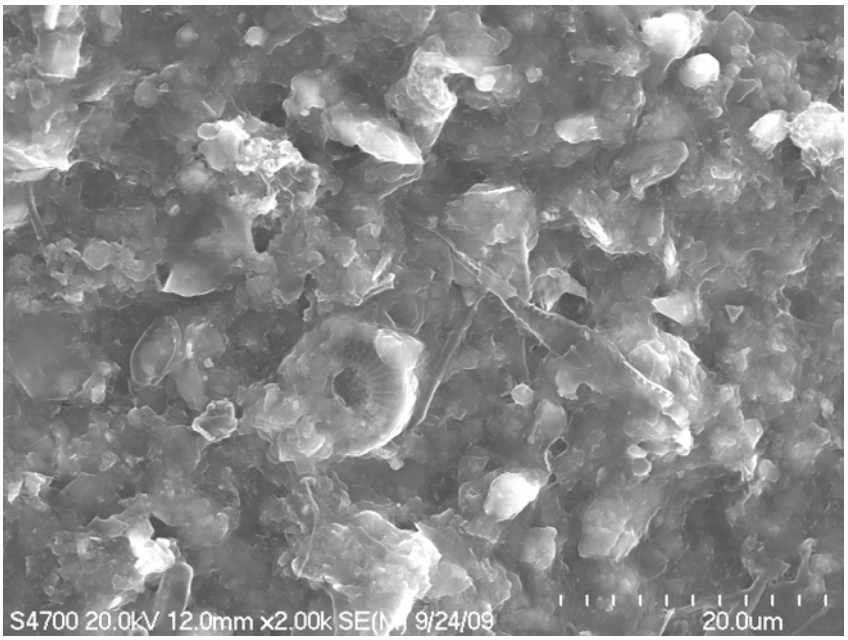

Figure A.14 SEM picture of the schmutzdecke material (7)

Figure A.15 SEM picture of the schmutzdecke material (8)

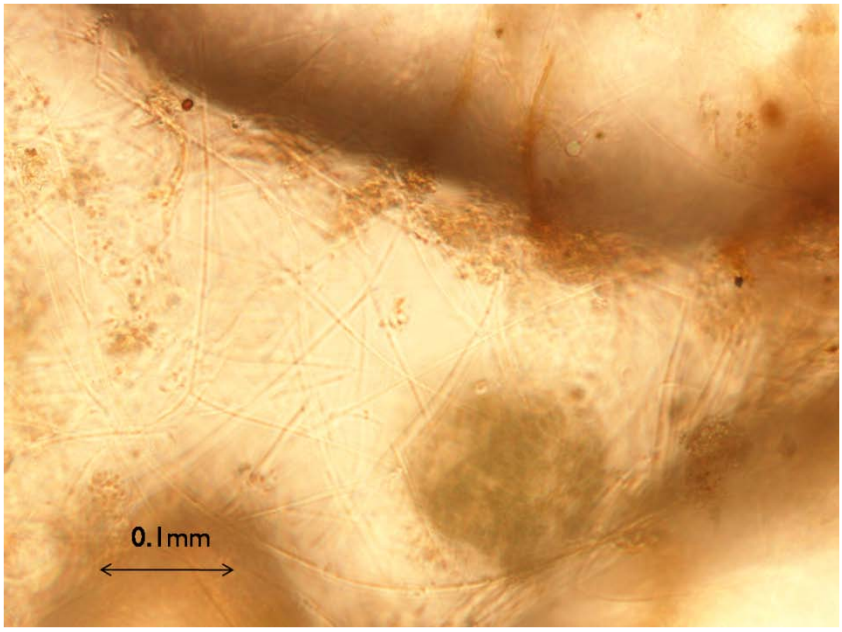

Figure A.16 "Net" of fungal hyphae stretching between the sand grains

Appendix B

ADDITIONAL TABLES AND FIGURES FOR CHAPTER 4

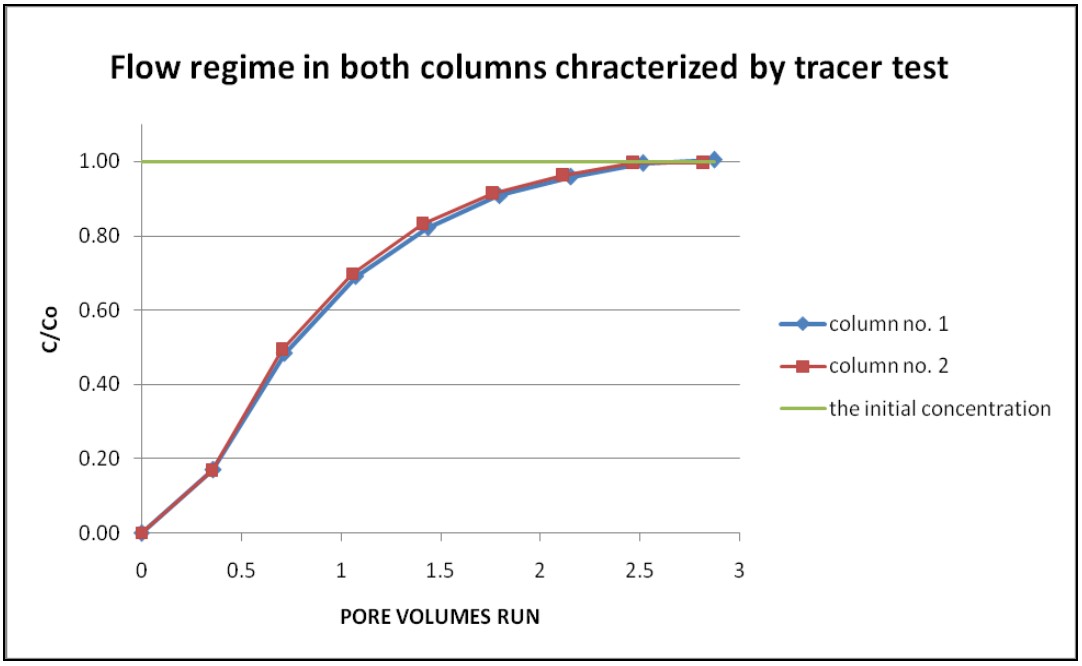

Figure B.1 Flow regime in both columns characterized by tracer curves (preliminary experiment)

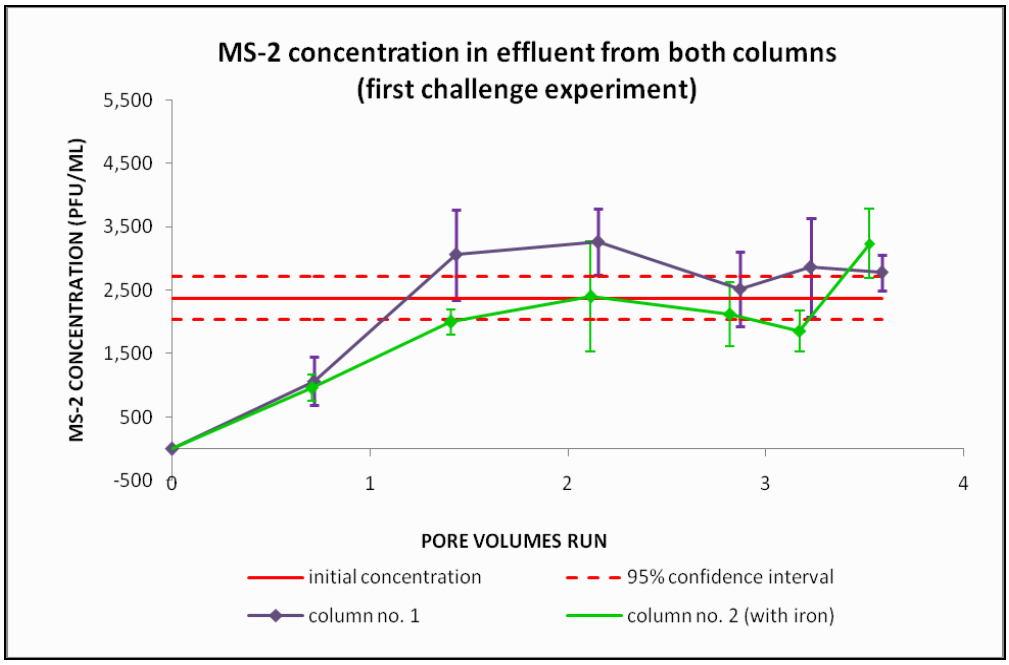

Figure B.2 MS-2 removal in both columns on the 2nd day of filters operation (preliminary experiment – samples analyzed one day after the experiment)

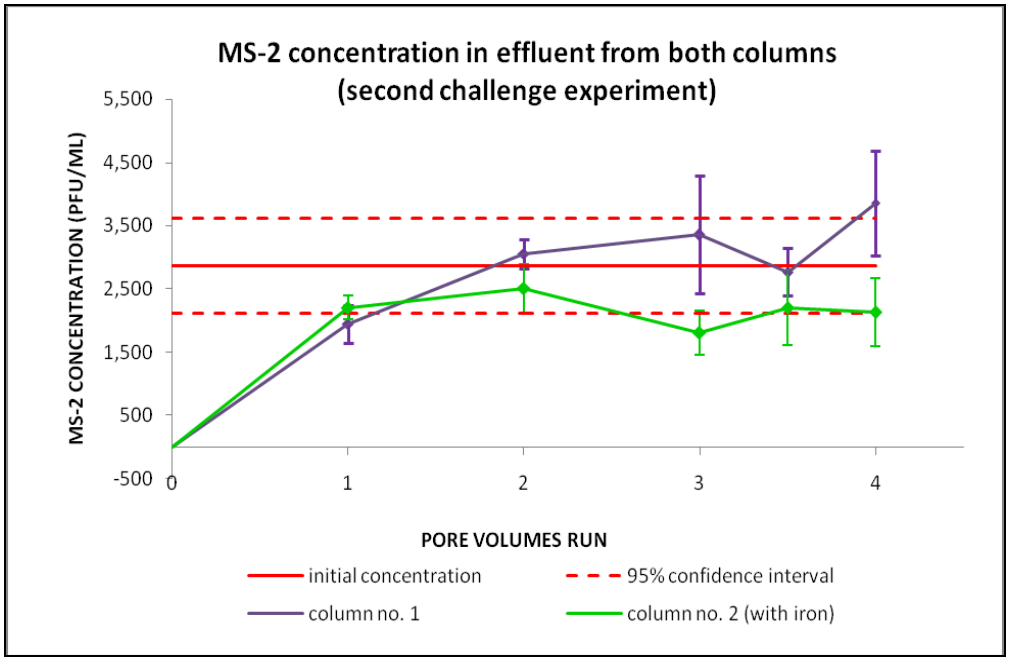

Figure B.3 MS-2 removal in both columns on the 10th day of filters operation (preliminary experiment – samples analyzed one day after the experiment)

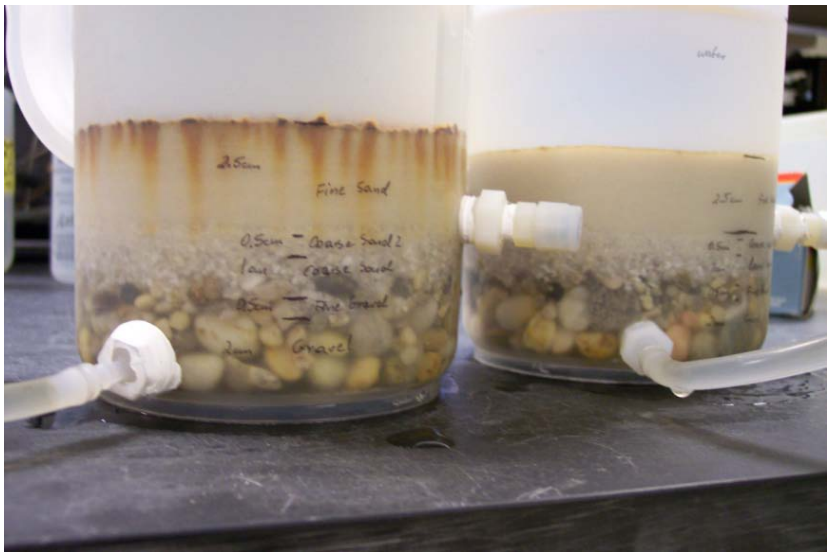

Figure B.4 Uneven ripening of sand below the iron filings – 1

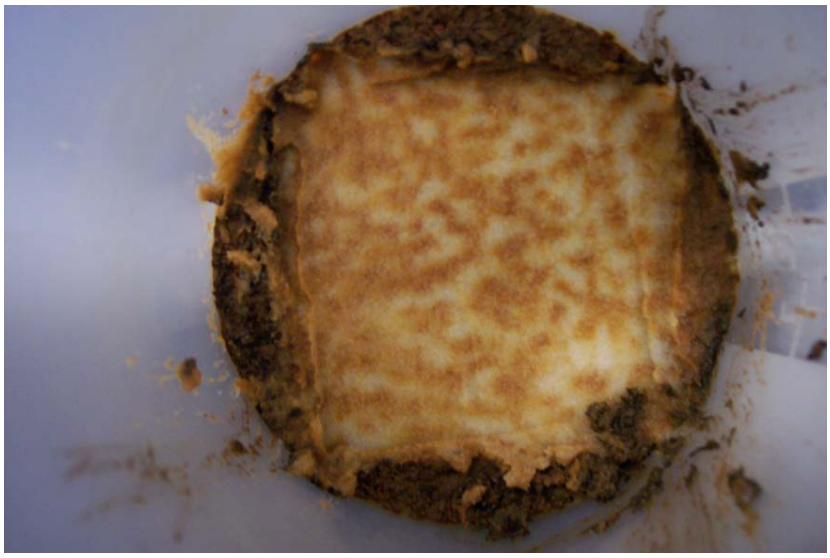

Figure B.5 Uneven ripening of sand below the iron filings – 2

Figure B.6 Pros and cons of different iron configurations in the column (Adapted from CAWST, 2008)

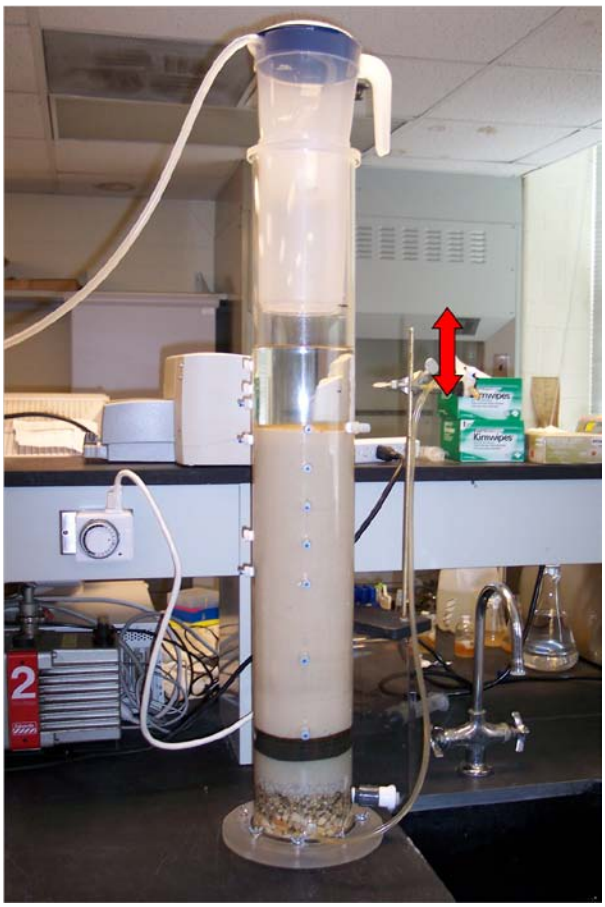

Figure B.7 Pressure head in the first challenge experiment

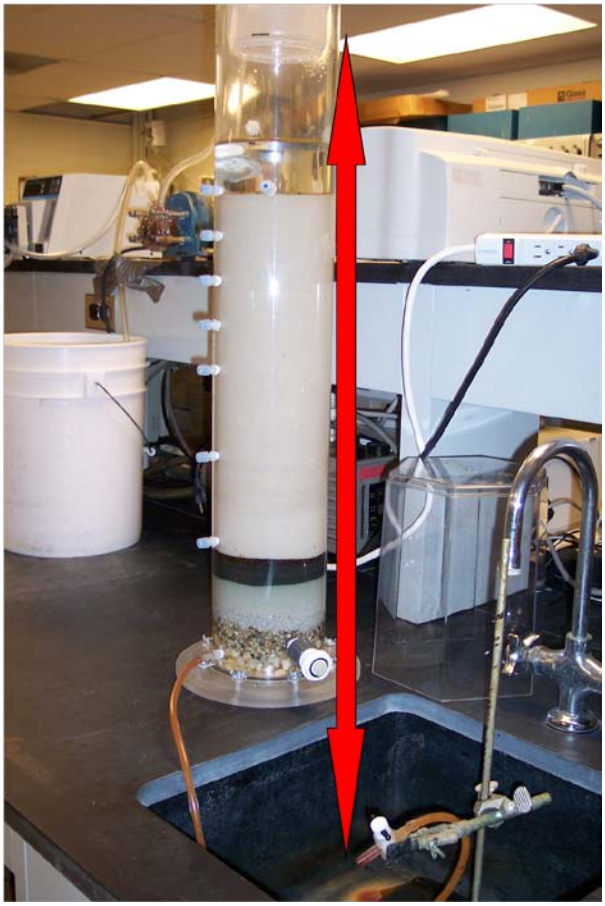

Figure B.8 Pressure head in the second challenge experiment

Appendix C

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