FILTRATION OF BACTERIAL COLIFORMS INCLUDING *Escherichia coli* IN WATER TREATED BY SLOW SAND FILTRATION

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ABSTRACT

Slow sand filtration is a method of water purification by means of percolating water through a column of fine granulated sediment and in some cases a layer of biological growth at the sand/water interface called the schmutzdecke. A series of nine slow sand filters (six of which were operational) were each connected to a main flow pipe which was attached to a roughing filter filled with gravel to remove solid waste from the untreated water. Raw untreated water was shunted through the roughing filter and then through each of the six operational slow sand filters for a period of roughly four months. The data indicate that there was a significantly greater amount of total coliforms including *Escherichia coli* present in the raw untreated reservoir water compared to the treated effluent water filtered by each of the six slow sand filters. However, because there was no observable schmutzdecke growth (studies show that the schmutzdecke aids in the removal of coliforms from untreated water (Weber-Shirk, Chan 2006)) in any of the slow sand filters, the significant removal of coliforms and *E. coli* from the raw untreated water must therefore be attributed to the kinetic adhesion of the bacterial cells to the sand particulates and the microscopic holes and divots located on sand particles. The three cleaning methods that were applied to the slow sand filters were harrowing, scraping and fabric application; however, there was no significant difference among the cleaning methods pertaining to the amount of coliforms present in their respective filtered water. Furthermore, the data suggest that there is no correlation between the influent flow rate (ml/s) of water into the slow sand filters and the prevalence of total coliforms including *E. coli* in the treated effluent water.

INTRODUCTION

Slow sand filtration is one of the earliest forms of water treatment and is still considered a very effective means of controlling and reducing water contamination (Rooklidge et al. 2004). Although the usage of slow sand filters have decreased in recent years due to the introduction of more technologically sophisticated filtration machinery, the efficacy of slow sand filtration to cleanse unpurified water still remains (McConnell et al. 1984). The discovery of the presence of harmful bacteria in aquatic systems has increased concerns for public health especially in rural communities where more advanced remediation techniques are not common (Rooklidge et al. 2004). Although slow sand filters lack the chemical treatment often administered in more modern filtration systems, their ability to remove not only solid matter but biological micro and macro organisms as well rivals that of chemically treated water systems. It's basic and simple design makes slow sand filtration a very inexpensive and efficient form of filtration which can be implemented in almost any environment provided there is a constant flow of raw untreated water. In addition to its simple design comes its unique ability to remove unwanted and harmful biological contaminants via mechanical obstruction by sand particulates and biological intervention through the schmutzdecke (Weber-Shirk, Chan 2006). During the slow sand filtration process, raw water which hasn't been treated flows by gravity through a column of sand on top of a layer of support gravel and flows out an underdrain collection grid where the effluent water is then shunted to some other means of containment (Rooklidge et al. 2004). As the sand becomes clogged and saturated with sediment from the raw water and as the biomass of the biological matter grows (more so in the schmutzdecke) the flow rates of these filters continues to decrease and the supernatant volume increases which are signs that the filters must be cleaned. One of the microorganisms of interest in this study involved the presence of coliforms in general and more specifically *Escherichia coli*. Although an in depth study regarding the efficacy of slow sand filters at removing microorganisms such as *E. coli* has not been has not been investigated, there seems to be much evidence of the proficient removal of such organisms through this method and it has been proposed that the schmutzdecke, which is a thick layer of biological growth at the top of the sand column, may be responsible for a large part of it (Campos et al. 2002). The specific objective of this study involved getting a better understanding of how slow sand filter coliform removal efficacy changed over time between cleaning intervals as the filters began developing the schmutzdecke and as the filters began clogging.

MATERIALS AND METHODS

Material for the filters. All nine slow sand filters were assembled using large plastic silo-like tanks set up in a series-like fashion. Each tank had polyvinyl chloride (PVC) piping connecting from each of the slow sand filter inlets to a main flow pipe which was connected to the roughing filter. At the inlets of each of the nine slow sand filters were valves to control the influent flow rate of roughing filter-treated water into each of the slow sand filters. A larger tank was used for the roughing filter which was connected directly to a hose which supplied the raw untreated reservoir water located at California Polytechnic State University in San Luis Obispo. Once the raw untreated reservoir water had been run through the roughing filter, it was then shunted through to the main flow pipe which fed the nine slow sand filters simultaneously. Once the water had been run through the slow sand filters, the effluent was transferred to a common effluent pipe in which all nine slow sand filters were connected to.

Slow sand filter design. Each of the nine slow sand filters contained an underdrain collection grid at the bottom of the tank made of PVC piping with small holes punched into it for the purpose of collecting the treated water that had percolated through the filter. On top of the collection grid was a small layer of coarse gravel which served as a protective agent for the small holes in the collection grid against the much finer sand that lay on top of the gravel which had the potential to clog the small holes. The much finer sand that lay on top of the gravel occupied roughly 95% of the tank and served as one of the primary means of filtration (mechanical filtration) for the slow sand filters. Above the sand was the water supernatant which flowed in through the main flow pipe.

Cleaning methods. Only six of the nine filters were used in the study due to technical difficulties. All of the slow sand filters were cleaned using three different methods: Harrowing (filters 3 and 5) which involved the plowing and thus homogenization of the top portion of the fine granulated sand below the supernatant; Scraping (filters 6 and 8) which involved removing a small portion of the top of the fine granulated sand using a shovel below the supernatant; and Fabric (filters 7 and 9) which involved the laying down of a piece of cotton fabric which served as a substrate for algal growth, which lay on top of the fine granulated sand and was removed at each cleaning.

Sampling technique. Water samples were taken weekly starting 8 November 2007 and ending 12 February 2008. 100ml samples of the effluent treated water from each of the six slow sand filters were stored in 120-ml vessels containing sodium thiosulfate and placed into a cooler for preservation until analyzed. A 100ml water sample was taken from the roughing filter and from the hose connecting to the reservoir which supplied the raw untreated water. The samples were then taken to be analyzed at the Environmental Biotechnology Institute (EBI) located at California Polytechnic State University, San Luis Obispo. A coliform detection reagent called Colilert®, manufactured by IDEXX Laboratories, was poured into each of the sample vessels and dissolved. After the Colilert® completely dissolved in each of the samples they were poured into separate coliform quantifying tools called Quanti-Tray® 2000 which are 97-well trays manufactured by IDEXX Laboratories Inc. Once the 100ml samples were poured into individual Quanti-Tray® 2000s they were then sealed by the Quanti-Tray® sealer and placed into an incubator at 35°C for roughly 24 hours.

Analysis. The Quanti-Tray® 2000 contains 49 large wells and 48 small wells all of which were occupied by sample water. Yellow wells indicated a positive for the presence of coliforms and a most probable number (MPN) was calculated based on the number of positives in large wells and small wells. A long wave ultraviolet lamp (365nm) was utilized for the specific detection of *E. coli* in the sample. Any of the wells that fluoresced under ultraviolet light was a positive for *E. coli* and an MPN was calculated based on the number of large and small wells fluorescing.

RESULTS

Each of the six of the slow sand filters produced treated water which had a significantly lower Most Probable Number (MPN) of total coliforms present in their respective effluent treated water than the raw untreated water from the reservoir and as a result all three cleaning methods implemented on the filters produced treated water that contained statistically less coliforms than that of the raw untreated water (Table 1). However, there were no statistically different MPN values of total *E. coli* present in effluent treated water for each of the six slow sand filters and the raw untreated water.

MPN data arrays used in t-test	*P* value Total Coliform	*P* value *E. coli*
Raw-Filter 3	0.021	0.25
Raw-Filter 5	0.020	0.25
Raw-Filter 6	0.022	0.26
Raw-Filter 7	0.020	0.26
Raw-Filter 8	0.020	0.26
Raw-Filter 9	0.020	0.26
Raw-Roughing	0.075	0.66

Table 1. *P* values calculated from MPN data arrays from raw water and each of the six filters.

The greatest average MPN for total coliform observed was in water treated by filter 6 (cleaned by Scrape) which had a value of 14.12 MPN/100ml and the lowest average MPN for total coliform observed was for water treated by filter 5 (cleaned by Harrow) which had a value of 2.80 MPN/100ml.

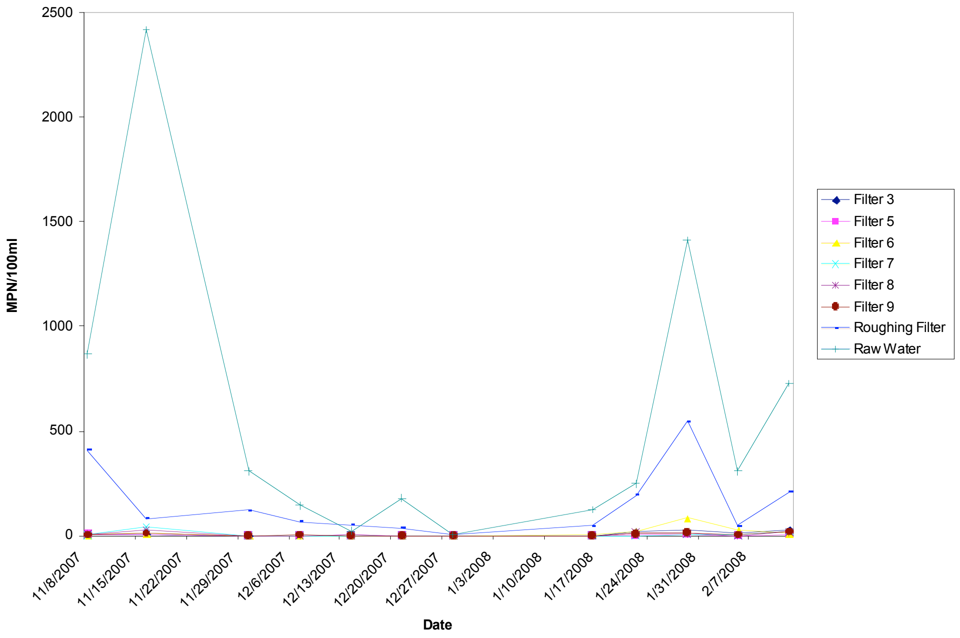

Figure 1.

Total coliform CFU/100ml for all six slow sand filters and for raw untreated water and roughing filter water.

On the other hand the greatest average MPN for total *E. coli* observed was in water treated by filter 8 (cleaned by Scrape) which had a value of 0.684 MPN/100ml and the lowest average MPN for total *E. coli* was in water treated by filter 3 (cleaned by Harrow) which had a value of 0.167 MPN/100ml.

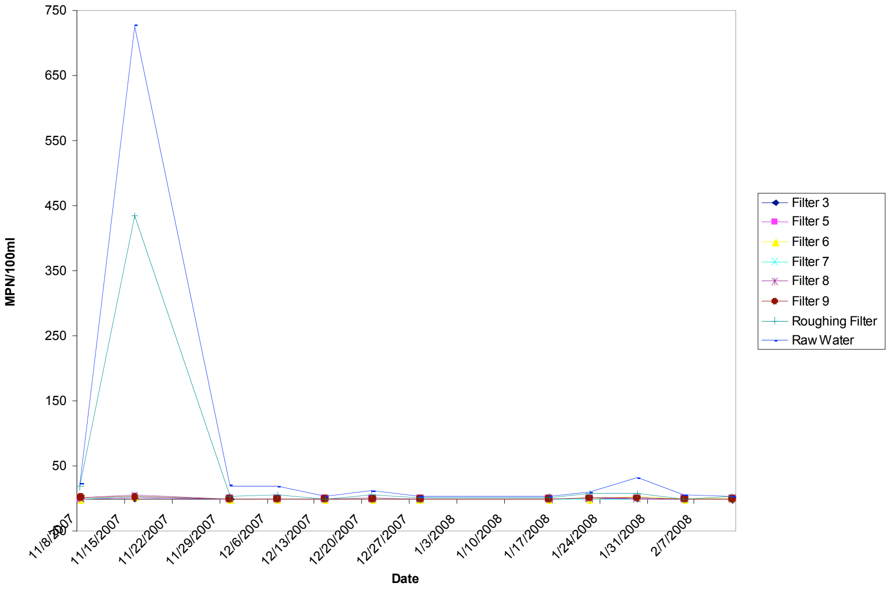

Figure 2.

Total *E. coli* CFU/100ml for all six slow sand filters and for raw untreated water and roughing filter water.

Between all three cleaning methods implemented, scraping had the greatest average MPN with a value of 10.34 MPN/100ml and harrowing had the least average MPN with a value of 5.62 MPN/100ml. The same trend seemed to be true with *E. coli* prevalence with scraping producing treated water containing the greatest average *E. coli* MPN with a value of 0.638 MPN/100ml and harrowing producing treated water containing the least average *E. coli* MPN with a value of 0.209 MPN/100ml.

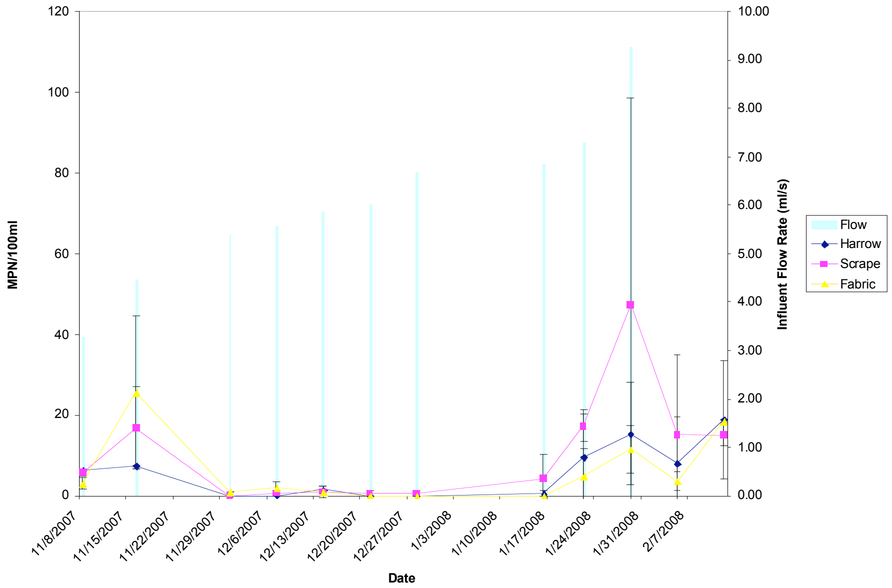

Figure 3.

Total coliform CFU/100ml for each cleaning method taking into account the average influent flow rate (ml/s). Error bars are standard deviations of the mean.

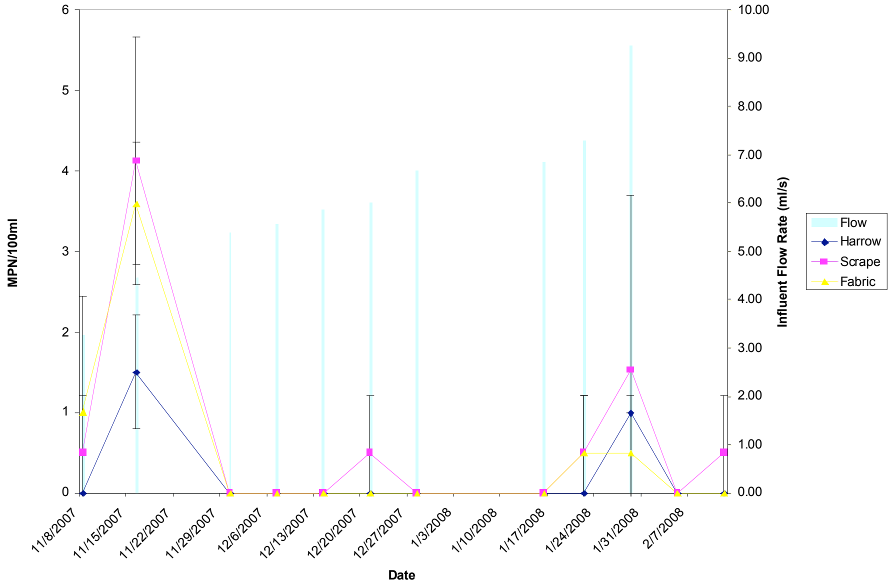

Figure 4.

Total *E. coli* CFU/100ml for each cleaning method taking into account average influent flow rate (ml/s). Error bars are standard deviations of the mean.

Further calculation indicated that there is no statistically significant difference between the cleaning methods and the efficiency of removing coliforms determined by a student's T-test with an alpha level of 0.05.

Total Coliform	*P* value	Total *E.coli*	*P* value
Harrow-Scrape	0.30	Harrow-Scrape	0.27
Harrow-Fabric	0.92	Harrow-Fabric	0.45
Scrape-Fabric	0.35	Scrape-Fabric	0.71

Table 2.

P values for all each filter against one another for total coliform and total *E. coli*.

In addition, there seemed to be no trend pertaining to the influent flow rate of raw untreated water into each of the filters and the removal of coliforms including *E. coli*. An average influent flow rate of 5.39 ml/s resulted in the greatest number of coliforms and an average influent flow rate of 4.48 ml/s resulted in the least amount of coliforms. The roughing filter treated water that did not have a significant difference in total coliform and total *E. coli* MPN with that of raw untreated water (*P* value = 0.07). No observable schmutzdecke growth was observed even after the filters had been running for roughly four months.

DISCUSSION

It has been proposed that the schmutzdecke is partly responsible for removal of coliforms (Campos et al. 2002), however no schmutzdecke ever developed in any of the six slow sand filters over the course of the study and therefore no correlation between coliform removal and schmutzdecke development could be inferred. The removal of coliforms including *E. coli* from raw untreated water must therefore be attributed to some other mechanism inside the slow sand filters that remains unseen and that lies within the sand column itself. The causative agent most likely responsible for the vast amount of coliform removal from untreated water within the slow sand filters is kinetic adhesion of the bacterial cells to the sand itself. A number of variables are responsible for the adhesion of cells to the particulates within the sand column including Lifshitz-van der Waals, Lewis acid-base and electrostatic interactions. The shape and size of the bacterial cell also contributes to the efficacy of particulate adhesion including the porous media that is used (Jacobs et al. 2007). A study conducted by Jacobs et al. measured the zeta potentials from sand and bacteria in an effort to study their interaction. Although negative zeta potentials between the sand and bacteria resulted in overall electrostatic repulsion and therefore delayed bacterial adhesion, it was observed that sand, on a very microscopic level, contains “caves and valleys” in which microorganisms have enhanced collision and wedging tendencies. The vast variety of physiochemical bacterial strains that Jacobs et al. used in their study illustrates how both surface thermodynamics and porous media effects influence the overall bacterial adhesion to sand particulates (Jacobs et al. 2007). The data that Jacobs et al. obtained from their bacteria-sand kinetic adhesion study explain the mechanism that removed much of the bacterial coliforms from the untreated water that was shunted through each of the slow sand filters and how a multitude of electrostatic forces and mechanical wedging influenced the filtration.

The influent flow rate (ml/s) of raw untreated water being shunted into the slow sand filters doesn't seem to have any correlation with the prevalence of coliforms including *E. coli* in the effluent treated water. According to both figures 3 and 4, there is no trend that indicates that as the influent flow rate increases, the abundance of coliforms and *E. coli* also increases and vice versa. In the data analyses of both total coliforms and *E. coli*, there are two peaks in CFU/100ml on 11 November 2007 and 29 January 2008, however the average influent flow rates on these days are quite different: 4.48ml/s being the average influent flow rate on November 11, and 9.28ml/s being the average influent flow rate on January 29. Furthermore, the average influent flow rate steadily increased throughout the sampling period and even with this increasing variation in the influent flow rate, there is still no discernable trend indicating that influent flow rate affected coliform abundance in treated slow sand filter water.

Slow sand filter cleaning methods seemed to have no significant efficiency over one another in regards to the prevalence of coliforms in their respective treated water as compared to raw untreated water. Water from all three methods of cleaning (harrow, scrape and fabric) was tested using a student's t-test analysis and all produced *P* values much greater than the alpha level of 0.05 (Table 2). Although it seems that the scraping and fabric methods have greater quantities of coliforms and *E. coli* throughout the sampling period, all three follow the same general trend of coliform abundance over time and are not different enough to consider them statistically different from one another.

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