BREAKTHROUGH OF INDICATOR ORGANISMS FROM SLOW SAND FILTERS AS PART OF A DRINKING WATER PRODUCTION SYSTEM FOR SUB-SAHARAN AFRICA

by

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of a thesis submitted by

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DEDICATION

I very humbly dedicate this work to all the needy people still lacking access to the most basic life need: potable water.

ACKNOWLEDGEMENT

This work wouldn't be done without the great support of many people who dedicated their energy, time, and money to my education in the U.S. I am very grateful to all of those starting with Dr. Warren Jones whose support, patience and generosity will be remembered forever. The same acknowledgement goes to Dr. Anne Camper and her lab's group who adopted me memorably, the CBE community, Dr. Florence Dunkel and her husband Bob Diggs, Dr. Kadidiatou Toure Gamby and many other, here unnamed, nice people on the MSU campus.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

ABSTRACT

Sub-Saharan Africa has the lowest proportion of population in the world with access to potable water, so that there is a dire need for low-cost, low-energy robust treatment technologies for drinking water. Constructed wetlands followed by optimized slow sand filtration has the potential for improving raw surface water quality to an acceptable level. A laboratory study examined the removal of *Enterococcus durans* and environmental coliforms with associated heterotrophic bacteria from slow sand columns operating with different sand sizes and flow conditions. *E. durans* removal far exceeded 90% in most systems, with better performance from a sand column with finer sand size (0.425 mm) and continuous flow. A column with 0.425 mm sand also performed better than a column with 0.6 mm when both were operated intermittently. Removal of environmental coliforms followed the same trends as observed with *E. durans,* but with roughly four times the overall breakthrough. Correction of the column breakthrough data to remove the effect of decay observed in control columns demonstrated that, in most cases, *E. durans* removal was accomplished by more than static loss of culturability. In the case of the environmental coliforms, corrected breakthrough was still below 100%, but much higher than with the *E. durans,* suggesting that extrapolation of results from a single species could produce erroneous estimates of removal of other organisms.

INTRODUCTION

Many Sub-Saharan Africa countries suffer for lack of a good drinking water supply system. In fact, Sub-Saharan Africa is the region in the world with the lowest level of improved drinking water coverage (UNDP, 2006). The problem is more crucial in small towns and villages where the resources are not available to implement classical drinking water treatment systems requiring "sophisticated" technologies, and the villages and countless camps along the Niger River in Mali are no exception. The problem is exacerbated by the practice during dry season, while the river is at its lowest level, of many fishermen and herdspeople (with many hundred thousands of cattle) settling the riparian zones. This practice is especially acute in the Inland Niger Delta, known as one of the biggest natural wetlands in the world. These settlements leave significant waste organics in that zone. In addition, all the wastewater from various sources of industrial pollution (from cities) and agricultural fertilizers and pesticides flow into the river upstream of the wetland area. The combination of these two main phenomena produces a dire situation for villagers living along the river who get their drinking water without any treatment, particularly at the beginning of rainy season. As the water level increases, the river picks up waste organics, dissolved chemicals and different kinds of trash with their quota of harmful microorganisms. Consequently, at the same period of every year there is outbreak of diarrheic diseases in the population from those areas. Morbidity and mortality are high.

There is a pressing need for low-cost, low-technology drinking water treatment systems as alternatives to classical systems that could reduce the threat of waterborne diseases in such poor communities. Constructed wetlands have been proven, as wastewater treatment systems, to remove organic content, solids content, and significant microorganism loads from water. Slow sand filters have been shown to further provide excellent treatment when operated with a consistent feed water quality. Therefore, the potential may exist to supplement constructed wetland systems with gravel and slow sand filters that could do the polishing operation necessary to produce potable water.

Interest Zone: the Inner Niger Delta

The Inner Niger Delta (also called Inland Niger Delta) is one of the largest riverine floodplains in the world, a natural wetland covering an area of about 4,119,500 ha. Located in the central part of Mali in a semi-arid Sahelian zone, just south of the Sahara Desert, rainfall in the Inland Niger Delta is very limited, so that it is highly dependent on the Niger River flooding (see Figure 1).

Annual flooding in the delta enabled farming, livestock and fishing in the area for centuries. Nowadays, a combination of factors such as diminishing river floods, drought, and increasing population size has greatly magnified the pressure on the wetland system. More than a million people live seasonally in the Inner Delta and its riparian zones as farmers, cattle breeders and fishermen. More than 70,000 ha in this region are being exploited for rice production with its quota of tons of fertilizers and pesticides. More than

5 million head of cattle and hundreds of thousands of sheep and goats pasture the grassland of the zone in low flood season leaving behind them huge amounts of animal waste. Some 80 000 fishermen (rasmar.org, 2006) produce other kinds of wastes, and these are added to other wastes and wastewater drained into the river from cities and towns (Bamako, Koulikoro, Segou, …) upstream. All of these contributions degrade the quality of the water in the Delta during the dry season. One may easily deduce how polluted the water in the Delta can be, especially at the beginning of the rainy season coinciding with the rising of the river while reoccupying its riparian zones. The dissolution of all the diverse matters occurs at that period, leading to the resuspension of various microorganisms and thus waterborne pathogens. All along its length, the Niger River provides -- directly or indirectly through subterranean sources fed by its waters -- drinking water supplies to the population alongside. Whereas larger cities and towns have good facilities to make their water potable, numerous villages and countless camps alongside the River and in the Inland Delta have no water treatment system. Hence, there is an annual outbreak of waterborne diseases such as cholera, typhoid, gastroenteritis and other dysenteric diseases, causing high rate of morbidity and mortality among children and other immunodeficient people.

It becomes obvious that any low-cost, low-technology water treatment system able to significantly reduce pathogens may be an alternative to explore for the above affected population in the Inner Niger Delta. Constructed wetlands (CW) combined with a slow sand filter might be a good option but still needed to be thoroughly examined.

Experimental trials in the greenhouses at MSU failed to produce a way to realistically scale down a model constructed wetland for microbial transport studies, so that in this thesis, the study of the potential for CW as water pretreatment for microbial removal was limited to a literature review. The laboratory portion of this study was focused on the performance of slow sand filters (SSF) operated in a fashion that might be expected in a rural setting. Background information about SSF will follow the material on CW prior to presentation of the experimental SSF results.

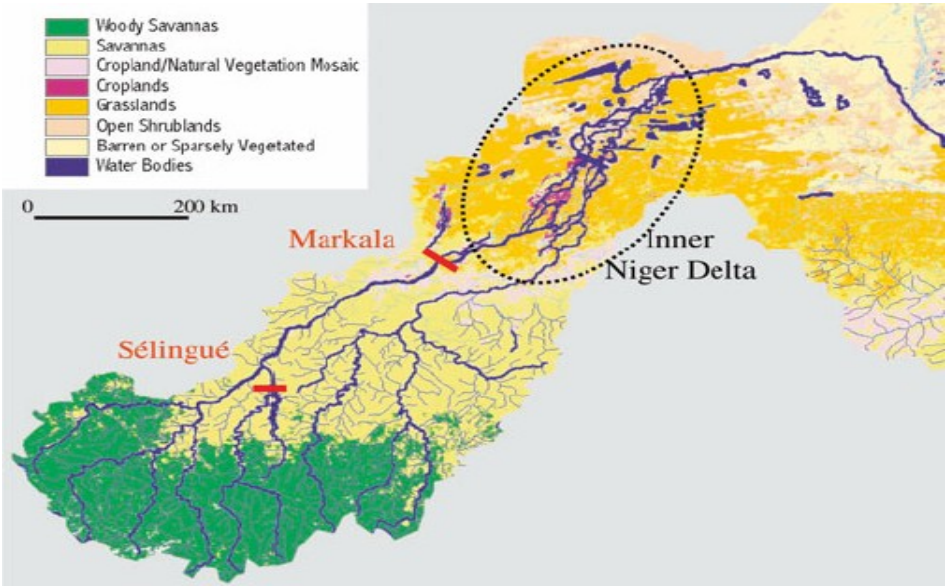

Figure 1. Map of the Inner Niger Delta, Mali

(http://www.waterfoodecosystems.nl/?page=1890)

Removal of Microorganisms in Constructed Wetlands

According to Khatiwada and Polprasert (1999): *"Research conducted in the past has shown that constructed wetlands are effective in removing pathogenic micro-organisms such as bacteria and viruses."*

The removal of organic content, suspended matter and microorganisms for water quality improvement through wetlands has been demonstrated to involve different factors and mechanisms. In particular, the fate of pathogens -- and by extension all other microorganisms in wetlands -- is controlled by physical factors including filtration, sedimentation, aggregation and ultra-violet light exposure; biological mechanisms such as antibiosis, ingestion by nematodes, protozoans or cladocera, lytic bacteria and bacteriophage attacks and natural death; and chemical factors: oxidation, adsorption and exposure to toxins given off by other microorganisms and plants (Gersberg et al., 1989 and Vincent et al., 1994 reported by Ottova et al., 1997; Khatiwada and Polprasert, 1999; Thurston et al., 2001) .

In the next few paragraphs the results of studies on the removal of microorganisms of public health significance in different types of wetlands are reported.

Removal of Coliforms, Fecal Coliforms

Different studies have been done upon the removal of coliforms and fecal coliforms in wetlands used as wastewater treatment systems. In a study conducted in five different subsurface flow constructed wetlands in the Czech Republic, removals of

coliforms and fecal coliforms averaged 96.3 and 98.2%, respectively (Ottova et al., 1997). Average removal of total coliforms and fecal coliforms at rates of 98.8% and 98.2% have been found in a subsurface flow constructed wetland receiving secondary sewage effluent (Thurston et al., 2001). In two subsurface flow constructed wetlands designed to remove contaminants from dairy wastewater, total coliforms and fecal coliforms were reduced by an average of 99% and 99.9%, respectively, between the raw wastewater pond and the wetland effluent (Ibekwe et al., 2003). Those two systems consisted of beds containing coarse and finer gravel plus a bed of reeds (*Phragmites communus*) and bulrush (*Scirpus validus*). A natural wetland in the semi-arid region of Northeastern Brazil comprising a flood plain on a river covered with *Typha spp, Eicchornia crassipes,* and *Juncus spp* was shown to remove 89% to 91% of fecal coliforms with 5 to 10 days of hydraulic retention time (HRT) during six weeks of observation (de Ceballos et al., 2001). Twelve tanks of subsurface horizontal-flow constructed wetlands with thick stone gravel beds planted with *Typha spp* from the natural wetland and fed with its effluent also showed 90% removal of fecal coliforms during a 12 week period. In a field-scale surface flow constructed wetland, designed to treat swine wastewater with a HRT of 4 to 17 days and planted with *Spariganium americanuum* and *Typha angustifolia* and *Typha latifolia*, 96% of *Salmonella*, 98% of *fecal coliforms*, and 99% of *E. coli* were removed (Hill and Sobsey, 2001). For the same swine wastewater treatment at laboratory-scale, a free water surface wetland reactor removed 70% of *Salmonella*, 90% of fecal coliforms and 90% of *E. coli* whereas

subsurface flow constructed wetland reactor removed 80% of *Salmonella* and 90% of fecal coliforms; results for *E. coli* were not given. Both systems operated at 2 to 4 days of HRT.

In a full-scale constructed wastewater wetland in Sweden planted with *Carex sp*, *Phragmites australis* and *Elodea Canadensis* and transected with sediment traps, fecal coliforms were reduced by up to 99.9% with a HRT of 7 days (Stenström and Carlander, 2001). In the system, settleable particulates were captured in sediment traps placed in transects across the wetlands. According to the authors, the fecal microorganisms analyzed (fecal coliforms and enterococci) showed prolonged survival times in the sediment. Thus, there is a potential for re-suspension of those microbes in the water. However, sedimentation of particles and associated organisms is reported to be a critical factor for the reduction of microorganisms from the water phase in the wetland.

In a multi-species (bulrush, cattail, black willow and cottonwood) wetland system (the water flow system: subsurface or open water and HRT not specified), Karpiscak et al. (1996, as cited by Karim, et al, 2004)) found a removal of 98% and 93% of total and fecal coliforms, respectively. In the same report, reductions of 57% of total coliforms and 62% of fecal coliforms from a duckweed-based wetland system were found. These results appear to be relatively low compared to the other total and fecal coliform reductions through different types of wetlands reported by many authors. This could be a result of the relatively shallow depth of root material present in duckweed compared to other plant species.

Coliphages and Bacteriophages

Removals of coliphage and bacteriophage have been studied by several groups. The interest in these viruses is due, in part, to their potential use as a surrogate for viruses pathogenic to humans.

Thurston, et al (2001) studied two subsurface flow wetland cells planted with cattail (*Typha domingenisis*), bulrush (*Scirpus olneyi*), black willow (*Salix nigra*) and cottonwood (*Populus fremontii)*. One received disinfected groundwater while the other received unchlorinated secondary treated wastewater that had previously flowed through a duckweed-covered pond. For average detention times of 3.8 days in both of multi-species wetland cells and 6.6 days for the wastewater in the pond, coliphage with an influent concentration of $2.5x10^2$ PFU/ml was reduced by 95.2%.

In a study mentioned previously (de Ceballos et al., 2001) both coliphage and bacteriophage removals reached 94% in the natural wetland at a final concentration of $4x10^4$ and $8x10^4$ PFU/ml respectively, whereas the constructed wetland showed a removal up to 96%. The Swedish full-scale constructed wastewater wetland mentioned previously showed coliphage reductions of approximately 70% for 7 days of HRT (Stenström and Carlendar, 2001).

Thirty eight percent of coliphage have been observed by Karpiscak et al. to be removed from a duckweed-based wetland system (Karim et al., 2004). The highly variable removals documented in these studies suggest that removal mechanisms can vary significantly.

Giardia and *Cryptosporidium*

Protozoan parasites, mainly *Giardia* and *Cryptosporidium*, have been the subject of many studies in wetlands by different authors.

From the same subsurface flow wetland mentioned above, Thurston et al. (2001) found an average reduction of 87.8% and 64.2% for *Giardia* cysts and *Cryptosporidium* oocysts, respectively. Karim et al. (2004) reported a removal of 98% of *Giardia* and 87% of *Cryptosporidium* observed by Karpiscak et al. in duckweed-based wetland system. In the same report Karpiscak et al. reported 73% and 58% of *Giardia* and *Cryptosporidium* reduction from a multi-species subsurface flow wetland comprised of bulrush, cattail, black willow, and cottonwood.

Furthermore, wetlands have been demonstrated to remove many other types of microorganisms including fecal enterococci, *Clostridium perfringens* (Stenström and Carlendar, 2001) as well as more than 70% of various chemical compounds such as ammonia, nitrogen, and phosphorus (Ibekwe et al., 2003; de Ceballos et al., 2001).

In light of the results presented above, it is clear that constructed wetlands can produce significant removal of various pathogenic microorganisms ranging from viruses to protozoa. Plants, gravels and hydraulic retention time play important roles in the removal efficiency. Thus, there is a real potential for a properly designed and operated CW to provide consistently stable water that may be used as influent to a SSF system for drinking water treatment.

Removal of Microorganisms in Slow Sand Filtration

Slow Sand Filtration (SSF) has been used in drinking water treatment since in the early 19th century when first investigated by the Scotsman John Gibb. Long considered as a mechanical process to strain out turbidity and suspended matters from drinking water, SSF's biological filtration capability was established with the discoveries of pathogenic bacteria by Pasteur, Koch, Escherich , and others during the 1860s and 1870s (Huisman and Wood, 1974). Although biological interactions within sand filters are only partially understood, there is no doubt about the ability of SSF to significantly remove waterborne microorganisms without use of chemicals in drinking water treatment (Graham and Collins, 1996). This property of SSF makes it a low-cost, simple technology suitable for drinking water treatment in rural communities like those alongside the Niger River where sand is an abundant natural material.

SSF have been known to be effective for removal of different types of microorganisms since their early application in drinking water treatment. Indeed, Graham and Collins (1996) cite Hendricks et al. (1991) to have found 2-log to 4-log removal of pathogenic viruses, bacteria and cysts in a mature filter-bed. The bed was considered mature when the schmutzdecke -- the biological top layer resulting from the degradation of organic particles of the raw water-- is formed on the top of the bed and biofilm is well-developed through all its thickness.

Under optimum temperature conditions for microorganism growth, Bellamy et al. (1985) and Bryck et al. (1987) have been cited in the book edited by Gary Logsdon

(1991) to have found percentage removals for total coliforms greater than 99% for mature filters, whereas percentage removal may be less than 60% for immature filters (when filters are first put into operation). Still in Logsdon (1991), Tanner has been cited to have found average removals of 90% and 99.9% for total coliforms and fecal coliforms, respectively, in a study done on three SSFs in Northern Dakota in 1987. The experimental conditions were not given.

The biosand filter (BSF), a household-scale slow sand filter system operating intermittently, gave an *E. coli* reduction of 90% at filter startup and 95-99.5% when the sand bed was ripened (Elliott et al., 2006). The same experiment showed a low reduction of coliphages MS2 and PRD-1 (only 69%) while echovirus 12 removal reached an average exceeding 95%. In another study with BSF, mean *E. coli* removal varied from 94% at filter startup to 99% at sand maturation in a laboratory setting, (Stauber et al., 2006). In the same paper, *E. coli* reductions averaged 93% from 55 BSF units operating at field scale.

An experiment by Bellamy et al. (1985) produced the values for *Giardia* cyst removal shown in Table 1 in six different plants (three mature and three immature filters) with .615 mm sand.

Table 1. Average removal of Giardia from SSF (Bellamy, et al, 1985)

Biological Condition of filter	**HLR[a] (m/h)**	**Infl. Cyst conc. (cyst/L)**	**Effl. Cyst conc. (cyst/L)**	**Removal (%)**
Mature	0.12	2100	0	99.98
New	0.355	2921	8.67	99.68

[a]Hydraulic Loading Rate

An experiment using full scale filters at McIndoe Falls, VT (Pyper, 1985) gave greater than 99.98% removal of *Giardia* cyst under warm temperatures, while removal was between 99.36 and 99.91% when the temperature dropped to less than 7°C (Logsdon, 1991).

Experiments conducted during four years using two filters fed with river water inoculated with a concentration of 400-600 PFU/mL polio viruses gave removal of 99.997% at a HLR of 0.2m/hr and a temperature between 16 and 18°C (Poynter and Slade, 1977). The same experiment's results were removals of 99.865% at HLR 0.4m/hr for 16-18°C and 99.8% at HLR 0.2 m/hr for 6°C.

Overall, the parameters shown to have a demonstrable effect on the performance were temperature (better removal at higher temperature) and maturity (well-ripened filters consistently perform better). While improved performance with lower HLR would be an expected result, Logsdon (1991) cites experiments by Walton and Hampton showing no effect between 0.12 m/hr up to 0.6 m/hr.

Thesis Goal and Objectives

Goal: optimize intermittent SSF performance on simulated CW effluent for drinking water treatment in a developing country.

Objectives:

Quantify the attenuation of a model pathogen (*Enterococcus durans*) through a slow sand filter.

Evaluate the effect of sand size and flow conditions (intermittent and continuous) on the breakthrough of the model pathogen from a slow sand filter.

Evaluate the breakthrough of an environmental coliform with associated heterotrophic population.

MATERIALS AND METHODS

Experimental Design

Three sand filter columns were used to simulate various aspects of the potential operation of slow sand filters in a rural setting. A model pathogen, *Enterococcus durans,* was used as a tracer through the columns. Two of the columns were packed with nominal 0.425 mm sand and one was packed with 0.6 mm sand. For the first two experiments (runs 1 and 2), only two columns were used – one with 0.425 mm and the other with 0.6 mm sand – and both were operated in intermittent mode for 8 hr/day followed by 16 hours of no flow. In the second set of experiments (runs 3, 4 and 5) the original 0.425 mm column was operated in continuous mode (24 hr/day flow), and a third column was added, packed with 0.425 mm sand. The additional column and the 0.6 mm sand column were operated for 8 hr/day. In the third set of experiments (run 6), the continuous flow system was switched back to intermittent flow as a replicate. In the final experiment, an undefined mixed culture of heterotrophs including coliforms was used as the tracer, and both coliforms and heterotrophs were monitored. The conditions tested in all runs are summarized in Table 2.

Model Slow Sand Filters

Three columns of PVC pipes of 4” diameter and 5’ length were used as filter units. Each column was loaded with 12 cm of underdrain gravel (size of 12 mm), 5 cm of

pea gravel (size of 4 mm), and 80 cm of fine sand forming the filter bed. The sand was purchased from a local store and sieved based on the U.S. standard sieve trays; the effective sizes (d_{10}) of 0.425 mm (columns 1 and 3) and 0.6 mm (column 2) with a uniformity coefficient (d_{60}/d_{10}) of 2.1 were used for the filter bed. With such sand characteristics, columns 1 and 3 meet the recommendation that d_{10} should fall between 0.15 and 0.44 mm for a full scale SSF plant, whereas column 2 does not. The uniformity coefficient for these systems fits into the range of 1.5-5 required (Barrett et al., 1991). The media bed was topped with 5 cm of pea gravel to prevent the small surface bed of a column from clogging too rapidly.

Table 2. Summary of experimental runs

Parameters	**Column 1**	**Column 2**	**Column 3**	**Run and nature of spike**	
ES[1] (mm)	0.425	0.6	0.425		
Flow regime HLR[2] (m/hr)	Intermittent 0.2	Intermittent 0.2	N/A	1	*Enterococcus durans*
Flow regime HLR (m/hr)	Intermittent 0.1	Intermittent 0.2	N/A	2	
Flow regime HLR (m/hr)	Continuous 0.03	Intermittent 0.3	Intermittent 0.2	3	
Flow regime HLR (m/hr)	Continuous 0.02	Intermittent 0.2	Intermittent 0.1	4	
Flow regime HLR (m/hr)	Continuous 0.1	Intermittent 0.2	Intermittent 0.2	5	
Flow regime HLR (m/hr)	Intermittent 0.2	Intermittent 0.2	Intermittent 0.1	6	
Flow regime HLR (m/hr)	Intermittent 0.2	Intermittent 0.2	Intermittent 0.1	Environmental coliforms	

[1] Effective Size of sand
[2] average Hydraulic Loading Rate

Two buckets (53 L and 55 L volume) were used as containers for the feed water to the columns, and were located to provide gravity flow to the columns (Figure 2). The feed water was made with 89% of laboratory tap water and 11% of wetland effluent to provide organic matter for the development of biofilms for the sand bed and to simulate a typical microbiological surface water quality in a developing country (Elliott et al., 2006). The mixture was monitored to ensure that no residual chlorine remained from the tap water; no residual chlorine was measured in the feed water after 15 hr. Another bucket of 40 L was used to collect the filter effluent. Both influent and effluent lines employed needle valves by which flow could be finely regulated.

The system was modified following the completion of the continuous flow experiments, and the modified version was used for run 6 with the *Enterococcus* and for the environmental spike (explained in more detail later in this chapter). First, the feedwater composition was modified due to the inaccessibility of CW effluent to mix with the tap water. 100% tap water was used, modified with 10 mg/L of protein extract (Primatone, Inc) and allowed to incubate overnight before application to the columns. This process also ensured removal of residual chlorine prior to introduction to the columns. The second modification was the addition of individual collection buckets (5.0 L) to the effluent of each column. During normal operation (when not sampling the system), these were allowed to fill to a volume of just over 4 L and overflow to the drain, and were not completely emptied even at night when there was no flow through the columns. During sampling events, the buckets were drained prior to the start of the day,

and were completely emptied at each sampling time while measuring the collected volume. This mixture was then sampled for microbial analyses. At the end of each 8-hour flow period, the lids were replaced on the buckets and they were allowed to sit empty overnight.

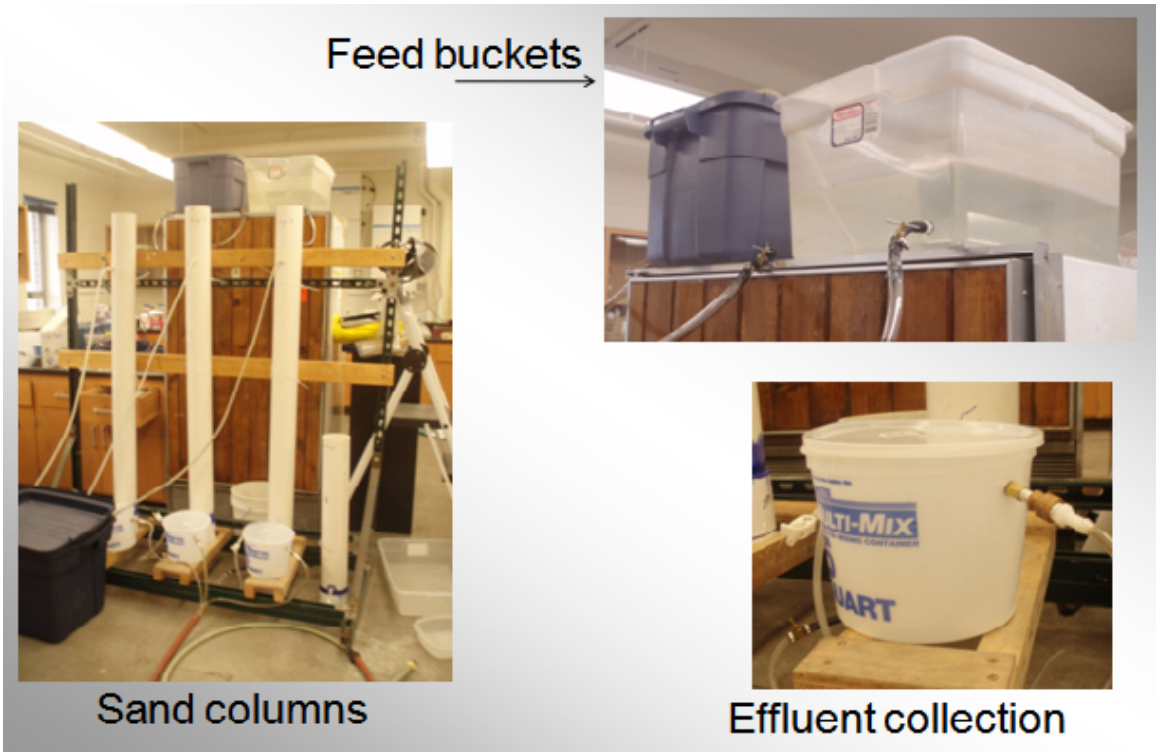

Figure 2. Experimental setup during the final two experiments (after the addition of collection buckets). W. Jones photo

For runs 3, 4 and 5, column 1 operated in continuous flow by regulating inflow and outflow valves whereas columns 2 and 3 operated in intermittent flow condition. Hydraulic loading rates (HLR) varied between 0.02 and 0.1 m/hr for column 1, between 0.2 and 0.3 m/hr for column 2, and between 0.1 and 0.2 m/hr for column 3 (table 2).

During the final studies (run 6 and for environmental coliforms) columns 1 and 2 were operated at a target filtration rate of 0.2 m/h, while column 3 was operated at 0.1 m/h It is noteworthy that the recommended range for the HLR for a full scale SSF is 0.06-0.3 m/hr (Barrett et al., 1991). All three columns operated in this range except column 1 during runs 3 and 4 which is a result of its bed maturation that led to the drop of its HLR (column 1 was more than a month older than the two other others).

Experimental Control

An experimental control consisting of two liters of feed water in a section of pipe with no sand or flow was performed during runs 5 and 6 and with the environmental spike.

Test Organism: *Enterococcus durans*

Enterococcus durans was used as model pathogen. Enterococci in general are bacteria found in the human intestine and are recognized to be very efficient bacterial indicator of fecal contamination of recreational surface waters (surfrider.org, 2007). The *Enterococcus durans* (ATCC® 11576™) species used is a gram-positive facultative anaerobe bacterium acquired in freeze-dried pure culture condition from ATCC.

The culture rehydration was undertaken in accordance with the American Type Culture Collection (ATCC) procedure. A freeze-dried culture of *Enterococcus durans* ATCC® 11576™ was revived by adding 1ml of Brain Heart Infusion (BD 237500) into a

pellet of the culture using a pasteur pipette. The aliquot was aseptically transferred into a 50 ml sterile centrifuge tube. After incubation at 37°C for 24 hours, the rehydrated bacteria were transferred into different microcentrifuge tubes. In each 2 ml microcentifuge tube, 0.7 ml of the culture was mixed with 0.3 ml of 70% glycerol to protect the membrane of the bacteria before storage at -70 °C for preservation for future use.

Inoculum Preparation

A few drops of the preserved *E. durans* ATCC® 11576™ were inoculated onto an *m-Enterococcus* agar plate and incubated at 37°C for 24 hours. A colony of organisms was picked from the plates and put into 10 ml of BD 237500 in 50 ml centrifuge tubes. The solution was shaken at 37°C and 180 rpm for 48 hours. Cells, after reaching their stationary growth phase, were harvested (usually after about 20 hours of growth) by centrifugation at 4°C and 5000xg for 25 min and transferred into 10 ml of the experiment feed water to acclimate the bacteria for about 30 min before their input into the columns. Stationary phase was determined by performing a growth curve on the organism under the conditions described above, and taking measurements of the absorbance at 600 nm (see Figure 3). Stationary phase was determined to be the point at which the absorbance reached its peak value. Plating of the inoculum prior to each spiking revealed the input of *E. durans* to be at concentrations varying from 6.1×10^7 to 2.4×10^9 CFU/mL.

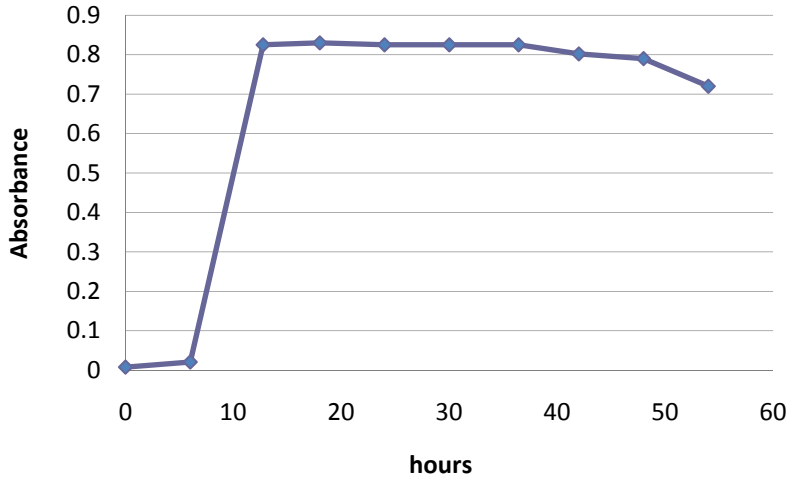

Figure 3. Growth curve for *E. durans* measured in the spectrophotometer.

Culturability Study of *E. durans*

Based on early observations of the decay of *E. durans*, culturability or decay studies were performed to evaluate the loss of culturable cells during incubation. Three solutions were utilized for this study:

(1) Feed water solution, as described above.

(2) Feed water solution after autoclaving at 121 °C for 30 minutes

(3) Phosphate buffered saline.

Ten ml of a stationary phase culture of *E. durans* was added to 2000 mL of the solution indicated, and samples were taken over an 8-24 hour period and enumerated using Enterococcus medium. Assuming that the organisms underwent exponential decay (first order decay), the resulting data were fit to a ln(number) versus time curve, and the slope reported as the decay rate coefficient k_d (hr^{-1}).

Water Spiking, Sampling, and Analysis

Bacterial breakthrough monitoring was conducted as a tracer study. Within a half hour of starting flow to the columns, a dose of *E. durans* of 6.1×10^8 to 2.4×10^{10} total CFU was spiked directly into headwater of each filter column to allow good dispersion of the inoculum within the column. Samples were taken for eight hours a day as grab samples as follows: every thirty minutes for the first two hours, then every hour for the next two hours followed by every two hours for the last four hours. As described above, the intermittent columns were then shut off for 16 hours, while the continuous column was allowed to continue to flow. No samples were taken during the 16-hour period. Sampling resumed the following day on the same schedule as described above. This process of daily sampling continued until no more colonies were detected from effluent samples.

Bacterial counts were determined by using the Drop Plate method (Herigstad et al., 2001). Samples were assayed on Difco™ m-Enterococcus Agar media incubated at 37 °C for 48 hours. *E. durans* produced colonies appearing as reddish spots with a dark center.

The environmental spike was prepared as follows. A sample of Mandeville Creek water was obtained from behind Roskie Hall on the MSU campus on or about March 12. This water was highly turbid as sampled, but after 24 hours of settling appeared fairly clear. One mL of this water was plated on a single McConkey's agar plate, and produced several typical coliform colonies. In each of two 500 mL Erlenmeyer flasks, 250 mL of

the water sample was then mixed with 250 mL of 0.1 strength Lactose Broth (0.5 g lactose, 0.8 g peptone and 0.5 g $MgSO_4$ per liter), leaving very little head space at the top, and placed in the 30°C incubator unmixed for 1 week. At the end of this period, the solutions contained what appeared to be microbial clumps, and a sort of scum had formed on the water surface. In addition, a significant odor had developed, and the overall solution was a turbid, milky white.

In preparation for inoculation, 150 mL was placed into each of six 200 mL centrifuge tubes, and centrifuged at 1000xg for 20 minutes. As the third of these six tubes was being decanted, it was noticed that the supernatant being discarded was still quite turbid. Fearing the further loss of active biomass, the remaining tubes and the pellet from the previously drained tubes were combined into a single solution which was vigorously shaken. The initial enumeration of the spike was done by serial dilution and spread-plating in triplicate on McConkey's agar.

After a half hour of flow, each of the columns received 100 mL of this mixture, with the exception of the control column, which received 35 mL. Samples were taken on about a 2 hour interval for microbiological analyses. Enumerations of the samples from this experiment were as follows: Coliforms were enumerated using the spot drop method, with 10 μL spots, 10 spots per dilution. Plating was on McConkey's Agar. Heterotrophs were enumerated using the spread plate method with 0.1 mL per plate, 2 plates per dilution, using R2A medium.

RESULTS AND DISCUSSION

Culturability Study of the Model Pathogen

A culturability study of *Enterococcus durans* was conducted to determine this organism's survival in different water solutions. Using the same sampling protocol described above, decay rates were computed as the slope of a regression line through data plotted as ln(count) versus time (Figure 4). The results are shown in Table 3.

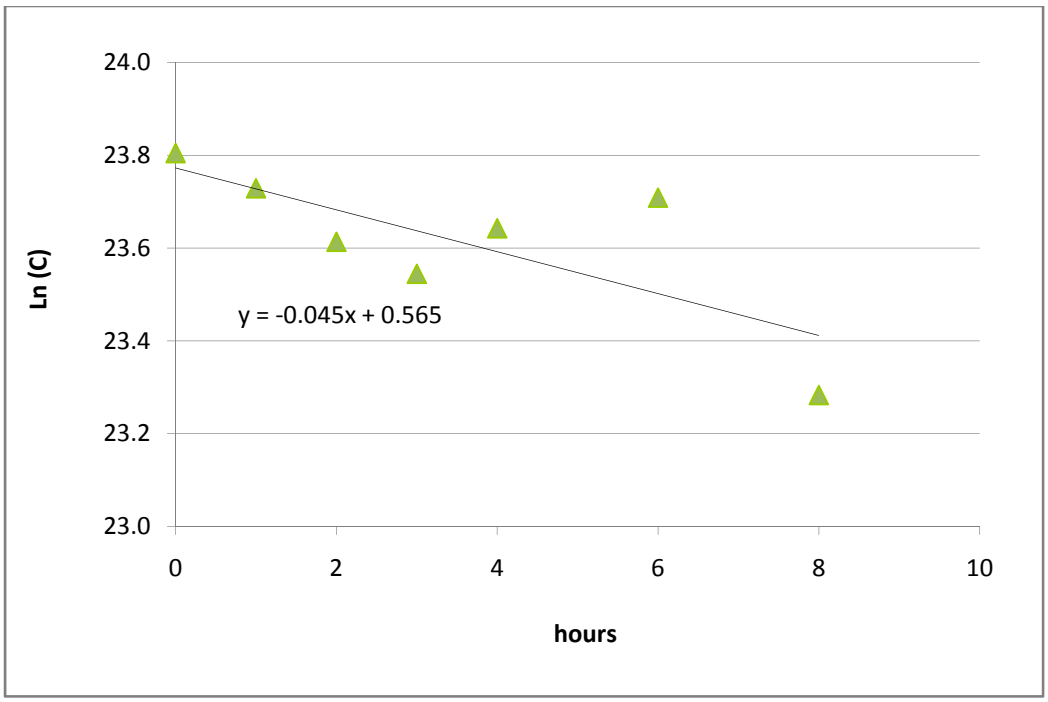

Figure 4. Sample decay rate curve for *E. durans* in feed solution showing kd = 0.045 hr^{-1}

Table 3. Decay rates for *Enterococcus durans* for the three different solutions

Water solutions		*feed solution*	*PBS*	*autoclaved feed*
Decay rates (hr^{-1})	test1	0.105	0.019	n/a
	test2	0.045	0.012	0.015
	test3	0.019	0.006	0.029
	test4	0.073	n/a	n/a
	mean	0.061	0.012	0.022
	Std. Deviation	0.037	0.007	0.010

The decay rate of the bacteria in the unaltered feed solution was always higher than that in the PBS and autoclaved feed except for the latter during test3. Enterococci are common inhabitants of the gastrointestinal tract of warm-blooded animals, and used as valuable bacterial indicator for fecal contamination of recreational marine water (APHA, 1995). *Enterococcus durans* may have survived better in the PBS due to the saline character of the solution. The lower decay rate of the bacteria in autoclaved feed may have resulted from a number of factors. Autoclaving is expected to have inactivated any organism that may have competed against the *E. durans* for its food source (nutrients); in fact, the process could have even made more nutrients available to the subsequently added organisms through cell lysis of the original population. It is also possible that the autoclaving eliminated all the bacteriovores present in the feed water that prey on the *E. durans*, or inactivated any antibacterial toxins or toxin-producing species in the

feedwater. A combination of the above factors is likely, but could not be determined without extensive and complicated further testing.

Values of the standard deviation follow the same trend as the means, and this is not unexpected; the feed solution can contain different populations on different days, and if competition/toxicity issues are important, significant variation could result. In the PBS and autoclaved solutions, less variation in composition is expected, with the collateral stability in k_d values.

Following the same protocol used for *E. durans* and described above, the culturability for coliforms and heterotrophs, determined from the control column during that study, gave decay rates of 0.074 hr^{-1} and 0.044 hr^{-1}, respectively. These two values for k_d are within the same range as those reported in Table 3.

Assessment and Analysis of Breakthrough Curves for *Enterococcus durans*

The breakthrough of a spike dose of organisms added to a sand column will occur over a period of time that exceeds several residence times of the column. In the case of the experiments described here, especially those experiments run with intermittent flow, temporal variations in column phenomena make detailed modeling of transport and reaction untenable. In the absence of any growth, degradation or decay reactions affecting the added cells, the total number of cells leaving the column over the experimental period should equal the total number of cells added, minus those

permanently attached to the column. This mass balance approach can be used to compute a fraction of cells recovered as follows:

$$\%breakthrough = \frac{\sum_{0}^{t} c(t) Q \Delta t}{c_{spike} V_{spike}} \times 100\% \quad (1)$$

The term QΔt in equation (1) is the amount of volume leaving the column during the previous interval (since the last sample). In runs 1 through 5, prior to the installation of the effluent collection buckets, these were computed from the average flowrate during the day multiplied by the time interval. Following the installation of the buckets (for run 6 of the breakthrough study and for the environmental spike), the product QΔt is really ΔV, the volume collected in the bucket since the last interval, and these were measured directly. The breakthrough computed in this manner reflects the portion of the total amount of cells that exit the column up to a point in time. Over the course of the experiment, once the cell concentration drops below detection, the equation reflects the total recovery of cells from the experiment.

The experiments performed to observe loss of culturability provide some insight into the fate of cells inside the column. Every colony forming unit detected in the column effluent had been in the column for the time between the inoculation and the sampling event. During that time, cells originally introduced into the column decayed or otherwise lost culturability in addition to the other myriad processes occurring inside the column. As a result, the actual observed breakthrough curves reflect the processes of cell death by predation, toxicity from other cells, transport by advection and dispersion,

adsorption and desorption to media surfaces and biofilms, plus a net loss of culturability that would have occurred in solution alone. To account for this loss of culturability, a control column operated at the same time the transport studies were performed serves as a way to measure that loss of culturability. During just the last three studies (runs 5 and 6 for *E durans* and the environmental test with coliforms), the data from the control column were used to compute a decay coefficient for that experiment (for the *E. durans* or the environmental coliform). The decay coefficient was used to compute a modified breakthrough curve, reasoning that if the control column organisms were reduced by a factor of e^{-kdt}, so were the organisms leaving a sand column at the same time (all were introduced simultaneously). A modified concentration, c*, is computed by multiplying the actual concentration by e^{+kdt},

$$c^* = c(t)e^{k_d t} \tag{2}$$

producing a breakthrough curve of the form:

$$corrected\ \%breakthrough = \frac{\sum_0^t c(t)Qe^{k_d t}\Delta t}{c_{spike}\,V_{spike}} \times 100\% \tag{3}$$

Using this computation approach, percentage breakthroughs versus time and percentage breakthroughs versus processed volume were performed for all three columns. Results for *Enterococcus* breakthrough curves for the six sets (equivalent of six spike doses) show different trends over the course of the experiment.

For run 1, % breakthrough curves peak at only about 0.45% for column 1 (nominal 0,425 mm sand) and 0.10% for column 2 (nominal 0.6 mm sand). This

contradicts the expectation that the smaller sand should perform better, since both columns at this point were operating in intermittent flow condition and same hydraulic loading rate (0.2 m/hr).

During run 2, when the HLR for both columns were different (0.1 m/hr for column 1 and 0.2 m/hr for column 2) but both columns still operating in intermittent flow condition, the results of the breakthrough curves show almost a switch in the maxima of total breakthrough percentages for the run (0.10% and about 0.40% for columns 1 and 2, respectively). Contrarily to run 1 when both columns had equal total processed volume (figure 5), the total processed volume for column 1 during run 2 is more than 4 times lower than that for column 2 (figure 6). This results from the fact that total processed volume is proportional to the HLR (which is higher for column 2 during run 2) and the time it takes for the bacteria to keep coming out of a column (column 2 released bacteria two days longer than column 1).

From run 3, the third column was added to operation, the characteristics of columns were:

(1) Column 1 with 0.425 mm sand size run continuously at 0.03, 0.02, and 0.1 m/hr HLR for runs 3, 4 and 5, respectively.

(2) Column 2 with 0.6 mm sand size run intermittently at 0.3 and 0.2 m/hr HLR

(3) Column 3 with 0.425 mm sand size run intermittently at 0.2 and 0.1 m/hr HLR

For run 3, percentage breakthroughs peak nearly at 0.001%, 9.50%, and 0.02% for columns 1, 2, and 3 respectively over three days of filtration. The same breakthrough pattern is found for run 4 with an overall breakthrough rate of column 1 much lower than for the rates shown by columns 3 and 2, respectively (figures 7 and 8).

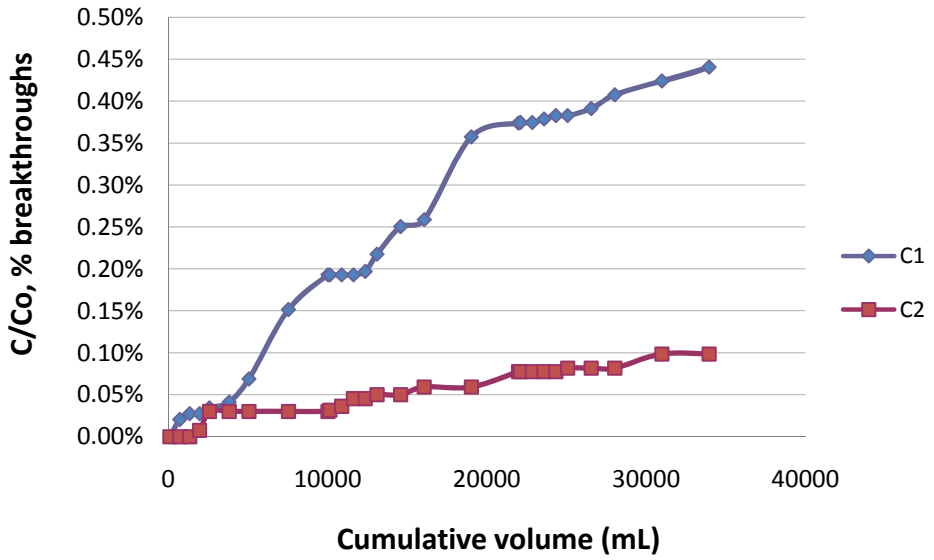

Figure 5. Breakthrough curves of *E. durans* for columns 1 and 2 during run 1

Without performing any control test to determine decay rates corresponding to the recovery of the bacteria from run 1 through run 4, there are no corrected breakthrough curves for those runs. On the other hand, for breakthroughs study during runs 5 and 6, controls were performed in parallel which enabled the corrected breakthrough computation.

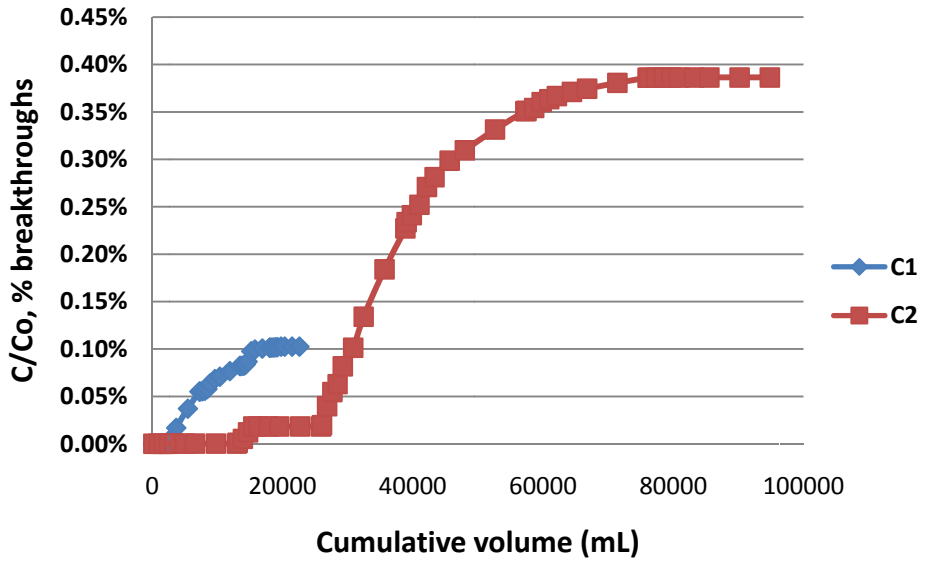

Figure 6. Breakthrough curves of *E. durans* for columns 1 and 2 during run 2

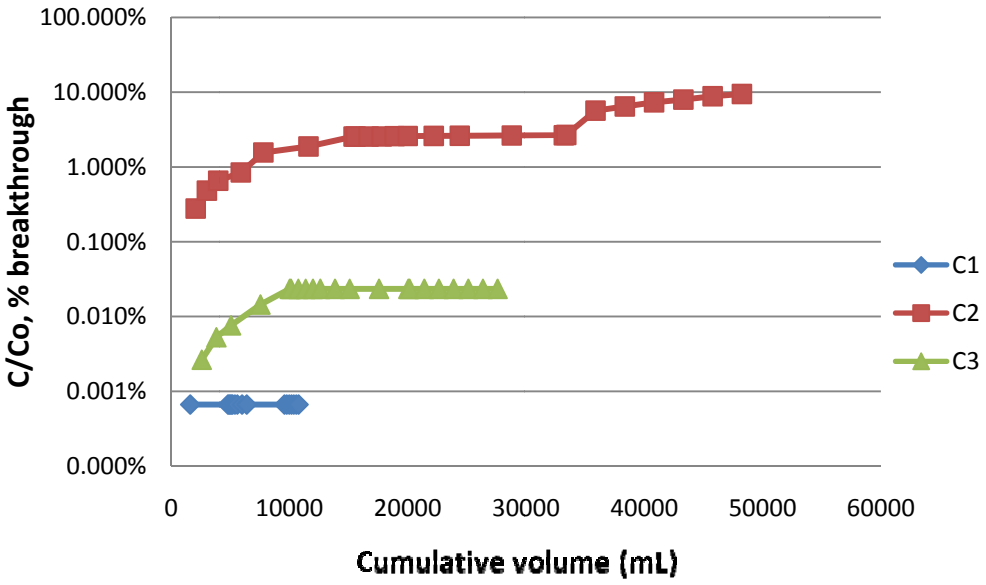

Figure 7. Breakthrough curves of *E. durans* for the three sand columns during run 3

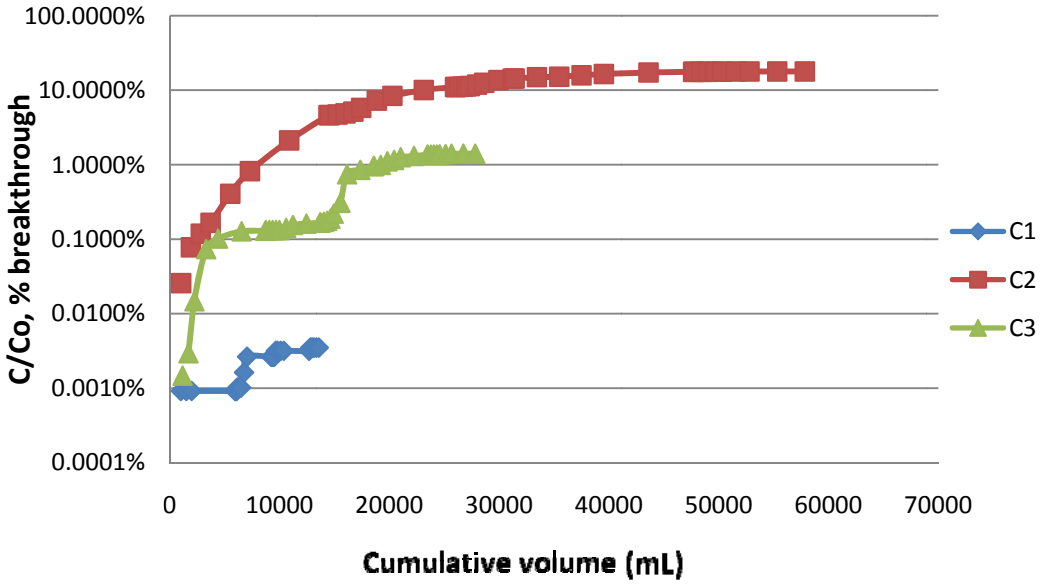

Figure 8. Breakthrough curves of *E. durans* for the three sand columns during run 4

Percentage breakthroughs for run 5 show the trend that column 1 produced lower recovery rate than columns 2 and 3 as observed previously. On the other hand, column 3 had a higher breakthrough rate than column 2 contrarily to the previous two runs (figures 9 and 10).

The corrected breakthrough curves follow the same trend as the original breakthrough curves, but with nearly three times the total breakthrough. In this run, however, the overall breakthrough is still less than 10%, indicating that the natural die-off or loss of culturability of the bacteria accounts for less than the other set of phenomena governing the bacteria removal within the sand columns.

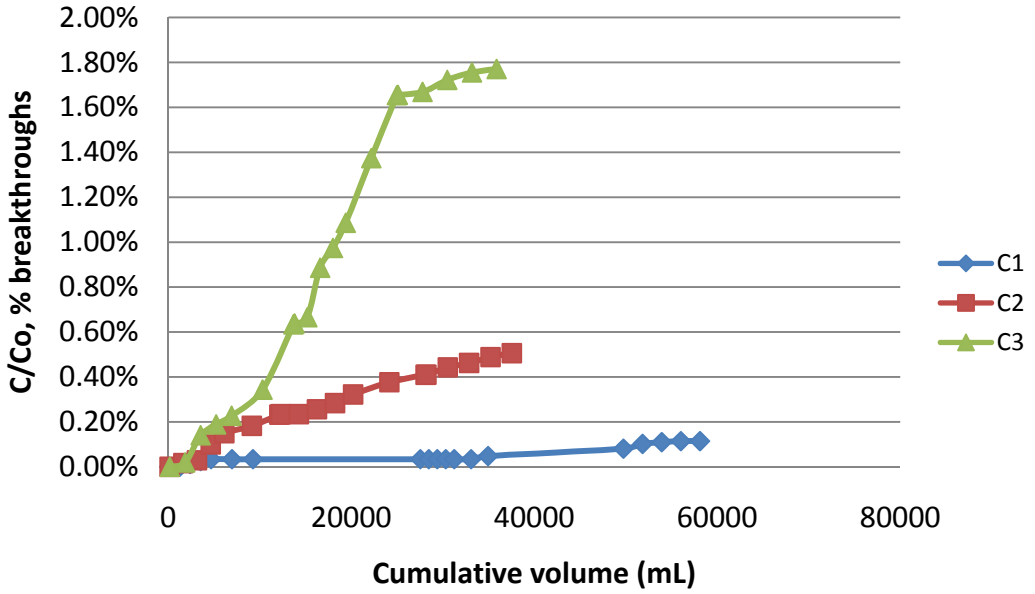

Figure 9. Breakthrough curves of *E. durans* for the three sand columns during run 5.

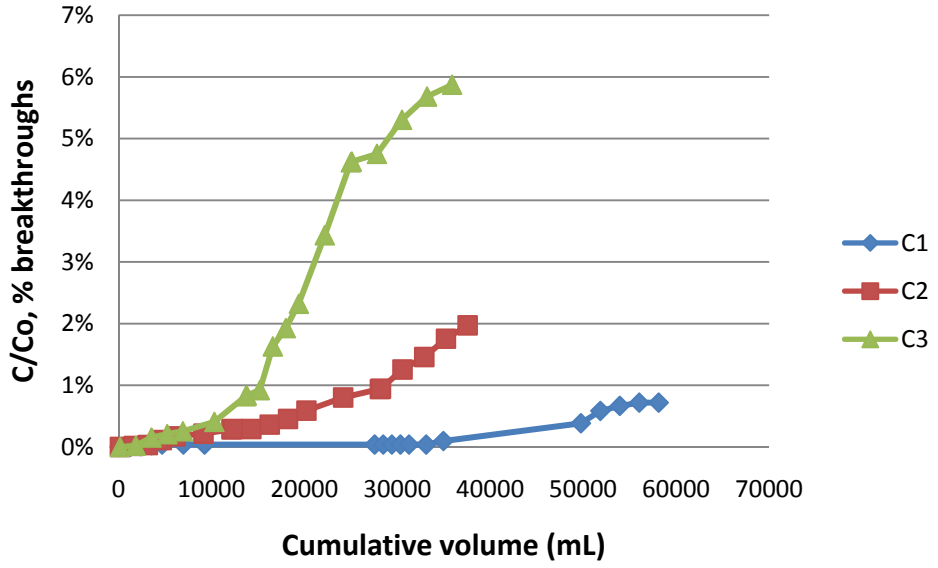

Figure 10. Breakthrough curves of *E. durans* for the three sand columns during run 5, corrected to account for static loss of culturability in the control columns.

During run 6, especially the curve for column 2, is a good opportunity to reflect on the effect of the stagnation period. Right around 21 liters of produced volume (end of day 2), there's a barely noticeable jump in the uncorrected breakthrough, but a large jump in the corrected counts. This should be pointed out as one of the artifacts that occur in this attempt to factor out the effect of continuous loss of culturability.

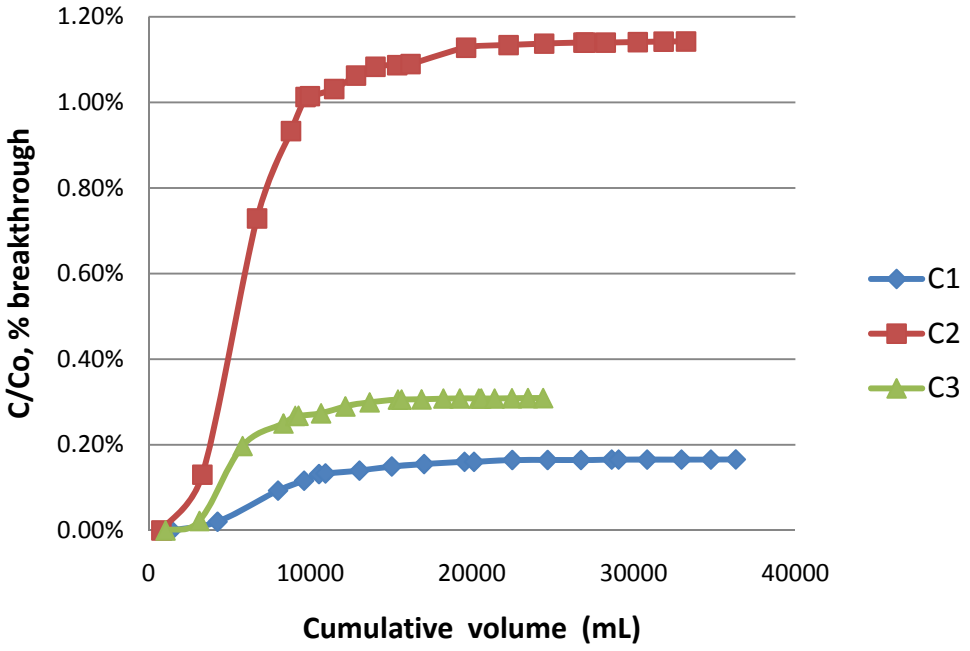

Figure 11. Breakthrough curve for *E. durans* by produced volume during run 6

Whether corrected or not, the breakthrough of *E. durans* in the above breakthrough studies are small, as shown in Table 4.

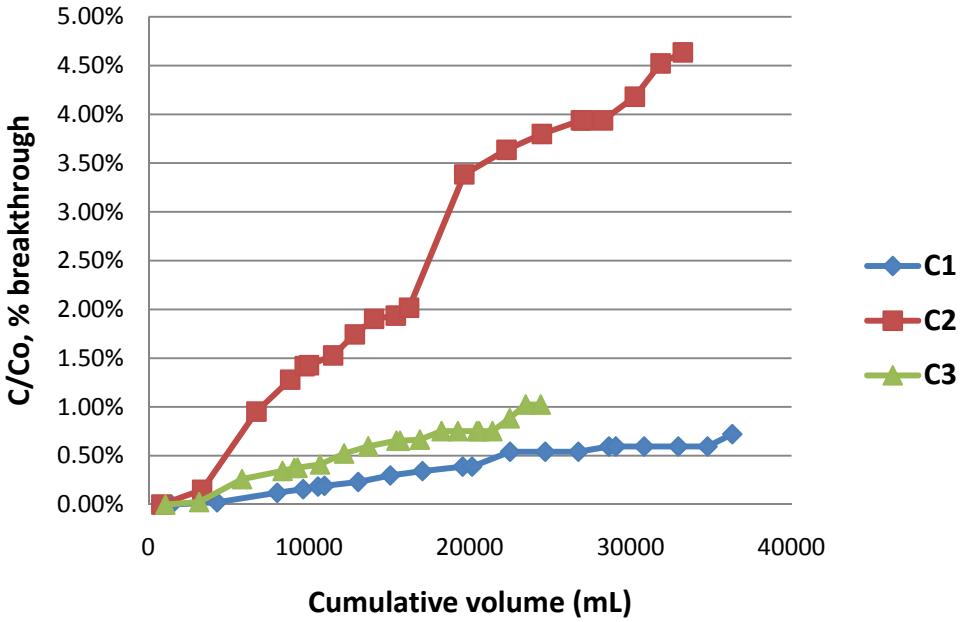

Figure 12. Corrected breakthrough curve for *E. durans* by produced volume during run 6

Table 4. Summary of total percentage breakthroughs of *E. durans*

	Total Breakthrough at end of data collection by experiment (%)					
Run #	Column1 Total	Corrected	Column2 Total	Corrected	Column3 Total	Corrected
1	0.44	n/a	0.10	n/a	n/a	n/a
2	0.10	n/a	0.39	n/a	n/a	
3	0.0007	n/a	9.48	n/a	0.02	n/a
4	0.0035	n/a	17.85	n/a	1.40	n/a
5	0.11	0.72	0.51	1.97	1.77	5.87
6	0.17	0.72	1.14	4.64	0.31	1.02

From this table, we clearly notice that the sand size and flow condition have significant impacts on the breakthrough of the bacteria. This can be seen by comparing columns 1 and 3, with equal sand size (0.425 mm). During runs 3 to 5, column 1 operated under continuous flow conditions and produced a lower breakthrough rate than column 3, operated intermittently. Column 3, in turn, having smaller sand size than column 2 (0.6 mm) while operating at same intermittent flow condition, had a consistently lower breakthrough rate. Therefore, a combination of smaller sand size and continuous flow condition favors the efficient removal of the bacteria in the sand column. During run 6, when all columns were operated intermittently, and column 3 operated at a lower average HLR (0.1 m/h) than columns 1 and 2 (operated at about the same average HLR of 0.2 m/h), column 2 performed the worst, while columns 1 and 3 were fairly near equivalent in performance. Likewise, during run 2 when both columns 1 and 2 were operating intermittently, column 1 performed better. However, during run 1 when column 1 operated intermittently at same HLR as column 2 (0.2 m/h), the breakthrough rate was lower for column 2 than column 1.

Assessment and Analysis of Breakthrough Curves for Coliforms

The same mass balance approach used for *E durans* is used to determine the recovery rate of coliforms from sand columns. Breakthroughs of coliforms obtained from columns are higher than what we saw for *Enterococcus*. Indeed, while the breakthrough percentage for column 2 (with the most breakthrough) barely approached 1.2% for *Enterococcus*, the

same column produced a coliform breakthrough of nearly 8%, as illustrated in figure 13. This is not unexpected, since the coliforms were selected from an environment where competition, low temperature and fairly oligotrophic conditions predominated, while the *E. durans* is a purified laboratory strain with a growth optimum near 37 °C (American Type Culture Collection). The effect of column operation and medium on coliform breakthrough was similar to that for the *Enterococcus*, although columns 1 and 3 switched places somewhat, with column 3 performing better than column 1.

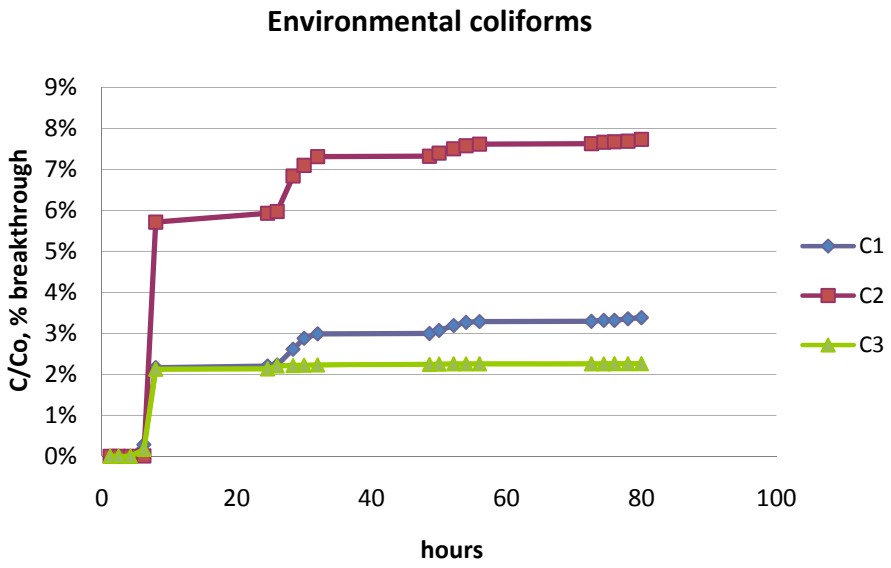

Figure 13. Breakthrough curve for coliforms over time

The "corrected" breakthroughs, expressed by filtrate volume production in figure 14, show a significant increase (nearly 10 times greater) over the uncorrected breakthroughs. This situation may be due to the fact that the breakthrough results from continued presence of colonies in the effluent long past the residence time of the column.

Because of the exponential correction factor (e^{+kdt}) those few colonies become very important in the overall corrected breakthrough curves. In other words, the corrected breakthrough curves show that it may be possible for organisms to survive longer in sand column than they would in the fluid, and perhaps, in some instances they do grow. It is also noteworthy that the timing of the breakthrough is important; columns 1 and 3 had about the same uncorrected breakthrough, while the corrected curves have column 1 nearly as high as column 2.

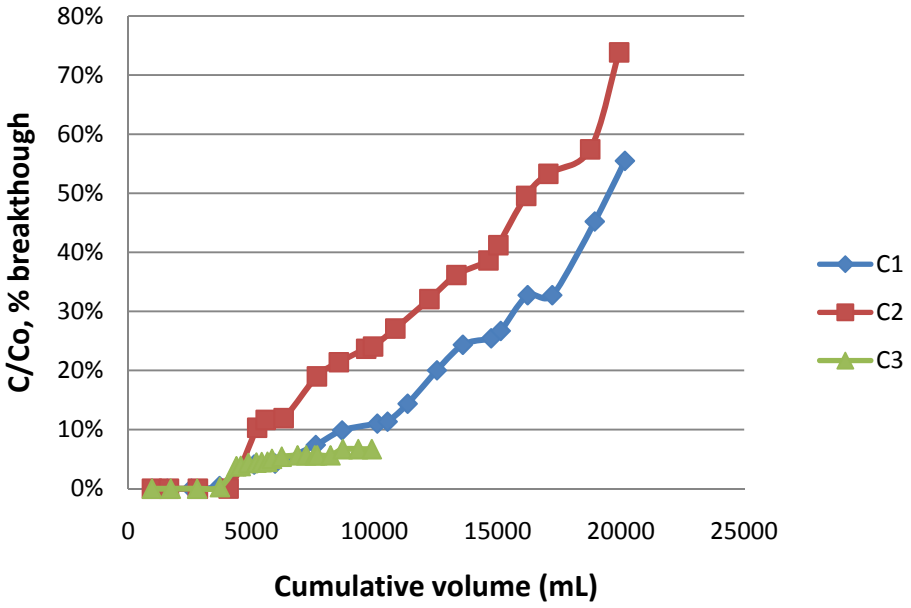

Figure 14. Breakthrough curve for coliforms by total volume

Environmental Heterotrophic Plate Count (HPC) Study Analysis

The heterotrophic plate count (HPC) results are illustrated in Figure 15. The inlet and control solutions contained significantly higher counts than the column effluents and

this trend persisted over the course of the sampling period. Column 1 shows a smaller concentration of heterotrophs in the filtrate than columns 2 and 3 almost at any given time which attests to its higher performance, although the difference is less than one log throughout. Other than the peak in concentration during the first day following the spike addition (most noticeable in Figure 16), the concentration of the bacteria in columns tends to also be quite stable over time, decreasing gradually by about a log. In contrast, the control shows a significant drop of its concentration over time, an order of magnitude that reaches about 2 log units. The inlet behaves similarly to the control with more variability.

Further investigation is, however, necessary to confirm whether or not the same population of heterotrophs remains identical over the course of sampling periods.

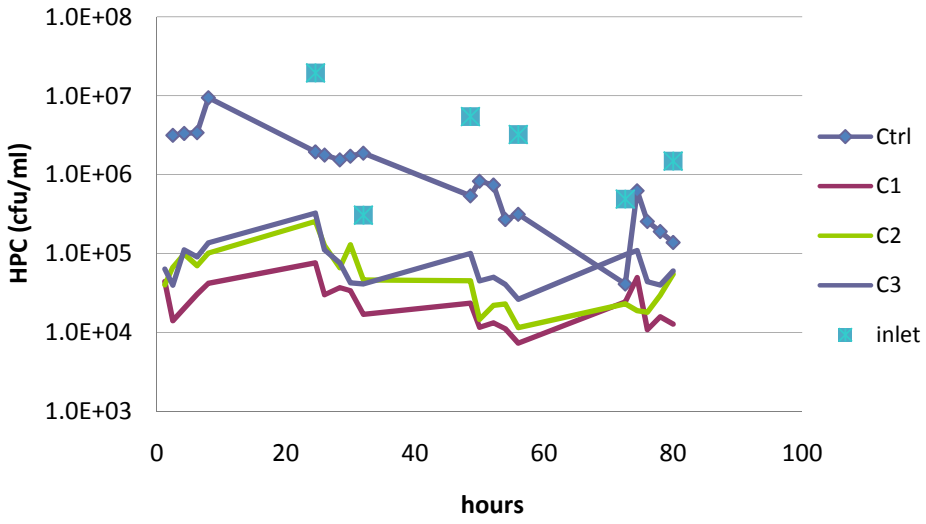

Figure 15. Environmental heterotrophs concentration over time

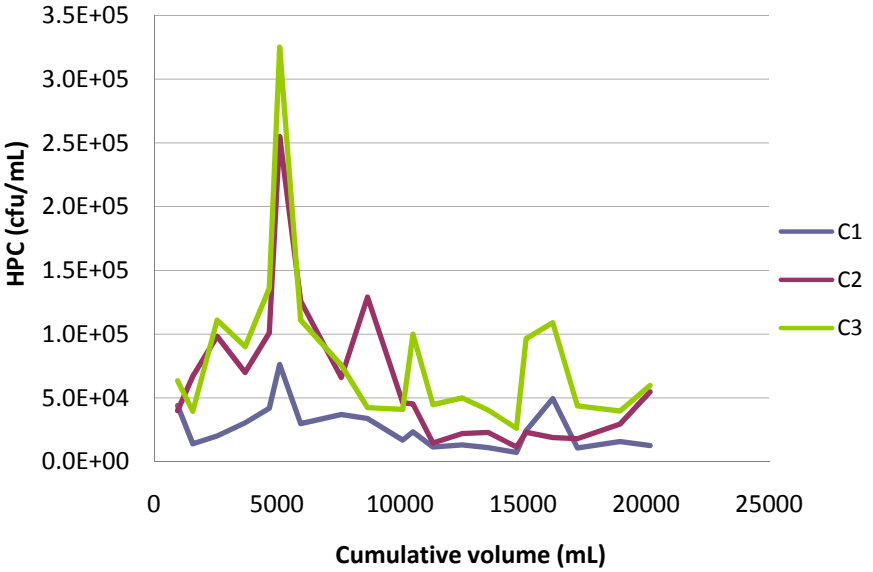

Figure 16. Environmental heterotrophs concentration by volume production

CONCLUSIONS AND RECOMMENDATIONS

- In many cases, the percent removal of the model pathogen (*Enterococcus durans*) from the slow sand columns far exceeds 90%
- Column with finer sand size and operating at continuous flow condition provide the least breakthrough rate for *E. durans*
- Column with finer sand size and operating at intermittent flow condition in turn provides lesser breakthrough rate for *E. durans* than column with same flow condition but higher sand size
- Breakthrough of environmental coliforms with an associated heterotrophic population spike produced higher breakthroughs of coliforms than with *E. durans.* Lower hydraulic loading and smaller sand again produced lesser breakthrough than higher flow and larger sand.

The results of this laboratory study have significance for the implementation of the system in Mali. As shown over the course of the experiments, effective sand size and flow regime produced consistent trends on the removal of the bacteria from the water: smaller sand size and continuous flow led to a higher removal rate. Of the two factors, sand size appeared to be more important, since the difference between the columns with small sand size operated continuously versus intermittently was less than the difference between the two intermittently operated columns with different sand sizes. As a consequence, in the field in Mali, emphasis will be made on having a filter bed with sand size of 0.425 mm or less. Additionally, a compromise may have to be made on the choice

of the flow regime: a study based on a comparison of what we gain to run a filter plant continuously and the operational benefit of running it intermittently will determine the final choice. From experience, we know that a better technical option is not always the best choice if that does not take into account the cultural aspect of the population served. Hence, even if running a plant continuously provides better water quality to the consumers but which might lead to a reluctance to take ownership of the investment (perhaps due to the additional time and effort constraints), then design for some intermittent use would provide for a long term functioning of the plant.

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APPENDICES

APPENDIX A:

DETERMINATION OF THE AVERAGE HYDRAULIC LOADING RATES (HLR)

The hydraulic loading rate (m/hr) is determined as follows:

$$HLR = \frac{Q}{A}$$

where Q is the average flow rate in mL measured from effluent collection and A, the surface area of a column that is,

$$A = \pi r^2 = \pi\left(\frac{4}{2} \times 2.54 \times 10^{-2}\right)^2 \cong 0.008\, m^3$$

Hence, we get the below table summarizing the HLR of the columns for different runs

Runs & columns #		Average flow rate (mL/hr)	Average HLR (m/hr)
1	Column 1	1360	0.2
	Column 2	1507	0.2
2	Column 1	743	0.1
	Column 2	1963	0.2
3	Column 1	199	0.03
	Column 2	2194	0.02
	Column 3	1265	0.2
4	Column 1	160	0.3
	Column 2	1431	0.02
	Column 3	689	0.2
5	Column 1	1038	0.1
	Column 2	1548	0.2
	Column 3	1480	0.2

Note: the computation for runs 6 and for environmental coliforms was actually based on volume measurement.

APPENDIX B:

COMPUTATION FOR BREAKTHROUGH DATA TABLES

Table 6. Breakthrough computation for column 1 during run 1

Time	C (CFU/mL)	Q*Δt (mL)	cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	sum C/Co
0	0.0E+00	103	103	0.0E+00	0.0E+00	0.00%
0.5	4.0E+01	620	723	2.5E+04	2.5E+04	0.02%
1	1.3E+01	620	1343	8.3E+03	3.3E+04	0.03%
1.5	0.0E+00	620	1963	0.0E+00	3.3E+04	0.03%
2	1.3E+01	620	2583	8.3E+03	4.1E+04	0.03%
3	6.7E+00	1240	3823	8.3E+03	5.0E+04	0.04%
4	2.7E+01	1240	5064	3.3E+04	8.3E+04	0.07%
6	4.0E+01	2480	7544	9.9E+04	1.8E+05	0.15%
8	2.0E+01	2480	10024	5.0E+04	2.3E+05	0.19%
24	0.0E+00	123	10147	0.0E+00	2.3E+05	0.19%
24.5	0.0E+00	740	10887	0.0E+00	2.3E+05	0.19%
25	0.0E+00	740	11628	0.0E+00	2.3E+05	0.19%
25.5	6.7E+00	740	12368	4.9E+03	2.4E+05	0.20%
26	3.3E+01	740	13108	2.5E+04	2.6E+05	0.22%
27	2.7E+01	1480	14588	3.9E+04	3.0E+05	0.25%
28	6.7E+00	1480	16069	9.9E+03	3.1E+05	0.26%
30	4.0E+01	2961	19030	1.2E+05	4.3E+05	0.36%
32	6.7E+00	2961	21991	2.0E+04	4.5E+05	0.37%
48	6.7E+00	123	22113	8.2E+02	4.5E+05	0.37%
48.5	0.0E+00	740	22854	0.0E+00	4.5E+05	0.37%
49	6.7E+00	740	23594	5.0E+03	4.5E+05	0.38%
49.5	6.7E+00	740	24334	5.0E+03	4.6E+05	0.38%
50	0.0E+00	740	25074	0.0E+00	4.6E+05	0.38%
51	6.7E+00	1480	26555	9.9E+03	4.7E+05	0.39%
52	1.3E+01	1480	28035	2.0E+04	4.9E+05	0.41%
54	6.7E+00	2961	30996	2.0E+04	5.1E+05	0.42%
56	6.7E+00	2961	33957	2.0E+04	5.3E+05	0.44%

Table 7. Breakthrough computation for column 2 during run 1

Time	C (CFU/mL)	Q*Δt (mL)	cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	sum C/Co
0	0.0E+00	113	113	0.0E+00	0.0E+00	0.00%
0.5	0.0E+00	679	792	0.0E+00	0.0E+00	0.00%
1	0.0E+00	679	1471	0.0E+00	0.0E+00	0.00%
1.5	1.3E+01	679	2150	9.1E+03	9.1E+03	0.01%
2	4.0E+01	679	2829	2.7E+04	3.6E+04	0.03%
3	0.0E+00	1358	4188	0.0E+00	3.6E+04	0.03%

4	0.0E+00	1358	5546	0.0E+00	3.6E+04	0.03%
6	0.0E+00	2717	8262	0.0E+00	3.6E+04	0.03%
8	0.0E+00	2717	10979	0.0E+00	3.6E+04	0.03%
24	1.3E+01	137	11116	1.8E+03	3.8E+04	0.03%
24.5	6.7E+00	827	11944	5.5E+03	4.4E+04	0.04%
25	1.3E+01	827	12771	1.1E+04	5.5E+04	0.05%
25.5	0.0E+00	827	13598	0.0E+00	5.5E+04	0.05%
26	6.7E+00	827	14426	5.5E+03	6.0E+04	0.05%
27	0.0E+00	1655	16080	0.0E+00	6.0E+04	0.05%
28	6.7E+00	1655	17735	1.1E+04	7.1E+04	0.06%
30	0.0E+00	3309	21045	0.0E+00	7.1E+04	0.06%
32	6.7E+00	3309	24354	2.2E+04	9.3E+04	0.08%
48	0.0E+00	125	24479	0.0E+00	9.3E+04	0.08%
48.5	0.0E+00	753	25232	0.0E+00	9.3E+04	0.08%
49	0.0E+00	753	25986	0.0E+00	9.3E+04	0.08%
49.5	0.0E+00	753	26739	0.0E+00	9.3E+04	0.08%
50	6.7E+00	753	27492	5.0E+03	9.8E+04	0.08%
51	0.0E+00	1507	28999	0.0E+00	9.8E+04	0.08%
52	0.0E+00	1507	30505	0.0E+00	9.8E+04	0.08%
54	6.7E+00	3013	33518	2.0E+04	1.2E+05	0.10%
56	0.0E+00	3013	36531	0.0E+00	1.2E+05	0.10%

Table 8. Breakthrough computation for column 1 during run 2

Time (hr)	C (CFU/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	Sum C/Co
0	0.0E+00	74.1	74	0.0E+00	0.0E+00	0.00%
0.5	0.0E+00	446	520	0.0E+00	0.0E+00	0.00%
1	0.0E+00	446	967	0.0E+00	0.0E+00	0.00%
1.5	0.0E+00	446	1413	0.0E+00	0.0E+00	0.00%
2	4.7E+01	446	1859	2.1E+04	2.1E+04	0.00%
3	1.3E+03	892	2751	1.2E+06	1.2E+06	0.01%
4	3.1E+03	892	3644	2.8E+06	4.0E+06	0.02%
6	2.7E+03	1785	5429	4.9E+06	8.9E+06	0.04%
8	2.4E+03	1785	7214	4.3E+06	1.3E+07	0.06%
24	5.3E+01	64	7278	3.4E+03	1.3E+07	0.06%
24.5	2.2E+02	385	7662	8.5E+04	1.3E+07	0.06%
25	2.9E+02	385	8047	1.1E+05	1.3E+07	0.06%
25.5	1.2E+03	385	8431	4.6E+05	1.4E+07	0.06%
26	3.5E+03	385	8816	1.4E+06	1.5E+07	0.06%
27	1.5E+03	769	9585	1.2E+06	1.6E+07	0.07%
28	7.8E+02	769	10354	6.0E+05	1.7E+07	0.07%
30	9.2E+02	1538	11892	1.4E+06	1.8E+07	0.08%

32	8.5E+02	1538	13430	1.3E+06	2.0E+07	0.08%
48	2.7E+01	47	13477	1.3E+03	2.0E+07	0.08%
48.5	1.4E+02	283	13760	4.0E+04	2.0E+07	0.08%
49	9.3E+01	283	14044	2.6E+04	2.0E+07	0.08%
49.5	1.1E+03	283	14327	3.1E+05	2.0E+07	0.08%
50	2.5E+03	283	14610	7.0E+05	2.1E+07	0.09%
51	4.7E+03	566	15176	2.6E+06	2.3E+07	0.10%
52	7.5E+02	566	15742	4.3E+05	2.4E+07	0.10%
54	2.5E+02	1132	16874	2.8E+05	2.4E+07	0.10%
56	1.8E+02	1132	18006	2.0E+05	2.4E+07	0.10%
72	2.0E+02	47	18053	9.4E+03	2.4E+07	0.10%
72.5	0.0E+00	283	18336	0.0E+00	2.4E+07	0.10%
73	1.3E+02	283	18619	3.8E+04	2.4E+07	0.10%
73.5	1.3E+02	283	18902	3.8E+04	2.4E+07	0.10%
74	1.3E+02	283	19185	3.8E+04	2.4E+07	0.10%
75	2.7E+02	566	19751	1.5E+05	2.5E+07	0.10%
76	0.0E+00	566	20318	0.0E+00	2.5E+07	0.10%
78	0.0E+00	1132	21450	0.0E+00	2.5E+07	0.10%
80	0.0E+00	1132	22582	0.0E+00	2.5E+07	0.10%

Table 9. Breakthrough computation for column 2 during run 2

Time (hr)	C (CFU/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	Sum C/Co
0	0.0E+00	133.0	133.0	0.0E+00	0.0E+00	0.0000%
0.5	0.0E+00	801.1	934.1	0.0E+00	0.0E+00	0.0000%
1	0.0E+00	801.1	1735.1	0.0E+00	0.0E+00	0.0000%
1.5	0.0E+00	801.1	2536.2	0.0E+00	0.0E+00	0.0000%
2	5.3E+01	801.1	3337.3	4.3E+04	4.3E+04	0.0002%
3	TNTC	1602.2	4939.4		4.3E+04	0.0002%
4	TNTC	1602.2	6541.6		4.3E+04	0.0002%
6	TNTC	3204.3	9745.9		4.3E+04	0.0002%
8	TNTC	3204.3	12950.2		4.3E+04	0.0002%
24	1.5E+03	133.0	13083.2	2.0E+05	2.4E+05	0.0010%
24.5	1.2E+03	801.1	13884.2	1.0E+06	1.2E+06	0.0052%
25	2.0E+03	801.1	14685.3	1.6E+06	2.9E+06	0.0120%
25.5	1.9E+03	801.1	15486.4	1.5E+06	4.4E+06	0.0182%
26	TNTC	801.1	16287.5		4.4E+06	0.0182%
27	TNTC	1602.2	17889.6		4.4E+06	0.0182%
28	TNTC	1602.2	19491.8		4.4E+06	0.0182%
30	TNTC	3204.3	22696.1		4.4E+06	0.0182%
32	TNTC	3204.3	25900.4		4.4E+06	0.0182%
48	2.6E+03	133.0	26033.3	3.5E+05	4.7E+06	0.0197%
48.5	6.0E+03	801.1	26834.4	4.8E+06	9.5E+06	0.0397%
49	4.4E+03	801.1	27635.5	3.6E+06	1.3E+07	0.0546%

49.5	2.5E+03	801.1	28436.6	2.0E+06	1.5E+07	0.0629%
50	5.6E+03	801.1	29237.6	4.5E+06	2.0E+07	0.0817%
51	2.9E+03	1602.2	30839.8	4.7E+06	2.4E+07	0.1013%
52	4.9E+03	1602.2	32441.9	7.9E+06	3.2E+07	0.1340%
54	3.7E+03	3204.3	35646.2	1.2E+07	4.4E+07	0.1841%
56	3.2E+03	3204.3	38850.5	1.0E+07	5.5E+07	0.2272%
72	8.2E+03	192.9	39043.4	1.6E+06	5.6E+07	0.2338%
72.5	2.2E+03	801.1	39844.5	1.8E+06	5.8E+07	0.2412%
73	2.3E+03	1161.8	41006.3	2.6E+06	6.1E+07	0.2521%
73.5	3.9E+03	1161.8	42168.1	4.5E+06	6.5E+07	0.2708%
74	2.1E+03	1161.8	43329.9	2.5E+06	6.7E+07	0.2812%
75	1.8E+03	2323.6	45653.6	4.2E+06	7.2E+07	0.2986%
76	1.1E+03	2323.6	47977.2	2.6E+06	7.4E+07	0.3096%
78	1.1E+03	4647.3	52624.5	5.3E+06	8.0E+07	0.3315%
80	1.0E+03	4647.3	57271.8	4.6E+06	8.4E+07	0.3509%
96	2.2E+02	192.9	57464.6	4.2E+04	8.4E+07	0.3511%
96.5	6.5E+02	1161.8	58626.4	7.5E+05	8.5E+07	0.3542%
97	1.3E+03	1161.8	59788.3	1.5E+06	8.7E+07	0.3605%
97.5	6.3E+02	1161.8	60950.1	7.3E+05	8.7E+07	0.3635%
98	6.7E+02	1161.8	62111.9	7.8E+05	8.8E+07	0.3668%
99	4.5E+02	2323.6	64435.5	1.1E+06	8.9E+07	0.3712%
100	3.5E+02	2323.6	66759.2	8.1E+05	9.0E+07	0.3745%
102	3.2E+02	4647.3	71406.4	1.5E+06	9.1E+07	0.3807%
104	2.8E+02	4647.3	76053.7	1.3E+06	9.3E+07	0.3861%
120	1.2E+02	192.9	76246.6	2.3E+04	9.3E+07	0.3862%
120.5	3.3E+01	1161.8	77408.4	3.9E+04	9.3E+07	0.3864%
121	0.0E+00	1161.8	78570.2	0.0E+00	9.3E+07	0.3864%
121.5	0.0E+00	1161.8	79732.0	0.0E+00	9.3E+07	0.3864%
122	0.0E+00	1161.8	80893.8	0.0E+00	9.3E+07	0.3864%
123	0.0E+00	2323.6	83217.5	0.0E+00	9.3E+07	0.3864%
124	0.0E+00	2323.6	85541.1	0.0E+00	9.3E+07	0.3864%
126	0.0E+00	4647.3	90188.4	0.0E+00	9.3E+07	0.3864%
128	0.0E+00	4647.3	94835.7	0.0E+00	9.3E+07	0.3864%

Table 10. breakthrough computation for column 1 during run 3

Time (hr)	C (CFU/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	Sum C/Co
0	0	16.5	16.5	0.0E+00	0.00E+00	0.0000%
0.5	0	99.6	116.1	0.0E+00	0.00E+00	0.0000%
1	0	99.6	215.7	0.0E+00	0.00E+00	0.0000%
1.5	0	99.6	315.3	0.0E+00	0.00E+00	0.0000%
2	0	99.6	414.8	0.0E+00	0.00E+00	0.0000%
3	0	199.1	614.0	0.0E+00	0.00E+00	0.0000%
4	0	199.1	813.1	0.0E+00	0.00E+00	0.0000%

6	0	398.3	1211.4	0.0E+00	0.00E+00	0.0000%
8	2.7E+01	398.3	1609.7	1.1E+04	1.06E+04	0.0007%
24	0	3186.4	4796.1	0.0E+00	1.06E+04	0.0007%
24.5	0	99.6	4895.7	0.0E+00	1.06E+04	0.0007%
25	0	99.6	4995.3	0.0E+00	1.06E+04	0.0007%
25.5	0	99.6	5094.8	0.0E+00	1.06E+04	0.0007%
26	0	99.6	5194.4	0.0E+00	1.06E+04	0.0007%
27	0	199.1	5393.6	0.0E+00	1.06E+04	0.0007%
28	0	199.1	5592.7	0.0E+00	1.06E+04	0.0007%
30	0	398.3	5991.0	0.0E+00	1.06E+04	0.0007%
32	0	398.3	6389.3	0.0E+00	1.06E+04	0.0007%
48	0	3186.4	9575.7	0.0E+00	1.06E+04	0.0007%
49	0	199.1	9774.8	0.0E+00	1.06E+04	0.0007%
50	0	199.1	9974.0	0.0E+00	1.06E+04	0.0007%
51	0	199.1	10173.1	0.0E+00	1.06E+04	0.0007%
52	0	199.1	10372.3	0.0E+00	1.06E+04	0.0007%
53	0	199.1	10571.4	0.0E+00	1.06E+04	0.0007%
54	0	199.1	10770.6	0.0E+00	1.06E+04	0.0007%

Table 11. Breakthrough computation for column 2 during run 3

Time (hr)	C (CFU/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	Sum C/Co
0	0.0E+00	158.3	158.3	0.0E+00	0.00E+00	0.00%
0.5	0.0E+00	953.5	1111.8	0.0E+00	0.00E+00	0.00%
1	4.9E+03	953.5	2065.3	4.6E+06	4.63E+06	0.28%
1.5	3.6E+03	953.5	3018.8	3.4E+06	8.04E+06	0.48%
2	3.0E+03	953.5	3972.3	2.9E+06	1.09E+07	0.65%
3	1.7E+03	1907.0	5879.3	3.2E+06	1.41E+07	0.84%
4	6.3E+03	1907.0	7786.3	1.2E+07	2.60E+07	1.56%
6	1.4E+03	3814.0	11600.4	5.3E+06	3.14E+07	1.88%
8	2.9E+03	3814.0	15414.4	1.1E+07	4.25E+07	2.55%
24	0.0E+00	182.5	15596.9	0.0E+00	4.25E+07	2.55%
24.5	0.0E+00	1099.3	16696.2	0.0E+00	4.25E+07	2.55%
25	9.3E+01	1099.3	17795.5	1.0E+05	4.26E+07	2.56%
25.5	4.3E+02	1099.3	18894.8	4.7E+05	4.31E+07	2.59%
26	1.5E+02	1099.3	19994.1	1.7E+05	4.33E+07	2.60%
27	8.7E+01	2198.6	22192.7	1.9E+05	4.35E+07	2.61%
28	1.0E+02	2198.6	24391.3	2.2E+05	4.37E+07	2.62%
30	6.7E+01	4397.2	28788.5	2.9E+05	4.40E+07	2.64%
32	6.0E+01	4397.2	33185.7	2.6E+05	4.42E+07	2.65%
48	2.6E+03	205.5	33391.2	5.3E+05	4.48E+07	2.69%
49	2.0E+04	2475.7	35866.9	5.0E+07	9.46E+07	5.68%
50	5.2E+03	2475.7	38342.6	1.3E+07	1.07E+08	6.44%
51	5.9E+03	2475.7	40818.3	1.5E+07	1.22E+08	7.33%

52	4.3E+03	2475.7	43294.1	1.1E+07	1.33E+08	7.97%
53	5.9E+03	2475.7	45769.8	1.5E+07	1.47E+08	8.85%
54	4.2E+03	2475.7	48245.5	1.0E+07	1.58E+08	9.48%

Table 12. Breakthrough computation for column 3 during run 3

Time (hr)	C (CFU/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	Sum C/Co
0	0.0E+00	102.973262	103.0	0.0E+00	0.00E+00	0.00%
0.5	0.0E+00	620.3	723.3	0.0E+00	0.00E+00	0.00%
1	0.0E+00	620.3	1343.6	0.0E+00	0.00E+00	0.00%
1.5	0.0E+00	620.3	1963.9	0.0E+00	0.00E+00	0.00%
2	2.3E+02	620.3	2584.3	1.4E+05	1.41E+05	0.00%
3	1.1E+02	1240.6	3824.9	1.4E+05	2.81E+05	0.01%
4	1.0E+02	1240.6	5065.5	1.2E+05	4.05E+05	0.01%
6	1.5E+02	2481.3	7546.8	3.6E+05	7.69E+05	0.01%
8	1.9E+02	2481.3	10028.1	4.8E+05	1.25E+06	0.02%
24	0.0E+00	103.0	10131.1	0.0E+00	1.25E+06	0.02%
24.5	0.0E+00	620.3	10751.4	0.0E+00	1.25E+06	0.02%
25	0.0E+00	620.3	11371.7	0.0E+00	1.25E+06	0.02%
25.5	0.0E+00	620.3	11992.0	0.0E+00	1.25E+06	0.02%
26	0.0E+00	620.3	12612.4	0.0E+00	1.25E+06	0.02%
27	0.0E+00	1240.6	13853.0	0.0E+00	1.25E+06	0.02%
28	0.0E+00	1240.6	15093.6	0.0E+00	1.25E+06	0.02%
30	0.0E+00	2481.3	17574.9	0.0E+00	1.25E+06	0.02%
32	0.0E+00	2481.3	20056.2	0.0E+00	1.25E+06	0.02%
48	0.0E+00	103.0	20159.2	0.0E+00	1.25E+06	0.02%
49	0.0E+00	1240.6	21399.8	0.0E+00	1.25E+06	0.02%
50	0.0E+00	1240.6	22640.5	0.0E+00	1.25E+06	0.02%
51	0.0E+00	1240.6	23881.1	0.0E+00	1.25E+06	0.02%
52	0.0E+00	1240.6	25121.8	0.0E+00	1.25E+06	0.02%
53	0.0E+00	1240.6	26362.4	0.0E+00	1.25E+06	0.02%
54	0.0E+00	1240.6	27603.0	0.0E+00	1.25E+06	0.02%

Table 13. Breakthrough computation for column 1 during run 4

Time (hr)	C (CFU/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*C (CFU)	Sum C (cfu)	Sum C/Co
0	0.0E+00	20.5	20.5	0.0E+00	0.0E+00	0.0000%
0.5	0.0E+00	123.6	144.1	0.0E+00	0.0E+00	0.0000%
1	0.0E+00	123.6	267.7	0.0E+00	0.0E+00	0.0000%
1.5	0.0E+00	123.6	391.3	0.0E+00	0.0E+00	0.0000%
2	0.0E+00	123.6	514.9	0.0E+00	0.0E+00	0.0000%
3	0.0E+00	247.2	762.1	0.0E+00	0.0E+00	0.0000%

4	3.3E+02	247.2	1009.3	8.2E+04	8.2E+04	0.0009%
6	0.0E+00	494.4	1503.7	0.0E+00	8.2E+04	0.0009%
8	0.0E+00	494.4	1998.1	0.0E+00	8.2E+04	0.0009%
24	0.0E+00	3955.1	5953.2	0.0E+00	8.2E+04	0.0009%
24.5	0.0E+00	68.3	6021.5	0.0E+00	8.2E+04	0.0009%
25	0.0E+00	68.3	6089.8	0.0E+00	8.2E+04	0.0009%
25.5	1.3E+02	68.3	6158.0	9.1E+03	9.2E+04	0.0010%
26	0.0E+00	68.3	6226.3	0.0E+00	9.2E+04	0.0010%
27	0.0E+00	136.6	6362.9	0.0E+00	9.2E+04	0.0010%
28	0.0E+00	136.6	6499.4	0.0E+00	9.2E+04	0.0010%
30	2.0E+02	273.1	6772.5	5.5E+04	1.5E+05	0.0016%
32	3.3E+02	273.1	7045.6	9.1E+04	2.4E+05	0.0026%
48	0.0E+00	2184.9	9230.5	0.0E+00	2.4E+05	0.0026%
48.5	0.0E+00	72.1	9302.7	0.0E+00	2.4E+05	0.0026%
49	0.0E+00	72.1	9374.8	0.0E+00	2.4E+05	0.0026%
49.5	0.0E+00	72.1	9446.9	0.0E+00	2.4E+05	0.0026%
50	0.0E+00	72.1	9519.1	0.0E+00	2.4E+05	0.0026%
51	3.3E+02	144.3	9663.4	4.8E+04	2.9E+05	0.0032%
52	0.0E+00	144.3	9807.6	0.0E+00	2.9E+05	0.0032%
54	0.0E+00	288.6	10096.2	0.0E+00	2.9E+05	0.0032%
56	0.0E+00	288.6	10384.7	0.0E+00	2.9E+05	0.0032%
72	0.0E+00	2308.4	12693.2	0.0E+00	2.9E+05	0.0032%
73	2.7E+02	110.0	12803.2	2.9E+04	3.1E+05	0.0035%
74	0.0E+00	110.0	12913.2	0.0E+00	3.1E+05	0.0035%
75	0.0E+00	110.0	13023.3	0.0E+00	3.1E+05	0.0035%
76	0.0E+00	110.0	13133.3	0.0E+00	3.1E+05	0.0035%
78	0.0E+00	220.1	13353.4	0.0E+00	3.1E+05	0.0035%
80	0.0E+00	220.1	13573.4	0.0E+00	3.1E+05	0.0035%

Table 14. Breakthrough computation for column 2 during run 4

Time (hr)	**C (CFU/mL)**	**Q*Δt (mL)**	**Cumul vol (mL)**	**Q*Δt*C (CFU)**	**Sum C (cfu)**	**Sum C/Co**
0	0.0E+00	148.5	148.5	0.0E+00	0.0E+00	0.0000%
0.5	2.2E+03	894.8	1043.3	2.0E+06	2.0E+06	0.0257%
1	4.5E+03	894.8	1938.0	4.0E+06	6.0E+06	0.0777%
1.5	3.5E+03	894.8	2832.8	3.1E+06	9.1E+06	0.1182%
2	4.1E+03	894.8	3727.6	3.6E+06	1.3E+07	0.1656%
3	1.0E+04	1789.5	5517.1	1.8E+07	3.1E+07	0.4066%
4	1.8E+04	1789.5	7306.6	3.2E+07	6.3E+07	0.8186%
6	2.8E+04	3579.0	10885.6	9.9E+07	1.6E+08	2.1152%
8	5.4E+04	3579.0	14464.7	1.9E+08	3.5E+08	4.6184%
24	0.0E+00	118.4	14583.0	0.0E+00	3.5E+08	4.6184%
24.5	1.1E+04	713.1	15296.2	7.7E+06	3.6E+08	4.7188%
25	1.8E+04	713.1	16009.3	1.3E+07	3.7E+08	4.8817%

25.5	2.9E+04	713.1	16722.4	2.1E+07	4.0E+08	5.1494%
26	6.9E+04	713.1	17435.5	4.9E+07	4.4E+08	5.7887%
27	7.9E+04	1426.2	18861.8	1.1E+08	5.6E+08	7.2559%
28	6.1E+04	1426.2	20288.0	8.7E+07	6.4E+08	8.3909%
30	4.5E+04	2852.5	23140.5	1.3E+08	7.7E+08	10.0638%
32	2.5E+04	2852.5	25993.0	7.2E+07	8.4E+08	11.0080%
48	4.5E+04	53.9	26046.9	2.4E+06	8.5E+08	11.0397%
48.5	8.3E+03	324.7	26371.6	2.7E+06	8.5E+08	11.0747%
49	1.4E+04	324.7	26696.3	4.5E+06	8.5E+08	11.1334%
49.5	2.4E+04	324.7	27020.9	7.8E+06	8.6E+08	11.2352%
50	3.0E+04	324.7	27345.6	9.8E+06	8.7E+08	11.3627%
51	6.1E+04	649.4	27995.0	4.0E+07	9.1E+08	11.8784%
52	8.0E+04	649.4	28644.4	5.2E+07	9.6E+08	12.5593%
54	6.4E+04	1298.7	29943.1	8.3E+07	1.0E+09	13.6460%
56	4.0E+04	1298.7	31241.9	5.2E+07	1.1E+09	14.3218%
72	2.3E+03	168.7	31410.6	3.9E+05	1.1E+09	14.3270%
73	2.4E+04	2032.4	33443.0	5.0E+07	1.1E+09	14.9733%
74	6.5E+03	2032.4	35475.4	1.3E+07	1.2E+09	15.1445%
75	2.6E+04	2032.4	37507.9	5.3E+07	1.2E+09	15.8403%
76	2.4E+04	2032.4	39540.3	4.9E+07	1.3E+09	16.4795%
78	1.5E+04	4064.9	43605.2	6.1E+07	1.3E+09	17.2776%
80	7.2E+03	4064.9	47670.1	2.9E+07	1.4E+09	17.6590%
96	2.0E+02	104.5	47774.6	2.1E+04	1.4E+09	17.6593%
96.5	1.0E+03	629.5	48404.0	6.3E+05	1.4E+09	17.6675%
97	2.6E+03	629.5	49033.5	1.6E+06	1.4E+09	17.6889%
97.5	6.6E+03	629.5	49663.0	4.2E+06	1.4E+09	17.7430%
98	3.9E+03	629.5	50292.5	2.5E+06	1.4E+09	17.7753%
99	2.7E+03	1259.0	51551.4	3.4E+06	1.4E+09	17.8190%
100	1.5E+03	1259.0	52810.4	1.8E+06	1.4E+09	17.8431%
102	3.3E+02	2517.9	55328.3	8.4E+05	1.4E+09	17.8540%
104	0.0E+00	2517.9	57846.2	0.0E+00	1.4E+09	17.8540%

Table 15. Breakthrough computation for column 3 during run 4

Time (hr)	**C (CFU/mL)**	**Q*Δt (mL)**	**Cumul vol (mL)**	**Q*Δt*C (CFU)**	**Sum C (cfu)**	**Sum C/Co**
0	0.0E+00	89	89	0.0E+00	0.0E+00	0.00%
0.5	0.0E+00	539	628	0.0E+00	0.0E+00	0.00%
1	2.0E+02	539	1166	1.1E+05	1.1E+05	0.00%
1.5	2.0E+02	539	1705	1.1E+05	2.2E+05	0.00%
2	1.6E+03	539	2243	8.6E+05	1.1E+06	0.01%
3	4.0E+03	1077	3320	4.3E+06	5.4E+06	0.07%
4	1.9E+03	1077	4397	2.1E+06	7.5E+06	0.10%
6	8.7E+02	2154	6552	1.9E+06	9.3E+06	0.13%
8	6.7E+01	2154	8706	1.4E+05	9.5E+06	0.13%

24	4.6E+03	51	8757	2.4E+05	9.7E+06	0.13%
24.5	0.0E+00	308	9065	0.0E+00	9.7E+06	0.13%
25	0.0E+00	308	9373	0.0E+00	9.7E+06	0.13%
25.5	0.0E+00	308	9681	0.0E+00	9.7E+06	0.13%
26	0.0E+00	308	9989	0.0E+00	9.7E+06	0.13%
27	1.1E+03	616	10605	6.6E+05	1.0E+07	0.14%
28	1.6E+03	616	11221	9.9E+05	1.1E+07	0.15%
30	4.0E+02	1232	12453	4.9E+05	1.2E+07	0.16%
32	3.3E+02	1232	13685	4.1E+05	1.2E+07	0.17%
48	3.3E+02	50	13735	1.7E+04	1.2E+07	0.17%
48.5	8.0E+02	302	14037	2.4E+05	1.3E+07	0.17%
49	1.3E+03	302	14338	4.0E+05	1.3E+07	0.18%
49.5	2.9E+03	302	14640	8.9E+05	1.4E+07	0.19%
50	7.8E+03	302	14942	2.4E+06	1.6E+07	0.22%
51	1.1E+04	604	15545	6.4E+06	2.3E+07	0.31%
52	5.3E+04	604	16149	3.2E+07	5.5E+07	0.74%
54	6.6E+03	1207	17356	8.0E+06	6.3E+07	0.85%
56	6.5E+03	1207	18563	7.8E+06	7.0E+07	0.96%
72	4.0E+02	50	18613	2.0E+04	7.0E+07	0.96%
73	4.2E+03	606	19220	2.5E+06	7.3E+07	0.99%
74	1.4E+04	606	19826	8.5E+06	8.1E+07	1.11%
75	6.1E+03	606	20433	3.7E+06	8.5E+07	1.16%
76	1.2E+04	606	21039	7.2E+06	9.2E+07	1.26%
78	3.2E+03	1213	22252	3.9E+06	9.6E+07	1.31%
80	3.0E+03	1213	23465	3.6E+06	1.0E+08	1.36%
96	0.0E+00	45	23510	0.0E+00	1.0E+08	1.36%
96.5	3.3E+02	270	23780	9.0E+04	1.0E+08	1.36%
97	6.7E+02	270	24050	1.8E+05	1.0E+08	1.36%
97.5	1.3E+03	270	24320	3.4E+05	1.0E+08	1.37%
98	1.7E+03	270	24590	4.5E+05	1.0E+08	1.37%
99	1.6E+03	540	25130	8.6E+05	1.0E+08	1.39%
100	1.9E+03	540	25669	1.0E+06	1.0E+08	1.40%
102	3.3E+02	1080	26749	3.6E+05	1.0E+08	1.40%
104	0.0E+00	1080	27828	0.0E+00	1.0E+08	1.40%

Table 16. Breakthrough computation for column 1 during run 5

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
0	0.0E+00	95	95	0.0E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
1	0.0E+00	1144	1239	0.0E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2	2.0E+02	1144	2382	2.3E+05	2.29E+05	0.01%	2.50E+05	2.50E+05	0.01%
3	2.0E+02	1144	3526	2.3E+05	4.57E+05	0.03%	2.62E+05	5.12E+05	0.03%
4	1.3E+02	1144	4670	1.5E+05	6.10E+05	0.03%	1.83E+05	6.95E+05	0.04%

6	0.0E+00	2287	6957	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
8	0.0E+00	2287	9244	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
24	0.0E+00	18299	27544	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
25	0.0E+00	925	28469	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
26	0.0E+00	925	29394	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
27	0.0E+00	925	30319	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
28	0.0E+00	925	31244	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
30	0.0E+00	1850	33093	0.0E+00	6.10E+05	0.03%	0.00E+00	6.95E+05	0.04%
32	1.3E+02	1850	34943	2.5E+05	8.57E+05	0.05%	1.04E+06	1.74E+06	0.10%
48	4.0E+01	14800	49743	5.9E+05	1.45E+06	0.08%	5.13E+06	6.87E+06	0.38%
50	1.8E+02	2093	51836	3.8E+05	1.83E+06	0.10%	3.57E+06	1.04E+07	0.58%
52	6.7E+01	2093	53929	1.4E+05	1.96E+06	0.11%	1.45E+06	1.19E+07	0.67%
54	4.0E+01	2093	56021	8.4E+04	2.05E+06	0.11%	9.51E+05	1.28E+07	0.72%
56	0.0E+00	2093	58114	0.0E+00	2.05E+06	0.11%	0.00E+00	1.28E+07	0.72%

Table 17. Breakthrough computation for column 2 during run 5

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
0	0.0E+00	124	124	0.0E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
1	2.7E+02	1499	1624	4.0E+05	4.00E+05	0.02%	4.18E+05	4.18E+05	0.02%
2	2.0E+02	1499	3123	3.0E+05	7.00E+05	0.03%	3.28E+05	7.46E+05	0.03%
3	1.1E+03	1499	4622	1.7E+06	2.40E+06	0.10%	1.94E+06	2.69E+06	0.11%
4	8.0E+02	1499	6122	1.2E+06	3.60E+06	0.15%	1.44E+06	4.13E+06	0.17%
6	2.7E+02	2999	9120	8.0E+05	4.40E+06	0.18%	1.05E+06	5.17E+06	0.22%
8	4.0E+02	2999	12119	1.2E+06	5.60E+06	0.23%	1.72E+06	6.89E+06	0.29%
24	2.7E+02	164	12283	4.4E+04	5.64E+06	0.23%	1.29E+05	7.02E+06	0.29%
25	0.0E+00	1978	14261	0.0E+00	5.64E+06	0.23%	0.00E+00	7.02E+06	0.29%
26	2.7E+02	1978	16240	5.3E+05	6.17E+06	0.26%	1.70E+06	8.72E+06	0.36%
27	3.3E+02	1978	18218	6.6E+05	6.83E+06	0.28%	2.22E+06	1.09E+07	0.45%
28	4.7E+02	1978	20196	9.2E+05	7.75E+06	0.32%	3.25E+06	1.42E+07	0.59%
30	3.3E+02	3957	24153	1.3E+06	9.07E+06	0.38%	5.09E+06	1.93E+07	0.80%
32	2.0E+02	3957	28110	7.9E+05	9.86E+06	0.41%	3.34E+06	2.26E+07	0.94%
48	1.0E+02	97	28207	9.7E+03	9.87E+06	0.41%	8.40E+04	2.27E+07	0.94%
50	3.4E+02	2335	30542	7.9E+05	1.07E+07	0.44%	7.53E+06	3.02E+07	1.26%
52	2.0E+02	2335	32877	4.7E+05	1.11E+07	0.46%	4.85E+06	3.51E+07	1.46%
54	2.7E+02	2335	35213	6.3E+05	1.18E+07	0.49%	7.16E+06	4.23E+07	1.76%
56	1.8E+02	2335	37548	4.2E+05	1.22E+07	0.51%	5.22E+06	4.75E+07	1.97%

Table 18. Breakthrough computation for column 3 during run 5

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
0	0.0E+00	140	140	0.0E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
1	2.7E+02	1691	1831	4.5E+05	4.51E+05	0.02%	4.72E+05	4.72E+05	0.02%
2	1.7E+03	1691	3522	2.8E+06	3.27E+06	0.14%	3.08E+06	3.56E+06	0.15%
3	6.7E+02	1691	5213	1.1E+06	4.40E+06	0.19%	1.29E+06	4.85E+06	0.21%
4	5.3E+02	1691	6904	9.0E+05	5.30E+06	0.23%	1.08E+06	5.93E+06	0.25%
6	8.0E+02	3382	10286	2.7E+06	8.00E+06	0.34%	3.54E+06	9.47E+06	0.41%
8	2.0E+03	3382	13668	6.8E+06	1.48E+07	0.63%	9.69E+06	1.92E+07	0.82%
24	8.0E+02	116	13784	9.3E+04	1.49E+07	0.64%	2.73E+05	1.94E+07	0.83%
25	4.7E+02	1398	15183	6.5E+05	1.55E+07	0.67%	2.01E+06	2.14E+07	0.92%
26	3.7E+03	1398	16581	5.1E+06	2.06E+07	0.89%	1.65E+07	3.80E+07	1.63%
27	1.5E+03	1398	17979	2.1E+06	2.27E+07	0.97%	6.91E+06	4.49E+07	1.93%
28	1.9E+03	1398	19378	2.6E+06	2.53E+07	1.09%	9.20E+06	5.41E+07	2.32%
30	2.4E+03	2797	22175	6.7E+06	3.20E+07	1.37%	2.59E+07	8.00E+07	3.43%
32	2.3E+03	2797	24972	6.5E+06	3.85E+07	1.65%	2.75E+07	1.08E+08	4.61%
48	2.9E+02	112	25084	3.3E+04	3.86E+07	1.66%	2.85E+05	1.08E+08	4.63%
50	1.1E+02	2699	27783	3.1E+05	3.89E+07	1.67%	2.90E+06	1.11E+08	4.75%
52	4.6E+02	2699	30482	1.2E+06	4.01E+07	1.72%	1.29E+07	1.24E+08	5.30%
54	2.9E+02	2699	33181	7.7E+05	4.09E+07	1.76%	8.79E+06	1.32E+08	5.68%
56	1.3E+02	2699	35881	3.6E+05	4.13E+07	1.77%	4.47E+06	1.37E+08	5.87%

Table 19. Breakthrough computation for column 1 during run 6

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
0	0.00E+00	1415	1415	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2	2.50E+02	2850	4265	7.13E+05	7.13E+05	0.02%	8.25E+05	8.25E+05	0.02%
4	6.80E+02	3740	8005	2.54E+06	3.26E+06	0.09%	3.41E+06	4.23E+06	0.12%
6.5	5.00E+02	1615	9620	8.08E+05	4.06E+06	0.12%	1.30E+06	5.53E+06	0.16%
7.5	5.90E+02	920	10540	5.43E+05	4.61E+06	0.13%	9.38E+05	6.47E+06	0.18%
22.5	1.30E+02	400	10940	5.20E+04	4.66E+06	0.13%	2.69E+05	6.74E+06	0.19%
24.5	1.10E+02	2100	13040	2.31E+05	4.89E+06	0.14%	1.38E+06	8.12E+06	0.23%
26.5	1.70E+02	2000	15040	3.40E+05	5.23E+06	0.15%	2.35E+06	1.05E+07	0.30%
28	9.90E+01	2000	17040	1.98E+05	5.43E+06	0.16%	1.53E+06	1.20E+07	0.34%
30	7.20E+01	2500	19540	1.80E+05	5.61E+06	0.16%	1.61E+06	1.36E+07	0.39%
47	0.00E+00	585	20125	0.00E+00	5.61E+06	0.16%	0.00E+00	1.36E+07	0.39%
49	6.30E+01	2365	22490	1.49E+05	5.76E+06	0.16%	5.33E+06	1.89E+07	0.54%
51	0.00E+00	2190	24680	0.00E+00	5.76E+06	0.16%	0.00E+00	1.89E+07	0.54%
53	0.00E+00	2060	26740	0.00E+00	5.76E+06	0.16%	0.00E+00	1.89E+07	0.54%
55	1.80E+01	1900	28640	3.42E+04	5.79E+06	0.17%	1.90E+06	2.08E+07	0.60%

72	0.00E+00	425	29065	0.00E+00	5.79E+06	0.17%	0.00E+00	2.08E+07	0.60%
73	0.00E+00	1765	30830	0.00E+00	5.79E+06	0.17%	0.00E+00	2.08E+07	0.60%
75	0.00E+00	2125	32955	0.00E+00	5.79E+06	0.17%	0.00E+00	2.08E+07	0.60%
77	0.00E+00	1820	34775	0.00E+00	5.79E+06	0.17%	0.00E+00	2.08E+07	0.60%
79	9.00E+00	1540	36315	1.39E+04	5.80E+06	0.17%	4.43E+06	2.53E+07	0.72%

Table 20. Breakthrough computation for column 2 during run 6

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
0	0	800	800	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2	1.80E+03	2535	3335	4.56E+06	4.56E+06	0.13%	5.28E+06	5.28E+06	0.15%
4	6.20E+03	3380	6715	2.10E+07	2.55E+07	0.73%	2.81E+07	3.33E+07	0.95%
6.5	3.40E+03	2100	8815	7.14E+06	3.27E+07	0.93%	1.15E+07	4.48E+07	1.28%
7.5	3.10E+03	900	9715	2.79E+06	3.54E+07	1.01%	4.82E+06	4.96E+07	1.42%
22.5	2.50E+02	270	9985	6.75E+04	3.55E+07	1.01%	3.49E+05	5.00E+07	1.43%
24.5	3.90E+02	1500	11485	5.85E+05	3.61E+07	1.03%	3.50E+06	5.35E+07	1.53%
26.5	8.10E+02	1350	12835	1.09E+06	3.72E+07	1.06%	7.57E+06	6.11E+07	1.74%
28	6.00E+02	1200	14035	7.20E+05	3.79E+07	1.08%	5.56E+06	6.66E+07	1.90%
30	9.90E+01	1350	15385	1.34E+05	3.80E+07	1.09%	1.19E+06	6.78E+07	1.94%
47	1.10E+02	825	16210	9.08E+04	3.81E+07	1.09%	2.80E+06	7.06E+07	2.02%
49	3.90E+02	3430	19640	1.34E+06	3.95E+07	1.13%	4.78E+07	1.18E+08	3.38%
51	8.10E+01	2630	22270	2.13E+05	3.97E+07	1.13%	8.82E+06	1.27E+08	3.64%
53	5.40E+01	2200	24470	1.19E+05	3.98E+07	1.14%	5.69E+06	1.33E+08	3.80%
55	3.60E+01	2430	26900	8.75E+04	3.99E+07	1.14%	4.85E+06	1.38E+08	3.94%
72	0.00E+00	135	27035	0.00E+00	3.99E+07	1.14%	0.00E+00	1.38E+08	3.94%
73	0.00E+00	1240	28275	0.00E+00	3.99E+07	1.14%	0.00E+00	1.38E+08	3.94%
75	1.80E+01	1980	30255	3.56E+04	3.99E+07	1.14%	8.51E+06	1.46E+08	4.18%
77	2.70E+01	1600	31855	4.32E+04	4.00E+07	1.14%	1.19E+07	1.58E+08	4.52%
79	9.00E+00	1385	33240	1.25E+04	4.00E+07	1.14%	3.98E+06	1.62E+08	4.64%

Table 21. Breakthrough computation for column 3 during run 6

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
0	0	1050	1050	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2	3.70E+02	2100	3150	7.77E+05	7.77E+05	0.02%	8.99E+05	8.99E+05	0.03%
4	2.30E+03	2665	5815	6.13E+06	6.91E+06	0.20%	8.21E+06	9.11E+06	0.26%
6.5	7.30E+02	2525	8340	1.84E+06	8.75E+06	0.25%	2.96E+06	1.21E+07	0.34%
7.5	8.50E+02	730	9070	6.21E+05	9.37E+06	0.27%	1.07E+06	1.31E+07	0.38%
22.5	5.40E+01	200	9270	1.08E+04	9.38E+06	0.27%	5.58E+04	1.32E+07	0.38%
24.5	1.40E+02	1400	10670	1.96E+05	9.58E+06	0.27%	1.17E+06	1.44E+07	0.41%

26.5	3.80E+02	1500	12170	5.70E+05	1.01E+07	0.29%	3.94E+06	1.83E+07	0.52%
28	2.20E+02	1500	13670	3.30E+05	1.05E+07	0.30%	2.55E+06	2.09E+07	0.60%
30	1.30E+02	1750	15420	2.28E+05	1.07E+07	0.31%	2.03E+06	2.29E+07	0.65%
47	0.00E+00	225	15645	0.00E+00	1.07E+07	0.31%	0.00E+00	2.29E+07	0.65%
49	9.00E+00	1235	16880	1.11E+04	1.07E+07	0.31%	3.98E+05	2.33E+07	0.67%
51	5.40E+01	1350	18230	7.29E+04	1.08E+07	0.31%	3.02E+06	2.63E+07	0.75%
53	0.00E+00	1015	19245	0.00E+00	1.08E+07	0.31%	0.00E+00	2.63E+07	0.75%
55	0.00E+00	1175	20420	0.00E+00	1.08E+07	0.31%	0.00E+00	2.63E+07	0.75%
72	0.00E+00	150	20570	0.00E+00	1.08E+07	0.31%	0.00E+00	2.63E+07	0.75%
73	0.00E+00	825	21395	0.00E+00	1.08E+07	0.31%	0.00E+00	2.63E+07	0.75%
75	1.80E+01	1075	22470	1.94E+04	1.08E+07	0.31%	4.62E+06	3.09E+07	0.88%
77	1.80E+01	990	23460	1.78E+04	1.08E+07	0.31%	4.92E+06	3.58E+07	1.02%
79	0.00E+00	940	24400	0.00E+00	1.08E+07	0.31%	0.00E+00	3.58E+07	1.02%

Table 22. Breakthrough computation for column 1 for environmental coliforms

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
1.25	0	960	960	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2.5	0	620	1580	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
4.25	0	985	2565	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
6.25	300	1140	3705	3.42E+05	3.42E+05	0.29%	5.43E+05	5.43E+05	0.45%
8	2300	980	4685	2.25E+06	2.60E+06	2.16%	4.07E+06	4.62E+06	3.85%
24.583	110	425	5110	4.68E+04	2.64E+06	2.20%	2.88E+05	4.91E+06	4.09%
26	36	850	5960	3.06E+04	2.67E+06	2.23%	2.10E+05	5.12E+06	4.26%
28.367	280	1650	7610	4.62E+05	3.14E+06	2.61%	3.77E+06	8.88E+06	7.40%
30	300	1070	8680	3.21E+05	3.46E+06	2.88%	2.96E+06	1.18E+07	9.87%
32	90	1430	10110	1.29E+05	3.59E+06	2.99%	1.37E+06	1.32E+07	11.01%
48.583	27	415	10525	1.12E+04	3.60E+06	3.00%	4.08E+05	1.36E+07	11.35%
50	110	815	11340	8.97E+04	3.69E+06	3.07%	3.63E+06	1.72E+07	14.37%
52.17	120	1190	12530	1.43E+05	3.83E+06	3.19%	6.78E+06	2.40E+07	20.03%
54	91	1050	13580	9.56E+04	3.92E+06	3.27%	5.20E+06	2.92E+07	24.36%
56	18	1150	14730	2.07E+04	3.94E+06	3.29%	1.31E+06	3.05E+07	25.44%
72.583	18	390	15120	7.02E+03	3.95E+06	3.29%	1.51E+06	3.20E+07	26.70%
74.417	27	1090	16210	2.94E+04	3.98E+06	3.32%	7.25E+06	3.93E+07	32.74%
76	0	1000	17210	0.00E+00	3.98E+06	3.32%	0.00E+00	3.93E+07	32.74%
78	27	1725	18935	4.66E+04	4.03E+06	3.36%	1.50E+07	5.43E+07	45.21%
80	27	1225	20160	3.31E+04	4.06E+06	3.38%	1.23E+07	6.66E+07	55.47%

Table 23. Breakthrough computation for column 2 for environmental coliforms

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
1.25	0	920	960	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2.5	0	690	1650	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
4.25	0	1175	2825	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
6.25	9.00E+00	1245	4070	1.12E+04	1.12E+04	0.01%	1.78E+04	1.78E+04	0.01%
8	5.90E+03	1160	5230	6.84E+06	6.86E+06	5.71%	1.24E+07	1.24E+07	10.32%
24.583	7.40E+02	350	5580	2.59E+05	7.11E+06	5.93%	1.60E+06	1.40E+07	11.66%
26	7.20E+01	725	6305	5.22E+04	7.17E+06	5.97%	3.57E+05	1.43E+07	11.95%
28.367	7.70E+02	1350	7655	1.04E+06	8.21E+06	6.84%	8.48E+06	2.28E+07	19.02%
30	3.50E+02	890	8545	3.12E+05	8.52E+06	7.10%	2.87E+06	2.57E+07	21.41%
32	2.30E+02	1110	9655	2.55E+05	8.77E+06	7.31%	2.73E+06	2.84E+07	23.68%
48.583	4.50E+01	280	9935	1.26E+04	8.79E+06	7.32%	4.59E+05	2.89E+07	24.06%
50	1.00E+02	905	10840	9.05E+04	8.88E+06	7.40%	3.66E+06	3.25E+07	27.12%
52.17	9.10E+01	1380	12220	1.26E+05	9.00E+06	7.50%	5.96E+06	3.85E+07	32.09%
54	8.20E+01	1100	13320	9.02E+04	9.09E+06	7.58%	4.91E+06	4.34E+07	36.17%
56	3.60E+01	1300	14620	4.68E+04	9.14E+06	7.62%	2.95E+06	4.64E+07	38.63%
72.583	3.60E+01	400	15020	1.44E+04	9.15E+06	7.63%	3.10E+06	4.95E+07	41.21%
74.417	3.60E+01	1130	16150	4.07E+04	9.19E+06	7.66%	1.00E+07	5.95E+07	49.57%
76	1.80E+01	900	17050	1.62E+04	9.21E+06	7.67%	4.49E+06	6.40E+07	53.30%
78	9.10E+00	1700	18750	1.55E+04	9.23E+06	7.69%	4.97E+06	6.89E+07	57.45%
80	4.50E+01	1175	19925	5.29E+04	9.28E+06	7.73%	1.97E+07	8.86E+07	73.85%

Table 24. Breakthrough computation for column 3 for environmental coliforms

Time (hr)	Cc (cfu/mL)	Q*Δt (mL)	Cumul vol (mL)	Q*Δt*Cc (cfu)	Sum C (cfu)	Sum C/Co	C' (cfu)	Sum C' (cfu)	Sum C'/Co
1.25	0	1175	960	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
2.5	0	765	1725	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
4.25	0	1070	2795	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
6.25	2.20E+02	925	3720	2.04E+05	2.04E+05	0.17%	3.23E+05	3.23E+05	0.27%
8	3.50E+03	670	4390	2.35E+06	2.55E+06	2.12%	4.24E+06	4.56E+06	3.80%
24.583	6.30E+01	175	4565	1.10E+04	2.56E+06	2.13%	6.80E+04	4.63E+06	3.86%
26	2.80E+02	305	4870	8.54E+04	2.64E+06	2.20%	5.85E+05	5.21E+06	4.35%
28.367	3.60E+01	330	5200	1.19E+04	2.66E+06	2.21%	9.69E+04	5.31E+06	4.43%
30	4.50E+01	215	5415	9.68E+03	2.67E+06	2.22%	8.91E+04	5.40E+06	4.50%

32	2.70E+01	235	5650	6.35E+03	2.67E+06	2.23%	6.77E+04	5.47E+06	4.56%
48.583	9.10E+01	180	5830	1.64E+04	2.69E+06	2.24%	5.97E+05	6.07E+06	5.05%
50	2.70E+01	400	6230	1.08E+04	2.70E+06	2.25%	4.37E+05	6.50E+06	5.42%
52.17	9.10E+00	640	6870	5.82E+03	2.71E+06	2.25%	2.77E+05	6.78E+06	5.65%
54	0.00E+00	350	7220	0.00E+00	2.71E+06	2.25%	0.00E+00	6.78E+06	5.65%
56	0.00E+00	350	7570	0.00E+00	2.71E+06	2.25%	0.00E+00	6.78E+06	5.65%
72.583	0.00E+00	100	7670	0.00E+00	2.71E+06	2.25%	0.00E+00	6.78E+06	5.65%
74.417	0.00E+00	540	8210	0.00E+00	2.71E+06	2.25%	0.00E+00	6.78E+06	5.65%
76	9.10E+00	500	8710	4.55E+03	2.71E+06	2.26%	1.26E+06	8.04E+06	6.70%
78	0.00E+00	625	9335	0.00E+00	2.71E+06	2.26%	0.00E+00	8.04E+06	6.70%
80	0.00E+00	550	9885	0.00E+00	2.71E+06	2.26%	0.00E+00	8.04E+06	6.70%

Table 25 computation of heterotrophs monitoring for column 1

Time (h)	Cc (CFU/mL)	V (mL)	Tot V (mL)
1.25	4.40E+04	960	960
2.5	1.40E+04	620	1580
4.25	2.02E+04	985	2565
6.25	3.05E+04	1140	3705
8	4.19E+04	980	4685
24.583	7.62E+04	425	5110
26	2.99E+04	850	5960
28.367	3.70E+04	1650	7610
30	3.38E+04	1070	8680
32	1.69E+04	1430	10110
48.583	2.35E+04	415	10525
50	1.16E+04	815	11340
52.17	1.32E+04	1190	12530
54	1.11E+04	1050	13580
56	7.29E+03	1150	14730
72.583	2.43E+04	390	15120
74.417	4.95E+04	1090	16210
76	1.08E+04	1000	17210
78	1.58E+04	1725	18935
80	1.27E+04	1225	20160

Table 26. Computation of heterotrophs monitoring for column 2

Time (hr)	C (CFU/mL)	V (mL)	Tot V (mL)
1.25	4.00E+04	920	920
2.5	6.69E+04	690	1610
4.25	9.83E+04	1175	2785

6.25	7.00E+04	1245	4030
8	1.01E+05	1160	5190
24.583	2.55E+05	350	5540
26	1.26E+05	725	6265
28.367	6.60E+04	1350	7615
30	1.29E+05	890	8505
32	4.62E+04	1110	9615
48.583	4.52E+04	280	9895
50	1.46E+04	905	10800
52.17	2.20E+04	1380	12180
54	2.29E+04	1100	13280
56	1.15E+04	1300	14580
72.583	2.30E+04	400	14980
74.417	1.89E+04	1130	16110
76	1.80E+04	900	17010
78	2.95E+04	1700	18710
80	5.48E+04	1175	19885

Table 27. computation of heterotrophs monitoring for column 3

Time (h)	**C (CFU/mL)**	**V (mL)**	Tot V (mL)
1.25	6.35E+04	1175	1175
2.5	3.95E+04	765	1940
4.25	1.11E+05	1070	3010
6.25	9.02E+04	925	3935
8	1.36E+05	670	4605
24.583	3.25E+05	175	4780
26	1.11E+05	305	5085
28.367	7.57E+04	330	5415
30	4.24E+04	215	5630
32	4.10E+04	235	5865
48.583	1.00E+05	180	6045
50	4.48E+04	400	6445
52.17	5.00E+04	640	7085
54	4.05E+04	350	7435
56	2.62E+04	350	7785
72.583	9.64E+04	100	7885
74.417	1.09E+05	540	8425
76	4.38E+04	500	8925
78	3.98E+04	625	9550
80	5.98E+04	550	10100

Table 28. heterotrophs monitoring for control and inlet

Time (hr)	Control C (CFU/mL)	Inlet C (CFU/mL)
1.25	n/a	
2.5	3.15E+06	
4.25	3.33E+06	
6.25	3.40E+06	
8	9.43E+06	
24.583	1.94E+06	4.34E+31
26	1.77E+06	
28.367	1.54E+06	
30	1.71E+06	
32	1.87E+06	3.06E+05
48.583	5.38E+05	1.22E+55
50	8.24E+05	
52.17	7.38E+05	
54	2.70E+05	
56	3.14E+05	1.70E+62
72.583	4.10E+04	2.63E+78
74.417	6.24E+05	
76	2.55E+05	
78	1.90E+05	
80	1.38E+05	9.89E+85