Evaluation of Enhanced Ceramic Water Filtration (ECWF) Systems for the Removal of Turbidity and Bacteria for Households in Developing Countries

By

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Abstract:
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Evaluation of Enhanced Ceramic Water Filtration (ECWF) Systems for the Removal of Turbidity and Bacteria for Households in Developing Countries

Thesis Directed by Associate Professor Angela Bielefeldt

Ceramic water filters are used for point-of-use drinking water treatment in many developing countries. However, the typical ceramic pot filters (CPF) produce a limited amount of water per family. This research explored a simple method to increase the water volume treated per day by adding a second 5-gallon plastic bucket inserted and sealed onto the top of the standard CPF. This so-called enhanced ceramic water filtration system (ECWF) was then evaluated to characterize flow rates and clogging over time, turbidity removal, and E. coli disinfection. The research tested two CPFs from Nicaragua and two CPFs from Cambodia in both a standard ceramic water filtration system (CWF) and ECWF.

The amount of water treated over the first hour when the system was initially filled were 2.4 to 3.4 times more for the Nicaragua CPFs and 3.7 to 4.2 times more for the Cambodia CPFs when operated in the ECWF compared to the CWF. The ECWF was successful at removing up to 500 NTU of turbidity and increased the first hour flow rates and cumulative volume filtered >300%. Overall, the enhanced flow rates of the ECWF system did not significantly affect the ability of the ceramic to remove *E. coli* compared to the CWF.

There were operational difficulties that could not be overcome to a level that would be desired to implement in the field. The different CPF dimensions from different factories and the inconsistency in the filters made it difficult to find buckets that fit exactly for the ECWF system. The ECWF system also resulted in water short circuiting through the glue and around the gasket when the top bucket did not seal correctly. The risk of cracking increased tremendously under

the enhanced flow rates due to the added stress on the ceramic. During these experiments with 5 different filters, 2 filters cracked while being used in the ECWF system. Though increased flow rates would be desired for household use, the ECWF system is not recommended without further investigation of the leakage issues and quality control during production to ensure uniform geometry.

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Chapter 1 Problem Statement

More than one billion people worldwide still lack access to clean drinking water (WHO, 2011a). Even those who are provided with access to quality water are sometimes unable to get the quantity they need. As part of the Millennium Development Goals (MDGs), the United Nations set multiple targets focused on improving access to water and sanitation and alleviating related diseases by 2015. Goal 7C states "Halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation" (UN, 2000). Reaching this target implies tackling both the quantity of water including the lack of access to sufficient amounts of water and the quality of water including the safety for consumption (WHO, 2010). Although water issues have been put in the spotlight, poor water quality continues to pose a major threat to human health mostly through diarrheal disease.

The disability-adjusted life year, also referred to as DALY, is a method of evaluating the overall burden of disease using a time-based measurement that combines years of life lost due to premature mortality and years of life lost due to time lived in unhealthy states (WHO, 2009). The most predominant waterborne disease, diarrhea, has an estimated annual incidence of 4.6 billion episodes per year (WHO, 2010) and is responsible for 1.8 million deaths every year, mainly in children under 5 years of age (WHO, 2008). Diarrhea alone amounts to an estimated 4 % of the total DALY global burden of disease. It was projected that 88% of the diarrhea burden is attributable to unsafe water, lack of proper sanitation, and poor hygiene practices (WHO, 2007). These deaths are completely preventable given access to clean drinking water, sanitation and hygiene. Therefore, improved water quality could also help reach MDG Goal 4A which states "Reduce by two thirds the under-five mortality rate" (UN, 2000).

Point of use (POU) or household water treatment and safe storage (HWTS) interventions can lead to remarkable improvements in drinking water quality and a reduction in diarrheal disease (WHO, 2011b). HWTS are a cost-effective alternative to conventional methods of water treatment, distribution, and storage (Clasen, 2008). These interventions can result in immediate positive differences in the lives of those relying on water from polluted rivers, lakes, unsafe wells or piped water supplies (WHO, 2011b). In many developing communities both the quantity and quality of drinking water are frequently insufficient. Correspondingly, the quantity of water for personal and domestic hygiene use has been shown to be more important than the quality of drinking water on reducing diarrheal disease occurrence (Gadgil, 1998).

Ceramic pot filters (CPFs) are used for household point-of-use drinking water treatment in over 25 countries, and as of 2008 there were 36 operating factories producing them in developing countries around the world (The Ceramic Manufacturing Working Group 2010). These CWFs have a number of different manufacturing methods depending on where they are made and as a result the CPFs can have various dimensions. One example of a standard ceramic pot filter (CPF) is shown (left) and the ceramic water filter (CWF) system (right) in Figure 1-1.

Figure 1-1Typical CPF produced by Potters for Peace Factory in Nicaragua (PFP 2006)

Ceramic pot filters have been shown to be the most effective HWTS method at reducing diarrhea in the long term (Hunter, 2009), but they are limited by the quantity of water they can filter due to the slow flow rate of initially approximately 1-3 liters per hour which decreases over time as water drains out of the filter. The minimum amount of clean water required daily is 20-50 liters per person to ensure basic needs for drinking, cooking, and cleaning (UN, 2010). The CPF alone produces around the 25 liters per day at top filtering rates; not nearly enough for a family of 2-5 people in the developing world.

This research project explored a simple method to increase the flow delivered by the CPF which was proposed by Chris Schulz (P.E., BCEE, senior vice president CDM; 2009), and determined whether the treated water quality under enhanced flow rates remained consistent with typical CWF use. This thesis will first present relevant background information on CPFs' effectiveness for removing both turbidity and bacteria, using CPFs with and without silver. Other approaches to enhance the flow rates of the CPFs and the comparative effect of these changes on effluent water quality will also be summarized. Chapter 3 defines the project specifications including the proposed technology and the research objectives. Chapter 4 presents the research methods and research approach, with supporting details provided in the Appendix. The experimental results for the turbidity tests and *E. coli* experiments with and without silver are presented in Chapters 5 and 6; respectively. Finally, Chapter 7 summarizes the significant results and conclusions from the research, discusses practical implications, and identifies key areas for future research.

Chapter 2 Literature Review

This chapter examines the related literature and research that has been done previously in the laboratory and the field. First, section 2.1 identifies the need for household water treatment and the different types of water treatment that have been researched for application in developing communities. Secondly, section 2.2 highlights ceramic water filters as an effective technology for drinking water treatment at the household level. Section 2.3 gives an overview of the CPF and section 2.4 describes how the manufacturing process varies depending on the location and the organization producing the CPFs. Section 2.5 discusses the flow rate and volume treatment capacity of CPFs and other attempts at increasing their flow rates. Section 2.6 summarizes previous bacterial disinfection research, including only physical removal (assuming no silver) and disinfection by inactivation and other means (with new silver or re-applied silver), both in the laboratory and in the field. Finally, 2.7 addressed the recontamination issues of the CPF technology.

2.1 Identifying the need for developing community water treatment

The need for safe drinking water worldwide is vast. Over 1/6 of the world's population still lacks access to safe drinking water and around 2 million deaths a year are caused by diarrheal disease, mostly in children under five years of age (WHO 2011a). Most of these illnesses and deaths are completely preventable given access to improved drinking water source, adequate sanitation, and hygiene. The World Health Organization has defined the following water sources as improved: public or private stand pipes, tube wells, protected dug wells, protected springs, and rainwater harvesting (WHO 2008b). It is important to note that a technically improved water source does not necessarily ensure microbial safety in the drinking water. When collected water is not protected by residual disinfectant such as chlorine, recontamination is a major risk as the water gets handled from the source to the household to the

mouth for consumption (Clasen & Bastable 2003, Wright 2003). Therefore, household-based interventions are typically more effective than water quality interventions at the source (Clasen et al. 2006). Further, safe treatment and storage at the household level is very important to eliminate these contamination risks and reduce the illnesses related to the consumption of unsafe drinking water.

2.2 Types of household water treatment

Household water treatment and safe storage (HWTS) has proven to be an effective alternative to conventional improvements in water supplies in developing communities (Clasen 2008). HWTS could be a major contributor to meeting the UN's Millennium Development Goals (MDGs) for safe drinking water. There are many options for household drinking water treatment in developing communities. Some of the more well-known options include disinfection by SODIS (solar disinfection), chlorination or a combination of coagulant and chlorine, filtration by ceramic filter, and bio-sand filter or slow sand filter.

Household water treatment technologies are already being promoted worldwide as successful interventions to decrease waterborne diseases in developing communities. A meta-analysis verified that point-of-use water treatment is an effective solution; 37 treatment technologies were evaluated for a range of characteristics, including improving microbial water quality and maintaining disinfection, the health impacts including diarrheal incidence, and costs. Of the technologies studied, ceramic filters were proved to be one of the five most promising technologies (Fewtrell et al 2005). A study by Hunter aimed to quantify the benefits of household water treatment at reducing diarrhea. Though difficult, the study made an effort to look past the potential impact of recall bias or lack of study "blindness". There still remains the challenge of providing houses with "blanks" or "control" filters due to the ethical implications of

telling someone the water was being purified when it was not. This research investigated 28 separate studies and the results showed that the ceramic filter was the most effective of the household water treatment interventions. The Monte Carlo model predicted that over 12 months the CPF was more likely to be still effective at reducing diarrheal disease while SODIS, chlorination, and combined coagulation and chlorination had little long term health benefits (Hunter 2009).

Ceramic water filtration has been proven to remove bacteria and reduce diarrheal disease. In a study in Bolivia, an intervention group of 25 households was given silver impregnated candle ceramic filters and compared to 25 households that continued to drink their original source water. The intervention group showed a 64% reduction in diarrhea prevalence (Clasen 2004). Another study in Colombia showed that the diarrhea amount in households using ceramic filters was 60% less prominent that those not using the filters (Clasen 2005). Brown (2007) concluded that ceramic water filters resulted in a 46% reduction in diarrheal disease in households studied in Cambodia when comparing users versus non-users in a randomized study.

The reduction of child mortality is one of the United Nations Millennium Development Goals: "reduce by two thirds the under-five mortality rate" (UN 2000). With diarrhea being the second largest cause of death in children under-five, implementing means to produce safe drinking water is imperative to meeting this goal. Ceramic water filtration is an inexpensive solution that can enable low income households to treat and maintain the microbiological quality of drinking water and can help reduce diarrheal disease especially when paired with sanitation and hygiene efforts (Brown 2007, Clasen 2004, Hunter 2009).

2.3 Point of Use Water Treatment: Ceramic Pot Filters

Ceramic pot filters have become increasingly used around the world based on a design originally developed by Potters for Peace in Nicaragua in the 1990s (PFP 2006). CPFs are now used around the world as a low cost method to treat water for potable use. CPFs are produced in small factories around the world using locally available materials. A local clay soil is combined with a fine organic material (such as sawdust, ground rice husks, etc.) and water, pressed into the desired shape, and then fired in a brick kiln. When fired at high temperature the organic material burns away leaving small, interconnected pores. After firing, the CPFs are generally subjected to basic quality assurance testing before being sold. CPFs without visible cracks are tested for flow rates and then coated with silver; those with cracks are broken down and recycled.

For the standard ceramic pot filters promoted by Potter's for Peace (PFP), the CPF should produce 1-2 L of treated water over one hour when the filter is initially full and clean. The RDI filters are tested to have an initial flow rate of 1.5-3 liters per hour (Hagan, 2009). It is assumed that any more than the above recommended flow rates indicates cracks or large pore spaces in the filter which would decrease the quality of treated water achieved. Flow rates that are too low would not produce sufficient water for consumers. If the CPF passes this flow test, a liquid, colloidal silver solution is brushed onto the inside and outside of the ceramic filter to provide disinfection capabilities beyond filtration mechanisms. In some factories, the CPFs are submerged in a tank of colloidal-silver water. In other cases, silver nitrate solution is used in place of colloidal silver (The Ceramics Manufacturing Working Group 2010). Differences in the production of CPFs are outlined in Section 2.4. Production procedures vary from country to country as do the end products. Filters are known to vary in size, materials, porosity, pore size, tortuosity, and other characteristics (van Halem 2006).

Detailed descriptions of the manufacturing process for the factories supported by Potters for Peace can be found in the Factory Startup Manual (Nardo, 2005), and the Potters for Peace Managua Production Manual (PFP, n.d.) and Current Practices (Rayner, 2009). The manufacturing procedure for the RDI filters from Cambodia can be found in the RDI- Ceramic water filter handbook (Hagan et al. 2009).

During typical use, the CPF is placed into a bottom receptacle which stores the treated water and has a spigot to access to the stored water. The most common receptacle used globally is a standard 19-L (5-gallon) plastic bucket. Batches of raw or pre-settled water are poured into the filter to fill it (~8-10 L). The water is gravity fed through the ceramic and collects in the bottom receptacle. This concept is illustrated in Figure 2-1 with example of flow over time. These types of filters have been acquired from Managua, Nicaragua and used in laboratory tests at the University of Colorado at Boulder (CU) since 2000. New ceramic filters were also shipped from Cambodia in 2009 for this research project.

One of the limitations of the current ceramic water filtration system (CWF) is the fairly low water yield. To obtain a sufficient water yield, users must frequently refill the CPF in order to maintain maximum head and the active filtration area. This is a significant inconvenience to the household user. The filters also clog over time with the removal of solids.

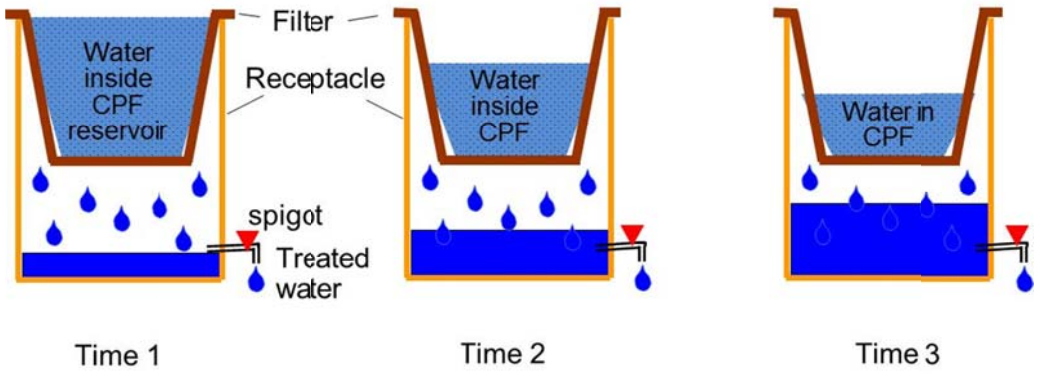

Figure 2-1 CWF; treating batch of water; over time water head level in the CPF drops and the active area of the filter also decreases

Ceramic water filters use both physical and chemical mechanisms to increase water quality from the source water. Physical mechanisms include pore size exclusion, cake filtration, diffusion, adsorption, and sedimentation with the main physical characteristic being the limiting pore size. The chemical mechanism is the inactivation of bacteria and potentially other pathogens by silver. Though the majority of the bacterial disinfection is due to the silver, smaller pore sizes can physically block the *E. coli* from breaking through the filter (Lubick 2008). Silver changes the bacterial cell membrane structure and interacts with nucleic acids. One example of disinfection by colloidal silver is the reaction of silver with the enzymes in the bacteria. Another mechanism is the attachment of the silver to the bacteria's cellular membranes causing the cells to increase in size and eventually results in death of the cell (PFP 2006). Silver's interaction with bacteria is still not widely understood though is assumed to be an important part of the ceramic water filter chemistry (see Section 2.6).

2.4 Widespread variation of CWF

As of 2008, point-of-use ceramic water filters (CWF) had been implemented in more than 25 countries with over 36 factories and interest in starting projects in 23 more locations (The Ceramics Manufacturing Working Group 2010). This section will detail some differences in the production methods of ceramic filters as well as the cost and capacity of filter manufacturing facilities.

While Potters for Peace (PFP) was the organization that got ceramic water filters on the map in developing communities starting in 1998, there are many other organizations that are making their own filters with their own methods. Production practices can vary between factories and while some factories have ongoing quality control and research corresponding to their products, many do not (Best Practices 2010). Nicaragua (PFP) and Cambodia (RDI) and

the Dominican Republic/Tanzania (FilterPure) are locations/organizations that have well documented manufacturing methods and testing. The filters used in this research project were from Nicaragua (designed and quality control by Potters for Peace) and Cambodia (design and quality control by RDI-C). Because ceramic water filters are locally produced, the clay source, organic material, and water source will differ between manufacturing sites.

2.4.1 Potter for Peace ceramic pot filters

Potters for Peace operates by training other organizations to make ceramic water filters using a calculated ratio of local terra-cotta clay and sawdust or other organic combustibles and water. The ratio of clay to sawdust is usually 1 to 1.5 and 1 to 3 for volume and weight, respectively. The clay and combustibles are sieved through a screen to ensure very fine particles. Depending on characteristics of the material, a ratio of 1:1 clay to sawdust by volume is a good place to start and then a 30% by weight addition of water (Nardo 2005). Filters are formed using a hand operated hydraulic truck jack or a hydraulic press and a two piece aluminum mold. Filters are left out to dry completely before being fired in a kiln at around 860 degrees Celsius. Filters are tested for acceptable flow rates 1-2 L per hour initially and then dried and impregnated with colloidal silver (Potters for Peace, n.d.). The actual filter unit costs between $4-$6 and the receptacle can add another $10 to $20 dollars to the price. A basic CPF facility with three or four workers has a production capacity of about 50 filters per day (PFP 2006).

2.4.2 Resource Development International- Cambodia ceramic filters

Resource Development International-Cambodia (RDI-C) has been making ceramic water filters since 2003. RDI-C mixes a local clay powder with laterite and ground rice husk and water at specific ratios that can be found in the RDI-C CWF practices website (RDI-C 2011). The clay

mix is pressed into filter form with a hydraulic press, dried and fired up to 866 degrees Celsius. Following firing and cooling, filters are soaked and tested for initial flow rates after 1 hour. Ideally the initial flow rates of the RDIC filters are between 1.8 and 2.5 liters per hour, but an acceptable range has been defined as 1.5 to 3 liters per hour (RDIC 2011). If the filter passes the flow rate test, it is left to dry and then impregnated with silver nitrate solution by painting. RDI filter range in cost depending on location but are typically around $8 for the system and new ceramic filters can be purchased for ~$3 (RDIC 2011).

2.4.3 Variation in manufacturing methods

The main difference that is apparent from region to region is the type of clay and organic material used to produce the CPF. Clay characteristics vary from region to region and it is necessary to find a local clay source. Easily available, combustible material also depends on the regions and types of organic waste available. The Managua, Nicaragua factory used sawdust as the combustible material while the Cambodian factory that the RDI filters were produced at prefers to use rice husks due to their availability. Cambodia is one of the few places that also add laterite to their clay mixture. Laterite has been known to provide viral bonding sites due to its high concentration of iron (Hagan et al 2009).

The silver used to impregnate the filters can also differ depending on location and preferred methods of the factory. Potters for Peace suggests using colloidal silver by mixing 1 ml of 3.2% liquid silver with 300 ml filtered water (PFP, n.d.). RDI-C uses silver nitrate ($AgNO_3$) instead of colloidal silver due to its known formulation, effectiveness, availability, and affordability (Hagan et al 2009). The methods for applying the silver vary as well. Most factories paint a silver solution on, while others dip the filters into a silver solution, and some integrate the silver mixture into the clay mixture before firing (The Ceramics Manufacturing

Working Group 2010). Overall, the different locations offer alternatives in materials and methods but aim to reach the same goal of providing households in developing communities a means for clean drinking water.

2.5 Flow rate limitations and attempts to increase the flow

Ceramic pot filters have been shown to be the most effective HWTS method at reducing diarrhea in the long term (Hunter 2009, Fewtrell 2005), but they are limited by the quantity of water they can filter. The initial flow rates of full filters are slow (approximately 1-3 liters per hour) which then decrease drastically over time due to both the drop in hydraulic head and the active filtering surface area of the filter as water drains out of the filter. The estimated daily volume of drinking water needed is 2.9 L, 2.2 L, and 1.0 L for adult males, adult females, and children, respectively (Howard &Bartram 2003). Drinking water is only a fraction of the amount of clean water needed daily to ensure healthy living standards. Many times user practices can create contamination potential even immediately before consumption with an unclean glass or dirty hands, which suggests that more water is needed for hygiene and sanitation purposes (Hwang 2002). The United Nations suggest that the minimum amount of clean water required daily is 20-50 liters per person to ensure basic needs for drinking, cooking, and cleaning (UN, 2010). The CPF alone produces around the 25 liters per day at top filtering rates; which would not meet the needs for the drinking, cooking, and hygiene needs of a family of 2 to 5 in the developing world. The slow flow rates of CPFs have been recognized as a main factor limiting their use (Clasen 2004, CDC 2008, Lantagne 2005, Sobsey 2008).

Filters are also subject to irreversible clogging with the removed particles (Gilver 2005). Previous research has shown that flow rates of the ceramic filters decrease significantly when treating surface water (Lantage 2001a, Hwang 2003, van Halem et al. 2007). During one study,

all filters studied had flow rates less than 0.5 liters per hour after only 12 weeks of operation (van Halem et al 2007). Another study reported that flow rates decreased between 39% and 64% over only one year of use (Lantagne 2001a). Lantagne also found that even over short testing periods (6 weeks) and a turbidity of 30 NTU, filters experienced a steady decline in the flow rate due to the build-up of organic material in the filters. Initially, flow rates ranged from 0.78 to 1.69 L/hour and decreased to 0.43 to 1.28 L/hour after 6 weeks of testing (Lantagne 2009). Van Halem found that over 12 weeks of testing the CPF flow rates decreased to half their initial flow rates (van Halen 2008).

Manufacturers suggest removing the filter from the bottom receptacle and scrubbing it with a brush to help increase the flow rate (The Ceramics Manufacturing Working Group 2010, Hagan 2009, Rayner 2009, Lantagne 2001b). While the first scrubbing was found to double the flow rates of filters used to treat surface water, the second scrubbing had much less of an effect suggesting that long term clogging is not preventable with this cleaning method (van Halem 2008). Furthermore, removing the CPF from the receptacle increases the risk for contamination and breakage and even with scrubbing, flow rates could not be completely restored and continued to decrease over time of use (Fahlin 2003, van Halem 2006, van Halem 2008, Gilver 2005).

Although the flow rate is the most limiting characteristic of the CPFs, little research has been done on increasing the flow rate while keeping the same manufacturing procedure. This research project is unique by not altering the current manufacturing procedure at all. The CWF system can be converted to the ECWF by only adding a few parts at a small cost. Other research efforts have examined different ways to try to increase the flow rates while still maintaining a consistent effluent water quality. Two studies successfully increased the flow rates of the

ceramic filters by changing the production methods and significantly altered the physical pore structure in the ceramic by changing the ratios of clay to combustibles (Lantagne 2009, Klarman 2009, Bloem et al. 2009).

Klarman constructed eight new filter designs by changing three variables: type of combustible material, ratio of combustible material to clay, size of screen used for combustible material. Silver was applied to these filters after firing. The initial flows of these filters in their first week of use after production ranged from 0.25 L/hr to 10.17 L/hr but generally increased over the five week study. The combined average flow rate increase from the first week to the fifth week of all filter designs was 44.1% or an increase of 1.075 L/hr, which is an opposite trend from other studies that showed the flow rates decrease over time (van Halen 2006, Lantagne 2001a). A likely explanation for this increase in flow rate was that bits of clay and combustible materials that were blocking the pores after firing were slowly washed out of the pores over the course of the experiment. Also, the turbidity in the inlet water was low, ranging from 1.16 NTU to 4.8 NTU with a mean of 3.0 NTU ± 1.0 NTU. The total coliform (TC) concentrations ranged between 535 CFU/100 mL and 11,567 CFU/100mL with a mean of 4,610 CFU/100 mL ± 4036 CFU/100 mL. Total coliform reductions ranged from 88.4% to >99.9% with an average of 98.47% (Klarman 2009). Over the 5-6 week test period, it was apparent that the decrease in clay to sawdust ratio during production also decreased the filters effectiveness. The filters made with rice husks and coffee husks were less effective at removing TC when compared to the filters that used sawdust as the combustible material. This study established that a flow rate of approximately 1.7 liters per hour was the upper limit for flow while maintaining good treated water quality. At flow rates higher than this the total coliform reduction fell below 99% and

caused the filter to lose its ability to reduce TC levels consistently, although disinfection still remained above 88% (Klarman 2009, Lantagne 2009).

Another study by Bloem et al. successfully increased the flow rates to 5-10 liters per hour by increasing the rice husk percentage and laterite percentage in the RDI-C filters. Flow rates varied over time but generally decreased over the 2400 L of cumulative volume filtered. Filters with an initially higher flow rate experienced a larger decrease over time compared to filters with an initially lower flow rate. However, the higher flow rate filters still exceeded the lower flow filters after 6 months of testing. For this study the enhanced flow rate did not affect the filters' ability to remove *E. coli*. Inlet water was spiked with *E. coli* concentrations ranging from 10^3 CFU/mL to 10^6 CFU/ml. Effluent samples showed disinfection averages of ~3 log without silver and ~6 log with silver for both control filters and enhanced flow rate filters (Bloem et al 2009). These results speak to the importance of silver for the deactivation of *E. coli* which is examined in Section 2.6.

Previous research at the University of Colorado has used filter cores to test if faster flow rates had an effect on the removal of *E. coli.* Inlet water was pumped through the filter cores at 32 ml/hour, increased from 16 ml/hour. On a flow rate per surface area basis, these rates correspond to full filter flow rates of 2 L/hr and 1 L/hr . The results showed no effect of flow rate on the disinfection capabilities of the filter cores (Kohler 2009).

There are still some concerns about the implications of raising the flow rates of ceramic water filters. PFP filters and RDI-C filters were purposefully manufactured to filter 1-3 L/hr (RDI-C 2011, Rayner 2009). These values are somewhat based on the residence time for bacterial interactions with silver. Silver contact time is dependent on the flow rate of the filter and affects the ability of silver to act on pathogens (Hagan 2009). Microdyn, the manufacturer

of a common colloidal silver that was historically used on CPFs, suggested a 20 minute contact time to achieve desirable inactivation (such as 99.9%) (Fahlin 2003). By changing the flow rates, the effectiveness of the silver could be at risk due to a decreased contact time. Higher flow rates could also affect filtration removal mechanisms such as diffusion, advection, etc. Also, the higher amount of organic in the ceramic mixture causes higher porosity which may result in a more fragile ceramic and a higher risk of breakage (Klarman 2009, Bloem et al 2009).

2.6 Removal of bacteria at standard flow rates

This section covers previous disinfection data and research on the removal of bacteria by ceramic pot filters caused by physical removal (assuming no silver) and disinfection by inactivation and other means (with new silver or re-applied silver). Ceramic water filtration has been proven to remove bacteria both by physical mechanisms (sedimentation, diffusion, cake filtration, pore size exclusion, etc.) and by deactivation from silver.

Different manufacturing groups and facilities have different standards for the type of silver used and the methods for applying it to the filter (see section 2.2). Silver is either applied to the ceramic after firing by painting or dipping or is integrated into the filter mixture prior to pressing and firing. Either silver nitrate or colloidal silver is used as the silver disinfectant (The Ceramics Manufacturing Working Group 2010). This section discusses previous research on the removal of bacteria first, without silver and then with the added disinfection benefits of silver.

2.6.1 Removal of bacteria without silver

Laboratory testing has proven that most of the bacteria (up to 99.99%) are removed mechanically by size exclusion due to the filters' small pores (0.6-3.0 microns) (Lantagne, 2001a). Other research has found much larger pore sizes, around 40 microns (van Halem 2007) that would make the ceramics' disinfection ability by physical filtration alone insufficient. Some

laboratory tests have shown that filters without silver also achieve high bacterial removal efficiency (Brown 2007), while others say silver is a necessary addition (Oyanedel- Craver & Smith 2008, van Halem 2006, Bloem 2009). Section 2.6.2 will discuss research that has examined the role of silver as a disinfecting agent.

2.6.2 Importance of silver for *E. coli* removal: laboratory tests

The second disinfection mechanism that takes place in the filter is the role of silver. Former laboratory studies have shown that silver is a necessary additive to the filter to reach 100% disinfection of bacteria and prevent bacterial growth within the filter (Lantagne 2001a). Silver has been proven to be an important aspect of the removal of bacteria; both by deactivating cells but also by preventing biofilm from developing in the pores which has been shown to cause a decrease in flow rates (Bloem et al. 2009). It is known that silver nano-particles demonstrated antibacterial properties by the inactivation of bacteria and the inhibition of cell growth (Dror-Ehre 2009), although it is still unclear the extent to which these different inactivation mechanisms work (Bielefeldt 2009).

Numerous experiments have quantified the removal of bacteria with the additive disinfection properties of silver and shown that the application of silver leads to higher microbiological removal efficiencies; filters with silver consistently performed better at microbial reduction than filters without it (van Halem 2006, Oyanedel-Craver & Smith 2008, Lantagne 2009). Brown (2007) loaded 600 L of water in a laboratory setting with ~100 CFU/mL of *E. coli* to three filters produced in Cambodia and observed an average of 99% (2 log) disinfection. Results from the field showed an extremely variable disinfection range with up to 99.9999% (6 log) disinfection and also negative removal. Silver also has been proven to inhibit

biological growth on the filters and in the receptacles (Oyanedel-Craver and Smith 2008; Bloem *et al.* 2009); this could be essential in limiting contamination potential in the bottom receptacle.

Both Aqua Pure and Potters for Peace filters removed all spiked *E. coli* in all samples up to log removal of 6.1 and 6, respectively, during a six week study, proving that both filters are highly effective at removing bacterial during the initial phase of use (Lantagne 2009). Multiple laboratory tests have shown very high log removals with silver, though it is important to note that the filters tested in lab are usually new with fresh silver and pure water spiked with a single species of bacteria or poorly characterized water quality (Bielefeldt 2009). Families have been known to use filters for 5 or more years with highly variable water quality (Lantagne 2001b; Campbell 2005). Some water sources may be impacted by human and agricultural wastes, with associated high concentrations of many different bacteria and organics. Therefore, it is of interest to look into long term effectiveness of the filters under conditions that simulate impaired water quality, and compare the performance of filters with no silver to filters with silver to quantify the significance of *E. coli* removal by silver disinfection/inactivation.

Laboratory experiments by van Halem (2006) were performed on filters from Nicaragua (with and without the colloidal silver), Cambodia (with silver) and Ghana (with silver). Filters were challenged daily with canal water and biweekly with *E. coli* spikes in the canal water at inlet concentrations of ~10^5 to ~10^7 CFU/mL. The filters were able to achieve log reductions between 2 and >6 log, 4 and >7 log, and 2 and >5 log for the Cambodia filters, the Ghana and silver-coated Nicaraguan filter, and the uncoated Nicaraguan filter, respectively. For the Nicaragua filter with colloidal silver, 67% of its effluent samples contained no *E. coli* while the filter without silver had *E. coli* colonies in all of its effluent samples proving that silver does have a positive effect on *E. coli* removal.

Previous studies at CU have shown that *E. coli* removal was highest directly after reapplication of silver and decreased soon after (Kohler 2009, Kowalski 2008) proving that *E. coli* is important and has the potential decrease in effectiveness after heavy bacterial loading. Kohler (2009) made filter cores by drilling out 5.85 cm diameter cores from a ceramic water filter that had been used for household treatment in Nicaragua for ~3 years. *E. coli* removal was examined with no silver and then later with reapplied silver. To simulate long term use of the filters and determine the long-term effectiveness, *E. coli* spiked water (~10^5 CFU/mL) was pumped through the filter cores for 47 hours at two different flow rates (32 ml/hr and 16 ml/hr). Without silver the cores had an average bacterial disinfection of 3.27 log (±0.46 log) observed after 3 hours of loading compared to 0.93 log (±0.14 log) observed after 47 hours of loading. This indicates that filter performance is determined by loading duration. After recoating the cores with colloidal silver, results show that silver improved the disinfection capabilities of the cores at longer loading durations. The average log disinfection at 47 hours was 0.93 log and 1.99 log for no silver and after silver recoating, respectively (Kohler 2009).

In the study by Kowalski (2008), six Nicaraguan filters with various histories and use (two lab tested, two lab-tested no silver, and two used in households in Nicaragua for more than 3 years) were used. This study evaluated the effectiveness of the filters at reducing *E. coli* in water at inlet concentrations ~$5x10^5$ to $4x10^6$ CFU/mL. Three batches of spiked water was loaded into the filters before recoating the filters with silver, immediately after recoating the filters, and after the effluent silver concentrations were stabilized. Results showed that before recoating the filters with silver there was between 0.84 to 4.2 log reductions of *E. coli* concentrations. Immediately after recoating with silver, log reduction values reached 2.5 to 4.3 log. After the silver concentrations stabilized in the effluent waters the log reductions were

comparable to those before recoating silver on the filters. This demonstrates that recoating the ceramic with silver has the potential to rehabilitate the filters removal efficiency for a short time (Kowalski 2008).

The reapplication of the silver did not appear to offer continuous rehabilitation of the filters capacity to remove high levels of bacteria. Also, silver concentrations in the effluent increased from 0.04-1.75 ppb to 36-45 ppb immediately after recoating the filters with silver, which was expected. Initial disinfection efficiencies ranged from 3 - 4.5 log, but the treatment efficiency decreased to 0.2 - 2.5 log with subsequent batches of spiked water (Bielefeldt et al 2009).

2.6.3 Effect of silver application methods on removal efficiency

Different methods of silver application have been researched to try and determine the most effective method. Silver application methods include painting or dipping after firing and mixing silver into the clay mixture before firing (see Section 2.4). Some research shows a more beneficial application method (Lantagne 2001a) while others showed no difference for disinfection between methods (Oyandel-Craver & Smith 2008, Klarman 2009, Napotnik 2009).

Research studying the effectiveness of the different application methods of silver has concluded that silver needs to be applied to both the inside and outside of the filter to reach 100% removal (Lantagne 2001a). It has been suggested that filters be dipped in colloidal silver rather than painted with colloidal silver to ensure all pores are coated and the bacteria is exposed to more silver as it passes through the ceramic (Fahlin 2003). In contrary, experiments by Oyanedel-Craver found the method of application (painting or dipping after firing) to not be important but rather it was the quantity of silver applied that dictated the removal efficiencies.

The filters removed between 97.8% and 100% of the applied bacteria with the colloidal silver treated filters out performing the filters without silver.

Lantagne (2009) reported on the effect of changing production variables as a follow up to Klarman's work designing new filters with higher flow rates. Results showed that changing the clay to sawdust ratio and combustible materials used, led to an increase in flow rate but a decreased in effectiveness of the filter in the first 5 weeks of use. Other changes in production methods, such as the silver application and the shape of the filter did not impact the filter's effectiveness over the course of the study (Klarman 2009, Lantagne 2009).

Napotnik (2009) tested three types of filters: 1) flat bottom with silver applied before firing 2) flat bottom with silver applied after firing 3) round bottom filters with silver applied before firing. Results showed that all three types were successful at reducing turbidity and bacteria for the first six weeks of use. There was no suggestion that the shape of the filter or the timing of silver addition had any effect on the filter performance. This implies that the method of silver application will not make a big difference in performance, it is just imperative that silver be present.

2.6.4 Field studies and filter life span

Long term studies in the field are rare and raise concern on the sustainability of effluent water quality from the ceramic water filters over their life time. Limited field studies so far have showed the effectiveness of CPFs at removing inlet microbiological contaminants when used correctly over long periods of time (≥5 years) (Campbell 2005, Lantagne 2001b).

Field results have rarely shown the same effectiveness as laboratory results due to the environmental factors and potential contamination sources in the home. Lantagne tested 24 pre and post treated samples in the field and found that only six percent of the filters fully removed

total coliform, 25 percent of the filters fully removed H2S-producing bacteria, 53 percent of the filters fully removed *E. coli.* Samples from seven homes showed higher effluent concentrations of total coliforms and *E. coli* (Lantagne 2001b) proving that recontamination in the field is definitely an issue that needs to be addressed.

Three studies by Clasen (2004, 2006, and 2007) showed that candle filters with impregnated silver were effective against *E. coli* and thermo tolerant coliforms. In one study, candle filters were distributed randomly to half of the 50 households participating in rural Bolivia. Houses without ceramic filters continued to use the local source of water and were considered controls. Four rounds of water samples taken from both the control houses and the experimental houses showed that treated water from 100% of the households with filters were free of thermo tolerant coliforms (TTC) compared to only 16% of the control households (Clasen et al 2004). The second study, also in Bolivia, reported intervention households had average TTC counts of 0.13 TTC/100mL, while control houses had an average of 108 TTC/100mL (Clasen et al 2006). The last study, in India, examined the reduction of *E. coli* and found > 4 log reduction in the field using silver impregnated candle filters (Clasen et al. 2007).

Some field studies show that CPFs are effective in the field but the cumulative time of the studies differ. Brown (2007) examined the microbiological effectiveness and health impacts up to 44 months in households in Cambodia and found that the CPFs reduced *E. coli* up to 6 log with average reductions of 2 log in both laboratory and field testing. During these 44 months, there was a disuse rate of 2% per month; 67% of this due to breakage while the average use time was 2 years (Brown 2007). Filters as old as 7 years were tested and found to still remove 100% of total and fecal coliform, indicating that the lifespan of the colloidal silver and the filters is longer than initially thought (Lantagne 2001a). In a study examining the PFP filters over the

first 6 months in the field after manufacturing, there were no obvious decreases in the filter performance in relation to flow rate and microbiological removal during the 6-month period (Hwang 2003).

The life span of ceramic filters and colloidal silver are still unknown and may be dominated by the conditions of the inlet water and seems to differ from study to study. High turbidity water has been proven to clog the filter pores and it was concluded that the suggested lifespan of 1–2 years might be compromised by scrubbing the filter element over twice a week (van Halem 2008). The PFP website claims that the effective life expectancy of the filter to be at least forty months (PFP 2006). Longer studies have to be examined to see if filters can sustain turbidity removal and the reduction of bacterial contaminated waters in field use.

2.7 Recontamination potential

Recontamination of post-filtered water is definitely a concern when implementing any means of household water treatment in the developing world. The occurrence of higher bacterial concentrations in treated effluent water compared to the inlet or source water has been reported in previous research both in the field and the laboratory work by Brown (2007), Lantagne (2001a and 2001b), Hwang 2002), Kowalski (2008), van Halem (2006) and Kohler (2009).

Previous lab studies at the University of Colorado aimed to determine the potential of the filters to contaminate clean de-chlorinated tap water after the batches of challenge water were loaded. Results showed that the filters could contribute bacteria into clean water after treating water with high *E. coli* concentrations (Kowalski 2008, Kohler 2009, Bielefeldt et al. 2009). The filter core tests that followed ~2 days of loading 10^5 CFU/mL *E. coli* plus 50 mg/L TSB found that the contaminated filter cores contributed up to 2.93 log CFU/mL into the effluent water when inlet concentrations were zero. It is also important to note that there was no distinct

change in contamination over time while loading the cores with "clean" water which means that the bacteria seem to be surviving at a steady state concentration instead of "flushing" out of the filter cores over time as was expected (Kohler 2009). Van Halem (2006) also saw higher effluent concentrations from the Nicaraguan filter with no silver in a twelve week lab study.

Some field work has also found higher effluent concentrations than inlet concentrations. In the field study by Lantagne (2001b) one home had source water concentrations 124, 70, and 0 CFU/100 mL of total coliform, fecal coliform, and *E. coli*, respectively, compared to 4900, 4320, and 1920 CFU/100 mL in the post-treated water. Brown (2007) showed 17% of all filter samples had higher concentrations of *E. coli* in the treated water than the inlet water with up to a 3 log increase of *E. coli* in field tests in Cambodia.

Most researchers have attributed this phenomenon to contamination in the bottom receptacle after the water has been filtered. Recontamination of filtered water due to contaminated receptacles was found in 33% of cases (Hwang 2002). There is dead space in the bottom receptacle that may be the perfect conditions to support bacterial growth after filtration. This bottom receptacle should be cleaned with soap to eliminate the potential for growth of bacteria (The Ceramic Manufacturing Working Group 2010) but the cleaning procedure also adds contamination potential. Recontamination of the filter and storage receptacle through improper handling could be a huge limiting factor to the success of this HWT technology (Brown 2007). Hwang (2002) found that user practices lead to high potential for contamination by not storing filters in hygienic places, using dirty containers to transport water from the source to the home, and using a contaminated cup to drink from. Susan Murcott even mentioned that sometimes families would overfill the filters which led to untreated water short circuiting around the ceramic and into the bottom bucket (Murcott 2009).

Chapter 3 Project Specifications

This chapter describes the proposed solution to enhancing the flow rate of the CPFs and details the research objectives and hypotheses related to these objectives.

3.1 Introduction to the ECWF system

It is of interest to see if the CWF can be modified to increase the flow rate and decrease the refill frequency to increase convenience without compromising water quality. One potential method to enhance the water treatment capacity of the CPF element is shown in Figure 3-1; the so-called "enhanced ceramic water filtration" system (ECWF). This concept was proposed by Chris Schulz of CDM (Schulz 2009). The enhanced water filtration system is a simple addition of a gasket to the CWF and a 5 gallon bucket, with holes drilled in the base that seals to the top (shown in Figures 3-1 and 3-2). The top buckets hold ~19 L of water that is expected to increase the hydraulic head by >200%, raising the initial flow rates to around 3-6 L/hr. The hydraulics of the ECWF will be tested for flow rates and cumulative volume filtered. The head still decreases over time but starts at a higher initial amount and the active area of the CPF stays constant as long as the top bucket does not empty completely. The ECWF adds an additional ~32 cm of head to the ~20 cm in the CWF. So, for example, if a user maximally filled the CWF or ECWF three times per day at 7 am, 2 pm, and 9 pm the water yield over a 24 hour period would be ~17 L and ~65 L, respectively. Costs and other general pros and cons of the two systems are summarized in Table 3-1 (Schulz 2009).

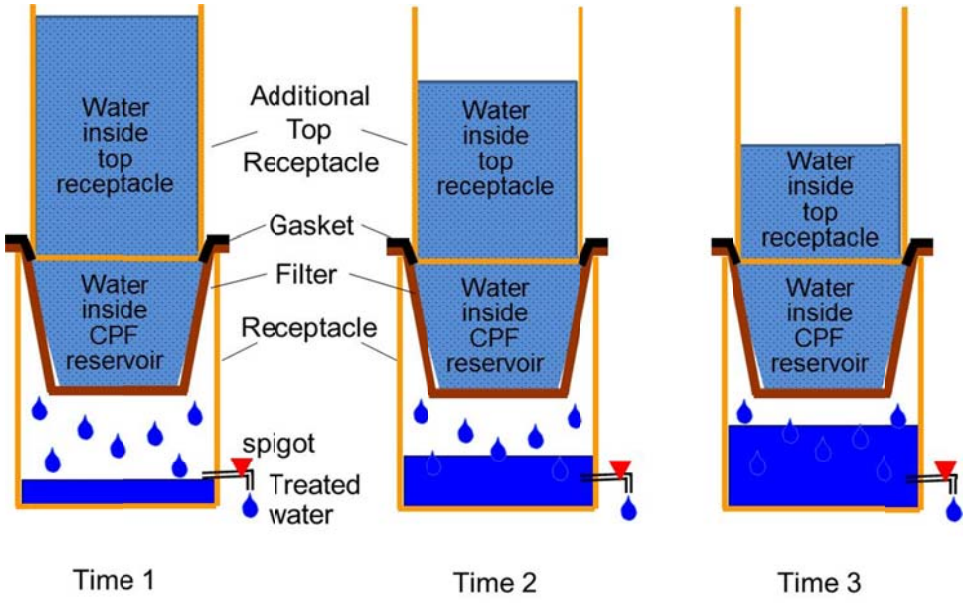

Figure 3-1 ECWF; over time water drains out of the additional top bucket

Figure 3-2 Enhanced Ceramic Water Filtration System.
Left: Nicaragua ECWF. Right: Cambodia ECWF.

Table 3-1 Comparison of Basic Characteristics of the CWF and ECWF (Schulz 2009)

	CWF	ECWF
Cost	Cost ~$10.20	Higher Cost ~$14.40 (41% increase)
Treated water capacity	~ 17 L/d	~65 L/d
Contamination concerns	Bottom receptacle contamination (below spigot)	Bottom receptacle contamination (below spigot)
Breakage Potential	May be more breakage potential due to removing CPF from receptacle for cleaning	May be greater risk of cracking due to greater pressure on ceramic in the ECWF
Other observations and concerns		May reduce some risk to CPF since some settled turbidity may be captured in the top bucket
		May be difficulty achieving a water-tight seal with the gasket and preventing leakage
		May require better uniformity during CPF production to ensure good fit between top bucket and CPF

To test the performance on the ECWF system, this research project compared two filters from Nicaragua, used extensively in prior CU testing, and two new filters, shipped from Cambodia in December 2009. Two primary mechanisms, physical removal and chemical/biological inactivation, are considered important in the production of potable water by the filters for this research project. First, the physical filtration controlled mainly by straining and cake filtration for removing turbidity is analyzed in the turbidity testing. These may not be the only physical filtration mechanisms working in the ceramic filters, but they are the dominant ones for turbidity removal due to the size of the particles. Second, the importance of silver to microbial inactivation is examined for *E. coli* disinfection for both CWF and ECWF operation. Also, the potential for microbial growth in the filters without spiked bacteria but with a carbon source (tryptic soy broth) was briefly examined through "control" experiments. These results helped show the effect of the increased flow rate on the resulting water quality in order to assess

if the ECWF system is a viable solution for producing larger volumes of potable water for household use.

3.2 Research Objectives and Hypotheses

The objectives of this research project were:

1) Compare the flow rates and hydraulic capacity for CWF and ECWF systems over time, and determine how these change as the filters clog due to turbidity removal.
2) Compare turbidity removal of CWF and ECWF at 5 NTU, 50 NTU, and 500 NTU.
3) Compare *E. coli* removal of CWF and ECWF without silver at inlet concentrations of ~10^5 CFU/mL *E. coli* and 50 mg/L of TSB (Trypic Soy Broth).
4) Compare *E. coli* removal of CWF and ECWF when the ceramics had been treated with reapplied colloidal silver at inlet concentrations of ~10^5 CFU/mL *E. coli* and 50 mg/L TSB.

The hypotheses that correspond to these objectives were:

1) The ECWF would yield significantly higher flow rates than the CWF, but will clog in less time when treating water with high turbidity because of the increase in volume treated
2) The ECWF and CWF will have similar turbidity removal
3) Without silver the bacterial removal of the CWF and ECWF systems would be similar
4) With silver the bacterial removal of the ECWF system would be lower than the CWF due to shorter contact time with the silver for disinfection

Chapter 4 will describe the experiments that were designed to meet the research objectives and test the hypotheses.

Chapter 4 Research Approach and Methods

Chapter 4 will discuss, in detail, the research approach to meeting the objectives described in the previous chapter and the necessary methods used to reach these objectives.

4.1 Research Approach

The research tested two CPFs from Nicaragua and two CPFs from Cambodia in both a standard ceramic water filtration system (CWF) and enhanced ceramic water filtration (ECWF) system. The exact dimensions of the filters were measured and will be described in Section 4.5. Under standard conditions the ceramic filters from Nicaragua held approximately 8 L and the filters from Cambodia held approximately 10 L of water. The initial full flow rates under standard conditions were around 1-2 L/hr. The filters from Cambodia were produced by Resource Development International (RDI). The operating mode of each test was designed to simulate usage in practice. The reservoir in all four systems were filled and allowed to drain. The volume of water treated over the first hour was always measured. Experiments ran for 4 days for the CWF turbidity and *E. coli* tests and EWCF turbidity tests, but only 2 days for the ECWF *E. coli* experiments. Kaolin clay was selected as a surrogate for natural turbidity particles and *E. coli* was used for the microbial representative. The experiments that were conducted to meet each of the four research objectives are described in the following sections.

4.1.1 Flow rate

The first research objective was to compare flow rates and hydraulic capacity for CPFs operated under CWF and ECWF. This was determined by filling the filters to a selected, measured depth with Boulder tap water and then measuring the depth of water at multiple times as the water filtered through the system. Time "zero" was considered the time at which the filters were filled to the top (same depth every time for each filter). From the measured geometry of the filters and the water depth, the cumulative volume filtered and flow rates at any

time were calculated (see Section 4-6). The flow rates of CWF and ECWF and cumulative volumes filtered were measured at the times listed in Table 4-1 and were compared graphically. This tested the potential increase in flow rate capacity and filtered volumes of the ECWF system and compared them to the standard ceramic pot filter flow rates and filtered volume.

Table 4-1 Hydraulic Testing Water Depth Measurement Times

Experimental Conditions	Time in hours after start of experiment
CWF	t= 1, 2, 3, 6, 7.25
ECWF	t= 1, 2, 3.33, 4.66, 6

4.1.2 Turbidity Removal

The second objective was to compare the turbidity removal of the CWF and ECWF for water containing ~5 NTU, ~50 NTU, ~500 NTU. These tests were conducted by preparing turbid water using Boulder tap water and spiking in Kaolin (Acros Organics) in a controlled lab setting. The water was then loaded into the filters and the initial turbidity and effluent turbidities at certain times were measured. The flow rate over the first hour after refilling the filters was always measured and showed that over time the filters did experience clogging when loaded with high turbidity water ≥50 NTU. Filters were loaded for 4 consecutive days for both the CWF and ECWF experiments. Under CWF experimental conditions, filters were loaded twice a day 6-9 hours apart. Under ECWF experimental conditions, filters were loaded two or three times per day 4-6 hours apart.

4.1.3 Bacterial Disinfection Effects when Filters Contained No Silver

The third research objective was to compare the *E. coli* disinfection of the CWF and ECWF systems when the CPF contained negligible silver. These experiments were conducted with inlet concentrations of ~10^5 CFU/mL *E. coli* K12 spiked from a stock solution into de-

chlorinated Boulder tap water, with an added 50 mg/L tryptic soy broth (TSB). The concentrations of bacteria in the inlet water and effluents for each system were quantified using a Model D spiral plater on non-selected tryptic soy agar (TSA) plates. High concentrations of bacteria were used to increase the ability to quantify high log removals of the *E. coli* and to represent long term bacterial loading capacities of the ceramic filters in a short time. TSB was added to the water to simulate contaminated source water that would also contain high bacterial concentrations; the equivalent total organic carbon (TOC) concentration was ~15-20 mg/L. For example, if human excreta from latrines contaminated a water source, both bacteria and high carbon concentrations could be present; such is the case for the drinking water source in Myanmar, Inle Lake, that was impacted by pit latrines and agricultural activities with measured TOC concentrations of 32.9 to 51.9 mg/L (Akaishi et al. 2006).

Result from these tests showed contamination other than *E. coli* in the effluent samples. Therefore, more tests were conducted to find out if the de-chlorinated tap water with TSB but without spiked *E. coli* would also cause contamination or bacterial growth in the filter. The standard cleaning method for the CPFs that was used in the lab (which is more aggressive than what is typically used by households) used a diluted bleach solution. This treatment may not have removed all of the bacteria resulting in contamination from the filters. Alternatively, the non-sterile de-chlorinated tap water could contribute bacteria to the test systems, particularly given the TSB added to the water. The control tests were conducted by spiking in 50 mg/L TSB into de-chlorinated tap water and running a comparable experiment to the *E. coli* tests for both CWF and ECWF. The inlet water and effluent water was plated using the Model D Spiral Plater to see if there was microbial growth. TOC samples of the influent and effluent were analyzed using a Sievers 800 Portable Total Organic Carbon Analyzer to compare the total organic carbon

in the water. It was assumed that a decrease in TOC was the result of biological activity, although some sorption of TOC onto the ceramic filters may have also occurred. These experiments helped explain some of the microbial activity that was seen during the *E. coli* tests, particularly the colonies on the effluent plates that did not resemble *E. coli*.

Experimental testing times are shown in Table 4-2 and Table 4-3 and compared to the control tests and the *E. coli* tests with re-applied silver.

4.1.4 Bacterial Disinfection Effects when Filters Contained Colloidal Silver

The final research objective was to test for the significance of silver on bacterial removal. Typically new CPFs are coated with silver, so it is important to determine how the ECWF would function with silver. However, since no new filters with silver were available, colloidal silver was reapplied to the filters previously tested (see Appendix A.15 for procedure to make colloidal silver solution and section 4.10 for the silver application method used).

After the experiments described above in sections 4.1.1, 4.1.2, and 4.1.3, the filters were cleaned with tap water and extensive disinfection by soaking the filters for 1-2 days in a 10x dilution of household bleach (Clorox ~5%-10% NaOCl), and then air dried completely. Next, a liquid solution of colloidal silver was applied to the Nicaragua filters using standard methods (Rayner, 2009). Then experiments with *E. coli* were run on the enhanced and standard systems in a manner similar to those previously described above in section 4.1.3. This enabled comparison between the tests with the reapplied colloidal silver and the same tests on filters without any silver. Experimental sample times for refilling, flow rate measurements, inlet and effluent sampling, and silver samples are presented for easy comparison in the tables below. The control test, the no silver test, and the re-applied silver test are all included for CWF (Table 4-2) and ECWF (Table 4-3).

Table 4-2 CWF sampling times for all *E. coli* experiments

CWF: Times in hours after start of loading			
	No Silver CWF	No Silver control CWF	With Silver CWF
Filters were refilled	t= 0, 5, 22, 26, 30, 46, 50, 54, 70	t= 0, 7, 24, 31, 48, 55, 72	t= 0, 7, 23, 31, 46, 55, 73, 80,
Flow Rate Measurement	t= 1, 5, 6, 22, 23, 26, 27, 30, 31, 46, 47, 50, 51, 54, 55, 70, 71, 74	t= 1, 7, 8, 24, 25, 31, 32, 48, 49, 55, 56, 72, 73, 79	t= 1, 7, 8, 23, 24, 31, 32, 46, 47, 55, 56, 73, 74, 80, 81
Inlet and Effluent Samples for *E. coli*	t= 1, 5, 22, 26, 30, 46, 50, 54, 70, 74	t= 1, 7, 24, 31, 48, 55, 72, 79	t= 0, 7, 23, 30, 46, 54, 72, 80
Silver Samples	NA	NA	t= 7, 30, 54, 80, 104*

* =represents part of a rinse phase after *E. coli* loading

Table 4-3 ECWF sampling times for all *E. coli* experiments

ECWF: Times in hours after start of loading			
	No Silver ECWF	No Silver control ECWF	With Silver ECWF
Filters were refilled	t= 0, 3, 7, 12, 22, 30, 35, 46	t= 0, 5, 10, 24, 29, 34	t= 0, 4, 8, 22, 26, 30, 48*, 54*, 71*, 78*
Flow Rate Measurement	t= 1, 3, 4, 7, 8, 12, 13, 22, 23, 30, 31, 35, 36, 46, 47	t= 1, 5, 6, 10, 11, 24, 25, 29, 30, 34, 35, 48	t= 1, 4, 5, 8, 9, 22, 23, 26, 27, 30, 31, 48, 49*, 54*, 55*, 71*, 72*, 78*, 94*
Inlet and Effluent Samples for *E. Coli*	t= 1, 3, 7, 12, 22, 30, 35, 46	t= 5, 10, 24, 29, 34, 48	t= 0, 4, 8, 22, 26, 30, 54*, 78*
Silver Samples	NA	NA	t= 0, 1, 4, 8, 22, 32, 48, 72*, 104*

* = represents part of a rinse phase after *E. coli* loading

4.2 Experimental Test Matrix

An overview of all of the experiments conducted over the course of this research is provided in Table 4-4. The table includes all of the experiments of this research project in chronological order and the measurements that were recorded for data analysis. Unexpected clogging and breaking of the filters lead to some alternative filters joining the experiment later in the research. The "pre-tests" for the *E. coli* experiments are addressed in Chapter 6.

Table 4-4 Summary of Experimental Conditions

Experimental Conditions	Filters Used	Measurements	Notes
Initial flow rate tests CWF	18193, 11136, RDI 1, RDI 2 29027	First hour flow rate, cumulative volume filtered	All filters at CU lab were tested for initial flow rates during filter selection process for this research
5 NTU Turbidity CWF	18193, 11136, RDI 1, RDI 2	Flow Rate, Cumulative Volume Filtered, Inlet and Effluent Turbidities	
5 NTU Turbidity ECWF			
50 NTU Turbidity CWF			
50 NTU Turbidity ECWF			
500 NTU Turbidity CWF			
500 NTU Turbidity ECWF			
Pre-tests for *E. coli* disinfection	18193, 11136, RDI 1, RDI 2		Multiple experiments did not work but were imperative to determining best methods for future experiments
~10^5 CFU/mL *E. coli* influent ECWF	18193, 11136, RDI 1, RDI 2	Flow Rate, Inlet and Effluent Concentrations	
~10^5 CFU/mL *E. coli* influent CWF			
Tap water	18193, 11136, RDI 1, RDI 2	Flow rate, Cumulative Volume Capacities for CWF and ECWF	
CWF Control Test: De-chlorinated tap water with TSB	18193 11136	Flow Rate, Inlet and Effluent Concentrations, TOC	RDI filter flow rates out of acceptable range
ECWF Control Test: De-chlorinated tap water with TSB	11136	Flow Rate, Inlet and Effluent Concentrations, TOC	18193 cracked
~10^5 CFU/mL influent on	11136	Flow rate, inlet and	29027 replaced 18193.

re-applied silver ECWF, and new Nic ECWF	29027	effluent concentrations, silver analysis	
~10^5 CFU/mL influent on re-applied silver CWF, and new Nic CWF			

4.3 Test Filters

Five CPFs were evaluated in this study: three from Nicaragua and two from Cambodia. Each filter was operated under CWF and ECWF conditions, as described in section 4.2. The ceramic pot filters 18193, 11136 and 29027 were manufactured in Managua, Nicaragua and used in various laboratory tests at the University of Colorado prior to the current experiments. The used Nicaragua filters were selected by their initial flow rate after saturation. The goal was to find two filters used at CU that had a similar use history and a similar initial full flow rates (see Table 4-5). Filters 18193 and 11136 were used for the majority of the experiments until 18193 unexpectedly cracked while running enhanced flow rate conditions under a controlled lab setting; filter 29027 was brought in for the final experiments to replace this cracked filter. The CPFs were disinfected prior to the current study using a 100x dilution of household bleach for two batches (~1 day). This treatment and previous testing had removed silver from the CPFs (Stewart, 2010).

Table 4-5 Ceramic water filter history and use prior to current research

Filter	Date of Fabrication/ Shipment to CU	Silver	Summary of Laboratory Use Prior to Current Experiments	Initial First Hour Flow Rate L/hr
18193	Shipped new from Nicaragua in Nov 2001	No silver	2002-2003 flow studies (Fahlin), Pathogen Removal, 2007 *E. coli* tests (Kowalski).	1.8
11136	July 1999, used in the field and shipped in Oct 2003	Used, reapplied in 2008	Field used, periodically chlorine, Pathogen Removal, 2007 *E. coli* tests (Kowalski).	2.0

RDI 1	New from Cambodia, shipped Dec 2009	Assumed silver	New from Cambodia.	1.3
RDI 2	New from Cambodia, shipped Dec 2009	Assumed silver	New from Cambodia.	1.5
29027	New from Nicaragua Summer 2003	No Silver	Pathogen Removal, 2007 *E. Coli* tests (Kowalski).	1.4

CPFs with the serial numbers 18193 and 11136 were selected for the experiments. 18193 originally had no silver applied to it. It was acquired in November of 2001 on a trip to Managua, Nicaragua and used in Kate Kowalski's thesis research for *E. coli* testing and microsphere removal, as a filter with "no silver". 11136 was manufactured in July 1999 and used in Nicaragua by families for household water treatment for many years before being shipped to University of Colorado in October of 2003; it was used in Kate Kowalski's thesis research for *E. coli* testing and microsphere removal, as a filter "previously used". Filter 29027 was shipped new from Nicaragua in 2003 and was also used in Kate Kowalski's *E. coli* and microsphere testing as a filter with "no silver".

Two new CPFs from Cambodia were manufactured by RDI and shipped to Boulder in November of 2009 and considered new from Cambodia with silver. The flow rate tests, turbidity tests, and cleaning with bleach was assumed to remove all silver before starting the *E. coli* testing. RDI 2 was found to be slightly smaller than RDI 1. They were manufactured at the same facility in Cambodia and shipped over at the same time, though they did have slightly different dimensions and behaved somewhat differently in the testing. This was expected as every filter is structurally different due to variability in the clay, organics, and firing.

Different filter manufacturing facilities can produce different size filters depending on the mold they use. Each of the filters used in these experiments were unique in size and shape. The filters made in Cambodia were significantly larger than the ones manufactured in Nicaragua. The filters from Nicaragua have an internal capacity of ~8 L, while the Cambodia filters hold ~10 L. These size variations can affect the flow rate of each filter and the disinfection efficiency. Two different sized top buckets were used for the ECWF system, to accommodate the difference in filter size of the Nicaragua filters and Cambodia filters.

4.4 Experimental apparatus: CWF and ECWF

The CWF experimental apparatus included the ceramic filter placed in a plastic bucket with a spigot near the bottom. The lip bottom of the ceramic filter rests on the top edge of the bottom receptacle. The experimental apparatus for the Nicaragua filters and the Cambodia filters differed slightly. The RDI bottom receptacles were specifically made to fit the RDI filters and had a spigot coming out of the side resulting in a dead volume under the spigot of ~2.9 L, while the Nicaragua filters fit in a typical 5 gallon bucket that was made with the spigot coming out of the bottom in order to reduce dead space to ~0.8 L (Figure 4-1). This set up was recycled from Kowalski's research at CU. The lid from the plastic bucket was placed on top of the CPF in the manner recommended to household users; this helped to keep the unfiltered water closed to the air and minimize light exposure which could lead to algae growth.

Figure 4-1 Experiment apparatus (RDI: left, Nicaragua: right)

The ECWF apparatus added a gasket and top bucket to the original CPW apparatus to increase hydraulic head and flow rates. The CWF and ECWF experimental set ups are compared in Figure 4-2.

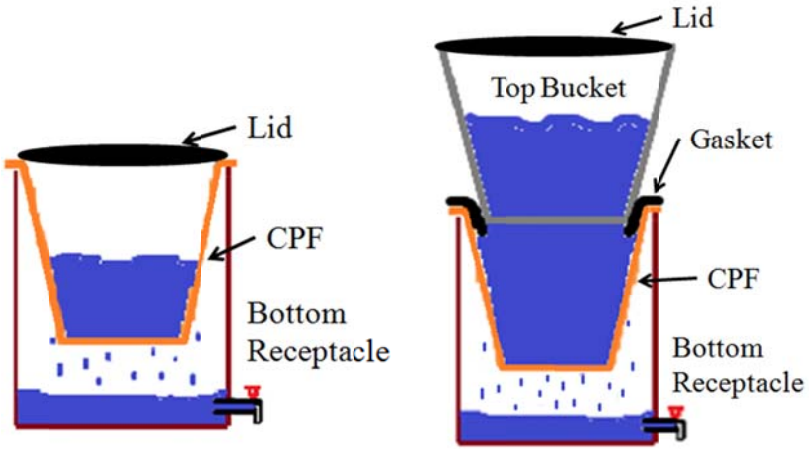

Figure 4-2 CWF and ECWF Experimental Apparatus

The bottom receptacle remained the same throughout the experiments: a 5-gallon plastic bucket for the Nicaragua filters and the RDI bottom receptacle for the Cambodia filters (Figure 4-3). The top receptacles for the ECWFs were similar; both were ~5 gallon plastic buckets but

with slightly different dimensions to accommodate the internal diameter at the top of the different CPFs.

Figure 4-3 ECWF experimental apparatus

The enhanced ceramic water filtration system was built in the lab at the University of Colorado. It was difficult to find top buckets that were the correct size to seal to the ceramic filters but multiple five gallon buckets were purchased and tested until the correct sizes were found. Approximately 9 holes of ~0.5 cm diameter were drilled out of the bottom of the buckets, enough that the holes did not restrict flow into the ceramic filter (Figure 4-4).

Figure 4-4 Top bucket (bottom view)

To secure the top bucket into the CPF without leakage, a gasket of 1/4" thick black sponge rubber (MetroGasket) was glued to the ceramic. It covered the top lip and extended down along the inside of the CPF (Figure 4-5). The gaskets were cut to size and glued directly to the ceramic following the directions provided by the glue source. Multiple types of glue and sealants were attempted throughout experiments including Liquid Nails, Devcon Flow-Mix 2 Ton Epoxy, and Gorilla Glue. Gorilla Glue was the most successful sealant to attach the gasket material to the ceramic filter, although it still was not efficient for long-term use and top bucket removal and re-sealing.

Figure 4-5 Gasket glued to the CPF

The gasket was glued completely to the top of the lip and let dry overnight. Then glue was placed ~2 cm down the inside of the ceramic and let dry with the bucket placed in the top to hold the gasket to the ceramic while the glue dried. Before any experiments, the top plastic buckets were placed and physically manipulated into the in the top of the ceramic filters until the gasket created a watertight seal between the ceramic and the plastic. This was tested for leaks using Boulder tap water before any turbidity or *E. coli* experiments.

4.5 Filter Dimensions

Each filter was measured a minimum of six times using the procedures used previously in both Fahlin's and Kowalski's research for each of the following parameters *S, d, D, h(max),*

L_b, L_s and *2r(max)* (Fahlin 2003). The dimension definitions and the geometry shown in Figure 4-6 are based on Fahlin's previous hydraulic research.

Since the filters are not completely symmetrical, the average and standard deviation for each dimension were calculated and are stated in Table 4-6. The inside side length of the filter from the bottom of the filter to the top of the lip was represented by *S*. The interior bottom diameter and top diameter were referred to as *d* and *2r,* respectively. With the filter completely full of water *h (max)* was measured as the max height of water the filter was able to hold. The thicknesses of the filter on the bottom and on the sides were introduced as L_b and L_s.

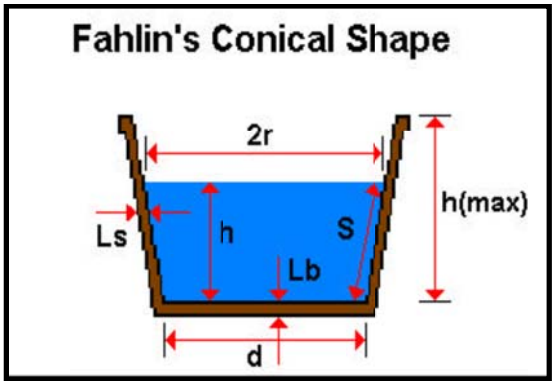

Figure 4-6 Fahlin's geometric model with dimensions, not to scale (2003)

Table 4-6 Measured Dimensions of Ceramic Water Filters (cm).

CPF Number	18193	11136	RDI 1	RDI 2	29027
Internal Side Length (S)	21.0±0.1	20.9±0.1	22.8±0.1	22.0±0.1	20.9±0.2
Interior Bottom Diameter (d)	18.9±0.1	19.2±0.2	20.4±0.1	20.3±0.1	19.3±0.1
Outer bottom Diameter	21.1±0.1	21±0.1	23.7±0.1	23.2±0.1	21.1±0.1
Height of water (h max)	20.7±0.1	20.8±0.1	21.4±0.1	21.2±0.1	20.6±0.1
Wall Thickness (Ls)	1.44±0.60	1.45±0.11	1.48±0.27	1.51±0.1	1.19±0.12
Bottom Thickness (Lb)	1.4±0.1	1.6±0.1	1.63±0.2	1.55±0.1	1.9±0.1
Inner Top Diameter	25.9±0.2	25.7±0.2	28.6±0.1	28.2±0.1	26.2±0.7

The geometry of the ceramic filters idealized as a frustum of a cone were used to calculate the water volume inside the CPFs, from which the flow rates were calculated between two water depth measurement times (described further in section 4.6).

The ECWF system used the dimensions of the top bucket to calculate flow rates. The top bucket was treated as the frustum of a cone, the same as the CPF in terms of geometric modeling and calculations. The buckets were assumed to be symmetrical but every measurement was taken a minimum of three times to ensure that the dimensions were consistent throughout. The parameters are represented by the same variables as the CPFs and are presented in Table 4-7.

Table 4-7 Enhanced Ceramic Water Filter Parameters

Parameter (cm)	**Home Depot (Nicaragua Top Buckets)**	**Lowes (Cambodia Top Buckets)**
Internal Side Length (S)	32.3	32.5
Interior Bottom Diameter (d)	23.4	25
Diameter at fill line (2r)	28.0	28.5
Height of water (h_{max})	32.1	32.45
Volume of Bucket (L)	16.7	18.2

4.6 Flow rate methodology

The geometric model used for the ceramic filters was a partial cone as shown in Figure 4-7 (drawing not to scale). The sides of the filter and the top buckets were considered to be the frustum of a cone. The variables used to calculate the volume and then flow rate are presented in Table 4-8. The same geometrical concept was used for both CWF and ECWF calculations. Similar triangles were used to find the unknown variable y. For CWF testing, the depth of water was measured from the bottom of the filter to the water surface. For ECWF tests, the distance was measured down from a manufactured line in the bucket to the surface of the water.

For all CWF and ECWF tests, the filters were filled to the same height to ensure constant starting volumes at each refill time. For the CWF experiments, the Nicaragua filters were filled to a water height of 19.8 cm and the Cambodia filters were filled to a water height of 21.1 cm. For the ECWF tests the top buckets were all filled to the same manufactured line in the buckets and the depth was measured down from there.

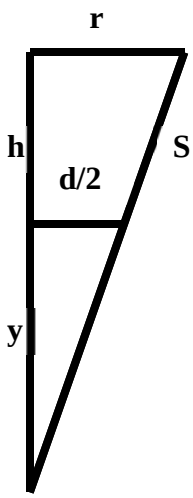

Figure 4-7 Triangular Cross section of internal cone (Fahlin 2003)

The ceramic pot filter and the top buckets were both treated as cones to find the volume or water shown in Figure 4-8. All parameters were then used to find the volume of water at any height in the filter or in the top bucket for the ECWF system using the equations below. Volume of the cone is represented as the variable V_C (Eq. 2) and stays constant as the volume of water in the filter or bucket, V_W, can vary depending on the water height.

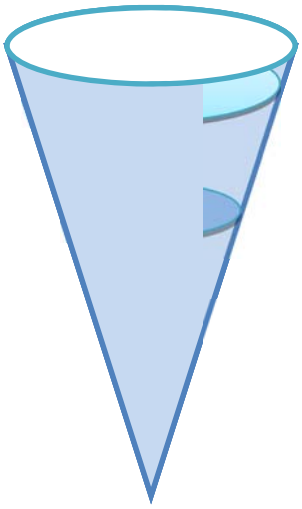

Figure 4-8 Cone geometry for determining water volume

$$y = \frac{h\left(\frac{d}{2}\right)}{(r - d/2)} \qquad (1)$$

$$V_c = \frac{1}{3} * \pi * (d/2)^2 * y \qquad (2)$$

Table 4-8 Calculated Ceramic Water Filter Parameters

Filter	**18193**	**11136**	**RDI 1**	**RDI 2**	**29027**
y (cm)	55.89	61.44	53.24	54.48	57.62
d/2 (cm)	9.45	9.6	10.2	10.15	9.65
V_c (cm^3)	5226.69	5929.56	5800.52	5877.57	5618.97
Max volume of water V_w (L)	8.22	8.29	10.18	9.88	8.42

To ensure acceptable flow rates, filters were tested between each experiment for first hour flow rates. All ceramic water filters were saturated with de-chlorinated Boulder tap water for two to three days prior to experiment start. This was to ensure that all pores in the filter were saturated with water and the flow rate reached its full capacity. There is a lot of pore space in the filter (~ 1 L of water (Fahlin 2003)) so it was necessary to saturate these pores before beginning any experiments. Flow rates of filters were compared by the first hour flow rate when the CWF or ECWF are completely full. The acceptable initial flow rate range for CWFs is between 1 to 2 liters per hour for the Nicaragua filters and 1.5 to 3 liters per hour for the Cambodia filters. The first hour represents the max flow rate capacity of the filters as this is when the hydraulic head is the greatest. As the water level decreases so does the flow rate. This is due to the decreasing hydraulic head but also as the level drops, so does the surface area of the ceramic that the water is passing through.

The first step in calculating the flow rates was to calculate *d/2* and *y* for each filter. The parameter *d/2* was simply the radius of the bottom of the filter and *y* was calculated using the measured dimensions (Eq. 1). Next, the radius as a function of height, r_h, was calculated using

$$r_{(h)} = \frac{d}{2}\left(\frac{h_w + y}{y}\right) \qquad (3)$$

where *d/2* and *y* are constants for each filter (Table 4-8) and h_w is the height of the water at any known time from when the filter was filled.

The volume of water, V_W, at any height, h_w, can be calculated with equation 4. Next, the flow rate can be calculated knowing two different water volumes and the cumulative time between the measurements (Eq. 5).

$$V_W = \left[\frac{1}{3} * \pi * r_{(h)}{}^2 * (h_w + y)\right] - V_C \qquad (4)$$

$$Q = \left(\frac{\Delta V_W}{time}\right) \qquad (5)$$

Hydraulic testing data was used to determine the increased flow rate under the enhanced conditions compared to the standard flow rate conditions. Testing was completed simply for flow rate comparisons between the CWF and ECWF. Boulder tap water was used for all hydraulic testing. Data analysis was used to quantify the possible increase of filtered water under the enhanced system compared to the standard system. The height of the water was recorded at known times to calculate the volume of water filtered. Plots were made for flow rate (L/hr) vs. time (hours) and cumulative volume filtered (L) vs. time (hours). Results can be found in Chapter 5.

4.7 Turbidity experiment details

The goal of these experiments was to quantify the decreases in water flow rates over time through the filters as well as the turbidity removal over time and the clogging potential of the ceramic. The CPFs were saturated with Boulder tap water for three days prior to the experiments. A batch of test water at a selected turbidity was prepared in a clean 55-gallon drum by spiking kaolin clay (Fisher Chemical) into Boulder tap water. Three levels of turbidity were evaluated: 5 NTU, 50 NTU, and 500 NTU; in that order. The 500 NTU turbidity water seems

high, but such turbid water is a drinking water source in northern Ghana where Susan Murcott of MIT is starting a new CPF filter factory. A single batch of this water was used to fill all 4 CPFs to the same depth in the system each cycle. The depth of water in the experimental system was measured over time, and geometry used to calculate the treated water volume over the time intervals (as described previously in section 4.6). For the CWF tests, the filters were refilled two times per day for four consecutive days. In the ECWF system, the top receptacle was refilled three times each day for four consecutive days. The turbidity of the water in the mixing drum, water inside the CPF, and effluent water was measured with a Hach Model 2100N Laboratory Turbidimeter. Before every experiment the Hach Turbidimeter was calibrated using standards to make sure all experiments were consistent in measurements (Model 2100N Instruction Manual, 1999).

Turbid water was prepared in a clean 55 gallon drum with Boulder tap water. Kaolin clay was spiked into the water and thoroughly mixed before each measurement with a large stir rod. To achieve the correct turbidity for each test, a calibration curve was made by spiking a known mass of Kaolin into a known volume of water and measuring the turbidity (Figure 4-9). Inlet turbidities were confirmed using a Hach Model 2100N Laboratory Turbidimeter and then the mixture was adjusted by either adding more water or more Kaolin clay until the mixture was between ~5-8 NTU, 50 NTU ± 5 NTU, and 500 NTU ± 15 NTU for the 5 NTU tests, 50 NTU tests and 500 NTU tests; respectively. All inlet turbidities were recorded from a sample in the drum and assumed to be completely mixed and constant throughout. The inlet turbidity in each filter was also measured but was often much higher than the turbidity in the drum because the kaolin clay accumulated in the filters over the span of the experiments and was dislodged into the

water when a new batch was poured into the filter. A full turbidity experiment schedule is outlines in Appendix A.1.

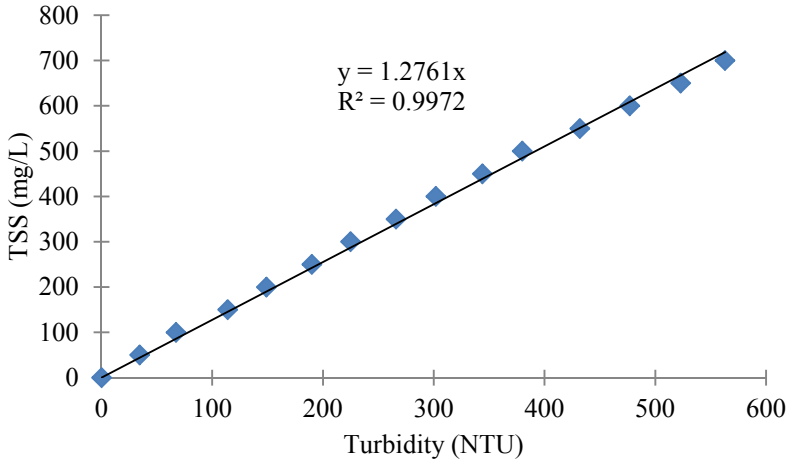

Figure 4-9 Calibration curve total suspended solids (mg/L) vs. turbidity (NTU)

For turbidity data analysis, the calibration curve and Equation 6 were used to evaluate the mass of Kaolin removed over time

$$mg\ removed = (C_{in} - C_{out}) * Q * t \qquad (6)$$

where the C_{in} and C_{out} are the concentrations in mg/L of the inlet water and the effluent water, Q is the flow rate and t is the time. The concentrations were found using the equation from the calibration curve (Equation 7) to find the total suspended solids (TSS) in mg/L at any measured turbidity from Figure 4-9.

$$TSS\ (mg/L) = 1.28 * Turbidity(NTU) \qquad (7)$$

All ceramic water filters were scrubbed with a brush after every turbidity experiment (see Figure 4-10), sometimes multiple scrubbings were needed until the filters appeared relatively clean. The kaolin is white and easily visible in the pores of the ceramic. To clean, the filters were filled ¼ full with clean tap water and with a brush the bottom and sides were scrubbed.

The water was dumped out and the process repeated until the filter had minimal kaolin visible. The gasket made it difficult to easily dump out all of the rinse water.

Figure 4-10 Brushes used to scrub the CPFs

The first hour flow rates under CWF operation were tested after each experiment and cleaning using tap water. The goal was to try and get the filters back to their original flow rate before the next experiment to minimize the cumulative clogging of the filters. First hour full flow rates were used as the basis comparison between filters and in between experiments. It is more likely that the Cambodia filters would experience some irreversible clogging, since they were new prior to the experiments. By comparison, all of the Nicaragua filters had experienced tests with turbidity prior to the current tests and dead end pores were more likely to have already been clogged.

A few times the flow rates were still very low after scrubbing so more cleaning was necessary. After the 500 NTU tests, there was almost ~0.8 cm of kaolin caked onto the inside of the filters. This cleaning process involved physically scraping off the kaolin before attempting to scrub out the pores. It can be assumed that the household users will not take this much time to clean the filters, if they even have enough water to waste on rinsing the turbidity from the filter.

4.8 Control (Blank) test details

Control tests were run for CWF and ECWF to see if the filters would support bacterial growth with only de-chlorinated tap water and 50 mg/L of TSB. The sample times and methods

were supposed to mimic the experiments with *E. coli.* Prior to the experiments, the filters were soaked with bleach water at a 10x dilution for over 24 hours and then the bleach water was allowed to drain through. After the bleach soak, about 1 day of tap water batches were run through followed by a day of de-chlorinated tap water batches to rinse out all of the bleach. Prior to the start of the experiment, the bottom receptacles were also bleached using methods described in section 4.9.1. Tap water was de-chlorinated using 4 mg/L of sodium sulfite (Kohler 2009) and spiked with 50 mg/L of powdered tryptic soy broth (TSB) and then run through the filters. The CWF experiments lasted 4 days with loading two batches per day and the ECWF experiments lasted 2 days loaded with three batches per day. These experimental durations resulted in similar cumulative volumes of water treated. Effluent samples were plated for bacterial counts and total organic carbon (TOC) samples were also taken for analysis (see Section 4.11.2).

4.9 *E. coli* experiment details

Escherichia coli K-12 strain was used in the laboratory bacterial disinfection experiments. The strain was maintained from previous research done by Kowalski (2008) and Kohler (2009). A centrifuged, concentrated stock of *E. coli* was spiked into de-chlorinated tap water and then put in the ceramic filters. See Appendix A.10 for the centrifuging procedure. Clean glassware, sterilized by autoclaving (Appendix A.5), was also filled with the inlet batch at every refill and left on the bench to represent what was going on in the filters in terms of bacterial growth or decay. Samples from within each filter were not taken to avoid the requirement to sample each filter separately and the desire to exclude bacteria from within the filters that might have added additional bacteria to the samples. Samples were taken of both the new batches at the times of the refill and of the bench culture (to represent spiked inlet

concentrations) and effluents from each individual filter at all sampling times and then spiral plated on tryptic soy agar plates (100 x15 cm). Typically a 10x dilution of the inlet was plated and an undiluted effluent sample was plated until it was determined that dilution was required to count the colonies. Each sample dilution was plated in triplicate, and sometimes multiple dilutions were plated. The plates were counted after incubation for 24 hours at ~35 degrees Celsius. The concentration of *E. coli* in the sample was calculated using the plate counts. A detailed experiment schedule is outlined in Appendix A.3.

A fresh stock of *E. coli* strain K12 was grown on liquid TSB (at ~35ºC for 15-18 hours before centrifuging and re-suspending the concentrated stock in 250 mL of sterilized water. The concentration of the centrifuged stock was consistently ~10^9 CFU/mL. Further details on preparation of TSB, TSA, spiral plating, and disposal of agar plates can be found in Appendix A.9 through A.13. All bacterial waste was disposed of in accordance to Appendix A.6. An appropriate amount of *E. coli* stock was spiked into de-chlorinated tap water containing 50 mg/L TSB to achieve ~10^5 CFU/mL. This *E. coli*-spiked water was treated by the CWF and ECWF systems. During the experiments, the plastic lids were placed on top to minimize light and ambient bacteria exposure. Based on the preliminary tests, there was an apparent decrease in effluent contamination when the lids were placed on top. The taps on the bottom receptacles were also left open to minimize the residence time of the water in the bottom receptacles (in particular the dead volume below the level of the tap). Bacterial growth in the bottom receptacles has been found in previous studies (Huang, 2002) and was significant in a preliminary study when the water was allowed to accumulate over time in the bottom receptacles.

4.9.1 Cleaning before *E. coli* experiments

Before every experiment the bottom receptacles were filled with approximately 5 L of tap water and 50 milliliters of household bleach (Clorox household bleach at 5.95% sodium hypochlorite). A brush was then used to scrub the inside of the plastic receptacles with the bleach water. The bleach water was then run through the taps. The buckets were rinsed 2-3 more times with tap water and allowed to drain through the taps as well. Filters were soaked in 10x household bleach solution for 24 hours and one full "batch" of household bleach water was allowed to run through the filter approximately 3 days before the start of the experiment. Three to four more batches of regular Boulder tap water were run through the filters over the preceding 2 days to ensure all the bleach was rinsed from the pores in the filter. Initial effluents of the tap water were plated before the first batch of spiked *E. coli* water was run through to make sure that there was no bacterial contamination evident prior to the beginning of the experiment. The reservoir that the spiked *E. coli* water was prepared in was bleached and rinsed out three to four times between the preparations of each new batch of *E. coli* water. This eliminated possible bacteria carry-over from the previous batches.

4.9.2 *E. coli* stock preparation

Preparation of *E. coli* stock involved preparing a flask of liquid TSB at concentration of 30 g/L, spiking in *E. coli* colonies and incubating overnight at 35 °C. This procedure was done the two days prior to the start of the experiment and every day during the experiment. At around 5 pm, a colony was taken off the streak plate (prepared from a frozen glycol stock- see Appendix A.7) and placed into the sterile TSB using sterile technique (see Appendix A.8 for streak plate procedure). The TSB with *E. coli* was placed on a stir plate in the incubator at 35 degrees Celsius overnight. The next morning at around 8 am the stock was removed from the incubator;

this allowed time for the *E. coli* to reach exponential growth rate (around 15-18 hours). The *E. coli*/TSB stock was placed in five 50 mL plastic centrifuge tubes and then centrifuged once for 10 min at the maximum centrifuge speed of 3725 rpm. The TSB was decanted off the top and replaced with sterile water and then mixed using a mini-vortex. The centrifuged stocks were combined into sterile, autoclaved glassware and then plated at a dilution of 10^{-6} and placed in the refrigerator at ~5˚C to stabilize growth/death. Its concentration was consistently around 10^{9} CFU/mL. For more details see Appendix A.10. A new *E. coli* stock was grown every evening on liquid TSB and centrifuged and plated every morning, stored in the refrigerator overnight and used to spike the de-chlorinated tap water the next day after the plates were counted and the concentration calculated (See section 4.10.2 for *E. Coli* counting methods).

4.9.3 *E. coli* spike preparation

Boulder tap water was de-chlorinated using a diluted 4 mg/L sodium sulfite solution. A high concentration solution was made at 4 g/L and then diluted by factor of 1000 to get the correct dose of 4 mg/L of sodium sulfite in the inlet water. For example, if 30 L of tap water needs to be de-chlorinated, it would require 30 mL of the 4g/L solution. This method was used throughout the experiments.

Centrifuged stocks were consistently around 10^{9} CFU *E. coli*/mL. Inlet concentrations were aimed to be 10^{5} CFU/mL. For each morning refill, the reservoir was filled with Boulder tap water and sodium sulfite solution was added to de-chlorinate the tap water. TSB, in powder form, was added to the influent water at a concentration of 50 mg/L in order to keep the active *E. coli* alive at a close to steady state and to mimic potential carbon in a typical contaminated source water. After mixing, the centrifuged *E. coli* stock of known concentration was then spiked in using autoclaved pipette tips according to Equation 8,

$$V_{centrifuge} = \frac{C_{desired\ spike} * V_{spike}}{C_{centrifuge}} \quad (8)$$

where the $C_{desired\ spike}$ was a constant 10^5 CFU/mL, V_{spike} was the volume needed to fill the filters, and the $C_{centrifuge}$ is the concentration of the centrifuged stock in CFU/mL and determined by plate counts (see section 4.11.1).

The spiked solution was then mixed fully and poured into the filters. All inlet samples were then plated at 10x dilutions. A clean container was placed on the bench next to the filters and filled with inlet water and then plated every time effluent samples were plated to see if there was any change in influent concentration over time on the bench.

4.9.4 Plating procedure

The spiral-plating technique was used to determine the bacterial concentration of all samples (see Appendix A.12 for more detail). Samples were collected in sterile vials, diluted into sterile water if needed, and then plated on prepared tryptic soy agar plates using a Model D spiral plater manufactured by Spiral Biotech Inc. Between plating of different samples, the spiral plater stylus was sanitized using ethanol and sterile water. Each sample was plated in triplicate. See Appendix A.11 for detailed procedure on preparing the agar plates.

4.10 Silver-coated CPFs and *E. coli* experiment details

The ECWF and CWF experiments that followed the reapplication of silver were designed to determine the benefits of silver, and therefore used the same methods as the previous *E. coli* experiments. The major difference was the collection of silver samples at designated times to measure the amount of silver that leached off of the ceramic and into the effluent water.

The silver used was Argenol brand in powder form from Spain (Batch number 249). The elemental composition of this material was extensively characterized by Stewart (2010), see Table 4-9. He found that the Argenol product contained few contaminants and consisted of

primarily spherical nanoparticles with an average diameter 10-50 nanometers. The silver concentration was 70% of the mass of the silver powder. The other 30% was mostly casein to aid in suspension (Stewart 2010).

Table 4-9 Elemental composition of the Collargol colloidal silver solution as determined by ICP-MS (Stewart 2010)

Element	(ppb)	(% of total)
Ag	**178753**	**92.61%**
Na	10782	5.59%
Fe	2303	1.19%
B	1045	0.54%
P	85.63	0.04%
Br	35.69	0.02%
Au	6.37	0.00%
Zn	5.66	0.00%
Mo	2.15	0.00%
Be	1.91	0.00%
Sn	0.83	0.00%
Li	0.76	0.00%
Pb	0.60	0.00%
V	0.27	0.00%
Sb	0.19	0.00%
Co	0.18	0.00%
∑	193023	100.00%

The methods used to apply the silver to the filters were followed from the Potters for Peace manual and coincided with Best Practices and Current Practices (Rayner, 2009). The filters were completely dried before applying silver so that all of the colloidal silver would be absorbed into the pores of the filter (PFP manual n.d.). The filters were therefore air dried for 2 or more days (average relative humidity 54.0% for Boulder on dates of air drying: February 23rd to 25th, 2011). The primary silver solution at a concentration of 32000 ppm was made by adding 0.32 g of Argenol silver into 10 mL of DI water. Then 2 mL of this concentrated solution was added to 300 mL of DI water (The Ceramic Manufacturing Working Group 2010) resulting in a concentration of 213 ppm. This entire 300 mL of solution was painted on every part of the filter, inside and out with a clean brush (Figure 4-11). (Rayner 2006). It was easy to see where the

silver had been painted already as the ceramic absorbed the liquid very quickly and was visibly wet. The inside of the pot used about 2/3 of the paint (200ml) and then use the remainder of the paint was used to coat the outside (100ml) (Nardo).

Figure 4-11 Silver re-application

The two *E. coli* experiments with silver were run in sequence with the enhanced flow rate conditions preceding the standard conditions with two days of de-chlorinated tap water rinse between the experiments. Effluent samples were collected from both filters in plastic 6 mL centrifuge tubes and taken to the Geology department at CU for silver concentration analysis by ICP-MS. Samples were taken for silver analysis at the following times for the ECWF experiment: one prior to both experiments during the saturation phase, at t~1 hour, 4 hours, 8 hours, 24 hours, 32 hours, 48 hours, and after the experiment during the rinse at 70 hours and 104 hours from the start. At hour 104, the CWF experiment started and silver was collected at t~8 hours, 32 hours, 56 hours, 70 hours, 94 hours, and 118 hours from the start of this experiment. The measured silver concentrations are presented in Chapter 6.

4.10 Data Analysis

This section goes into detail on the analysis of the collected data during the experiments; including *E. coli* data analysis methods, TOC sample analysis, and silver concentration analysis. See Appendix A.2 for turbidity data analysis.

4.10.1 *E. coli* Data Analysis

Spiral plating was used for all influent and effluent samples to accurately determine the *E. coli* concentrations. All plates were incubated at ~35 degrees Celsius for 24 hours ± 1.5 hours before counting. Concentrations were calculated by multiplying the amount of bacteria colonies on the plate by the dilution (10x, 100x, etc.) and then dividing it by the volume of liquid that is plated by the spiral plater. If the full plate could be counted, the number of colonies would be divided by 0.049 mL. This resulted in a minimum countable "detection limit" of 20 CFU/mL (representing less than 1 colony on the plate). One colony on the plate corresponded to a concentration of ~20 CFU/mL. Therefore, if the plate was blank the only conclusion that could be made was that the sample had <20 CFU/mL. If only partial plates could be counted, the colony amount would be divided by the corresponding volumes for that area of the plate. Plates were considered countable when individual colonies were visible to the naked eye. All bacteria were counted, even if the colony did not appear to be *E. coli* (see Figure 4-12, middle). Notes were made on colony morphology. In some cases the colonies were too small to count after 24 hours, so they were incubated for longer until they could be counted.

Effluent plates were many times extremely difficult to count because of the significant amount of bacteria which were obviously not *E. coli* colonies; frequently these plates were determined to be "too numerous to count" (TNTC) which was assumed to correspond to more than 100,000 colonies (Figure 4-12, right).

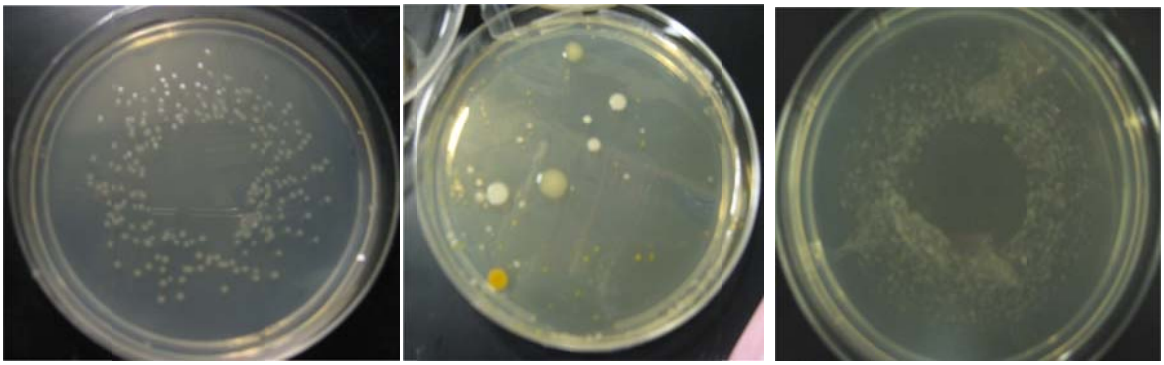

Figure 4-12 Effluent plates. Left picture: inlet, known *E. Coli* colonies. Middle picture: Effluent plate, unknown bacteria, Right: TNTC

After each plate was counted and raw colony counts were recorded, it was necessary to convert these counts to colony forming units (CFU) per milliliter. This was achieved by dividing the raw colony counts by the conversion factor for the counting grid used (Table 4-10) and multiplying it by the dilution factor. The following equation was used to convert raw counts into colony forming units per milliliter (CFU/mL)

$$\frac{CFU}{mL} = \frac{Raw\ count}{CF} * DF \qquad (9)$$

where CF is the conversion factor in milliliters deposited per template area (shown in Table 4-10) and the DF is the dilution factor. The dilution factor was usually 1, 10, or 10^6 for effluent samples, inlet samples, or centrifuged samples, respectively.

Table 4-10 Model D Spiral Plater conversion factors for counting grids

Template	Conversion Factor (mL deposited/template)
3c	0.00054
3b	0.00137
4c	0.00457
4a	0.0123
Total	0.0492

Percent removal and log removal were also calculated when analyzing the filters effectiveness as removing *E. coli* from the inlet water.

$$\% \, Removal = \frac{Spike - Effluent}{Spike} * (100) \quad (10)$$

$$Log \, Removal = \log(\frac{Spike}{Effluent}) \quad (11)$$

where the spike is equivalent to the concentration of the spiked inlet water in CFU/mL and the effluent is the concentration of the effluent water in CFU/mL.

4.10.2 TOC analysis

Total organic carbon (TOC) samples were also taken during the control tests and during the *E. coli* experiment with silver as an indication of biological activity. A decrease in TOC levels from the inlet to the effluent water would indicate biological activity taking place in the filter. A small decrease in TOC could be expected due to minimum sorption potential in the ceramic pores. TOC analysis was completed on a Sievers 800 Portable Total Organic Carbon Analyzer (Standard Method 5310C) with Automated Sampler. Samples were collected in sterile glass vials, preserved with phosphoric acid until the analysis was run, and wrapped in tinfoil and stored in a refrigerator at ~5 ºC. The minimum detection limit was 0.2 mg/L. Samples did not need to be diluted and TOC was recorded in triplicates for each sample. For further information see Appendix A.14.

4.10.3 Silver Concentration Analysis

Silver samples were taken to the Geology Building at the University of Colorado and analyzed there for silver concentrations. Samples of the filter influents and effluents were stored in plastic, 6 mL centrifuge tubes until analysis. Samples were analyzed with a Perkin Elmer SCIEX inductively coupled plasma mass spectrometer, model # Elan DRC-e. Indium was used as an internal standard. Four standards (blank, 100, 500, 1000 ppb) were used for calibration. Standards were made by accurately diluting certified standards. Results can be found in Chapter 6.

Chapter 5 Results of Flow, Turbidity, and Clogging Experiments

The goal of these experiments was to determine the amount of flow rate enhancement that could be achieved with the ECWF system; in particular, to determine how much water a family could expect under typical operating conditions of an ECWF system. It was important to confirm that the filters maintained their water treatment effectiveness, as quantified by turbidity removal, in the ECWF operation compared to CWF. The maintenance of flow under clogging conditions was also evaluated. In this chapter, the clean water flow rate results will be presented first, followed by the CWFs and ECWFs ability to remove turbidities of 5 NTU, 50 NTU and 500 NTU. Then, the potential for irreversible clogging and any clogging the filters experienced during the 4 day turbidity tests are addressed.

5.1 Clean Water Flow Rate Results

An initial set of experiments with clean Boulder tap were conducted to characterize the flow rates and cumulative volume filtered between the standard (CWF) and the enhanced (ECWF) systems. The key characteristics of the four test filters are summarized in Table 5-1. The dead volume in the bottom bucket is the amount of water that remains stagnant while the spigot is open. The spigots were kept open to limit this dead space but the design of the bottom receptacles do not allow for complete removal of dead volume. This dead volume made it difficult to limit the potential for bottom receptacle contamination, although recontamination results were inconclusive (see Chapter 7 *E. coli* Results).

Table 5-1 Volume and Flow Rate Characteristics of the Four CPFs used in the Study

	18193	**11136**	**RDI1**	**RDI2**
Internal volume at standard full depth, L	7.8	7.8	10.0	9.8
Internal top diameter, cm	25.9	25.7	28.6	28.2
Internal depth, cm	20.7	20.8	21.4	21.2
Dead volume under spigot, L	0.8	0.8	2.9	2.9
CWF initial full flow rate, L/hr	1.8	2.0	1.3	1.5

The hydraulic testing done for standard and enhanced systems took place over the course of one 8 hour day for both the CWF and ECWF experiment. The results for three of the four filters were averaged and presented in Figure 5-1 and 5-2. The RDI 2 results were significantly higher than the other three filters and thus left out of the graphs. RDI 2 had initial first hour flow rates of 2.47 L/hr and 7.03 L/hr for CWF and ECWF, respectively, compared to an average of 1.13 L/hr and 4.09 L/hr for the other three filters. The other three filters were extremely similar in flow rate performance as shown by the small error bars. It is important to note that this experiment was conducted after all the turbidity tests and *E. coli* tests without silver. The higher flow rate for RDI2 may therefore have been due to the development of a small crack during these tests. For the other three filters the overall flow rates may be slower than before all the tests, but the data still provides a good comparison of the CWF and ECWF systems even is not at ideal flow rates that represent new filters.

The enhanced system started with average flow rates at 4.1 L/hr decreased to 2.5 L/hr over the course of 6 hours. This decrease was due to the decrease in hydraulic head over time. It took approximately 6-7 hours for the top bucket of the ECWF system to empty completely. Meanwhile the ceramic filter is completely full this entire time until the top bucket empties and the water level in the ceramic filter continues to drop. The standard system started at an average full initial flow rate of 1.13 L/hr and dropped to 0.7 L/hr over the course of 6 hours, due to a combination of decreased head and lower active surface area as the filter empties. The cumulative volume filtered after 6 hours for ECWF and CWF averaged 15.2 L and 4.2 L for the three filters, respectively. Therefore, the enhanced flow rate system filter more than three times the amount of water over six hours as compared to the CWF.

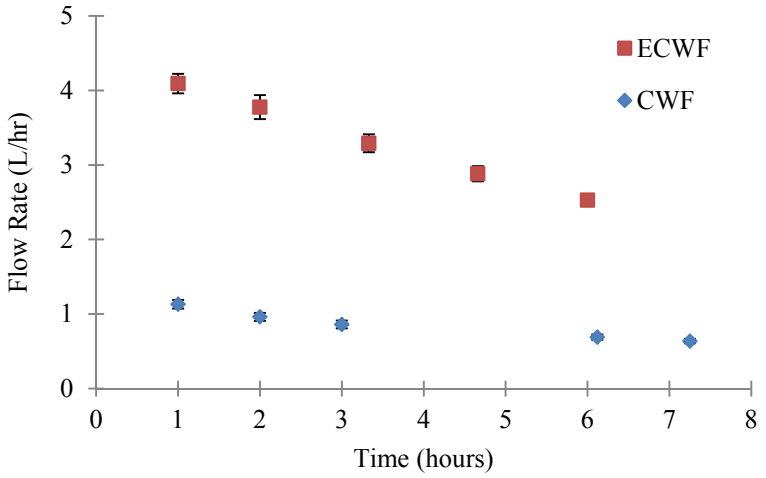

Figure 5-1 Average flow rate vs. time for three CPFs

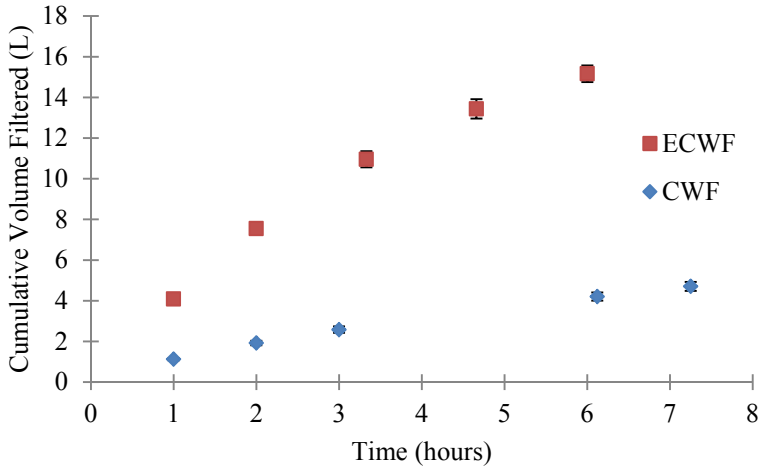

Figure 5-2 Average cumulative volume filtered vs. time for three CPFs

5.2 Removal of Turbidity by Ceramic Filters

In general the effluent turbidity decreased over the 4 day test period, as would be expected due to the accumulation of a cake layer of the turbidity on the inside of the filter which enhanced the filtration of particles beyond the pore structure of the ceramic itself. The four CPFs performed similarly during treatment of 5 NTU inlet water with the CWF system; effluent turbidity averaged 0.36 ± 0.21 NTU. When the inlet water contained ~50 NTU, the effluent turbidity values from the CWF were significantly different per CPF. The effluent turbidity was

lower for 50 NTU, averaging 0.25 ± 0.13 NTU. The effluent turbidity value was even lower when the inlet water contained 500 NTU during CWF operation, averaging 0.15 ± 0.08 NTU; the improved performance was again attributed to the cake layer development on the ceramic surface.

In the ECWF the effluent turbidity values were more variable. The effluent turbidity under ECWF was similar to the CWF for the 5 NTU inlet water, averaging 0.29 ± 0.18 NTU. The effluent turbidity values were much more inconsistent and higher for the ECWF compared to the CWF when treating 50 NTU and 500 NTU. At 50 NTU, the 18193 CPF had variable effluent turbidity ranging from 0.11 to 1.97 NTU and averaging 0.90 NTU; the variability may have been due to a leak in the gasket that was not visible. The other three CPFs performed more consistently, with an average effluent turbidity of 0.29 ± 0.21 NTU; not significantly different from the 5 NTU ECWF results or the 50 NTU CWF results. During the 500 NTU ECWF test, a visible leak under the gasket was noticed on the 18193 CPF, resulting in effluent turbidities ranging from 0.8 to 12.3 NTU. The RDI 2 CPF had effluent turbidity values of 0.12 to 6.6 NTU; this may have been due to a small leak in the gasket despite none being visible. Therefore, comparing only the two CPFs with consistent performance, there was not a statistically significant difference in the effluent turbidity during ECWF (0.26 ± 0.46 NTU) versus CWF; and 50 NTU versus 500 NTU. In summary, the enhanced flow rate did not definitely reduce the water treatment effectiveness, but some difficulties and risks with properly sealing the gasket did occur.

Both at standard flow rates and the enhanced flow rates, the filters were effective at removing turbidity. The error bars are quite large on the averages (Figure 5-3, 5-4, and 5-5) because the turbidimeter ranges greatly with such small values. It is important to note that the

average turbidity of the effluent water for all CWF tests was under 0.4 NTU. This is well below required turbidity for drinking water. The World Health Organization says drinking water should ideally be below 1 NTU (WHO 1993). The US EPA, on the other hand, requires drinking water in the United States to be below 0.3 NTU (US EPA 2011).

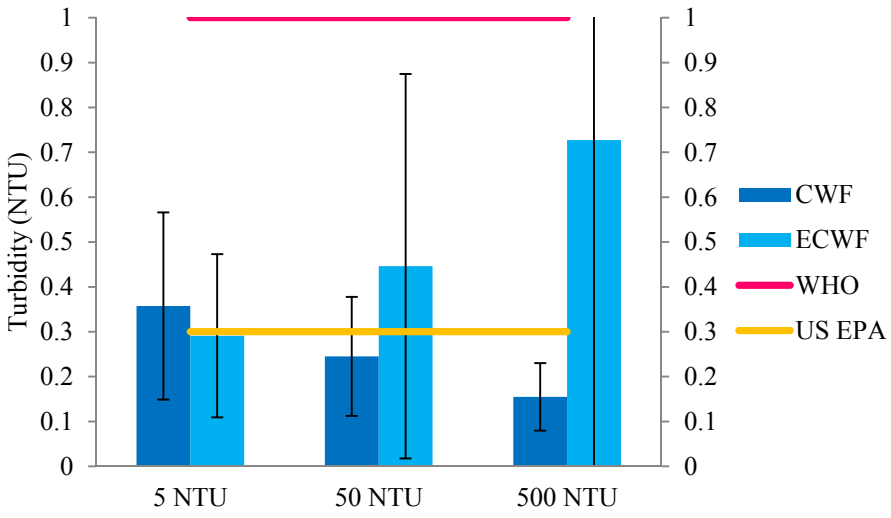

Figure 5-3 Comparing CWF and ECWF for 5 NTU, 50 NTU, and 500 NTU experiments; average of four CPFs shown with error bars representing the standard deviation of the data over time and across the filters

For all turbidity testing the effluent turbidity decreased over the four day test. It is expected that the larger pores get clogged with particles over time, decreasing the turbidity in the effluent as more and more batches of water are filtered. Figure 5-6 to 5-8 show the effluent turbidities over time for all four filters for CWF and ECWF for 5 NTU, 50 NTU, 500 NTU. Figures 5-9 to 5-11 show the effluent turbidities vs. cumulative volume filtered for CWF and ECWF for 5 NTU, 50 NTU, 500 NTU. The filters were effective at removing turbidity under both the standard and enhanced ceramic water filtration systems. During the 500 NTU ECWF test, a visible leak under the gasket was noticed on filter 18193 from Nicaragua (Figure 5-8) resulting in effluent turbidities around 10 NTU. RDI 2 also had increased turbidity in the

effluent for the 500 NTU enhanced set up; this could be due to a small leak in the gasket but none were visible.

The mass of turbidity removed for each test was also calculated and is presented for CWF in Figures 5-12 to 5-14 and ECWF in Figures 5-16 to 5-18. Figure 5-15 and 5-19 show the average mass removed for all four filters comparing each turbidity loading condition under CWF system and ECWF system, respectively.

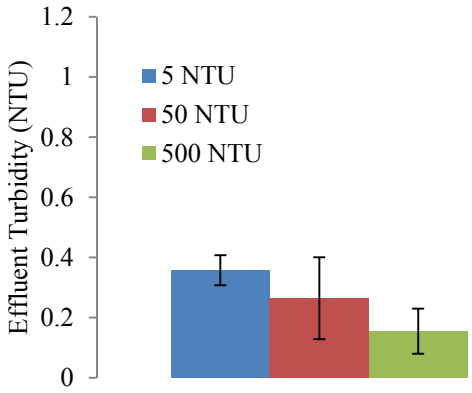

Figure 5-4 Average effluent turbidity for CWF

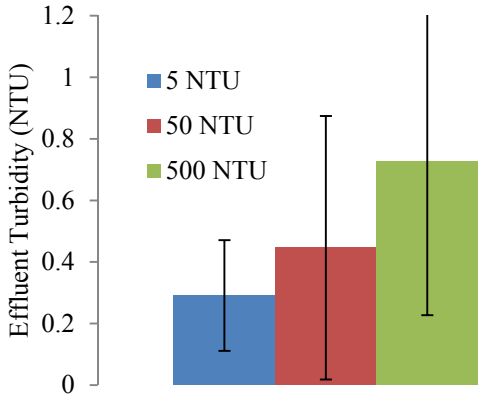

Figure 5-5 Average effluent turbidity for ECWF

Overall, under the ceramic water filter system the effluent turbidity decreased as the influent turbidity increased. This could be a result of the larger pores being blocked by the first batches of turbid water so less turbidity was let through during later batches. Results showed that for the CWFs, the higher the influent turbidity, the lower the effluent turbidity. Under enhanced flow rates, the trend was opposite. This could be due to small leaks in the gasket as it was tested more and more. It also could be due to the turbidity breaking through under enhanced flow rates and intense turbidity loading. The conditions of the filters after the 500 NTU ECWF experiment is shown in Figure 5-20. The filters were completely coated with a thick layer of kaolin clay.

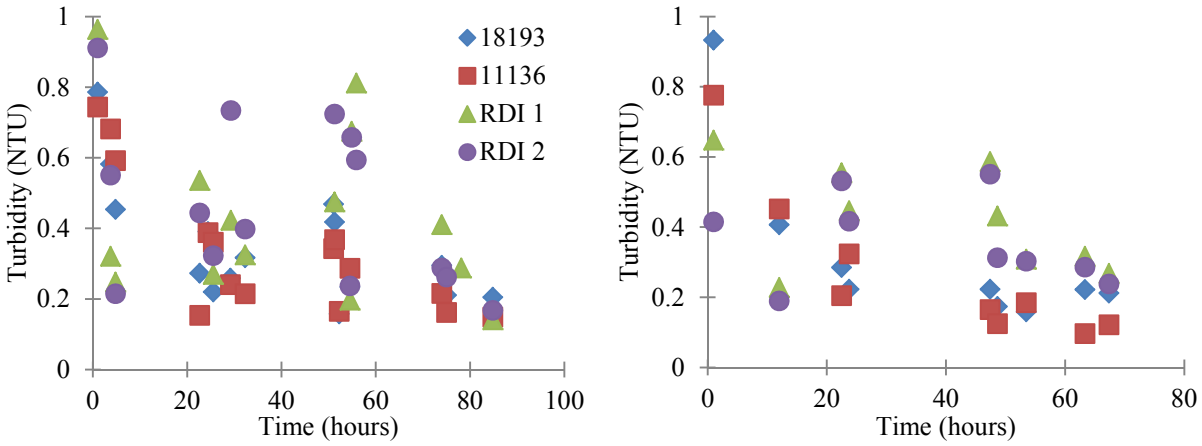

Figure 5-6: 5 NTU CWF (left) and ECWF (right) effluent turbidities vs. time

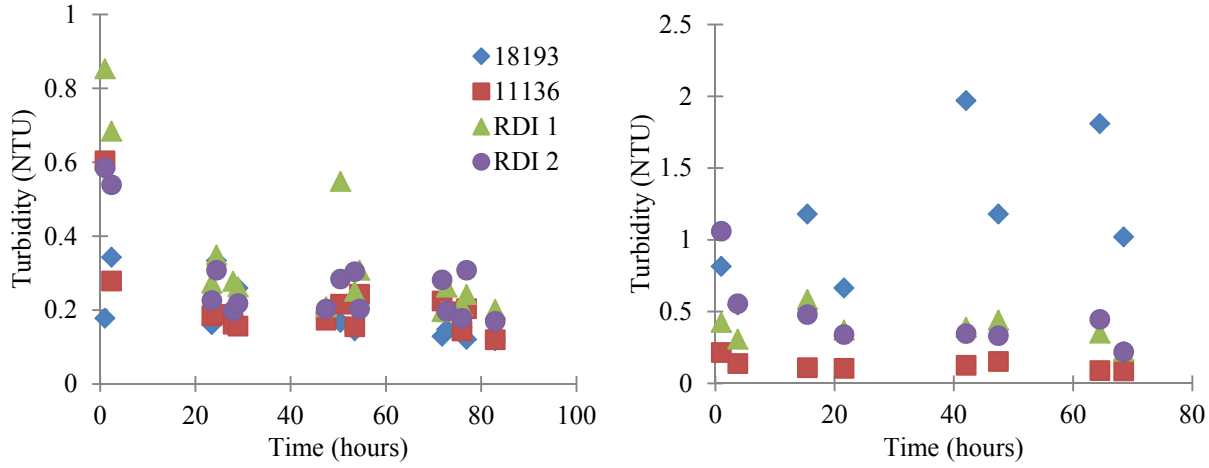

Figure 5-7: 50 NTU CWF (left) and ECWF (right) effluent turbidities vs. time

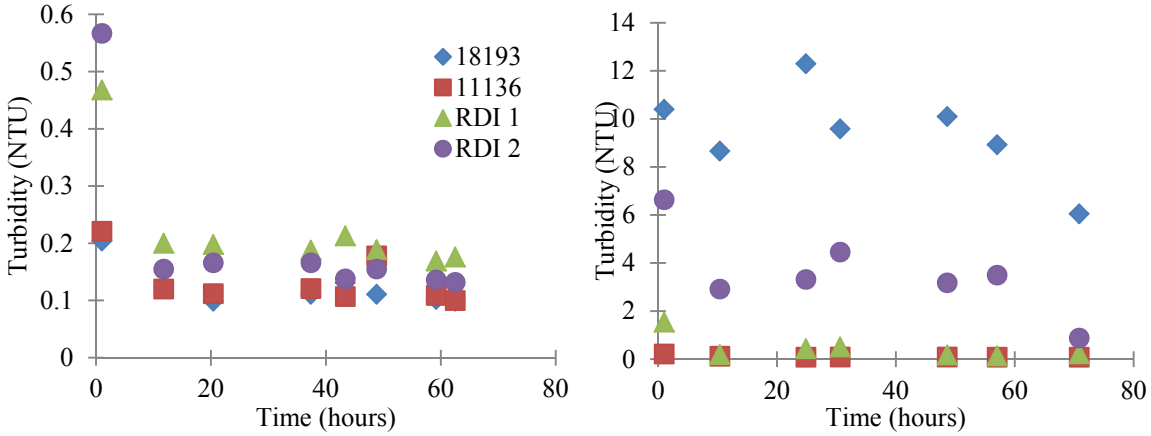

Figure 5-8: 500 NTU CWF (left) and ECWF (right) effluent turbidities vs. time

NOTE: The scale in the vertical axis is different for all the enhanced systems due to the significant differences in effluent turbidities as a result of potential leaks.

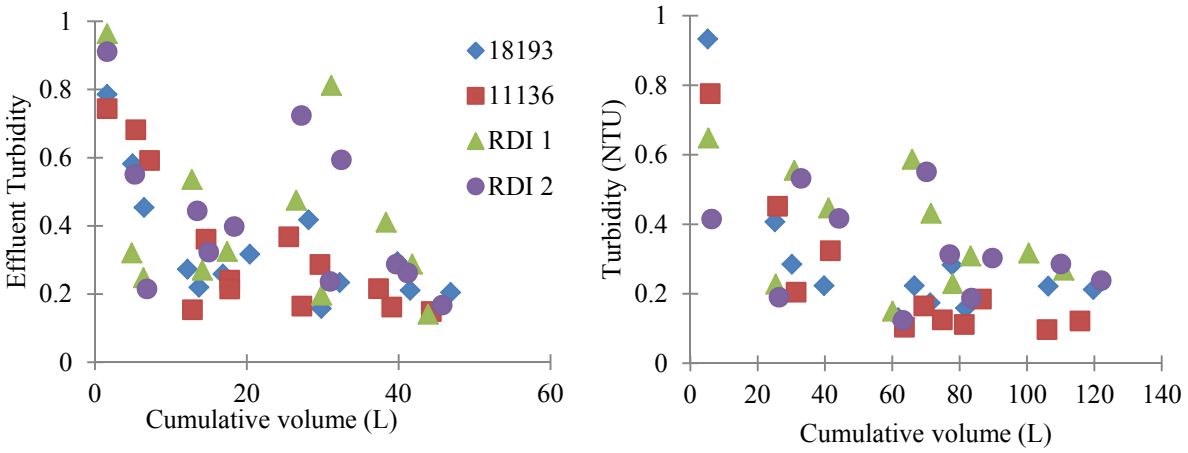

Figure 5-9: 5 NTU CWF (left) and ECWF (right) effluent turbidities vs. cumulative volume

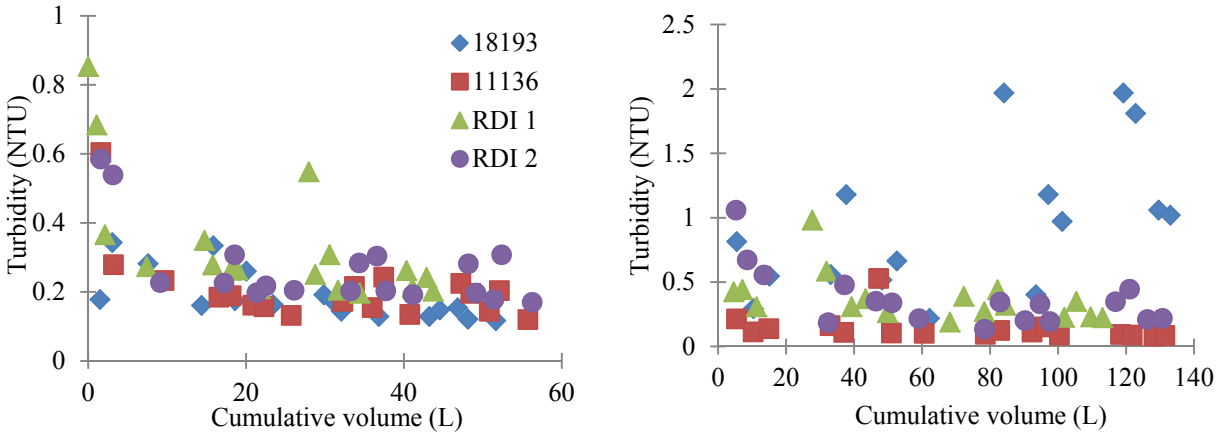

Figure 5-10: 50 NTU CWF (left) and ECWF (right) effluent turbidities vs. cumulative volume

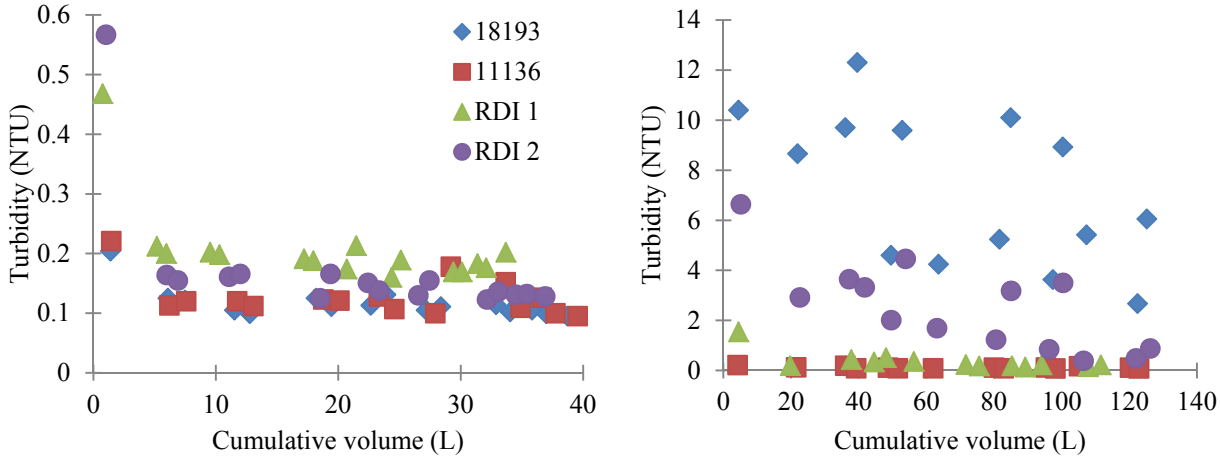

Figure 5-11: 500 NTU CWF (left) and ECWF (right) effluent turbidities vs. cumulative volume

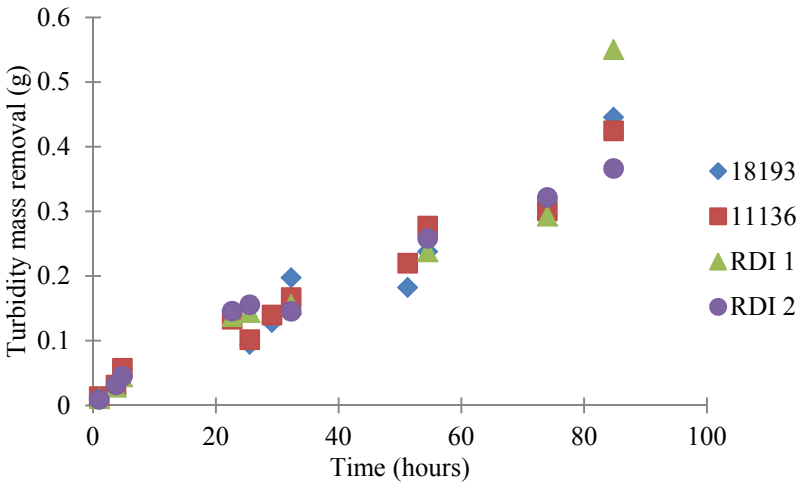

Figure 5-12: CWF 5 NTU Turbidity mass removal (g) vs. time

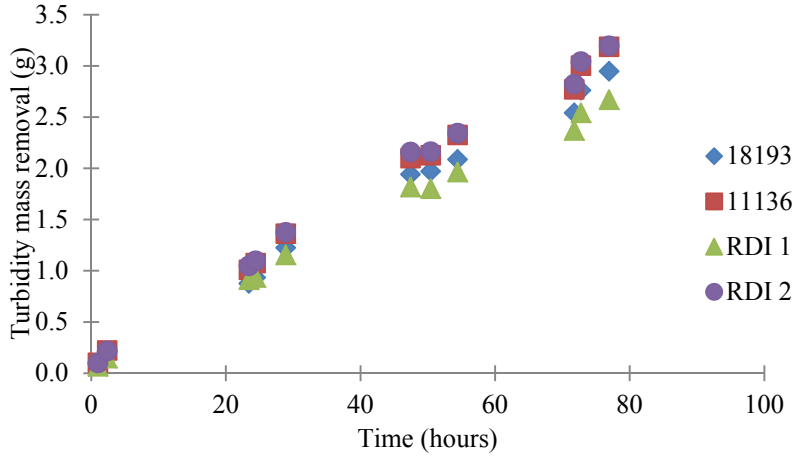

Figure 5-13: CWF 50 NTU Turbidity mass removal (g) vs. time

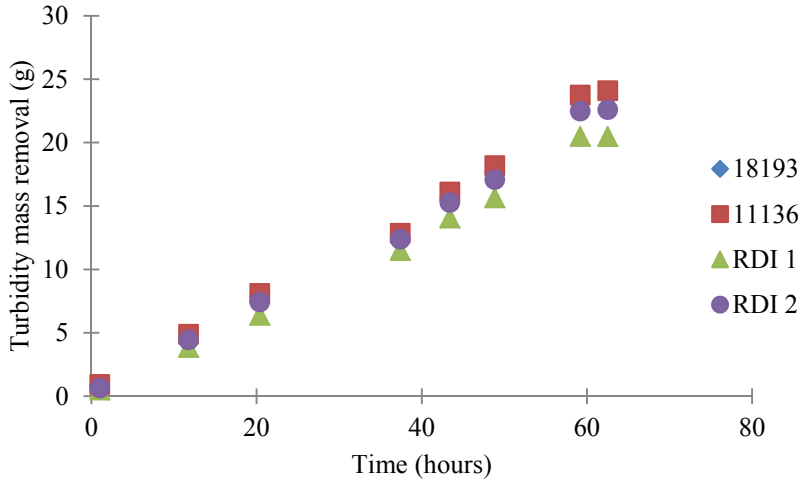

Figure 5-14: CWF 500 NTU Turbidity mass removal (g) vs. time

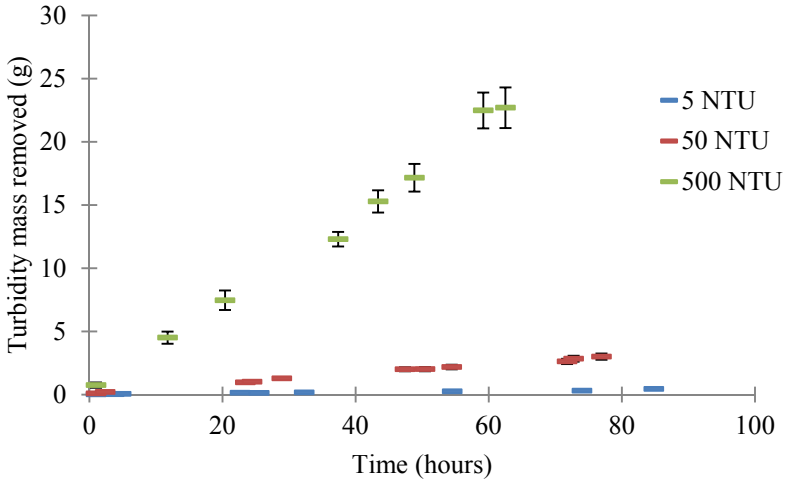

Figure 5-15: CWF Average turbidity mass removal (g) for 5, 50, and 500 NTU

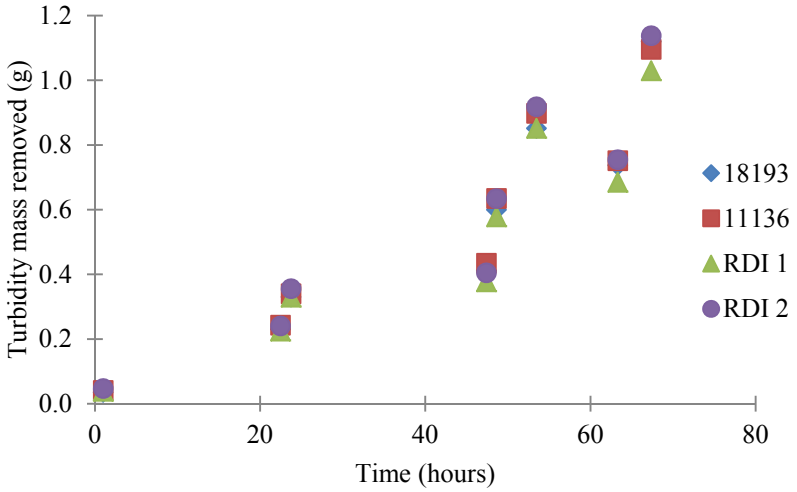

Figure 5-16: ECWF 5 NTU Turbidity mass removal (g) vs. time

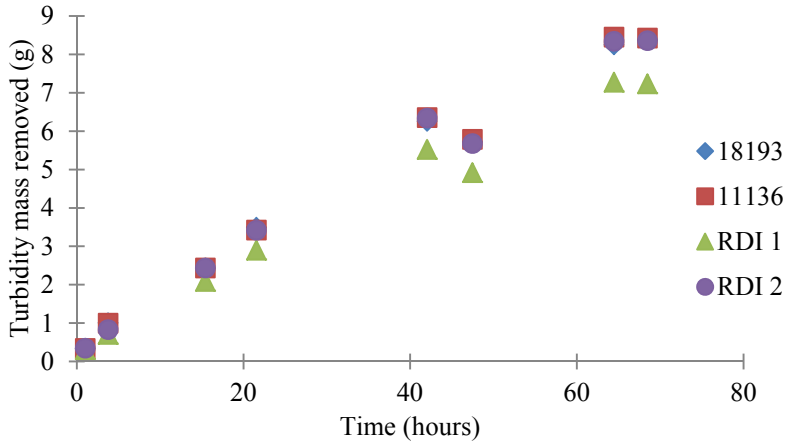

Figure 5-17: ECWF 50 NTU Turbidity mass removal (g) vs. time

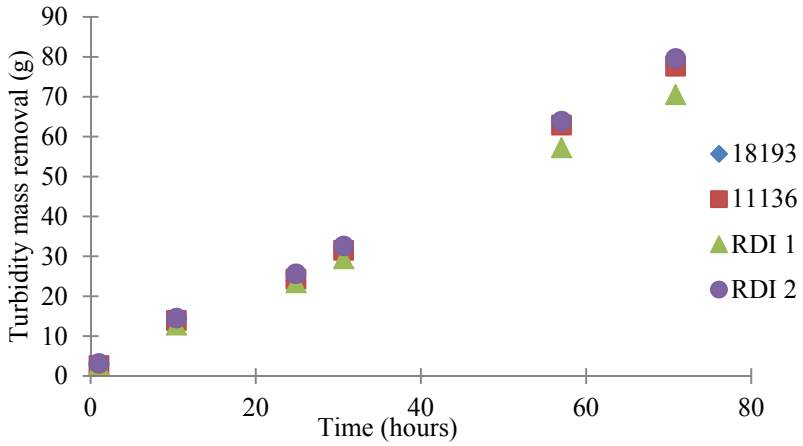

Figure 5-18: ECWF 500 NTU Turbidity mass removal (g) vs. time

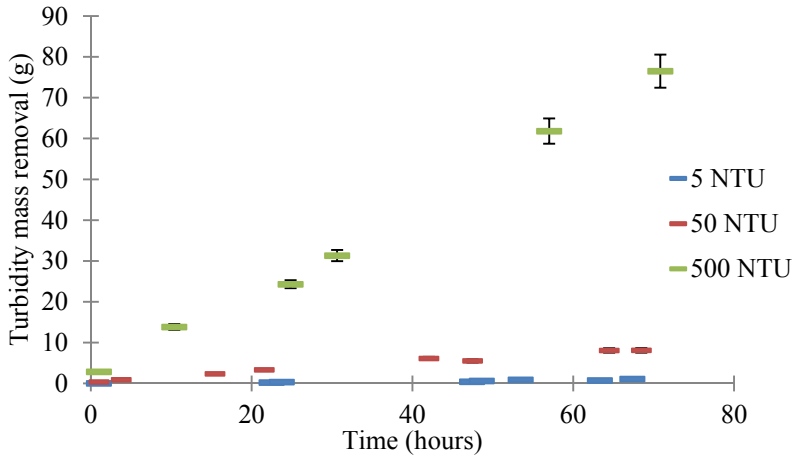

Figure 5-19: ECWF Average turbidity mass removal (g) for 5, 50, and 500 NTU

Figure 5-20: CWF before 500 NTU and after 500 NTU loading

The effluent turbidity values were much more variable and higher from the ECWF compared to the CWF when treating 50 NTU and 500 NTU. In the ECWF, there were some cases where small leaks were visible around the gasket seal between the CPF and the top bucket. Other cases, leaks were not visible but the effluent turbidity results prove that they were most likely present. Overall, when there were no leaks present, the filters were sufficient at removing large amounts of turbidity at both standard condition and enhanced flow rate condition.

5.3 Irreversible Filter Clogging During Treatment of Turbid Water

During treatment of water spiked with ca. 5, 50, and 500 NTU of kaolin, the first hour flow rates in the CWF and ECWF decreased by 5-16% and 6-18%, 18-29% and 20-37%, and 17-34% and 14-45% after treatment of ~50 L, ~40-50 L, and ~35-40 L of water, respectively. Thus, the clogging was similar per volume of treated water. Table 5-2 shows the percentage decrease in first hour flow rates each filter experienced for both the regular and enhanced systems. For the CWF tests these percentages take into account the first hour flow rate prior to the test starting and compared that to the last flow rate measurement taken on day four of the experiment. The

ECWF test have no prior test data for clean tap water so these percentages compare the first hour flow rate data from the first batch of turbid water to the last flow rate measurement from day four. These ECWF flow decrease percentages would be expected to actually be greater if there was pre-experiment data for clean water flow rates prior to turbidity loading.

Table 5-2 First hour flow rate percentage decrease over four day testing period for different turbidity loading

Flow Rate Decrease (Percentage)					
		18193	11136	RDI 1	RDI 2
CWF	5 NTU	10	16	12	5
	50 NTU	18	21	27	29
	500 NTU	27	29	34	17
ECWF	5 NTU	14	17	18	6
	50 NTU	37	35	24	20
	500 NTU	45	42	14	20

5.3.1 Clogging: first hour flow rates after each experiment

Overall, the two filters from Nicaragua had higher flow rates than the two from Cambodia. The first hour flow rates of all four ceramic water filters were measured after each experiment and intense cleaning (Figure 5-21). These results show that even after the final intense cleaning of the filters, the flow rates could not reach their original flows though they got close. 18193 decreased from 1.8 L/hr to 1.6 L/hr, 11136 decreased from 2 L/hr to 1.6 L/hr, RDI 1 decreased from 1.3 L/hr to 1.2 L/hr, and RDI 2 decreased from 1.5 L/hr to 1.3 L/hr over the course of all the turbidity tests and scrubbing.

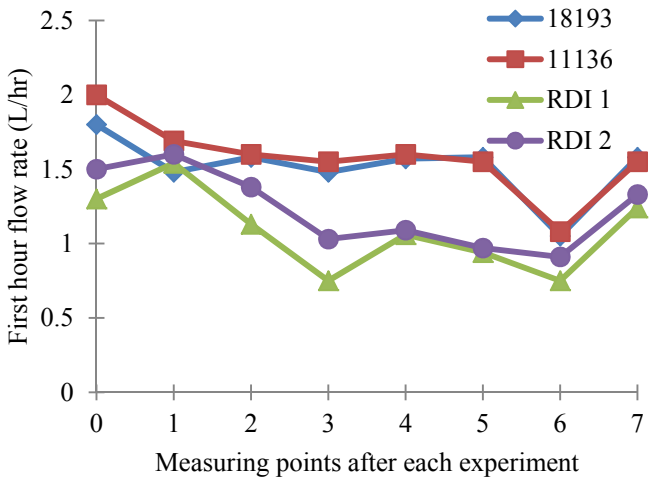

Figure 5-21 First hour flow rates tested with CWF system between each lab experiment with zero NTU (tap water) after scrubbing and cleaning

It is apparent that the performance of the ceramic is affected by its use history, making it difficult to restore it back to its initial flow rate once it has been clogged. Therefore, it is unlikely that filters will keep their initial flow rates after years of use in the field, especially if the untreated inlet water has high turbidity levels. Some areas using ceramic water filtration as the main source of water treatment have reported turbidities up to 1000 NTU (Murcott, 2009). Areas with high inlet turbidities are encouraged to use a pre-filtration method such as running the water through a cloth to remove some turbidity. Turbidities this high would likely clog the ceramic filters very quickly. Even if users are maintaining and scrubbing their filters, it is likely that the first use of the filter will be the fastest use of its lifetime.

5.3.2 Clogging: flow rate data over four day turbidity experiments

When comparing the first hour flow rates of the CWF to the ECWF over a four day testing period it became apparent that the enhanced systems experienced more clogging due to the increased loading of turbidity and volume of turbid water. Average first hour flow rates for all four filters are plotted vs. volume for both CWF and ECWF testing in Figure 5-22. Figure 5-23 to 5-25 compares the performance of all filters under CWF and ECWF for each of the three

testing conditions (5 NTU, 50 NTU, 500 NTU). These graphs show flow rates versus cumulative volume filtered to show the volume output difference between the filters. Figures 5-26 to 5-29 show the flow rates for each filter individually for both CWF and ECWF under each testing condition (5 NTU, 50 NTU, 500 NTU). These graphs show flow rates versus the first 8 batches for each experiment. As the turbidity was increased from 5 NTU to 50 NTU to 500 NTU the first hour flow rates decreased. The difference between 50 NTU and 500 NTU was not as significant as expected. Some filters experienced greater percentage decreases from the 50 NTU than the 500 NTU (see Table 5-2). This could due to the largest decrease of flow rate experienced from the first batch of turbid and then steadily decreasing after.

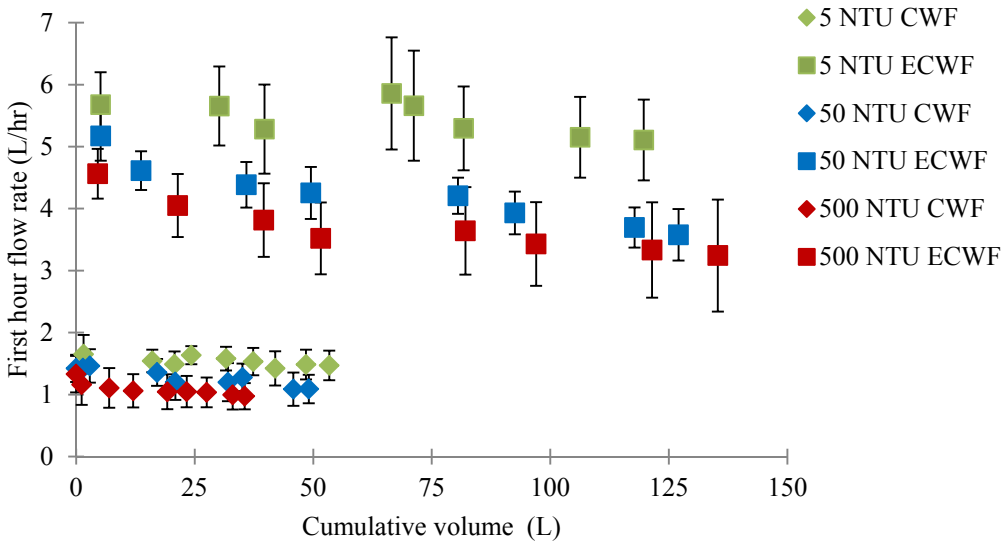

Figure 5-22: Average first hour flow rate (L/hour) vs. volume filtered for all four filters under CWF and ECWF at 5 NTU, 50 NTU, and 500 NTU.

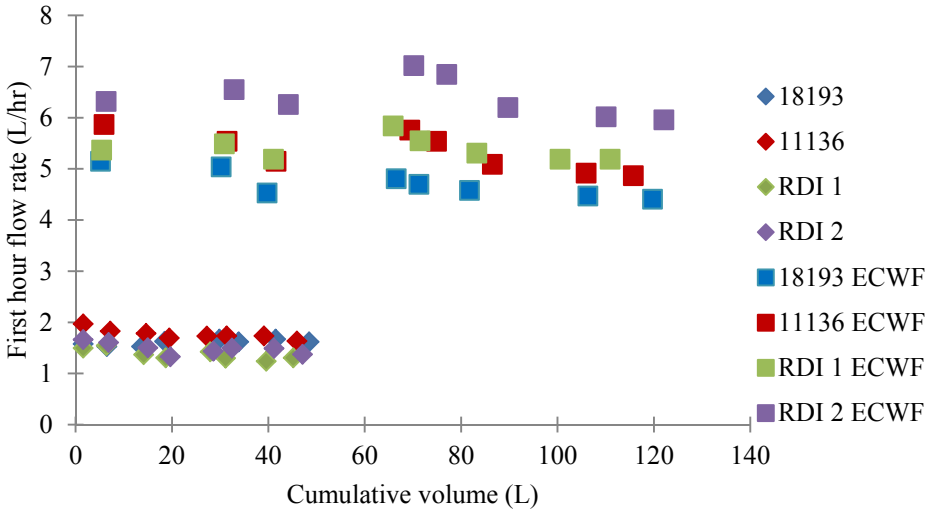

Figure 5-23 First hour flow rate for 5 NTU comparing CWF to ECWF

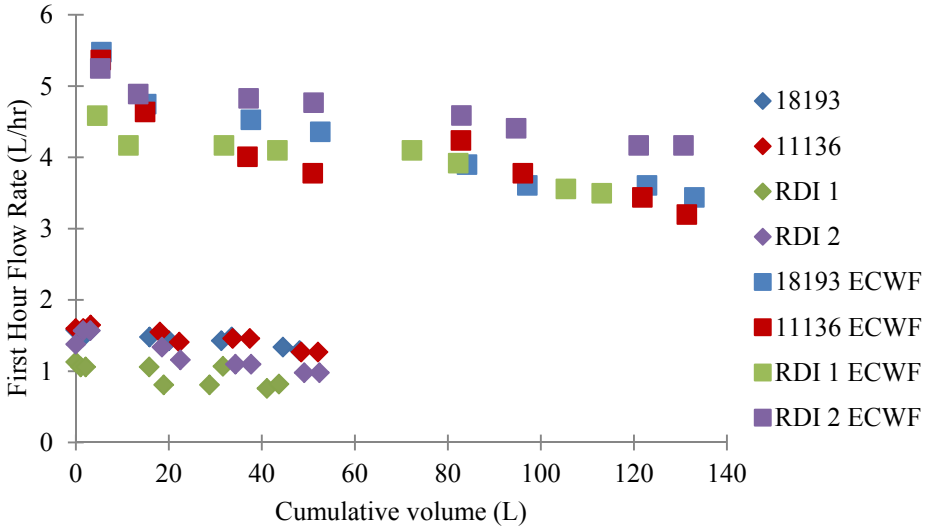

Figure 5-24 First hour flow rate for 50 NTU comparing CWF to ECWF

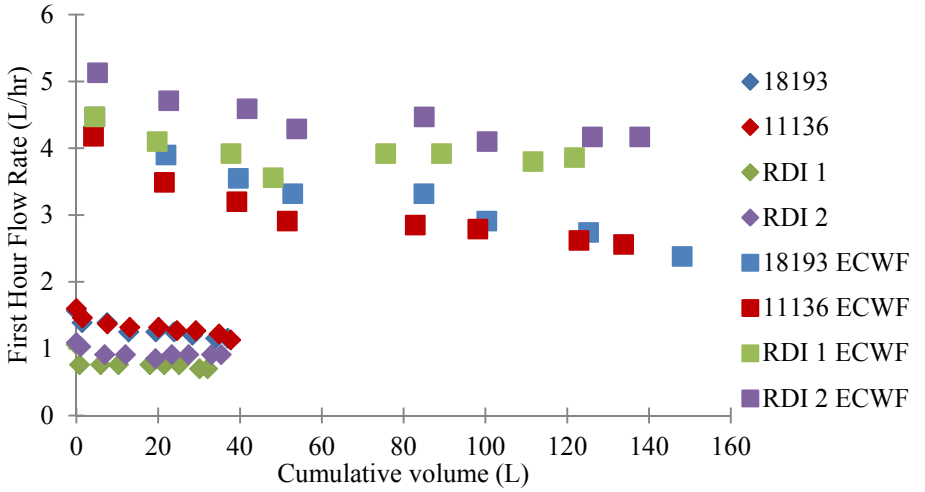

Figure 5-25 First hour flow rate for 500 NTU comparing CWF to ECWF

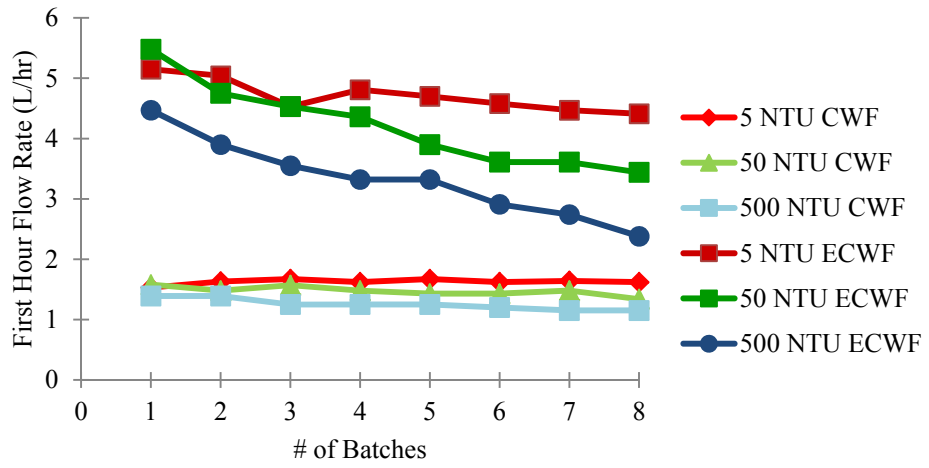

Figure 5-26 CWF vs. ECWF flow rates for 18193

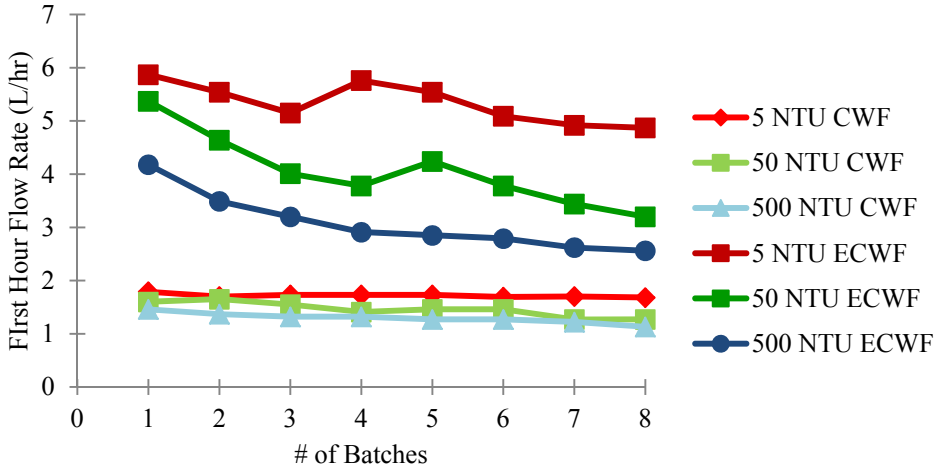

Figure 5-27 CWF vs. ECWF flow rates for 11136

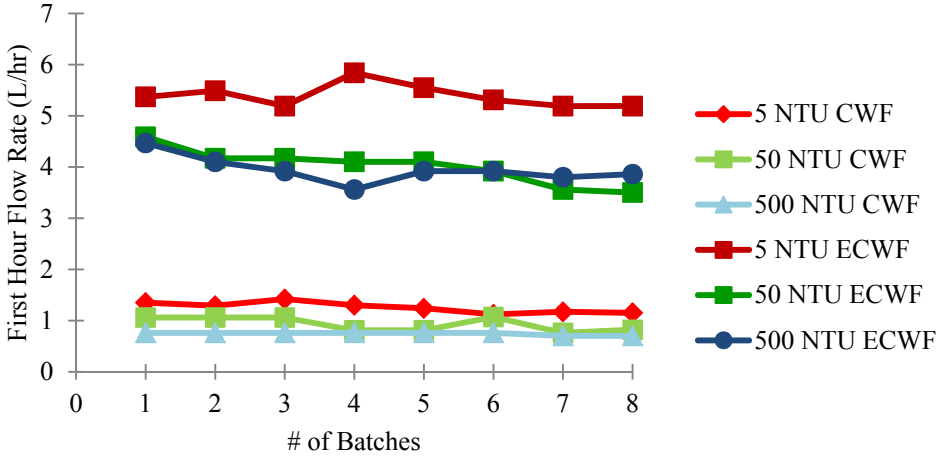

Figure 5-28 CWF vs. ECWF flow rates for RDI 1

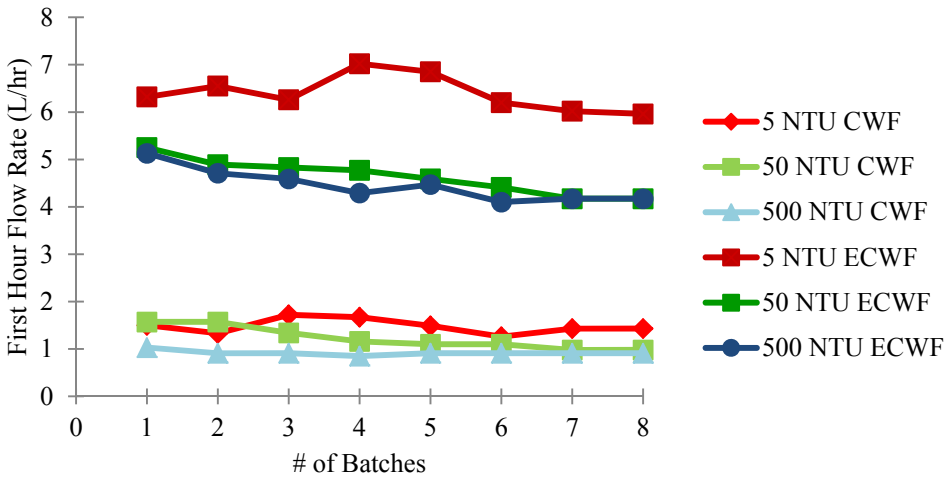

Figure 5-29 CWF vs. ECWF flow rates for RDI 2

During experiments treating tap water spiked with 5 NTU turbidity, the flow rates in the CWF and ECWF systems were somewhat variable over the 4 day test period, without severe decreases over the 8 batches of water treated (~50 L of water for the CWFs and ~130 L of water for the ECWFs). The RDI 2 filter experienced the least clogging, at only 5% decrease in the full first hour flow rate after 4 days. Clogging due to turbidity removal after 4 days ranged from 10-16% in the other three CPFs in the CWF system compared to 14-18% in the other 3 CPFs in the ECWF system. However, the ECWFs treated a greater quantity of water over this time period. When clogging is compared after a similar volume of water treated (~50 L) and turbidity removed, the percent flow rate reduction was 0 to 7% (the RDI 1 and RDI 2 CPFs had higher flow rates at the start of the 4th batch). Paired t-tests per batch showed no significant differences between the flow rates under CWF and ECWF.

During the experiments with 50 and 500 NTU turbidity, significant reductions in the full first hour flow rates occurred as the treated volume of water increased. Paired t-tests between CWF and ECWF by batch of water being treated showed significantly different flow rate reductions, but when the results were compared based on the volume of water treated the flow

rate reductions were not significantly different. This is logical because clogging was due to the amount of solids removed from the water which accumulated on and within the CPFs. After treating 43 to 53 L of water containing 50 NTU of kaolin, flow rates dropped by 18-29% and 9-30% in the CWF and ECWF. After treating ~32-42 L of water containing 500 NTU of kaolin, flow rates dropped by 16-34% in the CWF and 11-24% in the ECWF.

It was also found that the turbidity of water sampled from within the CPF for CWF operation or top receptacle for ECWF operation increased as more and more batches were loaded (data not shown). This was expected because the kaolin was physically stopped by the ceramic and left inside the filter so when more water was poured in, the kaolin from past batches was re-suspended and mixed with the new batch causing a rise in turbidity.

Chapter 6 Removal of E. coli by ceramic pot filters: CWF vs. ECWF

One of the main objectives of the experiments was to determine the bacterial disinfection capabilities of the CPFs under enhanced flow rates in order to determine whether disinfection was comparable to CWF operation. It was important to see if the faster flow rates had an effect on the disinfection ability; some filtration mechanisms and chemical disinfection are impacted by contact time issues. Numerous CWF tests have been previously conducted, but it was important to test identical filters under both ECWF and CWF to enable direct comparison. To accomplish this, *E. coli*-spiked water was loaded into four CPFs operated under CWF and ECWF modes, and effluent concentrations were measured. Multiple tests were performed over the 2010 summer which are referred to in this chapter as "pre-tests". These test results are not reported but the lessons learned and resulting method alterations are discussed in section 6.1.

Another important objective was to quantify the importance of colloidal silver to the CPF disinfection properties under enhanced flow rates. Disinfection can occur by either the physical removal of the bacteria by the ceramic or chemical inactivation of the bacteria by the colloidal silver. This chapter examines first the ceramic's ability to remove bacteria strictly through physical pore-size limitations and surface interactions. The filters were then recoated with colloidal silver and tested again under similar conditions to determine any added benefits to disinfection due to the silver.

6.1 *E. coli* pre-tests for CWF

E. coli loading tests were complicated as bacteria are living microorganisms and more difficult to control than turbidity. It was easy to aim for the inlet water to be a certain concentration but bacteria grow or die with variations in substrate (i.e. TSB), oxygen, etc. Experiments that resulted in non-ideal results were considered to be pre-tests. The lessons

learned from these pre-tests helped to ensure better results in the future experiments and are described briefly below.

6.1.1 Lids on, taps open

The first CWF pre-test in lab resulted in extremely highly contaminated effluents almost immediately. Also inlet concentrations were found to be contaminated after sitting on the bench for a few hours. This was due to the fact that the CPFs were open to the air and the bottom receptacle taps were only open when samples were collected. This allowed contamination from the air to effect the inlet water conditions and allowed for a large volume of water to be stagnant in the bottom receptacle. From this test, it was concluded that the plastic bucket lids should be placed over the CPF or top bucket at all times to limit contamination from the air and that the taps should be left open at all times to limit dead space and associated bacterial growth in the bottom receptacle.

6.1.2 De-chlorinated tap solution and TSB solution

Originally a concentrated de-chlorinated tap solution was made by putting 2 grams of sodium sulfite into 0.5 liters of tap water to de-chlorinate the tap water for the inlets for the entirety of each experiment. The concentrated solution was spiked into the inlet water at a volume of 1 mL for every 1 L of inlet water needed. Also, a TSB broth was made at a high concentration and used to spike in 50 mg/L into the inlet. During one experiment the inlets were extremely contaminated with something other than *E. coli* by the end of the second day and it was confirmed by plating samples that the sodium sulfite solution and the TSB broth were both contaminated.

From this point on, a new de-chlorination solution at a concentration of 4 g/L was made new every morning with sterile water and kept in the refrigerator to minimize bacterial growth

and was only used for one day. TSB was directly spiked in a powder form at 50 mg/L into inlet water instead of making a liquid stock that could easily get contaminated and support exponential bacterial growth.

6.1.3 Incubator Temperature Setting

Following the ECWF *E. coli* test the filters were tested under CWF conditions, and towards the end of the experiment it was noticed that the temperature of the incubator was at 42ºC instead of 35ºC. This elevated temperature appeared to inhibit the growth of non-*E. coli* bacteria, but the results were therefore inconsistent with normal test conditions. From this point on, the incubator temperature was always doubled checked when plates were incubated to ensure that was not another variable in the experiments.

6.2 Disinfection by CPFs without Silver: Comparison of CWF and ECWF

The third research objective was to compare the removal of *E. coli* without the disinfecting properties of colloidal silver for the CWF and ECWF for inlet concentrations ~10^5 CFU/mL. Prior to the start of the disinfection experiments, the CPFs and receptacles were treated with a 100x dilution of household bleach (~ 623 mg/L NaOCl). This likely removed silver from the ceramic surface (Stewart 2010). Therefore, these results assume that there was no removal of bacteria due to disinfection by silver and that all removal was strictly due to physical filtration mechanisms.

Directly before the start of each experiment, the effluents of the de-chlorinated tap "saturation phase" were plated to ensure that the filters were clean before the *E. coli* loading began. Most of the time these plate counts were zero, as expected. However, an agar plate with no visible colonies does not necessarily mean zero bacteria in the sample it just means that there was less than 1 colony per 0.049 mL or 20 CFU/mL. For undiluted samples, the minimum

detection limit of the spiral plater is then ~20 CFU/mL and the maximum counted in these experiments was ~100,000 CFU/mL.

6.2.1 Plate Counting Challenges and Contamination Potential

The bleach treatment prior to the experiments was expected to remove any bacteria from the system prior to the start of the experiments. An effluent sample of the saturation water was always taken prior to the start of *E. coli* loading; these samples were almost always zero colonies on the plates (therefore, less than 20 CFU/mL). Colonies that looked like *E. coli* were present in the effluent samples after only an hour of loading for every experiment. This shows that without silver ceramic pot filters are not efficient at removing *E. coli* strictly by physical filtration. Many of the effluent samples contained multiple bacterial colony morphologies (Figure 6-2 and 6-3), while the spiked inlet water only showed *E. coli*-like colonies (Figure 6-1). Because strict *E. coli* colonies could not be determined solely based on visual characteristics on the plates, reported plate counts include all countable colonies present on the plate as total colony forming units.

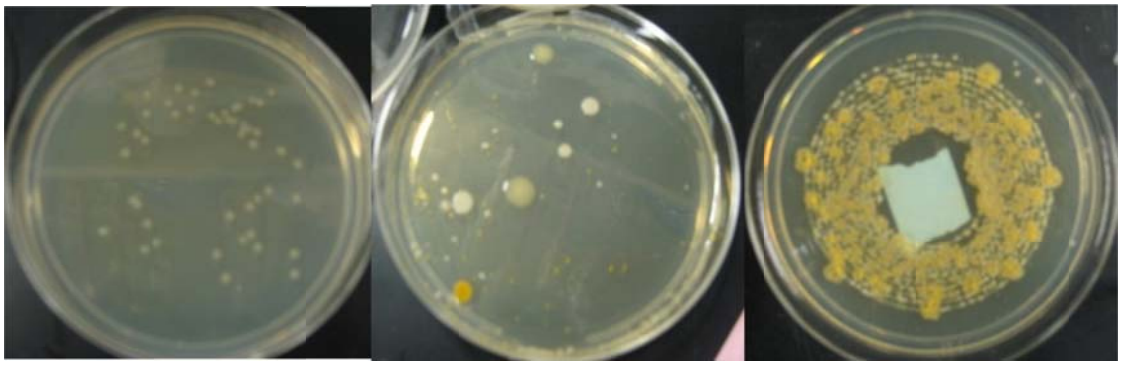

Figure 6-1 (left) *E. coli* inlet colonies
Figure 6-2 (middle) Effluent morphology colonies
Figure 6-3 (right) TNTC and apparent contamination

In addition, it was common that the effluent contained an extremely high number of very small colonies that were too numerous to count (TNTC); with the spiral plater that represents over ~100,000 CFU/mL (see Figure 6-4 and 6-5). It is possible that the bleach cleaning was insufficient to remove all of the bacteria that may have been present in the pores of the CPF. The

preceding pre-tests with TSB may have resulted in biofilms inside the filter, due to a combination of substrate, *E. coli* and other bacteria in the tap water. The incident of higher bacterial concentrations in treated effluent water compared to the source water has been reported in previous research in the field and laboratory work by Brown (2007), Lantagne (2001a) and Kowalski (2008).

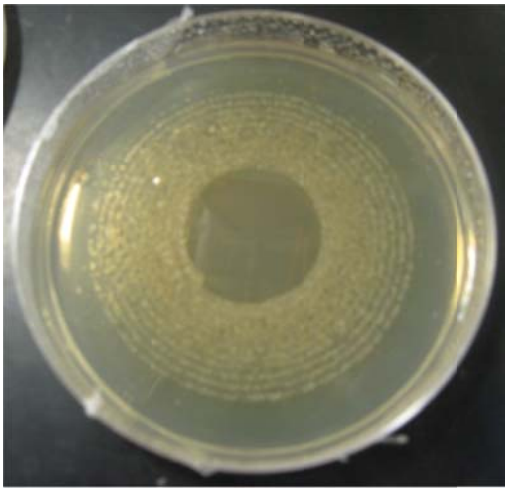

Figure 6-4 Example of plate TNTC (too numerous to count)

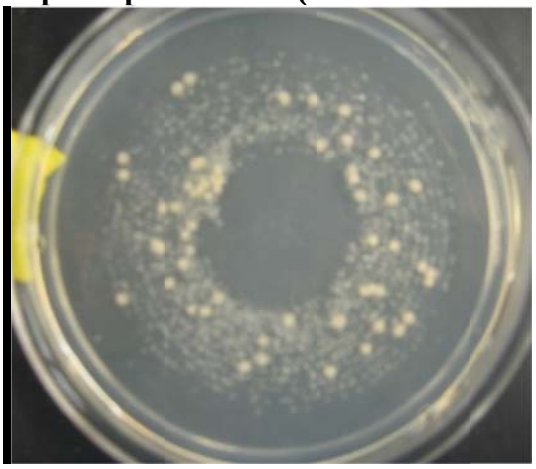

Figure 6-5 Example of plate with *E. coli* on a lawn of small bacteria (too numerous to count)

The effluent results (discussed in detail below) showed growth of bacteria at some point between when the *E. coli*-spiked tap water is put into the CWF or ECWF and when the effluent samples were taken out of the tap in the bottom receptacle. Along with the *E. coli* spike the water contained 50 mg/L TSB (equivalent to ~15-20 mg/L TOC). This carbon source may

stimulate the growth of bacteria within persistent biofilms within the pores of the ceramic. Any undetected bacteria that were present before the start of the loading would likely achieve exponential growth rate once the experiment started and the filters were loaded with high levels of bacteria and a food source of TSB. This could explain much of the bacterial contamination observed in the effluent.

6.2.2 CWF Disinfection

After the initial pre-tests and the non-silver ECWF tests (results reported in Section 6.2.3), the Nicaragua filters (18193 and 11136) and RDI filters (1 and 2) were loaded under standard flow rates with inlet *E. coli* concentrations of ~10^5 CFU/mL. The Nicaragua filters were loaded simultaneously under CWF mode with *E. coli* spiked water (~7.15E4 ± 1.05E4 CFU/mL) that also contained ~50 mg/L TSB (~ 15-20 mg/L TOC). The following week the same experiment was run on the RDI filters with *E. coli* spiked water (~$7.51 \times 10^4 \pm 1.03 \times 10^4$ CFU/mL). The RDI filters were only loaded for 3 days, so they lack data for later times and cumulative volumes, but they already showed higher effluent bacteria counts than the inlet *E. coli* by 3 days. The Nicaragua filters and RDI filters were loaded separately due to the overwhelming time commitment of each experiment. Testing two filters was found to be the limit for one person to achieve all the sampling times. The inlet concentrations were close for both experiments with an average of ~$7.34 \times 10^4 \pm 1.03 \times 10^4$ CFU/mL so the effluents for all the filters are compared on the same graph with average inlet concentrations.

The inlet and effluent bacterial counts versus time and cumulative treated water volume are shown in Figures 6-6 and 6-7, respectively. Initially, good removal of *E. coli* was achieved by the Nicaragua filters, with significantly higher effluent bacteria from the RDI filters. However, over the subsequent 24 hours to ~25 L the effluents became more contaminated than

the inlet water. The open symbols on the graphs represent that the effluent was TNTC and visually confirmed at least as bad as the influent and usually much worse but almost never with colony morphologies that only resembled *E. coli* (Figure 6-8). The effluent concentrations are very similar when compared versus volume filtered and time passed. The trend seems to be that after about ~24 hours or ~25 L of treated water the inlet concentration was assumed to break through completely. This was apparent when the inlet and effluents were plated and it could be confirmed visually that the effluents were worse, even at different dilutions.

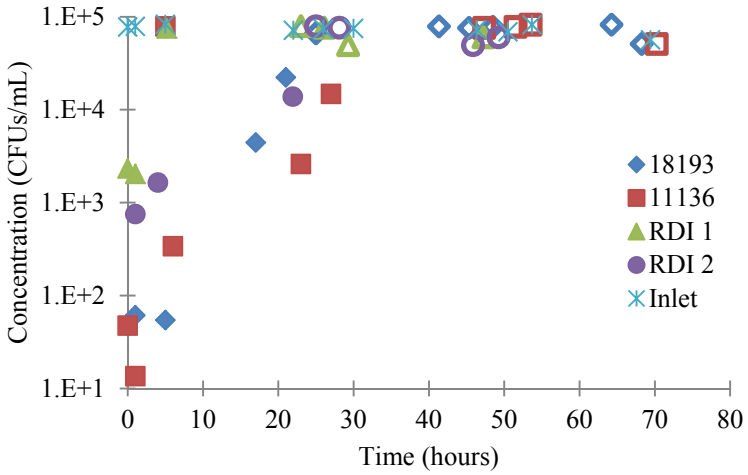

Figure 6-6 Effluent concentration vs. time for CWF *E. coli* experiments

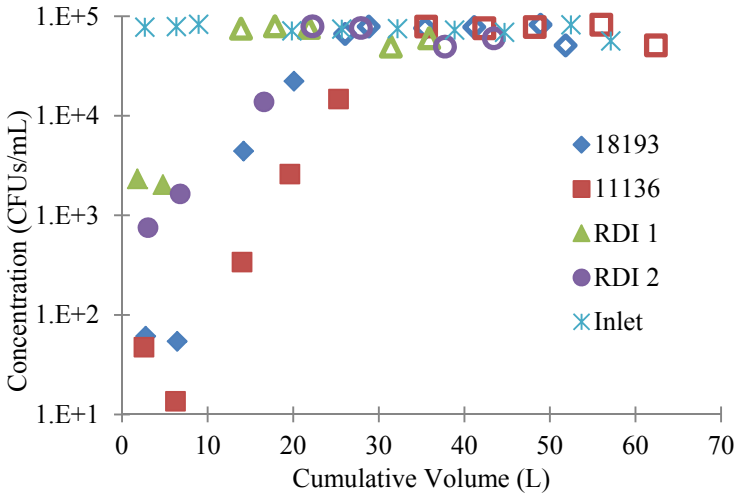

Figure 6-7 Effluent concentration vs. cumulative volume for CWF *E. coli* experiments

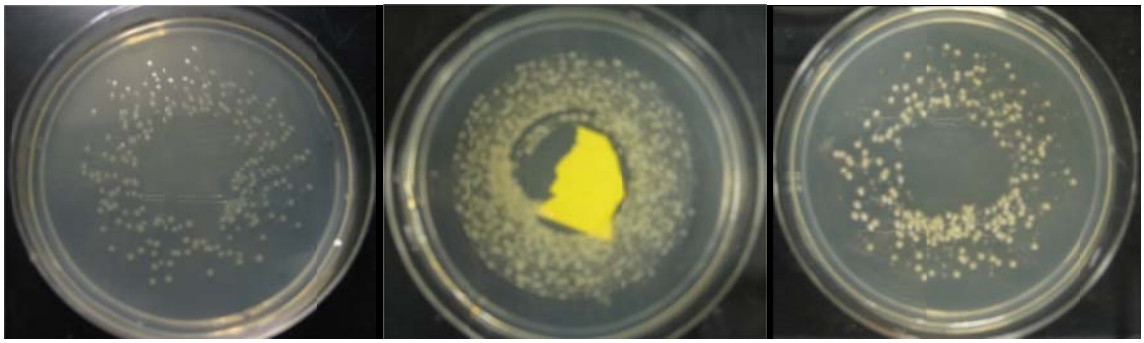

Figure 6-8 From left to right: Inlet (10x dilution), Effluent (no dilution), Effluent (100x dilution)

Figure 6-9 below shows the log disinfection at the sample points compared to the amount of water filtered for the CWF. Under CWF conditions there was almost complete removal of *E. coli* during the first batch of treated water (3 log removal) but the log removal quickly diminished.

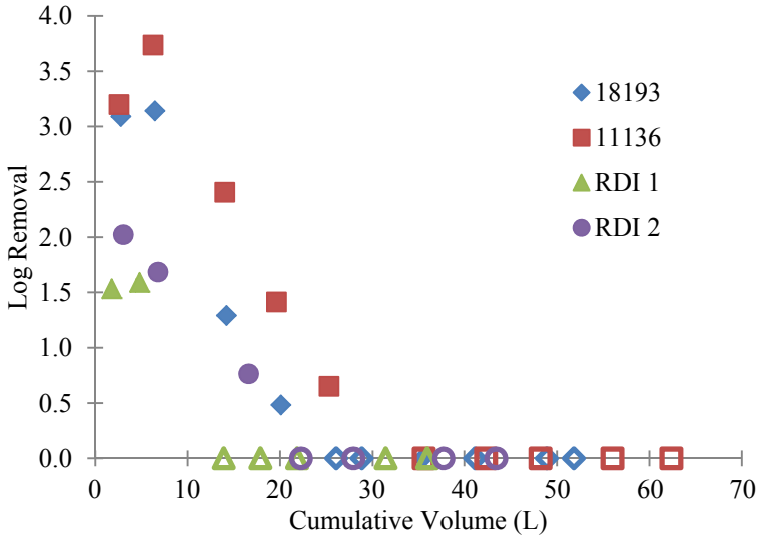

Figure 6-9 Log removal for CWF vs. cumulative volume (L)

The Nicaragua filters started off with over 3 log removal which decreased to zero log removal around 30 hours or 26 liters for 18193 and 46 hours or 42 liters for 11136. The RDI filters only had 1.5-2 log removal initially. This could be due to the fact that the initial effluents

of de-chlorinated tap at time zero already showed a few very small bacteria on the plates. Because the plates need to be incubated for 24 hours, day 2 of the experiment was already underway when it was found that the t=0 plates were not clean. In this case the filters were not initially clean with the confidence level that it could be assumed that the concentrations were <20 CFU/mL. From this point, the effluent concentrations increased drastically and RDI 1 had zero log removal after only 2 batches, ~20 hours, 14 liters. The RDI 2 bacteria effluent counts reached zero log removal after 3 batches, ~25 hours, and 23 liters. It is unknown why the RDI filters behaved so much worse under *E. coli* testing.

6.2.3 ECWF Disinfection

The ECWF systems were tested for removal of *E. coli* to compare to the CWF system. The inlet *E. coli* concentrations in these experiments were $1.47 \times 10^5 \pm 1.67 \times 10^5$ CFU/mL. The four ECWF filters were all loaded at the same times for the two consecutive days. At time zero, all the plate counts were zero from the saturation phase with de-chlorinated tap water except for RDI 1 which showed 1 colony on one of the three plates. In the ECWF systems, $\sim 10^3$ CFU/mL were measured in the effluent within the first hour (only 1 to 2 log removal), and the colonies were too numerous to count on the plate after ~ 20 hours or 60 L of treated water. This indicates that there was bacterial growth either in the filters or in the bottom receptacle for both the CWF and ECWF. Again, the open symbols on the graph signify that the effluents concentrations were assumed to be equal to or greater than the inlet concentrations. This occurred at the same sample time for all the ECWF systems (Figure 6-10). All four filters were declared contaminated with effluents TNTC after the loading of batch 5 around ~26 hours and cumulative volumes between 54 L and 75 L (Figure 6-11). Figure 6-12 and 6-13 show the log removal vs. time and cumulative volume for ECWF.

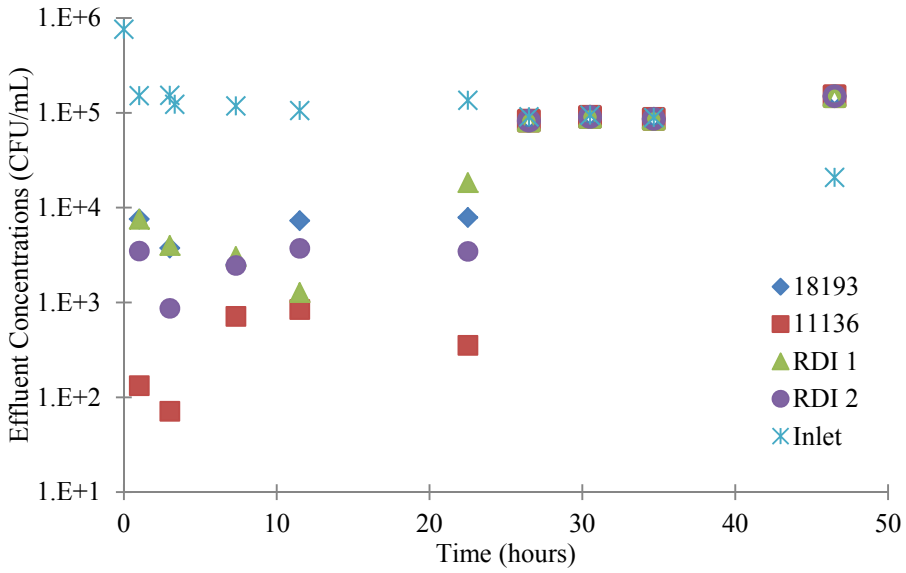

Figure 6-10 ECWF: Effluent bacteria concentrations vs. time

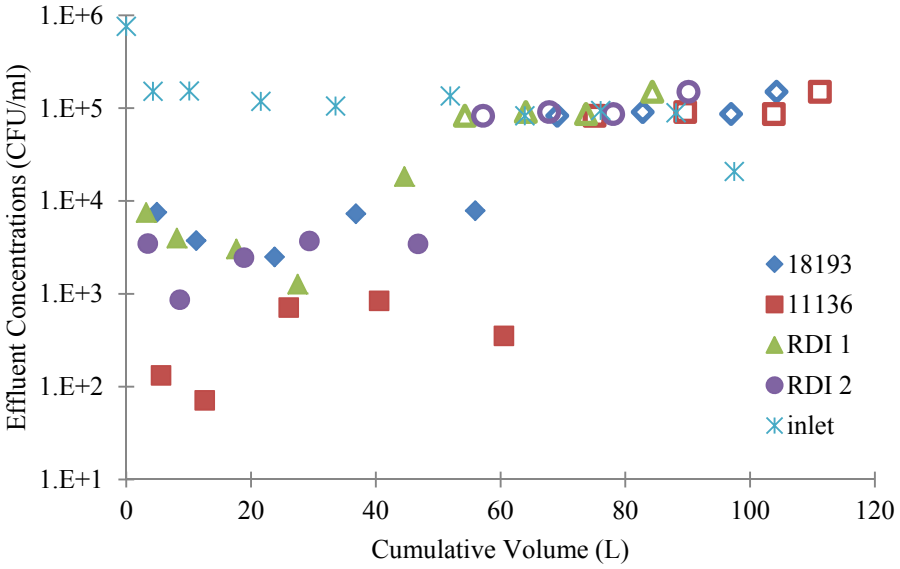

Figure 6-11 ECWF: Effluent bacteria concentrations vs. cumulative volume

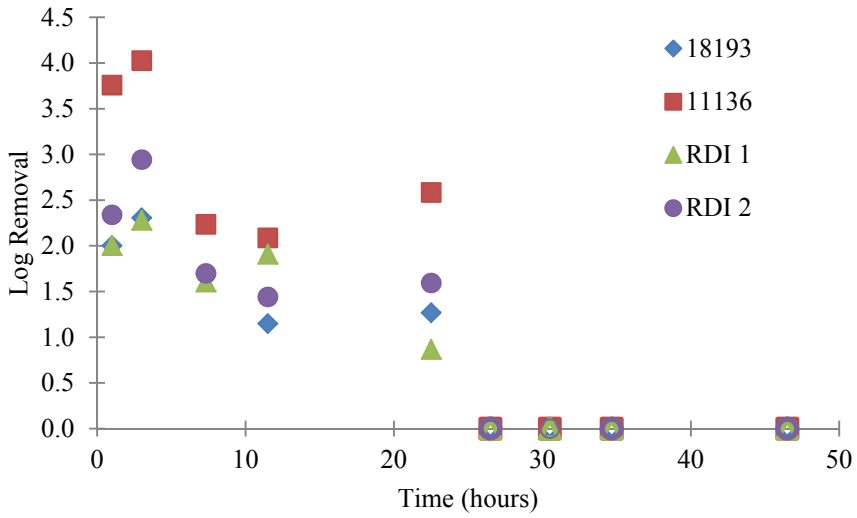

Figure 6-12 ECWF Log removal vs. time

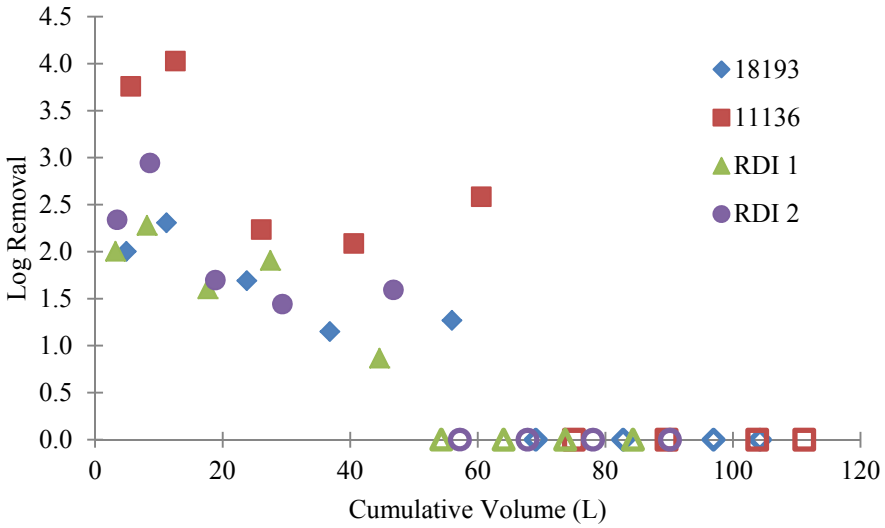

Figure 6-13 ECWF Log removal vs. cumulative volume

Filter 11136 started off around 4 log removal and was able to filter the highest volume of water before the log disinfection was reduced to zero. The other three filters had initially only around 2 log removal. It is important to note that the filters reached zero log removal at volumes ranging from 54 liters to 75 liters but this occurred all after the 5th batch of ~10^5 CFU/mL *E. coli* water was loaded, or ~26 hours.

Previous tests with six different Nicaragua CWFs without silver were conducted by Kowalski (2007) with *E. coli* spiked at ~ $1.78x10^6 \pm 2.58x10^6$ CFU/mL that contained residual TSB due to dilution of stock solution to spike concentration directly without centrifuging (however, TOC concentrations were not measured). In the previous tests, only three batches of ~10^6 CFU/mL *E. coli* were loaded into the CPFs over 2 or 3 days. Figure 6-14 shows Kowalski's data for log removal over the 3 batches of *E. coli* loading at concentrations near 10^6 CFU/mL; each bar for a "batch" and represents the average of sampling at 2 and 4 hr time points after loading the batch at one batch per day, so the overall time after start of loading represented by the 3 bars are: ~3 hours, ~27 hours, and ~32 hours. Figure 6-15 shows the log removal results for the CWF system per batch and the corresponding times. The CWFs were loaded with nine batches of *E. coli*-spiked water at concentrations near 10^5 CFU/mL. This bar chart shows the log removal after the first hour and after every batch loaded. The effluents were taken and plated after the first hour of every *E. coli* experiment. The first hour log removal is plotted because after an hour the first batch of *E. coli* spiked water will have forced the ~1 L of pore volume in the ceramic through and it was important to see if in that first hour there was already *E. coli* in the effluent. Only the non-zero log removals are reported. It can be assumed that for the batches above 4, the log removal was zero or less.

Two of the Nicaragua filters in Kowalski's study, "2 NS" and "1 Used" correspond to 18193 and 11136 in this research, respectively. Filter 18193 showed similar results to Kowalski's data with the first batch being the highest removal around ~4 log from her experiments and ~3 log and then decreasing to around ~1 log by the third batch. 11136 sustained around ~3 to 4 log removal for the first three batches in Kowalski's data and this research

showed a steady decrease in log removal from ~4 log for the first back to ~2.5 log for the second batch and ~1.5 log for the third batch.

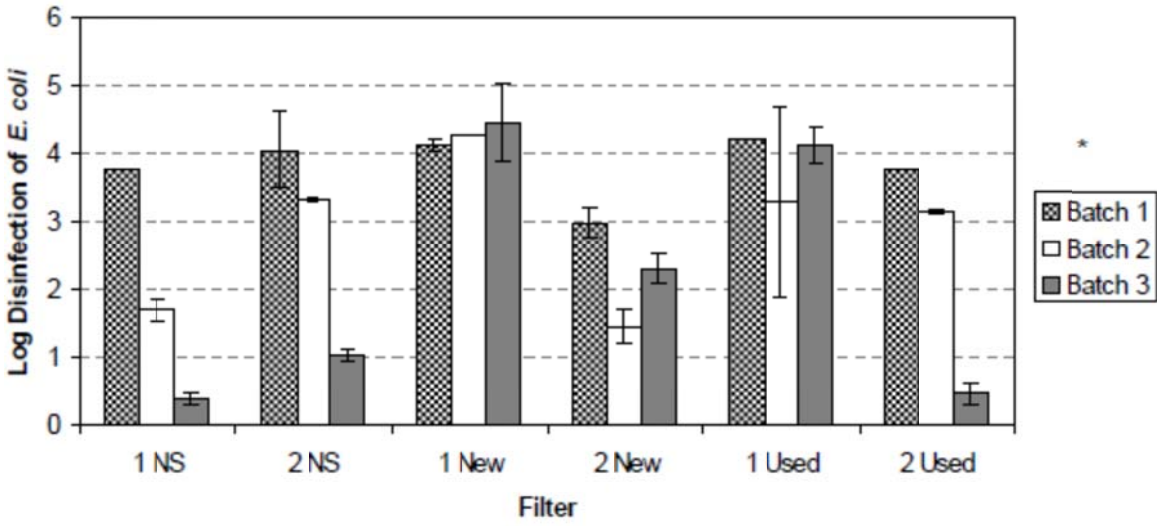

Figure 6-14 Log disinfection of *E. coli* for 3 batches (Kowalski, 2007)

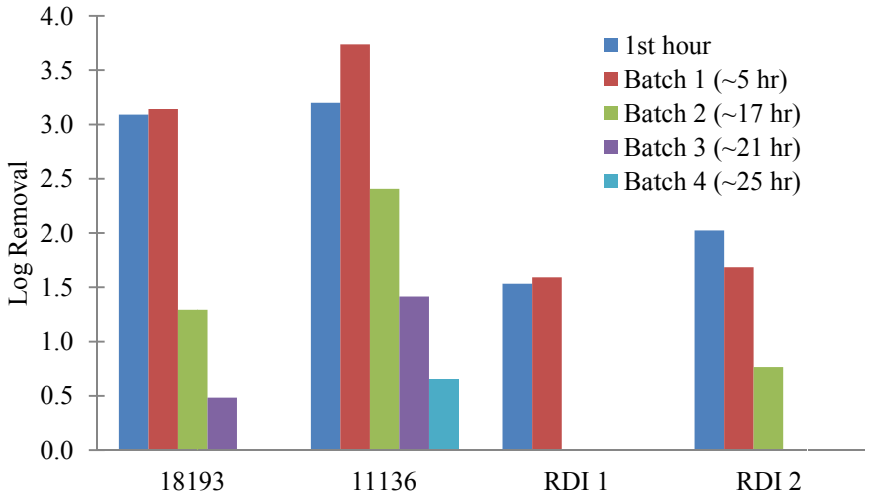

Figure 6-15 Log removal for first 4 batches CWF

The log removal for batches of the ECWF is reported in Figure 6-16. The ECWFs were loaded with 7 batches over two days. It is important to note that while the filters were loaded with a similar number of batches during the CWF (9 batched) and ECWF (7 batches) the enhanced test took place in half the time and accumulated about twice as much volume of

filtered water due to the much faster flow rates. The results showed that the CWF and ECWF systems became contaminated at similar times from the start of the experiments. The amount of cumulative volume filtered or number of batches of *E. coli*-spiked water did not seem to affect when the filter effluent samples became severely contaminated.

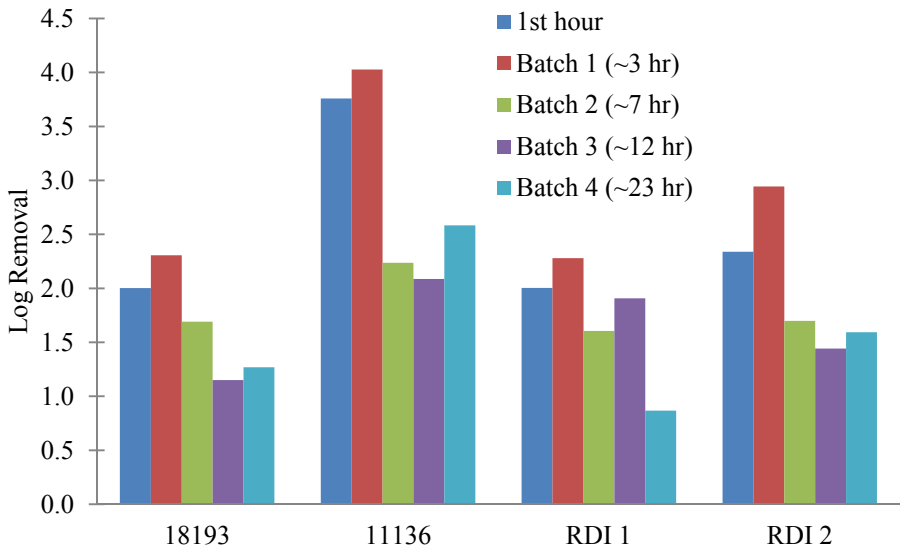

Figure 6-16 Log removal for first 4 batches ECWF

6.2.4 *E. coli* loading effect on flow rates

Under standard conditions of CWFs, the first hour flow rates did not show that the *E. coli* had a clogging effect of the CPFs over the four day test period (Figure 6-17). In fact, the results showed opposite the trend from what would be expected. The first hour flow rates actually increased over the four day loading; slightly for RDI 1 and most significantly for Nicaragua 11136.

The ECWF experiments showed that over two days of *E. coli* loading the first hour flow rates were generally stable for the first 40 hours of the experiment and then decreased from 3-5.5 L/hr down to 1.7- 4 L/hr. This shows that bacterial accumulation can also cause filters clogging at enhanced flow rates. It is still not clear why the CWF flow rates increased while the

flows for the ECWF decreased (Figure 6-18). First hour flow rates do not always consistently decrease and were found to be lower for the first batch of the day. This was presumably due to the ceramic drying out a bit as it was emptying overnight making the first new batch of the day the slowest because the ceramic needed to be re-saturated.

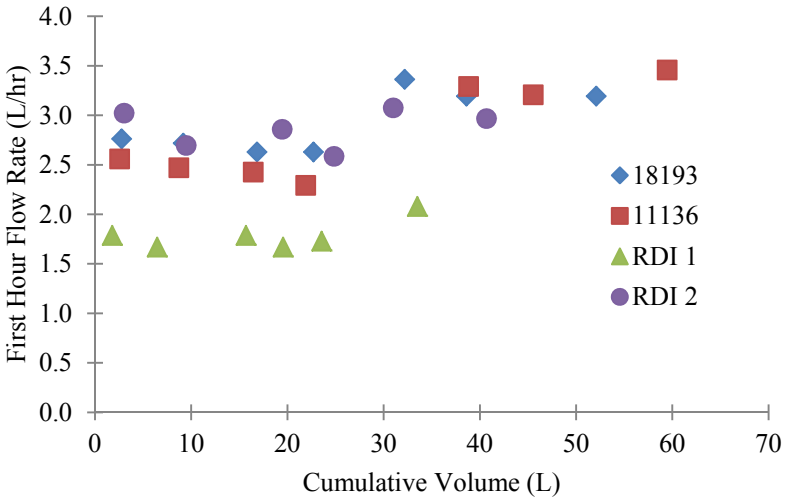

Figure 6-17 CWF: First hour flow rate vs. cumulative volume

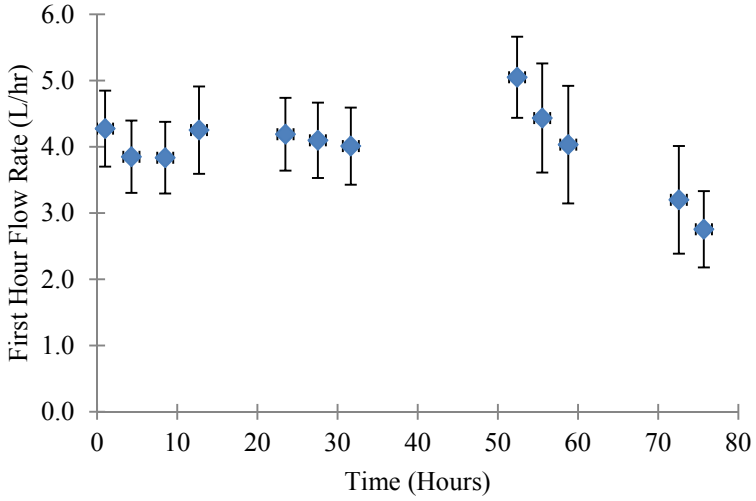

Figure 6-18 Average first hour flow rates for ECWF filters (error bars represent the standard deviation across all four filters)

6.2.4 Comparison of disinfection without silver: CWF vs. ECWF

The log disinfection for each of the four filters under CWF and ECWF operation are compared in Figures 6-19 through 6-26 for both time and cumulative volume filtered. Negative log removal was observed when there were more colonies in the effluent than in the inlet but is represented on these graphs as zero log because they could not be quantified due to plates that were TNTC. First, filter 18193 from Nicaragua is compared for its performance under the CWF system and the ECWF system. Then filter 11136 and RDI 1 and finally RDI 2. Graphs of effluent concentrations (CFU/ml) vs. time and cumulative volume filtered for each filter, comparing CWF and ECWF can be found in Appendix A.4.

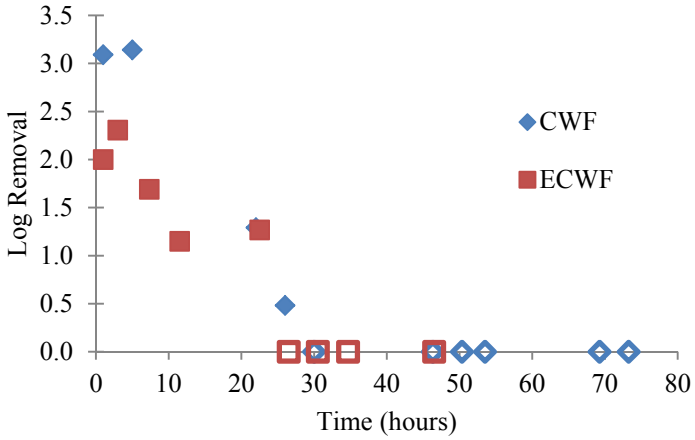

Figure 6-19 Log removal vs. time for filter 18193

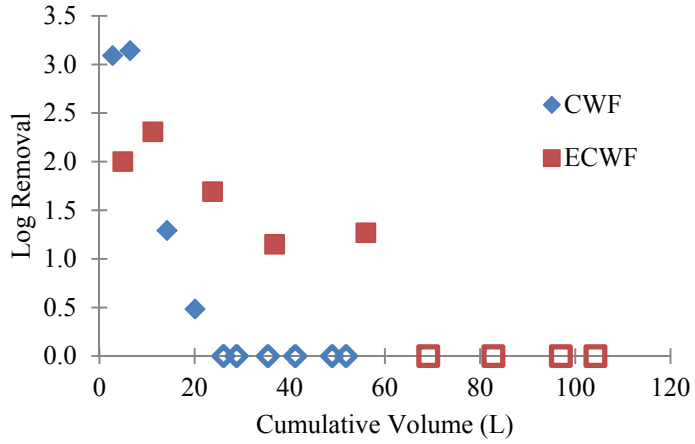

Figure 6-20 Log removal vs. cumulative volume for filter 18193

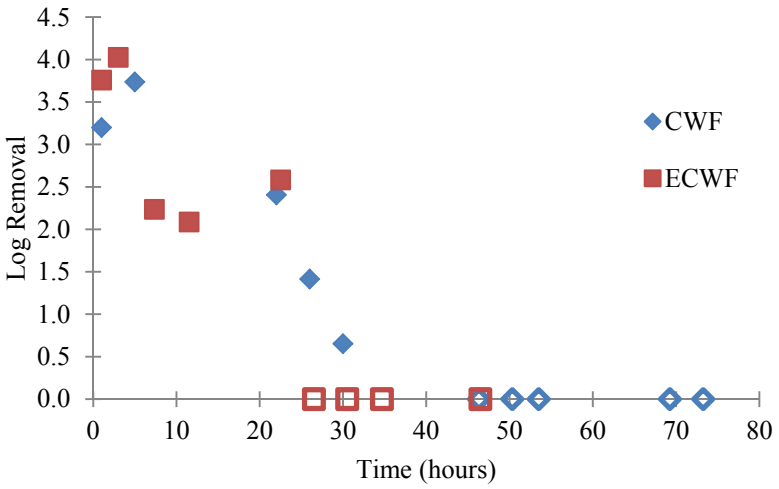

Figure 6-21 Log removal vs. time for filter 11136

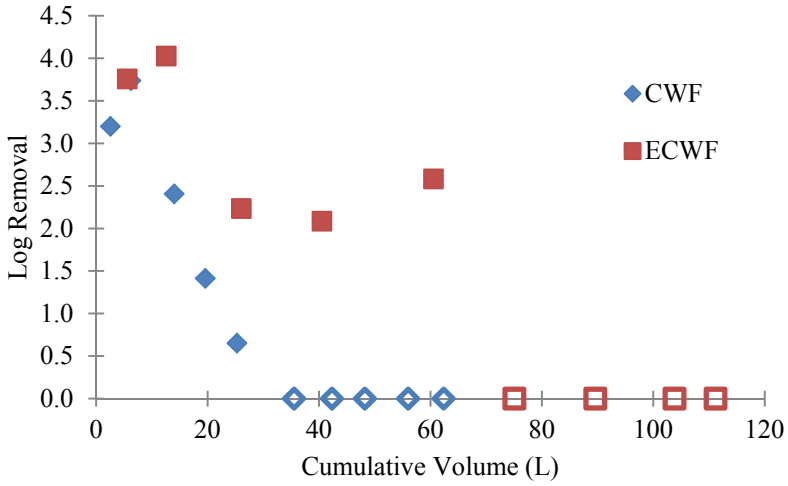

Figure 6-22 Log removal vs. cumulative volume for filter 11136

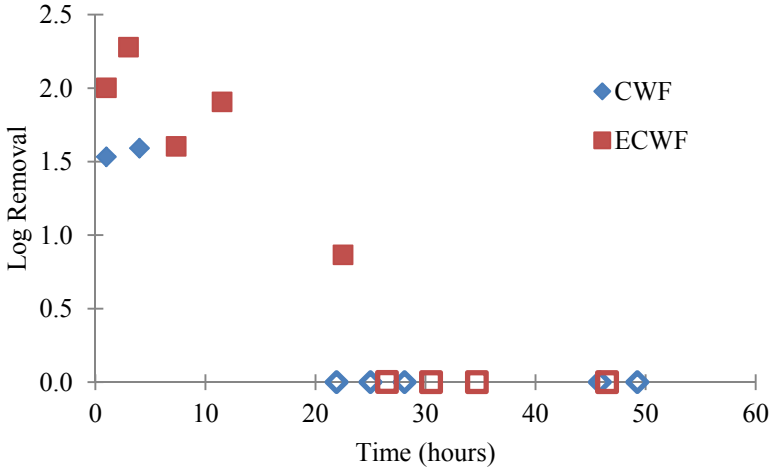

Figure 6-23 Log removal vs. time for filter RDI 1

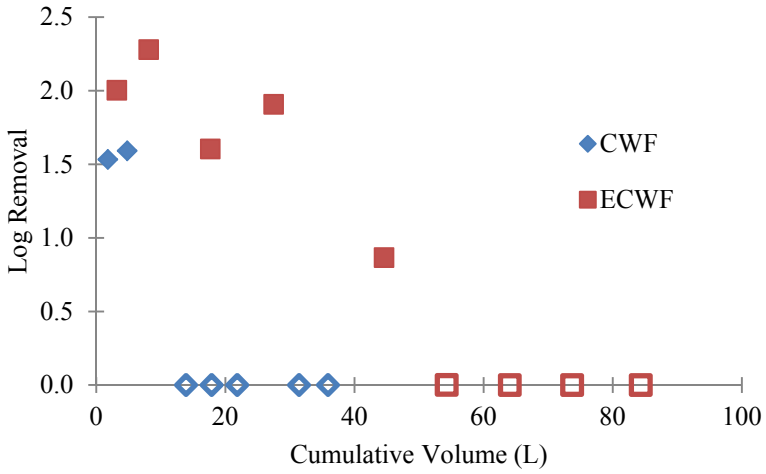

Figure 6-24 Log removal vs. cumulative volume for filter RDI 1

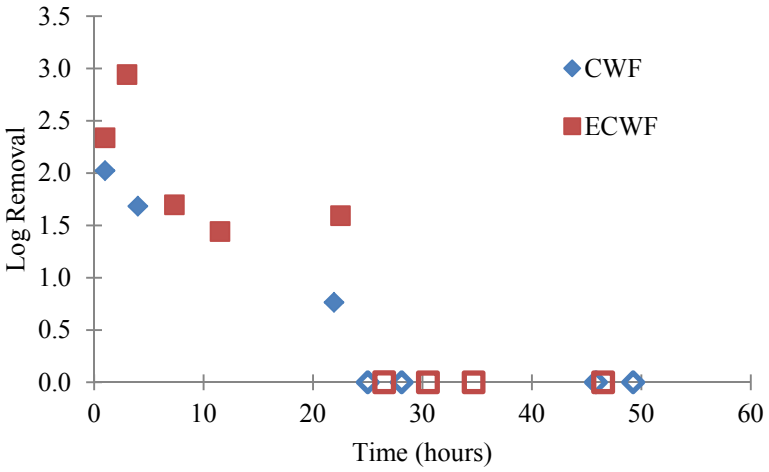

Figure 6-25 Log removal vs. time for filter RDI 2

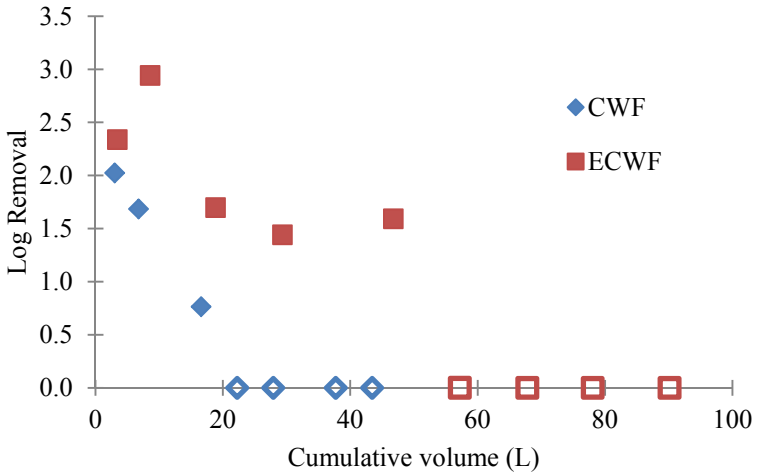

Figure 6-26 Log removal vs. cumulative volume for filter RDI 2

The log removal data versus time were very similar for ECWF and CWF. On a volume treated basis, the ECWF system was better because it was able to filter more water before becoming contaminated. This unusual result is likely due to the time required for bacteria growth, presumably due to the presence of the TSB. It is apparent that neither system was effective at removing bacteria under the loading conditions.

In all *E. coli* testing, the filters became contaminated with bacteria other than *E. coli* after the first 24 hours. Performance was therefore compared for the first 24 hours under standard and enhanced flow rates to compare the removal efficiency of *E. coli* before contamination. Figures 6-27 through 6-30 compare the log removal for CWF and ECWF in the first 24 hours. These graphs show that there may not be a huge difference in *E. coli* disinfection during the first 24 hours of the experiments. Filter 18193 had less log removal under CWF conditions than ECWF and 11136 had similar results under both flow conditions. The RDI filters were less effective under standard condition due to their initial contamination. Figure 6-31 compares the average log removal for the first 24 hours for each filter under CWF and ECWF systems. This graphs shows that there may not be a significant difference in the enhanced versus the standard flow rates for *E. coli* removal by CPFs without silver.

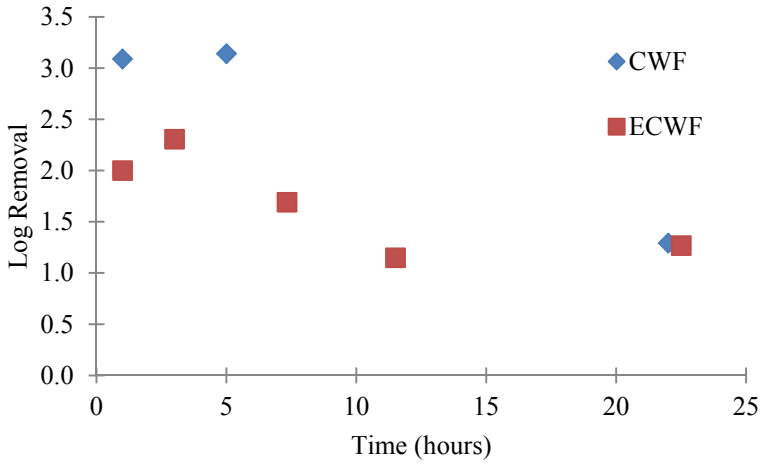

Figure 6-27: Log removal vs. time for filter18193 for the first 24 hours

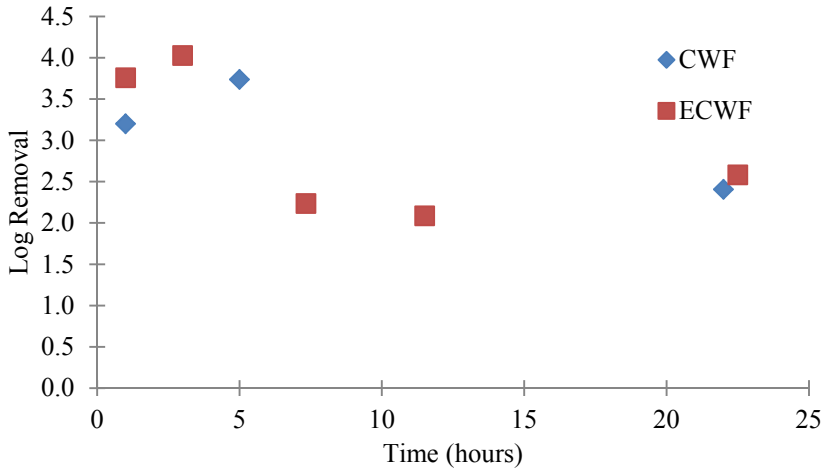

Figure 6-28: Log removal vs. time for filter 11136 for the first 24 hours

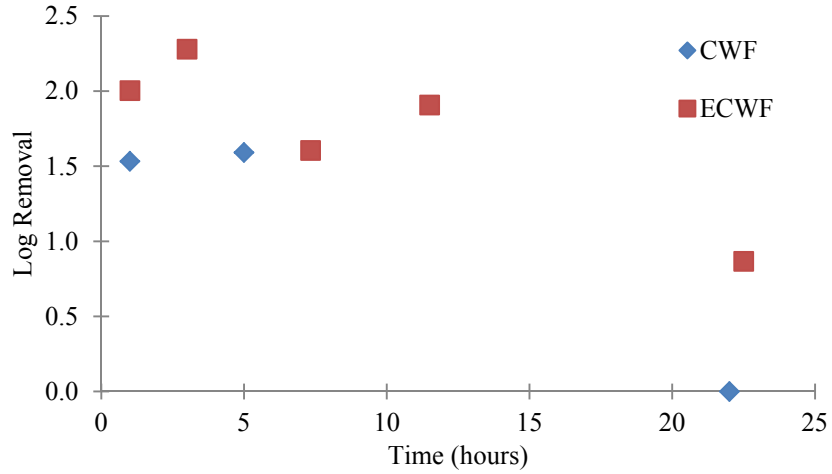

Figure 6-29: Log removal vs. time for filter RDI 1 for the first 24 hours

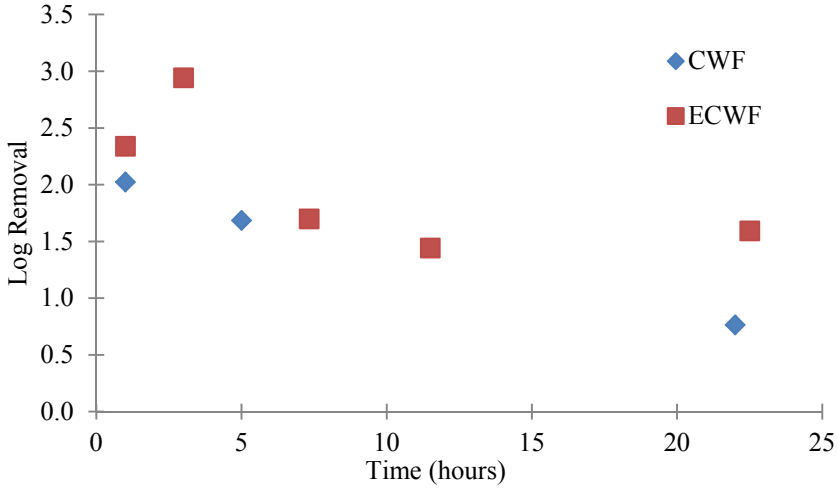

Figure 6-30: Log removal vs. time for filter RDI 1 for the first 24 hours

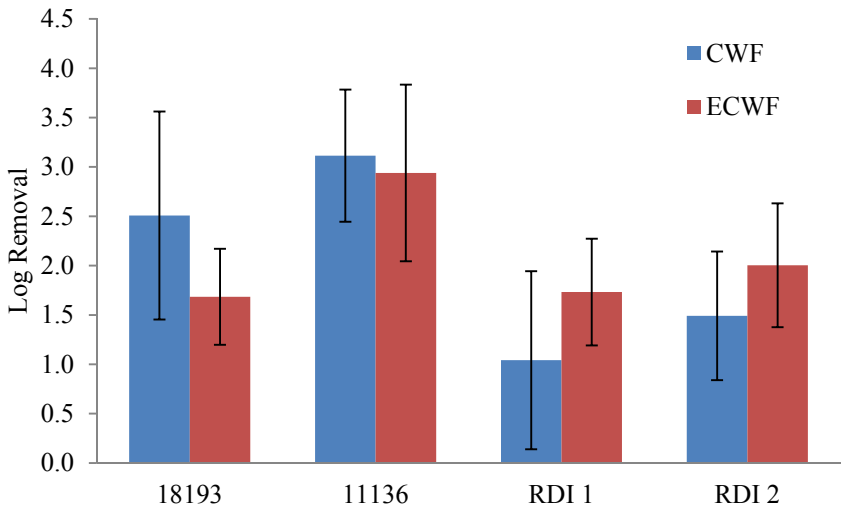

Figure 6-31: Log removal for first 24 hours for CWF and ECWF

Without silver the CWFs were not efficient at removing large concentrations of *E. coli*. ECWFs allowed the *E. coli* to breakthrough quicker than at standard flow rates, which was expected. Overall, strict *E. coli* removal was inconclusive because effluent sample plates had evidence of anywhere from 1 to 10 different kinds of bacteria. When comparing the CWF and ECWF without silver for all the filters it seems as though the time elapsed since the start of *E. coli* plus TSB loading was the most controlling factor of when the filters reached zero log removal. The effluents all seemed to become severely contaminated around the same time, regardless of the volume of *E. coli* inlet water that was treated by the filter. It was expected that the point where the bacteria broke through completely would be at a similar volume of water loaded in the filters, therefore the ECWF effluent was expected to become contaminated much faster because of the faster flow rates. This was not the case. The ECWF systems treated almost 3 times the volume that the CWF treated before the log removal was assumed to be zero or less than zero.

6.3 Control Tests

Control tests were run because the initial *E. coli* tests resulted in effluent plates with colonies other than *E. coli*, and sometimes the plates were covered with very small colonies. In order to better interpret the results from the *E. coli* tests it was essential to perform control tests to see if the bacteria growth that was witnessed could be explained by microbial activity in the filters. Control tests were done to address the microbial growth potential in filters. These experiments used Boulder tap water (TOC ~1-4 mg/L, Towler et al. 2007) de-chlorinated with sodium sulfite and an added carbon source of 50 mg/L of TSB. This inlet water had zero colonies on the plates throughout the entirety of the experiments (minimum detection limit of <20 CFU/mL). The objective was to determine if bacteria would grow in the ceramic or bottom receptacle when the inlet water contained zero detectable bacteria. If the effluent plates for the control tests showed some similar colonies to those with the *E. coli* spike, then it would be known that some other microbial activity was happening in the ceramic with the support of the TSB carbon source. The sample points were meant to mimic those from the summer *E. coli* experiments described in section 6.2 so that they could be compared.

The RDI filters were taken out of the experiments at this point because the flow rates differed by 300%. The first hour flow for RDI 1 had been reduced to less than 1 liter per hour and the RDI 2 filter had a first hour flow rate near 3 liters per hour. The Nicaragua filters had similar flow rates and performances at this point and were assumed to be a good representative for the control testing.

Before the CWF control test and before the ECWF control experiments the filters were soaked in a 10x solution of household bleach for >36 hours in an attempt to rid the ceramic pores of any residual bacteria from the previous experiments. This was a more extensive bleaching

process than performed prior to the previous experiments because of the high contamination measured in the effluent samples. Two days of rinsing with de-chlorinated tap followed the 24 hour bleach soak to make sure that all of the bleach was rinsed out of the filters before starting the experiment. The filters can be assumed to contain no silver at this point.

6.3.1 Control test results for CWF

The effluent bacteria counts during the CWF control tests are shown in Figures 6-32 and 6-33 for filter 18193 and Figures 6-34 and 6-35 for filter 11136 as a function of time since the start of the carbon loading and cumulative volume filtered. The TOC data is also shown in these figures as the decrease from the inlet water to the effluent samples. TOC was only measured on samples from day three and four of the CWF experiment starting with batch 5 of the entire experiment.

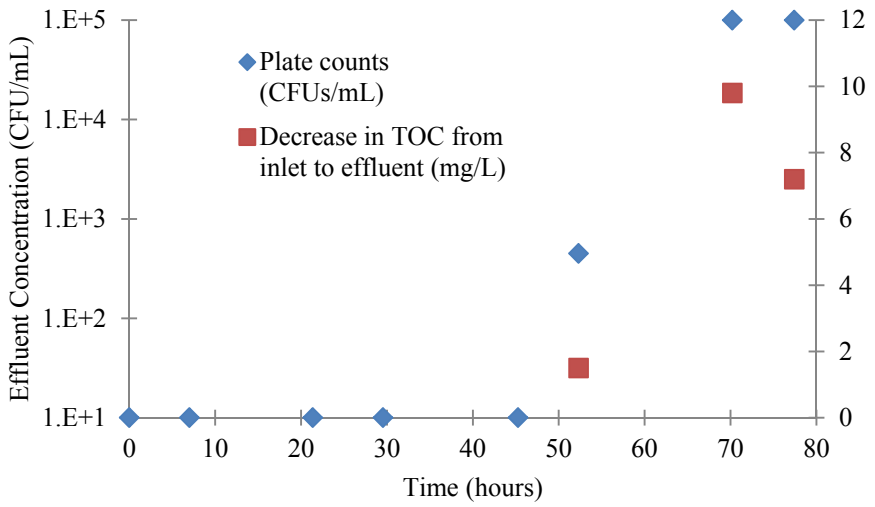

Figure 6-32 CWF 18193 Effluent concentrations and decrease in TOC vs. time

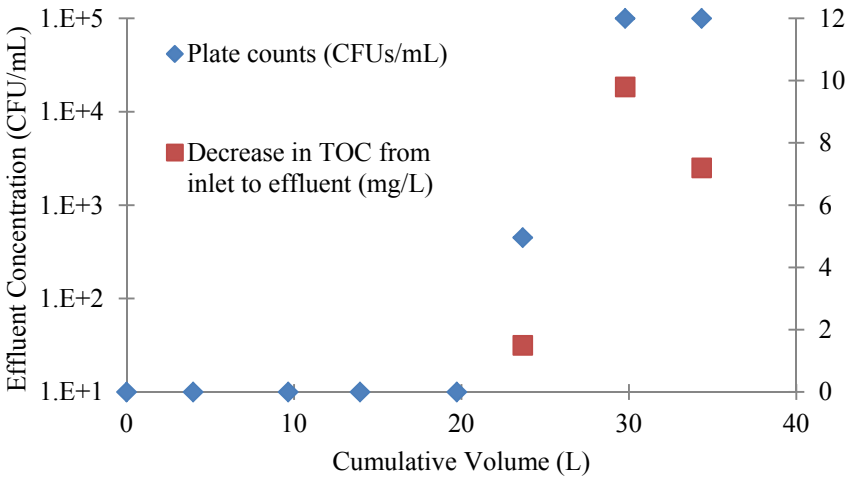

Figure 6-33 CWF 18193 Effluent concentrations and decrease in TOC vs. volume

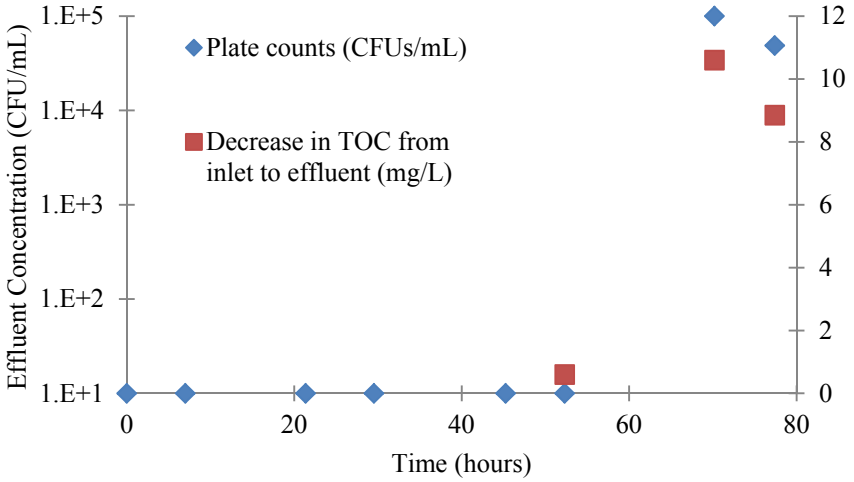

Figure 6-34 CWF 11136 Effluent concentrations and decrease in TOC vs. time

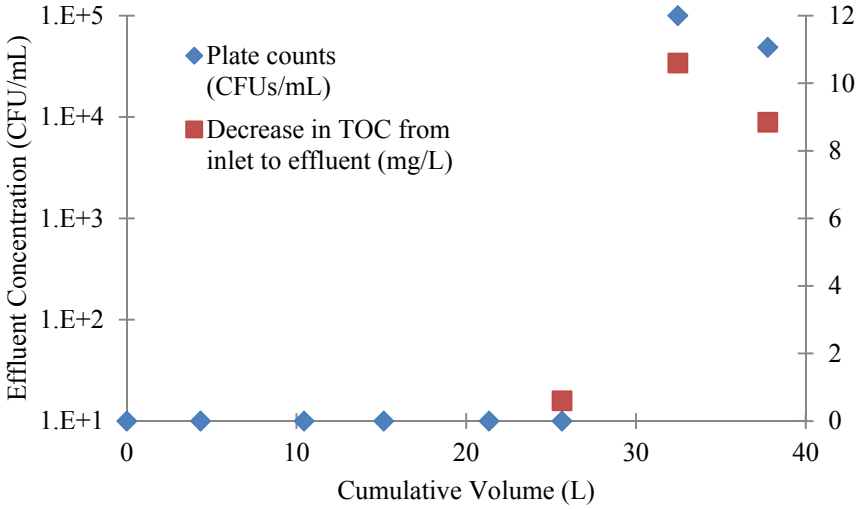

Figure 6-35 CWF 11136 Effluent concentrations and decrease in TOC vs. volume

The control experiments for CWF proved that there was bacterial activity occurring within the filter and/or in the bottom receptacle when there was a carbon source in the water (TSB). Bacteria appeared in the effluents after ~25 L or ~48 hours for both CWF filters. Assuming that TOC sorption to the ceramic was minimal, the TOC removal indicates microbial activity and respiration. These results show a direct correlation between high effluent plate counts and a large decrease in TOC.

The inlet TOC values ranged from 16 ppm to 20 ppm and the effluent TOC values decreased as the effluent bacteria concentrations increased. The tables below show the TOC data for the CWF control tests. Inlet water was also put in a container on the bench to represent the water within the filter at any time. To calculate the decrease in TOC, the effluent sample TOC was subtracted from the bench sample TOC at the corresponding time. The bench samples would vary slightly from the inlet samples over time but were representative of the inlet water. Previous research at CU with filter cores found similar results that when bacteria was present the TOC values would drop from the inlet to the effluent samples (Kohler 2009). Kohler's results showed that the filters can cause contamination of clean water after highly contaminated waters have been treated. These control tests showed that even with bleach cleaning between experiments, TSB in de-chlorinated tap water could cause bacteria to grow in the ceramic.

Table 6-1 CWF TOC data

Sample Time (hours)	Inlet TOC (mg/L)	Effluent TOC (mg/L)	Decrease in TOC from inlet to effluent (mg/L)
Batch 5	16.9		
18193 t=7		15.4	1.5
11136 t=7		16.3	0.6
Batch 6	15.9		
Morning Bench	20.8		
18193 morning		11	9.8
11136 morning		10.2	10.6
Batch 7	17.3		
18193 t=7		10.1	7.2
11136 t=7		8.45	8.85

6.3.2 Control test results for ECWF

Prior to these experiments, the filters were again disinfected with at least two batches of 100x household bleach water and rinse with de-chlorinated tap water. At the beginning of the ECWF control experiment, 18193 obtained a large crack down the inside of the filter while the top bucket was being sealed onto the filter and gasket. This filter was removed from the experiment and the ECWF test was continued with 11136 only. The effluent bacteria counts during the ECWF control tests are shown in Figures 6-36 and 6-37 for filter 11136 as a function of time since the start of the carbon loading and cumulative volume filtered.

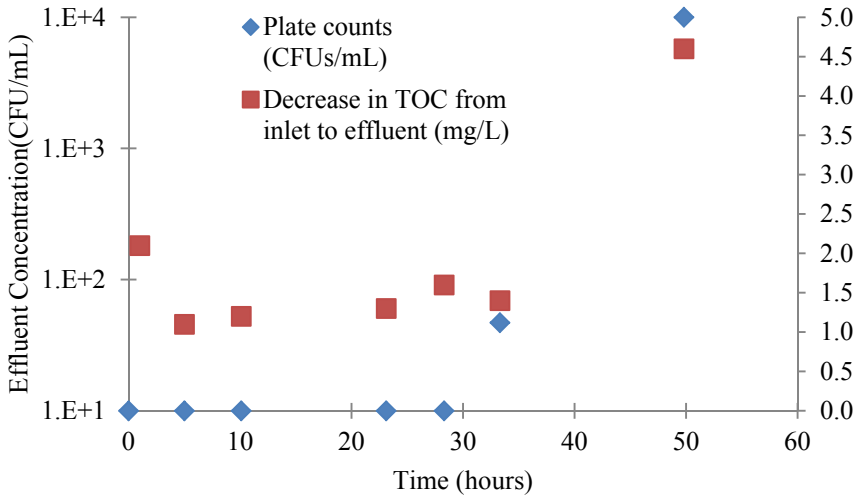

Figure 6-36 ECWF 11136 Effluent concentrations and decrease in TOC vs. time

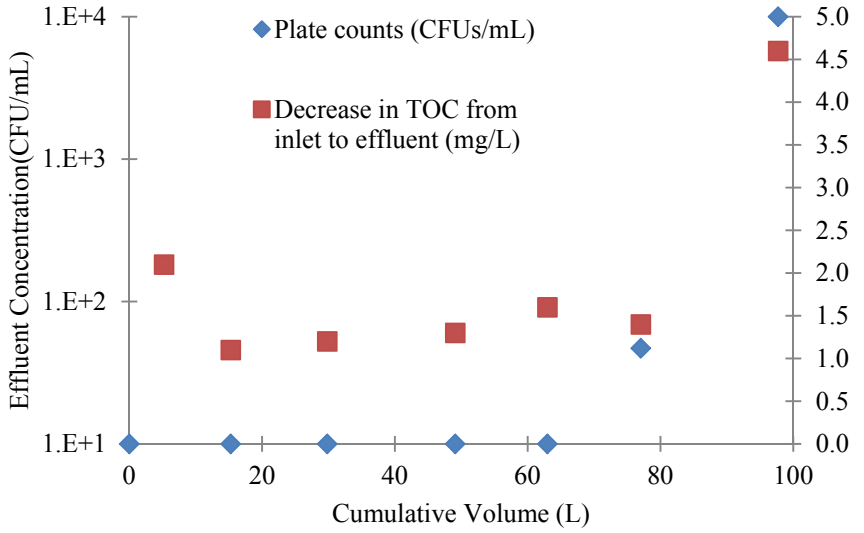

Figure 6-37 ECWF 11136 Effluent concentrations and decrease in TOC vs. volume

For the ECWF system, TOC samples were taken for all inlet batches at the fill time and again at the effluent sample times and for effluents at t~5 hours, 10 hours, 23 hours, 28 hours, 33 hours, and 49 hours. Bacteria seemed to become active in the filter or in the bottom receptacle after ~75 liters or ~30 hours for the ECWF. TOC data is reported in Table 6-2 for ECWF 11136. Once again, the filter effluent remained clean for 24 hours, with bacteria first detected in the

effluent after 33 hours. The bench samples again demonstrate minimal consumption of TOC in the tap water itself, which implies that biofilms were present in the ceramic filter.

Table 6-2 ECWF TOC data, all samples are for 11136

Time (hrs)	Sample	Cum volume treated (L)	Inlet TOC (mg/L)	Effluent TOC (mg/L)	Decrease in TOC from inlet to effluent (mg/L)
0	Batch 1	0	16		
1	t=1 hr	5.23		13.9	2.1
5	Bench 5 hr		15.7		
	t=5 hr	15.29		14.6	1.1
5.1	Batch 2		16.6		
6.1	t=1 hr	20.01			
10.1	Bench 5 hr		16.5		
	t=5 hr	29.81		15.3	1.2
	Batch 3		16.5		
23.1	Morning Bench		16.9		
	Morning Effluent	49.1		15.6	1.3
23.3	Batch 4		17.5		
28.3	Bench t=5				
	t=5	62.98	17.6	16	1.6
	Batch 5		17.2		
33.3	Bench t=5				
	t=5	77.04	17.6	16.2	1.4
	Batch 6		17.4		
49.8	Morning Bench		17.9		
	Morning Effluent	97.73		13.3	4.6

6.4 Impact of silver on *E. coli* Disinfection: Reapplied Silver

After the summer *E. coli* loading experiments and the control tests, the CPFs were tested under similar loading conditions with reapplied silver to observe the effect and importance of silver on the removal for the CWF and ECWF system. Because 18193 cracked during the ECWF control test, it was replaced with 29027 from Nicaragua (1 no silver from Kowalski's tests). CPF 29027 had a similar history and flow rate to the other Nicaragua filters. Before re-

applying silver to the filters, the filters were extensively treated with bleach in an attempt to ensure that all residual bacteria and/or biofilms were removed from the ceramic. The CPFs were soaked in ~10x dilution of household bleach in tap water for ~48 hours and then rinsed for a day with tap water and two days with de-chlorinated tap water. The filters were then assumed clean and left out in the lab to dry. The ceramic must be completely dry before re-applying silver (Rayner, 2009). After drying, the CPFs were each recoated with 300 mL of a liquid colloidal silver solution and tested again for *E. coli* removal. The liquid solution had an estimated silver concentration of 213 mg/L. The actual silver concentration was measured to be 106.1 mg/L based on ICP-MS analysis (see Section 6.5.3).

6.4.1 Disinfection

The silver-coated ceramic *E. coli* tests were run in sequence on both 11136 and 29027 starting with the 2 day ECWF test, then 2 days of rinsing with de-chlorinated tap water (no TSB) and bleaching the bottom receptacles, then immediately starting the 4 day CWF test. The graphs below show the increase in effluent bacteria concentrations as the experiment progressed (Figures 6-38 and 6-40). It is also important to note the TOC data (Figure 6-39 and 6-41). The TOC markers represent the decrease in TOC (ppm) from the inlet to the effluent samples. This trend shows that as the bacteria in the effluent increased the TOC in the effluent decreased, indicating that there was a correlation between effluent bacteria concentrations and a decrease in the TOC in the water.

The first effluent samples at t=0 after the saturation phase of the filters both had 0-40 CFU/mL at the beginning of the experiment. The filters may not have been completely clean after the re-application of silver and re-saturating with de-chlorinated tap water. The inlet concentrations are also reported on the graphs to show the *E. coli* concentrations that were being

loaded into the filters. For Figure 6-40, the cumulative volume was averaged for the two filters to plot the inlets at those points. The graphs also show when the rinse phase started and when the CWF test started. During the rinse phase the inlets all showed zero for the plate counts. They are plotted at 10 CFU/mL to represent that there were data for these points and they were known to be at the minimum detection limit for the spiral plater at <20 CFU/mL. The open symbols for CPF 29027 during the rinse represent that the plates were TNTC. During the CWF test 10x and 100x dilutions were plated of the effluents so those plate counts are all quantified values and the graphs show that the effluent concentrations were sometimes larger than the influent samples.

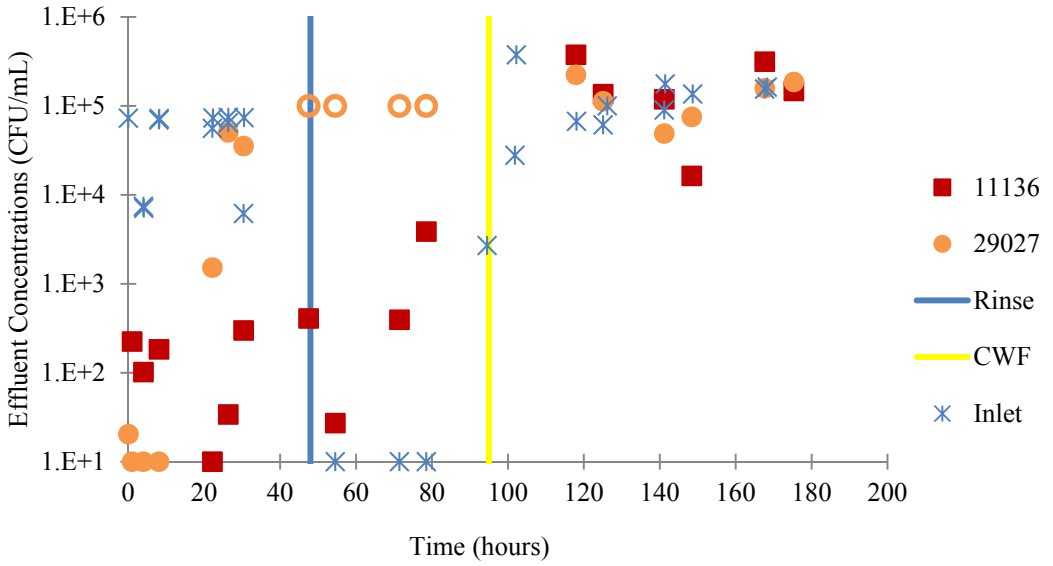

Figure 6-38 *E. coli* experiment series with reapplied silver showing effluent concentrations vs. time

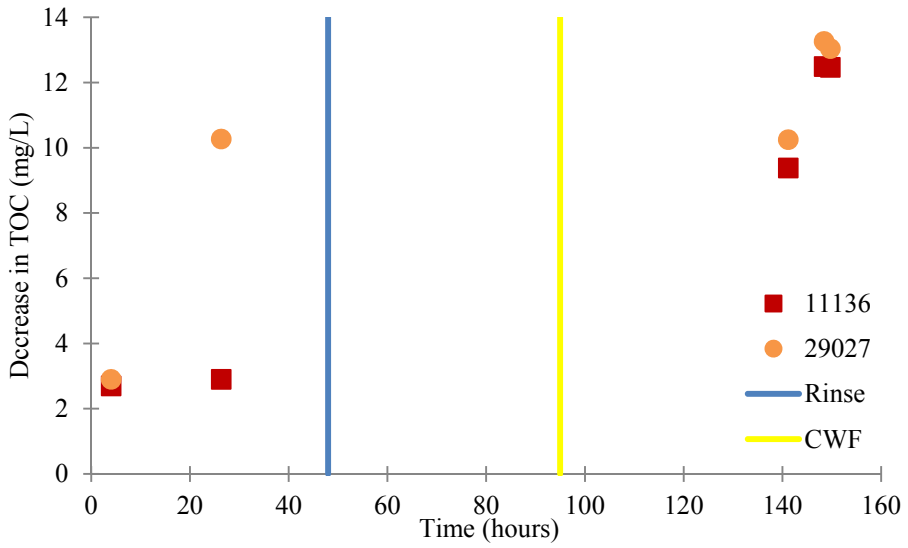

Figure 6-39 Decrease in TOC from the inlet to the effluent vs. time

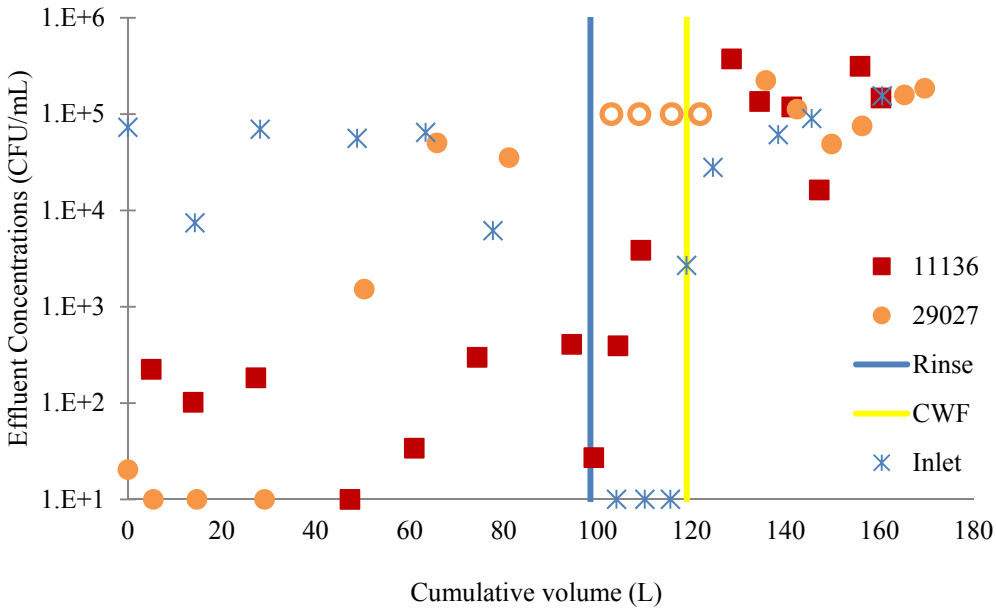

Figure 6-40 *E. coli* experiment series with reapplied silver showing effluent concentrations vs. cumulative volume

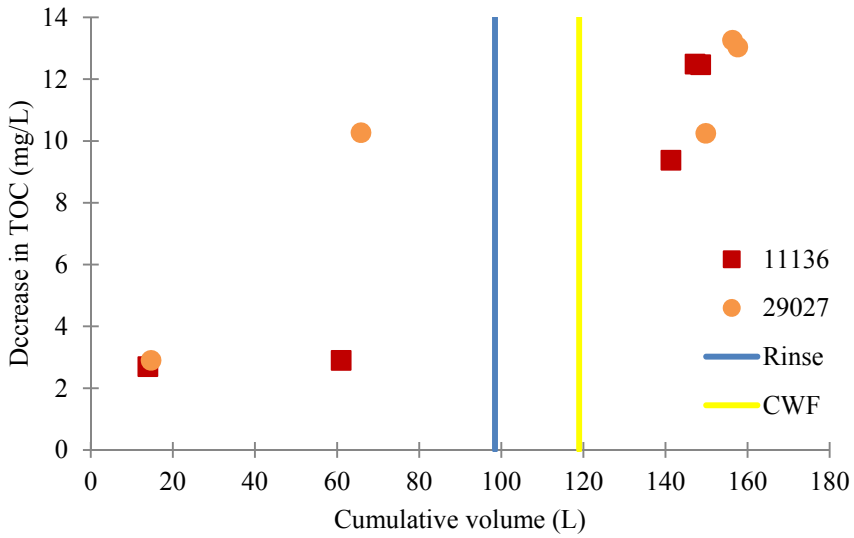

Figure 6-41 Decrease in TOC from the inlet to the effluent vs. cumulative volume

It is important to note the different behaviors in the two Nicaragua filters in the beginning of this test sequence. Filter 29027 showed effluent concentrations of zero (<20 CFU/mL) until ~50 L or ~22 hours and then broke through. From that point filter 29027 had much larger bacteria counts than 11136 (Figure 6-42). Previous tests by Kowalski (2007) also found that filter 29027 (1NS) had poorer disinfection than 11136 (1 Used) when both filters had no silver. There was also a much larger decrease in TOC from the inlet to the effluent for 29027 than for 11136 during the ECWF with silver experiment (Table 6-3), indicative of more bacterial activity in filter 29027.

Table 6-3 TOC Data from experiments with *E. coli* and re-applied silver for ECWF and CWF

	Inlet TOC mg/L	11136 effluent TOC mg/L	29027 effluent TOC mg/L
ECWF Batch 1, 4 hr	**17.0**	**14.3**	**14.1**
ECWF Batch 4, 4 hr	**17.5**	**15.0**	**7.23**
CWF Batch 4, morning	**14.5**	**5.12**	**4.25**
CWF Batch 5, 7 hr	**17.1**	**4.61**	**3.84**
CWF Batch 6, morning	**17.7**	**5.23**	**4.66**

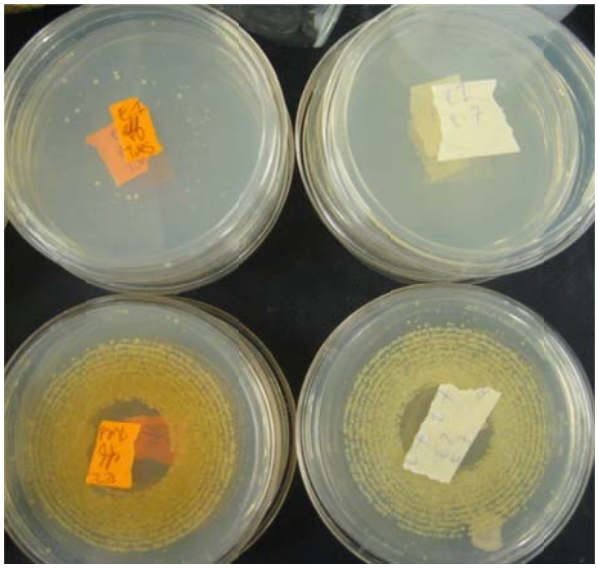

Figure 6-42 ECWF 11136 effluent plate (above) and ECWF 29027 effluent plate (below) after 2 day ECWF experiment at no dilutions

Overall the filters performed better initially with silver under the ECWF setup but once the effluents were contaminated the effluent concentrations remained high. During the rinse phase with de-chlorinated tap water between the two experiments the effluent concentration also increased. It is expected that filters with colloidal silver would be more effective at removing bacteria. Other research at CU concluded that generally, the highest *E. coli* disinfection occurred directly after recoating the filter with silver (Microdyne solution), when the silver concentrations were at a maximum (Kowalski, 2007). Over long term tests (44 months) ceramic filters with no application of silver were observed to be comparable in microbiological effectiveness to the filters with silver nitrate amendment (Brown, 2007).

6.4.2 Flow rates and clogging

First hour flow rates were measured throughout the silver-treated filter *E. coli* loading experiments and are presented in Figures 6-43 and 6-44 compared to the time (hours) and the cumulative volume filtered (L), respectively. The first hour flow rates for the ECWF system ranged between 4.56 L/hr to 5.01 L/hr for 11136 and 4.95 L/hr and 5.85 L/hr for 29027. For the

CWF system first hour flow rates ranged between 1.18 L/hr and 1.42 L/hr for 11136 and 1.29 L/hr and 1.76 L/hr for 29027. Overall there seemed to be no significant decrease due to clogging during bacterial loading.

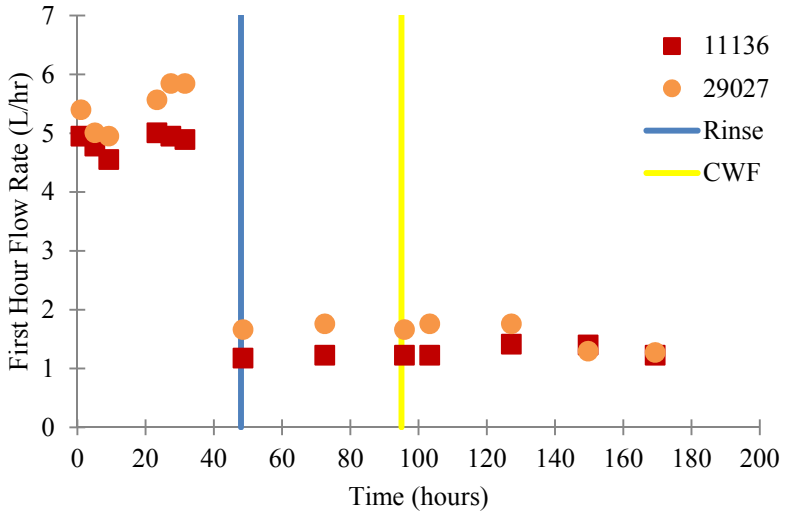

Figure 6-43 First hour flow rate vs. time (hours)

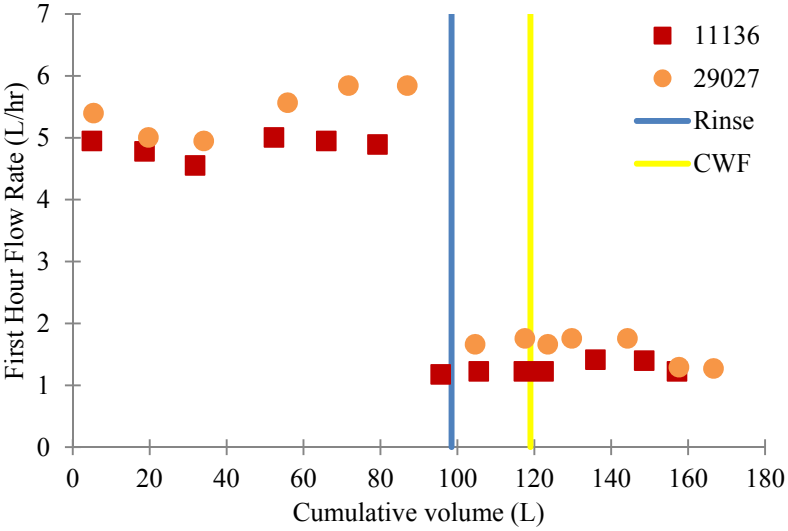

Figure 6-44 First hour flow rate vs. cumulative volume

6.4.3 Silver Effluent Concentrations

During the ECWF and CWF experiment sequence with re-applied silver, 6 mL effluent samples were taken at specific times and analyzed for silver concentration to measure the amount of silver that desorbed and/or dissolved from the ceramic. The measured amounts could include both nano-particulate silver and/or ionic silver. The solution that was re-applied to the ceramic was produced to have a concentration of 213 mg/L assuming a 3.2% solution of colloidal silver; however the analysis showed that this solution had a silver concentration of 106.1 mg/L. The actual measure silver concentration is almost exactly half the expected value.

Previous research at CU showed similar results. A colloidal silver solution was made to reapply to the cores. The expected concentration of was about 210 mg/L while the ICP-MS results for the paint solution showed a concentration of about 133 mg/L (Kohler 2009). Table 6-4 shows the silver concentrations in the effluents and the calculated cumulative mass of silver that came off of the filters. These samples were collected at selected times from the beginning of the ECWF test, the de-chlorinated tap rinse phase between the two experiments, the CWF test, and the final rinse. "DL" signifies that the concentration was lower than the ICP-MS minimum detection limit which was 0.43 ppb.

Table 6-4 Silver concentrations (ppb) in filter effluents

		11136		29027	
	Time (hours)	Ag conc. ppb	Cumulative Ag in effluent (µg)	Ag conc. ppb	Cumulative Ag in effluent (µg)
Rinse	t=0	0.55	0.03	2.57	0.13
ECWF	t=1	6.54	1.35	7.67	1.55
	t=4	0.63	1.39	4.11	1.83
	t=8	1.09	1.43	5.28	2.01
	t=22	10.52	1.66	3.30	2.08
	t=32	1.81	1.68	2.96	2.11
	t=48	2.04	1.70	2.43	2.13
RINSE	t=24	DL		DL	
	t=48	DL			
CWF	t=7	DL		1.78	2.15
	t=31	DL		4.18	2.18
	t=55	14.53	1.80	14.80	2.27
	t=79	4.5	1.83	DL	
RINSE	t=24	DL		DL	

The effluent concentrations of silver were expected to start off high during the saturation phase and then decrease over the course of the experiment. We did not see the expected desorption curve for silver that was previously seen with reapplied Microdyn silver (Kohler 2009). The mass balance indicates that >99% of the reapplied Argenol silver remained on the ceramic over the course of the experiment. This desorption of silver is shown as the % remaining in Figure 6-45 and Figure 6-46. The percent remaining that is shown in the graphs below is averaged between the two filters and does not drop below 99.95%. It also is calculated assuming that the concentration of silver in the material painted on the filter was 106.1 ppm.

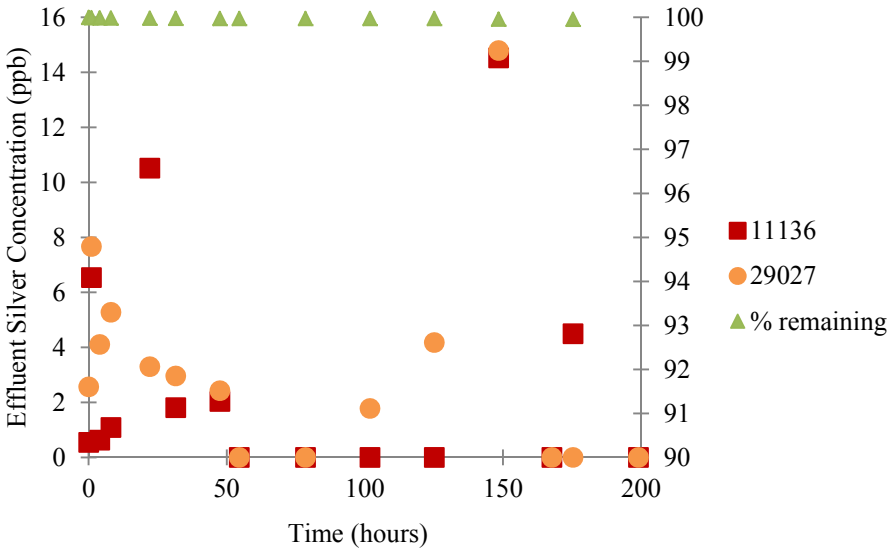

Figure 6-45 Effluent silver concentrations vs. time

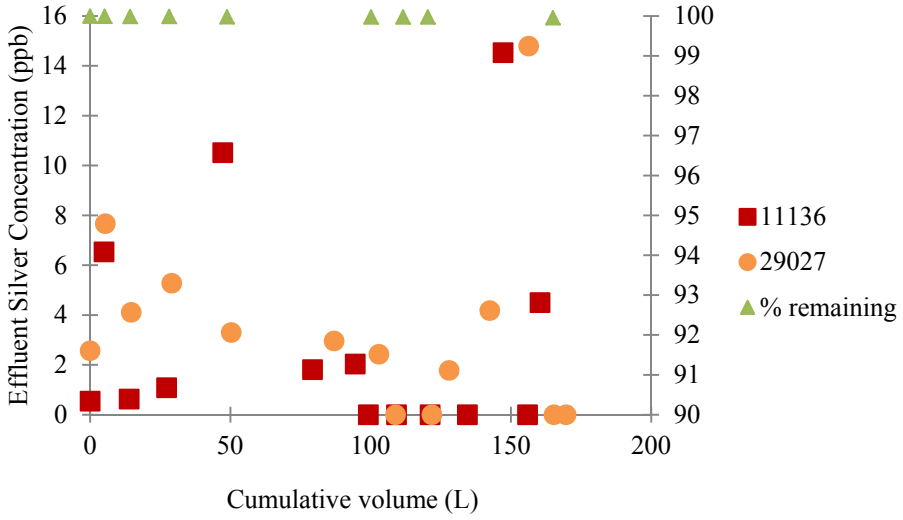

Figure 6-46 Effluent silver concentrations vs. cumulative volume

6.5 Comparison of ECWF with and without Silver

Overall there was less *E. coli* in the effluent over the whole experiment when silver was reapplied to the filters, although it was difficult to make direct filter performance comparisons because 11136 was the only filter that experienced all testing conditions. Figures 6-47 and 6-48 show the effluent concentration for 11136 versus the cumulative volume filtered and the time, respectively. Assuming inlet concentrations were ~10^5 CFU/mL the performance with the silver

initially was about the same as the filter without silver. Figures 6-49 and 6-50 illustrate the log removal versus the cumulative volume and the time, respectively. As the experiment progressed the silver-treated filter accomplished 2-3 log removal while the filter with no silver quickly became contaminated to ≤ 0 log removal.

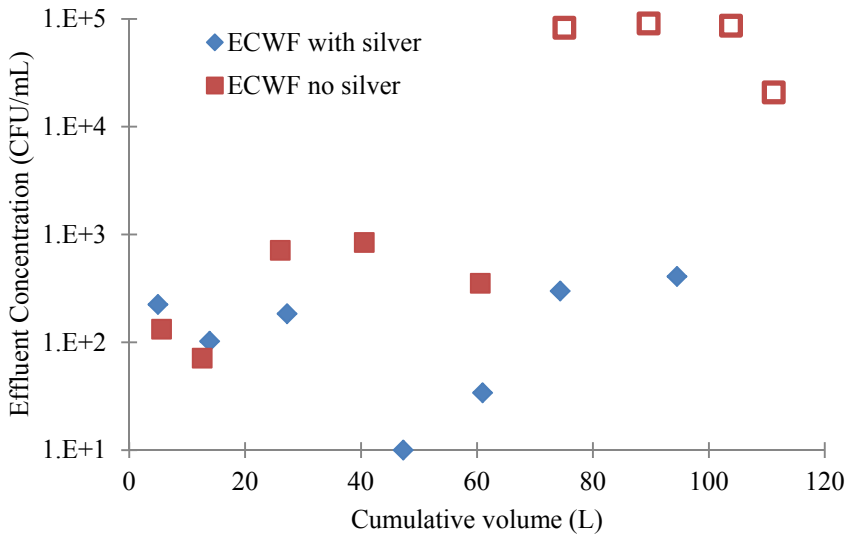

Figure 6-47 Comparison for 11136: ECWF with reapplied silver vs. ECWF with no silver for effluent concentrations vs. cumulative volume

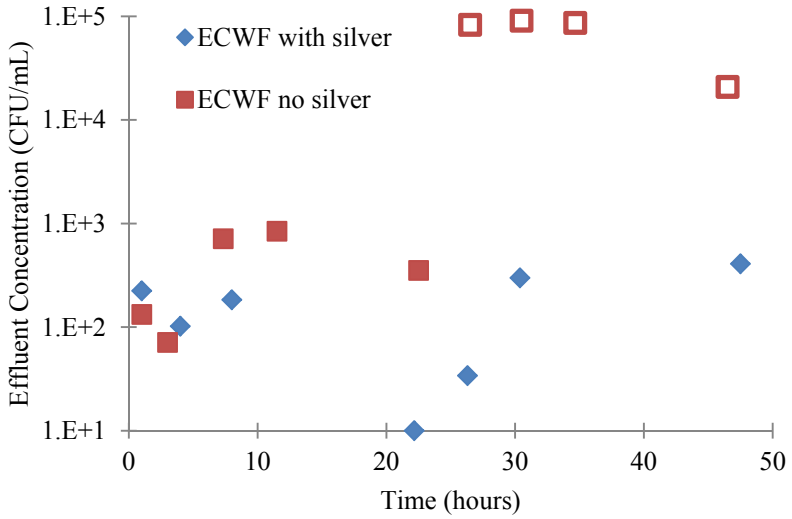

Figure 6-48 Comparison for 11136: ECWF with reapplied silver vs. ECWF with no silver for effluent concentrations vs. time

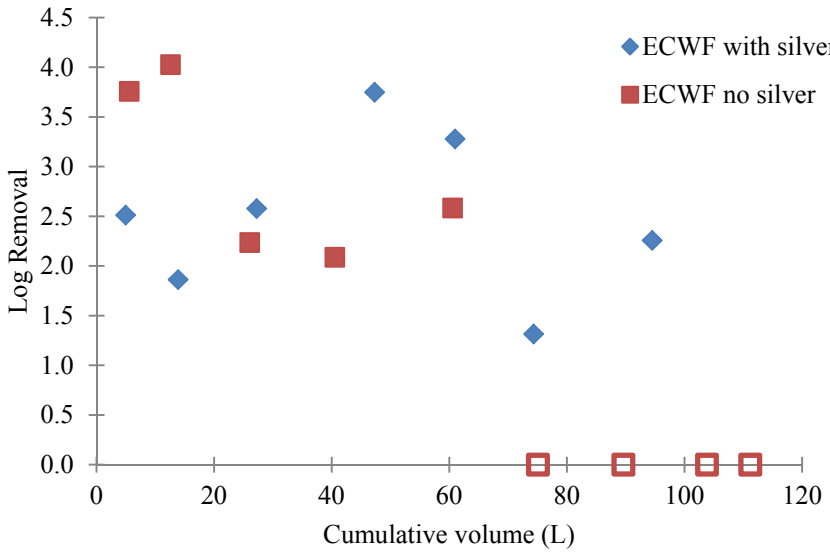

Figure 6-49 Comparison for 11136: ECWF with reapplied silver vs. ECWF with no silver for log removal vs. cumulative volume

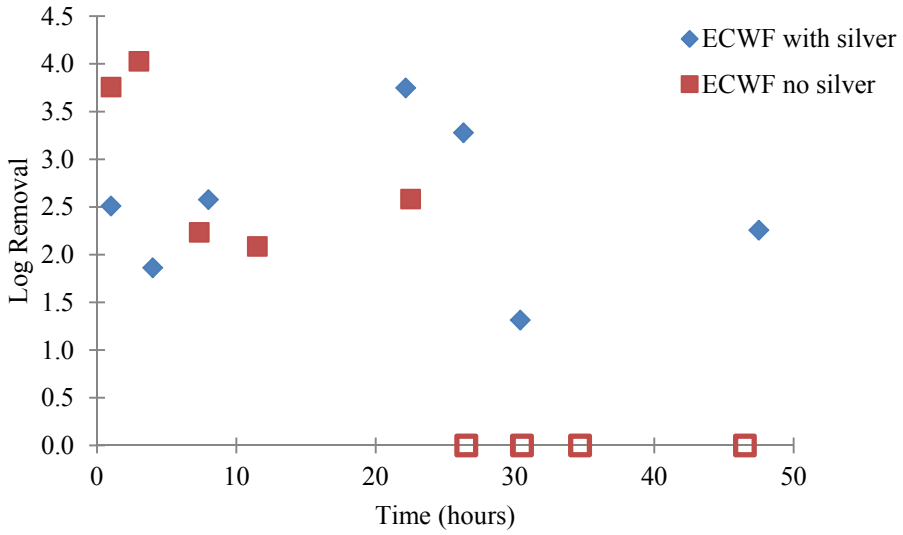

Figure 6-50 Comparison for 11136: ECWF with reapplied silver vs. ECWF with no silver for log removal vs. time

6.6 Operational Problems

The ECWF was difficult to use effectively even in a controlled laboratory setting. This section will discuss a variety of challenges associated with the operation of the ECWF and potential concerns for household use that arose throughout the research project.

6.6.1 Flow rate inconsistencies

Over the course of the entire research project the flow rates of the individual CPFs varied significantly. The flow rates were sometimes lower for the first batch of the day because the ceramic partially dried out overnight. Pores were also blocked by the high turbidity and bacteria loading. The inside and outside of the filters were scrubbed with a coarse brush before and after the experiments. This could have removed some of the ceramic itself which would cause the flow rates to increase. Flow rates may also be affected by the temperature of the water and how clean the filters were at that point. Flow rate measurements were taken during every test one hour after refilling and one hour after refilling during the saturation phase before the tests and the rinsing phase after the tests. Table 6-5 shows the variation in first hour flow rates measured for each filter at different time points during the research. Between experiments the first hour flow rates were measured to document any large changes.

Table 6-5 First hour full flow rates during entire research project in L/hr

	18193	11136	RDI 1	RDI 2	29027
Prior to all testing	1.8	2	1.3	1.5	1.4
After turbidity testing	1.58	1.55	1.24	1.33	
Before *E. coli* testing	1.76	1.6	1.24	1.27	
After *E. coli* testing ECWF no silver Before Bleaching and Scrubbing	2.18	1.93	1.72	3.12	
Between multiple *E. coli* tests CWF no silver	2.98	2.86	0.88	2.19	
After *E. coli* testing CWF no silver	2.49	2.73	2.07	2.96	
During Hydraulic Testing with Tap Water	1.19	1.08	1.12	2.47	
Before control tests	0.96	1.04			
After control tests	1.29	1.32			
Before *E. coli* testing with re-applied silver	1.18				1.66
After *E. coli* testing with re-applied silver	1.08				1.03

After the turbidity experiments the Nicaragua filters had first hour flow rates between 1.55 and 1.58 L/hr and the RDI filters were between 1.24 and 1.33 L/hr. After the *E. coli* tests without silver the RDI 1 first hour flow rate dropped below 0.9 L/hr while RDI 2 filter was above 2 L/hr. The RDI filters behaved similarly until the *E. coli* experiments. After the *E. coli* loading, RDI 2 was approximately twice as fast as RDI 1. RDI 2 was left out of the hydraulic testing results because it was ~2 times faster than the other CPFs, presumably due to a crack (although none was visible). The enhanced first hour flow rates were 4.05, 3.99, 4.24, and 7.03 L/hr for 18193, 11136, RDI 1, and RDI 2; respectively. The flow rates for each filter varied during all experiments not exclusively during the turbidity loading which was expected to clog the ceramic and decrease the flow rates.

Regardless of using the CWF or the ECWF system, cleaning the CPFs to restore the flow rates after clogging was important. During the turbidity experiments, the CPFs were intensely scrubbed to restore them to their initial flow rates between each experiment. This often required

multiple scrubbing and flow measurement cycles. Sometimes vigorous scrubbing was required, visibly removing ceramic particles off of the interior and exterior surfaces of the CPF.
It was extremely difficult to get the filters back to their original flow rate. Their flow rate characteristics seem to be affected by their loading history, so the initial flow rates decreased slightly from the previous tests. The filters were intensely scrubbed after each test so that the initial pre-test flow rates were as close as possible, making the data more comparable from test to test. It is inevitable that the filters will foul to a certain point because there is no easy way to backwash them. Scrubbing the inside of the ceramic filter is the best form of cleaning, but also involves physical labor and wastes clean water. One of the Cambodia filters unfortunately clogged irreversibly. It was bleached and scrubbed multiple times and the flow was significantly worse than ever before. That is the point where the RDI filters were taken out of the test matrix due to flow rate problems.

6.6.2 Gasket Sealing

The first practical challenge associated with the ECWF was achieving a seal between the top bucket and the gasket attached to the CPF. There were a few instances where the gasket sealing the top bucket to the CWF was visibly leaking, resulting in water pooling on the outside of the gasket and short circuiting around the outside of the CWF lip and down into the bottom bucket of treated water (Figure 6-51). This leaking water was untreated because it was forced between the gasket and the top bucket and then ran down the outside of the CWF into the bottom bucket. Every time a user needed to remove the top bucket to scrub the CPF, it would be a challenge to ensure that a proper seal was acquired when replacing the plastic top bucket. It was difficult to remove the top bucket to clean or take measurements and then replace with a sufficient seal. Many times when the top buckets were put back on and then filled up, there was

leak visible until the bucket could be manipulated against the gasket at the right depth and angle to create the proper seal.

Figure 6-51 Water pooling between the top bucket and the gasket

To help ensure a water-tight seal between the top bucket and the CPF it is important that the top opening of the CPF is a uniform circle. This requires good quality control at the factory. Also, in different countries the dimensions of the ceramic water filters vary. In the University of Colorado lab testing, two different types of five gallon buckets were used in order to properly fit the Nicaragua CPFs and the Cambodia CPFs. Therefore, it would also be necessary to ensure that appropriately sized plastic buckets could be found to fit the local CPF dimensions.

6.6.3 Gasket Leaking

Unlike gasket sealing, gasket leaking occurred when there was a leak in the glue attaching the gasket to the ceramic. Water could be seen running down the outside lip of the CPF between the gasket and the ceramic. This water was untreated because it was forced between the gasket and the CPF and then ran down the outside of the CWF into the bottom bucket. Multiple times this happened on all the ECWF systems. This was fixed by applying more “Gorilla Glue” at the visible location of the leak and applying pressure while waiting for it

to dry completely (about 1-2 hours or overnight, if convenient) before starting the next experiment. The gasket, when applied correctly could maintain a sufficient seal.

6.6.4 Fragile Ceramics

Another issue encountered in these lab experiments was the fragile nature of the ceramics. The ECWF system puts a different kind and amount stress on the CPF. First, the hydraulic head is increased drastically; initially ~53 cm in the ECWF compared to ~20 cm in the CWF. This puts stress on the contact point between the top bucket and the CPF. The adequate amount of pressure to seal the gasket also adds stress. RDI 1 developed a crack in the lip of the filter after only 15 days in the ECWF. 18193 completely cracked internally down the side during the *E. coli* testing when the top bucket was being placed on the CPF after a cleaning. Twelve new filters were ordered from the factory in December 2010 and all except one arrived cracked (Figure 6-52).

Figure 6-52 Broken ceramic pot filter after being shipped from Nicaragua

Chapter 7 Conclusions and Recommendations

This research examined an inexpensive addition to the already widely used ceramic pot filter to increase the flow rates and water treatment capacity. The ECWF as a system was examined for its potential increase the treated water volume for a household as well as its ability to remove high amounts of turbidity and *E. coli*. This chapter reiterates the key conclusions from the research and discusses directions for future research.

7.1 Enhanced water treatment capacity

The ECWF successfully increased the first hour flow rates of the ceramic pot filters from 1-2 L/hr to 4-6 L/hr. Hydraulic testing showed that the average initial flow rate of three CPFs increased from 1.13±0.06 L/hr to 4.1±0.15 L/hr when operated under ECWF compared to CWF. In a six hour period of treatment after refilling the CWF or ECWF once, the flow rates decreased from 1.13 to 0.7 L/hr and 4.1 to 2.5 L/hr due to the decreasing hydraulic head. It takes approximately 6-7 hours before the top bucket empties completely. It would be best not to let the top bucket empty fully so that the ceramic pot filter is always full and being used to its maximum capacity at all times.

The water treatment capacity over the first 6 hours after refilling the ECWF compared to the CWF produced 15.16 ± 0.41 liters versus 4.71 ± 0.22 liters. The ECWF treatment can produce approximately 3.2 times more volume of treated water for each refill. This is a significant increase in daily water produced for a household in the developing world. Assuming that a family can refill the system 2-3 times per day, the ECWF can produce >50 liters per day compared to a maximum of ~20 liters per day for the CWF system.

7.2 Turbidity Removal ECWF Summary

The ECWF system was sufficient at removing turbidity up to 500 NTU. The effluent turbidities decreased as the inlet turbidities increased when there were no leaks present in the gasket or the glue. When a leak was present the ECWF effluent turbidities were significantly higher than during CWF operation. The effluent turbidities also decreased over the course of the four day loading period. This was due to the Kaolin forming a "cake" layer on the inside of the ceramic and clogging larger pores with the initial batches. The first hour flow rates for the enhanced system decreased by 6-18%, 20-37% and 14-45% for 5 NTU, 50 NTU, and 500 NTU loading over ~120 L, ~130 L and ~135 L cumulative volume filtered, respectively.

7.3 *E. coli* removal ECWF Summary

After completing the *E. coli* experiments, it was concluded that although the ECWF system did increase the flow rates, it was unsuccessful at reliable bacteria disinfection of sufficiently high log removals. There were also overwhelming challenges that were discovered through laboratory experiments. These challenges would most likely be amplified in the field, a less controlled environment. Difficulties with use included the ability to properly seal the bucket and the gasket on the filter, avoiding leaks in the glue, and successfully removing the top bucket to empty/clean the CPF and then replacing it correctly. A few things were not measured and assumed consistent throughout each experiment. These include the temperature and properties of the tap water, the temperature of the room, and the temperature of the incubator (set at 37 degrees Celsius). These factors could affect the growth of bacteria during the experiment.

During the control tests with no inlet bacteria counts, bacteria grew on the effluent sample plates at the end of both the CWF and ECWF tests proving that there was microbial activity occurring between filling the filters and sampling from the bottom receptacle. Similar

bacteria growth in the effluent water was seen in both the control experiments and *E. coli* experiments. This proves that there was bacterial growth regardless if there were initially *E. coli* spiked in the inlet water.

In the control and *E. coli* experiments the taps were left open to limit the dead space in the bottom receptacle. This would not be realistic in a home environment because they would most likely have the taps closed until they needed water from the receptacle. Strict *E. coli* results were inconclusive because it was impossible to know if the colonies on the effluent plates were only *E. coli* and in many cases it was obvious they were not due to multiple colors and sizes of colonies.

The inlet plates showed strictly *E. coli* colonies where the effluent samples had in cases multiple types of bacteria and sometimes much larger amounts of bacteria. These colonies were smaller in size and believed to be something other than *E. coli*. The filters potentially could have had high *E. coli* removal which was masked by the unknown bacteria that appeared in the effluent samples that could have been competing with the *E. coli* for both agar on the plates or carbon source in the water (TSB). The microbial activity in the filter versus in the bottom receptacle is unknown but it is evident that it exists. Once the filters were declared contaminated and had effluent plate counts much higher than the influent plate counts, there was no way to reverse the growth without bleaching the filters. Even then, it appeared that a biofilm remained in the ceramic pores.

All of the *E. coli* tests were consistent in that there was never 100% removal and effluent plates got worse as the experiment continued and the cumulative volume filtered increased. There was either growth in the filters or in the bottom receptacle observed regardless of testing under CWF or ECWF conditions and silver or no silver. It is important to note that the duration

of the experiments, NOT the cumulative volume loaded, seemed to dictate when the filters became contaminated. Regardless of the difference in volume of water treated for the ECWF and CWF, both systems became severely contaminated around the same time. When the filters became contaminated a few things were observed: the effluent bacteria concentrations exceeded the inlet concentrations, the TOC in the effluent decreased compared to the TOC in the inlet water, and there was a noticeable smell coming from the ceramic filters.

7.4 ECWF Overall Performance

The ECWF treated about three times more water than the CWF. The turbidity removal of the two systems was comparable, but sometimes a leak in the ECWFs gasket seal or glue allowed the inlet water to short circuit around the filter and into the bottom receptacle of treated water. The CPFs without silver removed significantly less *E. coli* when operated as ECWFs compared to standard CWFs. However, contamination was noted in both systems after ~20 hours, presumably due to growth in the bottom receptacle. Due to shorter contact times with the silver-impregnated ceramic under enhanced flow rates, it was also expected that poorer disinfection would occur in CPFs with silver with operated as ECWF system.

The re-applied silver experiments show that even with silver the CPFs allowed for bacterial growth under standard and enhanced flow rates. These results were also inconclusive because the experiments were run in sequence with the ECWF completed first, followed by the CWF. There was no way to clean or disinfect the filters between the two experiments without striping the re-applied silver from the ceramic. Instead of bleaching the filters after the ECWF experiment they were rinsed with two days with de-chlorinated tap water. The CWF experiment started with already contaminated effluent samples that only got worse as the CPFs were loaded with more batches of ~10^5 CFU/mL.

7.5 Recommendations for Future Work

The point of this research was to quantify the removal efficiency of ceramic pot filters on turbidity and *E. coli* at enhanced flow rates. The results show that while the enhanced system was effective at removing turbidity, bacterial growth under experimental conditions with a readily available carbon source added at ~20 mg/L was an issue with or without silver. The user difficulty makes the ECWF not a recommended method of increasing the flow rate. Coupling the treatment results of the ECWF with the higher cost, sealing challenges, and enhanced cracking risks, alternative methods should be explored to enhance the water treatment capacity of CPFs. Using or distributing the ECWF system that was designed for this research is not recommended. Increasing the flow rate other ways should still be examined because it is already a culturally excepted and widely used technology.

7.5.1 Core experiments with silver

Already used cores should be recoated with the colloidal silver solution and tested for *E. coli* removal under enhanced flow rates. The new filters that arrived broken from Nicaragua could be cored and tested for *E. coli* removal under enhanced flow rates as new filters with silver. These results could be compared to the older, used filter cores to see if there is a difference between the effectiveness of "new" silver and reapplied silver. The core system also eliminates the variable of potential recontamination in the bottom receptacle and therefore could help us understand the biological activity within the ceramic walls.

Since the silver effluent data was also inconclusive, silver samples should be taken again from the core tests and analyzed for concentrations. This could prove if the reapplied silver comes off of the ceramic quicker than the new silver. Using the cores and pumps to regulate the flow rates and collect effluent samples is a much more controlled laboratory setting than the full

filter tests. With the core experiments the water can be sterilized because the volume needed is so much less than when testing full filters. The core tests can also control the flow rates of water through the ceramic by using pumps and there is no dead space after filtration because effluent samples are taken immediately after passing through the cores. The core tests could help answer some small scale questions about *E. coli* removal and the ability of the silver to stay on the ceramic surface.

7.5.2 Selective agar for *E. coli* experiments

The results of this research were inconclusive for strict *E. coli* removal. It is significant that more than just *E. coli* was growing on the non-selective agar plates during the *E. coli* experiments. Also, the control tests proved that even without *E. coli* in the inlet water, the ceramic filters still supported microbiological growth with a substrate of 50 mg/L of TSB. This proves that there was some growth occurring within the filter that was not strictly the *E. coli* which is important to know when examining the overall performance and behaviors of the filters. It would be helpful redo the *E. coli* experiments using both non-selective agar and selective agar to compare the actual removal of strict *E. coli* to the potential growth of other bacteria.

7.5.3 Testing different concentrations of TSB

The blank tests proved that with 50 mg/L of TSB added to the inlet water, the ceramic filters would begin to support bacterial growth in only a few days. Other concentrations of TSB have been tested at the CU laboratory using cores and shown that at 5 mg/L of TSB, bacteria will not start growing but that amount also does not support *E. coli* in the inlet water. Further work could examine the *E. coli* and control experiments with smaller concentrations of TSB. It is also imperative then to understand the characteristics of source water that would be found in the field. This includes but is not limited to the organic carbon that would appear in the inlet waters in the

field. Instead of using 50 mg/L of TSB, which corresponds to approximately ~20 mg/L of TOC, laboratory tests should be done that use a typical source water bacterial amount and organic carbon amount to see if this growth in the filters is something that is possible and highly likely in the field. Other amounts of substrate should be spiked into the inlet water. It is difficult to create water that would replicate something from the field because source water can vary immensely. Also, there would be potentially a lot going on in any source water such as different types bacteria, viruses, dirt, metals, chemicals, organics, etc. How all of these things interact with each other is also unknown many times though it is concerning that there may be a lot more going on in the water sources in the field compared to those prepared in a laboratory setting.

7.5.4 "Keg" system

A different enhanced ceramic water filtration system called the "keg" is being examined at CU in collaboration with Chris Schulz of CDM in Denver. In the "keg" system, two CPF are placed open end together and sealed with a gasket. Holes are drilled in the bottom of both CPFs and a PVC pipe and hose with holes in the side is placed into the "keg" and sealed on both ends. The keg system can then be dropped into a cistern of any size (bigger than the keg itself). The untreated water travels from the outside of the filter to the inside and is then pumped out through the PVC and hose. So far, creating a water tight seal around the holes in the ceramic and between the two filters has been a challenge. Once this is accomplished turbidity and *E. coli* testing will be completed.

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Appendices

A.1) Turbidity Test procedure

1) Rinse out bottom receptacle and spout with tap water
2) Place CWF into bottom receptacle
3) Prepare NTU batch in 55 gallon drum with tap water and kaolin mix, measure turbidity with Hach Model 2100N Laboratory Turbidimeter
4) Pour in prepared NTU batch
5) Measure initial water height
6) Measure turbidity in top of CWF or ECWF
7) Measure height after one hour
8) Measure turbidity in the effluent
9) Let water drain further (few hours)
10) Measure water depth and effluent turbidity
11) Repeat steps 3-10 for second batch (and third batch if applicable- during ECWF test)
12) Repeat all at the beginning of each day

A.2) Turbidity analysis

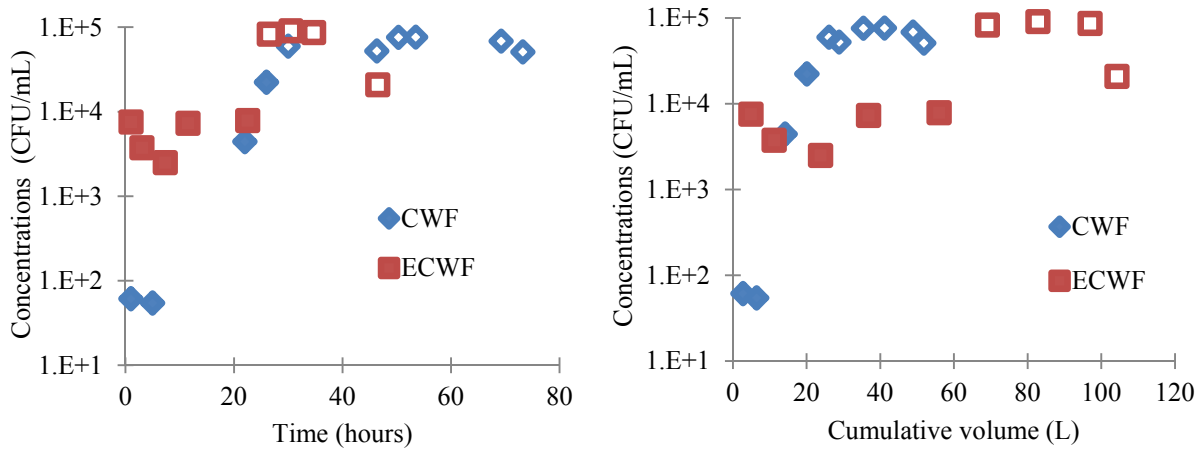
- Turbidity samples were analyzed using the Hach Model 2100N turbidimeter.
- Samples were measured with the signal average ON.
- When SIGNAL AVERAGE is on, the instrument's microprocessor compiles a number of readings and averages the result.
- The averaged value is calculated and displayed approximately once every second (Turbidimeter Manual 1999).
- Readings on the turbidimeter were taken once the displayed measurements steadied for a few seconds.

A.3) ECWF *E. coli* prep and schedule: example for 2 filters

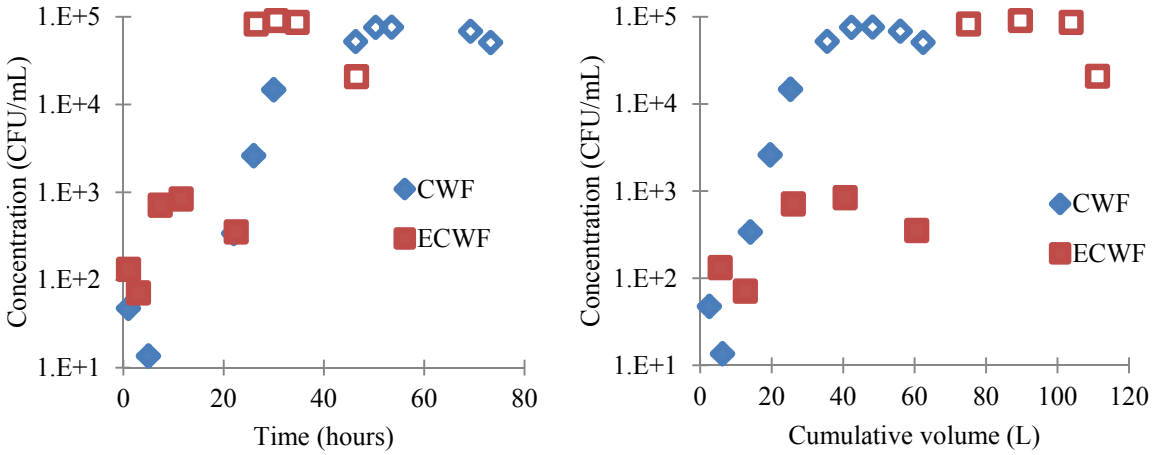
Day	Time	Immediate Task	Secondary Task	Third Tasks	# plates needed
-2		**flush filters with de-chlorinated tap water**	**All plates made**	**Reserve spiral plate for experiment day (full day!)**	
	evening!	**Streak Plate *E. coli***	**autoclave TSB for stock**	**Autoclave enough water/containers**	
-1		**flush filters with de-chlorinated tap water**			
	evening!	**Transfer colony to TSB, incubate**			
0	**7am**	**Centrifuge Stock**		**make new stock**	
	730am	**Plate centrifuged**	**Incubate plates, put**		**3**

		stock (10-6)	**stock in fridge**		
	8pm	**flush filters with de-chlorinated tap water**	**Transfer new colony to TSB, incubate**		
	9pm	**Take First hour flow rate**			
1	**DAY 1 OF EXPERIMENT**				
	7am	**bleach receptacle and bottom buckets.**	**Plate morning effluents**	**count colonies from yesterday's centrifuge stock**	
	7am	**centrifuge new stock and plate**			**6**
	730am	**dilute centrifuged stock into 40L de-chlorinated tap water**			
	8am	**fill CWFs**	**plate influent water (3 plates) 10x**		**3**
	9am	**first hour flow rate**		**Collect Silver Samples**	
	930am	**take effluent samples from each filter & bench (10x) t=1 hours**	**disposing of extra *E. coli* from first batch**		
	930am	**plate effluent samples**			**6**
	12:30pm	**plate inlet and effluent samples t~4 hours**	**De-chlorinating 35 L tap water & add centrifuged stock**		**9**
	1pm	**MEASURE WATER LEVEL then refill ECWFs**	**plate influent water (3 plates) 10x**	**Collect Silver Samples**	**3**
	2pm	**first hour flow rate**			
	530pm	**plate inlet and effluent samples t~8 hours**			**9**
	6pm	**MEASURE WATER LEVEL then refill ECWFs**	**Transfer new colony to TSB, incubate**	**Collect Silver Samples**	**3**
	7pm	**first hour flow rate**			

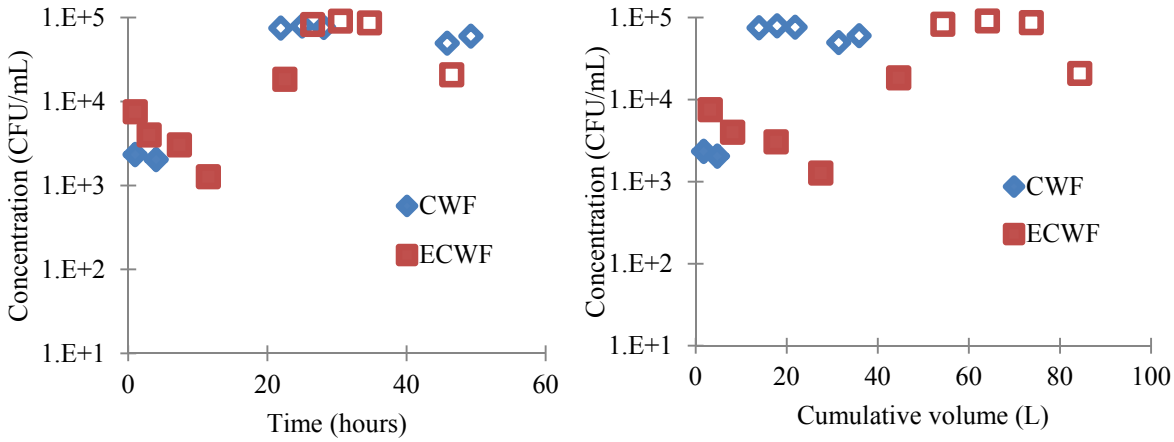
A.4) Effluent Concentrations vs. Time and Cumulative volume for CWF and ECWF without silver- individually



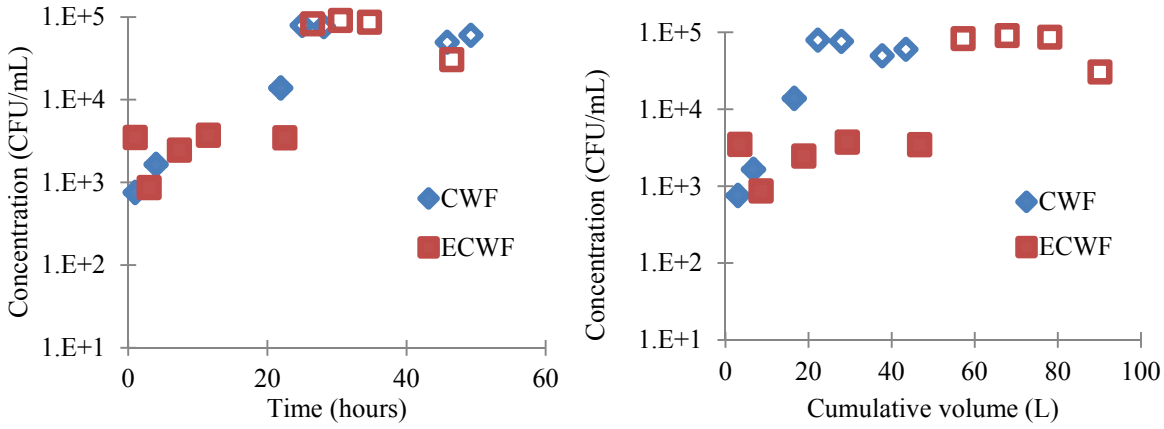
Effluent concentrations vs. time (hours) and cumulative volume (L) for filter 18193



Effluent concentrations vs. time (hours) and cumulative volume (L) for filter 11136



Effluent concentrations vs. time (hours) and cumulative volume (L) for filter RDI 1



Effluent concentrations vs. time (hours) and cumulative volume (L) for filter RDI 2

A.5) Sterile Glassware Cleaning Protocol (Kohler 2009)

Materials: Glassware (40mL vials with caps, 200ml collection jars, or other), cleaning brushes, aluminum foil, autoclave, autoclave indicator tape, autoclavable container and/or vial rack

Procedures:

• Dispose of any remaining sample using one of the methods discussed in A.4

• Scrub glassware with a cleaning brush under hot running tap water

Place glassware opening side down on a clean bench or drying rack and allow to air dry

• For 40 mL vials, loosely tighten screw caps onto the vials and place upright in autoclavable rack with a piece of indicator tape attached.

• For 200 mL collection jars, cover the tops of the jars with aluminum foil and place in the autoclavable container; attach a piece of autoclave indicator tape to the container

• For any other glassware being cleaned, secure caps loosely and/or wrap in aluminum foil.

• Place container containing jars and/or rack containing vials in the autoclave.

• Fill the autoclave with DI water and close the door to the autoclave.

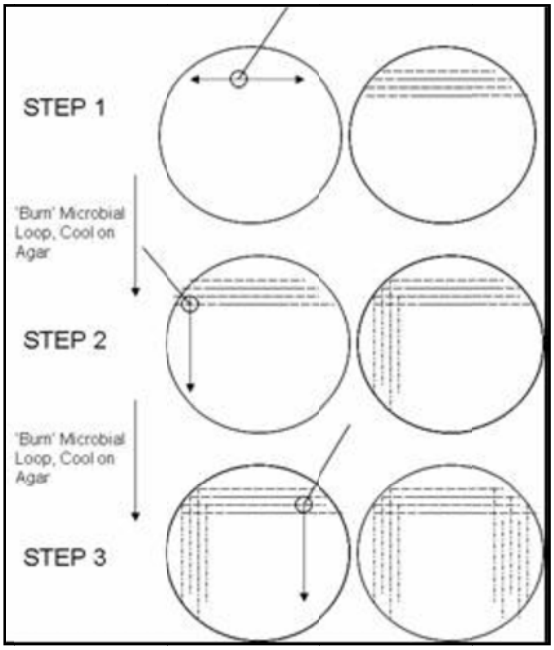
• If glassware only is being autoclaved set the autoclave to the "Fast" setting and set the timer to 15 minutes. If liquids are also being autoclaved the autoclave should be set to the "Slow" setting and the timer set to 25 minutes.
• After the autoclave has turned off and cooled down to less than 100°C, the autoclave can be emptied. Gloves should be worn when opening and removing items from the autoclave to prevent steam burns. The autoclave indicator tape should have changed indicating that everything has been properly autoclaved.

A.6) Disposal of Waste (Kohler 2009)
Method A: Autoclave
Materials: 1L flask, aluminum foil, autoclave indicator tape
Procedures:
• Empty liquid waste into the 1L flask, clean glassware as described in A.5
• When 1L flask is filled to about 900 mL, cover the top with aluminum foil
• Place a piece of indicator tap on the flask and autoclave for 25 minutes on the liquid setting
• When autoclave has cooled, remove flask from autoclave and ensure that the indicator tape has changed before placing on bench to cool
• When flask is cool, pour contents down the drain.
Method B: Chlorination
Materials: Waste bucket, bleach
Procedures:
• Empty waste into collection bucket and add a small amount of bleach
• Mix contents of bucket and let sit for 5 to 10 minutes
• Pour contents of bucket down the drain

A.7) Preparation of frozen glycol *E. coli* stock
Materials: 3 mL plastic tube, prepared liquid *E. coli* stock (see A.5), glycerin, sterile 1 mL pipette tips, 1 mL pipette
Procedures:
- Pipette 1 mL of liquid *E. coli* stock into the 3 mL plastic tube
- Pipette 1 mL of glycerin
- Label and place in -80 °C freezer

A.8) Preparation of Streak Plate Colonies
Materials: Agar petri dish, wire loop, freezer *E. coli* stock, Bunsen burner
Procedures:
- Flame the loop until it is red hot, touch it to the edge of the agar to cool
- Remove a loop full of K12 *E. coli* from the freezer stock
- Swab on new agar plate 5 times in one continuous motion; burn microbial loop, let cool, turn plate, and then swab 5 times in one continuous motion; burn microbial loop, let cool, turn plate, and then swab 5 times in one continuous motion
- Flame the loop again to disinfect it
- Parafilm the plate, label, and place streak plate upside down in the incubator at 35 °C for 24 hours

- Remove and place in the refrigerator. Streak plates are good to transfer colonies into fresh TSB to grow new *E. coli* stocks for around two weeks before it's necessary to make a new one.



A.9) Preparation of E. coli Stock

Materials: 250 mL Erlenmeyer flask, ultra-pure water (i.e. Milli-Q water), trypticase soy broth powder, 100 mL graduated cylinder, weigh dish, stir bar, stirring hot plate, autoclave indicator tape, sterile 50mL graduated cylinder, previous *E. coli* streak plate, masking tape, marker, wire hoop, Bunsen burner

Procedures:

- Place a clean 250 mL Erlenmeyer flask with fresh tin foil covering the opening, a stirring hot plate, drop in a stir bar and set to the lowest mixing speed.
- Measure out 250mL of ultra-pure water using the graduated cylinder and add to flask.
- Weigh out 7.5 g of trypticase soy broth powder in the weigh dish.
- Add the broth powder to the flask
- Increase the mixing speed and turn on the heating element to low
- Once the broth powder has completely dissolved in the water, place a piece of autoclave indicator tape on the bottle and autoclave for 25 minutes on the liquid setting
- Once the autoclave has cooled, remove the bottle of broth and make sure that the indicator tape has changed
- Set the bottle on the lab bench and allow to cool for 3 to 5 hours or until the bottle no longer feels warm to the touch
- Turn on Bunsen burner, and fire wire hoop until red hot
- Cool wire by touching to agar
- Scrap one colony off of agar plate gently with wire hoop and place colony into the TSB and visually confirm the stock being released into the TSB stock.
- Place TSB flask into incubator at 35ºC overnight on stir plate (~15-18 hours)

A.10) Procedure for Centrifuging *E. coli* stock

Materials: 6-50 mL centrifuge tubes, waste jar, clean Erlenmeyer flask

Procedure:

- Remove stock from incubator
- Pour stock into 5-50 ml centrifuge tubes
- Centrifuge stock for 10 min at 3725 rpm (centrifuge must be balanced- this requires 1-50mL centrifuge tube with water)
- Decant supernatant from each tube into waste container, add sterile water to centrifuge tube, vortex to mix.
- Place all mixed centrifuged stock into new sterile glassware and label "Centrifuge stock"
- Dilute centrifuge stock for plating 10^{-6}
- Plate centrifuge stock in triplicates and place in incubator to count the following morning.

A.11) Preparation of Agar Plates

Materials: Clean 1 L jar with screw cap, stir bar, Milli-Q water, volumetric flasks (1L and 500mL), tryptic soy agar, weighing dish, stirring hot plate, balance, disposable Petri dishes (size: 100mmx15mm), autoclave indicator tape, masking tape, marker

Procedures:

- Place the stir bar into the media/storage bottle and place on the stirring hot plate; turn on the stir mechanism so the stir bar is just stirring in the center of the jug's bottom.
- Mass out 30g of tryptic soy agar into a weighing dish on the balance.
- Measure out 0.75L of Milli-Q water using the volumetric flask.
- Add the powdered agar to the jug followed by the water.
- Increase the stirring speed so a vortex is just forming and turn on the heating element to a low level (2 on a scale of 10).
- Allow the agar to mix completely with the water.
- Loosely tighten the cap to the jug and place a piece of indicator tape to the jug
- Autoclave the jug containing the agar by placing the jug in the autoclave, filling the autoclave with water, closing the autoclave door, and setting the autoclave to the "Slow" setting and the timer to 25 minutes.
- After the autoclave has finished running and cooled to less than 100°C, the jug containing the agar is removed from the autoclave (gloves should be worn to prevent burns to the skin) and placed on the bench to cool for one to two hours.
- After the jug has cooled enough to handle, each Petri dish is filled with approximately 15 to 20mL of agar by pouring the agar into the smaller of the dishes. Exposure of the agar and plate to air should be limited.
- After the agar is poured into the plate, the plate is stored on a level surface for 24 hours before being placed media-side up in a plastic bag designed for storage of the poured plates. Approximately 20 plates can be stored per bag.
- Filled bags are then closed, labeled with date and contents, and then stored in the refrigerator until needed.

A.12) Spiral Plating (Kohler 2009)

Materials: Ethanol, sterile water, plating cups, Model D spiral plater, agar plates, permanent marker, 250mL flask for waste, Parafilm, incubator set to 35°C

Procedures:

- Label agar plates with the date, sample ID, sample dilution, and triplicate plate letter (ex: March 1st, t=4 hours, RDI 1, 1/3)
- Place the number of plating cups (number of samples+2) in the biosafety hood where the spiral plater is located; turn on blower and UV for the hood
- After 15 minutes, turn off the UV for the biosafety hood and turn on the light and outlet
- Attach the vacuum flask of the spiral plater to the vacuum valve in the hood
- Turn on the vacuum to the hood and ensure that a vacuum is being pulled on the vacuum flask
- Place ethanol, sterile water, 250ml flask for waste, samples, and labeled agar plates in the safety hood and turn on the spiral plater
- Fill one of the plating cups with ethanol and one with water, place filled cups in the cup holder of the spiral plater
- To clean the stylus:
    1. Move the cup holder so that the ethanol is under the stylus
    2. Lower the stylus into the ethanol using the stylus lift arm
    3. Rinse the stylus with ethanol by placing the valve in the on position for 5 seconds; turn off the valve
    4. Lift the stylus up using the lift arm, rotate the cup holder and place the stylus in the sterile water
    5. Rinse the stylus with ethanol by placing the valve in the on position for 5 seconds; turn off the valve
- To plate the sample:
    1. Fill one of the plating cups with the sample and place in the cup holder
    2. Move the cup holder so that the sample is under the stylus
    3. Lower the stylus into the ethanol using the stylus lift arm, make sure only the plastic portion of the stylus is submerged in the sample
    4. Rinse the stylus with sample by placing the valve in the on position for 5 seconds; turn off the valve
    5. Collect sample in the stylus by turning on the valve for 3 seconds (make sure the plastic portion of the stylus is submerged in the sample); ensure no bubbles are present in the sight glass
    6. Move the cup holder off to the side so that it is not over the turntable
    7. Place agar plate labeled with the sample ID on the turntable agar side down and remove the cover of the agar plate
    8. Lower the stylus onto the agar and start the automatic plating by pushing up (do not hold) on the start switch; the stylus will return to its start position after the sample has been plated
    9. Return the cover to the agar plate and place the plate agar side down to dry (once dry the plates can be stacked agar side up)
    10. Repeat steps 5 through 9, two more times for the triplicate plates
    11. Empty the sample cup into the 250mL flask and rinse with ethanol

- Repeat the process of cleaning the stylus and plating samples until all samples are plated; end with cleaning the stylus
- After all samples have been plated and the stylus cleaned a final time, turn off the spiral plater and empty the sterile water and ethanol cups into the waste flask, rinse cups with ethanol
- Turn off the vacuum to the hood, empty the contents of the vacuum flask into the waste flask
- Remove the samples, agar plates, waste flask and sterile water from the hood
- Wipe down the hood with ethanol and then remove the ethanol from the hood
- Turn off the hood outlet and turn on the UV light; leave on for 15 minutes
- Wrap the sides of the agar plates with Parafilm and then place in the incubator agar-side up
- After 15 minutes have passed, turn off the UV and blower on the biosafety hood then stack the plating cups and discard or place upside down on lab bench for future use.

A.13) Disposal of Used Agar Plates (Kohler 2009)

Materials: Agar plates for disposal, autoclave indicator tape, biohazardous autoclave bags, non-biohazardous certification tag, masking tape, autoclave bucket

Procedures:

- Place agar plates in the biohazardous autoclave bags, tape shut with masking tape and place a piece of indicator tape on the bag
- Place the bag in an autoclave bucket and autoclave for 25 minutes on the "liquid setting".
- Once autoclave has cooled, remove contents from autoclave and ensure indicator tape has changed
- Attach a completed non-biohazardous certification tag to the bag
- Remove the white copy for the tag and place in the pocket on the Disinfected Biological Waste trash can and place the bag of agar plates in said trash can.

A.14) Analyzing TOC Samples (Kohler 2009)

Materials: Sievers 800 Portable Total Organic Carbon Analyzer with Automated Sampler; computer loaded with DataPro v02.07; samples collected in sterile 40mL vials; sterile 40mL vials containing Milli-Q water (if diluting); 40mL vials containing deionized water as blanks (x4); phosphoric acid

Procedures:

- Transfer collected sample to an autoclaved 40mL glass vial
- If sample taken under spike conditions dilute the sample in Milli-Q water. For effluent samples dilute at a ratio of 1 in 3 (1 part sample to 3 parts dilution water) and for control samples dilute at a ratio of 1in 5. A sample of the Milli-Q water should also be collected for analysis
- Preserve samples by adding 2 drops of phosphoric acid
- If samples are to be analyzed at a later date, place in refrigerator; otherwise, go on to the next step
- Place samples in TOC automatic sampler rack from expected low to high concentration (Milli-Q, flushing phase samples, diluted control samples from spike phase, diluted

effluent samples from spike phase). Two blank samples should be placed at the beginning and end of the run.

- Set-up the analysis protocol on the computer using the DataPro (v02.07) software. The first two and last two blank samples should already be entered into the program. Leave these fields as they are.

For the samples to be analyzed enter into the following into the appropriate field: □□"Group Name": type sample identification into field
□□"TYPE": select "Sample" from the pull down menu
□□"VIALS": enter the number of vials into the field, typically "1"
□□"REPS": enter the number of desired repetitions, typically "4"
□□"REJECTS": enter the number of reject samples, typically "1"
□□"ACID RATE": enter the desired acid rate, for acidified samples this should be set to "0"
□□"OXID RATE": enter the desired rate of oxidizer addition, depends on expected TOC concentration. For Milli-Q water enter "0.2", for flushing condition samples enter "1.0", for spike condition samples enter "1.2".
Save the analysis set-up, include initials in the protocol file name.
Run the analysis by pressing "Run", entering name into "Analysts name" field and then pressing "OK".

- After the samples are done being analyzed the computer will print out the TOC results.

A.15) Colloidal Silver Solution Preparation (Best Practices 2010)
Materials:
Procedure: Mixing of the stock 3.2 percent solution was performed according to the following procedure:

- Add 0.32 g of Argenol silver into 10 mL of DI water. The primary silver solution at a concentration of 32000 ppm was made
- Then 2 mL of this concentrated solution was added to 300 mL of DI water resulting in a concentration of 213 ppm.
- This entire 300 mL of solution was painted on every part of the filter, inside and out with a clean brush (~200 mL inside and ~100 mL outside)