REMOVAL OF ESCHERICHIA COLI FROM STORMWATER USING MYCOFILTRATION

By

ALICIA ANN FLATT

A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN CIVIL ENGINEERING

WASHINGTON STATE UNIVERSITY
Department of Civil and Environmental Engineering

MAY 2013

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of ALICIA ANN FLATT find it satisfactory and recommend that it be accepted.

_____
Marc W. Beutel, Ph.D., Chair

_____
David Yonge, Ph.D.

_____
Jennifer Adam, Ph.D.

ACKNOWLEDGEMENT

First, I would like to thank the EPA and Fungi Perfecti for funding this research and their support throughout the last year; Paul Stamets, who first discovered mycofiltration's ability to remove fecal coliform from surface water, Alex Taylor, who has been monumental to the development of my thesis, and Morgan Wolff, Katie Brownson, and Regan Nally for all their help with this project. I would also like to thank Lisa Orfe, Dr. Doug Call and their staff at WSU vet med for truly going above and beyond in aiding us with our microbiological efforts. My advisor, Dr. Marc Beutel has also been paramount to my success at WSU, and I'd like to thank him for his encouragement and advice throughout this project. I'd also like to thank the other members of my board: Dr. David Yonge for showing me how to truly learn the logic behind the design and Dr. Jenny Adam for her advice and support over the years, and for encouraging me to transfer to WSU as an undergraduate. Also, I'd like to thank Louis Neira for his dedication to this project and willingness to work long, countless hours in the lab. I'd like to thank the other lab assistants who provided invaluable help on this project: Brian Beleau, Becca Kloster, and Jake Mullins. Thanks to the other CEE graduate students at WSU for their support and for being a sounding board for ideas. Lastly, I'd like to thank my family for their support, my brother Joshua for providing encouragement and comic relief, and my boyfriend Brian Floyd who has helped me through every peak and valley since the moment I stepped onto campus my freshman year. This research was supported, in part, by the National Center for Environmental Research, U.S. Environmental Protection Agency, under contract number: EP-D-12-010 with funding from the Small Business Innovative Research Grants Program. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Environmental Protection Agency.

REMOVAL OF ESCHERICHIA COLI FROM STORMWATER

USING MYCOFILTRATION

Abstract

By Alicia Ann Flatt, M.S.
Washington State University
May 2013

Chair: Marc W. Beutel

Pathogens from nonpoint sources are the leading cause of water quality impairments in US surface waters. Pathogen contamination causes millions of waterborne illnesses and tens of thousands of beach closures each year, and poses a serious threats to coastal shellfish harvesting—a critical economic activity in Washington State. Because of its low cost, ease of operation, and unique biochemical properties, there is growing interest in the use of mycofiltration to sustainably remove pollutants from urban and agricultural runoff. This study assessed the feasibility of mycofiltration to remove *E. coli* from synthetic stormwater as part of a Phase I EPA Small Business Innovative Research grant. Fungi species including *Irpex spp.*, *Stropharia spp.* and *Pleurotus spp.* were grown in mycofilters consisting of 5 gallon buckets with dense but permeable mycelium growth on wood chips and/or straw. Replicate mycofilters were loaded with dechlorinated tap water spiked with ~700 cfu/100 mL of *E. coli* at low (0.5 L/min) and high (2.2 L/min) hydraulic loading. Mycofilters were also tested in series (3 filters) at a hydraulic loading of 0.3 L/min. Influent and effluent was monitored for fecal coliform and *E. coli* using the EPA approved Coliscan membrane filter method. Biological monitoring was more of a challenge than anticipated due to the complex microbiology of the mycofiltration media.

Results generally confirmed that mycofilters had the capacity to remove *E. coli* under sediment-free conditions at a rate of roughly 20% per linear foot, with better removal at low hydraulic loading, increased filter media (series tests), and sediment-containing conditions. However, the mycofiltration media, in some cases, exported bacteria that caused false positives for fecal coliform (*Raoultella spp*. formally *Klebsiella spp*.) and *E. coli* (*Enterobactor spp.* and *Staphylococcus spp.*), which were identified via genetic testing. Results highlight the challenges of using traditional microbial indicator methods, such as enzyme-linked chromogenic media, to assess the capacity for ecotechnologies like mycofiltration to remove pathogens from polluted waters.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1 Background

Microbial pathogens from nonpoint source discharges are a significant public health concern. Pathogens, including those from nonpoint sources, are the primary cause of surface waters quality impairments in the United States (USEPA, 2012a). Pathogen-related impairments have substantial societal and environmental costs: millions of stormwater-attributable waterborne illnesses each year cost hundreds of millions of dollars (Gaffield et al., 2003). Pathogens pose a serious threats to shellfish harvesting, a special concern in Washington State where the commercial shellfish industry is valued at $80 million annual and where fishing- and shellfish-related license sales and recreational expenses exceed $900 million per year (Booth et al., 2006). Every year tens of thousands of beach closures nationwide cost local communities reliant on tourism and recreation thousands of dollars per day (NRDC, 2012).

Fecal coliform is a common indicator bacteria pollutant in storm water which can result from misconnected, leaking, or overflowing sanitary sewers and storm water contact with pet or animal waste (Clary et al., 2007; Thaddeus and McOliver, 2010). Indicator bacteria is a term used to generally describe fecal coliform bacteria, which are found in the intestinal tract of warm-blooded animals. Another common indicator organism is *Escherichia coli* (*E. coli*), a gram-negative, rod shaped organism that is useful for detecting fecal contamination in fresh waters in the US (USEPA, 2012b). Fecal coliform bacteria has historically been used to detect fecal contamination, however in the 1970's and 1980's the USEPA conducted a number of epidemiological studies which revealed that *E. coli* has a stronger correlation with fecal contamination in recreational waters (USEPA, 2012b). Direct contact with fecal contamination can increase the risk of adverse health effects such as fever, earache, sore throat, and

gastrointestinal illness and poses a particular threat to swimmers (Haile et al., 1999). The Washington State Department of Ecology regulates fecal coliform in surface waters of the State. Values are not to exceed 100 colony forming units per 100 mL of sample (cfu/100 mL) for primary contact recreation in freshwater, 200 cfu/100 mL for secondary contact recreation (e.g., wading or fishing), and 14 cfu/100 mL for shellfish harvesting areas (State of Washington, 2011).

A number of best management practices (BMPs) have been developed to decrease the level of pathogens in nonpoint sources. Extensive studies using the International Stormwater BMP database have been performed to assess the pathogen removal capabilities of various BMPs (Clary et al., 2010; Clary et al., 2007). Findings show that no BMP offers effective fecal indicator bacteria removal to standards for primary contact recreation (200 cfu/100 mL for fecal coliform). Retention ponds showed some capability of removing bacteria in regions with significant land area and adequate water rights or abundant rainfall, but are impractical in highly urban areas and arid/semi-arid climates due to the lack of space and water availability. Retention ponds also demonstrated exports of bacteria, likely due to deposits from waterfowl and wildlife they attract. Media filters and biorentention cells also showed some capability of removing bacteria at the site level, but can be expensive and require regular maintenance to maintain performance. Grass swales and manufactured devices also showed limited abilities of removing bacteria and, similar to retention ponds, often exported bacteria. Detention ponds exhibited some removal at low loading rates (< 200 cfu/100 mL), but were ineffective at higher loading rates (> 2,000 cfu/100 mL). Wetlands and porous pavement are suspected to have some effect on

bacterial removal, but the authors concluded that there was not enough data to support interpretations.

Sand filtration is possibly the only BMP that can consistently remove bacteria, but is limited by a low-loading rate and requires regular maintenance. Bright et al. (2010) concluded that sand filters could only effectively treat a loading rate of 3.45 cm/hr (0.054 L/min-ft^2). However, in their experiments, the sand columns loaded with the bacteria-spike stormwater were limited to a loading rate of ~0.5 in/hr (0.0197 L/min-ft^2) after 54 days due to clogging issues. Bacteria are also known to bind with sediment in natural systems, increasing their survivability and can also amplify clogging issues (Davies and Bavor, 2000; Clary et al., 2010).

1.2 Mycoremediation Biotechnology

Mycoremediation offers several unique mechanisms for removing bacteria from stormwater. Some species of fungi are known for their ability to attack bacteria and use them as a nutrient source (Fermor and Wood, 1981; Barron and Thorn, 1987; Hutchison et al., 1996; Hong et al., 2006). For example, *Stropharia spp.* has star-shaped cells, called acanthocytes, which grow from their hyphae and immobilize microbiota. Hong et al. (2006) experimentally showed that these cells inactivated 90% of nematodes within 15 min, with complete digestion of the nematodes in just 48 hours. Several other studies have shown other species of mycorrhizal fungi can lyse bacteria and use them as a sole source of nitrogen and carbon in an otherwise nutrient-deficient environment (Barron and Thorn, 1987; Fermor and Wood, 1981). Fungi are also uniquely capable of rapidly adjusting to changes in their environment through mechanisms such as the production of mycotoxins and altering their morphogenic courses (Ramos et al., 2008; Duran et al., 2010). Although the ability of fungi to attack bacteria has been well-documented, the extent

and specific mechanisms vary for each species of fungi and different forms of bacteria. For example, the ability of fungi to attack live bacteria is still unknown in some cases, and while some species like *Pleurotus* produce droplets of mycotoxins that can immobilize and attack bacteria, others like *Stropharia* can only degrade bacteria that comes into contact with its uniquely shaped hyphae (Fermor and Wood, 1981; Barron and Thorn, 1987; Hong et al., 2006). The diversity of fungal processes and abilities makes it difficult to predict *E. coli* removal rates in mycofilters, although they are expected to perform better than existing BMPs due to these unique removal mechanisms.

1.3 Previous Studies

There are only a handful of studies that have previously evaluated pathogen removal using mycofiltration. A pilot-scale mycoremediation study in the Dungeness Watershed, WA was conducted in 2009 to examine the *E. coli* removal of two myco-biofilters, one control and one containing fungi (Thomas et al., 2009). The myco-biofilters were loaded at approximately 1.4 L/min (0.0327 L/min-ft^2) with lightly contaminated influent from a lagoon. This study reported a 66% reduction in fecal coliform in the control biofilter and a 90% reduction in the experimental treatment biofilter. Another study performed at Evergreen State College looked at a lab-scale mycoremediation study using columns of alder sawdust seeded with *Pleurotus* mycelium (Rogers, 2012). This study showed a 20% reduction in the experimental mycofilters heavily loaded with coliform at hydraulic loading rates ranging from 2 mL/min to 20 mL/min (0.01 to 2 L/min-ft^2). Both studies used a wood-based substrate for fungal growth, which can be a source of *Klebsiella*, a non-fecal bacterium that is a false positive in fecal coliform tests (Caplenas et al.,

1981; Caplenas et al., 1984). Clearly, more research is needed to assess the feasibility of mycofiltration to remove pathogens from stormwater.

1.4 Project Objectives

The principal objective of this study was to evaluate the ability of mycofiltration to remove *E. coli* from synthetic stormwater. In measuring pathogens, the study used a chromogenic medium that differentiated between *E. coli* and general fecal coliform for enumerations. *E. coli* was chosen for the study because of its persistence in surface waters relative to other bacteria, and because regulating agencies are moving away from fecal coliform as an indicator bacterium. In addition, by focusing on *E. coli*, removal of bacteria could be measured without the interference of false positives from *Klebsiella*, a bacterium that is commonly found in the wood substrate used for fungal growth (Caplenas et al., 1981; Caplenas et al., 1984). This study, which was funded through the EPA Small Business Innovative Research Program, first examined the removal capabilities of one treatment of *Irpex spp*. and two treatments of *Stropharia spp.* in triplicate at two hydraulic loading rates. One of the two *Stropharia spp.* treatments was vigor tested by Fungi Perfecti LLC, the company which collaborated on this project, to assess the durability of the mycofilters under stressed conditions. The second part of this study looked at the effects of increasing the filter media volume to influent ratio by placing three filters of the same treatment in series. The series tests were conducted on vigor tested *Stropharia spp.*, non-vigor tested *Stropharia spp*., and non-vigor tested *Pleurotus spp*. The vigor tested *Stropharia spp.* and non-vigor tested *Pleurotus spp.* were additionally tested with a sediment/bacteria spiked influent to examine the effects of bacteria sorption onto sediment.

2. METHODS

2.1 Overview

Several variables were tested to evaluate the overall ability of mycofilters to remove *E. coli* from stormwater, including substrate and fungi type, increasing filter media volume, and the addition of sediment to the stormwater influent. The fungi and corresponding substrates were chosen by Fungi Perfecti LLC to be the most resilient species to climate variations and cost-efficient. Two main testing strategies were used to identify the removal efficiencies of the mycofilters: a single mycofilter test where one filter was tested at a time and a series mycofilter test where three filters were connected in series to triple the total treatment media volume (see Table 2-1). For each mycofilter, a 5 gallon bucket (1.2 ft high and 0.75 ft^2 cross-section) was prepared by drilling two rings with five 3/16-inch diameter holes in the center bottom of the bucket. Measured from the outside of the holes, the diameter of the inner ring is approximately 1 inch and the diameter of the outer ring is approximately 2 inches. To prevent the filter's substrate from clogging the holes, a wire mesh screen cut to a 4-inch diameter was placed over the holes on the inside of the bucket and tacked at four edges with silicon glue.

Each mycofilter was initially submerged in 9 L of dechlorinated tap water with no *E. coli* to achieve a uniform level of saturation, and then allowed to drain for 15 min prior to testing. The tap water available in the lab was advantageous because, in contrast to de-ionized water, it is extracted from an aquifer source and contains some minerals, which is more representative of stormwater runoff. Following the submersion period, each mycofilter was loaded from an individual 30 L batch of influent. For the single mycofilter tests, the influent tank was used for each filter and for the series tests, the influent tank was used for each replicate test. To prepare the influent, a large, clean plastic container was filled with 30 L of tap water, dechlorinated with

0.75 g of sodium thiosulfate, and allowed to mix for 15 min using an aquarium air pump with air stones. A 5 mL stock solution of *E. coli* ATCC 11775 inoculum was prepared by incubation in a 5 mL vial of Trypticase Soy Broth at 250 rpm and 37 ^{o}C for 16-18 hrs until the culture reached stationary phase, as determined by consistent cell densities on several drop-plate serial dilutions. The stock solution was then used to prepare a 1 mL diluted solution with a concentration of approximately $2 \cdot 10^{7}$ cfu/100 mL that was used to inoculate the influent. This produced a final influent volume of 30 L with an *E. coli* concentration of around 700 cfu/100 mL. This percolation solution preparation was repeated for each mycofilter percolation test. All of the mycofilters were tested with an *E. coli* solution inoculated from the same stock culture plate that was stored at 4^{o}C. One exception was the last series mycofilter tests-*Stropharia spp.* grown on wood chips, fine chips, and straw which used the same stock *E. coli* from lyophilization, but a fresh agar plate.

2.2 Single Mycofilter Tests

Fungi Perfecti LLC provided two treatments of *Stropharia spp.* (grown out on a mix of whole and shredded alder chips), one treatment of *Irpex spp.* (grown out on a mix of whole and fine alder chips and straw) and their corresponding control filters (substrate only) for this component of the study. Of the two *Stropharia spp.* treatments, one was "vigor-tested" by Fungi Perfecti LLC as part of a parallel study to test for biological resilience of the fungi to cycles of saturation, drying, heating, and freezing. When not being tested, mycofilters were stored in a 13 ^{o}C walk in cooler. To assure that testing is controlled for temperature, each mycofilter was acclimated in the laboratory at room temperature (~20 °C) for 48 hours before testing. The mycofilter was placed on a drainage basin held 8½ inches above the lab bench by two stacked

bricks on either side of the bucket. The bricks also supported the edges of a 5½ inch diameter plastic funnel with a ½ inch diameter, 2 foot long plastic tubing attached to neck of the funnel. During testing, the holes in the bottom of the 5 gallon bucket were aligned with the top of the funnel for effluent collection. A Masterflex 7523-20 peristaltic pump with a 7018-52 head and fitted with Masterflex L/S-18 tubing was used to pump the influent water from a feed tank into the mycofilter. Flow was distributed over the top of the mycofilter through a coiled discharge line placed on top of the mycofilter material. The line consisted of a coiled, ½ inch soft-walled tube with small holes every 2-4 inches along the tube. Material at the top of the mycofilter was also gently formed into a conical shape on the top of the filter to promote drainage into the center of the mycofilter.

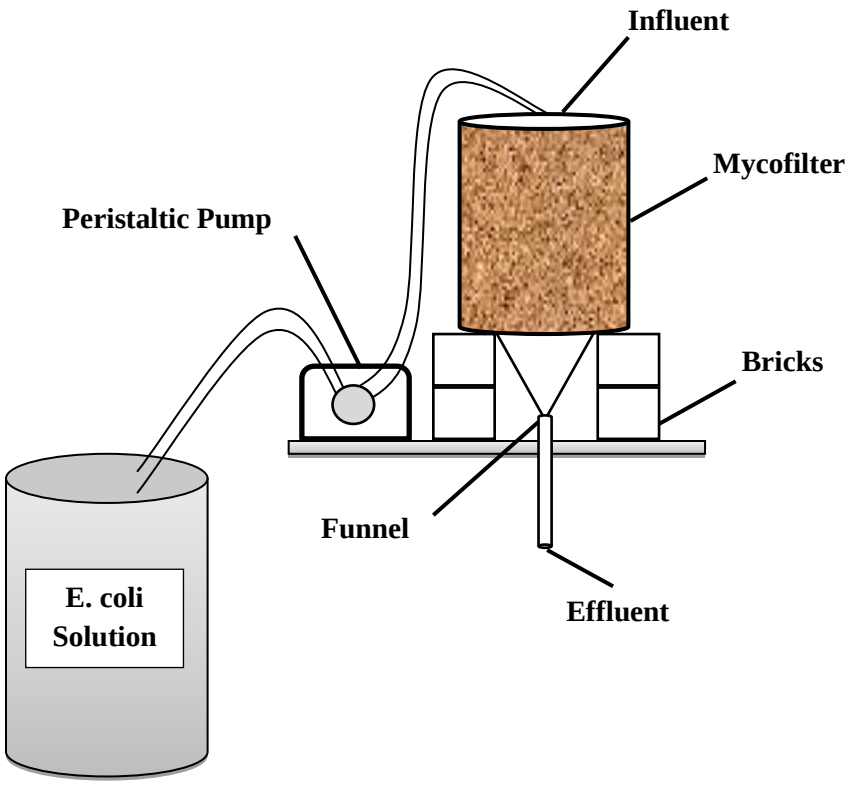

FIGURE 2-1 EXPERIMENTAL SET-UP FOR SINGLE MYCOFILTER TESTS

After the initial submerge and drain period, dechlorinated tap water containing ~700 cfu/100 mL *E. coli* was percolated through the mycofilter at a rate of 0.5 L/min (0.67 L/min-ft^2) with samples being collected at 0 (when outflow starts), 5, and 10 minutes. The mycofilter was allowed to drain for 15 minutes, and then loaded with 2.2 L/min (2.93 L/min-ft^2) of percolation solution. Again, samples were collected at 0, 5 and 10 minutes. Inflow samples were also collected at the beginning of each filter run. To confirm system cleanliness, water samples were also collected during the initial submerge period. So, for each filter test a total of 10 water samples were collected (2 samples during submerge period; 2 inflow samples; 3 outflow samples during the 0.5 L/min test; 3 outflow samples during the 2.2 L/min test).

2.3 Series Mycofilter Tests

The vigor-tested *Stropharia spp.* and corresponding controls from the single mycofilter tests were used again for the series mycofilter tests. Fungi Perfecti LLC provided new filters of *Pleurotus spp.* (grown out on a sterilized substrate of whole chips, fine chips, and straw), and a new set of *Stropharia spp.* (non-vigor tested) grown on wood chips, fine chips, and straw and its corresponding control filters (substrate only). The set-up for the series tests was nearly identical to the single mycofilter tests, with the schematic adapted to allow the effluent of the first filter to be the influent of the second filter and so on for a total of three filters in series. The same pre-soak batch of 9 L of dechlorinated tap water was run through the first filters, then allowed to percolate through the bottom-most filter prior to testing, with two influent samples and an effluent sample taken from each filter in series to confirm system cleanliness. The same influent batch and pump from the single tests were used with the hydraulic loading rate adjusted to 0.3

L/min (0.4 L/min-ft^2) to achieve a high removal efficiency in the system. The system was loaded for 30 min, with influent samples taken at 5, 15, and 25 min and effluent samples taken from each filter in series at 10, 20, and 30 min. Following this, the system was allowed to drain for 30 min, then the test was repeated for a total of 3 runs. Three of each of the "old" *Stropharia spp.*, its controls, and the new *Pleurotus spp.* filters were used for both the bacteria and the bacteria/sediment tests (a total of 6 tests were run on each filter). For the bacteria/sediment tests, silica sand with a mean diameter of 125 microns (Sil-Co-Sil 125) was added to the influent tank and kept in suspension using an air pump with air stones. The final sediment concentration in the influent was 20 mg/L. During testing, it was found that the filters can be extremely biologically active prior to testing, which can interfere with *E. coli* enumerations. In an effort to reduce this interference, the "new" *Stropharia spp.* filters (grown out on wood chips, fine chips, and straw) were pre-soaked individually with 9 L of dechlorinated tap water per filter. Additionally, the "new" *Stropharia spp.* filters and controls were swapped out for each run, so each filter was only tested once. Table 2-1 below summarizes the single and series mycofilter tests.

TABLE 2-1: SUMMARY OF EXPERIMENTAL SET-UPS

Single Mycofilter Tests	
Hydraulic Loading	0.5 L/min (0.67 L/min-ft^2) for 10 min 2.2 L/min (2.93 L/min-ft^2) for 10 min
Sampling	• Influent at t = 0 min for both loading rates • Effluent at 0, 5 and 10 min
Filter Media	• *Stropharia spp*. on wood chips and shredded chips (control, non-vigor tested, and vigor) • *Irpex spp*. on whole chips, fine chips, and straw (control and non-vigor tested)
Series Mycofilter Tests	
Hydraulic Loading	0.3 L/min (0.4 L/min-ft^2) for 30 min
Sampling	• Influent at 5, 12, and 25 min • Effluent at 10, 20, and 30 min
Filter Media	• *Stropharia spp.* on wood chips and shredded chips (control and vigor-tested)*† • *Pleurotus spp.* on whole chips, fine chips, and straw (non-vigor)* • *Stropharia spp.* on wood chips, fine chips, and straw (control and non-vigor)

*Filters were used for both bacteria and bacteria/sediment tests.
† *Stropharia spp.* filters and controls were previously used in single mycofilter tests.

2.4 E. coli and Fecal Coliform Enumeration

All influent and effluent samples were collected in sterile sample bottles and stored at 4 °C. Samples were tested within 6 hours of collection. Each sample was simultaneously monitored for *E. coli* and fecal coliform using the Coliscan C MF method, a U.S. Environmental Protection Agency (EPA) approved method (9222C) distributed by Micrology Laboratories (http://www.micrologylabs.com/Home). Fecal coliform was also measured to assess the potential for false positives due to presence of non-fecal *Klebsiella* species bacteria that are commonly found on decaying wood (Caplenas and Kanarek, 1984). For the single mycofilter tests, the pre-soak effluent was tested at a 1:5 and 1:10 dilution in duplicate and the remaining influent and effluent samples were tested at 1:20 and 1:10 dilutions in duplicate. For the series tests, the pre-

soak effluents were tested at a 1:10 dilution in duplicate and the influent and effluent samples were tested at a 1:20 dilution in duplicate.

Enumeration of *E. coli* and fecal coliforms followed the procedure outlined in *Standard Methods for the Examination of Water and Wastewater* (Hunt and Rice, 2005). In short, 100 mL of diluted water sample was filtered onto a 0.45 μm filter pad and then transferred to a petri dish containing an absorbent pad soaked with 1.75 mL of Coliscan MF medium. The dish was then incubated, inverted, at 35 ^{o}C for 24 hours. The Coliscan medium has two color producing chemicals, one that is activated by the enzyme galactosidase which is produced by general coliforms, and one that is activated by the enzyme glucuronidase which is produced by *E. coli* only (Micrology Labs). After the 24-hr incubation period, *E. coli* colonies were blue/purple in color and general coliforms were pink. Method blanks were also performed after approximately every 10 filters. Measurements of *E. coli* levels in effluent from the filtration experiments for each test type, loading rate, and each mycofilter type were then tabulated. Percent removal for each test was calculated by the following equation:

$$\text{Percent Removal} = \frac{C_{in}\text{-}C_{out}}{C_{in}} \times 100\%$$

Where C_{in} is the *E. coli* concentration in the influent and C_{out} is the *E. coli* concentration in the effluent.

2.5 False Positives and Bacterial Identification

The Coliscan MF method used to enumerate bacterial colonies uses a chromogenic media to distinguish *E. coli* (blue in color) and general fecal coliforms (pink in color). Prior to testing, it was expected that the wood substrate that the mycelium was grown on would produce non-fecal *Klebsiella* which are false positives for general fecal coliform (Caplenas and Kanarek, 1984).

Subsequently, all fecal coliforms were treated as false positives (later confirmed by bacterial identification) for the duration of the experiments. Some of the mycelium also presented colonies that were smaller in diameter than the influent *E. coli*, as well as teal in color. In some cases, these teal colonies were tested with Micrology Lab's Kovac's solution, an indole presence test. To use the Kovac's solution, a small drop was added to a blue/teal colony. If the colony is *E. coli*, the solution will turn bright red indicating a positive result, and if the colony is negative (not *E. coli*) the solution will remain yellow in color. In addition to on-site false positive identification efforts, some samples were sent to Microcheck (Northfield, VT) for bacterial identification. Samples were sent as the whole Coliscan MF plates, or in some cases specific colonies were struck out onto a brain heart infusion agar, incubated overnight, and then sent to Microcheck.

3. RESULTS

3.1 Single Mycofilter Tests

For the single mycofilter tests, the 0 min and 5 min effluent enumerations were low relative to the 10 min effluent counts. This suggested that the samples at 0 and 5 min were possibly a mix of pre-soak (*E. coli* free) solution and the actual bacteria-spiked influent. As such, only the10 min effluent enumerations were used in the percent removal calculation. Fungi-free controls showed virtually no removal at both the low and high hydraulic loading (Table 3-1). In contrast, the *Stropharia spp.* (single) mycofilters demonstrated capability to remove *E. coli* from the influent. The non-vigor tested mycofilters exhibited an average removal of 24% at low hydraulic loading and 4% at high hydraulic loading. The vigor tested mycofilters exhibited a similar removal rate of around 20% at both hydraulic loadings.

TABLE 3-1: *STROPHARIA SPP.* SINGLE MYCOFILTER RESULTS

		Low Flow (0.5 L/min)		High Flow (2.2 L/min)	
Replicate	Influent[a]	Effluent[b]	Percent Removal[c]	Effluent[b]	Percent Removal[c]
		Un-inoculated Controls			
1	759 ± 40	717 ± 38	6	755 ± 15	0
2	721 ± 28	688 ± 32	5	743 ± 43	-3
3	601 ± 37	575 ± 53	4	588 ± 29	2
Average ± Standard Error			5 ± 0		0 ± 2
		***Stropharia* Mycofilters (not vigor tested)**			
1	725 ± 57	535 ± 56	26	603 ± 40	17
2	679 ± 20	540 ± 21	20	665 ± 39	2
3	701 ± 40	530 ± 10	24	745 ± 15	-6
Average ± Standard Error			24 ± 2*		4 ± 7
		***Stropharia* Mycofilters (vigor tested)**			
1	933 ± 49	678 ± 25	27	783 ± 37	16
2	660 ± 49	630 ± 46	5	508 ± 47	23
3	781 ± 36	590 ± 29	24	588 ± 45	25
Average ± Standard Error			19 ± 7		21 ± 3

[a]Influent values are average plus/minus one standard deviation of quadruplicate bacteriological analyses conducted on two samples collected at the start of each run (low flow and high flow).

[b]Effluent values are average plus/minus one standard deviation of quadruplicate bacteriological analyses conducted on samples collected after 10 minutes.

[c]Percent removal is calculated as $(C_{in} - C_{out}) / C_{in} \times 100$.

Similar to the *Stropharia spp.* analysis, only the 10 min effluent samples were used in calculating the percent removal for the *Irpex spp.* mycofilter tests. The *Irpex spp.* controls, a mix of wood chips and straw, yielded an average increase in *E. coli* of 88% at the low hydraulic loading and 29% at high hydraulic loading (Table 3-2). The inoculated filters exhibited average removal rates of 5% at the low hydraulic loading and 3% at the high hydraulic loading. This was the first trial that showed increased levels of *E. coli* in the effluent of the controls, which was later hypothesized to be a result of false-positives, likely due to the straw media used in the mycofilters.

TABLE 3-2: *IRPEX SPP.* SINGLE MYCOFILTER RESULTS

		Low Flow (0.5 L/min)		High Flow (2.2 L/min)	
Replicate	Influent[a]	Effluent[b]	Percent Removal[c]	Effluent[b]	Percent Removal[c]
		Un-inoculated Controls			
1	700 ± 42	1263 ± 73	-80	705 ± 22	-1
2	838 ± 33	TNTC	N/A	1453 ± 113	-73
3	679 ± 31	1333 ± 97	-96	760 ± 24	-12
Average ± Standard Error			-88 ± 8		-29 ± 23
		***Irpex* Mycofilters (not vigor tested)**			
1	646 ± 21	568 ± 45	12	638 ± 62	1
2	706 ± 35	673 ± 62	5	638 ± 59	10
3	700 ± 59	713 ± 58	-2	723 ± 99	-3
Average ± Standard Error			5 ± 4		3 ± 4

[a]Influent values are average plus/minus one standard deviation of quadruplicate bacteriological analyses conducted on two samples collected at the start of each run (low flow and high flow).
[b]Effluent values are average plus/minus one standard deviation of quadruplicate bacteriological analyses conducted on samples collected after 10 minutes.
[c]Percent removal is calculated as $(C_{in} - C_{out}) / C_{in} \times 100$.

3.2 Series Mycofilter Tests

Following the single mycofilter tests, a new experimental set-up was devised to increase mycofilter volume to influent ratio, thereby increasing the contact time between synthetic stormwater and mycofilter media. The duration of the filter run was also increased to avoid any influence of the pre-soak step on effluent quality. The flow rate was decreased to 0.3 L/min and the test run time was increased to 30 min. The mycofilter volume was 'increased' by placing three filters in series, with 'Mycofilter 1' as the first in series, 'Mycofilter 2' in the middle, and 'Mycofilter 3' as the last in series. Percent removal was calculated based on influent samples taken at 5, 15, and 25 min and effluent samples taken from each bucket at 10, 20, and 30 min. The average removal of all three filters in series for each treatment was calculated to provide a

filter-by-filter breakdown of removal. In addition, the ‘overall removal’ or the composite average removal was calculated to provide a removal metric for the entire system (all three filters).

The first series mycofilter test included a control, the original vigor-tested *Stropharia spp.* mycofilters, and new non-vigor *Pleurotus spp.* The control filters showed average removal rates ranging from 12-19% and overall removal rates ranging from 31-48% (Table 3-3). The original *Stropharia spp.* mycofilters showed lower removal rates relative to their previous performance in the single filter trial, with average removal rates ranging from -3 to 4% and overall removal rates ranging from -11 to 15%. The new non-vigor *Pleurotus spp.* mycelium showed average removal rates ranging from 5 to 34% and overall removal rates ranging from 14-100%. These levels of removal were similar the *Stropharia spp.* mycelium that had been tested several months earlier.

TABLE 3-3: *STROPHARIA SPP.* AND *PLEUROTUS SPP.* SERIES RESULTS

	Run 1			Run 2			Run 3		
	Average	Stdev	% Conc Removal	Average	Stdev	% Conc Removal	Average	Stdev	% Conc Removal
CONTROL									
Influent	943	51		787	118		900	85	
Mycofilter 1 Effluent	600	207	36%	627	55	20%	637	60	29%
Mycofilter 2 Effluent	563	125	6%	583	50	7%	513	64	19%
Mycofilter 3 Effluent	490	151	13%	540	135	7%	510	60	1%
Average Removal			19%			12%			16%
Standard Error of Removal			9%			4%			8%
Overall Removal			48%			31%			43%
ORIGINAL VIGOR TESTED SR									
Influent	620	60		557	91		573	100	
Mycofilter 1 Effluent	547	45	12%	657	104	-18%	633	87	-10%
Mycofilter 2 Effluent	637	35	-16%	617	6	6%	577	85	9%
Mycofilter 3 Effluent	530	56	17%	607	150	2%	637	83	-10%
Average Removal			4%			-3%			-4%
Standard Error of Removal			10%			7%			6%
Overall Removal			15%			-9%			-11%
NEW NON-VIGOR TESTED *PLEUROTUS*									
Influent	747	81		873	87		703	40	
Mycofilter 1 Effluent	730	154	2%	803	76	8%	710	36	-1%
Mycofilter 2 Effluent	747	35	-2%	793	76	1%	697	96	2%
Mycofilter 3 Effluent	640	10	14%	450	139	43%	0	0	100%
Average Removal			5%			18%			34%
Standard Error of Removal			5%			13%			33%
Overall Removal			14%			48%			100%

Although the *Stropharia spp.* mycofilters lacked the performance observed in the first trials, observed *E. coli* colonies experienced a substantial decrease in size, pointing to a possible antibacterial mechanism at work. The reduction in colony size was observed as the bacteria

travelled through each successive mycofilter (Figure 3-1). This phenomena was also observed in the *Pleurotus spp.* mycofitler enumerations (Figure 3-2).

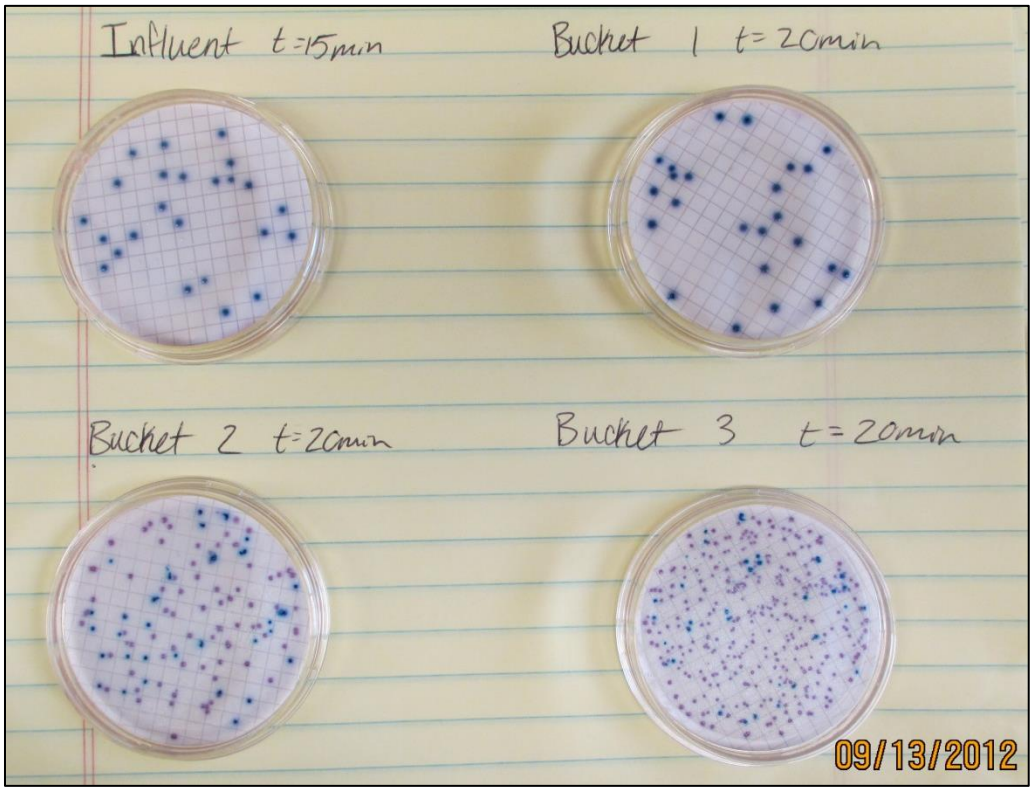

FIGURE 3-1: DECREASE IN SIZE OF COLONIES AFTER EXPOSURE TO *STROPHARIA SPP.* MYCELIUM (BLUE COLONIES ARE *E. COLI* AND PINK ARE FECAL COLIFORMS)

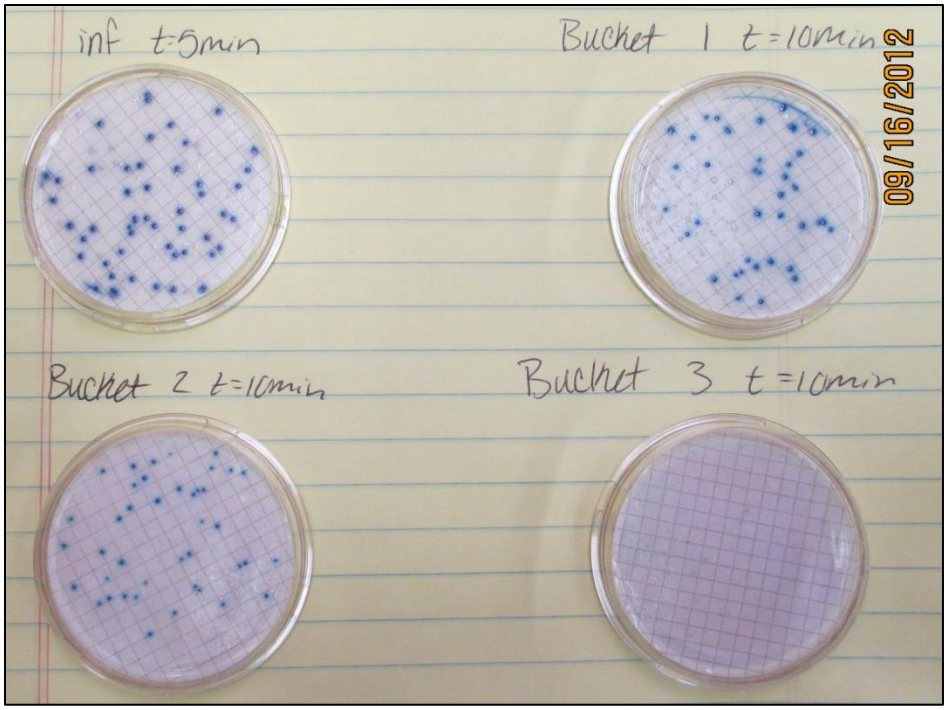

FIGURE 3-2: DECREASE IN SIZE OF COLONIES AFTER EXPOSURE TO *PLEUROTUS SPP.* MYCELIUM

For the second series mycofilter tests, a low level of suspended sediment was added to the influent tank to an approximate concentration of 20 mg/L. The original vigor-tested *Stropharia spp*. mycofilters exhibited poor performance during this trial, which was likely a result of senescence of the mycelium due to the extended periods of cold storage without supplemental water, then a drying period after the first series tests. Consequently, their removal rates were determined to be uncharacteristic and were not included in the analysis. For the sediment/bacteria trial, the controls showed average removal rates ranging from 13-18% and overall removal rates ranging from 38-45% (see Table 3-4). The *Pleurotus spp.* mycofilters had average removal rates ranging from 69-80% with overall removal rates of 100% for every replicate. The effluent enumerations from the *Pleurotus spp.* mycelium for the sediment/bacteria

tests were the second instance where false positives for *E. coli* were suspected (again, likely due to the straw used in the mycofilter media). Some effluent plates from the second and third filters in series had faint, teal blue-colored colonies that were tested with an indole presence test (Kovac's reagent). The testing showed that the teal colonies were not *E. coli*.

TABLE 3-4: *PLEUROTUS SPP.* SERIES WITH SEDIMENT RESULTS

	Run 1			Run 2			Run 3		
	Average	Stdev	% Conc Removal	Average	Stdev	% Conc Removal	Average	Stdev	% Conc Removal
CONTROLS									
Influent	877	67		853	116		833	29	
Mycofilter 1 Effluent	690	130	21%	587	92	31%	563	67	32%
Mycofilter 2 Effluent	597	176	14%	507	55	14%	477	59	15%
Mycofilter 3 Effluent	483	42	19%	507	49	0%	520	245	-9%
Average Removal			18%			15%			13%
Standard Error of Removal			2%			9%			12%
Overall Removal			45%			41%			38%
NEW NON-VIGOR TESTED PLEUROTUS									
Influent	917	101		940	62		1010	108	
Mycofilter 1 Effluent	750	75	18%	570	503	39%	930	87	8%
Mycofilter 2 Effluent	77	133	90%	0*		100%	0*		100%
Mycofilter 3 Effluent	0*		100%	0*		100%	0*		100%
Average Removal			69%			80%			69%
Standard Error of Removal			26%			20%			31%
Overall Removal			100%			100%			100%

0* - Blue colonies were observed but tested negative as E. coli.

The last experimental series testing effort evaluated a new batch of *Stropharia spp.* (non-vigor tested) and their corresponding controls in series (Table 3-5). All of the controls and two of the three inoculated runs showed bacterial 'production'. This posed the third instance of

suspected false positive results for *E. coli*. Roughly 20% of the plates from the inoculated filters were tested with Kovac's reagent, which tested positive for the effluent plates in runs 1 and 2, and negative for run 3. Overall, the results suggested that the mycofilters may be able to achieve 100% removal (as it did in run 3), but that the presence of straw in the mycofiltration media also likely contributed towards false positives for *E. coli*. Thus the Coliscan media was incapable of solely identifying actual *E coli*.

TABLE 3-5: NEW *STROPHARIA SPP.* SERIES RESULTS

	Run 1			Run 2			Run 3		
	Average	Stdev	% Conc Removal	Average	Stdev	% Conc Removal	Average	Stdev	% Conc Removal
CONTROL MYCOFILTERS									
Influent	620	73		687	109		650	94	
Mycofilter A Effluent	973	173	-57%	1,350	206	-97%	740	104	-14%
Mycofilter B Effluent	1,060	272	-9%	1,187	219	12%	>2000		
Mycofilter C Effluent	983	112	7%	1,400	185	-18%			
Average Removal			-20%			-34%			
Standard Error of Removal			19%			32%			
Overall Removal			-59%			-104%			
INOCULATED MYCOFILTERS									
Influent	730	125		630	134		740	166	
Mycofilter A Effluent	2,677	515	-267%	2,912	786	-362%	897	1,403	-21%
Mycofilter B Effluent	3,323	755	-24%	2,433	1,918	16%	0	0	100%
Mycofilter C Effluent*	2,197	1,834	34%	1,733	3,002	29%	0	0	
Average Removal			-86%			-106%			
Standard Error of Removal			92%			128%			
Overall Removal			-201%			-175%			100%

*Effluent from bucket 3 was negative for E coli in 20 min and/or 30 min samples.

4. DISCUSSION

4.1 E. coli Removal

Notable results of this study are primarily related to the series testing, with the exception of the ~20% removal realized in the initial single *Stropharia spp.* tests for both the vigor and non-vigor tested mycelium. The plated colonies from this trial were similar in size and color to the influent *E. coli*, and no significant export of *E. coli* was recorded relative to the influent. As such, the 20% removal realized in this trial may be the best representation of actual removal capabilities of each individual *Stropharia spp.* filter, as it was the only one where *E. coli* false positives were not suspected. (It was also notably the only trial where straw was not used in the mycofiltration media.) This removal rate also agrees with the ~20% removal of fecal coliforms noted by Rogers (2012) in a similar bench-scale study. In that study, glass columns (20 mm diameter and 200 mm in length) were seeded with *Pleurotus spp.* grown out on alder sawdust and loaded with a 100 ml solution of *E. coli* spiked water. The average influent *E. coli* concentration was 10^7 cfu/100 mL, significantly higher than the concentration used in this study (~700 cfu/100 mL). Hydraulic loading rates ranged from 2 mL/min to 20 mL/min (0.01 to 2 L/min-ft^2) in contrast to the 0.3 L/min to 2.2 L/min used in this study. By increasing the filter volume to the size of a 5 gallon bucket and reducing the *E. coli* concentration in the influent, this study should have experienced a higher removal rate than the Rogers study. One possible explanation is that the Rogers study used deionized water for the preparation of the influent and dilution of effluent samples in preparation for enumerations, which may cause the bacterial cells to become osmotically hypotonic and die. This study used dechlorinated tap water, which is rich in minerals and thereby preserves the cells during testing. Another notable difference between the two studies is the enumeration techniques used to count *E. coli* colonies. In the Rogers study,

enumerations were performed by serial dilutions onto agar plates which does not differentiate *E. coli* from general fecal coliform. Since both studies used a wood-based substrate, both could experience false positives from non-fecal *Klebsiella* (Caplenas et al., 1984; Caplenas et al., 1981). However, due to the enumeration method used by Rogers, false positives from *Klebsiella* could have been included in the *E. coli* counts, which is supported by the 20% increase in *E. coli* observed in this study's controls. Due to the high influent *E. coli* concentrations used, and the unknown effects from the dilution water and false positives, the Rogers study may be inconclusive about mycofilters' abilities to effectively remove pathogens from stormwater.

Another recent regional study of note is by Thomas et al. (2009). The study examined the *E. coli* removal of two biofilters, one control and one containing fungi. The two 3 m by 9 m biofilters were loaded at approximately 1.4 L/min (0.0327 L/min-ft^2) with the influent travelling through an array of native plants (seeded with a blend of mycorrhizal fungi for the experimental treatment), alder mulch (inoculated with *Stropharia spp.* and two types of *Pleurotus spp.* for the experimental treatment), sandy loam and organic compost, geotextile fabric, and gravel before being collected in an underdrain pipe. The cells were spiked with dairy lagoon waste to a fecal coliform concentration of approximately 30 cfu/100 mL. Although the study experienced a high reduction in the experimental treatment biofilters (90%), it is important to note that the control filter also experienced a reasonable removal rate of 60%. This could be explained by the other components of the biofilter (particularly the soil, geotextile fabric, and gravel) sorbing the bacteria in addition to the impacts from the fungi. Enumerations were conducted using the MF standard method SM18 9222D, in which a sample is filtered and then incubated on a selective media for fecal coliform growth. This technique also does not differentiate between *E. coli* and

general fecal coliforms, and was subjected to possible false positives from non-fecal *Klebsiella*. However, even considering potential false positives, the study demonstrated that biofilters seeded with mycorrhizal fungi, *Stropharia spp.*, and *Pleurotus spp.* are capable of removing pathogens from stormwater, notably even at low *E. coli* concentrations.

4.2 Non-lethal Effects on E. coli

In a number of cases, while mycofiltration did not exhibit complete removal of *E. coli*, it did have a size-reducing affect on the bacteria. For example, the non-vigor *Pleurotus spp.* filters in series, in addition to yielding overall *E. coli* removal rates of 14-100%, also resulted in size-reducing effect on the plated colonies as the bacteria travelled through each successive mycofilter. The same three filters were used for each run of this experiment, so it is possible that the change from 14% overall removal in the first run to 100% overall removal in the third run was a result of the mycofilters responding to repeated exposure to antagonizing organisms (*E. coli*). This same phenomenon also occurred during the series experiments on the vigor-tested *Stropharia spp.*, the sediment/bacteria tests on *Pleurotus spp.*, and the non-vigor *Stropharia spp.* in series. Fungi are uniquely capable of rapidly adjusting to changes in their environment through mechanisms such as the production of mycotoxins and altering their morphogenic courses (Ramos et al., 2008; Duran et al., 2010). One study reported that bacterial cell contents shrunk or disappeared, their cell walls reduced in thickness, and in some cases were even perforated by the hyphae when fungi was grown out on an agar plate with different bacteria colonies (Fermor and Wood, 1981). The reduction in cell size in this experiment was likely a result of the fungi activating their defense mechanisms to attack the *E. coli* in the influent.

4.3 Effects of Sediment on E. coli Removal

Stormwater is commonly high in suspended sediments, which bacteria are known to bind to, protecting the bacteria from ultraviolet radiation and predators and increasing their survivability once they have deposited onto surfaces or bottom areas. Several studies have shown that coliform bacteria concentrations are positively correlated with sediment concentration in natural waters (Schillinger and Gannon, 1985; Howell et al., 1996; Davies and Bavor, 2000; Karim et al., 2004; Struck et al., 2008). Schillinger and Gannon (1985) proposed two different mechanisms bacteria use to adsorb to suspended sediments in urban stormwater: exocellular polysaccharides and fimbriae (hair-like limbs). The study found that the adsorption attributes were sometimes suppressed under certain laboratory conditions, resulting in a bacteria-sediment sorption range from 15-47% for *E. coli*. The results of the study noted that more than 50% of the bacteria in their trials did not settle out, and may have associated with particles < 5 µm in size that remained in suspension.

Karim et al. (2004) reported that the number of fecal coliforms in wetland sediments were 1-2 orders of magnitude greater than in the water column. Although the study concluded that sedimentation is a key mechanism in removing pathogens from water in artificial wetlands, they also noted that bacteria experiences increased survival once deposited into the sediments. Davies and Bavor (2000) similarly found that concentrations of bacteria were higher in the sediment than the water columns in a constructed wetland; however they also noted that the bacteria associated primarily with finer particles (< 2 µm) which remained suspended during their experiments. Thus, the removal of bacteria in sedimentation ponds is limited to their ability to

settle fine particles, while the vegetation in wetlands is able intercept more of these fine particles. A study by Howell et al. (1996) showed that mortality rates of fecal bacteria were significantly less in finer sediments like clay, further supporting the need for a technology that can effectively remove these finer particles.

Sand filtration is possibly the only current BMP that is able to effectively remove bacteria that is sorbed to fine sediments. Bright et al. (2001) saw a reduction in *E. coli* from ~2,500 most probable number (MPN)/ 100 mL to 0.7 MPN/100 mL in a sand column experiment after 54 consecutive days of treating bacteria-spiked stormwater. The significant drawback of this technology is the ripening period and breakthrough associated with sand filters. In the Bright et al. (2001) study, the filters loaded with bacteria-spiked stormwater did not reach an effluent concentration of *E. coli* below the national standard until day 24. Furthermore, clogging issues completely incapacitated the filters by day 54 (the same day for with the aforementioned effluent concentration was taken). The sand filters are also only capable of managing a hydraulic loading rate of 0.3 mL/min (0.054 L/min-ft^2) less, which is very low for surface water treatment BMPs.

The *Pleurotus spp.* mycofilters in this study performed well under sediment conditions (20 mg/L), with an estimated 100% overall removal after the identification of *E. coli* false positives in the effluent using the indole presence test. The results of the sediment/bacteria study were difficult to interpret due to the overcrowding of pink non-fecal thermotolerant coliform colonies on the plates, which made it challenging to determine whether the Kovac's solution was turning red (indicating a positive result for *E. coli*) or the magenta haze of *Klebsiella* was deepening in color. Due to the ambiguity of the Kovac's test in this circumstance, combined with the fact that the blue colonies were significantly different in size and color compared to the known *E. coli*

plates from the influent, these colonies were determined to be false positives for *E. coli*, yielding a 100% removal rate in the third run. The mycofilters in this study notably removed *E. coli* from sediment-spiked water, which is a unique advantage of this technology. Also, unlike the sand filters in the Bright et al. study (2000), they did not require a long ripening period, and were able to treat water at an order of magnitude higher loading hydraulic loading (0.4 L/min-ft^2 areal loading compared to Bright et al.'s 0.054 L/min-ft^2). Additionally, mechanisms such as the production of mycotoxins will likely degrade the bacteria, which may offer an advantage over other technologies where bacteria survival in settled sediments is prolonged.

4.4 False Positive Identification

Upon the conclusion of the last mycofilter test, some unanswered questions about potential false positives remained. In an effort to make more sound conclusions about the results of this study, several colonies from the Coliscan plates were subcultured on brain heart infusion agar, incubated overnight at 35 ^{o}C, and then sent to Microcheck, an independent bacteriology identification laboratory (Northfield, VT) for bacterial identification. The first goal of this effort was to identify the 'fecal coliform' in the enumerations as false-positive *Klebsiella*. The second objective of this analysis was to determine the reliability of the Coliscan MF method and its use in tandem with the Kovac's reagent. For this, *E. coli* colonies were plated, and then the location where the colony grew on the Coliscan media was tested with Kovac's reagent. A combination of blue colonies with positive and negative results from the Kovac's reagent, as well as a culture of the stock *E. coli* used in the experiments was sent to Microcheck for analysis.

The results of the genetic identification are outlined in Table 4-1. The analysis identified the pink colonies as *Raoultella planticola*, which was formerly classified as *Klebsiella* until 2001

(Drancourt et al., 2001). Of the blue colonies that were sent to Microcheck, only the stock *E. coli* plate tested positive for *E. coli*. *Enterobacter aerogenes* tested both positive and negative with the Kovac's solution, with confirmation of four replicates for each result. Additionally, *Raoultella planticola* (formerly *Klebsiella*) elicited a positive result (with four replicates) from the Kovac's reagent.

TABLE 4-1: MICROCHECK RESULTS

Description	Microcheck Results
Pink Colony[2]	*Raoultella planticola* ATCC 33558
Blue Colony (Kovac's neg.)[4]	*Enterobacter aerogenes*
Blue Colony (Kovac's neg.)[1]	*Staphylococcus hominis hominis* ATCC 27844
Blue Colony (Kovac's pos.)[4]	*Raoultella planticola* ATCC 33558
Blue Colony (Kovac's pos.)[4]	*Enterobacter aerogenes**

Superscripts designate the number of replicates with the same description and results
*two of the replicates presented as a genus classification only

The reliability of membrane filter techniques using a chromogenic media is limited to relatively clean samples with low bacterial diversity (McLain et al., 2011). High false positive rates have also been correlated to crowded plates, which was a common occurrence in this study due to the *Raoultella* (*Klebsiella*) bacteria (Olstadt et al., 2007; Pitkänen et al., 2006). Olstadt et al. (2007) looked at the ability of different USEPA approved *E. coli* tests to suppress high levels of *Aeromonas spp.*, in an effort to mimic real-world conditions where there are numerous bacteria present in a given water sample. In that study, Coliscan was unable to suppress some strains of *Aeromonas spp.*, even at levels as low as 10 cells meaning that the Coliscan test could be less reliable when using highly populated bacterial samples. The Coliscan MF method uses

the detection of enzymes galactosidase and glucuronidase to identify fecal coliforms and *E. coli*. *Enterobacter aerogenes* and *Klebsiella pneumonia* (a species similar to *Klebsiella planticola*, now *Raoultella planticola*) are known to produce both of these enzymes under certain laboratory conditions (Kämpfer et al., 1991; Geissler et al., 2000). A study by Alonso et al. (1999) found that some strains of *Enterobacter* and *Klebsiella* produced the glucuronidase enzyme, which was assumed to be exclusively produced by *E. coli* in the Coliscan MF method. Furthermore, *Enterobacter* and *Klebsiella* have been shown to ferment lactose and produce indole in a laboratory study, meaning that the confirmatory reagent used in this study could have elicited a double false positive (Bernasconi et al., 2006). Additionally, the false positives for *E. coli* in this study seemed to have been correlated with the use of straw in the mycofilter substrate, which could easily be modified in future studies.

5. CONCLUSIONS

The principal objective in this study was to evaluate the ability of mycofilters to remove *E. coli* from synthetic stormwater. Five bench-scale tests were devised to test the effects of different species of fungi and mycofilter volume on removal rates. Notable results from the single-filter tests include a ~20% removal realized in the *Stropharia spp.* mycofilters for both vigor and non-vigor tested treatments. The *Stropharia spp.* mycofilters in series (three filters) yielded an estimated 100% removal in the third replicate under the assumption that the Kovac's negative plates were false positives. Bacterial identification showed that the Coliscan MF method and use of Kovac's reagent is inconsistent when identifying false positives, though there were no instances of false negatives. The *Pleurotus spp.* mycofilters tested in series also yielded removal rates as high as 100% in the bacteria/sediment trials, with confirmation of false positives using the Kovac's reagent. The *Pleurotus spp.* mycofilters also realized a 100% removal in one of the replicates from the non-sediment trials, with the other removal rates (14 and 48%) possibly being due to false-positives that were not correctly identified using the Kovac's reagent.

The Coliscan MF method is advantageous to other methods (e.g. spread plate, multiple-tube fermentation) because it has the capability of enumerating *E. coli* and general fecal coliform separately. This is true for most methods using a chromogenic media for the detection of galactosidase and glucuronidase enzymes, which are produced by fecal coliform and *E. coli*. The downfall to this method is that it is most effective under relatively clean and bacterially homogenous conditions (McLain et al., 2011). In biologically diverse systems, like mycofilters, the Coliscan MF media and supplementary indole presence test (Kovac's reagent) are not as effective for bacterial enumeration. This concern has also been documented in a study of false-

positive identification for *E. coli* in treated wastewater which concluded that microbial validation is of extreme importance in studies using chromogenic media to identify possible false positives (McLain et al., 2011). Overall, this study showed some evidence of mycofilters effectively removing *E. coli* from synthetic stormwater, but was unable to accurately quantify results due to hindrances from false positives. Future efforts should include bacterial identification in tandem with chromogenic media to estimate possible false positives and hopefully quantify their influence in overall removal rates. Additionally, avoiding the use of straw in the mycofilter substrate might reduce the influence from false positives. Mycofiltration is a promising biotechnology for the removal of pathogens from stormwater, which could yield better removal rates than some more conventional BMPs, particularly the most popular technologies like sand filters, if the impact from false positives can be more fully quantified.

6. REFERENCES

Alonso, J., A. Soriano, O. Carbajo, I. Amoros, H. Garelick. (1999). Comparison and recovery of *Escherichia coli* and thermotolerant coliforms with a chromogenic medium incubated at 41 and 44.5^{o}C. *App. and Env. Microbio.* August, 3746-3749

Barron, G. L., and Thorn, R. G. (1987). Destruction of nematodes by species of *Pleurotus*. *Botany*, 65 (4), 774-778.

Bernasconi, C., G. Volponi, L. Bonadonna. (2006). Comparison of three different media for the detection of *E. coli* and coliforms in water. *Water Science and Technology*. 54 (3), 141-145.

Booth, D., Visitacion, B., Steinemann, A. (2006). Damages and costs of stormwater runoff in the puget sound region. *The Water Center,* University of Washington.

Bright, T. M., Hathaway, J. M., Hunt, III W. F., Burchell, II M. R., & de los Reyes, III F. L. (2010). Impact of storm-water runoff on clogging and fecal bacteria reduction in sand columns. *Journal of Environmental Engineering.* 136 (12), 1435-1441.

Caplenas, N. R., Kanarek, M. S., & Dufour, A. P. (1981). Source and extent of *Klebsiella* pneumoniae in the paper industry. *Applied and Environmental Microbiology*. 42 (5), 779-785.

Caplenas, N. R., & Kanarek, M. S. (1984). Thermotolerant non-fecal source *Klebsiella pneumoniae*: validity of the fecal coliform test in recreational waters. *American Journal of Public Health*. 74, 1273-1275.

Clary, J., Jones, J. E., Urbonas, B.R., Quigley, M. M., Strecker E., Wagner, T. (2007). Can stormwater BMPs remove bacteria? New findings from the international stormwater BMP database. *Stormwater.* May. Retrieved from http://www.stormh2o.com/may-2008/bacterial-researchbmps.aspx

Clary, J., M., Leisenring, J., Jeray. (2010). Pollutant category summary: fecal indicator bacteria. *International Stormwater BMP Database*. Retrieved from http://www.bmpdatabase.org/Docs/BMP%20Database%20Bacteria%20Paper%20Dec%202010.pdf

Davies, C.M. and Bavor, H.J. (2000). The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. *Journal of Applied Microbiology. 9,* 349-360.

Drancourt, M., C. Bollet, A. Carta, P. Rousselier. (2001). Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella and Raoultella gen. nov.*, with description of *Raoultella*

ornithinolytica comb. nov., *Raoultella terrigena comb. nov.* and *Raoultella planticola comb.* Nov. *International Journal of Systematic and Evolutionary Microbiology.* 51, 925-932.

Duran, R., Cary, J. W., Calvo, A. M. (2010). Role of the Osmotic Stress Regulatory Pathway in Morphogenesis and Secondary Metabolism in Filamentous Fungi. *Toxins.* 2,367-381.

Fermor, T.R. and Wood, D.A. (1981). Degredation of bacteria by *Agaricus Bisporus* and other fungi. *Journal of General Microbiology*. 126, 377-387.

Gaffield, S., Goo, R., Richards, L., Jackson, R. (2003). Public health effects of inadequately managed stormwater runoff. *Amer. J. Pub. Health.* 93 (9), 1527–1533.

Geissler, K., M. Manafi, L. Amoros, J.L. Alonso. (2000). Quantitative determination of total coliforms and *Escherichia coli* in marine waters with chromogenic and fluorogenic media. *Journal of Applied Microbiology.* 88, 280-285.

Haile, R. W., Witte, J. S., Gold, M., Cressey, R., McGee, C., Millikan, R. C., Glasser, A., Wang, G. (1999). The Health Effects of Swimming in Ocean Water Contaminated by Storm Drain Runoff. *Epidemiology.* 10 (4), 355-363.

Hong, L., L. Xuan, L. Guohong, P. Yanbo, Z. Keqin. (2006). Acanthoaytes of *Stropharia spp.* function as a nematode-attacking device. *Journal of Appl. Environ. Microbiol.* 72 (4), 2982

Howell, J. M., Coyne, M. S., & Cornelius, P. L. (1996). Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. *Journal of Environmental Quality, 25,* 6, 1216-1220.

Hunt, M.E. and Rice, E.W. (2005). Microbiological Examination. In A. Eaton, et.al. (Eds.), *Standard Methods for the Examination of Water & Wastewater* (9-19-168). Washington D.C.: American Public Health Association.

Hutchison, L., S. Madzia, G. Barron. (1996). The presence and antifeedant function of toxin-producing secretory cells on hyphae of the lawn-inhabiting agaric *Conocybe lactea*. *Canadian Journal of Botany.* 74, 431-434.

Kämpfer, P., O. Rauhoff, W. Dott. (1991). Glycosidase profiles of members of the family *Enterobacteriaceae*. *Journal of Clinical Microbiology.* 29 (12), 2887.

Karim, M.R., Manshadia, F.D., Karpiscak, M.M., Gerba, C.P. (2004). The persistence and removal of enteric pathogens in constructed wetlands. Wat. Res. 38 (7), 1831–1837.

McLain, J., C. Rock, K. Lohse, J. Walworth. (2011). False-positive identification of *Escherichia coli* in treated municipal wastewater and wastewater-irrigated soils. *Canadian Journal of Microbiology.* 57, 775-784.

Micrology Laboratories, LLC. *Coliscan® MF*. Retrieved from http://www.micrologylabs.com/page/94/Coliscan-MF

National Resources Defense Council (NRDC) (2012). Testing the Waters: A guide to water quality at vacation beaches. New York, NY.

Olstadt, J., J. Schauer, J. Standridge, S. Kluender. (2007). A comparison of ten USEPA approved total coliform/*E. coli* tests. *Journal of Water and Health.* 05 (2), 267-282.

Pitkänen, T., P. Paakkari, I. Miettinen, H. Heinonen-Tanski, L. Paulin, M. Hanninen. (2006). Comparison of media for enumeration of coliform bacteria and *Escherichia coli* in non-disinfected water. *Journal of Microbiological Methods.* 68, 522-529.

Thaddeus G., McOliver, C.C. (2010). Improving the efficacy of wastewater polishing reed beds. *Water Environment Research Foundation & IWA Publishing*. Report DEC11U06.

Thomas, S.A., L.M. Aston, D.L. Woodruff, V.I. Cullinan. (2009). Field demonstration of mycoremediation for removal of fecal coliform bacteria and nutrients in the dungeness watershed, Washington. Final Report. *Pacific Northwest National Laboratory.* PNWD-4054-1.

Ramos, A. C., Façanha, A. R., Lima, P. T., Feijó, J. A. (2008). pH signature for the responses of arbuscular mycorrhizal fungi to external stimuli. *Plant Signaling & Behavior*. 3 (10), 850-852.

Rogers, T. (2012). Experimental evaluation of mycoremediation of *Escherichia coli* bacteria in solution using *Pleurotus ostreatus*. (Unpublished thesis dissertation). Evergreen State College, WA.

Schillinger, J.E., Gannon, J.J. (1985). Bacterial adsorption and suspended particles in urban stormwater. J. WPCF. 57 (5), 384–389.

State of Washington (2011). Water quality standards for surface waters of the state of Washington. Chapter 173-201A WAC, Washington State Department of Ecology, Olympia, Washington.

Struck, S.D., Selvakumar, A., Borst, M. (2008). Prediction of effluent quality from retention ponds and constructed wetlands for managing bacterial stressors in storm-water runoff. J. Irrig. Drain. Eng. 134 (5), 567–578.

United States Environmental Protection Agency (USEPA) (2012a). National summary of impaired waters and TMDL information. Office of Water, Washington, D.C. http://ofmpub.epa.gov/tmdl_waters10/attains_nation_cy.control?p_report_type=T#tmdl_by_pollutant

United States Environmental Protection Agency (USEPA) (2012b). Recreational water quality criteria. Office of Water, Washington, D.C. http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/RWQC2012.pdf

APPENDIX A:

Full Data Summary

Experimental Set-up #1a:

Date tested: Controls 1 & 2 tested 8-6-12, Control 3 tested 8-1-12
Test type: Filters were tested individually (not in series). Each bucket was flushed with a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the filter was loaded at 0.5 L/min for 10min, allowed to drain for an additional 30min, and loaded again at 2.2 L/min for 10min. The same influent batch was used for the entire duration of the test (at both hydraulic loading rates). The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.
Filter type: 3 control buckets containing 50% whole chips and 50% shredded chips
Comments: FP labels- Control 1 = SR-B-04, Control 2 = SR-B-01, and Control 3 = SR-B-03
Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together, orange highlight = data used in summary

CONTROL 1

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	2	2
Effluent	1:10A	0	0	78	780
	1:10B	0	0	78	780
	1:5A	0	0	TNTC	
	1:5B	0	0	TNTC	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	84	840	0	0
	1:10B	88	880	0	0
	1:20A	36	720	0	0
	1:20B	38	760	0	0
Effluent 0 min	1:10A	16	160	TNTC	
	1:10B	11	110	TNTC	
	1:20A	6	120	TNTC	
	1:20B	5	100	TNTC	
Effluent 5 min	1:10A	79	790	78	780
	1:10B	61	610	76	760
	1:20A	39	780	13	260
	1:20B	33	660	61	1220
Effluent 10 min	1:10A	75	750	49	490
	1:10B	64	640	25	250
	1:20A	41	820	18	360
	1:20B	38	760	20	400
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	67	670	0	0
	1:10B	90	900	0	0
	1:20A	28	560	0	0
	1:20B	37	740	0	0
Effluent 0 min	1:10A	59	590	confluent	
	1:10B	66	660	47	470
	1:20A	31	620	0	0
	1:20B	34	680	51	1020
Effluent 5 min	1:10A	73	730	9	90
	1:10B	70	700	10	100
	1:20A	39	780	6	120
	1:20B	50	1000	1	20
Effluent 10 min	1:10A	92	920	9	90
	1:10B	77	770	8	80
	1:20A	49	980	4	80
	1:20B	37	740	3	60

CONTROL 2

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	0	0
Effluent	1:10A	0	0	90	900
	1:10B	1	10	TNTC	
	1:5A	0	0	TNTC	
	1:5B	2	10	TNTC	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	70	700	0	0
	1:10B	74	740	0	0
	1:20A	40	800	0	0
	1:20B	28	560	0	0
Effluent 0 min	1:10A	10	100	confluent	
	1:10B	11	110	confluent	
	1:20A	8	160	TNTC	
	1:20B	6	120	TNTC	
Effluent 5 min	1:10A	79	790	78	780
	1:10B	89	890	67	670
	1:20A	29	580	47	940
	1:20B	46	920	38	760
Effluent 10 min	1:10A	73	730	30	300
	1:10B	74	740	35	350
	1:20A	30	600	17	340
	1:20B	34	680	15	300
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	71	710	0	0
	1:10B	68	680	0	0
	1:20A	40	800	0	0
	1:20B	39	780	0	0
Effluent 0 min	1:10A	58	580	68	680
	1:10B	73	730	29	290
	1:20A	35	700	41	820
	1:20B	37	740	21	420
Effluent 5 min	1:10A	81	810	6	60
	1:10B	66	660	9	90
	1:20A	37	740	5	100
	1:20B	37	740	4	80
Effluent 10 min	1:10A	75	750	6	60
	1:10B	80	800	1	10
	1:20A	31	620	3	60
	1:20B	40	800	2	40

CONTROL 3

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	1	1	0	0
Effluent	1:10A	0	0	48	480
	1:10B	0	0	32	320
	1:5A	0	0	102	510
	1:5B	0	0	81	405
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	61	610	0	0
	1:10B	65	650	0	0
	1:20A	30	600	0	0
	1:20B	30	600	0	0
Effluent 0 min	1:10A	3	30	110	1100
	1:10B	2	20	91	910
	1:20A	4	80	100	2000
	1:20B	1	20	58	1160
Effluent 5 min	1:10A	58	580	16	160
	1:10B	45	450	70	700
	1:20A	21	420	24	480
	1:20B	33	660	13	260
Effluent 10 min	1:10A	62	620	4	40
	1:10B	42	420	9	90
	1:20A	30	600	6	120
	1:20B	33	660	0	0
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	55	550	0	0
	1:10B	48	480	0	0
	1:20A	41	820	0	0
	1:20B	25	500	0	0
Effluent 0 min	1:10A	45	450	0	0
	1:10B	43	430	0	0
	1:20A	23	460	16	320
	1:20B	26	520	2	40
Effluent 5 min	1:10A	55	550	0	0
	1:10B	55	550	0	0
	1:20A	23	460	1	20
	1:20B	27	540	1	20
Effluent 10 min	1:10A	59	590	0	0
	1:10B	52	520	1	10
	1:20A	29	580	0	0
	1:20B	33	660	0	0

Blank 1: Taken after filtering pre-soak inf./eff., influent time 0 min, and 0.5L/min 0min, 5min, and 10min shows no general coliform or E.coli colonies

Experimental Set-up #1b:

Date tested: NV 1 & 2 tested 8-1-12, NV 3 tested 8-6-12
Test type: Filters were tested individually (not in series). Each bucket was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the filter was loaded at 0.5 L/min for 10min, allowed to drain for an additional 30min, and loaded again at 2.2 L/min for 10min. The same influent batch was used for the entire duration of the test (at both hydraulic loading rates). The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.
Filter type: 3 filters with Strophaira grown on 50% whole chips and 50% shredded chips (not vigor tested by FP)
Comments: FP labels- NV 1 = SR-B-07, NV 2 = SR-B-10, NV 3 = SR-B-06
Data: 1:1, 1:10, 1:20 represent diluttions performed , A/B designate duplicates of dilutions.TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together , orange highlight = data used in summary

NON-VIGOR 1

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	1	1
Effluent	1:10A	0	0	0	0
	1:10B	0	0	1	10
	1:5A	0	0	1	5
	1:5B	0	0	1	5
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	94	940	0	0
	1:10B	91	910	0	0
	1:20A	22	440	0	0
	1:20B	31	620	0	0
Effluent 0 min	1:10A	33	330	2	20
	1:10B	30	300	7	70
	1:20A	13	260	0	0
	1:20B	15	300	4	80
Effluent 5 min*	1:10A	79	790	0	0
	1:10B	53	530	0	0
	1:20A	14	280	1	20
	1:20B	25	500	0	0
Effluent 10 min	1:10A	68	680	0	0
	1:10B	42	420	0	0
	1:20A	24	480	0	0
	1:20B	28	560	0	0
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	70	700	0	0
	1:10B	77	770	0	0
	1:20A	38	760	0	0
	1:20B	33	660	0	0
Effluent 0 min	1:10A	57	570	0	0
	1:10B	54	540	0	0
	1:20A	18	360	2	40
	1:20B	30	600	1	20
Effluent 5 min	1:10A	64	640	0	0
	1:10B	55	550	0	0
	1:20A	35	700	0	0
	1:20B	35	700	0	0
Effluent 10 min	1:10A	66	660	0	0
	1:10B	55	550	0	0
	1:20A	34	680	0	0
	1:20B	26	520	0	0

* This time sample was not taken during initial test, so the filter was allowed to drain after the 10 min sample had been taken, then loaded again at 0.5 L/min for the 5 min sample

NON-VIGOR 2

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	0	0
Effluent	1:10A	0	0	0	0
	1:10B	0	0	0	0
	1:5A	0	0	0	0
	1:5B	0	0	0	0
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	64	640	0	0
	1:10B	69	690	0	0
	1:20A	34	680	0	0
	1:20B	37	740	0	0
Effluent 0 min	1:10A	21	210	0	0
	1:10B	16	160	0	0
	1:20A	13	260	0	0
	1:20B	9	180	0	0
Effluent 5 min	1:10A	50	500	0	0
	1:10B	69	690	0	0
	1:20A	25	500	0	0
	1:20B	25	500	0	0
Effluent 10 min	1:10A	57	570	2	20
	1:10B	57	570	0	0
	1:20A	24	480	0	0
	1:20B	27	540	0	0
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	68	680	0	0
	1:10B	56	560	0	0
	1:20A	36	720	0	0
	1:20B	36	720	0	0
Effluent 0 min	1:10A	44	440	0	0
	1:10B	65	650	2	20
	1:20A	30	600	0	0
	1:20B	30	600	0	0
Effluent 5 min	1:10A	48	480	0	0
	1:10B	59	590	0	0
	1:20A	29	580	0	0
	1:20B	25	500	0	0
Effluent 10 min	1:10A	58	580	0	0
	1:10B	72	720	0	0
	1:20A	31	620	0	0
	1:20B	37	740	0	0

NON-VIGOR 3

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	0	0
Effluent	1:10A	0	0	11	110
	1:10B	0	0	11	110
	1:5A	0	0	14	70
	1:5B	0	0	11	55
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	83	830	0	0
	1:10B	81	810	0	0
	1:20A	32	640	0	0
	1:20B	31	620	0	0
Effluent 0 min	1:10A	22	220	17	170
	1:10B	20	200	20	200
	1:20A	12	240	11	220
	1:20B	8	160	9	180
Effluent 5 min	1:10A	60	600	0	0
	1:10B	45	450	0	0
	1:20A	25	500	0	0
	1:20B	26	520	0	0
Effluent 10 min	1:10A	54	540	0	0
	1:10B	52	520	0	0
	1:20A	35	700	0	0
	1:20B	38	760	0	0
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	71	710	0	0
	1:10B	60	600	0	0
	1:20A	28	560	0	0
	1:20B	42	840	0	0
Effluent 0 min	1:10A	68	680	2	20
	1:10B	64	640	3	30
	1:20A	41	820	1	20
	1:20B	55	1100	1	20
Effluent 5 min	1:10A	78	780	0	0
	1:10B	67	670	0	0
	1:20A	37	740	0	0
	1:20B	35	700	0	0
Effluent 10 min	1:10A	73	730	0	0
	1:10B	76	760	0	0
	1:20A	45	900	0	0
	1:20B	39	780	0	0

Experimental Set-up #1c:

Date tested: Vigor 1 & 2 tested 7-26-12, Vigor 3 tested 7-30-12
Test type: Filters were tested individually (not in series). Each bucket was flushed with 9L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the filter was loaded at 0.5 L/min for 10min, allowed to drain for an additional 30min, and loaded again at 2.2 L/min for 10min. The same influent batch was used for the entire duration of the test (at both hydraulic loading rates). The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.
Filter type: 3 filters with Stropharia grown on 50% whole chips and 50% shredded chips (vigor tested by FP)
Comments: FP labels- Vigor 1 = SR-B-13, Vigor 2 = SR-B-09, Vigor 3 =SR-B-12
Data: 1:1, 1:10, 1:20 represent diluttions performed , A/B designate duplicates of dilutions.TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together , orange highlight = data used in summary

VIGOR 1

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	
	1:1B	0	0	0	
Effluent	1:10A	2	20	2	
	1:10B	2	20	5	
	1:5A	0	0	2	
	1:5B	0	0	2	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	86	860	0	
	1:10B	85	850	0	
	1:20A	44	880	0	
	1:20B	51	1020	0	
Effluent 0 min	1:10A	55	550	0	
	1:10B	36	360	0	
	1:20A	12	240	0	
	1:20B	26	520	0	
Effluent 5 min	1:10A	42	420	3	
	1:10B	58	580	0	
	1:20A	14	280	0	
	1:20B	24	480	4	
Effluent 10 min	1:10A	69	690	0	
	1:10B	74	740	0	
	1:20A	33	660	0	
	1:20B	31	620	0	
Loading Rate: 2.1 L/min					
Influent 0 min	1:10A	109	1090	0	
	1:10B	102	1020	0	
	1:20A	53	1060	0	
	1:20B	34	680	0	
Effluent 0 min	1:10A	27	270	1	
	1:10B	30	300	1	
	1:20A	9	180	0	
	1:20B	13	260	0	
Effluent 5 min	1:10A	72	720	0	
	1:10B	70	700	0	
	1:20A	39	780	1	
	1:20B	36	720	0	
Effluent 10 min	1:10A	86	860	0	
	1:10B	83	830	0	
	1:20A	36	720	0	
	1:20B	36	720	0	

VIGOR 2

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	3	3	148	148
	1:1B	0	0	17	17
Effluent	1:10A	10	100	142	1420
	1:10B	6	60	146	1460
	1:5A	14	70	TNTC	TNTC
	1:5B	21	105	TNTC	TNTC
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	86	860	0	0
	1:10B	82	820	0	0
	1:20A	30	600	0	0
	1:20B	31	620	0	0
Effluent 0 min	1:10A	65	650	13	130
	1:10B	68	680	4	40
	1:20A	20	400	5	100
	1:20B	27	540	108	2160
Effluent 5 min	1:10A	51	510	29	290
	1:10B	73	730	10	100
	1:20A	26	520	61	1220
	1:20B	33	660	26	520
Effluent 10 min	1:10A	72	720	8	80
	1:10B	57	570	7	70
	1:20A	42	840	3	60
	1:20B	30	600	6	120
Loading Rate: 2.1 L/min					
Influent 0 min	1:10A	52	520	0	0
	1:10B	64	640	0	0
	1:20A	17	340	0	0
	1:20B	28	560	0	0
Effluent 0 min	1:10A	37	370	18	180
	1:10B	42	420	12	120
	1:20A	16	320	9	180
	1:20B	17	340	11	220
Effluent 5 min	1:10A	61	610	0	0
	1:10B	56	560	2	20
	1:20A	28	560	9	180
	1:20B	31	620	0	0
Effluent 10 min	1:10A	54	540	0	0
	1:10B	47	470	0	0
	1:20A	20	400	0	0
	1:20B	31	620	3	60

VIGOR 3

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	1	1
Effluent	1:10A	0	0	19	190
	1:10B	0	0	28	280
	1:5A	1	5	41	205
	1:5B	1	5	60	300
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	83	830	8	80
	1:10B	70	700	7	70
	1:20A	42	840	2	40
	1:20B	31	620	3	60
Effluent 0 min	1:10A	36	360	54	540
	1:10B	35	350	34	340
	1:20A	22	440	confluent	confluent
	1:20B	16	320	67	1340
Effluent 5 min	1:10A	65	650	35	350
	1:10B	41	410	20	200
	1:20A	41	820	18	360
	1:20B	18	360	9	180
Effluent 10 min	1:10A	66	660	12	120
	1:10B	52	520	9	90
	1:20A	29	580	9	180
	1:20B	30	600	1	20
Loading Rate: 2.1 L/min					
Influent 0 min	1:10A	69	690	0	0
	1:10B	91	910	0	0
	1:20A	44	880	0	0
	1:20B	39	780	0	0
Effluent 0 min	1:10A	78	780	43	430
	1:10B	68	680	49	490
	1:20A	33	660	22	440
	1:20B	23	460	17	340
Effluent 5 min	1:10A	68	680	16	160
	1:10B	102	1020	10	100
	1:20A	38	760	0	0
	1:20B	41	820	13	260
Effluent 10 min	1:10A	58	580	3	30
	1:10B	59	590	4	40
	1:20A	35	700	2	40
	1:20B	24	480	4	80

Blank 1: Taken after filtering pre-soak inf./eff., influent time 0 min and influent time 0 min (run 2) shows no general Blank 2: Taken affter filtering 0.5 L/min samples for 0, 5 and 10 min shows no general coliform or E.Coli colonies

Blank 1: Taken after filtering pre-soak inf./eff., influent time 0 min and influent time 0 min (run 2) shows no general Blank 2: Taken affter filtering 0.5 L/min samples for 0, 5 and 10 min shows no general coliform or E.Coli colonies

Experimental Set-up #2a:

Date tested: 8-14-12

Test type: Filters were tested individually (not in series). Each bucket was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the filter was loaded at 0.5 L/min for 10min, allowed to drain for an additional 30min, and loaded again at 2.2 L/min for 10min. The same influent batch was used for the entire duration of the test (at both hydraulic loading rates). The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: 3 control buckets containing 25% whole chips, 50% fine chips, and 25% straw

Comments: The filter effluent was a dark yellow/brown that receded to clear by 10 min at 0.5 L/min. The dark color returned at the start of the second test (2.2 L/min) and again receded to clear by 10min.

Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC=too numerous to count Confluent=plate was unreadable, usually due to dyes bleeding together (Cells highlighted blue signify that some or all of the colonies were teal in color), orange highlight = data used in summary

CONTROL 1

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	0	0
Effluent	1:10A	0*	0*	TNTC	
	1:10B	0*	0*	TNTC	
	1:5A	0*	0*	TNTC	
	1:5B	0*	0*	TNTC	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	97	970	1	10
	1:10B	65	650	0	0
	1:20A	29	580	0	0
	1:20B	35	700	2	40
Effluent 0 min	1:10A	TNTC		confluent	
	1:10B	TNTC		confluent	
	1:20A	171	3420	confluent	
	1:20B	164	3280	confluent	
Effluent 5 min	1:10A	183	1830	TNTC	
	1:10B	181	1810	TNTC	
	1:20A	95	1900	TNTC	
	1:20B	95	1900	TNTC	
Effluent 10 min	1:10A	113	1130	TNTC	
	1:10B	128	1280	TNTC	
	1:20A	59	1180	TNTC	
	1:20B	73	1460	TNTC	
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	70	700	0	0
	1:10B	62	620	0	0
	1:20A	35	700	0	0
	1:20B	34	680	0	0
Effluent 0 min	1:10A	147	1470	TNTC	
	1:10B	136	1360	TNTC	
	1:20A	88	1760	TNTC	
	1:20B	88	1760	TNTC	
Effluent 5 min	1:10A	70	700	TNTC	
	1:10B	71	710	TNTC	
	1:20A	38	760	TNTC	
	1:20B	33	660	TNTC	
Effluent 10 min	1:10A	72	720	TNTC	
	1:10B	76	760	TNTC	
	1:20A	33	660	TNTC	
	1:20B	34	680	TNTC	

*several teal colonies present, but not counted

**blue colonies from all 2.2 L/min effluents look normal

CONTROL 2

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	5	5
	1:1B	0	0	1	1
Effluent	1:10A	0*	0*	TNTC	
	1:10B	0*	0*	TNTC	
	1:5A	0*	0*	TNTC	
	1:5B	0*	0*	TNTC	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	101	1010	0	0
	1:10B	67	670	0	0
	1:20A	40	800	0	0
	1:20B	43	860	0	0
Effluent 0 min	1:10A	TNTC		0	0
	1:10B	TNTC		0	0
	1:20A	TNTC		0	0
	1:20B	TNTC		0	0
Effluent 5 min	1:10A	TNTC		confluent	
	1:10B	TNTC		confluent	
	1:20A	TNTC		confluent	
	1:20B	TNTC		confluent	
Effluent 10 min	1:10A	TNTC		confluent	
	1:10B	TNTC		confluent	
	1:20A	212	4240	confluent	
	1:20B	TNTC		confluent	
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	81	810	1	10
	1:10B	87	870	0	0
	1:20A	42	840	0	0
	1:20B	42	840	3	60
Effluent 0 min	1:10A	TNTC		TNTC	
	1:10B	TNTC		TNTC	
	1:20A	187	3740	TNTC	
	1:20B	195	3900	TNTC	
Effluent 5 min	1:10A	139	1390	TNTC	
	1:10B	130	1300	TNTC	
	1:20A	58	1160	TNTC	
	1:20B	67	1340	TNTC	
Effluent 10 min	1:10A	128	1280	TNTC	
	1:10B	125	1250	TNTC	
	1:20A	86	1720	TNTC	
	1:20B	78	1560	TNTC	

*several teal colonies present, but not counted

**0.5 L/min all effluents: very small, numerous, teal colonies (not blue)

CONTROL 3

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	6	6
	1:1B	0	0	4	4
Effluent	1:10A	80	800	TNTC	
	1:10B	105	1050	TNTC	
	1:5A	63	315	TNTC	
	1:5B	45	225	TNTC	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	53	530	0	0
	1:10B	81	810	0	0
	1:20A	33	660	5	100
	1:20B	34	680	3	60
Effluent 0 min	1:10A	TNTC		TNTC	
	1:10B	TNTC		TNTC	
	1:20A	TNTC		TNTC	
	1:20B	TNTC		TNTC	
Effluent 5 min	1:10A	TNTC		TNTC	
	1:10B	TNTC		TNTC	
	1:20A	134	2680	TNTC	
	1:20B	125	2500	TNTC	
Effluent 10 min	1:10A	155	1550	TNTC	
	1:10B	144	1440	TNTC	
	1:20A	57	1140	TNTC	
	1:20B	60	1200	TNTC	
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	73	730	0	0
	1:10B	62	620	0	0
	1:20A	32	640	2	40
	1:20B	38	760	0	0
Effluent 0 min	1:10A	179	1790	TNTC	
	1:10B	179	1790	TNTC	
	1:20A	83	1660	TNTC	
	1:20B	80	1600	TNTC	
Effluent 5 min	1:10A	81	810	TNTC	
	1:10B	73	730	TNTC	
	1:20A	43	860	TNTC	
	1:20B	48	960	TNTC	
Effluent 10 min	1:10A	80	800	TNTC	
	1:10B	70	700	TNTC	
	1:20A	37	740	TNTC	
	1:20B	40	800	TNTC	

Experimental Set-up #2b:

Date tested: 8-16-12

Test type: Filters were tested individually (not in series). Each bucket was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the filter was loaded at 0.5 L/min for 10min, allowed to drain for an additional 30min, and loaded again at 2.2 L/min for 10min. The same influent batch was used for the entire duration of the test (at both hydraulic loading rates). The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: 3 buckets inoculated with Irpex on a substrate of 25% whole chips, 50% fine chips, and 25% straw

Comments: In contrast to the corresponding control filters, all of the effluent samples (including the pre-soak effluent) were clear

Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together (Cells highlighted blue signify that some or all of the colonies were teal in color), orange highlight = data used in summary

IL-F-01

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	0	0
Effluent	1:10A	0*	0*	0	0
	1:10B	0*	0*	0	0
	1:5A	12*	60*	TNTC**	
	1:5B	0*	0*	0	0
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	64	640	0	0
	1:10B	61	610	0	0
	1:20A	37	740	10	200
	1:20B	30	600	0	0
Effluent 0 min	1:10A	0	0	0	0
	1:10B	0	0	0	0
	1:20A	confluent		TNTC	
	1:20B	confluent		TNTC	
Effluent 5 min	1:10A	58	580	TNTC	
	1:10B	62	620	TNTC	
	1:20A	24	480	TNTC	
	1:20B	28	560	TNTC	
Effluent 10 min	1:10A	65	650	TNTC	
	1:10B	60	600	TNTC	
	1:20A	29	580	TNTC	
	1:20B	22	440	TNTC	
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	64	640	0	0
	1:10B	68	680	0	0
	1:20A	35	700	0	0
	1:20B	28	560	0	0
Effluent 0 min	1:10A	90	900	0	0
	1:10B	60	600	0	0
	1:20A	94	1880	0	0
	1:20B	45	900	0	0
Effluent 5 min	1:10A	56	560	TNTC	
	1:10B	54	540	TNTC	
	1:20A	31	620	TNTC	
	1:20B	38	760	TNTC	
Effluent 10 min	1:10A	75	750	TNTC	
	1:10B	48	480	TNTC	
	1:20A	30	600	TNTC	
	1:20B	36	720	TNTC	

*several teal colonies, but not as numerous as controls (20-40)

**entire filter is pink, hard to see individual colonies

Blank between IL-01 and IL-03 showed 3 magenta colonies and no blue/teal colonies

IL-F-03

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	8	8
Effluent	1:10A	0*	0*	TNTC	
	1:10B	36	360	TNTC	
	1:5A	14	70	TNTC	
	1:5B	15	75	TNTC	
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	66	660	0	0
	1:10B	84	840	0	0
	1:20A	43	860	0	0
	1:20B	37	740	0	0
Effluent 0 min	1:10A	116	1160	TNTC	
	1:10B	TNTC		TNTC	
	1:20A	72	1440	TNTC	
	1:20B	73	1460	TNTC	
Effluent 5 min	1:10A	68	680	TNTC	
	1:10B	72	720	TNTC	
	1:20A	42	840	TNTC	
	1:20B	23	460	TNTC	
Effluent 10 min	1:10A	82	820	TNTC	
	1:10B	73	730	TNTC	
	1:20A	28	560	TNTC	
	1:20B	29	580	TNTC	
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	61	610	0	0
	1:10B	62	620	1	10
	1:20A	31	620	0	0
	1:20B	35	700	0	0
Effluent 0 min	1:10A	TNTC		TNTC	
	1:10B	TNTC		TNTC	
	1:20A	54	1080	TNTC	
	1:20B	68	1360	TNTC	
Effluent 5 min	1:10A	61	610	TNTC	
	1:10B	60	600	TNTC	
	1:20A*	50	1000	TNTC	
	1:20B	27	540	TNTC	
Effluent 10 min	1:10A	70	700	TNTC	
	1:10B	75	750	TNTC	
	1:20A	31	620	TNTC	
	1:20B	24	480	TNTC	

*teal colonies (too small to count)

IL-F-04

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Pre-Soak					
Influent	1:1A	0	0	0	0
	1:1B	0	0	0	0
Effluent	1:10A	0*	0*	0	0
	1:10B	0*	0*	0	0
	1:5A	0*	0*	0	0
	1:5B	0*	0*	0	0
Loading Rate: 0.5 L/min					
Influent 0 min	1:10A	62	620	0	0
	1:10B	67	670	0	0
	1:20A	32	640	0	0
	1:20B	32	640	0	0
Effluent 0 min	1:10A	confluent**		confluent	
	1:10B	confluent**		confluent	
	1:20A	confluent**		confluent	
	1:20B	confluent**		confluent	
Effluent 5 min	1:10A	confluent**		confluent	
	1:10B	confluent**		confluent	
	1:20A	53	1060	TNTC	
	1:20B	25	500	TNTC	
Effluent 10 min	1:10A	69	690	TNTC	
	1:10B	58	580	TNTC	
	1:20A	36	720	TNTC	
	1:20B	43	860	TNTC	
Loading Rate: 2.2 L/min					
Influent 0 min	1:10A	73	730	TNTC	
	1:10B	62	620	TNTC	
	1:20A	55	1100	TNTC	
	1:20B	29	580	TNTC	
Effluent 0 min	1:10A	TNTC		TNTC	
	1:10B	TNTC		TNTC	
	1:20A	62	1240	TNTC	
	1:20B	70	1400	TNTC	
Effluent 5 min	1:10A	69	690	TNTC	
	1:10B	75	750	TNTC	
	1:20A	29	580	TNTC	
	1:20B	36	720	TNTC	
Effluent 10 min	1:10A	79	790	TNTC	
	1:10B	64	640	TNTC	
	1:20A	48	960	TNTC	
	1:20B	25	500	TNTC	

*teal colonies (too small to count)

**very teal and green, also pink haze

Blank after influent 0min (2.2 L/min): 0 pink/0 blue

Experimental Set-up #3a:

Date tested: 9-10-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min. The same three filters were used for each run, with 30 min of drain time allowed between tests. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: New control material fermented substrate (25/50/25- whole chips, fine chips, straw)

Comments:

Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together, orange highlight = data used in summary

RUN 1 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	52	1040	0	0
	1:20B	41	820	0	0
15 min	1:20A	45	900	0	0
	1:20B	45	900	0	0
25 min	1:20A	46	920	0	0
	1:20B	54	1080	0	0
Bucket 1 Effluent					
10 min	1:20A	20	400	14	280
	1:20B	18	360	11	220
20 min	1:20A	41	820	3	60
	1:20B	38	760	0	0
30 min	1:20A	29	580	1	20
	1:20B	34	680	1	20
Bucket 2 Effluent					
10 min	1:20A	25	500	7	140
	1:20B	31	620	6	120
20 min	1:20A	34	680	7	140
	1:20B	35	700	6	120
30 min	1:20A	16	320	4	80
	1:20B	28	560	7	140
Bucket 3 Effluent					
10 min	1:20A	25	500	2	40
	1:20B	38	760	3	60
20 min	1:20A	27	540	7	140
	1:20B	6	120	6	120
30 min	1:20A	25	500	5	100
	1:20B	26	520	6	120

Blank after R1B1- 0/0

RUN 2 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	36	720	0	0
	1:20B	29	580	0	0
15 min	1:20A	44	880	0	0
	1:20B	41	820	1	20
25 min	1:20A	42	840	0	0
	1:20B	44	880	0	0
Bucket 1 Effluent					
10 min	1:20A	35	700	1	20
	1:20B	33	660	2	40
20 min	1:20A	30	600	1	20
	1:20B	33	660	0	0
30 min	1:20A	32	640	0	0
	1:20B	25	500	0	0
Bucket 2 Effluent					
10 min	1:20A	27	540	4	80
	1:20B	26	520	0	0
20 min	1:20A	36	720	1	20
	1:20B	27	540	0	0
30 min	1:20A	31	620	0	0
	1:20B	28	560	3	60
Bucket 3 Effluent					
10 min	1:20A	38	760	5	100
	1:20B	30	600	4	80
20 min	1:20A	25	500	5	100
	1:20B	28	560	3	60
30 min	1:20A	23	460	4	80
	1:20B	18	360	2	40

Blank after R2B2 and R3B1- 0/0

RUN 3 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	52	1040	0	0
	1:20B	46	920	0	0
15 min	1:20A	43	860	0	0
	1:20B	38	760	0	0
25 min	1:20A	46	920	0	0
	1:20B	45	900	0	0
Bucket 1 Effluent					
10 min	1:20A	27	540	1	20
	1:20B	36	720	1	20
20 min	1:20A	21	420	0	0
	1:20B	37	740	0	0
30 min	1:20A	35	700	0	0
	1:20B	35	700	1	20
Bucket 2 Effluent					
10 min	1:20A	23	460	0	0
	1:20B	21	420	1	20
20 min	1:20A	27	540	2	40
	1:20B	29	580	3	60
30 min	1:20A	26	520	3	60
	1:20B	28	560	3	60
Bucket 3 Effluent					
10 min	1:20A	28	560	2	40
	1:20B	23	460	0	0
20 min	1:20A	34	680	4	80
	1:20B	23	460	4	80
30 min	1:20A	22	440	5	100
	1:20B	23	460	2	40

Blank after R3B2-0/0

Pre-Soak Influent					
	1:1A	0		0	
	1:1B	0		0	
Pre-Soak Effluent					
Bucket 1	1:20A	2	40	19	380
	1:20B	1	20	18	
Bucket 2	1:20A	1	20	18	
	1:20B	0	0	14	
Bucket 3	1:20A	1	20	20	
	1:20B	2	40	22	

*Blank after pre/post soak: 0/0

Experimental Set-up #3b:

Date tested: 9-13 and 9-14-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min. The same three filters were used for each run, with 30 min of drain time allowed between tests. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: Pleurotus grown on sterlizied substrate (25/50/25- whole chips, fine chips, straw) (new material-not used in previous testing)

Comments: (Bucket 1 = PC-S-02, Bucket 2 = PC-S-03, Bucket 3 = PC-S-01)

Data: 1:1, 1:10, 1:20 represent diluttions performed , A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together , orange highlight = data used in summary

RUN 1 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	41	820	0	0
	1:20B	29	580	0	0
15 min	1:20A	32	640	0	0
	1:20B	38	760	0	0
25 min	1:20A	52	1040	0	0
	1:20B	32	640	0	0
Bucket 1 Effluent					
10 min	1:20A	31	620	0	0
	1:20B	29	580	0	0
20 min	1:20A	34	680	0	0
	1:20B	35	700	0	0
30 min	1:20A	50	1000	1	20
	1:20B	40	800	0	0
Bucket 2 Effluent					
10 min	1:20A	41	820	0	0
	1:20B	34	680	0	0
20 min	1:20A	32	640	1	20
	1:20B	39	780	0	0
30 min	1:20A	34	680	0	0
	1:20B	44	880	3	60
Bucket 3 Effluent					
10 min	1:20A	31	620	1	20
	1:20B	32	640	0	0
20 min	1:20A	33	660	1	20
	1:20B	32	640	1	20
30 min	1:20A	31	620	2	40
	1:20B	33	660	0	0

Blank after eff. 10 min-0/0

Blank after eff. 20 min- 0/0

RUN 2 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	48	960	19	380
	1:20B	32	640	38	760
15 min	1:20A	46	920	1	20
	1:20B	39	780	3	60
25 min	1:20A	57	1140	13	260
	1:20B	40	800	0	0
Bucket 1 Effluent					
10 min	1:20A	38	760	0	0
	1:20B	37	740	0	0
20 min	1:20A	47	940	0	0
	1:20B	42	840	91	1820
30 min	1:20A	36	720	108	2160
	1:20B	41	820	TNTC	
Bucket 2 Effluent					
10 min	1:20A	41	820	0	0
	1:20B	45	900	0	0
20 min	1:20A	49	980	TNTC	
	1:20B	32	640	TNTC	
30 min	1:20A	33	660	TNTC	
	1:20B	38	760	TNTC	
Bucket 3 Effluent					
10 min	1:20A	10	200	0	0
	1:20B	19	380	7	140
20 min	1:20A	29	580	TNTC	
	1:20B	24	480	TNTC	
30 min	1:20A	24	480	TNTC	
	1:20B	29	580	TNTC	

Blank after eff. 10 min-0/0

Blank after eff. 20 min - 0 blue/ 11 pink

RUN 3 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	43	860	0	0
	1:20B	32	640	3	60
15 min	1:20A	38	760	35	700
	1:20B	30	600	37	740
25 min	1:20A	29	580	15	300
	1:20B	39	780	28	560
Bucket 1 Effluent					
10 min	1:20A	38	760	TNTC	
	1:20B	30	600	10	200
20 min	1:20A	29	580	5	100
	1:20B	41	820	20	400
30 min	1:20A	39	780	18	360
	1:20B	36	720	58	1160
Bucket 2 Effluent					
10 min	1:20A	29	580	TNTC	
	1:20B	39	780	TNTC	
20 min	1:20A	29	580	TNTC	
	1:20B	32	640	8	160
30 min	1:20A	40	800	TNTC	
	1:20B	40	800	TNTC	
Bucket 3 Effluent					
10 min	1:20A	0	0	TNTC	
	1:20B	0	0	TNTC	
20 min	1:20A	0	0	0	0
	1:20B	0	0	1	20
30 min	1:20A	0	0	TNTC	
	1:20B	0	0	2	40

Blank after eff. 10 min - 0 blue/1 pink

Blank after eff. 20 min- 0 blue/4 pink

Pre-Soak Influent					
	1:1A	0		0	
	1:1B	0		0	
Pre-Soak Effluent					
Bucket 1	1:20A	0		0	
	1:20B	0		0	
Bucket 2	1:20A	0		0	
	1:20B	0		0	
Bucket 3	1:20A	0		0	
	1:20B	0		0	

*Blank after pre-soak: 0/0

Experimental Set-up #3c:

Date tested: 9-12-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min. The same three filters were used for each run, with 30 min of drain time allowed between tests. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: Old vigor-tested Stropharia on 50/50 substrate (same filters that were used in single bucket tests)

Comments: (Bucket 1 = SR-B-12, Bucket 2 = SR-B-09, Bucket 3 = SR-B-13)

Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC=too numerous to count Confluent=plate was unreadable, usually due to dyes bleeding together, orange highlight = data used in summary

RUN 1 (VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	33	660	0	0
	1:20B	29	580	0	0
15 min	1:20A	28	560	0	0
	1:20B	28	560	0	0
25 min	1:20A	37	740	0	0
	1:20B	31	620	0	0
Bucket 1 Effluent					
10 min	1:20A	23	460	7	140
	1:20B	32	640	3	60
20 min	1:20A	24	480	4	80
	1:20B	26	520	5	100
30 min	1:20A	26	520	1	20
	1:20B	33	660	1	20
Bucket 2 Effluent					
10 min	1:20A	33	660	TNTC	
	1:20B	27	540	TNTC	
20 min	1:20A	48	960	TNTC	
	1:20B	19	380	TNTC	
30 min	1:20A	32	640	71	1420
	1:20B	CON	#VALUE!	CON	
Bucket 3 Effluent					
10 min	1:20A	14	280	TNTC	
	1:20B	33	660	TNTC	
20 min	1:20A	29	580	TNTC	
	1:20B		0	TNTC	
30 min	1:20A	26	520	TNTC	
	1:20B	28	560	TNTC	

Blank between R1B1 & R1B2- 0/0

RUN 2 (VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	23	460	0	0
	1:20B	23	460	0	0
15 min	1:20A	27	540	0	0
	1:20B	30	600	0	0
25 min	1:20A	35	700	0	0
	1:20B	29	580	0	0
Bucket 1 Effluent					
10 min	1:20A	37	740	48	960
	1:20B	37	740	2	40
20 min	1:20A	42	840	4	80
	1:20B	27	540	1	20
30 min	1:20A	20	400	1	20
	1:20B	34	680	1	20
Bucket 2 Effluent					
10 min	1:20A	39	780	106	2120
	1:20B	23	460	91	1820
20 min	1:20A	31	620	60	1200
	1:20B	CON		CON	
30 min	1:20A	40	800	51	1020
	1:20B	21	420	44	880
Bucket 3 Effluent					
10 min	1:20A	31	620	TNTC	
	1:20B	21	420	TNTC	
20 min	1:20A	39	780	TNTC	
	1:20B	CON		CON	
30 min	1:20A	26	520	TNTC	
	1:20B	blank		blank	

Blank between R2B1 & R2 Inf-0/0
Blank after R2B2 & R1B3-0/0

RUN 3 (VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	33	660	0	0
	1:20B	25	500	0	0
15 min	1:20A	43	860	0	0
	1:20B	24	480	0	0
25 min	1:20A	25	500	0	0
	1:20B	22	440	0	0
Bucket 1 Effluent					
10 min	1:20A	34	680	1	20
	1:20B	27	540	2	40
20 min	1:20A	32	640	1	20
	1:20B	24	480	0	0
30 min	1:20A	29	580	1	20
	1:20B	44	880	1	20
Bucket 2 Effluent					
10 min	1:20A	23	460	78	1560
	1:20B	25	500	76	1520
20 min	1:20A	33	660	58	1160
	1:20B	31	620	74	1480
30 min	1:20A	34	680	73	1460
	1:20B	27	540	51	1020
Bucket 3 Effluent					
10 min	1:20A	24	480	TNTC	
	1:20B	33	660	TNTC	
20 min	1:20A	29	580	TNTC	
	1:20B	32	640	TNTC	
30 min	1:20A	30	600	TNTC	
	1:20B	43	860	TNTC	

Blank between R3B2 t=10 and R3B3 t=10- 0/0

Pre-Soak Influent					
	1:1A	0		0	
	1:1B	0		0	
Pre-Soak Effluent					
Bucket 1	1:20A	0		TNTC	
	1:20B	0		TNTC	
Bucket 2	1:20A	0		confluent	
	1:20B	0		confluent	
Bucket 3	1:20A	0		confluent	
	1:20B	0		confluent	

*Blank between pre/post-soak and R1B2- 0 blue/6 pink

Experimental Set-up #4a:

Date tested: Run 1: 9-26-12, Runs 2 & 3: 9-27-12
Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min. The same three filters were used for each run, with 30 min of drain time allowed between tests. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.
Filter type: Control material fermented substrate (25/50/25- whole chips, fine chips, straw) (same material as used in the previous series testing)
Comments:
Data: 1:1, 1:10, 1:20 represent diluttions performed , A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together

RUN 1 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	45	900	0	0
	1:20B	41	820	1	20
15 min	1:20A	39	780	0	0
	1:20B	56	1120	0	0
25 min	1:20A	38	760	0	0
	1:20B	44	880	0	0
Bucket 1 Effluent					
10 min	1:20A				
	1:20B	27	540	0	0
20 min	1:20A	41	820	0	0
	1:20B	36	720	3	60
30 min	1:20A	41	820	0	0
	1:20B	35	700	1	20
Bucket 2 Effluent					
10 min	1:20A	22	440	22	440
	1:20B	28	560	10	200
20 min	1:20A	43	860	5	100
	1:20B	37	740	10	200
30 min	1:20A	23	460	1	20
	1:20B	26	520	2	40
Bucket 3 Effluent					
10 min	1:20A	18	360	26	520
	1:20B	27	540	6	120
20 min	1:20A	25	500	4	80
	1:20B	28	560	4	80
30 min	1:20A	29	580	4	80
	1:20B	18	360	7	140

Blank between eff. 10 min & eff. 30min- 0/0
Blank between inf 15min and eff. 20 min- 0/0

RUN 2 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	46	920	2	40
	1:20B	27	540	0	0
15 min	1:20A	52	1040	2	40
	1:20B	44	880	0	0
25 min	1:20A	39	780	0	0
	1:20B	48	960	0	0
Bucket 1 Effluent					
10 min	1:20A	24	480	2	40
	1:20B	24	480	2	40
20 min	1:20A	31	620	0	0
	1:20B	33	660	1	20
30 min	1:20A	33	660	0	0
	1:20B	31	620	0	0
Bucket 2 Effluent					
10 min	1:20A	33	660	0	0
	1:20B	24	480	1	20
20 min	1:20A	21	420	4	80
	1:20B	27	540	6	120
30 min	1:20A	19	380	1	20
	1:20B	28	560	1	20
Bucket 3 Effluent					
10 min	1:20A	25	500	0	0
	1:20B	20	400	0	0
20 min	1:20A	29	580	9	180
	1:20B	25	500	0	0
30 min	1:20A	23	460	4	80
	1:20B	30	600	7	140

Blank between eff. 10 min & eff. 30min- 0/0

RUN 3 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	37	740	0	0
	1:20B	43	860	1	20
15 min	1:20A	37	740	0	0
	1:20B	48	960	0	0
25 min	1:20A	44	880	0	0
	1:20B	41	820	0	0
Bucket 1 Effluent					
10 min	1:20A	27	540	2	40
	1:20B	26	520	0	0
20 min	1:20A	30	600	5	100
	1:20B	22	440	4	80
30 min	1:20A	37	740	0	0
	1:20B	27	540	2	40
Bucket 2 Effluent					
10 min	1:20A	31	620	0	0
	1:20B	21	420	2	40
20 min	1:20A	19	380	4	80
	1:20B	22	440	1	20
30 min	1:20A	22	440	0	0
	1:20B	28	560	1	20
Bucket 3 Effluent					
10 min	1:20A	15	300	0	0
	1:20B	13	260	1	20
20 min	1:20A	48	960	0	0
	1:20B	29	580	0	0
30 min	1:20A	30	600	2	40
	1:20B	21	420	0	0

Blank between eff. 10 min and eff. 20 min- 0/0
Blank between eff. 20 min and eff. 30 min-0/0

Pre-Soak Influent					
	1:1A	0	0	0	0
	1:1B	0	0	1	1
Pre-Soak Effluent					
Bucket 1	1:20A	0	0	7	140
	1:20B	1	20	10	200
Bucket 2	1:20A	0	0	59	1180
	1:20B	0	0	61	1220
Bucket 3	1:20A	0	0	TNTC	TNTC
	1:20B	0	0	TNTC	TNTC

*Blank after pre/post soak- 0 blue/0 pink

Experimental Set-up #4b:

Date tested: 9-24-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min . The same three filters were used for each run, with 30 min of drain time allowed between tests. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: Pleurotus grown on sterilized substrate (25/50/25- whole chips, fine chips, straw) (same material as used in the previous series testing)

Comments: (Bucket 1 = PC-S-02, Bucket 2 = PC-S-03, Bucket 3 = PC-S-01)

Data: 1:1, 1:10, 1:20 represent diluttions performed , A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together

RUN 1 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	39	780	0	0
	1:20B	41	820	1	20
15 min	1:20A	55	1100	0	0
	1:20B	43	860	10	200
25 min	1:20A	57	1140	4	80
	1:20B	40	800	3	60
Bucket 1 Effluent					
10 min	1:20A	33	660	pink haze	
	1:20B	34	680	pink haze	
20 min	1:20A	44	880	pink haze	
	1:20B	38	760	pink haze	
30 min	1:20A	33	660	pink haze	
	1:20B	43	860	pink haze	
Bucket 2 Effluent					
10 min	1:20A	13	260	pink haze	
	1:20B	10	200	pink haze	
20 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
Bucket 3 Effluent					
10 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
20 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	

TNTC* - to numerous to count, blue colonies were observed but tested negative for E. coli

Blank after eff. 10 min- 1 blue/0 pink
Blank after eff. 20 min - 0/0
Blank after eff. 30 min- 0 blue/6 pink

RUN 2 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	50	1000	0	0
	1:20B	42	840	0	0
15 min	1:20A	50	1000	5	100
	1:20B	51	1020	1	20
25 min	1:20A	51	1020	haze	
	1:20B	38	760	2	40
Bucket 1 Effluent					
10 min	1:20A	46	920	pink haze	
	1:20B	30	600	pink haze	
20 min	1:20A	56	1120	pink haze	
	1:20B	39	780	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
Bucket 2 Effluent					
10 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
20 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
Bucket 3 Effluent					
10 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
20 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	

TNTC* - to numerous to count, blue colonies were observed but tested negative for E. coli

Blank after eff. 10 min- pink haze
Blank after eff. 20 min- 0 blue/5 pink
Blank after eff. 30 min- 0 blue/1 pink

RUN 3 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	48	960	2	40
	1:20B	56	1120	2	40
15 min	1:20A	61	1220	4	80
	1:20B	49	980	3	60
25 min	1:20A	42	840	pink haze	
	1:20B	47	940	pink haze	
Bucket 1 Effluent					
10 min	1:20A	44	880	pink haze	
	1:20B	54	1080	pink haze	
20 min	1:20A	40	800	pink haze	
	1:20B	43	860	pink haze	
30 min	1:20A	59	1180	pink haze	
	1:20B	39	780	pink haze	
Bucket 2 Effluent					
10 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
20 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
Bucket 3 Effluent					
10 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
20 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	
30 min	1:20A	TNTC*	0	pink haze	
	1:20B	TNTC*	0	pink haze	

TNTC* - to numerous to count, blue colonies were observed but tested negative for E. coli

Pre-Soak Influent					
	1:1A	0	0	0	0
	1:1B	0	0	0	0
Pre-Soak Effluent					
Bucket 1	1:20A	2	40	pink haze	
	1:20B	0	0	pink haze	
Bucket 2	1:20A	NC	NC	pink haze	
	1:20B	NC	NC	pink haze	
Bucket 3	1:20A	NC	NC	pink haze	
	1:20B	TNTC*	TNTC*	pink haze	

*>200 colonies (NC=pink haze with blue areas- no clear colonies though)

Experimental Set-up #4c:

Date tested: 9-23-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min. The same three filters were used for each run, with 30 min of drain time allowed between tests. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: Old vigor-tested Stropharia on 50/50 substrate (same filters that were used in single bucket tests)

Comments: (Bucket 1 = SR-B-12, Bucket 2 = SR-B-09, Bucket 3 = SR-B-13)

Data: 1:1, 1:10, 1:20 represent diluttions performed , A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together

RUN 1 (VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	40	800	0	0
	1:20B	39	780	41	820
15 min	1:20A	41	820	41	820
	1:20B	40	800	58	1160
25 min	1:20A	49	980	2	40
	1:20B	58	1160	5	100
Bucket 1 Effluent					
10 min	1:20A	45	900	64	1280
	1:20B	34	680	30	600
20 min	1:20A			75	1500
	1:20B			47	940
30 min	1:20A	40	800	27	540
	1:20B	40	800	34	680
Bucket 2 Effluent					
10 min	1:20A	77	1540	TNTC	
	1:20B			TNTC	
20 min	1:20A			pink haze	
	1:20B			Blank*	
30 min	1:20A	53	1060	pink haze	
	1:20B	58	1160	pink haze	
Bucket 3 Effluent					
10 min	1:20A			Blank*	
	1:20B	43	860	TNTC	
20 min	1:20A	43	860	pink haze	
	1:20B			Blank*	
30 min	1:20A	61	1220	pink haze	
	1:20B	73	1460	pink haze	

Blank after eff. 10 min- 0 blue/~40 pink

Blank between R1 eff. 20 min and R2 eff. 10 min- 0 blue/4 pink (lots of pink dye w/ no colonies in addition

RUN 2 (VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	49	980	53	1060
	1:20B	48	960	67	1340
15 min	1:20A	47	940	21	420
	1:20B	42	840	0	0
25 min	1:20A	49	980	52	1040
	1:20B	47	940	0	0
Bucket 1 Effluent					
10 min	1:20A	41	820	35	700
	1:20B	41	820	51	1020
20 min	1:20A	40	800	18	360
	1:20B	52	1040	24	480
30 min	1:20A	51	1020	29	580
	1:20B	55	1100	42	840
Bucket 2 Effluent					
10 min	1:20A	56	1120	TNTC	
	1:20B	53	1060	TNTC	
20 min	1:20A	49	980	TNTC	
	1:20B	72	1440	TNTC	
30 min	1:20A	155	3100	TNTC	
	1:20B	55	1100	TNTC	
Bucket 3 Effluent					
10 min	1:20A	56	1120	TNTC	
	1:20B	71	1420	TNTC	
20 min	1:20A	71	1420	TNTC	
	1:20B	72	1440	TNTC	
30 min	1:20A	48	960	TNTC	
	1:20B	64	1280	TNTC	

Blank after eff. 10 min- 1 blue/11 pink

Blank after eff. 20 min- lots of pink smears

Blank after eff. 30 min- 0 blue/13 pink

RUN 3 (VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	40	800	47	940
	1:20B	44	880	54	1080
15 min	1:20A	51	1020	31	620
	1:20B	38	760	38	760
25 min	1:20A	51	1020	47	940
	1:20B	58	1160	40	800
Bucket 1 Effluent					
10 min	1:20A	50	1000	40	800
	1:20B	53	1060	30	600
20 min	1:20A	45	900	38	760
	1:20B	42	840	15	300
30 min	1:20A	53	1060	37	740
	1:20B	51	1020	45	900
Bucket 2 Effluent					
10 min	1:20A	41	820	TNTC	
	1:20B				
20 min	1:20A	TNTC		TNTC	
	1:20B	62	1240	TNTC	
30 min	1:20A	46	920	TNTC	
	1:20B	46	920	TNTC	
Bucket 3 Effluent					
10 min	1:20A	45	900	TNTC	
	1:20B	58	1160		
20 min	1:20A	54	1080	TNTC	
	1:20B	64	1280	TNTC	
30 min	1:20A	60	1200	TNTC	
	1:20B	59	1180	TNTC	

Blank after eff. 10 min- 0 blue/2 pink & pink smearing

Pre-Soak Influent					
	1:1A	0	0	0	0
	1:1B	0	0	0	0
Pre-Soak Effluent					
Bucket 1	1:20A	0	0	0	0
	1:20B	0	0	0	0
Bucket 2	1:20A	0	0	0	0
	1:20B	0	0	0	0
Bucket 3	1:20A	0	0	0	0
	1:20B	0	0	0	0

Pre-Soak Results Experimental Set-up #5					
	Dilution	Pink	Blue	Blue #/100 ml	Comments
Pre-Flush For Buckets 5, 12, and ?? 12/11/12					
Influent	1:1A	12	0	0	
	1:1B	1	0	0	
Bucket 1 Effluent	1:10A	TNTC	83	830	
	1:10B	TNTC	75	750	
Bucket 2 Effluent	1:10A	TNTC	>70	>700	
	1:10B	TNTC	>70	>700	blue streaks, as if there's a small trail of blue colonies
Bucket 6 Effluent	1:10A	TNTC	>70	>700	
	1:10B	TNTC	>70	>700	
Pre-Flush For Buckets 1, 2, and 6, 12/15/12					
Influent	1:1A	0	0	0	light green colonies ~40-60, not E coli
	1:1B	0	0	0	light green colonies ~40-60
Bucket 1 Effluent	1:10A	TNTC	~150	>1500	
	1:10B	TNTC	~150	>1500	
Bucket 2 Effluent	1:10A	TNTC	47	470	
	1:10B	TNTC	64	640	
Bucket 6 Effluent	1:10A	TNTC	6	60	
	1:10B	TNTC	13	130	
Pre-Flush For Buckets 10, 11, and 18, 12/15/12					
Influent	1:1A	0	0	0	light green colonies ~40-60
	1:1B	0	0	0	light green colonies ~40-60
Bucket 10 Effluent	1:10A	TNTC	20	200	
	1:10B	TNTC	21	210	
Bucket 11 Effluent	1:10A	TNTC	19	190	blue colonies testing negative for E coli
	1:10B	TNTC	18	180	
Bucket 18 Effluent	1:10A	TNTC	44	440	blue colonies testing negative for E coli
	1:10B	TNTC	~34	>340	small streaks, small, indistinguishable green(?)/blue colonies
Pre-Flush For Buckets 22, 40, and 42, 12/16/12					
Influent	1:1A	0	0	0	some pink staining, no pink colonies other than what was
	1:1B	1	0	0	recorded, though
Bucket 22 Effluent	1:10A	0	~150	>1,500	~150+ E. coli colonies-also tested positive with Kovac
	1:10B	0	~150	>1,500	
Bucket 40 Effluent	1:10A	TNTC	S		Confluent blue streaks, tested negative for E. coli
	1:10B	TNTC	S		
Bucket 42 Effluent	1:10A	TNTC	105	1,050	
	1:10B	TNTC	93	930	
Pre-Flush For Buckets 26, 32, and 37, 12/16/12					
Influent	1:1A	0	0	0	some pink staining, no pink colonies other than what was
	1:1B	1	0	0	recorded, though (Same as Influent for 22, 40 and 42)
Bucket 26 Effluent	1:10A	0	TNTC		200+ blue colonies/pink haze
	1:10B	0	TNTC		
Bucket 32 Effluent	1:10A	TNTC	~80	>800	approximation-colonies were faint and difficult to count
	1:10B	TNTC	~70	>700	approximation-colonies were faint and difficult to count
Bucket 37 Effluent	1:10A	TNTC	114	1,140	
	1:10B	TNTC	S		confluent-blue streaks
Pre-Flush For Buckets 28, 38, and 39, 12/16/12					
Influent	1:1A	0	4	4	
	1:1B	0	5	5	Very light colored, faint in comparison to effluent.
Bucket 28 Effluent	1:10A	TNTC	~120	>1,200	
	1:10B	TNTC	~100	>1,000	
Bucket 38 Effluent	1:10A	TNTC	~90	>900	
	1:10B	TNTC	~90	>900	
Bucket 39 Effluent	1:10A	S	S		blue streaks and 100's of blue/pink colonies
	1:10B	S	S		

*Blank after inf & eff for #22, 42, 26, and 40- 0 blue/1 pink

*Blank after in & eff for 2, 32, 37, 38, 39, 28- 6 blue/3 pink

Notes

Kovac test

negative for E. coli

positive for E. coli

mixed - One plate was positive, the other was negative.

When staining with kovak, it is not possible to dye individual colonies. It appears that the presence of a limited number of E. coli on a plate will yield a "positive" kovac test.

S = streaking of dye on plate, impossible to count colonies.

TNTC - too numerous to count

Experimental Set-up #5a:

Date tested: 12-16-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. Then, the system was loaded at 0.3 L/min for 30min. 3 different filters were used for each test (9 filters total for 3 runs). The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fres h batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: Control of 25/50/25- (whole chips, fine chips, straw)

Comments: FP Bucket labels in table

Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together

RUN 1 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	28	560	0	0
	1:20B	31	620	0	0
15 min	1:20A	31	620	0	0
	1:20B	29	580	0	0
25 min	1:20A	38	760	0	0
	1:20B	29	580	0	0
Bucket 1 Effluent (SR-H-05)					
10 min	1:20A	56	1120	TNTC	
	1:20B	55	1100	TNTC	
20 min	1:20A	45	900	TNTC	
	1:20B	53	1060	TNTC	
30 min	1:20A	50	1000	TNTC	
	1:20B	33	660	TNTC	
Bucket 2 Effluent (SR-H-??)					
10 min	1:20A	59	1180	TNTC	
	1:20B	70	1400	TNTC	
20 min	1:20A	58	1160	TNTC	
	1:20B	TNTC		TNTC	
30 min	1:20A	39	780	TNTC	
	1:20B	39	780	TNTC	
Bucket 3 Effluent (SR-H-12)					
10 min	1:20A	51	1020	TNTC	
	1:20B	43	860	TNTC	
20 min	1:20A	53	1060	TNTC	
	1:20B	57	1140	TNTC	
30 min	1:20A	48	960	TNTC	
	1:20B	43	860	TNTC	

Blank after eff. 10 min- 0/0
Blank after eff 20 min- 0 blue/1 pink
Blank after eff 30 min- 0/0

RUN 2 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	30	600	0	0
	1:20B	34	680	0	0
15 min	1:20A	37	740	2	40
	1:20B	31	620	3	60
25 min	1:20A	44	880	0	0
	1:20B	30	600	0	0
Bucket 1 Effluent (SR-H-18)					
10 min	1:20A	75	1500	TNTC	
	1:20B	76	1520	TNTC	
20 min	1:20A	75	1500	TNTC	
	1:20B	70	1400	TNTC	
30 min	1:20A	54	1080	TNTC	
	1:20B	55	1100	TNTC	
Bucket 2 Effluent (SR-H-11)					
10 min	1:20A	60	1200	TNTC	
	1:20B	70	1400	TNTC	
20 min	1:20A	56	1120	TNTC	
	1:20B	72	1440	TNTC	
30 min	1:20A	56	1120	TNTC	
	1:20B	42	840	TNTC	
Bucket 3 Effluent (SR-H-02)					
10 min	1:20A	71	1420	TNTC	
	1:20B	74	1480	TNTC	
20 min	1:20A	80	1600	TNTC	
	1:20B	68	1360	TNTC	
30 min	1:20A	53	1060	TNTC	
	1:20B	74	1480	TNTC	

Blank after Inf 5 min and eff 10 & 20 min for B2 & B3- small pink stain, but no blue
Blank after inf 15 min, 25 min and B1 eff. 10 & 20 min- 0/0

RUN 3 (CONTROL)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	36	720	0	0
	1:20B	38	760	0	0
15 min	1:20A	27	540	0	0
	1:20B	32	640	0	0
25 min	1:20A	35	700	14	280
	1:20B	27	540	1	20
Bucket 1 Effluent (SR-H-10)					
10 min	1:20A	34	680	TNTC	
	1:20B	45	900	TNTC	
20 min	1:20A	33	660	TNTC	
	1:20B	35	700	TNTC	
30 min	1:20A	42	840	TNTC	
	1:20B	33	660	TNTC	
Bucket 2 Effluent (SR-H-06)					
10 min	1:20A	81	1620	TNTC	
	1:20B	81	1620	TNTC	
20 min	1:20A	~100	2000	TNTC	
	1:20B	~100	2000	TNTC	
30 min	1:20A	86	1720	TNTC	
	1:20B	84	1680	TNTC	
Bucket 3 Effluent (SR-H-01)					
10 min	1:20A	TNTC		TNTC	
	1:20B	TNTC		TNTC	
20 min	1:20A	~120	2400	TNTC	
	1:20B	~120	2400	TNTC	
30 min	1:20A	~90	1800	TNTC	
	1:20B	~90	1800	TNTC	

Blank after eff. 10, 20, & 30 min for B2 & B3- 0 blue/2 pink
Blank after inf 25 min, and B1 30 min- 0/0

Experimental Set-up #5b:

Date tested: 12-17-12

Test type: Filters were tested in series (Bucket 1 was on top, Bucket 2 in the middle, and Bucket 3 at the bottom). The system was flushed with 9 L of a "pre-soak" influent of dechlorinated tap water that did not contain E. coli and was allowed to drain for 30min. 3 different filters were used for each test (9 filters total for 3 runs). Then, the system was loaded at 0.3 L/min for 30min. The same influent batch was used for all 3 runs, unless the runs were split across multiple days, in which case a fresh batch was made for each day. The influent was 30L of dechlorinated tap water spiked with E. coli (ATCC=11775) to acheive a concentration of ~700 cfu/100mL.

Filter type: Stropharia grown on 25/50/25- (whole chips, fine chips, straw)

Comments: FP Bucket labels in table

Data: 1:1, 1:10, 1:20 represent diluttions performed, A/B designate duplicates of dilutions. TNTC= too numerous to count Confluent= plate was unreadable, usually due to dyes bleeding together

RUN 1 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	40	800	3	60
	1:20B	44	880	0	0
15 min	1:20A	40	800	4	80
	1:20B	37	740	0	0
25 min	1:20A	28	560	12	240
	1:20B	30	600	6	120
Bucket 1 Effluent (SR-H-28)					
10 min	1:20A	167	3340	TNTC	
	1:20B	158	3160	TNTC	
20 min	1:20A	134	2680	TNTC	
	1:20B	122	2440	TNTC	
30 min	1:20A	96	1920	TNTC	
	1:20B	126	2520	TNTC	
Bucket 2 Effluent (SR-H-38)					
10 min	1:20A	140	2800	TNTC	
	1:20B	221	4420	TNTC	
20 min	1:20A	180	3600	TNTC	
	1:20B	194	3880	TNTC	
30 min	1:20A	130	2600	TNTC	
	1:20B	132	2640	TNTC	
Bucket 3 Effluent (SR-H-32)					
10 min	1:20A	151	3020	0	0
	1:20B	125	2500	0	0
20 min	1:20A	228	4560	TNTC	
	1:20B	155	3100	TNTC	
30 min	1:20A	0	0	11	220
	1:20B	0	0	22	440

RUN 2 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	22	440	12	240
	1:20B	35	700	7	140
15 min	1:20A	34	680	0	0
	1:20B	33	660	2	40
25 min	1:20A	25	500	2	40
	1:20B	40	800	3	60
Bucket 1 Effluent (SR-H-39)					
10 min	1:20A	198	3960	TNTC	
	1:20B	177	3540	TNTC	
20 min	1:20A	122	2440	TNTC	
	1:20B	122	2440	TNTC	
30 min	1:20A	S		TNTC	
	1:20B	109	2180	TNTC	
Bucket 2 Effluent (SR-H-42)					
10 min	1:20A	187	3740	TNTC	
	1:20B	213	4260	TNTC	
20 min	1:20A	163	3260	TNTC	
	1:20B	167	3340	TNTC	
30 min	1:20A	0	0	31	620
	1:20B	0	0	40	800
Bucket 3 Effluent (SR-H-37)					
10 min	1:20A	>150	3000	TNTC	
	1:20B	260	5200	TNTC	
20 min	1:20A	166	3320	0	0
	1:20B	155	3100	0	0
30 min	1:20A	144	2880	0	0
	1:20B	S		TNTC	

RUN 3 (NON-VIGOR)

		Blue Colonies		Pink Colonies	
		Count	cfu/ 100mL	Count	cfu/ 100mL
Influent					
5 min	1:20A	28	560	7	140
	1:20B	46	920	1	20
15 min	1:20A	45	900	3	60
	1:20B	27	540	49	980
25 min	1:20A	35	700	4	80
	1:20B	41	820	4	80
Bucket 1 Effluent (SR-H-26)					
10 min	1:20A	119	2380	TNTC	
	1:20B	150	3000	TNTC	
20 min	1:20A	152	3040	TNTC	
	1:20B	158	3160	TNTC	
30 min	1:20A	102	2040	TNTC	
	1:20B	150	3000	TNTC	
Bucket 2 Effluent (SR-H-22)					
10 min	1:20A	~350	7000	TNTC	
	1:20B	378	7560	TNTC	
20 min	1:20A	344	6880	TNTC	
	1:20B	358	7160	TNTC	
30 min	1:20A	300	6000	TNTC	
	1:20B	348	6960	TNTC	
Bucket 3 Effluent (SR-H-40)					
10 min	1:20A	385	7700	TNTC	
	1:20B	317	6340	TNTC	
20 min	1:20A	232	4640	TNTC	
	1:20B	284	5680	TNTC	
30 min	1:20A	358	7160	TNTC	
	1:20B	318	6360	TNTC	

Blank A1: 0/0 (after R1 eff. 10 & 20 min for B1 & B2)
Blank A2: 0 blue/25 pink (after Run 3 B2 & B3 eff. 10 min)
Blank A3: 1 blue/68 pink (after Run 3 inf 15 min and eff 20 min B1, B2, and B3)

Blank L1: 0 blue/1 pink (during Run 2)??
Blank L2: 1 blue/ 35 pink (during run 3??)

APPENDIX B:

Microcheck Results

	Name	% Match	Species	Comments
			October Microcheck	
1	Blue colony (influent)	97.5	Enterobacter Hormaechei	genus identification
2	Yellow colony (influent)	98.6	Sphingobacterium multivorum	genus identification (old culture plate, colony color may not be accurate)
3	Tan colony (influent)	100	Pseudomonas asplenii	see Microcheck analysis (old culture plate, colony color may not be accurate)
4	Blue colony (effluent)	97.69	Enterobacter Hormaechei	genus identification
5	Pink colony (effluent)	99.91	Serratia marcescens	species identification
6	Beige colony (effluent)	99.93	E. coli ATCC 11775	species identification (old culture plate, colony color may not be accurate)
7	Blue colony (effluent)	97.72	Enterobacter Hormaechei	genus identification
8	Purple colony (effluent)	99.31	Stenotrophomonas maltophilia	species identification
9	Yellow colony (effluent)	96.02	Sphingobacterium multivorum	genus identification (old culture plate, colony color may not be accurate)
			January Microcheck	
10	Our stock E. coli	99.94	E. coli ATCC 11775	species identification
11	Pink colony	99.95	Raoultella planticola ATCC 33558	species identification (Raoultella formerly classified as Klebsiella)
12	Pink colony	99.95	Raoultella planticola ATCC 33558	species identification (Raoultella formerly classified as Klebsiella)
13	Blue colony (Kovac's neg.)	100	Staphylococcus hominis hominis ATCC 27844	species identification
13a	Blue colony (Kovac's pos.)	99.66	Raoultella planticola ATCC 33558	species identification (Raoultella formerly classified as Klebsiella)
13b	Dup. Blue colony (Kovac's pos.)	99.97	Raoultella planticola ATCC 33558	species identification (Raoultella formerly classified as Klebsiella)
14a	Blue colony (Kovac's pos.)	96.71	E. coli FSIS=NA	genus identification
14b	Dup. Blue colony (Kovac's pos.)	96.7	Enterobacter pyrinus	genus identification
15a	Blue colonry (Kova's pos.)	99.96	Raoultella planticola ATCC 33558	species identification (Raoultella formerly classified as Klebsiella)
15b	Dup. Blue colony (Kovac's pos.)	99.87	Raoultella planticola ATCC 33558	species identification (Raoultella formerly classified as Klebsiella)
16a	Blue colonry (Kova's pos.)	99.88	Enterobacter aerogenes	species identification
16b	Dup. Blue colony (Kovac's pos.)	99.87	Enterobacter aerogenes	species identification
17a	Blue colony (Kovac's neg.)	99.88	Enterobacter aerogenes	species identification
17b	Dup. Blue colony (Kovac's neg.)	99.88	Enterobacter aerogenes	species identification
18a	Blue colony (Kovac's neg.)	99.88	Enterobacter aerogenes	species identification
18b	Dup. Blue colony (Kovac's neg.)	99.88	Enterobacter aerogenes	species identification