ABSTRACT

Title of Thesis: ATTACHMENT, GROWTH AND PERSISTENCE OF *CRONOBACTER* ON GRANULAR ACTIVATED CARBON FILTERS

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Several *Cronobacter* outbreaks have implicated contaminated drinking water. This study assessed the impact of granular activated carbon (GAC) on the microbial quality of the water produced. A simulated water filter system was installed by filling plastic columns with sterile GAC, followed by sterile water with a dilute nutrient flowing through the column at a steady rate. Carbon columns were inoculated with *Cronobacter* on the surface, and the effluent monitored for *Cronobacter* levels. During a second phase, commercial faucet filters were distributed to households for 4-month use. Used filters were backwashed with sterile peptone water, and analyzed for *Cronobacter*, total aerobic plate count, coliform bacteria and Enterobacteriaceae. *Cronobacter* colonized the simulated GAC and grew when provided minimal levels of nutrients. Backwashed used filters used in home settings yielded presumptive

Escherichia coli, *Pseudomonas* and other waterborne bacteria. Presumptive *Cronobacter* strains were identified as negative through biochemical and genetic test.

ATTACHMENT, GROWTH AND PERSISTENCE OF *CRONOBACTER* ON GRANULAR ACTIVATED CARBON FILTERS

by

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Dedication

Dedicated to Yin Yu, for her sweet company through the happiest of days.

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List of Abbreviations

E. coli - *Escherichia coli*

GAC - Granular Activated Carbon

DNA - Deoxyribonucleic Acid

NEC - necrotizing enterocolitis

FAO - Food and Agriculture Organization

WHO - World Health Organization

FDA – United States Food and Drug Administration

MLST - Multi-Locus Sequence Typing

CDC - Center for Disease Control and Prevention

CSO - Combined Sewer Overflow

EMB - Eosin Methylene Blue

EPS - Extracellular Polymeric Substances

PCR - Polymerase Chain Reaction

BAC - Biologically Activated Carbon

APHA - American Public Health Association

WSSC - Washington Suburban Sanitary Commission

DC Water – District of Columbia Water and Sewer Authority

TSB - Tryptic Soy Broth

BHI - Brain Heart Infusion

CFU - Colony Forming Unit

XLD - Xylose Lysine Desoxycholate

ESPM - R&F *Enterobacter sakazakii* Chromogenic Plating Medium

DFI - Brilliance *Enterobacter sakazakii* Agar

LST - Lauryl Sulfate Tryptone

Chapter 1: Introduction

Cronobacter is an opportunistic pathogen that is known to cause life-threatening infections in neonates, and contaminated infant formula has been epidemiologically linked with its infections. *Cronobacter* spp. have also been found in water and sewage treatment systems, suggesting it can also be waterborne. Additionally, antimicrobial resistant *Cronobacter sakazakii* isolates were identified in domestic kitchens, indicating that *Cronobacter* can be present at various sites in household environments.

Drinking water provided to households may not be properly treated and it contains minimal amount of organic compound that serves as nutrient for bacterial growth. Some regions have aged combined sewage system and heavy precipitation may cause sewage runoff and contaminate water bodies that provide drinking water. As people are paying more attention to water quality, GAC filled filters have been gaining its popularity among household users for cooking, drinking and reconstituting infant formula. However, previous studies concluded that microorganisms are able to colonize on GAC and release into finished water, therefore introducing a public health risk to immunocompromised populations such as infants. Bacteria attached to GAC are also able to metabolize nutrient compound from the water and grow extensively. Since *Cronobacter* spp. was previously isolated from water system, we were promoted to test whether the GAC filters can support the growth of *Cronobacter* and other pathogenic microorganisms by accumulating organic matter.

The goal of this study is to test to the hypothesis that the household GAC water filters can serve as source of *Cronobacter* spp. due to the growth of the pathogen on filter matrix.

Accordingly, the research project was designed as two phases. The first phase was to introduce *Cronobacter sakazakii to* a simulated home water filter system in order to assess the attachment, persistence and growth of *Cronobacter* on GAC water filters. The second phase was a small survey conducted by placing 24 faucet filters in households, with the used filters being evaluated for *Cronobacter* spp. and other microorganisms. Presumptive *Cronobacter* isolates were further analyzed by biochemical and genetic technologies.

Chapter 2: Literature Review

2.1 *Cronobacter*

Cronobacter is a genus of facultatively anaerobic, gram-negative, oxidase-negative, catalase-positive, asporogenic, motile, and rod-shaped bacteria within the family of Enterobacteriaceae *(54)*.

2.1.1 *Cronobacter* Taxonomy and Phylogeny

Cronobacter was previously known as "yellow-pigmented *Enterobacter cloacae*", and reclassified as *Enterobacter sakazakii* in 1980 based on the difference between *E. cloacae* and *E. sakazakii* in deoxyribonucleic acid (DNA) hybridization, biochemical reactions, pigment production and antibiotic susceptibility. Based on the 16 *E. sakazakii* biogroups and phenotypes, *Cronobacter* genus was then reclassified as a separate genus in 2008. *Cronobacter* genus currently includes seven species: *Cronobacter sakazakii*, *Cronobacter malonaticus*, *Cronobacter turicensis*, *Cronobacter muytjensii*, *Cronobacter condiment*, *Cronobacter dublinensis* (with three subspecies, *dublinensis*, *lausannesis*, and *lactaridi*) and *Cronobacter genomospecies (54, 57)*.

2.1.2 Epidemiology

Cronobacter have been associated with neonatal infections by the consumption of infant formula. Fatal *Cronobacter* infections of infants were associated with necrotizing enterocolitis (NEC), septicemia and meningitis *(48)*.

NEC has high case fatality rate of 15-25%, and it is generally characterized by bacterial colonization on the intestinal tract and increased protein level in the gastrointestinal lumen *(49)*.

Association between *Cronobacter* infection and NEC has been confirmed but the pathogenesis of the disease remains unrevealed.

Among *Cronobacter* related diseases, meningitis and septicemia are invasive, with bacterium attaching to and subsequently invading the intestinal epithelial layer after the infant is born. Meningitis caused by *Cronobacter* infection is characterized by cystic changes, abscesses, fluid collection, brain infarctions, hydrocephalus, necrosis of brain tissue, and liquefaction of white cerebral matter. Its mortality rate is 40-80%, which is the highest among all the other *Cronobacter* associated syndromes. Infection survivors often suffer severe neurological sequelae, such as hydrocephalus, quadriplegia and retarded neural development *(9, 46, 51)*.

Certain populations are more susceptible to *Cronobacter* infection *(79)*. Most infections occurred to infant within the very early stage of life. The number of reported cases of *Cronobacter* infection in young infants has been increasing in recent years, however, it is still a rare cause of severe infection compare to other infectious diseases *(78)*. The overall case-fatality rate of neonatal *Cronobacter* infection in infants was 33%, with meningitis being most often associated with fatalities. Additionally, *Cronobacter* infections have been recorded in all age groups, and several reported cases of *Cronobacter* infections were among adults (eight cases) or children aged >12 months (three cases). Elderly were especially susceptible due to an immunocompromised state, resulting in urosepsis and osteomyelitis *(60, 61)* .

Overall, it is estimated that the annual incidence rate of *Cronobacter* infection among neonates was 17.60 per million population during 1992 to 2007 worldwide, and the incidence rate decreases with age *(78)*. However, this result is obtained from limited data due to the lack of surveillance data for *Cronobacter* related illness. According to the FoodNetsurveillance sites, the annual rate of invasive *Cronobacter sakazakii* infection is estimated to be 1 per 100,000 infants,

which are children under 12 months of age, with the infection rate among low-birthweight neonates being significantly higher *(94)*. Therefore, children >12 months and adults are generally at lower risk than infants for *Cronobacter* infections *(10, 79)*.

Additionally, *Cronobacter* infections can be characterized by multiple strains, requiring the investigation of multiple isolates *(49)*. Previous research has proven that certain strains of *Cronobacter sakazakii* were more virulent *(20, 47, 57)*. For example, *Cronobacter sakazakii* colonal complex ST4 is strongly associated with the cases of meningitis, and it has been frequently isolated from powdered infant formula and processing plants in some countries *(23, 73, 82, 90)*.

2.1.3 Infection Source

The first two documented cases of neonatal meningitis by *Cronobacter* infection were reported in 1961 *(99)*. In the United States, four cases of *Cronobacter* infection were reported to the U.S. authority prior to 2011, and the investigation were conducted jointly by the Food and Drug Administration (FDA), the Center for Disease Control and Prevention (CDC), and state health departments. Within these cases, four infants were infected within the first five weeks of age by consuming powdered infant formula. Three infants developed meningitis and one had septicemia, and two of the patients died. *Cronobacter* were isolated from some of the powdered infant formula cans unopened or bottled nursery water, and no contamination were found during manufacturing or shipping. DNA fingerprinting investigation suggested that there was no genetic connection between *Cronobacter* strains involved in these incidents *(41)*.

Although the environmental sources of *Cronobacter* remains unknown, reported incidences of *Cronobacter* implicated that dry foods may serve as the reservoirs, such as

powdered infant formula, powdered milk, herbal teas, and starches. *Cronobacter* is able to survive in the desiccated state for an extended period of time Some capsulated strains of *Cronobacter* were able to be recovered from powdered infant formula after 2 years and then grow rapidly upon reconstitution *(5, 37)*.

Cronobacter can form biofilm and produce cellulose. It is able to attach to and persist on materials that are commonly used in infant feeding and food preparation such as silicone, stainless steel and glass, which may increase the risk of infection *(49, 54, 67)*. With the presence of organic matrices, *Cronobacter* are especially resistant to sanitizers and disinfectants. This has added difficulty in properly maintaining hygiene of food preparation equipment used to serve neonates *(8)*. Kilonzo-Nthenge and associates *(58)* tested *Cronobacter sakazakii* at 234 contact sites in 78 domestic kitchens in middle Tennessee, United States. *Cronobacter sakazakii* was recovered from 26.9% of kitchens tested. Multidrug resistance of the bacterial isolates was also observed.

Additionally, previous studies have also isolated *Cronobacter* from drinking water and water supply system, suggesting its waterborne characteristic *(41, 57, 69)*. *Cronobacter genomospecies* 1 (strain NCTC 9529) isolated from fresh water was characterized by genetic approach in various studies *(56, 57, 95)*. Liu and associates *(69)* evaluated a membrane filter method to qualitatively and quantitatively analyze *Cronobacter* spp. and applied the method in testing drinking water in China. The isolation rate of *Cronobacter* spp. from "small community water supplies on premises" water samples was 31/114, which is significantly higher than samples obtained from municipal water supply systems, suggesting the high prevalence of *Cronobacter* contamination in water with poor hygiene. Oger *et al. (76)* investigated the prevalence of *Cronobacter* in local drinking water supplies in Europe and found *Cronobacter* in

1.8% samples tested (10/564 strains). Schindler and colleagues *(86)* tested drinking water samples from south Bavaria, Germany; 256 coliform strains were isolated and 1 strain was confirmed as *Cronobacter*. Schindler was also able to isolate *Cronobacter* from bottled mineral water *(87)*. The profile of *Cronobacter* contamination associated with water source is shown in Table 2.1.

Infection Source	Origin	Reference
water	Great Britain	NCTC 9529 *(27)*
drinking water	France	Leclerc *et al.* and Oger *et al. (62)*
water	USA	Farmer *et al. (39)*
drinking water	USA	Camper *et al. (16)*
water	Belgium	Goullet and Picard *(44)*
water	France	Goullet and Picard *(44)*
drinking water	Germany	Schindler and Metz *(86)*
bottled mineral water	Germany	Schindler *(87)*
drinking water	Italy	Bartolucci *et al. (6)*
water	Great Britain	Oliver *(77)*
water	France	Leclerc *et al. (63)*
drinking water	Republic of Korea	Lee and Kim *(64)*
drinking water	Great Britain	Williams and Braun-Howland *(102)*
water	USA	Cruz *et al. (30)*
drinking water	China	Liu *et al. (69)*

Table 2.1 *Cronobacter* contaminations in water, drinking water and related products

2.2 Water Filtration and Granular Activated Carbon

As people are increasingly concerned about drinking water quality, household water filters have been gaining in popularity, claiming to remove unpleasant odors and provide safer drinking water. Consumers are advised to purchase and use water filter for drinking, cooking, watering plants, and preparing infant formula *(32)*.

Most water filters use granular activated carbon as their filtration medium. GAC can be embedded in various types of water filter products such as pitchers, bottles, under sink systems,

and faucet systems. It is able to absorb a variety of compounds from water, and these compounds generally include chlorine that causes unpleasant taste and odor, metallic compounds, (e.g. copper, cadmium, mercury, lead), pharmaceuticals such as antibiotics, microorganisms such as fecal coliforms, and other industrial pollutants.

The absorptive properties of GAC is characterized by its porous nature that results in an extensive surface area which ranges from a few hundred to 1,500 m^2/g *(15)*. However, mechanisms of adsorption are different depending on the nature of the compound. For example, residual disinfectants such as chlorine and chloramine may remain in finished water after disinfection process. They can be removed by GAC through a catalytic reduction reaction that transfers electrons from the carbon surface to the disinfectants. Decayed plant and organisms from soil releases water soluble organic material into the surface water, and they are generally absorbed by GAC because the carbon and the contaminants exert force to adhere to each non-polar surface, preventing organic matter being dissolved in water.

2.3 Dynamics of Bacteria on Granular Activated Carbon

GAC is able to filter microorganisms. However, the average size of a bacteria (1-2 μm) is larger than GAC pore size (less than 1 μm), the porosity of granular activated carbon still plays an important role in bacteria's colonization on carbon particles *(15)*. Figure 2.1 demonstrated the bacterial attachment on GAC using scanning electron microscope, and extracellular polymeric substances (EPS) presented on biofilms enhanced the attachment (Figure 2.2).

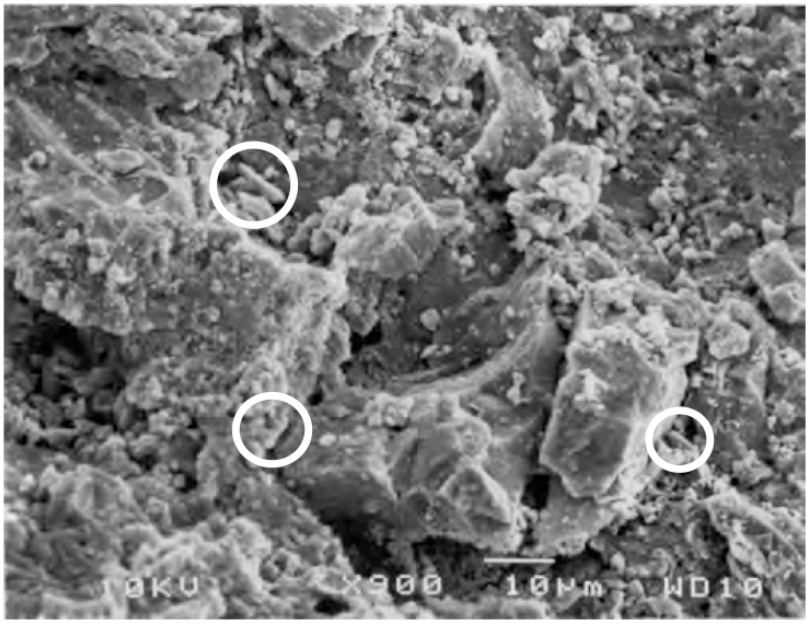

Figure 2.1 Scanning electron micrograph of microorganisms associated with the surface of granular activated carbon, as indicated by white circles *(15)*.

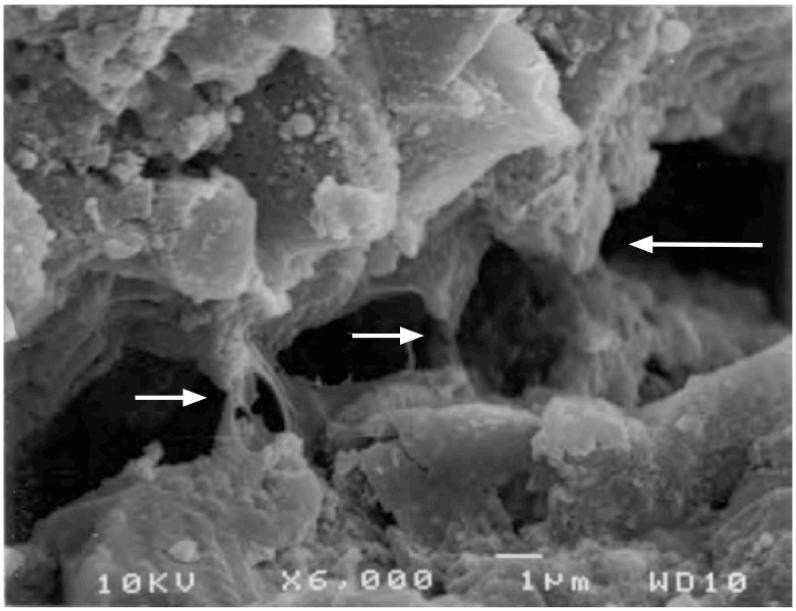

Figure 2.2 Scanning electron micrograph illustrating the presence of extracellular polymeric substances (EPS) attached to a granular activated carbon particle, as indicated by white arrows *(15)*.

2.3.1 Colonization

Bacteria are able to colonize GAC and it is impossible to maintain a sterilized activated carbon filter as long as there are bacteria in the water. Colonized bacteria can metabolize a fraction of organic compound in the water, thus increasing the filter's total capacity to remove organic compounds. Once colonized, the carbon inside the filter is considered as biologically activated carbon (BAC) *(13)*. It is a growing public health concern that pathogenic bacteria may colonize and proliferate on activated carbon, and previous studies have confirmed the strong affinity between these two *(15)*.

Camper *et al. (18)* confirmed bacterial attachment and persistence to granular activated carbon using three pathogens, *Yersinia enterocolitica* 0:8, *Salmonella* Typhimurium, and enterotoxigenic *E. coli*. Bacteria were inoculated into the water circulation that flowed through sterile GAC columns. Results demonstrated that the test bacteria were able to rapidly attach to either sterile GAC or BAC, with nearly 100% of *E. coli* attached to GAC within 30 minutes. Bacteria were also able to persist on carbon columns for an extended period of time even when unsterile river water was introduced into the circulation.

Rollinger and Dott *(85)*investigated the bacterial attachment to activated carbon and the bacterial regrowth on activated carbon through a bench scale circulated filtration system. Five different species of bacteria were applied to the system, and effluent and carbon columns were analyzed for bacterial count. The results have shown that all bacteria species have successfully absorbed by the filter within a few hours or a few days. *E. coli*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* were able to colonize and grow on sterile activated carbon filtered with tap water, increased to 10^6 to 10^7 CFU/g in the columns (Figure 2.3). *Kebsiella pneumoniae* and *Streptococcus faecalis* failed to colonize the carbon and died off shortly after inoculation.

However, all bacterial species died off quickly after inoculated on nonsterile carbon column, which is previously colonized with autochthonous microflora (Figure 2.4). This leads to the hypothesis that newly introduced bacteria are not able to proliferate due to limited nutrient and competition in BAC columns.

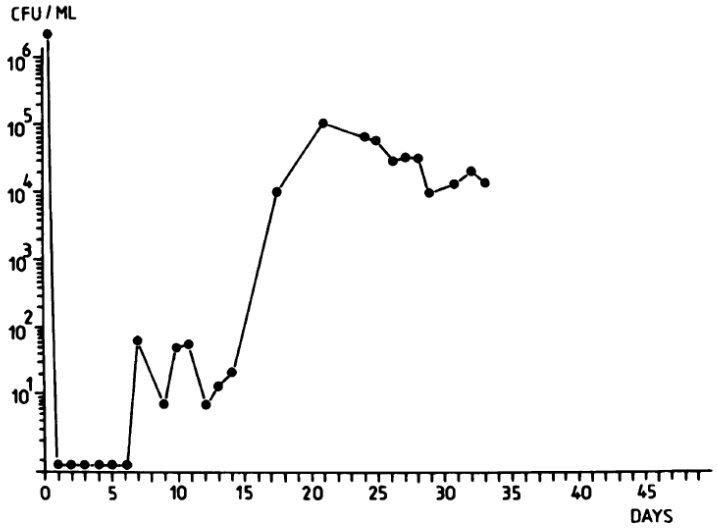

Figure 2.3 Bacterial counts in the effluent of a GAC filter inoculated with *E. coli*. The first point of the curve represents the total bacterial concentration of the inoculum (2×10^6 CFU/ml). 5-ml water samples were taken over a period of 33 days *(85)*.

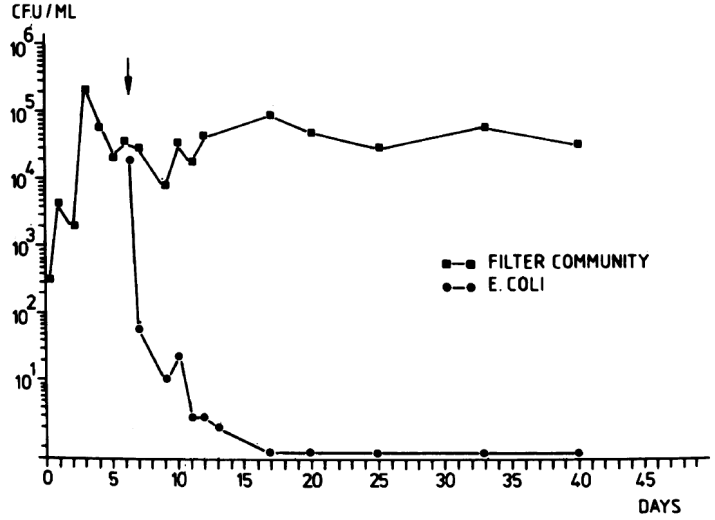

Figure 2.4 Stable heterotrophic plate count bacterial population (filter community) in the effluent of a biologically activated filter. The arrow indicates the inoculation with 4×10^6 CFU of *E. coli (85)*. *E. coli* was not able to compete with indigenous bacteria community.

2.3.2 Persistence And Growth

Carbon and energy sources present in the water supply may accumulate on GAC and supports the colonization and growth of bacteria from water and soil, including most nonpathogenic heterotrophs and autotrophs *(15)*. Previous researchers have isolated *Achromobacter*, *Arthrobacter*, *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Corynebacterium*, *Pseudomonas*, and *Moraxella* from either the filter medium or effluent *(14, 85, 89)*. Norton and LeChevallier *(75)* identified 19 Gram-negative and 5 Gram-positive bacterial genera in biofilms on GAC using fatty acid analysis. Most of the bacteria identified were the bacteria mentioned above. Typical coliforms were also isolated from water filter effluents, while the influent water always gave negative results. These coliforms generally included *E. coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Serratia*, *Hafnia*, and *Aeromonas (11, 16, 31, 98, 101)*. Many of the coliforms are not a natural component of the environment, and they generally habituate in the gastrointestinal system of a warm-blooded host. This indicated that these pathogens have successfully competed with heterotrophic bacterial populations on GAC and proliferated *(15)*. However, coliforms were not frequently isolated and identified, suggesting that colonization of coliform may be site-specific *(15)*.

The growth of these bacteria was observed, indicating that bacteria attached to GAC may utilize organic compound. For example, Buckin and associates confirmed the increase in coliform count by backwashing filter and enumerating isolated bacteria *(12)*. Davies and McFeters *(33)* applied glutamate as a carbon source to GAC and compared the bacterial growth rates between bacteria attached activated carbon and those suspended in the same level of nutrient. A significantly higher growth rate was observed with the bacteria on GAC. Bacteria were also more active on GAC based on tritiated thymidine and uridine uptake measurement.

Studies have also shown that nutrients attached to the carbon can be released and used by bacteria attached to the GAC, thereby favoring growth on GAC *(21, 22, 91)*.

2.3.3 Penetration

Bacteria can be released from GAC during filtration as single cells, aggregates or attached to carbon particles that were released into water, especially when the filter medium was biologically activated (colonized with heterotrophic bacteria) *(15)*. Stewart and associates *(93)* used a Swinnex with a polycarbonate filter to trap and collect carbon particles released from a pilot plant, and detected 36 particles per liter of filtrate. They observed 200-7000 viable count of bacteria every 1000 particles. Camper *et al*. *(16)* sampled 18,600 liters of filtered water and collected 201 carbon particle samples. 17% of the filter sites were isolated with coliform bacteria attached to released carbon particle, and 28% of these coliforms were fecal biotypes.

2.3.4 Effects of disinfection on bacterial activity

Stewart *et al. (93)* constructed a GAC pilot plant to study the bacteriological activity affected by disinfectants. Samples were collected from six carbon columns in the plant and analyzed for heterotrophic plate counts and total coliforms according to water testing standard of American Public Health Association. Filtered water was also exposed to disinfectants such as chloramines and free chlorine to quantify their effects on bacterial activity, and bacteria identification was performed by streaking plates and biochemical test kit. The study demonstrated that heterotrophic plate counts in the effluents from all columns increased significantly to 10^5 CFU/ml and maintained at 10^4 CFU/ml. Scanning electron microscope analysis was performed to determine the number of bacterial cells that had colonized carbon

particles released from the filtration process. Most of the carbon particles were colonized with 1 to 50 bacterial cells, including fecal coliforms. The study also compared efficacy of various chemical disinfectants, indicated that bacteria associated with carbon particles were extremely resistant to disinfection *(93, 96)*.

2.3.5 Commercial household filter test

Daschner *et al*. *(32)* conducted the first reported study of microbiological contamination of commercial household water filters. A total of 34 commercial water filters were collected from different households. Most effluent samples from the used filters had higher bacterial count than tap water, as shown in Figure 2.5. Six water filters were tested in the laboratory. The results suggested bacterial growth and biofilm formation at both room temperature and 4 ℃. A variety of bacteria, fungi and molds were isolated from the filter material. The study *(32)* failed to associate the bacterial activity with the degree that filter had been used.

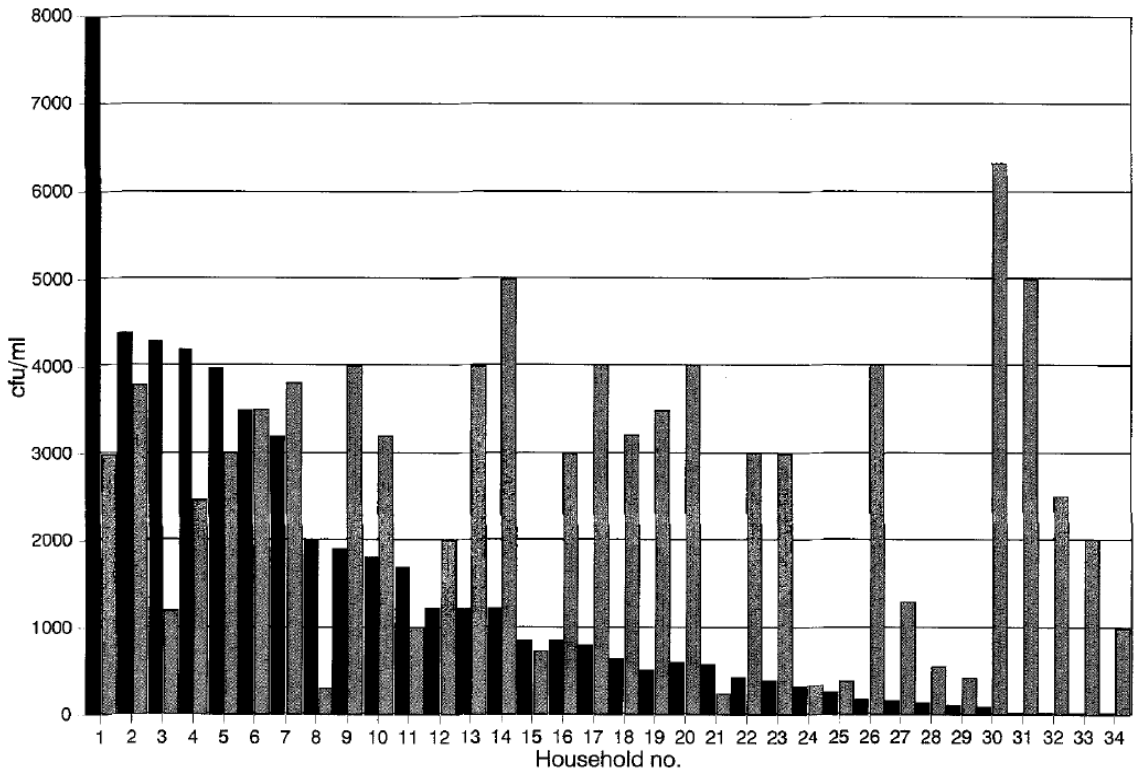

Figure 2.5 Comparison of bacterial concentration in tap water and filtered water in 34 German households. The black columns represent tap water and the grey columns represent filtered water *(32)*.

2.4 Nutrients In Water System

Once bacteria are attached to GAC, they are able to metabolize organic compounds from the water. Therefore, it is important to maintain water quality and minimize its nutrient content through wastewater treatment and water filtration system. However, water systems in some regions are extremely old and may pose a public health threat by providing improperly treated drinking water.

In Washington D.C., Potomac surface water is primarily treated with coagulation, sedimentation, and filtration process to remove most of the impurities, coliforms, virus and waterborne parasites. Water is then treated with disinfection agents such as chlorine and

orthophosphate so that the microbial load in water reaches acceptable levels *(28)*. However, organic compounds and microorganisms may still be found in finished water due to improper treatment or leaks in the sewage system after the treatment facility. The sewerage system that is currently used in Washington D.C. is one of the oldest in the U.S., with sections being more than 200 years *(4)*. It is a combined sewer system that collects rainwater runoff, domestic sewage and industry waste in one pipe that leads to water treatment plants. Combined sewage systems have serious flaws during extreme precipitation such as heavy storms. In such cases, wastewater is directly discharged into environmental water bodies when the volume exceeds system capacity, resulting in a combined sewer overflow (CSO) *(43, 65)*. The risk of bacterial contamination associated CSO has been previously studied in the U.S *(34, 71)* and around the globe *(80, 97)*. Additionally, a study conducted in Massachusetts has found statistical significance between the extreme precipitation and emergency room visits for gastrointestinal illness in the area with combined sewer system, indicating a considerably higher load of organic compounds and microorganisms in drinking water under specific circumstances *(55)*.

Overall, numerous bacteria have been isolated from GAC of used filters, indicating bacterial contaminations in drinking water system. Previous studies have demonstrated that a number of bacteria are able to attach to GAC filters and grow by utilizing nutrient in the water resulted from improper water treatment and distribution system. Additionally, bacteria can also release from GAC filters and contaminate effluent. Since several *Cronobacter* outbreaks have implicated contaminated drinking water, it is hypothesized that GAC filters could serve as a reservoir for *Cronobacter* by trapping low levels of nutrient upon which the organism could grow.

Chapter 3: Objectives

Based on the fact stated above, the goal of this project is to test the hypothesis that home water filters can serve as source of *Cronobacter* spp. due to the growth of the pathogen on the filter matrix as the filters accumulate organic matter.

The research project was designed to test the hypothesis in two phases:

The first phase was a proof of principle study using a simulated home water filter system. The general approach was to operate the system with water containers filled with low levels of nutrients in the form of 0.01% BHI broth. *Cronobacter sakazakii* was then introduced and its levels were monitored over time to assess attachment, persistence, growth and release into the water supply.

The second phase was a small survey conducted by placing 24 faucet filters in different households for 4-month use. The used filters were then assayed for *Cronobacter* spp. and other microorganisms, with presumptive *Cronobacter* isolates being further evaluated by biochemical and genetic techniques.

Chapter 4: Materials and Methods

4.1 Bacterial Strains

Cronobacter sakazakii strain 607 used in this study was obtained from Food and Drug Administration/Center for Food Safety and Applied Nutrition stock culture collection. *E. coli* K-12 (ATCC 23716) was supplied by Department of Nutrition and Food Science in University of Maryland. Cultures were stored at -80 °C in 40% glycerol and tryptic soy broth (TSB). Cells were resuscitated by culturing in TSB broth for 24 h at 37°C.

4.2 Plating Media

All bacterial media were prepared according to the manufacturers' instruction. Approximately 15mL of media was poured to bacteriological petri dishes with lid (Falcon, Cat. No. 08757), and dried upside down at room temperature for 48 hours. Then they are packaged in petri plate sleeves and stored at room temperature for less than a week to maintain its functionality.

4.3 Simulated Home Filter System

4.3.1 System Configuration

To simulate the household GAC water filter system, aquarium activated carbon was first applied. The carbon was filled into plastic pipette to its maximum volume. A water reservoir was placed at higher location to create water pressure for constant flow, and it is completed sealed to prevent microbial contamination. Sterilized rubber tubes were used to connect water source and

carbon columns. However, a large amount of carbon particles were flushed to the effluent during operation due to the loose structure of aquarium activated carbon. Since bacteria may attach to carbon particles, this may negatively affect microbial enumeration results. Because the water carboy was sealed, water stopped flowing after approximately one hour due to imbalanced pressures. Additionally, water leakage was frequently observed since each connection in the system was not fully airtight. According to Bernoulli's principle, this may also attract bacteria from outside environment since the flowing water created a lower pressure compared to outside. This may resemble the real world scenarios, which cracks in aged water pipes may create an inward force on nearby microorganisms, and eventually result in bacterial contamination in water. In the early attempts to operate this system, water effluent was highly contaminated.

After a number of revisions on the prototype, GAC obtained from commercial pitcher filters was applied in the system since it remains intact after long-term water filtration. It is also mixed with silicon beam to increase the ability to attract contaminants, as well as adding durability to the product. Additionally, polyvinyl chloride tubes were used due to its sturdy characteristics. Each connection was applied with epoxy putty to completely seal any potential leaks. Eventually, a fully designed water filter simulation system was installed in the laboratory in Department of Nutrition and Food Science, University of Maryland. A schematic diagram of the system is shown in Figure 4.1.

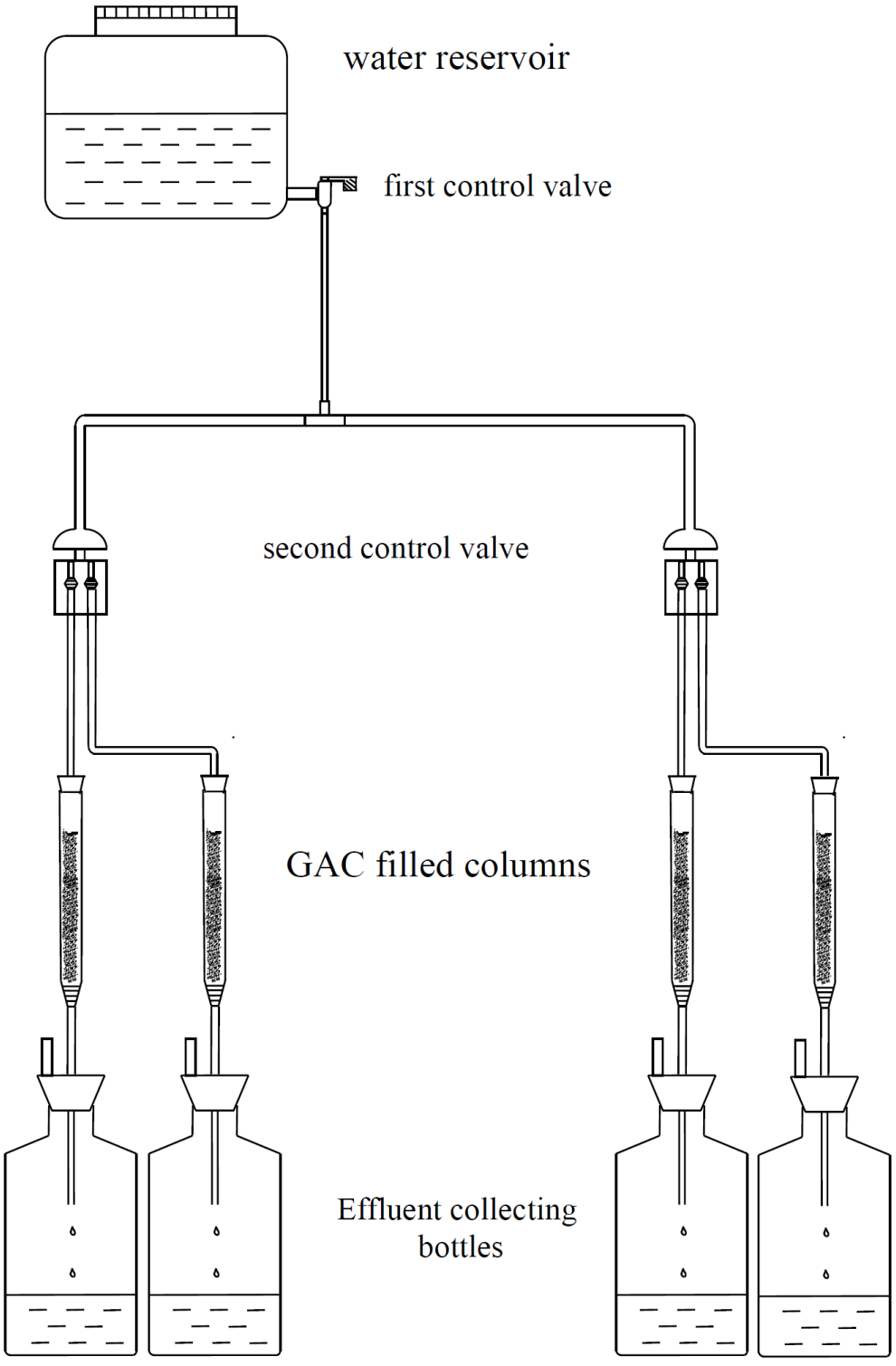

Figure 4.1 Schematic diagram of simulated home water filtration system.

The water reservoir (Fisherbrand, Cat. No. 03007) was located at the top shelf of laboratory bench, which was approximately 5 feet above the water-collecting bottle. The purpose was to create a steady gravitational pressure to maintain constant water flowing within the system. The spigot attached to the carboy serves as the first control valve of water flow rate. The second control valve was created using a 4-channel check valve purchased from a local pet store. The valve is originally designed for household aquarium usage, and it was applied to our simulation system, being airtight and adjustable in terms of flow rates. Additionally, it is made with durable, transparent plastic that could be sanitized by bleaching and water flow rates readily observed.

GAC was obtained from pitcher replacement filters (Brita LP, Oakland CA. Model No. 35503). It is constructed with plastic cover that contains granular activated carbon inside the main body. Built-in mesh screens are installed on both side of the filter to prevent carbon flecks release. Silicon beads is mixed with the carbon to serve as an ion-exchange resin that may improve the ability to remove residual disinfectants in the water.

Pitcher filters were autoclaved at 121 ℃ for 15 minutes to sterilize the granular activated carbon. Under a biosafety hood (Thermo Scientific 1300 Series Class II, Type A2, Waltham, MA), pitcher filters were cut open with sterilized scissors. Sterile 50mL disposable plastic pipettes (Thermo Scientific Nunc, Cat. No. 12567) were used as the columns in the simulated faucet filter. The upper portion of the pipettes was removed using a sterile handsaw. All tools used in this step were sanitized by submerging in 10% of hypochlorite (ACROS Organics, Cat. No. AC41955) for 24 h.

To simulate the household water filter construction, a small portion of micro-fine polyester pads was rinsed with 70% ethanol and air-dried, then inserted into the bottom of the

prepared column. It is used because it is durable in a long-term moist environment. It also traps small particles such as carbon flecks but allows bacteria in the water to pass.

The sterile GAC was then transferred to fill the columns up to the 25mL mark on the pipette. The column was weighed before and after filling with GAC. The average weight of added GAC was 17.8 g. The upper open end of the columns was sealed with one-hole rubber stopper (Fisherbrand, Cat. No. 14-135B) to prevent bacterial contamination from surrounding environment.

The effluent collection section of the system was comprised of a sterile 1-liter glass bottles sealed with a one-hole rubber stopper (Fisherbrand, Cat. No. 14-135G). To create constant air pressure within the system, as well as lowering the risk of airborne bacterial contamination, the rubber stoppers were cut with a 3mm hole and connected with a tube that is filled with polyester pad at the end.

Each component of the system was connected with polyvinyl chloride tubing (Fisherbrand Cat. No. 14169) which was previously submerged in 10% hypochlorite solution for 24h. Additionally, each connection was completed sealed by epoxy putty to prevent water leakage and bacterial contamination.

4.3.2 Preliminary Studies with *E. coli* K-12

During the first day of experiment, a water carboy was filled with 20 Liter of 0.01% (0.1 g/L) Brain Heart Infusion (BHI) broth to simulate the nutrient level that is associated with polluted water supply system. The BHI was autoclaved at 121 ℃ for 15 minutes. Tubing, stoppers and control valve was previously submerged in 10% bleach solution for 24h. Water carboy was then placed at room temperature overnight until completed cooled down. After 24

hours, the laboratory bench was sprayed with 70% ethanol following the schematic diagram (Figure 4.1).

E. coli K-12 was inoculated into 5ml of sterile TSB and incubated at 37℃. The inoculum was fully vortexed for 10 seconds, and then diluted to 10^{-1} by transferring 1ml of inoculum to 9.0ml dilution blank containing sterile 0.1% peptone water (BD Difco, Cat. No. DF1807). A 10^{-3} dilution were prepared by transferring 0.1 ml of the 10^{-1} dilution to a second 9.9-ml dilution blank containing sterile 0.1% peptone water, and the 10^{-5} dilution was prepared in the same manner. A 50-µl aliquot of each dilution were plated onto triplicate pre-poured BHI agar (BD Difco, Cat. No. DF0418) plates using a spiral platter (Eddy-Jet Spiral Plater; NeuTec Inc., Farmingdale, NY, USA). The plates were then incubated at 37 ℃ for 24 h. The number of colony forming units (CFUs) per ml on the BHI agar plates was determined by Automatic Colony Counter (Flash & Go; NeuTec Inc., Farmingdale, NY), and the MacConkey agar plates were used to confirm the identity of *E. coli*, which appears pink to red colony with bile precipitation. The level of *E. coli* in the 10^{-5} dilution was approximately 10^4 CFU/ml.

Two carbon columns were included in the first experiment. To operate the system, the spigot on the carboy was opened first, followed by the opening of control valve. To assure the volume of collected water is 1 liter per day, the water flowrate was adjusted to 1 drop of water every 4.8 – 5.2 seconds.

The system was operated for 30 minutes until the activated carbon is fully rinsed with water and carbon dust was flushed. Operation was paused, and 1 ml of the 10^{-5} dilution was quickly added to the top surface of GAC using a pipette. The inoculum was allowed to attach to the GAC for 15 minutes. The uninoculated control column received 1.0 ml of sterile water instead of the inoculum. The system was then initiated by slowly opening the spigot and valve

until the desired flowrate. The adjustment time was minimized to avoid timing errors. The goal was to adjust the flowrate before the first drop of effluent.

Timing started at the point when first drop of effluent dripped into collecting bottle, marked as 0 minutes. Stoppers attached to the collecting bottle were quickly removed and 1ml of dripping effluent was collected into sterile 1.5 Microcentrifuge tubes (Fisherbrand, Cat. No. 02681272) that have been previously autoclaved at 121 ℃ for 15 minutes. Microcentrifuge tubes were immediately transferred to ice bucket for spiral plating. Sampling for inoculated column was conducted every few minutes within the first hour of operation, every few hours of the first day of operation, and twice a day during the first week. Effluent samples were then plated onto BHI agar plates and counted using the same assay for testing inoculum. Effluent from the control column was sampled in the same manner. Detailed sampling times are shown in the Appendix 1-2. Final result is plotted by MATLAB 8.6 software (MathWorks, Natick, MA).

After seven days, the columns were disassembled from the system and examined. Under the biosafety hood, carbon columns were equally cut by a sterile handsaw. The length of each portion of GAC on the column was 8ml, which weighed 5.93g. Carbon inside the column was then flushed by 100-ml mixture of detergent using a pipet dispenser and collected into a 100-ml sterile beaker. The mixed detergent was described by Camper, *et al*., which is a solution of Tris buffer (0.01 M, pH 7.0) (Fisherbrand, Cat. No. BP1759), Zwittergent 3–12 (10^6 M) (EMD Millipore Cat. No. 6930155GM), ethyleneglycol-bis-(*β* amino-ethyl ether)-*N*,*N*^{1}-tetra acetic acid (EGTA, 10^3 M) (MP Biomedicals, Cat. No. ICN19517425) and peptone (0.01%). Each bottle was then homogenized at 16,000 rpm at 4°C using PRO250 homogenizer (PRO Scientific Inc., Monroe, CT) for 3 minutes.

Filter pads were also collected with 100ml of mixed detergent in a bottle and vigorously shaken for 3 minutes.

Finally, 50 µl of each homogenized solution (including columns and pads) were plated onto triplicate pre-poured BHI agar plates and single MacConkey agar plates using a spiral platter. BHI plates were counted using the automatic plate counter, and MacConkey plates were used to confirm the identity of *E. coli*.

4.3.3 Trial #1 (Water With Minimal Nutrient Test)

After the preliminary tests, simulated system was fully dissembled. Each component or connecting part was submerged in 10% bleach solution for 24h. Water carboy was emptied and refilled with 20 Liter of 0.01% BHI broth. It was then autoclaved at 121 ℃ for 15 minutes and placed at room temperature overnight. *Cronobacter sakazakii* 607 was inoculated in 5ml of sterile TSB and incubated at 37℃. The inoculum was fully vortexed for 10 seconds, and then diluted to 10^{-1}, 10^{-3}, and 10^{-5} following same protocol in the preliminary studies. A 50-µl aliquot of each dilution were plated onto triplicate pre-poured BHI agar plates using a spiral platter. The plates were then incubated at 37 ℃ for 24 h, and then placed at room temperature for 24 – 48h until the yellow-pigment is generated. *Cronobacter sakazakii* 607 is a strong pigment producer and this served as the indicator of *Cronobacter* colonies on BHI agar plates *(37)*. The number of colony forming units (CFUs) per ml was determined by Automatic Colony Counter (Flash & Go; NeuTec Inc., Farmingdale, NY). The level of *Cronobacter sakazakii* 607 in the 10^{-5} dilution was approximately 10^4 CFU/ml.

Simulated water filtration system was assembled in the same manner. Two carbon columns were included in the first experiment, with one being inoculated with 1-ml *Cronobacter*

inoculum and one serving as control. The system was initiated for 30 minutes by opening carboy spigot and control valve and water flowrate was adjusted to 1 liter per day, until the carbon columns were fully rinsed. Following the same experimental design in preliminary studies, 1-ml of the 10^{-5} dilution of *Cronobacter* inoculum was added and the water effluent was sampled. Detailed sampling times are shown in the Appendix 3.

After seven days, the columns were examined in the same manner. 50 µl of each homogenized solution were plated onto triplicate pre-poured BHI agar plates using a spiral platter, and plates were incubated at 37 °C for 24 h and placed at room temperature for approximately 48hr until yellow-pigment appears. Plates were counted using the automatic plate counter.

4.3.4 Trial #2 (Nutrient Deprived Water And Sterile Water Test)

In the second experiment, the simulation system was installed following the same protocol. Two water carboys were included, with one filled with sterile water with minimal nutrient and another one filled with sterile water. Four columns were installed. The first column was inoculated with 10^4 CFU of *Cronobacter* and filtered water with minimal nutrient. The second column served as the control that filters water with minimal nutrient. The third column was inoculated with same level of *Cronobacter*, and only sterile water was filtered. The fourth column serves as another control with only sterile water filtered. The effluent was collected and examined with the same method.

After seven days, the third and fourth columns were disassembled and analyzed. The first and second columns continued to filter water, and the water with minimal nutrient was switched

to sterile water only. At 14 days after the experiment started, all the columns were disassembled and analyzed using the same assay as in trial #1.

4.4 Phase Two: Commercial Faucet Filter Test

A popular brand household faucet water filter systems (Brita Basic Faucet Water Filter System, Model Number: 35214; Brita, Oakland, CA) were distributed to 24 households in Maryland and Washington D.C. suburban area for 4-month use. The zip code of each sampling location is shown in a map (Figure 4.2). Upon the time of collection, filter replacement indicators were checked for the degree of usage, and replacement filter cartridges were sealed and placed in a sterile bag while awaiting further examination. A brief survey was also conducted to understand the experience of using the product.

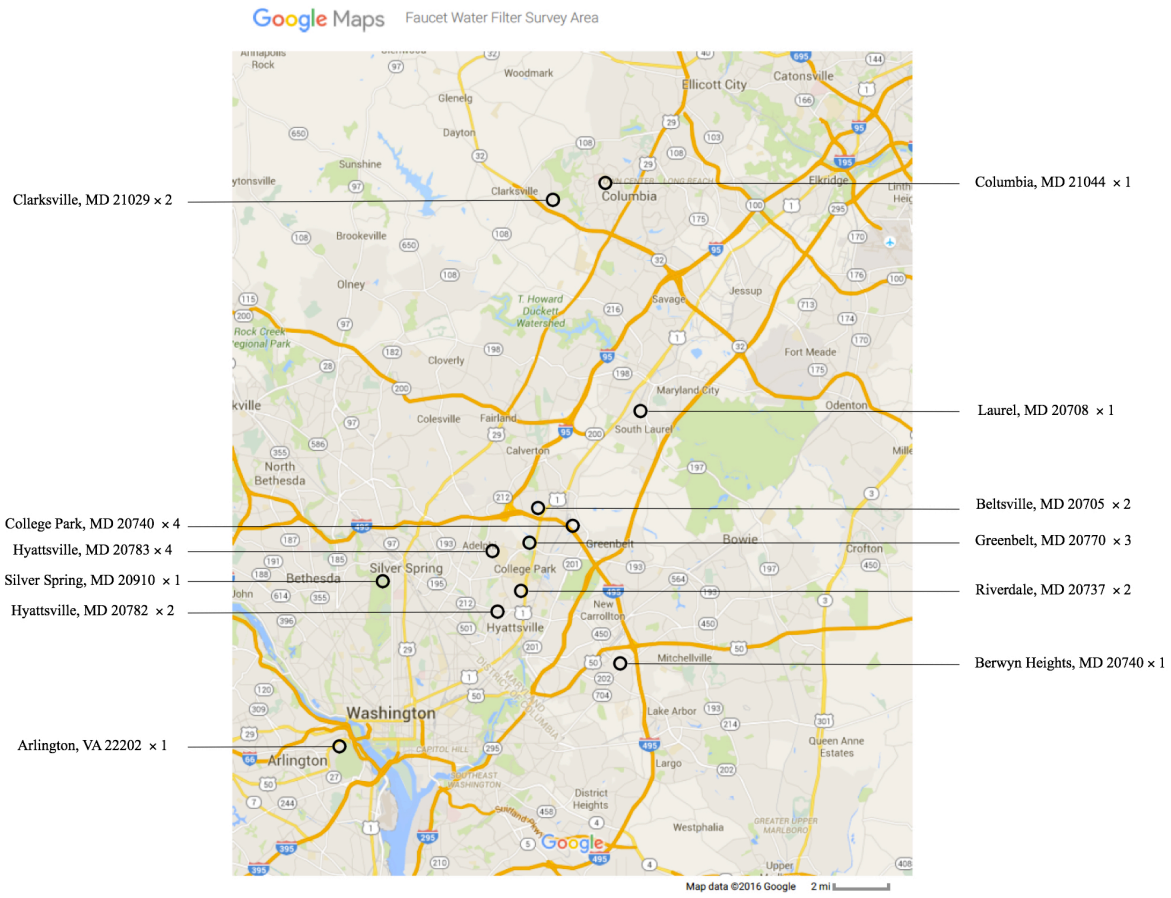

Figure 4.2 Map of faucet water filter survey area. Each survey location is circled and indicated with zip code. Number of surveyed filters in each location is also included.

The diagram of cross section of the filter is shown in Figure 4.3.

Under the biosafety hood, both water inlet and outlet of the replacement filter cartridge were connected with a short polyvinyl chloride tube that was previously submerged in 10% bleach for 24h. A pipet dispenser (Argos Technologies, Cat. No. 03391) and a sterile 50mL disposable plastic pipettes were used to push 50ml peptone water backwards from the water outlet. Effluent at the water inlet was collected into a 50ml centrifuge tube (Fisherbrand, Cat. No. 06443). Each centrifuge tube was then centrifuged at 3200 rpm at 4°C for 15 minutes by

Beckman GS-15R centrifuge (Beckman, Palo Alto, CA). Finally, 45 ml of supernatant was removed and the bacterial pellet was suspended in the remaining 5ml peptone water solution.

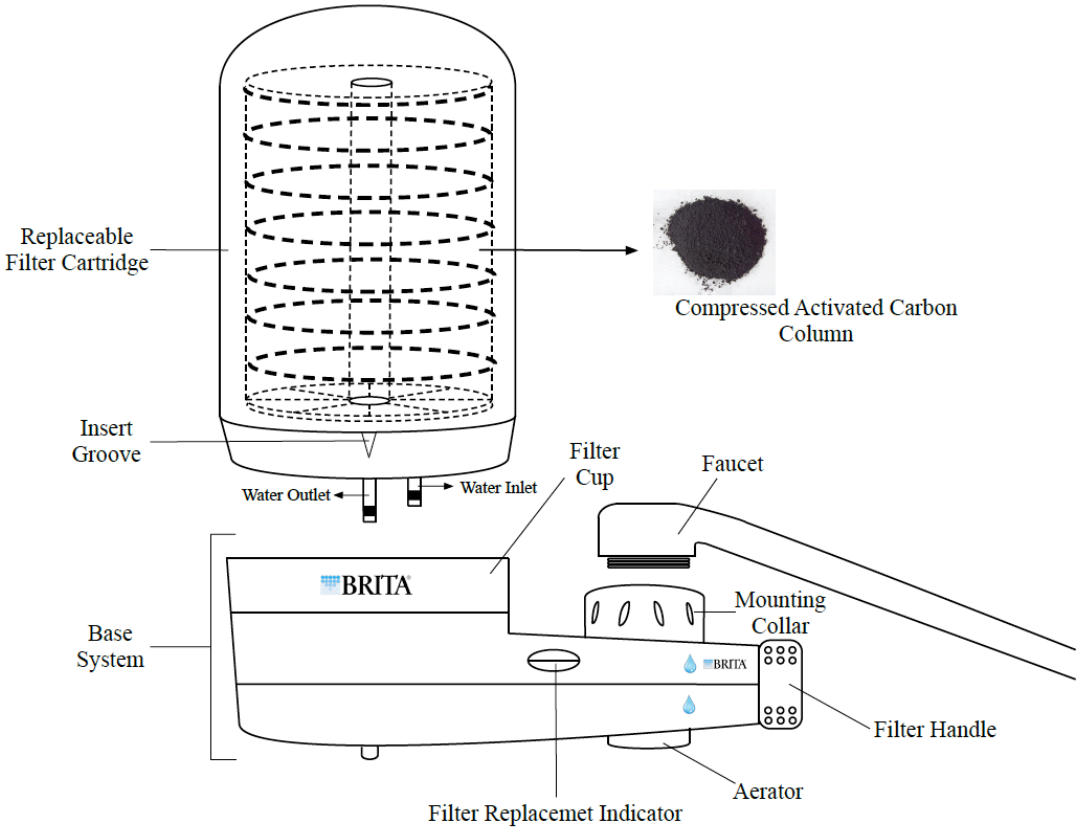

Figure 4.3 Configuration of household faucet water filtration system.

4.4.1 Bacterial Enumeration and Initial Identification

50-µl aliquots of each concentrated backflow sample was plated onto triplicate pre-poured BHI agar plates, Violet Red Bile Glucose agar (VRBG) agar (BD Difco, Cat. No. D186617) plates, and MacConkey agar (BD Difco, Cat. No. DF0075) plates. Bacterial suspension was also plated on Brilliance *Enterobacter sakazakii* agar (DFI formulation) (Oxoid, Cat. No. CM1055) *(52)*.

After incubation, BHI plates were counted by Automatic Colony Counter to calculate the total aerobic plate count. Enterobacteriaceae ferment glucose on VRBG plates, thus they produce acid products and form red to dark purple colonies surrounded by red-purple halos. Coliform bacteria present as brick red in color and maybe surrounded by a zone of precipitated bile due to lactose fermentation on MacConkey plates *(103)*. 51 colonies were picked by sterile inoculating loops (Fisherbrand, Cat. No. 22363602) and then streaked onto BHI plates for storage and further analysis. BHI plates were incubated upside down at 37 °C overnight. Colonies from BHI were then streaked onto four differential plating media, which were: Eosin Methylene Blue (EMB) Agar (Thermo Scientific Remel, Cat. No. R453402), Simmons Citrate Agar (Thermo Scientific Remel, Cat. No. R454652), Xylose Lysine Desoxycholate (XLD) Agar (BD Difco, Cat. No. DF0788179) and Pseudomonas Isolation Agar (BD Difco, Cat. No. DF0927)

EMB agar was applied for its ability to isolate gram-negative enteric bacteria, such as *E. coli*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella* and *Pseudomonas*; Simmons Citrate Agar was used for the differentiation of Gram-negative bacteria based on citrate utilization, which results in an intense blue color in the slant; XLD is effective in differentiating *Shigella* and *Salmonella*; and Pseudomonas Isolation agar was used to isolate *Pseudomonas* spp. and differentiate *Pseudomonas aeruginosa* since it may form greenish colonies after 18 hours incubation and turn blue to blue-green as incubation continues. Bacteria samples were also streaked onto DFI agar to further confirm their identity of *Cronobacter* spp..

4.4.2 Statistical Analysis

Among filters with different indicator status, differences in total aerobic plate count were examined by one-way Analysis of Variance (ANOVA). When differences were significant,

Tukey's test was used to compare the means. $P < 0.05$ was considered statistically significant. The same method was employed to analyze coliform counts and Enterobacteriaceae counts. Statistical analysis was performed by JMP® Pro 11.0.0 software (SAS Institute, Cary, NC).

4.4.3 Biochemical and Genetic Test

Isolates that produced chromogenic reactions on DFI agar consistent with *Cronobacter* spp. were re-streaked onto pre-poured BHI agar plates and incubated for 24h at 37 °C. Plates were then transferred to U.S. Food and Drug Administration laboratory in Laurel, Maryland for biochemical and genetic confirmation.

Colonies of the six presumptive *Cronobacter* isolates on BHI plates were inoculated into Tryptic Soy Broth (TSB) (Thermo Scientific Remel, Cat. No. R455052) with 1% NaCl and incubated at 37°C for 24h. Six isolates were also streaked onto pre-poured R&F *Enterobacter sakazakii* chromogenic plating medium (ESPM) described by L. Restaino et al *(35, 83)* to check the colony morphology, and Bovine Blood Agar (Hardy Dagnostics, Cat. No. 02686210) to prepare biochemical test.

After 24h, 2ml of vortexed bacterial cultures were loaded to 2ml Microcentrifuge tube and centrifuged (RCF 20,800, 3 min). Supernatant was then removed to get the bacterial pellet for genomic DNA extraction, which was conducted by QIAcube instrument (QIAGEN, Valencia, CA) according to manufacturer's instruction. The total amount of extracted DNA was quantified by Nanodrop 2000 instrument (Thermo Scientific, Wilmington, MA).

Polymerase Chain Reaction (PCR) analysis was based on the *Cronobacter* zinc-containing metalloprotease gene, *zpx*, using primer BAM 122 (5'-AWATCTATGACGCGCAGAACCG-3') and primer BAM 123 (5'-

AAAATAGATAAGCCCGGCTTCG-3') that amplifies a 350-bp fragment of the *zpx* gene *(59)*. PCR preparation was conducted by mixing 9.5 µl of nuclease-free water (Invitrogen Ambion, Cat. No. AM9938), 2 µl of primer set, 12.5 µl of Master Mix (Promega GoTaq, Cat. No. PRM5122) which supplied GoTaq Hot Start Polymerase, buffer, dNTPs, and MgCl2, and 1 µl bacterial cell lysate as DNA template. *Cronobacter* strain BAA 894 was applied as positive control, and nuclease-free water was used for negative control. *(59)*.

2720 Thermal Cycler (Applied Biosystems, Foster City, CA) was used to run the PCR, with temperature profile of:

95℃, 5 minutes to activated DNA polymerase, followed by 35 cycle of 1 minute at 95℃, 30 seconds at 62℃, 1 minute at 72℃, and final extension for 7 minutes at 72℃.

Electrophoresis was carried out for 30 minutes at 100 V using RunOne Electrophoresis Cell (Embitec, San Diego, CA), and a digital image was captured by SynGene apparatus (SynGene, San Diego, CA) under UV illumination.

Biochemical confirmation and characterization were carried out using a VITEK-2 Compact (Biomerieux, Hazelwood, MO) in conjunction with a "gram-negative card" according to the manufacturer's instructions. Vitek 2 Compact version 5.03 software was used to identify the six bacterial strains taxonomically through a slash-line protocol analogous that is designed for the identification of *E. coli*, *Salmonella* spp., and other enteric gram-negative bacterial pathogens.

Chapter 5: Results

5.1 Phase One

5.1.1 Preliminary Studies

The bacterial level in the inoculum was between 10^4 to 10^5 CFU, and detailed data is listed in Table 5.1.

During the first preliminary study, *E. coli* was first detected from the water effluent at 1 minute, and the bacterial level was 10^2 CFU per ml. A steady increase of bacterial concentration was observed until 4 minutes, reaching 10^3 CFU per ml. It indicates that bacterial level had reached the maximum adsorption ability of GAC at the moment, since the bacterial level dropped to 10^1 CFU/ml. Between 15 minutes and 3 days of sampling, concentration of water effluent demonstrated significant increase from 10^1 CFU/ml to 10^7 CFU/ml, suggesting that bacteria attached to GAC utilized nutrients from the water for growth. Bacterial level stayed fluctuating between 10^6 to 10^8 CFU/ml until 7 days. Overall bacterial growth curve is shown in Figure 5.1. Second preliminary study was conducted following the same protocol, and the inoculated bacterial level was slightly higher than the first study. Bacterial level in the water effluent showed the same trend with the first study (Figure 5.2) and it reached 10^6 to 10^7 CFU/ml at the end.

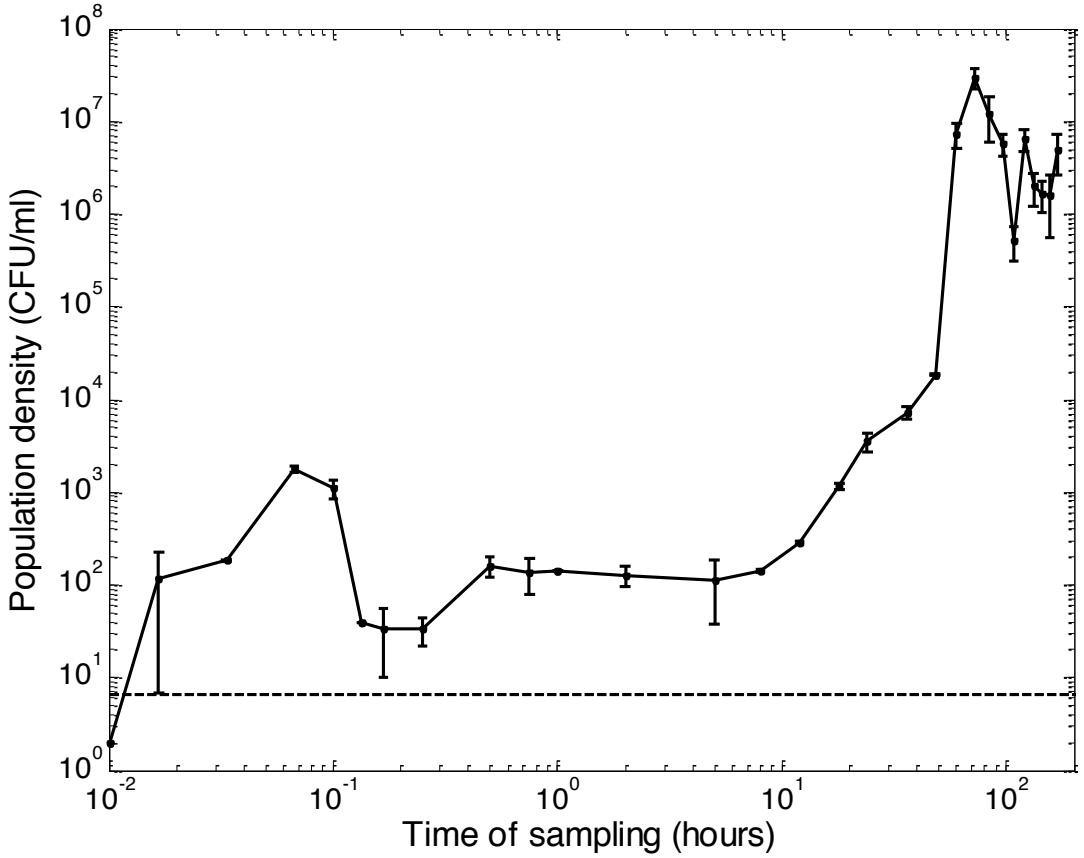

Figure 5.1 Preliminary study #1: *E. coli* concentration in the water effluent filtered by sterile granular activated carbon in 7 days. Water was added with minimal nutrient (0.01 % of Brain Heart Infusion Broth) then sterilized. Each data point represents average of triplicate bacterial count (± sd). Dotted line indicates the lower limit of detection.

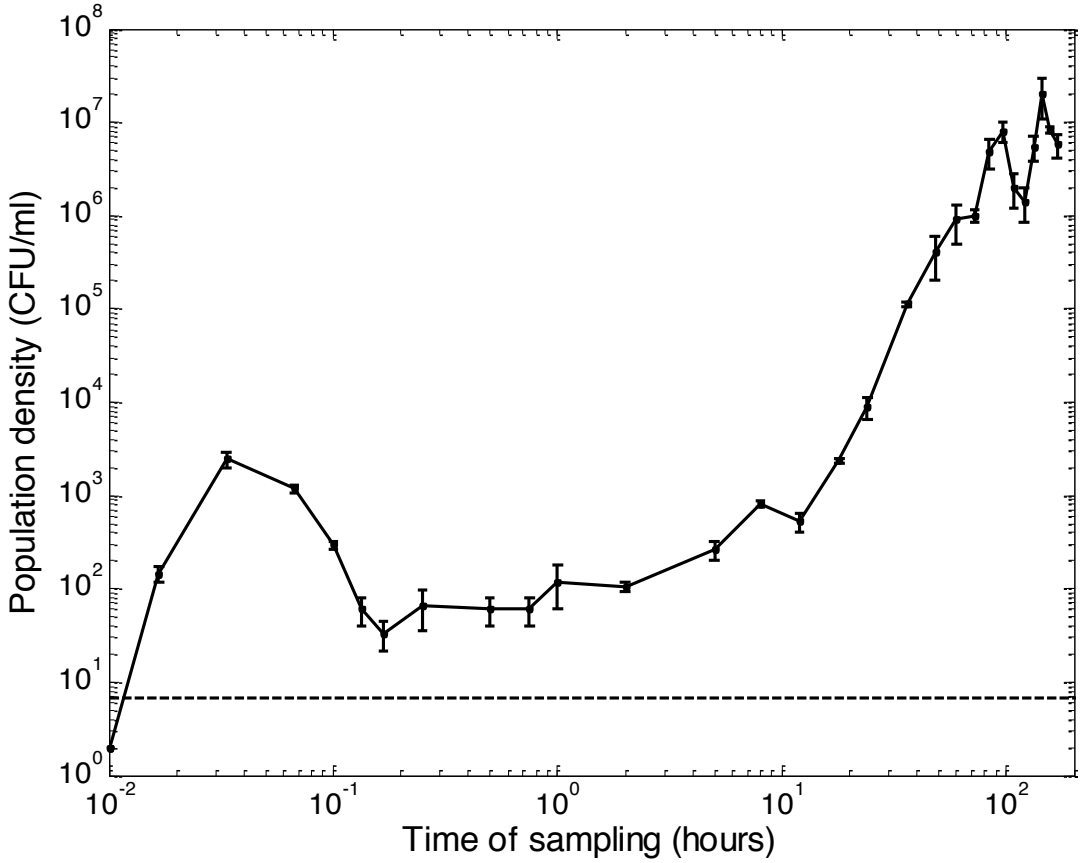

Figure 5.2 Preliminary study #2: *E. coli* concentration in the water effluent filtered by sterile granular activated carbon in 7 days. Water was added with minimal nutrient (0.01 % of Brain Heart Infusion Broth) then sterilized. Each data point represents average of triplicate bacterial count (± sd). Dotted line indicates the lower limit of detection.

In both studies, carbon columns were cut by sterile handsaw and analyzed for bacterial profile. No contamination was observed. The top part of the column absorbed most of the bacteria, while the bacterial count was lower at deeper portion of the column. The fewest bacteria were recovered from the polyester pad at the bottom of the column. Detailed data is shown in Table 5.2.

5.1.2 Trial #1

After inoculating the GAC columns with 10^4 CFU of *Cronobacter sakazakii* 607 and initiating the flow of water, *Cronobacter* was first detected during the second time sampling at 1 minute, and attained a level of 10^2 CFU/ml at 2 minutes (Figure 5.3). Bacterial level then gradually decreased and became not detectable between 45 minutes to 2 hours after the operation started, indicating that *Cronobacter* from the inoculum have successfully attached to the GAC and the flowing water was unable to desorb the bacteria.

After 2 hours, bacteria were released as more water flowed through, and the bacterial concentration demonstrated a fast increase and remained at the level of approximately 10^7 CFU/ml starting at 36 hours after the first sampling. This indicated that the attached *Cronobacter* on GAC has colonized and grew by utilizing nutrient from water. Since the adsorption capacity of GAC is limited due to the number and the size of the pores, *Cronobacter* were released significantly into effluent, and fluctuated between 10^6 and 10^7 CFU/ml. Since the GAC obtained from the pitcher filter was fairly new, carbon particles were observed during the first day of sampling. They may able to carry attached bacteria into effluent and recovered from plate culturing *(93)*.

Additionally, no microorganism growth was observed on BHI agar plate and MacConkey agar plates plated with samples from control columns.

After 7 days, effluent sampling was ceased and the carbon column were taken off and analyzed. Results have shown that the top part of carbon column had the highest level of adsorption, which was up to 10^9 CFU per gram of GAC, and bacterial concentration gradually declined with the depth of carbon. The polyester pad at the bottom of the column trapped the lowest level of bacteria, which was approximately 10^7 CFU in total (Figure 5.2).

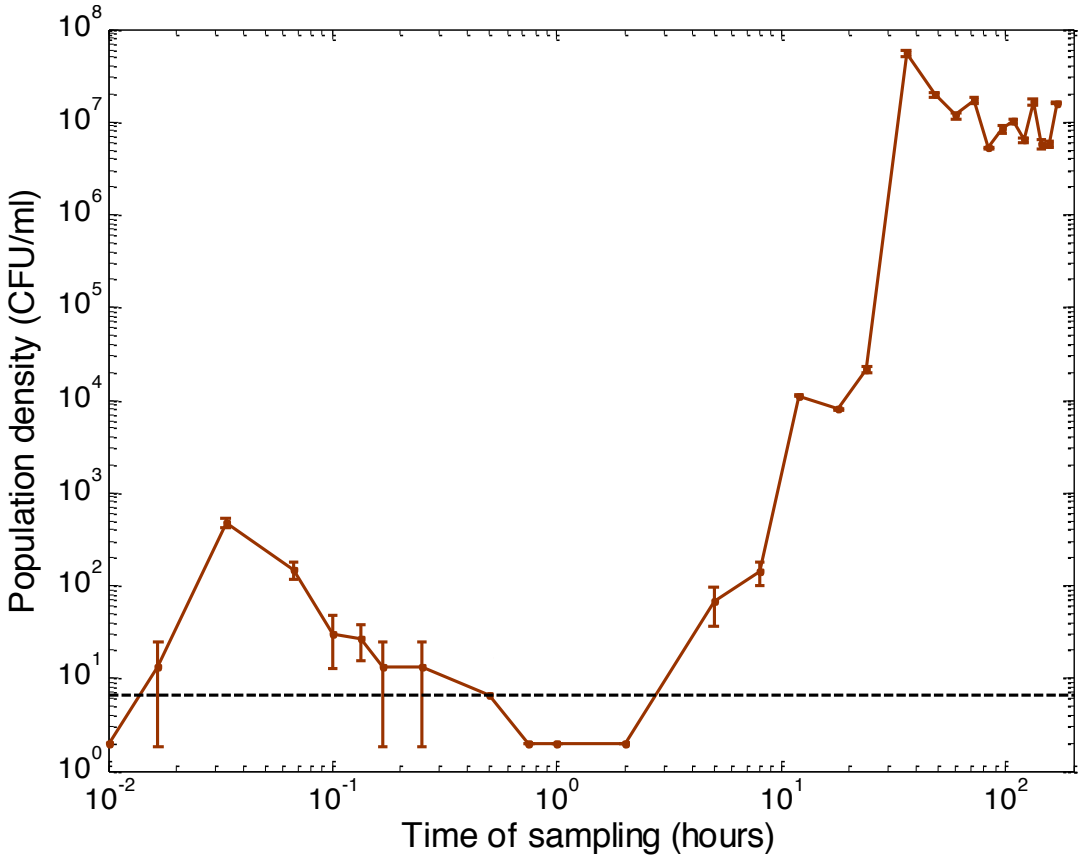

Figure 5.3 Trial #1: Bacterial concentration in the water effluent filtered by sterile granular activated carbon in 7 days. Water was added with minimal nutrient (0.1 % of Brain Heart Infusion Broth) and then sterilized. The lower limit of detection was approximately 20 CFU/ml, and samples with non-detectable *Cronobacter sakazakii* 607 were assigned a value of ≤ 20 CFU/ml. Each data point represents average of triplicate bacterial count (± sd). Dotted line indicates the lower limit of detection.

5.1.3 Trial #2

Two columns were installed and inoculated. One column was treated with sterile water with minimal nutrient, and another column was treated with only sterile water. Both columns were inoculated with the same level of *Cronobacter*. Accordingly, two control columns (inoculated with 1-ml sterile water) were also installed and monitored.

During the first week, similar results for column treated with minimal nutrient were observed (Figure 5.4). *Cronobacter* was detected after 1 minute of sampling, and its concentration gradually declined until undetectable levels at 45 minutes. *Cronobacter* concentration peaked and maintained at 10^7 CFU/ml after 4.5 days of sampling, which was about 3 days later than the time in the first experiment. It is likely because of the environmental temperature in the laboratory was lower than the one during previous test, and it may have restricted bacterial growth. For the column treated with sterile water, bacterial concentration in the effluent showed similar trend within the first hour (Figure 5.4). However, it had a gradual reduction in bacterial count after 2 minutes and remained undetectable after 24 hours. It indicated that *Cronobacter* was fully attached to GAC and likely entered a starvation state due to nutrient deprivation. This carbon column was removed after 7 days since there was no detectable *Cronobacter* in the effluent after 24 hours. Bacterial concentrations in the carbon were lower than the ones treated with minimal nutrient and deprived. After 7 days, the first column was switched to sterile water only. Bacterial level slightly declined, but still fluctuated between 10^5 and 10^6 CFU/ml, showing that *Cronobacter* was able to survive and grow for an extended period of time by utilizing the remaining nutrient that has been previously absorbed on GAC (Figure 5.5).

At 14 days, the remaining columns were cut apart following the same procedure, and the result for bacterial count are similar, as shown in Table 5.2. All the control columns in Trial #1 and Trial #2 were also enumerated for bacteria and they were found to be sterile since no bacterial growth was observed on incubated plates.

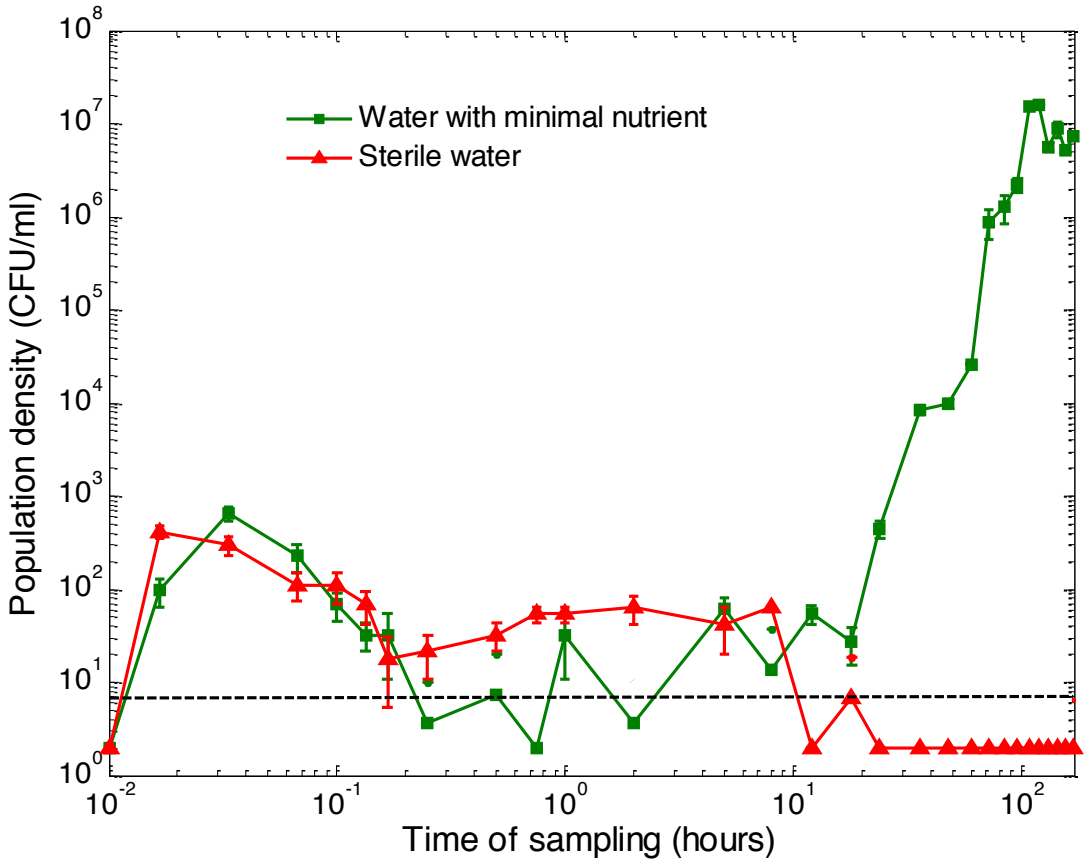

Figure 5.4 Trial #2: Bacterial concentration in the water effluent filtered by sterile granular activated carbon. The first column was given water with minimal nutrient, and the second column was given sterile water only. The lower limit of detection was approximately 20 CFU/ml, and samples with non-detectable *Cronobacter sakazakii* 607 were assigned a value of ≤ 20 CFU/ml. Each data point represents average of triplicate bacterial count (± sd). Dotted line indicates the lower limit of detection.

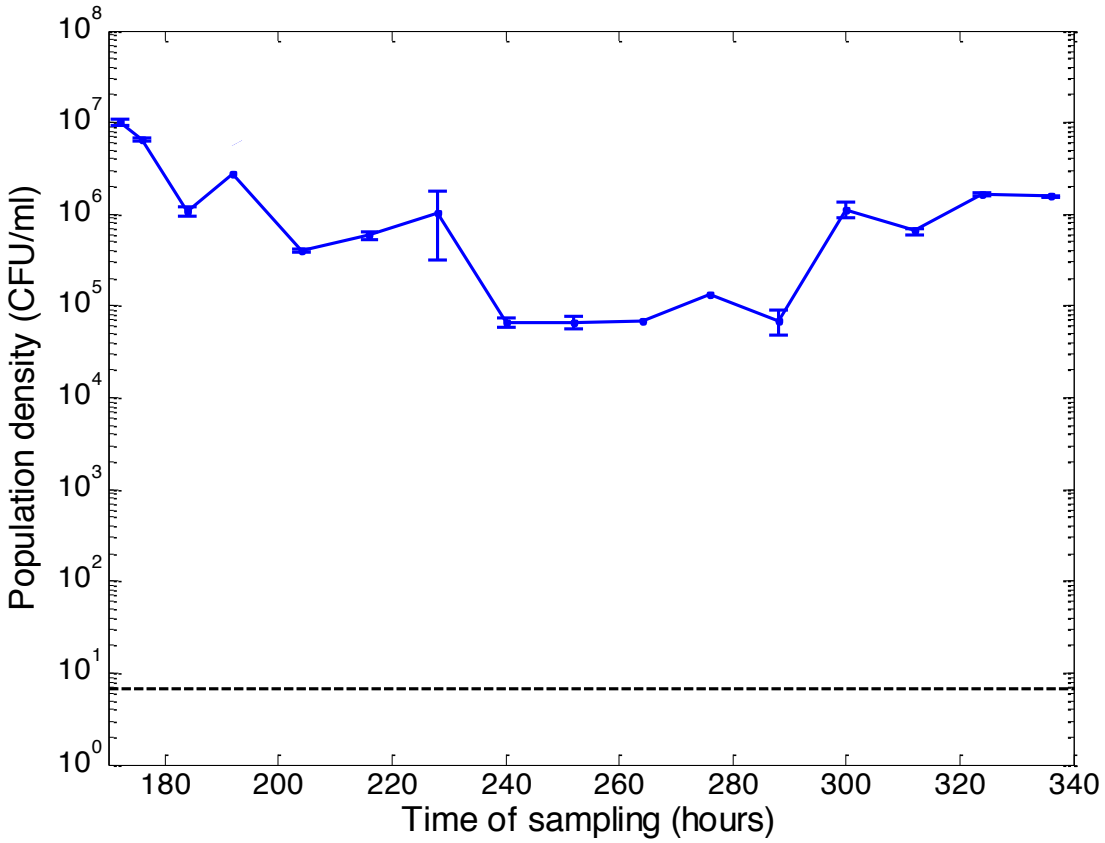

Figure 5.5 Trial #2: Bacterial concentration in the water effluent filtered by sterile granular activated carbon after 7 days. Water with minimal nutrient content was replaced with sterile water only. Each data point represents average of triplicate bacterial count (± sd). Dotted line indicates the lower limit of detection.

Bacterial concentration of the inocula*

Experiments number:	Original culture (average value from triplicate plate counts):	Diluted (10^{-5}) inoculum:
Preliminary Study #1	2.54×10^{9}	2.54×10^{4}
Preliminary Study #2	8.73×10^{9}	1.28×10^{4}
Trial #1 (Water with nutrient)	1.28×10^{9}	1.28×10^{4}
Trial #2 (Water with nutrient and then deprived)	1.91×10^{9}	1.91×10^{4}
Trial #2 (Water only)	2.53×10^{9}	2.53×10^{4}
Bacterial desorption comparison	1.89×10^{9}	1.89×10^{4}

*** Unit: CFU/ml**

Table 5.1 Bacterial concentrations (CFU/ml) in inocula. Inoculum was prepared by 10^{-5} dilution of original culture in each experiment.

Bacterial concentration in GAC[a]

Column number:	Top part	Middle part	Bottom Part	Filtration Pad[b]
Column #1 (Preliminary Study #1)	2.26×10^{9}	9.32×10^{7}	4.47×10^{7}	1.00×10^{6}
Column #1 Control (Preliminary Study #1)	< LoD*	< LoD	< LoD	< LoD
Column #2 (Preliminary Study #2)	2.62×10^{9}	4.72×10^{8}	4.94×10^{8}	2.36×10^{7}
Column #2 Control (Preliminary Study #2)	< LoD	< LoD	< LoD	< LoD
Column #3 (Trial #1: water with nutrient)	1.19×10^{9}	9.54×10^{7}	4.65×10^{7}	1.38×10^{6}
Column #3 Control (Trial #1: water with nutrient)	< LoD	< LoD	< LoD	< LoD
Column #4 (Trial #2: water with nutrient and deprived)	2.86×10^{9}	1.87×10^{8}	7.09×10^{7}	2.54×10^{6}
Column #4 Control (Trial #2: water with nutrient and deprived)	< LoD	< LoD	< LoD	< LoD
Column #5 (Trial #2: sterile water only)	2.82×10^{5}	4.72×10^{4}	4.97×10^{4}	1.15×10^{4}
Column #5 Control (Trial #2: sterile water only)	< LoD	< LoD	< LoD	< LoD
Column #6	5.68×10^{5}	3.24×10^{4}	2.77×10^{4}	4.82×10^{4}

(Desorption test: mixed detergent)				
Column #7 (Desorption test: sterile water)	8.20×10^{3}	1.12×10^{3}	2.25×10^{2}	1.12×10^{2}

[a]Unit: CFU per gram of GAC
[b]Unit: CFU per ml in filtration pad desorbed solution
*** LoD: Limit of Detection. In this study, LoD is 20 CFU/ml.**

Table 5.2 Bacterial concentrations in granular activated carbon. Column #1, #2, #3 and #4 were analyzed by mixed detergent and homogenization procedure from a previous study *(17)*. Sterile water was used to desorb bacteria on column #5 to compare desorption efficacy with mixed detergent on *Cronobacter* specifically.

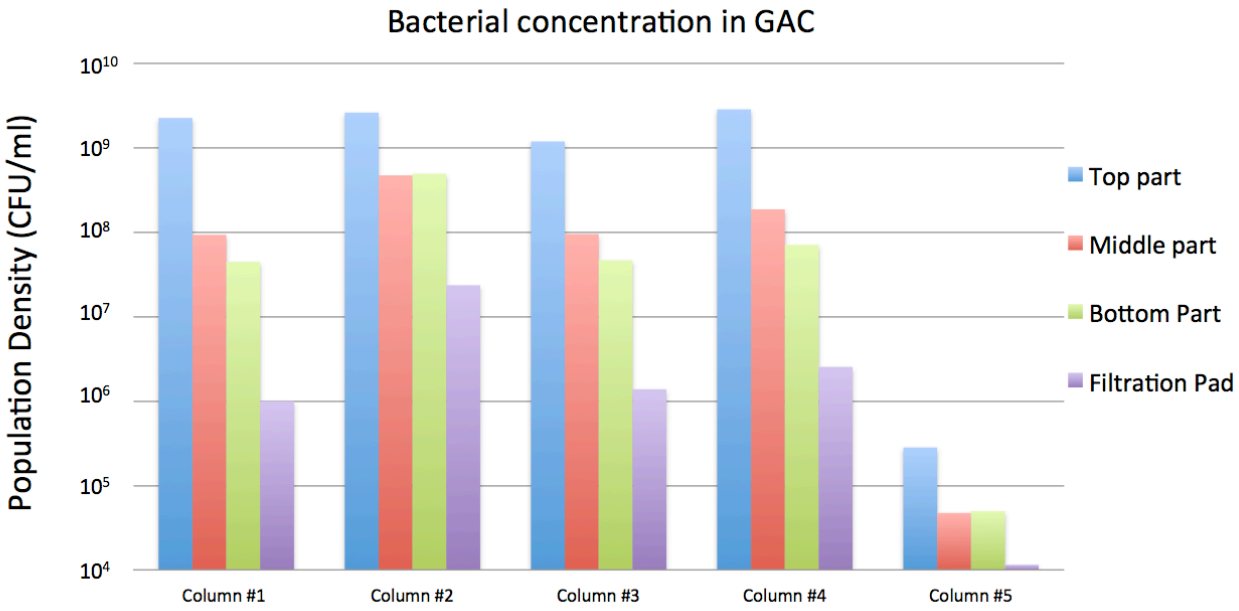

Figure 5.6 Bacterial concentrations in granular activated carbon. Column #1, #2, #3 and #4 were analyzed by mixed detergent and homogenization procedure from a previous study *(17)*. Sterile water was used to desorb bacteria on column #5 in order to compare desorption efficacy with mixed detergent on *Cronobacter* specifically.

5.2 Phase Two

5.2.1 Bacterial Enumeration

24 commercial faucet filters were collected after about 4 month use by residents near Washington D.C. Metropolitan area, and the indicator on the filter base measured the extent of usage. 7 filters (29.1%) were collected with red indicator (over 100 gallon of water filtered), 3 filters (12.5%) were collected with half red half green indicator (75 gallon to 100 gallon of water filtered), and 14 filters (58.3%) were collected with green indicator (less than 75 gallon of water filtered). The bacterial count on three different plates largely varied due to the different degree of usage. Backwash effluent from filters with red indicator generally showed a notably larger bacterial count on each type of medium (Table 5.3). The highest bacteria count was observed on BHI plates from 1 filter with red indicator, which was 10^5 CFU/ml, and fewer bacteria were recovered from all the other filters with red indicator. This is typically because the bacteria level has reached GAC's maximum adsorption capability, and newly introduced bacteria from water influent have successfully colonize and compete with the indigenous organisms or be retained in large numbers so they can be released into the effluent *(15)*.

Nevertheless, some filters with green indicators also demonstrated higher bacterial count of 10^4 on all types of medium, indicating that the carbon can be activated biologically and colonized with bacteria in a short period of time, despite of the filter replacing interval based on the manufacture's instruction. Thermotolerant Enterobacteriaceae were isolated from three filters, and only one had a red indicator.

Only rarely used filters had considerably low amount of bacteria isolated from backwash experiment. An unused filter was also tested for control and no bacteria was recovered. Details are shown in Table 5.3.

Filter NO.	Indicator Status	TAPC*	Coliforms*	Enterobacteriaceae*
1	Half Red Half Green	2.06×10^4	2.29×10^3	3.67×10^2
2	Green	7.89×10^4	4.73×10^4	4.30×10^3
3	Red	2.78×10^3	8.33×10^2	7.27×10^2
4	Half Red Half Green	6.31×10^4	6.44×10^3	1.85×10^4
5	Green	1.92×10^4	1.17×10^3	1.33×10^3
6	Green	9.25×10^4	1.12×10^3	1.57×10^4
7	Green	3.41×10^3	1.97×10^3	2.35×10^3
8	Green	5.70×10^3	3.33×10^2	8.87×10^2
9	Red	9.45×10^3	3.41×10^3	3.04×10^3
10	Half Red Half Green	2.66×10^4	4.20×10^2	7.67×10^2
11	Green	2.71×10^4	1.53×10^4	1.32×10^4
12	Green	6.34×10^2	< LoD	< LoD
13	Red	3.77×10^4	5.80×10^4	2.96×10^4
14	Green	2.73×10^4	1.42×10^4	7.39×10^3
15	Red	1.10×10^5	7.30×10^4	4.47×10^4
16	Green	1.96×10^4	2.43×10^3	8.00×10^2
17	Red	4.55×10^4	3.36×10^4	2.14×10^4
18	Red	3.02×10^4	9.89×10^3	1.21×10^4
19	Green	1.29×10^4	1.13×10^2	2.07×10^2
20	Green	1.33×10^3	< LoD	< LoD
21	Green	1.42×10^3	< LoD	< LoD
22	Red	5.75×10^4	4.17×10^4	4.73×10^4
23	Green	5.30×10^4	5.04×10^3	9.74×10^3
24	Green	2.66×10^3	< LoD	< LoD
Control	Green	< LoD	< LoD	< LoD

***Unit: CFU/ml**
TAPC: Total Aerobic Plate Count
LoD: Limit of Detection. In this study, LoD is 20 CFU/ml.

Table 5.3 Profile of collected filters after 3-month use. Filters were backwashed with sterile peptone water and counted for total aerobic plate count, total coliforms and Enterobacteriaceae by the spiral plating technique.

5.2.2 Statistical Analysis

Significant difference in coliform bacteria count was observed among filters with different indicator status (one-way ANOVA, p=0.0034), and Tukey's test shows that the level of bacterial concentration in filter with red indicator (mean = 3.10×10^4 CFU/ml) is significantly higher than bacterial concentration in filter with green indicator (mean = 3.32×10^3 CFU/ml).

Enterobacteriacea count also generated significant difference between filters with red indicator and filters with green indicator (one-way ANOVA, p=0.0084). Enterobacteriacea count is significantly higher in filters with red indicator (mean = 2.23×10^4 CFU/ml) than filters with green indicator (mean= 3.98×10^3 CFU/ml).

5.2.3 Bacterial Identification

Based on the morphology described by manufacture's manual, 51 colonies from VRBG and MacConkey plates were picked and streaked onto selective medium described previously.

Six blue-green colored colonies were identified as presumptive *Cronobacter* spp., because it contains enzyme a-glucosidase that hydrolyse the chromogenic substrate and produce blue-green pigment on pale yellow DFI medium. They were streaked onto BHI plates for further biochemical and genetic test.

Five colonies were identified as *Enterobacter aerogenes*. They demonstrated good growth of brown, dark-centered, slightly mucoid colonies on EMB agar, yellow to red colonies on XLD agar due to the degradation of xylose, lactose and sucrose and generation of acid products, fairly good growth on Simmons Citrate agar and turned medium into blue.

Four colonies were identified as *Psedomonas aeruginosa*. They have good growth on Pseudomonas Isolation agar and presented as greenish colonies after incubation for 18 hours and

blue to blue-green after incubation continues up to 24-48 hours. With magnesium chloride and potassium sulfate in the formula, these bacteria strains were able to produce pyocyanin, the blue-green pigment that will diffuse into the agar.

Two colonies were identified as *Klebsiella pneumoniae*. They have shown good growth of brown, dark-centered, extremely mucoid, slightly metallic sheen colonies on EMB agar, indicating lactose fermentation and acid production. Because they produces acid on XLD agar, they also turns XLD agar into yellow. Simmons Citrate agar plates turned blue after incubation, indicating their ability to utilize citrate.

One colony was identified as *Shigella flexneri*, as it grew fairly well on EMB agar and presented as colorless to light amber colonies. It formed red colonies on XLD agar, and it was inhibited on Simmon Citrate agar and maintained green medium.

Four colonies were identified as *E.coli*, since they have demonstrated large colonies that have green metallic sheen color on EMB agar. Because of its ability to utilize xylose and produce acid, it presented as yellow colonies on XLD agar and turned the agar into yellow. On Simmons Citrate agar, these colonies were unable to utilize citrate and maintained green color of the medium.

The identification results are summarized in Table 5.4.

Bacterial Isolate NO.	Original Filter NO.	Presumptive Identification Result	Colonial Morphology on Key Media
2	1	*Cronobacter* spp.	Produced blue-green pigment on pale yellow DFI media
3	2	*Pseudomonas aeruginosa*	Good growth on Pseudomonas Isolation agar, presented as greenish colonies after incubation for 18 hours and blue to blue-green after incubation continues up to 24-48 hours
4	3	*Pseudomonas aeruginosa*	Good growth on Pseudomonas Isolation agar, presented as greenish colonies after incubation for 18 hours and blue to blue-green after incubation continues up to 24-48 hours
7	3	*Cronobacter* spp.	Produced blue-green pigment on pale yellow DFI media
9	5	*Enterobacter aerogenes*	Good growth of brown, dark-centered, slightly mucoid colonies on EMB agar; yellow to red colonies on XLD; fairly good growth on Simmons Citrate agar and turned media into blue color
10	5	*Klebsiella pneumoniae*	Good growth of brown, dark-centered, extremely mucoid, slightly metallic sheen colonies on EMB agar; turned XLD agar into yellow; turned Simmons Citrate agar plates to blue
11	5	*Shigella flexneri*	Fairly good growth on EMB agar and presented as colorless to light amber colonies; formed red colonies on XLD agar; inhibited on Simmons Citrate agar and maintained green colored media
14	3	*Pseudomonas aeruginosa*	Good growth on Pseudomonas Isolation agar, presented as greenish colonies after incubation for 18 hours and blue to blue-green after incubation continues up to 24-48 hours
15	3	*Pseudomonas aeruginosa*	Good growth on Pseudomonas Isolation agar, presented as greenish colonies after incubation for 18 hours and blue to blue-green after incubation continues up to 24-48 hours

16	5	*E.coli*	Demonstrated large colonies that has green metallic sheen color on EMB agar; presented as yellow colonies on XLD agar and turned the agar into yellow; maintained green color of the Simmons Citrate agar
17	1	*Cronobacter* spp.	Produced blue-green pigment on pale yellow DFI media
21	8	*E.coli*	Demonstrated large colonies that has green metallic sheen color on EMB agar; presented as yellow colonies on XLD agar and turned the agar into yellow; maintained green color of the Simmons Citrate agar
27	10	*Cronobacter* spp.	Produced blue-green pigment on pale yellow DFI media
32	7	*Cronobacter* spp.	Produced blue-green pigment on pale yellow DFI media
33	10	*Cronobacter* spp.	Produced blue-green pigment on pale yellow DFI media
34	19	*Enterobacter aerogenes*	Good growth of brown, dark-centered, slightly mucoid colonies on EMB agar; yellow to red colonies on XLD; fairly good growth on Simmons Citrate agar and turned media into blue color
35	19	*Klebsiella pneumoniae*	Good growth of brown, dark-centered, extremely mucoid, slightly metallic sheen colonies on EMB agar; turned XLD agar into yellow; turned Simmons Citrate agar plates to blue
36	19	*Enterobacter aerogenes*	Good growth of brown, dark-centered, slightly mucoid colonies on EMB agar; yellow to red colonies on XLD; fairly good growth on Simmons Citrate agar and turned media into blue color
37	14	*Enterobacter aerogenes*	Good growth of brown, dark-centered, slightly mucoid colonies on EMB agar; yellow to red colonies on XLD; fairly good growth on Simmons Citrate agar and turned media into blue color

39	15	*E.coli*	Demonstrated large colonies that has green metallic sheen color on EMB agar; presented as yellow colonies on XLD agar and turned the agar into yellow; maintained green color of the Simmons Citrate agar
42	15	*E.coli*	Demonstrated large colonies that has green metallic sheen color on EMB agar; presented as yellow colonies on XLD agar and turned the agar into yellow; maintained green color of the Simmons Citrate agar
51	23	*Enterobacter aerogenes*	Good growth of brown, dark-centered, slightly mucoid colonies on EMB agar; yellow to red colonies on XLD; fairly good growth on Simmons Citrate agar and turned media into blue color

Table 5.4 Presumptive identification results based on bacterial morphology on selective media.

5.2.4 Biochemical test

Six presumptive strains of *Cronobacter* spp. demonstrated poor growth on ESPM agar. Only one strain presented as blue-black colonies, which is similar to the typical reaction of *Cronobacter* spp. according to manufacturer's manual. All the other strains grew blue-green colonies on ESPM agar, indicating that chromogenic substrates in the agar were hydrolyzed by enzymes that does not typically exist in *Cronobacter* spp..

Vitek 2 Compact generated six different bionumbers for six presumptive *Cronobacter* strains, which are: 4520101300220001, 6120501300220001, 4520105300220001, 6520105300220001, 6120715350220001, and 4120101300220001. However, they are identified as *Rhizobium radiobacter* with 99% probability and an "excellent identification" confidence level.

5.2.5 Genetic test

PCR test was also conducted for 5 strains and they all have shown negative results on the digital image. The sixth strain was not tested by genetic approach since it already can be biochemically identified as *Rhizobium radiobacter* with high probability.

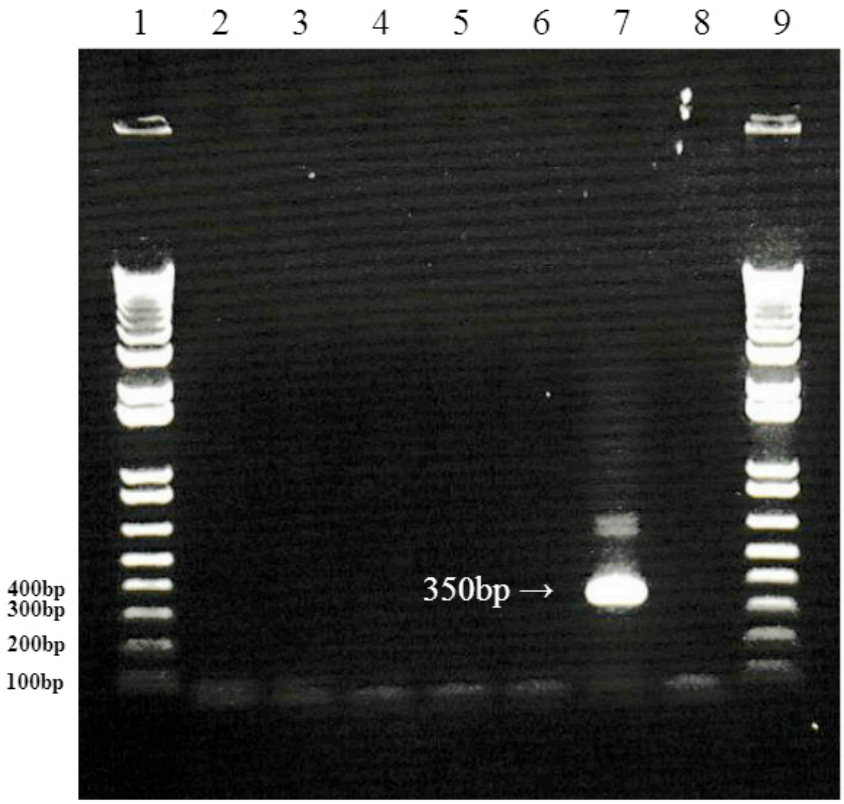

Figure 5.7 PCR results for *Cronobacter zpx* using primer BAM 122 and BAM 123 for presumptive *Cronobacter* strains and controls. Lanes 1 and 9, Bio-Rad 100bp molecular size DNA markers; lanes 2-6, presumptive strains JL7, JL17, JL27, JL32 and JL33; lane 7, *Cronobacter* strain BAA 894; lane 8, nuclease-free water.

Chapter 6: Discussion

Results from the first stage in this project have demonstrated similar trend of bacterial concentration in the effluent with previous studies. Rollinger *et al. (85)* installed filter test equipment that circulated water continuously through the GAC column. The water was taken from the municipal water system and refilled after each sampling. This may simulate minimal nutrient in water supply, which is similar to the method of sterilizing tap water with nutrient addition in this study. All the inoculated bacteria (10^7 CFU) were quickly absorbed by sterilized GAC within 1 hour. However, within the five bacterial strains inoculated, *E. coli*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* have shown the same bacterial concentration change in effluent as our study, while no growth was observed for *Klebsiella pneumoniae* and *Streptococcus faecalis*, indicating that the survival and growth on GAC is species-specific *(85)*.

Camper and associates introduced sterile water into virgin GAC filters that has been inoculated with *Yersinia enterocolitica*, *Salmonella typhimurium*, and *E. coli*. All three organisms readily colonized GAC and maintained population of 10^5 to 10^7 CFU per gram, which is similar to the result of our study when only sterile water was introduced to virgin GAC *(18)*. Rollinger and coworkers also mentioned another two studies conducted in Germany that also demonstrated a decline in *E. coli* counts in sterile tap water *(85)*. However, once the nutrient was given to the system, bacterial concentration on GAC was significantly higher, suggesting that bacterial has extensive ability to utilize nutrient in water.

Previous studies have also tested bacterial survival when coliforms were introduced to GAC that has been previously colonized with indigenous organisms. Competitive exclusion of

the coliform bacteria was observed on GAC that has been initially colonized with heterotrophs. It will be interesting to study the survival of *Cronobacter* on biologically activated carbon.

Bacteria tend to enter a starvation state when it is attached to GAC, characterized by decrease in cell size and increased resistance to acid shock and oxidative stress *(12, 15, 88)*. Functional steps that are common to isolate gram-negative pathogens general include a pre-enrichment step in a non-selective broth that allows stressed cells to recover from injury, a selective enrichment step by plating out samples onto a selective diagnostic medium to isolate targeted bacteria from the competitive microflora that have survived the pre-enrichment step, and an identification step that identifies targeted bacteria strain biochemically. One potential modification to our study could be to culture microorganisms in backwash effluent in peptone water for 1 day, and use Enterobacteriaceae enrichment (EE) broth to inhibit the growth of gram-positive organisms and increase specificity of the method. This is typically used to isolate *Cronobacter* from dehydrated infant formula *(24-26, 92, 104)*. Instead of using MacConkey and VRBG agar to enumerate coliform and Enterobacteriaceae, alternative media such as Lauryl Sulfate Tryptone (LST) has been proven to be effective for enumerating coliforms in water *(70, 72, 81)* since it contains sodium lauryl sulfate as a selective agent against most gram-positive microorganisms, and lactose as fermentable carbohydrate. Previous research used LST to enumerate coliforms in water after incubation at 35 °C, and enumerate thermotolerant bacteria such as thermotolerant *E. coli* after incubation at 44 °C *(2, 42)*. Because *Cronobacter* may grow at an elevated temperature *(74)*, Guillaume-Gentil and coworkers designed a modified LST (mLST) that contains high level of sodium chloride that inhibit the growth of many other members of Enterobacteriaceae but allow the growth of *Cronobacter* at 45 °C *(45)*. Considering the fact that in this study, a few bacillus-like and fungi-like colonies grew on both MacConkey

and VRBG plate due to their limited ability to inhibit gram-positive bacteria, and these colonies have to be manually deducted when performing a microbial enumeration, it will be interesting to use this newly developed media to culture potential *Cronobacter* isolated from water filters. However, this enrichment medium also has some limitations because it may inhibit some strains of *Cronobacter* at high temperature environment by its relatively high content of sodium lauryl sulfate *(45, 53)*.

Camper and associates *(17)* developed a procedure to desorb *E. coli* and heterotrophic plate count organisms, and it is used in our experiment. In this study, an additionally experiment was conducted to prove that the mixed detergent has a high removal efficacy of *Cronobacter* and ability to prevent readsorption. The simulation system was set up following the same schematic design. Two carbon columns were inoculated with 10^4 CFU of *Cronobacter,* and sterile water flowed through columns at a steady rate for 24 hours. One column was homogenized in mixed detergent and another column was homogenized in sterile water. Homogenized solution was enumerated for *Cronobacter* using the same experimental design. Results have shown that the mixed detergent demonstrated significantly higher efficacy of desorbing *Cronobacter* than sterile water (Table 5.2).

There are a number of reported waterborne disease outbreaks in the United States every year. Most outbreaks were associated with *Legionella*, which counted for 66% of total outbreaks during the year of 2011. Among all the other cases, *Shigella* and Shiga toxin-producing *E. coli* counted for the most hospitalizations *(7, 29)*. United States Environmental Protection Agency established national water quality standard that requires routine coliform test, and no fecal coliforms are allowed to be detected *(1)*. However, a variety of coliform bacteria has been isolated and identified in this study, thus it is a reflection of poor water quality in Washington

D.C. Metropolitan area. Additionally, fecal coliforms such as *E. coli* serve as a good indicator of overall water quality, since it can survive in water distribution system for extended period of time and the examination process is cost-effective *(36)*.

Cronobacter is ubiquitous in the environment *(60)*, and it has been previously found in water distribution systems *(41, 57, 69)*. Although there was no *Cronobacter* identified within 24 tested filters, it is likely that *Cronobacter* spp. would be isolated from household water filter if the number of tested water source significantly larger.

In this study, some collected filters with green indicators showed large number of total coliform and Enterobacteriaceae, suggesting that waterborne microorganisms can colonize GAC filters within a short period of time despite the recommended filter cartridge replacing interval by the manufacture.

The selectivity mechanism of DFI agar is based on the α-glucosidase reaction. The α-glucosidase presented in *Cronobacter* spp. is able to hydrolyses the chromogenic subtract in the formulation, resulting in blue-green colonies. Since α-glucosidase have been reported from many microorganisms, plants and mammals *(68, 100)*, and the colonies on DFI agar maybe misinterpreted as *Cronobacter* spp. since they have generated similar pigment. For example, *Proteus vulgaris* is weakly a-glucosidase positive and they grow as grey colonies on DFI agar. Based on our observation, the six presumptive strains were also grey in color and they required extended time before the color appeared.

For the genetic test, although the primers used for detecting zinc-containing metalloprotease is effective in identifying *Cronobacter* spp., it still has limitation. Some *Cronobacter genomospecies* do not test positive by using this primer, and the PCR method for detecting *Cronobacter condiment* has not been fully developed *(19, 59, 66, 95)*. However,

combining the result from PCR and Vitek, six presumptive strains can be confidently confirmed negative for *Cronobacter* identification.

The six presumptive strains were identified as *Rhizobium radiobacter* through biochemical approach. *Rhizobium radiobacter* is a gram-negative bacillus that is typically found in soil, and it was recognized as an opportunistic pathogen *(38, 50, 84)*. The presence of *Rhizobium radiobacter* indicated that the drinking water had previous contact with soil material during distribution process or urban storm water runoff.

Overall, our study have demonstrated that *Cronobacter* are able to colonize granular activated carbon filter, penetrate the treatment barrier and release into water effluent, introducing a safety risk of waterborne pathogens, and potentially contaminating the kitchen environment. These results suggest that the use of these filters should be considered a potential source of *Cronobacter* and other foodborne pathogens under certain circumstances.

Appendices

Appendix 1: Experimental data from Figure 5.1

Sampling Time	Effluent Bacterial Concentration (CFU/ml)			Average Value
0min	<LoD	<LoD	< LoD	< LoD
1min	1.68×10^{2}	1.48×10^{2}	1.14×10^{2}	1.43×10^{2}
2min	2.63×10^{3}	2.81×10^{3}	1.94×10^{3}	2.46×10^{3}
4min	1.30×10^{3}	1.19×10^{3}	1.08×10^{3}	1.19×10^{3}
6min	3.20×10^{2}	2.80×10^{2}	2.80×10^{2}	2.93×10^{2}
8min	4.00×10^{1}	6.00×10^{1}	8.00×10^{1}	6.00×10^{1}
10min	4.00×10^{1}	2.00×10^{1}	4.00×10^{1}	3.33×10^{1}
15min	6.00×10^{1}	1.00×10^{2}	4.00×10^{1}	6.67×10^{1}
30min	4.00×10^{1}	6.00×10^{1}	8.00×10^{1}	6.00×10^{1}
45min	8.00×10^{1}	4.00×10^{1}	6.00×10^{1}	6.00×10^{1}
1hr	1.20×10^{2}	6.00×10^{1}	1.80×10^{2}	1.20×10^{2}
2hr	1.00×10^{2}	1.00×10^{2}	1.20×10^{2}	1.07×10^{2}
5hr	3.20×10^{2}	2.80×10^{2}	2.00×10^{2}	2.67×10^{2}
8hr	7.60×10^{2}	8.40×10^{2}	8.80×10^{2}	8.27×10^{2}
12hr	4.20×10^{2}	5.20×10^{2}	6.60×10^{2}	5.33×10^{2}
18hr	2.36×10^{3}	2.20×10^{3}	2.50×10^{3}	2.35×10^{3}
24hr	7.91×10^{3}	7.12×10^{3}	1.14×10^{4}	8.81×10^{3}
1.5D	1.19×10^{5}	1.08×10^{5}	1.12×10^{5}	1.13×10^{5}
2D	6.34×10^{5}	2.74×10^{5}	3.00×10^{5}	4.03×10^{5}
2.5D	1.35×10^{6}	5.56×10^{5}	8.06×10^{5}	9.04×10^{5}
3D	1.16×10^{6}	8.59×10^{5}	9.65×10^{5}	9.95×10^{5}
3.5D	6.76×10^{6}	3.91×10^{6}	3.73×10^{6}	4.80×10^{6}
4D	8.00×10^{6}	1.00×10^{7}	6.00×10^{6}	8.00×10^{6}
4.5D	2.92×10^{6}	1.51×10^{6}	1.60×10^{6}	2.01×10^{6}
5D	2.06×10^{6}	1.08×10^{6}	1.12×10^{6}	1.42×10^{6}
5.5D	7.34×10^{6}	4.50×10^{6}	4.58×10^{6}	5.47×10^{6}
6D	3.10×10^{7}	1.23×10^{7}	1.78×10^{7}	2.04×10^{7}
6.5D	8.68×10^{6}	7.59×10^{6}	8.44×10^{6}	8.24×10^{6}
7D	5.25×10^{6}	7.59×10^{6}	4.43×10^{6}	5.76×10^{6}

*** LoD: Limit of Detection.**
In this study, LoD is 20 CFU/ml.

Appendix 2: Experimental data from Figure 5.2

Sampling Time	Effluent Bacterial Concentration (CFU/ml)			Average Value
0min	<LoD	< LoD	< LoD	< LoD
1min	3.25×10^{1}	7.59×10^{1}	2.39×10^{2}	1.16×10^{2}
2min	1.84×10^{2}	1.87×10^{2}	1.81×10^{2}	1.84×10^{2}
4min	1.78×10^{3}	1.64×10^{3}	1.94×10^{3}	1.79×10^{3}
6min	8.20×10^{2}	1.26×10^{3}	1.22×10^{3}	1.10×10^{3}
8min	4.00×10^{1}	4.00×10^{1}	4.00×10^{1}	4.00×10^{1}
10min	2.00×10^{1}	2.00×10^{1}	6.00×10^{1}	3.33×10^{1}
15min	4.00×10^{1}	4.00×10^{1}	2.00×10^{1}	3.33×10^{1}
30min	2.00×10^{2}	1.20×10^{2}	1.60×10^{2}	1.60×10^{2}
45min*	2.00×10^{2}	1.20×10^{2}	9.00×10^{1}	1.37×10^{2}
1hr*	1.40×10^{2}	1.40×10^{2}	1.40×10^{2}	1.40×10^{2}
2hr	1.00×10^{2}	1.60×10^{2}	1.20×10^{2}	1.27×10^{2}
5hr	2.00×10^{2}	8.00×10^{1}	6.00×10^{1}	1.13×10^{2}
8hr	1.38×10^{2}	1.42×10^{2}	1.50×10^{2}	1.43×10^{2}
12hr	2.80×10^{2}	3.00×10^{2}	2.84×10^{2}	2.88×10^{2}
18hr	1.08×10^{3}	1.28×10^{3}	1.16×10^{3}	1.17×10^{3}
24hr	4.44×10^{3}	3.15×10^{3}	3.02×10^{3}	3.54×10^{3}
1.5D	6.03×10^{3}	8.30×10^{3}	7.40×10^{3}	7.24×10^{3}
2D	1.87×10^{4}	1.81×10^{4}	1.84×10^{4}	1.84×10^{4}
2.5D	9.72×10^{6}	5.35×10^{6}	6.78×10^{6}	7.28×10^{6}
3D	3.80×10^{7}	2.74×10^{7}	2.42×10^{7}	2.99×10^{7}
3.5D	1.92×10^{7}	8.40×10^{6}	8.60×10^{6}	1.21×10^{7}
4D	4.40×10^{6}	5.40×10^{6}	7.40×10^{6}	5.73×10^{6}
4.5D	3.60×10^{5}	4.40×10^{5}	7.46×10^{5}	5.15×10^{5}
5D	8.40×10^{6}	6.20×10^{6}	4.80×10^{6}	6.47×10^{6}
5.5D	2.80×10^{6}	1.20×10^{6}	2.00×10^{6}	2.00×10^{6}
6D	1.80×10^{6}	2.20×10^{6}	1.00×10^{6}	1.67×10^{6}
6.5D	2.80×10^{6}	1.00×10^{6}	1.00×10^{6}	1.60×10^{6}
7D	7.40×10^{6}	2.80×10^{6}	4.60×10^{6}	4.93×10^{6}

*** LoD: Limit of Detection.**
In this study, LoD is 20 CFU/ml.

Appendix 3: Experimental data from Figure 5.3

Sampling Time	Effluent Bacterial Concentration (CFU/ml)			Average Value
0min	< LoD	< LoD	< LoD	< LoD
1min	2.00×10^{1}	2.00×10^{1}	< LoD	1.33×10^{1}
2min	4.40×10^{2}	5.40×10^{2}	4.60×10^{2}	4.80×10^{2}
4min	1.80×10^{2}	1.40×10^{2}	1.20×10^{2}	1.47×10^{2}
6min	4.00×10^{1}	4.00×10^{1}	1.00×10^{1}	3.00×10^{1}
8min	4.00×10^{1}	2.00×10^{1}	2.00×10^{1}	2.67×10^{1}
10min	2.00×10^{1}	2.00×10^{1}	< LoD	1.33×10^{1}
15min	2.00×10^{1}	2.00×10^{1}	< LoD	1.33×10^{1}
30min	2.00×10^{1}	< LoD	< LoD	6.67×10^{0}
45min*	< LoD	< LoD	< LoD	< LoD
1hr*	< LoD	< LoD	< LoD	< LoD
2hr	< LoD	< LoD	< LoD	< LoD
5hr	1.00×10^{2}	6.00×10^{1}	4.00×10^{1}	6.67×10^{1}
8hr	1.80×10^{2}	1.00×10^{2}	1.40×10^{2}	1.40×10^{2}
12hr	1.12×10^{4}	1.15×10^{4}	1.09×10^{4}	1.12×10^{4}
18hr	7.99×10^{3}	8.09×10^{3}	7.90×10^{3}	7.99×10^{3}
24hr	2.30×10^{4}	2.07×10^{4}	2.01×10^{4}	2.13×10^{4}
1.5D	5.20×10^{7}	6.00×10^{7}	5.26×10^{7}	5.49×10^{7}
2D	1.90×10^{7}	2.08×10^{7}	1.94×10^{7}	1.97×10^{7}
2.5D	1.26×10^{7}	1.20×10^{7}	1.08×10^{7}	1.18×10^{7}
3D	1.88×10^{7}	1.60×10^{7}	1.72×10^{7}	1.73×10^{7}
3.5D	5.29×10^{6}	5.38×10^{6}	5.04×10^{6}	5.24×10^{6}
4D	7.40×10^{6}	9.20×10^{6}	8.40×10^{6}	8.33×10^{6}
4.5D	1.06×10^{7}	9.40×10^{6}	1.02×10^{7}	1.01×10^{7}
5D	6.20×10^{6}	6.80×10^{6}	6.20×10^{6}	6.40×10^{6}
5.5D	1.62×10^{7}	1.50×10^{7}	1.74×10^{7}	1.62×10^{7}
6D	6.60×10^{6}	5.60×10^{6}	5.20×10^{6}	5.80×10^{6}
6.5D	5.40×10^{6}	6.40×10^{6}	5.60×10^{6}	5.80×10^{6}
7D	1.56×10^{7}	1.62×10^{7}	1.58×10^{7}	1.59×10^{7}

*** LoD: Limit of Detection.**
In this study, LoD is 20 CFU/ml.

Appendix 4: Experimental data from Figure 5.4 and Figure 5.5 (water with minimal nutrient)

Sampling Time	Effluent Bacterial Concentration (CFU/ml)			Average Value
0min	< LoD	< LoD	< LoD	< LoD
1min	1.30×10^{2}	9.76×10^{1}	6.51×10^{1}	9.76×10^{1}
2min	5.53×10^{2}	6.40×10^{2}	7.59×10^{2}	6.51×10^{2}
4min	3.15×10^{2}	1.95×10^{2}	1.74×10^{2}	2.28×10^{2}
6min	7.59×10^{1}	4.34×10^{1}	8.68×10^{1}	6.87×10^{1}
8min	2.17×10^{1}	3.25×10^{1}	4.34×10^{1}	3.25×10^{1}
10min	1.08×10^{1}	3.25×10^{1}	5.42×10^{1}	3.25×10^{1}
15min	1.08×10^{1}	< LoD	< LoD	3.60×10^{0}
30min	2.17×10^{1}	< LoD	< LoD	7.23×10^{0}
45min[a]	< LoD	< LoD	< LoD	< LoD
1hr	1.08×10^{1}	3.25×10^{1}	5.42×10^{1}	3.25×10^{1}
2hr	1.08×10^{1}	< LoD	< LoD	3.60×10^{0}
5hr	4.04×10^{1}	6.06×10^{1}	8.08×10^{1}	6.06×10^{1}
8hr	4.04×10^{1}	< LoD	< LoD	1.35×10^{1}
12hr	4.04×10^{1}	6.06×10^{1}	6.06×10^{1}	5.39×10^{1}
18hr	2.02×10^{1}	2.02×10^{1}	4.04×10^{1}	2.69×10^{1}
24hr	5.45×10^{2}	3.84×10^{2}	3.84×10^{2}	4.38×10^{2}
1.5D	8.30×10^{3}	7.63×10^{3}	9.65×10^{3}	8.53×10^{3}
2D	1.09×10^{4}	1.00×10^{4}	8.70×10^{3}	9.87×10^{3}
2.5D	2.66×10^{4}	2.53×10^{4}	2.69×10^{4}	2.63×10^{4}
3D	6.00×10^{5}	8.00×10^{5}	1.20×10^{6}	8.67×10^{5}
3.5D	1.40×10^{6}	8.00×10^{5}	1.60×10^{6}	1.27×10^{6}
4D	2.60×10^{6}	2.00×10^{6}	2.00×10^{6}	2.20×10^{6}
4.5D	1.64×10^{7}	1.52×10^{7}	1.44×10^{7}	1.53×10^{7}
5D	1.50×10^{7}	1.58×10^{7}	1.62×10^{7}	1.57×10^{7}
5.5D	6.40×10^{6}	5.20×10^{6}	5.40×10^{6}	5.67×10^{6}
6D	1.08×10^{7}	7.80×10^{6}	8.00×10^{6}	8.87×10^{6}
6.5D	4.80×10^{6}	5.20×10^{6}	5.60×10^{6}	5.20×10^{6}
7D	7.20×10^{6}	7.60×10^{6}	7.20×10^{6}	7.33×10^{6}
4hr[a]	1.06×10^{7}	1.07×10^{7}	9.04×10^{6}	1.01×10^{7}
8hr[a]	6.23×10^{6}	6.56×10^{6}	6.78×10^{6}	6.52×10^{6}
16hr[a]	9.60×10^{5}	1.20×10^{6}	1.02×10^{6}	1.06×10^{6}
24hr[a]	6.73×10^{7}	5.97×10^{5}	8.14×10^{5}	2.29×10^{7}
1.5D[a]	4.03×10^{5}	3.85×10^{5}	4.15×10^{5}	4.01×10^{5}
2D[a]	5.29×10^{5}	6.32×10^{5}	6.20×10^{5}	5.94×10^{5}
2.5D[a]	1.89×10^{6}	5.88×10^{5}	6.73×10^{5}	1.05×10^{6}
3D[a]	6.89×10^{4}	7.32×10^{4}	5.80×10^{4}	6.67×10^{4}
3.5D[a]	7.89×10^{4}	5.73×10^{4}	6.51×10^{4}	6.71×10^{4}

4D[a]	2.00×10^{3}	2.00×10^{3}	< LoD	1.33×10^{3}
4.5D[a]	< LoD	4.00×10^{3}	< LoD	1.33×10^{3}
5D[a]	7.32×10^{4}	4.65×10^{4}	8.93×10^{4}	6.97×10^{4}
5.5D[a]	1.32×10^{6}	8.75×10^{5}	1.20×10^{6}	1.13×10^{6}
6D[a]	5.98×10^{5}	6.80×10^{5}	6.93×10^{5}	6.57×10^{5}
6.5D[a]	1.68×10^{6}	1.69×10^{6}	1.60×10^{6}	1.66×10^{6}
7D[a]	1.54×10^{6}	1.53×10^{6}	1.60×10^{6}	1.56×10^{6}

*** LoD: Limit of Detection.**
In this study, LoD is 20 CFU/ml.
a: Time after the sterile water is introduced

Appendix 5: Experimental data from Figure 5.4 (sterile water only)

Sampling Time	Effluent Bacterial Concentration (CFU/ml)			Average Value
0min*	< LoD	< LoD	< LoD	< LoD
1min	3.90×10^{2}	3.69×10^{2}	4.88×10^{2}	4.16×10^{2}
2min	2.60×10^{2}	2.60×10^{2}	3.69×10^{2}	2.96×10^{2}
4min	7.59×10^{1}	1.08×10^{2}	1.52×10^{2}	1.12×10^{2}
6min	1.52×10^{2}	9.76×10^{1}	7.59×10^{1}	1.09×10^{2}
8min	9.76×10^{1}	6.51×10^{1}	4.34×10^{1}	6.87×10^{1}
10min	1.08×10^{1}	3.25×10^{1}	1.08×10^{1}	1.80×10^{1}
15min	2.17×10^{1}	3.25×10^{1}	1.08×10^{1}	2.17×10^{1}
30min	2.17×10^{1}	3.25×10^{1}	4.34×10^{1}	3.25×10^{1}
45min	6.51×10^{1}	5.42×10^{1}	4.34×10^{1}	5.42×10^{1}
1hr	6.51×10^{1}	5.42×10^{1}	4.34×10^{1}	5.42×10^{1}
2hr	3.91×10^{1}	7.59×10^{1}	7.59×10^{1}	6.36×10^{1}
5hr	2.17×10^{1}	3.91×10^{1}	6.52×10^{1}	4.20×10^{1}
8hr	6.52×10^{1}	6.52×10^{1}	6.52×10^{1}	6.52×10^{1}
12hr	< LoD	< LoD	< LoD	< LoD
18hr	< LoD	< LoD	2.02×10^{1}	6.73×10^{0}
24hr	< LoD	< LoD	< LoD	< LoD
1.5D*	< LoD	< LoD	< LoD	< LoD
2D*	< LoD	< LoD	< LoD	< LoD
2.5D*	< LoD	< LoD	< LoD	< LoD
3D*	< LoD	< LoD	< LoD	< LoD
3.5D*	< LoD	< LoD	< LoD	< LoD
4D*	< LoD	< LoD	< LoD	< LoD
4.5D*	< LoD	< LoD	< LoD	< LoD
5D*	< LoD	< LoD	< LoD	< LoD
5.5D*	< LoD	< LoD	< LoD	< LoD
6D*	< LoD	< LoD	< LoD	< LoD
6.5D*	< LoD	< LoD	< LoD	< LoD
7D*	< LoD	< LoD	< LoD	< LoD

*** LoD: Limit of Detection.**
In this study, LoD is 20 CFU/ml.

Appendix 6: Biochemical test results from Vitek 2 Compact instrument

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 4, 2011 19:59 GMT-05:00
Autoprint

Isolate Group: JL-2

Bionumber: 6520105300220001
Selected Organism: Rhizobium radiobacter

Comments:	

Identification Information	Card:	GN	Lot Number:	241351640	Expires:	Jul 30, 2016 12:00 GMT-05:00
	Completed:	Mar 4, 2011 20:10 GMT-05:00	Status:	Final	Analysis Time:	2.75 hours
Selected Organism	99% Probability		Rhizobium radiobacter			
	Bionumber:	6520105300220001			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 06.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 4, 2011 20:43 GMT-05:00
Autoprint

Isolate Group: JL-7

Bionumber: 4120101300220001
Selected Organism: Rhizobium radiobacter

Comments:	

Identification Information	Card: GN	Lot Number: 241351640	Expires: Jul 30, 2016 12:00 GMT-05:00
	Completed: Mar 4, 2011 20:55 GMT-05:00	Status: Final	Analysis Time: 3.50 hours
Selected Organism	99% Probability	Rhizobium radiobacter	
	Bionumber: 4120101300220001		Confidence: Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 06.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 4, 2011 19:44 GMT-05:00
Autoprint

Isolate Group: JL-17

Bionumber: 4520101300220001
Selected Organism: Rhizobium radiobacter

Comments:	

Identification Information	Card: GN	Lot Number: 241351640	Expires: Jul 30, 2016 12:00 GMT-05:00
	Completed: Mar 4, 2011 19:54 GMT-05:00	Status: Final	Analysis Time: 2.50 hours
Selected Organism	99% Probability	Rhizobium radiobacter	
	Bionumber: 4520101300220001		Confidence: Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 06.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 4, 2011 20:42 GMT-05:00
Autoprint

Isolate Group: JL-27

Bionumber: 6120715350220001
Selected Organism: Rhizobium radiobacter

Comments:	

Identification Information	Card: GN	Lot Number: 241351640	Expires: Jul 30, 2016 12:00 GMT-05:00
	Completed: Mar 4, 2011 20:54 GMT-05:00	Status: Final	Analysis Time: 3.50 hours
Selected Organism	99% Probability	Rhizobium radiobacter	
	Bionumber: 6120715350220001		Confidence: Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 06.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 4, 2011 19:57 GMT-05:00
Autoprint

Isolate Group: JL-32

Bionumber: 4520105300220001
Selected Organism: Rhizobium radiobacter

Comments:	

Identification Information	Card:	GN	Lot Number:	241351640	Expires:	Jul 30, 2016 12:00 GMT-05:00
	Completed:	Mar 4, 2011 20:09 GMT-05:00	Status:	Final	Analysis Time:	2.75 hours
Selected Organism	99% Probability		Rhizobium radiobacter			
	Bionumber:	4520105300220001			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 06.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 4, 2011 19:57 GMT-05:00
Autoprint

Isolate Group: JL-33

Bionumber: 6120501300220001
Selected Organism: Rhizobium radiobacter

Comments:	

Identification Information	Card:	GN	Lot Number:	241351640	Expires:	Jul 30, 2016 12:00 GMT-05:00
	Completed:	Mar 4, 2011 20:09 GMT-05:00	Status:	Final	Analysis Time:	2.75 hours
Selected Organism	99% Probability		Rhizobium radiobacter			
	Bionumber:	6120501300220001			Confidence:	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						

Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	+	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	-	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	+	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	+	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Installed VITEK 2 Systems Version: 06.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

Appendix 7: Water Filter Survey

Water Filter Survey

What's the status of the filter indicator?

(1) Green (2)Red (3)Half Green Half Red

How often do you use the filter?

(1) very often (2)often (3)sometimes (4)rarely (5)almost never

Regardless of the filter, how much water do you use from the faucet where the filter was installed?

(1) very Large (2)large (3)medium (4)small (5)almost zero

What's the main purpose of using water? (Please mark all that apply)

(1) washing hands/foods (2)cooking (3)drinking (4) other (please list below)

In what circumstances that you turn on the filtration function? (Please mark all that apply)

(1) water used for food (2)wash hand (3)drinking directly (4)always turned on (5) other (please list below)

In your opinion, what's the benefit of using a filter? (Please mark all that apply)

(1) water tastes better (2)water is safer (3)no particular reason

Do you have children in the family, if so, please list their ages.

(1)Yes_____

(2)No

Do you have infant (less than 12 month) or young children who consume infant formula? If so, please describe the method of feeding infant formula (would you use filtered water to feed the baby? Do you normally heat it to boil?)

What's your opinion on this filtration system? Is there anything can be improved based on your experience?

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