

DEVELOPMENT OF A SILVER ION-BASED WATER PURIFIER

by

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Abstract

Water purification methods that remove pathogens and harmful or distasting molecules make water potable. Recently, silver loaded ion-exchange resins have demonstrated a strong role in removing microbes. The goal is to make an effective silver ion-based water purifier that is portable, environmentally stable, and cost efficient.

The project was conducted as a collaborative effort with Safewater A/S, an up and coming entrepreneurial business located in Denmark that is interested in developing novel water purifiers for developing nations, adventurers and military personnel. Purolite, a prominent business in ion-exchange resins located in Whales, designed and provided Safewater A/S and our research team with experimental resins for water purification, which will be discussed in the body of this thesis.

The data reveals critical issues that may render this tool unavailable for commercial production in some countries due to the mode of action for killing the bacteria and the amount of silver leaching. Tests were conducted using *Escherichia coli* K12 and *Enterococcus faecalis* OG1SSp as model fecal organisms using different silver ion-exchange resins. Surveillance of leached silver ions, pH changes, and total dissolved solids (TDS) were also monitored to find correlations with capacity (liters of purified water produced) and effectiveness of microbicidal action.

Overall, one resin was found to contain properties consistent with the stated objectives; however its use in some countries as a water purifier for human consumption will be nullified due to extensive silver leaching. Although this resin could be used in the United States of America since it passes the Environmental Protection Agency (EPA) standards, Safewater A/S is interested in further developing it for countries with stricter regulatory constraints before mass production. The goal of the present thesis report is to address the stated objectives in the development of a water purifier.

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Since I became a Kansas State Wildcat in 2004, I have wanted to complete my bachelors and masters in Microbiology. It's very fulfilling now that I've finally made it. I am proud of myself for this great achievement, as is my family. Although my career in Microbiology comes to an end here, I will forever love this field and will forever follow it as a hobby. Now, I will take the reader through my journey here at KSU, as I acknowledge key personal here at the Division of Biology and Chemistry, as well as family and friends, which helped me through this journey.

Principles of Biology, Fall 2004, Dr. Gary Conrad and Dr. Pat Hook were my professors for my 7:30am class. I hated this time but unlike most of my peers, I loved learning about biology so I was caught in a love-hate relationship. I distinctly remember frequently asking Dr. Conrad questions about everything. He soon recognized my passion for the field and introduced me to Dr. Helmut Hirt because he needed a lab assistant. Dr. Hirt allowed me the uncanny privilege to work in his lab from 2004 to 2008. If he hadn't I wonder if I would be where I am at today. Initially, I worked as a lab assistant. cleaning lab equipment, making media, and overall just took care of the lab. After a year and a half, Dr. Hirt gave me my first experiment. I was so excited! I finally had my chance to work with *Enterococcus faecalis* and had something other than media to talk about with the graduate students in the lab. As time progress, Dr. Hirt allowed me to grow away from cleaning equipment and into becoming a Microbiologist. He developed small experiments for me to conduct, and went over the results with me so that I would better understand the bigger picture. Thank you Dr. Conrad and especially Dr. Hirt for helping me get into this amazing field. I am forever indebted to you.

Near the end of my undergraduate schooling in 2008, I sent out graduate applications to Kansas State University and other Universities. Dr. Sherry Flemming contacted me about a position in her lab. I shadowed her lab for a couple weeks and then decided to join. The science in her immunology lab was amazing, and I am so grateful that I had the opportunity to experience it. Although my time in her lab was short, I would still like to thank Dr. Flemming for the experience.

Soon after, I joined Dr. George Marchin's lab and began my thesis project. Dr. George Marchin was one of a kind. He was creative, intelligent, and very humorous. I loved everyday under Dr. Marchin. When he passed in June of 2009, I was deeply saddened. He allowed me the space to work as an individual, but also monitored me, ensuring minimal mistakes. He gave me great ideas and encouragement to continue work, and always kept the lab environment at a very low stress level, and with lots of laughter. I owe him so much, and I'm forever thankful for the time he was my Mentor.

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Project Beginnings

In 1993, Dr. George L. Marchin received a phone call from Jack Lambert at the National Aeronautics and Space Administration (NASA) concerning high yields of iodide from their polyiodinated resins used for water disinfection aboard the Space Shuttle. Jack asked George for his opinion on removing the iodide and he came up with using a silver ion resin because of silver's affinity towards halides. Jack agreed and thought of using a zirconium peroxide resin to oxidize iodide to elemental iodine. This collaboration between Dr. Marchin's team and NASA led to the development of four patents based on silver-chelex and zirconium peroxide resin. However, when Marchin's laboratory was testing the resin, they discovered that it also had antibacterial properties, which were published in a short note of the 1994 Annals of the Abstracts of Annual Meeting of the American Society for Microbiology and in a patent. Marchin's patents remained in the Kansas State University Research Foundation until the late 90's, which were then dropped. Then in 2007, Dr. Rob Fleuren, owner of Safewater AS (located in Denmark), called Dr. Marchin asking his opinion on pursuing and developing his silver resin as a water purifier. Marchin agreed and Safewater and investors provided research money for the project.

CHAPTER 1 - Literature Review

History

Water Supply, Treatment, and Management

Water is essential for all living things so it should come as no surprise that our earliest civilizations had intelligent methods and ideas for obtaining and using it. The earliest water purification, which is written in Sanskrit from 2000BC, describes multiple methods of purifying water such as: boiling, placing hot metal instruments in the water, and filtering water through crude sand or charcoal filters (Baker and Taras 1981). Five hundred years later, Mohenjo-Daro, an ancient but large civilization situated in Pakistan nearby the Indus River; has the worlds oldest water supply and wastewater management system known to man. However this was not discovered till the 1930's. Perhaps the most intelligent civilization of its time, it had homes equipped with toilets, bathing areas, and canals which transported rainfall back towards the Indus River (Wiesmann 2007). The Romans are perhaps the most widely known for their innovated water supply and waste management. In 321BC the first aquaduct was built and twenty-one years later there were a staggering fourteen, each delivering 40 million gallons of water a day to Rome, Italy (Cech 2010). The Cloaca Maxima or "the great sewer", is one the oldest and largest sewer systems and belonged to the Roman Empire. The ditch system allowed few private houses and public areas access. This winding tunnel is 1,600 meters (0.99miles) long, 4 meters tall and 3 meters wide ran pass the Roman Forum, and crossed the Velabrum between the Palatine and Capitoline hills, and empties in the Tiber (Aldrete 2004). Then, after the Dark ages, exploration began for water filtration due to a man named Sir Francis Bacon (Baker and Taras 1981). He was interested in desalinating seawater, so he designed an experiment, which consisted of a hole in the shore. His idea was, seawater would flow against gravity up through the bottom of the hole filtering out the salt and leaving desalinated water in the top layer of the stagnant water. Although his attempt failed, Bacon experiment is probably the most significant test done of his

time because it helped generate a cause that is still trying to be solved today for some developing regions (Baker and Taras 1981).

Silver in Water Treatment and Medicine

Middle-aged historian, Herodotus, is one of the first known men to record the use of silver in water purification although, silver had been known as early as 3000BC (Miessler 2004). During war times, Herodotus wrote that the King of Persia provided his men with boiled water contained in vessels of silver (Clement and Jarrett 1994). He also wrote that no Persian king would drink water that did not come from a silver container (Alexander 2009). This was an era when gold and silver became very important in religion. The King of Persia intent was most likely to bless the water since it was not until the 16th century that the first “animalcules” were found by Antoni van Leeuwenhoek (Fuchs 1984). In addition, Romans would use silver regularly and documented it in the Roman pharmacopeae (Silvestry-Rodriguez, Sicairos-Ruelas et al. 2007). The Macedonians may be the first to use silver to treat wounds since they placed silver plates to prevent or treat surgical infections. Then Gaber, in 702-705 A.D., reported the first use of silver nitrate as a medical agent. Two hundred years later, Avicenna in 908 A.D. used silver fillings as a blood purifier and a preventative for heart palpitations and treatment of offensive breath. Then seven hundred years later, Angelo Sala in 1614 administered silver nitrate as a counterirritant, purgative, and for brain infections. At one point, it was rumored that silver could stop epileptic seizures because a diseased patient once swallowed a silver coin, which was used to prevent him from biting his tongue during an episode. His episodes subsided and silver was given recognition and responsibility. Silver has also influence words used today. During this same era, Alchemist developed a system that correlated the seven planets with the seven days and with the parts of the body. The symbol for the moon and the brain was silver, which gave rise to “the silver moon” and “lunatic” (Alexander 2009). Two hundred years later, silver coins were still used in preventing spoilage, as American Settlers placed silver dollars in barrels of liquids and milk to avoid spoilage (Demling and DeSanti 2001; Robert H. Demling 2001; Alexander 2009). Little did they know of silvers very potent microbiocidal properties. Then in 1834, one-percent silver nitrate solutions were administered by a German obstetrician, F. Credé, to eliminate postpartum blindness. Nearly 100 years later, Nishida et al. published information showing the decline use of silver nitrate in newborns due to the high amount that had developed

chemical conjunctivitis and the increase in number of resistant organisms (Nishida and Risenberg 1975). Virtually all reports concerning silver in the medical field occurred prior to the 1940's due to the prevalence of antibiotics at post World War II era. Then after 1940, silver was combined with salts or in complexes in creams to improve shelf life. These new forms still maintained antibacterial properties (Demling and DeSanti 2001).

Raulin documented the first bactericidal action of silver in 1869. However, it was criticized and deemed unscientific. The first accredited study over the use of silver was in 1936 by Szniolis, which showed 100µg of silver could disinfect water in four hours (Sharma, Kumar et al. 1990). Although silver helped during World War II as a water disinfectant, interest in silver began to slip due to other drugs and treatment emerging, such as antibiotics (Sharma, Kumar et al. 1990). Then in 1965, C.A. Moyer used a 0.5% silver nitrate dampened cotton gauze on the burned patients wounds or grafts. This reduced infection and improved healing and began the evolution of silver usage for burn victims. A combination of Moyers 0.5% silver nitrate dressing and Sulphamylon ® (p-aminomethylbenzene sulfonamide hydrochloride) became a popular choice of burn treatment and lead to a very important discovery in 1969 by C.L Fox; silver sulfadiazine (Fox 1968). The combination of sodium sulfadiazine with silver nitrate undergoes a cation substitution of the outer sphere. The reaction results in the soft acid (silver) replacing the hard acid (sodium) making a powerful antimicrobial ointment and according to Hard Soft Acid Base Chemistry, a more favorable complex. Other combinations of silver and sulfa groups were tested in vitro but none were as effective as silver sulfadiazine. Today, silver sulfadiazine is still used but as a 1% hydrophilic cream for second and third degree burns (Klasen 1999).

Colloidal Silver in Medicine

Colloidal silver is an alternative medicine that has been used since the early 1900's. In addition, colloids of other transition metals for therapeutics or medicinal means have also been synthesized, such as: tin, copper, zinc, platinum, cobalt, vanadium, gold and even sulfur. Most colloidal suspensions consist of deionized water with silver particles that are between 5 and 200 nanometers (Levine 2001). This size is necessary to be named colloidal. In 1915, G.L. Rohdenburg experimented with colloidal silver against malignant tumors in Ehrlich mice (Rohdenburg 1914). The mice were treated subcutaneously with 0.5 cubic centimeters of the ointment or given intravenous injections that were isolated away from the tumor. The mice were treated every other day for two weeks. Data between the injected mice and their controls was not

significant; therefore Rohdenburg felt it was unnecessary to release the data. Colloidal silver alone could reduce malignant tumor growth by 16% and lecithin by 15% but colloidal silver in conjunction with lecithin would reduce tumor growth by 28% (Rohdenburg 1914).

The Rohdenburg experiment influenced the use of colloidal silver as a treatment. Many people today still use colloidal silver as a home remedy, believing it has true powers against many sicknesses such as headaches and arthritis. Although, there is no scientific data to support their claims. Below in Table 2.2 is a list of applications of how silver is used today.

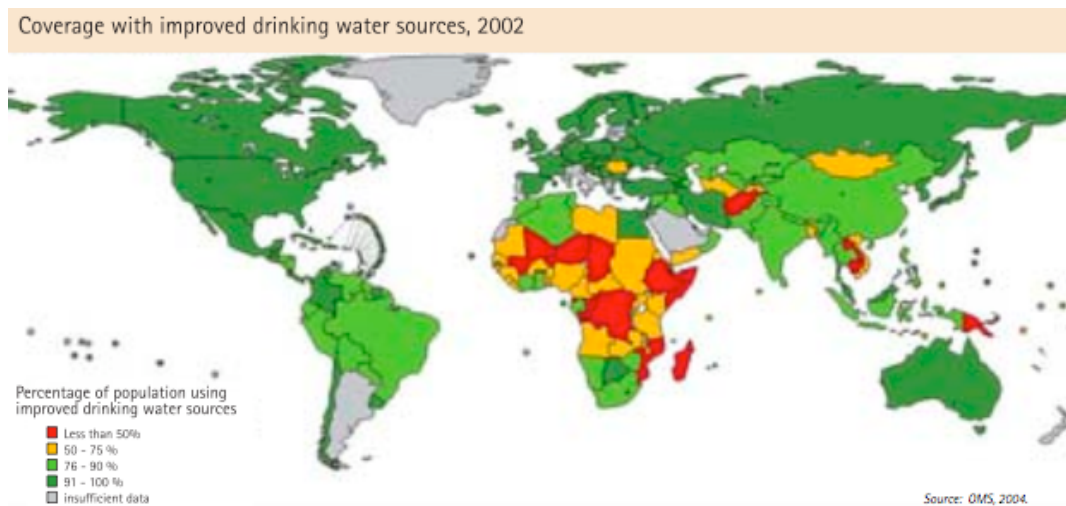
Table 1.1 Applications of Silver Today

- Water purification
 - Wound and burn care
 - Bone prostheses
 - Reconstructive orthopaedic surgery
 - Cardiac devices, such as heart valves
 - Catheters and surgical appliances
 - Dental hygiene (prevention and correction of pyorrhea, gingivitis, and bad breadth)
 - Eye conditions (primarily the prevention of ophthalmia neonatorum) (Alexander 2009)
 - Household cutting boards, table tops, surface disinfectants, and refrigerators.
-

Water Issues

Communities with limited sanitary water pertain to most of Africa and parts of South Asia (Figure 1.1). These areas may have access to clean drinking water but at an unaffordable price or they are located in a area with limited purification methods due to the lack of electricity, tools, or chemicals.

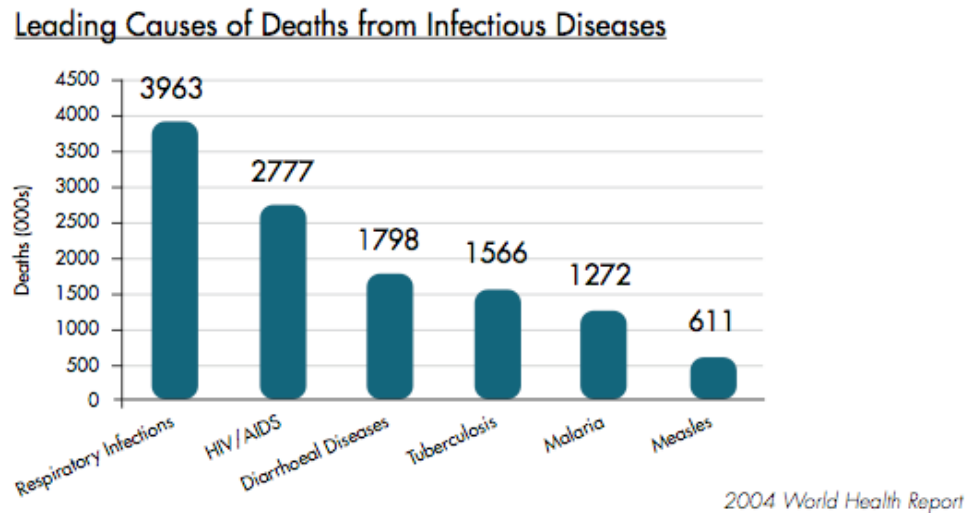
Figure 1.1 World Improved Drinking Sources



Source: (WHO 2007)

Many people in developing nations survive on less than U.S. \$2.50 a day. Many find it difficult to boil water because they do not have access to electricity. According to www.globalissues.org, 706 million in South Asia are without electricity, 547 million in Sub-Saharan Africa, and 224 million in East Asia (Shah 2009). Therefore, these societies will most likely suffer from the world's third largest cause of death (diarrheal infectious diseases; Figure 1.2). Southern Africans are willing to pay \$10 as an acceptable price for household water treatment (WHO 2007). However, this price would most likely be much lower as you went north in Africa, especially in the Sub-Saharan region.

Figure 1.2 Leading Causes of Deaths from Infectious Diseases



Source: (WHO 2007)

There are 4 billion annual cases of diarrheal disease with 1.8 million leading to death, 90% under the age of 5. The children have the highest occurrence of episodes, suffering an average of 3 times a year. Cholera, which causes a severe case of diarrhea, infects between 100,000 to 200,000 people annually (Nations 2006). These issues can be easily reduced by simple means that Americans, Europeans and other societies use, such as washing hands or correcting water supply and treatment. A review reported by the World Health Organization (WHO) showed that washing hands could reduce diarrheal episodes by 45%, and improved water supply may reduce up to 25%. In addition, using safer means of water storage and treatment could reduce these episodes by 39%, or 32% by just improving sanitation (WHO 2007). According to the United States Centers for Disease Control and Prevention (CDC), \$0.1-0.2 could provide enough bleach to for a family for one month, and \$3 a year could provide a ceramic pot filters for water filtration. Solar energy would be the best option if the overhead cost was lower (WHO 2007). The solar energy could power burners to boil water or UV lamps to sterilize water.

Boil it, Cook it, Peel it, or Forget it!

Waterborne diseases obtained from drinking water can be caused by bacteria, viruses, prions, fungi, microsporidia, protozoan, and helminths. Majority of these are zoonotic and cause serious illness in less developed countries. However in developed nations waterborne diseases can occur. In a surveillance summary of waterborne disease outbreaks in the U.S. from 2005-06, there were 28 waterborne disease outbreaks (WBDO) in the United States (Yoder, Roberts et al. 2008). Twenty WBDO were connected with drinking water (DW), six were connected to water not intended for drinking (WNID) and two were connected to water of unknown intent (WUI), as seen in Figure 1.3.

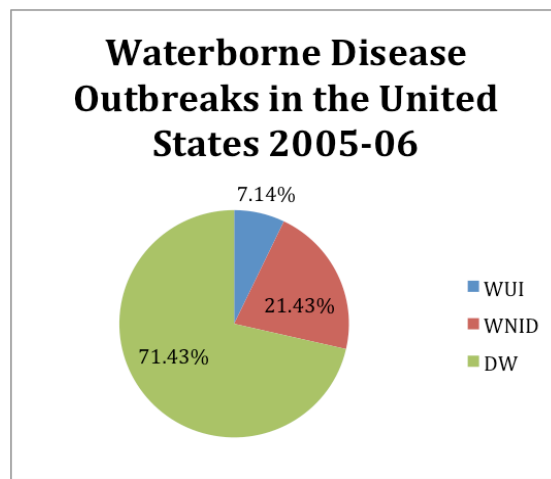


Figure 1.3 Waterborne Disease Outbreaks in the United States, 2005-06

The CDC and EPA reported that of the 20 waterborne disease outbreaks associated with drinking water in the United States, 60% of the waterborne disease outbreaks were due to bacterial infection (*Legionella* causing 50% of the outbreaks, followed by *Campylobacter jejuni* (5%) and *Escherichia coli* O157:H7 (5%)). Viral followed with 15% (norovirus G1 (10%) and hepatitis A (5%)) and then protozoan with 10% (*Giardia intestinalis* (5%) and *Cryptosporidium* (5%)). The remaining 10% were unknown. The drinking water outbreaks caused 50% with acute respiratory illness, 45% with acute gastrointestinal illness, and 5% with hepatitis (Yoder, Roberts et al. 2008).

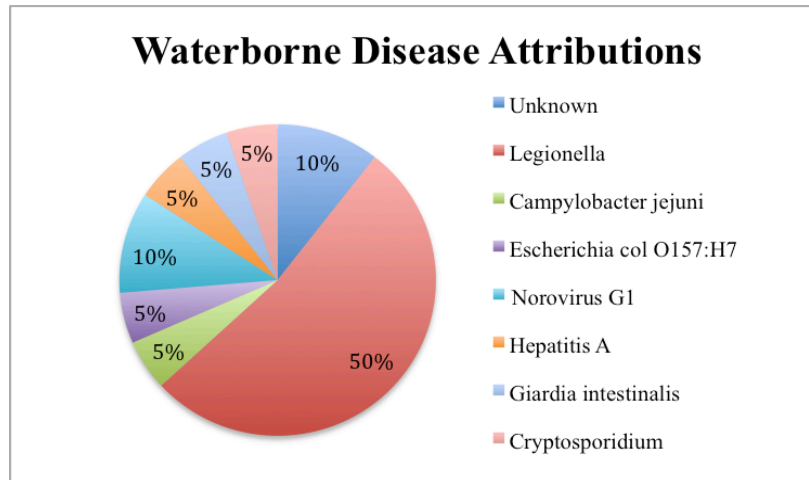


Figure 1.4 Waterborne Disease Attributions

Bacterial waterborne disease cause more deaths than any other microorganism. Leading by 1 to 1.5 billion infections a year (50% of the worlds infections), which greatly influences the annual 3 million deaths (Flach, Qadri et al. 2007). The inability to absorb food and minerals due to diarrhea greatly retards the ability of the human body to maintain nutrition and growth (Okeke 2009). Rotavirus has proven to be very efficient virus at causing diarrhea. However, on average symptoms clear very rapidly, within a day or two, like most viral cases of diarrhea (Adenovirus, Caliciviruses, Astrovirus). Albeit, this is not to say viral infections should be of no concern because viral gastroenteritis is the second most common illness in the United States (NDDIC 2010) and most likely higher in third world countries. There are serious viral diseases and some may last a prominent time, but on average bacterial infections possess a greater danger because of the longer time it takes to cure, especially if antibiotics are not available. Typically a bacterial gastroenteritis clears up in two to three days (MedlinePlus 2010). But the infection could last much longer and perhaps this is why more deaths are attributed to bacterial infections.

In 1993, the EPA released that the most common waterborne diseases were caused by: *Legionella*, *Salmonella typhi*, *Shigella*, and *Vibrio cholera* (EPA 1993). The following paragraphs will contain history, infection mechanisms, symptoms and other background information about each microorganism including *Campylobacter jejuni* and *E. coli* strains because they have recently become a major public health concern.

Legionella

Legionella pneumophila is the most commonly known pathogen for causing Legionnaires disease and Pontiac Fever. It is a gram negative, peritrichous, aerobic rod that can aerosolize and infect respiratory systems. *Legionella* was first recognized and named in 1976 at a American Legion convention held in Philadelphia, Pennsylvania as most of the attendants suffered from an outbreak of Legionnaires disease, a serious lung infection. Thus, it was named *Legionella* after the American Legion group (CDC 2008).

Legionella may be found in cooling towers, evaporators, condensers, showers, hot tubs, spas and other water sources due to its ability to form adherent biofilms (Prescott, Harley et al. 2005). In freshwater, the microbe will replicate inside amoeba and in alveolar macrophages located in the lungs of humans. Human immunosurveillance pathways are numerous, although the best characterized uses Naip5 and IpaF, which are both host proteins in the cells cytosol. These cytosolic proteins detect the presence of *Legionella pneumophila* flagellin. Presence leads to activation of caspase-1 followed by rapid apoptosis (Monroe, McWhirter et al. 2009).

According to the CDC, Legionnaires disease usually affects people over the age of 65; it develops signs of fever, chills, coughing, muscle aches, and headaches within 2 to 14 days after exposure. Pontiac Fever is a milder infection which leads to fever, headaches, muscle aches and last 2 to 5 days (CDC 2008).

Early environmental detection of *Legionella* in water sources is key. However, many factors may protect the microbe from being monitored or treated such as, optimal temperature (25 and 55C) allow for survival and proliferation, water stagnation, biofilms, presence of organic and inorganic matter, algae, and protozoa. Thus, the most effective treatment is targeted treatment which is dependent on the environment where *Legionella* is found (Carducci, Verani et al. 2010). The most common treatment is using a high concentration of chlorine, heat, and scrubbing the water container to remove biofilms (Prescott, Harley et al. 2005).

Between 1993 and 2004, *Legionella* infections increased annually in Europe. In 1993, there was an infection rate 4.14% and by 2004 it rose to 8.2% (Table 1.2). Although this infection rate may not seem very high for Europe, this BSL-3 microbe most likely has a higher infectivity in third world countries that do not have improved water supplies or sources.

Table 1.2 Reported cases of Legionnaires' disease in Europe, 1993-2004

Year	No. of cases	No. of countries contributing data	Population (millions)	Rate per million
1993	1242	19	300	4.14
1994	1161	20	346	3.35
1995	1255	24	339	3.70
1996	1563	24	350	4.46
1997	1360	24	351	3.87
1998	1442	28	333	4.33
1999	2136	28	398	5.38
2000	2156	28	400	5.38
2001	3470	29	455	7.60
2002	4696	32	466	10.1
2003	4578	34	468	9.8
2004	4588	35	557	8.2

Source: WHO 2007B

Salmonella

Salmonella is a gram negative, peritrichous, facultative anaerobe rod that is an inhabitant of the intestinal tract. It was named after the first Doctor of Veterinarian Medicine, Daniel Elmer Salmon by his assistant, in his honor. However, of the 2000 plus serotypes of *Salmonella*, only a small amount are virulent (Ibarra and Steele-Mortimer 2009). The most common health issues caused by *Salmonella* are gastroenteritis and typhoid fever (Prescott, Harley et al. 2005). An infection by *Salmonella* may be referred to as Salmonellosis. *Salmonella typhi* is the most common food-borne cause for gastroenteritis and one of the most common waterborne causes (EPA 1993; Ibarra and Steele-Mortimer 2009). The bacterium is found initially in the intestinal tract of birds and other animals. Infection begins by ingestion of contaminated water or food such as poultry, eggs, or beef (Prescott, Harley et al. 2005). The non-typhoidal *Salmonella* is usually present in mild gastro-enteritis but less fortunate areas suffer serious illness. There are an annual 3 million deaths of the 1.3 billion annual cases of *Salmonella* gastroenteritis with less than 5% mortality rate in developed countries and between 18-24% in underdeveloped countries (Chimalizeni, Kawaza et al. 2010). On average, 40,000 Americans are affected by Salmonellosis, which is approximately 0.013% of the U.S. population (NIAID 2009; Bureau 2010). General infection is highest between May through October due to the temperate climates.

Species of *Salmonella* that cause gastroenteritis will pass the acidic stomach and colonize the intestine by surviving and replicating in a variety of different cells, such as enterocytes, M cells, dendritic cells, and macrophages (Ibarra and Steele-Mortimer 2009; Owens 2010). *Salmonella* may stimulate a pro-inflammatory response in the intestine which may be beneficial for the microbe by infecting recruited cells to the site and also establishing home in the microflora that has been cleared by the immune response (Owens 2010).

Symptoms usually consist of diarrhea, fever, abdominal cramps, chills and nausea after an 8 to 48 hour incubation time. Sickness may last 3 to 7 days with possible bloody diarrhea (NIAID 2009; Owens 2010). Typhoid fever has a higher incubation period, consisting of 5 to 21 days but may last up to a month. Early symptoms of typhoid fever are fever, headache, abdominal pain, cough, constipation, diaphoresis, anorexia, weakness, and sore throat. Fever generally increases gradually over a two-week period. Following the initial symptoms are splenomegaly, abdominal distention and pain, bradycardia, rash, meningismus, and mental confusion. If late *S. typhi* infections are not treated (around the fourth week), intestinal perforation, endocarditis, pericarditis, pneumonitis, orchitis, and focal abscess can occur (Owens 2010).

Shigella

Shigella is a Gram-negative bacillus of the *Enterobacteriaceae*. It's a facultative anaerobe, intracellular pathogen that is closely related to *Escherichia coli* (Prescott, Harley et al. 2005; Carneiro, Travassos et al. 2009). *Shigella dysenteriae*, *Shigatoxicogenic Escherichia coli* (STEC), and enterohemorrhagic *E. coli* (EHEC) can cause dysentery and/or hemolytic uremic syndrome by producing a toxin called Shiga toxin.

Shiga toxin is composed of two major groups Stx1 and Stx2, which both work in an A, B fashion (Ramamurthy 2008). Meaning, the toxin has two moieties or two subunits that work in a step-wise fashion. Two different methods of Shiga toxin activation exist. Obata et al. 2008, describe their method as; unit B binds membrane glycolipid, globotriaosylceramide (Gb3) for entrance into the cell. Once inside the cytosol, the toxin is transferred to the endoplasmic reticulum. Next, unit A depurinates adenine 4324 of the 28S ribosomal RNA or the 60S subunit, thus preventing protein synthesis (Obata, Tohyama et al. 2008). A second method is described by Sandvig and Deurs. Processing the enzyme occurs by the enzyme furin, which is located in the Golgi apparatus or endosome. Subsequently, this allows the second moiety to inhibit protein

synthesis, which leads to cell death. Possible human cells affected by Shiga toxin are: M cells, macrophages, dendritic cells, neurons and endothelial cells (Sandvig and van Deurs 2000; Obata, Tohyama et al. 2008; Ramamurthy 2008; Carneiro, Travassos et al. 2009). It has been reported that one enterotoxin or a few may destroy a whole cell (Sandvig and van Deurs 2000).

Transmission typically occurs through the fecal-oral route and is most prevalent in day cares and custodial establishments (Prescott, Harley et al. 2005). Shigellosis is predicted by the CDC to affect over 400,000 Americans a year, which is 0.13% of the United States population (Bureau 2010; NIAID-B 2010). Exposure to 10 to 100 *Shigella* cells is enough to cause an infectious dose (Prescott, Harley et al. 2005). Symptoms generally occur 2 days after infection and include fever, tiredness, watery or bloody diarrhea, nausea and vomiting, and abdominal pain. Infection typically alleviates within 5 to 7 days (NIAID-B 2010).

Vibrio

Vibrio is a Gram-negative, facultative anaerobe, curved bacillus that is a natural inhabitant of the aquatic environment. *Vibrio cholerae* may be the most widely known species of *Vibrio* due to its pathogenicity, and pandemics throughout Asia, Africa and the Middle East (Prescott, Harley et al. 2005). However, of the 200 plus lipopolysaccharide O antigens, called serotypes/serogroups, pandemics are only caused by serotypes O1 and O139 (Senderovich, Izhaki et al. 2010).

Sources of *V. cholerae* can be found in fecal contaminated food (especially seafood) or fresh and blackish waters in tropical, subtropical and temperate regions. *Vibrio* vectors are water crustaceans, such as copepods, chironomids, fish, shellfish located in fecal contaminated water (Prescott, Harley et al. 2005). Fish feeding birds have also been shown to carry *Vibrio* to other water reservoirs, helping the spread of *Vibrio* (Prescott, Harley et al. 2005; Senderovich, Izhaki et al. 2010).

There are 1 million annual cases of severe dehydrating diarrhea with 120,000 leading to death (Sheahan, Cordero et al. 2004). Its route of disease is largely attributed to a bacteriophage, CTX ϕ , which encodes a virulent enterotoxin by the *ctxAB* genes. The enterotoxin, commonly known as cholera toxin (CT) or cholera toxin, has two functional units similar to *Shigella* toxin. However, it takes CT and toxin coregulated pilus (TCP) to cause disease. TCP is a pilus encoded by *tcpA* operon that aids in colonization of the intestinal epithelial cells by acting as a receptor of the bacteriophage to acquire the CT genes (Abuaita and Withey 2009).

Infection begins with attachment of subunit B of the CT, which is a homopentameric protein. Then invasion by subunit A (Flach, Qadri et al. 2007). Subunit A is a ADP-ribosylating enzyme that acts on intestinal epithelium G proteins, leading to continual activation of adenylate cyclase, which causes dysentery through excessive secretion of water and electrolytes (Abuaita and Withey 2009).

Cholera symptoms are generally vomiting, leg cramps, copious amounts of watery stool and shock due to extreme dehydration. If left untreated, progression could lead to death within hours (CDCb 2010). Endemic areas use cholera dipsticks to rapidly assess symptoms of a possible cholera infection. Results can show in as little as 10mins and have shown to have a specificity of 84 to 100% for O1 and O139 with a sensitivity of 94 to 100% (Bhuiyan, Qadri et al. 2003).

Campylobacter

Campylobacter is a Gram-negative, microaerophilic, spirillum is the most common food-borne illness from undercooked meat but is also being recognized as a waterborne pathogen too (CDC-C 2005; Prescott, Harley et al. 2005). Commonly found in chicken, turkey, and cattle, *Campylobacter* is transmitted by the fecal-oral route through water. *Campylobacter* can grow to 10^5 to 10^9 colony forming units/gram of intestinal contents (Snelling, McKenna et al. 2005).

Broilers, which are large breeding grounds for industrial chicken production, face a large issue with contaminated chicken. Broiler water chlorination is a common and effective treatment against *Campylobacter* but recent results show that *Campylobacter jejuni* can reside inside protozoan *Tetrahymena pyriformis* making *C. jejuni* 50 times more resistant to free chlorine when compared to *C. jejuni* alone (Snelling, McKenna et al. 2005). *T. pyriformis* acts as a transfer vector for *C. jejuni*, allowing it to not only resist unfavorable conditions, but also spread across more water reservoirs.

Human infection by *Campylobacter* (commonly known as Campylobacteriosis) will incubate for 2 to 10 days and produce the same symptoms from infection by food or water. Disease is caused by infection of epithelial cells and production of an antigenically cholera toxin (Prescott, Harley et al. 2005).

Symptoms include diarrhea bloody diarrhea abdominal pains, nausea, vomiting, fever, and tiredness. Symptoms typically last 2 to 5 days, but have been recorded as long as 10 days.

Healthcare advisors will typically treat this infection with water, electrolytes and antibiotics such as erythromycin, ciprofloxacin or azithromycin (NIAID-D 2007).

Escherichia

Escherichia coli is a Gram negative, facultative anaerobe, ampicillin-resistant bacillus that is a commensal of the human gut microflora (Prescott, Harley et al. 2005). Theodor Escherich in 1885 was the first to describe this microbe, which has become the top model bacterium for research laboratory experiments (Lim, Yoon et al. 2010). There are over 700 different strains of *E. coli* that are both beneficial and detrimental to human health (Todar 2008). For example, some can produce vitamin K and help our gut break down foods (Bentley and Meganathan 1982; Prescott, Harley et al. 2005). Others, like *E. coli* O157:H7, can cause profuse damage to the small intestine leading to extreme diarrhea. Scientists have organized these diarrhea causing *E. coli* into 6 different categories (Prescott, Harley et al. 2005):

- Enterotoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterohemorrhagic *E. coli* (EHEC)
- Enteroaggregative *E. coli* (EAaggEC)
- Diffusely adhering *E. coli* (DAEC)

Methods of infection range from the production of enterotoxins, which ultimately activate cyclic guanosine monophosphate (cGMP), or cyclic adenosine monophosphate (cAMP), to multiplying inside the epithelial cells to making effacing lesions, which can cause hemorrhagic colitis or hemorrhagic uremic syndrome (Prescott, Harley et al. 2005). Today, most concern is around *E. coli* O157:H7. This microbe was first recognized in 1982 as a human pathogen as it gained much hype during an outbreak in Oregon and Michigan, and it still remains a foremost public health concern in North America and Europe (Lim, Yoon et al. 2010).

Evolution of *E. coli* O157:H7 is likely to have branched from *E. coli* O55:H7, a less virulent and non-toxic strain. Through the four key evolutionary events listed below, O157:H7 became the pathogenic strain we fight against today (Lim, Yoon et al. 2010). These events include:

- Transduction of STx1 bacteriophage
- Transduction of STx2 bacteriophage

- Transformation of plasmid, p0157 and *rfb* region
- Loss of β -glucorindase and the ability to ferment D-sorbitol

Sources of *E. coli* O157:H7 have been found in bovine, sheep, pigs, goats, and turkeys feces. If infected, symptoms may begin 3 or 4 days later. Symptoms include diarrhea, abdominal cramping, nausea and vomiting (NIAID-C 2010). According to the May Clinic, fluids are the best treatment, however, if sickness is due an enterotoxin producing *E. coli*, they advise against anti-diarrheal medicine because it will not help remove the toxin (Mayo 2010).

Other Well-Known Causes

Table 1.3 lists other important non-bacterial waterborne diseases that also contribute to the 4 billion annual cases of diarrhoeal disease, and the 1.8 million deaths.

Table 1.3 Some Waterborne Diseases

Disease	Microbial Agent	General Symptoms
Amebiasis	Protozoan (<i>Entamoeba histolytica</i>)	Abdominal discomfort, fatigue, diarrhea, flatulence, weight loss
Cryptosporidiosis	Protozoan (<i>Cryptosporidium parvum</i>)	Diarrhea, abdominal discomfort
Giardiasis	Protozoan (<i>Giardia lamblia</i>)	Diarrhea, abdominal discomfort
Hepatitis	Virus (hepatitis A)	Fever, chills, abdominal discomfort, jaundice, dark urine
Viral Gastroenteritis	Viruses (Norwalk, rotavirus and other types)	Fever, headache, gastrointestinal discomfort, vomiting, diarrhea

Source: EPA 1993

Health and Silver

Routes of exposure to silver are many; therefore this topic will solely be based around ingestion of silver salts since this is the primary form a person could be exposed to. The best known medical condition from silver ingestion is argyria, which is an abnormal bluish-greying of the skin (Jacobs 2006). Argyria occurs due to the deposition of silver into the soft tissues, such as the perivascular regions, as black granules of silver sulfide and silver selenide (Lansdown 2007). Sunlight causes photoreduction of the silver complexes to metallic silver, which enhances melanin production and generation of the irreversible blue-gray color skin color (Drake and Hazelwood 2005). This condition is particularly noticeable in daylight and in blond haired individuals (Faust 1992).

Silver compounds are generally considered non-carcinogenic and non-toxic to the immune, nervous, reproductive, and cardiovascular systems (Drake and Hazelwood 2005; Lansdown 2006). However, the U.S. governed Food and Drug Administration (FDA) does not support the use of ingested silver for medical use, although they have shown in small and unsupported clinical experiments that colloidal silver can reduce nocturia, acne, skin lesion infections, throat mucus, coughing, irritation, colds, nasal discharge, sinus discomfort, abdominal pain, but improve sexual performance (FDA 1999).

However, prolonged intake of silver salts cause fatty degeneration of the kidneys and liver, and mutations in red blood cells (Drake and Hazelwood 2005). Another report suggest suggest that ingestion of silver may cause decreased kidney function and abdominal pain (Faust 1992). In mice, chronic exposure causes enlargement of the left ventricle, minimal thickening of the basement membranes of the renal glomeruli, and hypoactivity (Faust 1992).

Overall, silver is a relatively inert trace element that has been used for thousands of years and has shown to play a vital role in the decrease of infectious microbes as well as promoting human health.

Mechanism of How Silver Kills Microbes

Publications point to multiple methods silver inactivates bacteria. Silver has a strong affinity for sulfur and phosphate groups. This supports publications that suggest silver binding to and denaturing DNA, RNA and proteins (Lansdown 2002; Li, Mahendra et al. 2008). Silver ions

can impair the electron transport chain, inactivation of bacterial DNA, and cause cell membrane damage (Robert B. Thurman 1989; Lansdown 2002).

Silver in the form of a nanoparticle is reportedly able to interact with the transmembrane electron transfer of microbial cells, disrupt cell envelopes, oxidize cellular components, bind to thiol groups of proteins, prevent DNA replication, and produce reactive oxygen species (Li, Mahendra et al. 2008). This study will test some of the mechanisms in order to better understand how the silver ion-exchange resin operates.

Ion-Exchange Resins

Ion exchange resins are made up of inorganic or organic ion exchange materials with natural and synthetic subdivisions (Calmon and Keressman 1957). They work off of a basic chromatography principle by separating water-soluble ions due to charge and size. Positively charged resins will bind with anions, and vice versa. Ionic bonds are relatively easy to break, which allows for replenishing/washing the resin back to its original equilibrium, in theory, indefinitely. This technique began by Thompson and Way in 1850 because of an observation they made between ammonium ions switching with calcium and magnesium ions in soil. This was soon followed by a worker under Thompson named Spence, he was a pharmacist from York. Spence eluted gypsum (calcium sulfate dihydrate) by passing water through a glass column filled with clay treated with ammonium sulfate (Dorfner 1972). This gave rise to its first name “base exchange chemistry”, but most likely changed names in the 1870’s to “ion-exchange chemistry” due to Lembergs experiment. He demonstrated ion exchange through a negative charge porous membrane or sieve called a zeolite (Dorfner 1972). Since the late nineteenth century, ion-exchange chemistry has grown immensely and is used nearly in every field. Bulleted below in Table 1.4 is a brief list of some uses of ion-exchange resins.

Table 1.4 Applications of Ion-Exchange Resins

- Acid, base and salt preparation.
 - Water softening and desalination treatment.
 - Decontamination of wastewater.
 - Removal of coagulation factors to extend the lifespan of blood samples for medical purposes.
 - Separation of amino acids, antibodies, and other molecular compounds for research use.
- (Calmon and Keressman 1957; Dorfner 1972)
-

Matrix Types of Ion-Exchange Resins

Ion-exchange resins are produced in gel and macroporous forms. Appearance is one method of distinguishing the two forms. The gel form appears wet or shiny, and has tendency to stick. Macroporous resin beads are larger spheres; appear dry with no apparent adhesiveness.

The two different types are also much different at the molecular level. Gel beads are said to have “no true porosity” meaning the holes within the beads are not pores but are altering gaps that can change size (Shuey 1990). The gaps are made up by the freedom of movement between polymerized divinylbenzenes (DVB) thus influencing porosity. True pores can be found in macroporous resins. Macroporous resins, in a sense, are made up of gel resin beads. As monomers polymerize, tiny spherical resin particles form due to small polymer particles precipitating out. The resultant tiny sphericals are gel resin beads, that combine to form macroporous spherical beads with true pores (Shuey 1990).

Production of Functional Groups, Cationic & Anionic

Cellulose, dextran, and agarose are natural polysaccharides that are used to make ion-exchange resins. However, most ion-exchange resins are made up of styrenes and divinylbenzenes due to their durability and simple chemical modification to make functional groups (Shuey 1990). Originally, cation-exchange resins were prepared by reacting polyhydric phenols with formaldehyde, producing a phenolic resin. Then they were treated with phenolsulfonic acid, or phenolmethylenesulfonic acid to make a sulfonic functional group (Calmon and Keressman 1957). Conventional ways use concentrated sulfuric acid to sulfonate a styrene/DVB copolymer to produce the same functional group but it gives a higher concentration of sulfonic groups. Therefore, more ion-exchange can occur (Calmon and Keressman 1957; Shuey 1990). Hydrogen ions initially bound to the functional group and can be easily exchange with other cations at nearly any pH. Affinity for the functional group is typically determined by pH. Table 1.5 is a list optimal pH ranges for different types of ion-exchange resins.

Anion-exchange resins were first made by reacting formaldehyde with hydrochloride, which spontaneously combusted into a gel. The gel was then dried and grounded up to make small particulates. The resulting resin was a weakly anionic exchange resin with primary and secondary amino groups as their functioning groups. More recent production of anion exchange resins react chloromethyl methyl ether with the polymer followed by a reaction with a tertiary

amine. This results in a strong base anion exchange resin with a quaternary amine as the functional group (Calmon and Keressman 1957).

Anionic exchange resins also come in two general types, type I and type II. Type I has stronger basic properties, and resist heat better. However its basicity also makes it difficult to regenerate. Type II may not be as strong and stable as type I but it is better for more frequent use due to its regeneration ability. It requires less resources to recover to its original equilibrium thus is useful with a larger amount of ionic concentrations (Calmon and Keressman 1957).

Table 1.5 Resin pH tolerance

Type of Ion-exchange resin	pH range
Type I Strong base anion exchanger (Quaternary Amine)	1-12
Type II Strong base anion exchanger (Dimethylethanol Amine)	1-12
Strong acid cation exchanger (Sulfonic acid/ Sulfonate)	4-14
Weak acid cation exchanger (Carboxylic Acid)	6-14
Weak base anion exchanger (Tertiary Amine)	0-7

Source: Dofner, 1972

Ion-Exchange Property: Cross-linkage

Polymerized styrenes make a string of linear and stable complexes. However bridges must be made between each linear string in order to form a matrix, which makes up the bead. The bridge is referred to as a cross-linkage, which is typically a DVB. Degree of cross-linking can determine many other properties important in ion-exchange chemistry. For example, a resin with 2% cross-linkage will have a higher level of permeability than a higher cross-linked resin, which allows for acquisition of larger molecules. They are also easier to regenerate and have a lower resistance to shrinking or swelling (Bio-Rad 1997).

Swelling occurs when the resin comes in contact with a solution. This greatly affects the ability of the functional groups interaction with ions and complexes since it spreads or dilutes them out of an area (Bio-Rad 1997). As the resin loses hydration it begins to shrink. This may ruin the application of the resin since it may create a new chemical equilibrium with the new and lower hydration level. This may promote a release of the ions attached to the functional groups. Subsequently, this may decrease selectivity for certain ions.

Ion-Exchange Resins – A Home and Portable Purification System

Water purification has always been a topic of concern. Some businesses have applied ion-exchange resins in water purification. The Lifestraw® by the Swiss-based Vestergaard-Frandsen is a prime example. The Lifestraw® has a combination of an iodinated resin, an empty chamber, and granular activated carbon (GAC). These three phases are inside a plastic tube that is 31 centimeters long and 30 millimeters in diameter. This very affordable item (approximately \$5 plus shipping) allows the user to use it as a one-sided straw. All microbes, excluding *Giardia*, will die by passing the iodinated beads first, and then any additional microbes that survived will die in the empty chamber due to the high abundance of iodine still present in the solution. The last phase, granular activated carbon (GAC) will remove the iodine taste and also retain any larger microbes. Vestergaard-Frandsen claims one Lifestraw® can filter up to 700 liters of water, removing: 99.9999% of waterborne bacteria, 98% of waterborne viruses, and 15micron particles (Vestergaard-Frandsen 2006). Another water purifier that uses this similar method is Katadyne.

Recently health organizations have raised concerns about iodinated resins due to their release of iodine into water. Iodine plays a vital role in thyroid hormones. High levels of iodine are dangerous and can lead to hyperthyroidism. Symptoms of iodine toxicity are abdominal pain, coughing, delirium, diarrhea, fever, thirst, shock, stupor, vomiting, and lack of urine (MedlinePlus-B). Recently, the European Union has made a decision (Commission Decision 2008/809/EC) to ban all iodinated resins used as a disinfectant or in biocidal product as of October 25th 2009 (EU 2009). According to the United States Centers of Disease control and Prevention (CDC), the WHO recommends only using iodinated resins in emergency situations for a few weeks. No individuals with thyroid disease and iodine allergies or who are pregnant should drink iodine containing water (CDC-D 2009). Thus, a water purifier that uses silver instead of iodine would have a commercial advantage.

CHAPTER 2 - Project Summary and Objectives of Study

PROJECT SUMMARY

This project is aimed to develop a novel water purifier against waterborne pathogens that is portable, inexpensive, safe, and efficacious for developing nations, adventurous and military personal. Due to the high abundance of waterborne infections (4 billion/year), mortality (1.8million/year), and new national standards, a silver-ion exchange resin for water purification is novel, effective, and has recently gained large marketable recognition due to the ban of water purifiers that use iodinated resins in some countries. Although, studying the effect of this resin against all waterborne pathogens is necessary, this thesis will only discuss its capabilities against fecal model organisms *Escherichia coli* K12 and *Enterococcus faecalis* OG1SSp. We **hypothesized** that a silver loaded ion-exchange resin best fits our aims based on unpublished data and the wide use of silver in the medical field. I will use colony-forming unit (CFU) counts to determine kill percentages that have passed different silver water purifiers. The Kansas State University (KSU) nuclear reactor and Purolite conducted silver analysis. Total Dissolved Solutes (TDS) probes , pH probes, and temperature controlled environments were used to fulfill the following objectives:

1. Develop a water purifier that kills 99.9% of bacteria at a flow rate of 120mls/min.

A fecal bacterium present in contaminated water is the common cause for gastroenteritis and therefore is an indicator of unsafe drinking water. This project will use fecal bacteria, *Escherichia coli* K12 and *Enterococcus faecalis* OG1SSp, as a representative of fecal contamination. To pass Environmental Protection Agency (EPA) regulations as well as other countries, the purifier must kill 99.9% of all bacteria. In addition, the purifier must produce these numbers while pumping water at a rate of at least 120mLs/min since this is the average speed an individual sucks water up through a straw.

2. Limit silver leaching to 100ppb to meet EPA & WHO standards

The silver loaded ion-exchange resin may release silver during water flow and could thereby be

consumed by the user. Although publications discuss silver salts inert toxicity, the EPA and WHO limits 0.1mg of silver per liter of water in order to be sold for human use.

3. Determine limitations of the water purifier.

Water across the globe consist of different temperatures, turbidities, pHs, salt contents and many more factors. Therefore, our lab will simulate these conditions to determine what environments the purifier can operate in. In addition, we will determine the purifiers “life” or length of use against these conditions.

CHAPTER 3 - Materials and Methods

Culturing *Enterococcus faecalis* OG1SSp and *Escherichia coli* K12

Enterococcus faecalis OG1SSp

E. faecalis was grown in Difco Todd Hewitt Broth (THB) (Sparks, MD) overnight in a 37°C incubator. *E. faecalis* was plated on membrane Enterococcus Agar (mEA) and grown at 37°C.

E. coli was grown overnight in Difco Tryptone Yeast Extract (TYE) broth in a shaking water bath (New Brunswick) at 37°C. One liter of TYE broth is composed of 10g of Difco Tryptone peptone, 1g of Difco Yeast Extract, and 8g NaCl. After boiling, 10mLs of 10% glucose, 2mLs of 1M CaCl₂, and 1mL of 1% thiamine were supplemented. *E. coli* was plated on Difco membrane fecal coliform (mFC) agar supplemented with 10mLs of 1% rosolic acid (1g rosolic acid per 100 mL of 0.2N NaOH) per liter. Plates were grown at 37°C.

Determining Kill Percentages

Kill percentages represent amount of bacteria killed upon passage through a silver ion-column. Experiments designed to determine kill percentages used 500mLs of sterile reverse osmosis water with between 1000 to 1100ppm of NaCl and containing no less than 10⁶ bacteria/mL. The initial bacterial population in the 500ml water was determined by sampling 100µL and then serial diluting in 0.9% NaCl solution. The bacteria was plated in triplicates and spread by a glass L-rod or 5mm glass beads across 16 x 15mm polystyrene petri dishes containing the bacteria's respective agar discussed above. The remaining 499.9mLs were passed through the silver ion-exchange column and 100 ml aliquots were collected. Next, 100µL samples from each 100mL aliquot were serial diluted in 0.9% NaCL and immediately plated in triplicates. All plates were incubated at 37°C overnight. The following day, colony-forming units (CFU) were counted on all plates and averages were calculated to determine kill percentages.

Kill percentages were determined by using the following formula:

$$\text{Initial}^T = \text{Initial bacterial population before passing the silver ion-exchange column}$$

Remaining^T = Remaining bacterial population after passing the silver ion-exchange column

$$\text{Kill Percentage} = ((\text{Initial}^T - \text{Remaining}^T) / \text{Initial}^T) * 100$$

Measuring TDS, pH, and Temperature

Total dissolved solids (TDS) and temperature was calculated by the DiST 5 HANNA Conductivity/TDS/Temp tester. The amount of hydrogen ions were measured by Fisher Scientific Accumet pH meter 50 electrode.

Molecular Techniques

E. coli K12 chromosomal DNA was extracted and purified using a Mini-Prep kit, supplied by Qiagen (Valencia, CA). Amplification of gene *thrA* was achieved by custom primers produced by Integrated DNA Technologies (Coralville, IA). Further description of the primers may be found in Table 3.1. This gene is located between nucleotide 337 and 2799 and is 2425 nucleotides in length. This gene was used as a model for studying the inhibition capabilities of silver nitrate, reduced silver nitrate (silver nitrate solution exposed to light for a prolonged time will excite silver to gain an electron, becoming a silver metal), and silver-ion exchange resin beads on Taq DNA polymerase. The polymerase chain reaction (PCR) mix composed of 25 μ L of AccessQuick Master Mix (2x) by Promega (Madison, WI) with 23 μ L of template DNA and 1 μ L of Primer 1 (forward primer) and Primer 2 (reverse primer), followed by 80 μ L of mineral oil to prevent evaporation during cycling. PCR cycles were setup using the following program:

- 1.) Initial at 95°C for 3min
- 2.) Denature at 95°C for 1min
- 3.) Anneal at 55°C for 2min
- 4.) Rest at 72°C for 3min
- 5.) Go to step 2, repeat 19 times.
- 6.) Final at 72°C for 10min
- 7.) Overnight at 4°C

Gel electrophoresis used solidified 1% agarose gels with 10 μ L of 0.5 μ g/ml ethidium bromide. E-Gel® 96 High Range DNA Marker (Carlsbad, CA) was aliquoted in 12 μ L into

outside wells and used as a molecular weight ladder. Size can be viewed in Figure 3.1. DNA samples were stained with 2 μ L Blue/Orange 6x Loading Dye by Promega (Madison, WI) and placed in wells.

Figure 3.1 Picture of E-Gel® 96 High Range DNA Marker containing ethidium bromide from Invitrogen (Invitrogen).

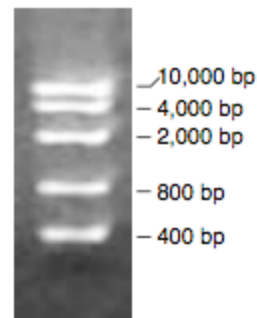


Table 3.1 Primer Description

	Region	Melting Point (T _m)
Primer 1	5'-CGG CGG TAC ATC AGT GGC -3'	59.0°C
Primer 2	5'-GCC CGC ACC ATA TCC GCG -3'	64.6°C

Transmission Electron Microscopy (TEM)

TEM was used on resins exposed to *E. coli* and on solutions of before and after exposure to the water purifier. The resin was fixed, dehydrated, infiltrated and embedded by Dr. Daniel L. Boyle. The following protocol is from Dr. Dan Boyle, he developed the pictures of the resin bead. The ultrastructural analysis was generated by immersing the resin beads in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2-7.4) for 1-16 hours at room temperature at constant shaking. Then 3 washes of 0.1 M sodium cacodylate buffer (pH 7.2-7.4) for 5 mins each at room temperature with constant rotation. Then post-fixing was done with 1-2% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2-7.4) at room temperature for 1 to 2 hours at constant rotation. The osmium tetroxide cross-links the fatty acids and amino acids, and stains to highlight membranes. Then increasing ethanol concentration was used to dehydrate the samples at constant rotation at room temperature:

1. Three, five minute washes of 50% ethanol
2. Three, five minute washes of 60% ethanol
3. Three, five minute washes of 70% ethanol

4. Three, five minute washes of 80% ethanol
5. Three, five minute washes of 90% ethanol
6. Three, five minute washes of 95% ethanol
7. Three, five minute washes of 100% ethanol
8. Three, five minute washes of 100% propylene oxide

Infiltration of samples was done with EMBED 812/Araldite resin at room temperature and constant rotation. Samples were exposed to 1:1 ratios of propylene oxide and Araldite resin for 10mins. Then expose to 1:2 of propylene oxide: resin for 10 to 20mins. Finally set in 100% Araldite resin for 10mins then fresh 100% Araldite for 1 to 16 hours. Lastly, the samples were set in a flat mold or BEEM capsule under a fume hood for approximately 1 hour at room temperature to remove previous resin. Then new resin was combined with the sample and any air bubbles were removed. The resin was allowed to polymerize for 24-48 hours at 60°C and then pictures were taken.

Solution samples of *E. coli* were prepared with copper formvar/carbon, 200 mesh grids (Ted Pella, CA). The grids were allowed to settle on a 5 μ L sample and then were placed in 2% uranyl acetate for 1 minute. Lastly, the grid was rinsed with water and placed in the TEM.

Water Purifiers

Many different silver ion-exchange resins were used in this project. Experimentation with amounts of resin as well purifier dimensions were variable. Combinations of mix bed resins and granular activated carbon were also experimented with the silver ion-exchange resin. A MasterFlex peristaltic pump by Cole-Palmer was used to pump water samples through the column for nearly all experiments. Velocity was set at 120mLs/min and water ran through against gravity.

This section will share a brief history of each type of water purifier made and used in the lab (in chronological order) to show the evolution of our product and current experimentation methods used today.

Start Small

Initially, Finntip plastic 10mL syringes were used to quickly produce results and to conserve resin materials. The syringes were 12cm long, had a 2.4cm wide mouth, which then narrows down to a 1.1cm diameter column, followed by a tip that is 1cm long. The first 4 trial water purifiers composed of any one of the following with silver: Chelex® 100; Chelex® 20 produced by BioRad Laboratories (Richmond, Ca); Dowex IDA-1 by Dow Chemical Company (Midland, Michigan). Varying concentrations of silver were bound to the resins in the laboratory. Chelex® 100 with 50% silver saturation produced the best results therefore silver analysis was conducted to observe silver leaching. Kansas State University Nuclear Reactor (Manhattan, Ks) conducted silver analysis on samples exposed to 5cc's of Chelex® 100 50% silver saturation resin. All samples were irradiated, detected and then analyzed by Gamma Acquisition and Analysis program (GENIE 2000 VDM) to develop a peak and NID report. Isotope, Ag110m, was 94.6% abundant around 657.76 keV peak. Therefore, Ag110m was monitored through each sample and revealed there was no greater than 0.001g/L runoff when the resin operated in -8°C, 25°C, or 44°C temperature environments. EPA limits 0.01g/L of silver therefore; gel type resins with 50% silver saturation passed all standards and produced promising results. However, this combination did not resist salts well. When this resin was exposed to MgCl₂, CaCl₂, and NaCl, silver chlorides would precipitate off the resin, producing a turbid white precipitate in the output. In addition, Chelex® 100 cost approximately \$500 for 700g. Therefore, silver precipitation and cost of the resin were our two biggest problems.

Prototype 1

Safewater A/S contracted Purolite International Limited (Llantrisant, Wales) to develop a resin that was similar to Chelex® 100 with 50% silver saturation but more affordable and salt resistant. A series of resins were produced that were gel and macroporous type resins with a low cross-linkage and had iminodiacetic acid (IDA) functional groups or aminophosphonic acid (PA) groups.

Purolite S939 Ag was a PA resin and it could tolerate salts the best but could not kill bacteria or viruses at high flow rate. Purolite D5134 was a macroporous, low cross-linked, IDA resin that could disinfect up to 700 liters of water but had issues with silver precipitation and viral inactivation. All of these resins were tested in a 15cm long, 3cm diameter PVC tubes, with

2cm diameter inlet and outlet caps and was Prototype I. Variations of these resins were combined with untreated 14-60 mesh granular activated carbon from Sigma (St. Louis, Mo), and mix bed resins consisting MB400 and C120E by Purolite. Combinations of the resins were kept separate from each other inside the PVC tube by customized mesh pieces.

Prototype II

Subsequent experiments followed and new protocols were tested. A new method was implemented which required any bacterial challenge to not be below 10^6 bacteria/mL, with no less than 1000ppm of NaCl in 500mLs of reverse osmosis water and pumped through at 120mLs/min by a peristaltic pump. Purolite developed a new resin known as D5194, and Safewater requested a change in dimensions so that the purifier could fit inside a water bottle. The new column was referred to as Prototype II. D5194 is very similar to D5134 but with classified minor changes. Prototype II is 23mm long with caps, internal diameter of 1.7mm and outer diameter of 2.0mm. Caps on each end narrow the diameter to 1.2mm. Mix bed resins and granular activated carbon were removed from the column since D5194 could produce good killing and resist 1000ppm NaCl.

D5194 Resin Killing Capabilities

Fresh D5194 resin filled Prototype II and was tested in 15°C, 23°C, 25°C, 28°C, and 30°C against 500mLs of 1000ppm NaCl and no less than 10^6 *E.coli*/mL.

Salt Tolerance

Presence of silver chloride was tested by challenging 3ccs of fresh D5194 resin with 50mLs of 1000ppm CaCl₂, 1000ppm NaCl or a combination of 1000ppm CaCl₂ and 1000ppm NaCl. Presence of a milky precipitate indicated silver chlorides. If no precipitate formed, 1mL of 1 M potassium iodide was added to ensure there was no silver present. If there was, a white precipitate formed which is silver iodide.

Silver analysis

Purolite conducted silver analysis on D5194 samples. They used a modified atomic adsorption (A.A.) assay for silver (Purolite PITM 17C). Below is a replicate of the information a Purolite ISO9000 officer released to me.

PITM 17C

ANALYSIS OF SILVER IN SOLUTION BY A.A.

Reagents: Silver Standard Solution 1000ppm
Nitric Acid Concentrated
A.A. Spectrophotometer
Reagent quality demineralised water.
Volumetric glassware

Dilute standard solution volumetrically to give an appropriate range of standard concentrations, this may vary with the expected sample concentration.

For samples in the higher range a minimum of three standards typically of 1, 2 and 3ppm concentration is used along with a blank (usually D.M. water) for zero point to produce a calibration curve.

For samples in the lower range a minimum of three standards typically of 0.1, 0.2 and 0.3ppm concentration is used along with a blank (usually D.M. water) for zero point to produce a calibration curve.

All standards, samples and blanks must be made up to a concentration of 5%v/v Nitric acid.

Silver is sensitive to light if samples and standards are to be stored they should be kept in the dark, alternatively amber glassware may be used.

General A.A. Set up:

Flame: Air/Acetylene

Wavelength: 328.1 nm

Slit: 0.7nm

Minimum detectable limit is 0.02 mg/L (ppm)

Maximum linear range is 3 mg/L (ppm)

Set up and quantitation limits may vary slightly between instruments, consult instrument manual as appropriate.

CHAPTER 4 - Results

Since my beginnings in this project in late 2008, I have collected a lot of data. We began this project in the dark, so we had many ideas on what methods to use. Through trial and error we discovered which resins and methods worked best. Therefore, Chapter 4 will share brief results on why previous resins failed (Table 4.1) in order to keep this section uncluttered and less confusing as well as results developed since Safewater A/S and our lab agreed on a feasible Statement of Work in the fall of 2009. The statement of work had two main experiments based around resin D5194, and they were based around questions:

1. How do the following temperatures effect resin D5194 ability to disinfect 2×10^6 bacteria/mL? Temperatures were: 15°C, 23°C, 25°C, 28°C, 30°C.

2. How many liters can resin D5194 disinfect before exhaustion?

Experiment one and silver-leaching analysis was conducted and revealed vital information concerning the importance of a time dependency of silver contact time with *E. coli*. Subsequent experiments were done to support our hypothesis that silver is stripped from the silver ion-exchange resin and needs time to react with the microbes in an output beaker or chamber. The new data canceled experiment 2, since a new problem arose as well as a broken agreement between Safewater A/S and Purolite, which eliminated our communication with Purolite.

Table 4.1 Unsuitable Resins for Water Purification

Phase 1 (Ion-Exchange Resin)	Phase 1 Functional Group	Phase 1 Type	Phase 2	Phase 3	Reason for discontinuing
SilverChelex®100	Iminodiacetic Acid	Gel	MB400	GAC	High Price
SilverChelex® 20	Iminodiacetic Acid	Macroporous			Inability to kill at high flow rate
D5134	Iminodiacetic Acid	Macroporous	MB400	GAC	Could no disinfect animal viruses or work in the presence of salts. Amount of MB400 needed is to large to prevent salts.
SilverChelex®100	Iminodiacetic Acid	Gel	GAC		High Price
D5134	Iminodiacetic Acid	Macroporous	GAC		Could no disinfect animal viruses or work in the presence of salts.
S950+Silver	Aminophosphonic Acid	Gel	MB400	GAC	Inability to kill animal viruses
S950+Silver	Aminophosphonic Acid	Gel	GAC		Inability to kill animal viruses
S939	Aminophosphonic Acid	Gel	MB400	GAC	Inability to kill animal viruses
S939	Aminophosphonic Acid	Gel	C120e	GAC	Inability to kill animal viruses
S939	Aminophosphonic Acid	Gel	IRA-400	GAC	Inability to kill animal viruses
S939	Aminophosphonic Acid	Gel	GAC		Inability to kill animal viruses
DOWEX-IDA1	Iminodiacetic Acid	Macroporous	GAC		Inability to kill at high flow rate.
DOWEX-IDA1	Iminodiacetic Acid	Macroporous	MB400	GAC	Inability to kill at high flow rate.

Effect of Temperature on D5194

Fresh D5194 was placed in Prototype II and was challenged with *E. coli*. The resin reached approximately 99% in each condition. Although some trials did not. Each trial would approximately take 1 hour and 30mins to finish because each sample was plated on an mFC with a glass L-rod. Time was essential; therefore 5mm glass beads were used to spread the bacteria. Experimental time was cut from 1 hour and 30mins to approximately 30mins. Unaware, this change dramatically influenced my results and revealed vital information. Trials below labeled with a star (★) were plated with 5mm glass beads and on average had significantly lower kill percents.

Figure 4.1 Temperature Effect: 15°C

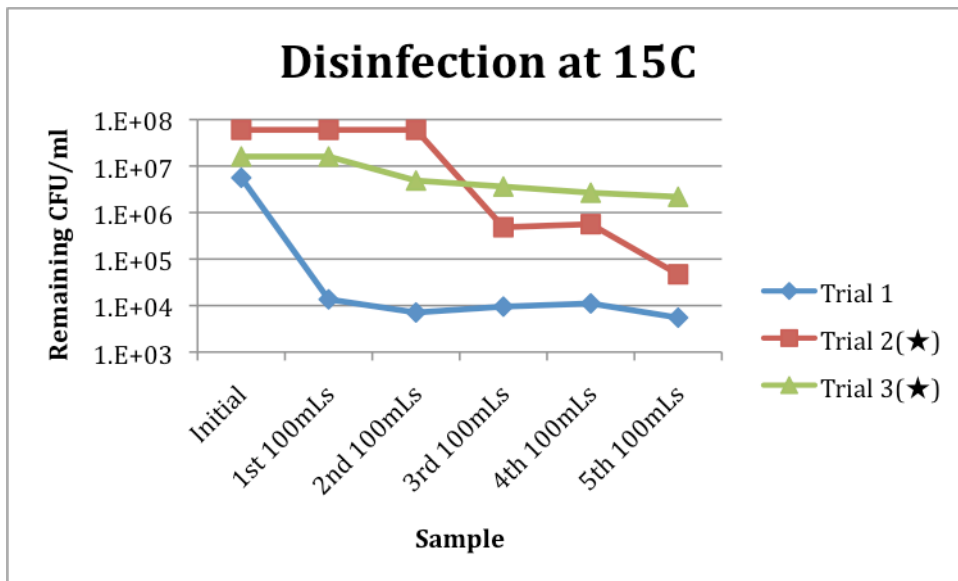


Figure 4.2 Temperature Effect: 23°C

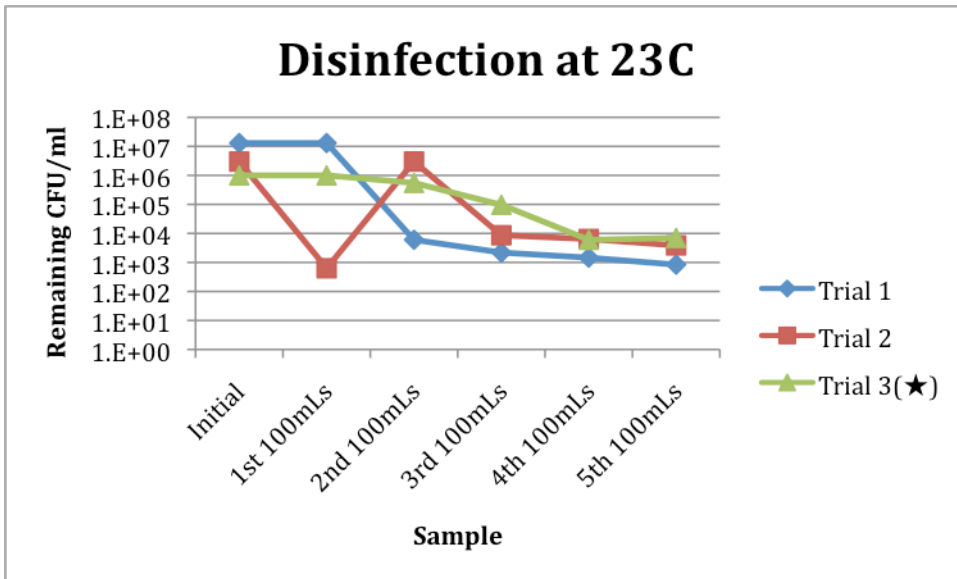


Figure 4.3 Temperature Effect: 25°C

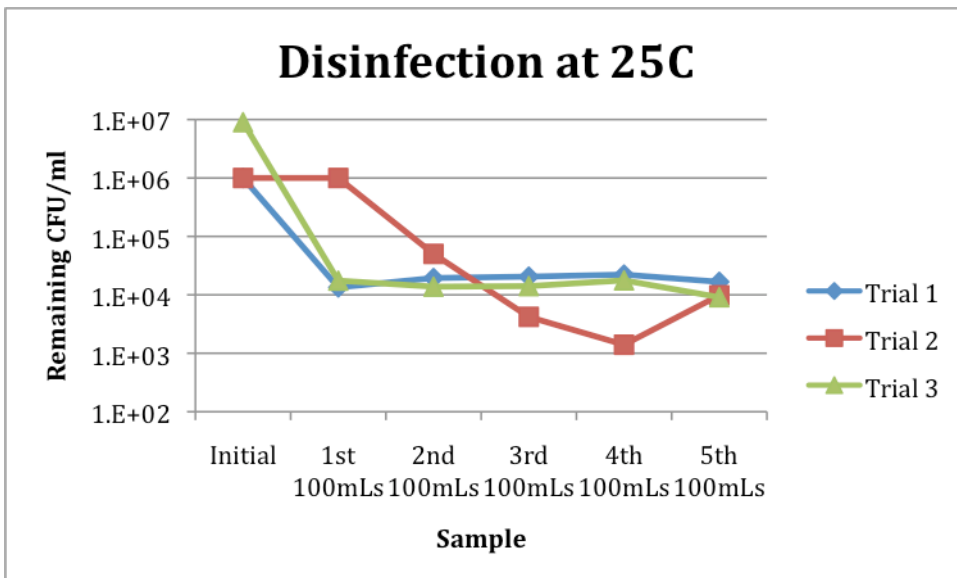


Figure 4.4 Temperature Effect: 28°C

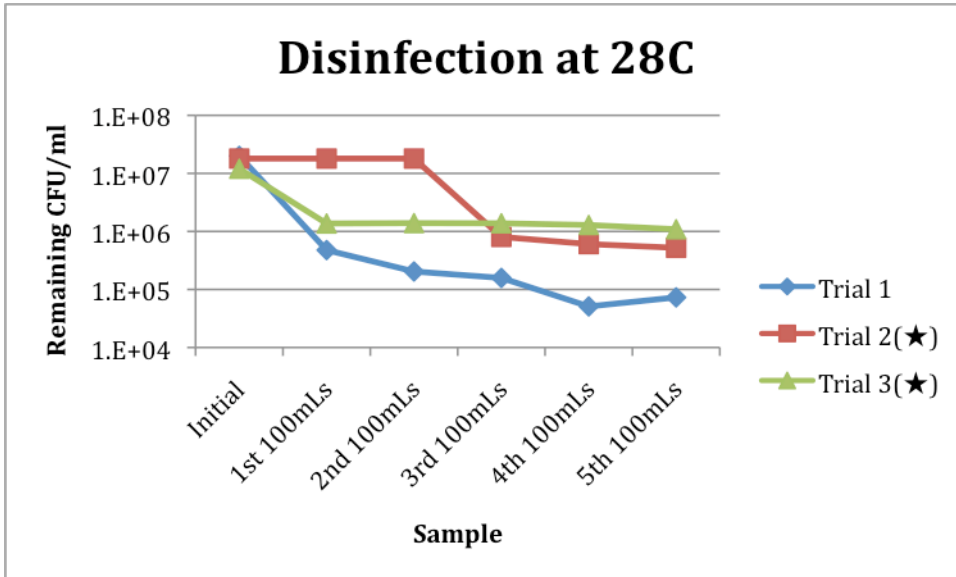
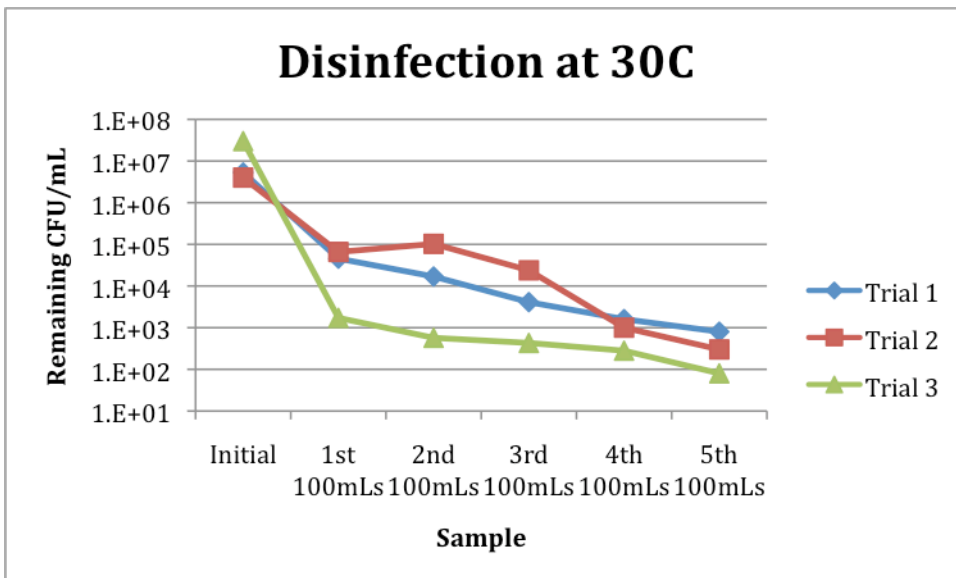


Figure 4.5 Temperature Effect: 30°C



Average Temperature Disinfection

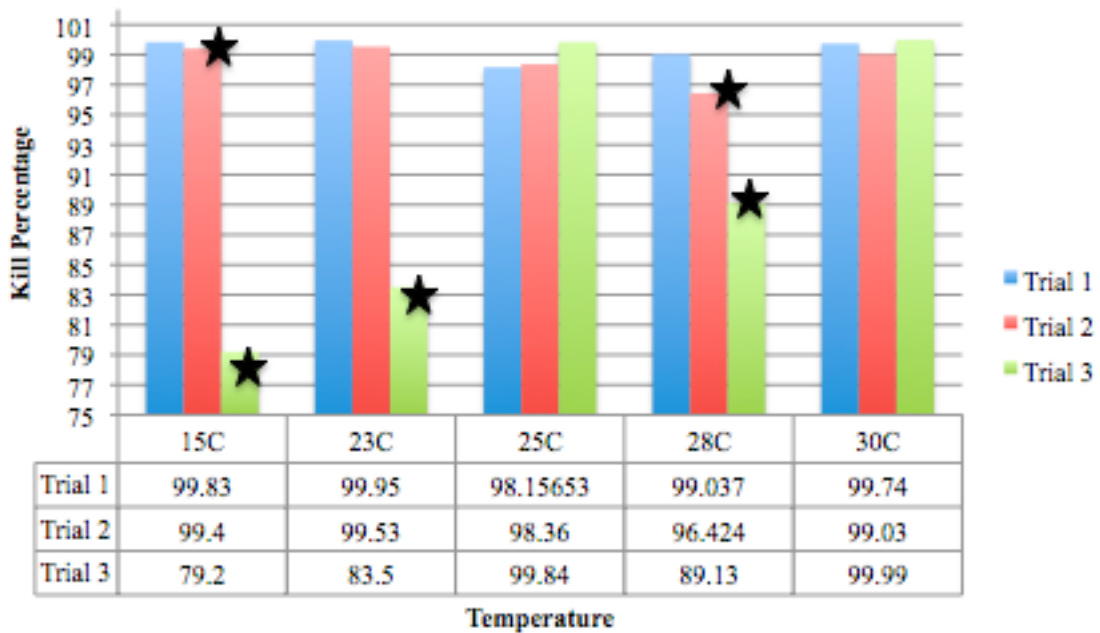


Figure 4.6 Overview of Temperate Effect

L-rod experiments had increasing kill percentages in the later samples collected because they were plated after a long delay. Reducing time before plating revealed that silver contact time was necessary because kill percentages dropped Fig. 4.6 shows the average kill percent for trials that used 5mm glass beads (★) were lower than trials that used L-rod . The change in technique brought up the importance of duration of contact between *E. coli* and silver ions in determining the kill percentage.

Silver Leaching Analysis

National standards for water purifiers that use silver as a disinfectant are typically 100ppb or 0.1mg/L. Silver analysis was conducted by Purolite on 10mL samples from water flushed through D5194 resin and water with *E. coli* flushed through D5194. Results reveal that the first liter that passed, which was the RO water with 1000ppm NaCl, had a substantially high amount of silver. We hypothesize that Purolite did not wash the resin after they reacted the ion-exchange resin with silver. Unbound silver or weakly chelated was easily washed off, thus producing a very high initial silver leaching. In addition, silver leaching is dependent on initial bacterial load, on average there was around 31ppb leaching when only water passed through. However, when 10^5 *E. coli*/mL passed the average is 77ppb. Silver increased to 120ppb when 10^6 bacteria/mL passed. Controls were 10mL samples of the initial 10L that did not come in contact with D5194. Results may be seen in Figures 4.7 and 4.8. Figure 4.7 is an overview of the entire experiment results, and Figure 4.8 is similar to 4.7 but with a smaller y-axis range.

Figure 4.7 Silver Analysis D5194

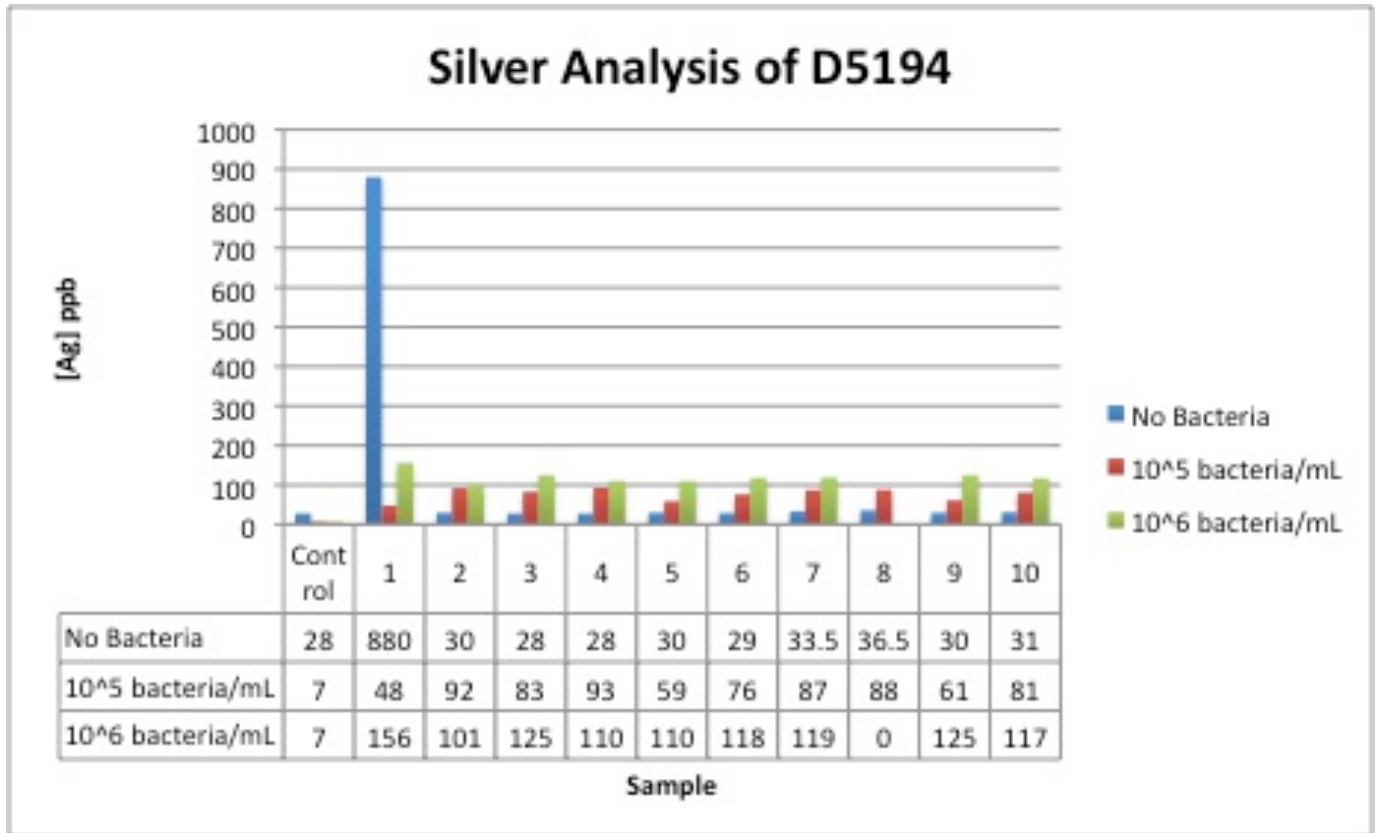
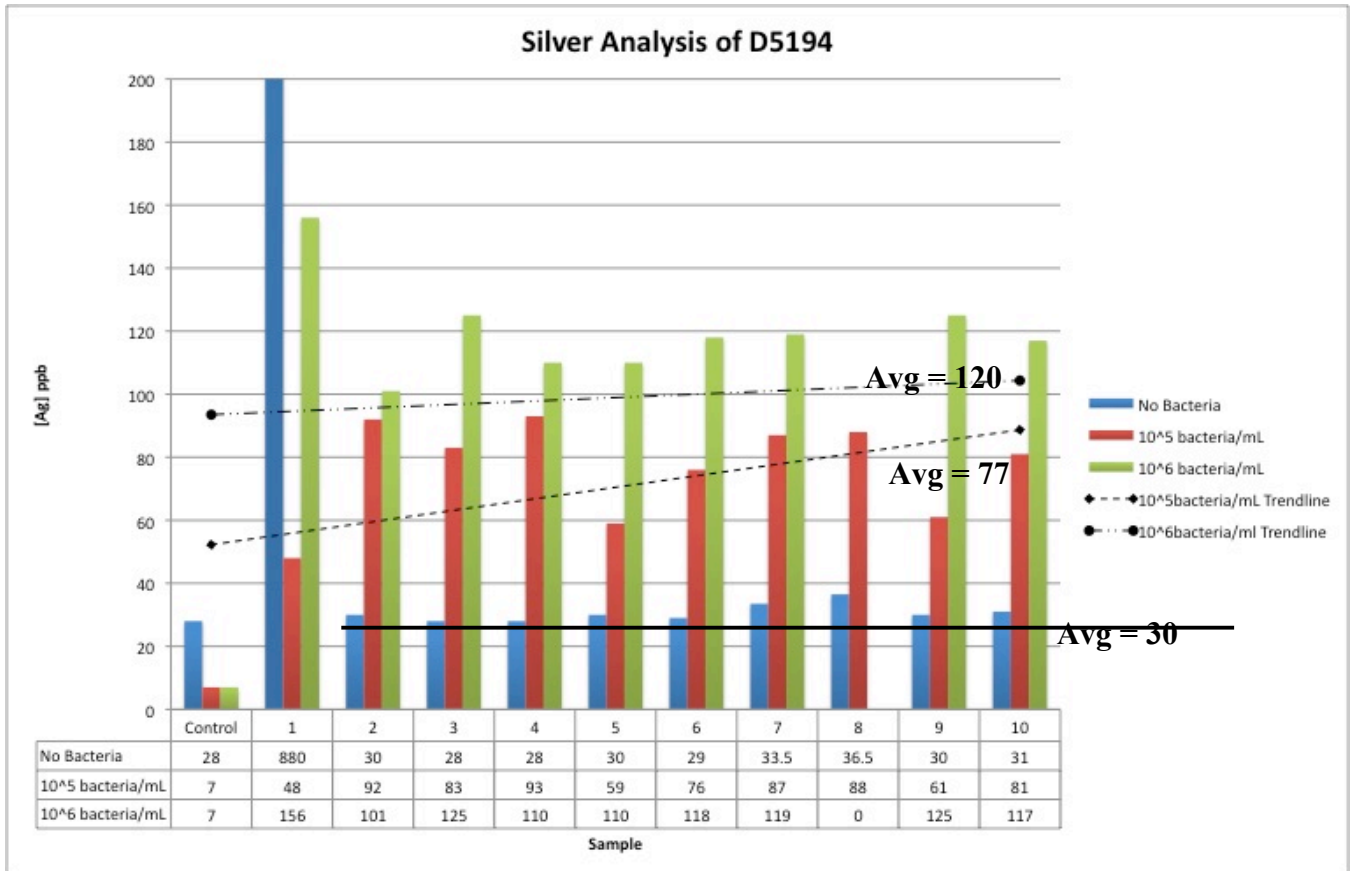


Figure 4.8 Silver Analysis of D5194; A Closer Look

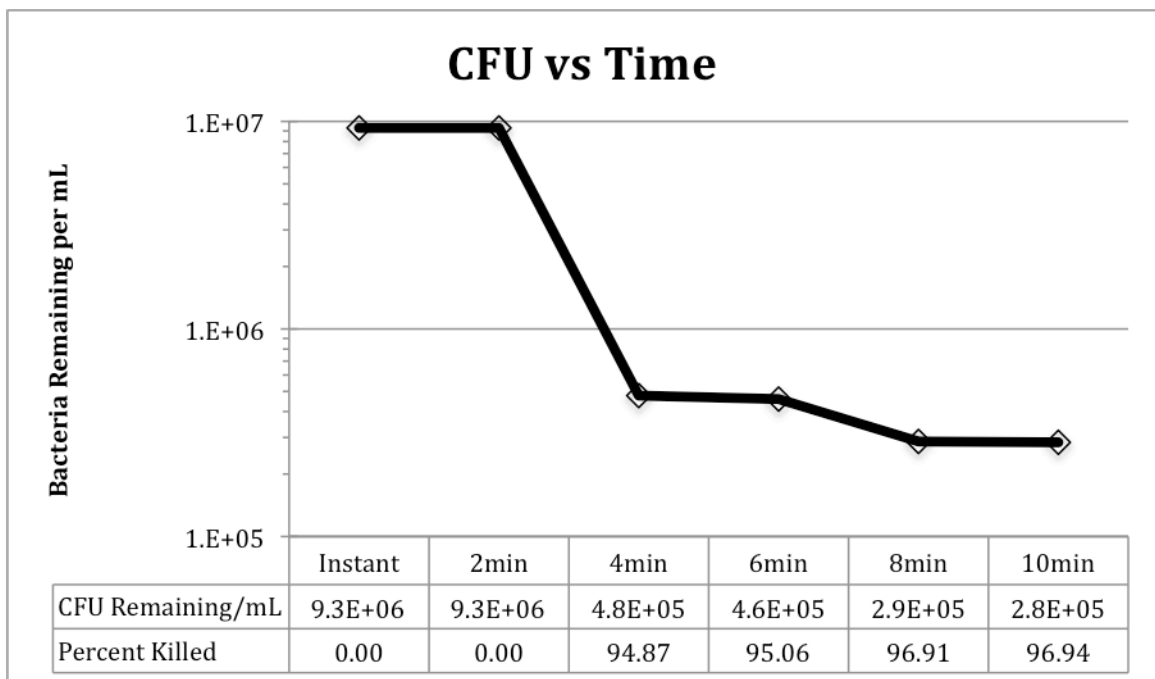


Silver Neutralizer Experiment

Silver ions that leach off of the resin need contact time with the bacteria in order to disinfect. A mixture of 1 gram of sodium thioglycolate with 1.46g of sodium thiosulfate in 1 liter of distilled water was used as a silver neutralizer developed by Tillton et al in 1978. This experiment used a modification of the study. 500mls of contaminated water was pumped through the water purifier. Once all 500mls passed, a timer was started. At 2, 4, 6, 8 , and 10 minute time points, 9mL samples were mixed with 1mL of silver neutralizer and then plated with glass beads, followed by incubation overnight. The following day CFU's were counted. Figure 4.9 graphs the importance of time as a function of bacterial disinfection.

Results from Figure 4.9 suggest that it takes approximately 4mins to disinfect 94% of water contaminated with 10^6 bacteria/ml and 96% by 10mins. Although this experiment never reaches 99.9999% killing, it is clear that it takes no longer than 1 hour and 30mins to reach since this was the typical length that it took to spread the bacteria using the glass L-rod in the temperature experiments (Figure 4.1 through 4.6).

Figure 4.9 CFU vs. Time



Silver Ion-Exchange Mechanism

TEM, PCR, and gel electrophoresis were done to help answer “How is the silver ion-exchange resin killing bacteria?” Visualization of the phenotypical changes before, and after exposure was captured. Enzymatic inhibition on Taq was tested to discover if a silver ion-exchange resin had the ability to inhibit DNA replication like many other forms of silver could. Gel electrophoresis provided a picture of gene amplification in the presence of silver.

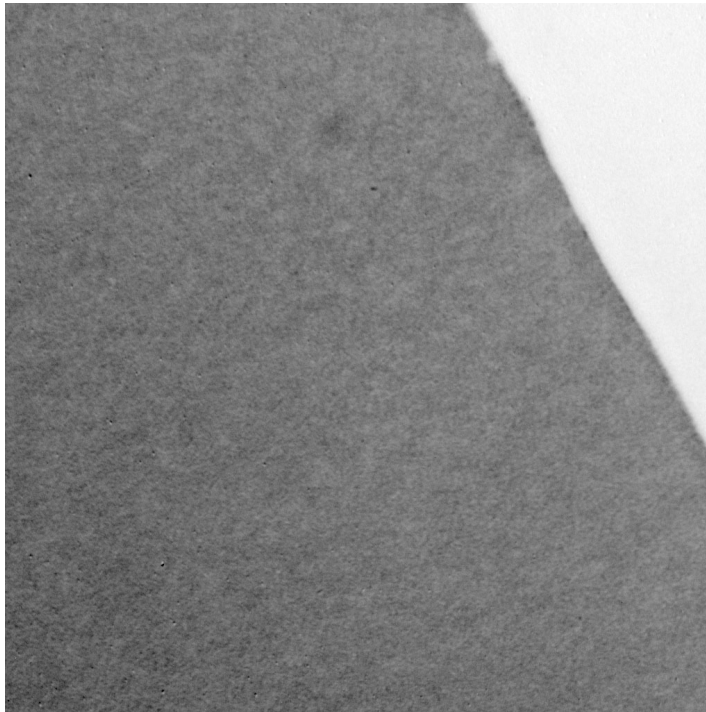
TEM

Pictures of *E. coli* before and after exposure to the silver ion-exchange resin as well as pictures of the resin bed were visualized. Pictures suggested that bacterial casualty happens after treatment in the output beaker. Microbes are not bound to silver in the resin (Figure 4.10 B). Instead, I hypothesize that silver ions are removed from the iminodiacetic acid groups and react with the bacteria in the output beaker (Figure 4.10 D). Figure 4.10 displays TEM pictures that support this hypothesis.

Figure 4.10 TEM

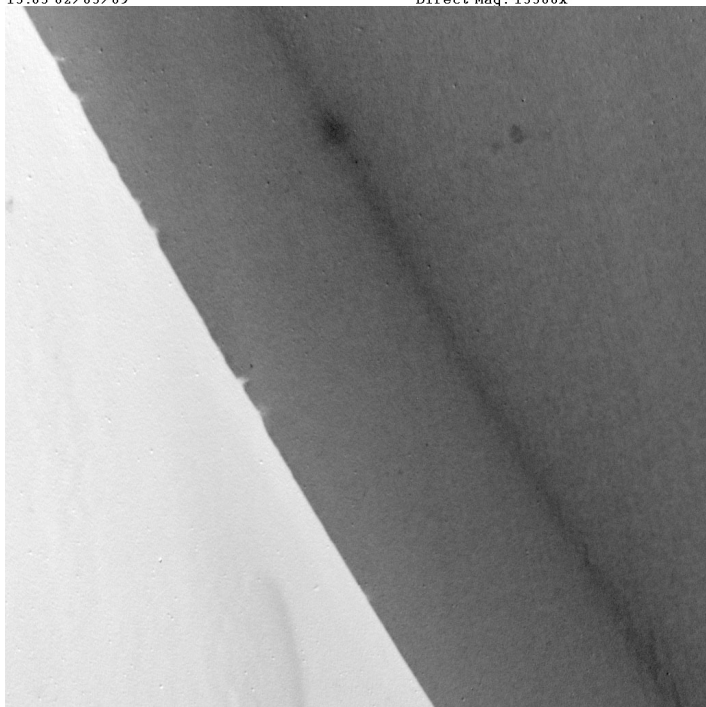
E. coli was flushed through Chelex® 100. The resin bead (shaded area) has no silver or bacteria attached to it (A). Silver attached to the iminodiacetic acid groups can be seen as dark granules, since silver is electron dense. Small amounts of bacterial debris is present on the beads (B). *E. coli* bacterium before exposure to a silver ion-exchange resin (C). *E. coli* after running through the water purifier lyses, allowing for the membrane to fold over itself and for cytosolic components such as DNA to float outside the cell (D)

A. *E. coli* through Chelex® 100.



chelbac02.tif
chel bacteria
surface
15:05 02/05/09

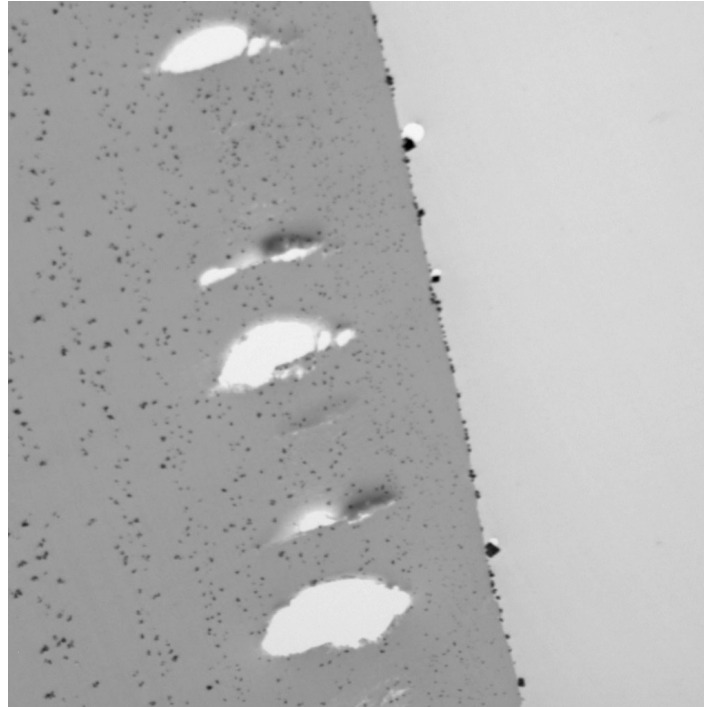
500 nm
HV=100kV
Direct Mag: 13500x



chelbac01.tif
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surface
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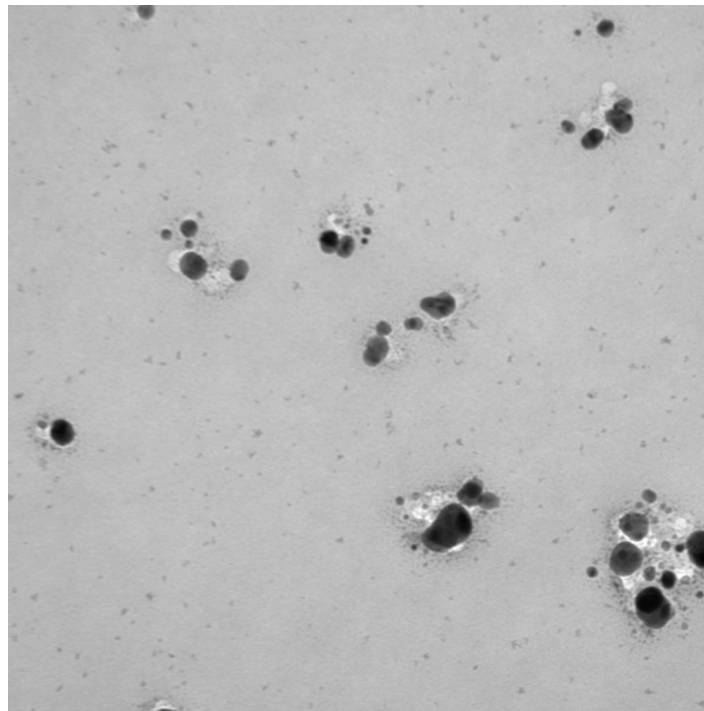
2 microns
HV=100kV
Direct Mag: 2600x

B. *E. coli* through silver saturated Chelex® 100



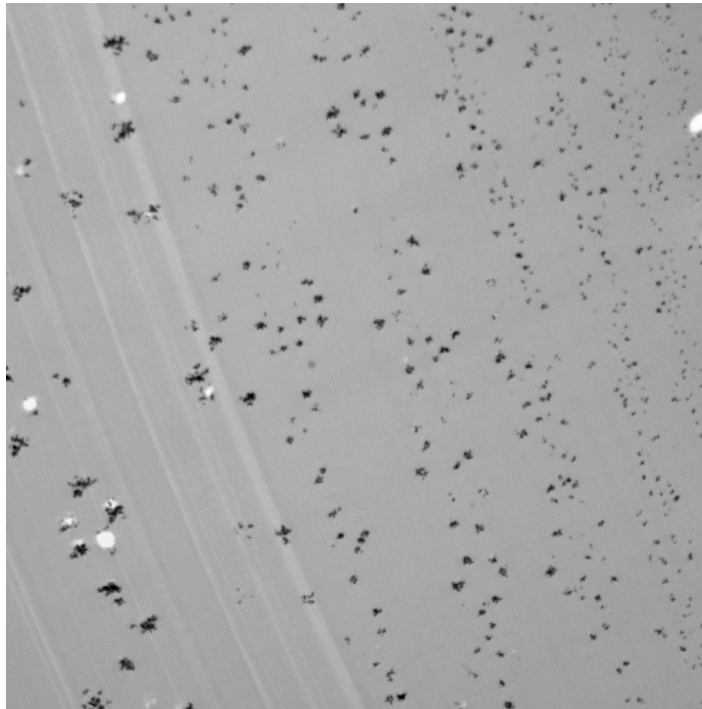
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2 microns
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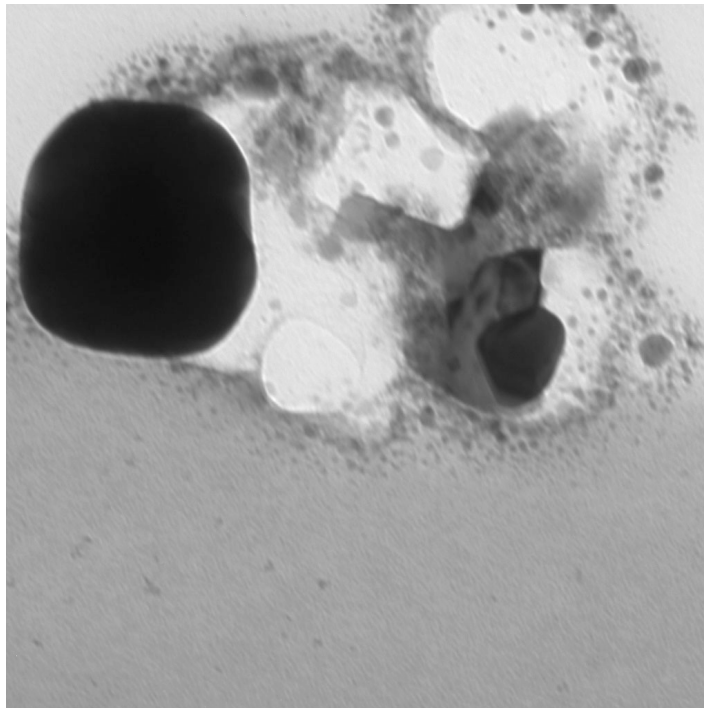
Agchelbac06.tif
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100 nm
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Agchelbac02.tif
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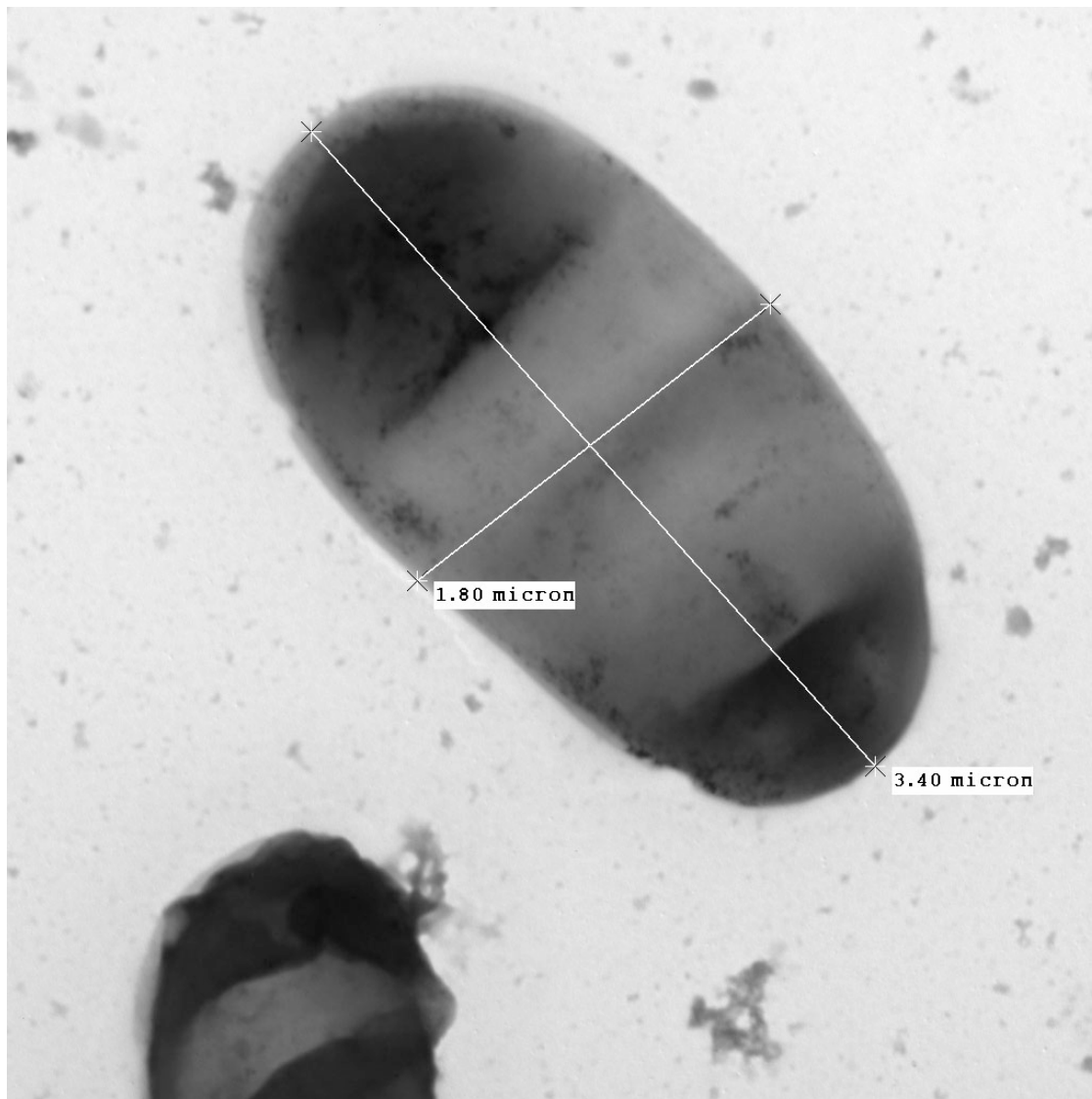
2 microns
HV=100kV
Direct Mag: 2600x



Agchelbac05.tif
Ag chel bac
surface
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100 nm
HV=100kV
Direct Mag: 130000x

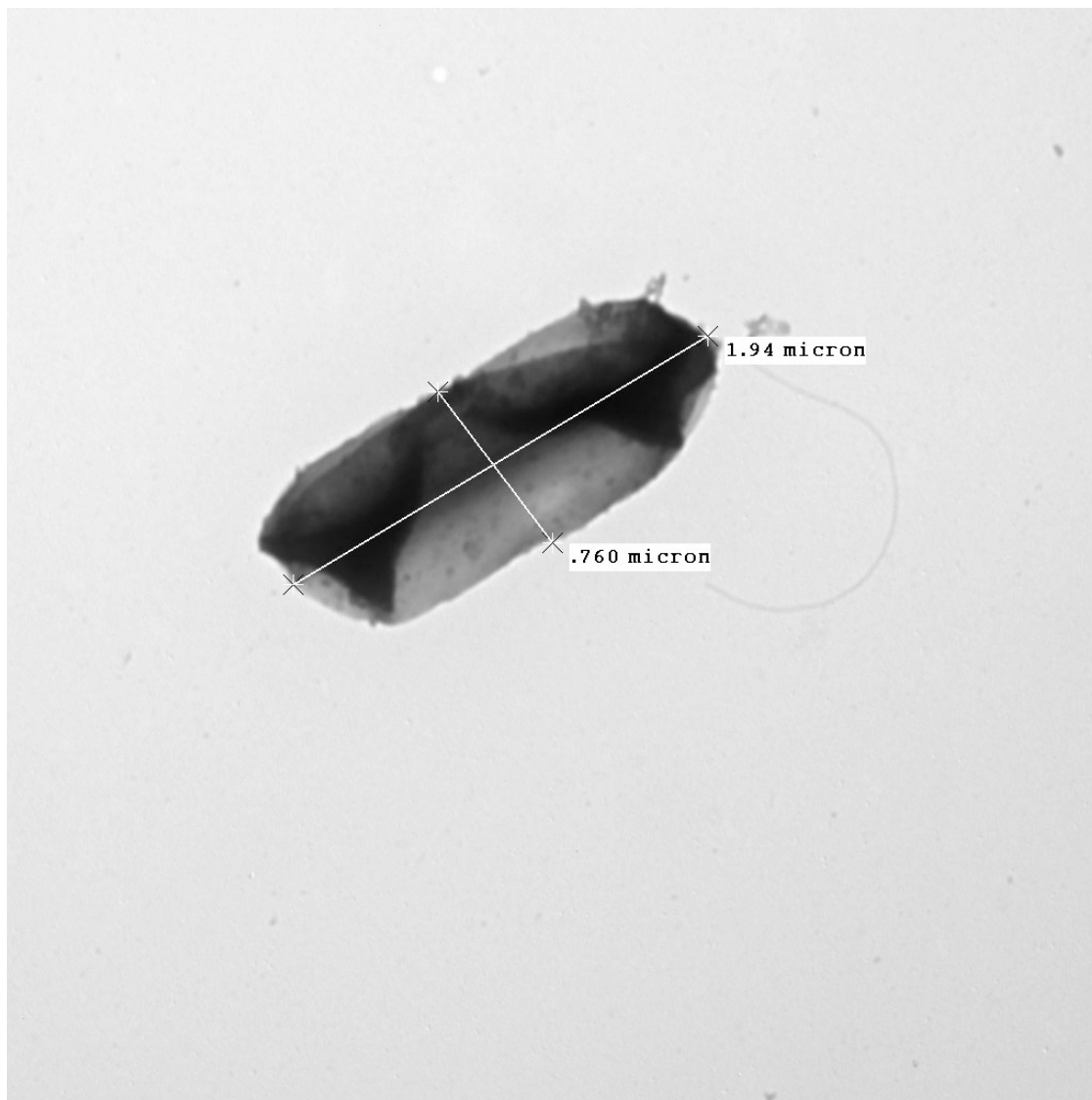
***C. E. coli* before passing through D5194**



ecoli precolum.tif
Ecoli pre column
14:41 02/09/10

500 nm
HV=100kV
Direct Mag: 13500x

D. *E. coli* after passing through D5194 (120mLs/min)



ecoli postcolumn SN1.tif
Ecoli post column SN
15:02 02/09/10

500 nm
HV=100kV
Direct Mag: 13500x



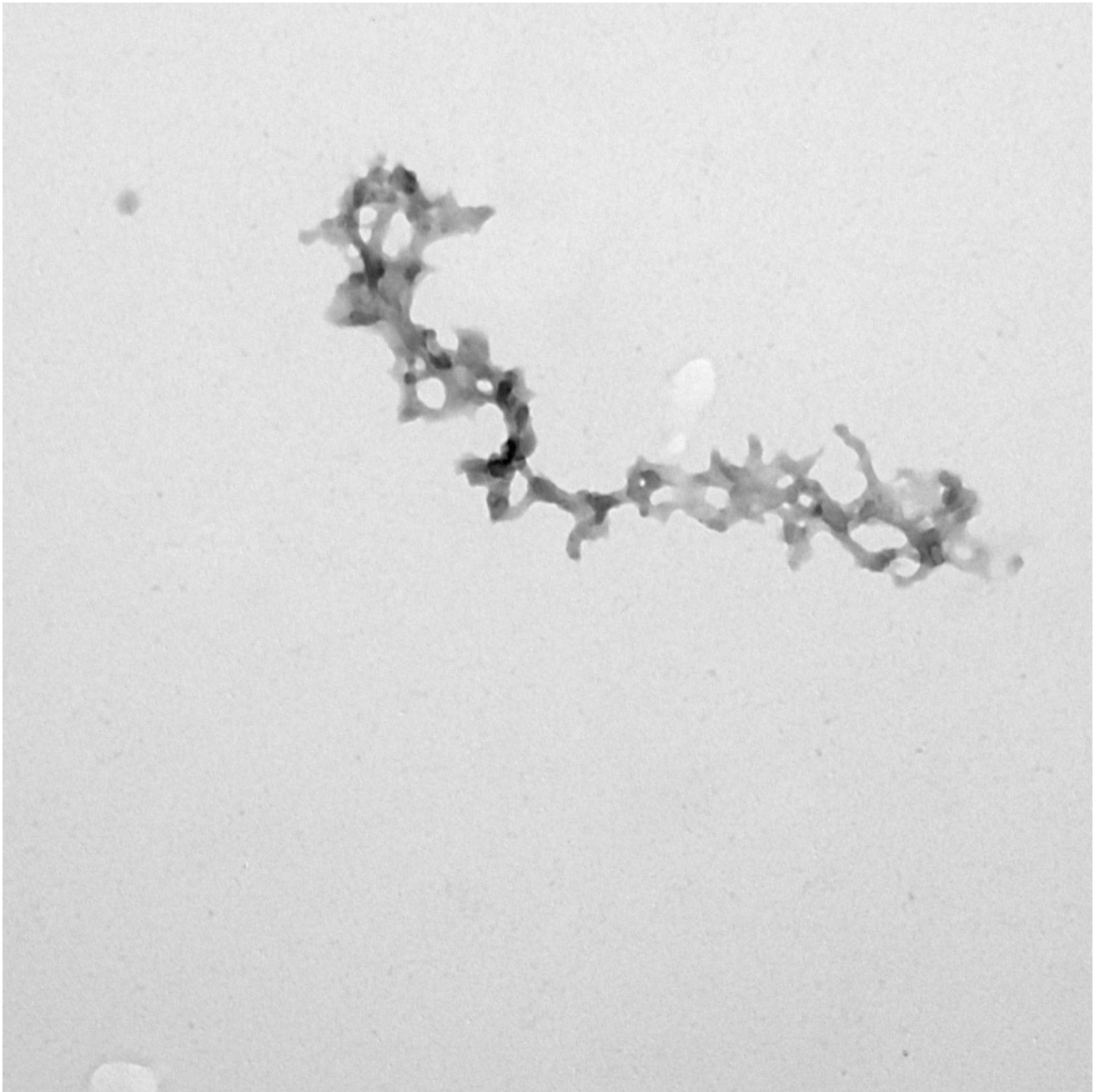
ecoli postcolumn SN.tif
Ecoli post column SN
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500 nm
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Direct Mag: 13500x



ecoli postcolumn SN2.tif
Ecoli post column SN
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500 nm
HV=100kV
Direct Mag: 13500x



chromosomal dna.tif
chromosomal DNA
15:12 02/09/10

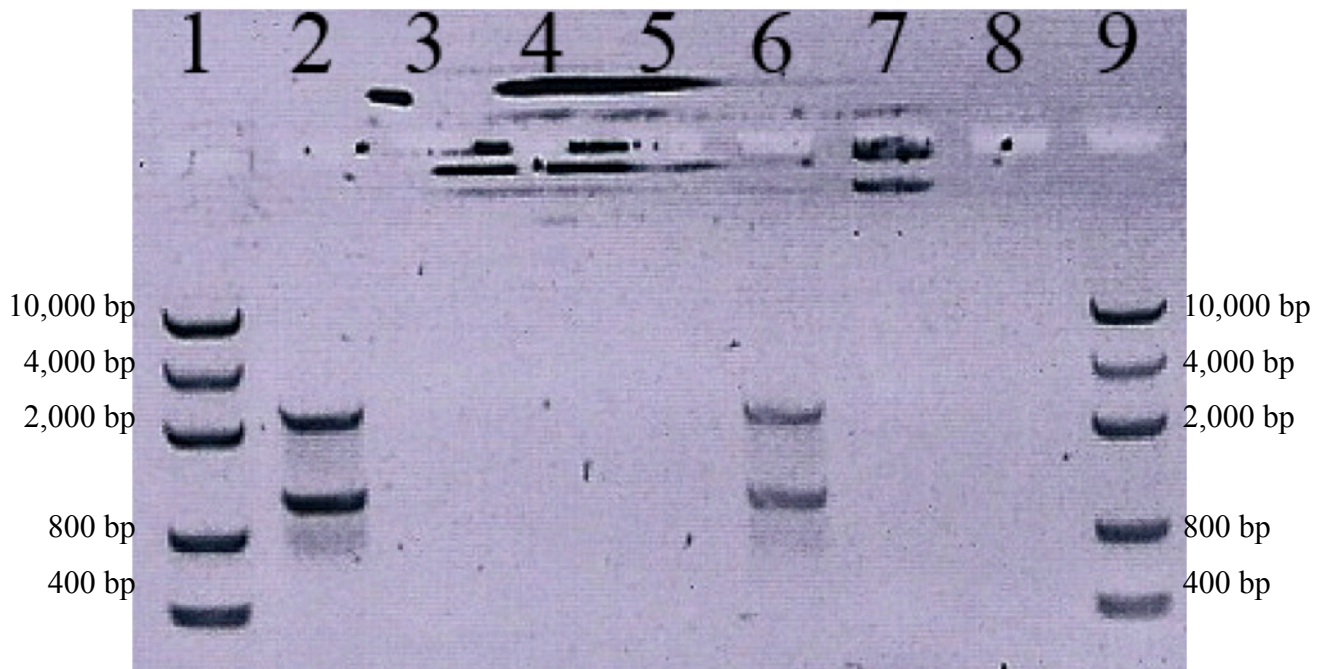
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Direct Mag: 34000x

PCR

Gel electrophoresis supported publication claims over silver inhibitory mechanisms. This assay demonstrates ionic silver and silver nitrates ability to inhibit enzymatic activity of Taq polymerase. In addition, the gel presents free chromatic material available for PCR after *E. coli* has passed through a silver ion-exchange resin (Lane 6).

Figure 4.11 Gel electrophoresis

Lane 1 and 9 are E-Gel® 96 High Range Ladders. Lane 2 is a positive control, which consist of *thrA* gene. Lane 3 is a negative control, which consist of 0.9% NaCl in place of bacterial DNA. Lane 4 is PCR of *thrA* in the presence of 10 D5194 beads. Lane 5 is PCR of *thrA* in the presence of reduced 1.5 μ L of 25mM silver nitrate. Lane 6 has 23 μ L of 3 day-old output in place of isolated bacterial DNA during PCR. Lane 7 has 23 μ L of an *E. coli* overnight sample in place of isolated bacterial DNA. Lastly, lane 8 is similar to lane 5 but with freshly made 1.5 μ L of 25mM silver nitrate (non-reduced).



CHAPTER 5 - Discussion

Silver may be the best transition metal in the medical field and perhaps the best element in general for killing microbes because of its high biocidal potency and low human toxicity. The lack of clean drinking water affects roughly around 4 billion people (WHO 2007). Therefore, developing a water purifier that is affordable, portable, effective and safe is in dire need. Thus a silver water purifier is fitting, especially since the ban of polyiodinated resins used in water purification in October 2009.

Producing a product that can disinfect large amounts of microbes and resist salts has been tedious. Subtle changes in the resin bed properties have proven to greatly influence its capabilities in water purification. Perhaps this project is going against the nature of silver ion-exchange resins because ideally we need a resin that does not exchange silver. Instead, we need a silver resin that can kill on contact, immediately, and be robust against salts.

Our project began with using Chelex® 100 with 50% silver bound. Bacterial challenges were in reverse osmosis (RO) water, lacking 1000ppm NaCl. The approximate TDS was between 10 and 50ppm. On average, 99.999% of the bacterial challenge was disinfected and according to results from the Kansas State University Nuclear Reactor there was no greater than 0.01mg/L silver leaching. Bacterial casualty by silver bound Chelex® 100 was initially hypothesized to only occur by contact with the resin. These experiments met many national standards until we tested it in the presence of salts. By challenging Chelex® 100 with 50% silver to one of the following: sodium carbonate; calcium carbonate; magnesium acetate we tested the abilities of cations (sodium, calcium, and magnesium) at displacing silver. Unpublished data showed sodium could force silver exchange but magnesium and calcium could not. Thus, the use of sodium chloride stemmed because this salt contains a cation that has a higher affinity for the iminodiacetic acid functional groups than silver and chloride as an anion that is reactive with free silver to form silver chlorides, which can be visible to the human eye.

Furthermore, silver chlorides have contact disinfection abilities (Adams, Santschi et al. 1999; Li, Zhang et al. 1999). This is may be a problem for our data because it is unknown how much is occurring due to silver contact inside the purifier and silver chlorides that form from leaching off the resin. In other words, it is unclear how much bacterial casualty is occurring

during passage (due to contact with the resin) versus after passing the column (due to silver chloride in the output beaker).

Silver bound Chelex® 100 could disinfect well but not in the presence of salts. In addition, the resin was unaffordable for personal use in developing nations. Dowex IDA-1 Cation, Chelex® 20 are macroporous resins that were tested in the beginning but neither of them could disinfect at high flow rates. Soon after, Safewater A/S began a contract with Purolite to help develop a resin that was similar to Chelex® 100 but more affordable and salt tolerable. Purolite released two different resins to us, and they were S939 and D5134. S939 was a gel type resin that used aminophosphonic acid to chelate silver. The resin tolerated salt exposure the best but could not disinfect viral or bacterial challenges. Therefore, the resin was dropped. D5134 was a macroporous resin that used iminodiacetic acid groups, similar to Chelex® 20. Although, it could disinfect bacterial challenges exceptionally well and last up to 700 liters, it could not operate in the presence of salts and could not combat viral loads.

Purolite then developed a new resin called D5194. It is similar to the D5134 but with classified changes. In general, the results showed that D5194 could disinfect at any temperature and against animal viruses but killed more bacteria as more solution flowed through, even in the presence of salts. Our first hypothesis concerning increasing kill percent, as the sample passed D5194 was that the resin bed was losing hydration when not in use, thus the silver on the functional groups did not immediately have available access to disinfect to its fullest capacity near the beginning of the bacterial challenge. However, this hypothesis failed because end caps placed on the prototype held in the hydration, and the results curiously produced the same trend. Little did we know, this began to expose how microbial disinfection was occurring.

Although, we saw no silver chloride precipitate in the output, Safewater suggested doing silver analysis in case of the presence of silver dichlorides, which are invisible to the human eye. The experiment used a total of 20 liters of RO water with 1000ppm NaCl. The first 10 liters were passed and sampled. Then the remaining 10L in the tank were supplemented with *E. coli* and then pumped through the column for samples every liter. The controls had a high amount of silver meaning our water supply has silver present. The results show that the first sample from the first 1L of the 10L was very high. Our hypothesis is that Purolite did not rinse the resin to remove any extra silver, thus leading to an abnormally high (880ppb) amount of silver release from the first sample. After the first liter passed, the average silver released into the output was

30ppb but increased once the 10 liters of *E. coli* contaminated water was processed through. Purolite did atomic adsorption assays and the results can be seen in Figures 4.7 and 4.8. Water containing approximately 10^5 bacteria/mL leached on average, 77ppb, and 120ppb when 10^6 bacteria/mL were pumped through. The increase in silver leaching is due to the lack of silver chelation strength by D5194. The increase in silver release could be attributed to the interaction of silver binding with phosphate and sulfur groups located on the outer most microbial membrane. Although D5194 can kill bacteria (Figure 4.1 through 4.7), and animal viruses, the large amount of silver release (Figure 4.7 and 4.8) may prohibit its suitability as a water purifier in some nations. United States EPA allows for 0.1mg/L (100ppb). This is the same standard WHO recommends. However some countries within the European Union have a stricter requirement of silver as low as 10ppb.

Initial plating techniques used a glass L-rod to determine D5194 disinfection. Then a change in technique to using glass beads, unknowingly made a dramatic impact and revealed how D5194 was killing the bacteria. This adjustment decreased spreading time from approximately 1 hour and 30mins to 30mins. Figures 4.1 through 4.5 that have a star used the new technique, and they on average exhibited poorer killing percentages. D5194 was then hypothesized to work differently. The resin's high disinfection abilities depended on the release of silver into the output. Greater time periods between water resting in the output beaker and plating on the agar gave better killing percentages because leached silver had a longer amount of time to react with viable bacteria in the output, thus increasing kill percentages. This helps explain the consistently higher kill percentages towards the end of each temperature experiment that used the glass L-rod (Figure 4.1 to 4.6).

A silver neutralizer, which was made up of sodium thioglycolate and sodium thiosulfate from Tilton and Rosenberg 1978 publication, was used to quantitate the amount of time needed for the leached silver to react with 10^6 bacteria/ml. A modification of Tilton and Rosenberg experiment suggest that it approximately takes at least 4mins to kill 94% of the initial 10^6 bacteria/ml and 10mins for 97% (Figure 4.8). It is currently unknown how long it takes for the silver from D5194 to react and reach 99.999% disinfection but it is not longer than 1hr and 30mins since this was the approximate time it took to spread temperature experiments using the glass L-rod, and those experiments typically reached that percentage.

Experimental support by TEM showed that the lack of bacteria bound to the silver granules on the resin, although some cellular debris may have been present (Figure 4-10B). In addition, TEM showed lysed *E. coli* cells with chromatic matter outside the cell (Figure 4-10D), which was still usable by Taq polymerase during amplification in PCR (Figure 4.11, lane 6). Meaning, either the ionic silver can bind the outer microbial membrane, causing the cell to lyse, or silver residues are interacting with outer membrane. The production of reactive oxygen species is possible too. However, results clearly indicate that the silver ion-exchange resin lyses cells (Figure 4.10D). In addition, the TEM revealed chromatic material that appeared unharmed (Figure 4.10D). PCR and gel electrophoresis revealed that it is still capable of DNA amplification, which disproves claims made by Lansdown, and Li, which stated that DNA replication is inhibited by silver (Lansdown 2002; Li, Mahendra et al. 2008). No data supports the two were bound together, but silver has a high reactivity with the phosphate groups, which are located in DNA's sugar-phosphate backbone, suggesting the two were most likely bound together.

Furthermore, Gel electrophoresis of PCR samples in the presence of D5194, reduced silver nitrate, and freshly made silver nitrated resulted in inhibition (Figure 4.11 has a lack of band in lanes 4, 5, and 8). However, this should be attributed to silver binding and inhibiting the Taq polymerase and not the DNA. This supports claims that silver can inhibit enzymatic activity.

In conclusion, our goal of developing a water purifier has not been fulfilled. D5194 has thus far been the closest we have come to a suitable resin and could be sold for use in some countries. However, it does not meet our project goals but much has been learned. Silver bound Chelex® 100 may have disinfected high amounts of bacteria, but no experiments challenged it with bacteria and salts because of the high amount of white precipitate that formed after salt exposure. In addition, those experiments were plated with a glass L-rod, allowing any leach silver to disinfect in the output beaker, which could have attributed to the large amount of disinfection. The phosphonic acid (PA) resin resisted the salts the best. Essentially it is a silver resin and not a silver ion-exchange resin because no precipitate formed when it was exposed to many different salts. The PA resin interaction with silver must be the strongest of any resin we tested, which is most likely true since according to Hard Soft Acid Base Chemistry, soft-soft interactions are the strongest. Results of the PA resin showed it could not kill bacteria, suggesting the strength of the interaction between the resin and the silver is very high. Perhaps,

the strong affinity will not allow the silver to interact with the bacteria rendering incapable as a water purifier. I hypothesize that silver must fall off the resin in order to produce strong disinfection, because silver needs at least 2mins to begin seeing large disinfection. Thus, leaving our project with the only choice of leaching off and reacting in the output to reach high disinfection. However, the amount of silver leaching occurring is unacceptable for many countries (eg. France, and Demark), and it is close to passing the EPA, EU, and WHO regulations when challenged with 10^6 bacteria/ml.

Currently, there is no silver resin that is good at both killing bacteria/viruses and also good at not releasing silver. A resin that kills bacteria/viruses at high speeds (eg. IDA resins) has to release silver to reach 99.999% killing. In addition, a resin that does not let go of silver (eg. PA resin) will not kill the bacteria very well. Therefore, invention of a resin with such nature would be a technological advancement.

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