3-30-2009

Reducing Sediment and Bacterial Contamination in Water Using Mucilage Extracted from the *Opuntia ficus-indica* Cactus

Audrey Lynn Buttice
University of South Florida

Reducing Sediment and Bacterial Contamination in Water Using Mucilage Extracted from the *Opuntia ficus-indica* Cactus

by

Audrey Lynn Buttice

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemical Engineering
Department of Chemical and Biomedical Engineering
College of Engineering
University of South Florida

Major Professor: Norma Alcantar, Ph.D.
Mark Jaroszeski, Ph.D.
Joyce Stroot, Ph.D.
Peter Stroot, Ph.D.

Date of Approval:
March 30, 2009

Keywords: flocculant, nopal, prickly pear, sustainability, drinking water, kaolin, *E. coli*, *Bacillus cereus*

This work is dedicated to my mother, Judy Smith, stepfather Mike Smith and brothers James and Jeffrey for their support, love and patience. Without a solid family structure to help promote and encourage my education I do not know where I would be or how I could ever have come this far.

I would also like to dedicate this work to the people who are currently struggling with water contamination and to all of those who seek to help them.

Acknowledgments

I would like to start by thanking my major professor, Dr. Norma Alcantar for her support, faith and guidance over the last two years. For her time, input, for always understanding and for putting her students first, I will be forever thankful.

- I would like to thank Dr. Joyce Stroot for all of her guidance in the bacteria related work documented here. I have been privileged with an exquisite microbiology teacher.
- I extended my gratitude to Dr. Peter Stroot for his help and use of his facilities.
- Thank you to Dr. Mark Jaroszeski, and Dr. Vinay Gupta for the assistance and usage of their laboratory equipment and Betty Loraamm for help with the TEM.
- Thank you to my fellow graduate students, and dear friends Eva Williams, Jeffy Jimenez, Cecil Coutinho, Bijith Mankidy and Samuel DuPont for all of their help and support.
- Thank you to my lab group, good luck all of your endeavors.
- The work documented in this thesis was funded by grant No. 0808002 from the National Science Foundation and Integrating Global Capabilities into STEM Education Critical Technologies, Strategies for Meeting the UN's Millennium Development Goals on Water and Sanitation Grant Focus on Sustainable Healthy Communities: WATER from Graduate School, USF and the State of Florida.

Table of Contents

List of Tables

List of Figures

Reducing Sediment and Bacterial Contamination in Water Using Mucilage Extracted from the *Opuntia-ficus indica* Cactus

Audrey Lynn Buttice

ABSTRACT

Throughout the past decade an increased amount of attention has been drawn to the water contamination problems that affect the world. As a result, a variety of purification methods targeted at communities in developing countries have surfaced and, although all have contributed to the effort of improving water quality, few have been accepted and sustained for long term usage. Case studies indicate that the most beneficial methods are those which use indigenous resources, as they are both abundant and readily accepted by the communities. In an attempt to make a contribution to the search for water purification methods that can serve in both developed and developing countries, two fractions of mucilage gum, a Gelling (GE) and a Non-Gelling (NE) Extract, were obtained from the *Opuntia ficus-indica* cactus and tested as a flocculating agent against sediment and bacteria suspended in surrogate ion-rich waters. Diatonic ions are known to influence both cell binding and mucilage properties, causing $CaCl_2$ to be tested as a flocculating agent alone and in conjunction with mucilage. Column tests were utilized to determine the settling rates of contaminant removal from the waters and the precipitated

flocs were then evaluated. In columns employing Kaolin as a model for sediment removal, settling rates as high as 13.2 cm/min were observed using GE versus a control (suspensions with no treatment) settling at 0.5 cm/min. *B. cereus* tests displayed flocculation initiation up to 10 minutes faster than columns treated with calcium chloride ($CaCl_2$) when using less than 10 ppm (GE) and 5 ppm (NE) of mucilage in addition to $CaCl_2$. *B. cereus* removal rates between 95 and 98% have been observed in high concentration tests (> 10^8 cells/mL). Tests on *E. coli* flocculation differed slightly from those seen using *B. cereus* with control columns requiring 5 to 10 minutes longer to begin flocculation and mucilage treated columns displaying signs of flocculation much earlier. Mucilage is an ideal material for water purification and contaminant flocculation because it grows abundantly, is inexpensive and offers communities a sustainable technology.

Chapter One: Introduction

1.1 Thesis Outline

This thesis presents the flocculant capabilities of a cactus common to many dry arid climates throughout the world. Two fractions of mucilage gum were extracted from the *Opuntia ficus-indica (O. ficus-indica)* cactus and tested for their ability to remove sediments and two types of bacteria from contaminated ion-rich water. Chapter One provides an introduction to the current state of water contamination around the globe, discusses several relevant and currently used removal methods, introduces the *O. ficus-indica* cactus and discusses the primary differences in the two types of bacteria that were studied during the course of this project. Chapter Two conveys details on the methodology and background used throughout the experimentation process. Chapter Three presents the results found using the techniques outlined in Chapter Two. Chapter Four summarizes the results and provides conclusions and recommendations for future work on this project.

1.2 Water Contamination and Regulation Policies

Over the past few decades an increasing amount of awareness has been drawn to the water contamination problems worldwide. Although water is a renewable resource, it is difficult to obtain for instance, in 2008 it was estimated that of the 70 percent of the

Earth's surface that is water, only one percent of is viable freshwater for drinking [1]. Water is vital to the health and life of every known organism on the planet and with so little freshwater available for consumption, a significant amount of energy, time and money are spent maintaining, cleaning, and distributing what little is available for consumption. In more developed countries technological advancements give way to new and more efficient, effective methods of cleaning water allowing large populations the health benefits of clean water access. However, less developed countries lack the money, technical equipment and education to build and sustain the same structures and continue to struggle with contaminated water supplies [2]. In these countries the quality of health is severely hindered by contaminated wells, unforgiving storage methods and a lack of proper sanitation [3]. The United Nations estimated that 1 billion people lacked access to potable water in 2006 and 2.6 billion people were neither educated regarding nor practiced safe sanitation techniques [4]. With so many people living on the brink of illness and death a wealth of attention has been devoted to developing new and innovative methods of water purification that could mitigate the needs of people throughout the world [5]. Case studies have demonstrated that the most effective tools presented for sanitation have relied mainly on indigenous resources as they are available and accepted by the communities that use them [4].

Due to the common direct use of both ground water and runoff water, a lack of proper sanitation and poor water storage units, a wide variety of contaminants have access to the community water supplies. These contaminants include microorganisms, sediments, chemicals and heavy metals [6]. These materials are likely to be found in the

same water supply demonstrating that the method of purification that is used needs to be capable of treating a combination of contaminants.

Although all the contaminants that afflict the world are dangerous and potentially life threatening, bacterial contamination is one pollutant that is commonly studied and assessed even in countries where water purification methods are of great resource and technology. The UN estimated in 2006 that an average of 1.8 million children die every year from diseases related to bacterial contamination which often cause severe diarrhea. Water is used in the household for activities ranging from bathing to growing crops, which give bacteria that have infiltrated the water supply direct access to the families that obtain water from nearby lakes, rivers or the community well [7]. By practicing inadequate sanitation, bacteria is often brought into the house and body through the water supply then systematically re-enters the groundwater where it will eventually reach the water supply again creating a cycle of bacterial contamination [8-11]. As a result of the connection between sanitation and bacteria contaminated water supplies, health organizations have gradually expanded water purification attempts to include education regarding the importance of practicing proper sanitation. Due to this cycle many sources responsible for safe water efforts, such as the World Health Organization (WHO), discuss the required need of community support and education leading to a variety of regulation and outreach programs [4, 12, 13].

For the past half century, the United States' water sources, treatment facilities, and distribution systems have been highly regulated by the Environmental Protection Agency (EPA), founded in 1970, under a series of policies and statues. For instance, the

Water Pollution Control Act was developed by the EPA in 1948 and regulates the quality of various bodies of water [14]. This act marked the beginning of a multitude of water quality control acts, including the Safe Drinking Water Act (SDWA), instituted in 1974, and the Clean Water Act (CWA), developed in 1977 [3]. To prepare the standards for microbiological contamination limits, the EPA did a series of case studies comparing several contaminants found in water supplies to local health, and a statistical analysis of the association between contaminant and disease rate resulted in the set limits. The CWA regulates the quality of surface water that is used for recreational activities. *Escherichia coli* (*E. coli)* and *Enterococci*, both found in the large intestine of mammals, were introduced as indicator organisms to develop standards and indicate whether or not the water had been polluted with fecal contaminants [7]. These standards were set at 126 cells/100 mL for *E. coli* and 33 cells/100 mL for *Enterococci*. The regulations outlined by the SDWA are naturally more stringent than those provided by the CWA because the regulated water sources will be consumed by the urban and rural communities. For the most part, the information provided by the SDWA is not exact limits to which regulations are upheld, but rather are methods and techniques required to reduce the amount of biological contaminant as much as possible aiming for zero viable cells [2, 3, 7]. One of the very few standards that are listed is for fecal coliforms, which restricts the number of positive tests to five percent when more than forty samples are taken in a month [15]. Both of these Acts also regulate the frequency that the water in question is to be tested and offers many suggestions to both clean and test the water samples. From the time of

their enactment, these guidelines have evolved with additional laws to better fit the improved technology and microbiological assessments of the current time [14].

Although not as strict as these U.S. laws, organizations such as the United Nations (UN) have also developed several programs designed to help developing countries gain access to clean water. The UN was developed in 1945 with the goal of spreading peace and helping the world gain basic human rights. A branch of the UN called the World Health Organization (WHO) was formed in 1948 and focuses on the health and quality of life of people throughout the world. A logical part of the WHO's contribution to the developing world is focused on water and sanitation improvements. Documents supported by the WHO report total coliform standards in a very similar manner to the EPA. For example, up to 95 percent of untreated water must test negative for these coliforms to meet the criteria for safe consumption [3]. In treated water, any positive detection is cause for reevaluation because additional contaminating sources may be present or the purification system being used is not performing optimally.

The Millennium Declaration was developed by the UN in 2000 which spawned the Millennium Development Goals (MDG). These goals outline eight human rights, including environmental sustainability and clean water access for developing countries. The goal devoted to sustainability and water aims to cut the population living without clean water and effective sanitation in half by the year 2015. Progress reports released in 2007 and 2008, composed by the UN, outline the progress accomplished in reaching the goals at the halfway point. It was discussed in the reports that for the goal of basic sanitation and clean water resources to be met, the success rate needs to increase

dramatically. Several areas showed signs of improvement while others were actually considered to have worsened [12, 13].

In addition to the WHO and MDG, the UN also began to organize Human Development Reports in 1990 [16]. Each yearly report is published with information relative to the most pressing issues of the year. The 2003 report was dedicated to the MDG and discussed in great detail the goals, the difficulties that would be faced, how they could possibly be achieved and the aspects of life that would be affected. The report also discusses the importance of individual country ownership of the goals, which has shown to play an extremely vital role in the use and maintenance of the purification systems installed [16]. The 2006 report focused on the quest to bring water to countries that struggle with contamination. Topics included the relationship between economics and water availability, progress witnessed in the MDG, strategies for significant impacts, water providers, and the importance of practicing good sanitation techniques [4].

1.3 Current Removal Methods

In the US, centralized water treatment systems are in place and utilized in water delivery to households and consist of fairly intricate technological purification methods. Developing countries do not have access to a centralized water system and are limited by a number of factors including low energy sources, unavailability of chemicals and equipment, and many have not been educated regarding water purification and sanitation.

In response to these limitations a variety of purification methods have been developed and tested for use in developing countries. Many of these systems fit in to one

of three different categories, including filtration, disinfection and coagulation/flocculation or are composed of a combination of these three methodologies [17].

1.3.1 Filtration Systems

Many filters designed for use in developing countries consist of raw materials that are naturally found in the targeted area. These materials include sand, rice hull, and coal. In their simplest form these filters are fairly simple, inexpensive, and easy to build and maintain, and have been found to be an effective tool for removing bacteria and other microorganisms from contaminated water. To extend the filters lifetime, it is recommended that the user allows the water to sit for up to four days prior to filtration, allowing turbidity in the water to settle naturally and microbiological content to die [17]. Figure 1 shows a schematic of the commonly used slow sand filter.

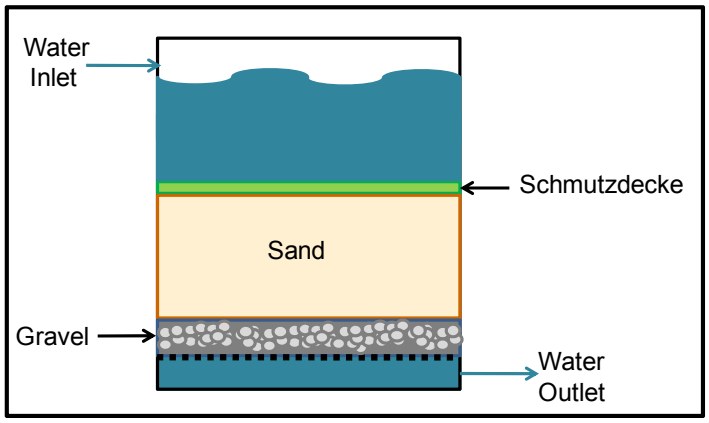

Figure 1. Schematic of a commonly used sand filter. Water enters in the top of the unit and is cleaned mostly by the schmutzdecke layer before passing through the sand and gravel then exiting from the bottom of the unit.

Almost any sand filter operates by introducing water into the filtration unit at the top of the column. A layer of bacteria, algae and various other living contaminants called the schmutzdecke gradually builds above the sand portion of the filter, which is added mainly for support. Case studies indicate that the schmutzdecke layer requires approximately three weeks to build to a sufficient level [18]. This layer is considered to remove a fairly large amount of living contaminants before the water even reaches the sand portion of the filter. Tests with *Escherichia coli (E. coli)* have shown that a significant amount of bacteria removal occurs during the movement of the water through the schmutzdecke layer, but the amount that is removed is greatly impacted by the design, and operational state of the unit [17-19]. Other work has shown a 39 percent increase of coliphage, viruses that infect bacteria, removal in sand filtration columns to 99 percent after the schmutzdecke has had time to build itself on the top of the packed sand. A limiting factor of slow sand filter use is that as the layer builds, it begins to inhibit the flow of water and has to be removed approximately every three months causing the performance of the filter to drop periodically [20]. In addition to slow sand filtration, rapid sand filtration systems have also been tested, but have proven to be more difficult to set up and function at a less efficient rate compared to slow sand filtration units [17]. Often the addition of coagulants is necessary to obtain removal rates equal to those observed with the slow sand filtration methodology, causing these systems to be less effective in developing communities [19].

Rice hull, which is another commonly used material for filter beds, has been known to contain approximately 90 percent silica which provides the ability to purify

contaminated water and reduce turbidity. These filters also demonstrate slightly higher removal rates of *E. coli* (90 to 99 percent removal) when compared to slow sand filters (60 to 96 percent removal) [17].

Coal and activated carbon, which has been treated with high temperatures and a lack of oxygen, are both materials that are also studied for their abilities to remove contaminants from drinking water [5]. In addition to being tested alone for removal, these materials are also often impregnated with other materials that are known to reduce contaminants, for instance aluminum sulfate (also known as Alum) or lime. Studies of coal impregnated with Alum the removal rates of viruses, rotaviruses and polioviruses were between 95 and 99 percent in certain pH ranges [17]. Activated carbon is also commonly used in water treatment due to its high porosity and high reactivity, which aids it in targeting water suspended organic substances and improve taste, odor, and turbidity [21].

A practical up-flow filtration system was designed by the United Nations Children's Fund (UNICEF) in 1987, which combines carbon and sand filtration into one unit that is easy to operate, maintain and produces a significant amount of clean drinking water. These filters, however, need to be cleaned regularly and often require the water to be free of harmful pathogens prior to filtration [17].

While the filter systems discussed so far are mainly composed of raw materials found in the community, other methods have also been developed using materials that the communities produce and use regularly. One such filter is used in Bangladeshi villages and utilizes a sari cloth. Studies showed that when folded between four and eight times,

the cloth resulted in a filter with a pore size of approximately 20 μm. The case study targeted cholera infection and with the use of the folded sari as a filtration mechanism infections decreased by 38 percent. It was also observed that 90 percent of the villagers used the sari with little opposition from the users [22].

1.3.2 Disinfectants

Disinfection is a water treatment that is commonly used in both developed and developing countries and works by chemically inactivating microorganisms [7]. Chlorine is the most commonly used chemical and is used in developing countries at concentrations of 0.2 to 0.5 mg/L of treated water. Clay pots available in the communities are filled with sand and the appropriate amount of chlorine and are then submerged in the water that is to be treated for at least one week [17]. Case studies in Uzbekistan have shown that in homes where chlorine was used as a disinfectant for drinking water, diarrhea cases decreased 67 percent among children compared to those without the disinfectant [23]. When using disinfectant chemicals alone, bacteria can attach to the surface of the pipes or containers in which the water is transferred or stored leading them to gain resistance to the treatment. By attaching to these surfaces, or to other particles in the water, the bacteria can potentially be shielded from the disinfectant allowing it to survive and grow. In studies with *Klebsiella pneumonia*, surface attachment was among one of several factors, including age of the biofilm, encapsulation and growth conditions, that inhibited the inactivation with chlorine by 2 and 10 times the rate of unattached cells [24]. Similar results are shown for the chlorination of *Bacillus anthracis* and *Bacillus*

thuringiensis spores [25]. Many times chlorine is utilized in conjunction with a filtration system to assist with turbidity reduction and the removal of material that is not living however, most natural materials will degrade with chlorine contact [17].

One concern associated with disinfectants is the possibility of disease from residual chemicals. In the past, chlorine has been monitored for its potential threat as a carcinogenic and possible effects on the reproductive tract [7]. Disinfection also does not remove the particles that are in the water.

In developing countries solar radiation is also commonly used to disinfect bacteria living in feces and water. Clear plastic bottles are filled with drinking water and then put in the sun between six hours and two days, depending on the climate at the given time. This can raise the temperature of the water to 55˚C reducing the amount of active organisms [7].

1.3.3 Coagulants/Flocculants

The final major form of water decontamination is coagulation and filtration. Coagulation involves an additive designed to change the chemical charge of the particles contaminating the water. By doing this the particles can be flocculated, creating a large volume of connected particles that will then be settled to the bottom of the column under the influence of gravity [26]. Although some particles settle due to gravitational forces alone, the establishment of aggregated particles can increase the sedimentation rate drastically. There are four different types of sedimentation currently being studied including discrete particle settling, flocculant settling, hindered settling and compression

settling. Discrete particle settling occurs when the suspended particles settle naturally without the addition of a coagulant or flocculant. When particles begin settling naturally but flocculate as they descend through the water, flocculant settling is considered to be taking place. Hindered settling occurs when the settling of the particles influences the settling of other particles in the solution and compression is considered to take place when there are a large number of particles present in the solution and settling is hindered. It is also common for more than one of these settling types to occur in a given system [26, 27].

Aluminum Sulfate (often called Alum) is commonly used as a coagulant in many developed countries as a method of water purification; however, it is not as easily accessed in developing communities.

With many case studies indicating the benefits of using indigenous materials for flocculation, attention has been drawn to the long time use of plant materials and clays as coagulating agents. The seeds from several plants, including the *Moringa oleifera*, *Moringa stenopetala*, and *nirmali* have traditionally been used by some communities for several hundred years and are beginning to be tested in laboratory settings for their abilities to remove contaminants [17, 28]. Removal with these organic materials have shown 90 percent reductions of turbidity in waters with suspended kaolin clay and 40 to 50 percent reduction of bacteria using concentrations as low as 2 ppm. Generally, the inner surface of the containers that hold contaminated water are coated with the material and a reduction of suspended particles can be observed overnight [17, 29].

Current studies of bacteria adhesion using ions, such as calcium (Ca^{2+}), have suggested that by introducing monovalent and divalent ions to solutions containing cells that a increase in binding was observed. In studies on the adhesion of *Lactobacillus (L.) reuteri* DSM 12246, *L. plantarum* Q47, *L. rhamnosus* GG, and *L. johnsonii* NCC 533 to epithelial cells from mammals, significant increases in binding were observed with the addition of calcium ions [30]. In addition, the importance of Ca^{2+} on the stability of activated sludge has also been studied. When Ca^{2+} is removed from the sludge, flocculation decreases as does the filterability, while turbidity increases, indicating that the Ca^{2+} plays a fairly considerable role in the binding of the sludge contents [31].

1.4 Project Objectives

The main objective of the project documented in this thesis is to test the use of mucilage gum, extracted from the *Opuntia ficus-indica (O. ficus-indica)*, as a flocculating agent for developing countries. Because the cactus is commonly found throughout the world, it offers the potential of serving people in many countries without the risk of community opposition. Treatment on two types of contaminants were tested separately and evaluated in surrogate hard and soft waters that mimic natural water sources.

1.4.1 Sediment Reduction from Ion-Rich Water Supplies

The first goal of this project was to evaluate the effects that ion-rich water has on the mucilage and its ability to reduce turbidity, suspended solids, in water. Kaolin clay was used as a representation of sediments and was suspended in deionized (DI), hard

(HW) and soft (SW) waters for treatment with two fractions of mucilage gum. The resulting flocculation was evaluated.

1.4.2 Bacteria Reduction from Ion-Rich Water Supplies

The second goal of the project was to study, and compare, the ability of mucilage to remove gram-positive and gram-negative bacteria from ion rich water. The ion-concentration, mucilage type and flocculation was studied and compared briefly to the removal ability witnessed with sediments. Gram-positive *Bacillus cereus (B. cereus)* and gram-negative *Escherichia coli (E. coli) HB101*, that has been transformed to contain a plasmid with a gene encoding the green florescence protein (GFP), were used to study removal and possibly also serve as surrogates for similar types and sizes of bacteria.

1.5 The *Opuntia ficus-indica* Cactus

1.5.1 Prevalence and Characterization

The *O. ficus-indica*, also known as the Nopal or Prickly Pear, is a cactus that is found in most areas of the globe that offer dry arid climates. Although native to Mexico, the *O. ficus-indica* has spread throughout the world and can currently be found growing in many regions including South America, North America, India, Africa and many of the countries surrounding the Mediterranean Sea [32]. Not only can the cactus be found all over the world, but it also grows at an extremely fast rate. A case study on a Nopal farm just outside of Mexico City reported that the fruit from the cactus could be harvested in as little as two to three months after the cactus is planted. In addition, this study also has

reported vegetation production (dry weight) from the plant to be as much as 20,000-50,000 kg/ha/yr (1ha = 1 hectare = 10,000m^2) and fruit production of 8,000-12,000kg/ha/yr [32].

Scientists predict that the pads (nopalitos) and fruit (tunas) were consumed as a food source by the community dating as far back as 9,000 – 12,000 years ago [33]. In addition to use as a food source, the cactus has also served many other uses in the community and has gained attention from the scientific world. The fruit from the cactus is often used to create dyes and indigenous knowledge indicates that the pads have been used as a water purification method. Past research and knowledge has also suggested that the pads could potentially serve a purpose in the medical field [33, 34].

Since the cactus grows abundantly in many areas and is currently used as a food source, the pads and fruit can be found at many local markets and is generally inexpensive. Figure 2 shows the tunas and pads from the *O. ficus-indica* for sale at the Red Barn Flea Market in Bradenton, Florida. The fruits are sold here three for a dollar and the pads, which contain mucilage, are sold five for a dollar.

Figure 2. Tunas and pads from the *Opuntia ficus-indica* cactus for sale at the Red Barn Flea Market in Bradenton, FL, USA.

The *Opuntia* genus in the cacti family has been known for its large production of mucilage, a complex used by the cactus to store water. Mucilage serves many purposes in the food industry. It has been used as an addition to house paint and is the product of the cactus that is used by some communities as a water purification method [34].

As shown in Figure 3, a method has been developed to extract two different fractions of mucilage gum, a Non-Gelling (NE) and Gelling (GE) Extract, from the *O. ficus-indica* [34].

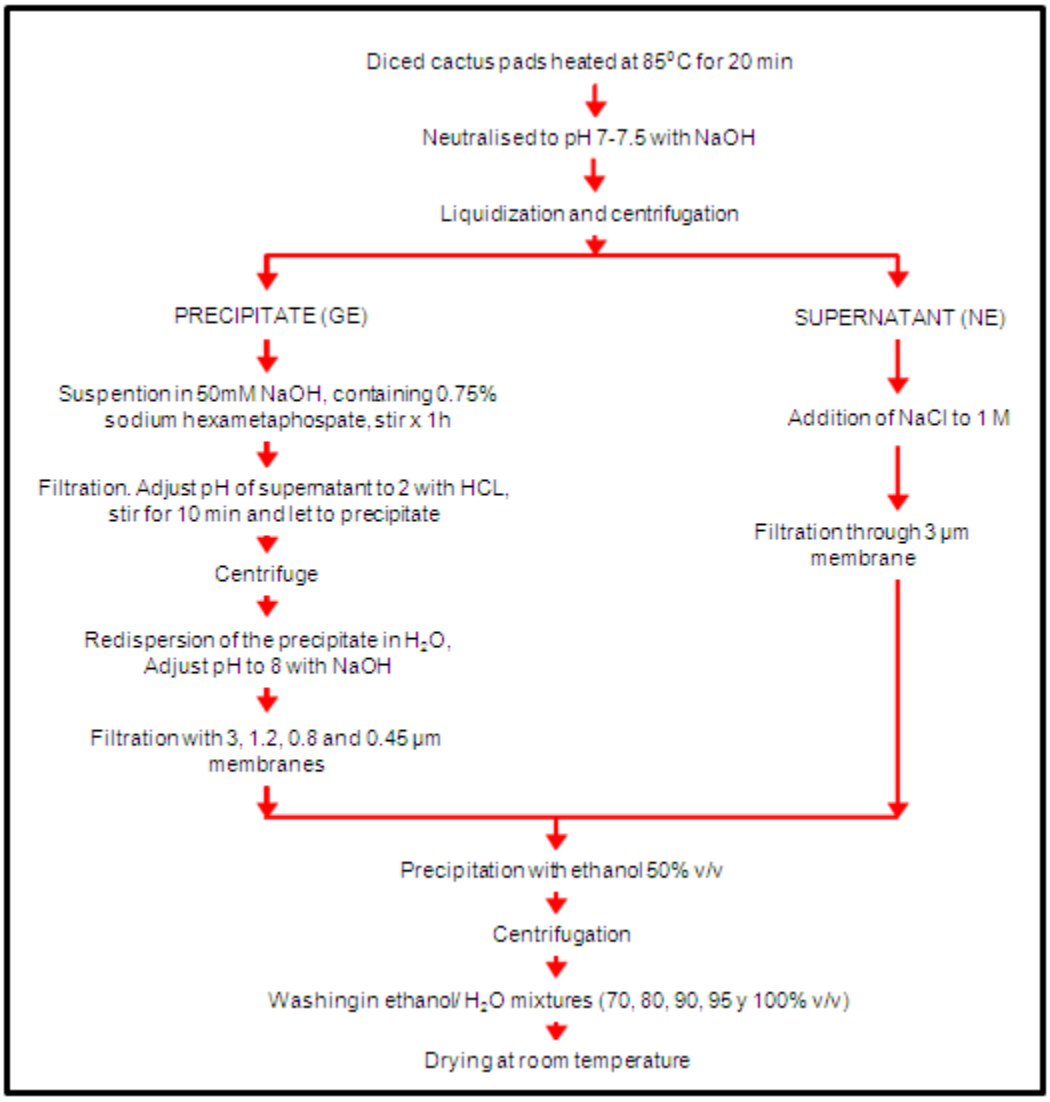

Figure 3. Outline proposed by F. Goycoolea and A. Cárdenas for extracting Non-Gelling and Gelling Extract from the *O. ficus-indica*. [34]

The chemical contents of the mucilage gum from the *O. ficus-indica* has been studied in the past and, although there have been some discrepancies with the reported contents, several main components have been identified. This mucilage is thought to

consist of approximately 55 sugars, mainly arabinose, galactose, rhamnose, xylose and uronic acids, the percentage of which varies with mucilage type [34-36]. Studies with the addition of divalent cations, such as Mg^{2+} and Ca^{2+}, to mucilage have resulted in property changes, such as increases in viscosity [34, 36]. While NE is reported to display higher viscosities than GE without any additions, GE was shown to have a higher percentage of uronic acids, which is thought to provide the extract with stronger gelling properties that are witnessed in the presence of monovalent and divalent cations [34].

1.5.2 Past Studies of Contaminant Removal

Young *et al.* demonstrated the use of NE and GE mucilage fractions for removal of sediment in DI water and concluded that both mucilage fractions act faster in sediment removal than the controls containing no flocculating agent, and solutions treated with the commonly used Alum [37-39]. These tests also concluded that in order to obtain the same settling rate as the GE extract, 300 times as much Alum would need to be used. Residual turbidity was also evaluated in these tests and appeared to rise with increasing mucilage concentration. At very low concentrations of mucilage treatment, however, the residual turbidity was relatively low.

In the same study arsenic removal with GE was evaluated and suggested that the arsenic is some how transported to the top of the column by the mucilage resulting in a 33 – 45% removal rate [37-39]. Further evaluation regarding effectiveness of the mucilage to remove heavy metals is currently under investigation.

In this thesis the work done by Young *et al.* was expanded upon by the testing of the removal of the sediment kaolin from DI and ion-rich water. In addition, the spectrum of contaminants that are being studied was broadened to include bacteria.

1.6 Bacteria Studied

For this project, two bacteria types were evaluated for flocculation when treated with mucilage. Two non-pathogenic bacteria, *Bacillus cereus (B. cereus)*, a gram-positive bacterium, and *Escherichia coli (E. coli) HB101*, a gram-negative bacterium were utilized. Table 1 lists some characteristics of these bacteria.

Table 1. Characteristics of *Bacillus cereus* and *Escherichia coli* HB101.

	Bacillus cereus	***Escherichia coli***
Type	gram-positive	gram-negative
Size, Shape	1 x 3 μm, Rod	1 x 2 μm, Rod
Location	Soil	Mammal Feces
Spore-forming	YES	NO
Optimal Growth Conditions	Temperature: 35-37°C, Stirring: 200 rpm	Temperature:35°C, Stirring: 200 rpm
Pathogenic	NO	NO

These bacteria were chosen for testing because of their availability, ease of use in the laboratory setting and because of their locations. Both of these bacteria could be a

point of concern in drinking water contamination. *B. cereus*, commonly found in soil, could potentially be washed into the aquifers that supply water with rain, and *E. coli* can easily enter water supplies if safe sanitation is not practiced [40, 41]. As previously discussed, drinking water is monitored for *E. coli* as part of the safe drinking water regulations, and is used as an indicator organism to detect fecal contamination. These bacteria could possibly also act as surrogates for other bacteria contaminants of similar size and characteristics if the flocculation affects of the mucilage is a result of surface interactions. Since this is commonly the case with flocculants, the surface characteristics of gram-positive and gram-negative bacteria was studied and compared.

Figure 4 shows a schematic of the cell wall of gram-positive bacteria. The outer layer of the cell wall consists of peptidoglycan, a combination of polysaccharides and amino acids, and contains teichoic acids, another kind of polysaccharide that links to lipids and maintain the attachment with the cell membrane [42].

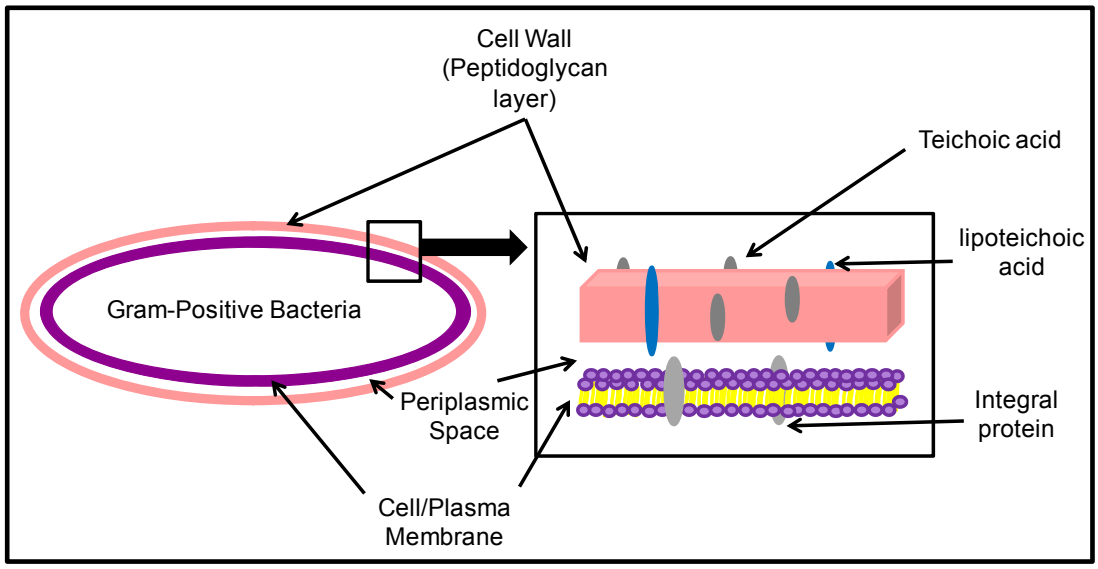

Figure 4. Cell wall of gram-positive bacteria. The outer layer consists of a thick peptidoglycan layer which is exposed to the external environment.

In Figure 5, components of the gram-negative bacteria that are different than those found in gram-positive bacteria are shown in red while those that are relatively the same are shown in black.

Gram-negative walls also have a layer of peptidoglycan, however, it is much thinner than those found in gram-positive walls and it is not directly exposed to the environment outside of the cell. Gram-negative bacteria have an additional bilayer that consists of phospholipids, channel proteins and an outer layer that consists of lipids attached to sugars, called lipopolysacharides or LPS.

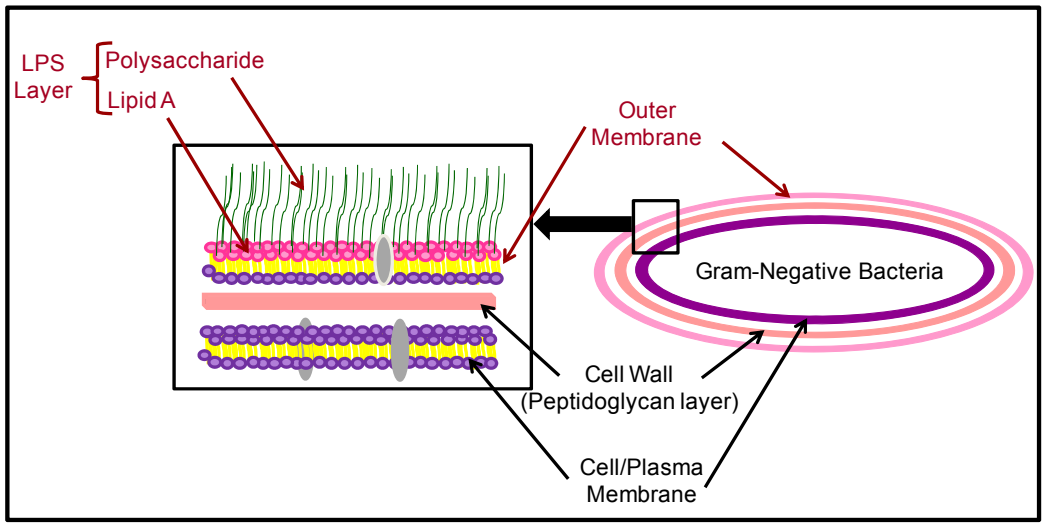

Figure 5. Cell wall of gram-negative bacteria. The outer cell wall consists of a lipopolysacharides layer, which is exposed to the external environment.

Lipid A, which is attached to the polysaccharide, is the cause of illness when gram-negative bacteria are killed while inside of the body. This extra layer in the cell wall also makes gram-negative bacteria more difficult to kill with antibiotic treatment, as it could potentially immobilize the movement of drug into the cell [43-45].

The differences shown in the outer portion of the bacteria cell walls could potentially be responsible for differences in the ability of mucilage to remove the bacteria.

Chapter Two: Experimental Procedures

2.1 Mucilage Extraction and Characterization

Mucilage was extracted for use in this project using a method very similar to that outlined by F. Goycoolea and A. Cárdenas discussed in section 1.5.1 of this thesis [34]. *O. ficus-indica* pads were originally purchased from Living Stones Nursery in Tucson, Arizona then replanted and grown in Tampa, Florida. Figure 6 shows a detailed outline of the method used for this purification and the alterations made to the previously discussed methodology.

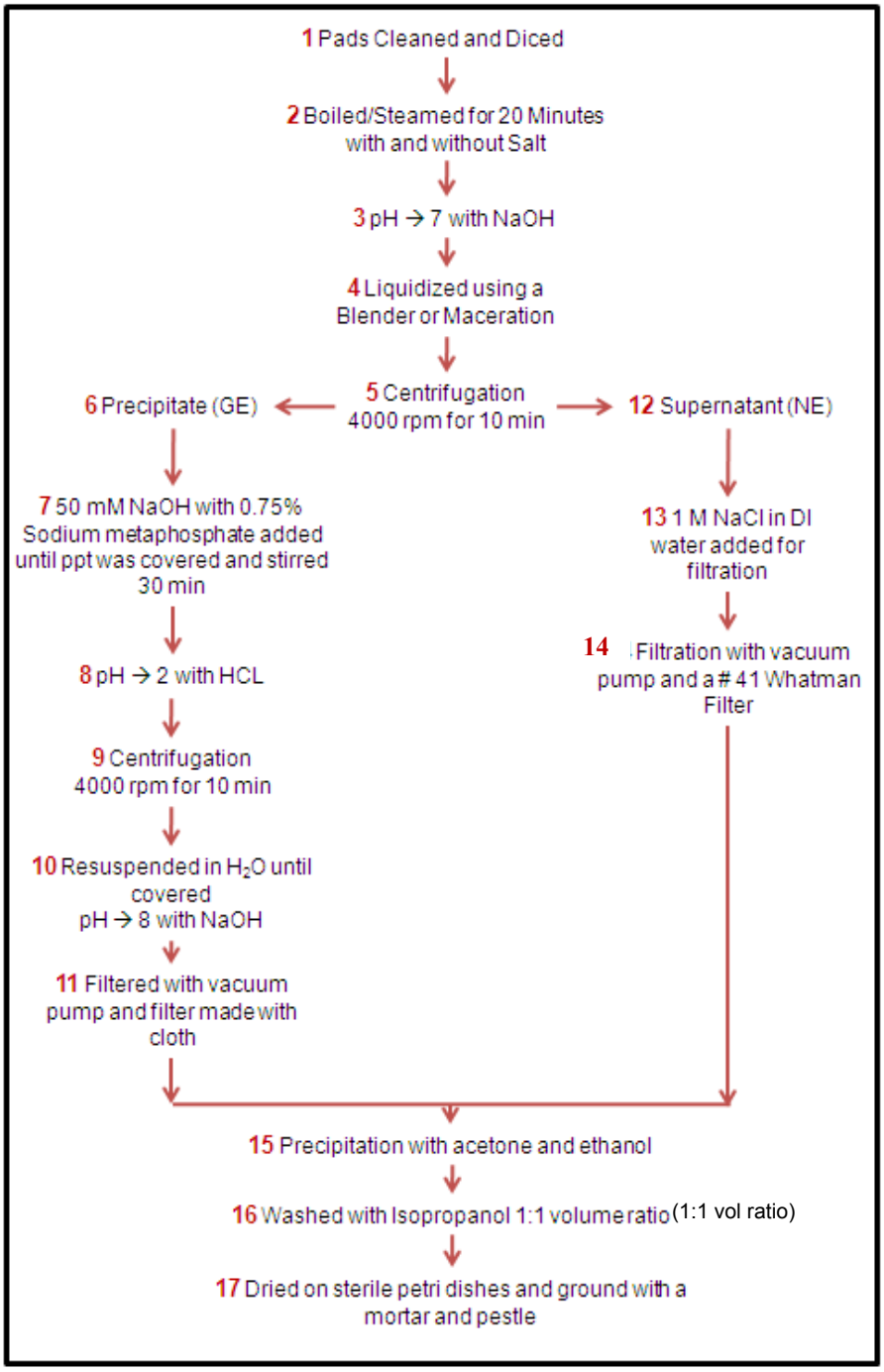

Figure 6. Detailed outline of extraction method for mucilage evaluated. Main differences from the protocol described by Goycoolea *et al.* include heating method, liquidization method, filter size, and precipitation and washing chemicals.

A total of four pads and four different heating/liquidization methods were used to determine the method that would produce the highest yield percent. Table 2 outlines the pads by number, the methodology used, and their mass both before and after cleaning.

Table 2. Pad heating/liquidization methods and initial mass.

PAD	Heating and Maceration Method	Initial Mass (g)	Mass after Cleaning (g)
1	Boiled with Salt and Blended	346.2	335.9
2&3	Steamed and Blended with Water	543.5	534.4
4 -1	Boiled and Macerated	439.5	438.5
4 - 2	Boiled and Blended		
Total		1329.2	1308.8

Aside from the heating and liquidization method, all pads follow the outline shown in Figure 6 to provide GE and NE mucilage fractions.

The final step of the extraction involves grinding the dried mucilage with a mortar and pestle to provide a fine powder for its use in mucilage experiments. Prior to experimentation this powder was added to DI water, with a final concentration of 500 ppm, and mixed with a tissue grinder to produce an even suspension. This solution was then diluted accordingly for experimentation and was stored in the refrigerator sealed with wax film until it was used.

Once the extraction was complete, the mucilage was tested with kaolin and compared to a control without mucilage, in order to indicate whether or not the mucilage could produce increased settling as observed in the work done by Young *et al.*[37-39].

2.2 Preparation of Synthetic Water and Calcium Chloride Solutions

In an attempt to closely evaluate how mucilage would react when used as a purification method in the ion-rich environment that is commonly found in real bodies of water, surrogate ion-rich waters were prepared for kaolin and bacteria suspensions. Smith, Davison and Hamilton-Taylor offer recipes for three major freshwater surrogates including hard, soft and acidic water [46]. For the purposes of this work, hard water (HW) and soft water (SW) were prepared and utilized in comparison with deionized water (DI). The water preparations presented by these authors are based off of samples taken from Esthwaite Water Lake (SW model) and Rostherne Mere Lake(HW model) both found in England.

The preparation of these waters include the mixing of several solutions of salts dissolved in DI water, resulting in high ion-concentration water (HW) and low ion-concentration water (SW). The materials used during the preparation of the surrogate waters can be found listed in Table 3.

Table 3. Synthetic water materials.

Name	Manufacturer	Catalog #	Lot #	Description
Calcium Nitrate Tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$	MP Biomedicals	193800	6343E	100g, Ultra Pure
Potassium Phosphate	Acros	205920025	A0240951	2.5kg, 99+%, Ultra Pure
Sodium Sulfate Anhydrous Sodium Sulfate (Na_2SO_4)	Acros	35425-0010	B0123472	1kg, Granular
Potassium Bicarbonate	MP Biomedicals	152557	5477H	1000g, Reagent Grade
Calcium Chloride Hexahydrate ($CaCl_2 \cdot 6H_2O$)	Acros	389250010	A0231511	1kg, Extra Pure
Sodium Bicarbonate ($NaHCO_3$)	Fisher Scientific	S233-500	073814	500g
Magnesium Chloride Hexahydrate ($Cl_2Mg \cdot 6H_2O$)	Acros	197530010	A0245268	1kg, 99%
Magnesium Sulfate Heptahydrate	Acros	423905000	A0237024	500g, 98+%
Calcium Chloride ($CaCl_2$)	MP Biomedicals	153502	9645E	500g
500 mL Filter	Fisher Scientific	S66128	638611	0.20 µm Cellulose Nitrate membrane, sterile
50 mL Filter	Corning	430320	30208505	0.22 µm Cellulose Acetate membrane, Sterile
Compressed Nitrogen	Airgas	NI HP300	-	Minimum Purity 99.995%, gas cylinder
Compressed Air	Airgas	AI UZ300CT	-	Ultra High Purity, Certified
Compressed Carbon Dioxide	Airgas	CD R300	-	Research Grade
Deionized Water (DI)	Millipore System	A10	F4BN74788	DI Feed Water, Ion-Exchange, Activated Carbon

The stock solutions prepared are outlined in Table 4 along with the amount of chemical added, the final concentration and the final ion-concentrations. The mixing concentrations and final pH is shown in Table 5.

Table 4. Concentrations of synthetic water stock solutions.

Chemical	**Amount (g)**	**Conc (M)**	**SS Ion Conc (g/L)**	
			C^{z+}	A^{z-}
Soft Water (SW)				
Stock Solution 1: 1 L prepared, 1/1000 dilution factor				
Magnesium Chloride Hexahydrate	12.17	0.060	1.455	4.244
Calcium Chloride Hexahydrate	17.49	0.080	3.200	5.661
Clacium Nitrate Tetrahydrate	3.541	0.015	0.601	1.859
Stock Solution 2: 5 L prepared, 1/1.1 dilution factor				
Calcium Oxide	0.094	0.0003	0.014	0.001
Stock Solution 3: 1 L prepared, 1/1000 dilution factor				
Sodium Sulfate	16.34	0.115	5.290	11.05
Potassium Bicarbonate	2.508	0.025	0.999	1.528
Sodium Bicarbonate	1.681	0.020	0.460	1.220
Hard Water (HW)				
Stock Solution 1: 1 L prepared, 1/100 dilution factor				
Calcium Chloride Hexahydrate	7.497	0.034	1.372	2.426
Calcium Nitrate Tetrahydrate	1.189	0.005	0.202	0.624
Stock Solution 2: 5 L prepared, 1/1.1 dilution factor				
Calcium Oxide	0.458	0.002	0.066	0.005

Table 4. (Continued).

Stock Solution 3: 1 L prepared, 1/100 dilution factor				
Sodium Sulfate	2.820	0.020	0.913	1.907
Potassium Bicarbonate	0.763	0.008	0.304	0.465
Sodium Bicarbonate	2.265	0.027	0.620	1.645
Potassium Phosphate	0.47	0.003	0.108	0.262
Stock Solution 4: 1 L prepared, 1/100 dilution factor				
Magnesium Sulfate	10.05	0.041	0.991	3.915

Stock solutions 1, 3, and 4 (for HW only) require the salts to be added to DI water using a stir bar. Smith *et al.* advises that, if kept in a cool shaded place, these solutions will not expire and can be used for future water preparations. Because Calcium Oxide (CaO) is harder to dissolve than the other salts and requires the water to be stripped of carbon dioxide (CO_2), the water was bubbled with compressed nitrogen (N_2) gas for one hour using a nitrogen blanket created with a grocery bag. The bubbling was achieved using standard tubing with holes punctured approximately six inches up from the bottom using a screw. This tube was submerged in the water while it was stirred with a stir bar allowing the bubbles to fill the container. The appropriate amount of CaO was then added to the water and was then bubbled with N_2 for another hour until the CaO was completely dissolved. The solution was then bubbled for 10 minutes, decreasing the pH and preventing the formation of any unwanted precipitates as outlined by Smith *et al.*. These stock solutions were then mixed according to Table 5, which also indicates the final pH of the solution after bubbling with compressed air.

Table 5. Characteristics of stock solutions for mixing 5 L of soft water (SW) and hard water (HW).

	SS 1 (mL)	SS 2 (mL)	SS 3 (mL)	SS 4 (mL)	DI Water (mL)	Total Vol (mL)	Final pH
Soft Water	5	4545	5	--	445	5000	7.43
Hard Water	50	4545	50	50	305	5000	8.34

Upon completion, the water was stored on the bench top at room temperature and, prior to use, 500 mL of the waters were filtered using a vacuum pump and bottle top filters with 0.20 μm membranes, sterilizing the water for use.

The SW and HW were also used to produce calcium chloride ($CaCl_2$) solutions that were tested with kaolin and used in bacteria tests. Stock solutions of $CaCl_2$ were prepared in both HW and SW and were then filtered using a vacuum pump and bottle top filters with 0.22 μm membranes, sterilizing the solution for later use. The solution was then diluted accordingly for each experiment.

2.3 Column Tests and Flocculation Evaluation

Column tests were used to evaluate the flocculation and removal of sediment and bacteria suspended in water. The column array was set up using 10 mL Fisherbrand pipettes which were broken between the -1 and -2 mL markers. The bottom of the pipettes were closed using parafilm and the column was taped together and hung in front of a dark sheet of paper so that the flocculation could be easily seen. Because of the flocculating abilities of the diatonic ion Ca^{2+}, and its affects on the mucilage, $CaCl_2$

solutions of various concentrations were prepared and tested with kaolin and bacteria. For both kaolin and bacteria tests the column contents were added together in 10 mL centrifuge tubes, then vortexed before being poured into the column array. Table 6 lists the materials utilized during column test experiments.

Table 6. Materials used for column tests.

Name	Manufacturer	Catalog #	Lot #	Description
Tissue Grinder	Fisher Scientific	08-414-10B	-	Medium grind, 7 mL
Kaolin	Fisher Scientific	S71954	200009608	500g
Aluminum Sulfate $(Al_2(SO_4)_3)\cdot 18H_2O$	Fisher Scientific	S70495	200305504	500g
Phosphate Buffer Saline (PBS)	Sigma	P-3813	047K8207	0.01 M, pH 7.4

Kaolin particle size was unknown from the manufacturer and was determined using Dynamic Light Scattering (DLS) and Transition Electron Microscopy (TEM). Kaolin suspensions with final concentrations of 50 g/L were used in the column tests evaluated in this thesis and mimic the possible mud-like conditions in water storage units. At this concentration, the kaolin solutions formed a clear interface while settling, allowing its height to be read every minute for sixty minutes. The settling rates were determined and plots were generated for comparison between different waters, mucilage type and concentrations. Tests were run with varying mucilage concentration in SW, HW and DI waters. In addition, the use of the common flocculant Alum was also evaluated for comparison.

Bacteria tests were evaluated with high bacteria concentrations of 10^8 cells/mL, in order to make the effect of mucilage addition easier to see. Unlike kaolin columns, bacteria columns do not form a clear interface, but rather small flocs, which can be seen forming and falling in the otherwise turbid water. The time from which these flocs began to form to the time that they completed their descent was recorded and compared for various treatments conditions.

All results shown are the average and standard deviation of at least three settling tests and all statistics were calculated using Origin 8.

2.4 Bacteria Storage, Growth and Evaluation

B. cereus and *E. coli* were grown and stored on glass beads with a mixture of LB media (including ampicillin and arabinose concentrations of 5 mg/mL and 100 μg/mL for *E. coli*) and glycerol at -80˚C. When ready for use, cultures were started from these beads on LB media agar plates. The plates were grown for 12 hours at 35˚C and were then stored in the refrigerator for future use. One colony was then selected from the plate using a sterile loop and immerged in a 5 mL LB media tube that was incubated at 37˚C shaking at 200 rpm for at least 9 hours. After 9 hours a 75 mL LB broth culture was inoculated from the 5 mL culture using a 1:1000 dilution. This culture was incubated over night at 35˚C shaking at 200 rpm and removed for use approximately 15 hours later at the same optical density reading for every experiment providing a stock solution concentration of 10^9 cells/mL. The bacteria were then washed once in PBS using a centrifuge running at 4,000 rpm for 5 min and a mini vortexer. Once washed, the final

stock solution cell count was determined using a direct counting chamber from Nexcelom Bioscience. Table 7 lists the materials that were used during bacteria growth and evaluation.

Table 7. Materials used in bacteria growth and evaluation.

Name	**Manufacturer**			**Catalog #**
Bacillus cereus	Frozen stock already in lab			ATCC 10876
Escherichia coli pGLO Bacterial Transformation Kit	BioRad	166-0003EDU	-	ATCC 33694
Yeast Extract	Fisher Scientific	BP1422-500	076094	500g
Tryptone	Acros	61184-5000	B0124145	500g
Sodium Chloride (NaCl)	Acros	42429-5000	B0113819	99+%
Agar	MP Biomedicals	100262	8388F	USP Grade, 80-100 mesh
Ampicillin Sodium Salt ($C_{16}H_{18}N_3O_4SNa$)	MP Biomedicals	194526	R21558	Crystalline, Cell Culture Reagent
L-(+)-Arabinose (C_5HO_6)	MP Biomedicals	100706	8590J	Crystalline, Purity: >98%
Petri-dishes	Acros	0875798	-	100x15mm
Orbital Incubator Shaker	Amerex	Gyromax 727	-	20-420 rpm Ambient Temp +5-80°C
Cellometer Cell Counting Chamber	Nexcelom Bioscience	CP2-002	-	Plastic Disposable Counting Grid

In bacteria tests where removal percentage was evaluated, plate counts were performed according to standard microbiology procedures [43-45]. One mL of solution was taken from the top of the column and dilutions of this sample were plated and the colonies counted 24 hours later. This count was subtracted from the initial count and divided by the initial count to provide the percentage of the bacteria that were removed from the solution.

The *E. coli* HB101 used in this study was transformed into *E. coli* GFP using a pGLO transformation kit purchased from Biorad. By heat shocking the bacteria with the pGLO plasmid present, the PGLO enters the cell wall resulting in *E. coli* cells that exhibit green fluorescence under UV light. In order to grow the transformed cells, the sugar arabinose and antibiotic ampicillin is added to the LB media or agar that is used. With final concentrations of 5 mg/mL arabinose the bacteria continued to produce the pGLO when multiplying and with the addition of 100 μm/mL of ampicillin, bacteria that did not contain the pGLO plasmid were eradicated from the media by the antibiotic.

2.5 Imaging Techniques

A combination of microscopy techniques, including Transition Electron Microscopy (TEM), Atomic Force Microscopy (AFM) and Optical Microscopy (OM), were utilized in the evaluation of the mucilage and floc formation of the particles evaluated. The equipment and materials used in sample preparation are listed in Table 8.

Table 8. Materials and equipment used for imaging.

Name	Manufacturer	Catalog #	Lot #	Description
Optical Microscope	Leitz Diaplan Wetzlar	-	-	10, 40 and 100 objectives
Camera for Microscope	Diagnostic Instruments Inc.	Model 15.2 64 Mp Shifting Pixel Serial # 241632		
Microscope Imaging Software	Diagnostic Instruments Inc.	SPOT version 4.5		
Microslides	Corning	2948-75 x 25	18907003	Single frosted, precleaned 75x75mm
Formvar/Carbon 150 Mesh Copper Grids	Electron Microscopy Sciences	FCF150-Cu-50	-	Formvar coated grid, 150 mesh
Transition Electron Microscope (TEM)	FEI Company	Morgagni 268 D	-	Resolution: 35-280000x High Voltage Range 40 to 100 kV
Mica	Axim Mica	ASTM D-351-97	-	Scratch Free, V-1 quality
Aluminum Cantilevers	Budget Sensors	TAP300Al	-	Al reflex coating, 30 nm thick, Resonance Frequency-300 kHz, Force Constant – 40N/m
Atomic Force Microscope (AFM)	PSIA	XE-100	-	Research-Grade AFM; Max scan range

The topography of the mucilage stock solution (500 ppm) was evaluated using the AFM. Scans of the mucilage were obtained using aluminum TAP300Al cantilevers in tapping mode on a fresh cleaved mica surface.

Internal mucilage structure and samples from taken from the top of kaolin columns were evaluated using a TEM. A 20 μL sample of the solution was deposited on a copper grid and left to adsorb for five minutes. The remaining liquid was then removed and the grid left to dry for approximately 1 hour prior to imaging.

Kaolin was removed from the column at the interface between the settled flocs and the water using a glass pipette and bulb and imaged on the optical microscope. Flocs were extracted from the bottom of the columns containing bacteria, using a small valve, and were also imaged by optical microscopy. For both kaolin and bacteria approximately 7 μL of sample was deposited on a glass microscope slide and covered with a glass coverslip for imaging. *E. coli* images were taken on the microscope using a GFP tube filter to produce florescent images.

Chapter Three: Results and Discussion

3.1 Mucilage Extraction and Evaluation

As discussed in section 2.2, four pads were processed providing a Gelling (GE) and Non-Gelling (NE) Extract for testing. Figures 7 and 8 exhibit images taken during the extraction process. The letters located in the upper left hand corners of the pictures correspond with the steps described on the flow chart provided in Figure 6.

Image A corresponds with step five and shows the precipitant that was used for the GE, left, and the supernatant which will produce the NE, right (step 5 in Figure 6). Image B demonstrates the filters, made by cutting circular shapes out of fabric, used during the precipitate filtration. Image C shows the precipitate before, during and after filtration and image D shows the supernatant as it is emerging from the filter funnel (steps 11 and 14 in Figure 6).

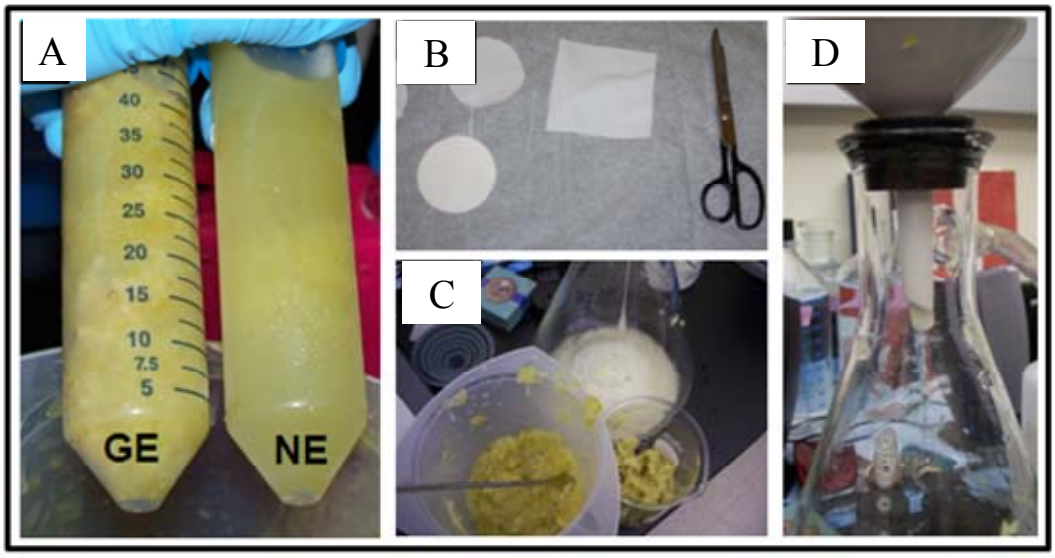

Figure 7. Images of extraction centrifugation and vacuum filtration. Image A shows the precipitate (produces GE) and supernatant (produces NE) obtained from centrifugation step of extraction method. B demonstrates the fabric filters that were cut for use in filtering the precipitate as shown in image C. Image D shows the supernatant as it emerges from the filter funnel.

Figure 8 shows images of the latter part of the extraction method where acetone and ethanol (volume ratio 1:1) were added to both mucilage fractions and left to precipitate (step 15 in Figure 6). Images A and B show the mucilage being drawn out of the solution as the water is evaporated. The mucilage was left to sit in this solution for two days before it was removed and washed with isopropanol. Image C shows the mucilage spread out on Petri dishes while being dried. The image on the bottom right (D)

shows the final powder state of the mucilage once it has been dried and ground with a mortar and pestle (step 17 in Figure 6). All steps shown were carried out in a fume hood.

Figure 8. Images of extraction precipitation, drying and resulting mucilage. Image A and B shows the mucilage being drawn out of solution as the water is evaporated using ethanol and acetone. Image C shows the washed mucilage spread on Petri dishes to dry and D shows the final product of the extraction.

Table 9 presents the results from this extraction including the mass of both GE and NE extracted as well as the percent yield, which represents the mass of the dried mucilage over the initial pad mass.

Table 9. Summary of Gelling Extract (GE) and Non-Gelling Extract (NE) extraction.

PAD	NE Extracted (g)	% Yield	GE Extracted (g)	% Yield
1	1.3835	0.40	N/A	N/A
2&3	2.7488	0.51	0.801	0.15
4-1	3.1976	1.46	N/A	N/A
4-2	2.2384	1.02	N/A	N/A
Total	9.5683	0.73	0.801	0.0612

In some instances, the GE amount obtained was small and during the evaporation step of the extraction procedure it was unintentionally dried completely and was therefore discarded as shown by N/A in the Table 9.

Although the mass of extracted mucilage appears to be relatively low, the amount of mucilage that was obtained has the potential of treating a large amount of water. The percent yield presented is also low which is mostly because the pad is composed of a large percentage of water and other material that is removed during the extraction process. As seen later in this document, the amount of mucilage preferred for contaminant removal is approximately 2 ppm (2mg/L), which indicates that an extracted weight of 1 g is capable of cleaning up to 500 L of water.

Figure 9 shows the removal rates of kaolin (50g/L) when treated with the mucilage obtained from the extraction with a final concentration of 2 ppm.

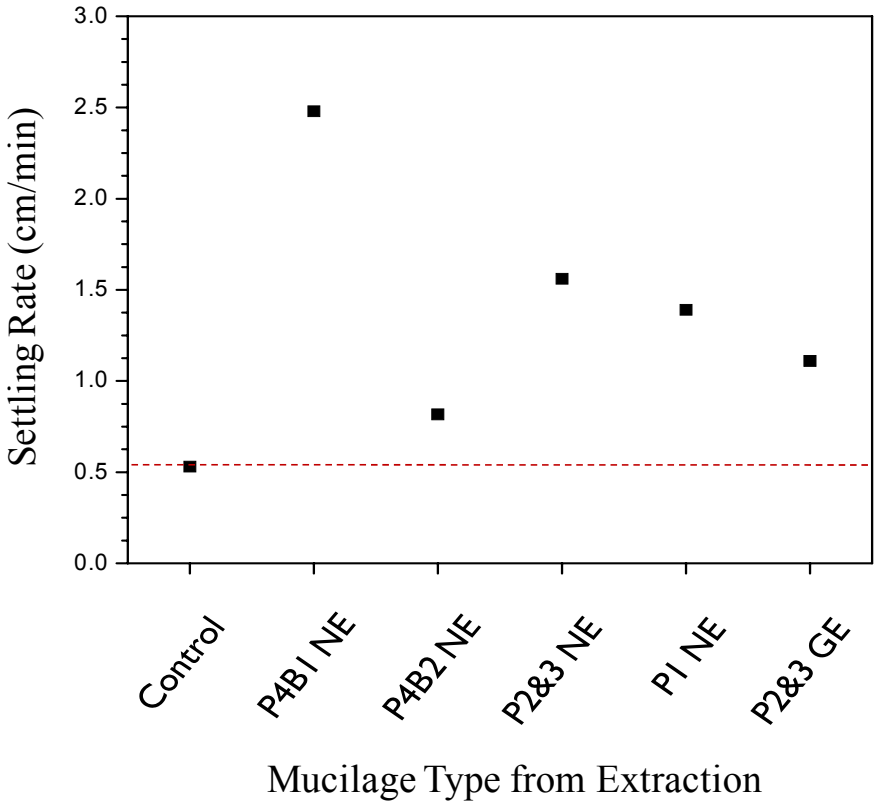

Figure 9. New mucilage tests on kaolin suspended in DI water (50g/L). Settling rates suggest that all mucilage induces settling faster than no treatment.

From Figure 9 it is seen that all of the mucilage obtained from the extraction induced higher settling rates in kaolin than the untreated control. The differences in settling rates could potentially be contributed to the purity of the extraction.

For comparison purposes, it was desired to test removal with GE and NE mucilage fractions obtained from the same pad and extraction method. Therefore the GE

and NE obtained from Pads 2&3, the only method that resulted in both GE and NE, was used for evaluation in all experiments.

In an attempt to better understand the different properties observed between the GE and NE, images were obtained of the stock solution using both the TEM and AFM.

Figure 10 shows the images taken with the TEM of the GE (A) and NE (B) with a magnification of 28,000x. The image of the GE displays an orderly chain-like structure with almost the same angle of orientation. Conversely, NE images show a denser net-like structure with cell sizes of approximately 200 nm.

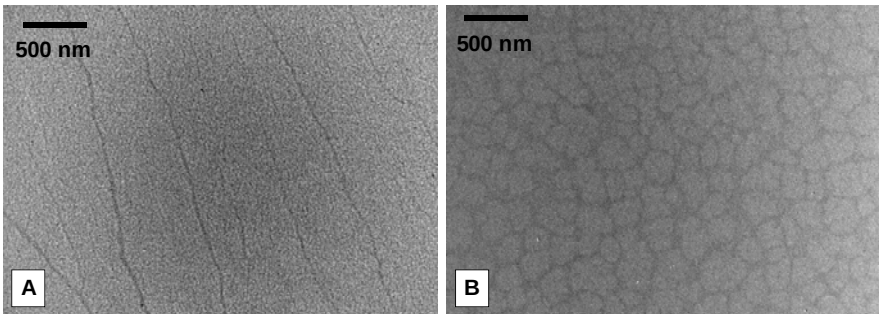

Figure 10. TEM images of the stock solution of A) GE and B) NE. GE displays an orderly structure with nearly the same angle of orientation while NE shows a much denser net-like structure. Samples extracted from 500 ppm stock solutions of mucilages and deposited on copper grids. The solution was allowed to sit for five minutes before the remaining water was removed and the grid left to dry for approximately one hour prior to imaging.

Figure 11 displays AFM scans of both GE (A) and NE (B) with imaged areas of 2 x 2-µm x-y and 0.5 x 0.5 µm x-y. The AFM pictures shown here are consistent with the internal differences shown in the images obtained from the TEM. Maximum heights of 2.01 nm (GE scans) and 1.42 nm (NE scans) were recorded by the AFM.

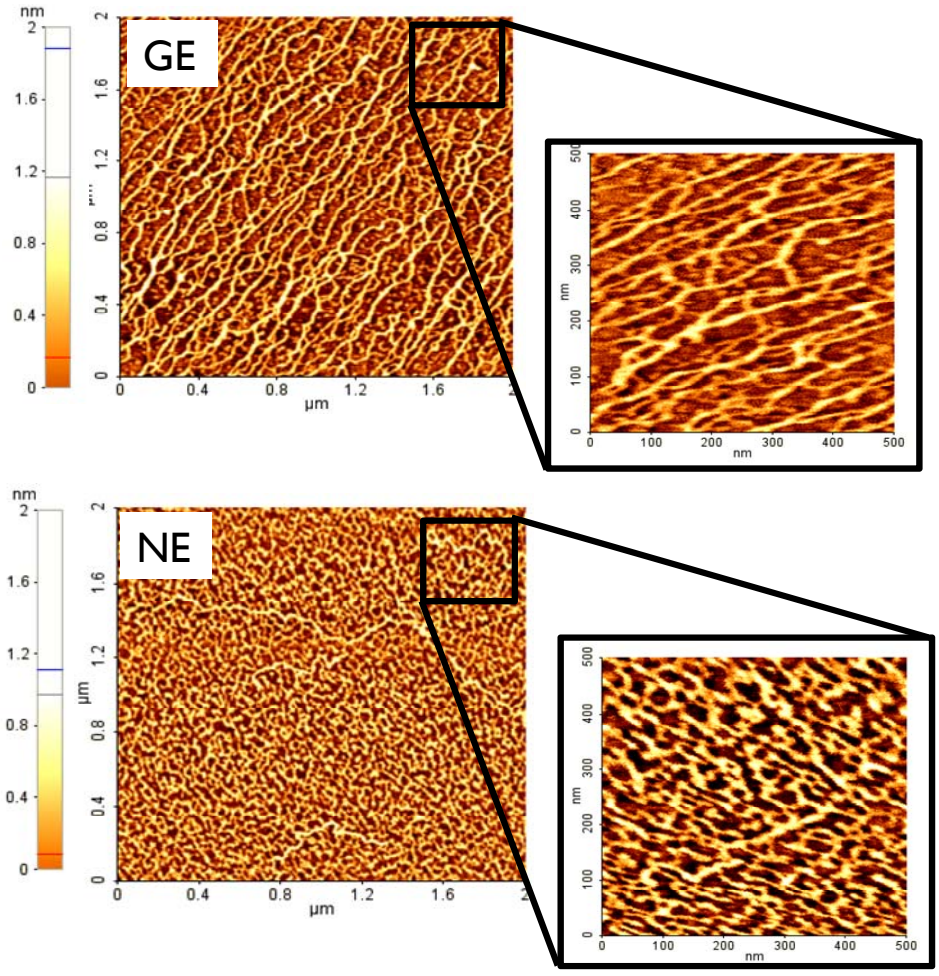

Figure 11. AFM scans of GE (A) and NE (B) stock solutions with imaged areas of 2 x 2-µm x-y. Zoom-in images provide a 0.5 x 0.5 µm x-y scan that is not necessarily from the indicated area of the larger scan.

The structural differences observed here could potentially be the cause of behavioral differences that were seen in the remaining results found in this section.

3.2 Sediment Settling Tests

3.2.1 Kaolin Size Evaluation

Figure 12 shows the DLS output with a particle diameter determined to be 518 ± 30 nm. The intensity is determined to be 100 percent which indicates that there is a relatively small, if any, distribution of sizes in the kaolin suspension. The TEM image located in the upper right hand corner confirms this size.

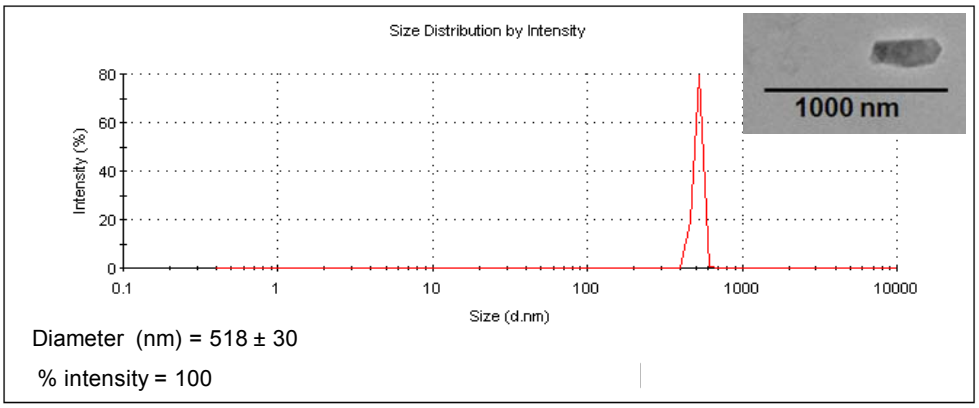

Figure 12. Kaolin particle size evaluation using DLS and TEM. Particle size was determined to be approximately 518±30 nm.

3.2.2 Flocculation with Gelling Extract, Non-Gelling Extract and $CaCl_2$

Experiments of settling using kaolin result in plots similar to the plot shown in Figure 13. The kaolin height is plotted as a function of time for several different mucilage concentrations. This plot was generated using the data collected from an experiment of kaolin suspended in DI water (50 g/L) that was treated with GE and is used here a representation of only one run of data and is shown primarily as an example.

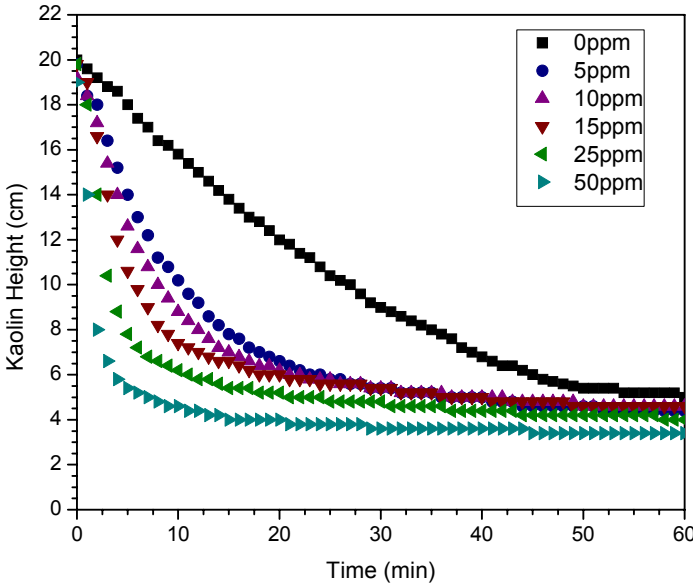

Figure 13. Full 60 minute plot of kaolin settling in DI water with GE.

Due to the dynamics of column tests, the kaolin reaches a point in the column where it slows its settling and begins to compress into the bottom of the column. From the plot shown in Figure 13, it is suggested that the mucilage columns settled faster as concentration increased. Figure 14 shows the same results as the above plot but here the

plots were truncated where the compression began leaving only a straight line. The dotted lines represent the data, while the red lines display the linear curve fit as calculated using Origin. The slopes of the lines represent the settling rate of the kaolin in cm/min, and are provided here for each concentration of GE.

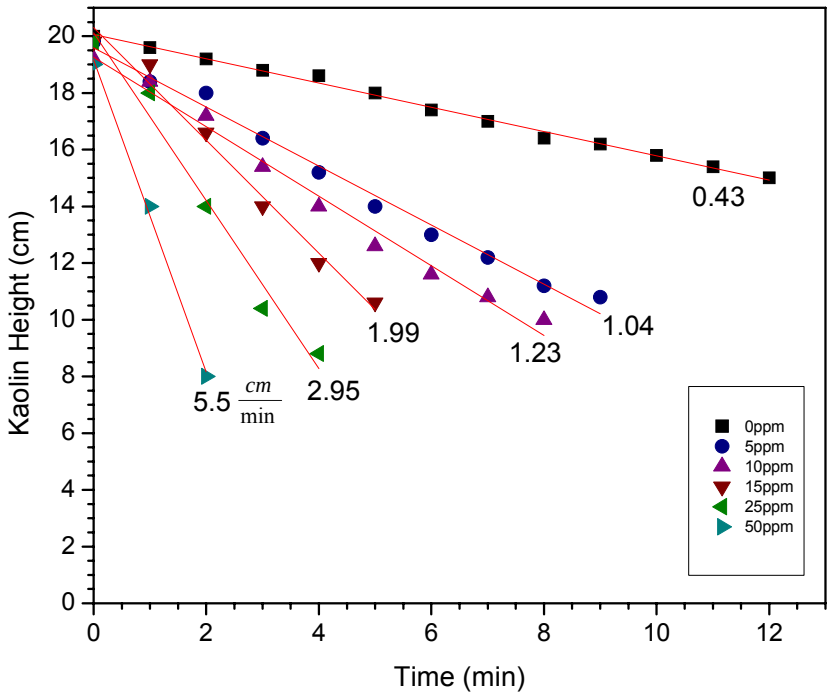

Figure 14. Truncated kaolin plot with linear curve fit slopes. Resulting slope represents the settling rate of the kaolin in cm/min.

All kaolin data was processed in this manner and the corresponding settling rates were plotted together in order to evaluate the consequence of different concentrations, water type and flocculants as seen in Figure 15. All standard deviations calculated are

comparisons of the settling rates determined according to the modeling described in Figure 14.

The NE and GE plots show the kaolin settling rates as a function of mucilage concentration ranges from 0 to 100 ppm in DI, SW and HW. The bottom graph shows the removal rate of kaolin suspended in HW when treated with the commercially used flocculant Alum. Alum was tested with kaolin suspended in HW (50g/L) because this is the water type that exhibited the best results with GE and NE. Three characteristics of the mucilage induced settling can be determined from the first two plots. First of all, regardless of the water type, the kaolin settles at the same rate of approximately 0.5 cm/min without the addition of mucilage. This is the first point on the plots, and indicates that any further differences in the settling are a result purely of the mucilage interaction with the kaolin clay and ions in the water.

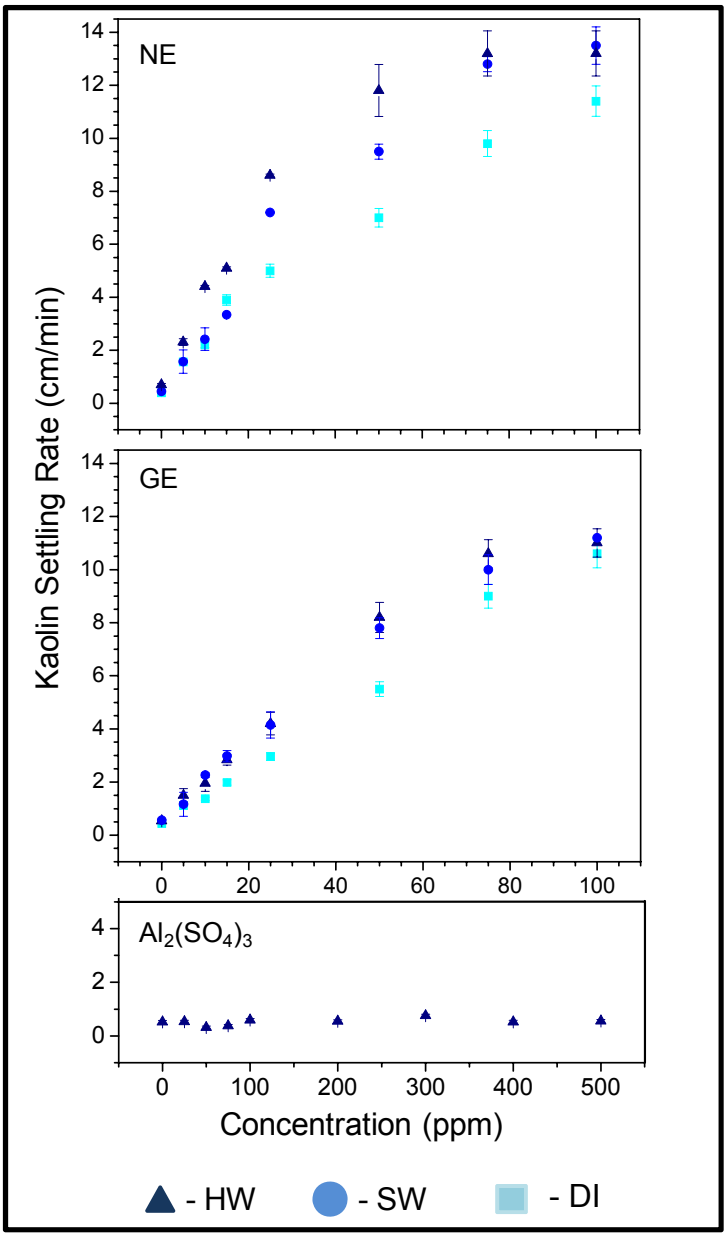

Figure 15. Kaolin sedimentation measurements with NE, GE and Alum. Top plot: Kaolin suspensions in hard water (HW), soft water (SW) and Deionized water (DI) treated with NE at a range of concentrations (50 g/L). Middle plot: Kaolin suspensions in hard HW, SW and DI water treated with GE at a range of concentrations. Bottom Plot: Kaolin suspension in HW treated with aluminum sulfate Alum.

Figure 15 shows the effect of ion-concentration in the water with NE, which is observed to significantly influence the kaolin settling rate. Columns containing HW exhibit settling rates higher than the SW, which in turn settled faster than the kaolin suspended in DI water. In columns treated with GE (Figure 15 middle) the same effect is observed concerning the ion-concentration of the water, however, here it is not as significant as in NE treated columns.

The settling rate of the kaolin is a function of the mucilage concentration, both NE and GE, and increases with concentration regardless of the water type. Initially the relationship between concentration and settling rate appears to be directly proportional, but as the concentration of mucilage increases, the settling rate eventually reaches a point where it does not react as dramatically to increases in mucilage concentration.

Finally, the NE has a more significant effect in removing the sediment kaolin from contaminated water than GE. In HW columns treated with 100 ppm NE, an average settling rate of 13.2 cm/min was achieved, while columns under the same conditions but treated with GE only reached an average settling rate of 11.0 cm/min. Columns containing SW and DI water display similar differences.

The variation in settling capabilities between the mucilage types observed here could possibly be attributed to the way that the different mucilage structures discussed in section 3.1 interact with both the kaolin and the ions in the water.

Figure 15 (bottom plot) also shows the settling rate of kaolin columns treated with $Al_2(SO_3)_4$. Kaolin suspended in HW was used to generate these results, as HW yielded the best results in columns treated with mucilage. Experimental results with $Al_2(SO_3)_4$

concentrations ranging from 0-500 ppm show little to no increase in settling rate when compared to the control, indicating that in the conditions provided mucilage is a more efficient and effective flocculating agent for sediment contaminated waters.

In columns treated with higher mucilage concentrations (approximately 15 – 100 ppm) textural changes of the kaolin were observed from the flocculation effects. The increased settling rates discussed above are a result of this flocculation. Figure 16 shows a photograph of this consistency difference. Column 1 is a control containing no mucilage, while columns 2, 3 and 4 are columns containing GE at concentrations of 15, 25 and 50 ppm. All columns shown are of kaolin suspensions in DI water, however, the same consistency changes were observed in columns of SW, HW and columns treated with NE.

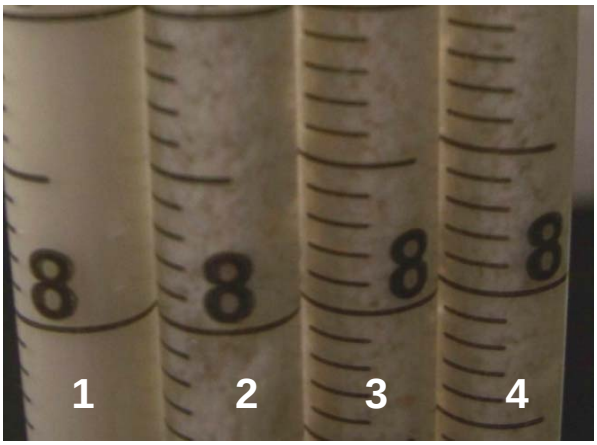

Figure 16. Kaolin flocs as seen in experimental columns. Column 1 presents a control column with kaolin suspended in DI water with no treatment. Columns 2, 3 and 4 are test columns containing kaolin suspended in DI water when treated with Gelling Extract (GE) concentrations of 15, 25 and 50 ppm. Flocculation can be observed in the columns treated with GE.

In an attempt to visualize the flocculation effects of the mucilage, TEM and optical microscopy images were prepared. Figure 17 illustrates microscope images of kaolin in HW both alone and treated with 50 ppm NE. Image A is an image of a sample taken from the liquid kaolin interface at the bottom of the control column. This column was not treated with any flocculating agent and the kaolin particles are seen to be freely floating in the solution. Image B shows the same liquid kaolin interface taken from a column that was treated with a final NE concentration of 50 ppm. Here, the flocs of kaolin can be seen confirming what was originally seen as flocculation.

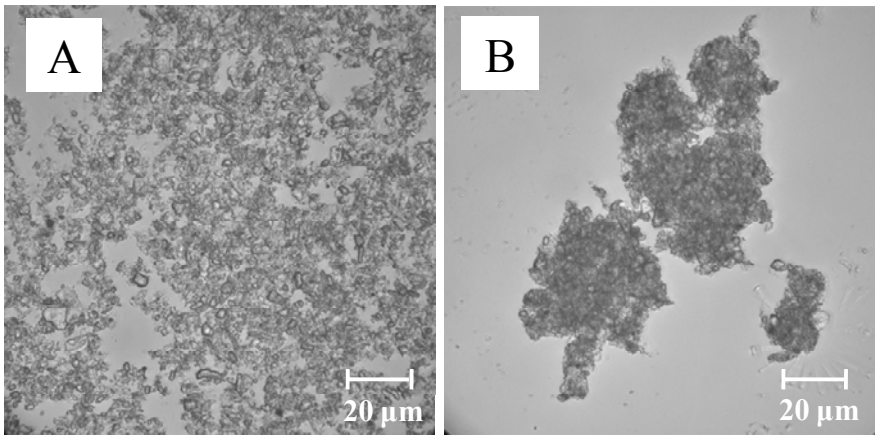

Figure 17. Microscope images of kaolin flocculation. Samples taken from columns containing kaolin suspended in hard water (HW) untreated (A) and with the addition of 50 ppm NE (B). This confirms the textural differences observed in the columns during experimentation.

Figure 18 confirms the flocculation observed in the columns and with the optical microscope and shows TEM imaging of the flocs formed during settling. The TEM images shown in Figure 18 were generated using samples taken from the top of the settled columns containing kaolin suspended in DI water both untreated (A) and with the addition of 50 ppm GE (B). Due to the difference in extraction points, the size and amount of the flocs and kaolin illustrated here are slightly different from those above. The image in Figure 18 (A) shows the sample taken from the top of the control column that was not treated with any flocculanting agent. All columns imaged of the control

exhibited similar images of lone kaolin particles. This indicates that there is no flocculation of kaolin, and the few particles that are left in the solution are freely floating.

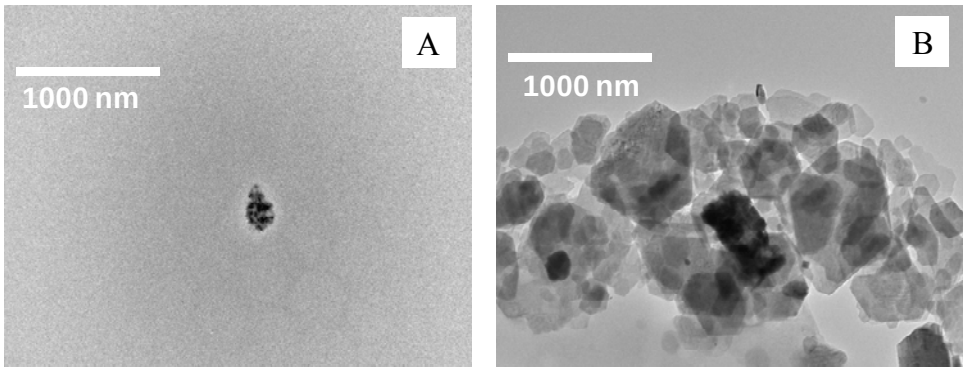

Figure 18. TEM images of kaolin flocculation. Samples taken from the top of columns containing kaolin suspended in DI water both untreated (A) and with the addition of 50 ppm GE (B). The control column exhibits no flocculation, while aggregation is observed in the mucilage treated column

The image on the right shows a sample from the top of a kaolin column treated with 50 ppm GE. Although this image was generated from a column that was treated with GE in place of NE and DI water in place of ion-rich, the same flocculation is observed.

In order to determine whether or not the diatonic ion Ca^{++} has an effect on kaolin binding as it has been projected to have on bacteria, $CaCl_2$ solutions were prepared and added to columns containing kaolin suspended in SW and HW with final concentrations of 0-50 mM. Figure 19 shows the results from these tests prepared using the same method

as the previously plotted kaolin results. Although at some concentrations the settling rate appears to have increased slightly compared to the control, these increases are not significant when compared to the increases observed with the addition of mucilage.

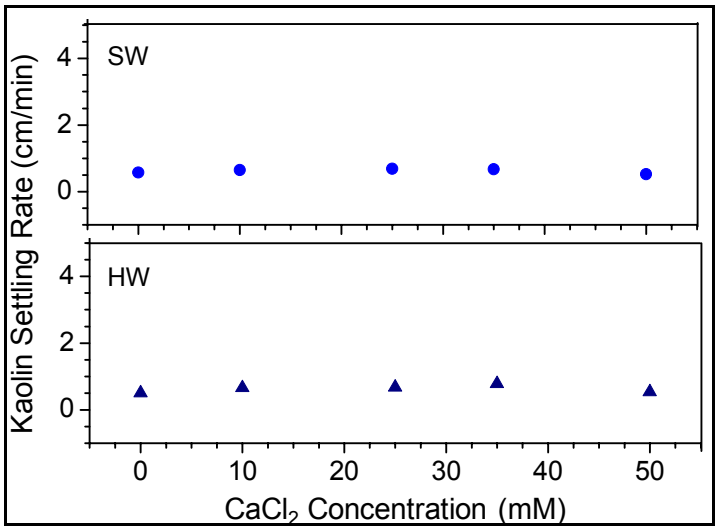

Figure 19. Kaolin treated with $CaCl_2$ in SW and HW. Results demonstrate the use of calcium chloride on the settling rate of kaolin suspended in soft water (SW) and hard water (HW). The addition of $CaCl_2$ is not seen to play a significant role on the settling rate when compared to the untreated column

3.3 Bacteria Flocculation Tests

In tests evaluating the removal of bacteria suspended in synthetic waters, it was observed that the mucilage alone did not display any significant settling. $CaCl_2$ did induce flocculation when tested alone and also when used in combination with both

fractions of mucilage. Because of this, all tests discussed here are treated with a combination of $CaCl_2$ and mucilage, and compared to a control column treated with only $CaCl_2$ and no additional flocculants.

Unlike the kaolin settling discussed in section 3.3, the bacteria flocculation studied does not form a clear interface that can be recorded every minute. Instead the treated bacteria form small white flocs in the otherwise turbid water, which then fall to the bottom of the column as they are formed. Figure 20 shows the flocs at the bottom of an experimental column that contains *B. cereus* in HW that has been treated with $CaCl_2$ and GE. Due to the difference, the mucilage evaluation discussed in this section was slightly different. Here, box plots are used to show the beginning (bottom of the box), duration (the space in between), and the completion (top of the box) of the floc formation and decent in the column. Dotted lines are used to represent the beginning (bottom dotted line) and the end (top dotted line) of the control column that contains no mucilage and only a specified amount of $CaCl_2$.

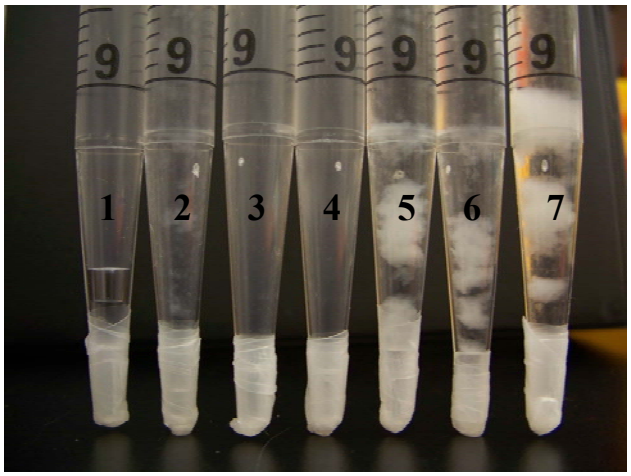

Figure 20. Image of *Bacillus cereus* settled flocs at the bottom of the test columns. From left to right, the columns contain the following treatments for *B. cereus* in hard water (HW). 1: no treatment, 2-7 all contain $CaCl_2$ at a concentration of 20 mM, and 3-7 contain the following concentrations of added Gelling Extract (GE): 3: 25 ppm, 4: 50 ppm, 5: 2 ppm, 6: 3 ppm and 7:4 ppm.

3.3.1 *Bacillus cereus* Flocculation and Evaluation

There are many factors that could potentially affect the removal rate of *B. cereus* from contaminated water such as $CaCl_2$ concentration, mucilage concentration and the ion-content of the water. Figure 21 provides a plot of *B. cereus* settling with $CaCl_2$ concentrations from 10-35 mM and these same concentrations with the addition of NE. From this plot it is observed that as $CaCl_2$ concentrations increase, so does the settling rate of the *B. cereus*. Results from columns treated with mucilage in addition to $CaCl_2$, exhibit flocs beginning and completing more quickly than columns containing only $CaCl_2$. This indicates that although the $CaCl_2$ causes flocculation, when combined with mucilage the speed of the reaction increases because of the GE or NE addition. All

mucilage treated columns shown contained a final NE concentration of 2 ppm, which indicates that only a small amount of mucilage is needed to increase the flocculation time by up to 10 minutes.

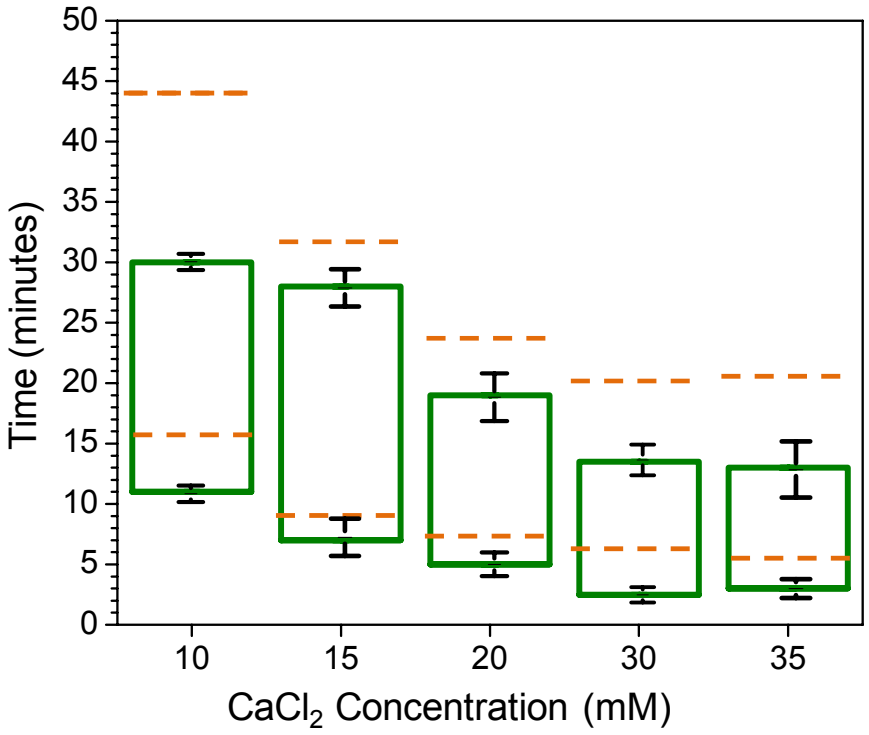

Figure 21. *B. cereus* settling time versus $CaCl_2$ concentration**.** Results represent the time at which flocs formed in the columns (the bottom of the box) and the time that they finished their decent to the bottom of the column (top of the box). The dotted lines represent the control begin and end time. Columns contained 10^8 cells/mL suspended in hard water (HW) treated with a range of $CaCl_2$ concentrations both alone and with the addition of 2 ppm Non-Gelling Extract (NE)

Figure 21 represents the flocculation of *B. cereus* as a function of the $CaCl_2$ concentration while the mucilage content is held constant.

In order to isolate the effects of the mucilage on the flocculation, $CaCl_2$ concentration was held constant over a range of mucilage concentrations. Figure 22 provides a plot of the settling of *B. cereus* for GE and NE concentration ranges of 0-50 ppm in HW.

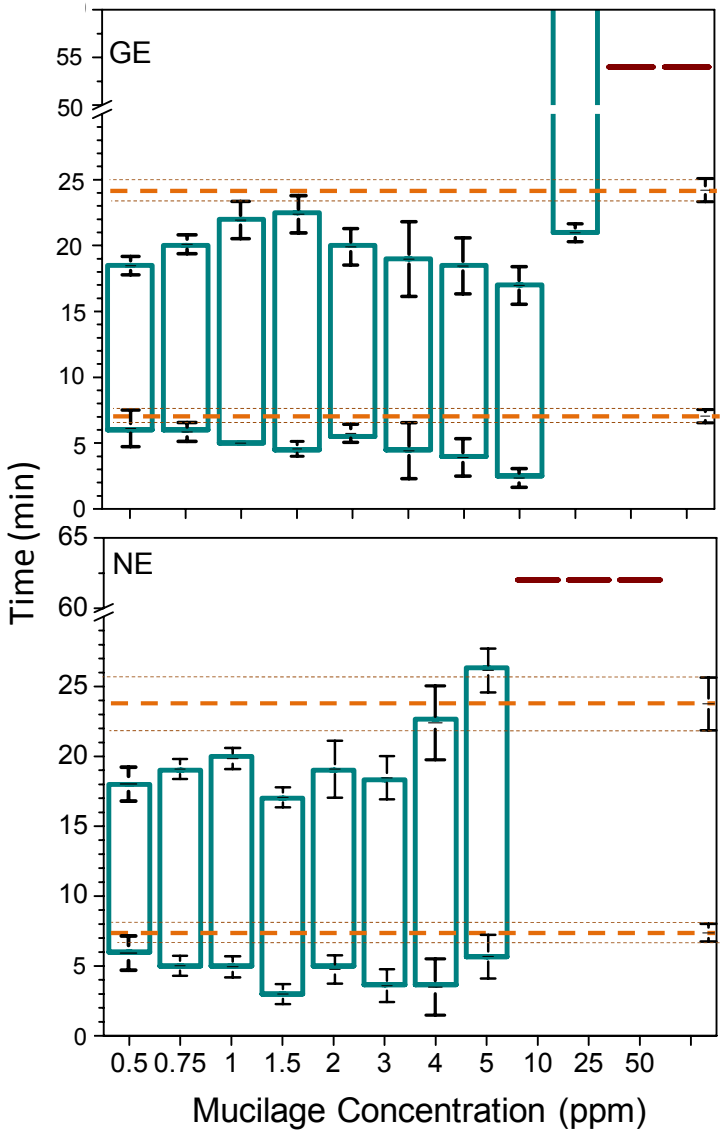

Figure 22. *B. cereus* flocculation using Gelling Extract (GE) and Non-Gelling Extract (NE) concentration ranges in hard water (HW) with final $CaCl_2$ concentrations of 20 mM. Experimental columns treated with GE exhibit increased flocculation time with concentrations ranging from 0.5 to 5 ppm. In columns treated with NE flocculation times occurring faster than the control were observed in columns treated with concentrations between 0.5 and 4 ppm. The higher mucilage concentrations (25 and 50 GE and 10, 25 and 50 NE) did not show signs of flocculation during the time frame of the experiment.

From the plots in Figure 22, it is observed that there is a concentration of mucilage, both GE and NE, where the flocculant is no longer more efficient or effective as the control. In columns treated with GE, the mucilage caused flocculation at a faster rate than the control until a concentration of 10 ppm was reached. At this concentration, the column treated with mucilage begins to form flocs earlier than the control, treated with only $CaCl_2$, but then the floc formation and settling continues after the control is complete and is still not finished after an hour. At GE concentrations of 25 and 50 ppm, flocculation had not even begun in the time scope of the experiment. This critical concentration of mucilage could be caused by a number of things including over activity of the mucilage when introduced to the column. The second plot provided shows the settling time of columns treated with NE under the same water and $CaCl_2$ conditions. These plots display similar concentration results as those seen in the GE treated columns, however, the mucilage stops settling more rapidly than the control at 5 ppm. At concentrations ranging from 0.5 to 4 ppm, the mucilage treated columns begin and end faster than the control but at concentrations of 5 ppm, the treated column takes more time to completely settle than the control, although it still begins faster and ends in the scope of the experiment. Then, at 10, 25 and 50 ppm, the mucilage fails to draw a reaction from the *B. cereus* in the time frame of the experiment. This effect is noticed to begin at much lower concentrations in columns treated with NE rather than GE. This is potentially due to the structural differences that were discussed in section 3.1 and could be caused by the size difference in the mucilage structure. Additionally, the lack of flocculation observed in high concentrations test could be caused by the mucilage interaction with itself at the

higher concentration. This plot also outlines the low concentrations of mucilage that are required, and actually preferred, for bacteria settling. At only 0.5 mg/L, effective and rapid flocculation is observed granting the mucilage extracted from one pad the ability to possibly treat a large amount of water.

It was observed that the SW required a higher concentration of $CaCl_2$ in order to provide similar results to those observed in HW at 20 mM, which is most likely caused by the ion difference in the waters. The HW contains a higher level of Ca^{2+} ions after it is prepared than the SW, leading it to need a smaller concentration of $CaCl_2$ during experimentation. In SW columns containing only 20 mM $CaCl_2$ and treated with mucilage ranges from 2 to 25 ppm, flocculation occurred slowly if at all. This observation implies that in order for the GE to be as effective as possible, additional ions may need to be added to the water with the mucilage. Because of this difference, HW columns were treated with 20 mM $CaCl_2$ while SW columns contained a final concentration of 50 mM. Figure 23 provides a plot of settling time versus GE concentration in SW.

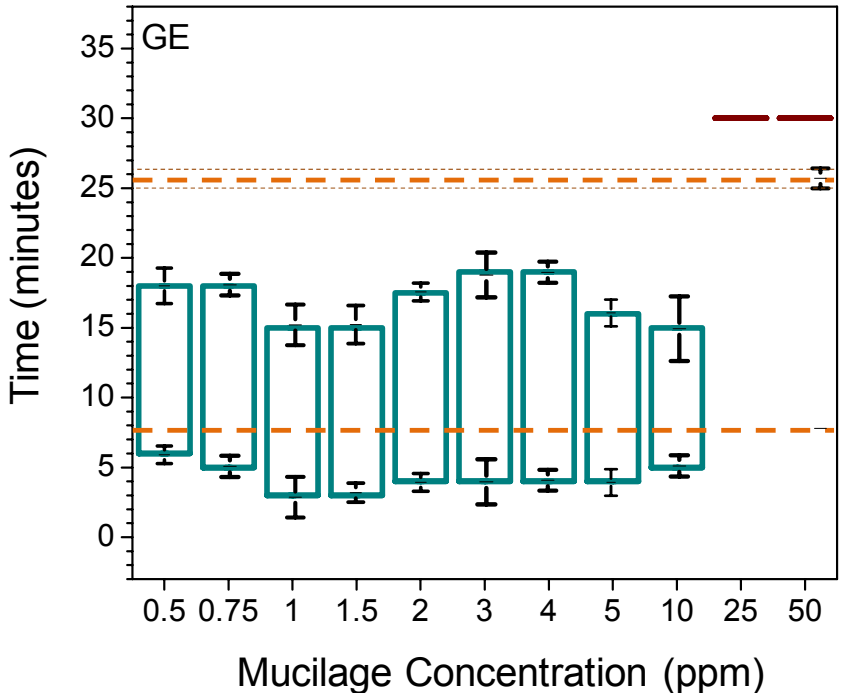

Figure 23. *B. cereus* settling times in SW with GE. Soft water columns treated with GE display similar results to hard water columns treated under the same conditions. Here, in the presence of 50 mM $CaCl_2$, GE concentrations of 0.5 to 10 ppm exhibit flocculation more rapidly then the control containing only $CaCl_2$. Concentrations of 25 and 50 display no flocculation in the time frame of the experiment.

This plot shows similar results to the plot shown in Figure 22 of the flocculation with GE. The main differences observed are that the column containing 10 ppm complete settling faster than the control, and it is not until concentrations of 25 and 50 are used that the mucilage fails to work as well as the control. This is most likely caused by the higher $CaCl_2$ concentrations used in tests with SW.

In regards to better understanding the differences observed in the settling times provided in Figures 21, 22 and 23, microscope images were taken of the control columns and the columns treated with mucilage. Figure 24 shows microscope images of two control columns. The first image (A) shows cells from a column that contained untreated *B. cereus* in HW and the second image (B) is from the same suspension as above, but treated with 20 mM $CaCl_2$.

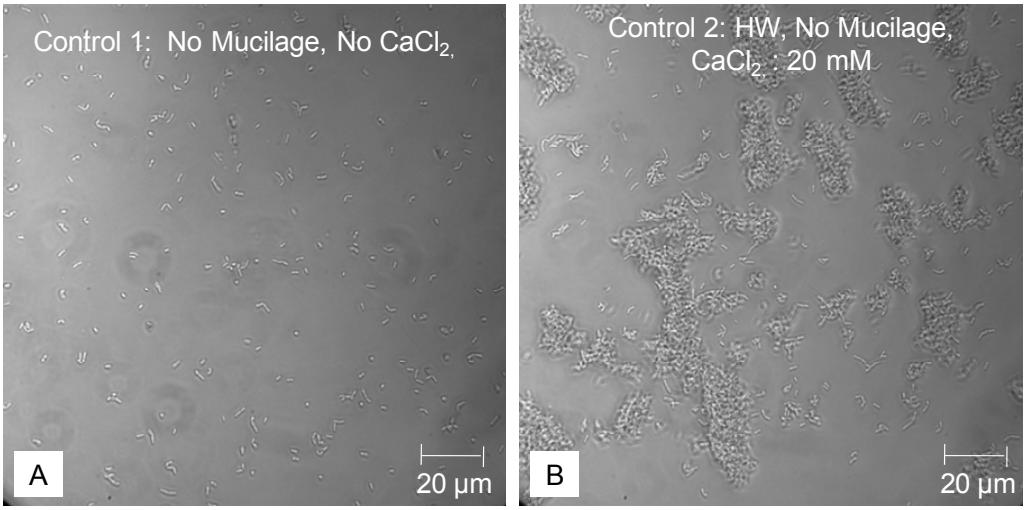

Figure 24. Microscope images of *B. cereus* control columns. Final cell concentration of 10^8 cells/mL. Image A taken of a sample obtained from an untreated column while the image B is cells from a column treated with $CaCl_2$. In untreated columns bacteria are dispersed and freely floating. With the addition of 20 mM $CaCl_2$ small flocs are observed.

Figure 24A shows that in the absence of $CaCl_2$ and mucilage, there is no flocculation and the bacteria are still freely floating in solution. Figure 24B shows that with the addition of $CaCl_2$ at a final concentration of 20 mM small flocs are formed, causing the settling that was discussed earlier. Figure 25 presents images of the *B. cereus* under the same conditions as the control columns, but solutions treated with GE (A) and NE (B) with final concentrations of 2 ppm.

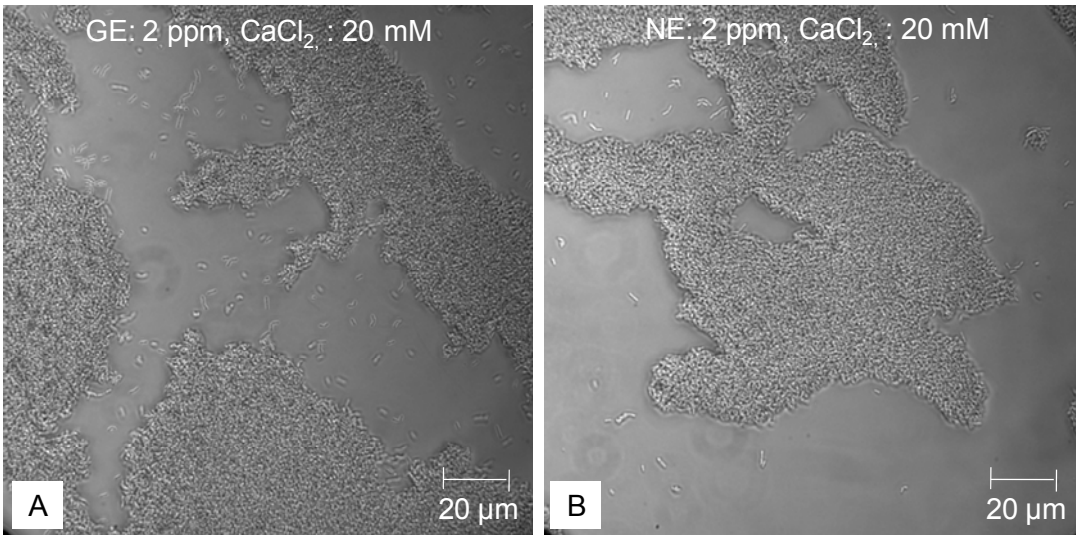

Figure 25. Microscope images of *B. cereus* columns treated with GE and NE in HW. The images here show the flocculation effects that the mucilage has on the *B. cereus*. Both images are from columns containing 20 mM of $CaCl_2$ and 2 ppm of GE (A) and NE (B). The flocs observed here are much larger than those seen in columns with only $CaCl_2$ treatment.

The column containing only $CaCl_2$ showed some flocculation, the effect was not as large or tightly packed as those observed in columns treated with GE and NE. Similar flocculation was observed in columns containing SW and can be seen in Figure 26. Image A shows the flocculation caused by the $CaCl_2$ at a concentration of 50 mM. The flocculation phenomena observed here is similar to that shown above in the columns containing only 20 mM $CaCl_2$, indicating that at a concentration more than double larger and more stable flocs are not formed. The images below show the flocs formed in columns treated with GE (B) and NE (C) with final concentrations of 2 ppm, and are very similar to those seen in Figure 25. This indicates that the flocs formed are generally much larger and more stable in both HW and SW than their $CaCl_2$ counterparts.

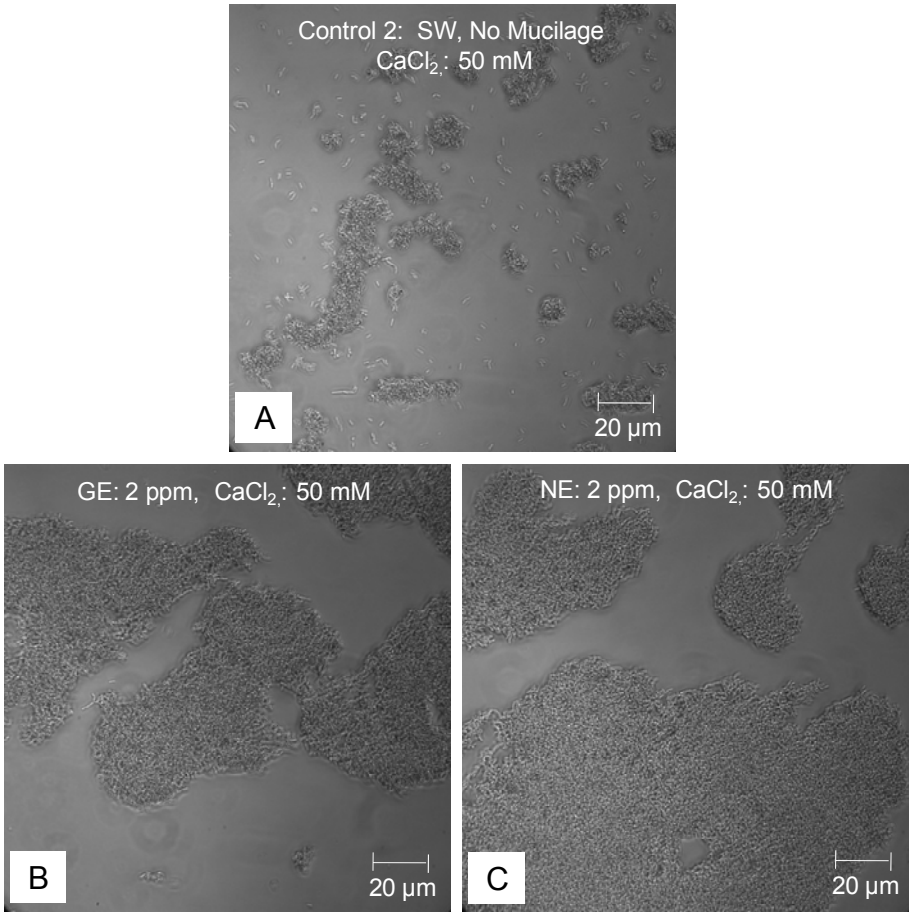

Figure 26. Microscope images of *B. cereus* treated in SW columns. Image A shows cells extracted from a column treated with 50 mM of $CaCl_2$ and no mucilage. Here, as in previous images, flocs are present, but they are small and do not appear to be very stable. GE (B) and NE (C) treated bacteria with a final concentration of 2 ppm show the flocs that are larger and more defined than those observed in $CaCl_2$ control columns.

Table 10 provides removal rates of *B. cereus* suspended at high concentrations (10^8 cells/mL) in SW with final $CaCl_2$ concentrations of 40 mM. GE and NE treatment concentrations evaluated include 0, 2, 3, 4 and 5 ppm. All removal rates are in the 95-99% range including those of the column containing only $CaCl_2$.

Table 10. Removal rates of *B. cereus* in soft water columns treated with GE, NE and 40 mM $CaCl_2$.

Mucilage Type	Mucilage Concentration				
	0 ppm	2 ppm	3 ppm	4 ppm	5 ppm
GE	98.21 %	98.50 %	98.06 %	98.59 %	95.69 %
NE	98.21 %	98.11 %	98.07 %	98.59 %	98.51 %

These results show that, although the addition of mucilage increases the flocculation reaction that takes place between the *B. cereus* and the $CaCl_2$, the resulting removal rate does not differ significantly. Although these removal rates appear high, the water treated in these experiments is not yet fit to consume. Due to the high initial cell count concentrations, removal rates of 99% still results in a significant number of viable cells in the solution.

3.3.2 *Escherichia coli* Flocculation and Evaluation

In the testing of mucilage for *E. coli* removal, HW, $CaCl_2$ concentrations of 20 mM, and GE were used due to their capabilities observed in *B. cereus* tests. Figure 27 provides a plot of flocculation time as a function of GE concentration in columns of HW with 20 mM $CaCl_2$.

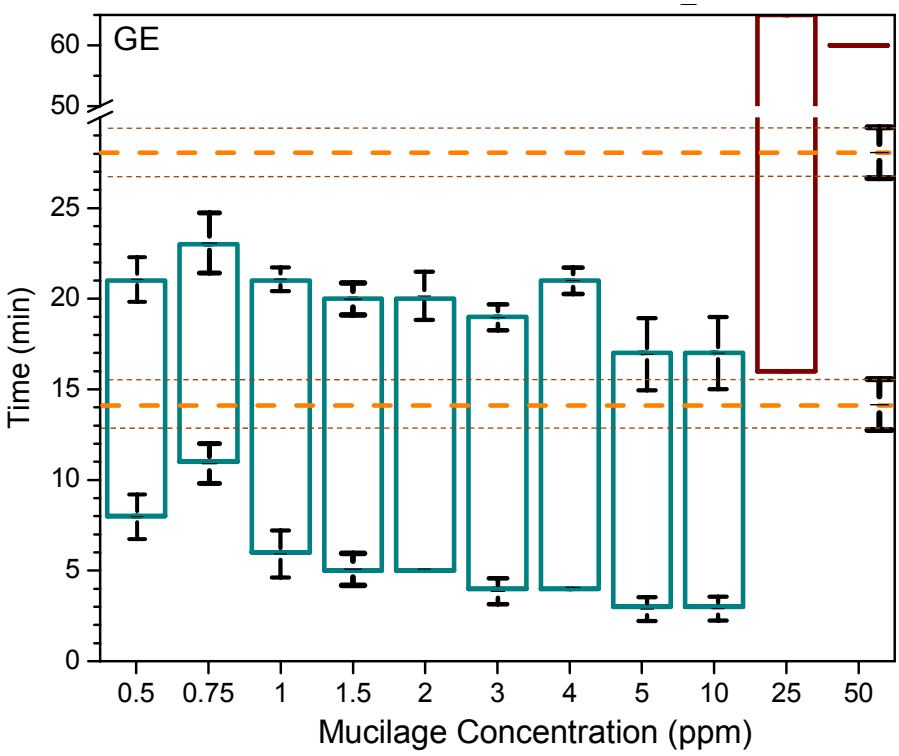

Figure 27. *E. coli* flocculation in HW at a range of GE concentrations. In columns containing 10^8 cells/mL, *E. coli* flocculation with the addition of mucilage was observed to be more efficient with the addition of 0.5 to 10 ppm GE. The control required twice as much time after column inoculation to show signs of settling when compared to the same test with *B. cereus*.

Figure 27 shows that for final GE concentrations of 0.5 to 10 ppm, the columns treated with mucilage begin and end much faster than the control. From this plot differences between *B. cereus* and *E. coli* can be observed. In columns of *B. cereus* in HW the control column flocculation was observed to begin in 7.5 minutes. Conversely, in

columns containing *E. coli*, flocculation does not begin in the control column until 14 minutes after column inoculation. *B. cereus* columns were also seen to complete settling 24 minutes into the experiment while *E. coli* columns took slightly longer.

Both bacteria exhibit differences to the kaolin studies performed with the same mucilage. In kaolin columns the higher mucilage concentrations worked better and an optimal concentration as reached where settling no longer increased. In columns of bacteria treated with mucilage the opposite effect is observed. The lower concentrations work better and at higher concentrations, no reaction is seen in the columns. This is potentially due to a number of things including the size and surface characteristic differences between the contaminant types. In kaolin suspensions, the ion concentration was observed to affect the settling rate; however, the mucilage did not rely on the presence of ions for the flocculation to occur with kaolin as it does with bacteria.

Figure 28 shows a picture of the flocs forming in columns that contain *E. coli* suspended in HW and treated with 20 mM $CaCl_2$ and GE concentrations of 0 ppm (1), 10 ppm (2), 25 ppm (3), 50 ppm (4), and 2 ppm (5) under UV light.

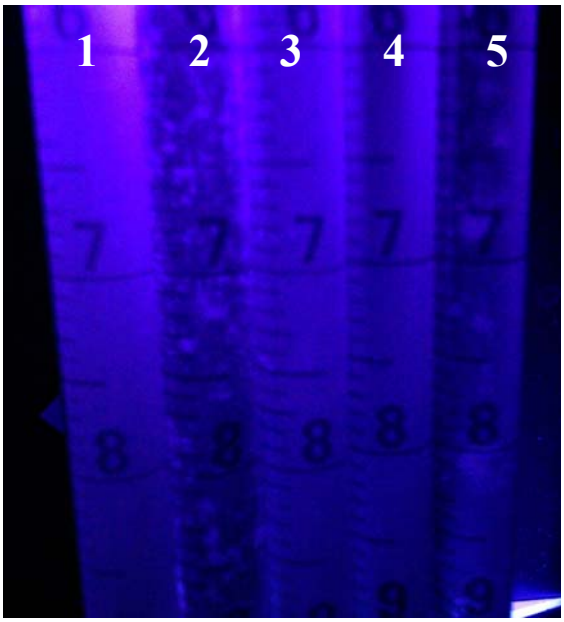

Figure 28. Picture of flocs forming in treated *E. coli* columns under a UV light. All columns contain *E. coli* suspended in hard water and treated with 20 mM $CaCl_2$. GE mucilage addition as follows: (1) 0 ppm, (2) 10 ppm, (3) 25 ppm, (4) 50 ppm, (5) 2 ppm. Columns 2 and 5 display signs of flocculation.

Since the *E. coli* were transformed to contain a pGLO plasmid, the cells fluoresce under UV light making the flocs formed easier to see. In Figure 28, columns 2 and 5 have begun to form flocs and the water around the flocs appears relatively clear when compared to columns 1, 3 and 4 that have not yet began to flocculate.

In addition to using the *E. coli* GFP's florescent qualities help to assess the removal from the water as the flocs are formed, but they also allow the flocs to be viewed under the microscope using a florescent filter. Figure 29 shows florescent microscope images *E. coli* suspended in HW and treated with no flocculating agent (A), with only 20

mM $CaCl_2$ (B), and with 20 mM $CaCl_2$ and 2 ppm GE (C). In Image A, of a column that was not treated, single *E. coli* bacteria can be seen floating freely in the solution. When treated with 20 mM $CaCl_2$ (B) flocculation can be observed, however, the flocs are not very large and do not contain a lot of bacteria. In the final image (C), from a sample treated with 2 ppm GE in the presence of 20 mM $CaCl_2$, a large cluster of bacteria are present, and covers the entire image. Here, the size and high bacteria content of the flocs formed using mucilage can be observed.

The flocculation observed in bacteria columns is similar to that seen in suspensions of kaolin treated with mucilage. The gathering of particles observed in both cases causes the density of the contaminating material to change, as it is becoming larger, and gravity induced settling occurs.

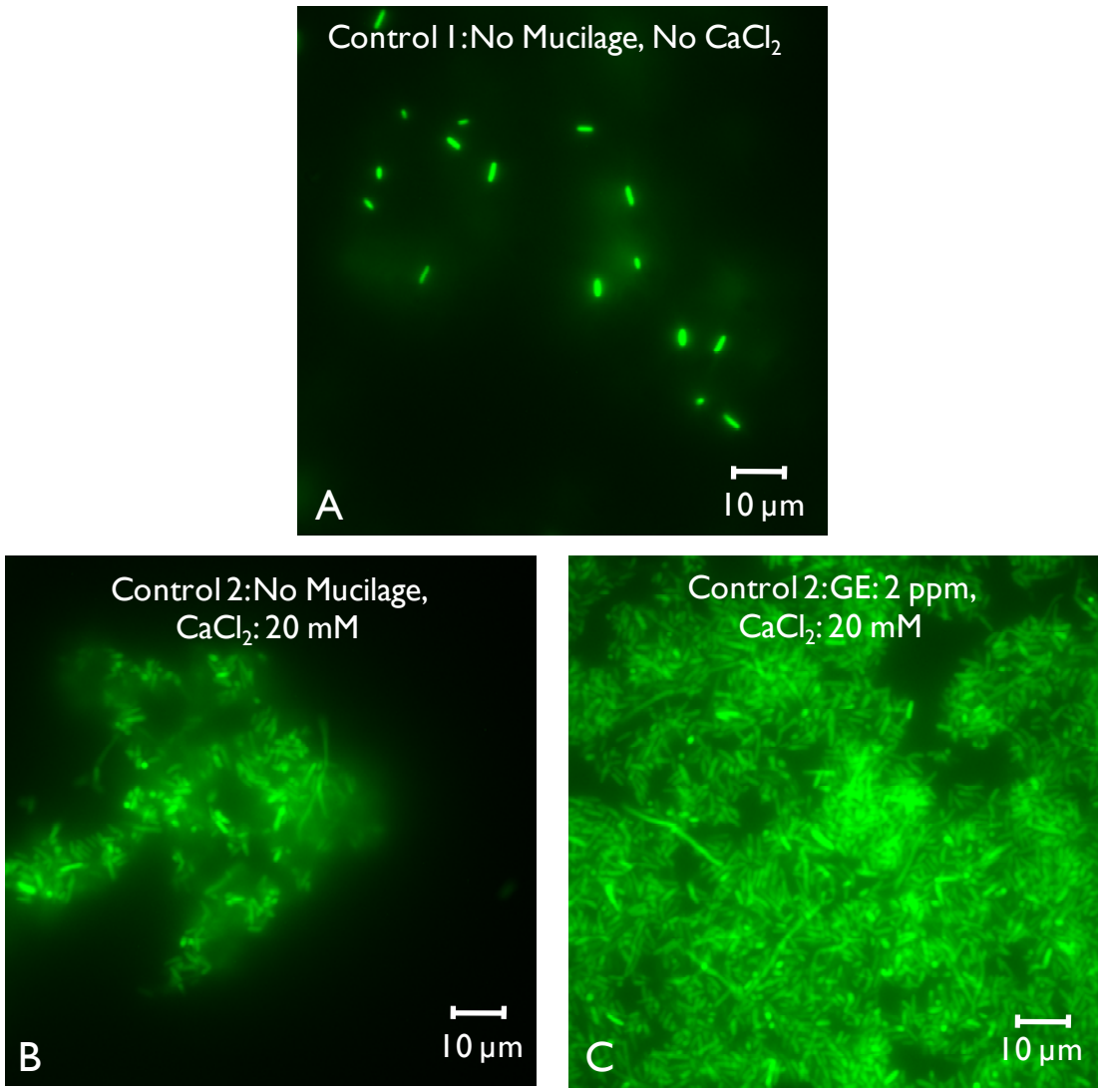

Figure 29. Florescent images of the flocs formed in columns containing *E. coli* suspended in HW. Not treated (A), treated with 20 mM $CaCl_2$ (B) and with the addition of 2 ppm GE (C). By comparing the images the differences in the size of the flocs formed can be observed.

Chapter Four: Conclusions and Future Work

4.1 Summary of Findings

The work presented in this thesis demonstrates that both the Gelling (GE) and Non-Gelling (NE) mucilage extracts obtained from the *O. ficus-indica* cactus are capable of removing sediment and bacteria. Different fractions of mucilage are more efficient with different contamination species, which is possibly due to the structural differences between the two mucilage compounds. Structurally, the GE exhibits a fiber like structure that is orderly and directional while the NE has a denser net like structure.

Kaolin has been observed to form a very clear interface as it settles that could be read every minute and plotted to observe the settling nature of the column. In water prepared at high ion-concentrations, referred to as HW, settling rates of kaolin increased with increasing concentrations of both NE and GE at a faster rate than SW, surrogate water with lower ion-concentration, which in turn settled faster than kaolin suspended in DI water. The concentration of both NE and GE gradually reach a point where the settling rate begins to level off and the change in rate versus concentration is no longer significant. In columns containing NE average settling rates of 13.5 cm/min were reached and in columns treated with GE average rates of 11.2 cm/min were observed. Columns containing $Al_2(SO_4)_3$ at high concentrations exhibited little increase in settling rate above the control settling of 0.5 cm/min.

Images of samples taken from columns of high GE and NE concentrations showed a clear aggregation where samples from the control columns showed particles that were free floating and separated. The flocculation phenomena is the cause of the increased settling rates with the addition of mucilage.

Gram-positive *B. cereus* demonstrated flocculation similar to kaolin when exposed to GE and NE coupled with $CaCl_2$. Bacteria flocculation, unlike kaolin was not observed to form a clean interface, rather the flocculation beginning and end time were recorded and evaluated compared to a control. Here, the mucilage was more effective at lower concentrations, which is opposite to what was observed in kaolin columns. For NE, concentrations of 0.5 to 4 ppm produced flocs that both developed and settled faster than the control column that contained only $CaCl_2$. At concentrations of 5 ppm the flocs took a greater amount of time to settle then those formed in the control column. At concentrations of 10, 25 and 50 ppm, signs of flocculation did not even appear in the time scope of the experiment. Columns treated with GE worked at slightly higher concentrations than the columns treated with NE and were not slower than the control until concentrations of 10 ppm. Concentrations of 25 and 50 ppm showed no signs of removal. In addition, the flocculation time frame was shown to decrease as $CaCl_2$ concentration increased over 10-35 mM, as well as columns ran with the addition of 2 ppm NE to these $CaCl_2$ concentrations.

Images of *B. cereus* untreated showed that the bacteria were still freely floating in solution and that no aggregation had occurred. In columns containing HW with 20 mM $CaCl_2$ and SW with 50 mM $CaCl_2$, a small amount of flocculation was observed, but it

appeared to be small and loosely structured. In images of HW (20 mM $CaCl_2$) and SW (50 mM $CaCl_2$) and the addition of 2 ppm NE or GE, flocs were observed to be both larger and seemingly more stable due to the tightness of the bacteria packing. From these treated columns removal rates of 95-99 percent were observed.

Settling results for gram-negative *E. coli* proved to be similar to those for *B. cereus* using HW, $CaCl_2$ concentrations of 20 mM and GE concentrations ranging from 0.5-10 ppm. Control columns were observed to require twice as long to begin settling after inoculation and mucilage columns did not appear to require more time. When observed under UV light, the water appeared to clear significantly while the flocs were being formed and in microscope images using florescence, large flocs were observed in columns treated with 2 ppm GE and 20 mM $CaCl_2$ when compared to those treated with only 20 mM $CaCl_2$ and a column that was untreated.

4.2 Future Work Recommendations

4.2.1 Continued Bacteria Studies

All work provided here focused on the use of a Gelling and Non-Gelling Extract from the *O. ficus-indica*. Although previous work with the third fraction of mucilage, Combined Extract (CE), suggests that its use will result in a removal somewhere between the two fractions studied in this work, it would be valuable to test these contaminants using this mucilage fraction as well. The testing of this extract could also potentially illustrate how the mucilage will work when it is used in developing countries as this is what would be most easily obtained from the pads.

In this work, only suspensions of *B. cereus* were evaluated for bacteria removal rates. Removal rates of *E. coli* also should be tested and both should be evaluated at low initial cell concentrations. In environmental bodies of water, concentrations of 10^5 cells/mL are more likely to occur and need to be evaluated to determine the effectiveness of mucilage at these low concentrations.

The concern of whether or not *E. coli* and *B. cereus* could feed on the sugars found in the mucilage will also need to be addressed.

4.2.2 Shelf-life Evaluation

The results provided in this thesis demonstrate the small amount of mucilage that is required for contaminant removal from surrogate waters. Because of the relationship between the amounts of mucilage used to the amount obtained from a single pad through the extraction process, it would be valuable to consider the shelf-life of the mucilage product. This would determine whether or not the fast settling rates observed above would change over time, and if so to what degree. The GE and NE used to generate the results described by Young *et al.* were extracted in 2004 and still have the ability to remove sediment suspensions from water, although it may not be as effective as it was at the time of its extraction. In order to evaluate and account for the possible differences in structure that occurs over time, prepared mucilage should be tested at intervals to determine whether or not efficiency is lost. This evaluation could also be done with equipment designed to evaluate the contents of the mucilage, as it structural differences could be measured there as well.

4.2.3 Contaminant Combination Analysis

This work studied two contaminants separately and provides data that suggests mucilage is a useful flocculating agent for both. However, because it is highly unlikely that a single contaminant will occur in a given body of water, it would be interesting and provide a better understanding of how the mucilage would suffice in real world situations if the mucilage was tested on a column that contains a combination of two or more contaminants.

4.3 Final Remarks

From this work it can be concluded that mucilage has the potential of removing some of the most common contaminants from ion-rich water supplies. The amount of mucilage that is required to cause significant flocculation is very low and a single cactus pad offers the ability to clean a large amount of water. Not only does the cactus provide a green technology for use in water purification, but it also avoids controversy that is often observed with current methods including community opposition, energy requirements, and inconsistent results. Due to its common use and abundant growth, the *O. ficus-indica* cactus could offer an inexpensive, easy to use and extremely valuable flocculant to countries that struggle with water contamination.

References

1. Thornton, J., *Water Loss Control Manual*. McGraw-Hill Professional: 2002.

2. Bartram, J.; Howard, G., Drinking-water Standards for the Developing World. In *The Handbook of Water and Wastewater Microbiology*, Mara, D.; Horan, N., Eds. Academic Press: San Diego, CA, 2003.

3. Gleeson, C.; Gray, N., *The Coliform Index and Waterborne Disease: Problems of Microbial Drinking Water Assessment*. E & FN Spon: Boundary Row, London, 1997.

4. UN *Human Development Report 2006; Beyond Scarcity: Power, poverty and the global water crisis*; EPA Office of Water: New York City, 2006.

5. Geldreich, E. E.; Reasoner, D. J., Home Treatment Devices and Water Quality. In *Drinking Water Microbiology*, McFeters, G. A., Ed. Springer-Verlag New York, Inc.: New York City, 1990 pp 147-167.

6. EPA *Bacterial Water Quality Standards for Recreational Waters*; Office of Water: Washington DC, 2003.

7. Bitton, G., *Wastewater Microbiology*. 3 ed.; John Wiley & Sons, Inc: Hoboken, NJ, 2005.

8. Jensen, P. K.; Ensink, J. H. J.; Jayasinghe, G.; Hoek, W. v. d.; Cairncross, S.; Dalsgaard, A., Domestic Transmission routes of pathogens: The problem of in-house contamination of drinking water during storage in developing countries. *Tropical Medicine and International Health* 2002, *7*, (7), 604-609.

9. Mintz, E.; Bartram, J.; Lochery, P.; Wegelin, M., Not Just a Drop in the Bucket: Expanding Access to Point-of-Use Water Treatment Systems. *American Journal of Public Health* 2001, *19*, (10), 1565-1570.

10. Quick, R. E.; Venczel, L. V.; Mintz, E. D.; Soleto, L.; Aparicio, J.; Grionaz, M.; Hutwagner, L.; Greene, K.; Bopp, C.; Maloney, K.; Chavez, D.; Sobsey, M.; Tauxe, R. V., Diarrhoea prevention in Bolivia through point-of-use water treatment and safe storage: a promising new strategy. *Epidemiol. Infect* 1999, *122*, 83-90.

11. Curtis, V.; Cairncross, S.; Yonli, R., Review: Domestic hygiene and diarrhoea - pinpointing the problem. *Tropical Medicine and International Health* 2000, *5*, (1), 22-32.

12. UN *The Millennium Development Goals Report*; United Nations: New York City, 2008.

13. UN *The Millennium Development Goals Report*; United Nations: New York City, 2007.

14. Miller, E. W.; Miller, R. M., *Water Quality and Availability*. ABC-CLIO, Inc: Santa Barbara, CA, 1992.

15. EPA *National Primary Drinking Water Standards*; EPA Office of Water: 2003.

16. UN *Human Development Report 2003; Millennium Development Goals: A compact among nations to end human poverty*; EPA Office of Water: New York City, 2003.

17. Chaudhuri, M.; Sattar, S. A., Domestic Water Treatment for Developing Countries. In *Drinking Water Microbiology*, McFeters, G. A., Ed. Springer-Verlag New York Inc: New York City, 1990; pp 168-184.

18. Unger, M.; Collins, M. R., Assessing Escherichia coli removal in the schmutzdecke of slow-rate biofilters. *American Water Works Association Journal* 2008, *100*, (12), 60.

19. Logsdon, G. S., Microbiology and Drinking Water Filtration. In *Drinking Water Microbiology*, McFeters, G. A., Ed. Springer-Verlag New York Inc.: New York City, 1990; pp 120-146.

20. Dizer, H.; Grutzmacher, G.; Bartel, H.; Wiese, H. B.; Szewzyk, R.; Lopex-Pila, J. M., Contribution of the colmation layer to the elimination of coliphages by slow sand filtration. *Water Science and Technology* 2004, *50*, (2), 211-214.

21. LeChevallier, M. W.; McFeters, G. A., Microbiology of Activated Carbon. In *Drinking Water Microbiology*, McFeters, G. A., Ed. Spinger-Verlag New York Inc.: New York City, 1990; pp 104-119.

22. Colwell, R. R.; Huq, A.; Islam, M. S.; Aziz, K. M. A.; Yunus, M.; Khan, N. H.; Mahmud, A.; Sack, R. B.; Nair, G. B.; Chakraborty, J.; Sack, D. A.; Russek-Cohen, E., Reduction of cholera in Bangladeshi villages by simple filtration. *Proceedings of the National Academy of Sciences of the United States of America* 2003, *100*, (3), 1051-1056.

23. Semenza, J. C.; Roberts, L.; Henderson, A.; Bogan, J.; Rubin, C. H., Water Distribution System and Diarrheal Disease Transmission: A Case Study in Uzbekistan. *American Journal of Tropical Medicine and Hygiene* 1998, *56*, (6), 941-946.

24. LeChevallier, M. W.; Cawthon, C. D.; Lee, R. G., Factors Promoting Survival of Bacteria in Chlorinated Water Supplies. *Applied Environmental Microbiology* 1988, *54*, (3), 649-654.

25. Morrow, J. B.; Almedia, J. L.; Fitzgerald, L. A.; Cole, K. D., Association and Decontamination of *Bacillus* Spores in a Simulated Drinking Water System. *Water Research* 2008, *42*, (20), 5011-5021.

26. Parsons, S. A.; Jefferson, B., *Introduction to Potable Water Treatment Processes*. Blackwell Publishing Ltd: Oxford, UK, 2006.

27. Montgomery, J. M., *Water Treatment Principles and Design*. John Wiley & Sons: New York City, 1985.

28. Ndabigengesere, A.; Narasiah, K. S., Quality of Water Treated by Coagulation using *Moringa Oleifera* Seeds. *Water Research* 1997, *32*, (3), 781-791.

29. Babu, R.; Chaudhuri, M., Home Water Treatment by Direct Filtration with Natural Coagulant. *Indian Journal of Water and Health* 2005, *3*, (1), 27-30.

30. Larsen, N.; Nissen, P.; Willats, W. G. T., The effect of Calcium Ions on Adhesion and Competitive Exclusion of *Lactobacillus ssp.* and *E. coli* 0138. *International Journal of Food Microbiology* 2007, *114*, (1), 113 - 119.

31. Sanin, D.; Vesilind, P. A., Bioflocculation of Activated Sludge: The Role of Calcium Ions and Extracellular Polymers. *Environmental Technology* 2000, *21*, (12), 1405-1412.

32. Russell, C. E.; Felker, P., The Prickly-Pears (Opuntia spp., Cactaceae): A Source of Human and Animal Food in Semiarid Regions. *Economic Botany* 1987, *41*, (3), 433-445.

33. Griffith, M. P., The Origins of an Important Cactus Crop, *Opuntia ficus-indica* (Cactaceae): New Molecular Evidence. *American Journal of Botany* 2004, *91*, (11), 1915-1921.

34. Goycoolea, F. M.; Cardenas, A., Pectins from *Opuntia spp*.: A Short Review. *Journal of the Professional Association for Cactus Development* 2003, *5*, 17-29.

35. Trachtenberg, S.; Mayer, A. M., Composition and Properties of *Opuntia ficus-indica* Mucilage. *Phytochemistry* 1981, *20*, (120), 2665-2668.

36. Medina-Torres, L.; Fuente, E. B.-D. L.; Torrestiana-Sanchez, B.; Katthain, R., Rheological Properties of the Mucilage Gum (*Opuntia ficus indica*). *Food Hydrocolloids* 2000, *14*, 417-424.

37. Young, K. A. The Mucilage of *Opuntia ficus indica*: a natural, sustainable and viable water treatment technology for use in rural Mexico for reducing turbidity and arsenic contamination in drinking water. Master's Thesis, University of South Florida, Tampa, 2006.

38. Young, K. A.; Alcantar, N. A.; Cunningham, A., Cactus goo purifies water. *Science News* 2005.

39. Young, K. A.; Anzalone, A.; Pichler, T.; Picquart, M.; Alcantar, N. A., Using the Mexican Cactus as a New Environmentally Benign Material for the Removal of Contaminants in Drinking Water. 2005, *2005*, (93), 965-966.

40. Geldreich, E. E., Microbiological Quality of Source Waters for Water Supply. In *Drinking Water Microbiology*, McFeters, G. A., Ed. Springer-Verlag New York, Inc.: New York City, 1990; pp 3-31.

41. Priest, F. G., Isolation and Identification of Aerobic Endospore-Forming Bacteria. In *Bacillus*, Harwood, C. R., Ed. Plenum Press: New York City, 1989; pp 27-56.

42. Archibald, A. R., The *Bacillus* Cell Envelope. In *Bacillus*, Harwood, C. R., Ed. Plenum Press, New York: New York City, 1989.

43. Bauman, R. W., *Microbiology*. Pearson Education, Inc.: San Francisco, 2004.

44. Ingraham, J. L.; Ingraham, C. A., *Indroduction to Microbiology*. 2 ed.; Brooks/Cole: Pacific Grove, CA, 2000.

45. Lim, D., *Microbiology*. 2 ed.; WCB/McGraw-Hill: 1998.

46. Smith, E. J.; Davison, W.; Hamilton-Taylor, J., Methods for Preparing Synthetic Freshwaters. *Water Research* 2002, *36*, (5), 1286-1296.