EVALUATION OF SURFACTANT-MODIFIED ZEOLITE FOR THE REMOVAL OF *GIARDIA LAMBLIA* FROM CONTAMINATED WATERS

By

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ABSTRACT

Batch and column experiments were conducted to test for preferential removal of *Giardia lamblia* from water by surfactant-modified zeolite (SMZ). *Saccharomyces cerevisiae* has similar size and surface characteristics to *G. lamblia* and was therefore used as a surrogate. The zeolite treatments had an aggregate size of 1.4 to 2.4 mm. Hydrophobic SMZ, cationic SMZ, and raw zeolite were used in all experiments. Concentrations of *S. cerevisiae* were determined through direct counting methods using a black, 0.45-μm filter membrane. The SMZ treatments yielded *S. cerevisiae* partition coefficients that were 10 to 25 times greater than those for raw zeolite. Column results showed removal efficiencies of 40.8% and 46.3% for the cationic and hydrophobic SMZ treatments, respectively, compared to a removal efficiency of 15.3% for raw zeolite. The results indicate that SMZ preferentially removes *S. cerevisiae* from solution and is likely to be similarly effective in the removal of *G. lamblia*.

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INTRODUCTION TO WORK

This document summarizes the results of a thesis project. The document contains a journal article and supporting appendices. The thesis project partially fulfills the requirements for the Master of Science in Hydrology degree at the New Mexico Institute of Mining and Technology. The research conducted in this study assessed the potential application of surfactant-modified zeolite (SMZ) for the removal of the waterborne pathogen *Giardia lamblia* from contaminated drinking water. The primary objective of this study was to evaluate the efficiency of pathogen removal by SMZ through the use of the model organism *Saccharomyces cerevisiae*. A subsequent objective of the study was to find and develop a method of enumeration that can be applied to both the model organism and the pathogen *G. lamblia*. Additionally, this study included the design, construction and field-testing of a prototype filtration system.

The following manuscript, entitled "Examination of the removal of *Giardia lamblia* from contaminated water through the use of (SMZ) and model organism *S. cerevisiae*," was prepared for submission to a scientific journal and follows the editorial guidelines set by the Elselvier journal *Applied and Environmental*

Microbiology. The manuscript outlines the results of laboratory batch and column experiments that were completed to accomplish the project objectives.

The appendices in this document contain information on the initial enumeration method used in this study, detailed descriptions of post-experimental studies, experimental procedures and the subsequent results.

Evaluation of surfactant-modified zeolite (SMZ) for the removal of *Giardia lamblia* from contaminated waters

Diane K. Agnew[1] and Robert S. Bowman[2]

Abstract

Batch and column experiments were conducted to test for preferential removal of *Giardia lamblia* from water by surfactant-modified zeolite (SMZ). *Saccharomyces cerevisiae* has similar size and surface characteristics to *G. lamblia* and was therefore used as a surrogate. The zeolite treatments had an aggregate size of 1.4- to 2.4-mm. Hydrophobic SMZ, cationic SMZ, and raw zeolite were used in all experiments. Concentrations of *S. cerevisiae* were determined through direct counting methods using a black, 0.45-μm filter membrane. The SMZ treatments yielded *S. cerevisiae* partition coefficients that were 10 to 25 times greater than those for raw zeolite. Column results showed removal efficiencies of 40.8% and 46.3% for the cationic and hydrophobic SMZ treatments, respectively, compared to a removal efficiency of 15.3% for raw zeolite. The results indicate that SMZ preferentially removes *S. cerevisiae* from solution and is likely to be similarly effective in the removal of *G. lamblia*.

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Introduction

Throughout the world, people are concerned about microbiological contamination of drinking water. There are many biological water contaminants, but of all of the candidates for microbiological contamination, *Giardia lamblia* (also known as *Giardia intestinalis*) is one of the most important because it poses a major threat to the global water supply (8). Over the past two decades, infection with *G. lamblia* has become the leading cause of waterborne disease in humans living in the United States (6). *G. lamblia* infects millions of people across the globe in both epidemic and sporadic forms. Giardia is ubiquitous in surface waters in the United States (8). Due to the serious global concerns that are presented by the persistence of *G. lamblia* in the environment, there is a worldwide demand for water treatment techniques that are inexpensive, efficient and effective for the removal of *G. lamblia* from drinking water supplies.

The life cycle of *G. lamblia* consists of two stages: the actively reproducing trophozoite (10-20 μm) and the dormant cyst (10-15 μm). The cyst form of this protozoan is the most relevant stage of the life cycle for drinking water treatment, as it is environmentally resistant and infectious. Under favorable environmental conditions, *G. lamblia* cysts can remain viable for several months. Once in the cyst form, *G. lamblia* is resistant to chlorination and disinfection by ultraviolet light. Infection can occur with the ingestion of fewer than ten cysts (17). The disease that results from the ingestion of *G. lamblia* cysts is giardiasis, which is a diarrheal illness. Symptoms of the disease appear one to two weeks after infection and can persist for several weeks, depending on the health of the infected host (6). Symptoms of giardiasis range from the asymptomatic passage of cysts to chronic diarrhea, malabsorption and weight loss (1). For infected

hosts with compromised immune systems, children or the elderly, giardiasis may result in death.

In 1989, the United States Environmental Protection Agency (EPA) enacted the Surface Water Treatment Rule to address the health risks posed by *G. lamblia*. The rule states that all drinking water systems must filter and disinfect water from surface water sources to reduce the occurrence of microbes, namely *G. lamblia*. The rule stipulates a maximum concentration limit of zero cysts per mL for *G. lamblia* cysts in drinking water, due to the health risk at any cyst concentration. Under the treatment rule, all water treatment systems must filter and disinfect their water to a minimum of 99.99 percent combined removal and inactivation of *G. lamblia* (10). Likewise, the World Health Organization (WHO) also stipulates that drinking water must contain no more than zero cysts per volume of treated water (25). Table 1 summarizes the most common water treatment techniques that are currently employed in the United States, with their respective removal efficiencies. The removal efficiencies range from 95 to 99 percent (10). In the United States, approximately 95 percent of the water treatment facilities use chlorination as their disinfection step; in some cases, treatment plants use hyper-chlorination as the final treatment step. Due to the robustness of the *G. lamblia* cyst, the use of chlorination and hyper-chlorination is not fully effective. The less-than-ideal removal efficiency for the removal of *G. lamblia* from drinking water, coupled with the serious health risks posed by the organism, emphasize the need for inexpensive and effective water treatments that can be applied to groundwater and surface water sources.

Zeolites are naturally occurring clay-like minerals that can be used in water treatment applications, including the removal of microorganisms. They have several

unique properties such as a cage-like structure, a high cation exchange capacity, ion selectivity, and adsorption, which make them useful in environmental applications (5). Natural, untreated zeolite has a negative surface charge, and, therefore, does not have an affinity for anions or many microorganisms, who also typically have a net negative charge. However, when treated with a cationic surfactant, an organic bilayer can form on the zeolite surface, resulting in a positively charged particle. Previous researchers have shown that surfactant-modified zeolites (SMZ's) are highly effective in the removal of inorganic anions, organic species and microorganisms (4) (20). Schulze-Makuch et al. (20) demonstrated that SMZ effectively removes viral and bacterial contaminants from water. The researchers observed removal of at least 99% the viruses and 100% of *E. coli* present in the water used in the experiments (20).

The goal of this study is to evaluate the efficiency of SMZ for the removal of the pathogen *G. lamblia*. The filter medium used in this study is composed of a zeolite that has been treated with the surfactant hexadeclytrimethylammonium-chloride (HDTMA-Cl), which is commonly found in hair conditioners and mouthwash. The cost of SMZ is relatively low (~$0.50/kg), and if it is found to be an effective mode of removal of *G. lamblia*, SMZ will be a viable water treatment technique. The SMZ material has hydrophobic properties and induces a positive surface charge on the zeolite surface. Many microorganisms possess a negative surface charge and therefore can potentially adsorb onto the positively charged surface of the SMZ. Two different SMZ formulations are tested in this study, and raw zeolite material is the control.

The use of pathogens in the laboratory setting is both difficult and dangerous. For this reason, a non-pathogenic model organism was selected as a surrogate for *G. lamblia*

for use in this study: *Saccharomyces cerevisiae*, the common baker's yeast. The morphology and basic surface properties of *S. cerevisiae* are similar to the waterborne pathogen *G. lamblia*. The organism *S. cerevisiae* has the added advantage that it can be easily cultured in the laboratory and made into large volume yeast solutions. Batch and laboratory experiments were completed in order to evaluate the adsorption of the model organism *S. cerevisiae*, which has size and charge characteristics similar to that of *G. lamblia*.

Materials and Methods

Zeolite Properties

The zeolite used in this study is a natural clinoptilolite-rich tuff from the St. Cloud mine, Winston, NM. An XRD analysis, performed by Sullivan et al. (22), found the zeolite composition to be 74% clinoptilolite, 5% smectite, 10% quartz/cristobalite, 10% feldspar, and 1 % illite. The raw zeolite has an external surface area of 14 m^2/g (19). The aggregate size of the material used in both the batch and column experiments is 1.4 to 2.4-mm (8-14 mesh size).

Bowman et al. (4) reported the maximum surfactant loading (MSL) of the raw zeolite to be 140-mmol hexadeclytrimethylammonium-chloride (HDTMA-Cl)/kg zeolite. The surfactant used in this study to obtain the cationic and hydrophobic formulations was Lonza HDTMA-Cl Casoquat solution (CT-429, Code No. 5330000618, CAS No. 112-02-7), which contains 29% by weight HDTMA-Cl.

SMZ Preparation

The scope of this study included the preparation of hydrophobic SMZ in the laboratory. Preparation of the cationic SMZ used in this study is described in Bowman et al. (4).

An up-flow system was used for the treatment of the raw zeolite. A 20-L plastic bucket was equipped with barb fittings located at the bottom (inlet) and near the top (outlet). The bucket was filled with 10 kg of zeolite and the system was then saturated with CO_2 gas for 4 h to displace air. Twenty liters of the appropriate surfactant solution was prepared, placed in a 20-L reservoir and then circulated through the material at a flow-rate of 30 mL/min using a CHEM-FEED pump (Blue White Industries, Westminster, CA). The initial and equilibrium HDTMA concentrations were determined. After 24-h of circulation, the treated zeolite material was rinsed with a total of 70 L (10 pore volumes (PV)) of Type I water, through a series of rinses. The rinse-solution was sampled every 5 L to determine HDTMA concentrations for loading calculations. Following the rinsing of the material, the SMZ was air dried.

To create hydrophobic SMZ material, an initial surfactant concentration of 46.7 mM HDTMA (93.3 mmol HDTMA-Cl/kg zeolite) was prepared. The maximum surfactant loading of the zeolite ranges from 140 to 150 mmol HDTMA-Cl/kg zeolite (4, 14). A monolayer of surfactant on the zeolite surface results in hydrophobic conditions. Li and Bowman (14) demonstrated that the formation of a surfactant monolayer is a function of the surfactant loading. The most complete monolayer coverage occurs at approximately 2/3 of the maximum, or 93 mmol HDTMA-Cl/kg zeolite (22).

The initial HDTMA concentration was determined to be 46.0 mM (91.9 mmol HDTMA-Cl /kg zeolite) for a volume of 20 L. The amount of HDTMA measured in

solution at the completion of the 24-h surfactant treatment period, the equilibrium solution, was relatively small (6.39 mmol HDTMA-Cl/kg zeolite) and the concentration of HDTMA was negligible in the final rinse solution (0.07 mmol HDTMA-Cl/kg zeolite). The total sum of the rinse concentrations was 6.27 mmol HDTMA-Cl/kg zeolite. The surfactant loading on the zeolite was calculated by subtracting the mass of HDTMA in the equilibrium and rinse solutions from the initial mass of HDTMA used in the treatment process. The surfactant loading for the hydrophobic SMZ made in this study was equal to 79.3 mmol HDTMA-Cl/kg zeolite, which is close to the target of 2/3 MSL.

The analysis of HDTMA concentrations was completed via high performance liquid chromatography (HPLC) using the method described by Li and Bowman (14). The HPLC set-up included a Waters 501 HPLC Pump (Milford, PA), Waters UV 486 Tunable Absorbance Detector set at 254 nm, and Agilent EZChrom Elite software (Palo Alto, CA). All samples were run using 25-µL injections into a 5-mM p-toluenesulfanate and methanol (45:55) by volume mobile-phase solution. The HDTMA chromatographic separation was achieved after 5 min at a pump flow rate of 1 mL/min, using a 150-mm x 4.6-mm Nucleosil CN 5-µm column, packed with 5-µm particles (Supleco, Bellefonte Park, PA).

The hydrophobic SMZ was qualitatively characterized by observing relative rates of particle sedimentation. Using raw zeolite and cationic SMZ as a basis for comparison, twenty grams of each material was crushed and placed in glass bottles. A 40-mL volume of Type I water was added to each bottle, which was then thoroughly shaken. The mixtures were allowed to sit for 2 h. At the end of the 2 h the bottles were visually inspected for relative differences among the materials. It was observed that zeolite

particles remained in suspension in the case of the raw zeolite and the cationic SMZ. In contrast, the hydrophobic SMZ particles had mostly settled out of solution, indicating that there was a decrease in repulsive electrostatic forces, which allowed for the aggregation of particles. This behavior is consistent with the formation of a hydrophobic surface.

Phosphate Buffer Solution (PBS)

A pH 7 phosphate buffer solution (PBS) was used to suspend all organisms used in this study. Equal parts of 0.015-M NaCl, 7.5-mM Na_2HPO_4, and 2.5-mM NaH_2PO_4 were mixed to form the PBS. Every batch of PBS was sterilized in the autoclave (250^{o}F, 15 psi for 15 minutes) prior to use in organism suspensions. The PBS was stored at 4^{o}C following sterilization.

Saccharomyces cerevisiae (common bread yeast)

G. lamblia cysts are round or oval in shape with a diameter of 11-14 μm (17). The cysts are strongly negatively charged, with zeta potentials ranging from –20 to –13 mV at a neutral pH (12). The buoyant density of *G. lamblia* cysts are 1.04 g/mL (15). The *S. cerevisiae* cells (Ward's Natural Science, Rochester, NY) are generally ellipsoidal in shape, with a major diameter of 5-10 μm and a minor diameter of 1-7 μm. The surface charge of the *S. cerevisiae* has been observed to be –23.3 mV to –21.1 mV at 25^{o}C at pH 7 (16). The buoyant density of *S. cerevisiae* cells is 1.11 g/mL (2).

Culturing of *Saccharomyces cerevisiae*

A sabouraud dextrose broth (SDB) (Difco ®, Krackeler Scientific, Inc.) was used as the medium for culturing *S. cerevisiae* used in the batch experiments. This medium is typically composed of 5.0 g/L peptone from meat, 5.0 g/L peptone from casein, and 20

g/L D(+) glucose (7). The medium was prepared by adding 36-g of powdered SDB to 1000-mL of Type I water. For the column experiments, *S. cerevisiae* was cultured in a potato dextrose broth (PDB) (Difco ®, Krackler Scientific, Inc.). The medium was prepared by adding 24-g of powdered PDB to 1000-mL of Type I water. The pH of the PDB medium was adjusted to 5.2, with 1% NaOH in order to optimize the growth conditions for the yeast and to minimize the growth of contaminant bacteria. Both media were stirred until complete dissolution was observed and were then autoclaved at 121^{o}C and 15 psi for fifteen minutes.

The liquid medium was removed from the autoclave and allowed to cool to approximately 30^{o}C. Small amounts of *S. cerevisiae* were added to the broth and put on the automatic shaker for 24 h. The yeast cultures were kept at 30^{o}C and 50 rpm to optimize yeast growth and to ensure even cell-size distribution in the suspended colony. At the end of the 24-hour growth period, the cultures were removed from the shaker and added to centrifuge tubes for rinsing. Each tube was rinsed with Type I water until the supernatant was colorless. The yeast cells were re-suspended in 1 L of PBS in a single sterile flask. The yeast suspension was kept at 4^{o}C before and after use in the experiments, in order to minimize budding of yeast cells. Yeast suspensions were used in experiment the same day that they were made in order to minimize the growth of additional cells.

In order to increase the homogeneity of the yeast suspensions for use in the experiments, the suspension was kept on a stir plate while the suspension was sampled for the batch and column experiments.

Enumeration Methods

The cell concentration in samples was determined by direct counting filter membrane methods (13). The filter membranes used in this study were Advantex MFS black membranes composed of a mixture of nitrocellulose and other cellulose esters (Hardy Diagnostics, Santa Maria, CA). The filters have a pore size of 0.45 μm and a diameter of 47 mm, with a contrasting grid surface. For the preparation of samples, the filters were cut to a diameter of 25 mm, using a precision Osborne arch punch (Campbell-Bosworth Machining Co., Yoakum, TX).

Stainless steel filter holders (Millipore, Billerica, MA) were used for the filtering of samples. The filter holder, with the filter membrane in place, was autoclaved for 15 min at 121^{o}C and 15 psi. Following sterilization, the unit was allowed to cool to room temperature. A sterile syringe and needle (Fisher Scientific, Hampton, NH) were used to input 3 mL of PBS into the filter holder. The initial injection of PBS assisted with the even distribution of the cells across the filter membrane.

Following the PBS, a volume of sample was input through the filter holder. The volume of sample filtered was dependent on the anticipated concentration of the sample. Higher volumes were filtered for samples that were expected to have relatively low concentrations (10^4 or less). A minimum of 1 mL of sample was filtered in all cases. For samples with a concentration of 10^5 cells/mL or higher, the suspension was diluted by a factor of 100, to decrease the density of cells on the filter, allowing for a more accurate count.

The samples were then stained, on the filter, by injecting 5 mL of Acridine Orange (AO) (Sigma-Aldrich, St. Louis, MO). The stain was prepared by adding 0.25 g

of AO powder to 250 mL of Type I water. The suspension was sterilized by filter sterilization methods. To stain the samples on the filter, 5 drops of the sterile AC suspension was added to 5 mL of PBS and allowed to sit for 3 minutes. The stain was then injected into the filter holder.

Following the staining step, the filters were allowed to drain by gravity for 15 minutes. The filter holders were then opened, and the filters removed and placed on a glass microscope slide and allowed to dry. A cover slip was mounted on the dry filter with one drop of immersion oil and placed in a light-tight box for counting.

Prepared slides were counted on the epifluorescent microscope (MC100, Zeiss Axioskop, Thornwood, NY). The cells counts for a given sample were obtained from randomly selected fields, covering a wide area of the filter. The outer edges of the filter were avoided during counting. A total of either ten field-of-view areas or a total of 250 cells were counted for each filter, depending on which count yielded more cells counted per filter. The field-of-view area was defined by what was visible under the microscope at a given magnification. The concentration of cells/mL for the sample was calculated using the ratio of the effective area filtered to the field-of-view area, the average number of cells counted, and the volume of sample filtered.

Batch Experimental Methods

Batch experiments were performed, using *S. cerevisiae*, on cationic SMZ, hydrophobic SMZ and raw zeolite. For the experiments, 10 g of material was added to 20 mL of yeast suspension. Two yeast suspension concentrations were used: a high concentration (~10^6 cells/mL) and a lower concentration (~10^5 cells/mL). Samples were

placed in 30-mL PTFE centrifuge tubes and were mixed for 8 h at 100 rpm on the automatic shaker at a temperature of 25^{o}C. At the end of the 8-h period, the tubes were removed from the shaker and allowed to settle for approximately 1 min in order to allow for the separation of zeolite and suspended yeast cells. The settling rate of *S. cerevisiae* at 25^{o}C and pH 7 is $3.1x10^{-4}$ m/min (5.16 μm/s) (26). After 1 min, the yeast cells are estimated to have settled approximately 0.31 mm (310 μm). This distance is much less than the expected settling distance of the zeolite in the same period of time. During the SMZ preparation phase of this project, it was observed that the hydrophobic SMZ completely settled out of solution in at least 2 h, which corresponds to an approximate sedimentation rate on the order of $3x10^{-4}$ m/min (40-mL volume in the glass bottle equaled a height of ~38 mm). Since the exact time of settling is unknown, the actual sedimentation rate is likely to be much more rapid. In the case of the cationic SMZ and the raw zeolite, some particles remained in suspension after 2 h, but the suspension appeared to be clearer, indicating that some sedimentation was occurring. The separation of the zeolite particles from the *S. cerevisiae* cells was done to minimize interference from zeolite particles during the enumeration of *S. cerevisiae*.

Sterile syringes and needles were used to extract 3 mL of the initial and equilibrium suspensions. The initial suspension samples were diluted by a factor of 100 for counting and only 1 mL of sample was filtered. The equilibrium samples taken from tubes containing SMZ treatments were concentrated for counting by filtering 2 mL of sample. The raw zeolite equilibrium samples were diluted by a factor of 100 for counting. The samples were then analyzed using the filter membrane enumeration method.

Duplicates were prepared for each of the batch samples. In addition to duplicate samples, blank samples were included for each treatment, at each concentration, to determine the influence of the settling time and relative rates of settling. The blank samples consisted of *S. cerevisiae* suspended in PBS in the absence of zeolite.

Column Experimental Methods

Duplicate columns made of borosilicate glass, with PTFE end-pieces (Omnifit, Supelco, Bellefonte, PA) were used for the flow-through experiments. The columns had an inner diameter of 25 mm and were equipped with one fixed end-piece and one adjustable end-piece, which was adjusted down to a packed bed height of 48 mm. The columns were packed with cationic SMZ, hydrophobic SMZ, or raw zeolite. The three material treatments were sieved to the same grain size range of 1.4 to 2.4 mm by dry sieving, prior to the packing of columns. A coarse nylon mesh (approximately 780-μm pore diameter) was used to retain the material within the column. Three-way valves with Luer fittings (Cole-Palmer, Vernon Hills, IL) were fitted to each end of the column.

The packed columns were purged with CO_2 for 1 h and then saturated from the bottom by immediately injecting 20 mL of PBS into the column. The columns were assumed to be at steady state after the 1-h continuous injection of CO_2 followed by the 20 mL of PBS. The PBS was allowed to sit in the column for approximately 5 min prior to the first injection of yeast suspension. The pore volume (PV) was calculated from the measurement of the dry and saturated weights of each column.

Flow-through experiments were conducted in the saturated columns. Sterile labware and equipment was used throughout the column experiments. The stock yeast suspension for each column was sampled to determine the initial concentration. Pipettes

were used to extract 10-mL initial concentration aliquots from the yeast suspension beaker, which were then placed in 20-mL scintillation vials for analysis. Syringes were used to inject 1 PV of yeast suspension into each column and the effluent was collected. The effluent associated with this first pore volume of yeast suspension composed one sample. The first PV was then followed by a second injection of 1 PV of yeast suspension and the effluent was collected to generate a new sample. The 2nd PV of yeast suspension was then followed by an injection of PBS and the effluent was collected in the same manner. The pulses of suspension were injected at an average rate of 2.8 mL/s. Effluent samples were collected in 20-mL scintillation vials.

At the completion of a column experiment, for a given material treatment, the column was drained and the material was placed in a sterilized glass beaker for further treatment. A fraction of the material was added to agar plates for 48 h. The agar plates were visually inspected for yeast colony growth. Fifty milliliters of PBS was added to the remaining material in the beaker, which was then placed on the shaker at 100 rpm for 8 h. Ten milliliters of the supernatant was sampled with a sterile pipette and was placed in vials for analysis (see Appendix A).

Results and Discussion

Batch experiments results for Saccharomyces cerevisiae

Batch experimental data are presented in Figure 1 and Figure 2. Linear adsorption isotherms were approximated for the batch experiment data on linear and log-log scales using the following equations:

$S = K_d C$ **Equation 1: linear isotherm**

$$\log(S) = \log(K_F) - N\log(C)$$ **Equation 2: linearized Freundlich isotherm**

where S is the number of cells sorbed per dry unit of weight of solid (cells/M), C is the equilibrium concentration of the species (cells/L^3), and K_d and K_F are the partitioning coefficients. The isotherms in Figure 1 are nearly linear, with R^2 values ranging from 0.59 to 0.98 (Table 2). Figure 2 shows that the raw zeolite isotherm is approximately linear with an N value close to 1 (N=1.19), and an R^2 value of 0.96. The isotherms observed for the cationic and hydrophobic SMZ treatments are roughly linear with N values of 4.38 and 2.46 and R^2 of 0.83 and 0.87, respectively. The isotherms for all three treatments are assumed to be linear, although more points are needed to confirm this assumption. The K_d values for the different materials vary between 33.6 and 773 mL/g (Table 2). The cationic and hydrophobic treatments yielded K_d values that are 10 and 25 times greater than the coefficient found for the raw zeolite. *S. cerevisiae* was removed by the hydrophobic SMZ more effectively than by either the raw zeolite or the cationic treatment, for both concentrations tested.

The raw zeolite batch results yield percent removal values much greater than what was observed in the zeolite-free blanks. One possible mechanism that could be contributing to this apparent increase in percent removal is that the zeolite particles are "pushing" cells out of suspension. There is a large difference between the size of a zeolite particle (1.4-2.4 mm), compared to that of a *S. cerevisiae* cell (5-10 μm) that could result in cells becoming "trapped" under the zeolite particle as it settles out of suspension, removing it from the sampled equilibrium suspension. The percent removal determined

from the raw zeolite, cationic SMZ and hydrophobic SMZ batch studies may therefore over-estimate the removal of cells from suspension.

Experiments show a general, decreasing trend in the percent removal of cells at the lower concentration for all three of the materials tested. At the higher concentration average ($6.20x10^6$cells/mL), the percent removal range from 98.9-99.9% for the SMZ treatments (Table 3). In contrast, the percent removal values decrease to 85.6-98.3% at the lower concentration average ($2.01x10^5$ cells/mL) (Table 3). This trend could be due to differences in settling rates of cell aggregates due to increased cell flocculation at the higher concentration. The flocculation of yeast cells (namely *S. cerevisiae*) has been defined as "the phenomenon wherein yeast cells adhere in clumps and sediment rapidly from the medium in which they are suspended" (21). Cell surface properties, as well as environmental conditions, can lead to the onset of flocculation. Nutrient starvation and/or stress conditions can induce flocculation of *S. cerevisiae* cells (24). The exact mechanism leading to flocculation of yeast cells is not known, but is thought to be primarily due to physiochemical cell surface interactions, including hydrophobicity of yeast strains (21). To create the yeast suspensions, the cells are deprived of necessary nutrients. The suspension solution is a simple phosphate buffer solution that did not contain any glucose or sucrose, which are necessary for cell health. The higher concentration of cells will increase the cell-cell interactions, thereby increasing the chances of the formation of cell aggregates in suspension. The cell aggregates will settle out of suspension quickly and therefore, would not be sampled and included in the analysis of equilibrium concentrations.

Sample microscope slides were evaluated by visual inspection to determine if cell aggregates were more dominant for equilibrium concentrations from the high concentration batch studies. No notable aggregation of cells was observed in the slides. However, if the aggregates had settled out of solution it is likely that they would not have been sampled during the preparation of slides for analysis.

There was minimal removal (0.0994% to 22.0%) observed for the blank samples that were run in conjunction with the batch experiment studies performed using the three material treatments (Table 4). This indicates that there was not significant settling of cells during the time between removal from the shaker and the sampling of vials.

Column experiments for Saccharomyces cerevisiae

The data collected for the column experiments in this study did not have the resolution necessary to determine breakthrough curve characteristics. Instead, the percent removal for each column was calculated in terms of total yeast cells collected relative to the total number of yeast cells input into the columns (Equations 3a through 3c).

$$N_{Input} = V_{Total} \cdot C_o \qquad \textbf{Equation 3a}$$

$$N_{Collected} = V_{Slug} \cdot C_{1PV} + V_{Slug} \cdot C_{2PV} + V_{Slug} \cdot C_{3PV} \qquad \textbf{Equation 3b}$$

$$\%\ \text{Removal} = \left(1 - \frac{N_{Collected}}{N_{Input}}\right) \times 100 \qquad \textbf{Equation 3c}$$

Where N_{Input} is the total number of yeast cells injected into a column (cells), V_{Total} is the total volume of yeast suspension injected (L^3), C_o is the initial yeast concentration (cells/L^3), $N_{Collected}$ is the total number of cells collected in the effluent samples (cells),

V_{Slug} is the volume of effluent collected for a given slug injected (L^3), and C_{PV} is the effluent concentration measured in the effluent samples (cells/L^3).

Equation 3 was used to determine the removal efficiencies for each of the six columns (Table 4). There was a notable difference among the percent removal observed for the raw zeolite treatments and the two SMZ treatments. The average removal seen for the raw zeolite columns was 15.3% (Table 4). The cationic and hydrophobic SMZ treatments yielded average percent removals of 40.8% and 46.3%, respectively (Table 4). The column experimental results therefore indicated that the surfactant-treated zeolite material preferentially removed *S. cerevisiae*, with slightly increased removal efficiency with the hydrophobic SMZ material. The data from the column experiments were consistent with the trends observed in the batch experiments, where the surfactant-treated zeolite material preferentially removed *S. cerevisiae* cells relative to the raw zeolite material. Also, in both the batch and column experiments, the hydrophobic SMZ material appeared to be slightly more efficient at the removal of yeast cells from suspension.

In addition to calculating the percent removal for each of the columns, the loading rate of the filter-pack material was determined. Each column contained approximately 21 g of material. The loading rate for the filter-pack material used in the column studies was calculated by subtracting the total number of cells collected from the number of cells injected into the system. The average loading rate for the raw zeolite material was found to be 1.57×10^6 cells/g zeolite. The average loading rates for the cationic and hydrophobic SMZ materials were 4.32×10^6 cells/g zeolite and 5.20×10^6 cells/g zeolite, respectively (Table 4). The loading rates from the column studies can be compared to those

calculated from the batch experiments at the high concentration ($6.97x10^6$ cells/mL). The batch experimental data yielded an average loading rate of $1.17x10^7$ cells/g zeolite for the raw zeolite material. The loading rates observed for the batch SMZ treatments were similar, with values of $1.23x10^7$ cells/ g zeolite and $1.24x10^7$ cells/ g zeolite for cationic and hydrophobic SMZ, respectively (Table 3). The loading rates calculated from the batch experimental data are an order of magnitude higher than those found from the column studies, for all three materials tested. The fact that higher loading was observed for the batch experiments could indicate that saturation was not achieved in the column studies and therefore, higher percent removal values are likely to be possible under different flow conditions. In both the batch and column experiments, the apparent loading rate is influenced by mechanisms other than adsorption, since phenomena such as physical filtration and bio-films can affect the equilibrium suspension of cells.

Breakthrough curves were predicted for each of the three materials used in this study with the 1-dimensional advection-dispersion equation (Equation 4), using CXTFIT 2.1 (23).

$$R\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \qquad \textbf{Equation 4a}$$

where:

$$R = 1 + \frac{\rho_b}{\theta} K_d \qquad \textbf{Equation 4b}$$

$$D = \alpha v \qquad \textbf{Equation 4c}$$

and C is the effluent concentration (cells/L^3), D is the dispersion coefficient (L^2/T), v is the average linear velocity (L/T), R is the retardation factor, ρ_b is the bulk density (M/L^3), θ is the porosity, and α is the dispersivity (L).

The CXTFIT 2.1 program is a nonlinear, least-squares optimization code developed by Toride et al.(23). The values for porosity and bulk density were set to 0.67 and 0.9 g/cm^3 (3), respectively. The average flow rate for all experiments was $2.88x10^{-6}$ m^3/s, yielding an average linear velocity $8.77x10^{-3}$ m/s for the system. The dispersivity was assumed to be equal to the length of the column (0.05 m) and the resultant value for D was $4.4x10^{-4}$ m^2/s for all three columns. Using the K_d values calculated from the batch experimental data, the retardation factors were determined to be 46 for the raw zeolite, 256 for the cationic SMZ, and 1039 for the hydrophobic SMZ.

The observed column data was compared to the predicted breakthrough curve data at 3 PV (Figure 3, Figure 4, and Figure 5). In the case of the predicted results for both cationic and hydrophobic SMZ, no breakthrough was observed at 3 PV. A value of 0.01 for C/C_o was predicted for the raw zeolite material at 3 PV, which is much less than the 0.50 observed in the column experiments. The predicted breakthrough results are significantly lower than what was actually observed in the column experiments. This difference could be a result of physical mechanisms not taken into account by the predictive model, such as turbulent flow and shear forces.

Since the material treatments consisted of similar grain size distributions, the small percent removal (10.6-20.1%) observed for the raw zeolite columns is a strong indication that physical filtration was not a dominant process in the flow-through experiments. Therefore, the difference observed between the treated zeolite and the raw

zeolite was likely due to interactions between the yeast cells and the surfactant layer on the SMZ.

The relatively high flow velocity used in the column experiments may have had a significant effect on how many cells were removed from contaminated waters. One resultant effect of the higher flow velocities could have been shear forces acting along the grain surfaces of the filter material, which could have dislodged cells that may have adsorbed to the grain surfaces. In addition, the cells suspensions were being moved quickly through the columns, reducing the mean residence time of the organism. At the higher flow velocities, cells may have moved too quickly through the column, never having time to come into contact with the surface of the treated zeolite. If high flow velocities significantly lower the removal efficiency of SMZ, this would affect the application of SMZ filters at drinking water wells. Typical flow velocities at pumping wells are 20 –1000 L/min (19). Assuming a typical pumping well diameter of 0.15 m (6.0 in) (11) and a screened interval of 6.1 m (20 ft) (11), the flow velocity at a pumping well would range from 0.41 to 41 m/d. The low-end flow velocity at a typical pumping well is very close to the average flow velocity used in the column experiments, which was approximately 0.35 m/d.

One possible application of SMZ in water treatment is through the implementation of a permeable barrier to treat contaminated groundwater. The flow rates used in this study are far greater than the groundwater flow velocities that exist in natural systems. Commonly, groundwater moves through aquifer systems at an average velocity of 15 m/d (18), compared to the flow velocity of 160 m/d used in the column experiments. It is possible that at lower flow rates, such as those observed in natural

systems, percent removal rates of *S. cerevisiae*, and its pathogenic surrogate *Giardia sp.*, would be greatly increased to levels much closer to those seen in the batch studies.

Conclusions

The batch experiments completed in this study demonstrate a strong, preferential removal of *S. cerevisiae* cells by the hydrophobic surfactant-modified zeolite material, relative to the raw zeolite material. The K_d values calculated for the cationic and hydrophobic treatments are 10 to 25 times greater than the K_d value for raw zeolite. The yeast cells are most likely being removed by electrostatic or hydrophobic interactions occurring between the organisms and the treated zeolite surfaces. In addition, the hydrophobic SMZ treatment demonstrates slightly more efficient cell removal, removing 97.9-99.8% of the *S. cerevisiae* cells in suspension relative to a range of 85.6-99.4% for the cationic SMZ treatment. There is a general decrease in the percent removal between the high and low concentrations tested in the batch studies. This decrease in removal could be the result of cell-cell interaction mechanisms, such as yeast flocculation.

The preferential removal of organisms from suspension by the treated zeolite materials is also observed in the column experiments. The raw zeolite material demonstrates significantly lower removal (10.6-20.1%), compared to the cationic and hydrophobic SMZ treatments (39.2-42.3% and 43.2-49.3%, respectively). The low removal observed in the raw zeolite columns indicates that physical filtration is not a primary factor in this set of experiments. The hydrophobic SMZ treatment columns result in slightly greater removal compared to the cationic treatment, mirroring the observations from the batch studies.

The increased removal observed for the hydrophobic SMZ in both the batch and column studies could be a result of hydrophobic interactions between the yeast cells and the SMZ dominating over the electrostatic interactions. Smit et al. (21) found that proteins on the surface of *S. cerevisiae* result in surface hydrophobicity for the cell. The degree of hydrophobicity is strongly dependent on environmental conditions, especially nitrogen availability. The experiment performed by Smit et al. (21) indicates a rapid increase in the hydrophobicity of the yeast cell surface with increasingly nitrogen-limited environments. In the case of this study, *S. cerevisiae* are in nitrogen-limited conditions and therefore likely demonstrate highly hydrophobic surface characteristics. The hydrophobic nature of the cells likely dominated the interactions with the hydrophobic SMZ.

The results of the batch and column studies completed with *S. cerevisiae* are analogous to the results one would expect with actual *G. lamblia* cysts. The physical properties of *S. cerevisiae* are similar to those of *G. lamblia*, with the advantage of being viable (active) organisms. Both organisms are negatively charged at a neutral pH and 25^{o}C, indicating that adsorption interactions occurring with *S. cerevisiae* would also likely occur with the *G. lamblia* cysts. The batch studies completed with *S. cerevisiae* indicate that SMZ treatments have high removal rates but are comparable to current water treatment techniques (e.g. slow sand filtration).

The enumeration method employed in this study for the determination of *S. cerevisiae* cell concentrations is recommended for microbiological flow-through treatments because of the ability to count a wide range of concentrations. The largest source of error in this method is in the sample preparation and counting yeast cells. A

homogeneous distribution of cells on the filter is crucial to obtaining counts that are close approximations of the true concentrations. Zones of high yeast density or clumping of cells on the filter surface will yield over-approximations of cell concentration.

Recommended future work includes flow-rate optimization studies to evaluate how the flow rate affects the percent removal of the column treatments. Based on the results of the batch experiments, we believe that if flow-through experiments were conducted at flow rates closer to those observed in nature, the column experiments would yield much higher removal efficiencies.

Characterization of the cell surface properties and the interactions between the SMZ surface and the cell is also recommended. The high removal rates observed in the batch studies only indicate that there is a strong difference between the treated and untreated zeolite, but do not answer the question of whether or not the cells are being adsorbed onto the grain surface. The other possible influences on the observed concentrations are flocculation of *S. cerevisiae* cells and that the surfactant on the SMZ is effectively killing the cells. In addition to the cell-surface interactions, it is important to determine how the cell surface properties change with age of the cell, as well as pH and temperature of the suspension. This cell characterization will increase the understanding of the interactions occurring, thereby increasing the ability of the SMZ filter systems to adapt to water treatment facilities.

The SMZ material can be used in multiple applications, within the context of water treatment for microbial contamination. If future studies determine that there is greater than 99% removal at low flow rates, then it is possible that portable filter configurations could be used in backpacking filters. Inexpensive, point-of-use filters can

also be constructed out of the SMZ material that can be placed in homes. A “tea-bag” configuration is another possible application where the user essentially “swirls” the bag in water to remove microorganisms. The results from this study indicate that the optimal applications of SMZ for water treatment are in conditions where the water flow rates are very low and contact times are long.

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Table 1: Typical removal efficiencies of *G. lamblia* and other pathogens for water treatment technologies that are currently used (9).

Organisms	Coagulation and sedimentation (% removal)	Rapid Filtration (% removal)	Slow sand filtration (% removal)
Total coliforms	74-97	50-98	>99.999
Fecal coliforms	76-63	50-98	>99.999
Enteric viruses	88-95	10-99	>99.999
Giardia	58-99	97-99.9	>99
Cryptosporidium	90	99-99	99

Table 2: Batch Experiment results (K_d, N, and R^2) for the *S. cerevisiae* adsorption isotherms

	(Equation 1)		(Equation 2)	
	K_d	**R^2**	**N**	**R^2**
Raw Zeolite	33.6	0.980	1.19	0.958
Cationic SMZ	192	0.592	4.38	0.826
Hydrophobic SMZ	773	0.686	2.46	0.871

Table 3: Summary table of the equilibrium concentration (C_e), sorbed concentration (S), and percent removal for the batch experiments.

		Initial Conc. (cells/mL)	C_e (cells/mL)	S (cells/g)	% Removal	Avg. % Removal	Std. Dev. (%)	CV*
Raw Zeolite								
C_o	*A*	$6.97x10^6$	$3.58x10^5$	$1.32x10^7$	94.9	94.3	0.783	0.0083
	B	$5.43x10^6$	$3.39x10^5$	$1.02x10^7$	93.8			
$0.03C_o$	*A*	$1.94x10^5$	$2.90x10^4$	$3.30x10^5$	85.1	89.0	5.62	0.063
	B	$2.07x10^5$	$1.45x10^4$	$3.85x10^5$	93.0			
Cationic SMZ								
C_o	*A*	$6.97x10^6$	$4.25x10^4$	$1.39x10^7$	99.4	99.2	0.314	0.0032
	B	$5.43x10^6$	$5.72x10^4$	$1.07x10^7$	98.9			
$0.03C_o$	*A*	$1.94x10^5$	$2.80x10^4$	$3.32x10^5$	85.6	87.5	2.69	0.031
	B	$2.07x10^5$	$2.20x10^4$	$3.70x10^5$	89.4			
Hydrophobic SMZ								
C_o	*A*	$6.97x10^6$	$1.01x10^4$	$1.39x10^7$	99.9	99.8	0.120	0.0012
	B	$5.43x10^6$	$1.71x10^4$	$1.08x10^7$	99.7			
$0.03C_o$	*A*	$1.94x10^5$	$3.33x10^3$	$3.81x10^5$	98.3	98.1	0.276	0.0028
	B	$2.07x10^5$	$4.36x10^3$	$4.05x10^5$	97.9			
Zeolite-Free Blanks								
C_o	*A*	$6.97x10^6$	$5.43x10^6$	N/A	22.1	N/A	N/A	N/A
	B	$5.43x10^6$	*Not measured*					
$0.03C_o$	*A*	$1.94x10^5$	$1.94x10^5$	N/A	0.0994	0.148	0.000683	0.0046
	B	$2.07x10^5$	$2.07x10^5$	N/A	0.196			

**CV denotes coefficient of variation; N/A denotes not applicable*

Table 4: Removal efficiencies of *S. cerevisiae* for each of the six columns tested.

	S (cells/g zeolite)	% Removal	Avg. % Removal	Std. Dev. (%)	CV*
Raw Zeolite					
Column A	2.11×10^6	20.1	15.3	6.70	0.44
Column B	1.03×10^6	10.6			
Cationic SMZ					
Column A	4.07×10^6	39.2	40.8	2.18	0.053
Column B	4.58×10^6	42.3			
Hydrophobic SMZ					
Column A	5.80×10^6	49.3	46.3	4.35	0.094
Column B	4.60×10^6	43.2			

**CV denotes coefficient of variation*

Figure Captions

Figure 1. Isotherms for *S. cerevisiae* and three zeolite treatments (raw, cationic, and hydrophobic SMZ) based on batch experiment results.

Figure 2. Linearized Freundlich isotherm for *S. cerevisiae* and three zeolite treatments (raw, cationic, and hydrophobic SMZ), based on the batch experiment results.

Figure 3. Predicted breakthrough curve for raw zeolite.

Figure 4. Predicted breakthrough curve for cationic SMZ.

Figure 5. Predicted breakthrough curve for hydrophobic SMZ.

Figure 1: Isotherms for *S. cerevisiae* and three zeolite treatments (raw, cationic, and hydrophobic SMZ) based on batch experiment results.

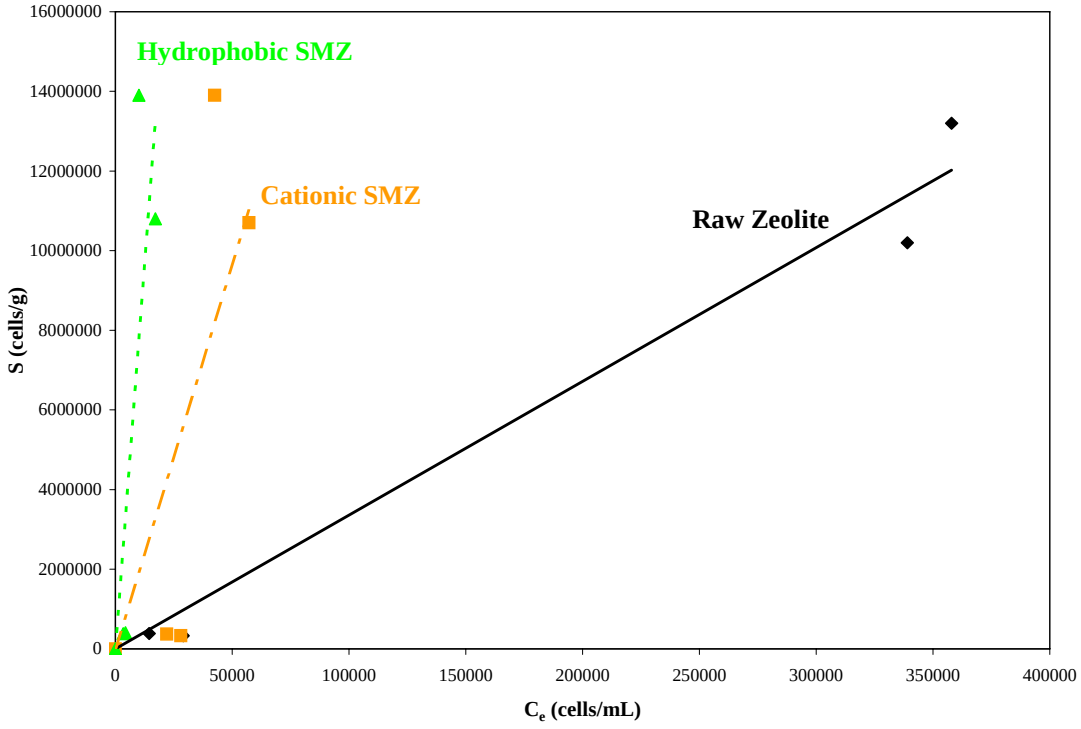


Figure 2: Linearized Freundlich isotherm for *S. cerevisiae* and three zeolite treatments (raw, cationic, and hydrophobic SMZ), based on the batch experiment results.

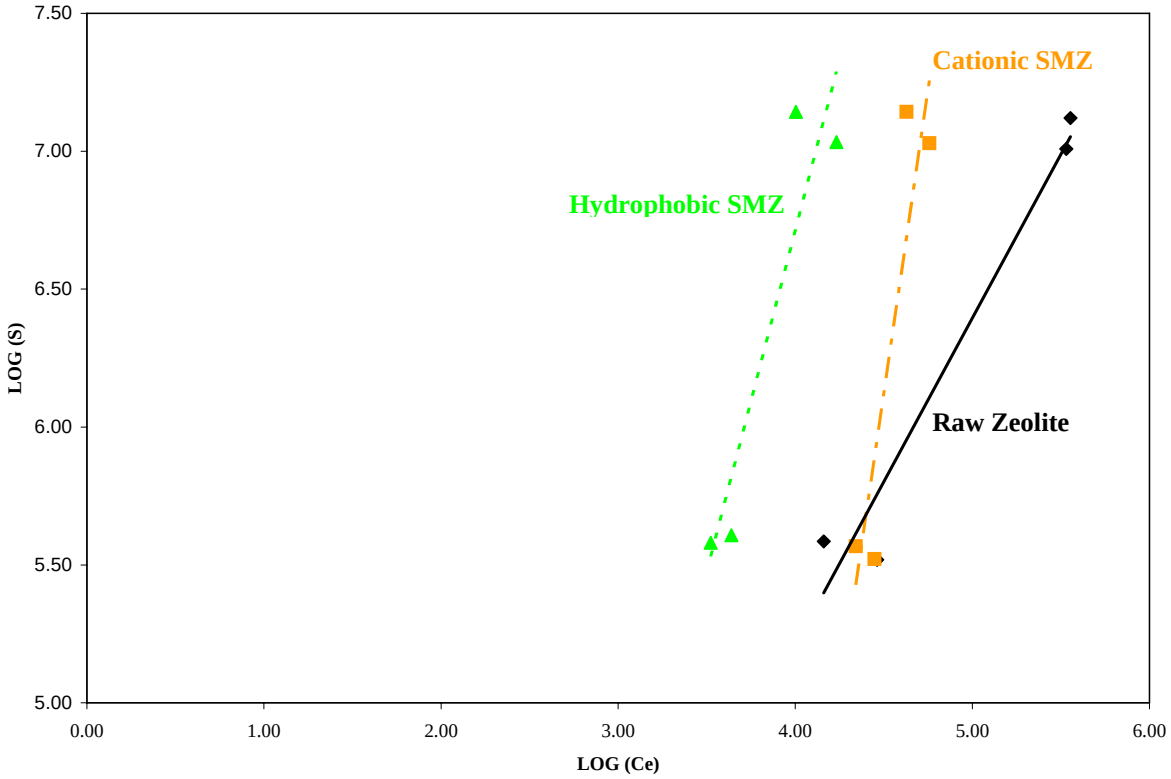


Figure 3: Predicted breakthrough curve for raw zeolite.

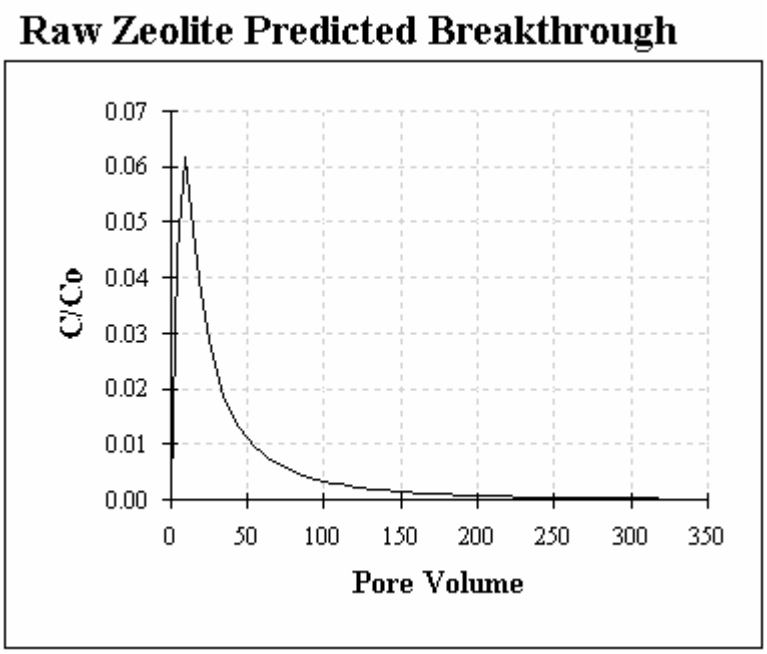


Figure 4: Predicted breakthrough curve for cationic SMZ.

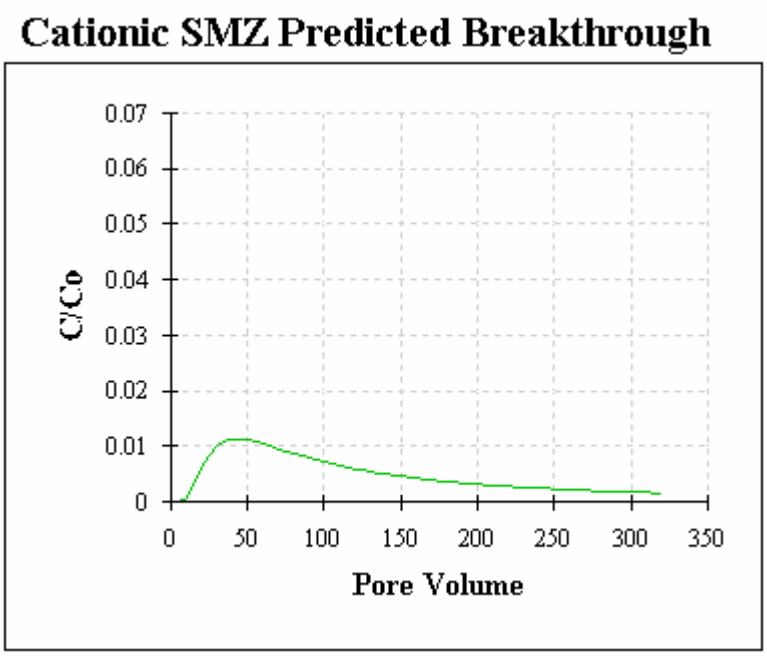
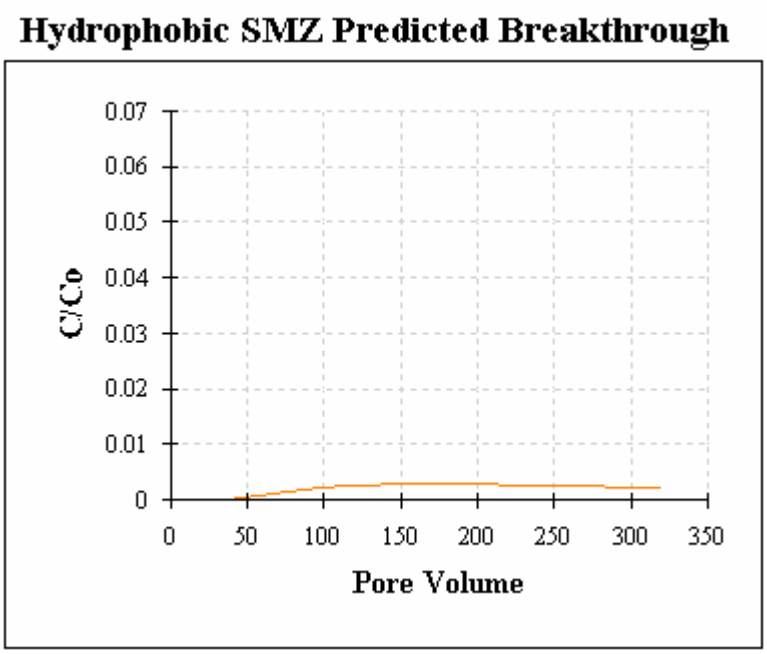


Figure 5: Predicted breakthrough curve for hydrophobic SMZ.



Introduction to Appendices

The following appendices provide descriptions and figures from post-column experiment work, previous enumeration attempts, and a prototype filter pack design tested in the field. The appendices are intended to provide information on methods used and the experimental data collected throughout the duration of the thesis project.

Appendix A contains descriptions of methods and results from post-column experiment studies: shake and sprinkle tests. Conclusions based on these tests are presented in this appendix.

Appendix B describes the concentration quantification method initially used in this thesis study, hemacytometry. The appendix describes the methods used, the scoping and method development work completed with hemacytometry, and reasons for discontinued use.

Appendix C describes the preliminary column experiment work that was completed. This appendix contains a description of the initial set-up, problems that were encountered and corrections that were made for the column experiments described in the manuscript.

Appendix D describes the filter pack that was designed and used in a field study conducted near Pullman, WA. The appendix presents proposed areas of improvement for future work with a filter pack design.

Appendix E contains the figures, data tables, and plots for the experiments described in the manuscript: pictures of the laboratory set-up used for the preparation the

hydrophobic SMZ, batch experiment tabulated data, and tabulated data for the column experiments.

Appendix F contains pictures of *G. lamblia* cysts and *S. cerevisiae* cells. This appendix also includes a snapshot of the epifluorescent microscope field of view for the enumeration of *S. cerevisiae*.

Appendix G contains the graphical outputs from the CXTFIT solution of the 1-dimensional advection-dispersion equation that was used to predict breakthrough curves for the column experiments. This appendix also contains the text output file with the predicted concentrations for each pore volume.

Appendix H contains all of the references cited in the appendices.

Appendix A: Shake and Sprinkle tests

Shake Test

Following the completion of the flow-through experiments, the used column filter material was emptied into duplicate, sterile glass beakers. A volume of PBS was added to the beaker, at a ratio of 2 units of volume to 1 unit of zeolite weight (Appendix Table A-1). The samples were then mixed at 100 rpm for 8 h at 25^{o}C. At the end of the mixing period, a volume of the supernatant was sampled and analyzed using the same enumeration methods employed in the batch and column experiments.

The beakers containing raw zeolite material yielded 10 times more cells per volume relative to both the cationic and hydrophobic treatments. Therefore, some cells were being held in the material by physical filtering, or gravitational setting, as indicated by the higher concentration in the raw zeolite. The lower numbers of cells in the supernatant associated with the SMZ treatments indicate that the physical filtering and gravitational settling phenomena are less important.

Sprinkle Test

Petri dishes were prepared with potato dextrose agar. A small portion of the used filter material was distributed on the agar plate and allowed to sit at room temperature for 48 h. The plates were visually inspected for growth of yeast (Appendix Figure A- 1, Appendix Figure A- 2, and Appendix Figure A- 3). The greatest amount of yeast growth was observed on the plate containing raw zeolite. There was minimal growth associated with the hydrophobic SMZ material and no growth was observed for the cationic SMZ.

The increased growth associated with the raw zeolite material correlates well with the results of the shake test, further indicating that a fraction of cells were being loosely retained in the raw zeolite material.

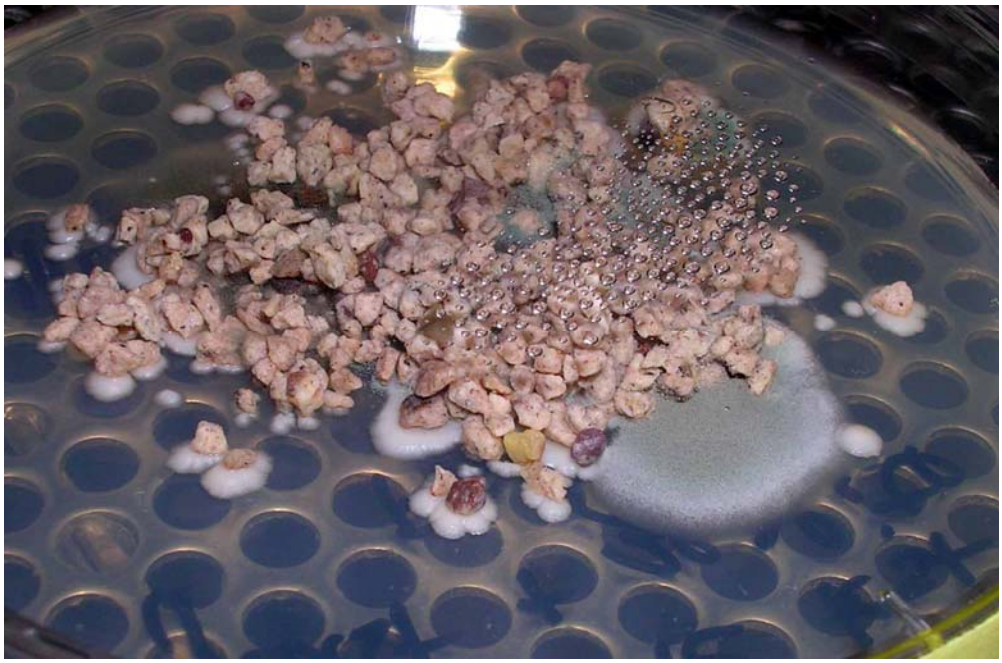
The lack of growth associated with the cationic material could be because the cells are strongly adsorbed to the material and therefore, were unable to grow on the agar. An additional explanation would be that the surfactant bi-layer on the surface of the zeolite is actually a biocide and effectively kills the organisms.

Appendix Table A- 1: Equilibrium concentrations measured in the supernatant of the shake test samples.

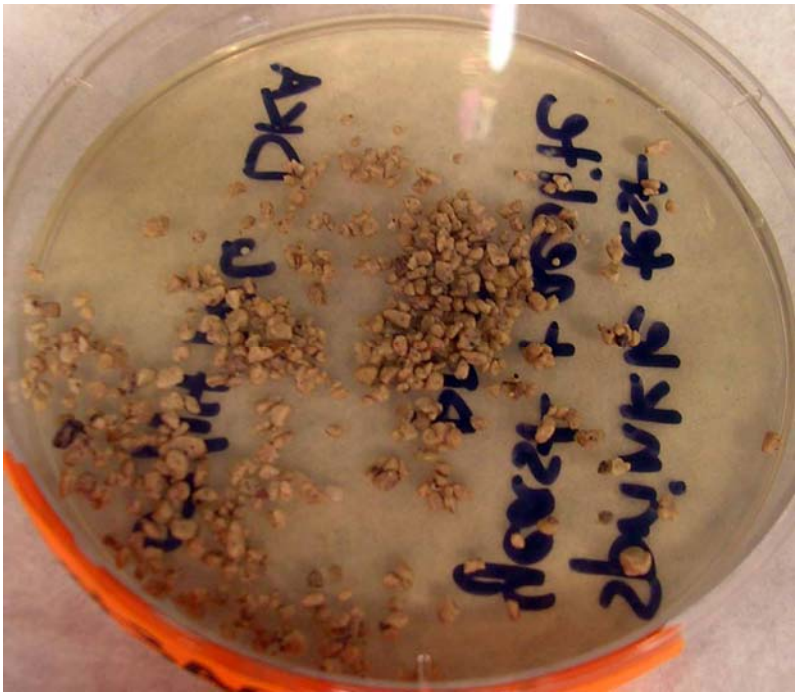
	Ce (cells/mL)	Avg. (cells/mL)	Std. Dev.	CV*
Raw Zeolite				
Beaker A	2.32×10^5	4.00×10^5	2.37×10^5	0.59
Beaker B	5.67×10^5			
Cationic SMZ				
Beaker A	4.16×10^4	3.72×10^4	6.22×10^3	0.17
Beaker B	3.28×10^4			
Hydrophobic SMZ				
Beaker A	3.40×10^4	2.58×10^4	1.16×10^4	0.45
Beaker B	1.76×10^4			

**CV denotes coefficient of variation*

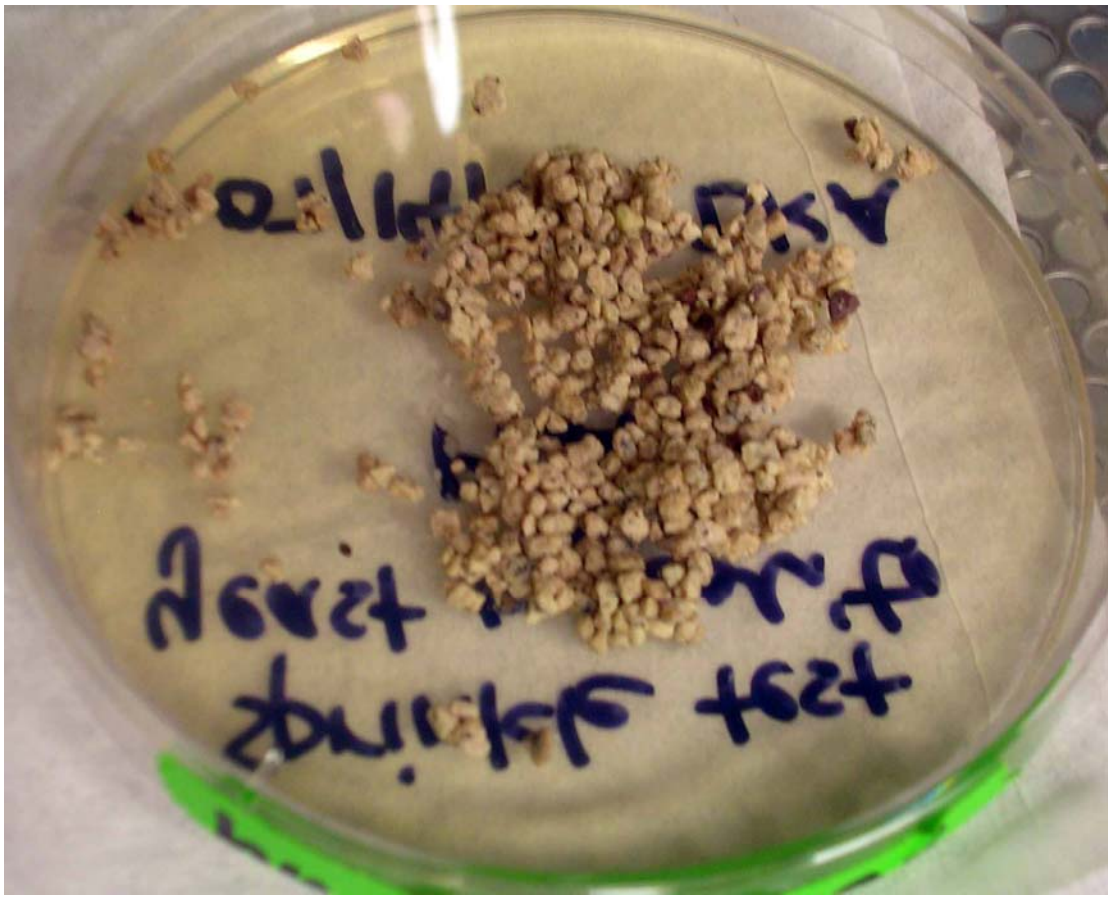
Appendix Figure A- 1: Raw zeolite with yeast growth (creamy, white substance) on an agar plate.



Appendix Figure A- 2: Cationic SMZ material, demonstrating no noticeable yeast growth on an agar plate.



Appendix Figure A- 3: Hydrophobic SMZ with minor yeast growth on an agar plate.



Appendix B: Alternative enumeration methods (hemacytometry)

Currently, microbiologists employ various methods for quantifying concentrations of *Giardia lamblia* in water samples. The proposed quantification methods in the current literature are flow cytometry, hemacytometry, and filter membrane enumeration. In this study, the initial mode of quantification was hemacytometry.

Suspensions containing non-viable *G. lamblia* cysts were obtained from Waterborne, Inc. (New Orleans, LA). The cysts came suspended in 5% formalin and 0.01% Tween 20. At the time of this study, the highest concentration of cysts available ($6.25x10^5$ cysts/mL) was purchased for quantification method development. The scope of the method development was to determine that counts could be reproduced over a range of dilutions, using a Bright Line Counting Chamber (Hausser Scientific, Horsham, PA).

A total of 90 μL of cyst suspension was sampled using a Rainin pipette (Rainin, Oakland, CA) and injected into a single well of a cell culture plate. A 10-μL aliquot of iodine stain was added to the cyst suspension in the well and mixed for thorough staining. From the stained suspension, 10 μL was extracted and was transferred to one side of the hemacytometer. An additional 10-μL volume was injected into the other half of the counting chamber. The hemacytometer was then placed on a light microscope and examined for the presence of cysts.

At the highest concentration of cysts used in the method development process, cysts were rarely observed on the surface of the counting chamber, among multiple aliquots taken from the same suspension. Despite the seemingly high concentration of cysts ($\sim10^5$ cysts/mL), this concentration is too low for hemacytometry applications.

High concentrations must be counted on the grid of a hemacytometer in order for the concentrations measured to be statistically accurate (2). Due to the small sub-sample collected (10 μL) for the hemacytometry methods, at lower concentrations, it is possible to sample fractions of the suspension that do not contain cysts.

The U.S. Environmental Protection Agency (EPA) has stipulated criteria for the enumeration of *G. lamblia* cysts through the use of hemacytometry (1). The EPA states that at least 50 cysts must be obtained from counting the four, 1-mm^2 corners of the hemacytometer grid (Appendix Figure B- 1) (1).

The calculation of total cell/cyst concentration for hemacytometry is a function of the depth of the plate, total area counted and the cells counted within that area. The depth of a Bright-Line hemacytometer is 0.1 mm. The volume of suspension contained in the four, 1-mm^2 regions of the hemacytometer grid is equal to 0.4 mm. Given the maximum concentration of G. lamblia cysts available (~10^5 cysts/mL), it would be expected that 40 cysts would be counted in the four, 1-mm^2 corners of the grid. A total count of 40 cysts is below the acceptable lower limit of 50 cysts,as stated by the EPA. In addition, the 10^5 cyst/mL concentration is in a vial containing a total of 8 mL which is a too small of a volume for batch and column experiment applications. Volumes on the order of 100's of mL to L are required to perform complete batch and column experiments. To obtain the volumes required for these experiments, the initial vial volume would need to be increased, thereby diluting the concentration to levels much lower than what can be feasibly counted with a hemacytometer.

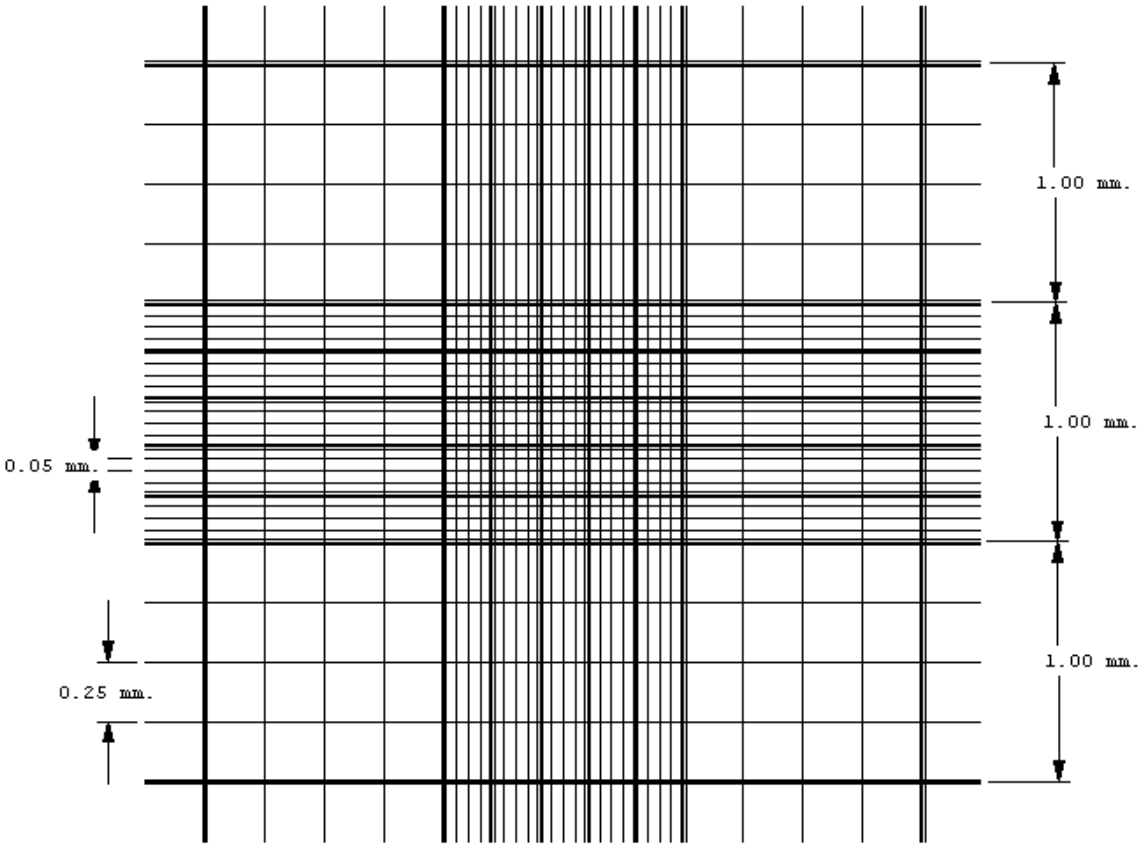
When this study was initiated, the maximum concentration of cysts available from Waterborne Inc. was the 10^5 cyst/mL concentration discussed in this section.

Waterborne, Inc., now offers a concentration of 10^8 cysts/mL. This initial concentration would need to be diluted before it could be counted on the cell plate because it would yield tens of thousands of cysts per plate. A concentration of 10^8 cysts/mL could possibly be applied to batch and column experiments that are done at small scales.

An additional concern when working with hemacytometry is the issue of homogeneity. If the organisms are not evenly distributed throughout the suspension suspension, the aliquots taken from the suspension do not represent the true concentration. In order to homogenize the suspension, suspensions would need to be constantly agitated (e.g. stirred on a stir plate) during sampling. Medema et al. (3) determined that the mode of mixing of *Giardia sp*. suspensions has a significant effect on the homogeneity of the cyst suspension. In their study, the researchers added a small volume (10-120 μL) of cyst stock to a large volume (100-150 mL) of suspension solution. This mode of mixing resulted in a wide range of concentration measurements for the same suspension. The second mode of mixing employed in Medema et al. (3) was combining the cyst stock and suspension solution in 1:1 volume basis until the required volume was reached. The concentrations determined for this method closely fit the expected Poisson distribution of counts.

For all enumeration techniques applied to the quantification of *Giardia sp*. concentrations, the issue of suspension homogeneity must be addressed. The method of mixing employed to create cysts suspensions should be considered to ensure the even distribution of cysts in suspension. In addition to proper mixing, cyst suspensions should be constantly agitated during sampling for enumeration to ensure that a representative volume is being collected.

Appendix Figure B- 1: Diagram depicting the grid that is etched onto the surface of the hemacytometer platform (7).



Appendix C: Preliminary column experiments

Previous work completed by Charlotte Salazar (6) examined the removal efficiency of treated zeolite materials, using microspheres as surrogates to *Giardia lamblia* and *Cryptosporidium parvum*. The batch experiments indicated that there was a strong removal of the microspheres by the surfactant treated zeolite material, but no significant differences were observed between the materials during the column experiments. The lack of difference in removal efficiencies was attributed to physical filtration by both the filter medium and the mesh used in the column end pieces. Using the lessons learned in the work completed by Salazar (6), preliminary column studies were completed such that physical filtration would be minimized, allowing for cell-zeolite interactions to be observed. The preliminary scoping work would also indicate what concentration of cells should be used in order to maintain concentration levels that would be statistically possible to count using the filter membrane method.

The diameter of the column was 25 mm. The bed height of the packed filter material reduced by approximately 2/3 from the work completed by Salazar (6). The microsphere column experiments were conducted in 150-mm long beds (6); the bed height used in this study was 48 mm. The mesh used in this study was coarser, with an approximate pore size of 780 μm. Appendix Figure C- 1 depicts the column experimental set-up used for the scoping experiments, as well as the column experiments presented in the body of the text.

Four columns were tested in the preliminary work to determine the influence of physical filtration on the removal of cells from suspension. Two raw zeolite columns and two hydrophobic SMZ columns were tested. The hydrophobic SMZ was used in the

scoping work because it was expected to have the highest removal of cells from suspension. The effluent concentrations from the hydrophobic columns would provide information on what minimum starting concentrations should be used.

The columns were saturated with CO_2 for 4 h, followed by an injection of 20 mL of PBS for complete saturation. A 2-PV slug was injected into the column and the effluent was collected for analysis. Initial concentration samples were also collected.

Appendix Table C- 1 is a tabulation of the concentrations observed for the preliminary column study. Appendix Table C- 2 summarizes the percent removal for the raw and hydrophobic columns. There is not data for the B column of the hydrophobic SMZ material. The samples associated with this column were not analyzed for one week, during which time they became contaminated with a bacteria that subsequently consumed the majority of the yeast cells in the samples.

Despite the lack of data for the B column of the hydrophobic material, it was decided that the information obtained from the preliminary work was enough to move forward with the complete column experiments. The very low percent removal for each of the raw zeolite columns indicated that physical filtration was very minor in the experiments. Also, the concentration in the hydrophobic effluent was easily counted using the filter membrane method.

Appendix Table C- 1: Concentrations measured in the initial and effluent samples during preliminary column experiments.

	RAW ZEOLITE				
	Column A (cells/mL)	**Column B (cells/mL)**	**Average (cells/mL)**	**Std. Dev. (cells/mL)**	**CV***
C_o	$2.08x10^6$	$2.65x10^6$	$2.36x10^6$	$4.01x10^5$	0.17
2 PV	$1.95x10^6$	$1.92x10^6$	$1.94x10^6$	$1.98x10^4$	0.01
	HYDROPHOBIC SMZ				
	Column A (cells/mL)	**Column B (cells/mL)**	**Average (cells/mL)**	**Std. Dev. (cells/mL)**	**CV**
C_o	$5.99x10^5$	***	$5.99x10^5$	N/A	N/A
2 PV	$1.20x10^3$	***	$1.20x10^3$	N/A	N/A

**CV denotes coefficient of variation*

Appendix Table C- 2: Percent removal values for the preliminary column studies.

	% Removal	**Average % Removal**	**Std. Dev. (%)**
Raw Zeolite			
Column A	6.15	16.75	14.98
Column B	27.34		
Hydrophobic SMZ			
Column A	99.80		
Column B	***		

Appendix Figure C- 1: Set-up used for the column experiments.

Appendix D: Pullman Field Filter-pack design

In October 2005, field study was completed in Pullman, WA to test a prototype filter pack for the removal of *Giardia lamblia* and *Cryptosporidium parvum*. The field-testing of the filter prototype was accomplished through collaboration with Colleen Rust and Dirk Schulze-Makuch, of Washington State University who headed the field study as part of the research requirements for a Master's thesis. Details of the field site and the results can be obtained from the thesis (5).

A filter pack was designed and constructed as part of the work completed for this thesis. Since the scope of the field study was to test a removal efficiency of a prototype filter pack, the filter was placed within the well at a depth below the lowest drawdown level. A submersible sampling pump was placed in the well, below the bottom of the filter pack, to sample the water entering the filter. The effluent water was sampled from the pump discharge (Appendix Figure D- 1).

It was decided that the majority of the filter pack should be made out of a fabric to hold the filter material. This decision was based on the need to have the flexibility to fit filter pack in the well, in addition to the electrical cords and sampling tubing associated with the submersible sampling pump. The fabric casing allowed for the filter pack to essentially seal the well to minimize leaks around the filter.

Appendix Figure D- 2 is a schematic diagram of the filter design that was constructed for the field test (Appendix Figure D- 3). The diameter of the filter was 4.25 in and was approximately 41 inches in length. The majority of the filter was made out of a 25-micron polyester mesh material that is typically used by the oilfield industry to remove particles from the recovered oil. The end caps of the filter were constructed out

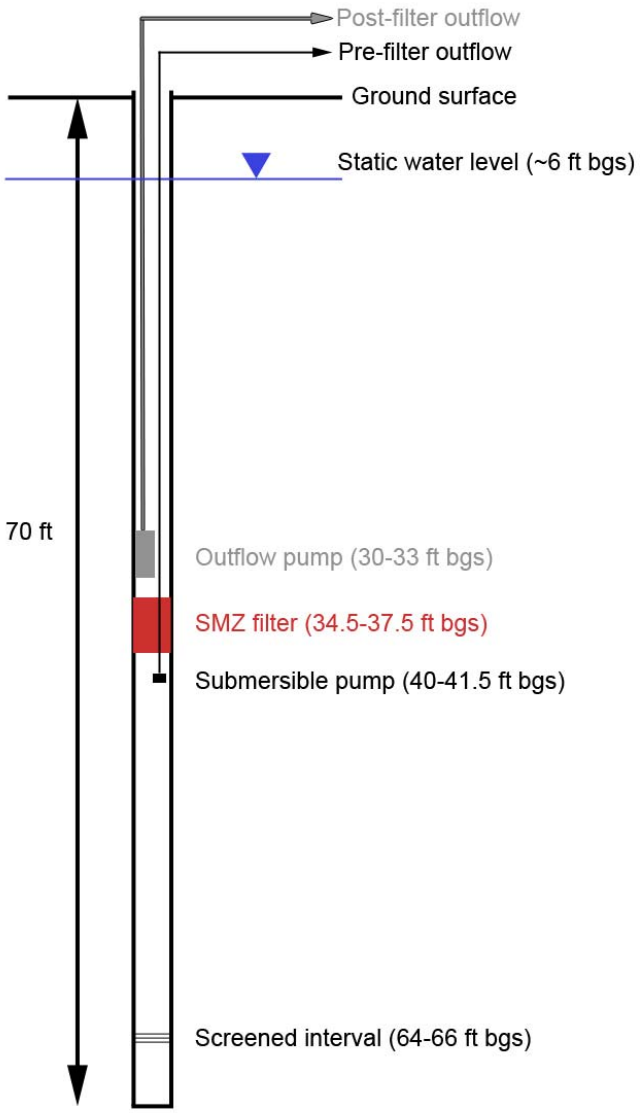
of a nylon window screen, backed by a nylon active wear mesh for durability. Two sections of PVC pipe were used at both ends of the filter as an anchoring point for both the filter material and the steel cables that were used to maneuver the filter in and out of the well. The steel cables used in the filter design were enclosed in the filter material and were coated with a durable polyurethane coating to prevent rusting of the cables in the well. Additionally, the tubing and cords associated with the submersible pump were run through the middle of the filter in order to prevent preferentially pathways between the filter and the sides of the well. Cationic surfactant-modified zeolite (8-14 mesh range, 1.4- to 2.4-mm grain size) was used as the filter pack material.

Prior to the field study, and the construction of the filter pack, the drawdown in the well and the surrounding observation wells was determined for the flow rates that would be used in the study. Based on the expected drawdown in the well, the height of the top of the filter pack was set to be 40 ft below the ground surface. From this, the length of the filter pack was designed such that it would be long enough to allow for a pumping rate of 20 gpm.

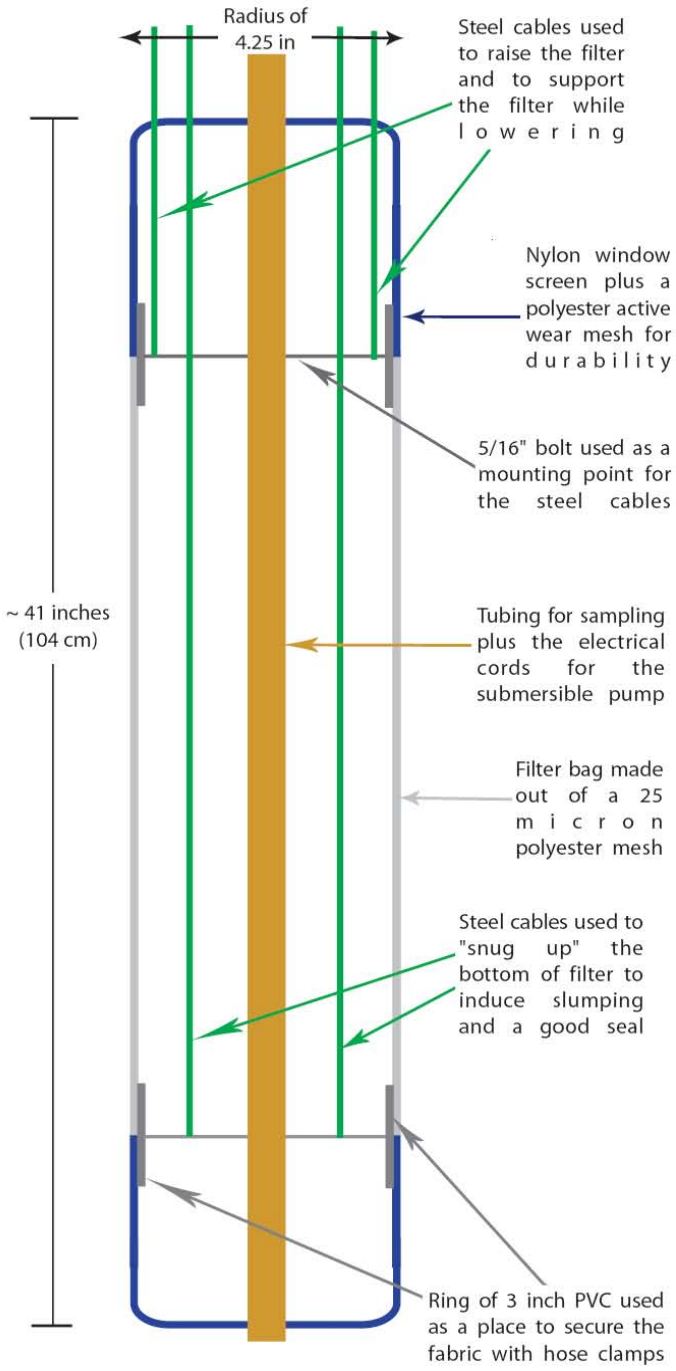
One the problems experienced with the filter design was that it was difficult to place into the well. The empty filter bag had to be lowered into the well and then filled with the zeolite material. After the bag was filled with zeolite, it was allowed to drop the desired depth by gravity, which took approximately 45 minutes. Appendix Figure D- 3 shows the filled filter bag, after it was removed from the well upon completion of the field test. Once the filter was in place, it took as many as three people to lower in the outflow pump: at least one person had to keep the steel cables, sampling tubing and pump electrical from getting caught up with the outflow pump tubing.

The nylon window screen mesh, in addition to the 25-micron polyester sides, was likely too small of a pore size for the filter application. In addition to too small of a pore size, the length of the filter was not optimal for the field conditions. The drawdown measured in throughout the duration of the field study was much greater than what was observed in previous pump test studies. For future field studies testing a prototype SMZ filtration system, it is recommended that the filter system be external to the well.

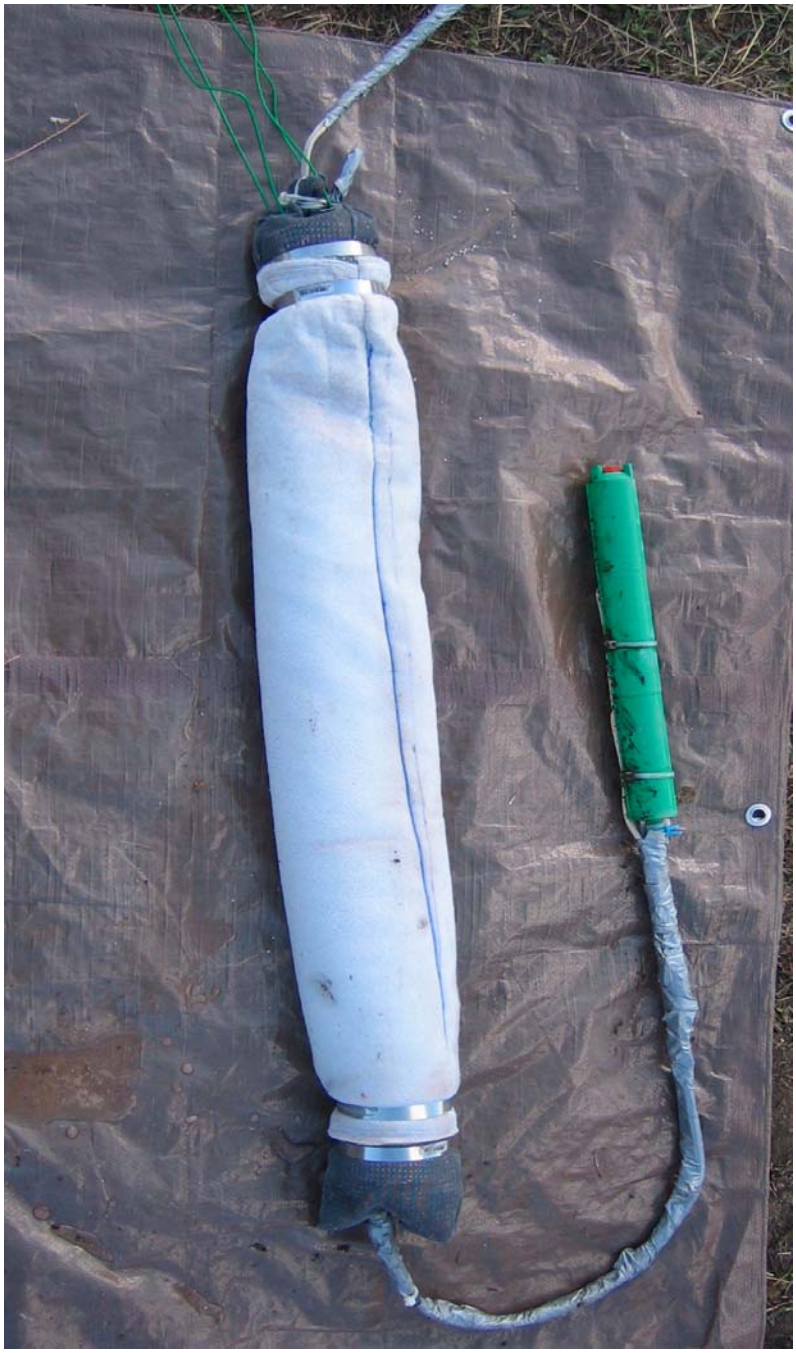
Appendix Figure D- 1: Schematic of the well set-up employed in the Pullman, WA field test. The diagram depicts the influent and effluence samples ports, as well as the location of the filter pack in the pumping well.



Appendix Figure D- 2: Diagram depicting the construction of the filter prototype tested in the field. The view is a cross-sectional view of the filter.



Appendix Figure D- 3: Picture of the actual filter tested in the field. This photo was taken at the end of the field test.



Appendix E: Data tables and plots for experiments described in the manuscript

Appendix Table E- 1, Appendix Table E- 2, and Appendix Table E- 3 are the tabulated concentrations for the column experiments. From these concentrations, the percent removal values were calculated for each of the six columns. The percent removal values are described in the manuscript in the discussion on the column studies.

Appendix Table E- 1: Concentrations measured for the samples collected from the raw zeolite column experiments.

	RAW ZEOLITE			
	Column A (cells/mL)	**Column B (cells/mL)**	**Standard Deviation (cells/mL)**	**Coeff. of Variation**
C_o	6.97×10^6	6.47×10^6	1.21×10^6	0.18
1 PV	1.57×10^6	1.50×10^6	5.42×10^5	0.35
2 PV	6.41×10^6	6.59×10^6	3.44×10^6	0.53
3 PV	3.16×10^6	3.49×10^6	8.07×10^5	0.24

Appendix Table E- 2: Concentrations measured for the cationic SMZ column experiments.

	CATIONIC SMZ			
	Column A (cells/mL)	**Column B (cells/mL)**	**Standard Deviation (cells/mL)**	**Coeff. of Variation**
C_o	6.87×10^6	7.16×10^6	1.40×10^6	0.20
1 PV	2.66×10^6	1.53×10^6	7.10×10^5	0.34
2 PV	3.02×10^6	3.76×10^6	9.46×10^5	0.28
3 PV	2.67×10^6	2.97×10^6	6.57×10^5	0.23

Appendix Table E- 3: Concentrations measured for the hydrophobic SMZ column experiments.

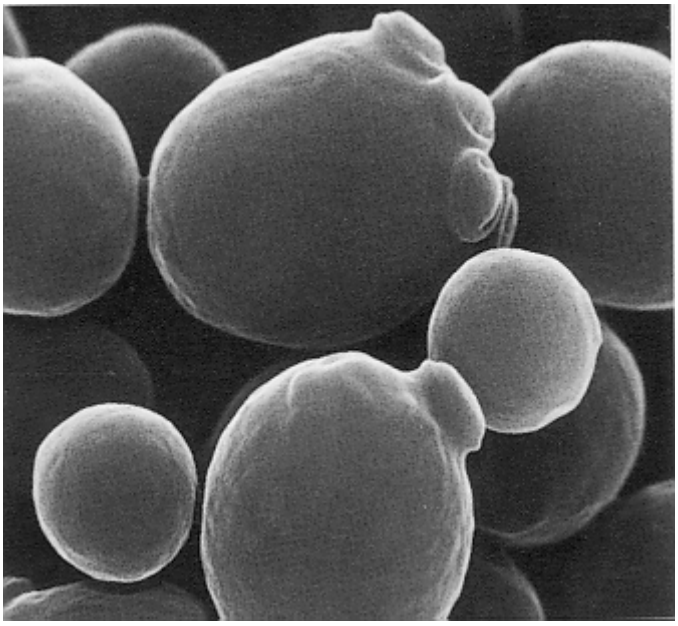
	HYDROPHOBIC SMZ			
	Column A (cells/mL)	**Column B (cells/mL)**	**Standard Deviation (cells/mL)**	**Coeff. of Variation**
C_o	7.79×10^6	7.06×10^6	2.80×10^6	0.38
1 PV	2.15×10^6	1.80×10^6	3.98×10^5	0.20
2 PV	3.42×10^6	3.88×10^6	7.52×10^5	0.21
3 PV	2.32×10^6	2.34×10^6	9.52×10^5	0.41

Appendix F: Relevant pictures of G. lamblia and S.cerevisiae

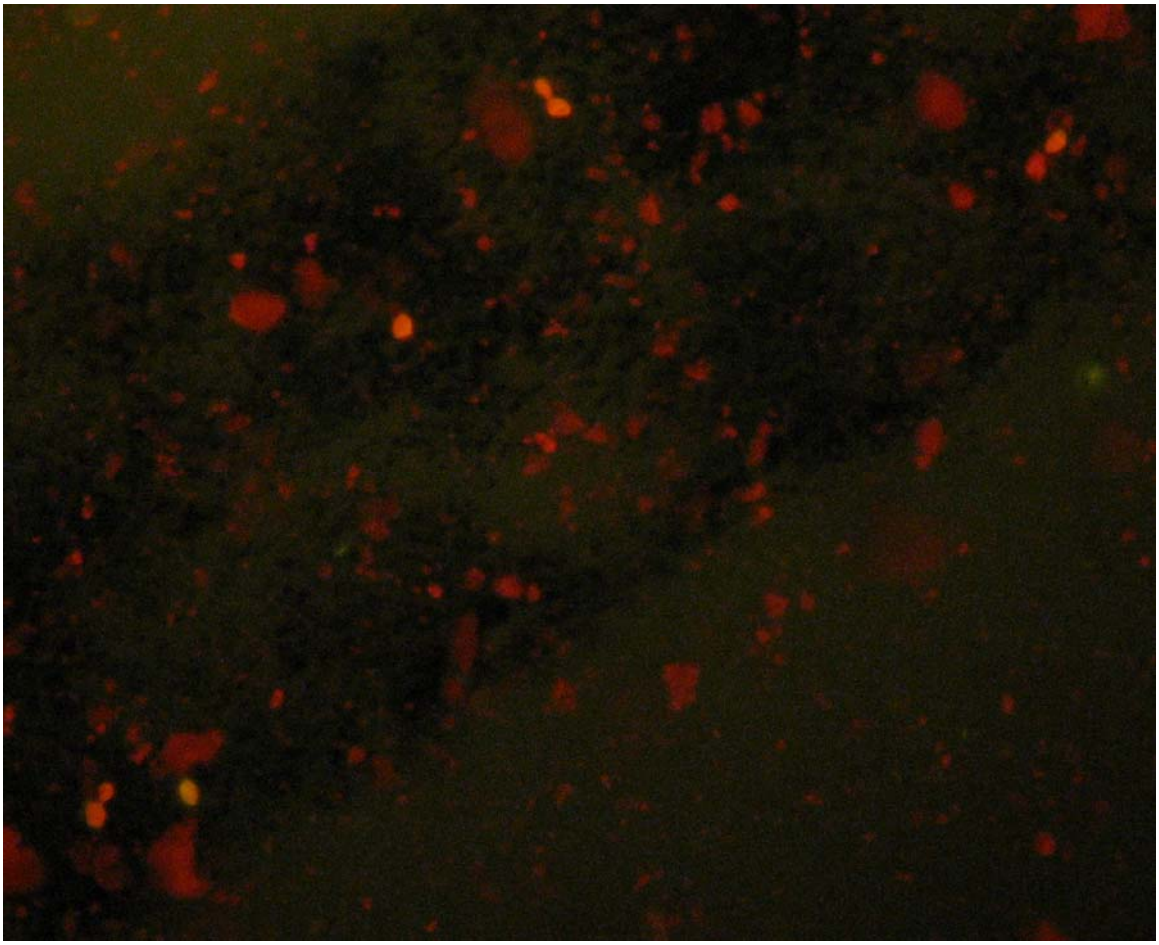
Appendix Figure F-1 is a scanning electron micrograph of *Giardia lamblia* cysts. A scanning electron micrograph of the *Saccharomyces cerevisiae* cells can be found in Appendix Figure F-2. Appendix Figure F-3 is a snapshot of the epifluorescent microscope field of view (400x) of the stained *S. cerevisiae* cells.

Appendix Figure F- 1: SEM of *G. lamblia* cysts (4).

Appendix Figure F- 2: SEM of *S. cerevisiae* cells (8).



Appendix Figure F- 3: Microscopic field of view of stained *S. cerevisiae*, taken for a column experiment sample.



Appendix G: Graphical and text outputs from CXTFIT predicted breakthrough curves

The graphical outputs illustrate the predicted breakthrough curves for the raw zeolite, cationic surfactant-modified zeolite (SMZ), and the hydrophobic SMZ. The parameters used for the solution of the 1-dimensional advection-dispersion equation can be found in the main body of this manuscript. In addition the graphical outputs, this appendix also contains the text output files for each of the three materials.

CXTFIT Text Output File for Raw Zeolite:

```
*********************************************************************
     *
*
     *     CXTFIT Version 2.1W (10/14/99)
*
     *     Analytical solutions for one-dimensional CDE
*
     *     Direct problem
*
     *
*
     *     Welcome to CXTFIT
*
     *     J.Simunek
*
     *
*
     *     Data input file:  CXTFIT.IN
*
     *
*

*********************************************************************


     Model description
     =================
        Deterministic equilibrium CDE (Mode=1)
        Flux-averaged concentration
        Reduced time (T), Dimensional position(Z)
          (All parameters except D and V are dimensionless)
        Characteristic length =     .0500
          for dimensionless parameters


     Initial values of coefficients
     ==============================
     Name        Initial value
      V........     .8770E-02
      D........     .4400E-03
      R........     .4600E+02
      mu.......     .0000E+00


     Boundary, initial, and production conditions
     ============================================
       Single pulse of conc. =    1.0000 & duration =    2.0000
       Solute free initial condition
       No production term


  Z=   .0500     (Flux conc. vs. time)
  Sum(C*dT)=       .3517
```

Time	C
.1000	.00000E+00
.2000	.15768E-25
.3000	.37965E-17
.4000	.61330E-13
.5000	.21031E-10
.6000	.10463E-08
.7000	.17231E-07
.8000	.14200E-06
.9000	.73677E-06
1.0000	.27635E-05
1.1000	.81822E-05
1.2000	.20281E-04
1.3000	.43847E-04
1.4000	.85099E-04
1.5000	.15143E-03
1.6000	.25115E-03
1.7000	.39305E-03
1.8000	.58599E-03
1.9000	.83862E-03
2.0000	.11591E-02
2.1000	.15547E-02
2.2000	.20322E-02
2.3000	.25969E-02
2.4000	.32537E-02
2.5000	.40060E-02
2.6000	.48567E-02
2.7000	.58075E-02
2.8000	.68594E-02
2.9000	.80123E-02
3.0000	.92649E-02
3.1000	.10614E-01
3.2000	.12056E-01
3.3000	.13583E-01
3.4000	.15186E-01
3.5000	.16857E-01
3.6000	.18584E-01
3.7000	.20355E-01
3.8000	.22159E-01
3.9000	.23983E-01
4.0000	.25815E-01
4.1000	.27647E-01
4.2000	.29467E-01
4.3000	.31266E-01
4.4000	.33037E-01
4.5000	.34773E-01
4.6000	.36468E-01
4.7000	.38117E-01
4.8000	.39715E-01
4.9000	.41260E-01
5.0000	.42749E-01
5.1000	.44181E-01
5.2000	.45553E-01
5.3000	.46865E-01
5.4000	.48117E-01
5.5000	.49309E-01
5.6000	.50441E-01

5.7000	.51514E-01
5.8000	.52529E-01
5.9000	.53487E-01
6.0000	.54390E-01
6.1000	.55238E-01
6.2000	.56033E-01
6.3000	.56777E-01
6.4000	.57472E-01
6.5000	.58119E-01
6.6000	.58719E-01
6.7000	.59275E-01
6.8000	.59788E-01
6.9000	.60261E-01
7.0000	.60693E-01
7.1000	.61088E-01
7.2000	.61447E-01
7.3000	.61771E-01
7.4000	.62062E-01
7.5000	.62321E-01
7.6000	.62550E-01
7.7000	.62751E-01
7.8000	.62924E-01
7.9000	.63070E-01
8.0000	.63192E-01
8.1000	.63290E-01
8.2000	.63366E-01
8.3000	.63420E-01
8.4000	.63454E-01
8.5000	.63468E-01
8.6000	.63464E-01
8.7000	.63443E-01
8.8000	.63406E-01
8.9000	.63352E-01
9.0000	.63284E-01
9.1000	.63202E-01
9.2000	.63106E-01
9.3000	.62998E-01
9.4000	.62879E-01
9.5000	.62748E-01
9.6000	.62606E-01
9.7000	.62455E-01
9.8000	.62294E-01
9.9000	.62124E-01
10.0000	.61946E-01

CXTFIT Text Output File for Cationic SMZ:

```
*********************************************************************
     *
*
     *     CXTFIT Version 2.1W (10/14/99)
*
     *     Analytical solutions for one-dimensional CDE
*
     *     Direct problem
*
     *
*
     *     Welcome to CXTFIT
*
     *     J.Simunek
*
     *
*
     *     Data input file:  CXTFIT.IN
*
     *
*

*********************************************************************


     Model description
     =================
        Deterministic equilibrium CDE (Mode=1)
        Flux-averaged concentration
        Reduced time (T), Dimensional position(Z)
          (All parameters except D and V are dimensionless)
        Characteristic length =     .0500
          for dimensionless parameters


     Initial values of coefficients
     ==============================
     Name        Initial value
      V........     .8770E-02
      D........     .4400E-03
      R........     .2590E+03
      mu.......     .0000E+00


     Boundary, initial, and production conditions
     ============================================
       Single pulse of conc. =    1.0000 & duration =    2.0000
       Solute free initial condition
       No production term


  Z=   .0500     (Flux conc. vs. time)
```

Sum(C*dT)= 1.5393

Time	C
.1000	.00000E+00
5.1000	.80146E-06
10.1000	.46513E-03
15.1000	.28648E-02
20.1000	.59785E-02
25.1000	.84621E-02
30.1000	.10057E-01
35.1000	.10924E-01
40.1000	.11280E-01
45.1000	.11301E-01
50.1000	.11112E-01
55.1000	.10797E-01
60.1000	.10410E-01
65.1000	.99877E-02
70.1000	.95519E-02
75.1000	.91171E-02
80.1000	.86924E-02
85.1000	.82830E-02
90.1000	.78918E-02
95.1000	.75203E-02
100.1000	.71688E-02
105.1000	.68371E-02
110.1000	.65247E-02
115.1000	.62307E-02
120.1000	.59542E-02
125.1000	.56941E-02
130.1000	.54494E-02
135.1000	.52191E-02
140.1000	.50023E-02
145.1000	.47981E-02
150.1000	.46056E-02
155.1000	.44239E-02
160.1000	.42523E-02
165.1000	.40902E-02
170.1000	.39369E-02
175.1000	.37918E-02
180.1000	.36543E-02
185.1000	.35239E-02
190.1000	.34002E-02
195.1000	.32827E-02
200.1000	.31710E-02
205.1000	.30648E-02
210.1000	.29637E-02
215.1000	.28673E-02
220.1000	.27755E-02
225.1000	.26878E-02
230.1000	.26042E-02
235.1000	.25242E-02
240.1000	.24478E-02
245.1000	.23747E-02
250.1000	.23047E-02
255.1000	.22377E-02
260.1000	.21735E-02
265.1000	.21118E-02
270.1000	.20526E-02

```
275.1000   .19958E-02
280.1000   .19413E-02
285.1000   .18889E-02
290.1000   .18385E-02
295.1000   .17900E-02
300.1000   .17433E-02
305.1000   .16983E-02
310.1000   .16550E-02
315.1000   .16132E-02
320.1000   .15730E-02
```

CXTFIT Text Output File for Hydrophobic SMZ:

```
*********************************************************************
     *
*
     *     CXTFIT Version 2.1W (10/14/99)
*
     *     Analytical solutions for one-dimensional CDE
*
     *     Direct problem
*
     *
*
     *     Welcome to CXTFIT
*
     *     J.Simunek
*
     *
*
     *     Data input file:  CXTFIT.IN
*
     *
*

*********************************************************************


     Model description
     =================
        Deterministic equilibrium CDE (Mode=1)
        Flux-averaged concentration
        Reduced time (T), Dimensional position(Z)
          (All parameters except D and V are dimensionless)
        Characteristic length =     .0500
          for dimensionless parameters


     Initial values of coefficients
     ==============================
     Name        Initial value
      V........     .8770E-02
      D........     .4400E-03
      R........     .1039E+04
      mu.......     .0000E+00


     Boundary, initial, and production conditions
     ============================================
       Single pulse of conc. =    1.0000 & duration =    2.0000
       Solute free initial condition
       No production term


  Z=   .0500     (Flux conc. vs. time)
```

Sum(C*dT)= .6373

Time	C
.1000	.00000E+00
5.1000	.11657E-22
10.1000	.13262E-11
15.1000	.72690E-08
20.1000	.48867E-06
25.1000	.55342E-05
30.1000	.26092E-04
35.1000	.75404E-04
40.1000	.16163E-03
45.1000	.28525E-03
50.1000	.44070E-03
55.1000	.61940E-03
60.1000	.81213E-03
65.1000	.10105E-02
70.1000	.12078E-02
75.1000	.13988E-02
80.1000	.15797E-02
85.1000	.17484E-02
90.1000	.19033E-02
95.1000	.20439E-02
100.1000	.21702E-02
105.1000	.22825E-02
110.1000	.23813E-02
115.1000	.24676E-02
120.1000	.25420E-02
125.1000	.26055E-02
130.1000	.26591E-02
135.1000	.27035E-02
140.1000	.27396E-02
145.1000	.27683E-02
150.1000	.27902E-02
155.1000	.28060E-02
160.1000	.28164E-02
165.1000	.28220E-02
170.1000	.28232E-02
175.1000	.28205E-02
180.1000	.28144E-02
185.1000	.28053E-02
190.1000	.27935E-02
195.1000	.27793E-02
200.1000	.27631E-02
205.1000	.27451E-02
210.1000	.27254E-02
215.1000	.27044E-02
220.1000	.26823E-02
225.1000	.26591E-02
230.1000	.26350E-02
235.1000	.26102E-02
240.1000	.25848E-02
245.1000	.25590E-02
250.1000	.25327E-02
255.1000	.25061E-02
260.1000	.24792E-02
265.1000	.24522E-02
270.1000	.24251E-02

```
275.1000   .23979E-02
280.1000   .23707E-02
285.1000   .23436E-02
290.1000   .23166E-02
295.1000   .22896E-02
300.1000   .22628E-02
305.1000   .22362E-02
310.1000   .22098E-02
315.1000   .21836E-02
320.1000   .21576E-02
```

Appendix H: Appendix references

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