



WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: An Investigation of the Factors Affecting Removal of *Cryptosporidium* and *Giardia* from Drinking Water Supplies by Granular Media Filtration

Keywords: *Cryptosporidium*, *Giardia*, Drinking Water, Filtration, Biofilm

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Statement of Critical Regional or State Water Problems

Cryptosporidium and *Giardia* are protozoan pathogens that are commonly found in drinking water supplies from surface sources (13, 15, 20, 25). LeChevallier and Norton (13) analyzed 347 raw water samples from 72 surface water treatment plants in the U.S. and Canada and reported *Giardia* and *Cryptosporidium* prevalence rates of 53.9 % and 60.2 %, respectively. An earlier study by the same researchers found that the Mississippi, Ohio, and Missouri rivers contain some of the highest concentrations of both *Cryptosporidium* and *Giardia* in the nation (15). The concentrations of *Cryptosporidium* in these rivers ranged from 10 to 480 oocysts/L. These parasitic protozoa, when ingested, can cause gastroenteritis in humans and can even result in the death of immunocompromised individuals (27). An outbreak of cryptosporidiosis, the illness caused by *Cryptosporidium*, occurred in Milwaukee, Wisconsin in 1993, affecting over 400,000 people (17). This episode was the largest recorded waterborne disease outbreak in the history of the United States. Many other outbreaks of cryptosporidiosis have been reported in the literature (e.g. 9, 16). The infectious dose for humans is not known, but is believed to be fewer than 10 oocysts (22) and could be as low as one oocyst (8). Clearly there is cause for concern in the upper midwestern United States over the potential for outbreaks of cryptosporidiosis and giardiasis.

Giardia and *Cryptosporidium* are protozoan pathogens that have two stages in their life cycles. The active, growing, reproducing form, present in the human or animal host, is called the trophozoite. In response to stimuli in the host's intestinal tract, *Giardia* and

Cryptosporidium are induced to produce the dormant or transmissive forms termed cysts and oocysts, respectively which are excreted into the environment. The cysts and oocysts are relatively hardy forms of the enteric protozoa that demonstrate excellent survival over a wide range of conditions (24) and are much more resistant to chemical disinfection than enteric bacteria and viruses (26). Therefore, the primary means of removal in most water treatment plants is through particle removal processes including chemical coagulation, flocculation, sedimentation, and filtration. Any factor that impacts the particle removal processes, especially filtration, will undoubtedly effect the removal of protozoa and ultimately the potential for outbreaks of giardiasis and cryptosporidiosis. One such factor that is especially relevant is the presence of biofilm in the filter bed, because changes in water treatment practice are likely to result in increased accumulation of biofilm in drinking water filters. Biofilms consist of bacteria and other microorganisms embedded in a matrix of extracellular polymers. Biofilm will alter the surface properties of the filter media and will most likely impact the deposition behavior of suspended particles in the filter bed. It is imperative that the effects of biofilm on removal of *Giardia* and *Cryptosporidium* be systematically evaluated in a controlled study in order to determine the potential health risks and to develop filter operational guidelines for optimum removal of protozoa.

Statement of Expected Results or Benefits

The main objective of this research is to evaluate the impact of filter operating conditions on the removal of *Giardia* cysts, *Cryptosporidium* oocysts, and other particles in drinking water filters. A 2⁵ factorial design study will be used to evaluate the impact of a variety of factors on the removal of protozoan cysts and oocysts in drinking water filters including: 1) the presence or absence of biofilm on the filter media, 2) coagulant type (alum and ferric chloride), 3) the presence or absence of dissolved natural organic matter (NOM), 4) hydraulic loading rate, and 5) the type of filter media (sand and GAC). Little is known about the impact of biofilm on particle removal in rapid filters, therefore, a key aspect of this research is to compare particle removal in “clean” filters and in filters containing biofilm. The goal of this research is to compare particle removal over a wide range of conditions in order to develop rapid filter operational guidelines that will provide for optimum removal of *Giardia* and *Cryptosporidium*. For example, if the presence of biofilm significantly impairs the removal of protozoa from the water supply, then this suggests that water utilities should consider elimination of biological activity in the filters (if possible) or consider a two-stage filter system. In a two stage filter system, the first stage filter would be operated as a biologically-active filter or biofilter and the second stage filter would have little or no biofilm in the filter bed. In addition, the use of a factorial design study will allow us to evaluate synergistic effects that may arise from various combinations of conditions. For example, if biofilm impairs deposition of particles, then a high hydraulic loading rate will most likely exacerbate the particle removal deficiencies by decreasing the residence time of the particles in the filter. The main benefit of this research will be recommendations for filter operation that will lead to improved filtration performance, especially with regard to the removal of *Giardia* and *Cryptosporidium*. This project will benefit the people of Minnesota and the nation as a

whole by providing information that is relevant to the prevention of waterborne disease and the production of safe, high-quality drinking water.

Nature and Objectives of the Research

Giardia and *Cryptosporidium* are pathogenic protozoa that are commonly found in drinking water supplies obtained from surface sources and their removal from water is highly dependent on filter performance. The main objective of this research is to evaluate the impact of various operational parameters on the removal of *Giardia* cysts, *Cryptosporidium* oocysts, and other particles in rapid filters. A key aspect of this research is to compare particle removal in “clean” filters and in filters containing biofilm to determine if removal of these particles is impaired or improved by the presence of biofilm. This research will lead to filter operational guidelines for achieving effective removal of protozoa from drinking water supplies.

Methods, Procedures, and Facilities

Phase I: Setup and Test Experimental Filtration Systems

A schematic diagram of the laboratory-scale filtration systems to be used in this research is shown in Figure 1. The laboratory-scale filter consists of a 2.54 cm (1 inch) ID x 61.0 cm (24 inch) PlexiglasTM column. The filter column will be packed with either 0.5 mm glass beads, filter sand (effective size of approximately 0.5 mm), or granular activated carbon (GAC) (effective size of approximately 0.7 mm). Feed solutions will be pumped into the filters using peristaltic pumps (Cole-Parmer Instrument Company) at loading rates within the range of typical rapid filter operation (5 to 25 m/h). Two parallel filters will be constructed for this research. One filter will be operated with a clean filter bed and the other will be operated as a biofilter with biofilm coated filter media. The biofilm will be established by seeding the filter with bacteria (a mixed population from the Mississippi River) and the biofilm will be maintained by pumping organic substrate (ozonated NOM from the Mississippi River) and nutrients into the filter. After the filters are constructed, the hydraulic characteristics of the filter systems will be evaluated using conservative tracer studies. Phase I of the project will be completed within the first year.

Phase II: Initial Filtration Studies with Latex Microspheres

Latex microspheres (Interfacial Dynamics Corp.) will serve as model particles and spherical glass beads (0.5 mm) will serve as model filter media (i.e. collectors) in the initial filtration studies. The purpose of the initial filtration studies is twofold. First, we will check our experimental methodology with a well-defined experimental system consisting of uniform spherical particles and clean spherical collectors. Then, we will add the complicating factor of biofilm and evaluate the effect of particle size on removal of particles in biologically active filters. Three different particle sizes will be selected to represent viruses (10 – 100 nm), bacteria (1 - 2 μm), and protozoan cysts (5 to 20 μm). Filter influent particle concentrations of at least $10^5/\text{mL}$ will be used so that accurate measurements of particle removal can be obtained. The concentration of latex

microspheres will typically be determined by light absorbance (460 nm). However, if the detachment of biofilm or bacteria during the filtration experiments with biofilm coated filter media interferes with the measurement of the latex microspheres, then latex microspheres containing fluorescent dye will be used along with epifluorescence microscopy for enumeration. Phase II will be completed within the first year of the project.

Phase III: Giardia and Cryptosporidium Filtration Experiments

The initial filtration experiments will be followed by experiments with virulent *Giardia* cysts and *Cryptosporidium* oocysts. The cysts and oocysts will be enumerated by the

Figure 1. Schematic diagram of the laboratory-scale filtration apparatus

immunofluorescence (IFA) assay technique (12). In natural water samples, there is typically interference from background debris during microscopic counting so extensive sample preparation is needed to extract the cysts and oocysts from the sample prior to fluorescent labeling and enumeration. Because the majority of the filtration experiments in this research will be performed with relatively clean water, a complicated sample preparation protocol will not be needed. The cysts and oocysts will simply be labeled with indirect fluorescent-antibody, mounted on a well slide, and counted by epifluorescence microscopy. The potential viability of the cysts and oocysts will be confirmed by identification of internal structures using differential interference contrast (DIC) microscopy (15).

Preliminary experiments with the cysts and oocysts will involve use of glass beads as filter media in order to compare results with the initial filtration studies using model particles in the protozoan cyst size range. The purpose is to determine if the protozoan-sized latex microspheres can serve as a surrogate for the infectious pathogens. Subsequent experiments will involve use of actual rapid filter media to better represent full-scale filter systems. The factors to be evaluated in these studies include: 1) the presence or absence of biofilm, 2) coagulant type (alum or ferric chloride), 3) the presence or absence of dissolved natural organic matter (NOM), 4) hydraulic loading rate (5 or 25 m/h), and 5) the filter media (sand or GAC). A 2^5 factorial design will be used as shown in Table 1. Since each experiment will be performed at least two times, this study will involve a minimum of 64 experiments. It is estimated that the filtration studies can be performed at a rate of approximately two per week. Therefore, we estimate a conservative completion time of approximately 1 year for Phase III, which will be initiated late in the first year and completed by the end of the second year of the project. In addition to the factorial design study, more experiments will be performed as time permits to evaluate the effects of other factors on removal of protozoa in filters such as: 1) other coagulants or coagulant aids (e.g. cationic polymers) and 2) additional filter media (e.g. anthracite coal, various types of GAC).

Table 1: *Giardia* and *Cryptosporidium* Filtration Experiments Included in the 2⁵ Factorial Design Study

Experiment No.	Biofilm		Coagulant		NOM		Hydraulic loading rate, m/h		Filter Media	
	Yes	No	Alum	FeCl ₃	Yes	No	5	25	Sand	GAC
1	X		X		X		X		X	
2	X		X		X		X			X
3	X		X		X			X	X	
4	X		X		X			X		X
5	X		X			X	X		X	
6	X		X			X	X			X
7	X		X			X		X	X	
8	X		X			X		X		X
9	X			X	X		X		X	
10	X			X	X		X			X
11	X			X	X			X	X	
12	X			X	X			X		X
13	X			X		X	X		X	
14	X			X		X	X			X
15	X			X		X		X	X	
16	X					X		X		X
17		X	X		X		X		X	
18		X	X		X		X			X
19		X	X		X			X	X	
20		X	X		X			X		X
21		X	X			X	X		X	
22		X	X			X	X			X
23		X	X			X		X	X	
24		X	X			X		X		X
25		X		X	X		X		X	
26		X		X	X		X			X
27		X		X	X			X	X	
28		X		X	X			X		X
29		X		X		X	X		X	
30		X		X		X	X			X
31		X		X		X		X	X	
32		X		X		X		X		X

Related Research

Most drinking water treatment facilities in the U.S. use chlorine for disinfection. Chlorine disinfection provides minimal inactivation of *Giardia* cysts and *Cryptosporidium* oocysts, therefore, the primary means of removal in water treatment plants is through conventional treatment which typically includes coagulation, flocculation, sedimentation, and filtration (1, 11, 16). Fortunately, conventional treatment is often effective at removing these pathogenic protozoan particles (14, 19). Nieminski and Ongerth (19) report 99 % (2 log) to 99.99 % (4 log) removal of *Giardia* and *Cryptosporidium* for two plants employing alum or polyaluminum chloride coagulation, flocculation, sedimentation, and dual media (anthracite-sand) filtration. The three plants surveyed in a study by LeChevallier and Norton (14) were typically able to achieve 2.5 log removals of both pathogens. Despite the potential for effective removal by conventional treatment, LeChevallier et al. (15) found that 17% of 83 filtered water effluents contained *Giardia* cysts and 27% contained *Cryptosporidium* oocysts. Therefore, conventional treatment provides a barrier but not an impenetrable one.

The attachment of a suspended particle to a surface or collector in a filter (e.g. a sand grain) is affected by short-range interactions including hydrodynamic retardation, London van der Waals attraction, and the overlap of electrical double layers (3). In addition, the hydrophobicity of the particle and the collector and the presence of polymers on either surface may also impact the potential for particle attachment (29, 30). Biofilms consist of bacteria and a surrounding matrix of extracellular polymeric substances, primarily polysaccharides, which are excreted by the bacteria (4). Two commonly used filter media are sand and GAC. A biofilm is chemically distinct from a sand grain (composed primarily of SiO₂) and the complex carbon surface of GAC. In addition to the significant chemical differences between the clean surface of sand, GAC, and biofilm, an amorphous biofilm coating could also influence the pattern of fluid flow near the surface of the collectors (4). Both of these factors could contribute to differences in particle removal for filters containing significant amounts of biofilm.

In fact, research has shown that biofilm accumulation on filter media affects particle deposition. Rittmann and Wirtel (23) report that biofilm colonization of granular activated carbon significantly increased cohesion efficiency of particles (1 μm milk solids) and the overall removal of particles in a fluidized bed reactor. Goldgrabe et al. (7) report lower particle removal in biological filters than in similarly operated non-biological (prechlorinated) filters. The impaired performance of the biological filter was either due to relatively poor attachment of influent particles, to production of particles in the biofilter (due to microbial growth), or to a combination of both effects. Little is known about the effect of biofilm on particle removal. The impact of biofilm on particle removal will most likely be affected by many factors including the characteristics of the particles, filter media, and biofilm, and the water chemistry. Hence, a thorough investigation of the impact of these parameters on particle removal is needed in order to determine the conditions needed for optimum filtration performance when biofilm is present.

Given the importance of filtration in preventing the occurrence of giardiasis and cryptosporidiosis, the effect of biofilm and other filter conditions on the removal of *Giardia* and *Cryptosporidium* in filters should be evaluated. This research is especially important since the occurrence of biofilm in drinking water filters is likely to increase in the future. This is due to several factors including: 1) the reduced use of prechlorination in drinking water treatment plants (2), 2) increased use of ozonation as a drinking water disinfectant (21), 3) increased use of GAC filter media (5), and 4) rising interest in biologically-active filtration or biofiltration (5). Many water treatment facilities are eliminating prechlorination, primarily to reduce the formation of chlorine disinfection by-products (DBPs) including trihalomethanes. The elimination of chlorine as a primary disinfectant (i.e. at or near the head of the plant) will result in more biological growth throughout the plant including the filters. Many water utilities are also considering switching to ozone as a primary disinfectant because it does not react with natural organic matter (NOM) to form chlorinated DBPs and because of its effectiveness at inactivating protozoan cysts and oocysts. Although ozone can provide effective *Cryptosporidium* oocyst inactivation, those organisms that survive or escape the disinfection process need to be removed by downstream processes in a so-called multiple barrier approach. Therefore, even a utility employing ozonation needs to be concerned about removal of protozoa in the filters. Those protozoa that escape the ozone contactor will encounter an increased amount of biofilm in the filters because ozone increases the biodegradability of the generally recalcitrant NOM (6, 10) which serves as the main substrate for bacteria in natural waters (18, 28). In addition, ozone reacts very rapidly in water so a disinfectant residual is not maintained for controlling microbial growth in the treatment works. GAC beds favor the growth of biofilms by destroying chemical oxidants such as chlorine and by providing an excellent surface for microbial growth (5). Finally, there is increasing interest in the use of biofiltration for removing biodegradable NOM from drinking water supplies to limit the formation of DBPs and to control microbial regrowth in distribution systems. DiGiano and Speitel (5) predict that biologically-active filters will become commonplace in the U.S. water industry over the next decade. Therefore, more research is needed to evaluate the impact of biofilm on particle removal in order to ensure the safety of treated drinking water supplies as the use of biofilters increases.

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