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Filtration Removals and Swim Diaper Retention of *Cryptosporidium* in Swimming Pools

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Synopsis

The ability of swimming pool filters to remove waterborne pathogens like *Cryptosporidium* oocysts and the ability of swim diapers to retain them are topics of great importance to public health. This research project was intended to evaluate some common swimming pool filtration technologies and swim diapers for removing (or retaining) *Cryptosporidium* oocysts and/or 5-µm diameter polystyrene microspheres, which were used as a *Cryptosporidium* surrogate. A spa with interchangeable sand, cartridge, and precoat filters was used for this research. Simulated pool water was created for each experiment from tap water. The results show that sand and cartridge filters have a limited capacity for *Cryptosporidium* removal, which was less than 37% on average for each. The use of a cationic polymer based clarifier with the sand filter improved oocyst removal to greater than 60%, but it was unclear how to use this product for maximum benefit. Precoat filters (e.g., diatomaceous earth and perlite) demonstrated significantly better pathogen removal capabilities than the other filters tested with mean removals of 2.35 to 4.33 Log (or 99.6 to 99.995%). The best pathogen removals were obtained in experiments with finer grades of precoat media. Swim diapers were not shown to be very effective at retaining *Crypto*-sized particles under the tested conditions with 25% to 70% release within 2 minutes.

Introduction

Relatively little is known about the capabilities of common swimming pool filters to remove waterborne pathogens. Recent research has found that sand filters typically remove approximately 25% of *Cryptosporidium* oocysts and less than 50% oocyst-sized microspheres per passage through the filter [1,2]. Drinking water treatment research has shown granular media filters can remove in excess of 6 Log (99.9999%) of *Cryptosporidium* oocysts [3]. Although the filters are similar for swimming pool and drinking water treatment facilities, there are two important differences between drinking water treatment and pool water treatment practice. First, drinking water treatment facilities perform chemical coagulation prior to filtration, which greatly enhances the ability of the filters to remove the pathogens by eliminating the natural electrostatic repulsion that exists between negatively charged pathogens and negatively charged filter media in water [4]. Second, the filter loading rates (flow per unit of surface area of filter) is typically at least 4 to 5 times lower in drinking water filters (i.e., less than 4 gpm/ft^2 (10 m/hr) instead of 15 to 20 gpm/ft^2 (37 to 49 m/hr)).

Diatomaceous earth (DE) is also used in drinking water treatment. The two important differences between sand filters used for drinking water and pool water treatment (stated previously) do not apply to diatomaceous earth filtration. The filter loading rates used for DE filters in both industries are similar, and neither industry necessarily practices coagulation as

pretreatment for DE filters. DE filters rely on the size exclusion principle to prevent pathogens from passing through the tiny pores in the DE media. Drinking water research has shown *Cryptosporidium* removal in excess of 6 Log for DE filters [5,6]. However, two important differences still exist between the DE filtration practices used in the drinking water and pool water industries. The grades of DE used in drinking water treatment are of a finer grade, which is typically measured as "hydraulic conductivity" of the media. Typical hydraulic conductivity values of the DE media used for swimming pools is 4-5 Darcys, but the hydraulic conductivities of the DE media used in drinking water treatment is typically less than 1 Darcy [4]. The finer-grained precoat media has smaller pores between the grains and removes smaller particles more efficiently, but this quality also makes it more difficult to force water through the filter reducing the hydraulic conductivity of the media. Second, the amount of DE media loaded into each filter per unit of support-leaf surface area is approximately double the value in pool water treatment (i.e., 20 lbs/100 ft^2 or 0.98 Kg/m^2) in drinking water treatment [4]. More media per unit surface area creates a thicker layer of DE cake that particles must pass through to penetrate the filter.

Pool filters are traditionally designed for keeping swimming pools looking clear and beautiful in the US, which is not the same as removing all of the waterborne pathogens. The swimming pool industry has traditionally relied on disinfectants, such as chlorine, to control the spread of waterborne diseases. The drinking water industry did largely the same thing until chlorine-resistant pathogens forced changes in the 1980's (for *Giardia*) and the 1990's and beyond (for *Cryptosporidium*). The chlorine-resistant pathogens forced the drinking water industry to put considerable emphasis on filtration optimization to achieve physical removal of these pathogens. US drinking water regulations are continuing to become more and more stringent on pathogen removal in order to safeguard public health. The swimming pool industry in the US could follow this approach.

This research project was intended to evaluate some common swimming pool filtration technologies for removing *Cryptosporidium* oocysts and 5-µm polystyrene microspheres from a simulated pool water. The filter types chosen were sand, cartridge, and precoat. Standard commercially-available filters and media were used in these experiments.

Methods

A 200 gallon (757 L) spa with a set of interchangeable sand, cartridge, and precoat filters was used at room temperature for this research. The filters and the pump were commercially-available products (Pentair Challenger 3 HP pump; Pentair Triton II TR-40 sand filter, Pentair Clean and Clear Plus 240 cartridge filter, and a Pentair FNS Plus 24 precoat filter). The three types of precoat media used in this study were standard DE (EP Minerals, Celatom Standard Pool Grade, permeability 4.5 Darcys), fine perlite (Proprietary expanded perlite product, permeability 1.5 Darcys), and fine DE (EP Minerals, Celatom Premium Pool Grade, permeability 1.2 Darcys). The spa system was capable of pumping water at 60 gpm (227 L/min), and the flow was measured with a digital flow meter (Scienco Products, Model SEM-40 electronic flow meter) and controlled with a 2" diameter PVC ball valve. Inline feeding of the oocyst/microsphere suspensions was made possible by a digital peristaltic pump (Watson Marlow, Model 505Di) feeding directly into the PVC pipe just upstream of the pump and filter. The oocyst/microsphere suspensions were made in a 1-L glass Erlenmeyer flask of simulated pool water and stirred continuously with a magnetic stirrer (Barnstead/Thermolyne,

Cimarec® Digital Stirring Hot Plate) and Teflon®-coated stir bar prior to and during the experiments.

The inline particle feeding system made it possible feed oocysts/microspheres without having to stop and start the precoat filters during the experiments. The sand and cartridge filters were operated differently because the stop/start process was not expected to alter their performance. For the sand and cartridge filter experiments, the flow of water was stopped just prior to beginning the oocyst/microsphere seeding, and a 3-way valve was repositioned to redirect the filtered water to the drain thereby preventing recirculation. Preliminary experiments revealed that the low removals associated with the sand and cartridge filters allowed rapid accumulation of particle in the spa in recirculation mode, which caused the influent number of oocysts/microspheres to increase rapidly.

Sand filters were backwashed with simulated pool water prior to experiments to prevent the pore water from changing the chemistry of the system during the experiments while the other two types of filters were simply drained completely and filled with simulated pool water at the beginning of the experiment. Pool clarifier (a commercially available cationic organic polymer based product) when used, was poured directly into the spa following an initial dilution in 10 L of simulated pool water and allowed to recirculate and mix completely prior to seeding oocysts and/or microspheres.

Simulated pool water was created for each experiment from 200 gallons (757L) of Charlotte, NC (US) tap water supplemented with sodium bicarbonate to an alkalinity of 150 mg/L as $CaCO_3$, with calcium chloride to a hardness of 250 mg/L as $CaCO_3$, with sodium hypochlorite to a free chlorine concentration of 2 mg/L, with hydrochloric acid to a pH of 7.5, and with a mixture of artificial sweat and urine to a final total organic carbon concentration of 20 mg/L as C. Experiments were performed in duplicate, and duplicate samples were collected during each experiment from the filter influent and filter effluent pipes. A maximum of approximately 10^8 heat-inactivated (55° C for 30 min) *Cryptosporidium* oocysts [7] and/or YG fluorescent carboxylate-modified polystyrene microspheres (Polysciences, Inc, Cat. #16592, 4.869 µm, std. dev. 0.246 µm) were used in each experiment to achieve a maximum filter influent concentration of approximately 132 oocysts/microspheres per mL of water. Influent samples of 50 mL were collected in sterile 50 mL conical-bottomed plastic centrifuge tubes (Falcon® Blue-Max™ Order #352074), and the volume of the effluent samples varied from 50 mL to 1 L with the larger samples collected in Wheaton glass media bottles.

Samples were stored at 4° C prior to analysis. Sample volumes analyzed were adjusted to obtain between 10 and 150 oocysts and/or microspheres per sample. Samples were filtered through 3-µm polycarbonate track-etched (PCTE) filters (GE, Order #K30CP02500) in 25-mm glass microanalysis filter funnels (Millipore Model xx10 025 00) by a regulated 3-place vacuum manifold. The staining method used for *Cryptosporidium* involved placing 600 µL of *Cryptosporidium*-specific monoclonal antibody (obtained from Dr. Mike Arrowood's lab at the CDC in Atlanta, GA) on top of the filter for 30 min at room temperature, rinsing twice with 750 µL of 1X PBS (pH 7.4)(Fisher Scientific, Order #BP399-500), applying 500 µL of a 1:150 dilution of a fluorescein isothiocyanate (FITC)-labeled goat anti-mouse antibody (Invitrogen Zymed® IgG+A+M (H+L) Order #65-6411) in the dark at room temperature for

30 min, and rinsing twice more with 1X PBS. The filters were mounted on glass micro slides (Gold Seal® Order #3058) with one drop of polyvinyl alcohol-DABCO solution [8] and a glass cover slip (Corning, 25-mm square, No. 1.5) for enumeration under epifluorescent microscope (Zeiss Standard 25 microscope) at 100X or 250X total magnification for microspheres and *Cryptosporidium* oocysts, respectively. The fluorescent filter set had a 450-490-nm excitation wavelength range, a 510-nm dichroic filter, and a 520-nm emission filter. The PCTE filters with 3-µm pores were used for the 5-µm microspheres and oocysts, which were used simultaneously in these experiments. The spa system was thoroughly cleaned between experiments with a minimum of three drain-and-fill rinses with recirculation at 60 gpm (227 L/min), and samples were collected prior to seeding in each experiment to measure any potential carryover between experiments. Control experiments were used to determine whether or not the oocysts and microsphere were destroyed by the pump and/or lost due to surface attachment within the system.

Swim diaper evaluations were performed with the same experimental setup, but there were some notable differences. First, no filter was used in these experiments, and the flow rate was held constant at 38 gpm (144 L/min). Second, artificial sweat and urine were not introduced, and the water was held at an elevated temperature of approximately 38° C. Due to the use of human subjects, no *Cryptosporidium* oocysts were used in the swim diaper experiments. Approximately 10^7 microspheres were used in these experiments, which were introduced in 30 mL of ultrapure water directly into the swim diapers. The subject and swim diaper were both wet prior to the simulated watery diarrhea accident. The subject(s) were allowed to play normally during experiments, but they were not allowed to leave the spa. Nine sets of duplicate samples were collected from the recirculation line over the 40 minute experimental period with 5 samples taken during the first 5 minutes.

Results

Percent *Cryptosporidium* removals and Log *Cryptosporidium* removals under varying conditions are shown in Figures 1 and 2, respectively. The error bars represent one standard deviation above and below the mean value. Control experiments showed that a significant amount of oocysts were not being lost to adhesion to the system surfaces or damaged by the pump and pipes. *Cryptosporidium* removals averaged 31.3% for the sand filter and 36.2% for the cartridge filter. Sand filter removals nearly doubled to a mean of 61.1% with the addition of clarifier to the system. Only one dose of clarifier (the minimum dosage recommended on the bottle) was used in this study. So, the clarifier dose was not optimized, and the clarifier was added and recirculated for a minimum of 4 hours prior to adding the oocysts. This practice seems consistent with what might actually occur with a fecal accident in a swimming pool in the US. No optimization was attempted in terms of dosage, point of application, or timing relative to the start of the filtration removal experiments. Adding clarifier immediately following a suspension of oocysts/microspheres (just prior to filtration) is another alternative (more typical of drinking water treatment practice) that was not explored in the present study. These two clarifier addition scenarios could be termed passive an active water treatment, respectively.

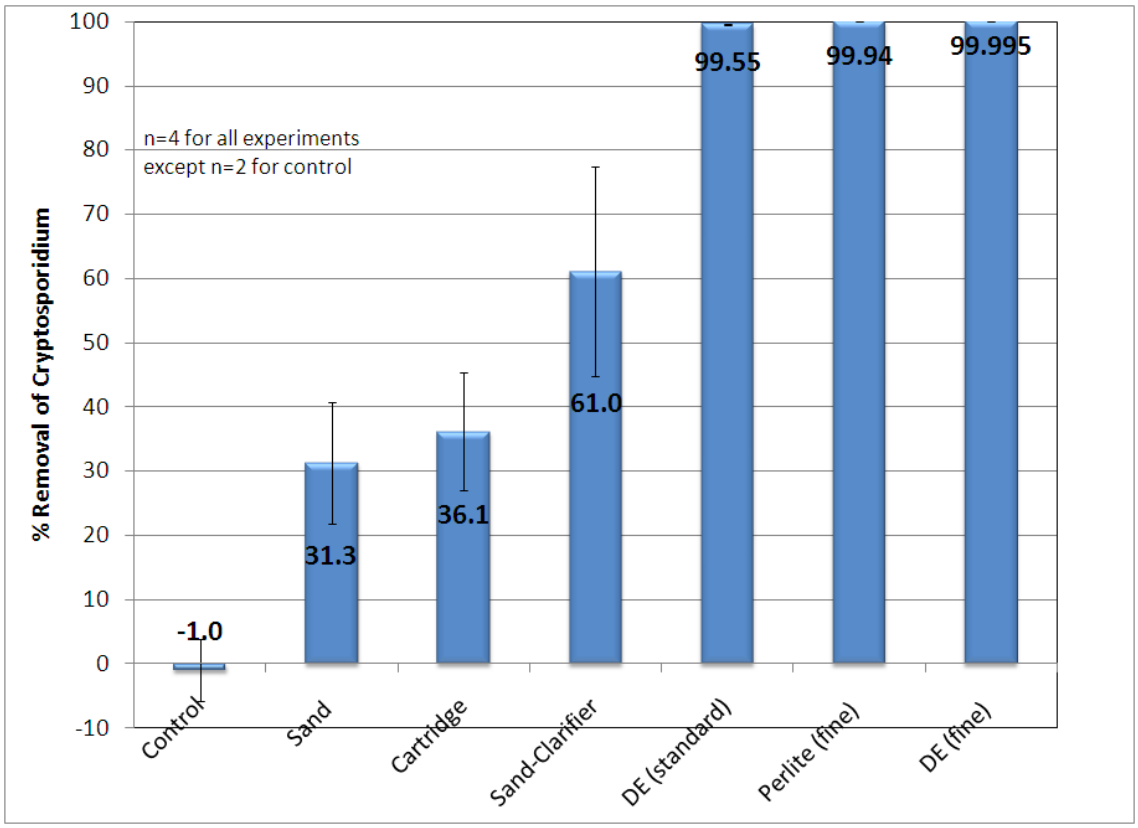

Figure 1. Percent *Cryptosporidium* removal for each pool filtration scenario

Precoat filter removals of *Cryptosporidium* oocysts were significantly higher than for the other two types of filters as shown in Figure 2. The standard grade of DE used in swimming pools removed 2.35 Log (99.55%) of oocysts. Two finer-grained types of precoat media each produced higher removals of oocysts than the standard DE media. Perlite and a finer grade of DE removed oocysts with an average efficiency of 3.23 and 4.33 Log, respectively. The filtration rate remained constant at 2.5 gpm/ft^2 (6.1 m/hr) throughout all of the precoat filtration experiments, but the amount of precoat media increased in some experiments. The weight of standard DE and perlite were identical in all experiments (i.e., 2.4 lbs for 10 lbs/100 ft^2 or 1.1 Kg for 0.49 Kg/m^2), but the lower density of perlite produced a precoat layer of approximately double the thickness of the DE precoat layer. Half of the data points with fine grade of DE were also produced at 10 lbs/100 ft^2 (or 0.49 Kg/m^2) but the other half were produced with 30 lbs/100 ft^2 (or 1.47 Kg/m^2) of DE. The thickness of the precoat layer did appear to make some difference in the *Cryptosporidium* removal (4.15 vs. 4.64 Log), but the number of replicate samples (n=2) was insufficient for statistical analysis. The other important factor impacting *Cryptosporidium* removal was likely the permeabilities of the precoat media, which were 4.5, 1.5, and 1.2 Darcys for the standard DE, perlite, and fine DE medias, respectively. A series of heteroscedastic, 2-tailed Student's t-tests (Microsoft Office Excel 2007) showed that the differences in oocyst removals between each of the treatment types were statically significant (α=0.05) with the exception of the sand filter versus the cartridge filter.

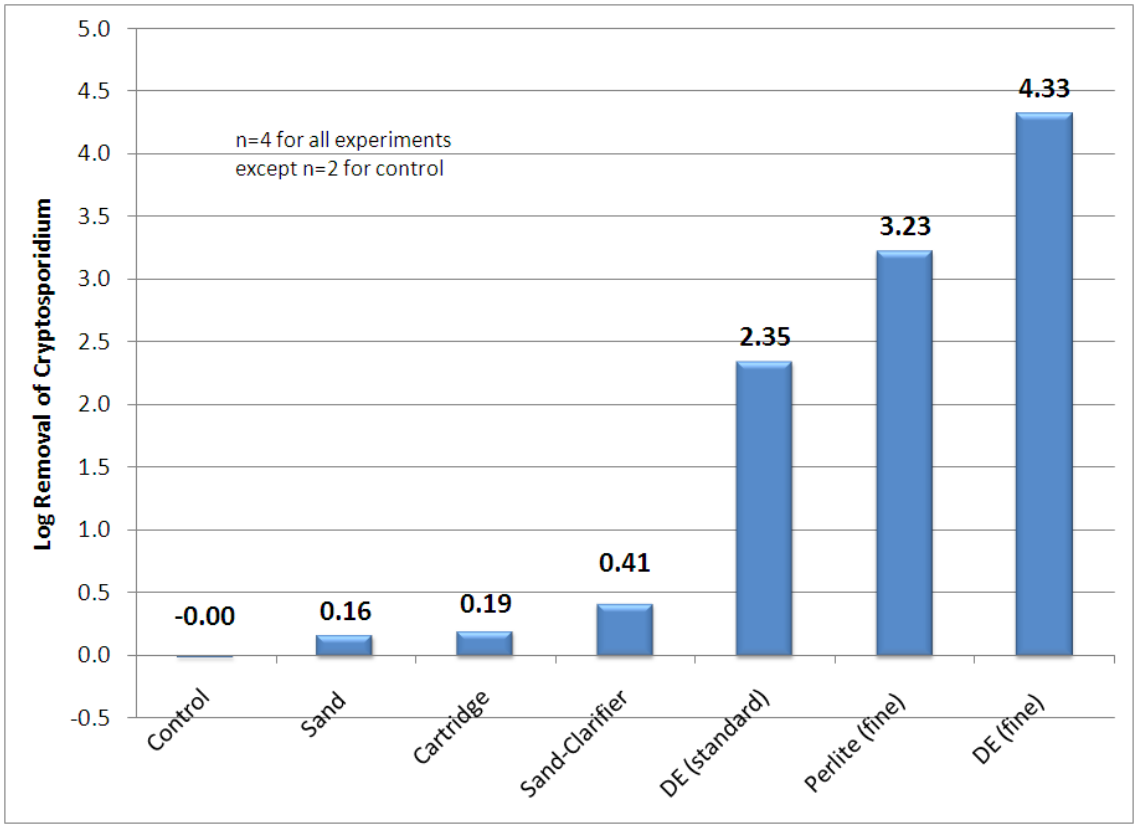

Figure 2. Log *Cryptosporidium* removal for each pool filtration scenario

The percent removals and Log removals of the 5-µm microspheres are shown in Figures 3 and 4, respectively. The microspheres were used as a potential non-infectious surrogate for *Cryptosporidium* oocysts since they have nearly identical size, shape, and density. The microspheres also serve as an internal control for oocysts since the microspheres do not have to be stained to be seen under an epifluorescent microscope, are not susceptible to excystation (breaking open), and are easier to identify under the microscope. Besides helping to verify the *Cryptosporidium* detection method worked properly, the microspheres could also be used as a surrogate to safely evaluate any pool filtration system without posing any risk to bathers. The *Cryptosporidium* removal results in Figures 1 and 2 are very similar to the microsphere removals in Figures 3 and 4. Log removals of microspheres and *Cryptosporidium* oocysts are shown side-by-side in Figure 5 to facilitate easier visual comparisons. A series of heteroscedastic, 2-tailed Student's t-tests (Microsoft Office Excel 2007) was not able to determine any statistically significant differences (α=0.05) between the oocyst and 5-µm microsphere removals for any of the experiments with identical treatment techniques. So, the microspheres appear to have been a good surrogate in this system.

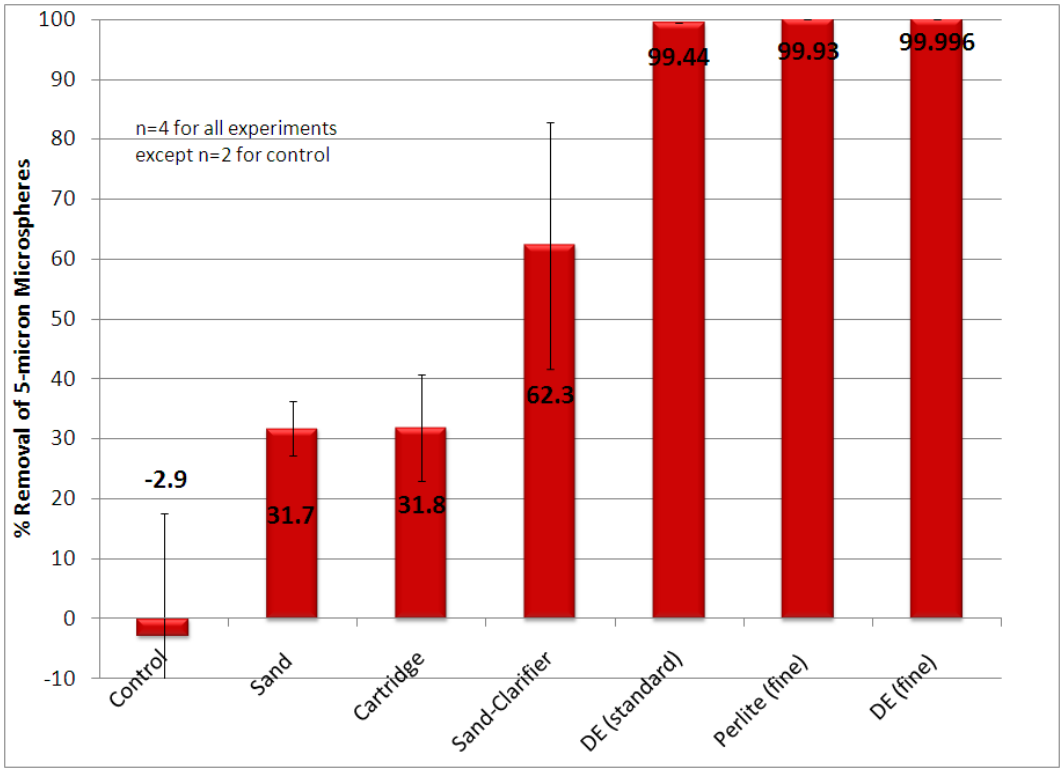

Figure 3. Percent 5-µm microsphere removal for each pool filtration scenario

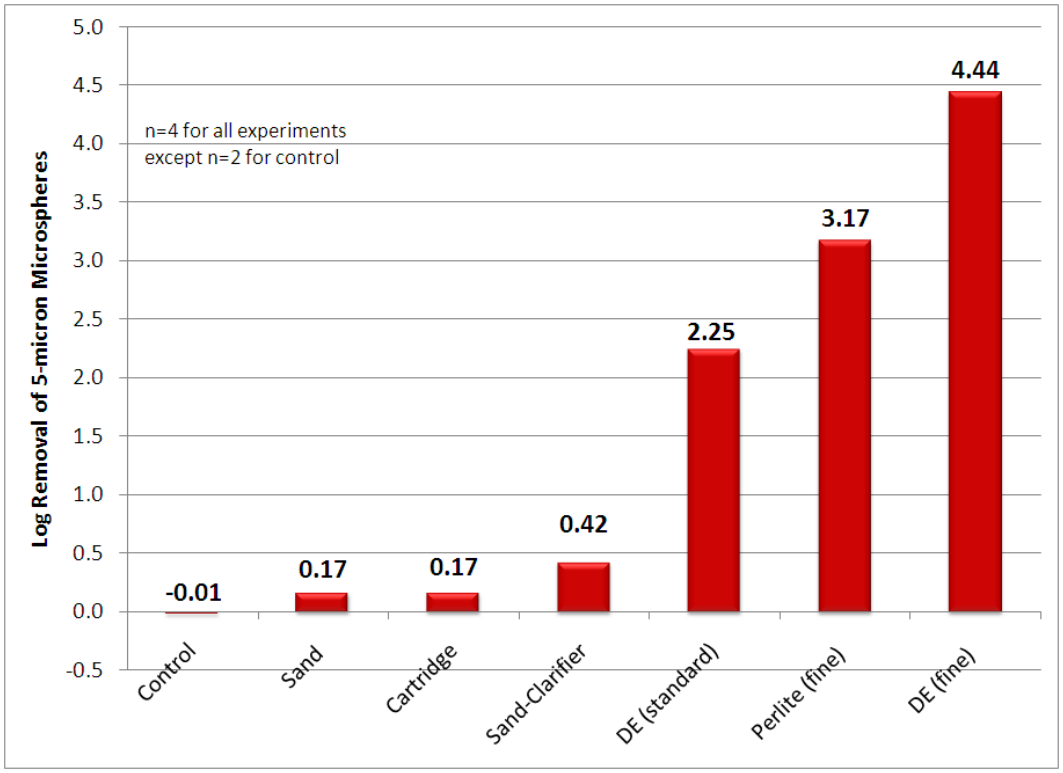

Figure 4. Log 5-µm microsphere removal for each pool filtration scenario

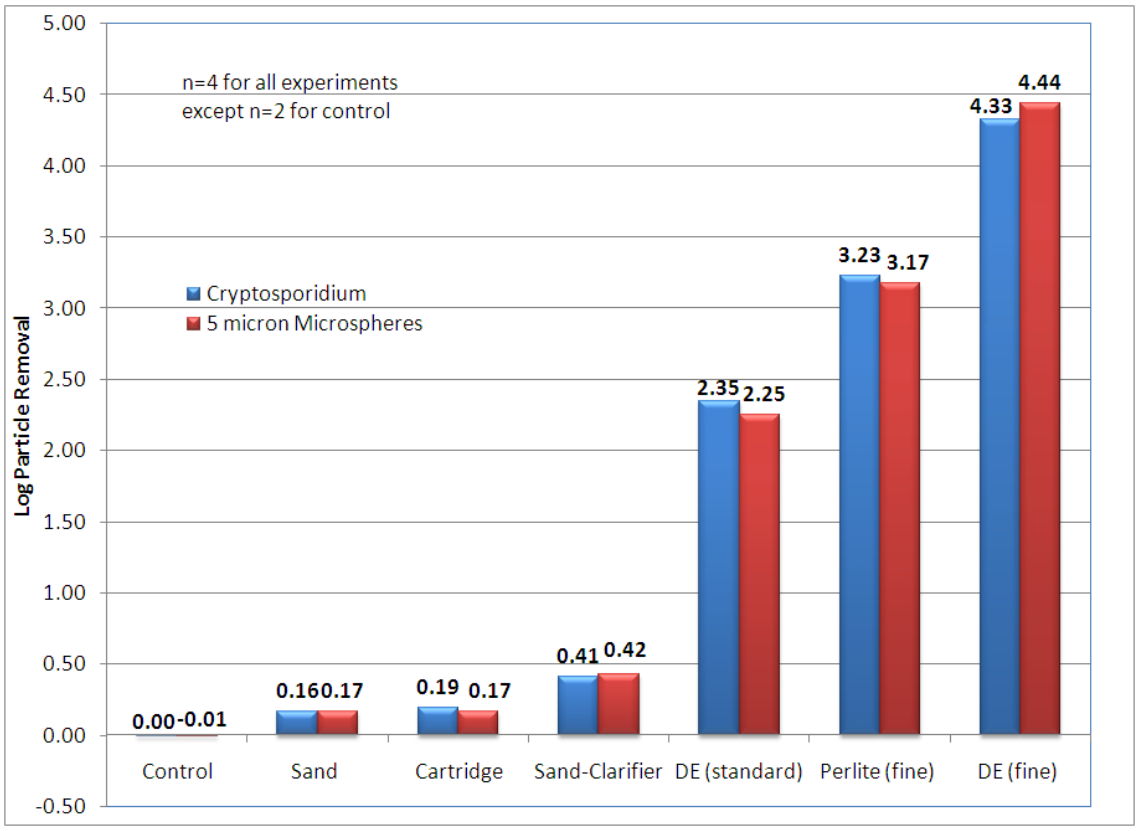

Figure 5. Log particle removal for each filtration scenario

Swim diaper results

Swim diapers are used around the world in swimming pools to contain solid fecal accidents, but the performance of swim diapers in containing watery diarrheal accidents common of *Cryptosporidium*-infected hosts is still in question. Since *Cryptosporidium* is responsible for the majority of waterborne disease outbreaks in the US, it is critically important that we understand how this pathogen behaves in the pool environment. The release of 5-micron microspheres from several types of swim diapers on human subjects is shown in Figure 6 along with the release of microspheres from swimming trunks (as a control). The swimming trunks seemed to be the worst case with almost 90% of the microspheres released within 1 min. However, none of the swim diapers tested performed very well with at least 50% of the microspheres released within 2 min. When dealing with 100 million or more parasites in a single accident, a 50% reduction is not going to eliminate the risk of waterborne disease outbreaks.

Putting a vinyl diaper cover over a disposable swim diaper did slightly improve the performance as shown in Figure 7. Alternate ways of using the vinyl diaper cover were explored such as putting the vinyl diaper cover under a pair of swimming trunks, and the results are shown in Figure 8. No substantial performance improvements were observed.

Figure 9 shows all of the swim diaper results. The use of trunks alone seemed to be the worst case, and the use of vinyl diaper covers over common disposable diapers seemed to be the best case. However, none of the swim diaper solutions tested actually held the majority of *Cryptosporidium*-sized particles for more than five minutes. In all cases, 25% or more of the microspheres were detected in the effluent samples within 2 minutes. So, extremely close supervision would be required to achieve even marginal reductions in pathogen levels in pools. It appears that improved swim diapers would be required to avoid introducing significant quantities of *Cryptosporidium* by diaper-aged hosts in pools.

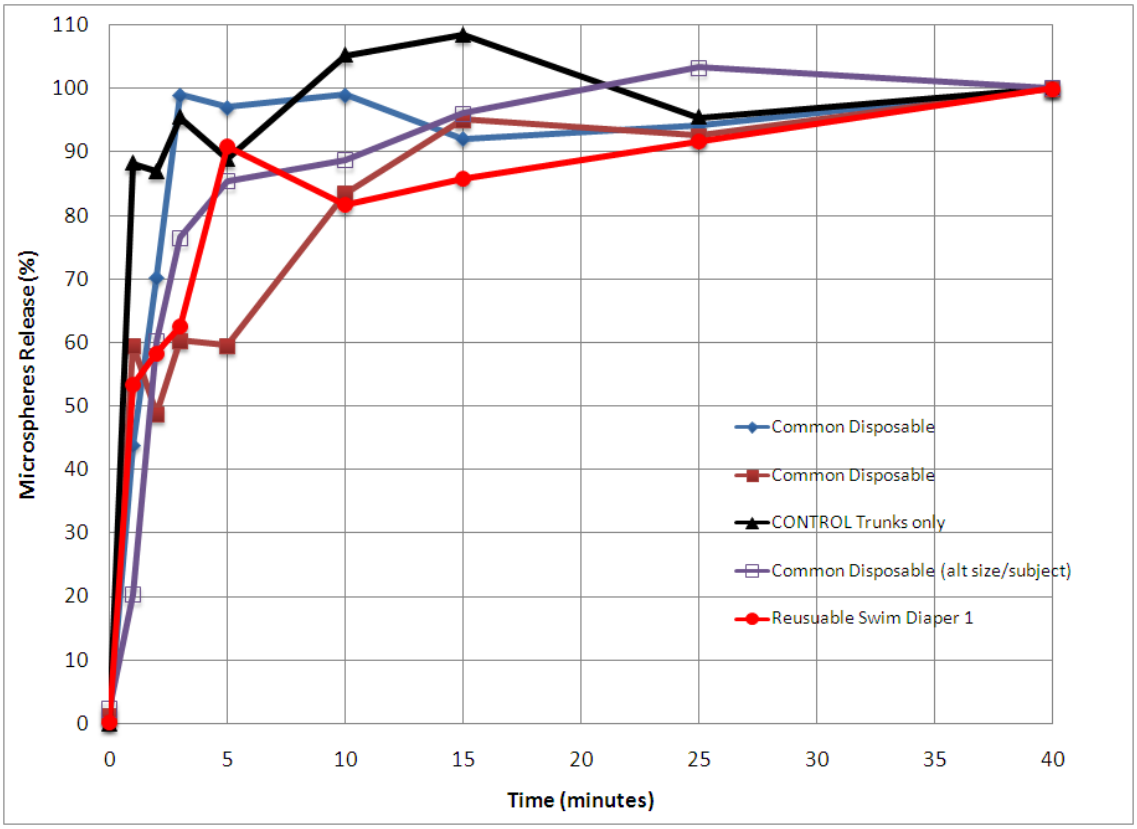

Figure 6. Swimming trunks and swim diaper releases versus time

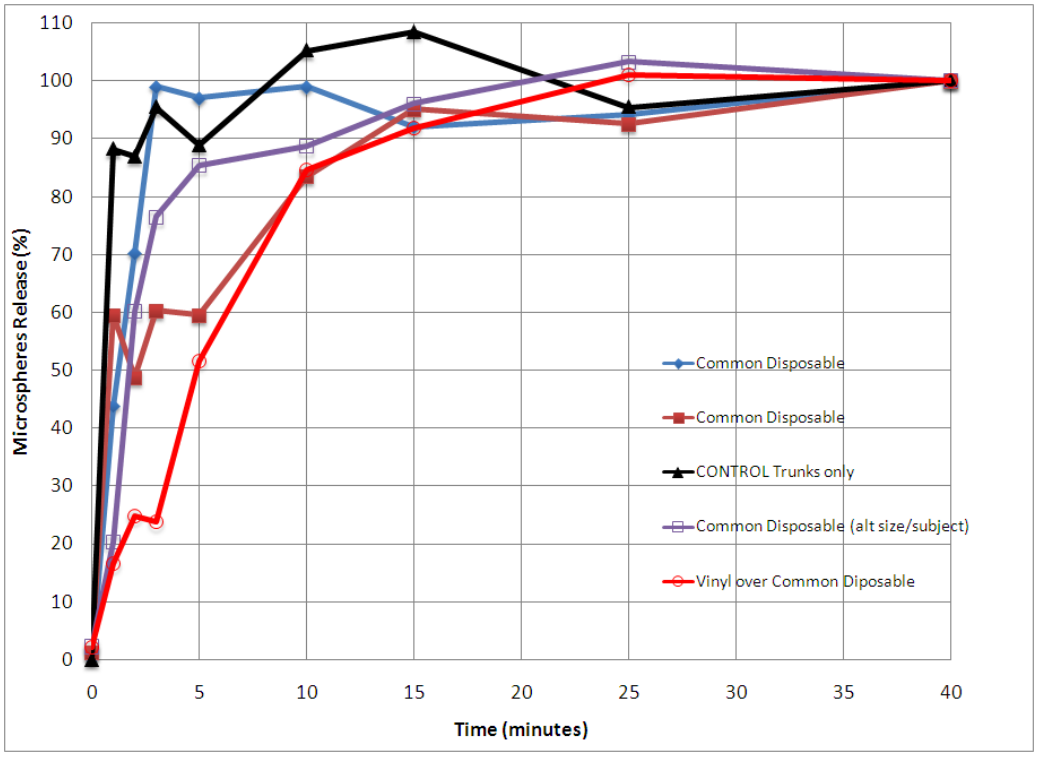

Figure 7. Swimming trunks and disposable swim diaper releases versus time

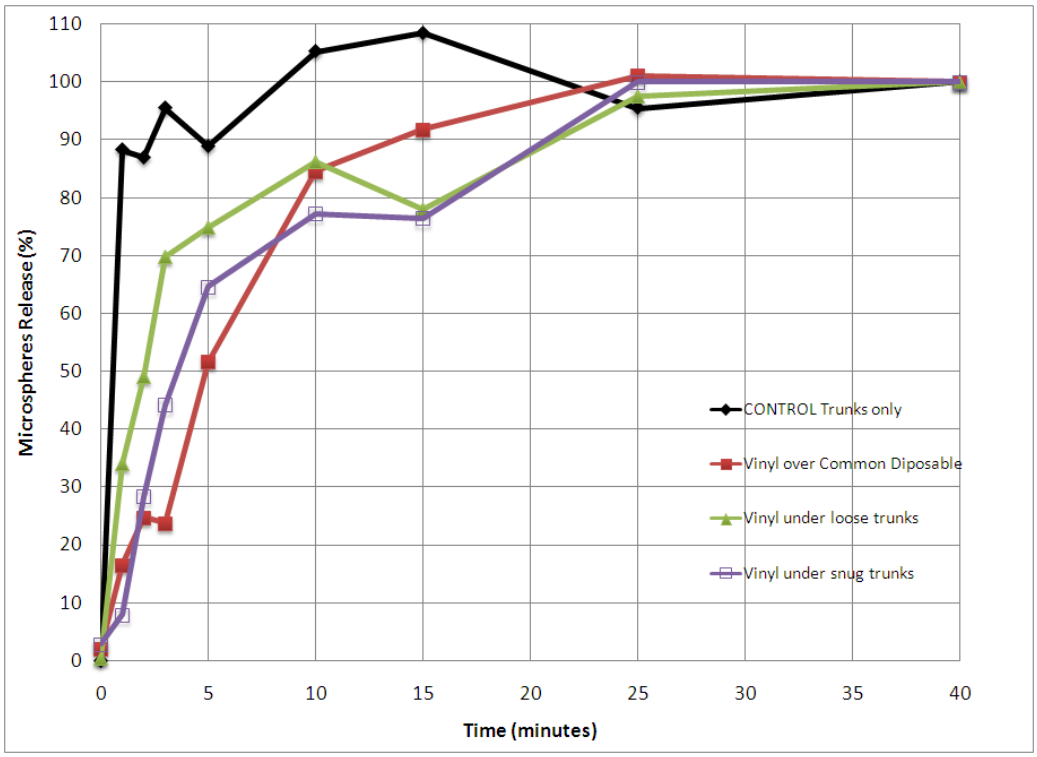

Figure 8. Swimming trunks and vinyl diaper cover releases versus time

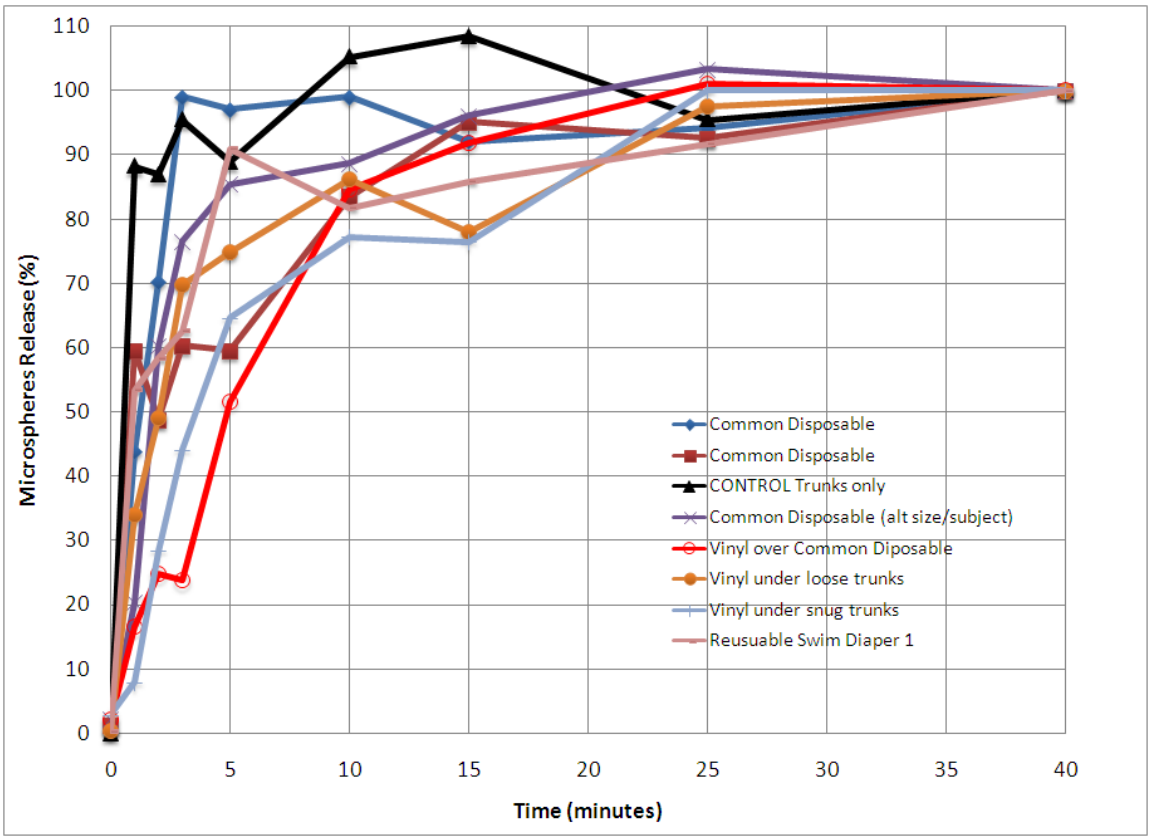

Figure 9. Combined swim diaper and swimming trunk release data versus time

Conclusions

The results show that sand and cartridge filters have a limited capacity for pathogen removal with removals averaging less than 36.2% for *Cryptosporidium* oocysts and microspheres. Diatomaceous earth and perlite have better pathogen removal capabilities with mean removals ranging from 2.25 Log (99.44%) to 4.44 Log (99.996%) with the higher pathogen removals obtained in experiments with finer grades of diatomaceous earth and perlite. The performance of the finer grades of precoat media appeared to be further enhanced by increasing the depth of the media layer in the filter. The performance of the sand filter with clarifier was not optimized, but it showed that clarifier use enhances sand filter performance. The use of chemical pretreatment (e.g., clarifier or coagulant) is standard practice in the drinking water treatment industry for sand filters, but the water treatment approach is to add the clarifier to the water containing oocysts as opposed to adding the oocysts to the water containing clarifier as was done in the present study. The DE media used in drinking water treatment tends to be of a finer grade than that commonly used in the swimming pool filters, and there appears to be a significant advantage in terms of *Cryptosporidium* removal for the finer-grained medias. Swim diapers offer only limited protection against the release of *Cryptosporidium* oocyst-sized particles into pools with 25% to 70% release rates within 2 minutes of an accident for all swim diapers and combinations tested.

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