# Full-Scale Evaluation of 3 Filtration Processes For Removing *Cryptosporidium*, *Giardia* & Surrogates

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The quality of reclaimed water has become an important public-health issue due to the application of reuse water for public-access uses such as irrigation in urban, environmental, agricultural, and recreational areas; groundwater recharge; and augmentation of potable supply.

"Reclaimed water" is defined as water that has received at least secondary treatment and basic disinfection at a domestic wastewater treatment facility and is then deliberately reused for beneficial purposes (OPPAGA 2000). Untreated wastewater contains a variety of microorganisms, including bacterial, viral and protozoan pathogens that may pose a health risk if the reclamation process is not properly controlled (Bitton 1994).

From 1986 to 2002, the use of reclaimed water in the state of Florida increased from 206 million gallons per day (MGD) to 584 MGD. Forty-six percent was being used for public-access areas by 2002 (FDEP 2003); therefore, it is important to assess water quality, particularly in regard to pathogens during the reclamation process.

This project evaluated filtration efficacy at two water reclamation facilities. Plant A has a design capacity of 43 MGD, and the effluent is reused for groundwater recharge, agricultural irrigation, and urban reuse. Plant B has a design capacity of 7.5 MGD, and the effluent is reused for groundwater recharge.

The wastewater treatment process at both plants includes primary and advanced secondary treatment with filtration. Primary treatment includes separating large solids from the waste stream through the processes of screening and sedimentation. Advanced secondary treatment involves chemical and biological nutrient removal and degradation of any remaining solids and reduction of pathogens.

Plant A utilizes rapid sand filtration and cloth media disk filtration for suspended solids removal before disinfection. Plant B utilizes upflow filtration before disinfection. For disinfection Plant A uses gaseous chlorine, while Plant B uses sodium hypochlorite.

*Cryptosporidium* and *Giardia* are two protozoa known to be waterborne pathogens. Both organisms are transmitted through the fecal-oral route, causing diarrhea and abdominal cramping. *Cryptosporidium* oocysts and *Giardia* cysts are shed through the feces of infected persons or animals and are resistant to environmental stresses and disinfectants such as chlorine (Fayer, et al 2002); therefore, physical removal of these organisms is important in the reclamation process prior to disinfection.

A study performed by Aqua-Aerobics demonstrated a 2-$\log_{10}$ removal of cysts and oocysts in biological treatment of wastewater (Aqua-Aerobic Systems Inc. 2003). As a result, treatment by filtration is the last step to remove any remaining cysts and oocysts before disinfection.

The objective of this study was to evaluate and compare the removal of *Cryptosporidium* oocysts and *Giardia* cysts by three different filtration technologies: cloth media disk, dual-media traveling bridge (sand/anthracite), and upflow continuous backwash filtration. In addition, the study looked at the effects of adding a polyaluminum chloride (PAC) as a coagulant to optimize the filtration process.

N-Nitrosodimethylamine (NDMA) has been found in treated wastewater and is formed in the presence of chloramines. (Luo et al 2003). NDMA is a carcinogen that is sensitive to light and degrades rapidly in the environment (Soroushian et al 2001). One of the goals of the project was to measure the effect of increasing polymer dose on NDMA formation.

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Table 1: Filter Comparison Study Parameters and Plant Performance

| Filter Conditions | Plant Flow Rate (MG) | SRT (Days) | Hydraulic Loading (g/min/ft²) | | | | | PAC Dose (ppm) |
|---|---|---|---|---|---|---|---|---|
| | | | Cloth Media | | Sand Filter | | Upflow Filter | |
| | | | Target | Actual | Target | Actual | Actual | |
| **Plant A** | | | | | | | | |
| Design Flow NO PAC | 20.31 | 6.5 | 4.00 | 4.13 | 2.00 | 2.12 | - | 0 |
| Design Flow ½ PAC Dose | 18.78 | 6.5 | 4.00 | 4.05 | 2.00 | 2.23 | - | 7 |
| Design Flow Recommended PAC Dose | 18.21 | 6.5 | 4.00 | 3.82 | 2.00 | 2.16 | - | 14 |
| Design Flow 1½ PAC Dose | 18.24 | 6.5 | 4.00 | 3.91 | 2.00 | 2.14 | - | 21 |
| 1½ Design Flow No PAC | 20.07 | 7.0 | 6.00 | 6.03 | 3.00 | 3.09 | - | 0 |
| 1½ Design Flow Optimal PAC Dose | 19.61 | 7.5 | 6.00 | 5.60 | 3.00 | 2.84 | - | 14 |
| **Plant B** | | | | | | | | |
| Design Flow No PAC | 4.44 | ND[1] | - | - | - | - | 3.85 | 0 |
| Design Flow ½ PAC Dose | 4.93 | ND[1] | - | - | - | - | 4.28 | 6 |
| Design Flow Recommended PAC Dose | 3.09 | ND[1] | - | - | - | - | 2.68 | 7 |
| Design Flow 1½ PAC Dose | 4.95 | ND[1] | - | - | - | - | 4.30 | 15 |

[1] ND = No Data

## Materials & Methods

### Collection

The study was conducted at Plant A in Orange County for a period of eight weeks. Each week of the study indicates an individual sampling event during which the loading rates of the sand filter, cloth-media filter, and PAC dosage were varied. A similar ongoing study is being conducted at Plant B.

The first six weeks of Plant A data and

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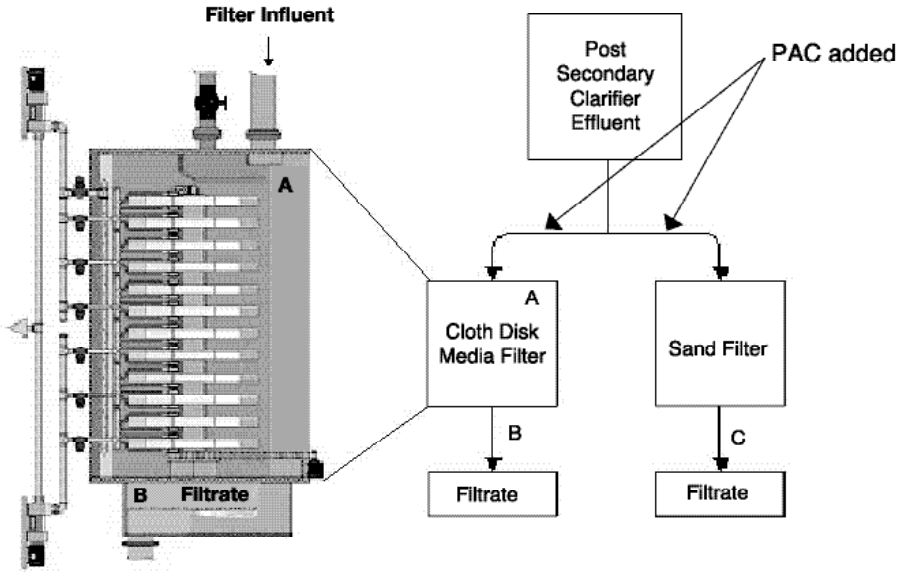
Figure 1: Schematic of Treatment Process with Collection Points

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first week of Plant B data are presented in this article. **Table 1** shows target filter loading rates and PAC doses. Filters were cleaned by backwashing after each event.

Samples were collected from three sites at Plant A (See **Figure 1** for collection sites). Site A collected post-secondary clarifier effluent (filter influent) from the influent water of the cloth media disk filter tank. Site B was the cloth media disk filtrate and C sand filtrate.

Samples for each site included two 1-L amber glass bottles preserved with 0.2 g sodium thiosulfate for NDMA analysis and on-site filtration to an Envirocheck HV™ filter for detection of *Giardia* cysts and *Cryptosporidium* oocysts. Matrix spikes were also collected at this time to determine the recovery efficiency of the analytical method in the sample matrix.

## Filter Description

Plant A: The cloth media disk filter (Aqua-Aerobic Systems Inc., Rockford, IL) is comprised of 12 disk filters contained in a 304-liter stainless steel tank. Under normal operating conditions, it can withstand an average hydraulic loading rate of 4 g/min/ft$^2$ with a maximum loading of 8 g/min/ft$^2$. The disks are lined up vertically in the filter tanks and allow water to pass through for filtration.

The backwashing system consists of suction headers lined up around each individual disk. The headers rotate around the disks and suction the filtrate back through the cloth media to dislodge particulate matter that is embedded within the media. Backwashing automatically occurs when the pressure differential on the cloth media exceeds a specific level or when the tank levels get too high, with a duration of 67 seconds. The filter tank also allows for large particulate matter to settle to the bottom, where the sludge produced is removed by a centrifugal pump.

The dual media traveling bridge filter, also at Plant A (Environmental Elements Corp., Baltimore, MD) is an automatic backwash gravity filter that uses silica sand and anthracite coal as the filter medium to remove solids suspended in the water. The filter is 114 feet long and 15.75 feet wide and consists of a partitioned filter bed grouped into 12-inch wide compartments. This filter has a designed hydraulic loading rate of 2 g/min/ft$^2$ and can handle as much as 4 g/min/ft$^2$.

The traveling bridge can travel across the entire length of the filter during backwashing, allowing the filter to remain in operation during the cleaning process. The duration of backwashing for the individual compartment is 22-60 seconds.

The upflow continuous backwash filter in Plant B (Dynasand, by Parkson Corporation, Fort Lauderdale, FL) is a continuously backwashed filter that uses silica sand to filter solids out of the water as the water flows up through the filter bed. During backwashing, the sand moves down to the bottom of the filter where an airlift pumps it to a sand washer that cleans the sand and lets it fall back to the top of the filter bed.

## *Cryptosporidium* Oocyst & *Giardia* Cyst Detection

A modified version of USEPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA (April 2001) was used for simultaneous detection of the cysts and oocysts. Since Method 1623 is a performance-based method, our laboratory's standard operating procedure was as follows:

- **Filtration**—Samples were filtered on-site using a diaphragm pump set up with a water totalizer and the Envirocheck HV™ filter (Pall Gelman Laboratory, Ann Arbor, MI). One hundred liters of sample was filtered on-site or until the Envirocheck HV™ filter clogged. The filters were labeled, bagged, packed on ice and returned to the lab for processing.
- **Matrix Spike**—To determine the recovery efficiency of Method 1623, a matrix spike was collected at each sample site for the first four weeks of the study. A matrix spike is a sample that is collected and then seeded with a known number of cysts and oocysts; it is then processed with the samples. During the first week, a 20-liter carboy grab was collected and returned to the lab to be seeded with Colorseed (BTF, Sydney, Australia). Colorseeds contain 100 cysts and oocysts each, which are stained with a red fluorescent dye to differentiate the spiked organisms from the indigenous cysts and oocysts present in wastewater. During the first PAC experiment, the Envirocheck HV™ filters clogged before 20 liters of the matrix water was filtered. Matrix spikes performed for subsequent experiments used the following procedure: Reagent water was seeded with Colorseed organisms and thoroughly mixed; the suspension was filtered prior to filtering the sample. In this manner, equivalent volumes of the samples and matrix spikes were collected. Recovery efficiency was determined for each matrix spike using **Equation 1**.

**Equation 1: Matrix Spike Recovery Efficiency**

$$\text{Recovery Efficiency} = \frac{\text{No. Colorseed cysts or oocysts counted}}{\text{No. Colorseed cysts or oocysts spiked}} \times 100$$

- **Elution/Concentration**—Elution buffer was prepared with Lauryl Sulfate and eluted by a wrist-shaker at 900 rpm. The samples were concentrated by centrifugation at 1,500 x *g* for 15 minutes and the supernatant was carefully aspirated without disturbing the pellet, leaving 5 milliliters of supernatant per every 0.5 milliliters of pellet. The pellet was resuspended in the remaining supernatant, divided into 5-milliliter aliquots, and transferred to a 16 x 125-millimeter Leighton tube with a 60 x 10-millimeter flat-sided capture area.
- **IMS**—Samples were further concentrated through immunomagnetic separation

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(IMS) using an Aureon CG Combo Kit (Aureon Biosystems, Vienna, Austria). Five milliliters of A-*IMS* buffer™ were added in three increments (2+2+1 milliliter) to the one final centrifuge tube and mixed thoroughly to ensure that all residual sample was suspended and transferred to the Leighton tube. One hundred microliters of *Cryptosporidium* A-*Beads* and *Giardia* A-*Beads* each were added to the samples in the Leighton tube and mixed on a rotating mixer at 23 rpm for 45 minutes. The samples were placed on a *MagnetOn* 4T™ (Aureon Biosystems, Vienna, Austria) with the capture window adjacent to the magnet and gently rocked for one minute. Without removing the tube from the magnet, the supernatant was decanted. The organism magnetic bead complex was transferred to a 2.0-milliliter centrifuge tube by suspending the complex in three increments of 330 microliter each of IT-*Wash* Buffer™. The complex was placed in a MPC-S magnet (Dynal, Lake Success, NY) and rocked gently for 30 seconds. The supernatant was carefully aspirated without disrupting the pellet. This wash step was repeated once more using 1 milliliter of IT-*Wash* Buffer™. Magnetic beads were removed from the organisms by acid dissociation, which is accomplished by adding 50 microliters of 0.1 N HCl to the complex, vortexed for 15 seconds, set for 10 minutes, vortexed again for 10 seconds, and then placed on the MPC-S magnet. The 50-microliter acid suspension was transferred to a Waterborne Superstick slide (Waterborne Inc., New Orleans, LA) with 15 microliters of NaOH. Acid dissociation was repeated two more times. Subsequent acid suspensions were added to the same well as the first. Positive and negative staining controls were prepared for quality assurance purposes.

- **Immunofluorescence Assay**—The slides were air-dried overnight and then stained using Aqua-glo G/C Direct fluorescein antibody kit (Waterborne, New Orleans, LA). One drop of Aqua-glo G/C Direct was applied to each sample well and incubated for 30 minutes at 35°C. Saline wash buffer was added to each well to rinse off the antibodies. Slides were tilted and reagents were wicked off with a paper towel. One drop of counterstain was applied to each well (to inhibit non-specific background fluorescence) and repeated once. Fifty microliters of 4', 6-diamidino-2-phenylindole (DAPI) was applied to each well and the excess wicked using a paper towel. One drop of 101 No-Fade mounting medium (Waterborne Inc., New Orleans, LA) was placed in the well and a cover slip applied, then sealed with clear nail polish. The slides were examined microscopically using an Olympus BX51 epifluorescent microscope (Olympus America Inc: Melville, NY). Cysts and oocysts were confirmed by fluorescence, size and shape, and presence or absence of DAPI stain and examined for internal features by differential interference contrast (DIC) at various magnifications (250-800X). Oocyst concentrations and method detection limits were calculated using **Equation 2**.

**Equation 2: Sample Recovery**

$$\text{No. cysts or oocysts/100 L} = \frac{T}{\text{No. liters filtered}} \times 100$$

- Where T=True value of Indigenous cysts or oocysts.
- When T = 0, detection limit is calculated by calculating T=1 and expressing sample recovery as "Less than" ("<").

- **Detection of N-Nitrosodimethylamine (NDMA)**—Analysis of NDMA was performed using a modification of the method developed by the California Department of Health Services (**www.dhs.cahwnet.gov**). One liter of sample was processed by a neutral extraction with methylene chloride and concentrated to a final volume of 1 milliliter. The extract was analyzed using a Gas Chromatograph/Mass Spectrometer (GC/MS) (Agilant Technologies, Palo Alto, California) in Selected Ion Monitoring mode to look for mass 74, which is indicative of NDMA using electron ionization.
- **Statistics**—Removal efficacy was calculated for oocyst levels using the following equation:

**Equation 3: Percent Removal for cysts and oocysts**

$$\text{Percent Removal (\%)} = \frac{\text{No. cysts or oocysts/100 L Filter Influent} - \text{No. cysts or oocysts/100 L Filtrate}}{\text{No. cysts or oocysts/100 L Post Secondary Clarifier}} \times 100$$

For *Cryptosporidium* and *Giardia*, percent removal was based on the actual counts and not those that were corrected for method recovery efficiency in order to reflect how the data would be reported for compliance purposes.

## Results

### Plant A Results

A total of 27 samples were collected over a period of three months (November 2003 through January 2004). Each week a different set of filter conditions and PAC dosage was evaluated (Table 1).

The removal of *Giardia* and *Cryptosporidium* at manufactured design loading rates with the various PAC doses is shown in **Figure 2**. The highest *Giardia* cyst removal was found using PAC addition at 14 parts per million (ppm). The percent removal was 99.62 percent on the sand filter and 92.69 percent for the cloth media filters. Based on these results, a PAC dose of 14 ppm was utilized for the balance of the study at various filter loadings. *Cryptosporidium* was effectively removed by both filters at PAC doses of 7 and 14 ppm.

The effect of the hydraulic loading rates on removal efficacy can be seen in **Figure 3**. Both *Cryptosporidium* and *Giardia* show highest removal at manufacturers' design loading rates for all three filter media. **Figure 4** shows the effects of the hydraulic loading rates on *Giardia* and *Cryptosporidium* at the optimal PAC dosage (14 ppm).

All samples were analyzed for NDMA in the filter influent and effluent. No NDMA was detected in any of the samples at the laboratory's instrument detection limit of 100 ng/L. The Environmental Protection Agency (EPA) estimates a $10^{-6}$ cancer risk for NDMA in drinking water at 0.7 ng/L. California has established an action level for NDMA in drinking water of 10 ng/L. Improvements in analytical methods are necessary to achieve lower detection limits before we can confirm that NDMA is not present in the reclaimed water.

### Plant B Results

Data for plant B were collected in May 2002. The flow rate for the plant was 3.54 million gallons with a hydraulic loading rate of 3.08 g/min/ft$^2$ to the upflow continuous backwash filter. Under these operating conditions, 96.83 percent of cysts and 86.67 percent of oocysts were removed by filtration.

## Discussion

The Florida Department of Environmental Protection's current guidance levels for *Cryptosporidium* and *Giardia* in reclaimed effluent are 5 oocysts per 100 liters and 22 cysts per 100 liters (York et al 2002). The variability of *Cryptosporidium* and *Giardia* concentrations in the filter influent are highlighted in this study.

It is noteworthy that *Giardia* cyst levels varied in Plant A, despite being collected at approximately the same time each day. *Giardia* concentrations ranged from 2,532 cysts/100 liters in Week 1 to 129 in Week 4. *Cryptosporidium* levels in the influent varied from 89 in Week 1 to <7 in Week 3. These variations affected the ability to evaluate the data.

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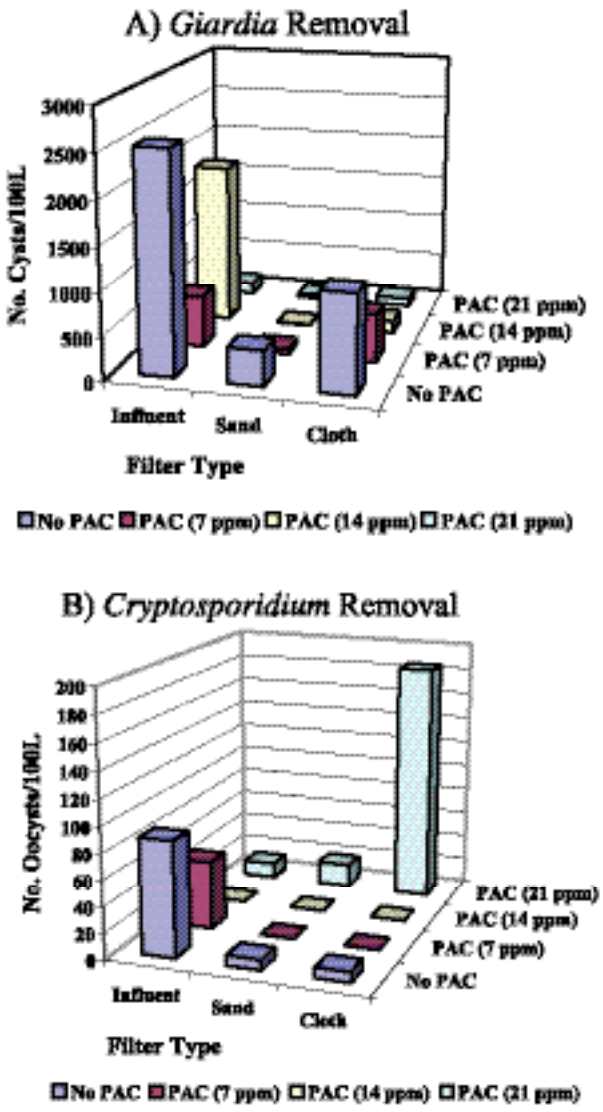


Figure 2: Effect of Different PAC Doses on Sand and Cloth Filter Removal Efficacy of *Giardia* & *Cryptosporidium* Manufacture Designed Hydraulic Loading

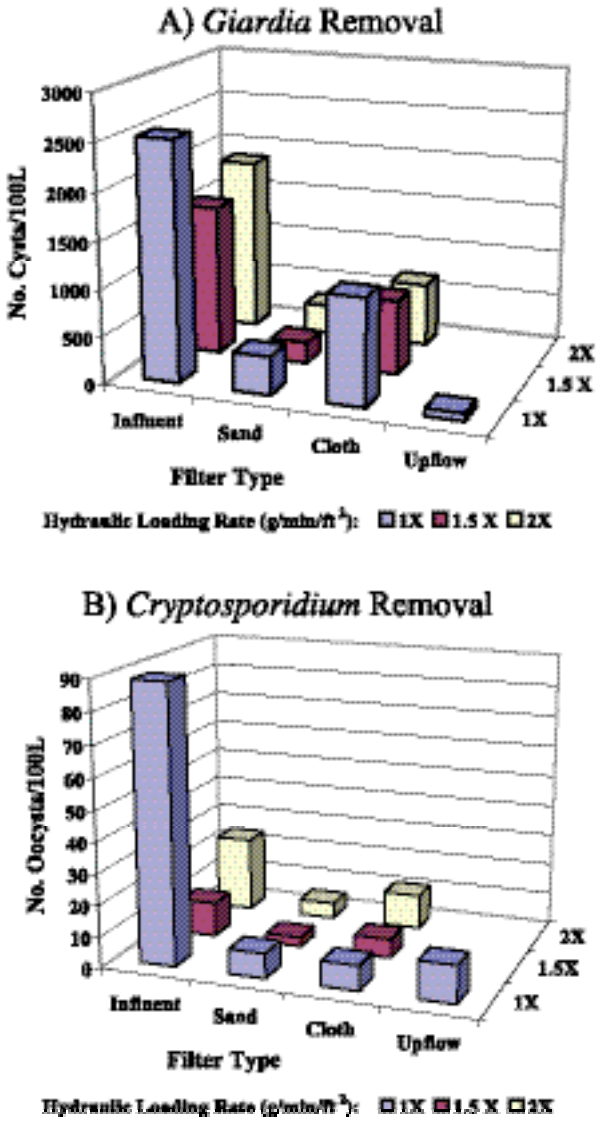


Figure 3: Effects of Hydraulic Loading Rates on *Giardia* and *Cryptosporidium* Removal Efficacy of Three Filter Media without PAC

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A 2003 study performed by Aqua-Aerobic Systems Inc. to evaluate *Cryptosporidium* and *Giardia* removal by cloth media filters showed similar results. In 50 percent of the sampling events of this study, *Cryptosporidium* was below the detection limit.

Though levels of *Giardia* and *Cryptosporidium* varied in influent and effluent waters, throughout the study, the sand filter removed a higher percentage of cysts and oocysts compared to the cloth media disk filter. Except for one sampling event, the percent removal of *Giardia* cysts remained between 85 and 99 percent for the sand filter, versus the cloth media disk filters, which fluctuated from 5 to 92 percent removal. The sand filter showed optimal removal of *Giardia* cysts (99.62 percent) at a PAC dosage of 14 ppm and hydraulic loading rate of 2.16 g/min/ft$^2$.

*Cryptosporidium* was not detected during this event; however, based on the removal of *Giardia* cysts (2.5-log$_{10}$), the PAC dosage of 14 ppm was chosen as the optimal dosage for the remainder of the study.

The highest dosage of PAC at 21 ppm resulted in difficulty with sample collection. The high turbidity and suspended solid levels in the influent waters caused the Envirocheck HV™ filters to clog at 8.5 liters. Debris and PAC that remained in the sample through processing for USEPA Method 1623 interfered with microscopy, showing a low percent recovery of 6-7 percent for all three samples. The levels reported for these samples were conservative, and data suggests increased numbers of *Cryptosporidium* in the filtrate. The difficulty in discerning cysts and oocysts from debris is highlighted by the fact that *Cryptosporidium* and *Giardia* could not be well differentiated from PAC and debris in the influent samples.

The cloth media disk filter consistently had a lower removal than the sand filter. There was only one sampling event that the cloth media disk filter was able to achieve a 1-log$_{10}$ removal (4.13 g/min/ft$^2$ and 14 ppm PAC). The addition of PAC doesn't appear to significantly improve protozoa removal in the cloth media disk filters.

Since this is an ongoing study, similar data collection still needs to be completed at Plant B. Initial testing on Plant B at design loading rate with no PAC indicates a 1.5-log$_{10}$ removal of *Giardia* and a 1-log$_{10}$ removal of *Cryptosporidium* by the upflow filters. Future projects will include additional testing at both plants.

Plant A intends to take all sand filtration off line and replace the sand filters with additional cloth media disk filters. The project will include testing the cloth media filters at full operation, as well as retesting with no PAC and lower PAC dosages at high flows.

In addition to the location at filter efflu-

ent, we will evaluate *Cryptosporidium* and *Giardia* levels post-disinfection. We plan to include infectivity assays of *Cryptosporidium* oocysts and *Giardia* cysts in the influent to filtration, effluent of filtration, and post-disinfection effluent.

## Acknowledgements

The authors would like to thank the Orange County Utilities staff for their assistance in performing the work: Chris Fasnacht, Tom Tompkins, Dean Cherry, John VonMutius, Andrew Altman, and the staff of Orange County Utilities Laboratory.

## References


- Aqua-Aerobic Systems, Inc. 2003. *Identification and Measurement of the Efficiency of* Giardia *and* Cryptosporidium *Removal from Wastewater Secondary Effluent Utilizing Cloth Media Filtration With and Without Chemical Addition.*
- Bitton, G. 1994. *Wastewater Microbiology*. Wiley-Liss, New York.
- California Department of Health Services. *http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/NDMA/NDMAlabs.htm#Acceptable Analytical Approaches* (accessed January 29, 2004).
- Fayer, R., Morgan, V. & Upton, S.J. 2000. *Epidemiology of Cryptosporidium: Transmission, Detection, and Identification.* Intl. J. Parasitol. 30:1305-1322.
- Florida Department of Environmental Protection (FDEP). 2003. 2002 Reuse Inventory. Chapter 62-610, Florida Administrative Code, Tallahassee, Florida. *http://www.floridadep.org/water/reuse/docs/pdf/2002Inventory.pdf* (accessed January 29, 2004).
- Luo, Xianghua, Clevenger, Thomas E., Gang, Dianchen. 2003. *NDMA Analytical Methods Comparisons and its occurrence in Missouri.* AWWA. 2003 WQTC Conference Proceedings.
- OPPAGA. 2000. *Office of Program Policy Analysis and Government Accountability Progress Report August 2000.* Report No. 00-04.
- Soroushian, F., Shen, Y. & Whner, M. 2001. *Evaluation and Pilot Testing of Advanced Treatment Processes for NDMA Removal and Reformation Prevention.* AWWA. 2001 Annual Conference Proceedings.
- York, D.W., Menendez, P., & Walker-Coleman, L., 2002. *Protozoan Pathogens and Reclaimed Water.* Proceedings 2002 Florida Water Resources Conference, March 26.


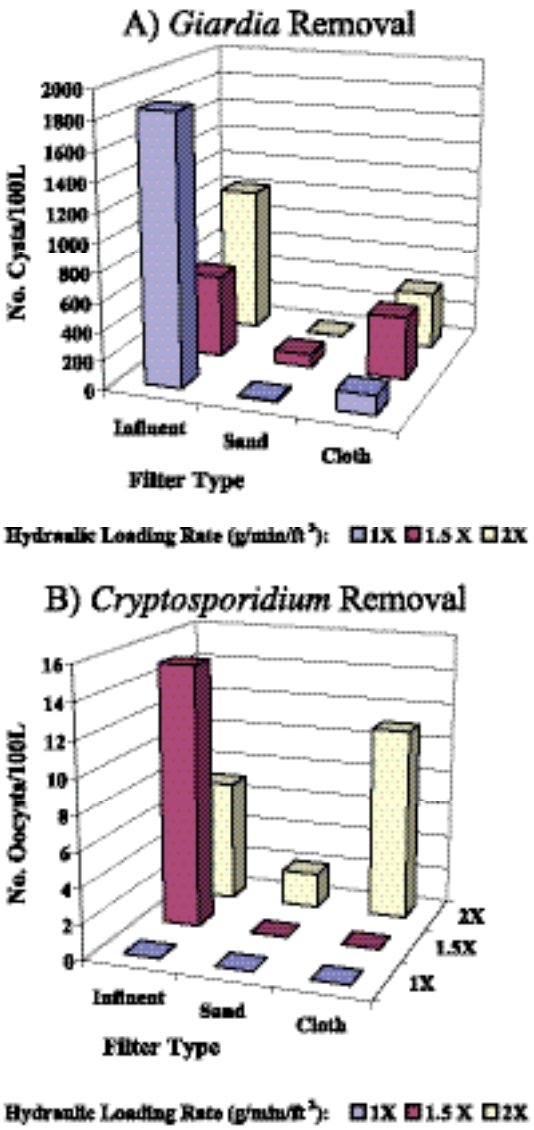


Figure 4: Effects of Hydraulic Loading Rates on *Giardia* and *Cryptosporidium* Removal Efficacy of Cloth and Sand Filter Media at Optimized PAC Dosage (14 ppm)