

Cryptosporidium Occurrence in Wastewaters and Control Using UV Disinfection

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ABSTRACT

This paper summarizes the conclusions of two recently completed projects that examined the occurrence of the protozoan pathogen *Cryptosporidium* in wastewater streams, and the efficacy of ultraviolet (UV) light for treatment of wastewaters to control *Cryptosporidium*. A 15-month survey of *Cryptosporidium* oocyst occurrence was conducted at ten US wastewater treatment plants. *Cryptosporidium* oocysts were found in all wastewater matrices from raw sewage to tertiary effluents. However, low doses of UV radiation (3 mJ/cm²) from either low or medium pressure UV lamps were effective in achieving an inactivation level of greater than three logs for *Cryptosporidium* oocysts in wastewater effluent as measured by cell culture. No evidence of oocyst DNA repair was observed. While the occurrence of *Cryptosporidium* oocysts is common in wastewater effluents, UV is highly effective for oocyst inactivation in wastewater.

Keywords: *Cryptosporidium*, disinfection, occurrence, oocysts, pathogens, particle association, ultraviolet (UV) irradiation, wastewater.

INTRODUCTION

Water bodies receiving wastewater effluents commonly serve as source waters for drinking water supplies. A current public health concern with wastewater effluents is the potential for transmission of infectious agents that may be present in human and animal feces. The diseases in the contributing communities are reflected by the varying numbers of pathogenic organisms including viruses, bacteria, helminths, and protozoa found in wastewater. Depending on the level of wastewater treatment, some of these pathogens may escape removal and/or inactivation and enter receiving waters.

Cryptosporidium is a significant concern to water suppliers worldwide, as this protozoan parasite forms highly resistant oocysts that can survive in moist environments for extended periods. The oocysts are highly resistant to environmental pressures and are difficult to remove in water treatment due to their small size (4 to 6 µm). *Cryptosporidium* oocysts are resistant to conventional chlorine based disinfectants, and oocysts that escape the coagulation/filtration process can remain viable in the distribution system. Dozens of outbreaks of cryptosporidiosis have occurred

worldwide, with resulting deaths (Lisle and Rose 1995; Kramer et al. 1998).

Animals and humans are reservoirs for this parasite, and it enters the environment through shedding of fecal material. Dozens of species harbor *Cryptosporidium* oocysts including mammals (cattle, horses, rodents, deer, dogs, cats), kangaroos, birds, reptiles, and fish. As such, there are many routes for this parasite to enter the environment including natural runoff (non-point sources), runoff from agriculture, effluents from industries such as meat processors, wastewater effluents, and combined sewer overflows.

DEVELOPMENT OF METHODS FOR RECOVERY OF CRYPTOSPORIDIUM FROM WASTEWATER MATRICES

Methods development for *Cryptosporidium* recovery from water has focused primarily on clean waters: source waters for drinking water plants such as lakes and rivers, and finished drinking water. Due to the differences in matrix composition from raw wastewater to tertiary effluents, different methods for recovery and enumeration of oocysts were needed to address matrix quality. The standard method in use in the US and generally worldwide is USEPA Method 1622 (USEPA, 2001). Using the basic format of Method 1622, a single method was developed for raw wastewater and primary influents that uses centrifugation to concentrate 500 mL samples. A second method was developed for secondary and tertiary effluents which includes the Pall Envirochek HV™ (Pall Corp. Ann Arbor, MI, product no. 12099) capsule for sample concentration. Similar to Method 1622, both methods rely on immunomagnetic separation (IMS) to separate oocysts from debris, followed by antibody staining and microscopy for identification and enumeration. The inclusion of ColorSeed™ (BTF, PO Box 599, North Ryde BC, NSW 1670, Australia), an internal positive control in each sample, provides information on method performance. ColorSeed contains flow cytometer-sorted, gamma-irradiated *Cryptosporidium* oocysts and *Giardia* cysts that have been permanently stained with a red fluorescent dye allowing differentiation between ColorSeed™ and naturally-occurring oocysts, which are not red. These wastewater-specific methods were developed and single-laboratory validated over a two year period.

OCCURRENCE SURVEY

Once method performance was established, these methods were used in a survey of *Cryptosporidium* occurrence in ten US wastewater treatment plants over a 15-month period. The wastewater treatment facilities sent monthly grab or composite samples to the laboratory (Clancy Environmental Consultants, Inc.) for analysis. Samples of econdary and tertiary effluents were filtered in the field, with ColorSeed™ added to the filter capsule prior to sample collection. ColorSeed™ was incorporated as an internal control to indicate method performance in each sample. Participating utilities were located in Alabama, California, Colorado, North Carolina, Pennsylvania, and Vermont. A total of 289 samples were analyzed in the 15-month survey. The summary data are presented in Table 1.

Table 1. Summary of *Cryptosporidium* Oocyst Recoveries and Occurrence in Wastewater Survey

Matrix (n = samples analyzed)	Volume Analyzed (L)	Range of Turbidities (ntu)	<i>Cryptosporidium</i> Concentration (#/L)	Percent of samples positive for <i>Cryptosporidium</i>
Raw Influent (95)	0.5	34.3 - 700	<2 - 24	29.5
Primary Effluent (84)	0.5	4.08 - 3.7	<2 - 86	45.8
Secondary Effluent (94)	4.2 - 22.7	0.75 - 19.3	<0.1 - 40.8	58.5
Tertiary Effluent (16)	22.7 - 131.3	0.75 - 23.62	<0.008 - 0.226	18.8

These data show the wide range of turbidities in each sample type, which contributes to the variability in the matrix itself and in the overall recovery of oocysts. Turbidities of raw influent ranged from 34.3 to 700 ntu, while primary influents ranged from 4.08 to 327 ntu. The range for secondary effluents was much narrower, 0.75 to 19.3 ntu, with tertiary effluents ranging from 0.75 to 2.62 ntu. As turbidity decreased through the treatment process, the ability to recover oocysts improved as water quality improved.

Another objective of the occurrence study was to examine the removal of oocysts across the treatment process. Since *Cryptosporidium* cannot reproduce outside of an animal host, the concentration of oocysts cannot increase through the treatment process. Regardless of differences in recovery efficiencies, the occurrence of *Cryptosporidium* oocysts in wastewater is significant. It is reasonable to assume that the concentration of oocysts should be continually decreasing across the treatment process, from raw influent to final effluent (secondary or tertiary) on a per volume basis. This was not always observed, as shown in data from one of the plants in Figure 1. This plant in Alabama operates at 2 mgd with a peak flow design of 3 mgd. The wastewater influent is comprised of 70% domestic and 30% industrial (food industry). Preliminary treatment of the influent includes screen and grit removal as well as pre-aeration. The pretreated wastewater enters the primary clarifiers

and the weir effluent is passed through biofilters, intermediate clarifiers, and then through trickling filters. The trickling filter effluent enters the secondary clarifiers. The effluent from the secondary clarifiers remains in a chlorine contact basin for 30 min before being discharged. This plant uses no chemicals, such as polymers, alum, or caustic for enhanced coagulation, phosphorus removal or pH adjustment.

Figure 1 shows the oocyst concentration per liter at the Alabama plant. In some months the concentration of oocysts recovered was higher in secondary effluent than in raw or primary (May, October, December, March). A factor that may contribute to this observation is the low overall levels of oocysts detected in this plant, making it difficult to show significant log₁₀ differences. The number of oocysts per liter in the Alabama data ranged from <1 to 4 across all matrices. Another factor is the improvement in recovery of oocysts in cleaner water matrices; hence greater numbers of oocysts were recovered from effluent samples than from raw or primary influent samples. *Cryptosporidium* oocysts were detected in 3 of 11 raw influent samples (27.3%) at a concentration of 2/L. In primary effluent samples, 18.2% (2/11) were positive for indigenous oocysts. In secondary effluents, 72.2% (8/11) samples were positive for oocysts, ranging from 1 to 36/10L. Similar trends in oocyst concentration variability across the treatment process were noted for other plants in the study.

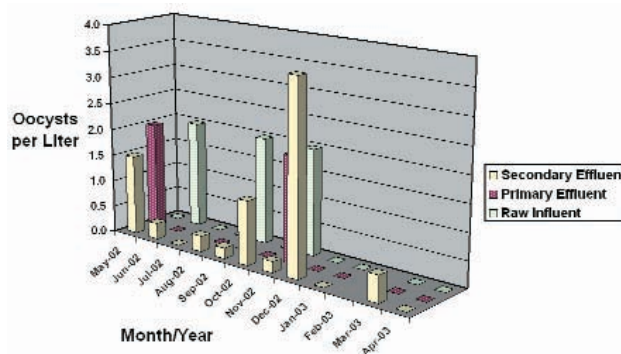


Figure 1. Oocyst concentrations in raw influent and primary and secondary effluents at Alabama wastewater treatment plant.

ULTRAVIOLET (UV) INACTIVATION OF *CRYPTOSPORIDIUM* IN DRINKING WATER

Given the presence of *Cryptosporidium* in wastewater and many surface waters, one of the major challenges for drinking water suppliers is the control of *Cryptosporidium*. Like *Giardia*, *Cryptosporidium* oocysts can be physically removed by the coagulation/filtration processes, although oocyst removals have been found to be slightly reduced likely due to their smaller size (Nieminski and Ongerth 1995; Ongerth and Pecoraro 1995). But unlike *Giardia*, *Cryptosporidium* oocysts are resistant to chlorine-based disinfectants at the concentrations and contact times practical for water treatment (Korich et al. 1990). This makes the physical removal process (coagulation, sedimentation, filtration) of

paramount importance in conventional water treatment where chlorine is relied on for disinfection.

UV has been used for drinking water treatment in Europe since the early 1900's, but until the mid-1990's, it was not considered to be an effective treatment for protozoan pathogens such as *Cryptosporidium*. Studies by Campbell et al. (1995) followed up with additional research (Bolton et al. 1998; Clancy et al. 1998; Bukhari et al. 1999; Clancy et al. 2000) showed *Cryptosporidium* to be extremely susceptible to UV. Dozens of studies have shown that UV is highly effective at relatively low UV fluences (10 mJ/cm²) for control of *Cryptosporidium* in drinking water applications. In response to this relatively new finding, in August 2003 the USEPA proposed the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA 2003) to require additional treatment of water sources with higher *Cryptosporidium* levels to protect public health. The use of UV for disinfection of drinking water derived from surface sources is a major aspect of this rule. Dozens of US cities have plans to incorporate UV into their treatment processes over the next decade, with the New York City proposal for a 2 billion gallon per day facility already in the design phase (Potorti et al. 2004).

UV, CRYPTOSPORIDIUM, AND WASTEWATER

UV is a viable alternative to chlorination for disinfection of wastewater. Although UV has been available as a technology since the early part of the 20th century, it did not experience widespread acceptance for wastewater treatment until the mid-1980's. Since this time, both research into UV disinfection and installations of UV facilities have increased dramatically. Some of the explanations for the increasing acceptance of UV irradiation for the disinfection of wastewater are:

1. proven ability to disinfect pathogenic bacteria and most viruses,
2. proven effectiveness in meeting federal wastewater effluent standards based on the reduction of indicator organisms in the finished effluents to meet permitted effluent discharge limits,
3. increased safety compared to the storage and handling of chlorine,
4. no known formation of toxic by-products,
5. increasing costs of chlorination due to regulations curbing chlorine discharge limits – thus mandating dechlorination, and
6. stringent and costly regulations regarding storage and transport of chlorine gas as part of the Uniform Fire Protection Code.

UV technology has become increasingly more reliable and predictable with regard to performance. Improvements in the lamp and ballast technology has led to the use of medium pressure UV sources for disinfection applications, thus expanding the range of

water qualities that can be treated with UV radiation. Future expansions in technology may allow for the design of UV lamps with optimized germicidal outputs for inactivation of specific pathogens in a given water quality matrix.

Cryptosporidium was not recognized as an important human waterborne pathogen until the mid-1980's and wastewater regulations have not incorporated removal or inactivation of oocysts in wastewater effluent standards. The current regulatory focus of wastewater disinfection is on bacteria – fecal coliforms and *E. coli*. The fate of specific pathogens in wastewater disinfection processes and the extent to which they elude disinfection or detection in wastewater are not well understood or documented. Previous literature reports indicate that *Cryptosporidium* is common in wastewaters worldwide (Madore et al. 1987; Stadterman et al. 1994; Robertson et al. 2000). At least ten cryptosporidiosis outbreaks between 1984 and 1998 have been attributed to sewage contamination of drinking water (McCuin and Clancy 2004). Since chlorination is ineffective in oocyst inactivation, the obvious question is – how well does UV perform for *Cryptosporidium* inactivation in wastewater matrices?

Questions regarding UV inactivation of *Cryptosporidium* in wastewater included:

1. how well is *Cryptosporidium* inactivated in wastewater matrices,
2. is there a difference between low and medium pressure lamps,
3. can oocysts undergo light or dark DNA repair,
4. how does particle association affect UV effectiveness?

The results of recent research indicate that both low and medium pressure UV irradiation are very effective for inactivation of *C. parvum* oocysts spiked into secondary wastewater effluent (Linden et al. 2004). Infectivity assays using cell culture indicated that inactivation levels greater than three log₁₀ can be achieved in wastewater with a UV dose of only 3 mJ/cm². These data agree with the results of Clancy et al. (2004) using animal infectivity and Shin et al. (2001) using cell culture. Rochelle et al. (2002) reported good correlation between the average infectivity of HCT-8 cells and CD-1 mice for untreated oocysts as well as oocysts exposed to UV light.

Inactivation of *Cryptosporidium* was most effective in the 250 to 270 nm range, which includes both the low and medium pressure output ranges. Despite the fact that many microorganisms exhibit the ability to repair UV damaged DNA and restore infectivity, it was found that UV inactivated *C. parvum* oocysts are not able to restore their infectivity in cell culture host cell lines following exposure to either light (photoreactivation) or dark DNA repair protocols.

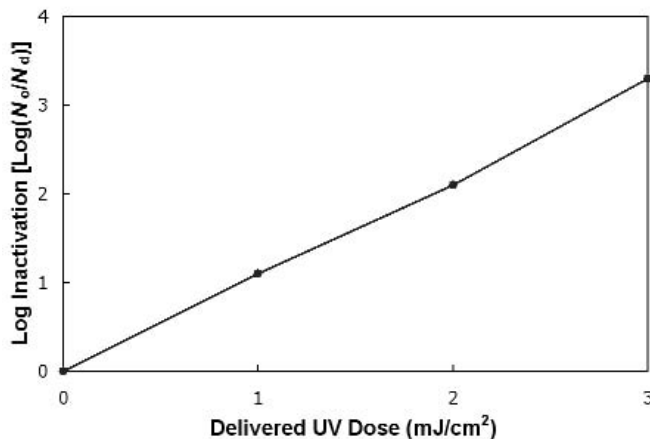


Figure 2. Inactivation of *C. parvum* oocysts in wastewater by low pressure UV radiation.

PARTICLE ASSOCIATION AND SEQUENTIAL DISINFECTION

As indicated above, the natural occurrence of *Cryptosporidium* in wastewater is too low to allow for the determination of log inactivation from UV exposure. Therefore, it is necessary to spike *Cryptosporidium* into the wastewater effluent to test for levels of inactivation. However, this may not represent the true physical state of occurrence of *C. parvum* in wastewater. A molecular biological approach was used to identify *C. parvum* in wastewater and visualize the degree of particle association. The method chosen for identification of pathogens associated with particles and flocs in wastewater was Fluorescent in-situ Hybridization (FISH). This method was modified to minimize the impact on particle/floc structure and allow visualization with minimal disturbance to the floc structure. It was visually shown that *C. parvum* oocysts were present and associated within wastewater flocs, in a fashion similar to that found for coliform bacteria. Although disinfection testing of these particle-associated pathogens was not possible, it may be assumed that these microbes could be protected from UV and chlorine disinfection. However, the extent of the significance of this particle association on disinfection could not be evaluated and would likely be source and process specific. This is a topic of future research.

Use of multiple disinfectants in series was hypothesized to be an effective strategy for inactivation of the wide range of pathogen types found in wastewater. The results indicated that a high level of inactivation of *C. parvum* oocysts in wastewater is achieved by application of low doses of low pressure UV 254 nm radiation. Therefore, an approach that utilizes UV disinfection followed by free chlorine dosing and subsequent formation of monochloramine (due to ammonia in the wastewater) along with long contact time should be capable of achieving significant inactivation of most microorganisms within a practical range of UV and free/monochloramine doses. Extended contact time with chlorine was also found to be effective in achieving inactivation of particle associated coliform bacteria in wastewater. However, because

protozoan pathogens such as *C. parvum* oocysts are highly resistant to chlorination, non-UV disinfected particle associated *C. parvum* oocysts will persist in wastewater following chlorination only. In addition, the formation of chlorinated byproducts may be a concern. Therefore, if complete inactivation of chlorine-resistant microbes is required, use of UV disinfection systems in wastewater treatment processes can be very effective but should be optimized or augmented (perhaps with filtration) to compensate for the reduction of disinfection efficacy due to aggregation and/or particle association.

The results of this research can be directly applied to the optimization of current wastewater disinfection practices with UV irradiation and chlorine. For example, a wastewater utility discharging treated effluent to a sensitive water body such as one used for drinking water, may want to employ a sequential UV-chlorine/chloramine disinfection scheme to ensure adequate inactivation of most protozoa, including *Cryptosporidium*. As another example, a wastewater utility experiencing problems with meeting effluent standards may need to investigate the issue of particle associated microbes and adjust their upstream processes to minimize formation of microbe-associated particles. Finally, although the use of free chlorine/chloramines sequential disinfection in a dynamic chloramination mode by a wastewater treatment plant could enhance the overall removal of bacteria and viruses with extended contact times, this scenario may not be capable of disinfecting *C. parvum* oocysts. Therefore, for some wastewater utilities that are particularly susceptible to *Cryptosporidium* oocyst contamination, addition of a UV disinfection process would be a prudent step to protect downstream users from the risk of *Cryptosporidium* exposure.

SUMMARY

The results from these studies demonstrate that *Cryptosporidium* occurs commonly in all types of wastewater matrices. Whether derived from wastewater, urban, or agricultural runoff, the percentage of wastewater samples positive for oocysts is high: 30% for raw sewage, 46% for primary influent, 59% for secondary effluent, and 19% for tertiary effluent. While occurrence is common, a critical question for risk assessment is whether or not the oocysts recovered are able to cause infection in humans or animals. This research did not answer this question, as the ability to detect infectious oocysts in wastewater samples is fraught with analytical difficulties. However, the research moved a step beyond to demonstrate that *Cryptosporidium* is easily inactivated in wastewater effluents using UV disinfection, with no evidence of DNA repair. UV wavelengths emitted from low and medium pressure lamps were equally effective for inactivation of *C. parvum* oocysts. That UV will play an important role in the future of wastewater disinfection for improved public health protection, is clear.

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