Assessing UV reactor performance for treatment of finished water

Z. Bukhari* and M. LeChevallier**
* American Water Works Service Company, Inc., Quality Control and Research Laboratory, 1115 South Illinois Street, Belleville, IL 62220, USA (E-mail: zbukhari@bellevillelab.com)
** American Water Works Service Company, Inc., 1025 Laurel Oak Rd., P.O. Box 1770, Voorhees, NJ 08043, USA

Abstract Recently, use of low levels of medium- and low-pressure ultraviolet light for successful inactivation of Cryptosporidium parvum oocysts has generated tremendous excitement in the water industry. Accurate delivery of the target dose, lamp performance, sensor stability and impact of water characteristics are some factors that could impact disinfection efficacy, in turn influencing decisions on application of this technology. To this end, American Water Systems, the largest investor owned water utility in the US, has responded to some of these challenges by ascertaining the long-term feasibility of applying UV for treatment of finished water. A 4 × 1 UV reactor with a 12 inch (0.3 m) diameter was installed after granular activated carbon filtration and was operated with a finished water flow rate of 600 gpm (2,700 L/min). Over a 12-month period, various chemical (THM, HAA, UV254, DOC, TOC, metals, nitrate, nitrites) and physical measurements (lamp voltage, current, sensor measurements) were monitored to define their impact (if any) on the operation of the reactor. MS2 bacteriophage challenge studies were conducted with various lamp configurations and lamp age. These inactivation data demonstrated high levels of correlation with controlled bench scale inactivation data. For C. parvum oocysts, bench scale studies were performed with a modified in vitro infectivity assay using HCT-8 cells, an enhanced infectivity protocol and with either immunofluorescence or quantitative PCR based detection. While both assays indicated increasing infections levels of HCT-8 cells with increasing oocyst inocula, UV treatment of oocysts produced markedly different infectivity responses. Based on the data generated in this study, one in vitro infectivity assay was selected to demonstrate >3 logs inactivation with low UV doses (5 mJ/cm² –10 mJ/cm²).

Keywords Cryptosporidium; drinking water; ultra violet

Introduction

Although ultra violet (UV) technology has been used for disinfection of water since the early 1900s, its application to treatment of drinking water remained uncertain until recently. This was due partly to the ambiguous nature of experiments performed for measurement of Cryptosporidium oocyst inactivation (Lorenzo-Lorenzo et al., 1993) and, subsequently, generation of data indicating the need for high UV doses to achieve appreciable inactivation of oocysts (Campbell et al., 1995) More recently, comparative studies examining viability and infectivity assays demonstrated significant differences between these procedures. Recognition of the fact that viability/infectivity assay selection was an important criterion for measuring oocyst inactivation served as a platform that led to discovery of UV treatment being a highly effective approach for Cryptosporidium oocyst inactivation (Bukhari et al., 1999). Since these findings, several other collaborative, as well as independent, studies have corroborated these data (Clancy et al., 2000), verifying that certain UV systems are capable of inactivating Cryptosporidium and Giardia, microbes responsible for numerous waterborne outbreaks (Craun et al., 1998). Additionally, data have also demonstrated effectiveness of UV for a wide variety of microorganisms and UV technology offers a further advantage of not producing unwanted by-products upon disinfection of water. The Stage 2 Microbial/Disinfection By-Product Agreement states
that UV technology is feasible and available provided certain design and operational issues are resolved.

The American Water System (AWS), the largest and most geographically diverse water service provider in the nation, owns and operates >80 surface water treatment plants and each plant is a possible candidate for application of UV technology. Therefore, a project was initiated to evaluate design and operational issues associated with the current state of UV technology and ascertain information on the feasibility of applying UV treatment as a disinfection barrier for protection from transmission of protozoan parasite infections; specifically human cryptosporidiosis, by the drinking water route. In order to evaluate the disinfection effectiveness of UV light, a commercially available pilot reactor, containing four 1 kw medium pressure lamps and capable of handling flow rates up to 700 gpm (3,150 L/min), was installed on the effluent to a granular activated carbon (GAC) filter. During the course of one year, various chemical and physical parameters of the influent water (pH, turbidity, disinfection by-products etc) were recorded. Primarily the objectives of these investigations were to determine whether the UV reactor under evaluation was capable of delivering adequate UV doses to inactivate appreciable levels (>2 logs) of oocysts. However, the pathogenic nature of oocysts, as well as the inability to generate sufficient quantities of oocysts to conduct pilot scale experiments, has necessitated the use of surrogate microorganisms to conduct biodosimetry experiments for UV dose verification.

To this end, several biodosimetry experiments were conducted using MS2 bacteriophage and various lamp configurations and lamp age. In order to corroborate the actual dose of UV light being delivered to the organisms in the pilot scale studies, MS2 bacteriophage inactivation levels were compared with bench-scale inactivation experiments under controlled experimental conditions with well-defined UV doses.

Bench-scale inactivation experiments were also conducted using C. parvum oocysts and in vitro infectivity assays. Presently, no industry standard procedures exist for in vitro infectivity assays for oocysts and mouse infectivity continues to remain a “gold” standard. In our studies, we examined the use of human ileocecal adenocarcinoma (HCT-8) cells in conjunction with infectivity measurements with either a quantitative polymerase chain reaction (q-PCR) or immunofluorescence (IF) microscopy to define a user-friendly in vitro viability assay for measuring UV associated inactivation of oocysts. This component of the study attempted to define an enhanced in vitro infectivity protocol that enabled determination of C. parvum inactivation following UV treatment and provided levels of inactivation that were similar to those reported previously with mouse infectivity assays.

Materials and methods

Bench and pilot scale UV inactivation experiments

For the inactivation experiments, a Rayox UV collimated beam unit (Calgon Carbon Corporation) was used. A medium pressure UV lamp (1 kW) was supplied with the instrument. A calibrated radiometer and detector (International Light) was used to measure irradiance from the collimated beam. The delivered UV dose was determined with the aid of a spreadsheet incorporating various correction factors associated with the experimental procedure. Briefly, microorganisms suspended in 15 mL PBS in a petri dish, were exposed to a known UV irradiance for a specified time and a mathematical equation was used to calculate the applied UV dose. A viability comparison between UV exposed microorganisms and untreated control organisms enabled calculation of organism inactivation at a specific UV dose.

A 700 gpm (3,150 L/min), 12 inch (30 cm) diameter UV reactor containing four 1kW medium pressure lamps was used for pilot scale studies. During spiking studies the reactor effluent was discharged to a filter to waste facility (Figure 1).
Enumeration of MS2 bacteriophage

USEPA Method 1601 was used for enumeration of MS2 bacteriophage before and after UV treatment. Briefly, soft agar containing antibiotics (4 mL) was dispensed into glass screw cap tubes, inoculated with 100 µL of log phase *E. coli* and 50 µL of the appropriate MS2 dilution. After thorough mixing, each sample was poured onto plates containing solidified TSA, gently swirled to allow even sample distribution over the surface, allowed to solidify and each inverted plate was incubated (37°C) for 16–24 h. Comparison of bacteriophage plaques before and after UV treatment enabled determination of the levels of inactivation at a given UV dose.

Measuring inactivation of *C. parvum* oocysts

An *in vitro* infectivity assay using HCT-8 cells and a modified pre-treatment/inoculation procedure to enhance infectivity was employed in these studies. Using confluent monolayers of HCT-8 cells, 100 µL suspensions of UV treated or control oocysts, containing a known number of organisms (between 1,000–10,000 oocysts), were added. The microtitre plates were placed in a 37°C CO₂ incubator for 72 h. Following incubation, each inoculated well was gently washed ×5 with PBS and detection of infection was performed either by q-PCR or IF microscopy.

Quantitative PCR (q-PCR). *C. parvum* DNA extracted from HCT-8 monolayers was detected with q-PCR using *Cryptosporidium*-specific primers targeting the heat shock protein gene. The thermocycling conditions consisted of initial denaturation (95°C; 10 min) followed by 45 cycles of denaturation (95°C; 30 s), annealing (59°C; 1 min) and extension (72°C; 30 s). The amplification process was followed by a single final extension (72°C; 10 min) and then samples were held at 4°C. Quantitation of the PCR amplified products used a fluorogenic probe that annealed specifically between the forward and reverse primers. In an intact probe, close proximity of reporter fluorogen (6-carboxy-fluorescein) and the quencher prevented dye fluorescence emission by Forster type energy transfer. However, during each extension cycle in PCR, the *Taq* DNA polymerase cleaved the reporter dye from the probe, increasing the distance between the reporter and quencher. This led to increased emission of a characteristic fluorescence signal which was associated with exponential increase of PCR product. The q-PCR reactions were carried out on the ABI Prism 7700 sequence detector, which provided software controlled reaction set up and data analysis. An argon ion laser (488nm) was used to carry excitation light through fibre optic cables into an optical multiplexer which distributed light to each of the 96-wells (containing PCR reactions). Fluorescence emission from each amplified product passed via fibre optic cables, through a spectrograph and was detected by a CCD camera (500–660 nm).
Immunofluorescence microscopy. Following 72 h incubation, unexcysted oocysts were washed from monolayers by using PBS (pH 7.2), cells were fixed with paraformaldehyde, then chilled methanol, and stained with 1/10 dilution of sporo-glo (Waterborne Inc). Excess antibody was washed from each well and mounting medium containing an antifadant was applied prior to visualisation using an inverted fluorescence microscope. During microscopy, a rationale was developed for determination of oocyst infectivity. This incorporated the use of current knowledge that oocysts undergoing UV treatment up to certain doses (i.e. <150 mJ/cm²) were still capable of undergoing excystation (Bukhari et al., 1999). Thus, it was possible that sporozoites from such oocysts could invade monolayers whereas sporozoites from untreated oocysts would not only invade cells but also differentiate into clusters of secondary infection. Using this rationale, infection was defined as instances where clusters of infection were present and this approach was used to develop a standard curve of number of clusters vs original oocyst inoculum.

Results

Bench scale inactivation of MS2 bacteriophage

A standard inactivation curve for MS2 bacteriophage was developed using three separate UV doses (Figure 2).

The data indicated good reproducibility in quintuplicate trials at each of the three doses with mean ± SD levels of log inactivation at 0.68 ± 0.13, 1.38 ± 0.24 and 2.47 ± 0.11 for 10 mJ/cm², 20 mJ/cm² and 40 mJ/cm² respectively (Table 1).

Pilot scale inactivation of MS2 bacteriophage

For the pilot scale studies, total MS2 bacteriophage spike doses ranged between 0.5 – 3.4 × 10¹³ organisms that were spiked continuously over 5 min duration. Initially samples pre- and post-UV treatment were collected at 1, 3 and 5 min intervals; however, no significant difference in bacteriophage inactivation was noted at these various time intervals of sample collection during the spiking regime. These data indicated rapid, uniform mixing of the

![Figure 2](https://iwaponline.com/wst/article-pdf/47/3/179/424084/179.pdf)

**Figure 2** Bench scale MS2 inactivation using various doses of UV

**Table 1** Comparison of bench and pilot scale MS2 inactivation data

<table>
<thead>
<tr>
<th>Anticipated UV dose based on lamp configuration (mJ/cm²)</th>
<th>Expected inactivation - bench scale (n=5)</th>
<th>Log inactivation - pilot scale (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>2.47 ± 0.11 (5)</td>
<td>2.59 ± 0.46 (6)</td>
</tr>
<tr>
<td>20</td>
<td>1.38 ± 0.24 (5)</td>
<td>1.92 ± 0.26 (5)</td>
</tr>
<tr>
<td>10</td>
<td>0.68 ± 0.13 (5)</td>
<td>1.21 ± 0.097 (3)</td>
</tr>
</tbody>
</table>
organisms. Based on this, additional spiking studies collected samples at the 3 min time interval only.

**Evaluation of *C. parvum* oocyst inactivation by *in vitro* infectivity assays**

Where cell culture was followed by detection of infection by q-PCR, log oocyst inactivation was determined to be 1.16, 1.24 and 1.84 at UV doses of 10 mJ/cm², 20 mJ/cm² and 40 mJ/cm² respectively. These values were significantly lower than those reported previously using mouse infectivity assay (Bukhari *et al.*, 1999, Clancy *et al.*, 2000). In contrast, using the enhanced infectivity protocol in conjunction with IF based detection of infection clusters, >3-log inactivation of *C. parvum* oocysts was calculated with UV exposures of 5 mJ/cm² and 10 mJ/cm².

**Discussion**

The bench scale inactivation studies for MS2 bacteriophage indicated excellent reproducibility between experiments conducted at each of the three UV doses that were examined. Furthermore the data generated in this study were in good agreement with previously published inactivation data for MS2 bacteriophage. During the pilot scale biodosimetry experiments, the target UV doses were achieved by selection of lamp configurations based on their pre-calibrated sensor readings as well as the computational fluid dynamics models used by the manufacturer. Although during this study, lamps of operational age between 16 h and 5,800 h were used, the inactivation data for a given target UV dose were very similar irrespective of lamp operation time. Furthermore, as the reactor was continuously operated for up to 5,800 h it also demonstrated that the lamp cleaning mechanisms performed effectively. Interestingly, the correlation between the pilot scale and bench scale log inactivation values was greatest at 40 mJ/cm²; however, the disparity between the log inactivation values between pilot and bench scale studies increased when the target UV dose values were reduced to 20 mJ/cm² or 10 mJ/cm². At both of these latter UV doses, the levels of inactivation were higher in the pilot studies compared to the bench scale studies. In the bench scale studies, both 10 mJ/cm² and 20 mJ/cm² demonstrated excellent reproducibility with respect to organism inactivation that would appear to suggest that the pilot unit actually delivered higher UV doses when targeting either 10 mJ/cm² or 20 mJ/cm².

During the course of these studies total THM and HAA were examined for both the finished water influent to the reactor as well as the effluent from the UV reactor. These data indicated disinfection by-product (DBP) values to be below their respective maximum contaminant limit (MCL) values and that employment of UV disinfection did not impact these DBP.

In order to develop bench scale inactivation data for *C. parvum* oocysts, initially the cell culture q-PCR format for enumerating *C. parvum* was examined as it had the advantage of providing a direct indication of the amount of amplifiable DNA with a specific set of primers. Furthermore, this assay also had the potential to help avoid the high variability associated with using the most probable number format of mouse infectivity assays. [However it is important to appreciate that mouse infectivity assays are currently recognised as the “gold” standard and other viability/infectivity assays need to demonstrate an acceptable level of agreement with this assay to evolve into procedures that generate meaningful data with respect to the ability of oocysts to perpetuate infection in susceptible hosts.] The cell culture q-PCR format used in these studies demonstrated increasing levels of oocyst inactivation with increasing doses of UV light (10–40 mJ/cm²). In previous studies, where *in vitro* viability assays (fluorogenic dyes, excystation) were used for measuring oocyst inactivation, little or no response was detected with these assays until the UV doses
exceeded 150 mJ/cm² (Bukhari et al., 1999). As the cell culture q-PCR demonstrated responsiveness at doses significantly lower than 150 mJ/cm², it suggested that the cell culture q-PCR had a higher level of sensitivity than these in vitro assays. The log inactivation values determined by the q-PCR procedure were 1.16 logs, 1.24 logs and 1.84 logs for 10 mJ/cm², 20 mJ/cm² and 40 mJ/cm² respectively. These inactivation values for *C. parvum* oocysts were considerably lower than those reported in previous studies using mouse infectivity assays (Bukhari et al., 1999; Clancy et al., 2000).

It is known that the UV doses examined in this study would not impact the ability of the oocysts to respond to excystation stimuli. This would suggest that UV treated oocysts inoculated onto cell monolayers have the potential to excyst and then invade cell monolayers. Should this happen, it could result in generation of a “background” signal which may be derived from either the sporozoites themselves or from DNA of trophozoites (should the invasive sporozoites undergo further differentiation). A number of different oocyst pre-treatment steps were used to enhance the differential between UV affected and unaffected sporozoites; however, these did not help to reduce the background signal detected by the q-PCR procedure. Consequently, an infectivity enhancing oocyst pre-treatment step in conjunction with IF-based microscopic detection was used to develop an approach for measuring inactivation of UV treated oocysts with in vitro infectivity assays. Whereas UV treated oocysts resulted in excystation and subsequent invasion of HCT-8 cells, leading to discrete pinpoints of invasion, untreated organisms continued to differentiate further to generate clusters of secondary infection. By enumerating clusters of infection only, a dose response curve was developed for various inocula of oocysts and which was then used to extrapolate the numbers of infectious oocysts present in a given inoculum of UV treated oocysts administered onto monolayers. Based on this in vitro infectivity rationale and UV treated (5 mJ/cm² or 10 mJ/cm²) oocyst inocula of 1,000 or 10,000, >3-log inactivation was determined. Current studies are examining higher inocula of UV treated organisms to determine actual levels of inactivation measurable by this in vitro infectivity assay format.

In conclusion, pilot scale biodosimetry data indicated the performance of the UV reactor to be stable and disinfection efficacy was not influenced significantly by lamp age <5,800 h operation. Also a good correlation between bench and pilot scale data was obtained at a target UV dose of 40 mJ/cm² and, while this declined with lower UV doses, nonetheless higher levels of inactivation were observed with the pilot scale data. Also, a simple, effective cell culture procedure in conjunction with IFA was used to demonstrate >3-log inactivation of oocysts in bench scale studies. Further studies are underway to define whether increasing the original oocyst inoculum administered onto monolayers can help to establish higher levels of inactivation for given UV doses.

**References**


