Efficacy of Low-Pressure Ultraviolet Irradiation for Inactivating Cryptosporidium parvum Oocysts in Turbid Water

Tsuyoshi HIRATA¹, Shigemitsu MORITA¹, Hitomi SUGIMOTO¹, Hiromi TAKIZAWA¹ and Takuro ENDO²

¹Water Environment Laboratory, School of Environmental Health, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229-8501, Japan (e-mail: hirata@azabu-u.ac.jp) ²Department of Parasitology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan

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ABSTRACT
To examine the effects of turbidity on the inactivating efficacy of low-pressure UV against oocysts of Cryptosporidium parvum HNJ-I strain, two experimental systems, a separated system and a mixed system, were designed. In the separated system, the UV was irradiated in a quartz chamber filled with turbid water and the UV passed through the quartz chamber was irradiated onto the oocysts suspended in purified water. In the mixed system, UV was directly irradiated onto the oocysts suspended in turbid water. As a source of turbidity, sludge from a water purification facility was used. Infectivity was determined by the animal infectivity test using SCID mice. UV was assumed to decrease its intensity in the mixed system in accordance with the Lambert-Beer’s Law, and therefore the average UV dose delivered to the oocysts was estimated by integrating the UV intensity from the surface to the bottom of the suspension. UV intensity showed a significant depletion in turbid water and resulted in turbidity-dependent decrease in infectivity; however, little difference was found among the relationships between the average irradiation dose delivered and infectivity reduction. Thus, it can be concluded that the inactivating efficacy per unit dose of UV delivered to the oocysts was identical in purified water and in turbid water.

INTRODUCTION
Waterborne outbreaks of cryptosporidiosis associated with contaminated surface water supplies have been documented in many countries (Atherton et al., 1995, Kramer et al., 1996). Nowadays, it is believed that Cryptosporidium parvum oocysts are ubiquitous in river environments and the concentrations in source river waters can be as high as 104 oocysts/100 L (LeChevallier et al., 1991, Rose et al., 1991, Hashimoto et al., 2002), the removal rate may be insufficient to eliminate the risk in case the source water is strongly contaminated. Chlorination has been used as a final disinfection process in many water supplies, but the oocysts of C. parvum were verified to be insensitive to the disinfection levels routinely used (Korich et al., 1990, Gyurek et al., 1997, Hirata et al., 2000). Thus, there is a great interest in developing an alternative and more effective disinfectant to inactivate such recalcitrant microorganisms.

UV irradiation has been considered ineffective for inactivating C. parvum oocysts because of extremely high resistance of their viability to UV, as assessed by in vitro excystation and vital dye methods (Campbell et al., 1995, Lorenzo et al., 1993, Ransome et al., 1993). Recently, the infectivity of the oocysts was verified as highly sensitive to UV. Reported UV doses required for a 2 log10 reduction in infectivity of C. parvum oocysts were extremely low; around 2 mJ/cm² for cell infectivity assessed by cultured HCT-8 or MDCK cells (Landis et al., 2000, Linden et al., 2000, Shin et al., 1999) and around 1 mJ/cm² for animal infectivity assessed by SCID mice (Morita et al., 2002). Thus, UV irradiation has emerged as a promising disinfection technology for reducing the infectivity of C. parvum oocysts in water.

There are some issues to be clarified prior to practical introduction of UV irradiation for water disinfection. They include photoreactivation, dark repair (Harris et al., 1987, Lindenauer et al., 1994, Water Environmental Federation, 1996) and adverse effect caused by suspended matters in water and wastewater. As for the photoreactivation and dark repair, Oguma et al. (2001) and Morita et al. (2002) have demonstrated that the UV irradiation produced pyrimidine dimers on the DNA of C. parvum and the induced dimers returned to the original monomer through the processes of photoreactivation (fluorescent light irradiation) and dark repair (storage in the dark), as deter-
mined by endonuclease sensitive site assay. Nevertheless, any recovery was never observed in mouse infectivity in every trial; more exactly, any difference was not observed in mouse infectivity between the oocysts just after IJV irradiation and the oocysts followed by photoreactivation or dark repair treatment, as evaluated by excretion of oocysts in feces (Morita et al., 2002).

The remaining issue to be made clear is the adverse effect of turbidity on the efficacy of UV irradiation (Jolis et al., 2001). Therefore, in this study, experimental assessment was carried out to examine the effect of turbidity on the efficacy of UV for reducing animal infectivity of C. parvum oocysts.

**MATERIALS AND METHODS**

**Cryptosporidium parvum Oocysts**
The C. parvum HNJ-I strain (genotype II, originally isolated from a human patient by Dr. M Iseki, Osaka City University of Medicine at that time), which was passaged in SCID mice (C.B-17/Icr, CLEA Japan Inc, Tokyo, Japan) at the Research Institute of Biosciences, Azabu University, was used in this study. Fresh feces from several infected mice were collected in 500 mL of purified water, emulsified, and filtered through a 0.1-mm mesh nylon sieve. The crude oocyst suspension (20 mL) was underlaid with sucrose solution (specific gravity of 1.10 at 20°C) to centrifuge at 1,500 x g at 4°C for 15 minutes. The interface and upper layer were recovered, diluted with 150 mM of pH 7.4 phosphate-buffered saline (PBS; 0.20 g of potassium dihydrogen phosphate, 0.20 g of potassium chloride, 1.15 g of disodium hydrogen phosphate and 8.0 g of sodium chloride in 1 liter of distilled water) containing 0.10% (v/v) Tween 80 and then, centrifuged at 1,500 x g at 4°C for 15 minutes. The precipitate was diluted with PBS containing 0.1% (v/v) Tween 80 to a final volume of 20 mL. The suspension was underlaid with sucrose solution and centrifuged at 1,500 x g at 4°C for 15 minutes. Then the interface was recovered and centrifuged to remove fecal matter. The stock of purified oocysts was stored in PBS at 4°C and used in experiments within 5 days.

**Table I. The conditions of UV irradiation for the separated system**

<table>
<thead>
<tr>
<th>Trial No</th>
<th>Turbidity of the sludge suspension (NTU)</th>
<th>Absorbance at 254 nm in the sludge suspension (cm⁻¹)</th>
<th>Transmittance of the sludge suspension (%)</th>
<th>Irradiation dose at the oocysts suspension (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Surface</td>
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<td>Bottom</td>
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<tr>
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<td>0.00</td>
<td>100</td>
<td>0.50</td>
</tr>
<tr>
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<td>100</td>
<td>1.00</td>
</tr>
<tr>
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<td>1.50</td>
</tr>
<tr>
<td>4</td>
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<td>75</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
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<td>50</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>290</td>
<td>0.60</td>
<td>25</td>
<td>1.50</td>
</tr>
</tbody>
</table>

**Pre-Treatment of Sludge**
Sludge from a water purification facility treating river water with a rapid filtration system (coagulation with alum, flocculation, sedimentation and rapid sand filtration) was used for the experiment as a source of turbidity. The sludge was diluted with distilled water and sterilized with chlorination (at 3,000 mg/L for 5 minutes) and UV irradiation (over 20,000 mJ/cm²) before use. Treated sludge suspension was diluted using sterilized tap water to an arbitrary concentration. Turbidity of the sludge suspension was determined by measuring the absorbance at 660 nm with a spectrophotometer (UV-150-02, Shimadzu Co., Tokyo, Japan) and converted to the nephelometric turbidity units (NTU).
Table II. The conditions of UV irradiation for the mixed system

<table>
<thead>
<tr>
<th>Trial No</th>
<th>Turbidity of the suspension (NTU)</th>
<th>Absorbance at 254 nm in the suspension (cm⁻¹)</th>
<th>Irradiation dose at the suspension (mJ/cm²)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Surface</td>
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<tr>
<td>8</td>
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<td>0.00</td>
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</tr>
<tr>
<td>9</td>
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<tr>
<td>10</td>
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<tr>
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</tr>
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<td>2.28</td>
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<td>190</td>
<td>0.39</td>
<td>0.90</td>
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<td>17</td>
<td>190</td>
<td>0.39</td>
<td>1.80</td>
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<td>19</td>
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<td>0.90</td>
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<td>1.80</td>
</tr>
<tr>
<td>21</td>
<td>560</td>
<td>1.14</td>
<td>4.80</td>
</tr>
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</table>

EXPERIMENTAL CONDITIONS
Two experimental designs, a 'separated' system and a 'mixed' system, were used for evaluating the effects of turbidity on the efficacy of UV for inactivating *C. parvum* oocysts. The experimental conditions are summarized in Table I for the separated system and Table II for the mixed system. Every run was conducted at the temperature of 20°C.

UV Irradiation in the 'Separated' System
Experimental equipment for the 'separated' system is shown in Figure 1. In this system, a quartz chamber (light pass length 10 mm) was placed just above a petri dish. The quartz chamber was filled with various concentrations of sludge suspension. A small portion of a purified *C. parvum* oocyst suspension was put into the petri dish (inner diameter of 56 mm) containing 20 mL of 150 mM PBS to give a final concentration of 106 oocysts per mL. The sludge concentration of water in the quartz chamber was adjusted to three different turbidities of 63, 140 and 290 NTU to allow 75, 50 and 25% transmission of UV254 through the chamber, respectively. A 5W low-pressure mercury lamp (QCGL5W-14 97D, Iwasaki Electric, Co., Ltd., Tokyo, Japan) was placed at the height where the intensity of delivered UV to the surface of the oocyst suspension through a filled quartz chamber with purified water was to be just 0.10 mW/cm². During UV irradiation, both the oocyst suspension in petri dish and the sludge suspension in quartz chamber were gently agitated using electromag-
Netic stirrers. UV intensity delivered to the oocysts at arbitrary depth of the oocyst suspension in purified water was considered identical to the UV intensity at the surface of the suspension. UV irradiation dose to the oocyst suspension was adjusted by controlling the exposure time.

**Figure 1. UV irradiation equipment for the separated system.**

**UV Irradiation in the ‘Mixed’ System**

Experimental equipment used for the ‘mixed’ system is shown in Figure 2. In this system, the oocysts suspended in turbid water were directly irradiated with an intensity of 0.10 mW/cm² of UV at the surface of the suspension. The turbidity of the mixed suspensions was adjusted to three different degrees of 63, 190 and 560 NTU to give UV transmittances of 0.14, 0.39 and 1.14 cm⁻¹, respectively. Differed from the separated system, Lambert-Beer’s Law expressed by following equation was applied to estimate the UV intensity in a given depth of the mixed suspension, as the UV was significantly absorbed by the mixed suspension.

\[ I(x) = I_0 \exp(-Ax) \]  \[ \text{[1]} \]

where, \( I(x) \) is UV intensity (mW/cm²) at an arbitrary depth \( x \) from the surface of mixed suspension, \( I_0 \) is UV intensity at the surface of the mixed suspension, and \( A \) is the extinction coefficient of UV at wavelength 254 nm. Average UV dose \( I_{av} \) delivered to the oocysts was estimated by integrating the UV intensity from the surface to the bottom of the mixed suspension, given by the equation.

\[ I_{av} = \frac{1}{R} \int I(x) \, dx \]  \[ \text{[2]} \]

where, \( R \) is the thickness of the mixed suspension, and \( I(x) \) is UV intensity at an arbitrary depth \( x \) in mixed suspension. The mixed suspension was constantly agitated throughout the UV irradiation. Complete mixing in the oocyst suspension was assumed to calculate the average dosage \( I_{av} \) delivered to the oocysts.

**Infectivity**

Infectivity was determined in animal infectivity tests using SCID mice in a specific pathogen-free area at the Research Institute of Biosciences, Azabu University. This study was approved by the Animal Research Committee of Azabu University. Five-week-old SCID mice were used for the tests after one-week conditioning for adaptation to the new cage. Five mice were used per dilution. Five-fold serial dilutions were made using sterilized tap water.

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An aliquot (0.5 mL) of the selected dilution series was administered orally to each mouse. Four weeks after oral administration, fresh feces from individual mice were collected, suspended in 50 mL of purified water and emulsified using a vortex mixer. A 5-mL sample of the suspension was overlaid on 8 mL of sucrose solution and centrifuged at 1,500 x g at 4°C for 15 minutes. The interface was recovered, and approximately 1 mL of the supernatant was filtered through a 25-mm cellulose acetate membrane filter (pore size 0.8 micrometers). The filter then was stained with immunofluorescent antibodies against oocyst wall protein (Hydrofluoro Combo Kit, Strategic Diagnostics Inc., DE, USA) and observed under an epifluorescent differential contrast microscope (BX-60, Olympus, Tokyo, Japan) at magnification of 400x in order to determine the presence or absence of oocysts. The Most Probable Number (MPN) was calculated from the ratios of oocyst-positive mice to administered, using the MPN program developed by Hurley and Roscoe, 1983. The relative infectivity (Ir) of each sample was calculated using the equation

\[ Ir = \frac{[\text{MPNa}]}{[\text{MPNo}]} \]

where, MPNa is the Most Probable Number of oocysts after UV irradiation, and MPNo is the Most Probable Number of oocysts before UV irradiation.

**RESULTS AND DISCUSSION**

**Pre-Evaluation of Inactivation Efficacy of UV**

To assess the inherent efficacy of direct UV irradiation to the oocysts, the oocysts suspended in purified water were placed in the petri dish and directly irradiated with UV using the experimental equipment for the mixed system. Results are shown in Figure 3. Infectivity decreased exponentially as UV dose increased; i.e., the number of log10 reduction increased linearly with irradiated UV doses. The UV dose required for 1, 2 and 4 log10 reduction in infectivity was 0.48, 0.97 and 1.92 mJ/cm², respectively.

![Figure 3](image)

**Relative infectivity** Ir, i.e., the ratio of remaining infectivity of C. parvum oocysts after UV irradiation to the initial infectivity, can be described by the following expression:

\[ Ir = e^{-kD} \]

where, k is kinetic inactivation rate (cm²/mJ), and D is UV dose irradiated to the oocysts (mJ/cm²).

UV was highly effective for reducing mouse infectivity of C. parvum oocysts, showing the value k in purified water was as large as 4.378 cm²/mJ. The UV dose required for a 2 log10 inactivation was as small as 1 mJ/cm², much smaller than the minimum dose of 16 mJ/cm² recommended by the U. S. Department of Public Health for a 2 log10 reduction of bacteria.

**Efficacy of UV Passed Through Turbid Water**

Figure 4 shows the reduction in infectivity of the oocysts suspended in purified water in the 'separated' system. UV dose delivered to the oocyst suspension at the turbidities of 63, 140 and 290 NTU was 0.50, 1.00 and 1.50 mJ/cm² and observed log10 reductions in infectivity were 1.08, 2.04 and 3.01, respectively. The kinetic inactivation rate was calculated to be 4.618 cm²/mJ; almost the same value observed in the direct irradiation with UV to the oocysts in purified water (shown in Figure 3). The observed relationships between UV dose delivered to the oocysts and
resulting infectivity reductions were almost the same as those found in direct irradiation, as far as the oocysts that were suspended in purified water. Thus, any adverse effect was not observed in the remaining UV passed through the turbid water.

Figure 4. Relationship between relative infectivity of C. parvum oocysts and measured UV dose irradiated to the oocysts in the separated system.

Efficacy of UV in Turbid Water

Figure 5 shows the results of the reduction in infectivity in the 'mixed' system. Estimated kinetic inactivation rates were 4.125, 3.910 and 3.707 cm²/mJ at the turbidities of 63, 190 and 560 NTU, respectively (shown in Table III). Compared with UV efficacy in purified water, achieved reduction in infectivity showed a tendency to decrease the efficacy of UV as the turbidity of oocyst suspension increased. However, the adverse effect of turbidity was small, maximal 20% in kinetic inactivation rate at the highest turbidity of 560 NTU.

Table III. The kinetic inactivation rate of C. parvum by UV irradiation.

<table>
<thead>
<tr>
<th>Turbidity of the suspension (NTU)</th>
<th>Kinetic inactivation rate (cm²/mJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.378</td>
</tr>
<tr>
<td>63</td>
<td>4.125</td>
</tr>
<tr>
<td>190</td>
<td>3.910</td>
</tr>
<tr>
<td>560</td>
<td>3.707</td>
</tr>
</tbody>
</table>

To make clear the possibility of insufficient agitation, the average infectivity reduction in the stationary state was calculated assuming that (1) UV intensity at a given depth in mixed suspension decreases in accordance with Lambert-Beer's Law, (2) individual oocyst stands throughout the duration of irradiation at the place where it is at the beginning of UV irradiation, (3) the kinetic inactivation rate is 4.378 cm²/mJ (observed rate in direct irradiation to the oocysts in purified water), (4) infectivity reduction depends only on the net dose of UV delivered to the oocysts. From these assumptions, the delivered UV dose Dₓ at arbitrary depth x can be expressed by the following equation:

\[ Dₓ = Iₓt \]

where, t is the irradiation time of UV.

Relative infectivity Iₓ exposed to UV dose of Dₓ can be expressed as follows:

\[ Iₓ = e^{-4.378 Dₓ} \]

where 4.378 is the kinetic activation rate (cm²/mJ).

Then, the predicted average relative infectivity Iᵣav is given by following equation:

\[ Iᵣav = 1 / \int_0^8 Iₓ dₓ \]

Predicted results of average reduction in infectivity are shown in Figure 6. Differences in infectivity reduction between those observed in the mixed system and predicted assuming stationary state were insignificant at the turbidity of 63 and 190 NTU. However, it is apparent that predicted infectivity at the turbidity of 560 NTU was sig-
significantly smaller than observed in the mixed system, suggesting that the agitation given in the mixed system was insufficient to assume complete mixing. Thus, the observed lower inactivation in the mixed system would be due to insufficient agitation.

Figure 6. Relationship between estimated relative infectivity of C. parvum oocysts and estimated average UV dose irradiated to the oocysts in the mixed system.

From these results, it may be possible that the turbidity may not bring out any adverse effect on the relationship between net dose of delivered UV and expected inactivation of C. parvum oocysts.

CONCLUSIONS
The results of the present study can be summarized as follows:

1. The infectivity of C. parvum HNJ-I oocysts decreased exponentially, as the UV irradiation dose increased. The UV dose required for a 2 log10 reduction in infectivity was only 1.0 mJ/cm² in purified water, when infectivity was determined by mouse infectivity.

2. UV intensity decreases in turbid water and the achieved inactivation level was reduced depending on UV-absorbing nature of the water. However, the inactivating efficacy per unit dose of delivered UV to a given depth where the oocysts are present can be considered to be identical in purified water and in turbid water.

Acknowledgments
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References


