UV Light Inactivation of Bacterial Biothreat Agents

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Seven species of bacterial biothreat agents were tested for susceptibility to UV light (254 nm). All gram-negative organisms tested required <12 mJ/cm² for a 4-log₁₀ reduction in viability (inactivation). Tailing off of the B. anthracis spore inactivation curves began close to the 2-log₁₀ inactivation point, with a fluence of approximately 40 mJ/cm², and 3-log₁₀ inactivation still was not achieved with a fluence of 120 mJ/cm².

The security of our nation’s water supply is a concern for water providers and public health officials. Questions have been asked regarding the possibility of our drinking water becoming contaminated with biothreat agents and the efficacy of current disinfection practices for the reduction in viability (inactivation) of biothreat agents (5, 8, 14). The use of UV irradiation as a supplemental water disinfection practice is increasing for several reasons, among them improving control of protozoa, such as Cryptosporidium spp., and decreasing disinfection by-products created by chemical disinfectants (21). This study employed a bench-scale collimated beam test to determine the UV fluence (dose) required to inactivate seven representative bacterial biothreat agents.

Seven species, two isolates each, from the Health and Human Services and U.S. Dept. of Agriculture lists of select agents (http://www.selectagents.gov/resources/List%20of%20Select%20Agents%20and%20Toxins_111708.pdf) were used in this study: Bacillus anthracis Ames spores, B. anthracis 34F2 (Sterne) spores, Brucella melitensis ATCC 23456, B. melitensis IL195, Brucella suis KS528, B. suis MO562, Burkholderia mallei M9, B. mallei M13, Burkholderia pseudomallei ATCC 11688, B. pseudomallei CA650, Francisella tularensis LVS, F. tularensis NY98, Yersinia pestis A1122, and Y. pestis Harbin. B. anthracis was grown on soil extract-peptone-beef extract agar (SEA) (1) or in Schaeffer’s sporulation medium (SSM) (10) for 7 days, resulting in >99% spores as determined by phase-contrast microscopy. The cells and spores were then washed by centrifugation (8,000 × g), resuspended in ultrapure water, transferred to centrifuge tubes, treated with 50% ethanol for 1 h at room temperature, and washed five times with sterile ultrapure water before being stored in reverse-osmosis water at −70°C. F. tularensis isolates were grown on cysteine heart agar (1) and all other isolates on Trypticase soy agar with 5% sheep blood (TSA II; Becton Dickinson Microbiology Systems, Sparks, MD) for 24 h before testing. B. anthracis spores were adjusted to 10⁷ CFU/ml and other bacterial suspensions to 10⁶ CFU/ml in Butterfield buffer (3 mM KH₂PO₄, pH 7.2; Becton Dickinson Microbiology Systems), and then isolates were sonicated (40-Hz ultrasonic cleaner; VWR, Suwanee, GA) for 1 min to disperse aggregates. The suspensions were diluted 1:100 in Butterfield buffer for final test concentrations. Five milliliters of each suspension were placed into a small petri dish (50-mm diameter) along with a small sterile stir bar, and the petri dish was placed on a stir plate.

UV irradiation was performed by using a collimated beam apparatus (Calgon Carbon, Pittsburgh, PA) equipped with a low-pressure lamp (254 nm) according to the standard method developed by Bolton and Linden (2). The surface of the suspension was placed 5 cm from the end of the collimating tube. The UV intensity was measured with a radiometer at 0.5-cm intervals across the test area and variability compensated for according to the UVCalc software directions (International UV association [http://www.iuva.org]). The fluences (UV doses) were determined using the UVCalc software, and the petri dishes were placed under the beam for at least five time periods to deliver a range of appropriate fluences to the organisms. Each irradiation test was conducted at room temperature (23 ± 2°C) in triplicate and in a random order of fluorences. After exposure, 10-fold serial dilutions were performed, and the dilutions were spread plated in triplicate. Plates were placed in a dark incubator within 10 min of plating and incubated at the temperature appropriate for the organism, and the colonies were counted at 24 h and 48 h for B. anthracis and at 3 and 5 days for the remaining organisms. Colonies were counted, and the log₁₀ inactivation at each fluence was determined for each organism. A linear regression of the fluence response data determined the fluence required for 2-, 3-, and 4-log₁₀ inactivation.

The UV fluences required for inactivation of each organism are reported in Table 1. Little difference in UV susceptibility was seen between the gram-negative organisms. B. suis KS528 and B. melitensis ATCC 23456 required the greatest UV fluence of the gram-negative organisms for 4-log₁₀ inactivation (10.5 and 10.2 mJ/cm², respectively), while the two Y. pestis isolates required the lowest UV fluence (4.1 and 4.9 mJ/cm²) for the same 4-log₁₀ inactivation (Table 1). Generally, the two isolates of each species differed no more than 3 mJ/cm² in the UV fluence required for 4-log₁₀ inactivation.

The spores of the two B. anthracis isolates were more resistant to UV than the gram-negative organisms tested but were similar to each other in UV susceptibility (Fig. 1). B. anthracis Sterne and B. anthracis Ames spores were inactivated by 90% (1 log₁₀) with fluences of 23.0 and 25.3 mJ/cm², respectively. B. anthracis spores produced on SEA and plated after UV ex-

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posure on TSA II required more than 40 mJ/cm² for a 2-log₁₀ inactivation, and further exposure to UV light did not inactivate the sample further (Fig. 1), as seen in the tailing off of the inactivation curve. In order to investigate this tailing off further, *B. anthracis* spores produced in SSM were also challenged with the same UV fluences and found to require 40 mJ/cm² for a 2-log₁₀ inactivation as well, but they continued to be inactivated to a slightly greater degree than the spores produced on SEA (Fig. 1). An additional experiment was conducted in which spores produced on SEA were grown on two media (SSM with 1.7% agar and TSA II) after UV exposure, and no difference in recovery was observed (data not shown).

The inactivation results for *Y. pestis*, *F. tularensis*, *Brucella* spp., and *Burkholderia* spp. reflect findings similar to those of other waterborne pathogenic organisms, such as *Escherichia coli*, *Shigella sonnei*, *Yersinia enterocolitica*, and *Campylobacter jejuni* (3, 4). These reported values ranged from 1.8 to 6 mJ/cm² for a 3-log₁₀ inactivation (Table 1).

Previous work established that bacterial spores are 10 to 50 times more resistant to UV at 254 nm than vegetative cells (11, 12). The DNA in spores is saturated with /H₉₂⁵¹/H₉₂⁵₂-type small acid-soluble proteins during the sporulation process. This bound small acid-soluble protein suppresses the formation of pyrimidine dimers (as seen in vegetative cells) when irradiated with UV and instead promotes formation of a unique spore photoproduction, 5-thyminyl-5,6-dihydrothymine. During germination, light-independent repair occurs by lyase activation of the spore photoproduction and nucleotide excision repair, restoring the two thymines (6, 18, 19). Variations in resistance to UV may be attributed to differences in sporulation conditions, such as the availability of metal ions present during sporulation, or germination conditions (10, 11, 13, 18).

The susceptibility of *B. anthracis* spores grown on SEA in this study can be compared to the results found by Knudson (6), in which a fluence of 120 mJ/cm² was not sufficient to achieve a 2-log₁₀ reduction. However, Nicholson and Galeano (12) did not observe tailing off of the disinfection curve occurring after a 2-log₁₀ reduction. We therefore produced spores in the same manner as Nicholson and Galeano to determine if the difference in spore preparation could account for the differences in UV susceptibility. Though this study noted a greater susceptibility of SSM-produced spores than SEA-produced spores, we did not see as great a reduction as did Nicholson and Galeano (12) (Fig. 1). Rice and Ewell (15) also reported tailing off of the inactivation curve in a similar study using *Bacillus subtilis* spores and were unable to determine if

### Table 1. UV fluence required for given log₁₀ inactivation of each organism

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fluence (mJ/cm²) for log₁₀ inactivation of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>B. anthracis</em> Ames</td>
<td>25.3 (5.1)</td>
</tr>
<tr>
<td><em>B. anthracis</em> Sterne</td>
<td>23.0 (0.7)</td>
</tr>
<tr>
<td><em>B. suis</em> OS562</td>
<td>1.7 (0.0)</td>
</tr>
<tr>
<td><em>B. suis</em> K1528</td>
<td>2.7 (0.2)</td>
</tr>
<tr>
<td><em>B. melitensis</em> ATCC 23456</td>
<td>2.8 (0.2)</td>
</tr>
<tr>
<td><em>B. melitensis</em> IL1095</td>
<td>3.7 (0.2)</td>
</tr>
<tr>
<td><em>B. pseudomallei</em> ATCC 1168</td>
<td>1.7 (0.2)</td>
</tr>
<tr>
<td><em>B. pseudomallei</em> CA650</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td><em>B. mallei</em> M-9</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td><em>B. mallei</em> M-13</td>
<td>1.2 (0.5)</td>
</tr>
<tr>
<td><em>F. tularensis</em> LVS</td>
<td>1.3 (0.0)</td>
</tr>
<tr>
<td><em>F. tularensis</em> NY98</td>
<td>1.4 (0.1)</td>
</tr>
<tr>
<td><em>Y. pestis</em> A1122</td>
<td>1.4 (0.5)</td>
</tr>
<tr>
<td><em>Y. pestis</em> Harbin</td>
<td>1.3 (0.1)</td>
</tr>
<tr>
<td><em>Bacillus anthracis</em> Sterne</td>
<td>27.5</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
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<tr>
<td><em>E. coli</em></td>
<td>3.0</td>
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<tr>
<td><em>Cryptosporidium</em></td>
<td>2.5</td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>2.1</td>
</tr>
<tr>
<td><em>Virus</em></td>
<td>58</td>
</tr>
</tbody>
</table>

| a | Data from reference 12 (estimated from graph). |
| b | Data from reference 4. |
| c | Data from reference 21. |
| d | 4-log₁₀ inactivation not achieved with a fluence of 60 mJ/cm². |

### FIG. 1. UV inactivation curves of *B. anthracis* spores. *B. anthracis* Sterne was grown and sporulated on SEA and SSM, and *B. anthracis* Ames was grown and sporulated on SEA.
the tailing off indicated the presence of a resistant subpopulation of organisms or was an artifact of the testing protocol. Subsequent work by Mamane-Gravetz and Linden (7) demonstrated that the tailing off of UV inactivation curves is a result of the presence of spore aggregates in the suspension, and the degree of aggregation is directly related to the hydrophobicity of the spores. The hydrophobicity of the spores used in this study was tested in the same manner as in the study by Mamane-Gravetz and Linden (7) and found to correlate with the inactivation curves in Fig. 1. The SSM-produced spores were less hydrophobic (P = 0.25) at 64.1% (standard deviation [SD], 5.6%) than the SEA-produced spores at 76.2% (SD, 2.8%), whereas the SEA-produced Ames spores were closer (P = 0.03) in hydrophobicity to the SEA-produced Sterne spores at 79.6% (SD, 3.4%). These observations agree with the previous publication (7) in that the more hydrophobic spores tend to aggregate together to a greater extent, shielding a greater number of spores from exposure to UV radiation, thereby creating a more pronounced tailing off of the inactivation curve.

Since the finding that UV irradiation can control protozoa much more effectively than chlorine, installation of UV technology in water treatment facilities has been on the rise, with more than 150 treatment plants in North America currently using the technology or planning installations in the near future (22).

The latest Environmental Protection Agency surface water treatment rules require drinking water systems to document their ability to provide a 2- or 3-log10 inactivation (for unfiltered systems) of Cryptosporidium (depending upon source water monitoring results and treatment practices in place at the facility), a 3-log10 inactivation of Giardia, and a 4-log10 inactivation of viruses (21). No two treatment facilities are alike, but some systems use cotreatment with other disinfection methods. However, it is possible that the clumping of spores may increase the efficacy of the facility’s coexisting available treatment, such as flocculation and filtration. Further examination of these practices would be necessary.

In the event that a biothreat agent is intentionally released into the distribution system after water treatment, and no disinfectant residual (chlorine or monochloramine) is provided by the treatment facility, a point-of-use (POU) or point-of-entry (POE) UV system may prove to be effective. NSF/ANSI standard 55 (9) establishes the requirements for two classes of POU and POE UV systems. The class A systems, designed to disinfect contaminated clear water, are required to deliver a minimum UV fluence of 40 mJ/cm². The class B systems offer supplemental reduction in pathogens and are required to deliver a UV fluence of 16 mJ/cm². Both class A and B POU/POE devices would be effective in providing a 4-log10 inactivation of the gram-negative organisms tested. Only the class A device would prove effective against B. anthracis spores prepared in this manner, though only in providing 2-log10 inactivation.

These data, along with previous investigations of the efficacy of chlorine and monochloramine against bacterial bioterrorism agents (16, 17), provide public health officials and water treatment facility operators essential information to better prepare for protecting public health in the event of a water contamination incident.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

REFERENCES