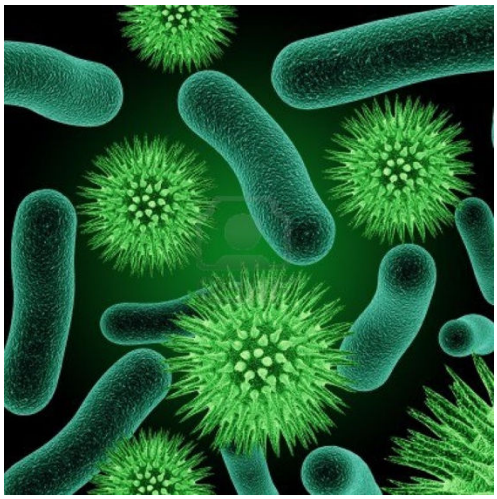

Killing of Bacillus Spores by High-Intensity Ultraviolet Light

STUDY ON EFFECTS OF PULSED LIGHT

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Rationale

Bacterial endospores, of the type by *Bacillus* and *Clostridium* species, are known to be highly resistant to various forms of radiation and other physical and chemical agents. High-intensity ultraviolet light, however, was suspected to overcome such resistance and to kill spores efficiently. To test this hypothesis, I irradiated samples of *Bacillus subtilis* spores (in collaboration with Roger Williams, Xenon Corporation) and measured the loss of viability as a function of UV dose and position with respect to the axis of the lamp.

Protocol

Four 2-L flasks, each containing 500 ml of DS medium (a nutrient broth-based growth and sporulation medium for *Bacillus subtilis*), were inoculated with *B. subtilis* strain SMY (a standard wild-type strain) and incubated with vigorous shaking for 36 hrs at 37^{o}C. Spore formulation was verified microscopically. Spores were harvested by centrifugation and washed twice with sterile, deionized water. The stock of spores was stored in water at 4^{o}C.

The spore stock was diluted in sterile, deionized water to give concentrations of approximately 1 x 10^{9}, 1 x 10^{8} or 1 x 10^{7} spores per ml. Fifty-microliter samples of each dilution were placed at three different locations with respect to the UV source (see below) and irradiated with one-to-four pulses of light. The samples were recovered, diluted as necessary with sterile water, and spread on agar plates containing a nutrient medium that supports growth of *B. subtilis.* After overnight incubation at 30^{o}C, the colonies that arose were enumerated. Based on the number of colonies obtained at a given dilution of the irradiated spores, the surviving titer of each sample was calculated.

The UV source was a SteriPulse-XL 3000[1] provided by Xenon Corporation and operated by Roger Williams (Applications Engineer/Lamp Engineer; Xenon Corporation). The samples were placed as follows:

Position 1 – on the lamp axis and at the midpoint of the lamp;
Position 2 – 1 cm above the lamp axis and on the midpoint of the lamp;
Position 3 – 1 cm above the lamp axis and 6.803 (172 mm) to the right of the midpoint of the lamp.

Results

As shown in the accompanying table and figures, killing of spores was observed for all dilutions of the spore preparation at all positions with respect to the axis and midpoint of the lamp. Killing was most effective, however, when the sample was on the lamp axis and at the midpoint of the lamp. The rate of killing was similar for all dilutions at a given position, although the most concentrated suspension may be killed slightly less effectively. If borne out by further experiments, such a result might imply that spores shield each other when they are above a certain concentration.

[1] *XENON Corporation model SteriPulse-XL 3000 has been renamed model Z-1000*

Microscopic analysis after irradiation (Sample A, 4 pulses) revealed that most of the spores had disintegrated.

Conclusions

1) The SteriPulse-XL 3000 machine is an effective device for reducing the viability of *B. subtilis* spores in suspension. Killing is rapid (1 second or less) and reduces viability by a significant factor. Starting with spore suspensions at 1 x 10^8 (Sample B) or 1 x 10^7 spores (Sample C) per ml, it was possible to completely eliminate viability with three pulses of UV light.
2) The most concentrated sample (A) was reduced in viability by 100,000-fold with three pulses, but a fourth pulse gave no further killing. The basis for the lack of additional killing is unknown and may warrant further experimentation.
3) Killing at position 1 was much faster than at positions 2 or 3. Thus, the most effective sanitization occurs on the lamp axis. The exact relationship between killing and the midpoint of the lamp remains to be determined. Since there was only a small difference between the results obtained at positions 2 and 3, it is likely that irradiation is equally effective across nearly the entire width of the lamp.
4) Since other species of *Bacillus* and *Clostridium* are thought to have similar responses to UV light, it is reasonable to assume that the methods described here would give similar results with spores, including *Bacillus anthracis*.

Viable Cells per ml						
Sample	Position	0 Pulse	1 Pulse	2 Pulses	3 Pulses	4 Pulses
A	1	1.4 X 10^6	4.4 X 10^7	8.8 X 10^5	8.9 X 10^3	6.7 X 10^3
A	2	1.4 X 10^6	2.0 X 10^8	9.0 X 10^7	>6.0 X 10^6	5.6 X 10^6
A	3	1.4 X 10^6	6.0 X 10^8	1.7 X 10^8	>1.5 X 10^7	>9.0 X 10^6
B	1	1.1 x 10^8	4.5 x 10^5	3.3 x 10^3	<30	<30
B	2	1.1 x 10^8	1.0 x 10^7	1.0 x 10^6	4.0 x 10^5	1.9 x 10^4
B	3	1.1 x 10^8	3.6 x 10^7	2.0 x 10^6	1.9 x 10^6	4.4 x 10^5
C	1	1.3 x 10^7	1.2 x 10^5	<3.0 x 10^3	<30	<30
C	2	1.3 x 10^7	9.8 x 10^5	1.9 x 10^5	1.5 x 10^4	1.2 x 10^4
C	3	1.3 x 10^7	1.5 x 10^6	3.8 x 10^5	1.5 x 10^5	6.4 x 10^4

Table 1: Viable counts of spores before and after irradiation

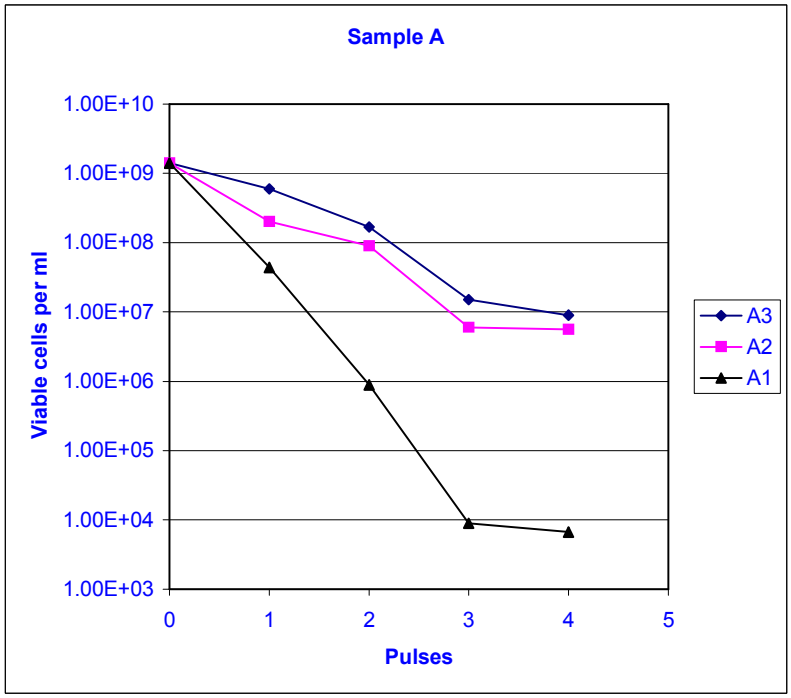

Figure 1: Sample A at positions 1, 2, and 3

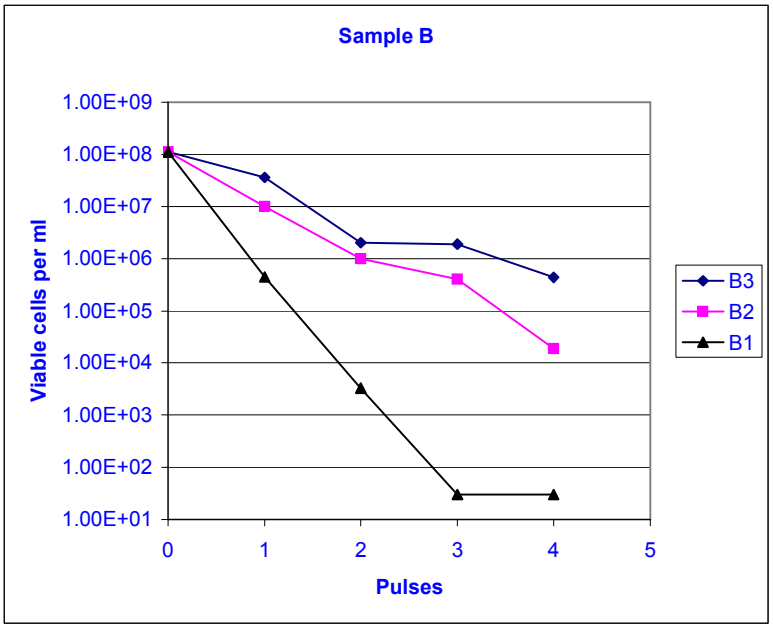

Figure 2: Sample B at positions 1, 2, and 3

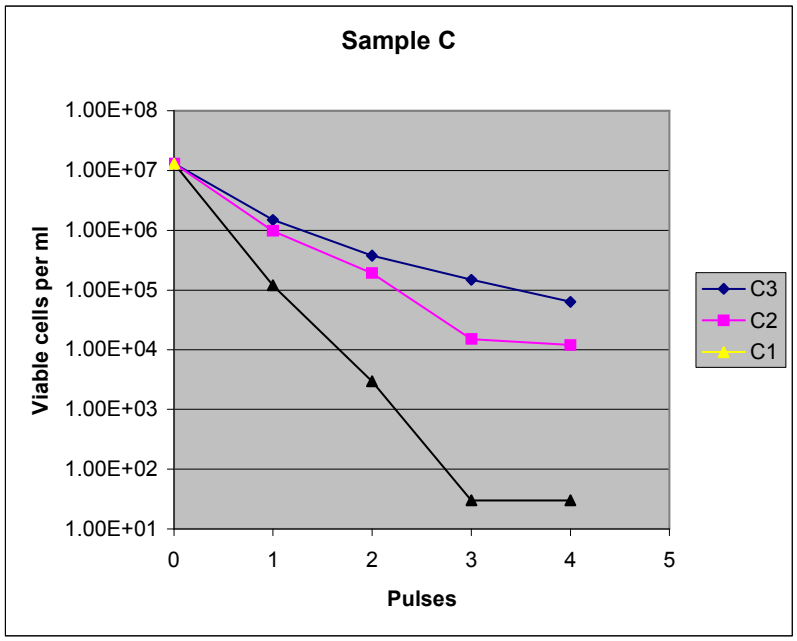

Figure 3: Sample C at positions 1, 2, and 3

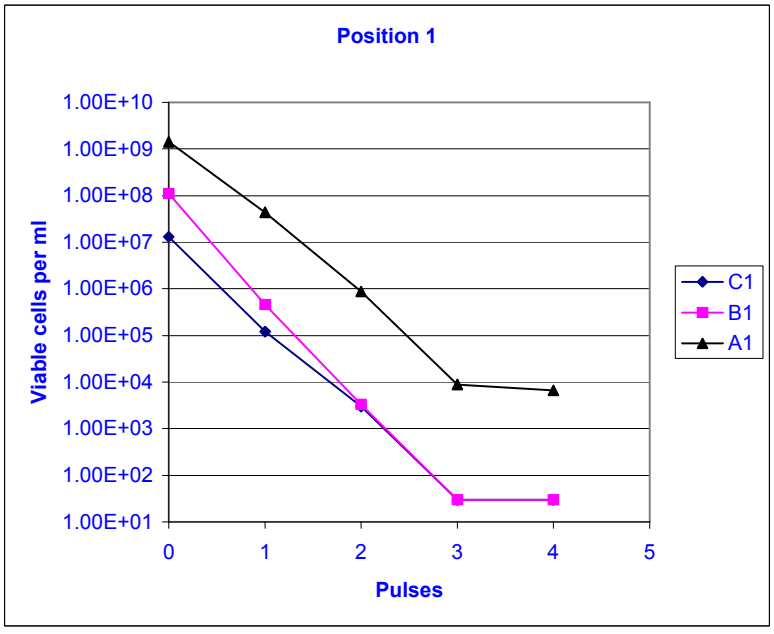

Figure 4: Samples A, B, and C at position 1

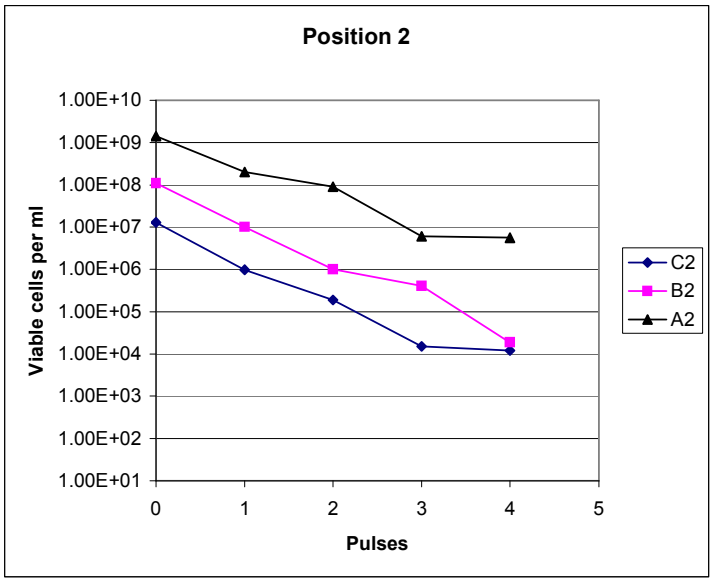

Figure 5: Samples A, B, and C at position 2

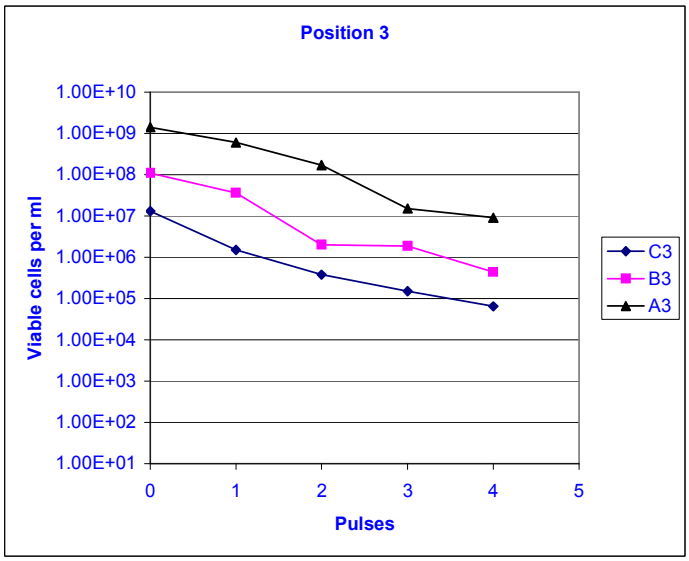

Figure 6: Samples A, B, and C at position 3

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