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EFFECTS OF REPEATED EXPOSURE TO FILTERED AND UNFILTERED BROADBAND LIGHT RADIATION ON *ESCHERICHIA COLI* GROWTH AND PROPAGATION



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14. ABSTRACT There is a high probability biological organisms will become inactive after exposure to non-ionizing radiation. Although continuous wave, low-pressure Hg lamps that emit UV-C radiation are recognized as effective for inactivating various microorganisms, there are other light sources that may be as effective. The objective of the experiments conducted during this study was to measure the inactivation efficiency of pulsed non-ionizing radiation on <i>Escherichia coli</i> using a broadband light system, broadband bandpass filters, and (in the UV range) narrow bandpass filters. The effectiveness of pulsed UV-C radiation was also compared to that from a continuous wave, low-pressure Hg light system. <i>E. coli</i> was plated onto tryptic soy agar and exposed to the filtered and unfiltered light sources. After exposure, all plates were incubated and then examined for growth or lack thereof to determine the inactivation effectiveness of broadband light and continuous wave light systems on <i>E. coli</i> .						
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PREFACE

The work described in this report was started in March 2010 and completed in July 2011.

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CONTENTS

1.	INTRODUCTION	1
2.	MATERIALS AND METHODS.....	2
2.1	Experimental Design.....	2
2.2	Pulsed Lamp Setup	2
2.3	CW UV Lamp	3
3.	RESULTS	4
4.	CONCLUSIONS.....	12
	LITERATURE CITED	17
	ACRONYMS.....	19

FIGURES

1.	Pulsed lamp setup with filter hood.....	3
2.	CW lamp setup.....	4
3.	Effect of filtered pulsed light (200 nm) on growth of <i>E. coli</i>	5
4.	Effect of unfiltered pulsed light on growth of <i>E. coli</i> (~8 log kill).....	6
5.	Effect of filtered pulsed light (220.5 nm) on growth of <i>E. coli</i>	7
6.	Effect of filtered pulsed light (248.9 nm) on growth of <i>E. coli</i>	7
7.	Effect of filtered pulsed light (260 nm) on growth of <i>E. coli</i>	8
8.	Effect of filtered pulsed light (270 nm) on growth of <i>E. coli</i>	8
9.	Effect of filtered pulsed light (280 nm) on growth of <i>E. coli</i>	9
10.	Effect of filtered pulsed light (302 nm) on growth of <i>E. coli</i>	9
11.	Effect of filtered pulsed light (320.7 nm) on growth of <i>E. coli</i>	10
12.	Effect of filtered pulsed light (400–700 nm) on growth of <i>E. coli</i>	10
13.	Effect of filtered pulsed light (700+ nm) on growth of <i>E. coli</i>	11
14.	Effect of CW UV (plus 254 nm filter) on growth of <i>E. coli</i>	11
15.	Effect of unfiltered pulsed light on growth of <i>B. cereus</i>	12
16.	Chart comparing log kills vs wavelength (220–340 nm, ▲ = CW)	13
17.	Comparison of measurements with the relative germicidal effectiveness curves	14

TABLES

1.	Pulsed Lamp Results.....	15
2.	CW Lamp Results	16
3.	<i>B. cereus</i> Exposure to Pulsed Lamp	17

EFFECTS OF REPEATED EXPOSURE TO FILTERED AND UNFILTERED BROADBAND LIGHT RADIATION ON *ESCHERICHIA COLI* GROWTH AND PROPAGATION

1. INTRODUCTION

Broadband pulsed light sources such as xenon arc lamps produce intense and short duration pulses of broad-spectrum “white light”. The spectrum of light from pulsed light systems extends from the UV (UV-C at 100–280 nm, UV-B at 280–315 nm, and UV-A at 315–400 nm) to the visible (400–700 nm) and near infrared (NIR; >700 nm). This wide spectrum allows a large amount of power to be contained in a single pulse. Energy, stored in capacitors, is released in a fraction of a second through the lamp, which further enhances the energy density delivered to the substrate. For most applications, a few flashes applied in a fraction of a second can provide a high level of microbial inactivation (1). Pulsed light systems do not contaminate surfaces because there are no chemicals involved; therefore, surfaces remain clean and unaffected.

A high-energy pulsed light system can rapidly decrease a population of microorganisms with low electrical power. There is minimal heating of sample materials because the duty cycles of the pulsed systems are very short with no continuous thermal load. The absence of ionizing radiation eliminates the need for heavy shielding around samples. The lack of surface contact eliminates any surface changes to substrates and does not require surface repairs. Additionally, pulsed light decontamination has potential for transitioning into laboratories in the Homeland Defense, U.S. Food and Drug Administration, U.S. Department of Agriculture, Department of Defense, and U.S. Environmental Protection Agency communities. The manageable size would position the system well for mobile and remote facilities. Ease-of-use, with a lack of chemical residue, could allow this system to transition to field-ready use.

UV radiation is a proven technique for inactivating biological organisms when properly applied (2,3). It has been used to combat potentially infectious agents such as the *Tuberculosis bacillus* for decades. However, the efficacy of the technique depends upon the organism. UV radiation used for disinfection of surfaces can be highly effective, as demonstrated by the disinfection processes for food and disinfection hoods for laboratory equipment.

This study examined light energy from pulsed radiation as well as continuous wave (CW) radiation when applied to samples of *Escherichia coli*. Samples were exposed to unfiltered light from the systems, as well as specific wavelength bands of the emitted spectrum, which were transmitted through optical filters.

2. MATERIALS AND METHODS

2.1 Experimental Design

The effectiveness of the use of pulsed light to inactivate *E. coli* and *Bacillus cereus* was examined in this study. Samples were plated on tryptic soy agar (TSA) then exposed to unfiltered and filtered pulsed radiation. Broad and narrow bandpass optical filters were placed in line with samples to transmit specific wavelengths and attenuate the energy from the pulsed light system.

The dose that passed through the bandpass filters was measured using an International Light Technologies, Inc. (Peabody, MA) SED033 detector, with a QNDS2 neutral density filter. The following narrow bandpass filters were used, each with a 15 nm pass band around the center wavelengths: 200, 220.5, 248.9, 260, 270, 280, 302 and 320 nm. Broad bandpass filters that transmitted 400–700 nm and 700+ nm were also used. The transmissions of the filters were characterized using a PerkinElmer (Waltham, MA) Lambda 900 spectrophotometer. A UV Products, LLC (Upland, CA) Sterilaire model XX-15S CW low pressure Hg UV lamp system with a 254 nm narrow bandpass filter in line was also used. The unfiltered emissions from the pulsed light system and the CW system were measured using the International Light detector and QNDS2 filter.

Liquid cultures of *E. coli* or *B. cereus*, grown overnight, were subsequently prepared in tryptic soy broth (TSB) and used to seed TSA plates as follows: (1) *E. coli* or *B. cereus* was serially diluted 1:10 in phosphate buffered saline (PBS) from 10^{-1} to 10^{-6} dilution. (2) Then 10 μ L spots from each dilution were plated onto the TSA plate, representing final dilutions of 10^{-3} to 10^{-8} . (3) Agar plates, with the top covers removed, were then placed in the pulsed light system and exposed to filtered or unfiltered light energy. (4) After exposures, all plates were incubated overnight at 37 °C. (5) Colonies were counted to determine the killing effect of the unfiltered pulsed light. The correlation between the doses required and the log inactivation was determined.

2.2 Pulsed Lamp Setup

The pulsed lamp was housed in a 24 × 18 × 20 in. stainless steel enclosure, centered on the inner top panel, and parallel to the opening. The enclosure was interlocked to disarm the control panel in the event the door was opened. The electronics for activating the lamp were adjacent to the enclosure. The control panel allowed the repetition rate to be set from one to nine pulses. More pulses could be activated by reactivating the start button. Cultured samples were centered under the lamp in the enclosure. A filter hood was used in all experiments to allow the placement of various filters in the light path to examine the effects of different wavelengths. The filter hood schematic and distance from the lamp to sample are shown in Figure 1.

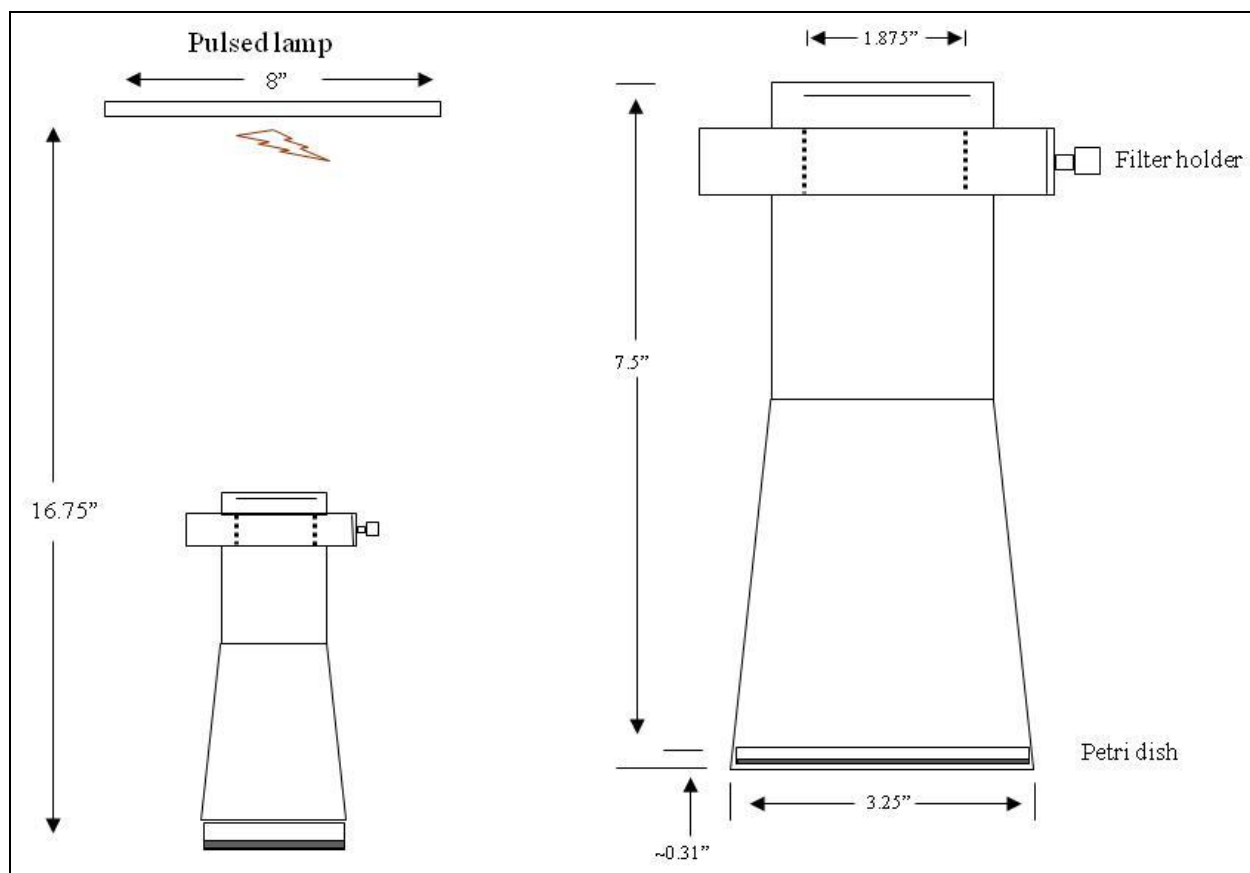


Figure 1. Pulsed lamp setup with filter hood.

2.3 CW UV Lamp

The model XX-15S CW Hg lamp system contained two Hg lamps enclosed in a housing, which was placed on a raised shelf above the samples. Samples were placed on the center of a shelf below the Hg lamps. Drawings of the CW system and distance from the lamps to the samples are shown in Figure 2. Opaque drapes were placed over the system to shield the operators from exposure to UV radiation.

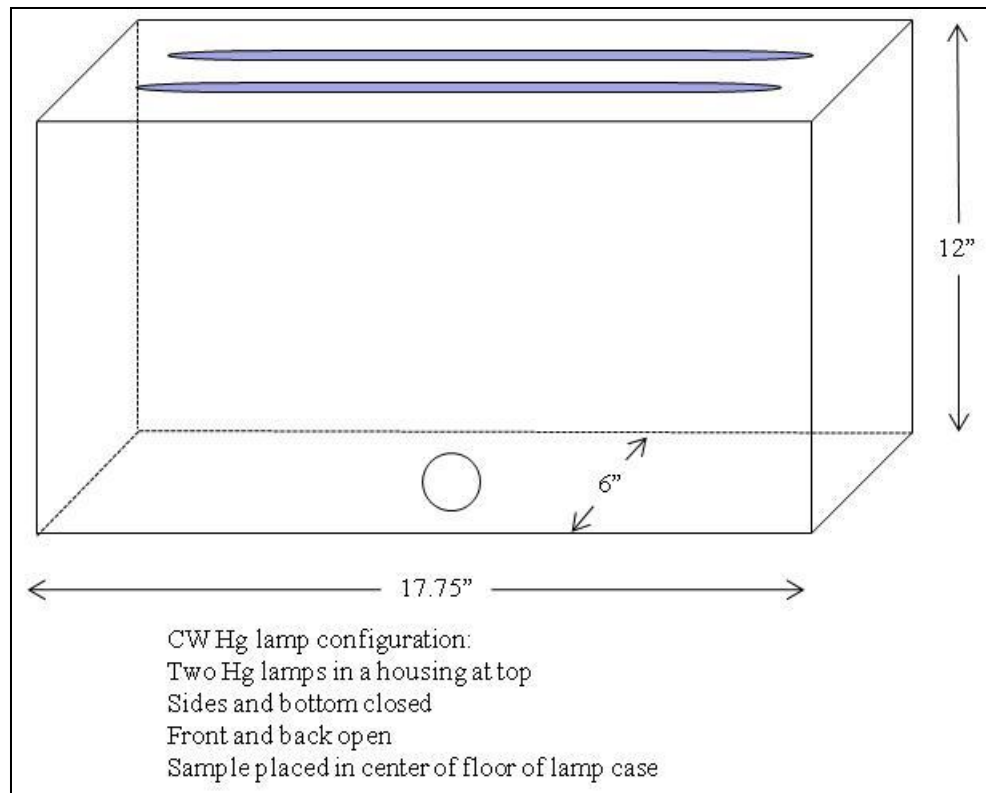


Figure 2. CW lamp setup.

3. RESULTS

The following results were obtained as a result of this study:

- Energy was delivered much faster when the pulsed lamps were used in comparison with the CW system. The pulsed lamp operated at ~ 1 Hz. The most pulses delivered to samples during testing were 40, which were delivered in less than 1 min.
- It appeared that air completely attenuated the emitted doses at the 200 nm wavelength. This wavelength was at the upper edge of the “vacuum” UV range, which commonly requires a vacuum chamber for studies involving these wavelengths. The radiometer used to measure energy did not register when the 200 nm filter was in place. Evaluation of the plates exposed to pulsed energy at 200 nm (Figure 3) showed only a 0.2 log kill, even after 40 pulses. Because there was no energy measured by the radiometer due to air attenuation, this small log kill may have been because of variability in the growth of *E. coli* on different plates.

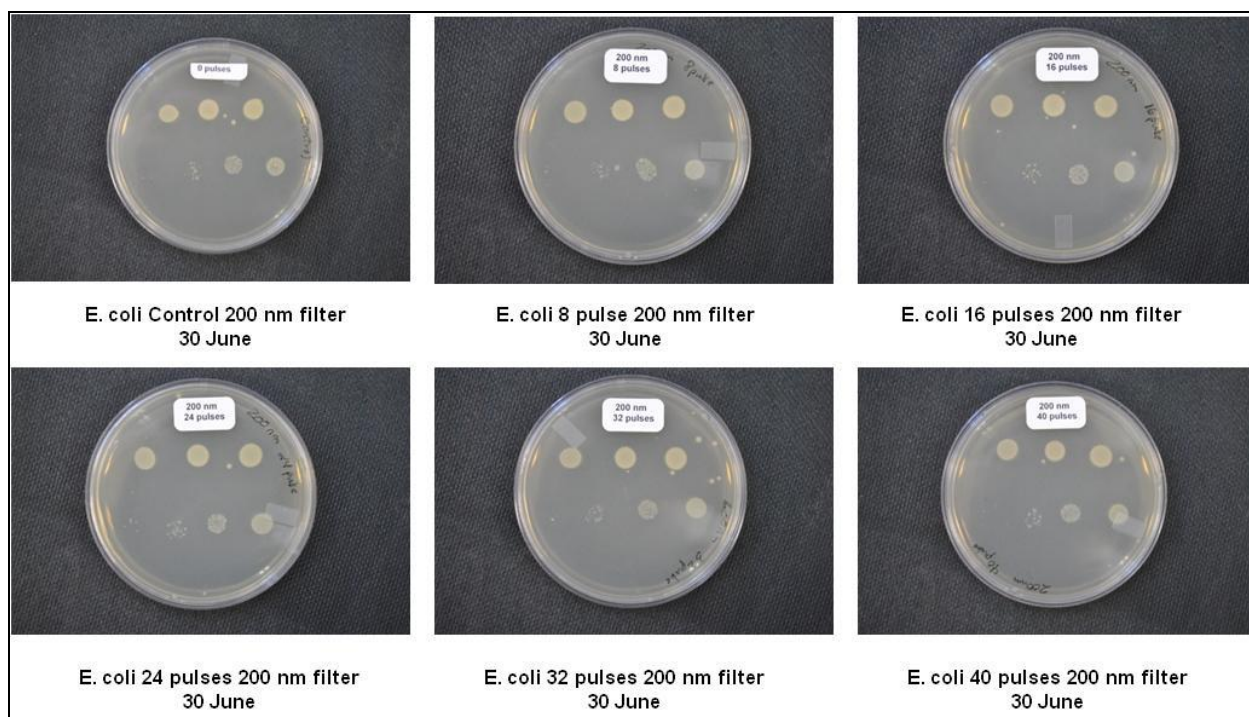


Figure 3. Effect of filtered pulsed light (200 nm) on growth of *E. coli*.

- The most effective wavelengths for decontamination (measured in log kill vs millijoules per square centimeter) were in the UV-C range. The CW lamp's emissions at 254 nm and the pulsed emissions in the same wavelength range showed similar levels of effectiveness. In terms of the specific wavebands that were tested, the most effective emissions were transmitted through the 248.9 and 260 nm filters, which were more effective than the other pass bands in the following order: 260 > 280 > 270 > 220 > 302 nm. The results for the 320 nm filter were consistent with studies of CW disinfection, which indicated that the killing mechanism changes with the use of longer wavelengths. For long wavelength UV and visible radiation, the inactivation appeared to be more of a "threshold" mechanism, and below the killing threshold, little, if any, reduction in colony formation was observed (4,5). No appreciable reduction in colony formation was observed with the 400–700 nm and 700–1100 nm filters; the dose delivered through the 400–700 nm filter may have been too low to overcome the killing threshold, based on other studies using CW disinfection (5). The pulsed doses at 400–700 and 700–1100 nm were about 3 orders of magnitude greater than in the UV-C range, where the greatest log kills occurred. Figure 4 shows an unfiltered exposure of *E. coli* to one pulse from the pulsed lamp system. Figures 5–14 show results from the CW and pulsed wavelengths. Tables 1–3 provide a compilation of the dose and log kills for the experiments.

- It has been speculated in the literature that pulsed UV radiation can inactivate the DNA of irradiated microorganisms (6), and the pulsing action can provide an additional photophysical killing mechanism. Further study is needed to determine the effectiveness of the photophysical killing mechanism for varying types of pulsed emissions because this study did not address the determination of killing mechanisms in the samples.
- The *B. cereus* (vegetative cells) survived multiple unfiltered pulsed exposures (Figure 15), although *E. coli* did not survive even one unfiltered pulse exposure. Based on these preliminary results with *B. cereus*, further experiments are needed to investigate the susceptibility of vegetative and spore forms of bacillus to unfiltered and filtered pulsed radiation.

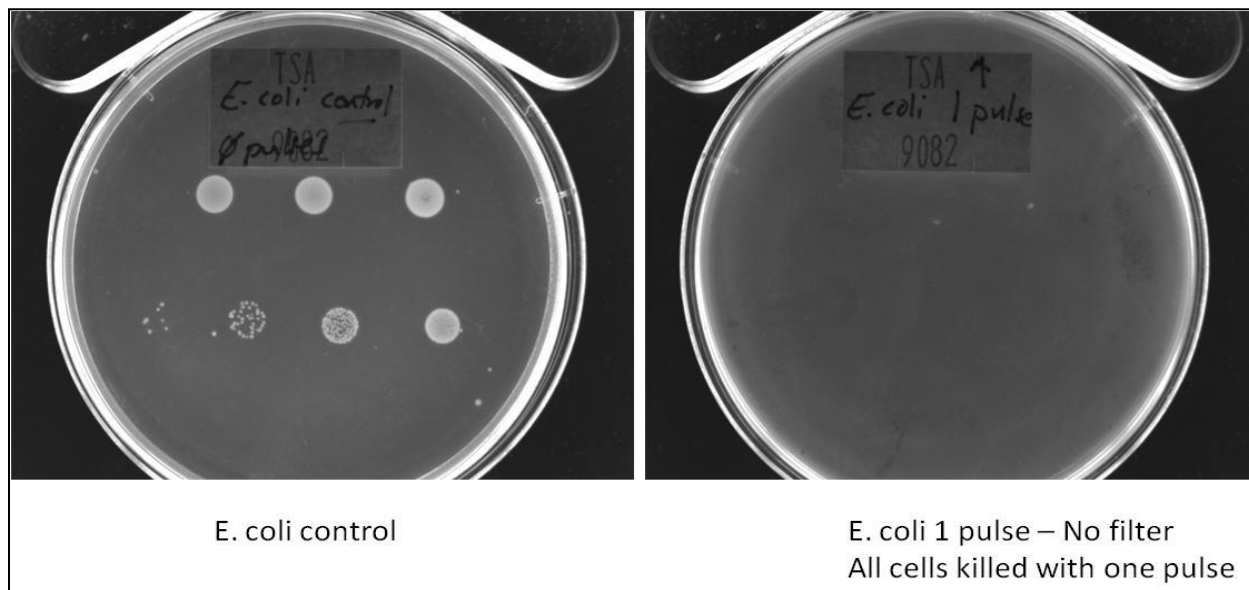


Figure 4. Effect of unfiltered pulsed light on growth of *E. coli* (~8 log kill).

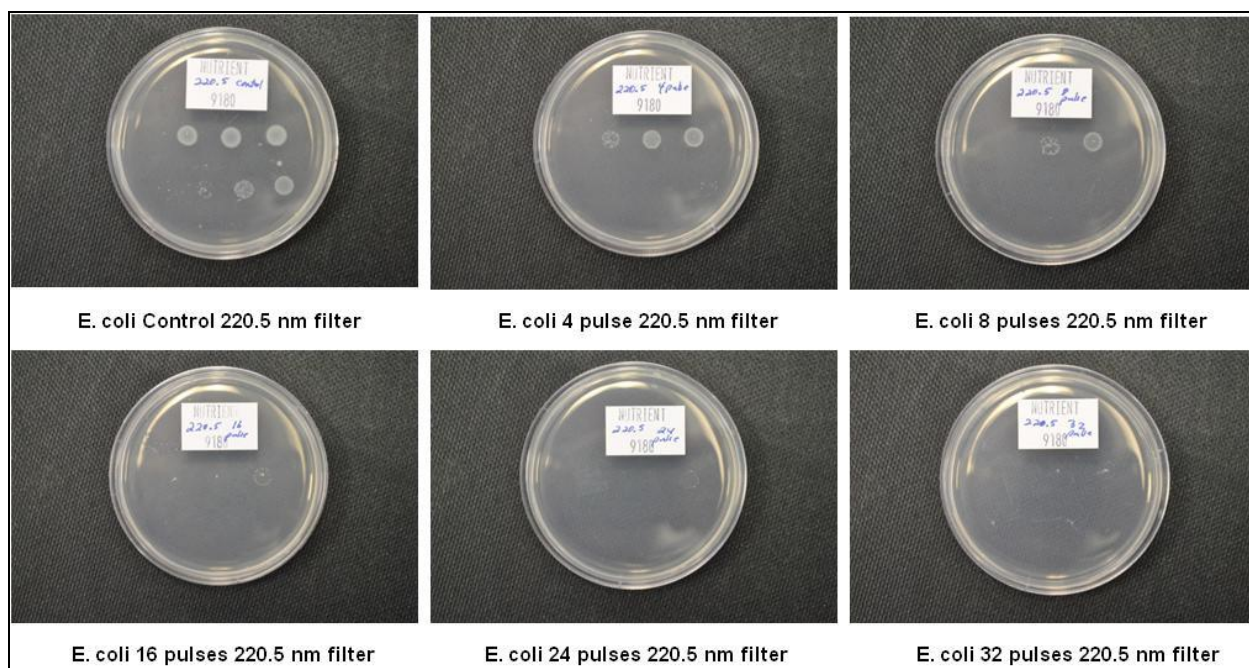


Figure 5. Effect of filtered pulsed light (220.5 nm) on growth of *E. coli*.

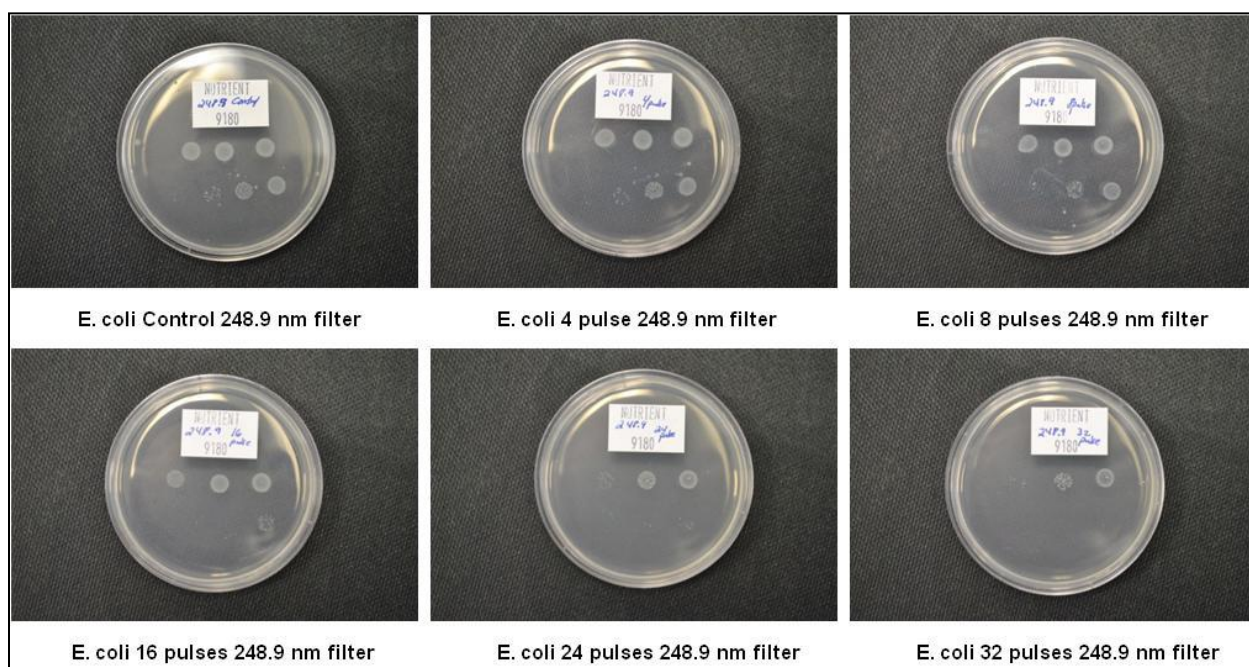


Figure 6. Effect of filtered pulsed light (248.9 nm) on growth of *E. coli*.

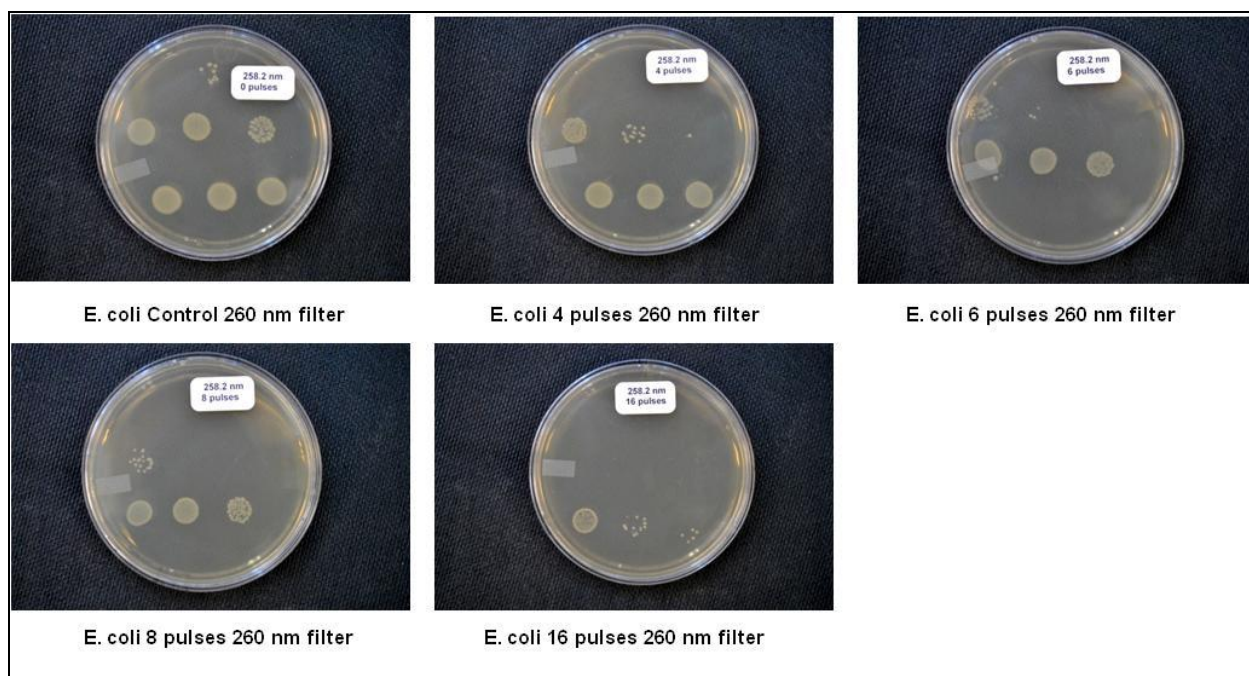


Figure 7. Effect of filtered pulsed light (260 nm) on growth of *E. coli*.

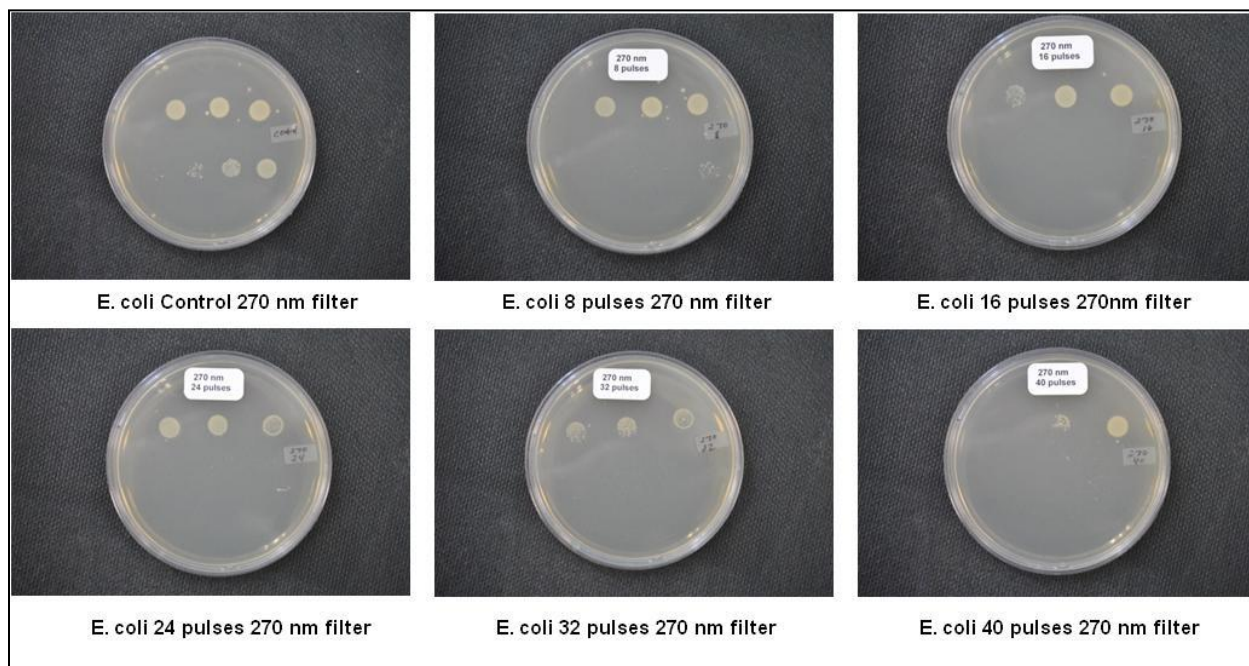


Figure 8. Effect of filtered pulsed light (270 nm) on growth of *E. coli*.

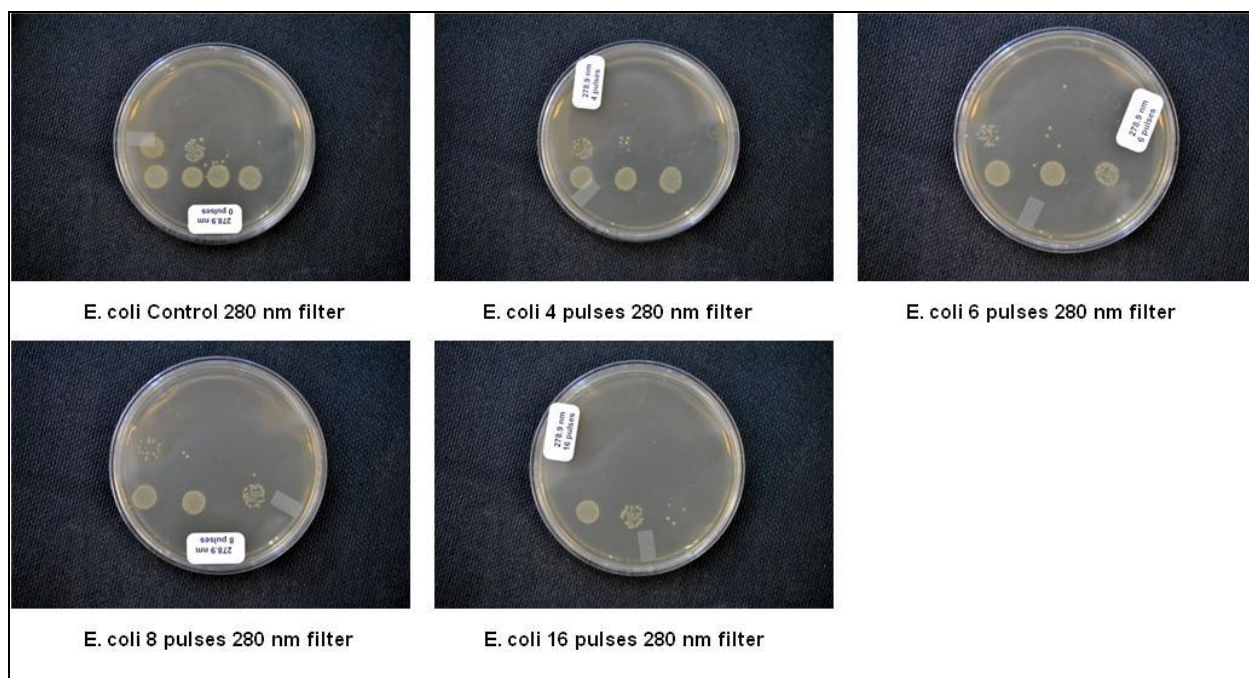


Figure 9. Effect of filtered pulsed light (280 nm) on growth of *E. coli*.

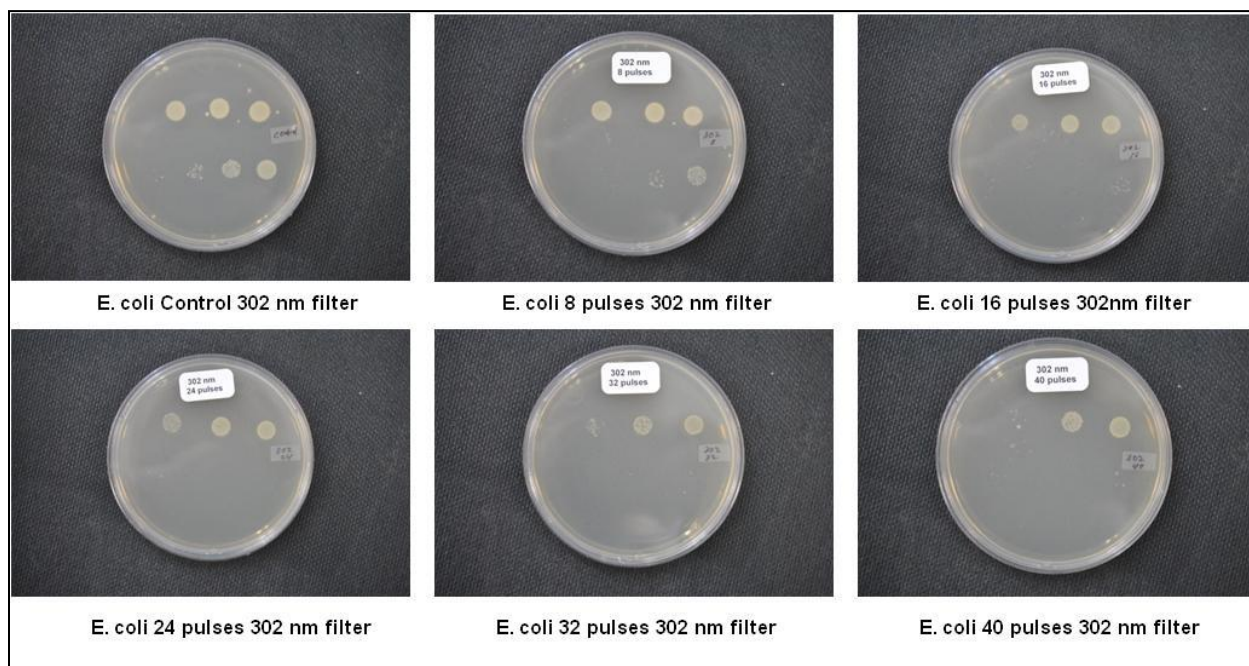


Figure 10. Effect of filtered pulsed light (302 nm) on growth of *E. coli*.

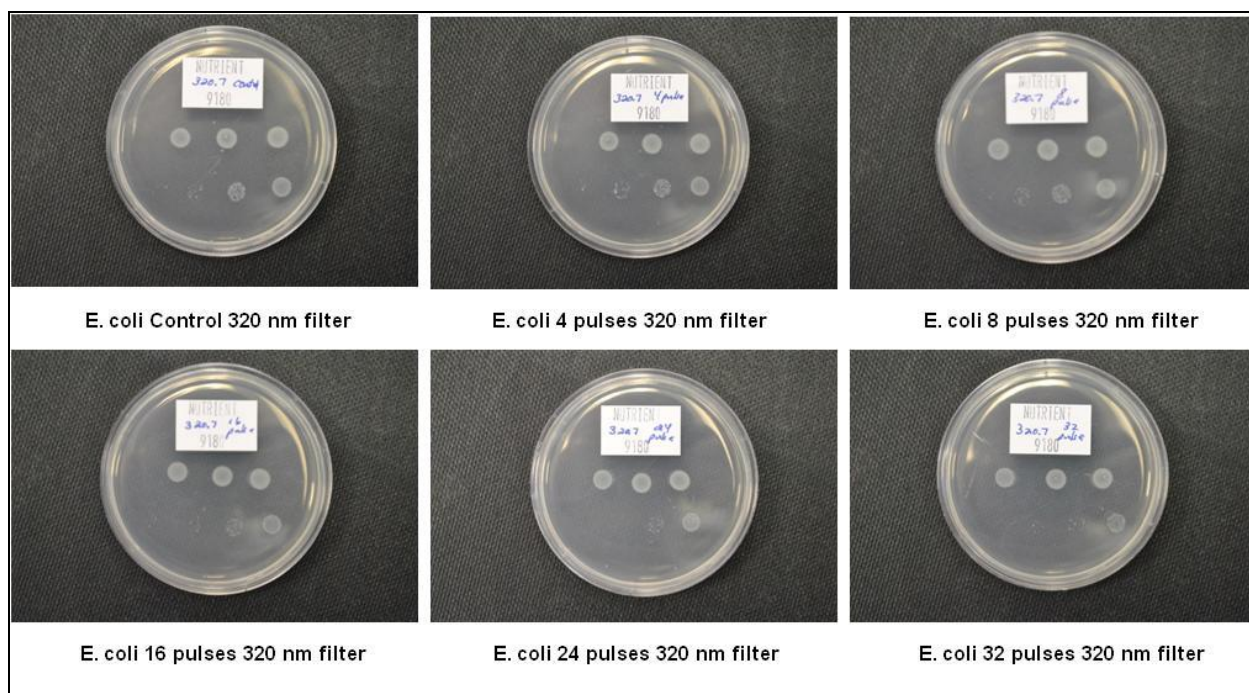


Figure 11. Effect of filtered pulsed light (320.7 nm) on growth of *E. coli*.

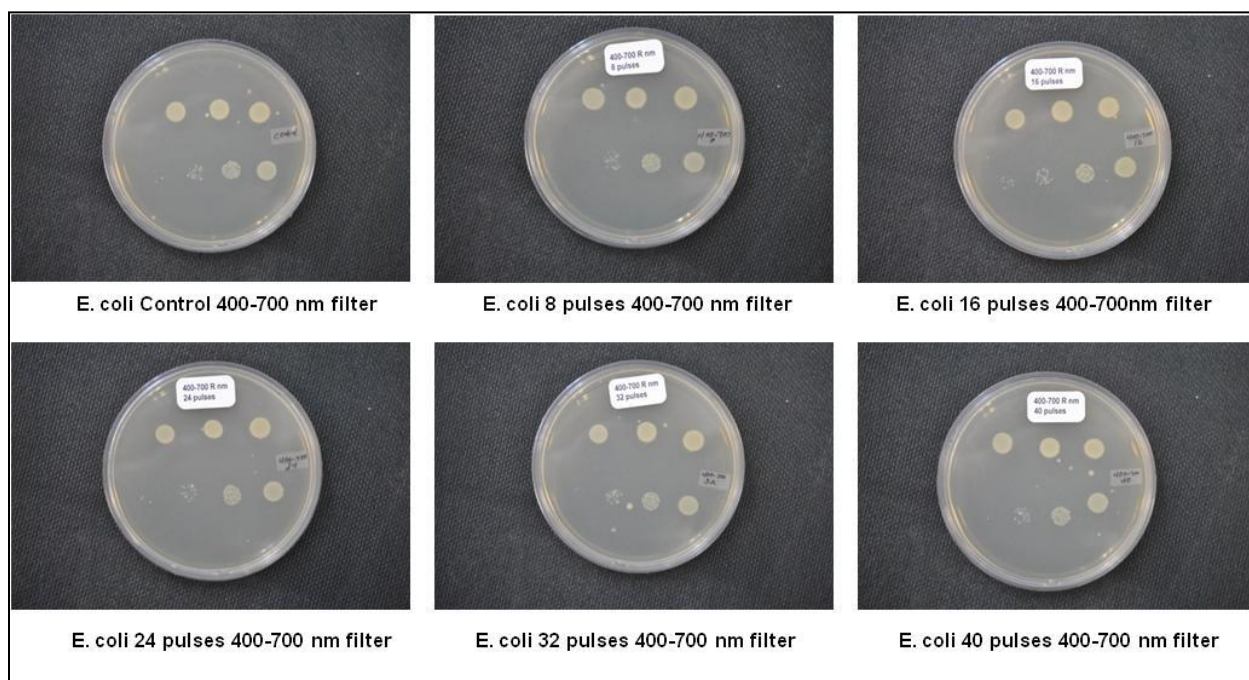


Figure 12. Effect of filtered pulsed light (400–700 nm) on growth of *E. coli*.

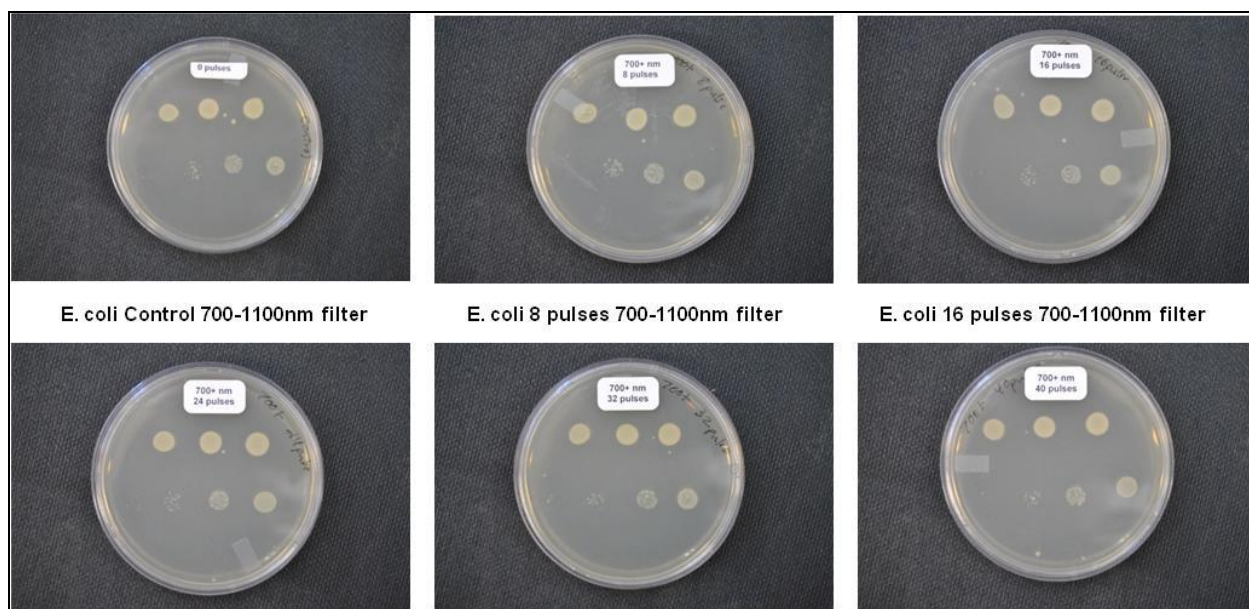


Figure 13. Effect of filtered pulsed light (700+ nm) on growth of *E. coli*

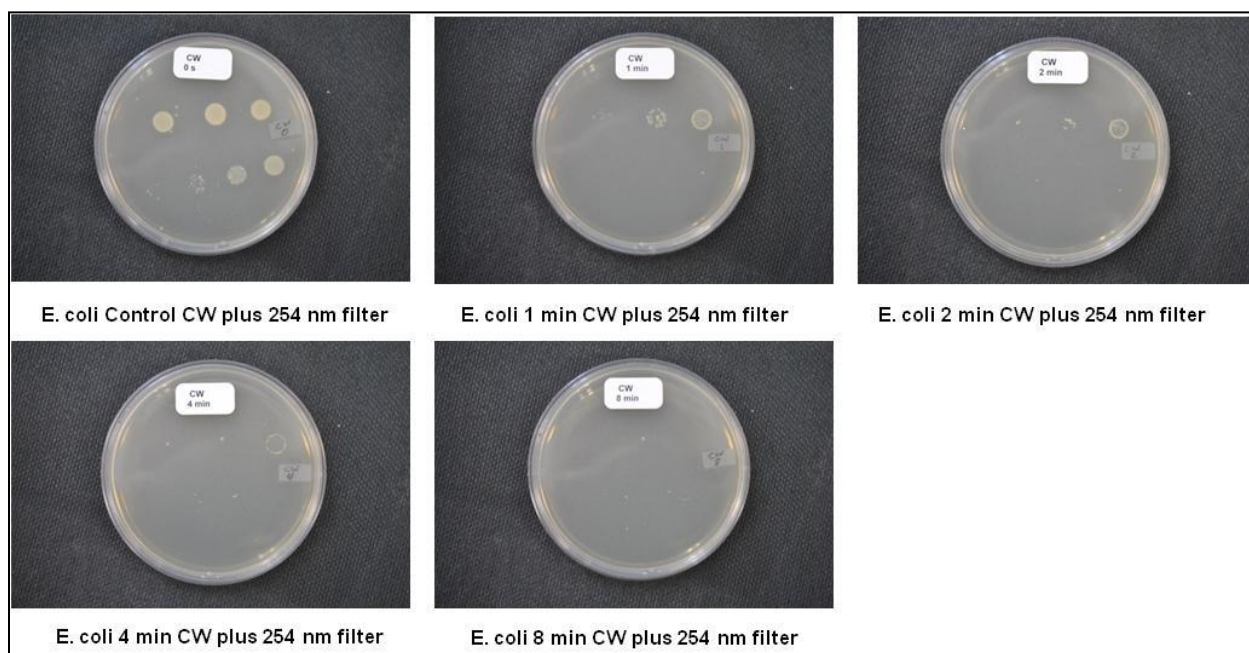


Figure 14. Effect of CW UV (plus 254 nm filter) on growth of *E. coli*.

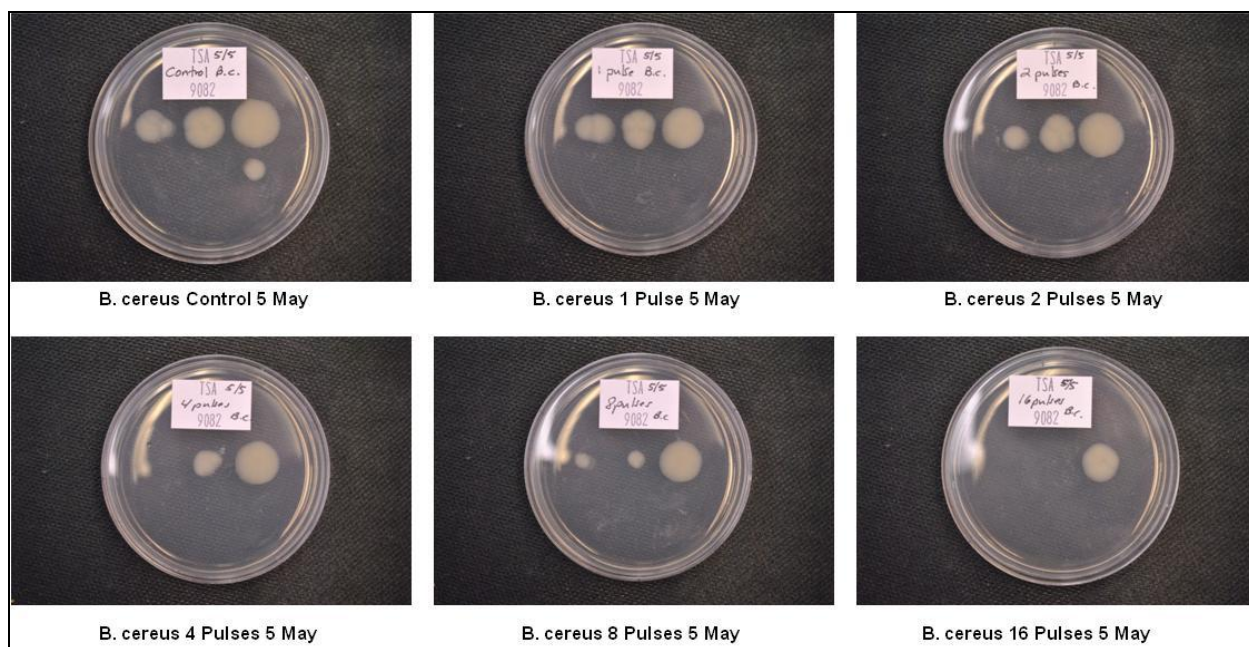


Figure 15. Effect of unfiltered pulsed light on growth of *B. cereus*.

4. CONCLUSIONS

The following conclusions have been drawn based on this study:

- Wavelengths in the UV-C range from 250–270 nm are those most effective in the killing of microorganisms by pulsed radiation. Figure 16 provides a compilation of the log kills for the experiments conducted during this study.
- Killing efficiency drops off dramatically when using wavelengths over 280 nm. Figure 17 provides a comparison of the current measurements with the relative germicidal effectiveness curves adopted by the Illuminating Engineering Society of the Deutsches Institut für Normung (7,8) and the pulsed UV disinfection curve, measured in 2005 by MacGregor et al. (2), normalized to 1.0 at peak effectiveness.
- Long wavelength UV and short wavelength visible radiations show some germicidal effectiveness; our results were consistent with other studies that indicated a threshold-type of killing mechanism was dominant at longer wavelengths.

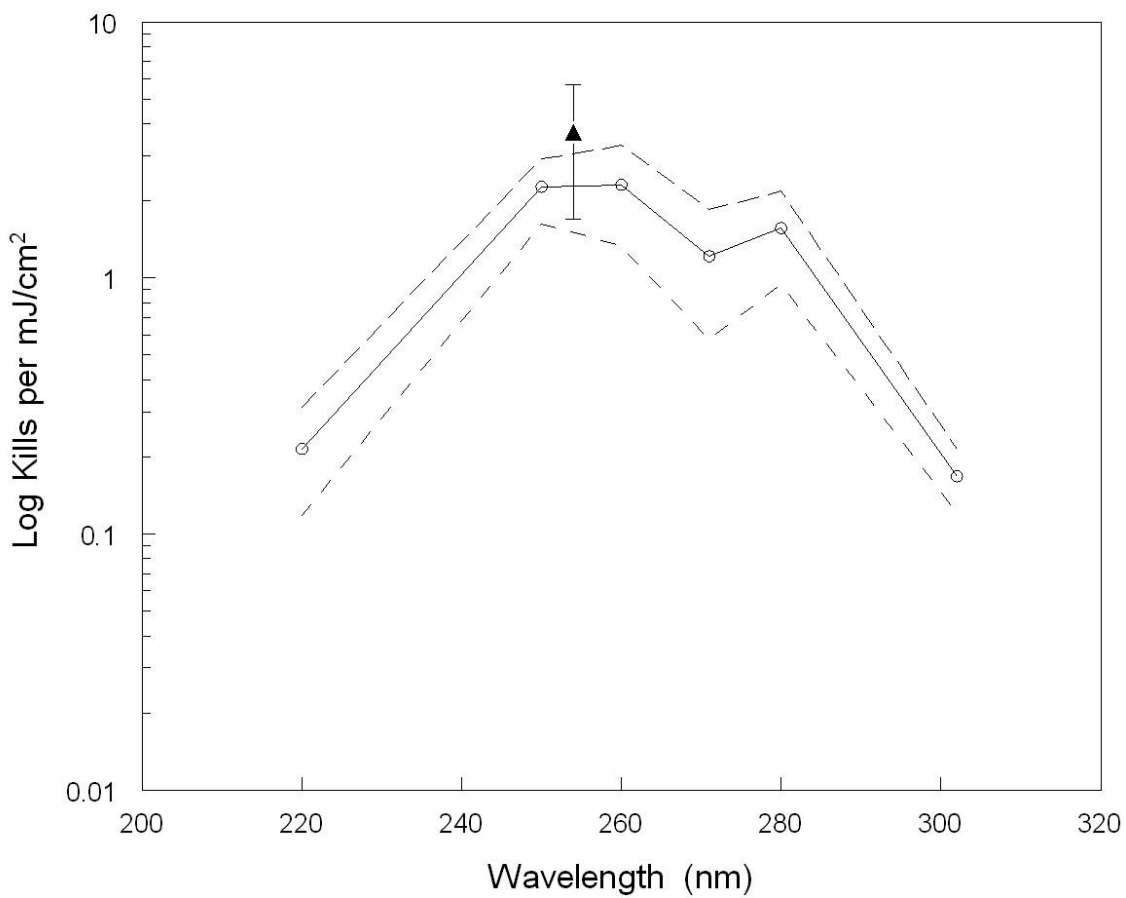


Figure 16. Chart comparing log kills vs wavelength (220–340 nm, ▲ = CW). Dashed lines indicate the 90% confidence range.

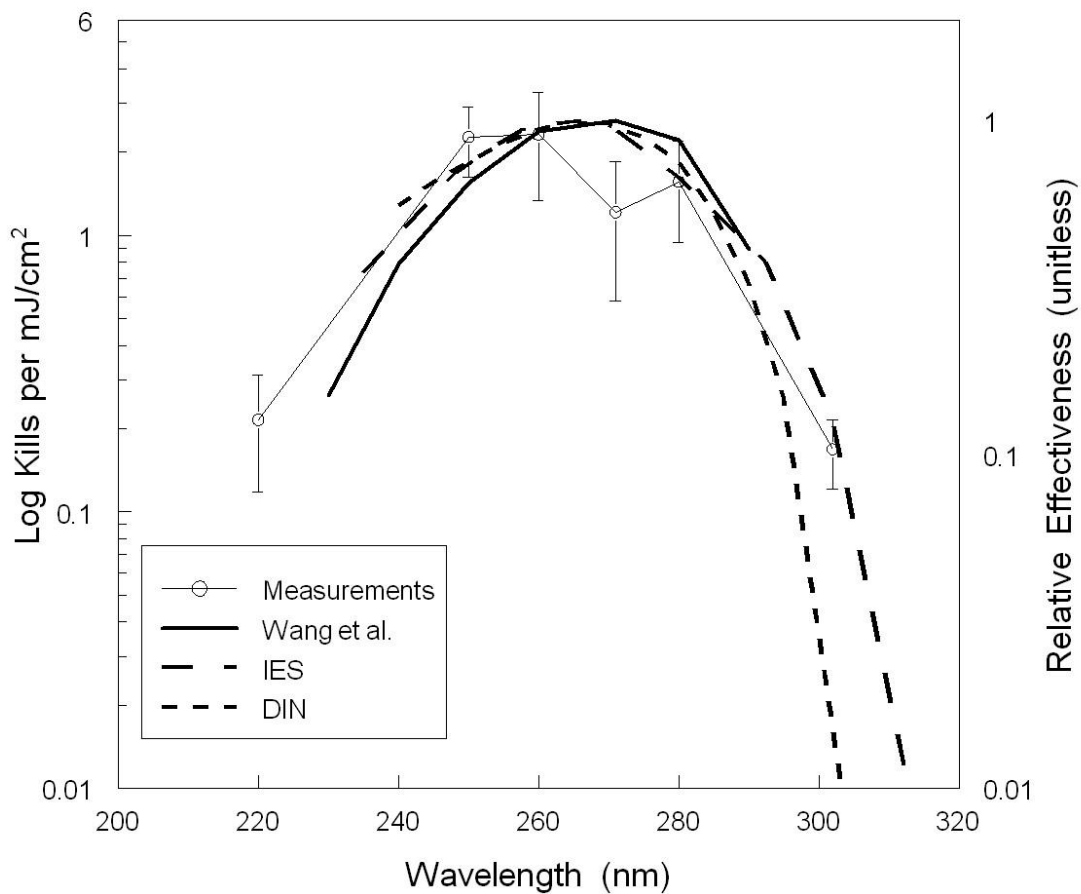


Figure 17. Comparison of measurements with the relative germicidal effectiveness curves. Standard adopted by the Illuminating Engineering Society of the Deutsches Institut für Normung, and the pulsed UV disinfection curve, measured in 2005 by MacGregor et al. (also normalized to 1.0 at peak effectiveness).

Table 1. Pulsed Lamp Results

200* nm			
Pulses	Dose (J/cm²)	Log Kills (–log[N/N₀])	<i>E. coli</i> (cfu/mL)
8	0	0.0	3.0E+08
16	0	0.2	1.8E+08
24	0	0.0	3.9E+08
32	0	0.2	2.0E+08
40	0	0.2	1.7E+08
220.5 nm			
4	8.06E-03	2.2	1.40E+06
8	1.60E-02	3.8	3.30E+04
16	1.70E-02	3.2	2.00E+03
248.9 nm			
4	2.00E-04	0.3	1.50E+08
8	4.00E-04	0.6	8.00E+07
16	8.05E-04	2.5	1.00E+06
24	1.21E-03	2.2	1.70E+06
32	1.61E-03	3.8	5.00E+04
260 nm			
4	5.14E-04	2.0	1.00E+07
6	7.70E-04	2.5	3.00E+06
8	8.05E-04	2.8	1.70E+06
16	2.05E-03	4.3	5.00E+04
270 nm			
8	6.48E-04	1.9	3.50E+06
16	1.30E-03	3.0	3.00E+05
24	1.94E-03	2.5	1.00E+06
32	2.59E-03	2.5	1.00E+06
40	3.24E-03	4.0	3.00E+04
280 nm			
4	6.89E-04	1.9	5.00E+06
6	1.03E-03	2.1	3.00E+06
8	1.38E-03	2.3	2.00E+06
16	2.76E-03	4.0	4.00E+04
302 nm			
8	4.50E-03	1.2	2.00E+07
16	9.00E-03	2.1	2.60E+06
24	1.35E-02	2.9	4.00E+05
32	1.80E-02	3.0	2.80E+05
40	2.25E-02	3.4	1.30E+05
320 nm			
4	1.61E-03	0.0	2.00E+08
8	3.20E-03	0.0	2.00E+08
16	6.40E-03	0.5	7.00E+07
24	9.60E-03	0.8	3.00E+07
32	1.28E-02	1.1	1.50E+07

*No energy detected; the 200 nm wavelength is at the edge of the “vacuum UV” range, and the air attenuation of UV radiation is very significant.

Table 1. Pulsed Lamp Results (continued)

400–700 nm			
Pulses	Dose (J/cm ²)	Log kills (–log[N/N ₀])	<i>E. coli</i> (cfu/mL)
8	1.70E-01	0.0	2.80E+08
16	3.40E-01	0.0	8.00E+08
24	5.10E-01	0.0	3.00E+08
32	6.80E-01	0.1	2.40E+08
40	8.49E-01	0.1	2.30E+08
700–1100 nm			
8	1.22E-01	0.1	2.50E+08
16	2.45E-01	0.0	3.00E+08
24	3.66E-01	0.0	4.00E+08
32	4.88E-01	0.1	2.50E+08
40	6.13E-01	0.5	1.00E+08

Table 2. CW Lamp Results

CW, 254 nm			
Time (min)	Dose (J/cm ²)	Log kills (–log[N/N ₀])	<i>E. coli</i> (cfu/mL)
1	6.65E-04	3.6	8.00E+04
2	1.33E-03	4.6	8.00E+03
4	2.66E-03	5.1	2.80E+03
8	5.32E-03	8.5	0

Table 3. *B. cereus* Exposure to Pulsed Lamp

Number of Pulses Unfiltered	Log Kills (–log[N/N ₀])	Colonies <i>B. cereus</i> (cfu/mL)
0	0	1E+05
1	0.5	3E+04
2	1.0	1E+04
4	2.0	1E+03
8	1.0	1E+04
16	3.0	1E+02

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ACRONYMS

CW continuous wave

NIR near infrared

PBS phosphate buffered saline

TSA tryptic soy agar

TSB tryptic soy broth

