Journal of Food Protection, Vol. 67, No. 5, 2004, Pages 1027–1030

Research Note

Inactivation of *Staphylococcus aureus* by Pulsed UV-Light Sterilization

KATHIRAVAN KRISHNAMURTHY,[1] ALI DEMIRCI,[1,2]* AND JOSEPH IRUDAYARAJ[1]

[1]*Department of Agricultural and Biological Engineering and* [2]*The Huck Institutes of Life Sciences, The Pennsylvania State University, University Park, Pennsylvania 16802, USA*

MS 03-405: Received 10 September 2003/Accepted 5 January 2004

ABSTRACT

Pulsed UV light is a novel technology to inactivate pathogenic and spoilage microorganisms in a short time. The efficacy of pulsed UV light (5.6 J/cm^2 per pulse) for the inactivation of *Staphylococcus aureus* as suspended or agar seeded cells was investigated. A 12-, 24-, or 48-ml cell suspension in buffer was treated under pulsed UV light for up to 30 s, and 0.1 ml of sample was surface plated on Baird-Parker agar and incubated at 37°C for 24 h to determine log reductions. Also, 0.1 ml of cell suspension in peptone water was surface plated on Baird-Parker agar plates, and the plates were treated under pulsed UV light for up to 30 s. The treated and untreated plates were incubated in the conditions described above. A 7- to 8-log CFU/ml reduction was observed for suspended and agar-seeded cells treated for 5 s or longer. In the case of suspended cells, the sample depth, time, treatment, and interaction were significant ($P < 0.05$). In the case of agar-seeded cells, the treatment time was significant ($P < 0.05$). Our results clearly indicate that pulsed UV technology has potential for the inactivation of pathogenic microorganisms.

Foodborne diseases are estimated to cause ~76 million illnesses, 325,000 hospitalizations, and 5,000 deaths annually in the United States *(14).* Therefore, foods contaminated with pathogenic microorganisms, such as *Salmonella* spp., *Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni, Escherichia coli* O157:H7, and *Listeria monocytogenes* remain a major public health concern. Among these pathogens, *S. aureus* is one of the most common causes of disease *(12).* According to the Centers for Disease Control and Prevention (CDC), there were 17,248 and 1,413 cases of *S. aureus* illness reported during 1973 through 1987 and 1993 through 1997, respectively, accounting for 14 and 1.6% of all illnesses caused by bacterial pathogens *(3, 15).*

Several technologies have been evaluated for the inactivation of these pathogens, such as heat treatment, cold-temperature treatment, irradiation, microwave radiation, pulsed electric field, magnetic field, high pressure, and ohmic heating treatments *(10).* In the present study, the effect of pulsed UV-light treatment on inactivation of a pathogen, *S. aureus* was investigated. Although, UV-light treatment is effective in the inactivation of pathogens, it may have several disadvantages, such as the oxidation of unsaturated fats, the poor penetration capability of UV light, and the destruction of some vitamins. However, these may be reduced or eliminated by shorter treatment time and thin-layer treatment.

UV light has been used as a bactericidal agent as early as 1928 *(21).* UV light is a portion of electromagnetic spectrum ranging from wavelengths of 100 to 400 nm. UV light in the wavelength range of 100 to 280 nm has germicidal capabilities *(8).* UV-light sterilization provides a cost-effective alternative to the existing heat pasteurization techniques and preservation methods. Also, taste degradation of food material subjected to UV light treatment is minimal, because UV-light treatment can be accomplished at an ambient temperature *(9).* UV light damages the DNA by forming pyrimidine dimers *(13).* This dimer prevents the microorganism from DNA transcription and replication, which leads to cell death. Several researchers have demonstrated that UV light can be used for the inactivation of pathogens without adversely affecting the quality of food *(1, 16, 18, 22).*

UV-light treatment can be accomplished in two modes—continuous or pulsed. Continuous UV light has several disadvantages, such as poor penetration depth and low dissipation power, whereas pulsed UV-light sterilization has a higher penetration depth and dissipation power. Pulsed UV-light treatment is more effective and rapid for microorganism inactivation than continuous UV-light sources, because the energy is multiplied manyfold *(4, 7).* Power dissipation from a continuous UV-light system ranges from 100 to 1,000 W *(6)*; however, a pulsed UV-light system can produce peak power distribution as high as 35 MW *(11, 13).* In pulsed UV-light treatment, the energy is stored in a high-power capacitor and released constantly during a short period of time (often in nanoseconds). This produces several high-energy flashes per second; hence, the

* Author for correspondence. Tel: 814-863-1098; Fax: 814-863-1031; E-mail: demirci@psu.edu.

microorganisms are inactivated effectively by the high UV content during the flash and the constant disturbance caused by pulses. Moreover, the pulsed UV light provides cooling periods between pulses and hence reduces the temperature build-up than continuous UV light because of the short pulse duration and cooling period between pulses. McDonald et al. *(13)* compared the effectiveness of continuous UV light source and the pulsed UV-light source for the decontamination of surfaces. The authors reported that the almost identical level of inactivation of *Bacillus subtilis* was obtained with an energy level of 4×10^{-3} J/cm^2 of the pulsed UV-light source instead of a 8×10^{-3} J/cm^2 continuous UV-light source.

Chang et al. *(5)* investigated the effect of continuous UV light on the inactivation of *E. coli, Salmonella* Typhi, *Shigella sonnei, Streptococcus faecalis,* and *S. aureus. E. coli, S. aureus, S. sonnei,* and *Salmonella* Typhi exhibited similar resistance to the UV light and required $\sim 7 \times 10^{-3}$ J/cm^2 energy to get a 3-log reduction. However, the resistance exhibited by *S. faecalis* was higher and required 1.4 times higher dose than the above-mentioned microorganism to get a 3-log reduction.

On the other hand, a 6-log reduction of *L. monocytogenes, E. coli, Salmonella* Enteritidis*, Psudeomonas aeruginosa, Bacillus cereus,* and *S. aureus* seeded on agar surface were obtained after 200 pulses of UV light *(16).* Sonenshein *(19)* investigated the effect of high-intensity pulsed UV light with an energy level of 15.8 J/cm^2/s on inactivation of *B. subtilis* spores. A complete inactivation (7- to 8-log reduction) was obtained with a 1-s exposure of UV light when the samples were placed at the lamp axis and at the midpoint of the lamp. The effectiveness of pulsed UV light for the inactivation of *E. coli* O157:H7 on inoculated alfalfa seeds was demonstrated by Sharma and Demirci *(17).* A complete inactivation of *E. coli* O157:H7 (4.80 log CFU/g) was obtained after 30 s of treatment when the thickness of alfalfa seeds was kept at 1.02 mm.

Stermer et al. *(20)* investigated the effect of pulsed UV radiation on the inactivation of bacteria in lamb meat. With 4×10^{-3} J/cm^2 energy, 99.9% of the naturally occurring flora of lamb (mostly *Pseudomonas, Micrococcus,* and *Staphylococcus* sp.) was inactivated. The bactericidal effectiveness of pulsed UV light was investigated by Bank et al. *(2).* Bacterial monolayers of *S. epideridis, P. aeruginosa, E. coli, S. aureus,* or *Serratia marcescens* on Trypticase soy agar plates were exposed to pulsed UV light. A 60-s treatment time at 31 cm distance from the light source resulted in a 6- to 7-log reduction in the viable bacterial population ($\sim 4 \times 10^{-4}$ J/cm^2).

In contrast, Bank et al. *(2)* and Rowan et al. *(16)* investigated the effect of pulsed UV light on the inactivation of *S. aureus* on solid surface. There is a need to investigate the effect of pulsed UV light on liquid medium, which represents liquid food systems such as milk. In addition to liquid medium, the effect of pulsed UV light on a solid agar surface was also investigated, because the system used in the present study is different from the one used by Bank et al. *(2),* and this can be used as a comparative method. The overall purpose of the present study was to investigate the possibility of using pulsed UV light as an alternative method for the inactivation of *S. aureus* and to investigate the effect of various parameters such as depth of sample, time of exposure, and medium of introduction (solid [agar-seeded cells] or liquid [suspended cells]).

MATERIALS AND METHODS

Microorganism. *S. aureus* (ATCC 13311) was obtained from the Penn State Food Microbiology Culture Collection. Cells were grown in 150 ml of tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) at 37°C for 24 h, and harvested by centrifugation (Sorvall STH750; Kendro Lab Products, Newtown, Conn.) at 3,300 $\times$ *g* for 25 min at 4°C.

Sample preparation. The cells to be treated by pulsed UV light were prepared as suspension cells and agar-seeded cells, which represent liquid and solid food systems. For suspended cells, after centrifugation, the pellet was resuspended in 100 ml of sterile 0.1 M phosphate buffer (pH 7.0) to obtain viable cell populations of approximately 7 to 8 log CFU/ml of *S. aureus.* A 12-, 24-, or 48-ml cell suspension in buffer was transferred to sterile aluminum containers with a diameter of 7 cm (VWR International, Buffalo Grove, Ill.).

For agar-seeded cells, the pellet was resuspended in 100 ml of sterile 0.1% peptone water. The resulting inoculum solution had approximately 7 to 8 log CFU/ml of *S. aureus,* and the cell suspension was serially diluted up to 10^{-5} dilution. A 0.1-ml sample of cell suspension from each dilution and inoculum was surface plated on Baird-Parker agar plates. To facilitate the direct counting of treated and untreated plates, regular plastic petri dishes were used rather than aluminum containers.

Pulsed UV-light treatment. Pulsed UV-light treatment was carried out with a laboratory scale, batch-pulsed light sterilization system (SteriPulse-XL 3000; Xenon Corporation, Woburn, Mass.). The system generated 5.6 J/cm^2 per pulse on the strobe surface, for an input voltage of 3,800 V and with three pulses per second, as per the manufacturer's instructions. The output from the pulsed UV-light system followed a sinusoidal wave pattern, with 5.6 J/cm^2 per pulse being the peak value of the pulse. Power values of UV-light treatments used in our study were based on the peak value of the pulse (5.6 J/cm^2 per pulse) generated on the strobe surface. The pulse width (duration of pulse) was 360 μs. The suspended cell samples with varying volumes were treated under pulsed UV light for 1, 2, 3, 4, 5, 10, 15, or 30 s at a distance of 8 cm from the UV strobe. Similarly, agar-seeded plates are treated under pulsed UV light for 1, 2, 3, 4, 5, 10, 15, or 30 s at a distance of 8 cm from the UV strobe. During the pulsed UV-light treatment, the temperature of agar plates and the suspended cell liquid were monitored using a type K thermocouple (Omegaette HH306, Omega Engineering, Inc., Stamford, Conn.).

Microbiological analysis. For suspended cells, untreated samples and samples immediately after pulsed UV-light treatment were analyzed for surviving populations of *S. aureus.* A 1-ml sample from the sample cup was serially diluted with 0.1 M phosphate buffer. This is followed by surface plating of a 0.1-ml sample on Baird-Parker agar. After incubation at 37°C for 24 h, the colonies were enumerated. The log reduction was calculated by subtracting the log value of control from that of treated sample. Each experiment was repeated three times.

For agar-seeded cells, pulsed UV-light–treated agar plates were incubated for 24 h at 37°C. The higher dilution plates with no colonies were discarded, and the colonies in the low dilution

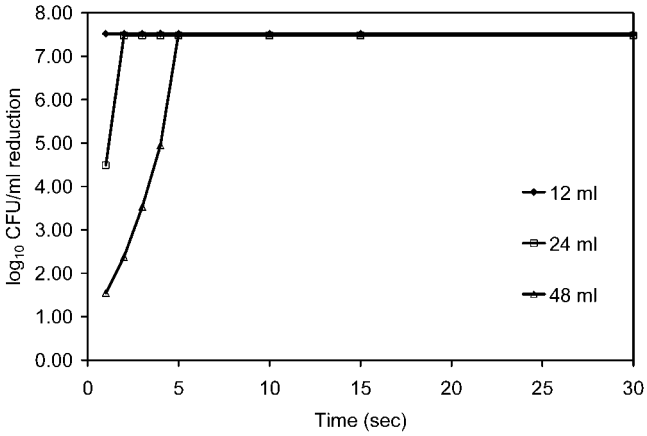

FIGURE 1. *Log reductions of* S. aureus *after pulsed UV-light sterilization in suspended cells.*

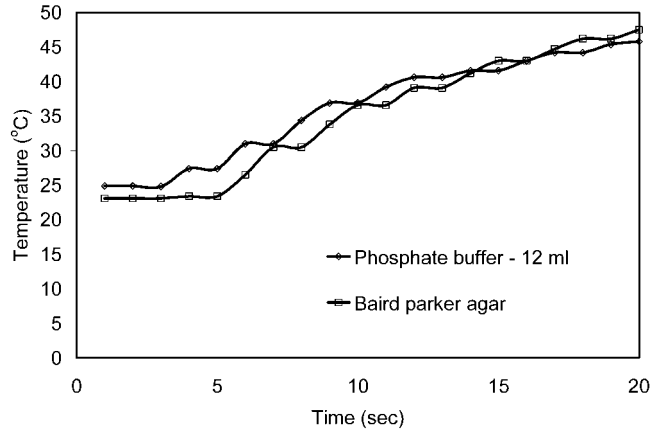

FIGURE 2. *Temperature profile of phosphate buffer and Baird-Parker agar base during pulsed UV-light treatment.*

plates were counted and reported. Three replicates for each experiment were performed.

Statistical analysis. Statistically significant differences between treated and untreated cells were tested using the general linear model of analysis of variance with two-way interaction with MINITAB software (version 13.3, Minitab Inc., State College, Pa.). A 95% confidence interval was used.

RESULTS AND DISCUSSION

Suspended cell treatment. To demonstrate effectiveness of pulsed UV light on a liquid medium, *S. aureus* cells were suspended in 0.1 M phosphate buffer and treated by pulsed UV light up to 30 s at a distance of 8 cm from a UV strobe. The corresponding power of pulsed UV light at the surface of the UV strobe were 16.8, 33.6, 84, and 504 J/cm^2/s for 1, 2, 5, and 30 s of treatment time, respectively. A complete inactivation was obtained for samples treated for 5 s for all sample sizes. The average corresponding log reduction was 7.50 log CFU/ml (Fig. 1). The pulsed UV light was very effective in inactivating *S. aureus* in suspension. Complete inactivation was also observed for 1- and 2-s treatments of 12- and 24-ml samples, respectively. However, after the 1-s treatment, 4.6- and 1.5-log reductions were obtained in 24- and 48-ml samples, respectively. For the 48-ml sample, the log reduction increased exponentially during 5 s. As expected, the log reduction increased with treatment time because of the increase in the number of pulses. The effect of the sample depth was significant ($P < 0.05$). The temperature of the sample increased as the treatment time increased after several seconds (Fig. 2); however, no significant temperature increase was observed during the first 5 s. Pulsed UV-light treatment is considered to be nonthermal, but this holds only for treatments of short duration. Temperature increases as absorbed energy accumulates during longer treatments. During a 20-s treatment time, the temperature increase was ~20°C for a 12-ml phosphate buffer sample. Finally, statistical analysis suggested that the effect of treatment time and the interaction with depth (treatment time × depth) were significant ($P < 0.05$).

Agar-seeded cells. To demonstrate the effectiveness of pulsed UV light on solid surfaces, agar-seeded *S. aureus* cells were treated by pulsed UV light for up to 30 s at a distance of 8 cm from the UV strobe. The corresponding power of pulsed UV light at the surface of the UV strobe was 16.8, 33.6, 84, and 504 J/cm^2/s for 1-, 2-, 5-, and 30-s treatment times, respectively. A 5-s treatment inactivated all *S. aureus,* to yield about an 8.5-log reduction (Fig. 3), which contributed to an effective inactivation of *S. aureus* ($P < 0.05$) on the agar surface when the distance between agar-seeded cells and the UV strobe was 8 cm. Bank et al. *(2)* obtained a 6- to 7-log reduction of agar-seeded cells after a 60-s treatment when the distance from the UV strobe was 31 cm. The corresponding power obtained by the sample was 6.67×10^{-6} J/cm^2/s. Because the distance of the UV strobe from the sample for our system was different from that of Bank et al., comparing the log reduction from both the cases would be futile. A 5-log reduction was obtained after a 1-s treatment. As expected, the log reduction of *S. aureus* increased exponentially with the treatment time, similar to suspended cell solution, and complete inactivation was achieved within 5 s. The temperature of the agar increased for longer treatment times (Fig. 2). However, there was no significant increase in the temperature during the first 5 s, and the inactivation was hypothesized to occur

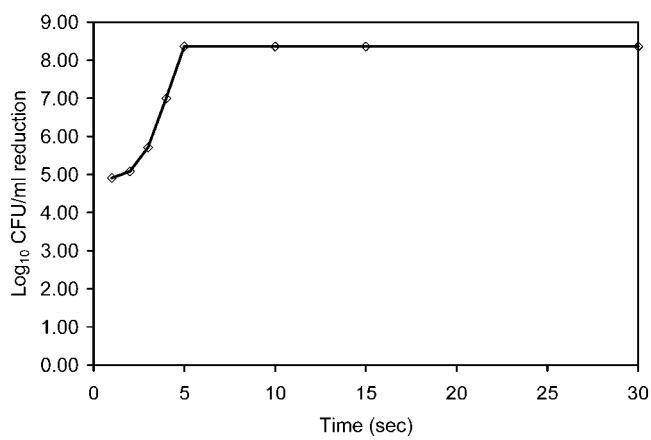

FIGURE 3. *Log reductions of* S. aureus *after pulsed UV-light sterilization in agar-seeded plates.*

primarily because of the pulsed UV light and not the synergistic effect due to the temperature increase. Experiments indicated that a few colonies survived along the edge of the plate, possibly because of the obstruction of light by the elevated edges of the plate. The intensity of UV light reduced with the radial distance from the central axis of the lamp.

Pulsed UV-light treatment is more effective for surface sterilization than for the sterilization of a liquid medium. Log reductions of 5.0 and 1.35 CFU/ml were obtained for agar-seeded cells and suspended cells (when the sample volume was 48 ml), respectively, after a 1-s treatment. However, as expected, when the depth of the suspended cell was kept to a minimum by having a smaller suspended cell volume, suspended cell inactivation yielded similar results as agar-seeded cells. As the sample depth of suspended cell increases, the inactivation level of *S. aureus* decreases, because of the poor penetration capacity of pulsed UV light. Therefore, one can increase the effectiveness of pulsed UV light on the inactivation of suspended cells by minimizing the sample depth and/or increasing the treatment time.

The present study clearly shows the potential of pulsed UV light for inactivating *S. aureus* in liquid and solid systems. The complete inactivation of *S. aureus* can be achieved within seconds with pulsed UV light. Therefore, pulsed UV light can be used as an alternative to thermal sterilization processes. However, there is a need for optimizing the experimental parameters to achieve the target inactivation level for specific applications. Further research is in progress to evaluate the applicability of pulsed UV light for the sterilization of various food products.

ACKNOWLEDGMENTS

This project was funded by a U.S. Department of Agriculture Special Research Grant (2002-34163-11837) and the Pennsylvania Agricultural Experiment Station. The pulsed UV-light system was provided by the NASA Food Technology Commercial Space Center.

REFERENCES

1. Allende, A., and F. Artes. 2003. UV-C radiation as a novel technique for keeping quality of fresh processed "Lollo Rosso" lettuce. *Food Res. Int.* 36:739–746.
2. Bank, H. L., J. L. Schmehl, and R. J. Dratch. 1990. Bacteriocidal effectiveness of modulated UV light. *Appl. Environ. Microbiol.* 56: 3888–3889.
3. Bean, N. H., and P. M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles, and trends. *J. Food Prot.* 53:804–817.
4. Center for Food Safety and Applied Nutrition, Food and Drug Administration. 2000. Kinetics of microbial inactivation for alternative food processing technologies: pulsed light technology. Available at: http://vm.cfsan.fda.gov/~comm/ ift-puls.html.
5. Chang, J. C. H., S. F. Ossoff, D. C. Lobe, M. H. Dorfman, C. M. Dumais, R. G. Qualls, and J. D. Johnson. 1985. UV inactivation of pathogenic and indicator microorganisms. *Appl. Environ. Microbiol.* 49:1361–1365.
6. Demirci, A. 2002. Novel processing technologies for food safety. *J. Assoc. Food Drug Officials* 66(4):1–8.
7. Dunn, J., T. Ott, and W. Clark. 1995. Pulsed light treatment of food and packaging. *Food Technol.* 49:95–98.
8. Environmental Protection Agency. 1999. Ultraviolet radiation: alternative disinfectants and oxidants guidance manual. Available at: http://www.epa.gov/safewater/mdbp/pdf/alter/chapt_8.pdf.
9. Hollingsworth, P. 2001. Niche players pushing UV light applications. *Food Technol.* 55(12):16–16.
10. Juneja, V. K., and J. N. Sofos (ed.). 2002. Control of foodborne microorganisms. Marcel Dekker, Inc., New York.
11. MacGregor, S. J., N. J. Rowan, L. Mcllvaney, J. G. Anderson, R. A. Fouracre, and O. Farish. 1997. Light inactivation of food-related pathogenic bacteria using a pulsed power source. *Lett. Appl. Microbiol.* 27:67–70.
12. Martin, S. E., E. R. Myers, and J. J. Iandola. 2001. *Staphylococcus aureus,* p. 345–382. *In* Y. H. Hui, M. D. Pierson, and J. R. Gorham (ed.), Foodborne disease handbook, 2nd ed., vol. 1. Marcel Dekker, Inc., New York.
13. McDonald, K. F., R. D. Curry, T. E. Clevenger, K. Unklesbay, A. Eisenstrack, J. Golden, and R. D. Morgan. 2000. A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces. *IEEE Trans. Plasma Sci.* 28:1581–1587.
14. Mead, P. S., L. Slutsker, and V. Dietz. 1999. Food related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
15. Olsen, S. J., L. C. MacKinon, J. S. Goulding, N. H. Bean, and L. Slutsker. 2000. Surveillance for foodborne disease outbreaks—United States, 1992–1997. *Morb. Mortal. Wkly. Rep.* 49(SS-1):1–66.
16. Rowan, N. J., S. J. MacGregor, J. G. Anderson, R. A. Fouracre, L. McIlvaney, and O. Farish. 1999. Pulsed-light inactivation of food-related microorganisms. *Appl. Environ. Microbiol.* 65:1312–1315.
17. Sharma, R. R., and A. Demirci. 2003. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling. *J. Food Sci.* 68:1448–1453.
18. Smith, W. L., M. C. Lagunas-Solar, and J. S. Cullor. 2002. Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk. *J. Food Prot.* 65:1480–1482.
19. Sonenshein, A. L. 2003. Killing of *Bacillus* spores by high-intensity ultraviolet light, p. 15–19. *In* Sterilization and decontamination using high energy light. Xenon Corporation, Woburn, Mass.
20. Stermer, R. A., M. Lasater-Smith, and C. F. Brasington. 1987. Ultraviolet radiation—an effective bactericide for fresh meat. *J. Food Prot.* 50:108–111.
21. Xenon Corporation. 2003. Sterilization and decontamination using high energy light. Xenon Corporation, Woburn, Mass.
22. Yaun, B. R., S. S. Sumner, J. D. Eifert, and J. E. Marcy. 2003. Response of *Salmonella* and *Escherichia coli* O157:H7 to UV energy. *J. Food Prot.* 66:1071–1073.