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Pulsed-Light Inactivation of Food-Related Microorganisms

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The effects of high-intensity pulsed-light emissions of high or low UV content on the survival of predetermined populations of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* were investigated. Bacterial cultures were seeded separately on the surface of tryptone soya-yeast extract agar and were reduced by up to 2 or 6 $\log_{10}$ orders with 200 light pulses (pulse duration, ~100 ns) of low or high UV content, respectively ($P < 0.001$).

Despite significant advances made towards a better understanding of bacterial transmission and pathogenicity in foods, the incidence of reported food-borne illnesses associated with bacterial enteropathogens continues to be a major problem in the United Kingdom (5) and North America (7). It is generally accepted that contamination of both unprocessed and uncooked foods with pathogenic bacteria is a major source of concern, and any method of either reducing or eliminating food contamination will have a significant effect on the incidence of food-borne disease (5). A possible approach to reducing the level of microbial contamination on food surfaces in slaughterhouses, and in other food preparation environments, is UV irradiation (1–3, 5, 8, 9). Stermer et al. (8) indicated that the bacterial load on fresh meat can be effectively reduced by UV irradiation, while Wallner-Pendleton and coworkers (9) showed that this method of disinfection reduced *Salmonella* surface contamination without adversely affecting poultry carcass color or increasing meat rancidity. These studies indicate that if an effective and economic method of UV generation can be developed, then UV irradiation may have a practical application in the disinfection of food and contact surfaces.

While conventional alternating-current systems produce light with a power dissipation in the range of 100 to 1,000 W per device, a pulse power energization technique (PPET) can dissipate many megawatts of electrical power in the light source (4). PPET also tends to produce a greater intensity of the shorter bactericidal wavelengths of light, and, by using this approach, it is possible to design an extremely short energization time of the light source (~100 ns). For modest energy input (e.g., 3 J), this results in high peak power dissipation (~35 MW). Here we present evidence that PPET may lend itself to surface disinfection since it significantly reduces large populations of various food-related microorganisms on laboratory-based media.

The effectiveness of PPET, with two different light sources, in reducing predetermined microbial numbers on agar surfaces was determined by using a variety of proven bacterial pathogens, namely, *Escherichia coli* NCTC 12079 (serotype O157:H7), *Listeria monocytogenes* NCTC 11994 (serotype 4b), diarrheagenic *Bacillus cereus* NCTC 11145, *Salmonella enteritidis* NCTC 4444, *Staphylococcus aureus* NCTC 4135, *Pseudomonas aeruginosa* NCTC 8203, and the yeast *Saccharomyces cerevisiae* NCTC 10716, obtained from the National Collection of Type Cultures, Colindale, London, United Kingdom. Bacterial cultures were grown at 30°C and maintained on tryptone soya agar supplemented with 0.6% yeast extract (TSYEA). The yeast culture was grown at 25°C and maintained on malt extract agar supplemented with 0.3% yeast extract, 1% glucose, and 0.3% mycological peptone (MYGPA). Analysis of variance, balanced model (Minitab software release 11; Minitab Inc., State College, Pa.), was used to compare the effects of pulsed-light irradiation, number of pulses applied, and the type of microorganism treated. The studies were performed in quadruplicate, and all significant differences are reported at 95% ($P < 0.05$) and 99.9% ($P < 0.001$) confidence intervals.

Pulsed-light inactivation of food-related microorganisms. The bacterial test strains were inoculated into 100 ml of tryptone soya broth supplemented with 0.6% yeast extract (TSYEB) and cultivated on a shaker at 125 oscillations per minute to a population density of ~10^9 cells ml^{-1} (confirmed via plate counts). A 0.1-ml aliquot of a 10^{-5} dilution of this culture (the diluent used was 0.01 M sodium phosphate [pH 7.2]–0.15 M NaCl) was transferred to 100 ml of fresh TSYEB. The bacterial test strains were again grown to a density of ~10^9 cells ml^{-1}. *S. cerevisiae* was grown to a cell density of ~10^9 cells ml^{-1} (confirmed via plate counts) in 100 ml of malt extract broth supplemented with 0.3% yeast extract, 1% glucose, and 0.3% mycological peptone (MYGPB) at 25°C. Ten samples, each containing 20 μl of a bacterial or yeast test culture, were surface inoculated on separate TSYEA or MYGPA plates, respectively, by using the Miles and Misra method (6).

The test assembly consisted of a rectangular polyvinyl chloride housing, a pulse generator, and associated switching with controlling circuitry as shown in Fig. 1. The light source was mounted 4.5 cm above two sample holders that were set at a position 45 degrees to the horizontal. This allowed two samples to be irradiated simultaneously, with each sample receiving the same average exposure. Two light sources were employed. The first of these was a Heraeus Noblelight XAP series (type NL4006) constructed from a clear fused-quartz tube (UV transparent) filled with xenon to a pressure of 59 kPa. The second light source was a Heraeus Noblelight XFP series (type NL4320) with a cerium-doped quartz envelope, again filled with xenon to a pressure of 59 kPa. The envelope of this tube (NL4320) restricted the light output in the UV region. Both light sources produced a broad spectrum of white light with peak spectral emissions at wavelengths of ~550 nm, as shown in Fig. 2A for the low-UV-content tube and in Fig. 2B for the

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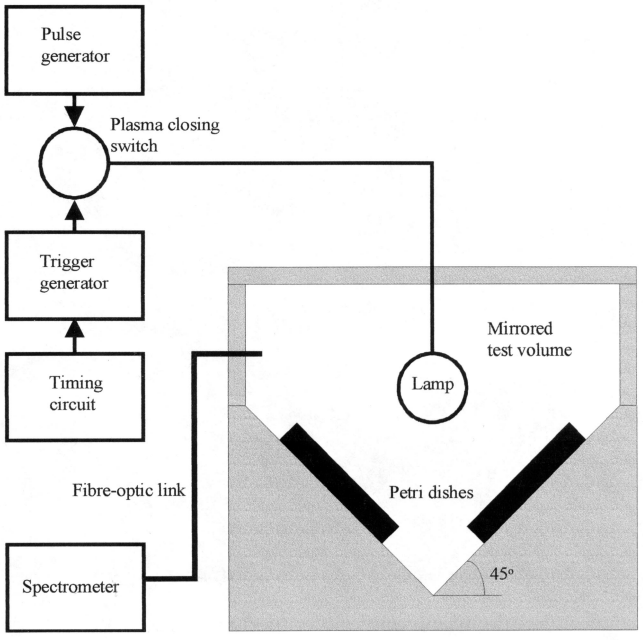

FIG. 1. Schematic layout of experimental facility for microbial inactivation with a pulsed-light source.

high-UV-content tube. The major differences in emission spectra occur between 200 and 450 nm.

A single-stage, inverting, pulse-forming-network Marx generator was used to create a high-peak-power discharge. The generator was charged to a DC voltage of 30 kV for all experiments and was discharged directly into the light source by using a plasma switch triggered via a high-voltage autotransformer. The generator source capacitance was 6.4 nF, and the source impedance, when fired, was 6.25 Ω. A fiber-optic link and timing control circuit were used to activate the pulse generator at a pulse repetition rate of 1 pulse per s. The generator was charged by using a Brandenburg 50-kV, 1-mA DC supply, and, at full voltage, the pulse-forming-network Marx generator contained a stored energy of 3 J. The nominal duration of the output pulse was 85 ns, representing an average peak electrical

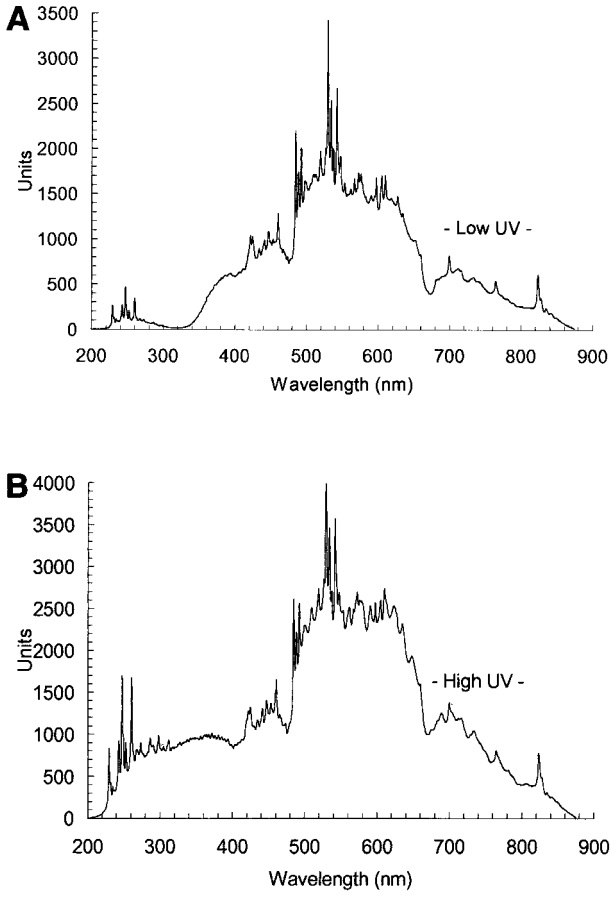

FIG. 2. Emission spectra (range, 200 to 900 nm) from two different light sources; one shows a low UV content (A), and the other shows a higher UV content (B).

power, per pulse, of 35 MW. This should be compared with the ~100-W average power rating of the light source when operated continuously. At 1 pulse per s, the average power consumption of the system was 3 W, and consequently no discern-

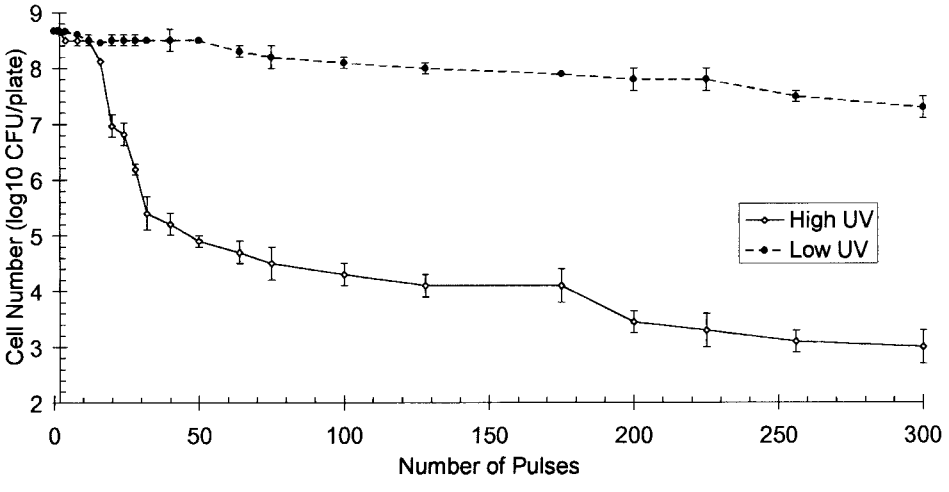

FIG. 3. Pulsed-light inactivation of surface-inoculated *E. coli* using two light sources which contained either a low- or high-UV content.

TABLE 1. Influence of two different pulsed-light sources on the viability of a variety of microbial populations

Treatment		No. of food-related microorganisms present (log_{10} CFU per plate [mean ± SD])[a]						
Light Source	No. of pulses	*B. cereus*	*L. monocytogenes*	*S. aureus*	*E. coli*	*S. enteritidis*	*P. aeruginosa*	*S. cerevisiae*
Untreated control	0	8.3 ± 0.1 B	9.4 ± 0.2 A	9.4 ± 0.1 A	9.6 ± 0.3 A	9.7 ± 0.2 A	8.7 ± 0.1 B	8.4 ± 0.2 B
High UV	100	4.5 ± 0.3 G	5.8 ± 0.4 E	5.2 ± 0.3 F	4.5 ± 0.1 G	5.2 F	4.3 ± 0.2 G	4.7 ± 0.2 G
	200	3.4 ± 0.3 H	5.0 ± 0.2 F	4.3 ± 0.1 G	3.4 ± 0.1 H	4.1 ± 0.2 G	2.9 ± 0.3 I	3.5 ± 0.1 H
Low UV	100	8.0 B	9.3 ± 0.1 A	8.9 ± 0.2 A	9.1 ± 0.1 A	9.1 ± 0.2 A	6.9 ± 0.3 D	8.0 ± 0.3 B
	200	7.9 ± 0.4 B	9.2 ± 0.1 A	8.1 ± 0.4 B	7.8 ± 0.2 C	8.1 ± 0.1 B	6.5 ± 0.1 D	7.7 ± 0.2 C

[a] Measured as log_{10} CFU per plate, where counts are averages of four replicate trials. Values followed by the same letter do not differ at the $P < 0.05$ level, whereas values followed by different letters differ at the $P < 0.05$ level.

ible increase in sample temperature was observed during treatment. The electrical diagnostics consisted of a high-voltage DC probe, used to measure the charging voltage, and a high-speed transient probe to monitor the pulsed voltage applied to the light source. With the line-source geometry employed, the light intensity profile varied by −30% from the center to the edge of the sample. The light emission was monitored with a four-channel Ocean Optics SQ2000 fiber-optic spectrometer. The spectral resolution was 1.25 nm for each channel (50-mm slit width), and the detectors (Sony 1LX511) were enhanced to allow UV detection. Continuous monitoring of the optical emissions verified that the emission spectra were constant throughout the experiment.

Surface-inoculated TSYEA and MYGPA plates containing approximately 10^9 cells ml of test culture^{-1} were positioned in the PPET assembly (Fig. 1). Samples of each test culture were treated with either 100 or 200 pulses of high-intensity light which contained either a low or high level of UV. Following treatment, the plates were wrapped in aluminium foil to prevent photoreactivation and were incubated for 48 h at 30°C. The study was carried out in quadruplicate with duplicate plates for each set of exposures. The surviving populations were enumerated and expressed as log_{10} CFU per plate.

Initial experiments with *E. coli* (Fig. 3) showed that the type of light source used had a significant effect on the level of inactivation ($P < 0.001$). A 5- and 6-log_{10}-order inactivation occurred after treatment with 100 and 200 pulses, respectively, with the higher UV light source, whereas 300 pulses of low-UV light gave a reduction of only 1 log_{10} order. Subsequently, 100 and 200 pulses were selected to test *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Listeria monocytogenes*, and the yeast *Saccharomyces cerevisiae*.

The results showed with each test culture that significantly greater levels of microbial inactivation ($P < 0.001$) occurred with light pulses of high-UV content. With 200 pulses of high-UV light, all of the microbial populations treated were reduced by 5 to 6 log_{10} orders, whereas with low-UV light, only a 1- to 2-log_{10}-order reduction in cell numbers occurred. It was found that there were variations in the susceptibility of test cultures (Table 1). The levels of resistance of the following bacteria differed (and are listed in order of decreasing resistance): *L. monocytogenes*, *Staphylococcus aureus*, *Salmonella enteritidis*, *E. coli*, *B. cereus*, *Saccharomyces cerevisiae*, and *P. aeruginosa* (the levels of resistance between *S. aureus* and *S. enteritidis* and those between *B. cereus*, and *S. cerevisiae* did not significantly differ at the $P < 0.05$ level). These findings are in agreement with the work of Jay (2), in which gram-positive bacteria were shown to be more resistant to the effects of UV light than gram-negative bacteria and pseudomonads and flavobacteria were shown to be the most sensitive.

By using this PPET approach for high-intensity light generation, it was possible to produce significant levels of peak power in the light source which are not achievable under conventional continuous excitation (4). This in turn results in a greater relative production of light in the shorter biocidal wavelengths. It has been well documented that UV is effective in killing microorganisms contaminating the surfaces of a variety of materials, including food (2, 3, 8, 9). The lethal action of high-intensity broad-spectrum light is due predominantly to either photothermal and/or photochemical mechanisms (e.g., the formation of lethal thymine dimers on the microbial DNA) (1, 2). Since only a negligible rise in temperature (i.e., less than 1°C) occurred in the treated agar, there were no appreciable photothermal effects. Therefore, it is likely that the lethality of this PPET approach can be attributed to the photochemical action of the shorter UV wavelengths. This is supported by the data in Fig. 2, which compares the spectral emissions produced by both PPET light sources and shows that they differ in the level of the shorter wavelengths in the range of 200 to 450 nm.

Bank and coworkers (1) have shown previously that a 6-log_{10} decrease in viable bacterial numbers, which had been surface inoculated onto trypticase soy agar plates, could be achieved by using a computer-controlled modulated UV-C light source (100 to 280 nm). Their light system used exposures of up to 60 s at 40-W peak power compared to an 85-ns exposure at 35-MW peak power in the present study, demonstrating the antimicrobial effectiveness of the PPET. This approach may be further improved by optimizing the emission spectra to enhance the antimicrobial UV-C content.

In conclusion, this study has shown that a 6-log_{10} reduction in microbial populations was achieved after exposure to 200 pulses of light containing high-intensity UV. The energy delivery system has modest energy requirements, ~3 J, and if this is delivered in a short period of time, with a high repetition rate, then rapid treatment can be achieved.

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