Light inactivation of food-related pathogenic bacteria using a pulsed power source

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The effects of high intensity light emissions, produced by a novel pulsed power energization technique (PPET), on the survival of bacterial populations of verocytotoxigenic *Escherichia coli* (serotype 0157:H7) and *Listeria monocytogenes* (serotype 4b) were investigated. Using this PPET approach, many megawatts (MW) of peak electrical power were dissipated in the light source in an extremely short energization time (about 1 μs). The light source was subjected to electric field levels greater than could be achieved under conventional continuous operation, which led to a greater production of the shorter bacteriocidal wavelengths of light. In the exposure experiments, pre-determined bacterial populations were spread onto the surface of Trypone Soya Yeast Extract Agar and were then treated to a series of light pulses (spectral range of 200–530 nm) with an exposure time ranging from 1 to 512 μs. While results showed that as few as 64 light pulses of 1 μs duration were required to reduce *E. coli* 0157:H7 populations by 99·9% and *Listeria* populations by 99%, the greater the number of light pulses the larger the reduction in cell numbers ($P < 0{\cdot}01$). Cell populations of *E. coli* 0157:H7 and *Listeria* were reduced by as much as 6 and 7 $\log_{10}$ orders at the upper exposure level of 512 μs, respectively. Survival data revealed that *E. coli* 0157:H7 was less resistant to the lethal effects of radiation ($P < 0{\cdot}01$). These studies have shown that pulsed light emissions can significantly reduce populations of *E. coli* 0157:H7 and *L. monocytogenes* on exposed surfaces with exposure times which are 4–6 orders of magnitude lower than those required using continuous u.v. light sources.

INTRODUCTION

The number of reported food-borne illness associated with bacterial enteropathogens continues to rise, despite significant advances made towards a better understanding of bacterial transmission and pathogenicity in foods and the introduction of good manufacturing practice (GMP) and other quality assurance schemes in many UK food industries (PHLS 1998; SCIEH 1998). In 1997, surveillance statistics from the Public Health Laboratory Service for England and Wales revealed that the total number of laboratory reports of illness attributed to *Salmonella* spp., *Campylobacter* spp. and verocytotoxigenic *E. coli* were 32 169, 50 201 and 1088 reports, respectively (PHLS 1998). This increased trend in food-borne disease associated with these enteropathogenic organisms was also evident in Scotland; of particular concern is the prevalence of verocytotoxigenic *E. coli* with this pathogen implicated in 422 reported food-related illnesses (i.e. >nine reports per 100 000 population) in 1997 (SCIEH 1998). In view of these statistics, it is not surprising that governing bodies and the consumer population are demanding immediate action to deal with this problem.

In addition to a host of other contributing factors, it is now generally accepted by the scientific community that contamination of unprocessed or uncooked food with animal faecal material is a major cause for concern and any method of either reducing or eliminating faecal food contamination will have a significant effect on the incidence of food-borne disease. In the recently published 'Pennington Report' (Pennington 1997), it was recommended that further consideration be given to the potential use and benefits of end-process disinfection treatments for slaughterhouses.

A possible method for reducing the level of bacterial con-

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tamination on food surfaces in slaughterhouses, butchers etc. is ultraviolet (u.v.) irradiation. It is well documented that u.v. light is effective in killing micro-organisms contaminating the surfaces of a variety of materials e.g. contact lenses (Gritz *et al.* 1990). The effectiveness of u.v. for reduction of *Salmonella* on eggs has been reported (Gao *et al.* 1997: Kuo *et al.* (1997). Stermer and co-workers (1987) indicated that the bacterial load on fresh meat can be effectively reduced by u.v. irradiation, while Wallner-Pendleton *et al.* (1994) revealed that this method of disinfection reduced *Salmonella* surface contamination without negatively affecting poultry carcass colour or increasing the rancidity of the meat. These studies indicate that if an effective and economic method of u.v. generation can be developed, then u.v. irradiation may have sufficient biocidal activity for practical application in the disinfection of food and food contact surfaces.

Using a pulsed power approach to high intensity light generation, significant levels of peak power can be produced at a level which is not achievable under continuous excitation. While conventional ac systems produce light with an electrical energy input generally of the order of 100–1000 W per device, a pulse power energization technique (PPET) can deliver many MWs of electrical power to the light source. This is achieved as a result of generating a significantly greater electric field level in the light source than can be achieved under conventional continuous operation. The PPET also tends to produce a greater intensity of the shorter, bactericidal wavelengths of light and, by using this pulsed power approach, it is possible to design the energization time of the light source to be very short (e.g. 1 μs). The objective of this inter-disciplinary study was to establish whether the application of a novel prototype pulsed power light source could significantly reduce populations of known bacterial pathogens to commercially acceptable levels using such short exposure times.

MATERIALS AND METHODS

Bacterial strains

The following bacteria used in this study were obtained from the National Collection of Type Cultures, Colindale, London: *Escherichia coli* NCTC 9001 (type strain), *E. coli* NCTC 12079 (serotype 0157:H7) and *Listeria monocytogenes* NCTC 11994 (serotype 4b). Both *E. coli* NCTC 12079 and *L. monocytogenes* NCTC 11994 have been implicated previously in incidents of food-borne illness. Cultures were grown at 30 °C and maintained on Trypone Soya Agar supplemented with 0·6% Yeast Extract (TSYEA); they were subcultured every 2 weeks.

Preparation of cultures for pulsed power light inactivation studies

The test strains were inoculated into 50 ml of Tryptone Soya Broth supplemented with 0·6% Yeast Extract (TSYEB), and were cultivated at 30 °C under rotary conditions (i.e. 125 rev min^{-1}) for 20 h. Afterwards, a 0·1 ml sample of the 10^{-5} dilution was transferred to 50 ml of TSYEB (giving a starting inoculum of 10–100 cells ml^{-1}). The test strains were again grown into stationary phase (30 °C for 20 h at 125 rev min^{-1}), yielding a homogenous, well distributed cell suspension. A 0·1 ml sample of the neat, 10^{-4} and 10^{-6} dilutions was surface plated onto TSYEA using the spread plate method.

The pulsed power light source

The test assembly used for the experiments consisted of a rectangular PVC housing, a pulse generator and associated switching and controlled circuitry as shown in Fig. 1. Internally, the light source was mounted 4·5 cm above two sample holders which were set at 45 degrees to the horizontal. This arrangement permitted two Petri dish samples to be located at right angles and irradiated simultaneously, with each sample receiving the same average exposure. The light source employed throughout the experiment was a Heraeus Noblelight XAP Series which was constructed from a clear fused quartz tube filled with Xenon to a pressure of 450 torr. The dimensions of the tube were such that it had a 3 mm diameter bore and 7·5 cm arc length. The tube had an earthed line trigger along the length of the envelope (but this was not

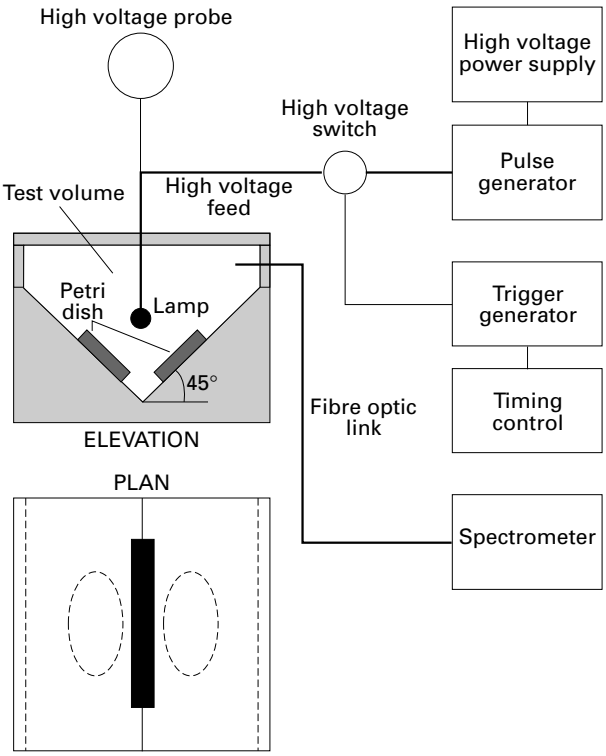

Fig. 1 Schematic lay-out of experimental facility for bacterial inactivation using a pulsed light source

utilized when discharging the system) and was capable of being operated with an average power of about 100 W.

The pulse generator was a single stage, inverting PFN Marx generator which was charged to 30 kV dc. The generator was fired using a trigatron via a high voltage auto transformer. The source capacitance of the generator was 6·4 nF and the source impedance, when fired, was 6·25 Ohms. A fibre optic link and timing control circuit was used to fire the pulse generator at a pulse repetition rate of one pulse per second. The generator was charged to 30 kV from a Brandenburg 50 kV, 1 mA dc charging supply and at full volts, the PFN Marx generator contained a stored energy of 3 Joules. The total electrical discharge time was approximately 1 μs, and this represented an average peak electrical power, per pulse, of 3 MW and a power density in the light source of 380 kW cm^{-2}. At a pulse repetition of 1 pulse s^{-1}, the average power consumption of the system was only 3 W. It should be noted that at this average power level, no discernible increase in sample temperature occurred. The electrical diagnostics consisted of a high voltage dc probe to measure the charging voltage of the system and a high speed transient probe which could monitor the voltage profile applied to the light source. As the emission intensity associated with a cylindrical source is not uniform and varies with the inverse of radial distance, this resulted in an intensity profile for the sample which represents a planar surface cutting across a source with cylindrical symmetry.

The optical emissions from the u.v source were monitored using a four channel Ocean Optics SQ2000 fibre optic spectrometer. The spectrometer channel in use had a spectral range of 200–530 nm and a resolution of 1·25 nm. A typical emission characteristic, recorded during the test sequence, is shown in Fig 2. The relative magnitude of the shorter wavelength light is not completely accurate because of increased absorption which takes place in both the air and optic fibres at these wavelengths. Monitoring of the optical emissions from the source verified that the emission spectra were consistent throughout the duration of the experiment.

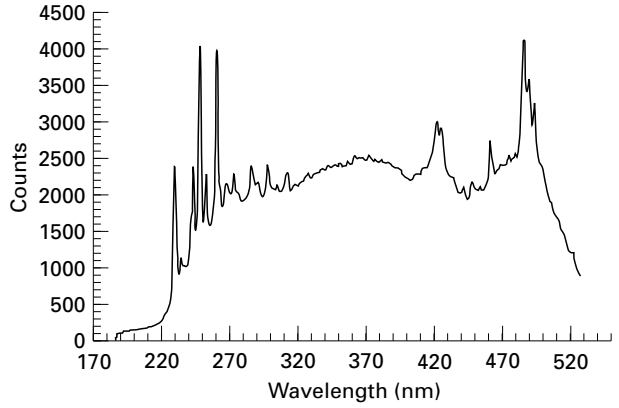

Fig. 2 The light emission spectrum produced by the pulsed power system

Treatment of test strains with the pulsed power light source

In the first series of experiments, the test cultures seeded on the TSYEA plates (at about 5 $\log_{10}$ cfu per plate) were positioned in the PPET assembly as described earlier (Fig. 1) and exposed to 0, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 pulses of high intensity light (i.e. 1 pulse is equivalent to 1 μs of exposure) with a relative intensity, in the spectral range 200–530 nm, as shown in Fig. 2. Afterwards, the treated TSYEA plates were wrapped in aluminium foil to prevent photo-reactivation and were then incubated for 48 h at 30 °C. Survival data were treated according to Chick's Law as log N_s/N_0 *vs* dose, where N_0 was the initial concentration of organisms and N_s was the density of survivors. In later exposure studies, the test cultures were seeded on TSYEA plates at a higher cell density of about 8 $\log_{10}$ cfu per plate and treated at the upper exposure level of 512 μs of pulsed-light. The treated plates were incubated and surviving populations were determined as described earlier. The study was carried out in triplicate using duplicate plates for each set of exposures; surviving populations were expressed in $\log_{10}$ cfu per plate.

Statistical analysis

Fisher's exact test was used to compare levels of bacterial inactivation ($\log_{10}$ cfu per plate). All significant differences were reported at the 95% ($P < 0{\cdot}05$) and 99% ($P < 0{\cdot}01$) levels of confidence.

RESULTS AND DISCUSSION

Cell numbers of each test culture inoculated on TSYEA were significantly decreased by exposure to pulsed light emissions from the PPET (Fig. 3). As little as 16 μs of light pulsing had a detrimental effect on the viability of *E. coli* 0157:H7, while a duration of 64 μs was required to reduce populations of *L. monocytogenes*. At the latter exposure, a 4 $\log_{10}$ reduction in *E. coli* 0157:H7 cell numbers was obtained. Results showed that the greater the number of pulsed light exposures, the larger the reduction in cell numbers. Survival data revealed that *E. coli* 0157:H7 was less resistant ($P < 0{\cdot}01$) to the lethal effects of radiation than to the type strain of *E. coli* and *L. monocytogenes*. At the upper exposure level of 512 μs of pulsed light, cell populations of *E. coli* 0157 and *L. monocytogenes* were reduced by approximately 7 and 6 log orders, respectively (Table 1).

As shown in Fig. 2, the spectral emission produced by

Table 1 Survival data for test cultures treated at the upper exposure level of 512 pulses

Number of light pulses (μs)	Number of cell survivors*		
	Escherichia coli 0157:H7	*E. coli*	*Listeria monocytogenes*
0	8·30 (0·2)†	8·53 (0·2)	8·38 (0·1)
512	1·48 (0·2)	2·36 (0·3)	2·13 (0·3)

* Measured as $\log_{10}$ cfu per plate, where counts are averages of three replicate trials.
† Numbers in parenthesis refer to variability about the mean.

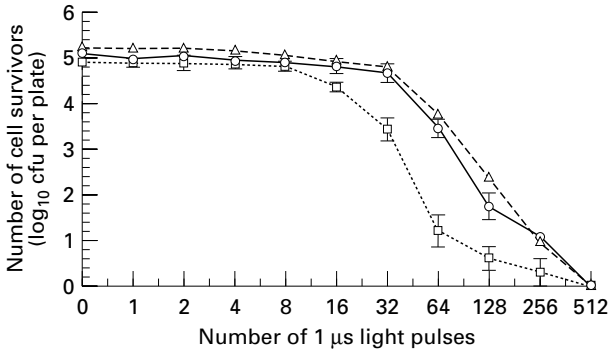

Fig. 3 Influence of the number of 1 μs light pulses on the viability of exposed bacterial cells ($\log_{10}$ cfu per plate). △, *Escherichia coli*; □, *E. coli* O157:H7; ○, *Listeria monocytogenes*

PPET (i.e. ranging from 200–530 nm) encompassed the shorter u.v. wavelengths of light. It has been well documented that u.v. is effective in killing micro-organisms contaminating the surfaces of a variety of materials including food. This biocidal effect is due in part to the formation of lethal thymine dimers on the bacterial DNA. By using this pulsed power 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. By raising the electric field levels applied to the light source, the PPET approach can dissipate many MWs of peak electrical power in the light source, which in turn results in a greater relative production of light with shorter bactericidal wavelengths. This study has shown that a 7 $\log_{10}$ reduction in pathogenic bacterial populations was achieved using an exposure of only 512 μs of pulsed light, with the high voltage pulse generator containing a stored energy of only 3 joules per pulse. This high voltage pulsed power approach would appear to be an effective and economic technique for bacterial destruction compared with the conventional techniques of continuous u.v. excitation. As PPET systems can be developed to operate at 100–1000 pulses s^{-1} (MacGregor *et al.*, 1997), as opposed to 1 pulse s^{-1} used in the present study, this approach lends itself to commercial applications which require a significant throughput of material (e.g. the food industry).

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