

ORIGINAL ARTICLE

Advanced high-power pulsed light device to decontaminate food from pathogens: effects on *Salmonella typhimurium* viability *in vitro*

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Keywords

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Abstract

Aims: The aim of this study was to construct an advanced high-power pulsed light device for decontamination of food matrix and to evaluate its antibacterial efficiency. Key parameters of constructed device-emitted light spectrum, pulse duration, pulse power density, frequency of pulses, dependence of emitted spectrum on input voltage, irradiation homogeneity, possible thermal effects as well as antimicrobial efficiency were evaluated.

Methods and Results: Antimicrobial efficiency of high-power pulsed light technique was demonstrated and evaluated by two independent methods – spread plate and Miles–Misra method. Viability of *Salmonella typhimurium* as function of a given light dose (number of pulses) and pulse frequency was examined. According to the data obtained, viability of *Salmonella typhimurium* reduced by 7 log order after 100 light pulses with power density 133 W cm^{-2} . In addition, data indicate, that the pulse frequency did not influence the outcome of pathogen inactivation in the region 1–5 Hz. Moreover, no hyperthermic effect was detected during irradiation even after 500 pulses on all shelves with different distance from light source and subsequently different pulse power density ($0\text{--}252 \text{ W cm}^{-2}$).

Conclusion: Newly constructed high-power pulsed light technique is effective nonthermal tool for inactivation of *Salmonella typhimurium* even by 7 log order *in vitro*.

Significance and Impact of the Study: Novel advanced high-power pulsed light device can be a useful tool for development of nonthermal food decontamination technologies.

Introduction

Despite tremendous progress in food microbiology, the number of reported food-borne diseases associated with bacterial enteropathogens continues to rise (PHLS 1998). Health experts estimate that every year all food-borne illnesses in USA cost five to six million US dollars in direct medical expenses and lost productivity. Infections with the bacteria *Salmonella* alone account for one million dollars yearly. Every year, CDC receives reports of 40 000 cases of salmonellosis in USA, and 1000 people deaths each year (Archer and Kvenberg 1985; Sala *et al.* 1995; Alzamora *et al.* 2000). Thus, food-borne diseases are extremely costly.

A good many effective chemical and physical methods for decontamination of food from pathogens exist: heat treatment, ionizing radiation, chemicals et cetera (Devlieghere *et al.* 2004; Luksiene 2005; Manas and Pagan 2005). Meanwhile, being effective decontamination tools, most of them make different undesirable changes in food matrix; for instance, they can alter the structure of proteins and polysaccharides, causing changes in the texture, produce free radicals, change physical appearance and functionality of food, affecting the flavour of fruit-based or high-fat food (Devlieghere *et al.* 2004). In addition, higher doses of ionizing radiation, for instance, may cause slight colour changes in

beef, pork and poultry (Gómez-López *et al.* 2005). Application of natural compounds, such as essential oils, chitosan, nisin or lysozyme is hampered because of the changed organoleptic properties of the food (Jay 1997).

The data presented serve as evidence that the methods recently applied for inactivation of pathogens in foods are not always efficient, ecologically friendly and cost-effective.

Pulsed UV-light-based effect might serve as a background for disinfection of different surfaces, air, water and decontamination of foods. Moreover, this treatment is environmentally benign, does not leave volatile organic compounds or produces suspended airborne particulates, is cost effective and generally does not change the characteristics of food matrices. The benefits include reducing public health risk from food-borne pathogens, extending shelf life of different foods and improving economics during food distribution (Ozer and Demirci 2005). Despite this long list of advantages, scientific knowledge in this field and development of technological principles are still not enough and need deeper scientific investigations and novel physical approaches in order to use this technique for industrial purposes.

The present work is focussed on the construction and evaluation of advanced high-power pulsed light device dedicated to decontaminate the food matrices from harmful and pathogenic micro-organisms. Thus, the viability of food pathogen *Salmonella typhimurium* as function of different light doses (number of pulses) and pulse frequency was investigated.

Materials and methods

Bacterial strain

Target bacteria *Salmonella enterica* serovar Typhimurium strain DS88 (SL5676 Sm² pLM32), resistant to tetracycline, was kindly provided by Prof. D.H. Bamford (University of Helsinki, Finland).

Preparation of *Salmonella enterica* serovar Typhimurium DS88 strain

The bacterial test strain was grown overnight at 37°C in 20 ml of LB medium, with aeration of 120 rev min⁻¹ (Environmental Shaker – Incubator ES-20; Biosan, Latvia). One millilitre of an overnight culture was transferred to 20 ml of fresh medium and was grown at 37°C to the logarithmic phase (OD₅₄₀ = 0.5 × 10⁸ cells per millilitre) in a shaker (120 rev min⁻¹). Then the test culture was plated onto LB Agar using two methods – spread plate and Miles–Misra methods.

Pulsed-light treatment of the agar-surface inoculated bacterial cells

The test culture inoculated on the LB Agar plates placed in the middle shelf of the chamber and exposed to 0–100 pulses of high-power pulsed UV light. In the first series of experiments 1 Hz pulse frequency was employed and in later series 5 Hz. In all cases, pulse power density was 133 W cm⁻², and the second shelf was chosen for the main irradiation experiments. Each treated plate was wrapped in aluminium foil to prevent photoreactivation and incubated for 24 h at 37°C.

Evaluation of viability of microbes

The effect of high-power pulsed light on *Salmonella typhimurium* viability was evaluated by two microbiological tests: spread plate and Miles–Misra methods (Roberts *et al.* 1995). Thus, 100 µl of appropriate dilutions of bacterial test culture (10⁸ cells per millilitre), exploiting the spread plate method, and 20 µl of appropriate dilutions of a bacterial culture was surface inoculated on the separate LB Agar plates. After treatment, all plates were placed in the thermostat for 24 h at 37°C. The surviving cell populations were enumerated and expressed as log₁₀ CFU ml⁻¹. The experiments were carried out in triplicate for each set of exposure (Gudelis *et al.* 2006).

Light dose estimation

In order to estimate the dose of total light energy delivered to the biological object, several mathematical calculations have to be performed. The pulse power density is a pulse energy (mJ) deposited per square centimetre of the treated surface during pulse duration ($\tau = 112 \mu\text{s}$), so it is a ratio of the pulse energy to the pulse duration (J cm⁻² s⁻¹). Traditionally, photobiological processes depend on the total light energy dose delivered to the object, and are expressed as J cm⁻². In the case of pulsed light delivery, the total dose (D) is the energy of one pulse e_1 (J cm⁻²), multiplied by a number of pulses during the whole treatment (t):

$$D = e_1 (\text{J cm}^{-2}) \cdot t(\text{s}) \cdot f(\text{Hz})$$

where f is the pulse frequency.

Spectral measurements

Light power density measurements were performed with a light energy measure by 3 Sigma metre ('Coherent') equipped with piroelectrical detector J25LP04.

Temperature measurements

The LM35 precision Celsius temperature sensors were used for temperature measurements as they have an advantage over linear temperature sensors calibrated in Kelvin; the user is not required to subtract a large constant voltage from its output to obtain convenient temperature scaling, and the sensor does not require any external calibration or trimming to provide typical accuracies of $\pm 1/4^\circ\text{C}$ at room temperature.

Statistical analysis

Bacterial populations in CFU ml^{-1} were transformed into $\log_{10} \text{ml}^{-1}$. Analysis of variance (ANOVA) was performed ($P < 0.05$). In addition, Bonferroni tests were performed between means. Two sample-independent *t*-tests were performed to compare 1 and 5 Hz pulse frequency samples.

Results

Construction of the device

The most efficient UV light source is xenon flash lamp, which creates the UV radiation generated in the low pressure gas (xenon) by a powerful electric discharge. While constructing the high-power pulsed light device, we made our choice on xenon flash lamp FL-75.

As presented in Fig. 1, the device consisted of a chamber, a reflector with a flash lamp, and a power supply unit. The chamber had three shelves with different distances from the flash lamp. Every shelf was $16 \times 16 \text{ cm}$ in order to irradiate simultaneously four Petri dishes with diameter 80 mm in the identical exposure conditions.

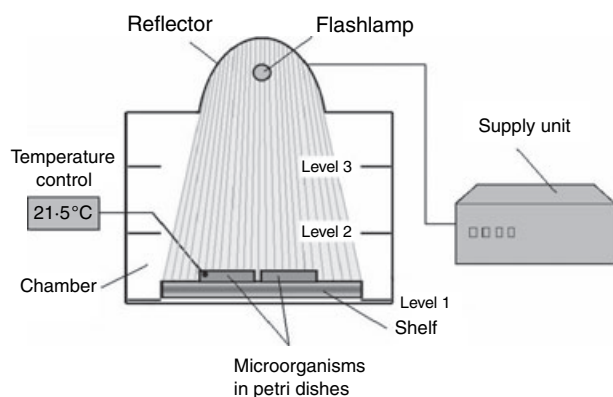


Figure 1 Schematic presentation of high-power pulsed UV light generating device to decontaminate foods: block diagram of device.

The temperature measurements were performed with computerized thermocouple, which was incorporated inside the chamber and fixed in plate with the agar.

Spectral characteristics of high-power pulsed light technique

Figure 2 represents typical spectrum of pulsed xenon flash lamp. The major differences in emission spectra can be observed between 200 and 600 nm. UV light radiation is the highest at the beginning of the discharge pulse (when the discharge current increases), and just after that the visible (VIS) and infrared light (IR) spectral components appear. Therefore, in order to gain more UV radiation, it is necessary to decrease the discharge pulse duration in the Xe gas. Using the above mentioned power supply equipment, we get the light pulse duration $\tau = 112 \mu\text{s}$ and the power of each pulse ranges from 0.07 to 0.9 MW. Eventually, the pulse power density varied between 0 and 252 W cm^{-2} depending on the distance from the flash lamp (three shelves) and discharge current (0–1600 V) (Table 1).

Thermal characteristics of high-power pulsed light technique

One of the main tasks of this work was to develop such a construction of high-power pulsed light technique, which would allow avoiding thermal effects on food matrix selected for treatment. Temperature kinetics was measured using special thermocouple, with digital indications of temperature. According to our data, the increase of temperature in the shelf, which is in the shortest distance from the light source, and even after 500 pulses did not

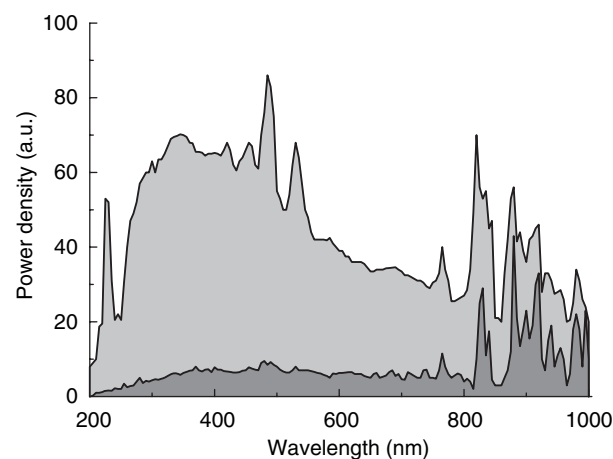


Figure 2 Typical spectrum of pulsed xenon flash lamp FL-75 and its dependence on input voltage (www.heraeus.com). Discharge power density (■) 24 kW cm^{-2} and (■) 3 kW cm^{-2} .

Table 1 Interrelation of voltage, pulse energy, pulse power density and pulse energy density and distance from the lamp in the device used for experiments

Shelf	Input voltage, U (V)	Pulse power density, P ($W\ cm^{-2}$)	Pulse energy density, E ($mJ\ cm^{-2}$)
1	500	18	2.0
	800	49	5.5
	1000	73	8.2
	1200	99	11.1
	1400	125	14.0
	1600	152	17.0
2	500	27	3.0
	800	70	7.8
	1000	105	11.8
	1200	143	16.0
	1400	180	20.2
	1600	224	25.1
3	500	34	3.8
	800	87	9.7
	1000	130	14.5
	1200	175	19.6
	1400	221	24.8
	1600	270	30.2

exceed $36^{\circ}C$. The main experiments were performed using the middle shelf (No. 2). Thus, the increase of temperature in the plate with agar placed on this shelf did not exceed $32^{\circ}C$ (Fig. 3). It is important to note that vari-

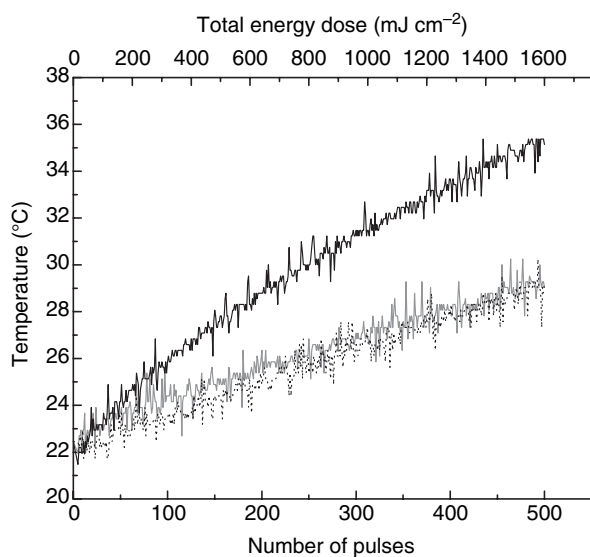


Figure 3 Increase of temperature on the surface of agar in Petri dish, placed inside the chamber as a function of pulse number (total energy dose $mJ\ cm^{-2}$); experimental conditions: input voltage 1200 V, frequency 5 Hz. —, Shelf 1; ·····, shelf 2; - - -, shelf 3.

ation of pulse frequency from 1 to 5 Hz had no influence on the agar temperature (data not shown). It means that novel possibility to reduce the duration of treatment five times, increasing pulse frequency from 1 to 5 Hz, might be taken into account.

Several optical technologies to increase the area of homogenous irradiation

One of the important tasks constructing new device was to use the light radiation in most effective and economic way. Thus, in order to gain homogeneous light radiation, we placed the Xe flash lamp in the focus of reflector with parabolic form. Moreover, the reflector was made from Al with raster (rough, patterned) surface (Fig. 4a). Such construction ensures the achievement of the light energy density variations no more than 10% in all irradiation area ($16 \times 16\ cm$) (Fig. 4b) and to find better decontamination conditions, if compared with the existing pulsed high-power device, described by Gómez-López *et al.* (2005). Moreover, the same irradiation homogeneity ($\pm 10\%$) was observed at all three distances from the light source (shelves).

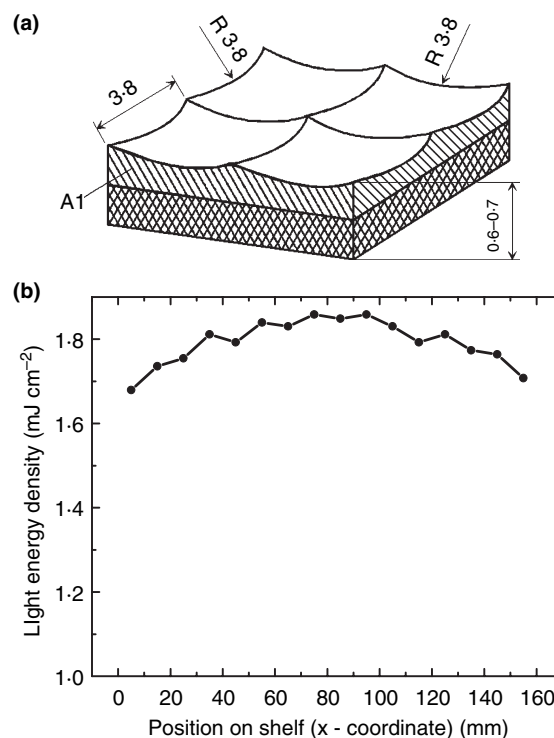


Figure 4 Schematic presentation of raster surface of reflector (a), which increases the irradiation homogeneity; variation of light energy density in the irradiation area ($16\ cm \times 16\ cm$) in the chamber (b).

Dependence of pulse power density on input voltage and distance from the light generating system

Pulse power density is one of the most important characteristics of this light technology. Varying energy density parameters enables to reveal additional mechanisms of microbe inactivation, which can lead to the development of much more efficient and nonlinear inactivation processes (Rice and Ewell 2001). It is important to note that pulse power density from 0 to 252 W cm^{-2} can be controlled by changing input voltage (0–1600 V) or distance from the light source (Fig. 5). As mentioned earlier, the higher values of voltage increase pulse power density as well as percentage of UV in broad spectrum of emitted light.

Evaluation of antimicrobial efficiency of constructed device

First, experiments were carried out in order to identify the significance of delivered pulsed light energy, achieved by changing the number of pulses on inactivation of *Salmonella typhimurium*. So far, clear dependence of *Salmonella* viability on the pulse number was detected by spread plate and Miles–Misra methods. Data presented in Fig. 6 indicate that inactivation of pathogen is a function of delivered light dose. A 7 log order reduction was observed after treatment with 100 pulses of 133 W cm^{-2} at high voltage (1200 V).

In our previous study, we analysed the dependence of pathogen viability on the frequency of light pulses (Gudelis *et al.* in press). According to our results, light pulses delivered with 1 Hz frequency have the same effect as

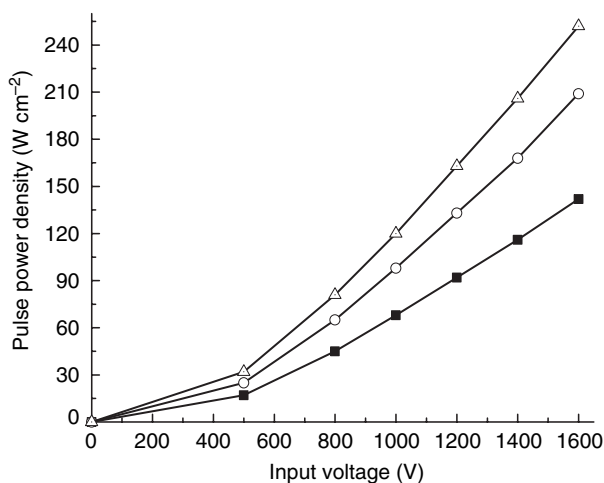


Figure 5 Dependence of pulse power density on the voltage and distance from the light generating system. —■—, Shelf 1; —○—, shelf 2; —△—, shelf 3.

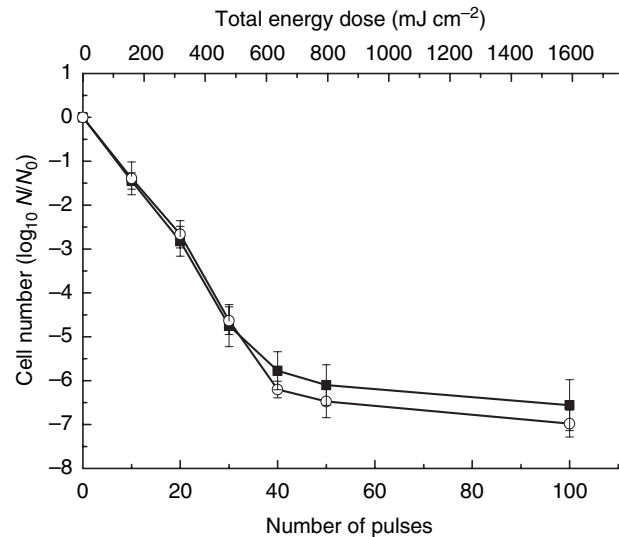


Figure 6 Inactivation of *Salmonella typhimurium* as function of pulse number (evaluated by spread plate (—■—) and Miles–Misra methods (—○—), experimental conditions: 5 Hz, pulse power density 133 W cm^{-2} , input voltage 1200 V, shelf two).

pulses delivered with 5 Hz frequency, while the exposure time in the latter case is five times shorter. Thus, we observed no discernible effect of pulse frequency on survival of micro-organisms. It means that the inactivation of bacteria depends on the total accepted light energy but not on the mode of pulse frequency, when frequency varied in between 1–5 Hz.

Inactivation modelling

The dependency of surviving bacteria on the emitted energy dose exhibits nonlinear sigmoidal behaviour. The dynamic model of microbial inactivation, described in (Geeraerd *et al.* 2000) seems to be a good approach in the case of light pulse-based inactivation as well (Fig. 7).

This model was successfully applied in the work (Marquenie *et al.* 2003) to describe the inactivation of fungal *conidia*, treated by pulse light. According to this model, the reduction of number of bacteria population N and the number of protective components C over the time t can be described by these two differential equations:

$$\frac{dN}{dt} = -kN \quad (1)$$

$$\frac{dC}{dt} = -k_{\max}C \quad (2)$$

where

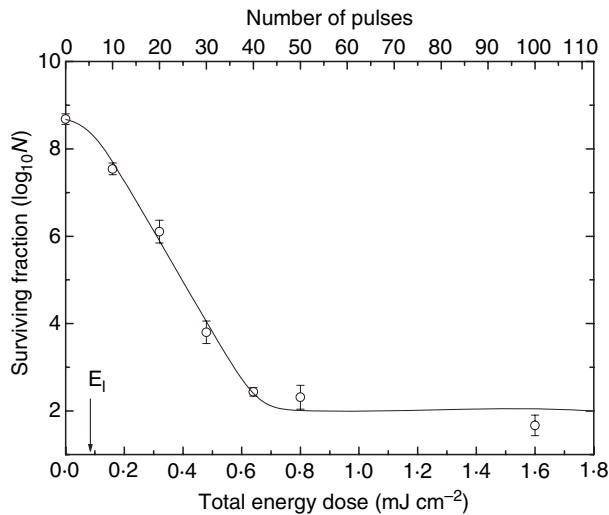


Figure 7 Modelling curve of salmonella viability as function of total energy dose (5 Hz spread plate method data and modelled curve by GlnaFIT). Arrow E_1 indicates the amount of light energy required to destroy cell protective components, which is $0.08 \pm 0.05 \text{ mJ cm}^{-2}$.

$$k = k_{\max} \left(\frac{1}{1 + C} \right) \left(1 - \frac{N_{\text{res}}}{N} \right) \quad (3)$$

k_{\max} is the inactivation rate of the protective component and N_{res} is the number of bacteria resistant to this treatment. So far, inactivation efficiency does not depend on the irradiation time in the range 20 s (5 Hz) to 100 s (1 Hz), only on the total energy dose. Thus, the more relevant independent variable would be the energy dose.

As the pulse light energy emission is linearly dependent on time, we substitute the time t with the energy dose E . After the few simple steps of integration, the number of surviving bacteria N after receiving a particular energy amount E can be expressed by the following equation:

$$N = \frac{(N_0 - N_{\text{res}}) \exp(-k'_{\max} E) (1 + C_0)}{1 + C_0 \exp(-k'_{\max} E)} + N_{\text{res}} \quad (4)$$

where N_0 is the initial bacteria count, C_0 is the initial amount of protective components, k'_{\max} is the inactivation rate in energy scale (expressed as $\text{cm}^2 \text{ J}^{-1}$).

As we care not about the absolute values but about the ratio of survived and initial bacteria number, we can also calculate the ratio:

$$f_{\text{res}} = \frac{N_{\text{res}}}{N_0} \quad (5)$$

The biological meaning of f_{res} is the percentage of the resistant bacteria strain in the initial population and it describes the tail of the sigmoidal curve. According to

Geeraerd *et al.* (2000), the shoulder of the curve is determined by the formula, which we slightly modified to use energy amount instead of time.

$$C_0 = \exp(k'_{\max} E_1) \quad (6)$$

The meaning of E_1 is an amount of light energy required to destroy the cell protective components. We will call it shoulder energy amount. The model by Geeraerd *et al.* (2000) uses a modified shoulder energy expressed by this mathematical transformation, and E'_1 is a better representation of the true shoulder energy on a sigmoidal curve:

$$C_0 = \exp(k'_{\max} E_1) = \exp(k'_{\max} E'_1) - 1 \quad (7)$$

which can be substituted into eqn (4).

By using the Log-Linear method with shoulder and tail of a GlnaFIT tool described in Geeraerd *et al.* (2005), the 5 Hz spread plate on a second shelf data was fitted. The calculated shoulder energy $E'_1 = 0.08 \pm 0.05 \text{ J cm}^{-2}$, $k'_{\max} = 26.7 \pm 2.9$, $\log_{10}(N_{\text{res}}) = 2.00 \pm 0.23$, $\log_{10}(N_0) = 8.67 \pm 0.33$, $f_{\text{res}} \approx 2 \cdot 10^{-7}$. It should be noted that k'_{\max} can easily be transformed back to time scale using simple relationship:

$$k_{\max} = k'_{\max} \cdot e_1 \cdot f \quad (8)$$

where $e_1 = 0.016 \text{ J cm}^{-2}$ is an energy density of one pulse, $f = 5 \text{ Hz}$ – pulse repetition rate. Similar transformation can be used to obtain the time required to destroy cell protective components. Shoulder length S_1 (in time scale) is:

$$S_1 = \frac{E'_1}{e_1 \cdot f} \quad (9)$$

For our experiment conditions and time scale, the parameters are $k_{\max} = 2.13 \pm 0.23 \text{ s}^{-1}$, $S_1 = 1.00 \pm 0.63 \text{ s}$ (Fig. 7). It is clear that some minimal energy dose exists in our case, 0.8 mJ cm^{-2} , after which subsequent irradiation is inefficient.

Discussion

Despite tremendous progress in microbiology and bacterial pathogenicity in foods, food-borne diseases continue to be a major problem in the developed world. Thus, every ecologically friendly, not chemical and cost effective technology is of great interest for scientists, producers and consumers. As a role, present disinfection and sterilization methods are not enough effective, either thermal or involve the use of biocidal chemicals that pose a potential occupational hazard and require a lot of working time to achieve the result (McDonald *et al.* 2000).

Actually, high intensity pulsed light technology is absolutely unique because of several characteristics. First, it is effective antimicrobial tool. Second, it has no harmful effects on surrounding. Third, this technology is 4–6 orders of magnitude faster than continuous UV light, what is remarkable advantage and makes it cost effective (MacGregor *et al.* 1998).

Of importance is to note that, according to our investigations, high-power pulsed light device constructed in our laboratory is not inducing hyperthermic effects. As a rule, high temperature is changing many food characteristics, which is absolutely undesirable. One of the most important advantages of this novel technique is that the reduction of microbe viability by 7 log order was achieved without any thermal effect. Temperature on the surface of agar does not exceed 36°C even at highest light power density (252 W cm⁻²) and 500 light pulses, whereas other authors obtained just 1 log microbe viability reduction in nonthermal irradiation conditions. Even Rowan showed just negligible rise in agar temperature during treatment, but the reduction of pathogens (*Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Listeria monocytogenes* and yeast *Saccharomyces cerevisiae*) viability was just 2–6 log order. Moreover, it was achieved even after 200 light pulses treatment (Rowan *et al.* 1999).

In addition, possibility to irradiate comparatively big areas at identical conditions with very low variation of light power density (10%) seems very important advantage of this device. Eventually, most important characteristic of newly constructed high-power pulsed light device is its antimicrobial efficiency. Data presented indicate that viability of *Salmonella* thyphimurium might be reduced by 7 log even after 100 pulses with 133 W cm⁻² light power density. This characteristic, as the most important, was evaluated by two independent microbiological methods – Miles–Misra and plating. As shown in Fig.7, both methods confirm the same sigmoidal dependence of *Salmonella* viability on the number of emitted light pulses. The model of pathogen inactivation clearly indicates that the destruction of protective cellular components depends on irradiation dose. According to our calculations, E'_1 – amount of light energy required to destroy cell protective components, is 0.08 ± 0.05 (mJ cm⁻²). Thus, further attempts have been performed to evaluate the resistant fraction of bacteria. According to our evaluations, the number of the resistant bacteria in the initial population is $f_{res} \approx 2 \cdot 10^{-7}$. It is very low.

So far, our results are in line with data obtained by other investigators. Anderson *et al.* (2000) indicated, that *Salmonella enteritidis* was enough susceptible to high-power pulsed light treatment in comparison with *Bacillus cereus*. Wallner-Pendleton *et al.* (1994) showed that this

method reduced surface contamination without any effect on poultry colour or meat rancidity. Gómez-López *et al.* (2005) used this treatment for decontamination of minimally processed vegetables and found reduction in the initial microbial load.

Thus, data obtained with novel high-power pulsed light technique constructed in our laboratory confirm the idea that pulsed light technique might be really promising and effective nonthermal tool to increase microbial control.

Conclusions

Unique properties of high-power pulsed UV light from theoretical point of view makes it an ideal technology to decontaminate food surfaces from different pathogens. Despite this, scientific knowledge and development of technological principles are still not sufficient and need novel physical approaches in order to use this technique for industrial purposes. High-power pulsed light device, constructed in our laboratory, has several technical advantages and works as a good, fast and safe antimicrobial tool. For instance, experimental study indicates that pulsed light technique can significantly reduce *Salmonella* thyphimurium population by 7 log even after 100 pulses of 133 W cm⁻² pulsed light treatment without any thermal effects. Moreover, variation of pulse frequency from 1 to 5 Hz had no impact on the temperature of agar. It means that costs of the technology might be lowered by reducing the duration of treatment five times. Eventually, pathogen inactivation modelling proved that no resistant *Salmonella* fractions occur after this treatment. Taking into account all mentioned above, it is possible to draw a conclusion that a novel nonthermal food decontamination technology with a high potential of being useful might emerge.

Acknowledgements

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