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Impact of treatment parameters on pulsed light inactivation of microorganisms on a food simulant surface

B. Kramer[a,b,*], J. Wunderlich[a], P. Muranyi[a]

[a] Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany
[b] Technical University of Munich, Freising-Weihenstephan, Germany

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ABSTRACT

The impact of the fluence regulation on the inactivation efficiency of pulsed light (PL) surface disinfection treatments was investigated. *E. coli* and *L. innocua* were exposed to PL on a gel surface under variation of the applied voltage, the number of light flashes as well as the distance between the flash lamp and the sample surface. The results revealed deviations from the reciprocity law when the total fluence striking the sample surface was not applied at once, but subdivided into several successive light flashes. No differences were found when the fluence was delivered with only single light flashes, irrespective of the applied voltage. The pulse frequency did not have an impact on the microbial reduction within 1–5 Hz. Furthermore, the sensitivities of various bacterial strains, endospores and conidiospores were compared. Differences occurred for vegetative bacteria without a clear pattern, while bacterial endospores were more resistant. Dark pigmented mold spores were slightly more resistant than bacterial endospores. All dose-response curves exhibited a downward concavity, except for *P. aeruginosa*.

Industrial relevance: This study shows that the inactivation of bacteria on e.g. food surfaces by pulsed light systems depends on the way of fluence dosage. While it is irrelevant whether the fluence is regulated by the discharge voltage or the distance between the flash lamp and the treated surface, it is more effective to apply only single light flashes of high fluence instead of several consecutive light flashes. There is furthermore no distinct trend regarding the sensitivity of bacteria to PL, variations occur on species and strain level. Bacterial spores are in general more resistant while pigmented conidiospores show a slightly higher resistance than bacterial endospores.

1. Introduction

The application of intense short light pulses of a broad spectrum represents an emerging non-thermal surface sterilization technology. Its suitability to inactivate a wide range of microorganisms including bacteria, mold spores, endospores, viruses or parasites has been shown in various studies so far (Farrell, Garvey, Cormican, Laffey, & Rowan, 2010; Farrell, Garvey, & Rowan, 2009; Gómez-López, Devlieghere, Bonduelle, & Debevere, 2005). The main inactivation mechanism attributes to UV-induced destruction of nucleic acids although some differences to continuous UV-light treatments are assumed (Cheigh, Park, Chung, Shin, & Park, 2012; Takeshita et al., 2003). The disinfection of surfaces, like thermal-sensitive plastics, as well as liquids is possible within very short time, wherefore this technology has already been adopted for the sterilization of packaging material on industrial scale. In case of the treatment of food, a lot of research activity is currently performed and it seems that the efficiency strongly depends on the specific food properties, treatment conditions and the equipment used (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010; Rowan, Valdramidis, & Gómez-López, 2015). Concerning the relevance of different influence parameters on microbial inactivation, some studies have been performed so far (Artíguez & de Marañón, 2014; Lasagabaster & de Marañón, 2013; Levy, Aubert, Lacour, & Carlin, 2012). The efficiency of pulsed light inactivation treatments depends on the applied fluence, which may be controlled by the applied discharge voltage, the distance between sample and flash-lamp as well as the number of applied light flashes. However, it is still not clarified how the applied fluence affects microbial inactivation. Some studies have stated that UV-inactivation of microorganisms is in line with the Bunson-Roscoe law (Rice & Ewell, 2001), which claims that the total number of photons determines photochemical reactions (Gómez-López & Bolton, 2016). In theory, the microbial inactivation effect obtained in UV or pulsed light treatments should therefore be independent from the way of energy dosage. However, other studies reported about significant

* Corresponding author at: Department Retention of Food Quality, Fraunhofer Institute for Process Engineering and Packaging (IVV), Giggenhauser Str. 35, D-85354 Freising, Germany.
E-mail address: bernd.kramer@ivv.fraunhofer.de (B. Kramer).

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deviations from the reciprocity law in UV inactivation experiments (Bosshard, Berney, Scheifele, Weilenmann, & Egli, 2009; Sommer, Haider, Cabaj, Heidenreich, & Kundi, 1996; Sommer, Haider, Cabaj, Pribil, & Lhotsky, 1998). To our knowledge, there is no study where the efficiency of pulsed light inactivation of bacteria on a surface has been investigated by directly comparing the impact of three ways of energy dosage. The objective of this study was a quantitative assessment of the inactivation of *E. coli* and *L. innocua* on an anionic polysaccharide gel, in consideration if the way of fluence dosage has an impact and if the reciprocity law is valid for PL inactivation. For this reason, the microbial data of various challenge tests was fitted to a Weibull model and the obtained curve parameters were compared. The impact of the pulse frequency on bacterial inactivation has been investigated as well. Furthermore, it was studied if there is a trend in the sensitivity of various microorganisms as differing results have been reported so far (Farrell et al., 2010; Gómez-López et al., 2005).

2. Material and methods

2.1. Bacterial strains, growth conditions and preparation of inoculums

All test strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) except for *Salmonella enterica* ATCC BAA-1045, which was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, US). The bacteria (15 different strains, Table 5) were initially grown in 100 ml tryptic soy broth (Oxoid, Hampshire, UK) at 37 °C for 16–18 h in a shaking bath. Tryptic soy agar (Oxoid, Hampshire, UK) was subsequently inoculated with the bacterial suspension by use of an inoculating loop, incubated for 24 h at 37 °C and stored at 5 °C. Working cultures were made by inoculating 100 ml of tryptic soy broth with cell material from the agar surface and following incubation for 16–18 h at 37 °C in a shaking bath until early stationary phase. 20 ml of the culture were subsequently centrifuged at 9000*g* for 10 min and washed twice with sterile deionized water in order to remove all solutes. The cell count was determined in a counting chamber by microscopic methods and finally adjusted to approximately $2 * 10^8$ cells/ml. Spore suspensions of *B. atrophaeus* (DSM 675), *B. subtilis* (DSM 4181) as well as conidiospores of *A. niger* (DSM 1957) and *A. brasiliensis* (DSM 1988) were prepared according to the methods reported by Muranyi, Wunderlich, and Heise (2007). In brief, endospores were obtained by inoculation of plate count agar (Oxoid, Hampshire, UK) containing 10 mg/L manganese sulfate with vegetative bacteria from an overnight culture. After cultivation for 10 days at 30 °C, sporulation was confirmed by phase contrast microscopy. Spores were harvested with sterile ringer solution, washed several times, pasteurized (80 °C, 30 min) and then stored at 5 °C in the dark. Conidiospores were obtained from inoculated yeast extract glucose chloramphenicol agar (Oxoid, Hampshire, UK) after incubation for 10 days at 30 °C. The spores were harvested by tapping the agar plates with sterile sea sand and after suspension in sterile ringer solution and ultrasonic treatment for 1 min, the spore containing supernatant was separated from the sea sand and stored at 5 °C. Previous to the trials, the spore count of the prepared stock suspensions was determined by plate counting as described below. Working suspensions of spores were prepared as described for vegetative bacteria and a concentration of approx. $2 * 10^7$ spores/ml was adjusted.

Test bacteria as well as spores were PL treated on Gelrite (Roth, Karlsruhe, Germany), an anionic polysaccharide gel, which forms clear and heat stable gels in the presence of soluble salts. Viability loss due to desiccation is prevented by the residual moisture of the gel matrix. 1% Gelrite and 0.1% $CaCl_2 \times 2\ H_20$ (VWR, Darmstadt, Germany) were boiled in sterile deionized water until complete solvation before 15 ml were immediately poured into sterile petri dishes. After solidification, discs with a diameter of 1.5 cm and a thickness of 2–3 mm were cut out and placed on glass slides. Three discs were used for each sample. The gel surface of each disc was spot inoculated with 50 µl of the prepared suspension containing either vegetative bacteria or spores and dried for 1 h in a safety cabinet at room temperature before treating the samples in the PL chamber. The resulting cell/spore density on the gel surface was about $1 * 10^7$ bacterial cells/cm^2 and about $1 * 10^6$ spores/cm^2.

2.2. Pulsed light set-up

The prepared samples were treated in a lab-scale PL chamber (dimensions: $50 * 50 * 30$ cm) (Claranor, Avignon, France), which was equipped with a three Xenon tubes reflector (emission window: 120 cm^2). The lamps are connected to a capacitor and emit broad spectrum intense light flashes between 200 and 1100 nm. The applied voltages ranged from 1 to 3 kV, which corresponded to fluences between 0.10 and 1 J/cm^2 (± 5%) at a distance of 10 cm from the reflector. The distance between reflector and sample table was adjustable between 1 and 20 cm. The applied fluence was determined with a Solo2 Power and Energy Meter with a QE25LP-S-MB detector head (Gentec, Quebec City, Canada) prior to the experiments for all different settings. The UV-contents of PL at different voltages were determined by measuring the fluence on top of the detector head without and with a FGL400S longpass filter (Thorlabs, Newton, USA) transmitting only wavelengths above 400 nm. The difference between the determined values gives the respective amount of UV radiation. UV proportions ranged between 16.3 and 25.5% depending on the applied voltage.

2.3. Treatment conditions

Prepared samples were PL treated at various fluences with the respective energy dose being adjusted by three different parameters (Table 1). For these trials, *E. coli* DSM 498 and *L. innocua* DSM 20649 were used as test bacteria. The first way of dose regulation was done by the variation of the distance between the samples and the flash lamp at fixed voltages and application of single light flashes respectively. The distances were varied between 1 and 15 cm for 1 kV, 1.25 kV, 1.5 kV, 1.75 kV and 2 kV respectively (Table 1).

The second way of dosage control was done by applying several successive light flashes at a frequency of 1 Hz, a medium distance of 10 cm and fixed voltage respectively. The following adjustments were used. 1 kV: 1–15 flashes; 1.25 kV: 1–6 flashes; 1.5 kV: 1–4 flashes; 1.75 kV: 1–2 or 1–3 flashes; 2 kV: 1–2 flashes (Table 1).

The third way of dosage regulation was done by tuning of the voltage discharge at a distance of 10 cm and application of only single light flashes. 1 kV, 1.25 kV, 1.5 kV, 1.75 kV, 2 kV, 2.25 kV, 2.5 kV, 2.75 kV and 3 kV were applied at a distance of 10 cm (Table 1).

Table 2 shows the fluences resulting from the different settings. Voltages of 2.25 kV, 2.5 kV, 2.75 kV and 3 kV at a distance of 10 cm resulted in fluences (± 5%) of 580, 700, 840 and 950 mJ/cm^2.

The impact of the pulsing frequency on microbial count reduction was assessed by applying 5 and 10 successive light flashes at a voltage of 1.25 and 1 kV with a frequency of either 1 Hz or 5 Hz. The respective count reductions were compared.

The PL susceptibility of 15 different vegetative bacterial strains,

Table 1
Overview of settings used for PL inactivation studies of bacteria on a gel surface.

Way of energy dosage	Distance between flash lamp and sample surface [cm]	Number of successively applied light flashes [1 Hz]	Applied voltage [kV]
Distance triggered	1,3,5,8,12 and 15	1	1, 1.25, 1.5, 1.75 or 2
Pulse number triggered	10	Variable: 1–15	1, 1.25, 1.5, 1.75 or 2
Voltage triggered	10	1	Variable: 1–3