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Factors affecting microbial inactivation by Pulsed Light in a continuous flow-through unit for liquid products treatment

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Abstract

Pulsed light (PL) could be an effective alternative technology to traditional thermal treatment in order to assure the microbial quality and safety of liquid food products. However, there is no published research about the factors affecting microbial inactivation in a continuous flow-through unit, which must be elucidated in order to successfully apply this novel technology for liquid products treatment at industrial level. The aim of this work was therefore to evaluate the impact of several PL process parameters on microbial inactivation in flow-through treated liquid systems. Reduction in *L. innocua* culturability increased with the number of light pulses and with the total fluence. Independently of treatment conditions, total fluences of 10 J/cm^2 induced more than 5 Log reductions in *L. innocua* culturability whereas no important rise in water temperature (< 7 ºC) was detected. For treatments of identical applied total fluences, *L. innocua* inactivation was higher at lower liquid thickness (2.15 mm) and faster flow-rate (5 L/min). The level of inactivation was also affected by the voltage input since a higher microbial inactivation was observed in treatments performed at 1000 V than at 3000 V. The results of this study demonstrate the high potential of PL for microbial inactivation in flow-through treated liquid systems.

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Keywords: Pulsed light; flow-through unit, liquid decontamination; microbial inactivation; *Listeria*

1. Introduction

The current need to assure the microbiological safety of liquid food products without affecting their sensory and nutritional quality (e.g. colour, vitamins contain…) has led to improvement in the existing processes and development of suitable non-thermal decontamination technologies, such as pulsed light (PL).

This novel technology consists of a successive repetition of short duration and high power flashes of broadband emission light (200-1100 nm) with approximately 40 % of the emitted light corresponding to the ultraviolet (UV) region. PL has been shown to be effective in inactivating a wide broad of microorganisms involved in food spoilage and foodborne pathogens [1,2]. Its antimicrobial effect has

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been attributed mainly to DNA damage (e.g. formation of pyrimidine dimers and other photoproducts) caused by its high content in UV wavelengths [3]. Other structural damages induced in cell walls, membranes and some internal structures could be involved in PL inactivation efficiency [3]. The antimicrobial effectiveness of PL is influenced by some process related factors as reported previously by some works carried out with static prototypes [4, 5]. To the authors' knowledge, there is not published research about the factors affecting microbial inactivation in a continuous flow-through unit, which must be elucidated in order to successfully apply this novel technology for liquid products treatment at industrial level. The aim of this work was therefore to evaluate the impact of several PL process parameters (voltage input, flow-rate, liquid thickness, number of pulses and total fluence) on microbial inactivation in flow-through PL liquid systems.

2. Material and Methods

2.1. Microorganism and samples inoculation

Listeria innocua was used as a surrogate for *L. monocytogenes* [6], which has been reported as one of the most resistant microorganisms to PL [7]. The strain of *L. innocua* CECT 910 was stored at -80 ºC in Brain Heart Infusion broth (BHI; Pronadisa, Conda Laboratories, Madrid, Spain) supplemented with 20 % glycerol. Thawed stock cultures (200 µL) were transferred to 10 mL of BHI and incubated at 37 ºC for 20 h. After this preculture, *L. innocua* was inoculated at 10^3 CFU/mL in BHI tubes and incubated at 37 ºC until early stationary growth phase (20 h, 109 CFU/mL). Cells were then harvested by centrifugation (5804R centrifuge, Eppendorf AG, Hamburg, Germany) at 10000 xg for 15 min at 4 ºC, washed twice in Potassium Phosphate Buffered Saline (KPBS; 0.01M K2HPO4, 0.01M KH2PO4, 0.15M NaCl; pH 6.8) and finally resuspended in this buffer to a cell density of approximately 10^9 CFU/mL. Samples containing 1.5 L of sterile distilled water were inoculated with adequate volumes of *L. innocua* suspensions (prepared as described above) in order to achieve approximately 10^7 CFU/mL.

2.2. Pulsed light treatment

PL treatments were performed by a dynamic flow-through pilot unit (Maria PUD system, Claranor, Manosque, France). Inoculated water samples were pumped through the unit, PL treated under different treatment conditions and finally recollected in sterile bottles for their posterior analyses. The impact of several flow-through process parameters, such as voltage input (1000 and 3000 V), flow-rate (1 and 5 L/min), liquid thickness (2.15 and 6.23 mm), number of pulses and total fluence (up to 10 J/cm^2), on *L. innocua* inactivation was evaluated. Prior and following each treatment session the unit was cleaned and disinfected in order to avoid any possible cross-contamination. Water samples temperature was measured prior and following each treatment condition by using a digital Checktemp 1 thermometer (Hanna Instruments). Each experiment was repeated at least three times.

2.3. Microbial analyses

Culturability of *L. innocua* in inoculated untreated (control) and PL treated samples was determined immediately after each treatment. Liquid samples were serially diluted in 1 % buffered peptone water (Pronadisa, Conda Laboratories, Madrid, Spain) and 0.1 mL of appropriate dilutions were surface plated onto BHI 1.5 % agar. After incubating Petri dishes at 37 ºC for 48 h, plates containing 0-300 colonies were enumerated and results expressed as Log CFU/mL. Inactivation of *L. innocua* after each treatment condition was expressed as Log (N/N0), where N represents post-treatment cell culturability and N_0 initial cell culturability. Student's t-tests with $p<0.05$ and n-1 degrees of freedom ($n \geq 3$) were used to determine the significance of the differences between samples.

3. Results and Discussion

The impact of a number of PL process parameters on *L. innocua* inactivation by a continuous flow-through PL treatment was evaluated. In agreement with previous results carried out with static PL units [4; 7] reduction in *L. innocua* culturability increased with the number of light pulses (Fig. 1). Taking into account the number of pulses as well as the pulse fluence value for each tested treatment conditions, it was calculated the total fluence or the amount of photons striking on the liquid per area unit. As previously suggested for liquids treated by static PL units [7; 6] microbial inactivation increased with total fluence (Fig.1). Independently of treatment conditions, total fluences of 10 J/cm^2 induced more than 5 log reduction in *L. innocua* culturability whereas no important rise in water temperature (< 7ºC) was detected (data not shown).

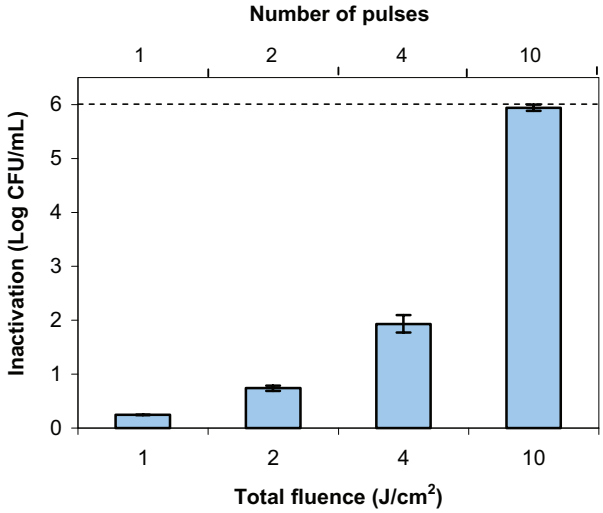

Fig. 1. Impact of the number of light pulses and total fluence on *L. innocua* inactivation in a flow-through unit. Liquid samples of 2.15 mm thickness were treated at 3000 V and 1 L/min. Error bars indicate confidence intervals ($\alpha = 0.05$) and dotted line the maximum detectable inactivation level

For the same total fluence, the impact of the voltage input on *L. innocua* inactivation by a continuous flow-through PL treatment was evaluated. Previous studies with static PL units reported an increase in microbial inactivation as the PL treatment voltage increased [8; 9]. Contrary to our expectations, microbial inactivation by our flow-through PL unit was higher at 1000 V than at 3000 V (Fig. 2), probably due to the higher number of pulses emitted at 1000 V in order to apply treatments of identical total fluence. This fact could ensure higher exposure of microorganisms to light within liquid sample, which could explain the higher effectiveness observed for PL treatments at lower voltage input.

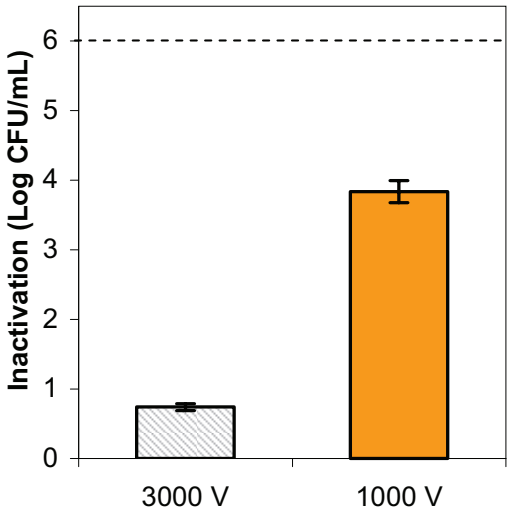

Fig. 2. Impact of the voltage input on the PL inactivation of *L. innocua* in a flow-through unit. Liquid samples of 2.15 mm thickness were treated at 2 J/cm^2 and 1 L/min. Error bars indicate confidence intervals ($\alpha = 0.05$) and dotted line the maximum detectable inactivation level

For the same treatment conditions (voltage and total fluence), *L. innocua* inactivation was higher when liquid thickness was of 2.15 mm than when it was of 6.23 mm (Fig. 3). These results suggest that liquid treatments in thin layer could ensure an adequate exposure of microorganisms to incident light improving thereby the effectiveness of PL. In that way, previous studies on dynamic continuous wave UV (CW UV) processing of liquid food products also reported higher microbial reductions as the reactor thickness decreased [10].

Liquid flow-rate was also shown to be an important factor affecting *L. innocua* inactivation by a continuous PL treatment. As shown in Fig.3, reduction in cell culturability was higher when PL treatments were carried out at a flow-rate of 5 L/min than of 1 L/min. The only previous study on flow-through PL treatment [11] reported no clear influence of flow-rates ranging from 20 to 40 mL/min on *Staphylococcus aureus* inactivation in PL treated milk. The fastest flow-rate tested in the present work (5 L/min) could favour mixing of the pumped liquid, maximizing therefore exposure of microbial cells to the incident light, which would explain the higher effectiveness. In fact, it has been shown that the use of turbulence enhances the level of microbial inactivation in CW UV treatments [12].

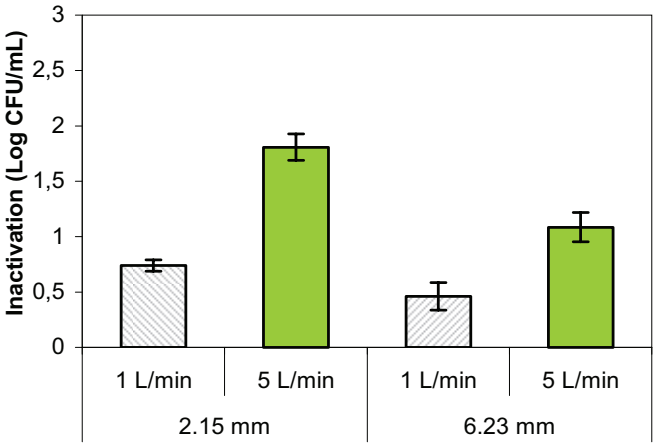

Fig. 3. Impact of liquid thickness and flow rate on *L. innocua* inactivation in a flow-through unit. Liquid samples were treated at 3000 V and 2 J/cm^2. Error bars indicate the confidence intervals ($\alpha = 0.05$)

4. Conclusions

The results of this study demonstrate the high potential of PL for microbial inactivation in flow-through treated liquid systems. It clearly seems that PL technology could be potentially adapted to a commercial setting for continuous treatment of liquid products. Although further research is needed to corroborate effectiveness of PL for liquid products preservation/decontamination, this technology appears as a promising non-thermal process which could be applied as an alternative to pasteurization in order to improve the microbial safety and quality of this kind of products (e.g. liquid food products and beverages).

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