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Microbial inactivation and effects of interrelated factors of intense pulsed light (IPL) treatment for *Pseudomonas aeruginosa*

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ABSTRACT

Intense pulsed light (IPL) inactivation of *Pseudomonas aeruginosa* for different pulse repetition rates (2–15 Hz) and widths (0.15–1.5 ms) were described using the double Weibull model and their energy incidents were compared. The values of the regression coefficient (R^2), RMSE (root mean sum of squared errors), accuracy factor (A_f), and bias factor (B_f) strongly suggested that the model provided a good fit to the data, and they were coupled with the fluence for the first log reduction (F_R) to compare the energy incidents of different treatments. The incident was higher for a lower pulse repetition rate or a longer pulse width. Moreover, in order to examine the effects of interrelated factors on the IPL fluence in terms of energy efficiency, we proposed several terms: the V_F value is defined as the increase in the voltage required for a 1-J/cm^2 increase in the fluence, and the z_{prr} and z_{pw} values are defined as the increases in repetition rate and width of the pulses, respectively, that result in one unit increase in the V_F value. By using these terms, the effects of pulse repetition rate and width on the IPL fluence were analyzed and predicted for further investigation.

1. Introduction

Intense pulsed light (IPL) is an effective nonthermal technology for the microbial inactivation of foodborne pathogens (Dunn, Ott, & Clark, 1995; Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010), and its kinetics has been investigated extensively to assess the potential of this method (Bialka, Demirci, & Puri, 2008; Buzrul & Alpas, 2004; Buzrul, Alpas, & Bozoglu, 2005; Chen & Hoover, 2004; Coroller, Leguérinel, Mettler, Savy, & Mafart, 2006; Ross, 1996; Rowan, Valdramidis, & Gómez-López, 2015). The Weibull model has been suggested to provide a better fit than the traditional log-linear model (Bialka et al., 2008; Buzrul et al., 2005; Chen & Hoover, 2004; 5 msMafart, Couvert, Gaillard, & Leguérinel, 2002), where the factor effects on the inactivation capability could be interpreted using linear regressions (Buzrul et al., 2005; Chen & Hoover, 2004).

IPL involves the emission of short-duration, high-energy pulses from a light source such as a xenon lamp, whose broad-spectrum light includes irradiation in the UV-C range of 200–280 nm (Dunn et al., 1995; Oms-Oliu et al., 2010). UV-C plays an important role in the lethality (Wang, MacGregor, Anderson, & Woolsey, 2005), as does the photochemical effect, a formation of dimers that impairs DNA and cell replications (Bolton & Linden, 2003), and the photothermal effect small increases in temperature (Wekhof, 2000).

A conventional log-linear model theory of the IPL inactivation curves was based on the assumption that they reflect first-order kinetics (Mafart et al., 2002; Peleg, 1999). However, actual microbial populations have not generally shown log-linear relationships, which has prompted several suggestions for modified models (Bialka et al., 2008; Buzrul et al., 2005; Ferrario, Alzamora, & Guerrero, 2013; Rowan et al., 2015). Buzrul et al. (2005) reported that since individual microorganisms rarely have the same sensitivity to a lethal source, the inactivation time conforms to a particular distribution, and the Weibull distribution that is based on the engineering principle of failure is widely used (Peleg, 2006). The Weibull model can be used to describe the nonlinear microbial inactivation curve (Bialka et al., 2008; Ferrario et al., 2013; Mafart et al., 2002; Uesugi, Woodling, & Moraru, 2007). This model includes scale and shape parameters, which show different scales and concavity, respectively, according to the combination of the type of microorganism and the lethal source intensity (Buzrul et al., 2005; Rowan et al., 2015). Moreover, Coroller et al. (2006) used two Weibull distributions to produce a double Weibull model that

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assumes that microbial populations can be divided into two subpopulations that exhibit different resistances toward the lethal source. While different scale parameters are assigned to each subpopulation, the same shape parameter is used for both subpopulations in order to simplify the curve equation. The inclusion in this model of different resistances of microbial subpopulations has resulted in it describing inactivation curves more accurately (Crook, Rossitto, Parko, Koutchma, & Cullor, 2015; Ferrario et al., 2013).

Various factors can affect the inactivation efficiency of IPL, and it is important to understand the concept of the incident energy in the IPL treatment of target samples since microbial inactivation capabilities vary even for the same input energy (Gomez-Lopez, Ragaert, Debevere, & Devlieghere, 2007). The distance from the light source, inoculated microbial strains, initial population, and minor properties such as the sample composition and thickness have been reported to influence the inactivation efficiency of IPL (Gomez-Lopez et al., 2007; Levy, Aubert, Lacour, & Carlin, 2012). The effects of interrelated factors including the input voltage and the number of pulses in IPL treatments of *Pseudomonas aeruginosa* that is found on different foodstuffs (Caldera et al., 2016) have also been examined (Farrell, Garvey, Cormican, Laffey, & Rowan, 2010). Moreover, several studies have used a concept of the classical z value with linear regression to describe the effect of external factors on the inactivation curve when fitting the Weibull model (Buzrul et al., 2005; Chen & Hoover, 2004). However, the effects of interrelated factors on IPL fluence using a predictable model have not been reported previously.

The aim of this study was to describe the IPL inactivation curves of *P. aeruginosa* at different pulse conditions using the double Weibull model, to compare energy efficiencies by introducing the concept of the F_R value, and to determine the effects of pulse conditions on the IPL fluence by proposing the V_F, z_{prr}, and z_{pw} values: the F_R value is defined as the fluence for the first log reduction of the initial microbial population. The V_F value is defined as the increase in the voltage required for one unit increase in the fluence, and the z_{prr}, and z_{pw} values are defined as the increases in repetition rate and width of the pulses, respectively, that result in one unit increase in the V_F value.

2. Materials and methods

2.1. Microbial inoculation

We conducted experiments using *P. aeruginosa* ATCC 10145 at an initial count of 10^9–10^{10} CFU/ml. *P. aeruginosa* samples cultured on Tryptic Soy Agar (Oxoid, Basingstoke, Hampshire, UK) were acquired from the Korean Culture Center of Microorganisms (Seoul, Korea), and then cultivated with a Tryptic Soy Broth (TSB; Oxoid) to produce the initial bacterial inoculum. A colony of the microorganism was transferred to 25 ml of sterile TSB and cultured at 37 °C for 24 h in a shaking incubator. After the preculture, a 1-ml aliquot was inoculated to 100 ml of TSB and cultivated for 10 h at 37 °C in the same incubator to attain the early stationary phase. The microorganisms were then separated by centrifugation at 8000 × g for 10 min at 4 °C, followed by washing twice in sterile 0.85% saline solution.

2.2. IPL treatments

2.2.1. Device

Treatments were performed using a self-designed, laboratory-scale IPL device in our laboratory. This device consists of a pulse generator connected to a treatment chamber (Fig. 1), and was the same device used in our previous study (Yi, Lee, & Chung, 2016)

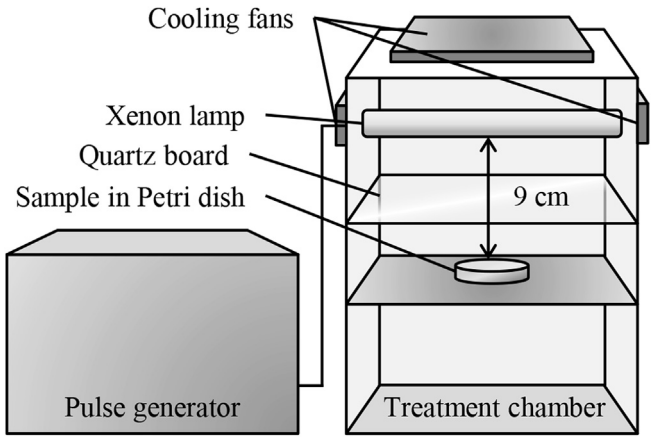

Fig. 1. Schematic diagram of the intense pulsed light (IPL) system used in this study.

except for the capability of the power supply that generates a maximum triggering voltage of 30 kV. The spectral distributions generated by the xenon lamp (200–1100 nm) used in the IPL device under different treatment conditions were measured using a spectroradiometer (ILT-900, International Light Technologies, Peabody, MA, USA) and are shown in Fig. 2. The IPL fluence was calculated from the corresponding spectral distribution by

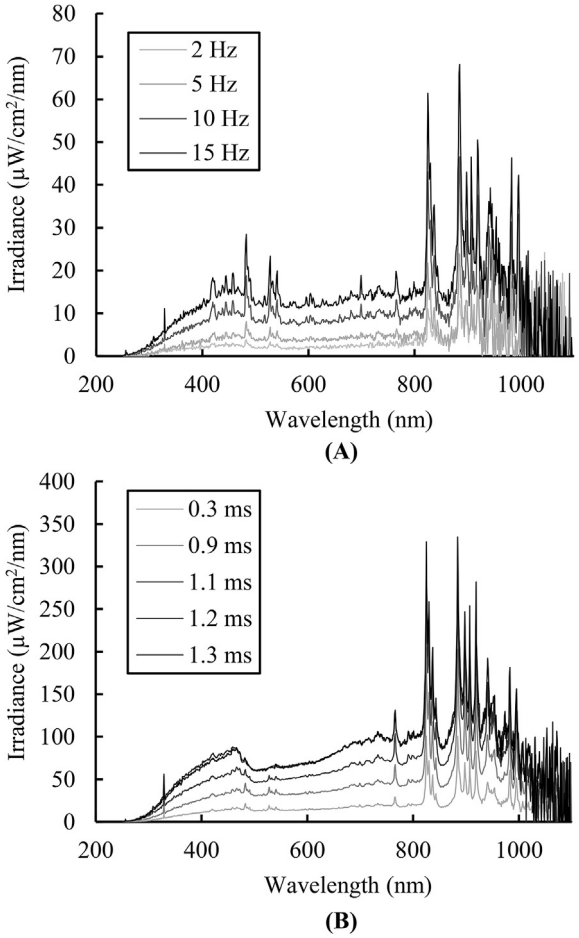

Fig. 2. Emission spectra of the xenon lamp used in this study for different (A) pulse repetition rates at 1800 V with a pulse width of 0.15 ms and (B) pulse widths at 900 V with a pulse repetition rate of 5 Hz.