Journal of Bioscience and Bioengineering
VOL. 117 No. 1, 81–85, 2014

www.elsevier.com/locate/jbiosc

Microbial inactivation and pesticide removal by remote exposure of atmospheric air plasma in confined environments

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Received 25 March 2013; accepted 7 June 2013
Available online 11 July 2013

Microbial inactivation and pesticide removal by remote exposure of atmospheric air plasma were investigated in confined environments, including an airtight box and commercial refrigerator. The relative sterilization ratios of remote plasma exposure in an airtight box were found to be affected by the distance from the plasma generator, the volume of box and the time of irradiation; however, over 99% saturation was obtained within only 120 s in all experiments. The sterilization of microorganisms and the removal of pesticide in a refrigerator with a volume of 292 l were also successfully achieved, resulting in over 99% inactivation or decontamination in a few minutes. Considering the reported results by direct plasma exposure and circulation, it can be concluded that the confined environment enhances the efficient irradiation of plasma by eliminating air flow. This system can be applied to the storage to keep agricultural products freshly and exclusion of harmful materials on the products.

[**Key words:** Plasma beam; Atmospheric air plasma; Anti-microbial sterilization; Removal of harmful chemicals; Storage of agri-products]

Food spoilage and food poisoning are mainly caused by microorganisms, which usually exist in processed as well as unprocessed foods. Because of these issues, controlling the presence of microbes is a highly important factor during food processing and preservation. Therefore, microbial inactivation is a key to enhancing microbiological safety and shelf life.

Pasteurization and thermal sterilization have been the conventional methods used for microbial inactivation. Meanwhile, other food preservation techniques such as microwave exposure and ultrasonic treatment have been suggested, and developed to reduce the unwanted side-effects of the thermal treatment methods such as the loss of color, flavor, taste and nutrients (1). It has also been reported that microwave exposure can produce thermal effects and microwave-stress, which helps eliminate microbial cells (2,3). Furthermore, Earnshaw et al. reported that ultrasonic treatment can enhance heat damage and cell death; thus, this approach has been used for commercial food-processing (4). Since these technologies require a relatively long time for sterilization and storage, high hydrostatic pressure (5,6), pulsed electric field (7,8), and ozone in water (9,10) have been examined as potential alternatives.

However, these techniques are only adequate for canning processes in factory and cannot be used on fresh fruits and vegetables. Recently, a cold-atmospheric air plasma inactivation method has emerged for microbial inactivation (11–16) and decomposition of pesticides (17,18) on heat-sensitive foods. This approach works because plasma generates many different reactive species, such as O, O_2^+, O_3, OH^-, NO, NO_2, which have strong oxidative effects that can help remove contaminants, such as microbes and pesticides, from the surface of food products.

Actually, plasma is ionized gas, besides ions and electrons, which also consists of uncharged particles, such as atoms, molecules and radicals. In this study, as is a common practice, we used the term plasma to designate as well an ionized gas. The use of the plasma radiation offers an original alternative way to sterilization, which consists in exposing microorganisms from an electrical discharge in an ambient condition. Unless the general gases are activated by the electrical discharge, they have no biocidal effect. Furthermore, these reactive species are no longer present after the electric field has been turned off. Therefore, there is no need for vent time and very little danger to us. The operating conditions of the plasma have to be set for an efficient inactivation of the microorganisms, while minimizing the damage to the other materials (19). The microbial inactivation mechanisms with such a plasma sterilization technique were very little known. The specific characteristics of the survival curve, the logarithm of the number of survivors as a function of exposure time to the plasma, show that the kinetics of such process is quite different from that of classical sterilization methods (20).

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1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2013.06.007

To date, several studies have evaluated the effects for the exposure of atmospheric air plasma, but remote exposure has yet to be systematically examined. In this paper, we report the effects for remote exposure of atmosphere plasma over a short period (e.g., only a few minutes) on microbial inactivation against various pathogens and the decontamination of pesticides in an airtight box and a refrigerator within a limited space. These environments were selected because they are typically where fresh fruits, and vegetables lose commercial value.

MATERIALS AND METHODS

Microorganisms and cultivations Two gram-negative strains, *Escherichia coli* (KCTC1041) and *Salmonella typhimurium* (KCTC2054), and two gram-positive strains, *Bacillus subtilis* (KCTC2217) and *Staphylococcus aureus* (KCTC1916), were used microbial inactivation experiments. All strains were cultivated overnight in 10 ml Luria–Bertani (LB) broth, spread at a concentration of 3.3×10^9 colony-forming unit (CFU) per milliliter on LB agar plates of 100 mm in diameter, and then serially diluted one to 10 times of 1/10 dilution steps and sequentially incubated for 16 h at 37°C after plasma exposure to estimate the number of surviving microbes on the LB agar plates.

Plasma generation system and sterilization The plasma generation system (Femtoscience, Hwaseong, Korea) for microorganism sterilization and pesticide removal consisted of a dielectric barrier discharge (DBD) electrode and a miniaturized radio frequency AC generator. For the sterilization test, a 4 kW plasma generator was installed on the top of an acrylic airtight box, which was specially designed for this experiment (Fig. 1), while two 2 kW plasma generators were placed on the top left edge and top right edge, respectively, for the tests performed in a commercial refrigerator (UDS-30RIR, Unique Daesung, Pocheon, Korea).

Remote plasma irradiation The prepared 500 μl suspension of four microorganisms (*E. coli*, *S. typhimurium*, *B. subtilis*, and *S. aureus*) were spread at a concentration of 1.3×10^3 CFU/ml onto the LB agar plate with 100 mm in diameter, simply air-dried to prevent leak out of the flow of suspension for 10–15 min at room temperature on a clean bench, and placed at a 100 mm distance from the plasma generator in an airtight box with a volume of 20, 86, and 110 l for 5, 10, 15, 20, 30, 60, 90, and 120 s, respectively. These conditions were used for the sterilization tests at room temperature. The distance from the plasma generator to the agar plate was 100 mm, 180 mm and 280 mm for the 20 l airtight box, respectively, and the plasma treatments were fixed at a generation voltage of 4.0 kV with 99.9% pure air at a flow rate of 5.0 l/min for all experiments. After the test in the airtight box was complete, the number of living microorganisms was monitored at 4°C under the same plasma exposure conditions used for the commercial refrigerator with two plasma generators. The volume of the refrigerator was 292 l, and the direct distance from the plasma generator to the samples was 450 mm.

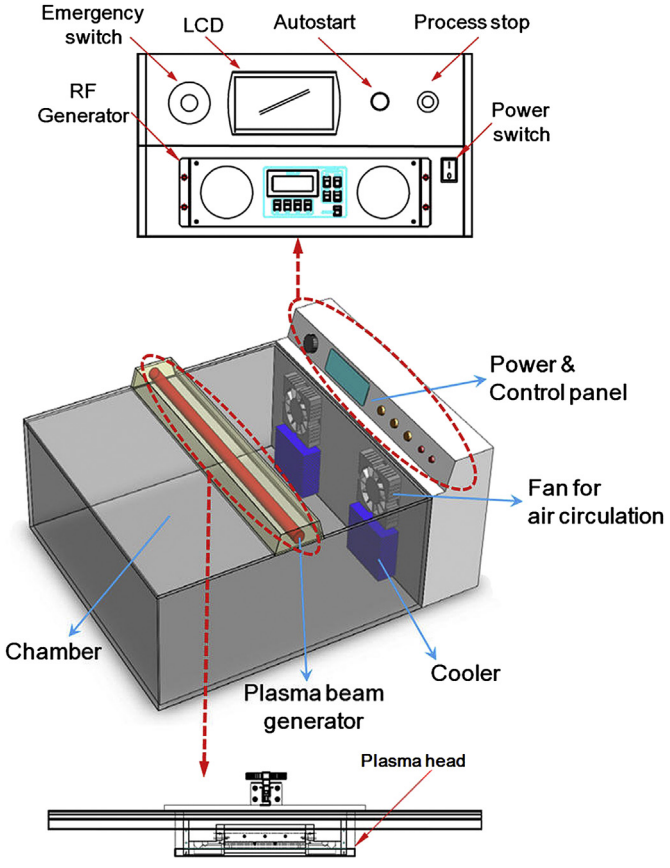

FIG. 1. Schematic illustration of an atmospheric-pressure air plasma device.

Removal of agricultural pesticides The surface of a fresh apple was coated by using spray-out with the representative pesticide, paraoxon, in a 10% (v/v) methanol solution at 0, 10, 100, 1000, 10,000 and 100,000 ppb, respectively. The apples were then exposed to the plasma in the commercial refrigerator that was utilized for the sterilization test. The remote plasma irradiation conditions were identical to those of the airtight box test. After rubbing 5 times a cotton tip on the apple surface in accordance with the manufacturer's instruction (Mecasys, Daejeon, Korea), the tip was dipped for 10 min in methanol solution as an extraction solvent to extract the agrichemicals. The amount of agrichemicals was determined by high-performance liquid chromatography (HPLC) analysis.

High-performance liquid chromatography Samples were analyzed by reverse-phase HPLC using a Supelcosil LC-18 column (4.6 mm × 250 mm; Agilent) for the quantitative analysis of pesticides. HPLC runs consisted by two mobile phases, 50% methanol and 50% acetonitrile, which were mixed using a linear gradient (0.5–1.0% change per minute) at a flow rate of 1.0 ml/min. The column temperature was maintained at 25°C. The peaks were detected using a UV detector at 276 nm. The identity of the peaks was confirmed by chromatography analysis of standard samples. The standard sample was paraoxon, which was purchased from Fluka (USA).

RESULTS AND DISCUSSION

Remote plasma irradiation in an airtight box The sterilization by remote plasma irradiation in a confined environment was examined as a function of the exposure time using *E. coli*. In addition, the effect of the distance from the plasma beam generator to the specimen on inactivation in the confined environment was also examined, while experiments in an open environment were conducted at a close distance due to unstable irradiation by the non-negligible air current. Relative sterilization ratios after plasma exposure in the airtight box are plotted in Fig. 2. Similar to the results observed in the open environments (11–16), the relative sterilization ratios in the confined environment rapidly increased with an increase in plasma exposure time and reached over 99% within 90 s at 100 mm irradiation distance. Considering that the

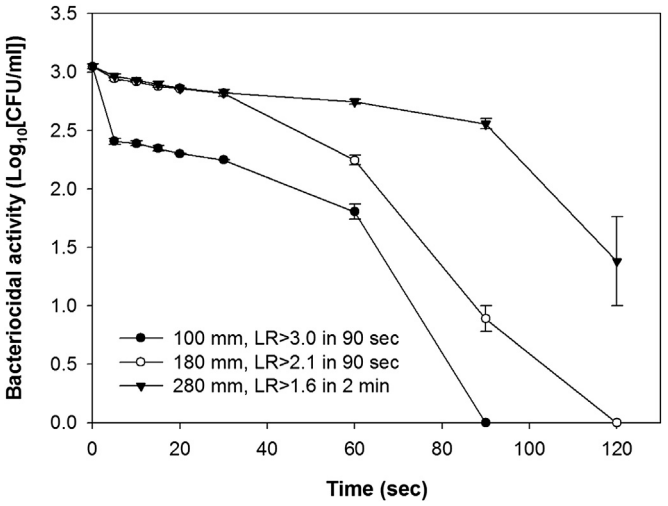

FIG. 2. The sterilization effects of atmospheric air remote plasma irradiation against *E. coli* cells on the agar plates in an airtight box as a function of the plasma exposure time and the distance from the plasma generator to samples. Each Log_{10} reduction (LR) represents its specific antibacterial activity *via* the distance from the plasma beam. Filled circle, 100 mm distance with LR > 3.0 in 90 s; opened circle, 180 mm distance with LR > 2.1 in 90 s; triangle, 280 mm distance with LR > 1.6 in 2 min. The results are an average value generated from three different experiments with their time profile and error ranges.