Vacuum xxx (2016) 1–7

Contents lists available at ScienceDirect

Vacuum

journal homepage: www.elsevier.com/locate/vacuum

Inactivation of bacteria on plant seed surface by low-pressure RF plasma using a vibrating stirring device

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ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form
5 July 2016
Accepted 12 July 2016
Available online xxx

Keywords:
Low-pressure plasmas
Plant seeds
Pathogenic bacteria
Xanthomonas
Bacteria inactivation
Vibrating stirring device

ABSTRACT

Studies on applications of plasmas in agriculture have been conducted actively in many countries. The inactivation effect of low-pressure RF oxygen and air plasmas on seed-borne bacteria on plant seed surfaces was investigated. Active species generated in low-pressure plasmas could reach all seed surfaces using a vibrating stirring device, and the inactivation effect would be improved. When cabbage seeds contaminated artificially by *Xanthomonas campestris pv. campestris* (Xcc), one of seed-borne bacteria that have high pathogenicity, were irradiated by low-pressure oxygen and air plasmas, the inactivation effect with the vibrating stirring device was more than approximately 10 times higher than that without the device. Although the surface of cabbage seeds suffered the crack-like damage, germination rates of cabbage seeds at 3 and 7 days after seeding were not affected by low-pressure oxygen and air plasmas. Also, abnormal cotyledon and root on germinating cabbage were almost not observed after the seeds irradiated by low-pressure plasmas were cultivated for 7 days. Furthermore, occurrence of mold and rot on germinating cabbage was not observed when cabbage seeds were irradiated by low-pressure plasmas and cultivated for 7 days.

1. Introduction

Plant diseases are caused by biotic factors such as pathogenic bacteria and viruses [1]. These biotic factors mainly infect plants due to seed-borne and soil contamination. Some bacteria that have high pathogenicity exist in seed-borne bacteria. For example, *Xanthomonas Campestris pv. Campestris* (Xcc) is a gram-negative bacillus and has high pathogenicity, causing plant disease like a black rot [2]. Chemical sterilization and dry heat sterilization are conventional methods for inactivation of bacteria on plant seed. However, these methods have some disadvantages. Chemical sterilization damages both humans and the environment [3,4]. Dry heat sterilization would cause the disorder of germination of seeds [5,6]. Establishment of new inactivation methods that do not damage humans, the environment, or plant seeds is necessary.

Recently, many studies on application of plasmas in agriculture and medicine have been reported [7–11]. The inactivation of bacteria on plant seeds and other agricultural products by plasmas also has been reported in these studies [12,13]. The effect of atmospheric and low-pressure plasmas on pathogenic fungi on the seed has been investigated [14]. Mold spores and bacteria on agricultural products can be inactivated by active oxygen species generated by an atmospheric plasma [15]. These studies indicate that pathogenic bacteria on seeds could be inactivated by active oxygen species generated in a low-pressure plasma. However, active species generated in a low-pressure plasma are not expected to reach contact areas of each plant seed and the ground when the seeds are irradiated by these active species. Bacteria that exist in the contact area of each seed and the ground would not be inactivated. Therefore, a vibrating stirring device is used to improve the inactivation of bacteria on plant seeds. Active species generated in a low-pressure plasma could reach all surfaces of plant seeds due to the vibrating stirring device, and high inactivation for bacteria is expected. The effect of low-pressure plasmas and a vibrating stirring device on the inactivation of pathogenic bacteria on plant seeds was evaluated in this study. Furthermore, damage to the seed surface, a decrease in germination rates, occurrence of abnormal cotyledon, root, mold and rot are expected when plant seeds are irradiated by active species generated in low-pressure plasmas. Thus, influences of low-pressure plasmas on the germination rate, the condition of surface of plant seeds and germinating plant were investigated.

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http://dx.doi.org/10.1016/j.vacuum.2016.07.017
0042-207X/

2. Experimental method

Fig. 1 shows the schematic of a plasma inactivation device for seeds. The device had a width of 490 mm, inner diameter of 210 mm, and internal volume of approximately 17 L. An RF electrode was set inside the chamber and constructed in a wave shape in order to produce spatially uniform active species with low electrical power. Cabbage seeds were used as test plant seeds to evaluate bacterial inactivation on the seed surface, and the seeds were contaminated by Xcc artificially. The cabbage seeds were placed on a plastic pot set on a vibrating stirring device below the RF electrode. The vibrating stirring device used in this study was a handmade product. The distance between the seeds and the RF electrode was approximately 120 mm. The air in the chamber was evacuated with a rotary pump, and oxygen or air gas supplied. RF power (13.56 MHz) was supplied to the RF electrode, and low-pressure plasma was generated. The gas pressure was 60 Pa and the RF power was 100 W. The number of cabbage seeds irradiated by low-pressure plasmas was 1000. The cabbage seeds were stirred by a vibrating stirring device once approximately every 30 min. The stirring period was approximately 2–3 s. Light emission spectra of low-pressure oxygen and air plasmas were measured by a spectroscope owing to the identification of active species generated in the plasmas. The light emission spectra around the location of cabbage seeds set on the device were measured.

Evaluation of inactivation of Xcc on the seed surface was based on those of the Seed Health Method (7-019a) of the International Seed Testing Association (ISTA). Cabbage seeds irradiated by low-pressure plasmas were put in 10 mL normal saline solution and soaked. This mixture was shaken for 2.5 h at room temperature at 125 rpm. Aliquots of 100 μL were extracted and dropped on nutrient agar (mCS20ABN) by pipette and spread by a sterile bent glass rod. Then, the agar plates were incubated at 28 °C. The number of colonies was counted after 3 days incubation.

The germination rate and the surface condition of cabbage seeds were evaluated to investigate the influence of low-pressure plasmas on the seeds. Cabbage seeds were irradiated by low-pressure oxygen and air plasmas for 0–3 h. The oxygen and air pressures were 60 Pa and the RF power was 100 W. Germinating seeds were defined as having a root tip emerging from the seed coat, and the germination rate was defined as the number of germinating seeds out of all seeds seeded. Two filter papers were set on a petri dish with a diameter of 90 mm, then cabbage seeds were placed on the papers. 50 cabbage seeds were placed on a petri dish. Two petri dishes with 50 cabbage seeds were used, and the germination rate was shown by normalizing each germinating rate. The germination rate was evaluated at 3 and 7 days after seeding for the cabbage seeds. The condition of cotyledon and root of the germinating cabbage with plasma irradiation was evaluated after 7 days cultivation. Furthermore, the surface condition of cabbage seeds irradiated by low-pressure oxygen and air plasmas was evaluated by scanning electron microscopy (SEM).

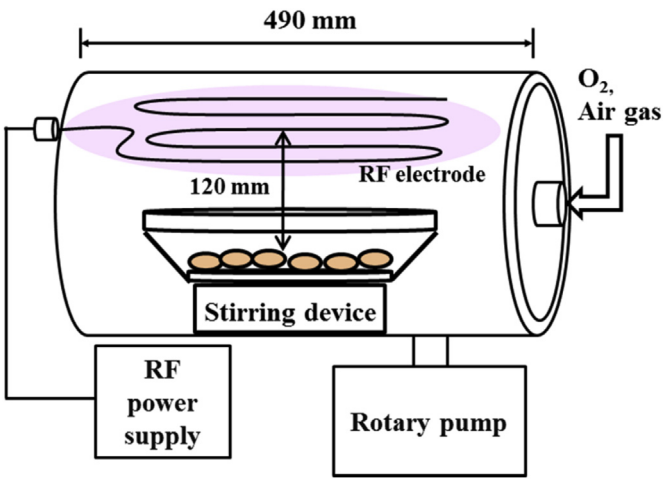

Fig. 1. Schematic of experimental apparatus.

3. Results and discussion

3.1. Evaluation of inactivation of Xcc on cabbage seed surface by low-pressure RF plasmas using vibrating stirring device

Cabbage seeds contaminated artificially by Xcc were irradiated by low-pressure oxygen and air plasmas, and the inactivation effect of the plasmas on the Xcc on the seed surface was compared with and without the vibrating stirring device, as shown in Fig. 2. The number of Xcc, shown on the vertical axis, was normalized by that of initial number of Xcc. The number of Xcc before plasma irradiation with or without a vibrating stirring device was 3.4×10^4 cfu/seed and 1.9×10^4 cfu/seed, respectively. The experimental day for evaluation of the inactivation effect with a vibrating stirring device was different from that without the stirring device. Cabbage seeds contaminated artificially by Xcc were prepared each experimental day, and the number of Xcc on the seeds differed slightly for each of the prepared seeds. As shown in Fig. 2, the number of Xcc on the cabbage seed surface without the stirring device did not decrease to 1/10 of the initial number after an oxygen plasma irradiation for 3 h. On the other hand, when the stirring device was used, the number of Xcc on the seed surface decreased to approximately 1/130 of the initial number after an oxygen plasma irradiation for 3 h. Also, the number of Xcc on the seed surface irradiated by a low-pressure air plasma for 2 h with a vibrating stirring device decreased to approximately 1/170 of the initial number. These results indicate that the inactivation of Xcc on the seed surface with the stirring device is more than 10 times higher than without the device. The number of Xcc on the seed surface irradiated by low-pressure air plasma for 3 h did not decrease more than that for 2 h irradiation.

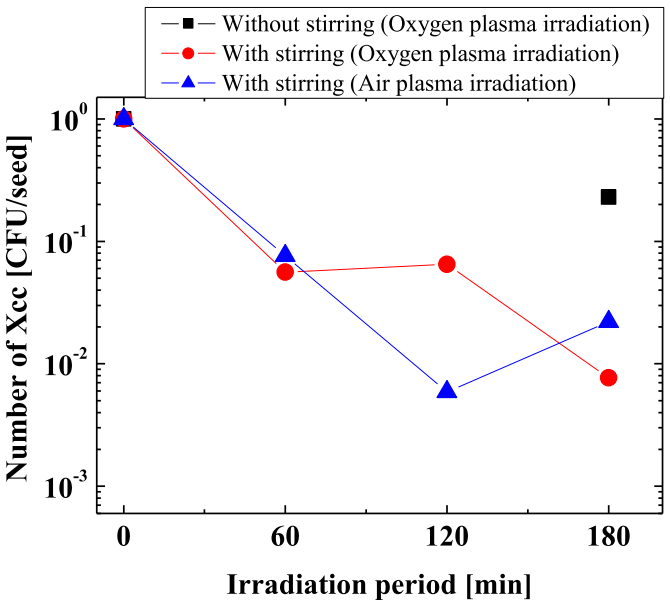

Fig. 2. Relationship between plasma irradiation period and number of Xcc on cabbage seed surface with or without vibrating stirring device.

Please cite this article in press as: R. Ono, et al., Inactivation of bacteria on plant seed surface by low-pressure RF plasma using a vibrating stirring device, Vacuum (2016), http://dx.doi.org/10.1016/j.vacuum.2016.07.017