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Inactivation of Bacteria using Discharge Plasma under Liquid Fertilizer in a Hydroponic Culture System

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ABSTRACT: We developed a discharge plasma reactor under liquid fertilizer for inactivating bacteria in the recirculation system of hydroponics. The plasma reactor consisted of a wire electrode that was placed in an insulating circular cylinder and a grounded electrode on a cylinder outside. The reactor was sunk under liquid fertilizer when used. Atmospheric air was injected into the cylinder using a gas pump and released through arrayed tiny holes of the reactor. Repetitive nanosecond high-voltage pulses were applied to the wire electrode using a magnetic pulse compression pulsed-power generator. The performance of the developed reactor was evaluated using tomato (*Solanum lycopersicum* L., Rinka 409) seedlings in hydroponics. In this study, 15 L of liquid fertilizer was contaminated with *Ralstonia solanacearum*, a plant pathogenic bacterium, after 40 min of discharge plasma treatment. The discharge plasma treatment was then continued for 100 min. Results showed that the number of colony forming units (CFU) of *R. solanacearum* in the liquid fertilizer decreased from 10^7 to 10^2 CFU/mL using the discharge plasma treatment. Seedlings with discharge plasma treatment were relatively healthy; in contrast, all seedlings in the positive control wilted and died from infection of *R. solanacearum* after 12 d. Disease severity was also suppressed after discharge plasma treatment.

KEY WORDS: discharge plasma under water, plant pathogenic bacteria, *Ralstonia solanacearum*, inactivation, hydroponics

I. INTRODUCTION

In recent years, hydroponics (a method of growing plants without soil) using liquid fertilizer has been widely used in agriculture.[1] Two systems of hydroponics include the recirculation system and the run-to-waste system. The recirculation system is more acceptable due to its reduced impact on environmental load, avoiding contamination of lakes, marshes, well water, and ground water. Other advantages of the recirculation system include higher production, energy conservation, better control of growth, and independence of soil quality.[2]

In general, the strategy in hydroponics has been to keep cultivating systems as clean as possible. Due to the use of clean substrates, soil-borne pathogens such as *Ralstonia solanacearum*, *Fusarium oxysporum*, and *Verticillium dahlia*; nematodes; and many others that can survive in the deeper soil layers can be circumvented. However, during the entire period of plant growth, contamination with pathogens can never be excluded,

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because they can be introduced via the water supply, air, insects, or inadvertently by the grower directly.[3] Zoospores actively swim to their hosts and infection can occur within minutes. Under favorable conditions, the rate of multiplication of these pathogens can be explosive. Once even one plant is infected, the entire cultivation bed is subjected to significant risks. Bacterial disease is responsible for serious crop losses, attacking more than 50 plant families, including such major crops as tomato, potato, and banana.[4] The bacteria that can spread through the cultivating system by recirculation of the irrigation water have been reviewed elsewhere.[5]

Traditionally, liquid fertilizer is treated with technologies such as heating,[6,7] ultraviolet irradiation,[8–12] and/or membrane filtration.[13,14] However, these treatments are limited in practice owing to their high running cost, including periodic cleaning. Thus, a discharge plasma under water, a promising technique to reduce infection risks, has attracted attention.[15,16] Discharge plasma produces chemically active species such as ozone[14,17] and hydroxyl[18] radicals. These chemical species have an important role in inactivating various pathogenic bacteria in liquid fertilizer.[19–23] The performance of a gas-liquid phase discharge plasma reactor was improved by using a magnetic pulse compression (MPC) pulsed-power generator.[24] In previous reports, the discharge plasma reactor increased the growth of *Brassica rapa* var. *perviridis* (Chinese cabbage)[25] and dry weight of cropped *Fragaria* × *ananassa* (strawberry).[26] In this experiment, the discharge plasma reactor was extended for practical use, and its performance for inactivating bacterium of the liquid fertilizer was evaluated using *R. solanacearum*, a plant pathogenic bacterium, and tomato (*Solanum lycopersicum* L., Rinka 409) seedlings.

II. EXPERIMENT

To extend the area in which a discharge plasma occurs, a gas-liquid phase discharge plasma reactor[24–26] was developed, as shown in Fig. 1. The reactor consisted of a wire electrode, that was placed in an insulating circular cylinder, and a grounded electrode placed on a cylinder outside. The distance between wire electrode and gas-liquid phase was approximately 10 mm. The reactor was sunk under the liquid fertilizer. Atmospheric air was injected into the cylinder using a gas pump and the air was released through

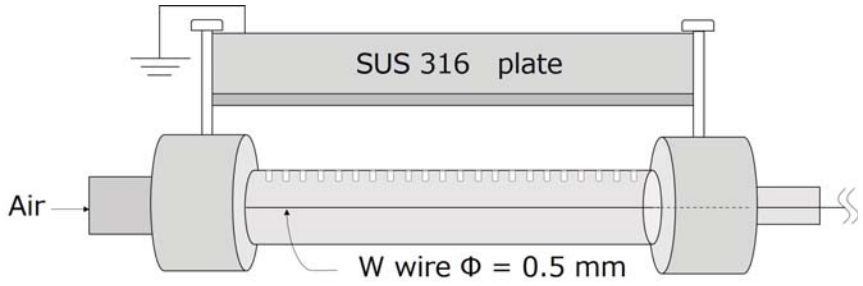

FIG. 1: Schematic of the discharge plasma reactor

holes in the cylinder that were 0.5 mm in diameter. The cylinders were separated from one another by 2.0 mm. Repetitive nanosecond pulses were applied to the wire electrode using an MPC pulsed-power generator. Figure 2 shows a schematic of the MPC circuit. Figure 3 shows the waveforms of applied voltage to the wire electrode and the flowed current to the ground through the plate electrode, as shown in Fig. 1. Peak voltage was ~10 kV. Pulse width and repetition rate were 150 ns and 2000 pulses per second (pps), respectively.

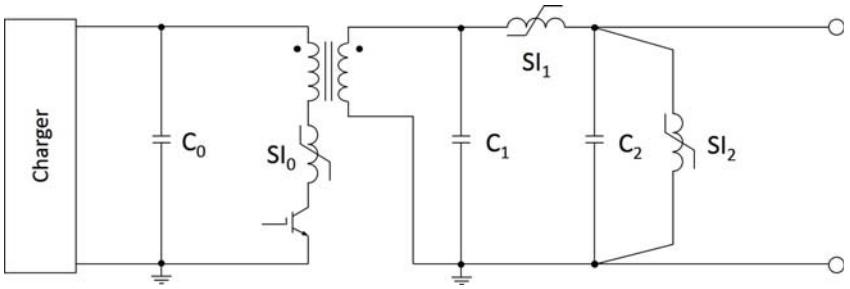

FIG. 2: Circuit of MPC

The performance of the developed reactor on inactivation of bacteria was evaluated in a recirculation system using the discharge plasma system, as shown in Fig. 4. In this study, the liquid fertilizer was heavily contaminated with *R. solanacearum*, a plant pathogenic bacterium, after 40 min of discharge plasma treatment. After that, discharge plasma treatment was continued for 100 min. The plant used was tomato (*S.*

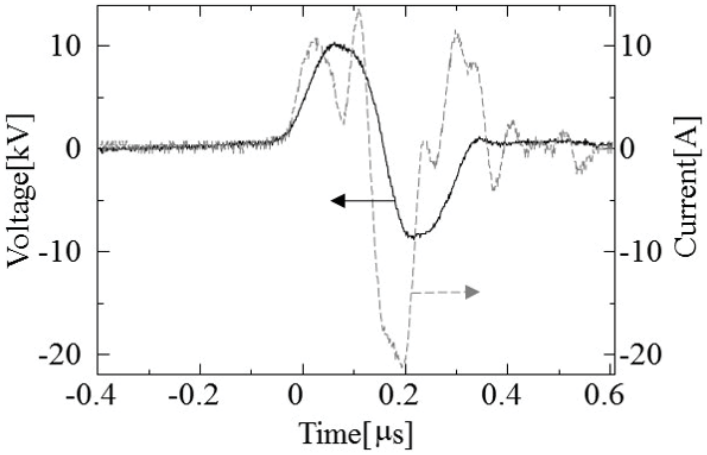

FIG. 3: Waveforms of applied voltage to a wire electrode in the reactor and discharge current flowing to the ground through a plate electrode

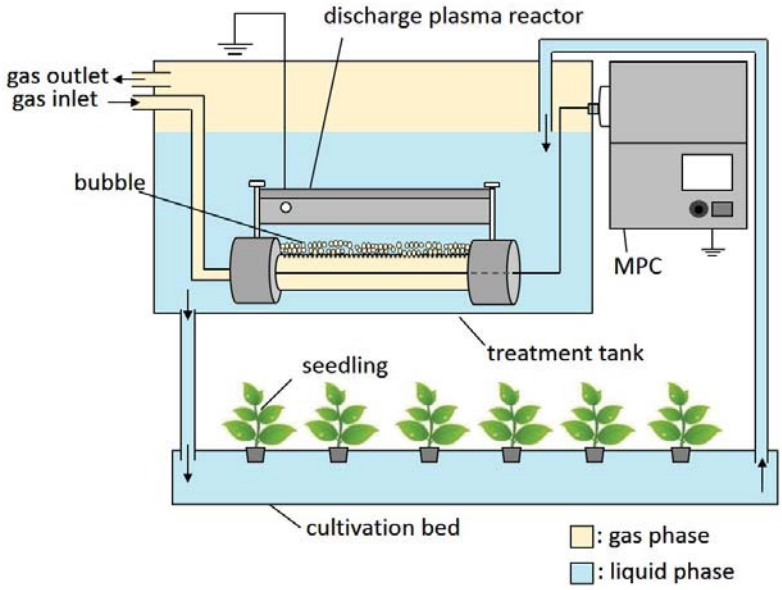

FIG. 4: Schematic of the discharge plasma reactor in the recirculation system of hydroponics

lycopersicum L., Rinka 409), the number of seedlings were 15, and the volume of the liquid fertilizer was ~15 L. We prepared two more experimental sections: one a negative control and the other a positive control. The liquid fertilizer in the negative control was neither contaminated with *R. solanacearum* nor treated with discharge plasma, and that in the positive control was contaminated but not treated with discharge plasma. The number of colony forming units (CFU) of *R. solanacearum* in the liquid fertilizer was obtained using Hara and Ono's selective medium.[27] Seedlings were monitored, and disease severity[28] was evaluated. Disease severity was obtained using the following equation:

$$\text{Disease severity} = 100 \times (4A+3B+2C+\text{D})/4n,$$

where *A*, *B*, *C*, and *D* are the numbers of tomato seedlings with <10%, 10%–33%, 33%–67%, and >67% infected stumps, respectively, and *n* is the total number of seedlings examined.

III. RESULTS AND DISCUSSION

Figure 5 shows photographs of the seedlings of experimental sections of negative and positive control, and discharge plasma on the initial, sixth, and 12th d. On the initial day, all seedlings were healthy. On the sixth day, all seedlings healthily grew as those in the negative control, but almost all seedlings in the positive control developed symptoms of

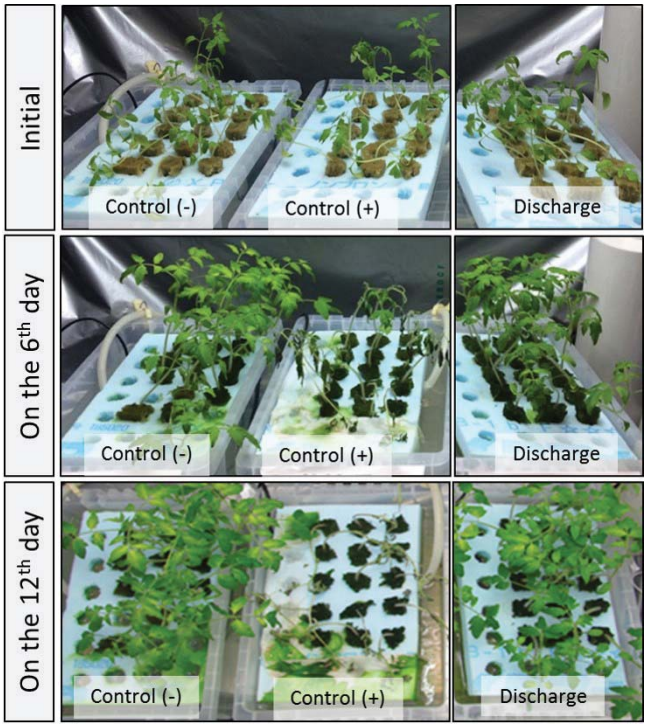

FIG. 5: Photographs of the seedlings of experimental sections of control. (–) Negative control; (+) positive control; and discharge plasma treatment on the initial, sixth, and 12th d

wilt disease. On the 12th d, all seedlings in the positive control clearly wilted and died by infection by *R. solanacearum*, unlike those in other experimental sections. Growth inhibition due to the oxidative stress by discharge plasma treatment was not found in seedlings undergoing discharge plasma treatment; bacterial wilt disease was significantly suppressed by discharge plasma treatment.

Figure 6 shows the disease severity of seedlings in experimental sections of negative and positive control and discharge plasma treatment. The disease severity was zero for 10 d for the seedlings in the negative control. The disease severity and in discharge plasma treatment increased on the sixth day and was 20% on the eighth day and after. In contrast, the disease severity of the positive control increased on the fourth day and continuously increased to 100% on the eighth day. The disease severity of seedlings was suppressed by discharge plasma treatment.

Figure 7 shows the number of CFU of *R. solanacearum* in the liquid fertilizer. Figure 7 shows that no *R. solanacearum* was detected in the liquid fertilizer of the negative control. This result has no contradictions with the results shown in Fig. 6. The number of CFU of *R. solanacearum* in the liquid fertilizer of the negative control was above 10^7 CFU/mL; in contrast, that of the discharge plasma treatment was $<10^2$ CFU/mL. Discharge plasma treatment inactivated *R. solanacearum*. Infection generally occurs when

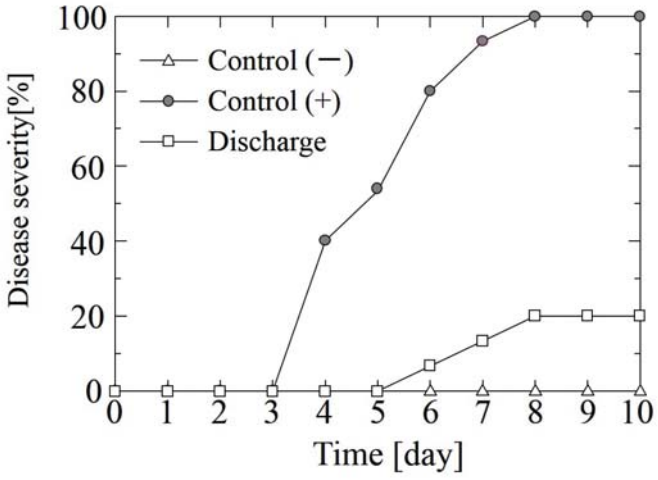

FIG. 6: Disease severity of seedlings in experimental sections of control. (–) Negative control; (+) positive control; and discharge plasma treatment

the number of CFU of *R. solanacearum* in the liquid fertilizer is $>10^4$ to 10^5 CFU/mL.[29] However, some seedlings in the discharge plasma treatment were infected, as shown in Fig. 6. This may be a result of bacterium density around the roots of some seedlings that was temporary but then sharply concentrated after adding *R. solanacearum* to the liquid fertilizer.

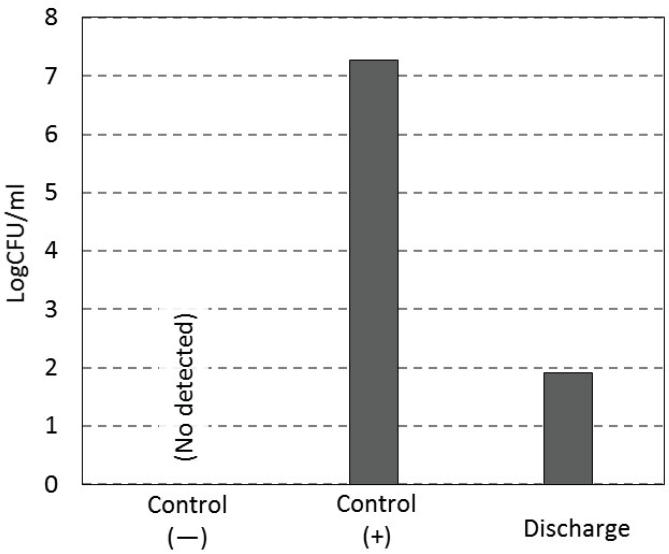

FIG. 7: Number of CFU of *R. solanacearum* in the liquid fertilizer of experimental sections of control. (–) Negative control (+); positive control; and discharge plasma treatment

IV. CONCLUSIONS

We developed a discharge plasma reactor under liquid fertilizer for inactivating bacteria in the recirculation system of hydroponics and evaluated the performance of the developed reactor using tomato (*S. lycopersicum* L., Rinka 409) seedlings in hydroponics. In this study, 15 L of liquid fertilizer was contaminated with *R. solanacearum*, a plant pathogenic bacterium, after 40 min of discharge plasma treatment. After that, discharge plasma treatment was continued for 100 min. Our main conclusions are as follows.

1. Almost all seedlings with discharge plasma treatment healthily grew, but all seedlings in the positive control wilted and died by infection from *R. solanacearum* on the 12th d.
2. Disease severity of seedlings in the positive control was 100%, but that in discharge plasma treatment was suppressed to 20% on the eighth day and beyond.
3. The number of CFU of *R. solanacearum* in the liquid fertilizer decreased from 10^7 to 10^2 CFU/mL by discharge plasma treatment. This result indicates that discharge plasma treatment inactivates *R. solanacearum*.

It can be concluded that bacterial wilt disease is significantly suppressed by the developed reactor.

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