Pulsed electric field disinfection treatment of *Fusarium oxysporum* in nutrient solution

Chongshan Zhong, Xinxing Guan, Zhe Fan, Weitang Song, Ran Chen, Yi Wang, Xiaoyan Sun and Shouxing He

ABSTRACT

The disinfection of recirculating nutrient solution is essential to avoid disasters due to the dispersal of pathogens in closed soilless culture systems. This work presents the development of a new technique to perform disinfection, based on pulsed electric field (PEF) treatment. Hoagland solution inoculated with *Fusarium oxysporum* was selected as the specimen to verify the effectiveness of PEF treatment. The results indicate that PEF deactivated most *Fusarium oxysporum* in nutrient solution within a few seconds with the maximum disinfection efficiency achieved being 99.84%.

The disinfection efficiency became higher with the extending of treatment time or the increase of PEF strength, while the initial microbial density was proven to play no role. Temperature rise indicates that PEF treatment played the dominant role in the process of disinfection. The energy efficiency depends mainly on treatment time and electric field intensity. The optimal operating conditions were found to be: treatment time less than 10 seconds and electric field strength around 5 kV/cm. This investigation implies that, in the future, PEF treatment has the potential to be applied to disinfect nutrient solution.

Key words | disinfection, *Fusarium oxysporum*, nutrient solution, pulsed electric field

Chongshan Zhong (corresponding author)
Xinxing Guan[†]
Zhe Fan[†]
Ran Chen
Yi Wang
Xiaoyan Sun
College of Information and Electrical Engineering,
China Agricultural University,
Beijing 100083,
China
E-mail: zhongchsh@cau.edu.cn

Weitang Song
College of Water Resources and Civil Engineering,
China Agricultural University,
Beijing 100083,
China

Shouxing He
The Institute of Alpine Economic Plants in Yunnan
   Academy of Agricultural Science,
Lijiang, Yunnan, 674100,
China

[†]These authors contributed equally to the work.

INTRODUCTION

A closed soilless culture system with recirculating nutrient solution features advantages over conventional drain-to-waste or open systems, such as reducing the cost of water and fertilizer, and avoiding environmental pollution (Ehret *et al.* 2001). However, the recycling use of nutrient solution could result in dispersal of pathogens in a growing system (van Os 1999). A few disinfection techniques are currently being utilized to avoid an outbreak of root-borne diseases due to contaminated nutrient solution: for example, ozone treatment (Kobayashi *et al.* 2011), chlorine or chlorine dioxide processing (Scarlett *et al.* 2016; López-Gálvez *et al.* 2017), UV radiation (Sutton *et al.* 2000), heat sterilizing (Runia & Amsing 2001a), and removing pathogens with slow filtration or constructed wetlands (Wohanka *et al.* 1999; Gruyer *et al.* 2013).

Each method has its noticeable merits, but also a few inherent weaknesses that also need to be considered. Ozone may react with specific iron chelates, manganese and organic matter in solution, hence, the effect of ozone treatment could be reduced. Moreover, the low dissolvability of ozone in solution implies potential health and environmental hazards (Stewart-Wade 2011). Interacting with some compounds (ammonium, organic nitrogen, etc.), chlorine forms chloramines (Raudales *et al.* 2014), which could hinder the growth of lettuce (Date *et al.* 2005). UV radiation most likely leads to photo-degradation of iron chelates (Albano & Miller 2001) and also the transparency of nutrient solution needs to be at least 60% to maintain the efficacy of UV (Yiasoumi *et al.* 2014). Heat sterilization not only consumes a large amount of energy but

also may have undesirable effects on some slightly soluble chemical compounds (Runia 1994). Tu & Harwood (2005) reported that slow filtration needs a large infrastructure and sediment clogging requires frequent maintenance.

The pulsed electric field (PEF) method has been widely investigated as a technology for the disinfection of liquid food and beverages (Sharma *et al.* 2014). The commonly accepted disinfection mechanism of PEF is the effect of electroporation and it has been demonstrated that this microorganism inactivation technology is both environmentally friendly and non-thermal (Barbosa-Cánovas *et al.* 1999). These advantages of the treatment could overcome some of the drawbacks of the presently existing methods. However, to the authors' best knowledge, the treatment of recirculating nutrient solution using PEF technology has not yet been reported.

In this work, to assess the PEF technique, a novel PEF-based disinfection system was developed to investigate various factors for disinfection efficiency. The study ends with positive conclusions.

MATERIALS AND METHODS

The preparation of the inoculated nutrient solution

Fusarium oxysporum is a serious pathogen in greenhouses, which may invade seedling and fruit production (Song *et al.* 2004; Elmer 2015), leading to a significant decrease in yield and a contamination of recirculating nutrient solution. Therefore, this study selected *F. oxysporum* as a typical pathogen. Since Hoagland solution is widely used in plant-growing (Hothem *et al.* 2003), it was chosen as the living environment of the pathogen.

The activated *F. oxysporum*, which was isolated from infected cucumber plants, was inoculated on potato dextrose agar medium (PDA, 50 g potato, 5 g dextrose, 5 g agar) in petri dishes, and then had 72 hours of cultivation at 28 °C in an incubator. To expand the cultivation, a 1-cm diameter colonized medium was cut from a petri dish and transferred into a 250 ml conical flask, which contained 80 ml potato dextrose broth (PDB, 50 g potato, 5 g dextrose). After that, the conical flask was put on an orbital shaker (SPH-200B, SHIPING, Shanghai,

China) at a speed of 150 rotations/min at an ambient temperature of 28 °C. The suspension in the conical flask was cultivated with shaking for 150 hours. In the last step, the diluted Hoagland solution was mixed with the suspension of *F. oxysporum* at a composition rate of 495 ml/5 ml, after which the inoculated nutrient solution for the disinfection experiments was ready (Huang *et al.* 2018).

Experimental setup

Figure 1 presents the scheme of the PEF treatment system, which was basically made from two distinctive parts: a high-voltage pulsed power supply and a series of treatment chambers.

The pulsed power supply contained a high-frequency transformer, a rectifier unit, a pulse-forming network (PFN) and a rotating spark gap switch (SGS). The PFN was made of a nine-stage L-C ladder with a characteristic impedance $(Z_C = (L_0/C_0)^{1/2})$ close to $33\,\Omega$, where $L_0 = 18.7\,\mu H$ and $C_0 = 18.1\,nF$. The repetition rate of the rotating SGS was 4.5 pulses per second. The pulsed peak voltage (V_p) could be adjusted from 4 kV to 8 kV.

The waveform of the pulsed voltage impulse was measured using a high-voltage probe (1000:1, P6015A, Tektronix, USA) connected to a digital oscilloscope (TDS2012C, Tektronix, USA). The pulse width is determined by $2n\,(L_0C_0)^{1/2}$, where the number of stages of the L-C ladder was $n = 9$. The same pulse width of 10 μs was applied in all the tests performed.

The PEF treatment chamber was designed as shown in Figure 1. The nutrient solution was filled into the space between the two circular electrodes, which were made of stainless steel with a diameter of 30 mm and a thickness of 5 mm. The distance (d) between the electrodes was 10 mm. The body of the treatment chamber was made of acrylic glass. In all tests, the resistance of each fully filled chamber was kept at $(60 \pm 1)\,\Omega$.

Evaluation of disinfection efficiency

The total plate count (TPC) method (Kobayashi *et al.* 2011) was adopted to detect the microbial density of the suspension. The
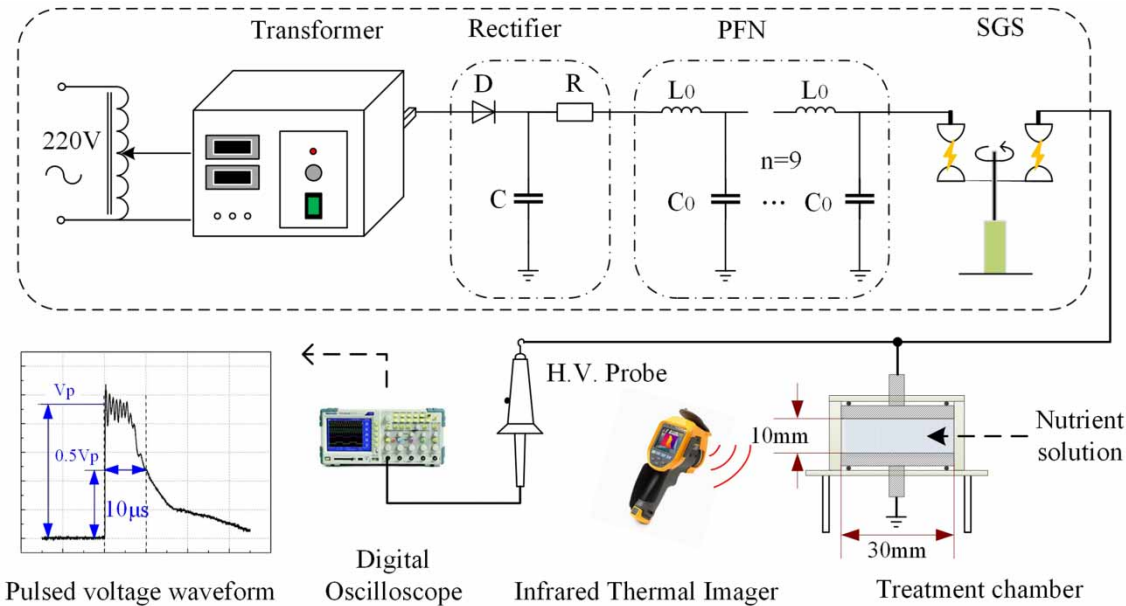
Figure 1 | Schematic diagram of the PEF disinfection system.

disinfection efficiency δ (%) was calculated by:

$$\delta(\%) = \frac{N_0 - N}{N_0} \times 100 \tag{1}$$

where N_0 and N are the colony numbers of *F. oxysporum* before and after PEF treatment, respectively. All experiments were carried out in triplicate.

Experimental design

Experiment of disinfection efficiency versus treatment time

The initial microbial density of the inoculated nutrient solution treated in the experiments was 1.04×10^6 colony-forming units per millilitre (CFU/ml). The 24 chambers were randomly divided into eight equal groups for different treatment times (0 s, 5 s, 10 s, 15 s, 20 s, 25 s, 30 s, and 60 s). The applied pulsed voltage impulse for all the groups was the same: 4.6 kV, 10 μs, 4.5 pulses per second.

Experiment of disinfection efficiency versus PEF strength

To investigate the effect of PEF strength on the disinfection efficiency, 18 treatment chambers were randomly divided into six

equal groups for different PEF strengths (*E*: kV/cm). Each treatment time was 10 seconds. The initial microbial density of nutrient solution for each experiment was 4.15×10^5 CFU/ml.

Experiment of disinfection efficiency versus initial microbial density

The experiment was to investigate the relationship between the initial microbial density and disinfection efficiency. In each experiment, the treatment time was 15 seconds, and the PEF strength was 6 kV/cm. The four initial microbial densities used for the experiments were 3.5×10^3, 3.5×10^4, 4.0×10^5 and 4.0×10^6 CFU/ml.

Experiment of temperature rise in the nutrient solution due to PEF treatment

The experiment was to confirm whether PEF treatment represents the dominant factor of disinfection. An infrared thermal imager (U5855A, Agilent Technologies, USA) was used to measure the temperature of the nutrient solution. The thermal imager captured temperature every 10 seconds during the 60 seconds of treatment. The PEF strength ranged from 4 kV/cm to 7 kV/cm. The measuring point was focused on the surface of the nutrient solution.

RESULTS AND DISCUSSION

Effect of treatment time on disinfection efficiency

Figure 2 shows that the residual microbial density decreased rapidly in the first few seconds. Meanwhile, the disinfection efficiency reached 95.38% under a 5-second treatment. The results demonstrate that the *F. oxysporum* in the nutrient solution was mostly deactivated within a few seconds. This result is very important and implies that, in the near future, a continuous-flow PEF treatment system can be developed.

The maximum disinfection efficiency of 99.84%, acquired during a 60-second treatment, is equivalent to a 2.80-order reduction. Disinfection efficiency increased with the increase of treatment time, however, the growth rate of the efficiency tended to saturate after 15 seconds. It follows that a treatment time of less than 10 seconds is preferable.

Effect of pulsed electric field strength on disinfection efficiency

Figure 3 shows that, as the PEF strength rose from 4 kV/cm to 8 kV/cm, the disinfection efficiency increased from 97.2% to 99.47%. The PEF across cells might lead to the generation of irreversible pores in the membrane, resulting in the leakage of intracellular compounds and even in the death of the cells. With the increase of PEF strength, the probability of electroporation increases correspondingly. The cells of *F. oxysporum* could tend to be inactivated due to the high
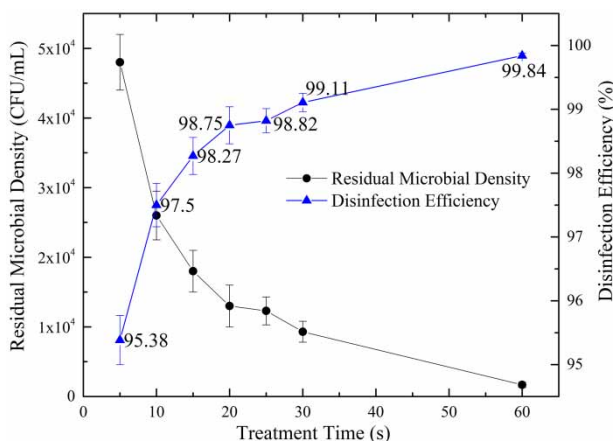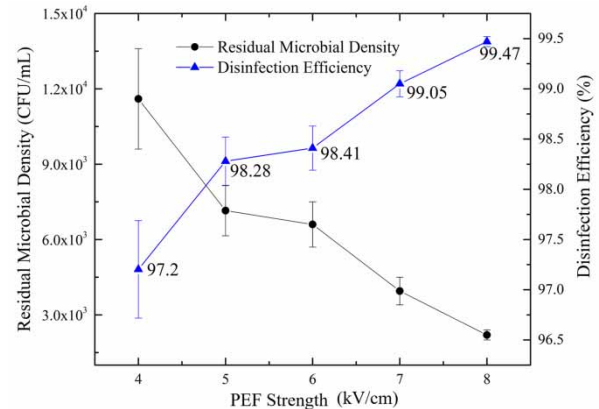

Figure 3 │ The variation of the residual microbial density and of the disinfection efficiency with PEF strength.

occurrence probability of irreversible pores. In practice, for an electric field of 5 kV/cm the resulting average disinfection efficiency was 98.28%.

Influence of initial microbial density

As shown in Figure 4, disinfection efficiency stayed around an average percentage of 97.5% with a fluctuation of less than 1%. This indicates the initial pathogen population has little influence on the disinfection efficiency of the PEF treatment. Since the density of pathogens in actual soilless culture systems varies over a large range, this result implies that the PEF treatment could work well practically in most conditions.
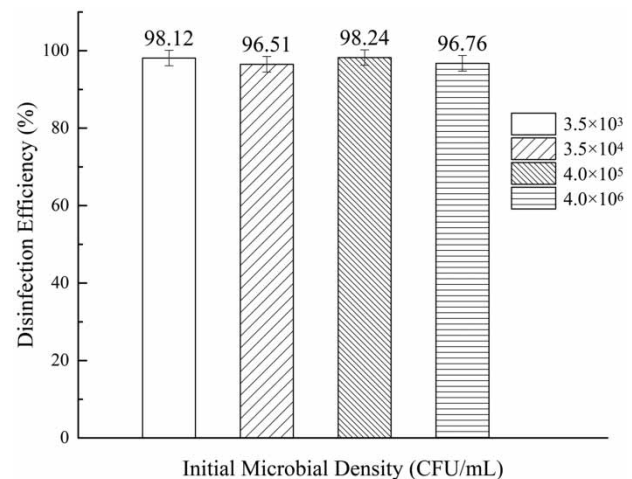

Figure 2 │ The variation of the residual microbial density and of the disinfection efficiency with PEF treatment time.

Figure 4 │ Disinfection efficiency for different initial microbial densities.

Temperature rise of nutrient solution caused by the PEF treatment

It can be observed from Table 1 that the temperature almost linearly increases with the treatment time. As expected, an increase in PEF strength increases the variation of the temperature rise. If no cooling systems are implemented, the temperature would keep rising with the treatment time.

The Joule's loss (Q, unit: J) and the temperature rise generated by a pulsed voltage can be calculated by:

$$Q = (U^2/R) \cdot t \tag{2}$$

$$\Delta T = Q/(C \cdot m) \tag{3}$$

where U (unit: V) is the peak of pulsed voltage, R (unit: Ω) is the resistance of the treatment chamber filled with nutrient solution, t (unit: s) represents the equivalent treatment time obtained by multiplying the pulse-number with the pulse-width, C (unit: $J\,kg^{-1}\,°C^{-1}$) is the heat capacity of the nutrient solution, m (unit: kg) is the mass of the treated liquid and finally ΔT (unit: °C) represents the temperature variation.

For a typical experiment: $U = 6,000$ V, $R = 60$ Ω, $t = 4.5 \times 10^{-4}$ s (45 pulses of 10 µs within 10 s), $C = 4,185.5\,J\cdot kg^{-1}\cdot °C^{-1}$ (using the data of water), 7.1 ml nutrient solution in the chamber. It follows that $Q = 270$ J and $\Delta T = 8$ °C. The corresponding temperature rise measured during the experiment (as shown in Table 1) was equal to 5.4 °C, close to the calculated value. The discrepancy is probably because the calculation did not consider the dissipation of heat. A more accurate calculation will require models based on finite element analysis (FEA).

In Table 1, for a peak pulsed voltage of 7 kV and a treatment duration of 60 seconds, the maximum temperature was 53 °C, corresponding to a temperature rise of about 25 °C. When the treatment time was less than 25 seconds the temperature did not exceed 40 °C, which is below 54 °C, the lethal temperature for *F. oxysporum* (Runia & Amsing 2001b). However, even under these conditions the disinfection efficiency was still high. Clearly this indicates that the PEF played the dominant role in the present disinfection experiments. This means that PEF treatment can be utilized as a non-thermal method for the disinfection of nutrient solution. However, cooling facilities will probably be required to be implemented in future practical PEF treatment systems, to control the temperature.

Energy consumption of PEF treatment method

For a practical PEF technique, energy efficiency and energy density are also two important factors to be considered. Since kilowatt-hour (kWh) is commonly used as a billing unit (1 kWh = 3.6 MJ, $ 0.072/kWh in China), the energy efficiency is defined as the volume (unit: L) of treated nutrient solution per kilowatt-hour (L/kWh). In addition, the definition of energy density adopted here is the electric energy of PEF per millilitre of nutrient solution (J/ml).

Table 2 shows the calculated injected electric energy, energy density, and energy efficiency for different parameters of the PEF treatment. The data in Table 2 indicate that energy consumption very much depends on the treatment conditions. The maximum energy efficiency in Table 2 is 322 L/kWh, for treatment parameters 4.6 kV/cm and 5 seconds. The design of future PEF systems need to consider the balance between two factors: the disinfection efficiency and the energy efficiency. On the condition that the disinfection efficiency is already high, it is reasonable to decrease both the treatment time and the PEF strength.

CONCLUSION

In this work, a PEF treatment method to disinfect recirculating nutrient solution of closed soilless culture systems has been investigated. A Hoagland solution inoculated with

Table 1 | Temperature rise (°C) with PEF strength and time

Treatment Time	PEF strength		
	5 kV/cm	6 kV/cm	7 kV/cm
0s	28	28	28
10s	32.2	33.4	35.2
20s	33.2	34.4	38.4
40s	41	41.4	45.2
60s	45.1	46	53.3

Table 2 | Calculated injected electric energy, energy density, energy efficiency and cost per unit for different parameters such as treatment time and applied voltage

Treatment time (s)	Applied voltage (kV)	Injected energy (J)	Energy density (J/ml)	Energy efficiency (L/kWh)	Cost per unit ($/m^3)	Disinfection efficiency (%)
5	4.6	79.35	11.17	322	0.22	95.38
10	4	120	16.90	213	0.34	97.2
10	4.6	158.7	22.35	161	0.45	97.5
10	6	270	38.03	94.67	0.77	98.4
10	7	367.5	51.76	69.55	1.04	99.05
10	8	480	67.61	53.25	1.36	99.47
15	4.6	238.05	33.53	107.37	0.67	98.27
15	6	405	57.04	63.11	1.15	98.24

F. oxysporum was treated by the novel PEF treatment system. The results indicate that the PEF technique is effective in deactivating most of the *F. oxysporum* in the nutrient solution. The increase of treatment time and electric field strength enhanced the disinfection efficiency, respectively. The maximum disinfection efficiency of 99.84% was obtained under the treatment condition: 4.7 kV/cm, 60 s, 10 µs. Initial microbial density seems not to have an obvious effect on the disinfection efficiency. PEF played the dominant role in the deactivation of *F. oxysporum*. From a practical perspective, the recommended operating conditions for PEF treatment should be short treatment time (less than 10 seconds) and low electric field strength (around 5 kV/cm). The results of this study demonstrate that PEF treatment implies a future technological alternative for the disinfection of nutrient solutions.

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