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Scalable Treatment of Flowing Organic Liquids Using Ambient-Air Glow Discharge for Agricultural Applications

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Received: 20 December 2019; Accepted: 21 January 2020; Published: 23 January 2020

Abstract: In this work, we developed a portable device with low production and operation costs for generating ambient-air glow discharge (AAGD) that is transferred to the surface of flowing liquid and demonstrated its applicability to practical use in agriculture. An experiment procedure that ensured the stable treatment of various liquids was established. Additionally, it was found that humidity did not have a significant effect on the treatment process, which makes the use of the developed device possible in various locations. It was found that an L-phenylalanine solution treated with AAGD allows simultaneous 40% hydroponic radish-sprout growth promotion with a bactericidal effect. Further, scalability and practical-application possibilities in hydroponic plant growth were discussed.

Keywords: glow discharge; plasma treatment; gliding arc; plasma agriculture; phenylalanine

1. Introduction

The fast development of low-temperature atmospheric-pressure plasma sources, which happened in last two decades, has led to the appearance of a huge number of new applications [1–6]. Developments of atmospheric pressure plasma sources allowed treatments of various targets by reactive species not employing vacuum systems; moreover, use of nonequilibrium or so-called “cold” plasmas made possible target treatment without thermal damage, which opened a door for use of plasmas in biomedical and agricultural applications [4,5,7–10]. A promising topic is liquid treatment by plasma and following the use of the treated liquid in medicine or agriculture [4,7,11–13]. During plasma treatment, reactive oxygen and nitrogen species (RONS) are delivered from the gas phase to the surface of the liquid with following dissolving and triggering various chemical reactions [14–16]. Additionally, in the case of organic liquids or other solutions, liquid components could be decomposed through an interaction with plasma or chemical reactions triggered by the plasma treatment [17]. The combination of RONS and other components present in the liquid treated with plasma could have a suppressing effect, such as bactericidal or insecticidal effect and the selective killing of cancer cells, or a promotion effect, such as enhancement of plant-seed germination, plant growth, and even chicken-growth promotion [14–23].

A promising field for the application of plasma technologies is hydroponic plant growth, where a plant grows in a nutrient medium without soil [24]. Considering numerous reports on plant-growth promotion and seed-germination enhancement by plasma treatment, the promotion effect could be
expected from the plasma treatment of a nutrient medium or fertilizers used for hydroponics. There are many reports on the use of organic fertilizers and amino acid fertilizers in particular [25,26]. L-Phenylalanine (L-Phe, C₉H₉NO₂) is a commonly used component of amino acid fertilizers; it was reported that use of L-Phe allows the production of tropane alkaloids to increase (up to industrial scale) as a secondary plant metabolite, and achieve folate (which is an important vitamin) biofortification in vegetables [27–30]. On the other hand, pathogenic bacterial presence in a hydroponic nutrient solution could interfere with plant growth and contaminate the collected crops; therefore, a bactericidal effect of the nutrient solution is desired [31]. A low pH level (below 4.7) is important for the bactericidal effect of plasma-treated liquids; however, a low pH level could be harmful to plants, and the pH of the nutrient solution is commonly kept in range from 5 to 6.5 [20,24]. It was reported that a bactericidal effect could be achieved by the treatment of solutions of organic compounds with a benzene ring (such as L-Phe) with plasma-generated neutral oxygen reactive species (O₃, [O(3P)], OH, and HOO; hereafter oxygen radicals); additionally, L-Phe treated with oxygen radicals allowed a simultaneous bactericidal effect achievement and hydroponic-plant-growth promotion [18]. Reported results look promising for use in agriculture; however, for practical applications, a more effective and scalable way of plasma treatment is desired.

In most cases, low-temperature nonequilibrium atmospheric-pressure plasma jets (NEAPPJs) or dielectric barrier discharges (DBDs) are used for liquid treatments. However, in the case of NEAPPJ, the production rate of reactive oxygen and nitrogen species (RONS) is low, which plays a key role in the bactericidal effect and plant-growth promotion, and liquid irradiation takes a long time (from several minutes to several hours) due to the low density of the plasma [32–35]. A high-density nonequilibrium atmospheric-pressure radical source (NEAPRS), reported elsewhere, allowed the problem of low RONS concentration appearing in the case of APPJ to be solved [14,18,36]. However, noble gases and high voltages are typically required for plasma generation in the case of NEAPRS (as well as in the case of NEAPPJ), and a substantial amount of electrical energy is required to sustain the discharge, when only a limited volume (typically several milliliters) of the medium can be treated at one time [18,32–39]. Low energy efficiency, the requirement of noble gases, and the limited volume of liquid that can be treated make a plasma-activated medium (PAM) expensive, and its use in practical agriculture applications impossible [18]. Considering the cheapest price of ambient air compared to other gases, and the high concentration of nitrogen and oxygen required for RONS production, the most effective way to reduce the cost of plasma treatment is the use of ambient-air high-density plasmas for liquid treatments [16,40–49]. Energy-effective treatment could be achieved by employing the plasma treatment of electrosprayed liquid; however, the reported flowrate for this system is below 1 L/min, which could result in problems with scaling PAM production [16]. A possible way to scale up production and reduce the cost of PAM is use of plasmas generated in ambient air to treat a continuous flow of liquid [19,40]. Several works have reported on treating water flow; however, plasma sources used in these studies either had a considerably low RONS production rate or required the use of noble gases, which could limit the wide application of the produced plasma-activated water (PAW) [40,50–54].

In the case of using liquids treated with plasmas, a PAM could be stored after treatment to reduce temperature and be utilized after storage considering the presence of long-living RONS [14,55]. Additionally, PAM temperature could be reduced by employing liquid flow [19]. For the reason stated above, thermal and “warm” plasmas could be used for medium irradiation following PAM cooling, and use in biomedical applications [19,56,57].

There are many works on the generation of arc and glow discharges, and other types of plasmas inside or on the surface of various liquids; however, there is a limited number of works reporting on treatments of organic liquids for applications in agriculture, and there are no works about the scalable treatment of organic-compound solutions for hydroponic-plant-growth enhancement [3,6,18,58–60]. In this work, we applied a recently reported scalable ambient-air low-current arc-treatment system for treating flowing solutions of organic compounds following the use of the treated medium in hydroponic
plant growth [19]. The reported discharge burns in glow mode rather than in arc considering the discharge characteristics; therefore, in the present work, the authors use the term “glow discharge” instead of “low-current arc”, used in the previous work [19]. The use of a scalable plasma-treatment system in ambient air reported in the present work is a step towards the practical use of PAM in hydroponic plant growth and other applications in agriculture and medicine.

2. Materials and Methods

2.1. Experiment Setup

In the present work, the treatment system of flowing liquid with glow discharge in ambient air that was reported elsewhere was used [19]. The experiment setup is presented in Figure 1. Plasma discharge was generated in ambient air between needle electrode and liquid surface by applying positive high voltage to a needle electrode (anode). A grounded ring electrode (Ni wire, Ni-311386, Nilaco, Tokyo, Japan) was introduced into the liquid, and during discharge, the grounded liquid was used as a cathode. The use of a needle electrode as anode and liquid as a cathode allowed us to avoid electrode erosion and minimize sample contamination by electrode metals.

![Figure 1. Experiment setup.](image-url)

High voltage was produced by a custom power supply consisting of a regulated direct-current power supply (DC PSU), a push–pull generator, a high-voltage transformer, a diode rectifier, and a reservoir capacitor. The discharge gap between liquid surface and the pin of the needle electrode was set at 8.5 mm by using a micromanipulator.

The voltage and current waveforms during the discharge were monitored using a high-voltage probe (P6015A, Tektronix, Beaverton, OR, USA) and a current probe (2877, Pearson Electronics Inc., Palo Alto, CA, USA), and stored in a digital oscilloscope (TBS2000, Tektronix Beaverton, OR, USA). The optical-emission spectrum (OES) from the plasma was recorded using a multichannel spectrometer (HR4000GC-UV-NIR, Ocean Optics, Largo, FL, USA) and a quartz optical fiber.

Liquid flow was employed to maintain the low temperature of the liquid (below 37 °C, which could be used for hydroponics and is safe for human body) after treatment and to produce larger PAM volumes under identical conditions. The treated liquid was introduced to the dish at a flow rate of 10.25 mL/min, and pumped out at a flow rate of 10 mL/min to compensate for liquid evaporation during the plasma treatment and keep the same level of liquid and discharge gap. The use of flowing...
liquid allowed plasma stability to be ensured and provided a possibility to scale up production when
the commonly used treatment of a single small-volume sample (mL scale) is only suitable for laboratory
studies. Additionally, the treatment of small samples may result in deviations in PAM parameters
related to human errors and equipment accuracy) in the case of the preparation of a large volume of
PAM by the collection of numerous small samples treated by plasma.

In the present work, organic compound L-phenylalanine (L-Phe) was dissolved in 2 mmol/L (mM)
phosphate buffer (PB) to a concentration of 80 mM and treated with plasma, with the following use in
hydroponic plant growth or inactivation of bacteria. In all present experiments, we used a discharge
gap of 8.5 mm, 28 W of input power supplied to the push–pull generator, and 10 mL/min liquid flow,
which is referred in the present work as the standard condition.

2.2. Hydroponic Plant Growth

Radish-sprout growth promotion was studied in the same way as was reported elsewhere [18].
Radish-sprout seeds were placed on moistened nonwoven fabric and incubated for 48 h in a growth
chamber (Biotorn, NK System, Osaka, Japan), operated at a controlled temperature of 22 °C and 60%
humidity. A germinated shoot was replanted to a hydroponic growth cell filled with L-Phe, treated with
plasma, and diluted to various concentrations. Replanted shoots were supported by a meshlike plate
to ensure vertical growth. A cell with replanted shoots was placed to the growth chamber (Biotorn, NK
System, Osaka, Japan), operated under the same condition, and radish-sprout length was examined
using a Vernier caliper 48 h after replantation. The same procedure with radish shoots being replanted
to a pristine 80 mM L-Phe was used as a control.

2.3. Micro-Organism Culturing and Colony-Forming-Unit Assay

A survival test of Escherichia coli (E. coli) was performed to evaluate the bactericidal efficacy of
L-Phe treated with glow discharge. E. coli (O1:K1:H7) was precultured in 3 mL of nutrient broth
(DifcoTM, BD, San Jose, CA, USA) at 250 rpm with a regulated temperature of 30 °C for 17 h. Then,
E. coli was separated by a centrifugation at 5000× g for 3 min and diluted with deionized (DI) water to
obtain a bacterial concentration of approximately 1 × 10⁷ mL⁻¹.

Then, an 80 mM L-Phe sample was treated with plasma, and a 0.3 mL E. coli suspension was mixed
with 2.7 mL of the treated liquid and incubated at 250 rpm with a regulated temperature of 30 °C for up
to 48 h. The 0.3 mL E. coli suspension was mixed with 2.7 mL of pristine and 80 mM L-Phe as a control.

Then, the analyzed suspensions of E. coli were diluted, and samples of 0.1 mL were spread onto a
nutrient agar medium (DifcoTM, BD, San Jose, CA, USA) and cultured at 37 °C for 24 h. The number
of E. coli that survived after the treatment were estimated using a colony-forming-unit (CFU) assay.

2.4. Diagnostics of Liquids Treated With Plasma

A deep-ultraviolet (DUV) absorption spectroscopy of DI water treated with plasma was used
for observing the effects of operational conditions on RONS production in treated liquids. The
spectrophotometer (SolidSpec-3700 DUV, Shimazu, Kyoto, Japan) was operated with a fixed spectral
resolution of 0.2 nm and fixed scan speed. Measurements were performed in quartz cuvettes (S10-G-10,
GL Sciences Inc., Tokyo, Japan) with an optical path length of 10 mm. The transmittances of DI water
and PAW were measured with an empty cuvette as reference. The transmittance spectra of pristine DI
water (T) and PAW (T') were converted into absorbance using Equation (1):

\[ \text{Absorbance} = -\log(T'/T). \] (1)

The pH of the treated liquid was measured using a pH meter (S SevenCompactTM pH/Ion,
METTLER TOLEDO, Columbus, OH, USA) and a probe (InLab Pure Pro-ISM, METTLER TOLEDO,
Columbus, OH, USA) immediately after irradiation.
3. Results and Discussion

3.1. Plasma Diagnostics

Typical current and voltage waveforms, optical-emission spectra, and photo of the plasma used in the present work transferred to the surface of DI water using a discharge gap of 8.5 mm are presented in Figure 2.

![Figure 2](image_url)

**Figure 2.** Typical (a) current and voltage waveforms, (b) optical emission spectrum, and (c) photo of plasma transferred to surface of deionized (DI) water.

Figure 2a shows that discharge was repulsing at a frequency of about 60 kHz, with voltage varying in the range of 700–2700 V and current in the range of 0–19 mA. In the case of arc discharge, plasma is sustained by thermal electron emission from the cathode resulting in low voltages (below 100 V) during discharge, when the current is typically in order of tens or hundreds of amperes (more than 5 A even for microarc discharges) [61,62]. In the case of glow-discharge electrons, which sustain plasma, they are formed in the cathode fall area resulting in voltages across the discharge gap in order of hundreds or thousands of volts and currents of few tens of mA [62,63]. From the current and voltage waveforms, the discharge current dropped to 0 if voltage was below the threshold (about 700 V), and the current started to increase if voltage was above the threshold, suggesting that the electrons are produced by a certain electric field. Considering the peak current of 25 mA and peak voltage of 3000 V (which is too large for normal glow), the discharge have burnt in a subnormal glow regime. Moreover, with further increase of input power from 28 W used in the present work to 35 W, the average current starts to increase when the average voltage starts to decrease (data not shown), which is typical behavior for subnormal glow discharge.

Additional evidence that discharge burns in a glow regime was the presence of a $N_2$ second positive emission band in the optical emission spectrum (Figure 2b), which typically originates from the positive column of glow discharge [56]. Additionally, no continuum emission from black-body radiation typical for arc discharges could be observed in the spectra (mostly OH and $N_2$ emission on
Figure 2b), which is another piece of evidence that discharge in the present work was not arc discharge. The structure of the discharge (Figure 2c) ignited between two Cu rod electrodes (using identical conditions) recorded by high-speed camera features strong cathode emission, dark space, emission from plasma bulk, and strong anode emission which is a typical structure of glow discharge (cathode emission, dark Faraday space, positive column, anode emission) [53].

Initially, discharges similar to AAGD were called a gliding arc (in the case of sliding discharge between the electrodes) or low-current arc (in the case of stationary discharge); however, current and voltage waveforms together with optical emission spectra are suggesting that this type of discharge burns in a subnormal or normal glow regime. Similar observations and conclusions were reported elsewhere; therefore, in the present work, we use “ambient-air glow discharge” (AAGD) instead of “ambient air low-current arc” used in previous work [19,64–67].

3.2. Plasma-Treatment Stabilization

Reproducibility is one of the most crucial factors for the practical use of treatment with AAGD. A major factor that could have effects on the treatment process in ambient air is humidity, which could vary in a wide range with location and weather-condition changes. Therefore, it is necessary to verify the effect of humidity on experiment reproducibility before analysis of any experiment data. The expected effect of humidity variation is a change in RONS concentrations delivered to the treated liquid (e.g., density change of OH radicals produced in the gas phase from water molecules), which could have a significant effect on the bactericidal activity of treated liquid and plant-growth promotion. The other possible source of problems with reproducibility is discharge initiation and the change of RONS concentrations in the treated flowing liquid during the initial state.

Most common long-living RONS produced in liquid exposed to ambient air during plasma irradiation are H$_2$O$_2$, NO$_2^-$, NO$_3^-$, and dissolved oxygen O$_{2aq}$. All of these species have strong absorption in the deep ultraviolet and absorption spectrum of the liquid at a wavelength range between 190 and 400 nm, and could provide information about the concentrations of each species, as was reported elsewhere [32,33,45]. An additional advantage of DUV absorption spectroscopy is the short time required for measurement (below 5 min), and the possibility of sample analysis without special preparation (e.g., filtering in the case of HPLC). DUV absorption spectroscopy allows the sample to be analyzed almost immediately after treatment, avoiding the presence of a large number of chemical reactions in the sample before and during measurements. However, in the case of L-Phe, liquid components could be decomposed by the plasma and result in the production of a large number of new components that could interfere with the RONS absorption spectrum in the deep ultraviolet (DUV) range. To avoid the presence of additional absorption peaks in the deep ultraviolet, DI water was treated with ambient-air glow discharge at a controlled humidity to evaluate the effects of humidity and discharge stability on RONS concentrations. An example of the measured spectra of DI water after plasma treatment, fitting the absorption spectrum, and deconvolution of the fitting on RONS absorption peaks is presented in Figure 3.

RONS concentrations are proportional to absorption-peak intensity in deconvolution according to the Beer–Lambert law (Equation (2)):

$$\text{Abs}_x(\lambda) = \varepsilon(\lambda) \times l \times c_x$$

where $\text{Abs}_x(\lambda)$ is absorbance, $\varepsilon(\lambda)$ is the molar absorption coefficient, $l$ is the optical-path length, and $c_x$ is the concentration of the molecule X [34]. The small error in fitting (within 1.5%) observed in Figure 3 is related to the presence of other species in the treated water that had absorbance in the DUV wavelength region, but were not accounted for in the fitting. Mostly H$_2$O$_2$ and NO$_3^-$ absorption peaks were present in deconvolution when the NO$_2^-$ peak was negligible.
Figure 3. Fitting and deconvolution of deep ultraviolet (DUV) absorption spectrum of DI water treated with ambient-air glow discharge (AAGD).

After initiation of the discharge, some instability was observed for several seconds (up to 20 s depending on conductivity) in the case of a 5 mL sample of DI water without flow [19]. However, in the case of flowing liquid, it could take longer to achieve RONS concentration saturation in the liquid that passes through the area of plasma treatment, which means that liquid conductivity increases with an increase of RONS concentration until its saturation point, affecting plasma-discharge parameters. To find the treatment duration required for stable RONS concentration in liquid passing through the plasma-treatment cell, the flowing DI water was treated with AAGD operated at a standard condition. The treated liquid was sampled at various times after discharge initiation, and samples were examined using DUV absorption spectroscopy to observe the effects of treatment duration on absorption in the DUV wavelength region that is related to RONS concentration. The measured absorption spectra and absorption peak intensity at 200 nm are presented in Figure 4.

Figure 4. (a) DUV absorption spectra and (b) intensity of absorption peak at 200 nm of flowing DI water treated with AAGD, sampled at 1, 3, 4, 5, and 10 min after treatment initiation.

The absorption of the treated liquid increased with an increase of plasma-treatment duration, indicating the increase of RONS concentrations. However, after 4 min, the spectrum shape did not change (Figure 4a), and the absorption spectrum remained at the same level of intensity (Figure 4b), indicating the same level of RONS concentrations in the treated liquids.
To avoid absorption-spectrum saturation, PAW samples were diluted by 10-fold, and minor variations in the mean intensity and standard deviation of absorption spectra of PAW after 4 min originate from dilution inaccuracy caused by the used pipette (PIPETMAN Classic P1000, Gilson, Middleton, WI, USA) and human error. For fast measurement, 2.7 mL DI water was prepared in a DUV absorption spectroscopy measurement cell, and 0.3 mL of treated water was suspended to the cell immediately after treatment, resulting in 3 mL of the 10-fold-diluted PAW required for measurements. Pipette accuracy was ± 8 µL, resulting in about 2.5% possible error in suspending 0.3 mL of the liquid, which is represented in Figure 4b. Considering error bars reported in the Figure 4b, it could be concluded that absorbance was stable after 4 min of irradiation within experiment uncertainty. The stabilization of DUV absorption spectra after 4 min indicates that RONS concentrations reached the saturation level, leading to liquid-conductivity and plasma-discharge-parameter stabilization. Therefore, it was necessary to wait for more than 4 min after the initiation of discharge to achieve reproducible RONS concentration.

Absolute values of RONS concentrations in the flowing liquid after 4 min of treatment measured by high-performance liquid chromatography and ion chromatography were 282 µM for H$_2$O$_2$, 4.4 µM for NO$_2^-$, and 520 µM for NO$_3^-$ (same as reported elsewhere for identical conditions) [19]. Obtained concentrations of H$_2$O$_2$ and NO$_3^-$ were at least 1 order of magnitude higher than those reported for conventional He or Ar plasma jets or DBD systems (which are typically in order of µM, considering the volume of produced liquid and duration of the treatment) [32–35]. However, NO$_2^-$ concentration produced by AAGD was negligible compared to that of H$_2$O$_2$ and NO$_3^-$, which correlates well with the deconvolution of DUV absorption spectra in Figure 3. Interestingly, the ratio of RONS concentrations in the present work for AAGD was similar to that observed for spark discharge (mostly H$_2$O$_2$ and NO$_3^-$) and the opposite for glow discharge reported elsewhere (mostly NO$_2^-$), which could be related to differences in discharge conditions [57]. Obtained concentration of NO$_3^-$ was lower compared to conventional systems employing Ar or He, which could be a limitation for the use of AAGD in certain applications requiring large NO$_2^-$ concentrations.

On the other hand, concentrations of H$_2$O$_2$ and NO$_3^-$ in the present work were lower than those reported for other plasma sources employing water flow, such as an electrospray system with corona (about 450 µM of H$_2$O$_2$, 100 µM of NO$_2^-$, and 4500 µM for NO$_3^-$) and spark discharge (about 700 µM of H$_2$O$_2$, 750 µM of NO$_2^-$, and 10,500 µM of NO$_3^-$), and plasma over a flowing-water film (about 2000 µM of H$_2$O$_2$), or air DBD plasma (about 300 µM of H$_2$O$_2$, 150 µM of NO$_2^-$, and 2000 µM of NO$_3^-$, no water flow) reported elsewhere [16,48,54]. However, considering the higher flow rate in the present work (10 mL/min) compared to other systems (1 mL/min for electrospray system and 4 mL/min for flowing water film), it could be concluded that AAGD treatment is comparable to analogs employing liquid flow for RONS production [16,54].

3.3. Humidity Effect

The discharge setup was placed in an acryl chamber with controlled humidity to check the effect of humidity on RONS concentrations in the treated liquid. Generator and pumps were placed outside the chamber and connected to the discharge setup through connectors mounted in the chamber. The chamber (volume about 10 L) was sealed and attached to the air dryer equipped with pump to achieve circulation of the air between chamber and dryer. Additionally, a small flow of dry air was supplied to the chamber to provide higher pressure inside the chamber and ensure that ambient air did not enter the chamber. Air circulation was controlled using a valve allowing to regulate the rate of drying that, together with evaporation of the liquid by the plasma, allowed to control the relative humidity in a range from 30% to 90%. The flow of DI water was treated with AAGD at the same condition at varied humidity, and samples were collected 5 min after discharge initiation to ensure plasma stability and RONS concentration. DUV absorption spectra of the collected samples and absorption peak intensity at 200 nm are presented in Figure 5.
Some variation in the mean of intensity of absorption spectra could be observed with the change of humidity; however, the shape of the absorption peak remained the same (Figure 5a). As was discussed above, considering the error bars represented in Figure 5b, humidity did not have an effect on absorption intensity within the experiment uncertainty. Additionally, the shape of the absorption peak remained the same, suggesting the same ratio among concentrations of produced RONS. Humidity therefore did not have a significant effect on concentrations of produced RONS. The observed effect could be explained by the local evaporation of the liquid in the area contacting with the plasma (about 0.25 mL of liquid was evaporated per minute) [19]. Considering the relatively small gap (8.5 mm) and fast evaporation, the plasma volume and the surrounding area could be saturated with evaporated water, eliminating the effect of humidity in ambient air.

If deionized water was replaced on another liquid with different conductivity, it would mostly affect the duration of the unstable phase in discharge after initiation. In general, solutions of organic compounds (such as 80 mM L-Phe) have much higher conductivity compared to DI water, resulting in much faster discharge stabilization. Therefore, the use of plasma-treated solutions of organic compounds collected 5 min or later after initiation of the discharge ensures stable concentrations of RONS. Moreover, no effect of humidity should be observed in the case of the liquid containing water to compensate variation of humidity by the evaporation of liquid. The effect may not work for liquids not containing water; however, it would work in the case of 80 mM L-Phe.

The flow of liquid containing water could be reproducibly treated with AAGD irrespective of humidity if the treated liquid is collected 5 min or later after the initiation of discharge.

### 3.4. Hydroponic-Plant-Growth Promotion Effect of L-Phe Treated With AAGD

To investigate the effects of 80 mM L-Phe treated with AAGD on plant growth, a radish sprout was placed in L-Phe treated with AAGD under the standard condition. The radish sprout was planted in a pristine 80 mM with L-Phe as a control. Figure 6 demonstrates the length of the radish sprout after hydroponic growth in pristine L-Phe and L-Phe treated with AAGD diluted to various concentrations.

The length of the radish sprout did not change in the case of L-Phe treated with AAGD compared with the pristine L-Phe, indicating that plasma-treated L-Phe under the present condition was not harmful to the plant. Moreover, plasma-treated L-Phe diluted two- and four-fold promotes plant growth resulting in the increase in length of about 30% and 40%, respectively, compared to the control. The observed promotion effect was weaker than that reported elsewhere (up to 99% increase in plant length), where L-Phe was treated with oxygen radicals employing NEAPRS [18]. The difference in the promotion effect could be explained by the difference in concentrations of reactive species formed in L-Phe after the treatment using NEAPRS and AAGD [14,18,19]. Treatment using AAGD mostly forms NOx radicals in the liquid phase, whereas the treatment with NEAPRS using an Ar and O2 mixture,
reported elsewhere, resulted in the presence of mostly oxygen reactive species in the liquid phase. Additionally, L-Phe presented in the pristine solution was decomposed through the interaction with plasma and participated in chemical reactions, leading to the formation of different chemicals after treatment with AAGD or NEAPRS. A possible significant difference in chemical composition between L-Phe treated with AAGD and NEAPRS could be responsible for the decrease of the promotion effect in the case of treatment with AAGD. Treatment with NEAPRS is limited to laboratory use due to high equipment and processing costs, and problems with scaling. On the other hand, treatment of flowing liquid using AAGD does not require process gases (such as He or Ar) and could be scaled up for the production of larger amounts of liquids, which is promising for practical use due to the low cost of the treatment despite a reduced plant-growth promotion effect.

![Figure 6](image_url)

**Figure 6.** Plant-growth enhancement effect on radish sprout cultured in varying concentrations of L-Phe treated with AAGD.

### 3.5. Bactericidal Effect of L-Phe Treated With AAGD

It was reported that in the case of water and other liquids treated by plasma, a low pH of liquid (below 4.7) after treatment is important for a strong bactericidal effect [20]. However, in cases of hydroponic plant growth and other applications in agriculture, a low pH could be harmful for plants, and a bactericidal effect in the neutral pH region (between 5 and 6.5) is desired [18,24]. The bactericidal effect of L-Phe treated with oxygen radicals in a neutral pH region was reported elsewhere [18]. High RONS concentration in liquids treated with AAGD looks promising for a strong bactericidal effect. To investigate the bactericidal effect of L-Phe treated with AAGD, 10 mL/min flow of L-Phe was treated with AAGD under the standard condition. Then, *E. coli* bacteria at a concentration of $10^7$ mL$^{-1}$ were suspended in the treated liquid and incubated for various durations. After incubation, the number of survived bacteria was estimated by a CFU assay. Figure 7 demonstrates the effects of incubation time on the number of survived bacteria in pristine L-Phe, L-Phe treated with oxygen radicals, and L-Phe treated with AAGD.

In the case of L-Phe treated with AAGD, the number of bacteria gradually decreased with an increase of incubation time and then reached the detection limit of colony count (10 CFU/mL) after 48 h, indicating a 6 log reduction. Additionally, the pH of 80 mL L-Phe did not change after treatment with AAGD and remained at a value of 6.3 owing to the use of phosphate buffer. In the case of L-Phe treated with NEAPRS at conditions used for the plant-promotion experiments reported elsewhere, it required 96 h to achieve the same bactericidal effect [18]. L-Phe treated with AAGD had a higher bactericidal effect in a neutral pH region than that treated with NEAPRS owing to a higher RONS concentration and a presence of different plasma-treatment byproducts. Moreover, bacterial concentration did not
significantly change in pristine $L\text{-Phe}$ after 48 h of incubation, indicating that $L\text{-Phe}$ was not responsible for the observed bactericidal effect, and it should have originated from byproducts of plasma treatment and RONS.

**Figure 7.** Viability of *E. coli* incubated in pristine $L\text{-Phe}$, $L\text{-Phe}$ treated with AAGD, and $L\text{-Phe}$ treated with oxygen radicals using nonequilibrium atmospheric-pressure radical source (NEAPRS) [18] as function of incubation time.

Undiluted $L\text{-Phe}$ treated with AAGD did not have a hydroponic-plant-growth promotion effect; therefore, the bactericidal effect of diluted $L\text{-Phe}$ treated with AAGD is important for practical use in hydroponics to suppress various pathogenic bacteria. Even after four-fold dilution, $L\text{-Phe}$ treated with AAGD had a bactericidal effect, and a 3 log reduction in the number of survived bacteria could be observed after 48 h of incubation, which was similar to the bactericidal effect of undiluted $L\text{-Phe}$ treated with NEAPRS [18]. The observed results showed that the flowing $L\text{-Phe}$ treated with glow discharge at ambient air could have a simultaneous hydroponic plant promotion effect and bactericidal properties. Additionally, it was reported that $L\text{-Phe}$ treated by plasma could preserve the bactericidal effect for more than one week, which allows to store and transport treated $L\text{-Phe}$ before use [18].

The proposed method uses ambient air (which is the cheapest possible gas), utilizing an $L\text{-Phe}$ solution (which is a typical fertilizer component) as a base material, and could be scaled considering flowing-liquid treatment. Moreover, the amount of produced plasma-treated $L\text{-Phe}$ could be increased by tuning power supplied to the plasma and flow rate, and increasing generator number. If the PAM produced by AAGD is not immediately supplied to the target after treatment, a larger amount of PAM could be produced by high-power discharge (which results in heating PAM to temperatures above 37 ℃) and cooled down before use. The possibility of scaling, inexpensive devices, and low operational cost is promising for the practical use of plasma treatment in hydroponics and other agricultural or biomedical applications.

The AAGD system could be introduced to existing hydroponics systems in several ways. A possible approach is to produce a suitable amount of plasma-treated $L\text{-Phe}$, dilute it to the desired concentration, and supply it directly to a hydroponic-plant-growth cell, as was done in the present work. Another approach is to organize liquid circulation between hydroponic-plant-growth cell and plasma-treatment device, and continuously treat circulating liquid or during a fixed amount of time per day. Direct treatment of the liquid medium during plant growth is advantageous for on-site liquid sterilization considering the stronger bactericidal effect of direct plasma treatment of suspension containing bacteria compared to the bactericidal effect of liquid treated with plasma, as was reported elsewhere [19]. Bacteria suspended in DI water could be sterilized after 30 s of treatment of suspension with AAGD, when it requires 60 min for bacterial sterilization by suspending it to DI water treated by AAGD for
1 min, which could be related to the presence of short-living radicals (such as peroxynitrite and HOO\(^\bullet\)) and the strong electric field and ultraviolet emission during the plasma treatment [19].

Despite the use of air as a process gas, low cost and simple setup, and the high concentration of RONS in treated liquid, power consumption of the present system (28 W) is higher compared to that of analogs, which could be a problem in large-scale application [16,40,48,54,57]. A problem with power consumption could be partially solved by the improvement of plasma-generator efficiency (currently only about 50% of power is absorbed in plasma) and fine-tuning the treatment parameters (matching liquid-flow rate and discharge power to achieve desired RONS concentration in the liquid without following dilution). On the other hand, it is not yet clear which components of the treated solution are responsible for plant-growth promotion and which for the bactericidal effect. Treatment by plasma results in RONS delivery to the liquid, triggering the chemical reactions and decomposition of organic components [17,68]. Products of plasma treatment could vary in a wide range depending on plasma type and parameters, resulting in different properties of produced PAM (e.g., stronger bactericidal effect after treatment by AAGD compared to NEAPRS reported elsewhere). Therefore, it is necessary to perform precise analysis of products produced by plasma and components responsible for plant-growth promotion and bactericidal effect for reliable comparison to treatment with alternative plasma sources or chemical methods.

4. Conclusions

In this work, we developed a scalable device for the treatment of flowing organic solutions using a glow discharge in ambient air. Further, we found operational conditions that enable a stable discharge process with the same liquid parameters after treatment. Humidity did not have a significant effect on the treatment process owing to local evaporation of liquid by plasma. We used an 80 mM \(L\)-Phenylalanine (\(L\)-Phe) solution in phosphate buffer for the experiments. \(L\)-Phe treated with glow discharge diluted to certain concentrations could have a simultaneous hydroponic-plant-growth promotion effect and bactericidal effect. The proposed system could work in ambient air under various conditions and be easily integrated to existing systems for hydroponic plant growth, which looks promising for practical use. In the future, further improvement of energy efficiency and analysis of components produced by treatment of \(L\)-Phe with ambient-air glow discharge is desired.

Author Contributions: Experiments, investigation, and writing—review and editing, V.G.; Survival test of \(E\). \(coli\), N.I.; Plant-growth promotion G.I.; Formal analysis, M.H. (Masaru Hori); Funding acquisition, M.H. (Mineo Hiramatsu); Validation and supervision, M.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, under grant number S1511021; and JSPS KAKENHI, grant numbers 19H05462 and 19H01889; and Plasma-Bio Consortium project, grant number 01221907.

Acknowledgments: Authors are grateful to Jun-Seok Oh of Osaka City University for fitting and deconvolution of DUV absorption spectra.

Conflicts of Interest: The authors declare no conflict of interest.

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