Title

Electro-degradation of culture solution improves growth, yield and quality of strawberry plants grown in closed hydroponics

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Research highlights

- In recycled hydroponics growth and yield of strawberry plants inhibited by autotoxicity
- Strawberry plant roots exudates benzoic acid during autotoxicity
- Benzoic acid degradation was faster in electrode using alternate current than direct current
- AC-ED in non-renewed solution improved growth, yield and quality of strawberry

Electro-degradation of culture solution improves growth, yield and quality of strawberry plants grown in closed hydroponics

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Abstract

Strawberry plants grown in closed hydroponics accumulate root exudates and cause autotoxicity- a form of intra-specific allelopathy. Root exudate contains several allelochemicals and among them benzoic acid (BA) found as the most potent growth inhibitor. In this study we applied electro-degradation (ED) to the culture solution in order to degrade their root exudates and improving growth, yield and quality of strawberry. There were four types of nutrient solution used in this study viz. renewed, non-renewed, non-renewed with direct current electro-degradation (DC-ED) and non-renewed with alternative current electro-degradation (AC-ED). Every three weeks interval, culture solutions were changed with fresh 25% standard Enshi nutrient solution in renewed treatment, while DC- and AC-ED treatment were applied in non-renewed solutions. Significantly greater fruit yield (225.9 g plant^{-1}) was obtained from renewed

nutrient solution, which was statistically similar to fruit yield in non-renewed solution with AC-ED application. Compared to renewed solution, fruit yield was decreased to about half (114.0 g plant^{-1}) in non-renewed solution while non-renewed with DC-ED produced intermediate yield between non-renewed and renewed solution or non-renewed with AC-ED. In general, growth performance was greater in renewed solution followed by non-renewed with AC-ED, while in non-renewed solution decreased significantly similar to DC-ED. A similar trend was observed in vitamin C content while brix and citric acidity was not varied. Minerals such as calcium and iron concentration in the culture solution were significantly decreased in DC-ED, consequently their contents were also found lower in crowns and roots compared to other solutions used. Therefore, it is evident that growth, yield and quality of strawberry can be improved through application of AC-ED in non-renewed solution.

Key word: autotoxicity**,** strawberry plant, root exudates, benzoic acid, electro-degradation, direct current, accelerate current, recycled hydroponics

1. Introduction

Hydroponic culture of a wide variety crops has been practiced in many countries since the 1950s, and the use of closed hydroponic systems has been encouraged recently (Ruijs 1994; Van Os 1995) to reduce environmental pollution and the cost of supplementary nutrients. Strawberry has also been grown hydroponically for higher yield and better quality compared to soil cultivation. In protected cultivation technique, large-scale production of strawberry through open system hydroponics discharge once used nutrient solution to the environment causing pollution and wastage of costly fertilizers. Therefore, commercial strawberry growers practiced closed hydroponic system for sustainable production (Takeuchi 2000; Oka 2002). However, under this closed hydroponic culture technique, autotoxicity- a form of interspecific allelopathy develops due to continuous accumulation of allelochemicals in the culture solution (Asao et al. 2003, 2007; Kitazawa et al. 2005). It is known that, this autotoxicity phenomenon occurs when a plant releases toxic chemical substances into the environment that inhibit germination and growth of same plant species (Miller 1996; Singh et al. 1999).

In strawberry, autotoxicity from root exudates has been studied in closed hydroponics and benzoic acid was confirmed as the most potent growth inhibitor (Kitazawa et al. 2005). Other studies showed that, when root exudates accumulated in their growing medium, the growth and metabolism of strawberry roots were inhibited, which resulted in an increase in the percentages of electrolytes in cells, a decrease in the free radical scavenging activity of roots, and an increase in root lipid peroxidation (Zhen et al. 2003). Under autotoxicity condition, damaged strawberry roots hamper water and mineral nutrient uptake. As a result, the growth of shoot and root, number of flowers and harvested fruit per plant and fruit enlargement greatly reduced (Kitazawa et al. 2005).

Elimination of the accumulated root exudates or autotoxic growth inhibitors from closed hydroponic system would be of great interest to the strawberry grower. The removal or degradation of these accumulated autotoxic growth inhibitors in the culture solution would lead to sustainable strawberry production. Our research group applied several ways to detoxify these exudates including adsorption by activated charcoal (Asao et al. 1998; Kitazawa et al. 2005), degradation by microbial strains (Asao et al. 2004a), and auxin treatment (Kitazawa et al. 2007) etc. Degradation of toxic compounds by electronic means is another way to detoxify allelochemicals. Phenolic compounds in aqueous solutions were found to decompose when treated by electro-degradation (ED) such as phenol (Comninellis and Pulgarin 1991; Feng and Li 2003; Fleszar and Ploszynka 1985), catecol (Comninellis and Pulgarin 1991), and hydroquinone (Comninellis and Pulgarin 1991; Fleszar and Ploszynka 1985), in aqueous solutions and benzene (Fleszar and Ploszynka 1985). These compounds are oxidized rapidly at the anode and decompose to CO_2 (Comninellis and Pulgarin 1991; Feng and Li 2003; Fleszar and Ploszynka 1985). Therefore, ED can also be applied to decompose allelochemicals, including benzoic acid exuded into the culture solution from plants and could be useful to mitigate autotoxicity in the hydroponic cultivation of strawberry.

In our previous study, autotoxicity in hydroponically grown strawberry plant was reported to mitigate through application of ED of root exudates (Asao et al. 2008). In this process,

exogenously added benzoic acid to a culture solution was almost completely decomposed within 24 hours by direct current electro-degradation (DC-ED). Moreover, they showed that DC-ED application to the culture nutrient solution could result in the decomposition of toxic root exudates, including BA from strawberry plants, and mitigate the effect of autotoxicity under closed hydroponics. They also reported that a rapid decomposition of Fe-EDTA in culture solution due application of DC-ED. In the following study, it was also found that DC-ED can breakdown the benzoic acid in the nutrient solution but it also decreases the iron and calcium concentrations, pH and increase solution temperature (Asaduzzaman et al. 2012). In DC-ED, iron and calcium ions were thought to be precipitated to the anode.

In order to overcome these issues associated with DC-ED, we planned to change the power source from DC to AC. In case of AC electro-degradation (AC-ED), positive and negative charges of the electrodes (anode and cathode) changes frequently. Thus, iron and calcium ions might not be precipitated to the electrode (especially the central core). We hypothesized that, application of AC-ED instead of DC-ED would result in degradation of benzoic acid from the closed hydroponics without altering properties of nutrient solution. In this study, we applied AC-ED in order to investigate the ED conditions, growth, fruit yield and qualities of strawberry grown in closed hydroponics, where nutrient solutions are not renewed throughout the growth period.

2. Materials and methods

2.1. Plant material

Strawberry (*Fragaria* × *ananassa* Duch. cv. Toyonoka) plantlets produced through plant tissue culture were used for this experiment. Micro-propagated strawberry plantlets were transferred into cell trays (48 cm × 24 cm × 4 cm, 72 cells/tray) with vermiculite substrate and were kept there for about 60 days under control growth chamber condition at 20/15 ºC (day/night), 60% relative humidity, fluorescent light with intensity of 145 μmol m^{-2} s^{-1} and a 12 hours photoperiod

for the formation of new roots and leaves. 25% standard "Enshi" nutrient solutions were used for growing strawberry plants in the cell trays.

At five-seven leaf stage, strawberry plantlets were transferred to grow beds of hydroponic system for nursery in an environment control room. Thirty eight plantlets were accommodated in each grow bed and there were three grow beds placed vertically in hydroponic system. 300 L, 25% standard "Enshi" nutrient solutions were used for hydroponic system and solution was renewed bi-weekly. Nutrient solutions were supplied at 55/5 min. (recycle/stop) by an automatic pump (KP-101, Koshin, Kyoto, Japan) with an automatic timer (KS-1500, Iuchi, Osaka, Japan) and maximum discharge of 31 L/min. Strawberry plantlets were kept in the nursery until the flowering of first cluster. Then the clusters were removed and more homogenous plants were selected as planting materials.

2.2. Nutrient solution

Strawberry plants were cultured in 25% standard 'Enshi' nutrient solution [Table S1; pH 7.25 and electrical conductivity of 0.8 dS m^{-1}] throughout the growth period. The electrical conductivity and pH of the tap water used to prepare the nutrient solution were 0.22 dS m^{-1} and 8.18, respectively.

2.3. Electrode used for electro-degradation of nutrient solution

We used small AC and DC type electrode (designed and built by Yonago Shinko Co., Ltd., Tottori, Japan) for electro-degradation of benzoic acid or autotoxic chemicals in without plant nutrient solution or culture solution used for strawberry (Fig. S1). In case of DC-ED, an electrode having a central core made of ferrite with a surface area of 65.9 cm^2 (anode) which enclosed with cylindrical tube made of titanium with a surface area of 103.7 cm^2 (cathode) (Asaduzzaman et al. 2012). While in AC-ED, the electrode had a central core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) which enclosed with cylindrical tube also made of titanium with a surface area of 95.5 cm^2 (cathode/anode). The nutrient solution can pass

through the electrode where electro-degradation takes place. The electrodes were coupled with a digital AC power supplier (AD-8735D, AND, Japan).

2.4. Experiment I

2.4.1. Selection of AC frequency for electro-degradation of BA in culture solution

In order to select the suitable frequency for AC-ED, three different frequencies viz. 500, 1000, and 1500 Hz were tested in nutrient solution containing benzoic acid (BA). At first 10 L of 25% standard "Enshi" nutrient solution was prepared with tap water and then 0.4885 g of BA was added to reach concentration of 400 μmol L^{-1} BA. Plastic containers (450 mm × 370 mm × 100 mm) were used for each frequency. In all cases, the AC-ED electrode was applied at 50% duty ratio, 2.0 amperes alternate current, and 14.0 volts. Nutrient solution samples (25 ml) were collected at 0, 1, 3, 6, and 24 hours of AC-ED application for measuring concentration of benzoic acid. Conditions of nutrient solution such as temperature, EC, and pH were recorded at each sampling. EC was measured by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan) while, temperature and pH were measured using pH meter (D-12, Horiba, Ltd., Kyoto, Japan) at each sampling.

2.4.2. Determination of BA concentration in the AC-ED treated nutrient solution

The collected nutrient solution samples at 0, 1, 3, 6, and 24 hours of AC-ED application were filtered through HPLC filter (0.20 μM, DISMIC-13, HP Membrane filter, Toyo Roshi Co., Ltd. Japan). Each filtrate (25 μL) was injected into a high performance liquid chromatography (HPLC) system (column oven L-2350, detector L-2400, and pump L-2130; Hitachi, Tokyo, Japan) to measure the concentration of benzoic acid in the nutrient solution. The analytical conditions were as follows: column: ODS 4.0 × 200 mm (Wakosil 10C18; Wako Pure Chemical Industries, Ltd., Osaka, Japan); eluent: CH_3CN/10 mM H_3PO_4 = 30/70 (v/v); flow rate: 1.0 ml min^{-1} at 30 °C; and detection: ultraviolet 254 nm.

2.5. Experiment II

2.5.1. Electro-degradation of culture solution in without plant experiment

AC-ED at the selected frequency (500 Hz) was compared with DC-ED in nutrient solution following a without plant experiment. Following similar procedure as to experiment I (section 2.4.1), three sets of nutrient solution containing 400 µmol L^{-1} BA were prepared. Electro-degradations were applied as DC-ED, AC-ED and control (without ED) for 24 hours (Fig. S2). The DC-ED was applied at 2.0 ampere and 18.0 volts, while the AC-ED conditions were the same as previous experiment at frequency of 500 Hz. Nutrient solution samples were collected for measuring benzoic acid at 0, 1, 3, 6, and 24 hours of ED. Temperature, EC, pH and benzoic acid concentration in electro-degraded nutrient solution were measured following methods as described in section 2.4.2.

In plastic bottles 25 ml samples were collected after 24 hours of ED process for the analyses of major nutrients. Nutrient solution was filtered with qualitative filter paper (Advantec Grade no. 131; 125 mm). Major mineral nutrients such as K^{+}, Ca^{2+}, Mg^{2+}, and Fe^{3+} was measured with an atomic absorption photometer (Z-2000, Hitachi High-Technologies Corporation, Kyoto, Japan), NO^{3-} with a compact NO_3^- meter TWIN NO_3^- (B-343, Horiba, Ltd., Japan) and PO_4^{3-} using spectrophotometer at 720 nm (U-2900, Hitachi High Technology, Tokyo, Japan).

2.6. Experiment III

2.6.1. Cultivation of strawberry in non-renewed solution treated with DC- and AC-ED

Healthy strawberry plantlets selected from nursery were used for this culture. Plantlets were grown in control room by maintaining a relative humidity of 60%, CO_2 concentration of 800 ppm, fluorescent light with intensity of 145 µmol m^{-2} s^{-1} and a photoperiod of 12 hours. Plantlets were planted to three stage vertical growing beds (125 cm × 90 cm × 10.5 cm). On 20th February 2016, five plantlets were planted in each growing bed fixed with urethane cubes (23 mm × 23

mm × 27 mm) in a controlled room at 25/20 °C (day/night) temperature. Three growing beds were filled with 25% standard "Enshi" nutrient solution with each capacity of 50 L connected to a 300 L reservoir tank. Nutrient solutions were recycled at 55/5 min. (recycle/stop) by an automatic pump (KP-101, Koshin, Kyoto, Japan) with an automatic timer (KS-1500, Iuchi, Osaka, Japan) and maximum discharge of 31 L/min.

There were four types of culture solutions viz. renewed tri-weekly, non-renewed, non-renewed with DC electro-degradation tri-weekly for 24 hours and non-renewed with AC electro-degradation tri-weekly for 24 hours. In renewed culture system, nutrient solutions were renewed tri-weekly. While non-renewed nutrient solutions, major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Fe^{3+}) concentration were adjusted at every three weeks interval as close as possible to the initial concentration of the 25% "Enshi" solution based on the chemical analyses described previously in section 2.5.1. The DC- and AC-ED were applied in the nutrient solution for 24 hours at three weeks interval in the setting as it was applied in without plant experiment (Fig. S3). Pollination was carried out using a calligraphy brush every 2 or 3 days. Harvest was carried out when the whole fruit or 80% of the fruit turned to red color. First harvest was carried out on 5th April 2016 and final harvest on 7th July 2016. Data were collected on growth parameters, chlorophyll content (measured by SPAD, Konica Minolta, Tokyo, Japan), and yield attributes at the final harvest.

2.6.2. Determination of strawberry fruit qualities

Fruits were composited after each harvest and were frozen at –30 °C for subsequent analysis of soluble solids, titratable acids and ascorbic acid content. Fruit samples were kept out of freezer before analysis to obtain sufficient juice for determining the above qualities. The soluble solid content of the fruit was determined using a digital refractometer (PR-1, Atago Ltd., Japan). Titratable acid contents were determined by diluting each 2 ml aliquot of strawberry juice to 10 ml with 8 ml distilled water and added 2–3 drops of phenolphthalein then adjusted the pH to 8.2 using 0.1 N (w/v) NaOH. The quantity of NaOH (ml), and the amount for appropriate acidity was converted into citric acidity (%). Ascorbic acid content was measured with 2, 4-

dinitrophenylhydrazine (DNP) colorimetry. Strawberry fruit juice (0.5 ml) was taken in 50 ml test tube then 0.5 ml of 10% meta- phosphoric acid solution, 1 ml of distilled water, 1 ml of 0.03% 2,6-dichlorophenol-indophenol (DCP), 2 ml of thiourea and 1 ml of DNP was added to the samples following 3 hours incubation at 37 °C in water bath. After incubation 5 ml of 85% H_2SO_4 were added to each sample keeping in water cooled with iced water. After 30 minutes cooling, ascorbic acid content was measured at 540 nm by spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan).

2.6.3. Determination of mineral nutrient content in plant parts

Mineral nutrients content in strawberry plants were also recorded. Strawberry plant parts were separated into leaves, crown and roots and kept in a constant temperature oven (DKN812, Yamato Scientific Co. Ltd. Japan) for 72 hours at 80 °C. When the dry matter reaches constant weight, it was ground into powder with a mixer machine (National MX-X53, Japan). Samples weighing 0.25 g were mixed with 8 ml of HNO_3 and digested by microwave sample preparation system (ETHOS 1, Milestone S.r.l, Bergamo, Italy). After digestion samples were measured up to 50 ml of volumetric flask and then filtered with qualitative filter paper (Advantec Grade no. 131, 185 mm). The filtered sample solutions were analyzed for mineral nutrients by atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

2.6.4. Measurement of temperature, EC, pH and determination of mineral nutrients of culture solution

Conditions of culture solution such as temperature, EC, and pH were recorded at every three weeks interval after ED application following the procedure as described in section 2.4.1. Amount of mineral nutrient remains in the culture solution were determined following the analytical procedures as described in section 2.5.1. Data were taken five times throughout the growing period.

2.7. Experimental design and statistical analysis

In experiment I, three different frequencies of AC-ED were evaluated and repeated sampling was done for each sampling. Each data represented means of five observations. Similarly in experiment II, three types of ED were applied to decompose BA and each data is the mean of five observations. In experiment III, four types of culture solutions were arranged in a completely randomized design with three replications. Analysis of variance for all data was done using computer package MSTAT-C developed by Russel (1986). The mean differences of each culture solution were separated according to Tukey's test at $P < 0.05$.

3. Results

3.1. Selection of frequency for AC-ED of BA in the nutrient solution (Experiment I)

The degradation of BA in nutrient solution under three different frequencies of AC was investigated. The concentration of BA decreased gradually over time. The amounts of BA (initially 400 µM L^{-1}) in the nutrient solution were measured as 370, 339, 247 and 0 ppm after 1, 3, 6, and 24 hours of AC-ED, respectively at frequency of 500 Hz. Similarly, BA concentrations were decreased to 385, 320, 231 and 5 ppm after 1, 3, 6, and 24 hours, respectively at 1000 Hz; 392, 300, 245 and 5 ppm after 1, 3, 6 and 24 hours, respectively at 1500 Hz (Fig. 1). Results showed that BA in the nutrient almost completely degraded after 24 hours due to application of AC-ED at all three frequencies. Although EC and pH of the treated nutrient solution were not varied greatly, temperature of the solution increased with the increase of AC frequency (Fig. 2). It showed that, significantly higher temperature of nutrient solution was recorded at 1500 Hz followed by 1000 Hz and 500 Hz of AC-ED.

3.2. Electro-degradation of nutrient solution in without plant experiment (Experiment II)

DC-ED and AC-ED were applied in the nutrient solution following a without plant experiment to investigate the degradation of BA. The concentration of BA was decreased sharply until 6 hours

of ED while it was not decreased considerably in control where ED not applied (Fig. 3). Compared to DC-ED, AC-ED showed faster BA degradation in all sampling stage and it was completely degraded at 24 hours. After 24 hours DC-ED, about 100 ppm BA remains in the treated nutrient solution while it was remains about as initial (about 400 μM L^{-1}) in control condition. Results showed overall decreasing trend of BA concentration as 341, 243, 135, and 0 ppm after 1, 3, 6 and 24 hours, respectively by AC-ED whereas, 336, 314, 224 and 67 ppm after 1, 3, 6 and 24 hours, respectively by DC-ED application.

Physical and chemical conditions of nutrient solution were also affected by the application of ED (Fig. 4). EC and pH were not affected by the either type of ED applied and control. However, temperature of the nutrient solution varied greatly. In DC-ED, temperature was raised significantly (7.7 °C) compared to AC-ED after 24 hours. In control and AC-ED, it was not raised greatly rather remain similar as initial.

Application of DC-ED and AC-ED also influenced major mineral nutrient content in culture solution (Table 1). Nitrogen, phosphorous, potassium, and magnesium concentration in the nutrient solution was not affected by the ED application and control. Interestingly, calcium and iron concentration was decreased significantly in DC-ED compared to AC-ED and control after 24 hours.

3.3. Application of DC- and AC-ED on the culture solution used for growing strawberry plant (Experiment III)

3.3.1. Effect of DC- and AC-ED on the growth of strawberry

Several growth parameters of strawberry were significantly affected by the application of ED in the non-renewed culture solution (Table 2). Long root length, leaf length and width, SPAD value and crown diameter were not affected the ED treatment. Number of leaves was significantly decreased in plants grown in non-renewed solution compared to renewed solution. While application of either DC- or AC-ED showed statistically similar number of leaves as it was produced in renewed or non-renewed solution. Leaf fresh weight was highest (28.1 g $plant^{-1}$) in

renewed culture solution and non-renewed culture solution with AC-ED, which was followed by non-renewed culture solution with DC-ED. The lowest leaf fresh weight was observed in non-renewed culture solution. Crown fresh weight followed similar trend. The crown fresh weight was the lowest (9.1 g plant^{-1}) in non-renewed culture solution where no ED was applied. Renewed culture solution and non-renewed culture solution with AC-ED produced significantly higher crown fresh weight, which was followed by non-renewed culture solution with DC-ED. Correspondingly, the highest dry weight of leaf (7.7 g plant^{-1}), crown (2.6 g plant^{-1}) and root (4.1 g plant^{-1}) was obtained from renewed culture solution and they were statistically similar with plants grown in non-renewed solution with AC-ED followed by DC-ED. The lowest dry weight of leaf, crown and root was obtained from non-renewed culture solution.

3.3.2. Effect of DC- and AC-ED on the fruit yield and yield attributes of strawberry

Yield attributes and fruits yield was significantly affected by types of culture solution used (Fig. 5 A). Number of fruit per plant greatly decreased (about 50%) in non-renewed culture solution compared to renewed culture solution. Plants grown in non-renewed culture solution with AC-ED application produced statistically similar number of fruits as renewed solution. However, plants grown in non-renewed culture solution with DC-ED produced intermediate type of fruits number. Individual fruit weight followed similar trend as it was found in number of fruit per plant. It was highest in renewed culture solution which was identical to fruits obtained from plants grown in non-renewed culture solution with AC-ED. The lowest individual fruit weight (6.9 g plant^{-1}) was obtained in non-renewed culture solution.

Fruit yield in different culture solutions were corresponding to their yield attributes (Fig. 5 B). The lowest fruit yield (114.0 g plant^{-1}) was recorded from plant grown in non-renewed culture solution. While the highest fruit yield was recorded in plants from renewed culture solution, followed by plants grown in non-renewed culture solution with AC-ED. However, plants grown in non-renewed culture solution with DC-ED application did not improved fruit yield greatly. Results indicated that about 49% yield was increased due to application of DC-ED in non-renewed culture solution compared to non-renewed culture solution entirely. When AC-ED

360 applied to non-renewed culture solution about 86% fruit yield was increased compared to non-
361 renewed culture solution.
362
363 ***3.3.3. Effect of DC- and AC-ED on the fruit qualities of strawberry***
364
365 The qualities of strawberry fruits were not differed significantly until fourth cluster except
366 vitamin C content (Table 3). The highest vitamin C content fruits were found in plants grown in
367 non-renewed culture solution treated with AC-ED from cluster I to IV, which was statistically
368 similar with fruits obtained from plant in renewed culture solution. In general, the lowest vitamin
369 C content fruits were obtained from plants grown in non-renewed culture solution and non-
370 renewed culture solution with DC-ED in all four clusters.
371
372 ***3.3.4. Effect of DC- and AC-ED on mineral contents in strawberry plant parts***
373
374 Electro-degradation of non-renewed culture solution significantly affect the mineral nutrient
375 content especially calcium and iron in crown and root of strawberry plants (Table 4). Other
376 minerals like potassium and magnesium in all plant parts was not affected by ED application. In
377 root and crown, both calcium and iron content were decreased significantly in non-renewed and
378 non-renewed with DC-ED application.
379
380 ***3.3.5. Effect of DC- and AC-ED on temperature, EC, pH and mineral nutrient content of***
381 ***culture solution used for strawberry***
382
383 Temperature, EC and pH of the culture solution measured were not differed significantly
384 throughout the growing periods (Table 5). In non-renewed culture solution, the amount of
385 calcium and iron were also found to be decreased due to application of DC-ED. While amount of
386 other minerals (nitrogen, phosphorus, potassium and magnesium) were not decrease considerably
387 due to application of either DC- or AC-ED. In non-renewed culture solution, application of DC-
388 ED results in significant decrease in calcium and iron.
389

4. Discussion

In non-renewed hydroponic culture of strawberry, several allelochemicals were found to be exuded from roots and BA was one of them (Kitazawa et al. 2005). Due to continuous accumulation of these allelochemicals including BA in the culture solution, plant roots become injured impairing water and mineral nutrient uptake and thus growth and normal activity of roots are hampered. Subsequently, the growth and yield of strawberry decreased. Research reports suggested several ways to eliminate these allelochemicals from the culture solution (Asao et al. 1998; Asao et al. 2004a; Kitazawa et al. 2005, 2007; Asao et al. 2008; Asaduzzaman et al. 2012; Mondal et al. 2013, 2015).

Our previous studies suggested that ED of nutrient solution using direct current could mitigate autotoxicity of plants in closed hydroponic culture (Asao et al. 2008; Asaduzzaman et al, 2012), but these methods had some troubles such as degradation of Fe-EDTA, low concentration of Ca^{2+} in the treated culture solution, decrease in solution pH and increase in solution temperature. In order to overcome these problems, we modified the ED electrode and also power source from DC to AC. In our present study, we used AC-ED electrode to compare its efficiency with previously used DC-ED electrode to decompose autotoxic chemicals in non-renewed culture solution of strawberry.

Suitable electrolysis conditions (2.0 amperes and 18.0 volts) for DC-ED electrode to degrade BA were investigated in the earlier studies (Asaduzzaman et al. 2012). However, for AC-ED machine suitable electric condition was not determined. Therefore, we examined three frequencies (500 Hz, 1000 Hz and 1500 Hz) against the degradation of BA. In all cases frequencies 50% duty ratio, 2.0 ampere and 14.0 volts were maintained. All these three frequencies were equally effective for degradation of BA (Fig. 1). However, the gradual rise of culture solution temperature was recorded in the higher frequency (1500 Hz). This increased temperature may negatively affect the plant root growth and development. Recent studies reported that temperature at the root-zone influences the growth and chemical composition of many plants (Adebooye et al. 2010; Malik et al. 2013; Yan et al. 2013; Sakamoto and Suzuki

2015a, 2015b). The high root-zone temperature (about 30 °C) for strawberry in a deep flow technique hydroponic system decreased oxygen consumption and cell viability of the roots, resulting in withering of the plants (Sakamoto et al. 2016). Therefore, in our studies, ED of benzoic acid without an augmented temperature in culture solution, use of 500 Hz frequency would be suitable.

In the following study, we compared the efficiency of DC-ED and AC-ED electrode against the degradation of BA in without plant experiment. In both cases, degradation of BA was observed, but rate of degradation was faster in AC-ED and it was found that, after 24 hours, BA was completely degraded but there some residues (about 100 ppm) remained in DC-ED (Fig. 3). Other studies reported that, phenolic compounds in aqueous solutions can be degraded through electro-chemicals means (Comninellis and Pulgarin 1991; Feng and Li 2003; Fleszar and Ploszynka 1985). In nutrient solution without application of ED, BA concentration was found to decrease slowly after 24 hours, might due to the microbial degradation (Sundin and Watcher-Kristensen 1994). Although, EC and pH of the culture solution was not differed significantly, temperature was increased significantly due to application of DC-ED. The reason might be associated with the DC electrode with produce heat during the ED process. In earlier studies, increase in solution temperature and decrease in pH was observed due to DC-ED of strawberry culture solution under Wagner's pot hydroponics (Asaduzzaman et al. 2012). Concentrations of mineral nutrients such as calcium and iron in the nutrient solution were decreased significantly after 24 hours of DC-ED application (Table 1). In DC electrolysis, iron and calcium ions were thought to be precipitated to the anode. On the other hand, in the AC electrolysis, since the positive and negative charge of the electrode changed frequently and iron and calcium ions were not precipitated. Thus, it was thought that AC electrolysis might be more suitable for strawberry production by degradation of BA in the culture solution.

448 DC-ED and AC-ED were also applied to the culture solution of strawberry to investigate their
449 effects on culture solution, growth, fruit yield and quality of strawberry under recycled
450 hydroponics.
451
452 Results showed that, in non-renewed culture solution without ED treatment, growth and fruit
453 yield of strawberry were decreased significantly compared to plants grown in renewed culture
454 solution (Table 2, Fig. 5) due to accumulation of allelochemicals (Kitazawa et al. 2015). This
455 phenomenon was also observed in earlier studies (Asao et al. 2008; Kitazawa et al. 2005). In this
456 case, application of ED in non-renewed culture solution increased growth and yield of strawberry
457 (Asao et al. 2008; Asaduzzaman et al. 2012). In this present study, application of DC-ED to non-
458 renewed did not improve the growth parameters, fruit yield and fruit quality (vitamin C content)
459 significantly compared to the plant performance in non-renewed nutrient solution. Plants grown
460 in non-renewed culture solution had lower calcium and iron in leaves and crown might be due to
461 hindered nutrient uptake as a result of accumulation of growth inhibitors in the rhizosphere
462 (Singh et al. 1999). The accumulation of growth inhibitors was found in hydroponic nutrient
463 solution from the root exudates of many plants such as tomato (Yu and Matsui 1993), strawberry
464 (Kitazawa et al. 2005), cucumber, taro, some leafy vegetables and ornamentals (Asao et al. 1998,
465 2003, 2004b, 2007). While lower content of calcium and iron in leaves and crown of plant grown
466 in DC-ED treated non-renewed culture solution might be associated with their lower
467 concentration in that culture solution (Table 5).
468
469 On the other hand, application of AC-ED to non-renewed culture solution significantly increased
470 growth parameters (number of leaves per plant, fresh weight of leaf and crown, dry weight of
471 leaf, root and crown, number of fruits per plant, individual fruit weight, yield per plant and
472 vitamin C content of fruits) as compared non-renewed solution. The possible reason this
473 improved plant performance due to application of AC-ED in non-renewed culture solution might
474 include the faster rate of BA degradation, no negative effects on solution EC, pH and
475 temperature and mineral nutrient content (especially calcium and iron) (Fig. 3, 4; Table 1, 5).
476 Therefore, results of this study revealed that overall improvement of growth, yield, fruit quality

and nutrient solution conditions were better due to application of AC-ED than DC-ED in non-renewed culture solution of strawberry in recycled hydroponics.

5. Conclusion

Strawberry production in non-renewed hydroponics resulted in reduced growth and yield. DC-ED and AC-ED treatment to non-renewed nutrient solution increased growth and yield of strawberry. DC-ED treatment to non-renewed culture solution could recover yield of strawberry to some extent but not completely However, complete yield recovery was obtained from AC-ED treatment to non-renewed culture solution. Furthermore, AC-ED treatment to non-renewed culture solution could maintain better nutritional and environmental condition of growing medium. Hence, we suggested that AC-ED treatment to nutrient solution for 24 h at every three weeks intervals could be applied for complete recovery of strawberry yield grown in closed hydroponic culture.

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Figure captions

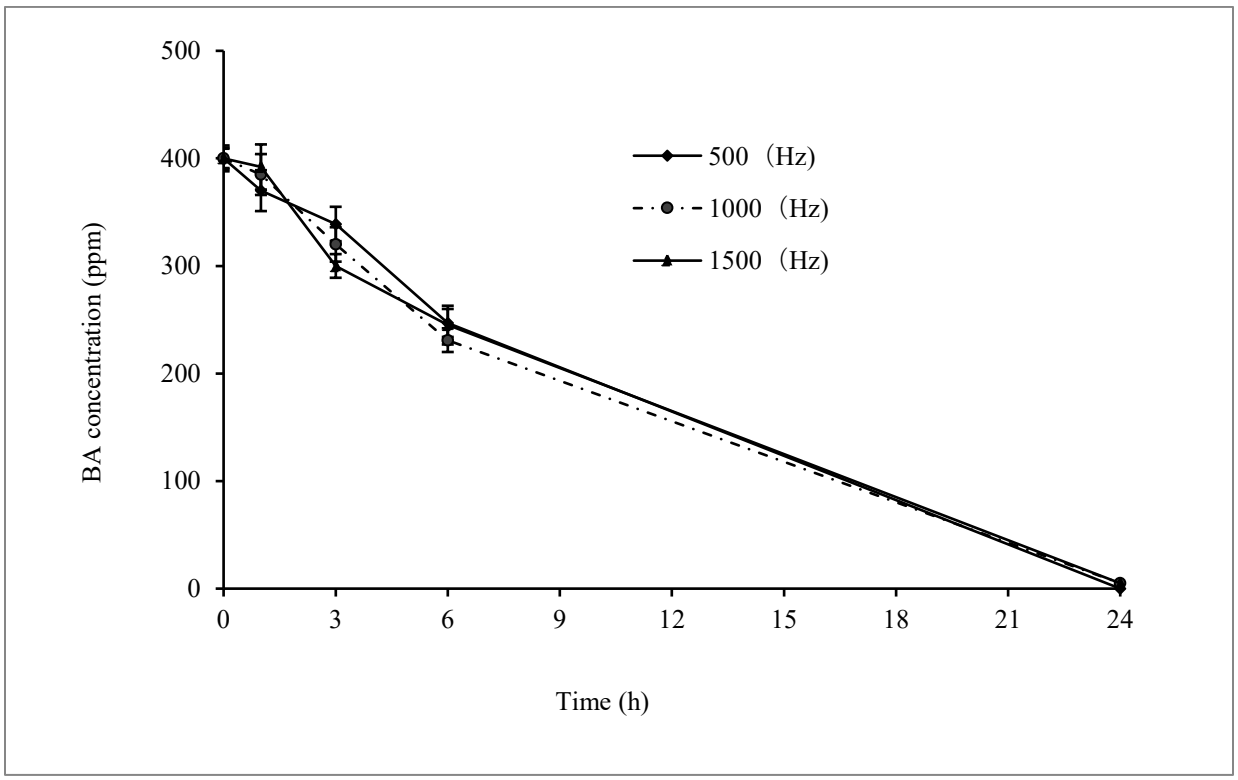
Fig. 1. Changes in benzoic acid concentration of the nutrient solution due to application of electro-degradation using alternate current (AC) at three different frequencies for 24 hours. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 µM L^{-1} benzoic acid. The vertical bars represent SE (n = 5). In AC supply 50% duty ratio, about 2.0 ampere and 14.0 volt were maintained for all frequencies. (Experiment I)

Fig. 2. Changes in electrical conductivity (A), pH (B) and temperature (C) of the nutrient solution due to application of electro-degradation using alternate current (AC) at three different frequencies for 24 hours. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 µM L^{-1} benzoic acid. In AC supply 50% duty ratio, 2.0 ampere and 14.0 volt were maintained for all frequencies. The vertical bars represent SE (n = 5). Different letters above each bar are significant and no letters are non-significant according to the Tukey's multiple range test at $P < 0.05$. (Experiment I)

Fig. 3. Changes in benzoic acid concentration of the nutrient solution due to application of electro-degradation using both direct current (DC) and alternate current (AC) for 24 hours in a no plant experiment. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 µM L^{-1} benzoic acid. The vertical bars represent SE (n = 5). In DC supply 18.0 volts and 2.0 amps were maintained for the entire period while in AC supply 500 Hz, 50% duty ratio, 14.0 volt and about 2.0 ampere were maintained. (Experiment II)

Fig. 4. Changes in electrical conductivity (A), pH (B) and temperature (C) of the nutrient solution due to application of electro-degradation using alternate current (AC) for 24 hours in a no plant experiment. Electro-degradation was applied in 10 L of 25% standard "Enshi" nutrient solution with 400 µM L^{-1} benzoic acid. In DC supply 18.0 volts and 2.0 amps were maintained for the entire period while in AC supply 500 Hz, 50% duty ratio, 14.0 volt and about 2.0 ampere were maintained. The vertical bars represent SE (n = 5). Different letters above each bar are significant and no letters are non-significant according to the Tukey's multiple range test at $P < 0.05$. (Experiment II)

Fig. 5. Effect of electro-degradation of non-renewed culture solution on yield attributes (A) individual fruit weight and number of fruit, and (B) fruit yield of strawberry plants grown under controlled environment condition. Electro-degradation was applied for 24 hours at every three weeks interval until final harvest. (Experiment III)



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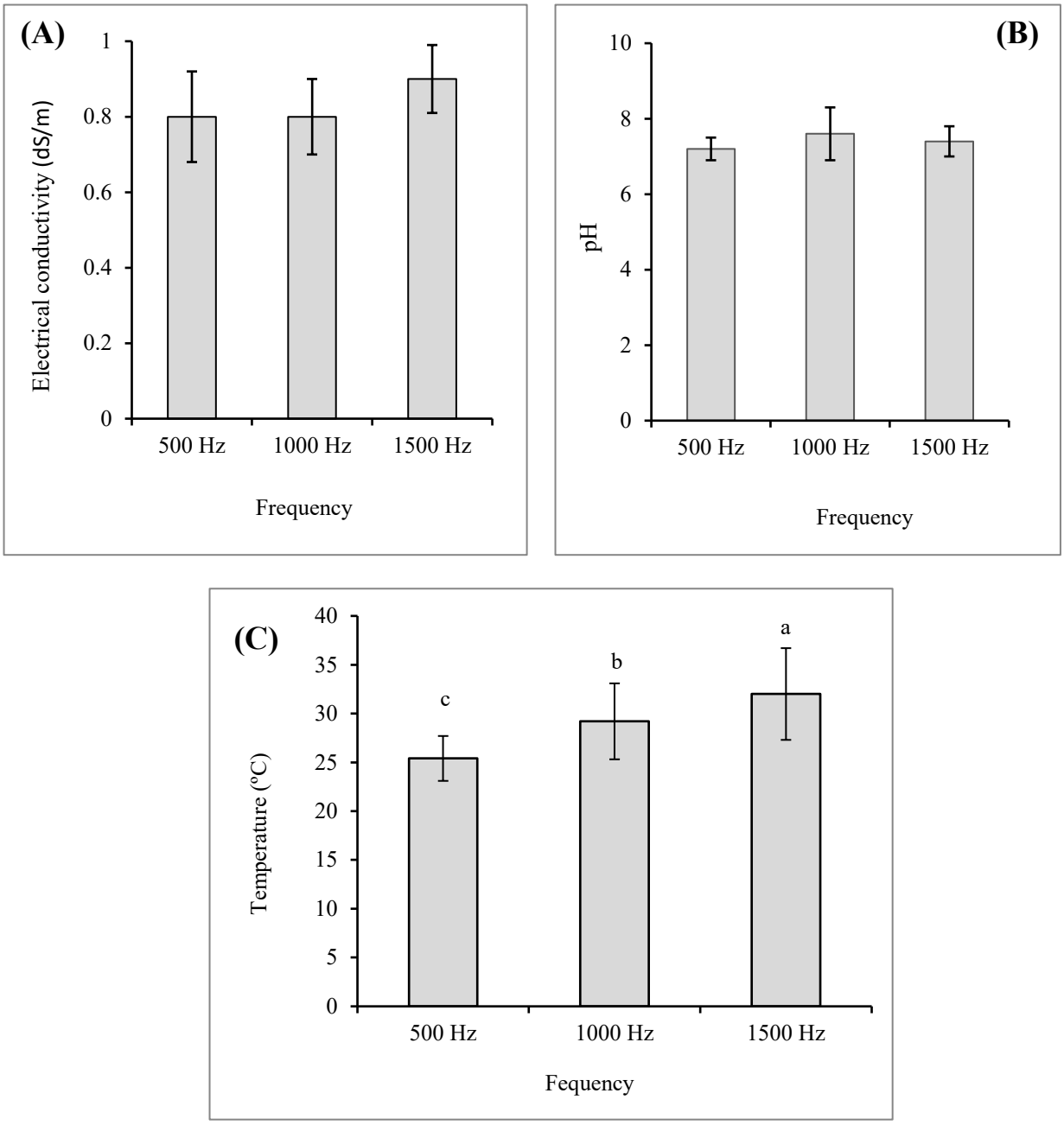

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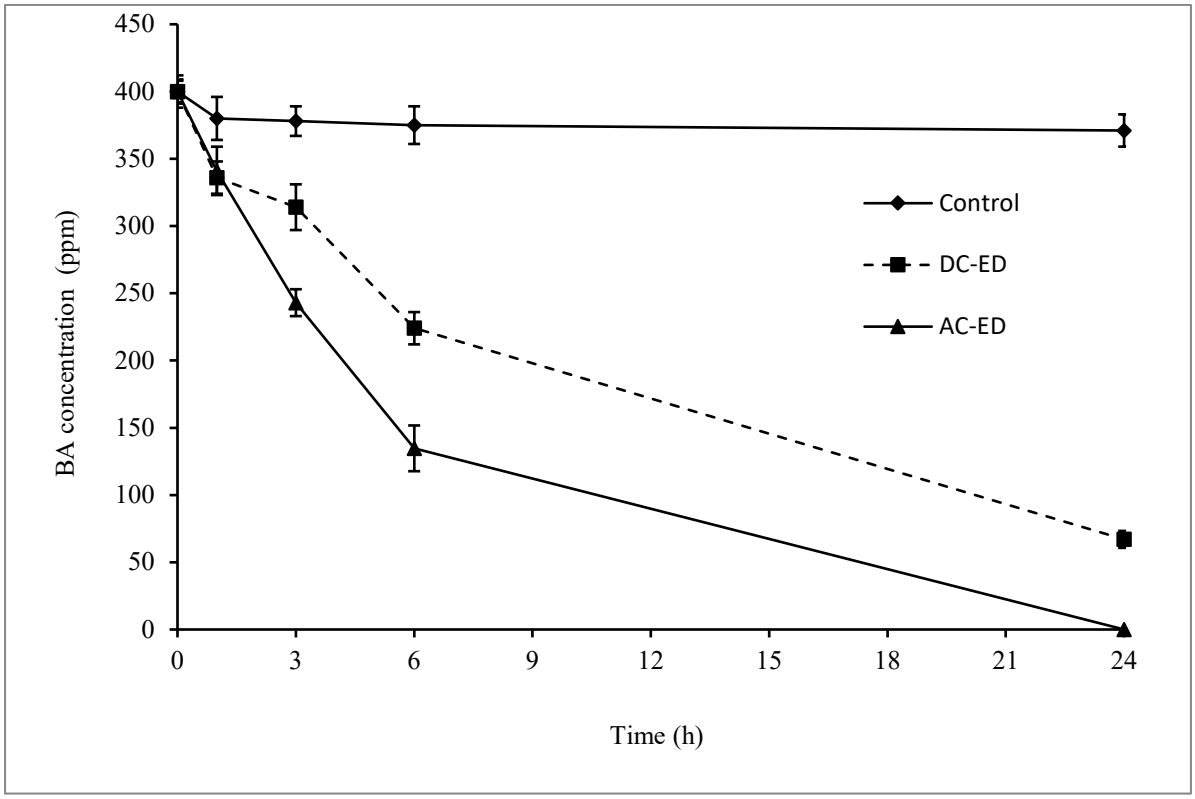

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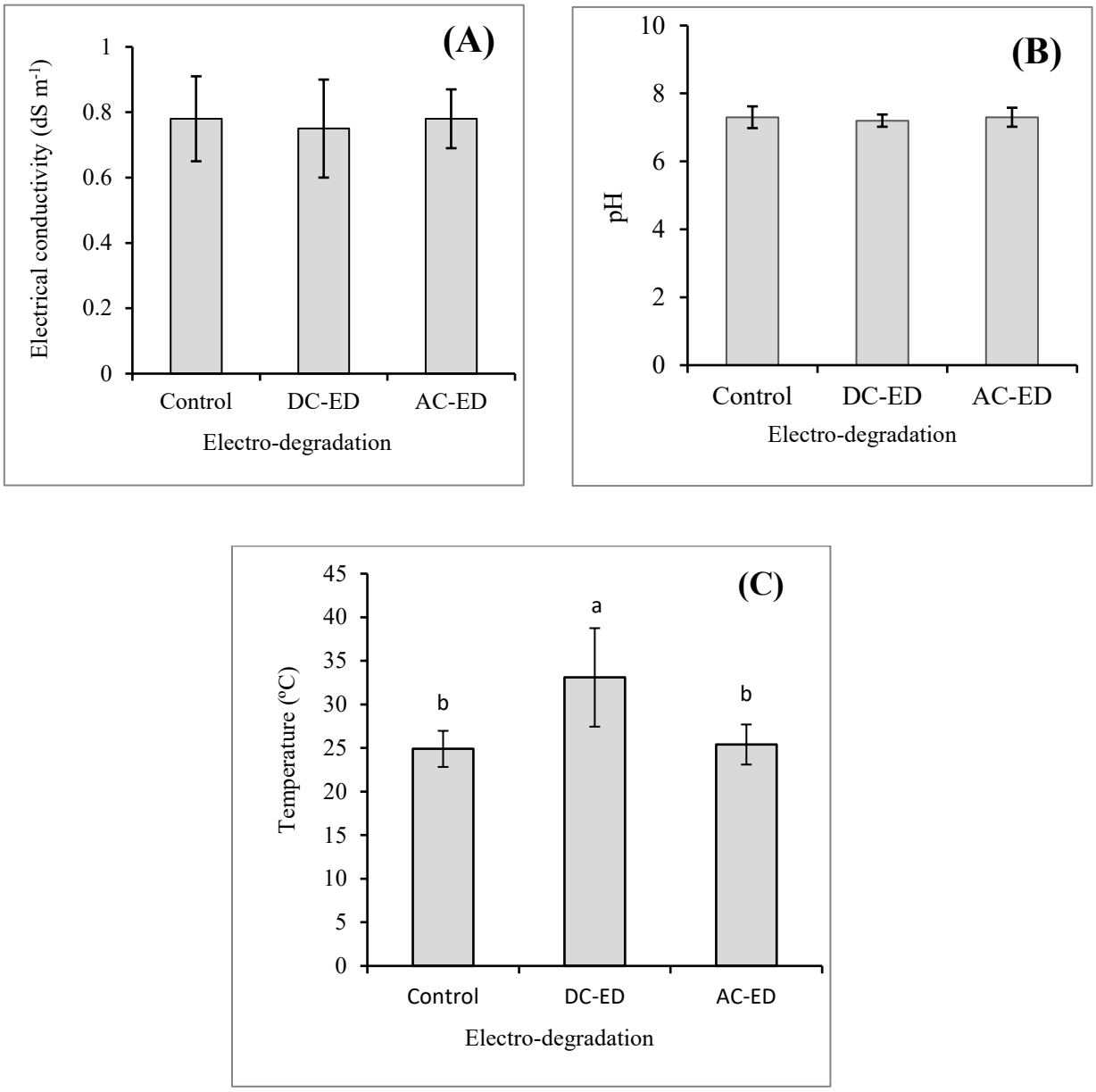

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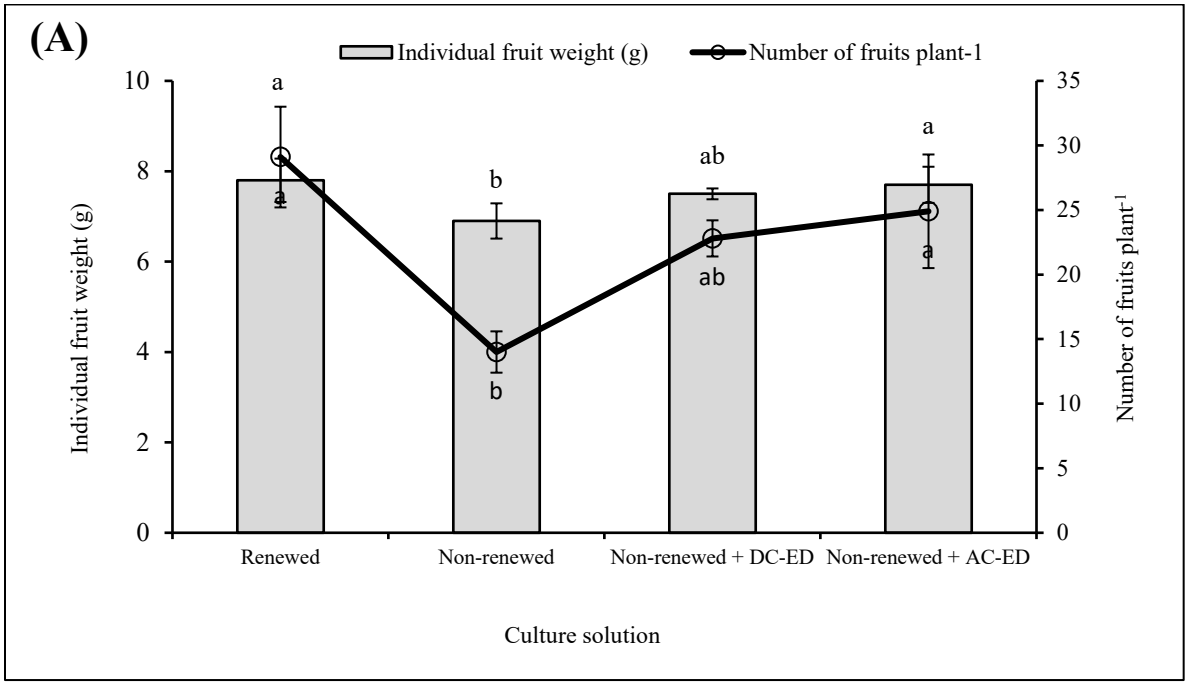

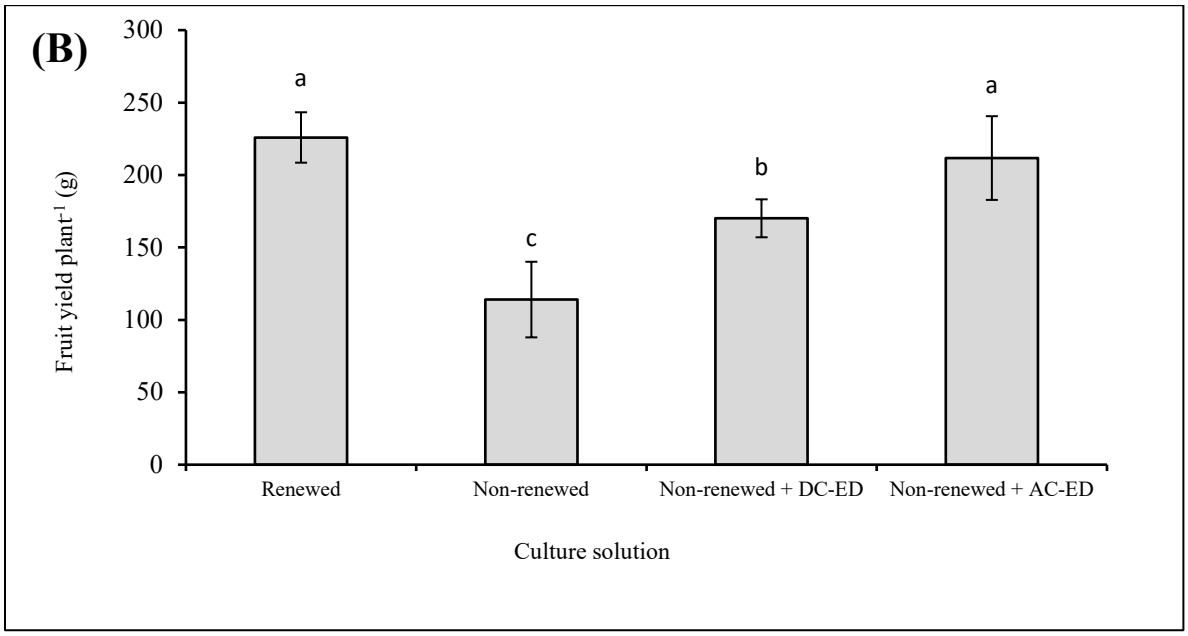

Fig. 5. Effect of electro-degradation of non-renewed culture solution on yield attributes (A) individual fruit weight and number of fruit, and (B) fruit yield of strawberry plants grown under controlled environment condition. Electro-degradation was applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Table captions

Table 1. Changes in mineral nutrients after application of electro-degradation of nutrient solution in no plant experiment. Electro-degradations were applied in 10 L of 25% standard "Enshi" nutrient solution with 400 μM L^{-1} benzoic acid for 24 hours. (Experiment II)

Table 2. Effect of electro-degradation of non-renewed culture solution on the growth of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Table 3. Effect of electro-degradation of non-renewed culture solution on the fruit qualities of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Table 4. Effect of electro-degradation of non-renewed culture solution on the mineral content in leaf, crown and root of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Table 5. Effect of electro-degradation of non-renewed culture solution on temperature, pH, electrical conductivity and residual nutrient content of nutrient solution at the final harvest. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Table 1. Changes in mineral nutrients after application of electro-degradation of nutrient solution in no plant experiment. Electro-degradations were applied in 10 L of 25% standard "Enshi" nutrient solution with 400 μM L^{-1} benzoic acid for 24 hours. (Experiment II)

Electro-degradation	NO_3^- (ppm)	$P_2O_5^-$ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	Fe^{3+} (ppm)
Control[z]	687	37.5	7.9	49.9 a[w]	16.2	3.5 a
DC-ED[y]	658	35.8	7.6	41.6 b	13.8	2.2 b
AC-ED[x]	669	37.5	7.2	52.6 a	15.4	3.4 a
Significance	NS	NS	NS		NS	

[z]Electro-degradation was not applied.
[y]Electro-degradation was applied using "Direct Current"
[x]Electro-degradation was applied using "Alternate Current"
[w]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Table 2. Effect of electro-degradation of non-renewed culture solution on the growth of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	No of leaves plant^{-1}	Longest root length (cm)	Leaf length (cm)	Leaf width (cm)	SPAD value	Crown diameter (mm)	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		
							Leaf	Crown	Leaf	Crown	Root
RW[z]	18.8 a[v]	58.7	13.9	13.9	57.6	16.9	28.1 a	16.6 a	7.7 a	2.6 a	4.1 a
NR[y]	14.2 b	54.2	13.5	13.1	55.2	15.5	21.7 c	9.1 b	6.1 b	1.8 b	2.9 b
NR + DC-ED[x]	15.1 ab	55.3	13.7	13.4	55.8	16.6	26.2 b	11.2 ab	7.0 ab	1.9 ab	3.0 ab
NR + AC-ED[w]	15.7 ab	57.8	13.8	13.6	56.2	16.8	28.1 a	14.9a	7.49 a	2.3 a	3.9 a
Significance		NS	NS	NS	NS	NS					

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Table 3. Effect of electro-degradation of non-renewed culture solution on the fruit qualities of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	Brix (%)				Citric acidity (%)				Vitamin C (ppm)			
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster I	Cluster II	Cluster III	Cluster IV
RW[z]	7.1	7.5	7.8	7.6	0.28	0.29	0.26	0.28	658.1 ab[v]	657.5 ab	656.0 ab	682.2 a
NR[y]	7.9	7.8	7.9	7.7	0.28	0.29	0.29	0.26	536.5 b	621.1 bc	597.0 b	616.2 b
NR + DC-ED[x]	7.5	7.5	7.7	7.5	0.28	0.31	0.30	0.30	593.3 b	603.4 c	616.4 b	623.8 b
NR + AC-ED[w]	7.7	7.7	7.2	8.0	0.31	0.31	0.29	0.28	693.4 a	681.5 a	698.0 a	686.5 a
Significance	NS	NS	NS	NS	NS	NS	NS	NS				

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Table 4. Effect of electro-degradation of non-renewed culture solution on the mineral content in leaf, crown and root of strawberry plants grown under controlled environment condition. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	Iron (mg kg^{-1} DW)			Calcium (mg g^{-1} DW)			Magnesium (mg g^{-1} DW)			Potassium (mg g^{-1} DW)		
	Leaf	Crown	Root	Leaf	Crown	Root	Leaf	Crown	Root	Leaf	Crown	Root
RW[z]	138	372 a[v]	238 a	26.7	22.7 bc	31.3 ab	7.3	7.3	14.7	35.7	21.0	25.8
NR[y]	131	279 b	194 b	20.7	20.7 bc	25.8 ab	7.1	7.2	12.9	39.5	17.7	22.4
NR + DC-ED[x]	122	209 c	183 b	22.4	19.0 c	24.0 b	7.5	7.3	12.8	35.9	18.2	23.9
NR + AC-ED[w]	149	302 b	246 a	30.2	24.2 a	34.0 a	7.7	6.8	14.5	41.7	23.0	25.0
Significance	NS			NS			NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Table 5. Effect of electro-degradation of non-renewed culture solution on temperature, pH, electrical conductivity and residual nutrient content of nutrient solution at the final harvest. Electro-degradations were applied for 24 hours at every three weeks interval until final harvest. (Experiment III)

Culture solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
RW[z]	19.4	7.22	0.77	3.9 a[v]	45.3 a	25.2	77.8	682.5	9.0
NR[y]	20.1	7.22	0.78	3.7 a	42.1 a	24.8	72.5	653.0	8.6
NR + DC-ED[x]	21.5	7.23	0.76	2.3 b	34.0 b	24.3	75.7	669.2	8.8
NR + AC-ED[w]	20.4	7.20	0.78	3.6 a	41.6 a	25.2	76.5	681.0	9.3
Significance	NS	NS	NS			NS	NS	NS	NS

[z]Nutrient solution was renewed at every three weeks interval.
[y]Nutrient solution was not renewed throughout the entire growing period but major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[x]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Direct Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[w]Nutrient solution was not renewed throughout the entire growing period and electro-degradation was applied using “Alternate Current” and major nutrients were adjusted to 25% “Enshi” nutrient solution at three weeks interval.
[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P < 0.05$.

Supplemental information

Table S1. Chemical composition of full strength "Enshi" nutrient solution used for this experiment.

Chemicals	Amounts[z] (g 1000 L^{-1})
$Ca(NO_3)_2.4H_2O$	950
KNO_3	810
$MgSO_4.7H_2O$	500
$NH_4H_2PO_4$	155
H_3BO_3	3
$ZnSO_4.7H_2O$	0.22
$MnSO_4$ .4H2O	2
$CuSO_4$.$5H_2O$	0.05
Na_2MoO_4 .$2H_2O$	0.02
NaFe-EDTA	25

[z]Amounts of salts per 1000 L of tap water (Hori 1966).

Fig. S1. Simple schematic diagram of the electrode used for electro-degradation.
(A) Different components of DC-ED electrode includes (1) pump, (2) plastic tube connecting pump with electrode, (3) anode, (4) cathode, (5) central ferrite core, (6) cylindrical titanium pipe and (7) nutrient solution flow. (Asaduzzaman et al. 2012);
(B) Different components of AC-ED electrode includes (1) pump, (2) plastic tube connecting pump with electrode, (3) anode/cathode, (4) cathode/anode, (5) central titanium core, (6) cylindrical titanium pipe and (7) nutrient solution flow.

Fig. S2. Electro-degradations of nutrient solution following without plant experiment for 24 hours. (A) AC-ED was applied at 50% duty ratio, 2.0 amperes alternate current, and 14.0 volts. (B) The DC-ED was applied at 2.0 ampere and 18.0 volts. (C) Control- without ED application, nutrient solution was flowed using pump only. (Experiment II)

Fig. S3. Three layered vertical growing beds used for cultivation of strawberry plants under controlled-environment. Each grow bed with 50 L nutrient solution capacity and three beds placed vertically were connected to a tank filled with 300 L nutrient solution. There were four different systems used for each types of culture solution such as **(A)** renewed, **(B)** non-renewed, **(C, E)** non-renewed with DC-ED, and **(D, F)** non-renewed with AC-ED. (Experiment III)

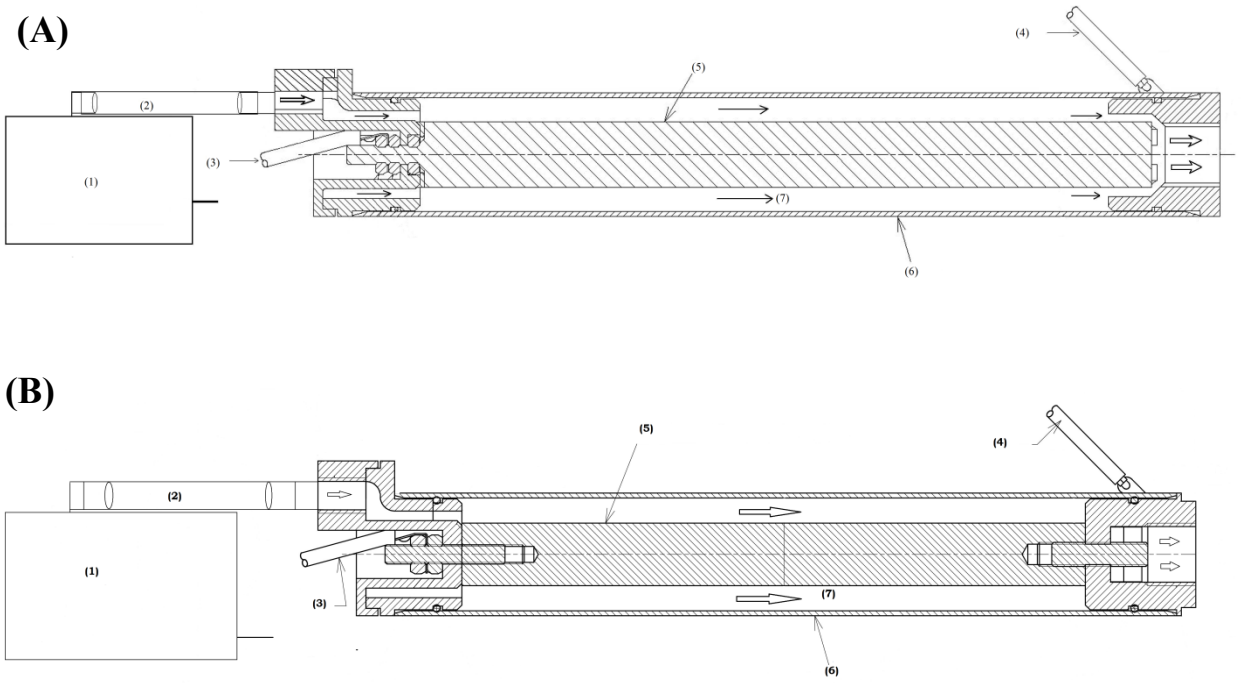

Fig. S1. Simple schematic diagram of the electrode used for electro-degradation.
(A) Different components of DC-ED electrode includes (1) pump, (2) plastic tube connecting pump with electrode, (3) anode, (4) cathode, (5) central ferrite core, (6) cylindrical titanium pipe and (7) nutrient solution flow. (Asaduzzaman et al. 2012);
(B) Different components of AC-ED electrode includes (1) pump, (2) plastic tube connecting pump with electrode, (3) anode/cathode, (4) cathode/anode, (5) central titanium core, (6) cylindrical titanium pipe and (7) nutrient solution flow.

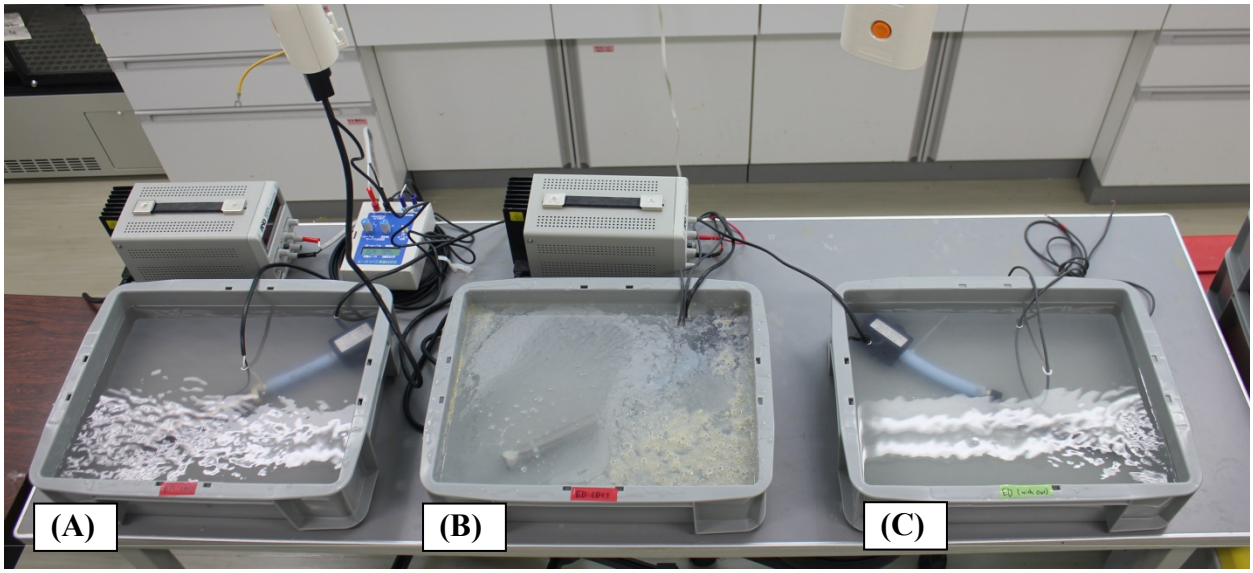

Fig. S2. Electro-degradations of nutrient solution following without plant experiment for 24 hours. (A) AC-ED was applied at 50% duty ratio, 2.0 amperes alternate current, and 14.0 volts. (B) The DC-ED was applied at 2.0 ampere and 18.0 volts. (C) Control- without ED application, nutrient solution was flowed using pump only. (Experiment II)

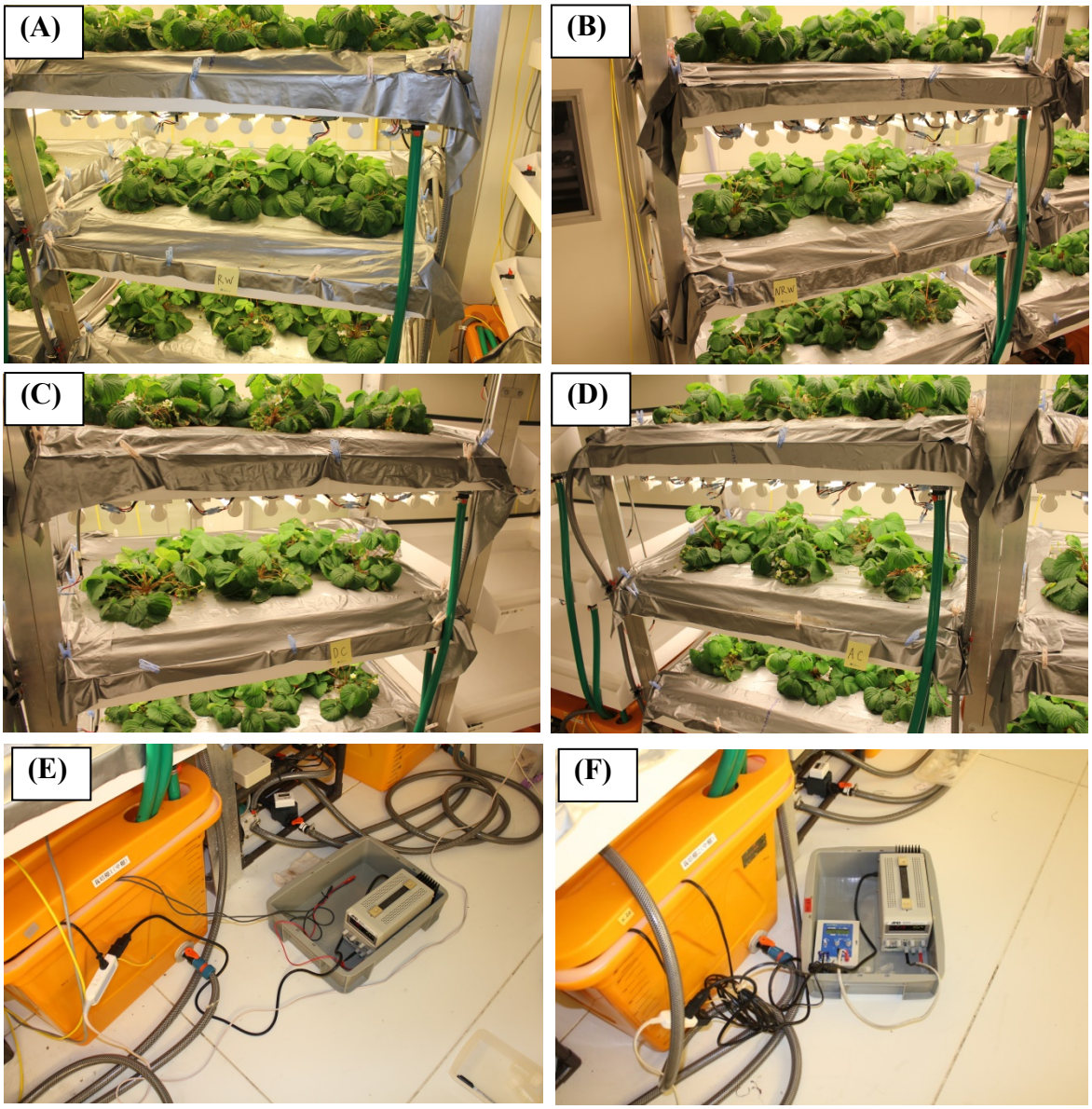

Fig. S3. Three layered vertical growing beds used for cultivation of strawberry plants under controlled-environment. Each grow bed with 50 L nutrient solution capacity and three beds placed vertically were connected to a tank filled with 300 L nutrient solution. There were four different systems used for each types of culture solution such as **(A)** renewed, **(B)** non-renewed, **(C, E)** non-renewed with DC-ED, and **(D, F)** non-renewed with AC-ED. (Experiment III)

Dear Editor-in-Chief
Scientia Horticulturae

We have revised our manuscript “Electro-degradation of culture solution improves growth, yield and quality of strawberry plants grown in closed hydroponics”.

We considered the suggestions given by the editor during revision. Responses to the reviewers are listed below this letter also highlighted in blue color.

Sincerely yours

T. Asao
Department of Agriculture,
Faculty of Life and Environmental Science,
Shimane University, JAPAN

Responses to the reviewers

(SUGGESTIONS)

The manuscript has eight big figures and siz tables. These are too many for general paper. They should be shown within 10 in total. At least, Figs 2 and 3 shold be shown as supplemental data.

(Response)

Thank you for the kind suggestion. We followed this suggestion and reduce the number of tables and figure in the revised manuscript.

Table 1 and Fig. 2 & 3 are given as supplemental information.