Title

Appropriate strategies of electrodegradation for the alleviation of growth retardation during autotoxicity of lettuce in recycled hydroponics

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Appropriate strategies of electrodegradation for the alleviation of growth retardation during autotoxicity of lettuce in recycled hydroponics

Abstract:

Recycled hydroponic solutions used for growing crops can accumulate allelochemicals that inhibit plant growth. We applied alternating current electrodegradation (AC-ED) to fresh nonrenewed nutrient solutions (i.e., solutions remaining unchanged throughout the culture period) and once-used nonrenewed solutions (i.e., solutions that had been used for a previous culture) for detoxifying autotoxic chemicals. Four experiments were conducted in which lettuce plants were grown in different nonrenewed solutions treated with AC-ED and in renewed solution that was not treated with AC-ED. Renewed solution (50% "Enshi" solution) was changed at 14-day intervals. In fresh starting solutions, no substantial difference was found in shoot fresh weight (SFW) between renewed and AC-ED-treated nonrenewed solutions at different intervals (experiment I) and frequencies (experiment III), but notably, the lowest yield was recorded in non-renewed solution. In contrast, in the case of once-used nonrenewed solution, weekly (experiment II) and thrice-weekly and continuous (experiment IV) AC-ED application showed significantly higher SFW compared to values for other solutions, and the lowest value was also found in nonrenewed once-used culture solution. Therefore, we recommend the application of AC-ED to non-renewed solution either thrice weekly or continuously for efficient detoxification of accumulated allelochemicals to improve the growth, yield and quality of lettuce under two or more successive cultivations in recycled hydroponics.

Keywords: *Lactuca sativa* L.; autotoxicity; plant factory; nonrenewed solution; mineral nutrients; root exudate

Introduction

Lettuce is a popular leafy vegetable that is generally consumed fresh worldwide. It is a good source of mineral nutrients, such as potassium, calcium, iron and copper, and vitamins A and C. Lettuce grown traditionally in soil may include pathogens and parasites resulting from the use of irrigation water and soil amendments (Jensen et al. 2013; Williams et al. 2013). Moreover, open field cultivation of lettuce faces challenges, especially from high temperatures, insect pests and diseases. In this regard, hydroponic systems provide better control over environmental factors and nutrient management practices for maintaining the quality of lettuce. The cultivation of leafy vegetables, including lettuce, in hydroponics is gaining popularity throughout the world and production in hydroponics has greatly expanded (Kozai 2013) . Hydroponic systems can lead to faster and maximized production of lettuce, as the plants are supplied with balanced nutrients throughout the culture period. After each lettuce culture, once-used nutrient solutions with unused residual nutrients are disposed of in the surrounding environment, causing pollution. Thus, recycled hydroponics are advantageous for sequential culture of lettuce with the same culture solutions after EC-based nutrient adjustment. This particular hydroponic system has been widely used in controlled environment agriculture and in plant factories (Ruijs 1995). This type of hydroponic production system maintains sustainability. In this system, both water and mineral nutrients are used efficiently; therefore, it minimizes the wastage of costly fertilizer and reduces environmental pollution.

However, under this managed culture system, crop yield has been found to be greatly affected due to the accumulation of inhibitory root exudates (Lee et al. 2006; Talukder et al. 2019b). Lower yield and yield characteristics (individual fruit weight, number of fruits/plant, fruit brix%) of strawberry were reported for nonrenewed nutrient solution than with renewed solution (Kitazawa et al. 2005; Talukder et al. 2019a). Similar results have also been evident in beans (Asaduzzaman and Asao 2012), taro (Asao et al. 2003), lettuce (Lee et al. 2006; Talukder et al. 2019b), several leafy vegetables (Asao et al. 2004) and certain ornamentals

(Asao et al. 2007). Recently, it was reported that non-recycled hydroponics may result in physiological disorders, such as yellowing of eggplant fruit (Singh et al. 2019). Retardation of the growth and yield of crops grown in recycled hydroponics occurs as a result of increasing concentrations of phytotoxic root exudates causing autotoxicity phenomena (Tang & Young 1982; Asao et al. 1998; Singh et al. 1999; Asao et al. 2003; Asao et al. 2004; Kitazawa et al. 2005; Asao et al. 2007; Asao et al. 2008; Asaduzzaman et al. 2012). As plant roots are first affected by phytotoxic chemicals in their rhizosphere, water and nutrient element uptake are most affected (Blum and Gerig 2005).

The growth and yield of lettuce have also been found to be reduced by the phenomenon of autotoxicity in successive cultivation with recycled nutrient solution (Lee et al. 2006). Therefore, cleaning nutrient solution by exclusion or detoxification of accumulated autotoxic chemicals may reduce the inhibition of crop growth in sequential production. Researchers have tried various methods for detoxifying autotoxic chemicals, such as activated charcoal (Asao et al. 1998), application of amino acids (Mondal et al. 2013) and electrodegradation in nutrient solution (Talukder et al. 2019b; Asaduzzaman et al. 2012; Asao et al. 2008), and have found positive results in terms of restricting the inhibitory effect of those allelochemicals. Alternating current electrodegradation (AC-ED) has been found to have a positive role in the growth retardation of lettuce grown in recycled hydroponic systems by detoxifying the allelochemicals accumulated in culture solution (Talukder et al. 2019b). In an AC-ED machine, the culture solution can pass through the electrode where ED takes place. At the anode, autotoxic compounds are oxidized and decomposed to carbon dioxide (CO_2) (Comninellis and Pulgarin 1991; Feng and Li 2003; Fleszar and Poszyńska 1985).

In recent research, the detrimental effect of autotoxicity in lettuce (Talukder et al. 2019b) and strawberry (Talukder et al. 2019a) under recycled hydroponics was alleviated by degrading allelochemicals through alternating current electrodegradation (AC-ED).

The present study was conducted to determine the appropriate interval and frequency of AC-ED application to nonrenewal solutions to minimize the inhibitory effect of autotoxicity on successive lettuce production in recycled hydroponics.

Materials and Methods

Preparation of planting materials

Lettuce seeds (*Lactuca sativa* cv. Souther) obtained from Takii Seed Company, Japan, were used as plant material. Seeds were grown in medium-sized (1-5 mm) vermiculite-filled cell trays (72 cells/tray) and put into a growth chamber (temperature: 25/20 °C day/night, relative humidity: 60%, intensity of fluorescent light: 140~160 μmol m^{-2} s^{-1}, photoperiod: 12 hours). After two weeks in experiments I, II and II or one week in experiment IV, seedlings were moved to hydroponic system nursery beds in plastic containers (68 cm × 53 cm × 23 cm) that contained 30 L of 50% Enshi solution and kept in a control room maintaining the same environmental conditions for seedling preparation, including 1000 ppm ambient CO_2 level. A pump (MX 808ST-W, Enomoto, Micro Pump Mfg. Co., Ltd., Japan; flow rate 25 L $min.^{-1}$) was used for continuous aeration of the solutions at the nursery. Nursery culture was continued for up to 14 days, and seedlings of similar size were chosen for final culturing.

Maintenance of hydroponic nutrient solution

Lettuce plants were cultivated in 50% "Enshi" solution (Hori 1966). The pH of the nutrient solution was 7.15, and EC was 1.4 dS m^{-1}. The recommended pH and EC for lettuce production in hydroponics are 6.0-7.0 and 1.2-1.8 dS m^{-1}, respectively (Singh and Bruce 2016). The EC of the culture solution was quantified by an EC meter (ES-51, Horiba, Ltd., Kyoto, Japan), and the pH was checked by a pH meter (D-12, Horiba, Ltd., Kyoto, Japan) at 7-day intervals. Weekly data on the pH, EC and temperature of the culture solutions for all experiments are included as supplemental information in Tables S1- S4. The nutrient solution was replaced at 14-day intervals with 50% "Enshi" solution in renewed treatments. For nonrenewed systems, however, the culture solution was unchanged throughout the cultivation period, but the concentrations of mineral nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Fe^{3+}) were adjusted to the original contents in 50% "Enshi" solution based on chemical analyses at 14-day intervals. Chemical analytical data during nutrient adjustment of the culture solutions for all experiments are included as supplemental information in Tables S5-S9. Similar nutrient solution adjustment has been successfully performed in strawberry (Asaduzzaman et al. 2012) and lettuce (Talukder et al. 2019b).

Process of lettuce cultivation

After completing the nursery period, almost identical lettuce seedlings were grown in three steps in vertical growing beds (125 cm × 90 cm × 10.5 cm) in a plant factory room. Each growing bed contained 20 lettuce seedlings fixed in place with urethane cubes (23 mm × 23 mm × 27 mm). Fifty percent "Enshi" nutrient solution was supplied into three beds, each of which had a 50-L capacity joined to a 300-L storage tank. Recycling of the nutrient solutions at 55/5 min (recycled/stopped) was performed by using an automatic pump (KP-101, Koshin, Kyoto, Japan) and timer (KS-1500, Iuchi, Osaka, Japan). The environmental conditions of the room were the same as those of the nursery hydroponic system, but the fluorescent light intensity was maintained at 250~280 μmol m^{-2} s^{-1} throughout the culture period. Pictures of plants at the start and end of culture are shown in Figure 1 and Figure 2.

Electrodegradation of culture solution

An AC-type electrode (Yonago Shinko Co., Ltd., Tottori, Japan) was used to degrade the accumulated autotoxic chemicals (mainly benzoic acid) in the lettuce culture solution. The AC-ED electrode had a middle core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) surrounded by a cylindrical tube of 95.5 cm^2 (cathode/anode) titanium. The solution was able to pass through the electrode where ED occurred. The electrodes were connected with a digital AC power provider (AD-8735D, AND, Japan), and 500 Hertz, 50% duty ratio, 1.8 amp and 24 volts were maintained during ED. These electrical conditions have been successfully applied in strawberry (Talukder et al. 2019a) and lettuce (Talukder et al. 2019b) to degrade allelochemicals.

Experimental treatments and data collection

Experiment I

Lettuce seedlings were transplanted on 24 April 2019 and harvested on 5 June 2019 at the age of 42 days. Lettuce plants were grown in five types of nutrient solutions: i) renewed, ii) nonrenewed, iii) nonrenewed + AC-ED (weekly), iv) nonrenewed + AC-ED (two-weekly) and v) nonrenewed + AC-ED (three-weekly). In the case of AC-ED-treated culture solution, AC-ED was applied for 24 hours according to the treatments. Data on growth attributes, yield, and chlorophyll content (measured by SPAD meter, Konica Minolta, Tokyo, Japan) were collected at harvest.

Experiment II

Lettuce plants were transplanted on 5 June 2019 and harvested on 10 July 2019 at the age of 35 days. There were five treatments in this experiment: i) renewed, ii) nonrenewed with once-used starting solution {used in Experiment I termed as nonrenewed 2C (second cycle)}, iii) nonrenewed 2C+AC-ED (weekly), iv) nonrenewed 2C+AC-ED (two-weekly) and v) nonrenewed 2C+AC-ED (three-weekly). AC-ED was applied for 24 hours according to the desired nutrient solution treatments. Data were collected at harvest on growth attributes, yields, and chlorophyll contents.

Experiment III

In the third experiment, we applied AC-ED more frequently per week. Lettuce seedlings were transplanted on 17 July 2019 and harvested on 21 August 2019 after 35 days. Five types of culture solutions were used in this experiment: i) renewed, ii) nonrenewed, iii) nonrenewed + AC-ED once weekly, iv) nonrenewed + AC-ED thrice weekly (Monday, Wednesday and Friday) and v) nonrenewed + AC-ED (continuously). Data on growth attributes, yields, and chlorophyll contents were collected at harvest.

Experiment IV

Lettuce seedlings were planted on 21 August 2019 and harvested on 25 September 2019 at the age of 35 days. In this experiment, lettuce plants were cultivated in five types of nutrient solutions: i) renewed, ii) nonrenewed with once-used starting solution (used in Experiment III, 2C solution), iii) nonrenewed 2C+AC-ED once weekly, iv) nonrenewed 2C+AC-ED thrice weekly (Monday, Wednesday and Friday) and v) nonrenewed 2C+AC-ED (continuously). Data were collected at harvest on growth attributes, yields, and chlorophyll contents.

Mineral nutrient determination in the shoots and roots of lettuce

At harvest roots and shoots of the plants were separated and placed in a steady temperature oven (DKN812, Yamato Scientific Co., Ltd., Japan), maintaining 80 °C temperature for 72 hours. When the dry matter reached constant weight, the material was ground into powder with a mixer machine (National MX-X53, Japan). Ground 0.25-g samples were digested by a microwave digestion system (ETHOS1, Milestone S.r.l, Bergamo, Italy), after mixing the samples with 8 ml of HNO_3 (60% conc.). After completing digestion, distilled water was added to each digested sample solution to a volume of 50 ml, and then the solution was filtered with filter paper (Advantec Grade no. 131, 185 mm). Finally, nutrient element content was determined by atomic absorption spectrophotometry (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

Determination of ascorbic acid in lettuce

Harvested lettuce shoots were stored in a freezer (at –30 °C) to determine ascorbic acid contents. Stored samples were removed from the freezer and pressed to obtain sufficient juice for analysis. Ascorbic acid content was calculated using 2,4-dinitrophenylhydrazine (DNP) colorimetry. After leaf extract filtering, 0.5 ml of juice from each sample was placed in a 50-ml test tube, and the following chemicals were added to the sample solution: i) 0.5 ml of 10% meta-phosphoric acid solution, ii) 1 ml distilled water, iii) 1 ml of 0.03% 2,6-dichlorophenol-indophenol (DCP) solution, iv) 2 ml thiourea solution and v) 1 ml DNP solution. After the addition of all chemicals, test tubes were incubated in a water bath at 37 °C for 3 hours. After completing incubation, 5 ml of 85% H_2SO_4 was poured into each test tube solution sitting in ice water. Afterwards, the ascorbic acid content was determined by a spectrophotometer (U-2900, Hitachi High Technologies Corporation, Tokyo, Japan) at 520 nm.

Experimental design and statistical analysis

In this study, AC-ED was applied in nonrenewed nutrient solution from successive cultures of lettuce grown in recycled hydroponics to determine the appropriate frequencies and intervals. Fresh nonrenewed culture solutions were used in experiment I and III on the other hand, for experiment II and IV these solution were prepared from the once-used nutrient solution from the previous culture of experiment I and III, respectively, and renewed nutrient solution was used as a control in every experiment. All experiments were structured in a completely randomized design of three replications. The Statcel 4 computer package (OMS publication, Tokorozawa, Saitama, Japan) was used to analyze the variance for all results. The Tukey-Kramar test (at $P<0.05$) was performed to determine significant differences in means. Finally, ANOVA of four experiments was organized based on the solution types nested with AC-ED using a heat map.

Results

Effect of AC-ED application intervals on nonrenewed fresh culture solution (Experiment I)

The use of AC-ED in nonrenewed culture solution at different intervals significantly affected lettuce growth performance (Tables 1; Figure 3). All growth features were substantially influenced except relative chlorophyll content and root dry weight. The highest leaf number was recorded in the treatment in which nonrenewed culture solution was subjected to AC-ED every three weeks. The plants in nonrenewed solution subjected to weekly AC-ED featured the greatest lengths and widths of leaves and root lengths, which were similar to those from renewed and other AC-ED-treated nonrenewed solutions. On the other hand, such growth features in untreated nonrenewed culture solution were reported to be the lowest. Shoot fresh weight was the highest (369.7 g plant^{-1}) in nonrenewed culture solution subjected to weekly AC-ED application, and this weight was similar to values for renewed solutions and nonrenewed solutions subjected to two-weekly or three-weekly AC-ED application (Figure 3). SFW was the lowest (230.1 g plant^{-1}) in untreated nonrenewed culture solution. The shoot dry weight was the highest in the renewed solution, which was also statistically identical to the nonrenewed culture solution subjected to weekly AC-ED application. The ascorbic acid content in lettuce was higher in nutrient solution subjected to three-weekly AC-ED application, which was analogous to renewed solution. Among the different culture solutions, the amounts of calcium, magnesium and zinc in the lettuce roots were noticeably different but did not affect the sodium and potassium concentrations in the lettuce plants (Tables 2).

Effect of different intervals of application of AC-ED to nonrenewed nutrient solution (Experiment II)

Lettuce growth in once-used nonrenewed (nonrenewed 2C) nutrient solution decreased considerably compared to that in nonrenewed solution (Tables 1; Figure 3). Weekly use of AC-ED in nonrenewed 2C nutrient solution improved development over that of other culture solutions. The AC-ED treatments improved the maximum lengths and widths of leaves and the dry weights of shoots and roots, while the numbers of leaves, longest root lengths and SPAD values were not remarkably varied. On the other hand, in plants cultivated in nonrenewed 2C solution, the overall leaf lengths and widths and shoot dry weights were reduced considerably compared to the AC-ED-treated nonrenewed 2C nutrient solutions. Among the three AC-ED-treated culture solutions, the weekly AC-ED solution showed better performance than did solutions subjected to two-weekly or three-weekly AC-ED. The most important growth parameter for leafy vegetables is the fresh weight of the shoot, which was recorded to be the highest (234.0 g plant^{-1}) in weekly AC-ED subjected nonrenewed 2C nutrient solution (Figure 3). The minimum (152.8 g plant^{-1}) fresh weight of shoots was found in nonrenewed 2C culture solution. No substantial change was recorded with respect to lettuce ascorbic acid content among all culture solutions. The AC-ED-treated nonrenewed 2C nutrient solution showed great variation in levels of mineral nutrients, in particular calcium and sodium in roots and shoots and potassium in roots, as well as zinc in lettuce shoots (Tables 2). The calcium and potassium contents in lettuce roots decreased significantly in nonrenewed 2C solution, but the sodium content was higher in nonrenewed 2C nutrient solutions.

Effect of AC-ED application frequency per week in nonrenewed fresh nutrient solution (Experiment III)

The frequency of application of AC-ED in nonrenewed solutions greatly influenced the growth of lettuce (Tables 1; Figure 4). The leaf number, maximum leaf width, longest root length and shoot dry weight were the lowest in the untreated nonrenewed culture solution. Plants in nonrenewed solution subjected to continuous AC-ED achieved the maximum numbers of leaves, leaf widths and shoot dry weights, which were similar to those in renewed and other AC-ED treated culture solutions. However, the lowest root dry weight was recorded in continuously applied AC-ED-treated culture solution, and the highest root dry weight was recorded in renewed culture solution. The maximum shoot fresh weight (267.4 g plant^{-1}) was found in nonrenewed culture solution subjected once weekly to AC-ED , which was identical to renewal and thrice weekly and continuous AC-ED treated culture solutions (Figure 4), and the minimum shoot fresh weight (130.2 g plant^{-1}) was found in recycled culture solution. The ascorbic acid content of lettuce was higher in nonrenewed nutrient solution than in the other treatments. In lettuce shoots and roots, the amounts of magnesium and zinc were noticeably different, but calcium, potassium and sodium concentrations did not differ between shoots and roots (Tables 2).

In both experiments I and III, the starting solution was fresh, but in the renewal treatment, lower lettuce yield was recorded in experiment III than in experiment I. This difference may have been due to the culture duration. The culture periods in experiment I and experiment III were 42 days and 35 days, respectively.

Effect of AC-ED application frequency per week on nonrenewed once-used (nonrenewed 2C) nutrient solution (Experiment IV)

In nonrenewed 2C nutrient solution, plant growth characteristics such as fresh weight of shoot, dry weight of shoot and root were reduced remarkably (Table 1; Figure 4). However, the numbers of leaves, maximum lengths and widths of leaves, longest root lengths and relative chlorophyll contents did not vary substantially among the nutrient solutions used. The highest dry weights of shoots (4.8 g plant^{-1}) and roots (0.41 g plant^{-1}) were found in once-used culture subjected thrice weekly to AC-ED. On the other hand, the lowest dry weights of shoots (2.7 g plant^{-1}) and roots (0.27 g plant^{-1}) were recorded in nonrenewed 2C nutrient solution. Shoot weight reached a maximum (108.8 g plant^{-1}) in nonrenewed 2C nutrient solution subjected thrice weekly to AC-ED and was similar under this condition to values observed for nonrenewed 2C nutrient solution subjected continuously to AC-ED. Shoot weight was found to be the lowest (64.8 g plant ^{1}) in nonrenewed 2C nutrient solution (Figure 4). The amounts of calcium, magnesium and zinc in the roots and zinc in the shoots of lettuce plants were notably affected by renewal and different AC-ED treatments, but concentrations of potassium, sodium and calcium in the shoots of the plants were not affected (Tables 2).

Lettuce growth performance and shoot fresh weight were lower in the once-used culture solution (experiment IV) than in the fresh culture solution (experiment III). A similar difference in lettuce performance was also observed between the once-used culture solution tested in experiment II and the fresh culture solution used in experiment I.

Discussion

The use of recycled hydroponics is limited due to the accumulation of phytotoxic compounds in the nutrient medium. Recent studies have stated that in nonrenewed solutions, allelochemicals are derived from root exudates in lettuce (Lee et al. 2006; Talukder et al. 2019b), strawberry (Asaduzzaman et al. 2012; Asao et al. 2008; Mondal et al. 2013; Kitazawa et al. 2005), cucumber (Asao et al. 1998; Yu and Matsui 1994), several leafy vegetables (Asao et al. 2004) and some ornamental plants (Asao et al. 2007) grown in recycled hydroponics. Efficient management of culture solutions for increasing lettuce yield in hydroponic systems should mitigate the inhibitory effect of allelochemicals. The application of AC-ED in nonrenewed nutrient medium was found to be an effective technique for removing allelochemicals from culture solution for continuous cultivation of lettuce (Talukder et al. 2019b). To apply AC-ED in nonrenewed nutrient solution, application intervals and frequencies need to be identified, and we conducted several experiments on successive cultivations of lettuce in hydroponic systems.

In fresh culture solution (experiment I), lettuce growth and yield declined considerably in the nonrenewed culture solution (Tables 1; Figure 3). Nutrient content in all types of culture solution was maintained as close as possible to that of 50% “Enshi” nutrient solution throughout the growing period. The contents of residual minerals in the cultivation solution did not fluctuate greatly. Therefore, the reduction in lettuce growth in the nonrenewed nutrient solution might be due only to inhibition by the accumulated allelochemicals.

Allelochemicals resulting from root exudation to the rhizosphere (Bertin et al. 2003) have been found to be responsible for influencing various physiological processes, such as transpiration, water consumption, photosystem II (PSII) output, nutrient absorption, dark respiration, adenosine triphosphate (ATP) production, cell cycling, phytohormone metabolism and gene expression. (Blum and Gerig 2005; Inderjit and Duke 2003). Therefore, in the nonrenewed solution, we found lower lettuce growth. Plants subjected to stressful conditions produce more reactive oxygen species (ROS), including allelochemicals that accumulate in the rhizosphere (Halliwell 2006; Rhoads et al. 2006; Yamamoto et al. 2003). These ROS are either toxic byproducts of aerobic metabolism or key growth, development and defense regulators (Laloi et al. 2004; Mehdy et al. 2008; Mittler et al. 2004). Several studies have shown that allelochemical stress can cause oxidative damage, as demonstrated by increased activity of ROS-scavenging enzymes and increased levels of lipid peroxidation of the membranes (Baziramakenga et al. 1995; Lara-Nuñez et al. 2006; Politycka 1996; Ye et al., 2004, 2006; Yu et al. 2003).

In addition, allelochemicals cause genome-wide gene expression changes and ultimately lead to root cell death (Bais et al. 2003). Hence, in the nonrenewed culture solution, we obtained reduced root growth. Damaged roots in turn impede water and the absorption of mineral nutrients. These effects resulted in reductions in leaf number per plant, leaf length and width, root length, shoot fresh and dry weight, etc. Lettuce cultivated in a nonrenewed nutrient solution showed lower contents of minerals; in particular, zinc was significantly lower in plant parts (Tables 2). This could be attributed to reduced nutrient uptake due to the accumulation of rhizospheric growth inhibitors (Singh et al. 1999). Autotoxic chemicals hamper the growth of roots; consequently, plant water and nutrient uptake declines considerably, resulting in low shoot dry weight.

In contrast, the application of AC-ED to the fresh nonrenewed culture solution at different intervals (weekly, two-weekly, and three-weekly) significantly increased lettuce growth, yield and nutritional quality, which were statistically similar to values from renewed culture. In case of once used culture solution weekly application of AC-ED showed better performance compare to other treatments. The mechanisms underlying this enhanced plant growth may include degradation of autotoxic substances, resulting in the elimination of deleterious effects on nutrient solution, due to application of AC-ED in nonrenewed culture media. Similar results have also been obtained for closed hydroponic strawberry (Asaduzzaman et al. 2012; Talukder et al. 2019a) and lettuce (Talukder et al. 2019b) production. No significant variation among the three AC-ED application intervals was observed, but the average value was highest in nutrient solution treated weekly by AC-ED

With fresh culture solution (experiment I), lettuce growth and yield did not substantially differ among weekly, two-weekly and three-weekly AC-ED-treated nonrenewed culture solutions. Similar results have also been reported in lettuce (Talukder et al. 2019b), for which yield did not vary substantially between weekly and two-weekly AC-ED-treated nonrenewed culture solutions. However, in the once-used culture solution in the present study (experiment II), different results were obtained; i.e., lettuce plant growth and yield were higher in nonrenewed 2C culture solution treated weekly by AC-ED. In the nonrenewed 2C solution, the calcium and potassium contents in the lettuce roots were markedly reduced, but the sodium content was higher in once-used nonrenewed nutrient solutions (Tables 2).

Lettuce growth and yield were remarkably lower in used culture solution (experiment II) than in fresh culture solution (experiment I). Relative chlorophyll content (SPAD value) was significantly decreased in the reused culture solutions (Figure 5). Lower chlorophyll content in leaves affects lettuce growth, resulting in reduced shoot fresh weight in reused culture solution compared to the fresh solution. This might be due to the accumulation of more autotoxic chemicals in the reused culture solution.

Many recent research findings have identified several organic acids, such as benzoic, phenylacetic, cinnamic, p-hydroxybenzoic, lauric, phthalic, vanillic, palmitic, and stearic acids, present in the root exudates of lettuce grown in nonrenewed solution (Asao et al. 2004; Lee et al. 2006), and these compounds have been recognized as key growth inhibitors. Lee et al. (Lee et al. 2006) also noted that the amounts and concentrations of these organic acids in nutrient solutions varied greatly with the frequency of reuse, usually suggesting an increasing trend with increasing reuse frequency. At comparatively low concentrations in the first culture, a few allelochemicals might be exuded from the roots. Afterwards, in the nonrenewed solution, the numbers of allelochemicals and their concentrations were found to be rising. As the level of allelochemicals was found to have increased in once-used nonrenewed solution, plant growth was severely affected by additive or synergistic means (Inderjit 1996). Consequently, further reduction of the growth and yield of lettuce plants was reported in once-used nonrenewed nutrient solution. For continuous and sequential production of lettuce by utilizing identical culture solutions in closed hydroponic systems, more frequent application of AC-ED is required to achieve a higher yield by reducing the negative effect of autotoxic chemicals.

As frequent (weekly) AC-ED application was found to be better for subsequent lettuce production in similar culture solutions, it was necessary to determine the most favorable frequency of AC-ED application per week in nonrenewed culture solution. We therefore attempted to identify a suitable frequency (number of times per week) for AC-ED application in nonrenewal culture (experiment III) and in once-used (nonrenewed 2C) nutrient solution (experiment IV). In both experiments, AC-ED was applied once weekly, thrice weekly, and continuously to culture solutions.

In experiment III, leaf number, maximum leaf width, maximum root length, and shoot fresh and dry weight were found to be lower in nonrenewed culture solution than in all other treatments (Tables 1; Figure 4). Lettuce showed better growth and yield in nutrient solutions treated by AC-ED at all frequencies and resembled lettuce grown in renewed nutrient solution. The reason for the growth enhancement in AC-ED-treated culture solution may be the detoxifying effect of AC-ED on allelochemicals. Similar results have also been achieved in closed hydroponic strawberry production (Asao et al. 2008). In the nonrenewed solution, root dry weight was recorded to be slightly higher, but nonrenewed solution showed shorter root length as well as lower shoots fresh and dry weights. This may reduce elongation of the primary root and induce the development of additional lateral roots due to the effect of allelochemicals. Similar results have also been reported in *Arabidopsis thaliana* by Abenavoli (Abenavoli et al. 2008). In experiment IV, lettuce was cultivated in once-used (nonrenewed 2C) nutrient solution (culture solution used in experiment III), including renewal solution. Plant growth and yield were greater in thrice-weekly AC-ED-treated nonrenewed 2C solution, which showed results similar to those for nutrient solution continuously treated by AC-ED (Tables 1; Figure 4).

Similar environmental conditions were maintained in all experiments (Experiments I, II, III and IV). However, growth and yield performance of lettuce varied greatly among experiments. This high variation may have been due to differences in seedling age and culture periods. The culture periods were different in experiment I (42 days) and experiment III (35 days). Both the seedling and nursery periods were 14 days in all experiments except in experiment IV. Seven-day-old seedlings were used in the nursery system for that experiment.

The shoot fresh weight of lettuce was higher in fresh culture solution (experiment III) than in the used culture solution (experiment IV). The SPAD value of leaves was also recorded to be significantly higher in fresh culture solution, resulting in increased growth and yield of lettuce (Figure 6). There was a positive correlation between the relative chlorophyll content and shoot fresh weight. This may be due to lower phytotoxic chemical accumulation in fresh culture solution in experiment III than in experiment IV.

In experiment III, all culture solutions were newly prepared to start with, and lettuce plant growth and yield did not vary remarkably among application frequencies of AC-ED. In contrast, more frequent (continuous or thrice weekly) application of AC-ED is better for lettuce growth and yield for cultivation in continuously used culture solution. These results may be due to the higher inhibitory effect of AC-ED on autotoxic chemicals that remained in the once-used culture solution.

The energy requirement for the AC-ED device is 0.144 megajoules (MJ) per hour. For detoxifying autotoxic chemicals in lettuce production, in practice, AC-ED machines need to be used for 24 hours at 14-day intervals. For one culture (4 weeks) of lettuce, two AC-ED applications are necessary, and the total energy requirement for that culture is 6.912 MJ.

For fresh culture solution (experiments I and III), the energy requirement (MJ/kg fresh weight) for AC-ED treatments was found to be lower than that of the once-used culture solution (experiments II and IV) for higher lettuce yield. The cost of energy is compensated for in the culture solution renewal process through continuous use without the reduction in yield of lettuce that occurs in nonrenewed culture solution.

AC-ED can be performed with an inexpensive and easily movable electric device in unchanged culture solution for hydroponic lettuce cultivation by using the same previously cultured nutrient solution. Thrice-weekly or continuous AC-ED application can improve lettuce yield under autotoxicity. On the other hand, nutrient solution renewal systems have a negative effect on our environment due to the disposal of used solution after each culture. Consequently, growing lettuce hydroponically in culture solution that has been previously used several times with AC-ED application thrice per week or continuously would be beneficial for lettuce producers. This would eventually reduce environmental pollution through the repeated use of the same culture solution for the production of lettuce in recycled hydroponic systems.

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Declaration of interest statement

The authors declare no conflict of interest.

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Figure 1. Pictures showing the status of lettuce plants at culture initiation (a) and before harvest (b) in 3-step vertical growing beds in the plant factory.

Figure 2. Pictures showing the variation in growth of lettuce plants under different treatments {Experiments I and II (from left to right): RW, NR, weekly AC-ED, two-weekly AC-ED, three-weekly AC-ED} & {Experiment III (from left to right): RW, NR, AC-ED once weekly, AC-ED thrice weekly, AC-ED continuously}.

Figure 3. Effects of interval of application of alternating current electrodegradation (AC-ED) to nonrenewed nutrient solution on yield (fresh weight of shoot) of lettuce cultivated in fresh (Experiment I) and once-used (Experiment II) culture solutions. The renewed solution was changed at 14-day intervals. Nonrenewed solutions were not changed, but nutrients were adjusted with 50% Enshi solution. (Bars represent standard errors of means, n = 9).

Significant differences among treatments are indicated by different letters according to Tukey-Kramar test at $P<0.05$.

Figure 4. Effect of frequency of application of alternating current electrodegradation (AC-ED) to nonrenewed fresh (Experiment III) and once-used (2C) (Experiment IV) culture solutions on fresh shoot weight of lettuce grown in recycled hydroponics. The renewed solution was changed at 2-week intervals. Nonrenewed solutions were not changed, but nutrients were adjusted with 50% Enshi solution. (Bars represent standard errors of means, n = 9). Significant differences among treatments are indicated by different letters according to Tukey-Kramar test at $P<0.05$.

Figure 5. Variation in the relative chlorophyll content (SPAD value) of lettuce leaves grown in fresh and once-used culture solution under different intervals (Experiments I and II) and of alternating current electrodegradation (AC-ED). Bars represent standard errors of the means, n=9. Significant differences among treatments are indicated by different letters according to Tukey-Kramar test at $P<0.05$.

Figure 6. Relative chlorophyll content (SPAD value) of lettuce leaves grown in fresh and once-used culture solution under different frequencies (Experiments III and IV) of alternating current electrodegradation (AC-ED). Bars represent standard errors of the means, n=9. Significant differences among treatments are indicated by different letters according to Tukey-Kramar test at $P<0.05$.

Table 1: Growth and yield performance of lettuce under different treatments which are rearranged with experiment runs nested within solution type (fresh vs used culture solution)

Solution	Run	Renewal	AC-ED (hr./week)	Experiment	Treatments	Shoot Fresh wt. (g/plant)	Energy (MJ/kg)[1]	Leaf no. /plant	Max. leaf length (cm)	Max. leaf width (cm)	Ascorbic acid (ppm)	Shoot dry wt./plant (g)	Root dry wt./plant (g)	Max. Root length (cm)	Root/Shoot ratio (%)
Fresh	24/04/2019	0	0	1	Nonrenewed	230[2]		16.7	25.6	17.1	67.2	7.3	0.25	43.1	3.4
		0	24	1	Nonrenewed+ AC-ED weekly	370	0.17	23.3	36.6	25.9	56.8	11.3	0.46	56.2	4.1
		0	12	1	Nonrenewed+ AC-ED two-weekly	350	0.10	23.8	35.0	24.2	69.3	8.6	0.37	53.4	4.3
		0	8	1	Nonrenewed+ AC-ED three-weekly	340	0.07	26.6	33.4	22.8	77.1	9.3	0.45	55.3	4.8
		1	0	1	Renewed	360		21.4	33.9	24.3	73.5	12.5	0.34	55.9	2.7
	17/07/2019	0	0	3	Nonrenewed	130		23.6	30.3	22.9	132.0	4.3	0.39	44.9	9.1
		0	24	3	Nonrenewed+ AC-ED once-weekly	267	0.17	22.3	33.3	25.6	58.1	7.1	0.33	52.0	4.6
		0	72	3	Nonrenewed+ AC-ED thrice-weekly	240	0.64	23.8	33.8	26.3	73.3	7.4	0.34	54.0	4.6
		0	168	3	Nonrenewed+ AC-ED continuously	260	1.26	27.1	33.0	27.7	78.5	8.8	0.30	53.9	3.4
		1	0	3	Renewed	230		16.2	20.4	17.0	81.4	7.6	0.48	52.0	6.3
Used	05/06/2019	0	0	2	Nonrenewed	153		16.9	24.2	22.1	73.6	5.7	0.23	51.4	4.0
		0	24	2	Nonrenewed+ AC-ED weekly	234	0.29	17.3	27.6	23.9	76.4	8.7	0.43	50.3	4.9
		0	12	2	Nonrenewed+ AC-ED two-weekly	210	0.20	16.3	24.8	21.2	78.1	8.7	0.42	51.0	4.8
		0	8	2	Nonrenewed+ AC-ED three-weekly	200	0.16	16.6	26.2	22.2	77.3	8.3	0.45	52.0	5.4
		1	0	2	Renewed	185		16.3	24.2	19.9	79.7	7.5	0.42	45.8	5.6
	21/08/2019	0	0	4	Nonrenewed	65		14.8	19.7	14.8		2.7	0.27	48.7	10.0
		0	24	4	Nonrenewed+ AC-ED once-weekly	70	4.49	15.9	20.7	15.6		3.4	0.39	45.2	11.5
		0	72	4	Nonrenewed+ AC-ED thrice-weekly	109	1.59	17.1	19.2	16.4		4.8	0.41	47.6	8.5
		0	168	4	Nonrenewed+ AC-ED continuously	100	4.64	15.7	20.2	16.0		3.5	0.35	48.2	10.0
		1	0	4	Renewed	80		16.2	20.4	14.7		2.8	0.33	48.6	11.8

[1] Amount of electrical energy (MJ/kg) required to impose each treatment of AC-ED

[2] Indicates the average value

The different colors represent the trend of variation among of the parameters of four experiments together. In each column, values highlighted in darker color represent higher value. All data of different parameters presented were tested at 5% level using Tukey-Kramar test.

Average of all cultures	**209**	**1.15**	**19.4**	**27.1**	**21.0**	**77.0**	**7.0**	**0.37**	**50.5**	**6.2**

Table 2: Nutrient content in shoot and root of lettuce under different treatments which are rearranged with experiment runs nested within solution type (fresh vs used culture solution)

Solution	**Run**	**Renewal**	**AC-ED (hr./week)**	**Experiment**	**Treatments**	**Ca Shoot (g/kg)**	**Ca Root (g/kg)**	**Mg Shoot (g/kg)**	**Mg Root (g/kg)**	**K Shoot (g/kg)**	**K Root (g/kg)**	**Na Shoot (g/kg)**	**Na Root (g/kg)**	**Zn Shoot (mg/kg)**	**Zn Root (mg/kg)**
Fresh	24/04/2019	0	0	1	Nonrenewed	18.8[1]	12.9	3.6	2.10	70.8	90.5	1.26	3.10	14.9	24.3
		0	24	1	Nonrenewed+ AC-ED weekly	17.6	12.2	4.0	2.60	60.9	85.5	1.50	2.90	16.9	57.3
		0	12	1	Nonrenewed+ AC-ED two-weekly	16.6	15.9	3.8	3.60	55.8	83.3	1.61	2.90	12.4	34.8
		0	8	1	Nonrenewed+ AC-ED three-weekly	15.3	11.7	3.2	2.10	59.3	89.5	0.93	2.80	14.7	43.6
		1	0	1	Renewed	16.0	11.1	3.2	2.40	63.6	81.2	0.83	1.70	14.2	44.7
	17/07/2019	0	0	3	Nonrenewed	11.4	5.4	3.2	3.90	54.9	82.8	0.69	1.68	30.3	51.4
		0	24	3	Nonrenewed+ AC-ED once-weekly	9.9	5.8	3.1	2.90	50.7	98.0	0.56	2.01	17.1	70.3
		0	72	3	Nonrenewed+ AC-ED thrice-weekly	10.5	6.2	2.6	2.10	48.6	84.1	0.62	1.87	20.4	66.2
		0	168	3	Nonrenewed+ AC-ED continuously	10.6	6.7	2.6	1.80	49.6	80.9	0.59	1.74	15.8	69.6
		1	0	3	Renewed	11.6	6.0	3.4	3.20	53.8	91.6	0.68	1.81	28.9	62.7
Used	05/06/2019	0	0	2	Nonrenewed	9.9	3.6	4.2	2.40	79.0	49.4	1.60	5.60	33.1	41.8
		0	24	2	Nonrenewed+ AC-ED weekly	7.8	4.2	4.8	2.70	66.8	88.0	1.30	2.50	35.6	70.0
		0	12	2	Nonrenewed+ AC-ED two-weekly	8.0	4.9	3.6	2.50	75.2	85.2	1.00	2.50	33.3	70.6
		0	8	2	Nonrenewed+ AC-ED three-weekly	6.5	6.8	4.3	2.50	78.0	96.8	1.40	2.50	29.8	67.1
		1	0	2	Renewed	7.3	5.5	3.5	2.80	68.1	88.4	1.40	1.90	22.4	68.0
	21/08/2019	0	0	4	Nonrenewed	13.8	7.3	3.6	3.70	69.5	51.3	1.00	1.60	19.6	33.3
		0	24	4	Nonrenewed+ AC-ED once-weekly	14.4	9.8	4.0	2.50	73.1	53.1	1.00	1.80	18.7	33.3
		0	72	4	Nonrenewed+ AC-ED thrice-weekly	15.7	5.1	3.5	2.10	66.6	60.2	1.00	1.80	25.1	40.7
		0	168	4	Nonrenewed+ AC-ED continuously	14.7	3.4	3.8	1.80	74.7	56.1	1.00	1.60	17.6	32.7
		1	0	4	Renewed	14.7	4.9	3.4	0.50	68.8	54.4	0.90	1.50	19.3	46.7

[1] Indicates the average value

The different colors represent the trend of variation among of the parameters of four experiments together. In each column, values highlighted in darker color represent higher value. All data of different parameters presented were tested at 5% level using Tukey-Kramar test.

Average of all cultures	**12.6**	**7.5**	**3.6**	**2.51**	**64.4**	**77.5**	**1.05**	**2.29**	**22.0**	**51.5**