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# 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 intraspecific 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 improve 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 it was decreased significantly in non-renewed solution with DC-ED similar to non-renewed solution. A similar trend was also 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.

## 1. Introduction

Hydroponic culture has been practiced for a wide variety of crops 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). Consequently, under autotoxicity condition, damaged strawberry roots hamper water and mineral nutrient uptake. As a result, the growth of shoot and root, number of

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flowers and harvested fruit per plant and fruit enlargement greatly reduced (Kitazawa et al., 2005).

Elimination of these accumulated root exudates or autotoxic growth inhibitors from closed hydroponic system would be of great interest to the strawberry grower leading 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 electro-chemical means is another way of detoxifying 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 h 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 to 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 in the same time it also decreases concentration of iron and calcium in the nutrient solution, lower the solution pH and increase solution temperature (Asaduzzaman et al., 2012). During DC-ED, iron and calcium ions were found to be precipitated on internal surface of anode resulting decrease in concentration.

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), both positive and negative charges of the electrodes (anode and cathode) change frequently. Thus, iron and calcium ions might not be precipitated to the electrode (especially in 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 were 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 °C (day/night), 60% relative humidity, fluorescent light with intensity of 145 $\mu mol\ m^{-2}\ s^{-1}$ and a 12 h 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 $min^{-1}$. 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 [Hori, 1966, 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 $\mu mol\ L^{-1}$ 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 A alternate current, and 14.0 volts. Nutrient solution samples (25 ml) were collected at 0, 1, 3, 6, and 24 h 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 h 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