DEVELOPMENT OF ENVIRONMENTALLY FRIENDLY SOILLESS CULTIVATION SYSTEM BY PHOTOCATALYTIC TREATMENT

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ABSTRACT

From the viewpoint of environmental conservation agriculture, it is desirable not only to switch nutrient solutions from opened to closed systems in soilless cultivation, but , （F_1） also to switch culture substrates from rockwool to organic substrates. This study investigated for development of closed soilless cultivation system using rice hull substrate by TiO_2 photocatalytic treatment of the waste nutrient solution.

First, performance of the photocatalytic treatment for closed soilless cultivation system using rice hull substrate was tested. The treatment successfully controlled the phytotoxicity eluted from the rice hull substrate and significantly increased growth and yield of tomato and rose comparing to closed system without the treatment. In addition, the yield was comparable with conventional open system using rockwool substrate at greenhouse under sun light.

Second, photocatalytic decomposition and detoxification of phytotoxic compounds released from only plant roots investigated. The phytotoxic activity of the phytotoxically active fraction extracted either from asparagus roots or from the recycled nutrient solution of an asparagus soilless cultivation system using inorganic substrate was decreased by photocatalytic treatment .

Finally, to enhance disease control in the closed soilless cultivation system using rice hull substrate, the suppression of bacterial wilt disease in a waste nutrient solution with photocatalytic and silver treatments was investigated. The untreated waste nutrient solution had a high Total Organic Carbon (TOC) concentration, and silver in the untreated solution had no antibacterial activity. By contrast, silver in the photocatalytically treated solution showed a high antibacterial effect via the low TOC of the treated solution. A similar trend of disease onset was observed when pathogenic bacteria were inoculated into the solutions used to cultivate tomato seedlings. From these results, it is expected that combining photocatalytic and silver treatments can contribute to stable crop production in closed soilless cultivation.

CHAPTER Ⅰ

Introduction

Ⅰ－1　BACKGROUND OF THE RESERCH

Soilless cultivation is a method used for growing crops without the use of soil as a root growing substrate where the nutrient solution comprising fertilizer dissolved in water is applied instead. This cultivation method enables stable cultivation over long periods without damage of plant yield caused by repeated cultivation of the same crop in the same soil. Furthermore, the method offers the benefits of being less labor-intensive than soil cultivation and helps establishing a safe and clean work environment (Japan Greenhouse Horticulture Association, 2002; Miyama et al., 2012a).

Soilless cultivation in Japan began in 1946. The area used for soilless cultivation accounted for only 4% of the facility-based horticultural land area in 2009, and this area, however, has increased significantly from 374 ha in 1989

to 916 ha in 1999, and 1,741 ha in 2009. The largest grown area among vegetables under soilless cultivation is for a tomato (Ito, 1999; Ministry of Agriculture, Forestry and Fisheries, 2011), accounting for 502 ha in 2009, whereas the most common ornamental crop under soilless cultivation is rose (Okada, 2003). In Kanagawa Prefecture, soilless cultivation has long been used, accounting for 5.5% of its facility greenhouse area in 2009, which was more than the national average of 3.4%.

Several different cultivation systems are currently used for soilless cultivation, the most common system is open system using rockwool substrate. Rockwool substrate has physical properties well suitable for crop cultivation. It is prepared by mixing silica stone with basalt or diabase and iron ore slag and further melting them at a high temperature to form a fibrous material. Open system is the method that the nutrient solution provides 20–30% more nutrients than a plant will absorb, and any surplus solution is discarded as runoff.

Recently, several approaches for cultivation are used in order to change to an environment-oriented agriculture (Miyama et al., 2002). Also, switching to the method to be environmentally friendly as well in soilless cultivation is desired (Van, 1995; Ruijs, 1994; Takemoto et al., 2010).

The open system using rockwool substrate results has two types of environmental impact. First, the use of rockwool as substrate entails changes in its physical properties after several years of use, eventually makings are unsuitable for crop cultivation and leading to the need for replacement. Used rockwool is disposed as industrial wastes; the amount disposed has been estimated to be 7.5 m^3 per 1,000 m^2 of tomato cultivation area, which affects the environment. The second environmental impact is due to the nutrient solution runoff, which has been estimated to be 750 metric tons per 1,000 m^2 of tomato cultivation area each year (Japan Greenhouse Horticulture Association 2002). This runoff contains fertilizer components such as highly concentrated NO_3^-, PO_4^{3-}, K^+ and other nutrients, which can cause eutrophication of the ground water and rivers. The standard value for the total concentration of nitrate nitrogen and nitrite nitrogen specified by Japan's environmental standards for water and ground water pollution is 10 mg L^{-1}. No studies have reported the occurrence of levels higher than this standard value in runoff from soilless cultivation; but, ideally, the amount of runoff draining into the environment should be reduced as much as possible.

Switching to soilless cultivation could also improve production. To lower production costs, growers need to reduce the expenditure incurred for substrate material and fertilizers. Lower production costs will enable growers who use the open system using rockwool substrate to maintain their agricultural businesses amid the decrease in the price of produce and the increase in the costs of materials and fertilizers.

Switching substrates and cultivation systems is desirable for both the reasons for environment and production. Rockwool, which becomes industrial waste after use, should be switched to a substrate that can be easily returned to environment such as rice hulls or coconut husks (Shinohara et al., 1999), and the conventional open system should be switched to the closed system that recycles the waste nutrient solution (Van, 1994; 2000; Kajihara et al., 2007). However, it is not enough to simply replace the closed system from the open system and to replace rockwool substrate with rice hulls or another organic substrate that enables easy return to the environment. These activities may increase the concentration of organic contaminates (Kato et al., 1973; Takahashi et al., 1976; Kato et al., 1977; Miyama et al., 2004; Chung et al., 2005; Kong et al., 2006; Kato-Noguchi et al., 2008) in the recycling nutrient solution and inhibit crop growth. Similarly, autotoxic compounds (Putnam et al., 1984; Yu and Matsui 1993; Pramanik et al., 2000; Singh et al., 1999; Asao et al., 2003; 2004) mixing into the nutrient solution from crop roots can inhibit crop growth by accumulating in the nutrient solution. Moreover, soil-borne diseases can occur even in soilless cultivation systems (Zinnen, 1988). Pathogen gets into the nutrient solution, disease occurs and rapidly spreads over the entire closed soilless systems,

causing serious damage and yield loss.

Closed systems using organic substrate, such as rice hull, therefore require a technique for decomposing organic contaminates and pathogenic microbes in waste nutrient solution. Currently, there are a heat treatment and UV (254 nm) treatment for method of sterilization of nutrient solution. However, decomposition of organic contaminates in the nutrient solution is not possible in these process (Japan Greenhouse Horticulture Association, 2002). Ozone treatment can be a powerful tool to perform the organic contaminants decomposition and sterilization. However, the problem remains in the safety of labors and the crop. Moreover, since soilless cultivation farms are relatively small in Kanagawa prefecture, installing the large-scale equipment is not cost-effective. To deal with these issues, a photocatalytic treatment of nutrient solution utilizes applying sun light as its light source have been developed in this study.

Ⅰ－2　PHOTOCATALYST

Ⅰ－2－1　Progress of research on photocatalysts

In 1972, Fujishima announced that by irradiating a titanium dioxide in water electrode with ultraviolet(UV) light, oxygen is generated while hydrogen is generated from the other electrode(platinum), resulting in the photolysis of water (Fujishima and Honda, 1972). This reaction is called the Honda–Fujishima effect after the names of the discoverers (Fujishima et al., 1999). Because the time of this discovery coincided with the first oil crisis, the above-mentioned publication on the generation of hydrogen from water attracted much attention.

Around 1980, a highly efficient reaction that generates hydrogen from water was discovered by adding organic substances to water (Hashimoto et al., 1984; 2005). By introducing an organic substance into water, hydrogen was found to be generated at a very high efficiency by the oxidation of the organic material and the reduction of water. At the time, it was believed that this discovery could relieve the energy crisis, and many researchers participated in this study from all over the world. However, the hydrogen generation from this reaction was low, and the practical application could not be achieved.

Around 1990, research aiming at adding the function of decomposing organic matter on the surfaces of buildings by coating the surfaces with Titanium dioxide had been started (Fujishima et al., 1999). From around 1995, antibacterial tiles, self-cleaning lighting covers, and so on started being implemented (Hoffmann et al., 1995; Watanabe, 1996; Herrmann, 1999; Lettmanna et al., 2001). By the year 2000, a novel Titanium dioxide surface with a thin film photocatalyst, which was responsive not only to UV light but also to light of a wide range of wavelengths up to the visible region (Irie et al., 2008). Thus, the application range of photocatalysts was expected to expand.

Currently, by taking advantage of the properties of a photocatalyst, non-staining lighting appliances, building materials, antibacterial tiles, air purifiers that decompose malodorous substances and bacteria, etc., are in practical use. Because this technology was created in Japan, our country is also the leader in its application and commercialization. However, in recent years, practical applications have also been progressing in China, Taiwan, Korea, Europe, North America, etc.

Ⅰ－2－2　Titanium dioxide (TiO_2)

Titanium dioxide is currently being used as a photocatalyst in recently marketed photocatalyst application products (Hashimoto et al., 2005). TiO_2 is a white powder that is widely used as a pigment for producing cosmetics and paints designed to exhibit as little response to light as possible. However, for photocatalyst applications, materials with the highest possible photoreactivity are used. TiO_2 has three types of crystal structures: rutile, anatase, and brookite. The rutile and anatase types are used for industrial applications. The anatase type has a higher photocatalytic activity than the rutile type; therefore, the rutile type is mainly used for white pigments and paints while anatase is primarily used as a photocatalyst. Although there are semiconductors other than TiO_2 with photocatalytic activity, TiO_2 is superior to other semiconductors in terms of durability, stability,

activity, and price. Furthermore, when TiO_2 is applied to building materials and air purifiers, it is coated onto the surface of the materials, rather than using it in its original powder-like form. Depending on this coating method, the intensity of the decomposition activity and duration of the effect vary significantly. Therefore, if a high decomposition activity is desired, it is necessary to conduct a search starting with the coating ingredients used with TiO_2.

I－2－3 Reaction principle of a TiO_2 photocatalysis

When irradiating a TiO_2 semiconductor with light at a wavelength with energy exceeding the band gap of TiO_2, i.e., UV light of 400-nm wavelength or less, electrons are generated in the conduction band, and positive holes are generated in the valence band. The electrons and holes react with the substance to promote an oxidation–reduction reaction (Hashimoto et al., 1996; Fujishima et al., 1999). This is called the photocatalytic reaction. The characteristic of the TiO_2 photocatalytic reaction is that the oxidizing power of the positive holes is stronger than the reducing power of the excited electrons. The oxidative degradation of environmental pollutants is performed using the strong oxidizing power of TiO_2. In a TiO_2 photocatalytic reaction, it is possible to use an UV lamp as a light source, but the UV region in sun light can also be used

I－2－4 Light sources used in TiO2 photocatalytic r eactions

Decomposition by a TiO_2 photocatalyst occurs when a substance to be decomposed is placed near the surface of TiO_2 and proceeds by light exposure (Fujishima et al., 1999; Hashimoto et al., 2005). The device can be made compact in the case of using an artificial light source in a cultured liquid purifying apparatus (Ollis et al., 1991; Herrmann 1999; Noguchi et al., 2003), but the operating cost of the irradiating light is high. However, solar energy is available in many agricultural areas regardless of the hydroponics, and solar energy does not have any cost. The intensity of UV light in sun light is, on average, approximately 1 mW cm^{-2}. When the photocatalytic reaction under sun light is used, efficiency is improved by increasing the surface area of the TiO_2 exposed to the light. For example, if 1 mol of trichloroethylene contained in 1 L of water is decomposed by irradiating it from above with sun light having an UV light intensity of 1 mW cm^{-2}, the decomposition would take approximately six years if the sample was in a 10 × 10 × 10 cm^3 container (light absorbing area 0.01 m^2). However, the decomposition would take only about 20 days in a 100 × 100 × 0.1 cm^3 container (light absorbing area 1 m^2)(Hashimoto, 2002).

Therefore, in this study, a porous material with a large surface area coated with TiO_2 in a shallow processing container was used as the photocatalytic material for processing. And sun light as the light source for the reaction of the TiO_2 photocatalyst was decided.

I－3 PUROPOSES OF THE PRESENT STUDY

From the viewpoint of environmental conservation in agriculture, it is desirable in soilless cultivation not only to the switch nutrient solution system from opened to closed, but also to switch culture substrates from rockwool to organic substrates. The purpose of the present study is to development of environmentally friendly soilless cultivation system with TiO_2 photocatalytic treatment under sun light. This system is a closed system using organic substrate such as rice hull instead of rockwool. The photocatalytically treatment equipment uses sun light as its light source, with no reliance on artificial lights.

I－4 ORGANIZATION OF THE PRESENT STUDY

This paper consists of the following sections:

In CHAPTER Ⅱ, the effectiveness of photocatalytic treatment of a nutrient solution, rice hull as the substrate, and a recycling nutrient solution on the growth and yield of tomatoes (the food crop most commonly grown by soilless cultivation in Japan)was investigated (Miyama et al., 2009).

In CHAPTER Ⅲ, the effectiveness of photocatalytic treatment on a nutrient solution, rice hull as the substrate, and a recycling nutrient solution on the growth and yield of roses (the ornamental crop most commonly grown by soilless cultivation in Japan)was investgated (Miyama et al.,

2012b).

In CHAPTER Ⅳ, photocatalytic decomposition and detoxification of phytotoxic compounds released from plant roots of asparagus as the experimental crop was investigated. (Sunada et al., 2008). Here, in order to investigate only the effect of phytotoxic compounds released from roots was used for the inorganic substrate, not the rice hull substrate.

In CHAPTER Ⅴ, to enhance disease control in the closed soilless cultivation system using rice hull substrate, the suppression of bacterial wilt disease of tomato in a simulated waste nutrient solution with photocatalytic and silver treatments was investigated (Miyama et al., 2013). Finally in CHAPTER Ⅵ, overall summary and conclusions considering about findings of this study were described.

CHAPTER Ⅱ

Closed soilless cultivation system of tomatoes planted on rice hull substrate with photocatalytic treatment of waste nutrient solution

Ⅱ－1 INTRODUCTION

Soilless cultivation is increasing because it offers a number of advantages, including the ability to control crop growth and the ease of expanding the scale of cultivation (Ito, 1999; Van, 1995; Ruijs, 1994). Almost all of the current soilless cultivation systems are open systems in which plants grown on inorganic substrate, such as rockwool, are supplied with a solution containing NO_3^-, PO_4^{3-}, K^+ and other nutrients. This approach, however, has 2 disadvantages from the viewpoint of conservation-oriented agriculture. The first is the eutrophication of soil and groundwater caused by the 10–30 % of the supplied nutrient solution that is not absorbed by the plants and is instead released into the environment as a waste product containing high concentrations of NO_3^- , PO_4^{3-} ,K^+ and other nutrients.The second drawback is the inevitable production of industrial waste when the rockwool substrate is renewed due to degradation of its physical properties over time (Daum and Schenk, 1998; Yu and Komada, 1999). Therefore, to realize conservation-oriented cultivation, it is desirable to use a closed soilless cultivation system where the waste nutrient solution is recycled rather than discharged into the environment (Pagliaccia et al., 2007). Additionally, it is also necessary to replace inorganic media such as rockwool with an organic substrate that can be decomposed in the environment.

Rice hull substrate is a promising alternative to rockwool because, rice hulls after use, can be returned to crop fields. In addition, they are readily available and inexpensive. However, the use of rice hull substrate in closed soilless cultivation causes growth inhibition and a decline in crop yield because phytotoxic compounds, such as momilactones, released from the rice hulls accumulate in the nutrient solution recycling in the system (Kato et al., 1973; Takahashi et al., 1976; Kato et al., 1977; Miyama et al., 2004; Chung et al., 2005; Kong et al., 2006; Kato-Noguchi et al., 2008).

It is well known that when titanium dioxide (TiO_2) absorbs ultraviolet (UV) light with a wavelength shorter than its band gap (approximately 380 nm), it exhibits a strong oxidation effect on organic compounds that are adsorbed to its surface to decompose them into CO_2 (Hoffmann et al., 1995; Heller 1995; Hashimoto et al., 2005; Fujishima et al., 1999). This oxidization power has been applied to purify environmental pollutants, mainly from the air and water (Ollis et al., 1991; Herrmann 1999; Lettmanna et al., 2001; Noguchi et al., 2003; Sun et al., 2003; Pichat et al., 2005; Lee et al., 2005). Therefore, TiO_2 photocatalytic treatment could also decompose and detoxify the phytotoxic compounds from rice hulls.

To test this hypothesis, in this study the effect of photocatalytic treatment of the recycling waste nutrient solution on the growth and yield of tomato plants in a closed soilless cultivation system using a rice hull substrate was investigated. As a model experiment, the phytotoxic activity of the rice hull extract was evaluated from the

germination rate of lettuce and tomato seeds before and after the photocatalytic treatment. Next, tomatoes planted on rice hull substrate in the closed soilless cultivation system were grown over six experiment terms for three years, and the growth and yield in the system where the waste nutrient solution was treated with a TiO_2 photocatalyst before being recycled back through the system (photocatalytically treated system; Fig. Ⅱ-1(a)) were compared with those in a system where untreated waste nutrient solution was recycled (untreated system; not shown) was compared.

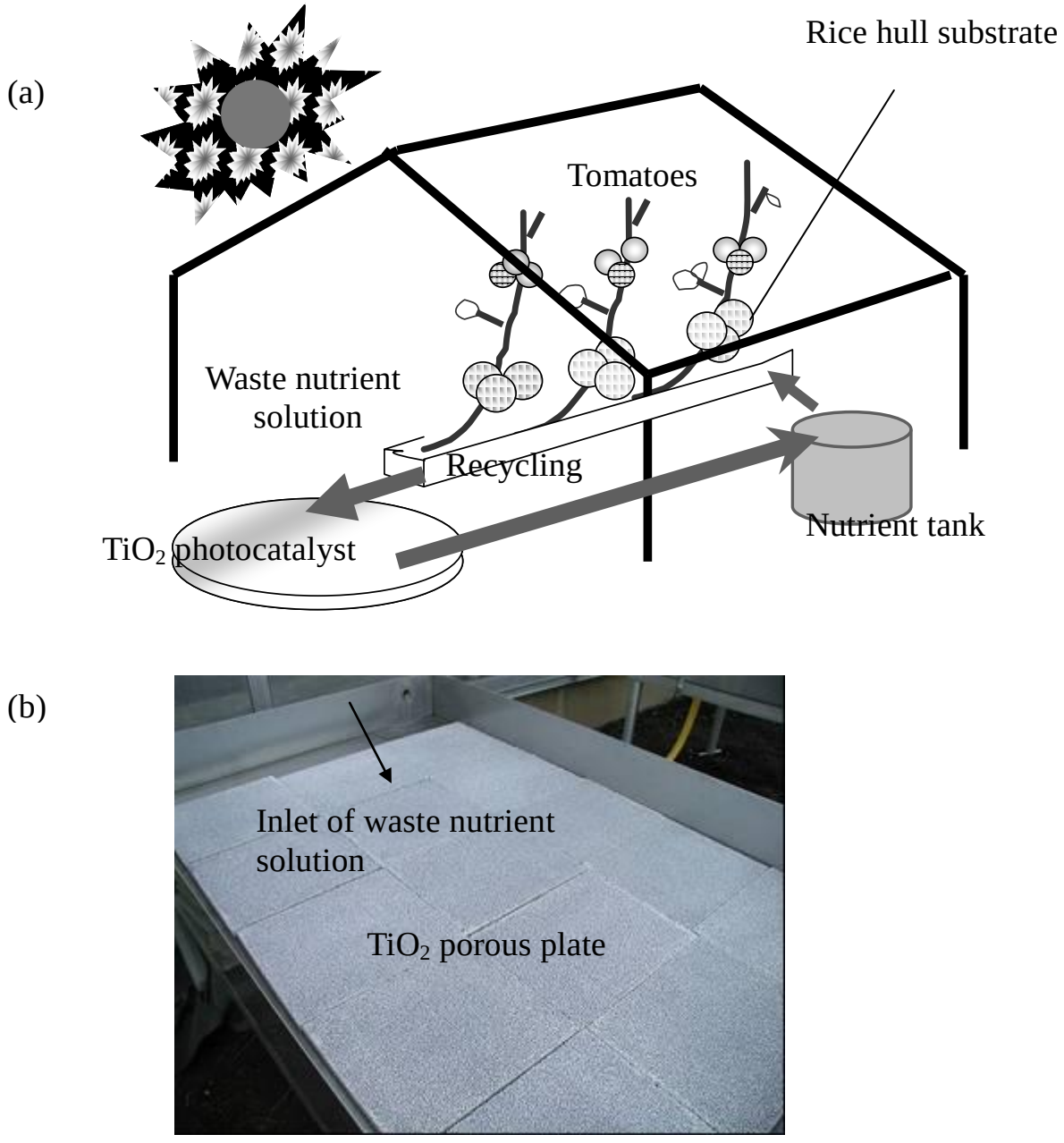


Figure Ⅱ-1 (a) Overview of a closed soilless cultivation system of tomatoes using rice hull substrate with photocatalytic treatment. (b) Photograph of TiO_2 porous plates set up outside a greenhouse in the closed soilless cultivation system.

Ⅱ－2　MATERIALS　AND　METHODS

Ⅱ－2－1　Photocatalytic material

A porous alumina plate coated with TiO_2 anatase-type nanoparticles (TiO_2 porous plate, 245 × 295 × 20 mm, Seiwa Kogyo Co.) was used to treat the rice hull extract and the waste nutrient solution from the closed soilless cultivation system (Fig. Ⅱ-1(b)).

Ⅱ－2－2　Model experiment: Effectiveness of photocatalytic treatment in rice hull extract

Pure water (500 mL) was added to 100 g of milled rice hulls in a glass bottle, and the mixture was shaken for 24 hours at 35 °C. The rice hull extract was prepared by removing the hulls from the mixture by filtering with a filter mesh size of ca. 0.3 mm. For comparison, extract from rockwool was also prepared by the same procedure. Each extract from rice hull or rockwool was conducted only once.

Photocatalytic treatment of the extract (300 mL) with a TiO_2 porous plate was conducted in a stainless steel container (250 × 300 × 20 mm) by irradiating the catalyst contained in the extract with UV light at an intensity of 2.0 mW cm^{-2} ,when the UV light intensity was measured by a UV photometer (UM-10 with UM360 sensor, Konica Minolta Co.) from black light tubes (Type FL15 BL-B, Hitachi Co. Japan). The concentration of total organic carbon (TOC) in a sample after being irradiated for a specified time was measured with a TOC analyzer (TOC-5000A, Shimadzu　Co.).

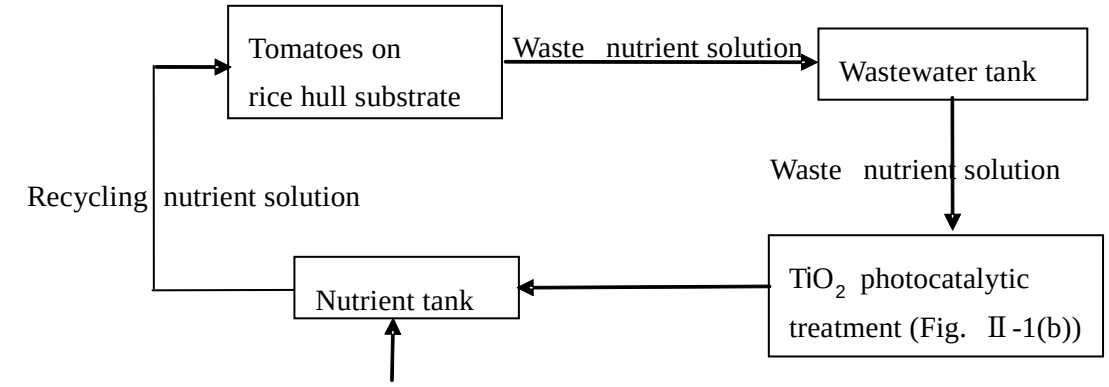
The phytotoxic activity of the extract was tested with a conventional germination method using lettuce seeds (*Lactuca sativa* L., cv Calmar MR, Nihon Nosan Seed Co.) (Kato et al., 1977; Yu and Matsui, 1993b). Briefly, 1.6 mL of the sample was poured over filter paper (Advantec No. 6) in Petri dishes (6 cm in diameter). Three dishes were used for the germination assay of each sample, and 15 lettuce or tomato seeds were sown in each dish. A control test using deionized water was conducted for each assay. In addition, the extract from rockwool was also assayed for comparison. After the dishes with lettuce seeds were incubated in the dark at 25 °C for 24 hours, lettuce seed germination rates for both the sample and control dishes were calculated as the average of three dishes for each sample. And the dishes with tomato seeds were incubated in the dark at 25 °C for 72 hours, tomato seed germination rates for both the sample and control dishes were calculated and radicle lengths were measured.

Ⅱ－2－3　Effectiveness of photocatalytic treatment in closed soilless cultivation system of growth and yield of tomatoes planted on rice hull substrate

To ascertain the effectiveness of the photocatalytic treatment in actual cultivation conditions under sunlight, experiments were conducted in closed soilless systems using tomatoes (*Lycopersicon esculentum* Mill., cv Hausu Momotaro, Takii Seed. Co.) grown on the rice hull substrate. There were six experiments over a three-years period with each consisting of 2 different treatment systems. In the photocatalytically treated system (Fig. Ⅱ-2(a)), the waste nutrient solution received photocatalytic treatment before being recycled back through the system, whereas in the untreated system (Fig. Ⅱ-2(b)), the　untreated waste nutrient solution was directly recycled through the system. Photocatalytic treatment of the waste nutrient solution was conducted by cycling the waste nutrient solution (40 L) through 16 porous TiO_2 plates in a shallow stainless steel container (900 × 1800 × 150 mm) set up facing west outside a greenhouse (Fig. Ⅱ-1(b)). Subsequently, the treated solution was returned to the nutrient tank for reuse.

In each system, the rice hull substrate (thickness, 75 mm) was placed into a styrofoam bed (0.3 × 11 m) aligned in a north-south direction in a glass greenhouse (area 150 m^2) located at the Kanagawa Agricultural Technology Center. Fifty tomato seedlings were transplanted in each bed at a density of 2.4 plants m^{-2} (planting interval; 230 mm) and fed with a conventional nutrient solution containing NO_3^-, PO_4^{3-}, K^+, and other nutrients (prescribed by Yamazaki formula nutrient solution for tomatoes). The nutrient concentration in the nutrient tanks of both system was controlled mainly by electrical conductivity (EC) in the range of 0.8─1.8 dS m^{-1} depending on growth stage.

(a) Photocatalytically treated system



(b) Untreated sy

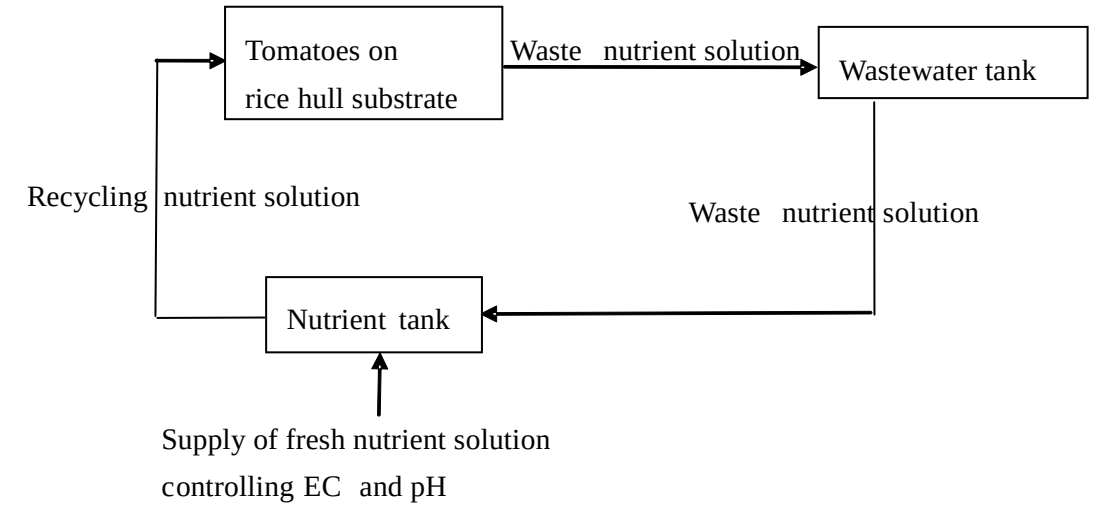


Figure Ⅱ-2 Schematic diagram for (a) the photocatalytically treated and (b) untreated closed soilless cultivation systems using rice hull substrate.

If the EC was higher than the set value, water was added, to the contrary, if it was lower, freshly concentrated nutrient solution was added. The pH in the nutrient tanks was also maintained at 5.5–6.5 by adding H_3PO_4 or KOH (Fig. Ⅱ-2).

The first transplantation was performed on August 29th, 2002, and the cultivation finished on January 23rd, 2003. The second transplantation was then performed and finished on July 24th, 2003. Plants in the same way twice a year for three years, using the same rice hull substrate continuously until the end of the 6th cultivation on July 21st, 2005 were cultivated. The concentration of TOC in the solution in the nutrient tank of each system was determined every other week for three years. To observe the tomato growth in the each system, 20 seedlings were selected as samples from the 50 transplanted seedlings, and the plant length, the number of leaves, and the stem circumferences just below every flower truss were measured during each of the six experiments. Furthermore, the stem weight and the number of trusses at the end of each experiment were compared for the two systems. The marketable tomatoes were also weighed as an index of the yield. The quality of the tomatoes was evaluated by the average Brix for five tomatoes obtained from every truss.

Ⅱ－3　RESULTS

Ⅱ－3－1　Model experiment: Effectiveness of photocatalytic treatment in rice hull extract

The concentrations of TOC in extracts from rice hulls and from rockwool were 1727 and 37 mg L^{-1}, respectively. The phytotoxic activity of the same extracts from the germination rate of lettuce seeds was determined. The germination rate for the rockwool extract was 100 % of the rate for deionized water used as a control. In contrast, the germination rate using the rice hull extract was 0 %, demonstrating strong phytotoxic activity of the extracts.

The rice hull extract showing strong phytotoxic activity was then subjected to either photocatalytic treatment with the TiO_2 porous plate or incubation in the dark with the same plate for the same period (ca. six days). Figure Ⅱ-3,4 shows the time change of the TOC concentrations of the extracts for each treatment. The TOC concentration decreased by 31 % when the extract was incubated in the dark, which was caused by the adsorption of organic constituents on the TiO_2 surface. In contrast, when it was irradiated with UV light, the concentration decreased with increasing irradiation time. The TOC concentration after four days of UV irradiation was approximately 99 % lower than the initial concentration. This result suggests that the organic constituents of the rice hull extract are oxidized to CO_2 by the TiO_2 photocatalytic reaction.

In parallel with this experiment, the phytotoxic activity of the same samples from the germination rate of lettuce seeds was determined. As shown in Fig. Ⅱ-3, the germination rate remained at 0 % for the extract incubated in the dark. However, the germination rate improved with UV irradiation, reaching the same level as the control after four days of UV irradiation. As shown in Fig. Ⅱ-4, the

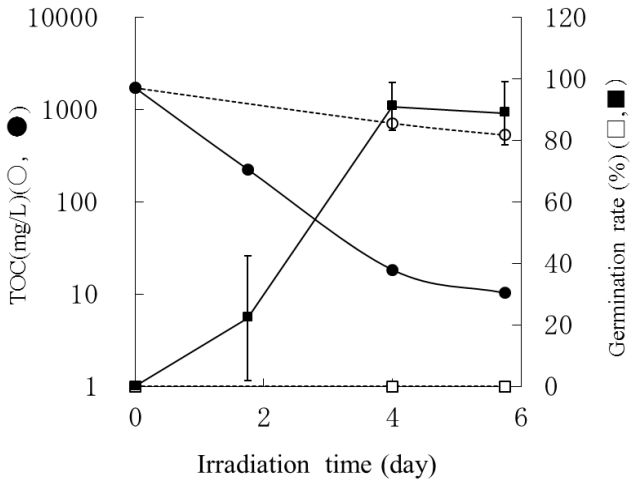


Figure Ⅱ-3 Temporal change of TOC concentration (○, ●) and lettuce germination rate (□, ■) for the rice hull extract. The solution was incubated with a TiO_2porous plate in the dark (○, □) or irradiated with UV light (●, ■). Error bars: Standard deviation of three replicates in the phytotoxic activity test.

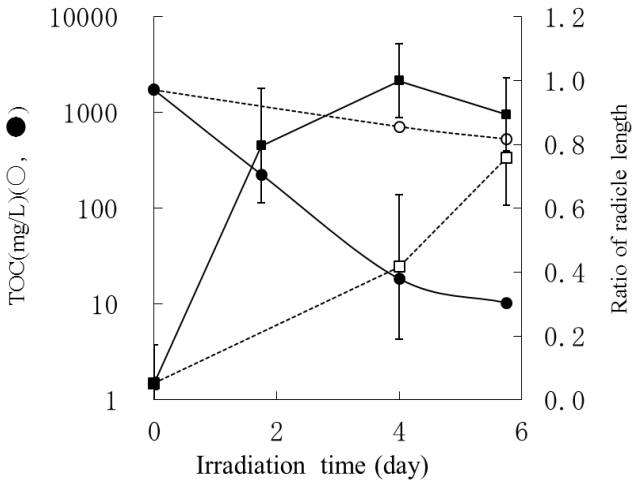


Figure Ⅱ-4 Temporal change of TOC concentration (○, ●) and tomato radicle length ratio (□, ■) for the rice hull extract. The solution was incubated with a TiO_2 porous plate in the dark (○, □) or irradiated with UV light (●, ■). Error bars: Standard deviation of three replicates in the phytotoxic activity test.

tomato seed radicle length was only 40% as long as the control for the extract incubated in the dark. However, when compared to the UV irradiation times, when radicle length reaching 100% of the control after four days of UV irradiation.

Ⅱ－3－2 Effectiveness of photocatalytic treatment in a closed soilless cultivation system of tomatoes

Figure Ⅱ－5 shows the temporal change of TOC concentrations in the solutions in each nutrient tank illustrated in Fig. Ⅱ- for the six experiments over three years. The TOC concentration in the untreated system shortly after transplanting the tomato seedlings in the 1st experiment term was high (800 mg L^{-1}). The high TOC concentration was considered to be due to the simultaneous extraction of organic constituents such as protein, sugar, and lipids from the rice hulls of the new substrate together with the phytotoxic compounds. The TOC concentration decreased to ca. 300 mg L^{-1} two weeks after transplantation and then varied between 100 and 200 mg L^{-1} in the 1st

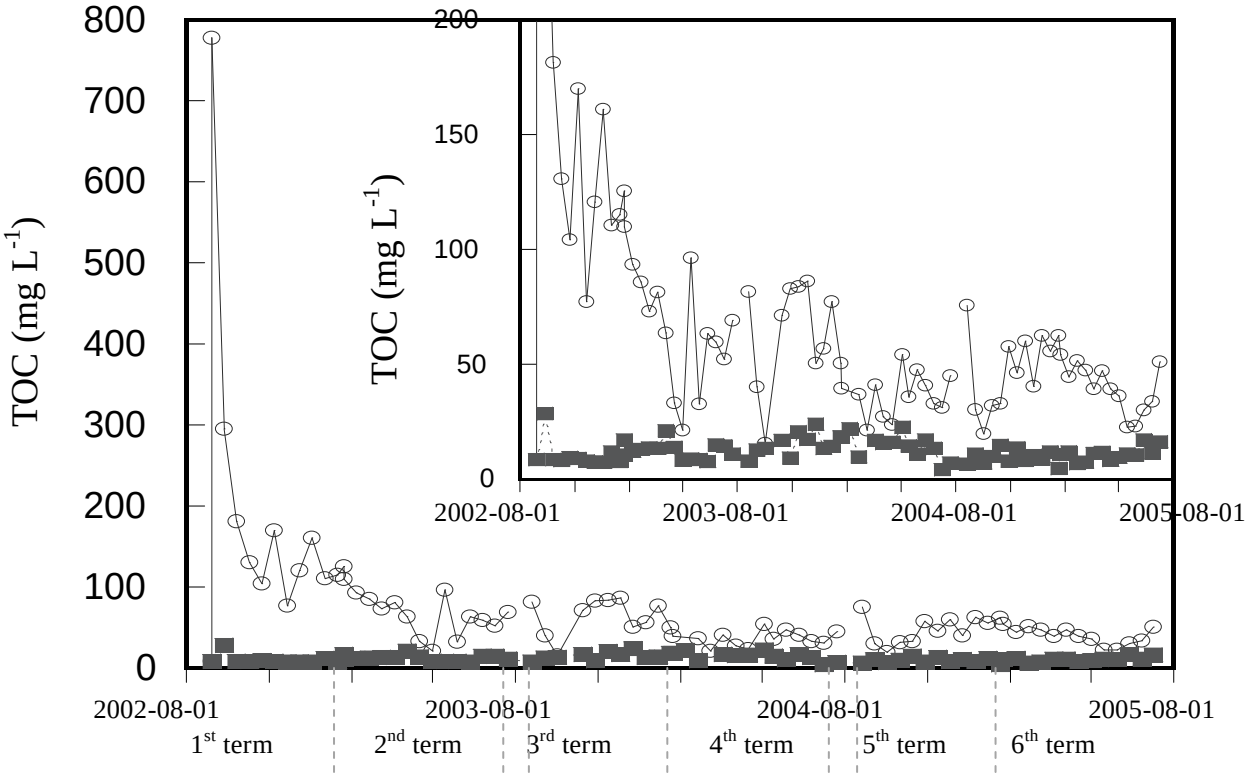


Figure Ⅱ-5 Temporal change of TOC concentration of the solution in the nutrient tank of the photocatalytically treated system (■) and the untreated system (○). The inset shows data plpted on the enlarged scale of TOC.

experiment term. In the photocatalytically treated system, on the other hand, a low TOC concentration of 8–30 mg L^{-1} was observed even after the transplantation, and the concentration subsequently remained at a low level of (approximately 5–20 mg L^{-1}) until the end of the 6th experiment. The TOC concentration of the freshly prepared nutrient solution remained constant at 5 mg L^{-1}, confirming that the low TOC concentration of the tank solution in the treated system was really due to the photocatalytic activity.

Next, growth indices such as plant length, the number of leaves, and stem circumference in the 1st experiment term were measured (Fig. Ⅱ-6(a)–(c)). A significant difference in plant length between the untreated and photocatalytically treated systems became apparent on the 28th day after transplantation (Fig. Ⅱ-6(a)). After 3 months, the plant length in the photocatalytically treated system was approximately 1.2 times longer than that in the untreated system. Photographs in Fig. Ⅱ-7 clearly show the difference in the plant length between the two systems. Similar trends were observed in the number of leaves (Fig. Ⅱ-6(b)) and stem circumference (Fig. Ⅱ-6(c)), demonstrating that the growth of tomato plants in the photocatalytically treated system was significantly higher than that in the untreated one (The unexpectedly short stem circumference average on the 84th day compared with that on the 70th day was due to the increase of slim stems at several measurement points). Similar changes in the growth indices were also observed in the second to 6th experiments; for example, the plant length in the photocatalytically treated system was approximately 1.1–1.6 times longer than that in the untreated system.

The growth in each experiment was further evaluated based on the stem weight and the number of trusses at the end of each experiment (Table Ⅱ-1). The cultivation in a greenhouse in the 2nd, 4th and 6th terms (seedling transplantation in January) hastens the time of the tomatoes' growth and yield compared with the soil cultivation outside the greenhouse, and thus the cultivation is called "forced cultivation". To the contrary, greenhouse cultivation in the 1st, 3rd and 5th terms (seedling transplantation in August)

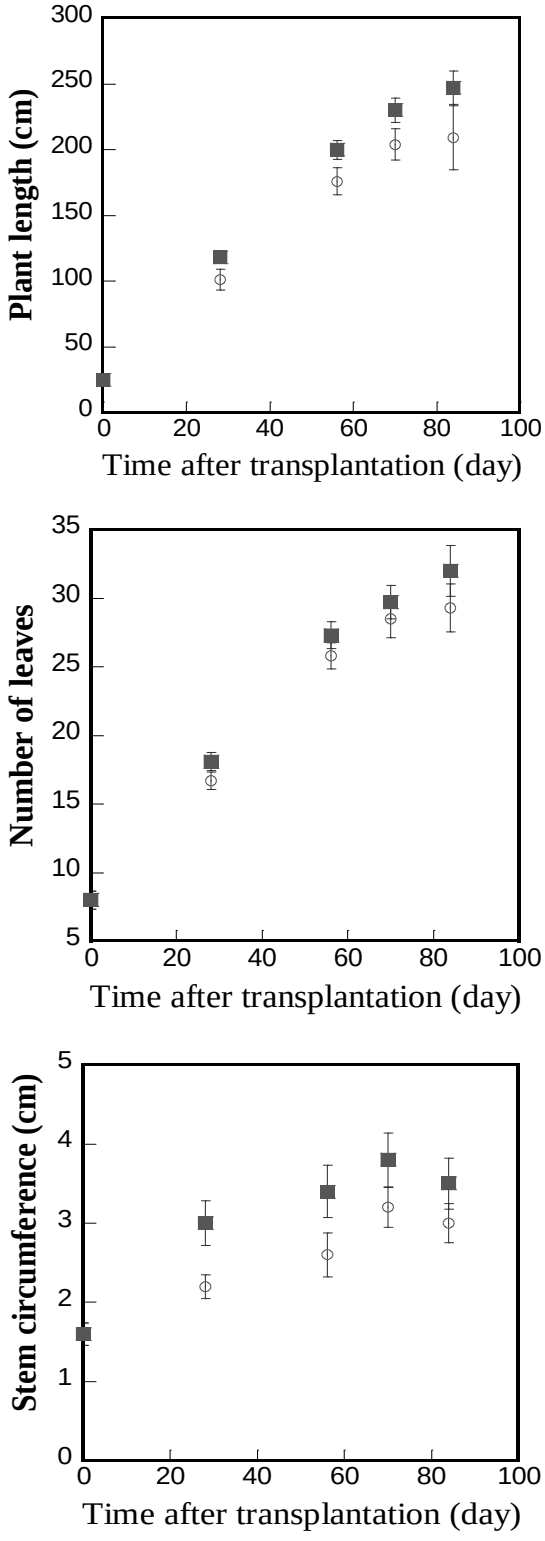


Figure Ⅱ-6 Comparison of (a) plant length, (b) number of leaves, and (c) stem circumference of tomato plants over time, after transplantation in either the photocatalytically treated system (■) or untreated system (○) in the first term. Error bar: Standard deviations of measurements from 20 plants. *Significance defined as $p<0.005$ in t-test.

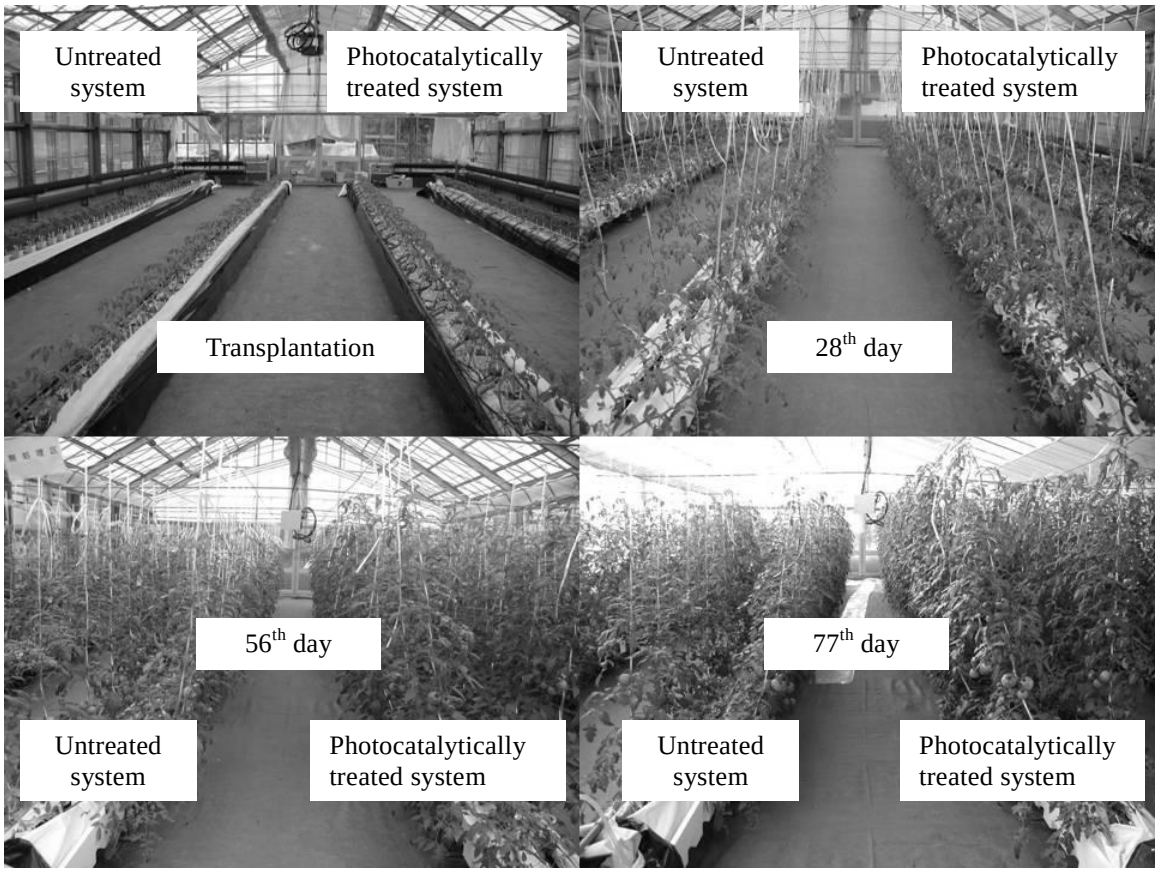


Figure Ⅱ-7 Tomato plants grown in either the photocatalytically treated or untreated closed soilless cultivation system in 1st term.

retards tomatoes' growth and yield, and is called "retarded cultivation". Since the experiment periods in the forced cultivation (2nd, 4th and 6th terms) were longer than those in the retarded cultivation (1st, 3rd and 5th terms), the growth in the forced cultivation was better than that in the retarded cultivation for both the untreated and photocatalytically treated systems. The stem weight in the photocatalytically treated system was approximately 1.4–2.5 times heavier than that in the untreated system in the six experiments. The number of trusses in the untreated system was 1.2–3.4 fewer compared with the photocatalytically treated system. As the number of trusses is directly related to the production level of tomato fruits, this difference has a large influence on the yield of tomatoes. Figure Ⅱ-8 shows the yield of marketable tomatoes in every experimental terms. The yield was 1.3–2.7 times greater in the photocatalytically treated

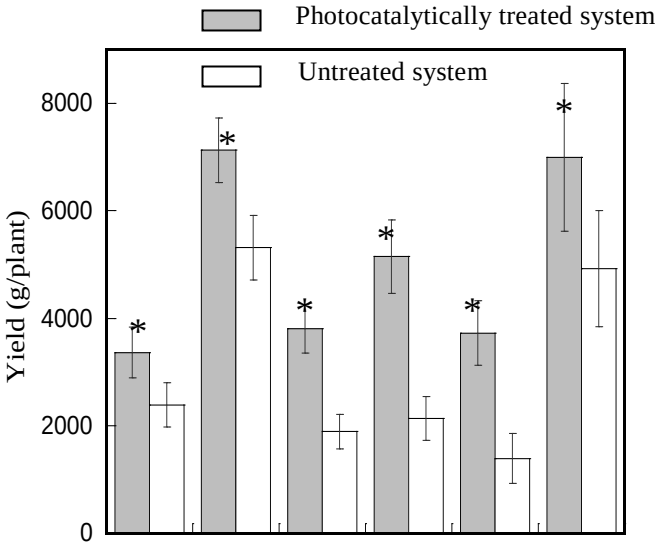


Figure Ⅱ-8 Yield of marketable tomato in either the photocatalytically treated or untreated closed soilless cultivation system. Error bar: Standard deviations of weights from 20 plants. *Significance defined as $p<0.005$ level in t-test.

Table Ⅱ-1. Tomato growth in the photocatalytically treated system versus the untreated system. Standard deviations of measurements from 20 plants (in parentheses).

		1st	2nd	3rd	4th	5th	6th
Stem weight (g)	(a) Treated	247 (38)	364 (61)	293 (47)	288 (71)	306 (36)	457 (60)
	(b) Untreated	176 (31)	243 (47)	129 (28)	119 (30)	123 (21)	219 (50)
	(a)/(b)	1.4	1.5	2.3	2.4	2.5	2.1
Number of trusses	(a) Treated	6.7 (0.5)	12.4 (0.6)	9.6 (0.5)	10.3 (0.7)	8.0 (0.7)	11.5 (1.2)
	(b) Untreated	5.5 (0.5)	11.0 (0.8)	7.7 (0.8)	6.9 (0.8)	5.5 (0.7)	9.6 (0.6)
	(a) – (b)	1.2	1.4	1.9	3.4	2.5	1.9

Significant difference between "(a)Treated" and "(b)Untreated" systems at $P<0.005$ in t-test was found in all the measurements.

Table Ⅱ-2. Tomato Brix(%) in the photocatalytically treated system versus the untreated system.

	1st	2nd	3rd	4th	5th	6th
Treated	4.9	4.7	5.0	5.4	5.7	5.6
Untreated	5.0	4.8	5.0	5.7	5.8	5.6

Measurement five fruits of each fruit trusses.

system, especially in the 3rd to 5th terms when the numberof trusses was much higher than in the untreated system. In addition, no significant differences between the catalytically treated and untreated systems from the 1st to 6th experiment terms were found in the Brix of the tomatoes (Table Ⅲ-2), which is an index of fruit quality.

Ⅱ－4 DISCUSSION

The germination rate of lettuce seeds on the rice hull extract in the model experiment showed that the extract contained phytotoxic compounds, most likely momilactones, as described in some reports (Kato et al., 1973; Takahashi et al., 1976; Kato et al., 1977; Chung et al., 2005). The decrease in TOC concentration and increase in the germination rate on the extract (Fig. Ⅱ-3) suggest that the phytotoxic compounds in the extract are detoxified with being decomposed to CO_2 by the photocatalytic reaction. Furthermore, when the phytotoxic activity was evaluated from the germination rate of the tomato seeds, the germination showed weaker inhibition than that for lettuce seeds (data not shown). The results of these model experiments indicate that if the TOC concentration of the rice hull extract decreases to a low level (a few tens of mg L^{-1}) by TiO_2 photocatalysis, the germination of both lettuce and tomato seeds are not inhibited.

In the closed soilless cultivation system of tomatoes planted on rice hull substrate, the results from TOC concentration changes and the tomato growth (Fig. Ⅱ－5, 6) in the six experiments over three years showed that the rice hull extract had a continuous influence from an early stage after transplanting the seedlings in each experiment term. However, the similar growth trend in the 1st to 6th experiment terms suggests that the high TOC concentration

shortly after transplantation in the 1st cultivation (Fig. Ⅱ－5**)** involved almost no inhibition of the tomato plant growth; the organic constituents having TOC concentrations of several tens of mg L^{-1} influenced the growth continuously through the six experiments. The lower TOC concentration in the photocatalytically treated system (less than tens of mg L^{-1}) shows that the inhibitive constituents from the rice hulls in the waste nutrient solution were almost completely decomposed to CO_2 by TiO_2 photocatalysis under sun light for 6–8 hours per day, and simultaneously the results of the above model experiments indicate that there is no inhibition in germination of both the tomato and lettuce seeds from.

The yield (Fig. Ⅱ-8) of marketable tomatoes in the photocatalytically treated system was almost identical to the open system using rockwool substrate where the waste nutrient solution was not recycled (5400 g $plant^{-1}$ in forced cultivation and 3300 g $plant^{-1}$ in retarded cultivation) (Kanagawa Agricultural Technology Center, 2008). The results of the growth and the yield(Fig. Ⅱ-6–8 and Table Ⅱ-1) suggest that by incorporating photocatalytic treatment under sun light into closed soilless cultivation systems, even crops planted on rice hull substrate which releasing phytotoxic compounds can be successfully grown without the risks of failed growth and yield declines. In addition, the treatment can reduce the environmental impact of the current soilless cultivation, causing the eutrophication of soil and groundwater by the waste nutrient solution and the industrial waste production by the used rockwool. The photocatalytically treated system is expected to be durable and effective for a long period of time because the photocatalytic material showed no apparent decline in performance and the photocatalytic effect lasting for the three year duration of the experiment. It is known that tomato plants release phytotoxic compounds from their roots (Yu et al., 1993a, 1993b; Jung et al., 2004). As the roots of the tomato plants in this study remained in the rice hull substrate as a residue at each experiment term for three years, it is predicted that the phytotoxic compounds from the roots are released in the waste nutrient solution. However, the autotoxic compounds released from asparagus roots are decomposed and detoxified by TiO_2 photocatalysis (This is described in Chapter Ⅳ, Sunada et al., 2008). Therefore, the long duration of photocatalytic effectiveness in this system suggests that TiO_2 photocatalytic treatment under sun light may be able to detoxify the phytotoxic compounds from both of rice hulls and tomato roots, in addition to lowering the environmental impact of soilless agriculture.

The amount of UV light absorbed by TiO_2 limits the photocatalytic treatment capacity. For example, as the sunlight contains only about 3 % UV light, only a small volume of wastewater can be treated. Nevertheless, the results from this study showed that wastewater from agricultural production can be effectively treated with TiO_2 photocatalysis using sun light. A capture area equal to about 5.7 % of the total cultivation area (ca. 21 m^2) for the photocatalyst was used. In addition, the effective irradiated area of the photocatalytic material was largely due to the porosity of the plate. This enabled the capturing of a large amount of sun light, allowing effective photocatalytic treatment of the agricultural wastewater. This study suggests that an environmentally friendly cultivation system can be realized by applying TiO_2 photocatalytic treatment with the use of a large catalyst area under sun light.

Ⅱ－5 SUMMARY

The effectiveness of photocatalytic treatment under sun light of waste nutrient solution emitted from a soilless cultivation system of tomatoes planted on rice hull substrate was investigated from the viewpoint of conservation-oriented agriculture. As a model experiment, the phytotoxically active extract from rice hulls was treated with a TiO_2-coated porous alumina porous plate irradiated with UV light at an intensity of 2.0 mW cm^{-2}, and the phytotoxic activities before and after irradiation were evaluated from the germination rate of lettuce and tomato seeds. The germination rate was 0 % before irradiation, demonstrating the strong inhibitory effect of the rice hull

extract, but recovered to 100 % after four days of irradiation. The growth of tomatoes planted on rice hull substrate under actual cultivation conditions was also observed in six repeated experiments over a three-years period by comparing tomatoes grown in a closed soilless cultivation system with photocatalytically-treated waste nutrient solution under sun light (photocatalytically treated system), with tomatoes grown in a closed system with untreated waste nutrient solution (untreated system). The results showed that tomato growth in the photocatalytically treated system was significantly higher than that in the untreated system in the six experiments over three years, and the yield in the photocatalytically treated system were comparable to those in a currently used open cultivation system using rockwool substrate. These results suggest that incorporating the photocatalytic treatment of waste nutrient solution under sun light will allow conservation-oriented closed soilless cultivation systems to be realized.

CHAPTER Ⅲ

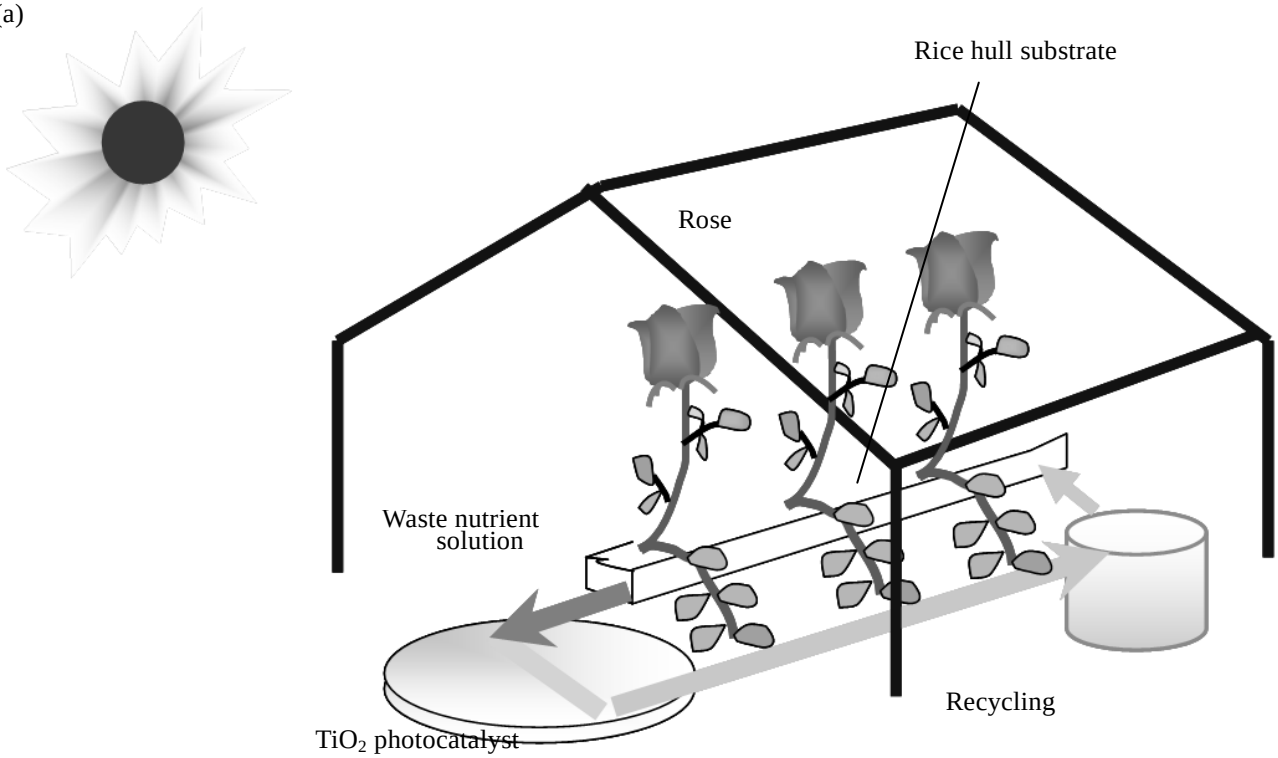
Closed soilless cultivation system of roses planted on rice hull substrate with TiO_2 photocatalytic treatment of waste nutrient solution

Ⅲ－1　INTRODUCTION

Soilless cultivation has increased in recent years for reasons such as ease in controlling crop growth and expanding the scale of production (Ministry of Agriculture, Forestry, and Fisherses, 2011; Ito, 1999). Ease of work and yield are good with the rose (*Rosa hybrida*) in particular., and the soilless cultivation area for rose accounts for a high ratio of about 45% of total cultivation of flower(Okano, 2003). This is the largest cultivation area in the field of soilless cultivation of flowers (Hayashi, 1990a,b). At present, almost all of the methods for soilless cultivation of roses used at production sites employ rockwool as the substrate, and nutrient solution is applied using an open system. However, in order to protect the environment, it is better to adopt a closed system which does not release waste nutrient solution produced by the open system(Van, 1995; Ruijs, 1994; Kajihara et al., 2005), and switch from rockwool to an organic substrate which can easily return to environment(Shinohara et al., 1999). Various substrates to substitute for rockwool have been examined including palm husk, rice hulll and cedar bark, but this research focuses on rice hulls which are easily obtained in a rice cultivating country such as Japan, and after use can easily be returned to the fields and examines their application to closed soilless cultivation. However, if rice hulls are used as the substrate and nutrient solution is supplied using a closed system, then it can be expected that phytotoxic compounds such as momilactone (Kato et al., 1973; Kato et al., 1977; Kato-Noguchi et al., 2008; Chung et al., 2005; Takahashi et al., 1976; Kong et al., 2006) will mix into the nutrient solution from the rice hulls, inhibiting growth and reducing yield.

It is well known that, if titanium oxide (TiO_2) is irradiated with UV light, a strong oxidative power is produced, and a photocatalytic reaction occurs which can achieve oxidative breakdown of various organic substances to carbon dioxide (CO_2) (Hoffmann et al., 1995; Heller 1995; Fujishima et al., 1999; Hashimoto et al., 2005). Attempts have been made to use this strong oxidative power to purify environmental pollutants in air and water (Ollis et al., 1991; Herrmann, 1999; Noguchi et al., 2003). Therefore, it was expected that phytotoxic compounds eluted by rice hulls would be broken down by this strong oxidative power, and when closed soilless cultivation of tomatoes was actually performed, growth and yield was greater in the case when water nutrient solution was photocatalytically treated than when there was no treatment (ChapterⅡ, Miyama et al., 2009). In addition, there may also be admixture of phytotoxic compounds (Fujii, 2000; Yu et al., 1993; Yu and Matsui, 1993) secreted by plants themselves into the waste nutrient solution, but in this case too, it has been shown using the example of substances secreted by the roots of asparagus that the compounds are broken down and detoxified by photocatalytic treatment(This is described in ChapterⅣ, Sunada et al.,

(a)



(b)

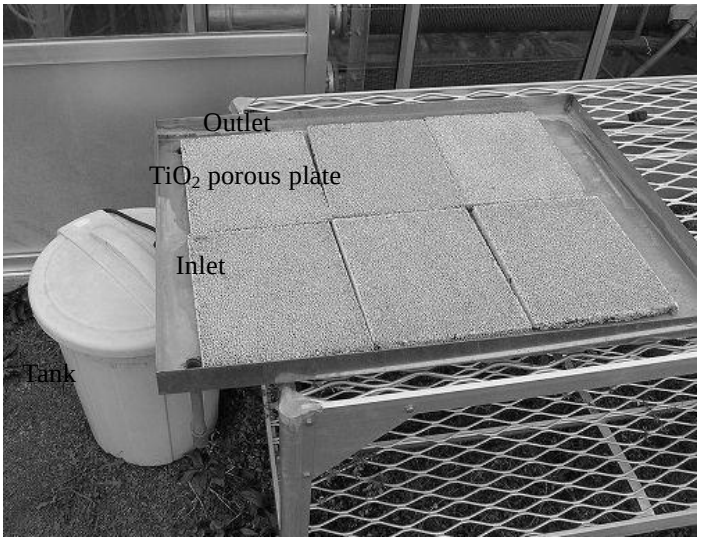
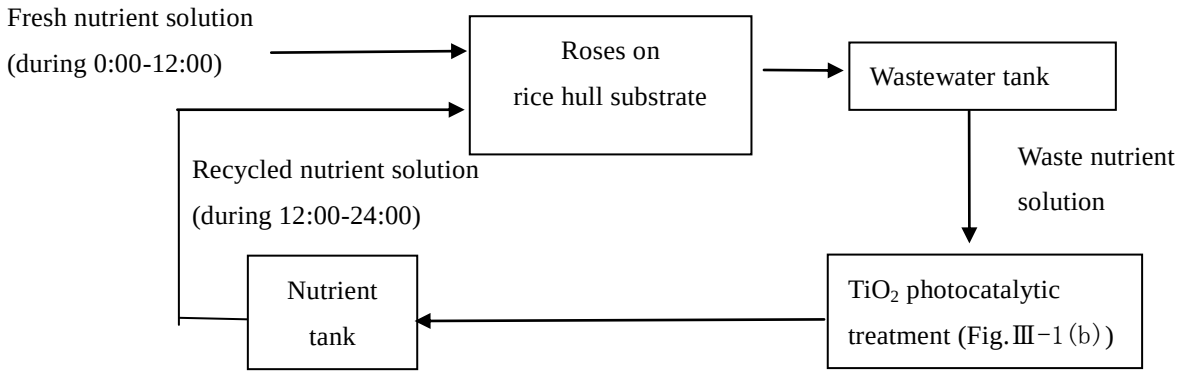
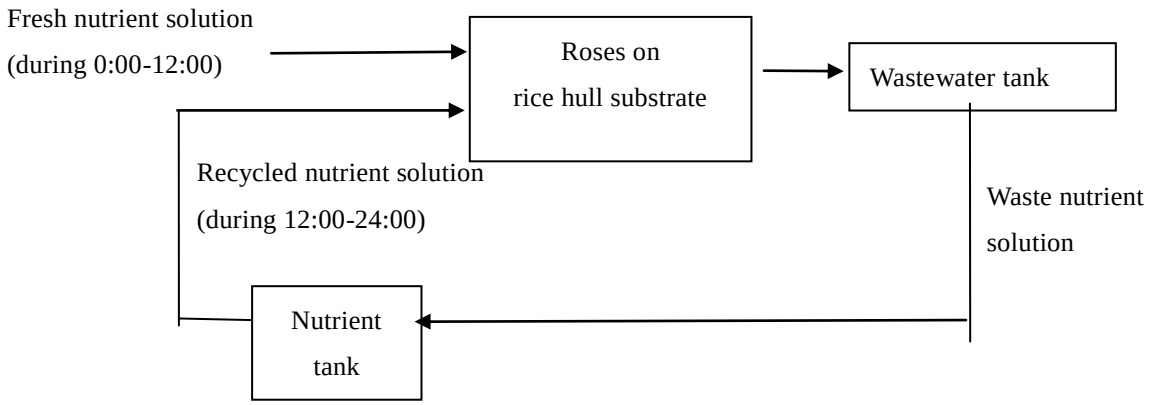


Figure Ⅲ-1 (a) Overview of TiO_2 photocatalytic treatment in a closed soilless cultivation system for rose.
(b) Photograph of TiO_2 porous plates (295 × 245 × 20 mm).

(a) Photocatalytically treated closed system using rice hull substrate
(Photocatalytically treated system)



(b) Untreated closed system using rice hull substrate (untreated system)



(c) Open system using rockwool substrate (Current system)

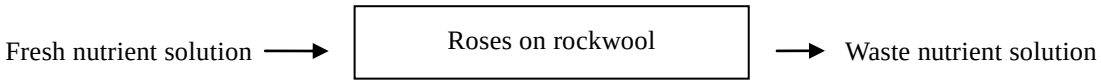


Figure Ⅲ-2 Schematic diagram of the (a) photocatalytically treated closed system using rice hull substrate (Photocatalytically treated system), (b) untreated closed system using rice hull substrate (untreated system), and (c) open system using rockwool substrate (current system).

2008). In soilless cultivation of roses using rice hull substrate, the waste nutrient solution is expected to contain phytotoxic compounds eluted from the rice hull substrate as well as those secreted by the roses themselves, but these could be broken down through photocatalytic treatment. However, the effects of the photocatalyst on growth are unknown for closed systems using practical cultivation conditions for roses, which are a woody plant rather than an herbaceous plant such as the tomato. It will also be necessary, from the standpoint of agricultural production, to investigate whether there are any differences with the current open system employing rockwool, in terms of growth and yield, when photocatalytic treatment of waste nutrient solution is performed in closed soilless cultivation using a rice hull substrate.

In this research, nutrient solutions were first prepared for the case where photocatalytic treatment is performed on the extract from rice hull, and the case where such treatment is not performed. Then rose seedlings were grown in each nutrient solution, and effects on rose growth were checked in comparison with the control case where an ordinary nutrient was used. Next, rose cultivation was carried out by actually using rice hull as the substrate, and employing a closed system for the nutrient solution. Cultivation was done using three systems – photocatalycally treated closed system using rice hull substate, untreated closed system using rice hull substate and current open system using rockwool substrate. The three systems were compared based on rose growth, yield and quality. As a result, it was confirmed that when photocatalytic treatment was performed, growth, yield and quality were on a par with the current system. The following reports on these results.

Ⅲ－2 MATERIALS AND METHODS

Ⅲ－2－1 Photocatalyst material

In all cases, a porous alumina plate coated with TiO_2 anatase-type nanoparticles (TiO_2 porous plate, 295 × 245 × 200 mm, Seiwa Kogyo Co.) was used for photocatalytic treatment (Figrue Ⅲ－1 (b)).

Ⅲ－2－2 Effectiveness of photocatalytic treatment of rose seedlings on rice hull substrate

In order to investigate whether substances eluted from rice hull affect rose growth, 450 g of rice hulls and 4.5 L of pure water were added to a glass container, placed in a water bath at 35℃ for 24 hours, and then the component eluted from the rice hull was extracted. The solution after removing the rice hull was taken to be the rice hull extract, and used for photocatalytic treatment.

Photocatalytic treatment of the rice hull extract was performed in a laboratory. First, an inlet and outlet were provided in a stainless steel container (300 × 250 × 30 mm) containing the aforementioned one TiO_2 porous plate. A five L tank was placed at the bottom of the shallow container, and 4.5 L of rice hull extract were poured. Treatment was performed by delivering liquid continuously from the inlet via a pump to the container at a rate of 3 L min^{-1}, and then returning the liquid from the outlet to the five L container. Irradiation was performed with a black light (FL15BL-B, Hitachi Co.) to achieve an UV light intensity of 2 mW cm^{-2} (Measured by UV photometer (UM-10 with UM360 sensor, Konica Minolta Co.) at the surface of the photocatalyst material. Solution irradiated for 1 week was taken to be photocatalytically treated solution, and used in growth experiments for rose seedlings.

One rose each (varieties ‘Roteroze’ and ‘Odorata’) were inserted into 50 mm cubes of rockwool (Nittobo Co.). These were placed in a greenhouse and sprayed with a water mist. After two months, the seedlings were used for the experiment. Twelve each of these rose seedlings (weight of leaves and shoots: 0.52±0.05 g $plant^{-1}$ for ‘Roteroze’, and 0.77±0.05 g $plant^{-1}$ for ‘Odorata’) were placed into stainless steel containers (300 × 250 × 30 mm), and immersed in 750 ml of test solution. There were three types of test solution: the rice hull extract (TOC: 400 mg L^{-1}), the solution obtained by photocatalytically treating the extract as described above (TOC: 15 mg L^{-1}); and pure water as a control. To each test solution were added 4.5 mL and 9.0 mL, respectively, of the commercial liquid

fertilizers High Spirit Ax and High Spirit By (Mitsubishi Plastics Agri Dream Co.). Growing was performed by placing these stationary in a growth chamber with an air temperature of 30℃, a light period of 12 hours and a dark period of 12 hours. During this time, when the amount of solution decreased due to evaporation, pure water was added to the shallow container.

Growth of rose seedlings in the three types of test solutions was compared by cutting off leaves and shoots from the stem before treatment and one week after treatment, and then determining and comparing their fresh weight. TOC was measured using a TOC analyzer (TOC-V, Shimadzu Co.) , and UV light intensity was measured using an UV photometer (UM-10 with UM360 sensor, Konica Minolta Co.).

Ⅲ－2－3　Method of photocatalytic treatment and evaluation of its effectiveness in closed soilless cultivation

Roses were cultivated with a closed nutrient solution system using rice hulls as the substrate. To investigate the effectiveness of photocatalytic treatment, cultivation testing was carried out using the three systems indicated in Fig. Ⅲ-2. First is the system where waste nutrient solution is photocatalytically treated, using sunlight as the light source, before recycling (Fig. Ⅲ-2(a), referred to below as the "photocatalytically treated system"). Second is the system where waste nutrient solution is recycled as is, without performing photocatalytic treatment (Fig. Ⅲ-2(b), referred to below as the untreated system). Third is the current open system using rockwool substrate (Fig. Ⅲ-2(c), referred to below as the "current system"). In current system, the waste nutrient solution is not recycled and is discharged as is.

In photocatalytic treatment in the photocatalytically treated system, it was decided to repeat a process during the day where six of the aforementioned materials were placed horizontally in a stainless steel shallow container (900 × 650 × 50(depth) mm) set on the south side outside of the greenhouse, and then 15 L of waste nutrient solution in a treatment tank were delivered from the inlet via a pump at a rate of 4 L min^{-1}, and returned to the tank from the outlet (Fig. Ⅲ-1(b)).

Nutrient solution was supplied using a controller (Mizumac, Sanshu Kogyo Co,). In the photocatalytically treated system and untreated system, newly prepared solution (mixed with the commercial liquid fertilizers High Spirit Ax and High Spirit By in a ratio (1:2); fresh nutrient solution in Fig. Ⅲ-2) was supplied during the morning. The waste solution rate was set to 30%, and waste nutrient not absorbed by the plants was temporarily stored in a storage tank (wastewater tank in Fig. Ⅲ-2). After that, in the morning, 100 L of solution – photocatalytically treated solution in the photocatalytically treated system (Fig. Ⅲ-2(a)), and waste nutrient solution as is in the untreated system (Fig. Ⅲ-2(b)) – was delivered to the fresh solution tank (nutrient tank in Fig. Ⅲ-2) and in the afternoon solution was supplied again from each nutrient tank. In the current system, nutrient solution newly prepared at the end of the day was supplied. EC of the newly prepared nutrient solution during the cultivation period was 1.9 dS m^{-1} maximum, 0.9 dS m^{-1} minimum, and 1.5 dS m^{-1} average. pH was 6.9 maximum, 5.3 minimum and 6.0 average. The EC and pH of recycled nutrient solution were set to be the same for the photocatalytically treated system and untreated system. For EC, values for the photocatalytically treated system were 2.6 dS m^{-1} maximum, 1.3 dS m^{-1} minimum, and 1.8 dS m^{-1} average; and values for the untreated system were 2.5 dS m^{-1} maximum, 1.2 dS m^{-1} minimum, and 1.8 dS m^{-1} average. For pH, values for the photocatalytically treated system were 7.6 maximum, 5.2 minimum, and 6.9 average; and values for the untreated system were 7.8 maximum, 5.7 minimum and 7.1 average. The TOC in the nutrient tanks for the photocatalytically treated system and the untreated system, and the TOC of fresh solution and waste nutrient solution in the current system, were measured once every two weeks during cultivation.

Cultivation was carried out in a north-south direction a glass greenhouse (area 80 m^2) located at the Kanagawa Agricultural Technology. In the greenhouse, minimum

temperature was set to 18℃ and ventilation temperature to 23℃, just as in the current system. In each test system, eight styrofoam soilless cultivation containers (inner width 30 cm, length 100 cm; Mitsubishi Plastics Agri Dream Co.) were lined up in series in the north-south direction. The inside of each soilless cultivation container was lined with root-blocking, waterproof sheet, and rice hull or rockwool slabs (300 × 910 × 75 mm) (Grodan Co.) were placed on top of that. The rose seedlings were placed after the surface was flattened. Grafted cutting seedlings raised for two months were used as seedlings. The variety was 'Roteroze', and the root stock was 'Odorata'. The number of seedlings planted for one system was 78. For the cultivation system, the intrarow spacing was set to 90 mm, the row spacing to 1400 mm, and the planting density to 7.9 plants m^{-2}. Arching was used as the training method. Planting was done on June 30, 2006. Cultivation ended on June 1st, 2007. Rose harvesting was conducted from September 2006 to May 2007.

Growth in the different systems was compared by measuring the weight of assimilation branches at the end of cultivation. Yield was compared by measuring the number of cut flowers, cut flower weight, and multiplication of number and weight. Quality of cut flowers was compared by measuring the cut flower length.

Ⅲ－3 RESULTS

Ⅲ－3－1 Effectiveness of photocatalytic treatment for rose seedling growth

Table Ⅲ-1 shows the fresh weight of shoots and leaves after one week of growth of rose seedlings, grown by adding fertilizer components to each of the following: untreated solution in which the rice hull extract was used as is, solution obtained by photocatalytically treating the rice hull extract, and pure water as a control. Compared with the case where growth was done by immersing in pure solution, which is the control for both 'Roteroze' and 'Odorata', in the case were immersion was done in untreated solution, the values were significantly lower ($p<0.05$). For 'Roteroze', the value was 61% of the fresh weight of the control, and for 'Odorata', the value was 72% of the fresh weight of the control.

On the other hand, no significant difference was evident when comparing the photocatalytically treated solution with pure water, and the fresh weight when soaked in photocatalytically treated solution was 87% of the fresh weight when soaked in pure water for 'Roteroze', and 83% for 'Odorata'.

Table Ⅲ-1 Fresh weight of the shoots and leaves of rooted rose cuttings after incubation for seven days soaked in pure nutrient solution (control), in phytotoxically active extract from rice hull (untreated), and photocatalytically treated extract (photocatalytically treated).

	Fresh weight of shoots and leaves (g plant^{-1})	
Test solution	'Roteroze'	'Odorata'
Photocatalytically treated	0.76	0.97
Untreated	0.53*	0.84*
Control	0.87	1.17

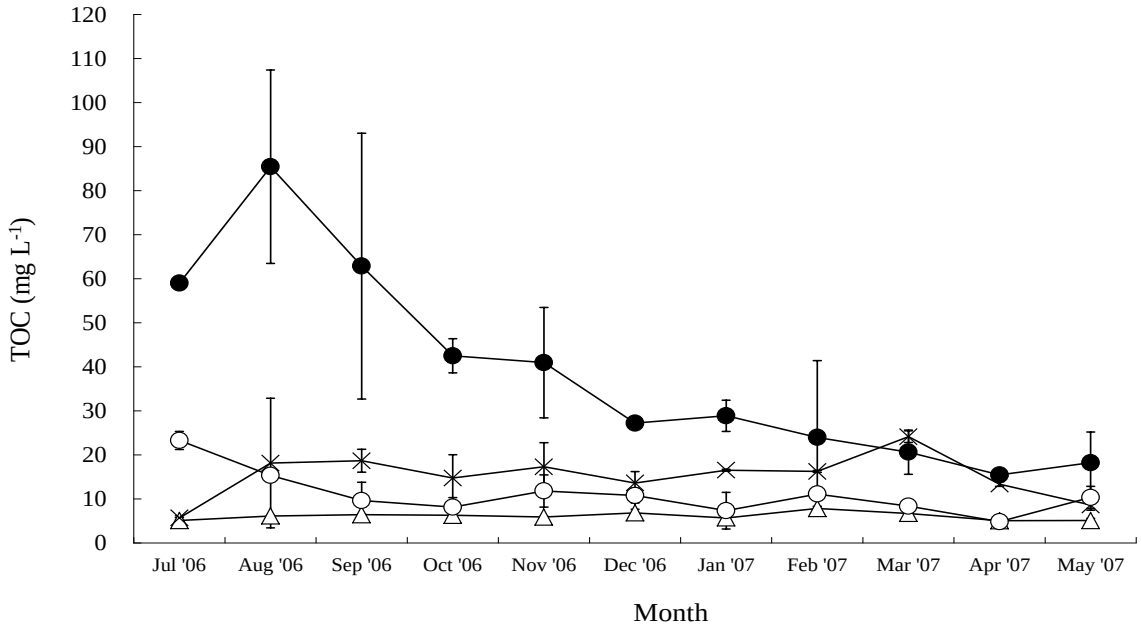
Total fresh weight of 'Roteroze' and 'Odorata' shoots and leaves before treatment was 0.52±0.05 and 0.77±0.05 g plant^{-1}, respectively. * Significance at $p<0.05$ compared to the control using Dunnett's multiple comparison test.

Ⅲ－3－2　Photocatalyst effectiveness in closed soilless cultivation of roses

Rose cultivation tests were conducted using closed nutrient solution systems with rice hull substrate incorporating photocatalytic treatment. That is, roses were actually cultivated using the three systems shown in Fig. Ⅲ-2.

Figure Ⅲ-3 shows the chronological change in average monthly TOC for nutrient solution in the nutrient tank in the photocatalytically treated and untreated system, and the fresh and waste nutrient solution in the current system. Whereas fresh solution in the current system varied in the range 5-8 mg L^{-1}, nutrient solution in the nutrient tank in the photocatalytically treated system varied in the range 5-23 mg L^{-1}. Nutrient solution in the nutrient tank in the untreated system was higher than that, and varied in the range 15-85 TOC of the waste solution in the current system varied in the range 6-24 mg L^{-1}, almost the same as the TOC of the nutrient solution in the treated system. TOC of the untreated system tended to be high during the period of high temperatures, and low during the period of low temperatures.

The weight of assimilation branches at the end of cultivation was significantly (p<0.05) lower in the untreated system than in the current system acting as the control, and growth was only 57% of the weight of assimilation branches in the current system. On the other hand, no difference was evident between the photocatalytically treated system and the current system (Fig. Ⅲ-4). In terms of yield, the number of cut flowers was 78% of the current system with the untreated system, and 94% with the photocatalytically treated system, which was almost on a par with the current system. In terms of cut flower weight, the untreated system was significantly (p<0.05) lower at 80% of the current system, while weight for the photocatalytically treated system was 91%, and not significantly different from the current system. As a result, the product of the number and weight of cut flowers was significantly (p<0.05) lower in the untreated system than in the current system and photocatalytically treated system,



FigureⅢ-3 Chronological change in TOC (Total Organic Carbon) concentration of the nutrient solution in the nutrient tank in photocatalytically treated (○)and untreated (●) systems, and fresh (△) and waste nutrient (×) solutions from the current system (Figure Ⅲ-2(c)). Error bars show the standard error of the TOC.

and no significant difference was evident between the current system and the photocatalytically treated system (Table Ⅲ-2). In terms of cut flower length, which serves as an indicator of the quality of rose cut flowers, the percentage with a length that the quality of cut flowers of at least 0.70 m, was at least 90% in all systems. When leaf color was measured using SPAD 502 (Konica Minolta Co.), no significant difference was evident among the three systems (data not shown).

Ⅲ－4 DISCUSSION

The results of using rose seedlings to investigate the effectiveness of photocatalytic treatment of rice hull extract showed that substances with an effect on rose seedling growth are contained in rice hull extract, and that those substances are broken down and detoxified through photocatalytic treatment. This applies to 'Roteroze' and to the root stock variety 'Odorata'. This is the same result as shown in Chapter Ⅱ relating to the germination rates of lettuce seeds and tomato seeds. That is, this suggests that, just like tomatoes (which are an herbaceous plant), roses (which are a woody plant) can have their growth impeded by substances such as momilactone when rice hull is used as the substrate and nutrient solution is recycled, and those phytotoxic compounds can be broken down and detoxified by a photocatalyst.

In rose cultivation tests conducted using closed nutrient solution systems with rice hull substrate and incorporating photocatalytic treatment, TOC of nutrient solution in the nutrient tank in the photocatalytically treated system varies at a lower level than in the untreated system. This shows that organic substances in the waste nutrient solution are broken down through photocatalytic treatment with sunlight for roughly 6-8 hours of the day. the TOC of nutrient solution in the nutrient tank in the untreated system was high during the period from July to September, immediately after planting, and the cause of this is thought to be the high elution from rice hulls at the start of use and during the high temperature period (Chapter Ⅱ, Miyama et al., 2009). If rice hulls are carbonized before use, then TOC of the waste nutrient solution will likely decrease, but it takes labor and energy to carry out carbonization. On the other hand, if a photocatalytically treated system is used, then rice hulls can be used as is as the substrate.

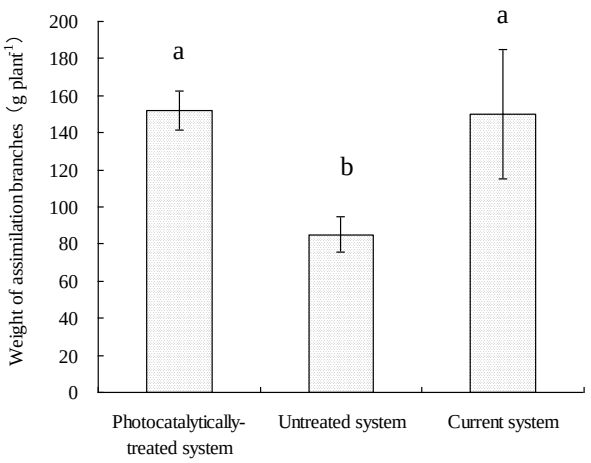


Figure Ⅲ-4 Weight of assimilation branches in each system. The error bars show the standard error of the weight. Different letters indicate significant differences using Tukey's multiple range test (p<0.05).

Next, the results in Fig. Ⅲ-4 and Table Ⅲ-2 showed that even in cultivation with recycled nutrient solution using a rice hull substrate, the growth, yield and cut flower quality of roses is on a par with the current system if waste nutrient solution is supplied after photocatalytic treatment.

The above results show that, in soilless cultivation of roses using rice hulls (which easily return to environment) as the substrate instead of rockwool (which becomes industrial waste), catalytically treating waste nutrient solution with sunlight, as in the case of tomatoes enables closed soilless cultivation with low environmental impact without reducing the growth, yield and quality of the product.

In recent years, rose producing growers in Japan have faced a difficult management environment due to issues such as rising material costs, and low prices for cut flowers. Therefore, they are making efforts to develop

Table Ⅲ-2 Yield of cut roses harvested from 2006 Sep. to 2007 March.

	Number of cut flower (plant^{-1}) A	Weight of cut flower (g) B	A×B (g plant^{-1})
Photocatalytically treated system	6.6 ab	65 a	426.9 a
Untreated system	5.4 b	57 b	312.1 b
Current system	7.0 a	72 a	502.1 a

Different letters within a column indicate significant difference using Tukey's multiple range test (p<0.05).

varieties (Hara, 2010) and reduce material costs. Switching to inexpensive rice hull substrate instead of rockwool, and using a closed system where waste nutrient solution is recycled, is advantageous for management because it reduces costs for substrate and fertilizer, which are part of materials costs.

This research has shown that cultivation can be done at a practical level, while protecting the environment and holding down materials costs, by applying a photocatalyst reaction to waste nutrient solution treatment in soilless cultivation.

Ⅲ－5　SUMMARY

From the viewpoint of conservation-oriented agriculture, this study investigated a closed cultivation system using a combination of rice hull substrate and TiO_2 photocatalytic treatment of the waste nutrient solution. First, an extract of rice hulls in pure water was treated with a TiO_2-coated porous alumina plate under UV light irradiation. Then, rose rooted cuttings were soaked in the treated extract, untreated extract, or pure nutrient solution. The total weight of the shoot and leaves soaked in the photocatalytically treated extract was heavier than that in the untreated extract, and was similar to that in the pure nutrient solution. Next, rose growth was compared in three practical cultivation systems: two closed systems using rice hull substrate with photocatalytically treated or untreated waste nutrient solution (photocatalytically treated and untreated systems), and an open system using rockwool substrate (current system). The growth and yield in the photocatalytically treated system were significantly greater than those in the untreated system and were comparable to those in the current system. These results show that the TiO_2 photocatalytic treatment of the waste nutrient solution is effective in closed soilless cultivation using rice hull substrate.

CHAPTER Ⅳ

Detoxification of Phytotoxic Compounds Released from Plant Root by Photocatalysis in a Closed Soilless Cultivation System .

Ⅳ－1　INTRODUCTION

Plants release phytotoxic chemicals into the soil where they accumulate and inhibit the growth and germination of other plants (Putnam et al., 1984; Yu and Matsui, 1993; Pramanik et al., 2000). Autotoxicity is a form of phytotoxicity that inhibits other individuals of the same species. Therefore, autotoxicity causes serious problems at agricultural production sites such as growth inhibition, yield declines and replant failures (Pramanik et al., 2000; Singh et al., 1999. Asao et al., 2003; 2004). Asparagus, in particular, has been reported to release strong autotoxic compounds which have been studied in detail (Kitahara et al., 1972; Young and Chou, 1985; Hartung et al., 1989; Blok and Bollen, 1993; Young, 1984; Motoki et al., 2006; Hartung et al., 1990; Heather et al., 1991; Motoki et al., 2006). For example, asparagusic acid (1,2-dithiolane-4-carboxylic acid) (Kitahara et al., 1972), methylenedioxy cinnamic acid (MDCA) (Hartung et al.,

1990), caffeic acid (Heather et al., 1991) and 3,4-dihydroxyphenyl acetic acid (3,4-DPAA) (Young, 1984; Hartung, 1991) have been isolated and characterized. Therefore, if the waste nutrient solution from soilless asparagus cultivation is recycled and reused in the system, these toxic compounds could inhibit the growth of the asparagus crop.

In this study whether plants that produce autotoxic compounds can be grown under a conservation-oriented cultivation system by allowing the recycling of the nutrient solution through TiO_2 photocatalytic treatment was investigated. First, the photocatalytic decomposition of the phytotoxic fraction extracted from asparagus roots will be described, followed by a description of the photodecomposition of the phytotoxic fraction found in the waste nutrient solution of an actual soilless asparagus cultivation system. Finally, the results of a comparative evaluation of asparagus growth in a system where the waste nutrient solution was treated with TiO_2 photocatalysts before being recycled versus a system where untreated waste nutrient solution was recycled were showed.

Ⅳ－2 MATERIALS AND METHODS

Ⅳ－2－1 Isolation of phytotoxic fraction from asparagus root

The phytotoxic fraction was extracted from asparagus (*Asparagus officinalis* L.) roots as previously described (Hartung et al., 1990). Briefly, fresh asparagus roots were dried in an incubator at 65 °C and then pulverized in a mixer. Deionized water was added to the powdered roots and this suspension was stirred overnight at 4 °C. Acetone (4:1 v/v acetone-sample) was added to the supernatant to precipitate the proteins and lipids and this suspension was once again stirred overnight at 4 °C. The precipitate was then filtered and the supernatant concentrated to a quarter of its original volume using a rotary evaporator. Chloroform equal in volume to the concentrate was added and the chloroform-soluble fraction was isolated and dried in a rotary evaporator. The dried sample was then dissolved in methanol and the methanol-soluble fraction was refined using octadecyl (C18) column (200 × 18 mm) chromatography and eluted with a step gradient of acetonitrile (100 %) to methanol (100 %) at 25 % intermediate steps.

Ⅳ－2－2 Phytotoxic activity test

The phytotoxic activity was tested by the conventional method previously described using lettuce seed (*Lactuca sativa* L., 'Calmar MR' Nihon Nosan Seed Co.) (Pramanik et al., 2000; Hartung et al., 1990). Briefly, 4 mL of each fraction was poured over filter paper (Advantec No. 2) in Petri dishes (9 cm in diameter). After the solvent (methanol, acetonitrile, etc.) in each fraction was completely evaporated from the filter, 4 mL of deionized water was added. 25 lettuce seeds were sown in each dish and each fraction was tested on two dishes. The dishes were incubated in the dark for 48 hours at room temperature. Control tests using deionized water and pure solvent instead of the fraction were conducted for each assay. After 48 hours, lettuce seed germination rates for both the sample and control dishes were calculated and the radicle lengths were measured. The indices for evaluating phytotoxic activity were germination rates and the ratios of radicle lengths between test samples and controls.

Ⅳ－2－3 Photocatalytic treatment of the phytotoxic fraction from asparagus roots

Photocatalytic treatment with TiO_2 of the phytotoxically active fraction extracted from asparagus roots was conducted as follows. 10 mL of the active fraction remaining after column chromatography had been evaporated and dried in a rotary evaporator, it was dissolved in 90 mL of mixed solvent comprised of methanol (5 mL) and pure water (85 mL). The TiO_2 aqueous suspension, which was prepared by dispersing a commercially available TiO_2 powder (ST-31, Ishihara Sangyo Co.) in pure water by sonication, was then added at a concentration of 10 g L^{-1}. This suspension was irradiated with UV light at an intensity of ca. 1.0 mW cm^{-2} from black light bulbs (Type FL15 BL-B, National) for 1, 2 and four days. The concentration of total organic carbon (TOC) was determined by a TOC analyzer TOC analyzer (TOC-5000A,

Shimadzu Co.) after the TiO_2 powders had been filtered from the suspension. The phytotoxic activity of this filtrate was assayed using the method described above. In addition, the concentration of 3,4-DPAA, a common asparagus phytotoxin, in the same samples was measured using HPLC (Shimadzu Co., Prominence).

Ⅳ－2－4　Photocatalytic treatment of the phytotoxic fraction from waste nutrient solution in a closed soilless asparagus cultivation

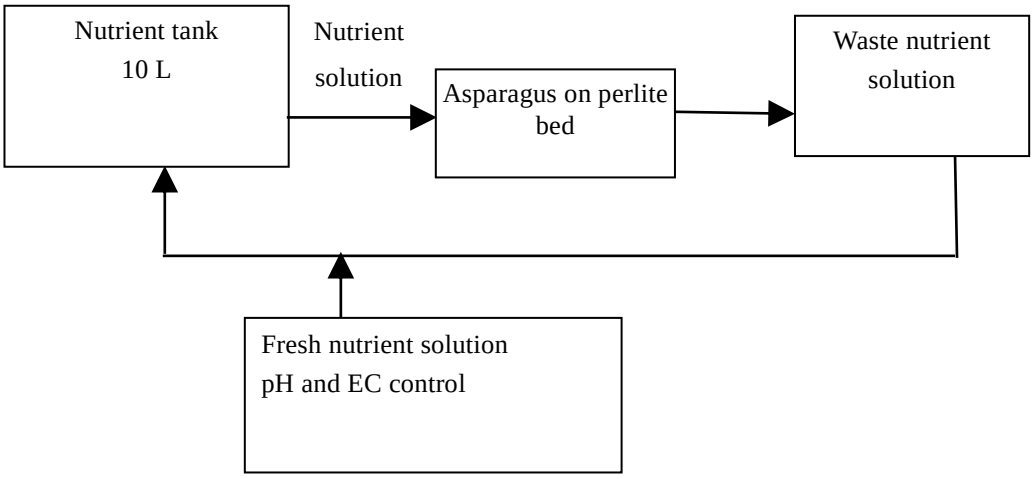
Asparagus was grown in a glass greenhouse in order to ascertain the effect of photocatalytic treatment on phytotoxic compounds found in the waste nutrient solution of an actual soilless cultivation system. In February 2005, nine asparagus seedlings were transplanted in each bed (350 × 1000 × 170 (depth) mm) of the inorganic substrate perlite and fed with a conventional nutrient solution (Otsuka-A fomula, Otsuka Kagaku Co.). The waste nutrient solution originated from the solution not absorbed by the asparagus. In order to obtain the phytotoxic fraction from the waste nutrient solution, the asparagus was grown for approximately three months with the recycled untreated nutrient solution through the soilless system so that the phytotoxic fraction would accumulate in the nutrient solution tank. Five L from the tank was concentrated to 100 mL in a rotary evaporator at 60 °C. To remove the majority of salt components from the concentrate, it was refined using the above-mentioned C18 column (Octa Decyl Silyl column) chromatography and eluted with acetonitrile (100%). After evaporating the acetonitrile and dissolving the remaining fraction in 90 mL of pure water,photocatalytic treatment of the fraction was conducted. After UV irradiation for 1, 2 and 4 days, the TOC of the sample was measured and its phytotoxic activity was assayed.

Ⅳ－2－5　Effect of photocatalytic treatment on closed soilless cultivation system of asparagus

To study the effectiveness of photocatalytic treatment under actual cultivation conditions and natural sunlight, asparagus in two soilless cultivation systems was observed over a 20 month period after transplantation of the seedlings. In the untreated system (Fig. Ⅳ-1(a)), waste nutrient solution without photocatalytic treatment was recycled through the system, and in the photocatalytically treated system (Fig. Ⅳ-1(b)), the waste nutrient solution received photocatalytic treatment before being recycled back through the system. In both systems, the nutrient solution was pumped from the nutrient solution tank through the perlite beds in which the asparagus was planted. In the untreated system, the waste nutrient solution was returned to the 10 L nutrient solution tank and reused. In contrast, in the photocatalytically-treated system, the waste nutrient solution was temporarily stored in a wastewater tank and then treated photocatalytically in the presence of sunlight and TiO_2 for two days. Subsequently, the treated solution was returned to the 20 L nutrient solution tank for reuse. Fresh nutrient solution was added to both systems as needed and the pH and electrical conductivity (EC) in the nutrient solution tanks were maintained at pH 7.2 and EC=2.0 dS m^{-1}, respectively. Photocatalytic treatment of the waste nutrient solution was conducted by cycling the waste nutrient solution through a porous plate coated with TiO_2 anatase-type nanoparticles (TiO_2 porous plate, 250 × 300 × 20 mm, Seiwa Kogyo Co.) under sun light (Fig. Ⅳ-2). The irradiated area by sunlight of the TiO_2 porous plate was 21 % of cultivation bed area, to capture a large amount of sun light for the treatment of the waste nutrient solution.

To observe the asparagus growth in the two systems, the tallest stem length, thickest stem diameter and total number of stems from an asparagus seedling were measured and the average for the nine seedlings in each bed was calculated and compared between the photocatalytically-treated and untreated systems. Asparagus spears were harvested once they reached a height of 250 mm in the second year after transplantation of the seedlings. The spears were cut to a uniform 250 mm in length if they were greater than 250 mm and weighed to determine the total yield.

(a) Untreated system



(b) Photocatalytically treated system

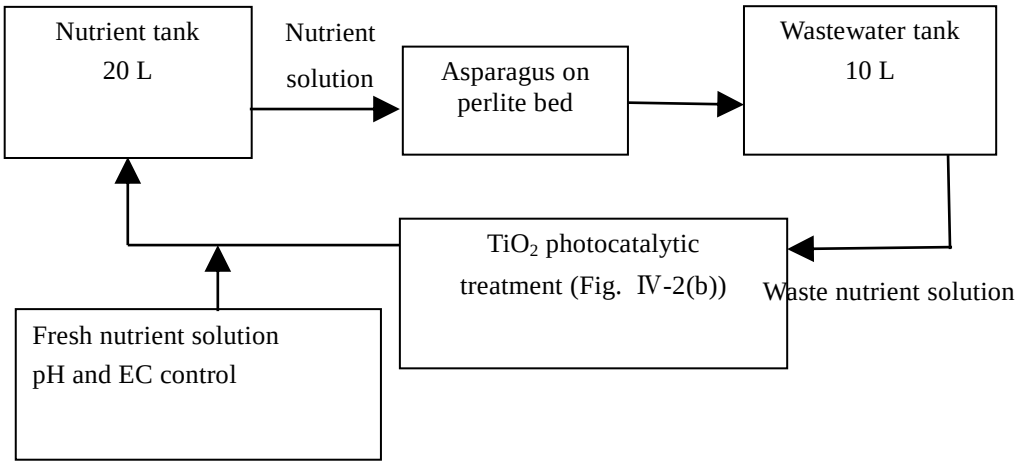


Figure Ⅳ-1 (a) Schematic diagram for the (a) untreated system and (b) photocatalytically treated system in closed soilless asparagus cultivation system.

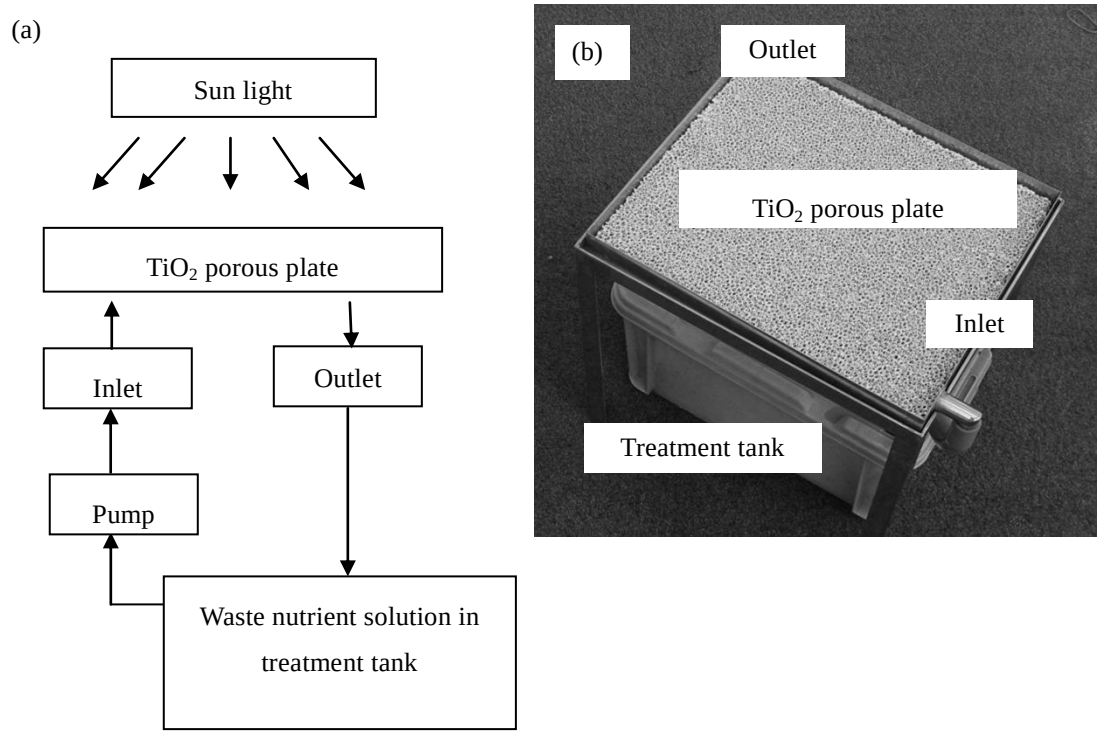


Figure Ⅳ-2 (a) Overview of the photocatalytic treatment method used in the closed soilless cultivation system. (b) Photograph of the TiO_2 porous alumina plate (250 mm × 300 mm × 20 mm) showing the inlet and outlet lines and location of the treatment tank.

Ⅳ－3　RESULTS

Ⅳ － 3 － 1　Photocatalytic effect on phytotoxic compounds obtained from asparagus roots

The phytotoxic fraction was extracted and isolated from asparagus roots, refined using column chromatography and eluted with acetonitrile (100 %). This fraction completely inhibited lettuce seed germination, showing strong phytotoxic activity. The fraction was then subjected to either TiO_2 photocatalytic treatment or incubation with TiO_2 in the dark. Figure Ⅳ-3(a) shows the time-dependent change of TOC concentration for each treatment. The TOC concentration initially decreased by 30 % before leveling off when incubated in the dark. This decline was caused by the adsorption of organic compounds on the TiO_2 surface. In contrast, when the fraction was irradiated with UV light, the TOC decreased with increasing irradiation time. The TOC concentration after four days of UV irradiation was more than 90 % lower than the initial concentration. This demonstrates that the organic constituents of the active fraction are oxidized to CO_2 by the TiO_2 photocatalytic reaction.

Parallel to this experiment, the phytotoxic activity was assayed of the same samples for which TOC concentrations were measured. As shown in Fig. Ⅳ-3(b), the lettuce seed radicle length was only 20 % as long as the control for the fraction incubated with TiO_2 in the dark. However, when compared to the UV irradiation treatment, the growth of the radicle improved with longer irradiation times, with the radicle length reaching 90 % of the control after four days of UV irradiation.

Several phytotoxic compounds have been isolated and identified from asparagus roots, including the common compound, 3,4-DPAA (Kitahara et al., 1972; Young, 1984; Hartung et al., 1990; Heather et al., 1991). In this study, the presence of 3,4-DPAA in the phytotoxically active fraction obtained from asparagus roots was shown *through HPLC*

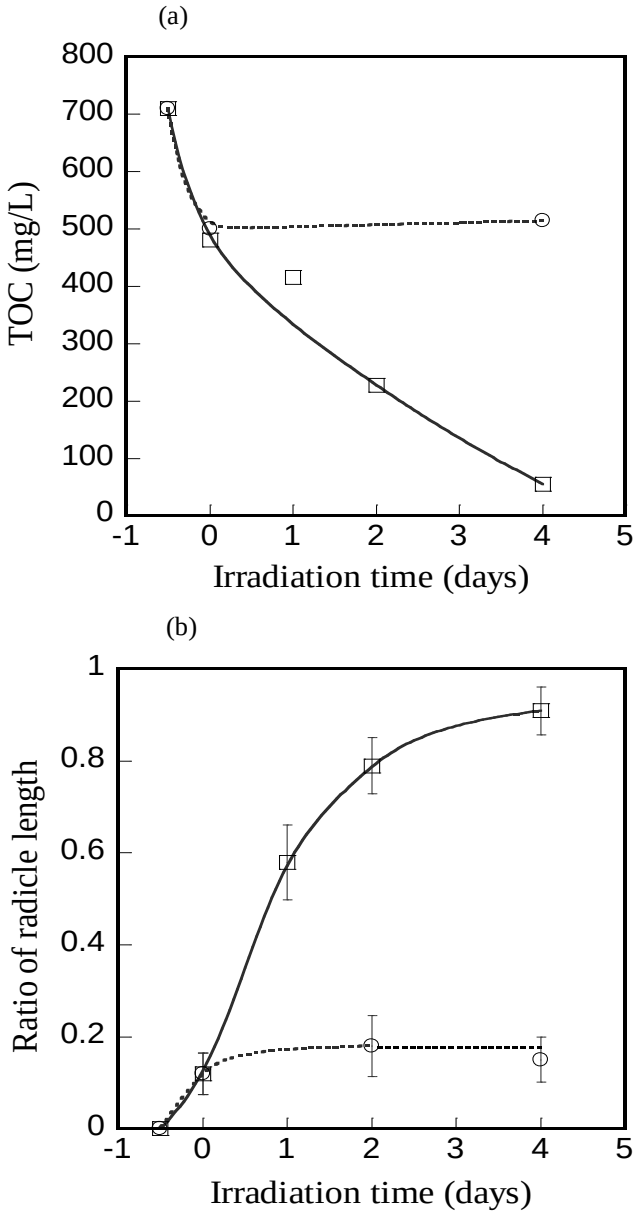


Figure Ⅳ-3 Changes over time in (a) TOC concentration and (b) lettuce radicle length ratio for the phytotoxically active fraction from asparagus roots with TiO_2 suspension as compared to controls. The suspension was incubated in the dark (○) or irradiated with UV light (□). Error bars: Standard deviations of two replicate evaluations in the phytotoxic activity test.

analysis. Concentrations of 3,4-DPAA during photocatalysis were measured for the same samples as shown in Fig. Ⅳ-3. When incubated in the dark, the

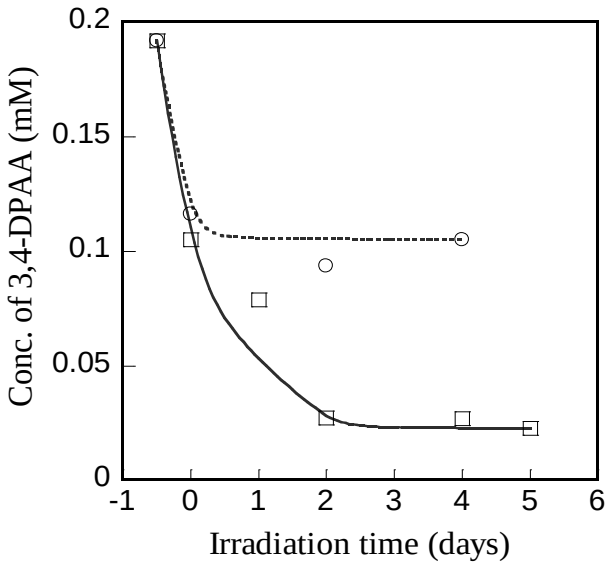


Figure Ⅳ-4 Changes in 3,4-DPAA concentration in the phytotoxically active fraction from asparagus root with TiO_2 suspension incubated in the dark (○) or exposed to UV irradiation (□). These were the identical samples as measured in Figure Ⅳ-3.

3,4-DPAA concentrations initially declined by nearly 40-45 % due to adsorption of the compound on the TiO_2 surface, but there were no subsequent changes in concentration (Fig. Ⅳ-4). In contrast, the 3,4-DPAA concentrations dramatically dropped to 85 % of the initial concentration after two days of UV irradiation. This decline was more rapid than the decrease in TOC concentrations, which was attributed to the following. While the TOC concentrations did not show a decline until the organic compounds had been decomposed completely into CO_2, in this experiment the 3,4-DPAA levels would decline as soon as 3,4-DPAA was oxidized into other compounds, without its complete oxidization into CO_2. The decomposition of different types of organic compounds by photocatalytic reactions is known to occur with little discrimination and essentially all organic compounds will ultimately be decomposed. Thus, from Fig. Ⅳ-4 it can be interpreted that phytotoxic compounds other than 3,4-DPAA in the phytotoxically active fraction are

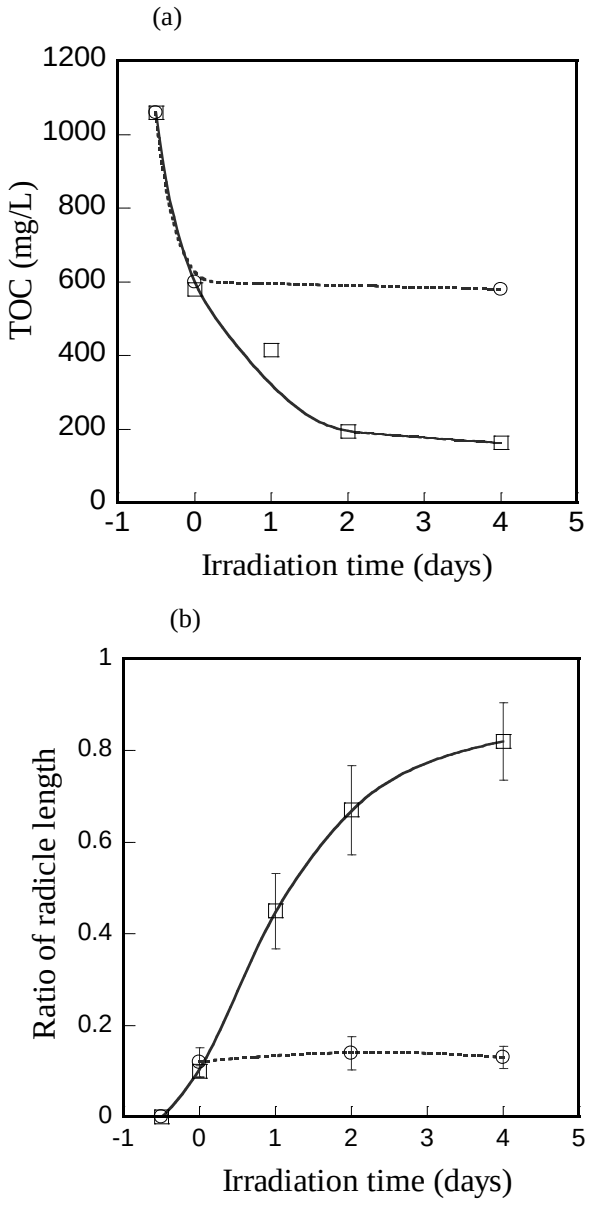


Figure Ⅳ-5 Changes over time in (a) TOC concentration and (b) lettuce radicle length ratio for the phytotoxically active fraction obtained from actual cultivation wastewater with TiO_2 suspension. The suspension was incubated in the dark (○) or irradiated with UV light (□). Error bars: Standard deviations of two replicate evaluations in the phytotoxic activity test.

similarly decomposed. This decomposition of phytotoxic compounds through photocatalytic reactions is posited to account for the improved growth of the lettuce radicle in the TiO_2 photocatalytically treated root extracts.

Ⅳ － 3 － 2　Photocatalytic effect on phytotoxic compounds in waste nutrient solution from soilless cultivation of asparagus

In order to ascertain whether phytotoxic compounds found in actual waste nutrient solution from soilless asparagus cultivation can be decomposed and detoxified by photocatalytic reactions, asparagus was cultivated

hydroponically with recycled untreated waste nutrient solution. The fraction from the nutrient solution tank that had been concentrated by 50 times completely inhibited lettuce seed germination, showing that phytotoxic compounds are indeed released into waste nutrient solution during actual soilless asparagus cultivation. When this concentrated fraction was treated photocatalytically, the TOC initially decreased when incubated in the dark due to adsorption on the TiO_2 surface, followed by no further changes over time, whereas under constant UV irradiation the TOC continued to decline over time (Fig. Ⅳ-5(a)). In addition, while in the non-UV exposed condition the length of the lettuce radicle was only around 15 % that of the control, after four days of UV irradiation, the radicle length was 80 % that of the control (Fig. Ⅳ-5(b)). The presence of 3,4-DPAA was also confirmed in the concentrated fraction. When subjected to the same photocatalytic treatment, the 3,4-DPAA concentrations of dark-incubated samples initially decreased only through adsorption on the TiO_2 surface, while with UV irradiation the concentrations continued to decline with increasing irradiation time (data not shown).

Ⅳ－3－3　Effect of photocatalytic treatment on closed soilless cultivation system

To study the effect of the photocatalytic treatment in a closed soilless cultivation system, the growth of asparagus in photocatalytically treated and untreated systems was observed (Fig. Ⅳ-1(a) and (b)). After 12 months of cultivation under closed soilless conditions, the growth of the asparagus fed from the photocatalytically-treated system was superior to the untreated system. When examined for changes in stem length during the first year after transplantation, the difference between the two

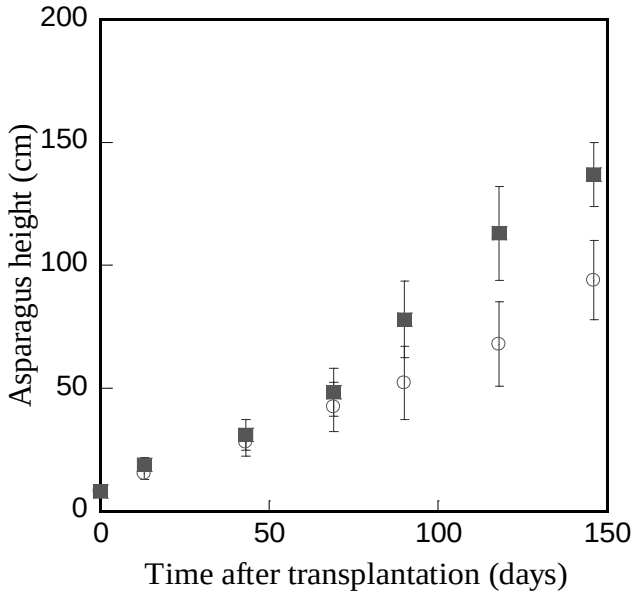


Figure Ⅳ-6 Comparison of asparagus height over time after transplantation in either the photocatalytically treated system (■) or untreated system (○) of the closed soilless cultivation. Error bar: Standard deviations of height measurements from nine seedlings

systems became apparent around the 90th day (Fig. Ⅳ-6).

Ultimately, the stem length of the asparagus in photocatalytically-treated system was about 1.3 fold longer than the stems in the untreated system. Table Ⅳ-1 shows for both systems the maximum stem diameter and the total number of stems from one seedling on the 146th day after transplantation as well as the asparagus spear yield over a nine-month period between the 12th and 20th month after plantation. For growth indices, the photocatalytically-treated system outperformed the untreated system, highlighted by a 1.6 fold increase in asparagus spear yield. The measurements of asparagus growth revealed that TiO_2 photocatalytic treatment under sun light removes hindrances to growth which is thought to be due to the decomposition and detoxification of phytotoxic compounds. In contrast, these phytotoxins accumulated in the nutrient solution tank of the untreated system and inhibited asparagus growth.

Ⅳ－4 DISCUSSHION

The results of TOC concentration and lettuce radicle length ratio shown in Fig. Ⅳ-3 suggest that the phytotoxic constituents of asparagus roots are decomposed by the photocatalytic reaction and their toxic activity can also be essentially abolished. The results of 3,4-DPAA concentration in Fig. Ⅳ-4 mirrored those from the photocatalytic treatment conducted on the active fraction obtained from asparagus roots in Fig. Ⅳ-3. The results in Fig. Ⅳ-5 show that the phytotoxic compounds released in waste nutrient solution under actual soilless asparagus cultivation are also decomposed and detoxified by TiO_2 photocatalytic reaction. The results in Fig. Ⅳ-6 and Table Ⅳ-1 suggest that by incorporating photocatalytic treatment into closed soilless cultivation systems, even crops that release autotoxins, such as asparagus, can be grown without the risks of yield declines or replant failures, and help lower the environmental impact.

The inorganic substrate perlite was used in the soilless cultivation system for this study, but from the viewpoint of conservation-oriented agriculture an organic substrate that can be easily returned to the environment such as rice hulls or coconut husks is more desirable. In a closed soilless system, however, phytotoxic compounds released by the rice hulls or coconut husks do in fact inhibit plant growth (Kato et al., 1977; Lee et al., 1999). It is hypothesized that TiO_2 photocatalytic treatment could also decompose and detoxify the phytotoxins released by organic substrate (such as momilactone from the rice hulls(Kato et al., 1977; Lee et al., 1999)).

It has been reported that replant failures and yield declines in asparagus are not due to phytotoxic compounds alone, but occur synergistically with root and crown rots caused by the fungal pathogens from *Fusarium oxysporum* f.sp. asparagi, *F. moniliforme* or *F. proliferatum* (Yang, 1982; Hartung and Stephens, 1983; Elmer, 1991; Schreuder et al., 1995; Blok and Bollen, 1996). Due to its antimicrobial effect, TiO_2 photocatalysis may be able to destroy these pathogens as well (Sunada et al., 1998; 2003). This study suggests that TiO_2 photocatalytic treatment

Table Ⅳ-1. Asparagus growth and yield in the photocatalytically-treated versus the untreated system of the closed soilless cultivation.

	Photocatalytically-treated system	Untreated system
Diameter of stem (mm) [a]	5.1 ± 1.3	3.0 ± 0.8
Number of stems from a seedling [a]	60 ± 17	38 ± 15
Asparagus spear yield (g) [b]	3211	1966

[a] Measurements made on the 146th day after transplantation.
[b] The asparagus were harvested in the 2nd year of cultivation.

under sunlight may be able to eliminate the problems of growth impediments to crops by phytotoxic compounds and pathogens, help increase the yield of plant biomass, in addition to lowering the environmental impact of soilless agriculture.

The application of TiO_2 photocatalysis for the treatment of water has been studied for more than a quarter of a century. However, the treatment capacity by photocatalysis is limited by the amount of UV light present in the light source. For example, if sunlight is used as the UV source, only a small volume of water can be treated due to the low amount of UV energy present in sunlight. This can be overcome by using an artificial light source, such as a Xe-Hg lamp, but the treatment costs become prohibitive. These factors have prevented TiO_2 photocatalysis from being used in practical applications to treat water. However, the results from this study showed that wastewater from agricultural production can be effectively treated with TiO_2 photocatalysis using sun light. It was achieved by capturing sun light using a large irradiated area of porous photocatalytic material in a shallow reaction tank. This study suggests that a water treatment system with a large catalyst area using only sun light can be applied for the treatment of wastewater and also be extended to environmental treatment applications in the cleanup of contaminated air and soil.

Ⅳ－5 SUMMARY

TiO_2 photocatalytic decomposition and detoxification of phytotoxic compounds released by the roots of asparagus (*Asparagus officinalis* L.) was investigated from the viewpoint of conservation-oriented cultivation. The phytotoxically active fraction was extracted either from dried asparagus roots or from the recycled nutrient solution of an asparagus soilless cultivation system. The phytotoxic activity gradually decreased in the fraction with TiO_2 powder under irradiation with ultraviolet (UV) light at an intensity of 1.0 mW cm^{-2} was found. The growth of asparagus plants under actual cultivation conditions was also investigated by comparing asparagus grown in a soilless system where recycled waste nutrient solution was photocatalytically treated with sun light and a system with untreated recycled waste nutrient solution. The results showed, as measured by growth indices such as stem length and stem thickness, that asparagus growth in the photocatalytically treated system was superior to the untreated one. Furthermore, the yield of asparagus spears was 1.6 fold greater in the photocatalytically treated system, demonstrating the detoxification effect on the phytotoxic compounds.

CHAPTER Ⅴ

Inactivation of bacterial wilt in closed soilless cultivation by photocatalytic treatment and silver

Ⅴ－1 INTRODUCTION

From the viewpoint of environmental conservation agriculture, it is desirable not only to switch nutrient

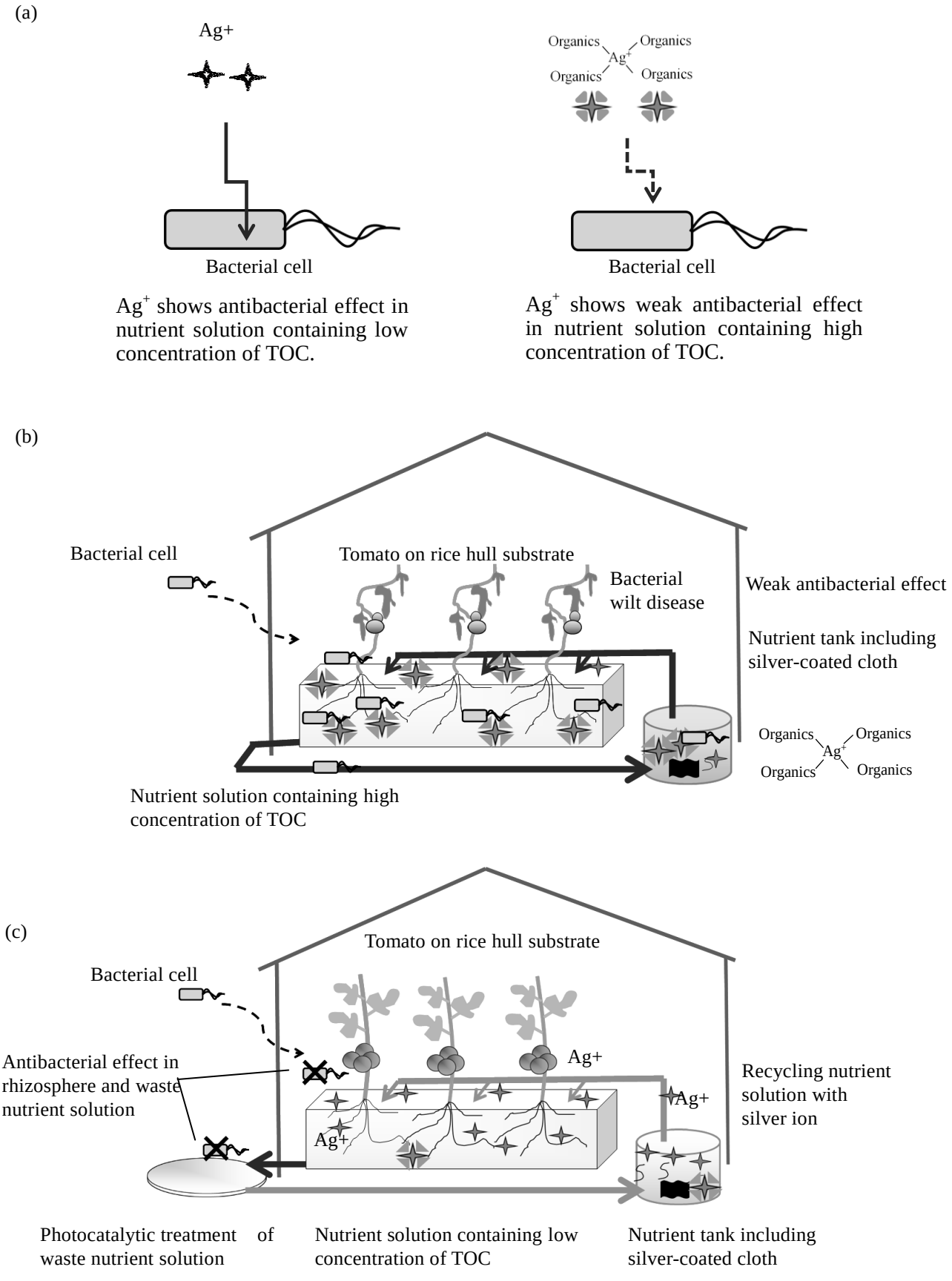


Figure V-1 Schematic diagram of (a) silver forms with and without organic, and (b) untreated and (c) photocatalytically treated closed soilless cultivation systems, using rice hull substrate with silver-coated cloth in an actual greenhouse.

solutions from opened to closed systems in soilless cultivation, but also to switch culture substrates from rockwool to organic substrates, such as rice hull or coconut husk. (Van Os, 1994; 1995; 2000; Shinohara et al., 1999; Urrestarazu et al., 2005). The switching to the closed system prevents groundwater and river from an eutrophication, and another switching to organic substrates do not emit used rockwools as industrial wastes, furthermore, organic substrates can easily return to the fields.

TiO_2 is a photocatalytic compound that has a strong oxidizing effect on irradiation with ultraviolet (UV) light and can convert various types of organic compounds into carbon dioxide by oxidative decomposition (Hoffmann et al., 1995; Fujishima et al., 1999; Herrmann, 1999; Hashimoto et al., 2005). Numerous researchers have attempted to exploit this photocatalytic reaction to decompose environmental pollutants in air and water (Ollis et al., 1991; Noguchi et al., 2003; Pichat et al., 2005). In the study mentioned in the previous chapters it has been confirmed that phytotoxic compounds from rice hulls substrate in waste nutrient solution are decomposed and detoxified through the treatment system with TiO_2 under sun light (ChapterⅡ,Ⅲ,Ⅳ, Sunada et al., 2008; Miyama et al., 2009; 2012b). Furthermore, the growth and yield of tomatoes in a closed cultivation with the treatment system were significantly higher than those with untreated system. It is well known that TiO_2 photocatalysts have antibacterial acivity (Sunada et al., 2003), however, no observation of disease suppression in the closed tomatoes cultivation system with photocatalytic treatment.

Soil-borne diseases can occur even in soilless cultivation systems (Zinnen, 1988). For example, the soil bacterium *Ralstonia solanacearum*, which causes wilt disease in tomatoes, can be introduced into soilless cultivation fields by carrier seedlings, aerosols, or dust (Hayward, 1991; Stanghellini and Rasmussen, 1994). As this bacterium multiplies in the rhizosphere and invades the roots of tomato plants (Jenkins and Averre, 1983), wilt disease occurs and rapidly spreads over entire closed soilless systems, causing serious damage and yield loss. Therefore, it is necessary to develop effective methods for preventing the infestation of crop plants in soilless cultivation beds.

Silver ions are well known to possess antibacterial properties. Silver-coated cloth, which consists of 100 % nylon fibers whose surfaces are uniformly loaded with fine particles of silver by electroless plating, is presently the only commercially available pesticide that can legally be added to nutrient solutions used for soilless cultivation in Japan. When silver-coated cloth is placed into a nutrient solution tank, silver ions are eluted and diffuse throughout the solution, providing an antibacterial effect on even the roots of crops cultivated in soilless beds (Kusakari et al., 1998; Zhao et al., 2000). However, the waste nutrient solution in closed soilless cultivation contains high concentrations of organic contaminants released from organic substrate materials and the crop itself (Sunada et al., 2008; Miyama et al., 2009). In particular, high concentrations of organic contaminants are extracted from organic substrates such as rice hull and coconut husk. It is thought that the antibacterial effects of silver ions decrease in the presence of organic contaminants in waste nutrient solution (Fig. Ⅴ-1(a)). As antibacterial activity typically involves the denaturation of functional biomolecules, which include enzymes and nucleic acids, resulting from their binding to silver ions (Thurman and Gerba, 1989), competitive organic contaminants present in nutrient solutions cause a potential reduction of the effects of silver. Therefore, it is likely to be difficult for silver ions, originating from silver-coated cloth, to exert antibacterial activity against pathogenic bacteria on plant roots in closed soilless cultivation systems (Fig. Ⅴ-1(b)). To obtain the antibacterial effect of silver ions, it is necessary to decrease the concentration of organic contaminants in the waste nutrient solution. In other words, if photocatalytic treatment decomposes the organic contaminants, silver ions in the waste nutrient solution could have antibacterial effect even in the closed system (Fig. Ⅴ-1(c)).

The purpose of this study is to examine the effectiveness of photocatalytically treated nutrient solutions containing

silver ions to maximize their antibacterial activity in a closed soilless cultivation system using a rice hull substrate. The relationship between the concentration of total organic carbon (TOC) and the antibacterial effects of silver ions was investigated by immersing silver nitrate in pure, untreated, and photocatalytically treated nutrient solutions. To evaluate the disease protection ability of silver ions in the three nutrient solutions, these were used to cultivate tomato seedlings inoculated with *R. solanacearum* disease severity and bacterial numbers were measured.

Ⅴ－2 METERIALS AND METHODS

Ⅴ－2－1 Preparation of test solutions

Three test solutions were prepared by adding nutrients (Otsuka-A formula, 0.5 units. Otsuka Kagaku Co.) to deionized water (pure nutrient solution), rice hull extract (untreated nutrient solution), or photocatalytically treated rice hull extract (photocatalytically treated nutrient solution), respectively. Rice hull extract was prepared by washing rice hulls twice with deionized water (100 g rice hulls L^{-1}). After adding a further one L deionized water, the rice hulls were allowed to soak at 35°C for 16 h, and were then removed by filtration through a six μm filter. To prepare the photocatalytically treated nutrient solution, 0.1g L^{-1} TiO_2 powder (P-25, Nippon Aerosil Co.) was added to a portion of the rice hull extract solution, which was then placed in a one L petri dish and irradiated with a blacklight (3.0 mW cm^{-2}) for two weeks. After adding nutrients to each of the three test solutions, the pH was adjusted to 6.5 by adding either phosphoric acid or potassium hydroxide, as appropriate. Inorganic nutrients in three test solutions were almost the same concentrations (data not shown).

Ⅴ－2－2 Silver-coated cloth

Silver-coated cloth (Satosen Co.) was used in the experiments. Silver-coated cloth pellets (1 × 1.5 cm) were placed into 500 mL of each test solution. The resulting mixtures were stored at 20°C for a specified period and samples periodically collected to measure the silver concentration. In this thesis, the term ‘silver’ to indicate silver ions or silver ions bound to organics was used(Fig. Ⅴ-1(a)).

Ⅴ－2－3 Determination of silver and TOC concentration

The concentration of silver, in the form of silver ions, organosilver complexes, etc. (Fig. Ⅴ-1(a)) in the nutrient solutions was determined using an HP 4500 inductively coupled plasma mass spectrometry (ICP-MS) system (Yokogawa Analytical Systems Inc.). Palladium (10 μg L^{-1}) was used as an internal standard of mass number 105, and an integration time of 0.1 s (performed five times) was used for all measurements. The concentration of TOC in test solutions was determined using a TOC analyzer (TOC-5000A, Shimadzu Co., Japan).

Ⅴ－2－4 Evaluation of the antibacterial effect of eluted silver against *R. solanacearum*

In the following experiments, $AgNO_3$ was used as the silver source instead of silver-coated cloth to obtain the constant concentration of silver in test solutions. *R. solanacearum* (strain KNOB53) was cultured in nutrient agar medium at 28 °C and then suspended in sterilized water at a concentration of 3.5×10^9 colony forming units (cfu) m L^{-1} as an inoculum for challenge experiments. Bacterial suspension (2 mL) was added to each test solution (18 mL) after addition of 42 μg L^{-1} silver nitrate ($AgNO_3$), and the resulting suspensions were incubated for 24 h at 25 °C. The number of bacteria in the suspensions was determined at scheduled times by counting colonies on a nutrient agar medium plate incubated for three days at 28°C.

Ⅴ－2－5 Measurement of wilt disease severity in tomato seedlings

An aqueous solution of $AgNO_3$ was added to the test nutrient solutions, at a silver concentration of 40 μg L^{-1}, and stirred overnight at room temperature. Bacterial suspension (20 mL, 2.0×10^8 cfu mL^{-1}) was added to the test solutions (1500 mL), and then stirred for an additional 3 h. 70 mL of each solution was added to separate 100 mL beakers. Two weeks old tomato seedlings (*Lycopersicon esculentum* Mill. cv. Hausu-Momotaro; Takii Seed Co.), grown in an artificially controlledenvironment were placed

Table Ⅴ-1 Silver concentration in untreated and photocatalytically treated rice hull extracts containing nutrients after the addition of silver-coated cloth.

Test nutrient solution	TOC (mg L^{-1})	Silver concentration (μg L^{-1})			
		0 day	1 day	2 day	4 day
Untreated	174 ± 1.2	0	19	27	55
Photocatalytically treated	14 ± 0.6	0	15	32	60

TOC; total organic carbon.

into each solution after cutting the roots in half lengthwise in order to facilitate the infection of pathogen (Ohata et al., 1995). The onset of wilt disease was evaluated after cultivating the seedlings in an artificially controlled environment for one week. The extent of disease was classified as “healthy” = 0, “etiolated” = 1, “wilted” = 2, or “dead” = 3.

Ⅴ－3　RESULTS

When silver-coated cloth was added to nutrient solution, a target silver concentration of 40 μg L^{-1}, which is effective against plant pathogenic bacteria without damaging crop roots, was set (Slade and Pegg, 1993; Kusakari et al., 1998). Here, silver was eluted similarly from the silver-coated cloth in both the untreated and photocatalytically treated nutrient solutions (Table Ⅴ-1)The concentration of silver reached 40 μg L^{-1} in less than four days after adding the cloth to the solutions.

Next, the antibacterial activity of silver against *R. solanacearum* was evaluated as evidenced by temporal change of bacterial concentration (Table Ⅴ-2). In pure nutrient solution (TOC = 5 mg L^{-1}) supplemented with silver, the concentration of bacteria decreased from an initial concentration of 3.5×10^8 cfu mL^{-1} to 1.9×10^4 cfu mL^{-1} after 1 h, representing an approximately 4.3 log decline, and levels were below the detection limit after 3 h. Conversely, in the untreated solution (TOC = 174 mg L^{-1}), the concentration of bacteria slowly decreased, only reaching an approximately 0.9 log reduction in cfu mL^{-1} after 1 h, and a 1.2 log decline after 6 h. By contrast, in the photocatalytically treated solution (TOC = 14 mg L^{-1}), the bacterial concentration displayed a 4.6 log decline from the starting value after 3h and became below the detection limit after 6 h. Thus, silver exhibited antibacterial effects in solutions with 5 and 14 mg L^{-1} TOC, but had little effect in the high TOC solution. Figure Ⅴ-2 clearly demonstrates the relationship between TOC and the antibacterial activity of silver.

The severity of disease in tomato seedlings grown in the three test solutions containing silver is shown in Table Ⅴ-3 and Fig. Ⅴ-3. When silver was added to the pure nutrient solution, no onset of wilt disease was observed in

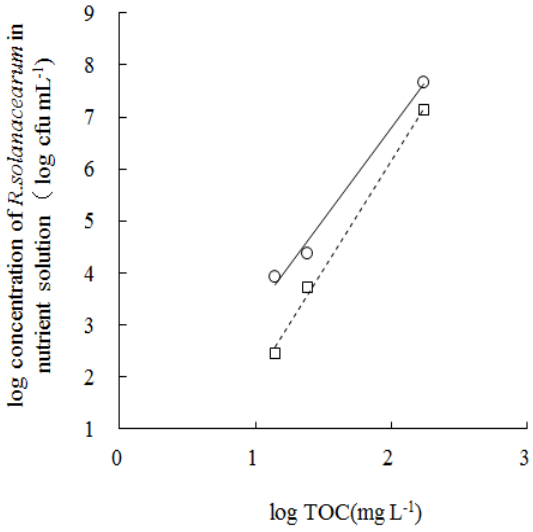


Figure Ⅴ-2 Relationship between TOC concentration and *R. solanacearum* cells in 3 test nutrient solutions. The nutrient solutions were incubated for 1 h (○) and 3 h (□) after the addition of 3.5×10^8 cfu mL^{-1} *R. solanacearum*. The concentration of silver in the nutrient solutions was 42 μgL^{-1}.

any of the tomato seedlings during the first week after transplantation; *R. solanacearum* bacteria were also not detected. By contrast, the onset of wilt disease was observed in all seedlings cultivated using the untreated solution, which was similar to the results in pure nutrient solution without added silver, and the number of bacteria increased approximately ten fold from the initial concentration (2.0×10^6 cfu mL^{-1}). The disease scores for seedlings cultivated using untreated and pure nutrient solutions were 2.0 ± 1.0 and 2.9 ± 0.4, respectively. For the photocatalytically treated solution, there was no onset of disease in the tomato seedlings in the first week after transplantation, a result that is similar to that for pure nutrient solution containing silver. In addition, a 4.2 log decline of bacteria from the initial concentration was observed.

Table Ⅴ-2 Concentration of *R. solanacearum* in photocatalytically treated and untreated solutions and pure nutrient solutions with and without silver [z].

Test nutrient solution	TOC (mg L^{-1})	Concentration of *R. solanacearum* (cfu mL^{-1})[y]			
		1 h	3 h	6 h	24 h
Pure nutrient solution without silver	5	—	—	—	1.4×10^9
Pure nutrient solution with silver	5	1.9×10^4	N.D.	N.D.	N.D.
Untreated nutrient solution with silver	174	4.7×10^7	1.3×10^7	1.8×10^6	4.7×10^3
Photocatalytically treated nutrient solution with silver	14	8.4×10^3	2.8×10^2	N.D.	N.D.

[z] The silver concentration was 42 μg L^{-1}.

[y] The initial concentration of *R. solanacearum* was 3.5×10^8 cfu mL^{-1}.

N.D.; non-detectable concentration, —; no measurement, TOC; total organic carbon

.

Table Ⅴ-3 Effect of photocatalytic treatment with silver on bacterial wilt incidence and disease index of tomato seedlings, and concentrations of silver and bacterial cells after growth of seedlings for one week

Test nutrient solution	TOC (mg L^{-1})	Wilt incidence [w] (%)	Disease score[x]	Concentration of	
				Silver (μg L^{-1})[y]	*R. solanacearum* (cfu mL^{-1})[z]
Pure nutrient solution without silver	5	100	2.9 ± 0.4	N.D.	2.6×10^7
Pure nutrient solution with silver	5	0	0	36.4	N.D.
Untreated nutrient solution with silver	144	100	2.0 ± 1.0	35.0	6.6×10^7
Photocatalytically treated nutrient solution with silver	14	0	0	36.8	1.9×10^2

[w] Wilt incidence = disease seedlings/total seedlings × 100 (%).

[x] Wilt disease was scored as "healthy" = 0, "etiolated" = 1, "wilted" = 2, or "dead" = 3.

[y] The initial concentration of *R. solanacearum* was 2.0×10^6 cfu mL^{-1}.

20 seedlings were tested for each solution.

N.D.; non-detectable concentration, TOC; total organic carbon.

Ⅴ－4　DISCUSSION

Based on the data presented in Table Ⅴ-2, the antibacterial effect of silver in pure nutrient solution used for the cultivation of tomato was confirmed. The antibacterial activity against *R. solanacearum* was higher in the pure and photocatalytically treated nutrient solutions than that in the untreated solution, even though the three solutions contained the same concentration of silver (42 μg L^{-1}). Notably, the activity was dependent on the TOC of the solutions (Fig. Ⅴ-2). In solutions with low TOC, silver is thought to exist mainly in the form of free silver ions, which are active against bacteria, whereas in the high TOC solution, silver ions form complexes with the organic compounds, and thus have lower antibacterial activity (Fig. Ⅴ-1(a)). It has been reported that silver ions likely interact with thiol groups derived from the amino acid cysteine and the phosphate groups of nucleic acids, resulting in impaired bacterial growth (Russell and Hugo, 1994). As organic contaminants present in the nutrient solution from rice hulls contain constituents possessing thiol and phosphate groups, these compounds are able to bind silver ions, leading to decreased antibacterial activity (Fig. Ⅴ-1(b)).

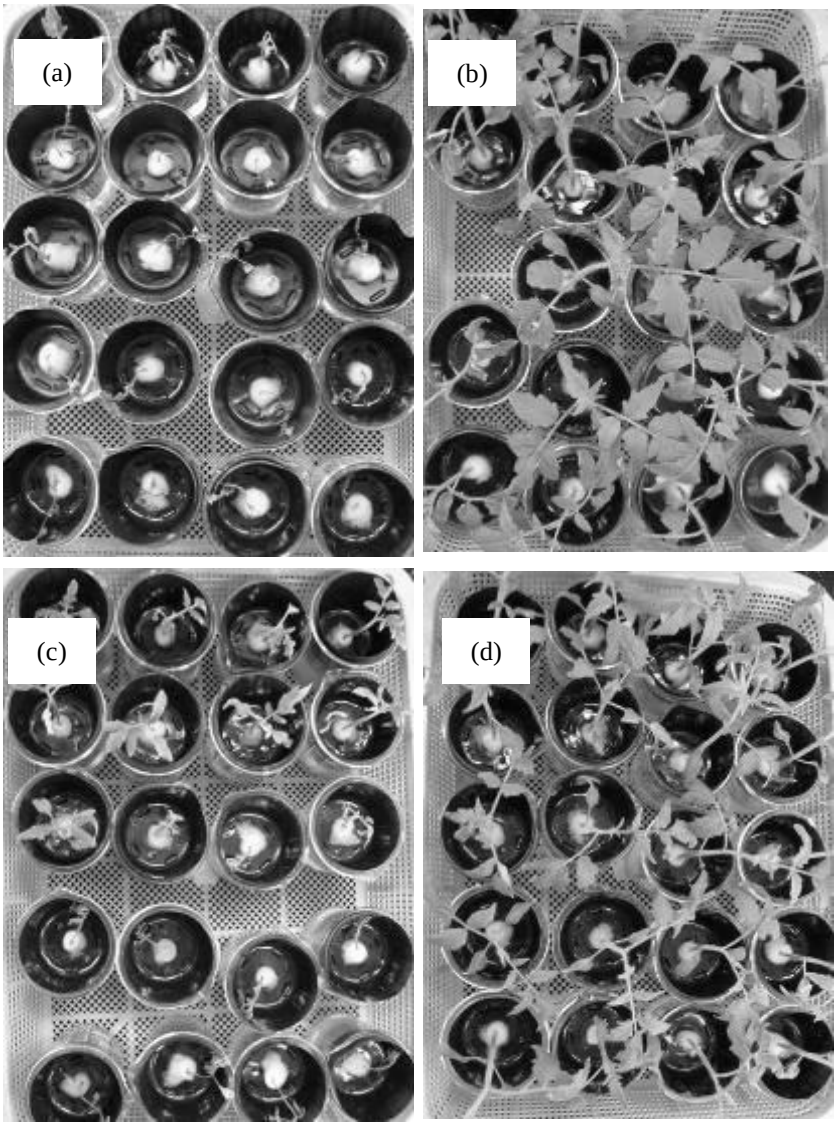


Figure Ⅴ-3 Photographs of bacterial wilt disease incidence in tomato seedlings in (a) pure nutrient solution without silver, (b) pure nutrient solution with silver, (c) untreated nutrient solution with silver, and (d) photocatalytically treated nutrient solution with silver.

Conversely, it has been found that photocatalytic treatment using TiO_2 powder decomposes the organic contaminants present in the nutrient solution, thereby improving the impaired antibacterial activity of the silver ions (Fig. Ⅴ-1(c)).

As shown in Table Ⅴ-3, the incidence of wilt disease in tomato seedlings was correlated with the number of bacteria detected among the four test solutions. Silver clearly suppressed the growth of *R. solanacearum* and the occurrence of wilt disease in the pure and photocatalytically treated nutrient solutions with low TOCs. These findings indicate that the antibacterial activity of silver against soil-borne bacteria around the tomato roots can be enhanced by lowering the TOC concentration through photocatalytic treatment. In addition, photocatalytic treatment itself has biocidal activity against microbes (Matsunaga et al., 1985; Sunada et al., 2003). Although TiO_2 photocatalytic treatment in this system has antibacterial effects on bacteria in the nutrient solution, it is not possible to show antibacterial activity in the cultivation substrate or rhizosphere of crop plants in cultivation fields where carrier seedlings, aerosols, or dust have been introduced. By contrast, silver eluted into nutrient solution can kill bacteria near the plant root in such cultivation systems. As shown in Fig. Ⅴ-1(c), when the waste nutrient solution was photocatalytically treated and silver-coated cloth was placed in the nutrient tank, the growth of bacteria in both the waste nutrient solution and in the rhizosphere of tomato roots was suppressed.

In the previous study, it have been confirmed that TOC of the recycling nutrient solution using rice hull substrate was kept for three years at the low concentration by photocatalytic treatment in the actual tomato soilless cultivation system (Chapter Ⅱ, Miyama et al., 2009). And, it is known that silver coated cloth can continue to release silver ion into the nutrient solution for half or one year. Therefore, it can be expected that the suppression effect on bacterial wilt disease in a laboratory scale (Fig. Ⅴ-3) is obtained even in the actual tomato soilless cultivation system over a long duration.

Although soil-borne plant diseases do not readily develop in soilless culture systems, the causal pathogens can be introduced when infected seedlings are planted in the cultivation field. Once disease occurs, the pathogens can rapidly spread, causing severe damage. Therefore, it is imperative that non-infected seedlings are grown in a greenhouse, and that the culture substrate, raw water, and nutrient solution are all disinfected prior to cultivation. In addition, any waste nutrient solution that will be recycled in closed soilless cultivation must also be disinfected (Vestergard, 1988). The disinfection methods of the waste nutrient solution, are treatments using ozone, hydrogen peroxide, and heating. However, the problems of the cost and risk that health hazard to workers or damage to the plant remain for these methods. The present findings suggest that the combined use of a temporary bactericide , such as the photocatalytic treatment of waste nutrient solution, with a continued bactericide such as silver-coated cloth added to the nutrient solution, is an effective approach for reducing the risk of soil-borne diseases in closed soilless cultivation. Moreover, such a strategy will minimize the energy costs associated with disinfection, because photocatalysis can be driven by sunlight. Therefore the combination of photocatalytic treatment and silver-coated cloth will allow stable crop production in closed soilless cultivation systems and is compatible with environmental conservation agriculture was proposed.

In conclusion, TiO_2 photocatalytic treatment for a simulated waste nutrient solution from closed soilless cultivation using rice hull substrate decreased the TOC concentration of the solution and enhanced the antibacterial activity of silver against wilt disease bacteria. The onset of wilt disease was also suppressed in tomato seedlings cultivated in the nutrient solution containing silver with photocatalytic treatment. Combining silver and photocatalytic treatments reduces the risk of soil-borne disease and achieves stable crop production, even in the context of closed soilless cultivation using an organic substrate.

Ⅴ－5　SUMMARY

From the viewpoint of conservation-oriented agriculture, a closed soilless cultivation using an organic substrate is desirable. To enhance disease control in the system, this study investigated the suppression of bacterial wilt disease in a simulated waste nutrient solution with photocatalytic and silver treatments. The untreated waste nutrient solution had a high total organic carbon (TOC) concentration, and silver in the untreated solution had no antibacterial activity. By contrast, silver in the photocatalytically treated solution showed a high antibacterial effect via the low TOC of the treated solution. A similar trend of disease onset was observed when pathogenic bacteria were inoculated into the solutions used to cultivate tomato seedlings. These results suggest that, even if the concentration of organic species in waste nutrient solution from a closed system is high, lowering the TOC by photocatalytic treatment promotes the suppression of bacterial wilt disease through the antibacterial activity of silver. It is anticipated that combining photocatalytic and silver treatments will help to stabilize crop production in closed soilless cultivation.

CHAPTER Ⅵ

Conclusions

The purpose of this research was to enable environmental preservation and reduce production costs by investigating the use of a photocatalytically treatment equipment designed to enable stable production with no decrease in yield when the substrate is switched from rockwool to rice hulls and open system is replaced by closed system that recycling waste nutrient solution.

Using a substrate such as rice hulls and recycling waste nutrient solution helps solve the following environmental problems: (1) conversion of used rockwool to industrial waste and (2) the possibility of drainage of NO_3^-, PO_4^{3-}, K^+ and other nutrients promoting substances, which are present in nutrient solutions, from cultivation systems, thereby leading to water pollution. Moreover, soilless cultivation growers can also reap the following business benefits: (1) using rice hulls (which are cheap due to their ready availability in Japan) reduces costs relative to those incurred when rockwool is used the substrate material and (2) fertilizers that would conventionally be discarded in nutrient solution can be saved. However, because using a substrate such as rice hulls and recycling the nutrient solution requires a purification treating process, the photocatalytic treatment to achieve these goals was utilized.

The photocatalytic treatment equipment established in this research was a simple device that was installed outside a greenhouse and was exposed to sun light. A material of porous alumina plate coated with TiO_2 anatase-type nanoparticles was set in a shallow-bottomed water container, and waste nutrient solution was drained into this container. The treatment equipment occupied an installation area that comprised just a few percent of the cultivation area.

First, the effectiveness of the photocatalytic treatment at decomposition organic constituents that accumulated in the recycling nutrient solution and contained g phytotoxic compounds was verified. As described in Chapters Ⅱ and Ⅲ, that the photocatalytic treatment resulted in the elution of phytotoxic compounds from rice hulls, thus preventing growth inhibition by these substances was observed. As described in Chapter Ⅳ, that photocatalytic treatment resulted in the decomposition of phytotoxic compounds that seep out from plant roots and reduced the growth-inhibiting properties of these substances was verified. Chapters Ⅱ and Ⅲ describe the results of actual soilless cultivation. Chapter Ⅱ shows that during soilless cultivation of tomatoes performed using rice hulls as the substrate and a recycling nutrient solution, subjecting the nutrient solution to photocatalytic treatment and then reusing it improved growth and yield relative to when the discharged nutrient solution was reused without

treatment. In this experiment, tomatoes were grown continuously for a total of six times over three years, and the same results were obtained for each time for each tomato. TiO_2 porous plate used this experiment was maintained its ability to break down organic constituents over the three-year period. Chapter Ⅲ shows that during soilless cultivation of roses, performed using rice hulls as the substrate and a recycling nutrient solution, subjecting the nutrient solution to photocatalytic treatment and then reusing it improved growth and yield relative to when the discharged nutrient solution was reused without treatment and provided the same yield as the conventional open system using rockwool substrate. Chapter Ⅴ describes the use of both photocatalytic treatment and silver ions (which have different disinfecting effects) as a method for disinfecting the nutrient solution during soilless cultivation of tomatoes. The effect of using photocatalytic treatment, which temporarily disinfects the discharged nutrient solution in the flow channel before reuse, along with silver ions for achieving a disinfecting effect by dissolving in the nutrient solution and recycling the solution near the roots was investigated. The decomposition of organic constituents caused by photocatalytic treatment enabled the silver in the nutrient solution to achieve a disinfecting effect, and the use of both approaches allowed production with a low risk of blight outbreak.

These findings indicate that the photocatalytic treatment equipment examined in this study was effective when using a substrate such as rice hulls and a recycling waste nutrient solution. Development of low-cost photocatalyst materials suitable for treating agricultural runoff is currently underway, and the use of photocatalytic treatment equipment on the farmland of crop producers is being tested.

The equipment described in this research uses only sun light as the light source for photocatalytically treatment. Because agricultural production generally takes place in sun light, there should be various facilities that can install this treatment equipment. Photocatalysis is similar to artificial photosynthesis and is highly applicable to agricultural production. Besides use in purification of waste nutrient solution for soilless cultivation, photocatalytic treatment using sun light also offers several applications for other areas of agriculture. For example, treatment methods for pesticides runoff are now being tested and their effects are being verified.

Japan’s agricultural production business is facing a difficult turning point, with a shortage of successors who will continue farming in the next generation. However, with its ability to enable safe cost-effective work in a clean environment, many eager successors have found soilless cultivation an attractive option. This research can be put to effective use at production sites for assisting in the establishment of environmentally-oriented and stable agricultural production.

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