ORIGINAL ARTICLE

Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics

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Abstract

Hydroponics is an excellent technique for the cultivation of vegetable crops and other plants, but organic fertilizers cannot be used in conventional hydroponic systems, which generally use only inorganic fertilizers, because organic compounds in the hydroponic solutions generally have phytotoxic effects that lead to poor plant growth. Few microorganisms are present in hydroponic solutions to mineralize the organic compounds into inorganic nutrients. In this article a novel and practical hydroponic culture method that uses microorganisms to degrade organic fertilizer in the hydroponic solution has been developed. Soil microorganisms were cultured by regulating the amounts of organic fertilizer and inoculum, with moderate aeration. The microorganisms mineralized organic nitrogen via ammonification and nitrification into nitrate at an efficiency of 97.6%. The culture solution containing the microorganisms was usable as a hydroponic solution, and organic fertilizer could be directly added to it during vegetable cultivation. Vegetables grew well in the organic hydroponic system. Organic hydroponics based on this method is therefore a practical tool for the utilization of organic sources of fertilizer.

Key words: biofilm, microbial nitrification, organic fertilizer, organic hydroponics, root hairs.

INTRODUCTION

Hydroponic culture promotes the growth of crops by allowing close control of fertilization. A closed recirculating hydroponic system conserves fertilizer and water; in contrast, soil culture represents an open system with a relatively low efficiency of water and fertilizer use (Hagin and Lowengart 1996). Conventional hydroponic systems cannot use organic fertilizer, which inhibits plant growth: organic compounds contained in hydroponic solutions have been regarded as phytotoxic (Garland and Mackoqiak 1990; Garland et al. 1993; Mackowiak et al. 1996; Garland et al. 1997; Atkin and Nichols 2004; Ehret et al. 2005; Lee et al. 2006). However, from the viewpoint of resource recycling, it is important to develop methods capable of using organic fertilizer sources in hydroponics.

The ability to use organic fertilizer in hydroponics (“organic hydroponics”) has been studied as a method to grow crops in space habitats (Garland and Mackoqiak 1990; Garland et al. 1993; Mackowiak et al. 1996; Stutte 1996; Garland et al. 1997). However, the direct use of organic fertilizer proved to be deleterious to plant growth (Garland et al. 1997). Therefore, organic fertilizer has been microbially pre-processed before incorporation into hydroponic solutions (Strayer et al. 1997; Atkin and Nichols 2004). The pre-processing has usually been conducted in two or more reaction tanks to provide ammonification and nitrification of organic nitrogen compounds. However, the efficiency of generating nitrate from organic nitrogen in the organic fertilizer was less
than 30% (Strayer et al. 1997). Nitrate is the desired form of nitrogen source for crop production, because many vegetable crops prefer this form over ammonium nitrogen (Ikeda and Osawa 1981; Strayer et al. 1997; Miyata and Ikeda 2005). Ammonium can be used as part of a mixed nitrogen source in hydroponic solutions, but crop failures are very real possibilities if ammonium is the primary nitrogen form (Trebst et al. 1960; Purich and Barker 1967; Givan 1979; Ikeda and Osawa 1981; Stutte 1996; Miyata and Ikeda 2005). For practical and successful organic hydroponics, it is necessary to efficiently generate nitrate from organic fertilizer, thereby allowing direct addition of organic fertilizer to the hydroponic solution during cultivation.

In soil culture, organic fertilizer is usually added directly during cultivation. The organic fertilizer incorporated into the soil is degraded by soil microorganisms, which generate nitrate via ammonification and nitrification, and this nitrate is absorbed rapidly by plants. If the microbial community that degrades organic fertilizer can be cultured in hydroponic solution, it should be possible to add organic fertilizer directly to the solution. However, past studies of organic hydroponics have not tried this.

In this paper, we describe fine-tuning of a previously developed method that can generate nitrate efficiently by culturing microorganisms capable of degrading organic fertilizer in the hydroponic solution (Shinohara 2006, 2007). Vegetables can then be grown in the resulting hydroponic solution. Furthermore, we successfully grew lettuce and tomato plants in the hydroponic solution by means of direct addition of organic fertilizer to the solution during cultivation. Fish-based soluble fertilizer, a by-product of the production of dried bonito fish flakes, was the main organic fertilizer.

**MATERIALS AND METHODS**

**Generation of nitrate from organic fertilizer by microbial degradation in water**

To find an appropriate source of microorganisms that could mineralize organic fertilizer, we added 5 g/L of field soil collected from a field of garden pea, *Pisum sativum*, at Japan’s National Institute of Vegetable and Tea Science. We also tested commercial “Nae-ichiban” nursery soil (Sumirin-Nousankougyo Co., Ltd, Kaifu, Aichi, Japan) and “Golden bark” compost (Shimizu-kou Mokuazai-Sangyou Kyoudou Kumiai, Shizuoka, Japan) as sources of inoculum. The soils were added to 100-mL flasks (n = 3 per treatment) of distilled water containing 1 g/L of fish-based soluble fertilizer (Makurazaki Gyokyo-Kumiai, Makurazaki, Kagoshima, Japan). As a control, we used distilled water containing the fish-based soluble fertilizer without inoculum. The fertilizer contained 4.5 mol/kg nitrogen (N), 0.019 mol/kg phosphorus (P), and 0.23 mol/kg potassium (K). We also tested 100-mL samples of sea water collected from Kinu-ura Harbor in Aichi Prefecture as inoculum, without soils or bark compost. The flasks were shaken (120 strokes/min) for 20 days at 25°C, and the nitrate and ammonium concentrations were then determined.

Based on the results of the previous experiment, we chose bark compost as the inoculum in the next experiment. To determine the optimum dose of inoculum, we added bark compost at 0, 0.5, or 5 g/L to 100-mL flasks (n = 3 per treatment) of distilled water containing 0.25 g of fish-based soluble fertilizer or corn steep liquor (CSL; Sakata, Yokohama, Kanagawa, Japan), which contained 2.4 mol/kg N, 0.48 mol/kg P, and 0.69 mol/kg K; the net concentration of fish-based soluble fertilizer or CSL was 2.5 g/L. The flasks were shaken (120 strokes/min) for 21 or 13 days, respectively, at 25°C, and then the nitrate and ammonium concentrations were determined.

To determine the optimum dose of organic fertilizer, we added fish-based soluble fertilizer at 0.5, 2.5, or 5 g/L daily for seven days from the start of the experiment to 2 L of water containing 5 g/L bark compost as microbial inoculum. The experiment was performed for 15 days at an ambient temperature of 25°C in buckets (n = 3 per treatment); during this time, the water was aerated (19.6 kPa) with a Nisso α-4000 aeration pump (Marukan Co. Ltd, Kasukabe, Saitama, Japan).

To determine the efficiency of conversion of organic nitrogen into nitrate, we added 1 g/L of canola oil cake, corn oil cake, CSL, soybean curd refuse, low-grade spirits distilled from sake lees, rice bran, fermented poultry manure, or fish flour to 50-mL flasks (n = 3 per treatment) of distilled water containing 0.5 g of bark compost. The flasks were shaken (120 strokes/min) for 17 days at 25°C, and the ammonium and nitrate concentrations were then determined.

In all these analyses, we used an RQ-Flex Plus Analyser (Merck, Frankfurt, Germany) to determine the concentrations of nitrate and ammonium ions. Microsoft Office Excel 2003 was used as the statistical software.

**Vegetable growth**

*Growth of tomato seedlings with the microbial culture solution as a hydroponic solution*

We conducted growth experiments with tomato seedlings using the microbial culture solution as the hydroponic solution. We raised four tomato (*Solanum lycopersicum*)...
cv. “Ponderosa”; Kobayashi Seed Co., Ltd, Kagawara, Hyogo, Japan) seedlings in a pot (ø 23 cm × height 23.2 cm) per replicate (n = 3 replicates per treatment). To prepare the microbial culture solution, we added fish-based soluble fertilizer at 300 g per 200 L of water containing 200 g of bark compost as a microbial inoculum, then aerated the water (19.6 kPa) for 33 days using two aeration pumps (Nisso α-4000). The prepared microbial culture solution contained 210 mg/L nitrate and 8.3 mg/L ammonium. Each hydroponic pot was filled with 1 L of a solution containing 3.98 mmol nitrate-nitrogen. As a control treatment, hydroponic solution was prepared with 1 L of distilled water containing 1 g/L of fish-soluble without inoculation of the microbial culture solution. The hydroponic solution contained 60 mg/L organic nitrogen. Each hydroponic pot was filled with 1 L of a solution containing 4.29 mmol organic nitrogen. We added 10 g of oyster shell lime (Urabe Industry Co., Ltd, Fukuyama, Hiroshima, Japan) suspended in each pot to provide additional nutrients and minor nutrients: 1.98 mmol Mg, 1.61 mmol Fe, 0.36 mmol B, 0.255 mmol Mn, 0.013 mmol Zn, 0.00188 mmol Cu, 0.0008 mmol Mo, and 10.5 mmol Ca. As another control treatment, conventional inorganic solution was used as a starter of hydroponic solution, which contained 1 g of Otsuka House TM No. 1 and 0.67 g of Otsuka House TM No. 2 (Otsuka Chemical Co., Ltd, Osaka, Japan). This provided the following nutrients: 11.9 mmol N (1.8% ammonium-nitrogen and 98.2% nitrate-nitrogen), 1.07 mmol P, 5.5 mmol K, 0.91 mmol magnesium (Mg), 0.013 mmol manganese (Mn), 0.013 mmol boron (B), 0.03 mmol iron (Fe), and 2.63 mmol calcium (Ca). We also added 60 g of oyster shell lime (Urabe Industry Co., Ltd, Fukuyama, Hiroshima, Japan) suspended in each pot to provide additional nutrients and minor nutrients: 11.9 mmol Mg, 9.67 mmol Fe, 2.17 mmol B, 1.53 mmol Mn, 0.077 mmol zinc (Zn), 0.0113 mmol copper (Cu), 0.005 mmol molybdenum (Mo), and 62.7 mmol Ca. We added fish-based soluble fertilizer at 3 g/pot (i.e. 0.5 g/L) daily for three days from the start of the experiment to the hydroponic solution in each pot. During cultivation, the solution was aerated (19.6 kPa) with a Nisso α-4000 aeration pump. The experiments were conducted in a glasshouse at Tsu, Mie Prefecture, November 12—29, 2010. The hydroponics systems using microbial and non-microbial solutions were each established in three pots per treatment.

**Growth of lettuce with the microbial culture solution as a hydroponic solution**

We also conducted growth experiments with butterhead lettuce seedlings using the microbial culture solution as the hydroponic solution. We raised four butterhead lettuce “Sarada” (**Lactuca sativa** var. **capitata**; Sakata Seed Corporation, Yokohama, Kanagawa, Japan) seedlings in a pot (width 9 cm × length 9 cm × height 15 cm) per replicate (n = 3 replicates per treatment). To prepare the microbial culture solution, we added fish-based soluble fertilizer at 300 g per 200 L of water containing 200 g of bark compost as a microbial inoculum, then aerated the water (19.6 kPa) for 170 days using two aeration pumps (Nisso α-4000). The prepared microbial culture solution contained 210 mg/L nitrate and 8.3 mg/L ammonium. Each hydroponic pot was filled with 1 L of a solution containing 3.98 mmol nitrate-nitrogen. As a control treatment, hydroponic solution was prepared with 1 L of distilled water containing 1 g/L of fish-soluble without inoculation of the microbial culture solution. The hydroponic solution contained 60 mg/L organic nitrogen. Each hydroponic pot was filled with 1 L of a solution containing 4.29 mmol organic nitrogen. We added 10 g of oyster shell lime (Urabe Industry Co., Ltd, Fukuyama, Hiroshima, Japan) suspended in each pot to provide additional nutrients and minor nutrients: 1.98 mmol Mg, 1.61 mmol Fe, 0.36 mmol B, 0.255 mmol Mn, 0.013 mmol Zn, 0.00188 mmol Cu, 0.0008 mmol Mo, and 10.5 mmol Ca. As another control treatment, conventional inorganic solution was used as a starter of hydroponic solution, which contained 1 g of Otsuka House TM No. 1 and 0.67 g of Otsuka House TM No. 2 (Otsuka Chemical Co., Ltd, Osaka, Japan). This provided the following nutrients: 3.98 mmol N (98.2% nitrate-nitrogen and 1.8% ammonium-nitrogen), 0.358 mmol P, 1.84 mmol K, 0.30 mmol Mg, 0.0043 mmol Mn, 0.0043 mmol B, 0.010 mmol Fe, and 0.88 mmol Ca. We added fish-based soluble fertilizer at 1 g/pot (i.e. 1 g/L) at the start of the experiment to the hydroponic solution in each pot. During cultivation, the solution was aerated (19.6 kPa) with a Nisso α-4000 aeration pump. The experiments were conducted in a glasshouse at Tsu, Mie Prefecture, July 21—29, 2010.

The hydroponics systems using microbial and non-microbial solutions were each established in three pots per treatment.

**Growth of tomato seedlings with oyster shell lime**

We conducted growth experiments with the tomato (cv. “Ponderosa”) seedlings, with or without oyster shell lime as a supplement to provide minor nutrients. We raised four seedlings in a pot (ø 23 cm × height 23.2 cm) per replicate (n = 3 replicates per treatment). To prepare the hydroponic solution, we added fish-based soluble fertilizer at 300 g per 200 L of water containing 200 g of bark compost as a microbial inoculum, then aerated the water (19.6 kPa) for 33 days using two aeration pumps (Nisso α-4000). The prepared hydroponic solution contained 164 mg/L nitrate ions and 9 mg/L ammonium ions. Each hydroponic pot was filled with 5 L of water and 1 L of a solution containing 3.8 mmol nitrogen as nitrate. We added 0 or 60 g of oyster shell lime (Urabe Industry Co., Ltd) suspended in the solution to provide additional nutrients and minor nutrients: 11.9 mmol Mg, 9.67 mmol Fe, 2.17 mmol B, 1.53 mmol Mn, 0.0771 mmol Zn, 0.0113 mmol Cu, 0.005 mmol Mo, and 62.7 mmol Ca. During cultivation, the solution was aerated (19.6 kPa) with a Nisso α-4000 aeration pump. The experiments were conducted in a glasshouse at Tsu from August 12
to September 2, 2010. We added 0.12 g ethylenediaminetetraacetic acid iron salt (FeEDTA) (Dojindo Laboratories, Kumamoto, Japan) in a pot as an Fe supplement eight days after the start of the experiment.

**Lettuce**

We conducted growth experiments with butterhead lettuce “Saradana” (*Lactuca sativa* var. *capitata*; Sakata Seed Corporation, Yokohama, Kanagawa, Japan) in a glasshouse at Tsu from October 15 to November 9, 2009. During these experiments, light and temperature were not controlled. We raised 10 butterhead lettuce seedlings in a polystyrene foam board floating in one hydroponic container (length 50 × width 20 × height 25 cm) per replicate (n = 3 replicates per treatment). To prepare the hydroponic solution, we added fish-based soluble fertilizer at 300 g per 200 L of water containing 200 g of bark compost as a microbial inoculum; the water was aerated (19.6 kPa) for a total of 48 days before cultivation using two aeration pumps (Nisso α-4000). The prepared hydroponic solution contained 235 mg/L of nitrate but no ammonium. Each hydroponic container was filled with 15 L of a solution containing 56.9 mmol nitrogen as nitrate. We also added 150 g of oyster shell lime (Urabe Industry Co., Ltd) suspended in the solution to provide additional nutrients and minor nutrients: 29.8 mmol Mg, 24.2 mmol Fe, 5.41 mmol B, 3.82 mmol Mn, 0.193 mmol Zn, 0.0283 mmol Cu, 0.0125 mmol Mo, and 157 mmol Ca. During cultivation, the solution was aerated (19.6 kPa) with a Nisso α-4000 aeration pump. As the control treatment, we used conventional hydroponics with inorganic fertilizer containing 4.78 g of Otsuka House TM No. 1 and 3.18 g of Otsuka House TM No. 2, which provided the following nutrients: 56.9 mmol N (1.8% ammonium-nitrogen and 98.2% nitrate-nitrogen), 5.1 mmol P, 26.3 mmol K, 4.35 mmol Mg, 0.06 mmol Mn, 0.063 mmol B, 0.14 mmol Fe, and 12.6 mmol Ca. We adjusted the amount of fertilizer added to the system to provide the same amount of nitrogen as was provided by the organic hydroponic system. To determine the concentration of nutrients in the hydroponic solutions the next day at the start of the experiment, the solutions were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) SPS-7700 (Seiko Instruments Inc. Chiba, Chiba, Japan). The organic and conventional hydroponics were each performed in three containers.

**Tomato**

We used a nutrient film technique (Wheeler *et al.* 1990) to grow cv. “Saturn” tomato plants (Takii & Co., Ltd, Kyoto, Japan; n = 24 per treatment) in a closed recirculating hydroponic system (M-shiki Suikou Co., Ltd, Yatomi, Aichi, Japan; length 465 × width 60 × height 18 cm) based on a deep-flow technique from June 7 to October 13, 2006. This experiment was conducted in a glasshouse at Taketoyo, Aichi Prefecture. To prepare the hydroponic solution, we added fish-based soluble fertilizer at 100 g or CSL at 200 g per 200 L of water containing 200 g bark compost as a microbial inoculum; the hydroponic solution was aerated with an MD-15R-N water pump (Iwaki Co. Ltd, Tokyo, Japan) by recirculation of the nutrient solution in the hydroponic system for 12 days.

The nitrate concentrations in the starter hydroponic solutions were 189 mg/L (fish-based fertilizer) and 420 mg/L (CSL); no ammonium-nitrogen was detected in either hydroponic solution. At the start of cultivation we also added 560 g of oyster shell lime to each hydroponic solution to provide minor nutrients and micronutrients: 111 mmol Mg, 90.2 mmol Fe, 20.2 mmol B, 14.3 mmol Mn, 0.720 mmol Zn, 0.106 mmol Cu, 0.047 mmol Mo, and 585 mmol Ca. For 24 days from the start of cultivation, we added fish-based soluble fertilizer at 0.5 g/plant daily or CSL at 1 g/plant daily to provide the equivalent of 30 mg N/plant daily; we subsequently added fish-based soluble fertilizer at 1 g/plant daily or CSL at 2 g/plant daily to provide the equivalent of 60 mg N/plant daily. The organic fertilizers were added directly to each hydroponic solution. We also added wood ash (JOY AGRIS Co., Ltd, Tokyo, Japan) containing 1.64 mol/kg K to the hydroponic solution as a potassium supplement during cultivation, at 0.34 g (containing 0.36 mmol K) per 1 g of CSL or 0.932 g per 1 g of fish-based soluble fertilizer. In the control treatment, we conducted conventional hydroponics with inorganic fertilizer solution that comprised a mixture of Otsuka House TM Nos 1, 2, and 5, at proportions of 60:20:1, respectively. This provided the following nutrient concentrations: 18.8 mmol/L N (1.8% ammonium-nitrogen and 98.2% nitrate-nitrogen), 1.69 mmol/L P, 8.69 mmol/L K, 1.49 mmol/L Mg, 0.04 mmol/L Mn, 0.04 mmol/L B, 0.1 mmol/L Fe, and 4.1 mmol/L Ca. The amount of fertilizer added to the system was adjusted to provide the same amount of nitrogen as in the organic hydroponic system. The concentration of nitrate ion in every hydroponic solution was daily analyzed just before addition of fertilizers in the hydroponic solution. The Brix values (a measure of the sugar content) of the fruits were measured in supernatant filtered from homogenized fruits, using an APAL-1 Refractometer (ATAGO Co., Ltd, Tokyo, Japan). We used an RQ-Flex Plus Analyser to quantify the ascorbic acid contents.
RESULTS

Generation of nitrate from organic fertilizer by microbial degradation in water

We chose field soil, nursery soil, bark compost, and sea water as inoculum sources for the microorganisms needed to mineralize organic nitrogen into nitrate in water in the presence of fish-based soluble fertilizer as a nitrogen source. The addition of nursery soil or bark compost as inoculum sources generated nitrate (Fig. 1). Sea water without soil or bark compost but with added fish-based soluble fertilizer also generated nitrate. The efficiency of generation did not differ significantly ($P > 0.05$) among the inocula; however, all three inocula were significantly ($P < 0.05$) more efficient than field soil. However, distilled water without inoculum but with added fish-based soluble fertilizer generated ammonium but no nitrate. These results indicate that the saprophytic bacteria in the fertilizer could generate only ammonium from the organic nitrogen in the fish-based soluble fertilizer, but microorganisms added to the water from the field soil, nursery soil, bark compost, or sea water were able to mineralize the organic nitrogen into nitrate (Fig. 1). We performed all subsequent experiments using bark compost, because it provided good results and is easily obtainable.

We examined the inoculum requirements with bark compost in 100-mL flasks of distilled water containing 0.25 g CSL (Fig. 2). The nitrate content increased as the ammonium content decreased in the solution with bark compost at 5 g/L, but no nitrate was detected in flasks when 0.5 g/L or less of bark compost was added. These results suggest that about 5 g/L of bark compost inoculum was needed to mineralize organic compounds into nitrate. However, nitrate was detected in flasks containing 0.25 g of fish-based soluble fertilizer per 100 mL when 0.5 g/L or more of bark compost was added (data not shown). These results suggest that the organic nitrogen in fish-based soluble fertilizer can be more efficiently transformed into nitrate than that in CSL.

We then examined the optimum dose of organic fertilizer (Fig. 3). We added fish-based soluble fertilizer daily for seven days from the start of incubation to water containing 5 g/L of bark compost inoculum. Nitrate was generated only in containers that received 0.5 g/L of fertilizer daily for seven days; higher application rates produced only ammonium. The efficiency of nitrate generation from the organic nitrogen was 97.6%. Nitrification was inhibited in containers that received fertilizer at 2.5 or 5 g/L daily for seven days. This suggests that nitrification can be inhibited by excessive amounts of organic fertilizer.

We then examined the efficiency of conversion of organic nitrogen into nitrate in various organic fertilizers. Nitrate was generated in each flask that contained CSL (at an efficiency of 99.7%), corn oil cake (91.3%), fish flour (77.7%), canola oil cake (74.5%),
soybean curd refuse (73.5%), and fermented poultry manure (30.5%). However, no nitrate was generated in the flasks that contained low-grade spirits distilled from sake lees or rice bran. The carbon-to-nitrogen ratios (C/N) of these organic fertilizers were 0.90 for CSL, 3.45 for fish-based soluble fertilizer, 4.66 for fish flour, 6.93 for canola oil cake, 7.0 for fermented poultry manure, 8.68 for corn oil cake, 10.43 for soybean curd refuse, 11.78 for the sake lees, and 18.1 for rice bran.

**Vegetable growth**

*Growth of tomato seedlings with the microbial culture solution as a hydroponic solution*

The tomato seedlings grew well when we used the microbial culture solution as the hydroponic solution (Fig. 4a). In contrast, all the tomato seedlings grown without the microbial culture solution died (Fig. 4b). Nitrate was detected in the hydroponic solution with the
microbial culture solution (206, 220, and 232 mg/L respectively, in the three media), but no ammonium was detected eight days after the start of the experiment. In contrast, only ammonium was detected (at 69, 70, and 80 mg/L respectively, in the three media) from the solutions that lacked any microbial culture solution.

**Growth of lettuce with the microbial culture solution as a hydroponic solution**

The lettuce seedlings grew well when we used the microbial culture solution (Fig. 5a). Similarly, the seedlings grew well in the system with conventional inorganic solution (Fig. 5c). In contrast, all the lettuce seedlings seriously wilted in the solution containing only the fish-based soluble fertilizer five days after the start of the experiment (Fig. 5b). Nitrate was detected in the hydroponic solution with the microbial culture solution (210, 230, and 235 mg/L respectively, in the three media) but no ammonium was detected seven days after the start of the experiment. Nitrate was detected in the solution with conventional inorganic solution (85, 115, and 180 mg/L respectively, in the three media) and ammonium was generated (at 28, 28, and 28 mg/L respectively, in the three media). Only ammonium was detected (at 38, 31, and 34 mg/L respectively, in the three media) from the solutions added with only fish-based soluble fertilizer.

**Growth of tomato seedlings with oyster shell lime provided**

The tomato seedlings grown in the solution containing oyster shell lime grew well (Fig. 6a). In contrast, leaf tips of the seedlings grown without oyster shell lime became...
yellow and the growth decreased (Fig. 6b and c). The addition of 0.12 g FeEDTA to each pot ameliorated but did not eliminate the symptoms (Fig. 6d).

**Lettuce**

We examined the growth of butterhead lettuce in a hydroponic nutrient solution in which the organic nitrogen contained in fish-based soluble fertilizer was optimally mineralized into nitrate (Fig. 7). The organic system produced significantly greater ($P < 0.05$) fresh head weight and root dry weight than in the conventional system. (We measured head fresh weight instead of dry weight, because fresh weight determines the commercial value of the lettuce.) The leaf nitrate ion content was 35.5% lower in the organic system, and the difference was significant ($P < 0.05$). The hydroponic solutions, the next day of the start, of organic system and conventional system contained 28.82 and 35.44 mg/L K, 66.7 and 7.24 mg/L Na, 66.32 and 37.22 mg/L Ca, 0.4988 and 0.285 mg/L Fe, 10.1 and 12.6 mg/L Mg, 0.086 and 0.4048 mg/L Mn, and 0.0598 and 0.1674 mg/L B, respectively. Neither nitrate nor ammonium was detected in the hydroponic solution of either system at the end of cultivation. The ascorbic acid content of the leaves did not differ significantly ($P > 0.05$) between the two systems.

**Tomato**

We also performed a tomato growth study (Fig. 8). The total fruit yields (24 plants per treatment) were 35,338 g with the conventional fertilizer, 32,974 g with the CSL, and 33,783 g with the fish-based soluble fertilizer, and the fruit weight per plant did not differ significantly ($P > 0.05$). The average Brix value of the fruits ($n = 32$ fruits per treatment) in the conventional treatment (4.8) did not differ significantly from the value in the CSL treatment (4.8), but both were significantly lower ($P < 0.05$) than the value in the fish-based soluble fertilizer treatment (5.2). The average ascorbic acid content of the fruits ($n = 32$ fruits per treatment) did not differ significantly among the treatments.
Biofilms of microorganisms also developed on the root hairs of roots submerged in the solutions containing CSL or fish-based fertilizer, but neither biofilms nor root hairs were observed in the conventional hydroponic system (Fig. 9).

The concentrations of nitrate ion in the hydroponic solutions from both organic systems were decreased gradually and the nitrate ion could not be detected in the solution 14 days after the start of cultivation (Fig. 10).

**DISCUSSION**

The addition of only fish-based soluble fertilizer without inoculum in distilled water resulted in the generation of ammonium but no nitrate (Fig. 1). In contrast, using inoculum such as nursery or field soil or bark compost...
Figure 9 Tomato growth in the conventional and organic hydroponic systems. The growth of tomato plants grown with three kinds of fertilizer was compared: (a) conventional inorganic fertilizer, and (b, c) microbial inoculum combined with (b) corn steep liquor and (c) fish-based soluble fertilizer. Photographs show (i) the tomato plants just before harvesting, (ii) the tomato roots on June 15, 2006 during cultivation, and (iii) the roots submerged in the hydroponic solution on June 27, 2006. Tomato roots were observed submerged in the hydroponic solution under a stereomicroscope. (a(iv)) Roots of tomato grown in the conventional hydroponic system with inorganic fertilizer had no root hairs. (c(iv)) Large numbers of root hairs developed on the roots of tomato grown in the organic hydroponic system with fish-based soluble fertilizer. The hairs were also covered with a biofilm.
resulted in nitrate generation. These results suggest that saprophytic microorganisms contained in fish-based soluble fertilizer cannot generate nitrate from its organic nitrogen, and that it is necessary to add inoculum to generate nitrate. Interestingly, the addition of only organic fertilizer without inoculum in sea water also resulted in generation of nitrate. This indicates that sea water contains microorganisms capable of degrading organic fertilizer to produce nitrate.

Control of the amounts of both inoculum and organic fertilizer is important in order to generate nitrate in water. Too small an amount of inoculum (≤0.5 g/L bark compost) resulted in the generation of only ammonium when 0.25 g of CSL was added per 100 mL (Fig. 2). Too much fish-based soluble fertilizer (≥2.5 g/L daily for seven days) also resulted in the generation of only ammonium (Fig. 3). The growth of nitrifying bacteria, such as the obligate chemolithoautotrophs Nitrosomonas spp. and Nitrospira spp., is particularly inhibited by the presence of organic compounds (Jensen 1950; Quastel and Scholefield 1951; Rittenberg 1969; Smith and Hoare 1977; Krummel and Harms 1982; Takahashi et al. 1992; Stutte 1996; Xu et al. 2000; Tomiyama et al. 2001). Therefore, to culture microorganisms capable of generating nitrate in water, it is necessary to add sufficient inoculum that contains nitrifying microorganisms and to restrict the amount of organic fertilizer that is added to the water.

However, it was not clear why adding 0.5 g/L bark compost generated no nitrate when 0.25 g of CSL was added per 100 mL, but generated nitrate when 0.25 g of fish-based soluble fertilizer was added per 100 mL. This may relate to differences in the form and biodegradability of the organic nitrogen between animal-based products (fish-based soluble fertilizer) and plant products (CSL).

The nitrate-generation efficiencies of the organic fertilizers were not clearly related to their C/N ratios. However, high-C/N organic fertilizers, such as low-grade spirits distilled from sake lees (11.78) and rice bran (18.1), were not appropriate for generating nitrate. Organic fertilizers with a C/N ratio of less than 11, such as soybean curd refuse (10.43), were mineralized into nitrate. These results suggest that the addition of organic fertilizer with a C/N ratio of ≥11 may cause nitrogen starvation of the microorganisms (Wang and Bakken 1997). We therefore suggest that organic fertilizers with a C/N ratio of <11 should be used to generate nitrate in an organic hydroponic system, or that a supplemental nitrogen source must be provided to decrease the C/N ratio.

The microbial culture solution was suitable for organic hydroponics (Fig. 4a). In tomato experiment, the nitrate-nitrogen concentration was increased by the addition of fish-based soluble fertilizer. In contrast, the addition of fish-based soluble fertilizer in the conventional inorganic hydroponic solution with no inoculum caused the death of all the tomato seedlings and generated only ammonium (Fig. 4b). As we mentioned in the Introduction, excessive ammonium can be deleterious to the growth of vegetables, such as tomato, and many vegetable crops prefer nitrate (Ikeda and Osawa 1981; Strayer et al. 1997; Miyata and Ikeda 2005; Cruz et al. 2006).

Interestingly, lettuce seedlings grew well in the conventional inorganic hydroponic solution added with fish-based soluble fertilizer (Fig. 5c) as observed in the microbial culture solution (Fig. 5a). These results suggest that lettuce is more susceptible to ammonium than tomato because lettuce is known as ammonium-phlic plant (Tadano and Tanaka 1976; Ikeda and Osawa 1981). However, lettuce seedlings seriously wilted in the solution added with only fish-based soluble fertilizer (Fig. 5b) and only ammonium was detected in the solution. In previous studies for organic hydroponics, application of hydroponic solution, derived from organic fertilizer, with ammonium-nitrogen without nitrate-nitrogen significantly suppressed lettuce growth (Schwartzkopf and Stroup 1993; Atkin and Nichols 2004). These results suggest that supplying nitrate by microbial nitrification or addition of conventional inorganic fertilizer is required for the growth of lettuce when ammonium nutrient solution derived from organic fertilizer is used in hydroponic cultivation. For successful organic hydroponics, it will therefore be important to use a microbial culture solution, such as the ones in the present study.

Figure 10 Nitrate concentration in the conventional and organic hydroponic solutions. The concentration of nitrate in the hydroponic solution with corn steep liquor fertilizer (CSL), fish-based soluble fertilizer (Fish), and conventional hydroponics with inorganic fertilizer (conv.) during tomato cultivation was measured.
that can generate nitrate from organic fertilizer, as the hydroponic solution.

The addition of oyster shell lime in the hydroponic solution can effectively supplement minor nutrients (Fig. 6). However, some leaf chlorosis occurs without this supplement, although the symptom of yellow leaf tips can be ameliorated by the addition of supplemental iron. These results suggest that the addition of oyster shell lime may be especially effective to eliminate iron deficiency. Shells such as those from oysters contain high levels of calcium carbonate (CaCO₃) as their principal component. The solubility of CaCO₃ in water is low (about 0.15 mmol/L at 25°C). This may explain why malabsorption of potassium or magnesium after the addition of oyster shell lime was not observed in the lettuce and tomato growth experiments (Hewitt and Smith 1975).

The fresh weight of lettuce was significantly greater in the organic system than in the conventional system. In previous studies of organic fertilizer in hydroponics, lettuce dry weights were similar to or less than those with conventional inorganic fertilizer but fresh weights were significantly lower (Anderson and Schmidt 2001; Atkin and Nichols 2004). It is not clear why higher root dry weight was produced in the organic system. Root development is promoted by the presence of certain microorganisms (Compant et al. 2005), so we should study the relationship between the action of the microorganisms present in the hydroponic solution and the resulting effect on root development.

It is not clear why the lettuce leaf nitrate content was significantly lower in the organic system than in the conventional system. It is assumed that it is attributed to larger growth of lettuce in the organic system (Fig. 7). In the hydroponic solution of the organic system, we only detected nitrate (235 mg/L nitrate); in the conventional inorganic hydroponic solution, the same amount of nitrogen was present, and comprised 1.8% ammonium-nitrogen and 98.2% nitrate-nitrogen. Nitrate ions at high concentrations in foods can harm the health of humans and animals (Blom-Zandstra 2008). These results suggest that the yield and quality of butterhead lettuce in the microorganism culture system were at least as good as those in the conventional inorganic chemical system.

It is interesting that in the tomato growth experiment, neither nitrate nor ammonium could be detected two weeks after the start of cultivation in the organic system (Fig. 8). In contrast, nitrate was detected up to one month after the start of cultivation in the conventional inorganic hydroponic system, despite the addition of the same amount of nitrogen. We found that biofilm collected from the surface of the roots was able to generate nitrate within a few days when supplied with organic fertilizer in water (data not shown). This suggests that the biofilm on the root surfaces was able to degrade the organic fertilizer into ammonium and then nitrate, which was then absorbed by the roots without diffusing into the hydroponic solution. This may be why inorganic nitrogen disappeared from the hydroponic solution two weeks after the start of cultivation in the organic system. The forms or composition of the absorbed nitrogen, such as ammonium or nitrate, were unclear because neither nitrate nor ammonium was detected in the hydroponic solution. In future research, we should study the mechanism of absorption of nitrogen in these systems.

The development of root hairs and biofilms on the roots submerged in the solutions is characteristic of the organic system (Fig. 9). It is known that few root hairs develop on roots submerged in a conventional inorganic hydroponic solution (Nakano et al. 2002). Little is known about the mechanism of root hair development (Michael 2001). The development of the root hairs in the organic system is therefore an important topic for future study.

From the biofilm present on the tomato roots in the organic system, we were able to isolate Arthrobacter spp., Ralstonia spp., Mycobacterium spp., Bordetella spp., and Rhodoferax spp. by means of dilution plating, but they were not detected in our 16S rRNA denaturing-gradient gel electrophoresis analysis (data not shown). This indicates that these isolates may be not major components of the biofilms. If we try to recreate the generation of nitrate from organic fertilizer using single isolates from biofilms, it will be necessary to isolate the major microorganisms responsible for the degradation of the organic fertilizer and to find an appropriate combination of those isolates. This will be an important issue for future study, since the development of a reliable organic hydroponic system will require consistency in the inoculum source.

Organic hydroponics appears to be a suitable medium for observations of the interactions between rhizobacteria and roots. This interaction occurs in soil but is difficult to observe without disruption of the rhizosphere structure. In conventional hydroponics, it is easy to observe roots, but there is little or no interaction between microorganisms and the roots because there are few microorganisms in the hydroponic solution. In contrast, our organic hydroponic method allows observation of these interactions, at any time, without disturbing the roots or microorganisms (Fig. 9). Biofilms are an important subject in studies of bacterial communication and interactions, and the possibility that the bacterial community in a biofilm interacts with plant roots is intriguing (Ramey et al. 2004).

The results of the tomato growth experiments suggest that the yield and quality of the tomato fruits produced by the organic fertilizer system were not inferior to those
produced by the conventional system. In previous studies of organic hydroponics, it was difficult to come up with an organic alternative to the conventional inorganic solutions used for growing tomatoes (Atkin and Nichols 2004; Miyata and Ikeda 2005). Inconvenient procedures to treat the organic fertilizer have been used, such as conducting the ammonification and nitrification reactions in separate bioreactor tanks, followed by eliminating the organic compounds and microorganisms from the mineralized solution before it was supplied to the plants (MacElroy and Bredt 1984; Tako et al. 1995; Garland et al. 1997; Atkin and Nichols 2004; Jewell and Kubota 2005; Miyata and Ikeda 2005). Furthermore, it was impossible to add organic fertilizer to the hydroponic solution during cultivation. In contrast, our method shows that it is possible to supply sufficient nutrients by adding organic fertilizer directly to the hydroponic solution during cultivation. It is therefore possible to use organic fertilizer in hydroponics.

In conventional hydroponics, microbial contamination of hydroponic solutions often results in root disease (Stanghellini and Rasmussen 1994; Vestergard 1994); this is why hydroponics researchers have traditionally regarded all microorganisms as deleterious. However, it is difficult and expensive to maintain axenic cultivation conditions in conventional hydroponics. In contrast, our results demonstrate that our novel technique is a feasible non-axenic alternative to conventional hydroponics. By culturing suitable non-pathogenic soil organisms, our cultivation system might be simplified and made less expensive. As seen in the tomato results, the ability to add organic fertilizer directly to the hydroponic solution is very convenient.

CONCLUSION

Bark compost, field soil, nursery soil, and sea water are appropriate sources of microorganisms to generate nitrate from organic fertilizer in water. About 5 g/L of bark compost inoculum is needed to mineralize organic fertilizer into nitrate; the addition of too little inoculum will prevent sufficient nitrate production. Nitrate was generated only in containers that received 0.5 g/L of fish-based soluble fertilizer daily for seven days; application rates of 2.5 g/L daily or higher for seven days produced only ammonium. The culture solution of microorganisms could be used as the hydroponic solution in our organic hydroponics system. The yield and quality of butterhead lettuce and tomato fruits were not significantly different from those in the conventional hydroponics system. Our method, which adds organic fertilizer directly into the hydroponic solution during cultivation, is convenient and practical. Unlike in previous studies of organic hydroponics, the organic fertilizer does not require pre-processing before it can be incorporated in the system.

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