Study on the Hydroponic Culture of Lettuce with Microbially Degraded Solid Food Waste as a Nitrate Source

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Abstract
It is important to use food waste as organic fertilizer because considerable energy is needed to produce inorganic fertilizer, while most food waste is burned and landfilled. Recently, a groundbreaking method, organic hydroponics, has been developed, which enables the cultivation of vegetables by directly adding organic fertilizers to the hydroponic solution. Organic fertilizer is degraded to mineral nutrients by a microbial method, multiple parallel mineralization, involving concurrent ammonification and nitrification reactions. Previous studies have shown vegetables thriving when raised on liquid organic fertilizers via organic hydroponics. However, cultivation using solid organic fertilizers has yet to be examined. In this study, we examined the feasibility of using solid food waste generated from a shopping center as a fertilizer for organic hydroponics. We confirmed nitrate ion generation from the food waste by multiple parallel mineralization. Using solid organic matter and the anaerobic conditions at the bottom of a rectangular container might stimulate denitrification. We improved the retrieval rate of inorganic nitrogen ions by filtrating the solution with a nonwoven fabric bag to remove undegraded organic matter and successfully cultivated butterhead lettuce using the food waste as fertilizer in the hydroponics. The results provided a methodological basis for a new effective means of recycling food waste.

Discipline: Soils, fertilizers and plant nutrition
Additional key words: biofilm, filtration, microbial ecosystem, multiple parallel mineralization, nitrogen fertilizer

Introduction

In conventional hydroponics methods, organic fertilizer cannot be used because organic matter should be phytotoxic and inhibit crop growth. The use of organic fertilizer in hydroponics would therefore be groundbreaking. Many previous attempts were made to use organic fertilizer for hydroponics, in which one or more reaction tanks were used for ammonification and nitrification before utilizing the solution as a hydroponic solution, since residual organic matter in the hydroponic solution was regarded as a phytotoxic factor for plant growth.

Conversely, the new organic hydroponics method allows organic fertilizer to be added to the hydroponic solution tank during the cultivation of vegetables. The hydroponic solution is made by a method called “multiple parallel mineralization”, which consists of two sequential microbial processes; ammonification and nitrification. The directly-added organic fertilizer can be degraded to inorganic nutrients in the hydroponic solution and has no harmful effects on vegetable growth.

Pollution due to the inadequate disposal of organic waste has become a significant issue in both developed and developing countries. It is also important to use food...
waste in agriculture efficiently, given, for example, that about 20 million tons of food waste was discarded in 2008 in Japan, but only about 5 million tons was recycled; mainly as compost and animal feed. In developing countries, despite the growing need for waste management systems following the concentration of population in urban areas and the development of industry accompanying economic growth, it is difficult to build many expensive incinerator plants immediately. We can expect to get organic fertilizer inexpensively from unused food waste. Organic hydroponics is also an anticipated low-cost method, which involves utilizing organic waste as fertilizer.

Previous studies succeeded in vegetable cultivation using two kinds of liquid organic fertilizers, fish-based soluble fertilizer, which is a by-product of bonito fish flake production, and corn steep liquor, which is that of cornstarch, as fertilizers in organic hydroponics. The yield was comparable to conventional inorganic hydroponics.

However, vegetable cultivation using solid organic fertilizer has yet to be examined, while the degradation of solid organic waste such as food scraps, crop residue and animal waste have not been examined sufficiently.

In this study we examined means of developing a method of organic hydroponics using solid food waste generated from a shopping center as nitrogen fertilizer. To do so, we examined how to develop an effective mineralization method using solid organic waste to cultivate lettuce.

Materials and Methods

1. Material and analytical method

Food waste generated from a shopping center was used as a fertilizer. It was milled and dried by vacuum heating in the shop and then ground to below 1 mm in diameter in the laboratory. It consisted of about 1/3 each of fish, vegetables and other leftovers and included 5.8% nitrogen by weight, with a carbon to nitrogen (C/N) ratio of 8.2 (Table 1).

In all subsequent analyses, we used a simplified reflectance spectrometer RQ-flex Plus Analyzer (Merck, Frankfurt, Germany) to determine the concentrations of ammonium, nitrite and nitrate ions. We estimated the efficiency of inorganic nitrogen recovery, calculated as the sum of ammonia-N, nitrite-N and nitrate-N in nutrient solution, divided by the nitrogen content of the added food waste.

2. The effect of filtration on the nitrate generation efficiency

We soaked 50 g of the compost (“Golden bark”, Shizukuoka city, Shizuoka prefecture, Japan) contained in a nonwoven fabric bag (“Toshanse”, Seiketsu network Co., Ltd, Osaka, Japan) as a microbial inoculum to two kinds of plastic containers; one of which rectangular and 64 cm long, 23 cm wide and 18.5 cm high (n = 6), and the other cylindrical; 28.3 cm high, 30 cm in diameter (n = 6) and filled with 10 L of tap water. The solution was aerated with an aeration pump (“Nisso innoβ-4000”, Marukan Co., Kusakabe, Saitama prefecture, Japan). The food waste was added to the solution at 1.0 g L⁻¹ day⁻¹ for 4 days from the start of the experiment. The compost bag was removed, when nitrite ions were first detected as exceeding 0.5 mg NO₂⁻ L⁻¹. In some cases, precipitation was removed by filtration using a nonwoven fabric bag (“Toshanse”, Seiketsu network Co., Ltd, Osaka, Japan) (n = 3 per treatment) at the same time. All the experiments were performed at 25°C. The dry weight of the removed precipitation was determined by drying at 60°C for exceeding 48 h.

3. Lettuce cultivation

The butterhead lettuce cultivar ‘Saradana’ (Lactuca sativa var. capitata, Atariya Noen, Co., Ltd, Chiba, Japan) was grown in an organic hydroponic system in a glasshouse at Nagoya, Aichi prefecture, Japan from October 12 to November 9, 2011. To prepare the hydroponic solution, we added food waste at 1.0 g L⁻¹ day⁻¹ for 4 days from the start of the experiment to a 1/5000 a Wagner pot filled with 3 L of tap water, containing 15 g of the bark compost contained in a nonwoven fabric bag; the hydroponic solution was aerated with the aeration pump (n = 6). When nitrite ions were first detected 13 days after first starting to prepare the solution, the compost bag of all pots was removed and the filtration procedure was

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>10.54</td>
</tr>
<tr>
<td>Total N</td>
<td>5.78</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>2.18</td>
</tr>
<tr>
<td>K₂O</td>
<td>1.17</td>
</tr>
<tr>
<td>CaO</td>
<td>2.53</td>
</tr>
<tr>
<td>MgO</td>
<td>0.17</td>
</tr>
<tr>
<td>Total C</td>
<td>47.16</td>
</tr>
<tr>
<td>C/N</td>
<td>8.16</td>
</tr>
</tbody>
</table>
conducted on 3 pots at the same time. It took 64 days to complete the nitrification when the concentration of nitrate ion stabilized. Both filtered and unfiltered solutions were diluted to about 41 mg N L\(^{-1}\) of total inorganic nitrogen with tap water. Lettuce seedlings were grown with vermiculite without fertilizers for 14 days. Subsequently, the roots of these seedlings were washed with tap water, and 3 heads of the seedlings were planted in a polystyrene foam board on the pot filled with 3 L of the solution with or without filtration (n = 3 replicates per treatment). A total of 0.59 g plant\(^{-1}\) (34 mg N plant\(^{-1}\)) of food waste was added for 28 days. For the first 12 days, 0.017 g plant\(^{-1}\) day\(^{-1}\) (1 mg N plant\(^{-1}\) day\(^{-1}\)) of food waste began to be added 5 days after transplanting, followed by adding 0.035 g plant\(^{-1}\) day\(^{-1}\) (2 mg N plant\(^{-1}\) day\(^{-1}\)) until harvesting. Fertilization was conducted 10 times (3 times per week like the arrows in Fig. 2) and 3 times fertilization supplied the weekly amount. We also added plant ash (JOY AGRIS Co., Ltd, Tokyo, Japan), suspended containing 2.1 mol kg\(^{-1}\) citric-soluble K and 1.9 mol kg\(^{-1}\) citric-soluble P as potassium and phosphate supplements, at 0.4 g per 1.0 g of food waste. Moreover we added 10 g plant\(^{-1}\) of oyster shell lime (Urase Industry Co., Ltd, Fukuyama, Hiroshima, Japan) suspended to provide the following nutrients: 1.74 mmol Mg, 0.061 mmol Fe, 0.207 mmol B, 0.055 mmol Mn, 0.014 mmol Zn, 0.003 mmol Cu and 85.9 mmol Ca. The filtration procedure was not conducted during the cultivation of lettuce. As a control, conventional inorganic hydroponics containing the same amount of nitrogen as in the organic hydroponics was conducted with inorganic fertilizer initially containing 0.23 g L\(^{-1}\) (23 mg N plant\(^{-1}\)) of Otsuka House\(^{TM}\) No. 1 and 0.16 g L\(^{-1}\) (17 mg N plant\(^{-1}\)) of Otsuka House \(^{TM}\) No. 2 (Otsuka Agri Techno Co., Ltd, Osaka, Japan) and additional fertilizer containing 0.20 g plant\(^{-1}\) (20 mg N plant\(^{-1}\)) of Otsuka House \(^{TM}\) No. 1 and 0.13 g plant\(^{-1}\) (14 mg N plant\(^{-1}\)) of Otsuka House \(^{TM}\) No. 2 in total with the same fertilization method as the organic hydroponic system. Those inorganic fertilizers provided the following nutrients in total per plant: 5.35 mmol (75 mg) N, 0.48 mmol P, 2.45 mmol K, 0.43 mmol Mg, 0.014 mmol Fe, 0.012 mmol B, 0.006 mmol Mn and 1.18 mmol Ca. Tap water was replenished in the pot at the time of fertilization. The dry weight of the lettuce was determined by drying at 60°C for exceeding 48 h.

**Results**

1. **The effect of filtration on the nitrate generation efficiency**

   The precipitation in the containers was removed from the rectangular and cylindrical containers by the filtration procedure on the 13th and 15th days, respectively, when nitrite ions were first detected. The precipitate was 7.7\(\pm\)0.2 g dry weight for the rectangular container, and 7.6\(\pm\)1.4 g dry weight for the cylindrical container, respectively, both of which amounted to 19% of the added food waste.

   The nitrate ion concentration with the rectangular container peaked on the 64th day with filtration (Fig. 1(a)) and on the 48th day without (Fig. 1(b)). In the two rectangular containers without filtration, the nitrate concentration and the efficiency of inorganic nitrogen recovery were abnormally low (Nos. 5 and 6 in Table 2), observed as a large standard deviation in the nitrate ion concentration (Fig. 1(b)). The efficiency of inorganic nitrogen recovery with filtration was 21.1\(\pm\)2.2% and without filtration 11.1\(\pm\)6.9%. The maximum nitrate ion concentration in a cylindrical container was achieved on the 62nd day with filtration (Fig. 1(c)) and on the 64th day without it (Fig. 1(d)). The efficiency of inorganic nitrogen recovery with filtration was 21.1\(\pm\)2.1%, while without filtration it was 16.2\(\pm\)1.8%.

2. **Lettuce cultivation**

   Ammonium ions disappeared within five days, and the nitrate ion concentration gradually declined from the start of cultivation (Fig. 2). The change in nitrate ion concentration showed a similar decrease for all treatments during the latter cultivation period, while the nitrate ion concentration of conventional hydroponics maintained a higher value than the organic treatments (Fig. 2). The lettuce grew well and was harvested on the 28th day (Fig. 3). The fresh weights of the lettuce shoots were 15.4\(\pm\)4.3 g without filtration, 12.4\(\pm\)3.1 g with filtration, and 16.7\(\pm\)6.1 g with the conventional solution (Table 3). There was no significant difference among these weights, nor any significant differences in the dry shoot and root weights (Table 3).

**Discussion**

Nitrate ions were generated using solid food waste via the organic hydroponic method. In this case, around 2 months is required for nitrate ion generation. Conversely, 2 weeks suffices to complete nitrification in the case of liquid organic fertilizer\(^{1,13}\). Nitrification of solid organic matter seems more difficult than that of a liquid equivalent. To shorten the period, we may need a solubilization process such as acid/alkali treatment and an explosion process as preprocessing.

The efficiency of inorganic nitrogen recovery on certain rectangular containers without filtration procedure was abnormally low (Nos. 5 and 6 in Table 2), and...
the peak concentrations of generated nitrite and nitrate ions were small compared to that of generated ammonium ions (Fig. 1 (b)). Anaerobic conditions and the supply of organic matter as the energy source for denitrifying microbes stimulate denitrification, while anaerobic conditions also stimulate anaerobic ammonia oxidation (anammox)\(^6\). Denitrification and anammox occurred in the inner zone of biofilm and sewage granules\(^6,14\). The decline in nitrite and nitrate ions suggested that denitrification and/or anammox occurred in thick biofilm and precipitation on the bottom of the container (Fig. 1(b)); and was prevented by filtration, due to the elimination of precipitation as a carbon source for denitrification microbes, and the breakup of thick biofilm under anaerobic conditions, unsuitable for denitrification and anammox microbes (Fig. 1(a)).

In the case of nitrification without filtration, the efficiency of inorganic nitrogen recovery was generally impaired when using a rectangular container compared to a cylindrical one (Table 2). This suggests that solid components are prone to lodge in the four corners of the rectangular container, which provide local anaerobic conditions suitable for denitrification and anammox. To generate nitrate ions efficiently, the use of cylindrical containers may be appropriate.

The efficiency of inorganic nitrogen recovery was relatively low, peaking at under 30%, regardless of the container form. In a previous study, the efficiency of a shaken flask using solid organic fertilizer varied from 0 to 91.3% due to the C/N ratio\(^13\). It was thought that organic matter with C/N ratio exceeding > 11 could not generate nitrate due to nitrogen starvation in the organic hydroponics\(^11,13\). The C/N ratio of the food waste in this study was 8.5, meaning the relatively high value around 11 was believed to explain the inefficiency of inorganic nitrogen recovery. To boost efficiency, it may be necessary to mix food waste with lower-C/N ratio organic matter. There may also be a need to reduce the size of food waste.

Fig. 1. Nitrate ion generation using two types of containers
The horizontal axis shows the number of elapsed days, and the vertical axis shows the inorganic nitrogen concentration (a) in a rectangular container with filtration, (b) in a rectangular container without filtration, (c) in a cylindrical container with filtration and (d) in a cylindrical container without filtration. The values are the means and standard deviations (n = 3). The thin arrows represent the addition of food waste, and the thick arrow represents the day of filtration.
waste particles, because small grains have a relatively large surface area, where microorganisms can attack and break down organic matter\(^1\).

The lettuce growth cultured by food waste as a nitrogen source via organic hydroponics was comparable to inorganic hydroponics with the same amount of nitrogen. In the nitrogen balance of inorganic hydroponics, 75 mg N plant\(^{-1}\) was fertilized and 27 mg N plant\(^{-1}\) (mg N L\(^{-1}\) (3 plants with 3 L = 1 plant with 1 L)) remained in the solution, assuming no denitrification occurred, 48 mg N plant\(^{-1}\) was absorbed by the lettuce (Fig. 2). In organic hydroponics, assuming equivalent nitrogen was absorbed, additional food waste as a nitrogen source may not contribute to lettuce growth because the nitrogen amount of 48 mg N plant\(^{-1}\) (mg N L\(^{-1}\)) is nearly equivalent to that of the first cultivation. In organic hydroponics, no inorganic nitrogen was generated from additional food waste and the nitrate ion concentration of conventional hydroponics exceeded that of organic hydroponics treatments during cultivation (Fig. 2). Considering these phenomena, nitrogen of additional food waste remained in organic form, was used for anabolism of microorganisms or lost due to denitrification. However, we believe the time required for nitrification is shorter than before cultivation due to the series of microbial ecosystems suitable for nitrification and already constructed in the container. In the case of liquid organic fertilizer, nitrification will be completed within 3 or 4 days of constructing the microbial ecosystem\(^1\). Future research is required to estimate the time in the case of solid organic fertilizer.

### Table 2. Individual results of nitrification

<table>
<thead>
<tr>
<th>Number</th>
<th>Container type</th>
<th>Filtration procedure</th>
<th>Nitrate concentration (mg N L(^{-1}))</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rectangular</td>
<td>○</td>
<td>46</td>
<td>23.1</td>
</tr>
<tr>
<td>2</td>
<td>Rectangular</td>
<td>○</td>
<td>42</td>
<td>22.1</td>
</tr>
<tr>
<td>3</td>
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<td>42</td>
<td>18.1</td>
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<tr>
<td>4</td>
<td>Rectangular</td>
<td>-</td>
<td>47</td>
<td>20.5</td>
</tr>
<tr>
<td>5</td>
<td>Rectangular</td>
<td>-</td>
<td>19</td>
<td>8.2</td>
</tr>
<tr>
<td>6</td>
<td>Rectangular</td>
<td>-</td>
<td>10</td>
<td>4.4</td>
</tr>
<tr>
<td>7</td>
<td>Cylindrical</td>
<td>○</td>
<td>56</td>
<td>24.0</td>
</tr>
<tr>
<td>8</td>
<td>Cylindrical</td>
<td>○</td>
<td>46</td>
<td>19.9</td>
</tr>
<tr>
<td>9</td>
<td>Cylindrical</td>
<td>○</td>
<td>45</td>
<td>19.3</td>
</tr>
<tr>
<td>10</td>
<td>Cylindrical</td>
<td>-</td>
<td>41</td>
<td>17.8</td>
</tr>
<tr>
<td>11</td>
<td>Cylindrical</td>
<td>-</td>
<td>40</td>
<td>17.1</td>
</tr>
<tr>
<td>12</td>
<td>Cylindrical</td>
<td>-</td>
<td>32</td>
<td>13.7</td>
</tr>
</tbody>
</table>

The efficiency listed is that of inorganic nitrogen recovery.

### Table 3. Yield of lettuce

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh shoot weight (g plants(^{-1}))</th>
<th>Dry shoot weight (g plants(^{-1}))</th>
<th>Dry root weight (g plants(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>With filtration</td>
<td>15.4±4.3</td>
<td>0.88±0.15</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>Without filtration</td>
<td>12.4±3.1</td>
<td>0.74±0.09</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>Conventional</td>
<td>16.7±6.1</td>
<td>0.90±0.09</td>
<td>0.12±0.02</td>
</tr>
</tbody>
</table>

The values of the results represent means and standard deviation (n=3). The weights did not differ significantly by the Steel-Dwass test.

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*Fig. 2. Inorganic nitrogen concentration during cultivation*

The horizontal axis shows the days elapsed since the start of cultivation, while the vertical axis shows the inorganic nitrogen concentration. The values represent the means and standard deviations (n = 3). The arrows represent the addition of fertilizers.*
The yield of lettuce regardless of filtration did not differ significantly from that in the conventional hydroponic system, although the inorganic nitrogen recovery efficiency remained low (Table 3). It seems that sufficient nitrate ions existed for lettuce growth during cultivation (Fig. 3). Based on this result, in the cylindrical container, the filtration procedure seems unnecessary when preparing the nutrient solution to harvest. However, for long-term cultivation, e.g. of tomatoes, a filtration procedure during cultivation may be necessary because of the accumulation of precipitations suitable for denitrification in a container.

Conclusion

In conclusion, the organic hydroponic system using solid food waste showed good nitrate ion generation and lettuce cultivation performance. Generating nitrate ions takes considerable time, about 2 months, compared to liquid organic fertilizers. The efficiency of inorganic nitrogen ion recovery may also tend to decrease when using rectangular containers. We recommend removing precipitates by filtering the solution using nonwoven fabric at the initial nitrite ion generation stage for efficient nitrification. The lettuce yields did not differ significantly from those in the conventional inorganic hydroponic solution. The effective utilization of food waste and other solid organic waste can be accelerated using the method developed in this study.

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