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Recycling of biodiesel fuel wastewater for use as a liquid fertilizer for hydroponics

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Abstract Wastewater is discharged during washing processes in the production of biodiesel fuel (BDF) using alkaline catalysts. It can be recycled as a liquid fertilizer for hydroponics by adding essential components for plant growth. The effects of the liquid fertilizer on plant growth were investigated. Liquid fertilizer containing a smaller amount of the BDF wastewater had a similar effect on plant growth as the standard nutrient solutions. This result reveals that BDF wastewater can be recycled for use as a liquid fertilizer for hydroponics. However, fertilizer with a larger amount of the BDF wastewater showed poor and varied plant growth due to the growth of microorganisms in the contaminated wastewater. Hence, when BDF wastewater becomes contaminated during storage, sterilization is necessary to recycle it as a liquid fertilizer. Moreover, contamination during storage should be avoided for successful recycling.

Keywords Biodiesel fuel · Plant factory · Hydroponics · Liquid fertilizer · Recycling

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Introduction

Biomass is a promising alternative energy source, because it is reusable and eco-friendly. Biodiesel fuel (BDF) produced from edible oil or its waste products and alcohol is one such biomass energy source. BDF can provide solutions not only to energy shortage issues but also to environmental problems, such as air pollution, caused by exhaust gases from diesel engines and water pollution from waste oil. The scheme of the BDF production using alkaline catalysts is shown in Fig. 1. When BDF is produced using alkaline catalysts, such as KOH, a glycerol by-product containing potassium, methanol, and oil is produced in the methanolysis reaction [1]. Moreover, alkaline wastewater (BDF wastewater) containing emulsified oil, glycerol, methanol, and potassium is discharged during a multi-step water washing process [2, 3]. The characteristics of BDF wastewater show variations for those processes: pH (3.3–11.2), chemical oxygen demand (COD, 11–590 g L^{-1}), biochemical oxygen demand (BOD, 1.6–300 g L^{-1}), grease and oil (0.4–22 g L^{-1}), total organic carbon (TOC, 1.7–40 g L^{-1}), and total suspended solids (0.3–8.9 g L^{-1}) [2]. Therefore, BDF wastewater must not be discharged into a drainage system without any treatment. Because raw BDF wastewater contains an extremely low amount of nitrogen (0.0647 g L^{-1}) [4] and has high BOD values [2], BDF wastewater is difficult to be treated by general biological treatment methods that use activated sludge.

In Japan, BDF wastewater is treated as an industrial waste, incinerated together with a glycerol by-product, or used as the dilution water in methane fermentation processes [5]. In Thailand, the treatment cost of BDF wastewater was estimated to be around 128.45–160.00 USD m^{-3} when it is sent to a treatment facility of a wastewater agency [6]. The cost of treatment by incineration in cement industry was estimated to be approximately

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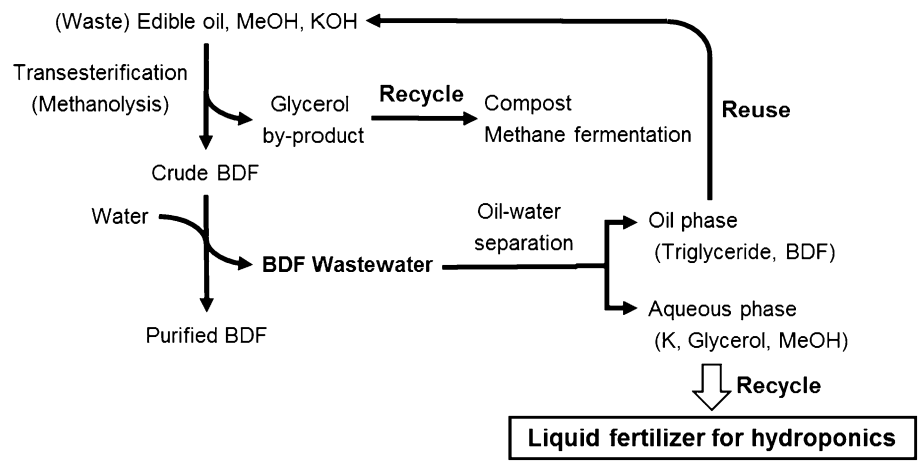

Fig. 1 Schematic view of the zero-emission BDF production process

60 USD m^{-3} [6]. To lower BDF production costs, the BDF by-product and wastewater should either be treated at a low cost or recycled. Wastewater treatment in the biodiesel production with alkali-catalyzed transesterification is summarized in some review articles [2, 3]. Regarding the recycling of the BDF production waste, hydrogen and ethanol production [7], compost fermentation [8], biological sulfate removal [9], and bioconversion to lipids and carotenoids [10] using the glycerol by-product were previously studied. Furthermore, many studies focused on recycling the glycerol by-product as a carbon source for fermentation or a source for bioconversion [11]. There have been some studies on the biological treatment and the recycling of BDF wastewater [2, 4, 12].

Plant factories can constantly produce fruits and vegetables by artificially controlling the environmental conditions, such as light, temperature, humidity, and the concentration of fertilizer and CO_2, regardless of season or location [13]. One of the advantages of plant factories is that the variation in the quality of products is small. Another benefit is that products are edible without the need for washing, because they are pesticide-free and the number of bacteria adhering to products is extremely low. On the other hand, plant factories that use artificial light sources have a disadvantage related to the cost of equipment and electricity charges. Therefore, they prefer to use cheaper liquid fertilizer for hydroponics. Hydroponics is a cultivation method of plants in liquid fertilizer, which nutrients are dissolved in water, without soil. It has been reported that supernatant solutions of jellyfish suspensions were used as a source of liquid fertilizer for ice plants [14].

BDF wastewater, which uses KOH as an alkaline catalyst, can be utilized as a liquid fertilizer, because potassium is essential for the plant growth. The oil phase in the BDF wastewater is reused in another BDF production process, and the aqueous phase is recycled as a liquid fertilizer, thereby a zero-emission BDF production process is achieved (Fig. 1). Advantages of recycling the BDF wastewater as liquid fertilizer are to obtain higher value-added products, such as vegetables, and to overcome the difficulties and costs of the BDF wastewater disposal. The main purpose of this study is to investigate the effectiveness of the recycling of BDF wastewater as a liquid fertilizer on plant growth.

Materials and methods

Preparation of liquid fertilizer from BDF wastewater

Wastewater discharged from the BDF production process was provided by the non-profit organization INE OASA (Kitahiroshima-cho, Japan). Potassium, calcium, and magnesium concentrations in the BDF wastewater were measured using an atomic absorption spectrometer (AA-6200, Shimadzu Corporation, Kyoto, Japan). Oil in the BDF wastewater was separated with 2 M HCl, and then, the pH of this oil-free BDF wastewater was adjusted around 6.0 with 1 M NaOH. Phosphate concentration in the oil-free wastewater was measured using the method of developed in a previous study [15]. Nitrate concentration in the pH-adjusted BDF wastewater was measured with F-kit nitrate (R-Biopharm AG, Darmstadt, Germany). Measured phosphate and nitrate concentrations were converted to those in the raw BDF wastewater.

The schematic flow diagram of preparation of liquid fertilizer from the BDF wastewater is shown in Fig. 2. The BDF wastewater was filtered using a quantitative filter paper (No. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The pH of the filtered BDF wastewater was adjusted to 2.0 by adding 2 M HNO_3. The pH of the solution was measured by a pH meter (F-73, HORIBA, Kyoto, Japan). After standing 5 days for the separation of the oil and water

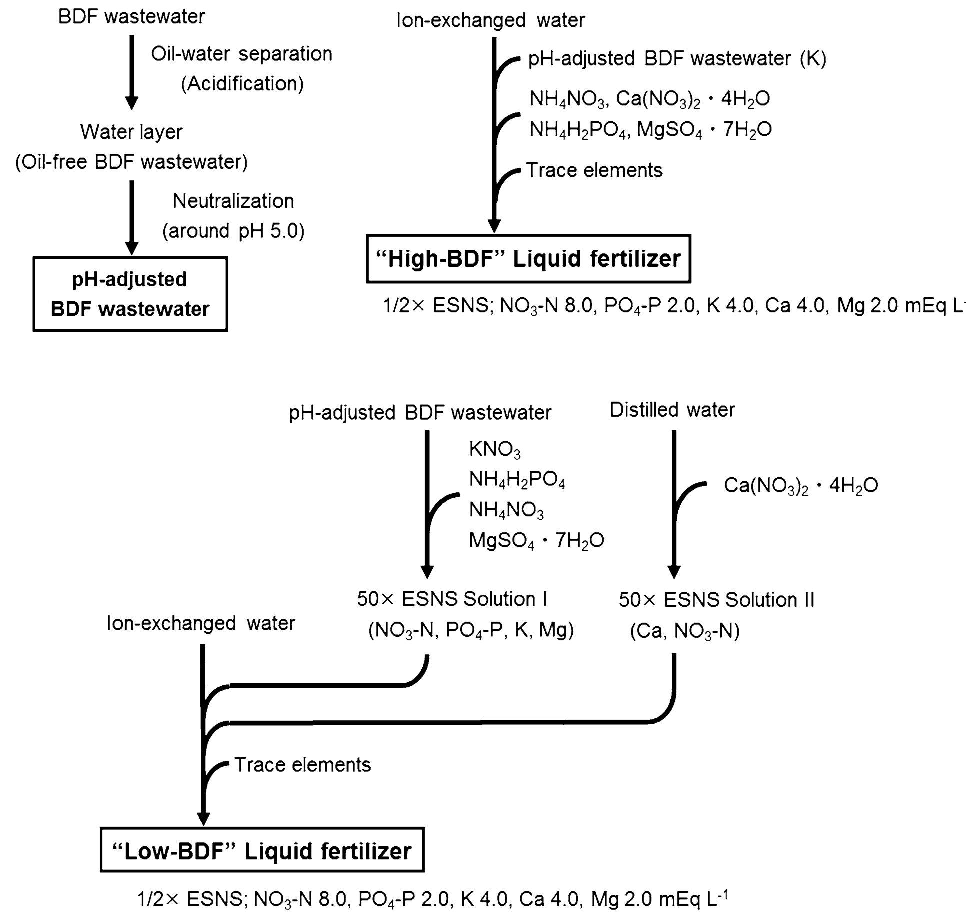

Fig. 2 Schematic flow diagram of the preparation of liquid fertilizer from the BDF wastewater

layers, 1 M KOH was added to the oil-free BDF wastewater to adjust its pH to around 5.0. Potassium nitrate, ammonium dihydrogen phosphate, ammonium nitrate, and magnesium sulfate heptahydrate were then added to the pH-adjusted BDF wastewater; their concentrations followed the 50-fold concentration of the Enshi Standard Nutrient Solution (ESNS, NO_3–N 400, PO_4–P 200, K 400, Mg 200 mEq L^{-1}). Calcium nitrate tetrahydrate was dissolved in distilled water to obtain Ca and NO_3–N concentrations of 400 mEq L^{-1}. Before hydroponics, these solutions were diluted with deionized water. This liquid fertilizer containing a smaller amount of the BDF wastewater was referred to as the low-BDF liquid fertilizer. Potassium and NO_3–N concentrations in the pH-adjusted BDF wastewater were calculated from the amounts of added 1 M KOH and 2 M HNO_3, respectively. The pH-adjusted BDF wastewater was diluted with deionized water to obtain a K concentration of 4.0 mEq L^{-1}. Ammonium dihydrogenphosphate, ammonium nitrate, magnesium sulfate, and calcium nitrate solutions were added to reach half the strength of the ESNS. This liquid fertilizer containing a larger amount of the BDF wastewater was referred to as the high-BDF liquid fertilizer. The amounts of pH-adjusted BDF wastewater added to prepare 1 L of the low-BDF and high-BDF liquid fertilizers were 10 and 207.3 mL on average, respectively. An ESNS prepared with distilled water instead of the BDF wastewater was used as a control. Trace elements containing Fe, Mg, Mn, B, Zn, Cu, and Mo (Tetsuriki Akua F14, Sakata Seed Corporation, Yokohama, Japan) were added to bring 10,000-fold dilution. The pH of the liquid fertilizer was adjusted to around 6.0 by adding 1 M KOH.

To confirm microbial contamination of the wastewater, the liquid fertilizer was shaken with a side-arm shake flask at 23 °C and 120 strokes per minute. Microbial growth was monitored by measuring the optical density of the liquid fertilizers at 600 nm (OD_{600}) using a spectrophotometer (BactoMonitor BACT-500, Intertech Inc., Tokyo, Japan). Microbial growth of the non-sterilized liquid fertilizers was compared with that of liquid fertilizers sterilized with an autoclave at 121 °C for 20 min.

Hydroponics of komatsuna

Komatsuna (*Brassica rapa* var. *perviridis*) (Misaki series, Sakata Seed Corporation, Yokohama, Japan) was used as the plant in this study. Plants were grown at 22.5 °C in 16 h of light and 8 h of darkness with fluorescent lamps in a growth chamber, using an average photosynthetic photon flux density (PPFD) of 106 μmol m^{-2} s^{-1} (MLR-350HT, SANYO, Osaka, Japan). Seeds were sowed into polyurethane foam soaked with tap water in a plastic pot and germinated for 3 days. Seedlings were raised by exchanging tap water for the liquid fertilizer 4–7 days after seeding. The concentration of the liquid fertilizers was 1/2 strength ESNS (NO_3–N 8.0, PO_4–P 2.0, K 4.0, Ca 4.0, Mg 2.0 mEq L^{-1}). Seven days after seeding, the polyurethane foam was planted into an expanded polystyrene board over a polypropylene container (inner dimensions of 119 (W) × 160 (D) × 70 (H) mm and inner volume of 1.6 L). Hydroponics was carried out in 1 L of the liquid fertilizer aerated with an air pump to which a valve was attached to avoid overflow. Deionized water was added to keep the volume of the liquid fertilizer at 1 L. The pH and electrical conductivity of the liquid fertilizer were measured with pH and conductivity meters (LAQUA twin B712 and B771, HORIBA, Kyoto, Japan), respectively. When the pH of the liquid fertilizer exceeded the range of 5.5–6.5, the pH was adjusted to 5.0–7.0 by adding 1 M KOH or 2 M HNO_3.

Twenty-one days after planting, the leaf length, width, and weight of harvested plants were measured. Chlorophyll content (SPAD value) of each leaf was measured with a chlorophyll meter (SPAD-502Plus, KONICA MINOLTA JAPAN, INC., Tokyo, Japan). Leaves were scanned with a scanner (ES-H7200, Seiko Epson Corporation, Suwa, Nagano, Japan) and the leaf area was measured with the ImageJ software version 1.50i [16]. The fresh weight of the above ground part of harvested plants, and the dry weight after drying with a circulation dryer (WFO-600ND, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 75 °C for more than 24 h were measured. Potassium, calcium, magnesium, nitrate, and phosphate concentrations in the liquid fertilizers were measured as described above. Glycerol concentrations in the liquid fertilizers were measured with F-kit glycerol (R-Biopharm AG, Darmstadt, Germany).

Results and discussion

Preparation of liquid fertilizer from BDF wastewater

In a previous study by Suehara et al. [4], physiological properties of the raw BDF wastewater discharged from the same plant as in this study were reported as follows: pH of 11.0, oil content of 15.1 g L^{-1}, carbon content of 14.8 g L^{-1}, nitrogen content of 0.0647 g L^{-1}, carbon to nitrogen (C/N) ratio of 229, and solid content of 2.67 g L^{-1}. Table 1 shows the composition of the BDF wastewater used in this study and typical liquid fertilizers. Although the amount of potassium in the BDF wastewater was comparable with those in liquid fertilizers, the amounts of Ca, Mg, NO_3–N, and PO_4–P in the BDF wastewater were much lower than those in liquid fertilizers. Therefore, it is necessary to add more of these components into the wastewater. As described below, acids and alkalis are required for oil separation and neutralization, respectively. To carry out separating the oil and supplying the insufficient elements simultaneously, both acids and alkalis should consist of those insufficient elements.

Although the BDF wastewater was originally discharged at an alkaline pH [4], its pH value decreased from 8.8 approximately 4.5 months after the discharge (Fig. 3a) to 6.5 approximately 14 months after the discharge (Fig. 3b). This is because carbon dioxide in the atmosphere is absorbed onto the BDF wastewater during storage in a polyethylene tank. Acidification is generally used to separate emulsified oil in BDF wastewater by destroying oil emulsion [2]. Considering their consumption as fertilizer components, nitric acid or phosphoric acid is desirable to decrease the pH of the BDF wastewater. The pH change by the addition of 2 M HNO_3 to 1.0 L of the BDF wastewater is shown in Fig. 3a. Figure 3b shows the change in pH by the addition of 2 M HCl, 2 M HNO_3, or 0.667 M H_3PO_4 to 0.1 L of the BDF wastewater. The initial pH of BDF wastewater did not greatly affect the relative amount of HNO_3 added to the BDF wastewater (triangles in Fig. 3).

Table 1 Composition of BDF wastewater used in this study and liquid fertilizers (mEq L^{-1})

	NO_3–N	PO_4–P	K	Ca	Mg
BDF wastewater	0.1[a]	0.0[b]	6.4[c]	0.4[c]	0.1[c]
Enshi standard nutrient solution (ESNS)	16.0	4.0	8.0	8.0	4.0
Yamazaki nutrient solution (tomato)	7.0	2.0	4.0	3.0	2.0
Yamazaki nutrient solution (lettuce)	6.0	1.5	4.0	2.0	1.0

[a] The pH of oil-free BDF wastewater was adjusted around 6.0 with 1 M NaOH. Measured concentration was converted to that in the raw BDF wastewater

[b] Oil was separated with 2 M HCl. Measured concentration was converted to that in the raw BDF wastewater

[c] Raw BDF wastewater

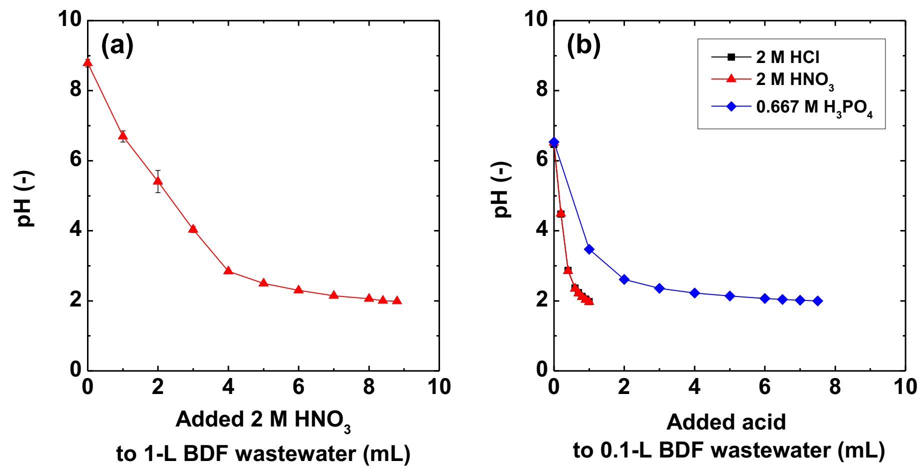

Fig. 3 **a** pH change in 1 L of BDF wastewater by adding 2 M HNO_3 and **b** pH changes in 0.1 L of BDF wastewater by adding 2 M HCl, 2 M HNO_3, and 0.667 M H_3PO_4. *Symbols* and *bars* represent mean and standard deviation of duplicate measurements, respectively

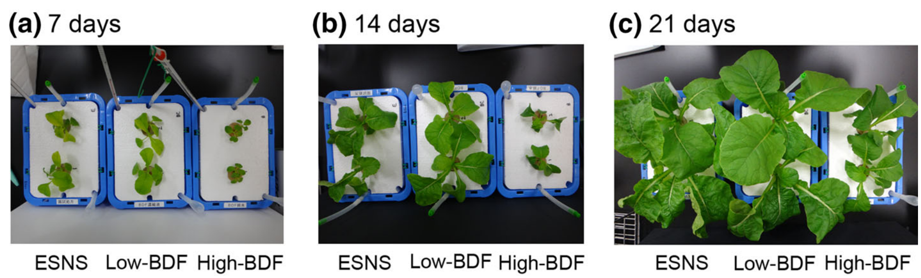

Fig. 4 Photographs of hydroponics **a** 7 days after planting, **b** 14 days after planting, and **c** 21 days after planting

The amount of H_3PO_4 required to adjust the pH to 2.0 was 7.5 times larger than that of HNO_3, whereas the amount of HCl added was similar to that of HNO_3. These results indicate that phosphoric acid can be used instead of nitric acid to decrease the pH of BDF wastewater, because phosphoric acid can be consumed as a fertilizer component and handled more safely than nitric acid.

Among insufficient components, $Ca(OH)_2$ and $Mg(OH)_2$ are not available to increase the pH, because their solubility in water are small. Aqueous K_3PO_4 solution was capable of increasing the pH of the oil-free BDF wastewater (data not shown). Although aqueous KOH solution and ammonia water can be used to increase the pH, it is known that higher NH_4^+ concentrations will inhibit the growth of some plants [17, 18]. In hydroponics, the ability of oxidizing ammonia nitrogen in culture to nitrate nitrogen is small, so nitrate nitrogen is generally used as a nitrogen source [18]. In this study, 2 M HNO_3 and 1 M aqueous KOH solutions were used for acidification and neutralization.

Morphological properties of komatsuna after hydroponics

Figure 4 shows the photographs of the plants during hydroponics. Table 2 presents the morphological properties of the harvested plants. Although komatsuna was able

Table 2 Morphological properties of harvested plants

	ESNS	Low-BDF	High-BDF
Fresh weight of above ground part (g)			
Max	20.95	21.36	11.28
Mean ± SD	15.13 ± 3.86	16.44 ± 4.03	5.41 ± 3.98
Min	10.17	8.29	1.39
Dry weight of above ground part (g)			
Max	1.32	1.46	0.81
Mean ± SD	1.01 ± 0.22	1.11 ± 0.26	0.41 ± 0.26
Min	0.76	0.62	0.12
Root length (mm)			
Max	563.7	525.2	293.2
Mean ± SD	351.5 ± 123.2	406.9 ± 93.2	127.1 ± 94.7
Min	208.5	226.3	Not determined
Stem diameter (mm)			
Max	8.1	7.9	6.7
Mean ± SD	6.2 ± 1.0	5.9 ± 0.9	4.2 ± 1.3
Min	4.7	4.6	2.4
Number of leaves			
Max	10	9	8
Mean ± SD	9 ± 1	8 ± 1	6 ± 1
Min	7	7	5

Two plant heads were cultivated on one board. Hydroponics were carried out four times

Table 3 Morphological properties of third and fourth true leaves containing a leaf stalk

	ESNS	Low-BDF	High-BDF
Fresh weight (g)			
Max	5.77	5.97	3.27
Mean ± SD	3.46 ± 1.00	3.87 ± 1.20	1.37 ± 0.89
Min	1.83	1.76	0.34
Leaf length (mm)			
Max	221.2	216.0	216.5
Mean ± SD	171.7 ± 20.9	182.8 ± 26.3	141.1 ± 43.7
Min	137.0	136.0	80.2
Leaf width (mm)			
Max	114.1	103.8	93.0
Mean ± SD	89.0 ± 12.4	91.1 ± 10.4	60.4 ± 17.5
Min	64.9	65.8	32.4
Leaf area (mm^2)			
Max	13128	12205	9212
Mean ± SD	8136 ± 2064	8842 ± 2142	4394 ± 2382
Min	4711	4622	1326
Average chlorophyll content (SPAD value)			
Max	39.5	44.5	46.2
Mean ± SD	35.8 ± 2.5	35.9 ± 3.8	38.2 ± 5.3
Min	30.6	27.7	27.4

Two plant heads were cultivated on one board. Hydroponics were carried out four times

to grow with all three kinds of liquid fertilizers, plants grown with the high-BDF liquid fertilizer were smaller than those grown with the ESNS and the low-BDF liquid fertilizer. The weight of above ground parts of plants grown with the ESNS and the low-BDF liquid fertilizer were 2.8–3.0 times heavier than those grown with the high-BDF liquid fertilizer. Plants grown with the ESNS and the low-BDF liquid fertilizer had a similar size and weight. Plants grown with the high-BDF liquid fertilizer showed growth variations. Table 3 presents the morphological properties of the third and fourth true leaves of harvested plants. True leaves of plants grown with the ESNS and the low-BDF liquid fertilizer were 1.9–2.0 times larger and 2.5–2.8 times heavier than those grown with the high-BDF liquid fertilizer. However, the chlorophyll contents in the third and fourth true leaves were similar for all liquid fertilizers used in this study. These results indicate that a large amount of BDF wastewater in the liquid fertilizers affected the plant growth, whereas BDF wastewater did not affect the chlorophyll biosynthesis.

Properties of the liquid fertilizers

Figure 5a, b show pH and electrical conductivity (EC) of the liquid fertilizers during cultivation, respectively. All

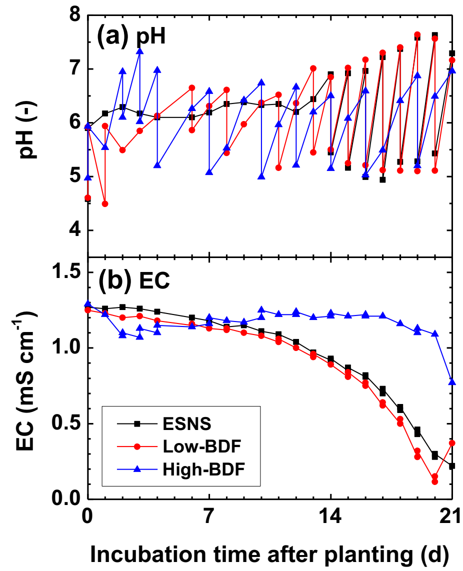

Fig. 5 **a** pH and **b** electrical conductivity (EC) of liquid fertilizers during hydroponics

data obtained from the four cultivations are given in Online Resource 1. When the BDF wastewater was included in the liquid fertilizer, the pH started fluctuating immediately after planting, and then rapidly increased 10 days after planting and onwards. In contrast, the pH of the ESNS varied gradually between 5.5 and 6.5 until the 14th day of planting, after which it changed rapidly. The EC of the high-BDF liquid fertilizer decreased slightly until the third day, and remained almost constant until the 20th day of planting. In contrast, EC values of the low-BDF liquid fertilizer and the ESNS gradually decreased at a similar rate during hydroponics. The variation in EC of the high-BDF liquid fertilizer is shown in Online Resource 1. To examine the differences of pH and EC variability in liquid fertilizers, the components of the fertilizers were measured. Figure 6 shows EC, NO_3–N, PO_4–P, K, Ca, and Mg concentrations in liquid fertilizers during hydroponics. Although EC of the liquid fertilizers was similar on the seventh day of planting, the concentrations of each component were different. In particular, NO_3–N, PO_4–P, and Ca concentrations of the high-BDF liquid fertilizer on the seventh day were lower than those of the ESNS and the low-BDF liquid fertilizer. On the other hand, K concentrations of the high-BDF liquid fertilizer on the seventh day were higher than those of the ESNS and the low-BDF liquid fertilizer. These results indicate that the BDF wastewater affected the pH values of the liquid fertilizer and that the immediate pH change of the high-BDF liquid fertilizer after planting was due to the consumption of NO_3–N and PO_4–P.

The high-BDF liquid fertilizer became turbid and markedly foamed around the seventh day of planting.

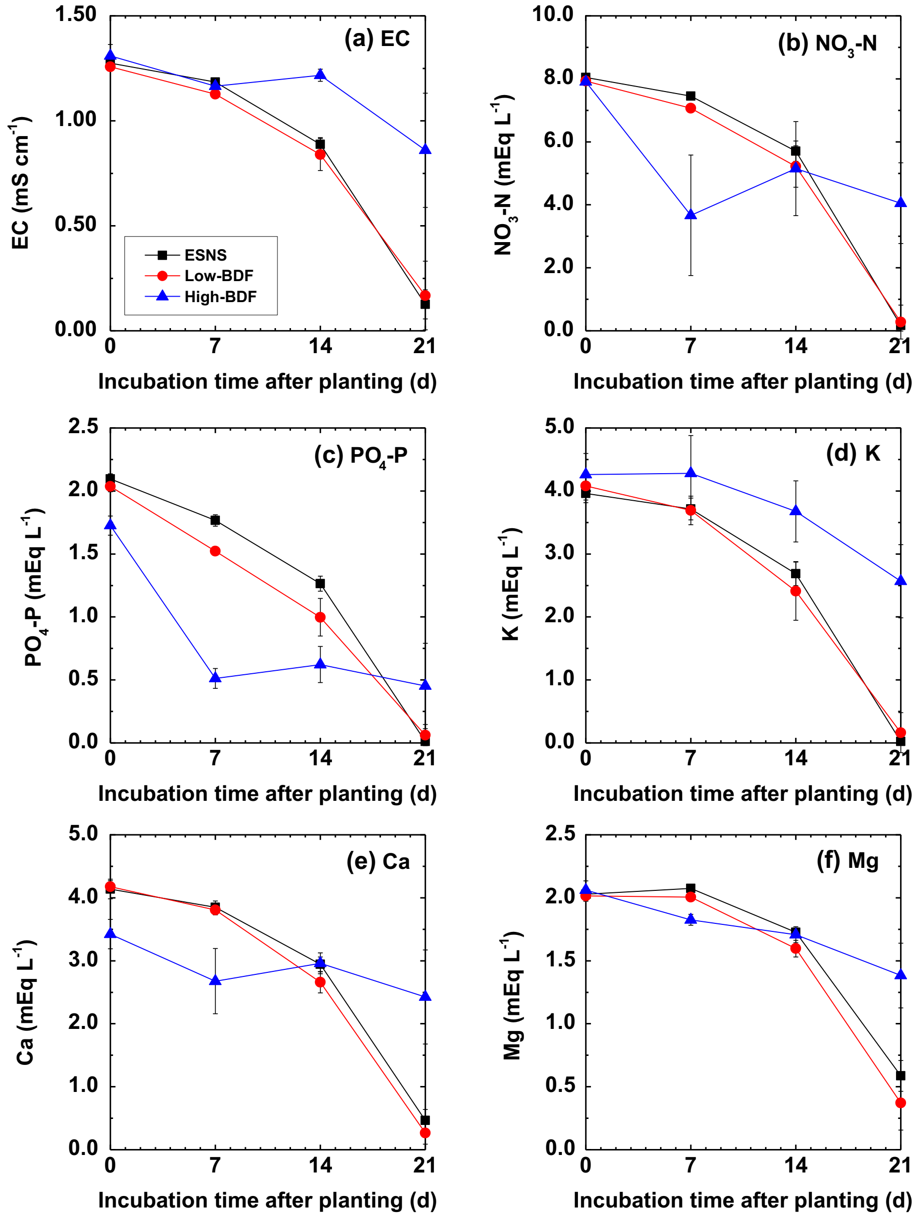

Fig. 6 **a** Electrical conductivity EC, **b** NO_3–N, **c** PO_4–P, **d** K, **e** Ca, and **f** Mg of liquid fertilizers during hydroponics. *Symbols* and *bars* represent mean and standard deviation of four cultivations, respectively

Figure 7 shows the effect of the sterilization of liquid fertilizers. The OD_{600} of the non-sterilized liquid fertilizers increased drastically as the amount of BDF wastewater was increased (open symbols in Fig. 7). On the other hand, the OD_{600} of the sterilized liquid fertilizers hardly changed regardless of the amount of BDF wastewater (closed symbols in Fig. 7). It has been reported that *Rhodotolura mucilaginosa* was able to grow in the nutrient-supplied BDF wastewater [4]. These results suggest that the BDF wastewater used in this study was contaminated with microorganisms that grew due to the addition of nitrogen and phosphorus and aeration.

Table 4 shows the glycerol concentrations in liquid fertilizers containing BDF wastewater during hydroponics. The high-BDF liquid fertilizer contained a larger amount of glycerol (0.475 g L^{-1}) than the low-BDF liquid fertilizer (0.022 g L^{-1}). Glycerol in both liquid fertilizers was almost consumed as a carbon source by the seventh day. Although microorganisms grew even in the low-BDF liquid fertilizer (Fig. 7), marked foaming was not observed during hydroponics, which suggests that the low-BDF liquid fertilizer did not affect plant growth. However, even if the small amounts of glycerol, NO_3–N, and PO_4–P were consumed (circles in Fig. 6b, c), the pH in the low-BDF liquid fertilizer would be immediately affected (circles in Fig. 5a). The plant growth was inhibited, because the limited amount of aeration necessary to avoid foaming due to microbial growth was insufficient for plant growth. These results suggest that (a) BDF wastewater changed the pH of the liquid fertilizer due to the consumption of NO_3–

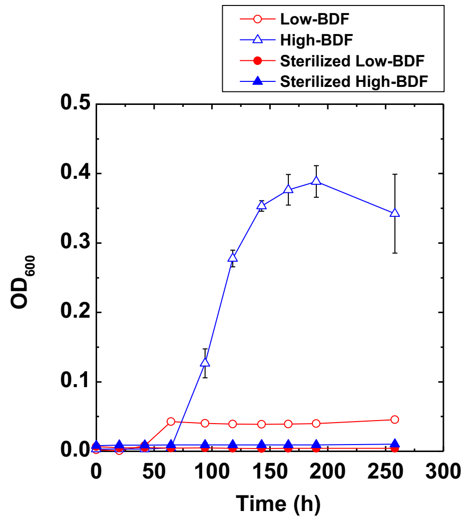

Fig. 7 Effect of the sterilization of liquid fertilizers. *Symbols* and *bars* represent mean and standard deviation of three measurements, respectively

Table 4 Glycerol concentration in the liquid fertilizers containing BDF wastewater during hydroponics

Incubation time after planting (days)	Low-BDF, mean ± SD ($g\ L^{-1}$)	High-BDF, mean ± SD ($g\ L^{-1}$)
0	0.022 ± 0.000	0.475 ± 0.014
7	<0.0004*	0.006 ± 0.002
14	Not tested	0.006 ± 0.001

Hydroponics were carried out four times

* Values below the detection limit

N and PO_4–P by microorganisms in the contaminated wastewater, and (b) a higher amount of BDF wastewater in the liquid fertilizer affected the amount of aeration due to microbial growth.

The carbon to nitrogen (C/N) ratio in the culture medium is an important factor affecting the growth of microorganisms. The high-BDF liquid fertilizer became turbid, probably because the C/N ratio of the high-BDF liquid fertilizer was more suitable for microbial growth than that of the low-BDF liquid fertilizer. *R. mucilaginosa* HCU-1 could grow using glycerol as carbon source and ammonia nitrogen [19, 20]. However, it could not grow using methanol as a carbon source (unpublished data) or nitrate nitrogen [21]. In a previous study, the ratio of glycerol by-product to nitrogen (G/N) was used as an indicator instead of the C/N ratio in the compost fermentation that uses the glycerol by-product [8]. Similarly, the ratio of carbon from the glycerol to ammonia nitrogen (gly–C/NH_4–N) can be used as an indicator to predict whether microbial growth can occur or not, in other words, sterilization of a liquid fertilizer is needed or not. Using the amount of added ammonium salts, the concentrations of NH_4–N in the low- and high-BDF liquid fertilizers were calculated to be 0.68 and 1.0 mEq L^{-1}, respectively. The difference was derived from the amount of added ammonium nitrate to adjust the NO_3–N concentration of the high-BDF liquid fertilizer. Using the calculated NH_4–N concentration, the gly–C/NH_4–N ratios of low- and high-BDF liquid fertilizers were estimated to be 0.9 and 12.7, respectively. The gly–C/NH_4–N ratio of the high-BDF liquid fertilizer is comparable with C/N ratios of the previous studies [4, 19, 20] (unpublished data), which suggests that the high-BDF liquid fertilizer is more preferable to grow microorganisms than the low-BDF liquid fertilizer. The oleaginous yeast *Rhodotolura glutinis* can also utilize nitrate nitrogen [21]. *R. glutinis* can grow in the BDF wastewater using glycerol as a carbon source [10, 22]. The nitrate nitrogen concentration of the high-BDF liquid fertilizer on the seventh day was lower than that of the ESNS and the low-BDF liquid fertilizer. This likely results from the contamination of microorganisms which can utilize nitrate nitrogen, such as *R. glutinis,* in the BDF wastewater. From the concentration of NO_3–N in the low- and high-BDF liquid fertilizers, the gly–C/(NH_4–N + NO_3–N) ratios were estimated to be 0.07 and 1.48, respectively. The gly–C/(NH_4–N + NO_3–N) ratio can be also used as an indicator to predict the need for the sterilization of liquid fertilizers.

Conclusions

BDF wastewater was shown to be suitable for use as a liquid fertilizer for hydroponics. Liquid fertilizer containing a smaller amount of BDF wastewater showed a similar effect on plant growth to the standard nutrient solution. However, a larger amount of BDF wastewater showed poor and varied plant growth due to the presence of microorganisms in the contaminated wastewater. Since the microorganisms also affect the pH of the liquid fertilizer, frequent management of the pH values is clearly necessary. Hence, if there is no pH control when BDF wastewater becomes contaminated during storage, the sterilization of the BDF wastewater is required to recycle it as a liquid fertilizer. Moreover, sterilization is also useful to recycle as much BDF wastewater as possible. The gly–C/NH_4–N and gly–C/(NH_4–N + NO_3–N) ratios can be used as indicators to predict the need for sterilization of the liquid fertilizer. Therefore, avoiding contamination during the storage of BDF wastewater is important to enable its use as a liquid fertilizer.

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