Bottom-to-top continuous irrigation of treated municipal wastewater for effective nitrogen removal and high quality rice for animal feeding

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ABSTRACT

A bench-scale experiment to cultivate rice for animal feeding with continuous irrigation of treated municipal wastewater (TWW) in six different conditions was carried out to examine nitrogen removal from TWW, yield and quality of harvested rice, and accumulation of heavy metals in soil and rice grains. A microbial fuel cell (MFC) system comprising graphite felt electrodes was also installed to generate electricity in the paddy field. The highest rice yield (9.0 ton/ha), dry mass (12.4 ton/ha), and protein content (13.1%), an important nutrient in animal feed, were obtained when a bottom-to-top irrigation (TWW was supplied to the underdrain pipe) was applied at the highest flow rate. The bottom-to-top irrigation achieved 79 to 91% removal of nitrogen in TWW, which was much higher than the top-to-top irrigation (58%). No accumulation of heavy metals was found in the experimental soils, and heavy metal concentrations in brown rice were lower than the allowable levels of current standards. The electric output from the MFC system was much lower than that reported in normal paddy fields, probably due to the poor connection between cables and electrodes. Further study is necessary to improve the electricity generation and to continuously monitor heavy metals in brown rice and the soil.

Key words | bottom-to-top irrigation, nitrogen removal, power generation, rice for animal feed

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ABBREVIATIONS

DO	dissolved oxygen
EC	electrical conductivity
MFCs	microbial fuel cells
MSD	midsummer drainage
SPAD	soil plant analysis development
TN	total nitrogen
TOC	total organic carbon
TWW	treated wastewater
WWTP	wastewater treatment plant

INTRODUCTION

The gap between freshwater demand and supply is widening significantly in many countries as a result of population growth, poor water management practices, and climatic variations (Lazarova *et al.* 2001). The agriculture sector is known to be the largest freshwater consumer with around 70% of the worldwide freshwater withdraw is used for irrigation (UNESCO 2003). As the consumption is predicted to reach approximately 8,515 km^3 per year by 2025 (The United Nations World Water Development 2012), it is essential to satisfy the growing demand for agriculture under increasing population, which requires more intensive water resources management (Gheewala *et al.* 2014). As a means of addressing this issue, municipal wastewater can be used for irrigation (Nyomora 2015). Municipal wastewater has been considered as an alternative resource of water, and the practice of reclaimed wastewater in agriculture is likely to become more commonly applied in many countries

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(UNEP 2005; Chung et al. 2011; Norton et al. 2013). At present, approximately 20 million hectares of arable land worldwide are reported to be irrigated with wastewater by 200 million farmers (Mateo-Sagasta et al. 2013). Municipal wastewater is composed of 99% water and 1% suspended colloidals and dissolved solids, including organic matter and nutrients (N, P, K), inorganic matter or dissolved minerals, toxic chemicals, and pathogens (Hanjra et al. 2012).

The reuse of wastewater in irrigation would potentially contribute to environmental conservation by reducing the direct discharge of pollutants to the natural water environment and the withdrawal of freshwater resources for irrigation, thereby decreasing the pressure on freshwater resources. By using wastewater for irrigation, organic matter and other nutrients can also be supplied, leading to higher crop yields, improved soil fertility and texture, and reduced use of synthetic fertilizers (Jiménez 2006; Mateo-Sagasta et al. 2013). However, wastewater irrigation may have negative effects on human health and on the environment because of its excessive contents of nutrients, pathogens, salts, and heavy metals (Hanjra et al. 2012).

Rice is cultivated in at least 95 countries across the globe, and is the staple food of more than half of the world's current population (Tsukaguchi et al. 2016). There are around 150 million hectares of rice fields worldwide, covering around 9% of the entire arable land on Earth. Of the total rice-harvesting area, 55% is under irrigated rice cultivation and contributes to 75% of the global rice production (IRRI 2002). A massive amount of water is used in rice cultivation because rice fields are flooded before sowing and the water level is normally kept at 4–10 cm in the growing season (Son et al. 2013), using a total of about 1,250 mm of water (Jang et al. 2012). Paddy rice is estimated to consume approximately 50% of the total irrigation water (Tuong & Bouman 2003; Muramatsu et al. 2014), and a reduction of 10% in water used for the irrigated rice would save 150,000 million m^3 of water. However, rice is very sensitive to water regime and attempts to reduce water use in rice cultivation may result in yield reduction and threaten food security (Tuong & Bouman 2003). Furthermore, the reuse of municipal wastewater for rice paddy irrigation can be considered an effective and sustainable way to save water resources only when rice yield is maintained or increased.

Irrigation of treated or untreated wastewater for rice paddy has been extensively practiced and investigated in several countries to evaluate the benefits or drawbacks (Yoon et al. 2001; Trang et al. 2006; Yang et al. 2006; An et al. 2007; Kang et al. 2007; Trang et al. 2007; Li et al. 2009; Papadopoulos et al. 2009; Chung et al. 2011; Rhee et al. 2011; Jang et al. 2013; Mukherjee et al. 2013; Son et al. 2013; Jung et al. 2014; Nyomora 2015). Jang et al. (2012) reported that nutrients and contaminants in wastewater can be removed through absorption by the rice plants and bacterial activities in the soil. In our previous study (Muramatsu et al. 2014), nitrogen removal of 95% from treated municipal wastewater (TWW) was achieved in a rice cultivation system with circulated irrigation with no accumulation of harmful metals in either rice or soil. In a subsequent study, we improved the circulated irrigation system by using a rice cultivar normally fed to animals, instead of that used for human consumption, and achieved not only increased yield of rice but also enhanced nitrogen removal (Muramatsu et al. 2015).

Besides the utilization of nutrients for rice production, another resource, organic matter, can be harvested from irrigated TWW to generate energy by installing microbial fuel cells (MFCs) to the rice cultivation system. MFCs are bioelectrochemical systems that convert chemical energy into electricity using living microbes as electrode catalysts to generate electricity from a variety of organic matter (Kouzuma et al. 2014). MFCs have been considered a promising and sustainable technology for power generation (Liu et al. 2013). Many studies have investigated the use of MFC systems for electric generation from organic matter in chemicals and wastes (Logan et al. 2005; Oh & Logan 2005; Wang et al. 2008; Behera et al. 2010), marine and river bed sediments (Tender et al. 2002; Reimers et al. 2006), wetlands (Ciria et al. 2005; Wang et al. 2012; Liu et al. 2013), and paddy fields within a wide range of scales from laboratory experiments to field practice (De Schamphelaire et al. 2008; Kaku et al. 2008; De Schamphelaire et al. 2010; Arends et al. 2014). The application of the MFCs to our rice cultivation system is based on the expectation that the electric output can be enhanced by using more organic matter for electrogenesis from TWW used in irrigation. Our first trial during the cultivation season in

2014 revealed that the power generated by the system was comparable to that observed in normal paddy fields (Watanabe *et al.* 2017).

In this study, we investigated the possibility of further improvements in the yield and quality of rice as animal feed and in the electric output through continuous irrigation of TWW instead of circulated irrigation. This challenge is supported by the observations in our previous study, which showed that rice yield and its protein content, as indicators of rice quality, could be increased by supplying a larger amount of TWW to the system (Watanabe *et al.* 2017). The aim of this study was to assess the performance of our animal-feeding-rice cultivation system with continuous irrigation of TWW. To this end, a bench-scale experiment was conducted, focusing on the effects of the direction and flow rate of TWW irrigation on nitrogen removal efficiency, yield and quality of harvested rice, and power generation. The accumulation of heavy metals in brown rice and paddy soil were also evaluated, as a negative impact of TWW irrigation, since it could be enhanced by supplying a larger amount of TWW with continuous irrigation.

MATERIALS AND METHODS

Experimental apparatus

The experiment was conducted using a bench-scale apparatus with a simulated 0.18 m^2 paddy field (Figure 1). This apparatus was used in our previous studies (Muramatsu *et al.* 2014; Muramatsu *et al.* 2015; Watanabe *et al.* 2017). At the bottom of the simulated paddy field, an underdrain pipe was equipped to supply water upward, and an overflow pipe was fixed at 20 cm height from the bottom. Six treatments (Runs A to F), without replicates, were applied with different experimental conditions (Table 1). TWW was used as irrigation water in Runs A, B, C, E, and F. In Runs A, B, C, and E, 'bottom-to-top' irrigation was applied, in which TWW continuously flowed through the underdrain pipe and infiltrated the paddy soil layer upward at flow rates of 2.0, 3.0, 3.0, and 4.5 L/day, respectively, and then flowed into the effluent tank. In Run F, where a 'top-to-top' irrigation was performed, TWW was incessantly supplied to the surface of the rice field at the same flow rate

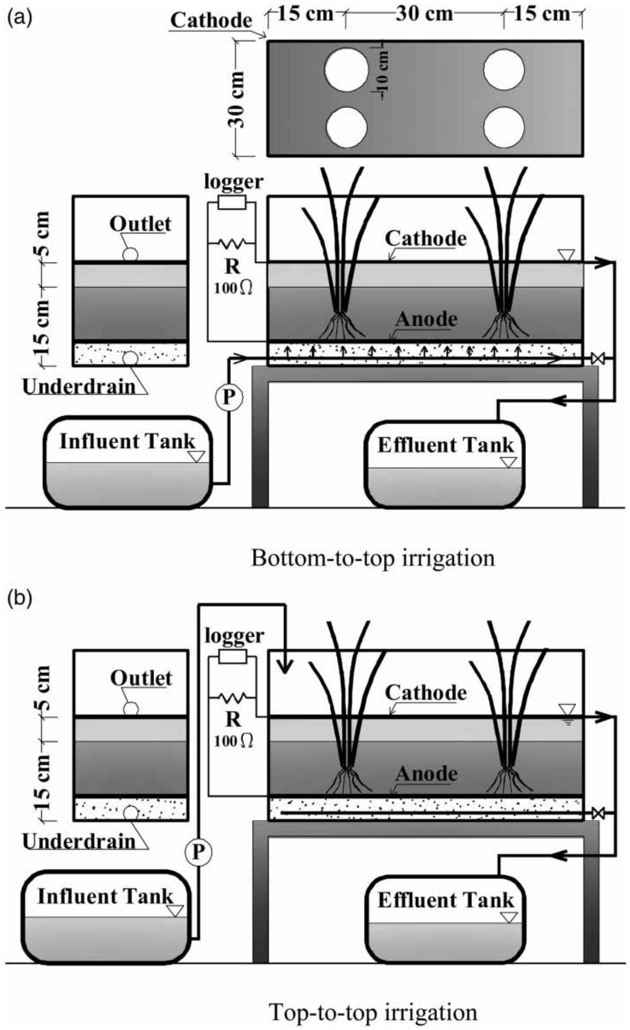

Figure 1 | Simulated paddy fields with different directions of continuous irrigation.

as run E and discharged horizontally from the top at the other side of the field. Run D was a control run, in which the paddy soil supplemented with N-P-K composite fertilizers was irrigated with tap water.

Schedule and conditions of the cultivation

TWW was obtained from a municipal wastewater treatment plant (WWTP) in Tsuruoka, Yamagata, Japan, which employs the standard activated sludge process followed by chlorine disinfection. To determine the fate of nitrogen, the stable isotope of nitrogen (^{15}N) was added to the TWW used for irrigation at 3 atm% of total nitrogen (TN). The soil used

Table 1 | Experimental conditions

Run	Irrigation condition A	B	C	D	E	F
Water		TWW		Tap water	TWW	
Flow rate (L/day)	2.0	3.0		Depend on evaporation	4.5	4.5
Flow direction		Bottom-to-top		No flow	Bottom-to-top	Top-to-top
Water supply		Continuous		As needed	Continuous	
Fertilizer		P		N, P, K (for basal); and N-K (before flowering)	P	
MFC circuit status	Close		Open		Close	

TWW: Treated municipal wastewater.

for the experiment was sampled on April 17, 2015, from the surface layer (0 to 20 cm) of a paddy field in the farm of Yamagata University (Tsuruoka, Yamagata, Japan).

Basal fertilizers were applied before transplantation to supply 160 kg/ha P-fertilizer for Runs A, B, C, E and F; and 160 kg/ha N-P-K fertilizer for Run D. In addition, a top-dressing was applied only for Run D with 100 kg/ha N-K fertilizer before the flowering stage on July 27. Rice seedlings of Bekoaoba, a large grain type high-yield variety, were transplanted at a rate of five plants per hill and four hills per run on May 28, 2015, and it was harvested on September 26, 2015. A water depth of approximately 5 cm was kept throughout the experiment, except in the midsummer drainage (MSD) period from July 27 to August 3, 2015. During the MSD, in order to enhance the rice root growth by supplying oxygen to the root region, the supply of TWW stopped and paddy soil was completely dried by removing all the water via the underdrain pipe.

MFC system

To generate electricity, an MFC system was installed into the simulated paddy field in all the runs. The MFC system, which was constructed using electrodes (0.6 m × 0.3 m) made of carbon graphite felt, was the same as the one used in the study of Watanabe *et al.* (2017). The anode was placed in the soil at approximately 10 cm depth below the soil surface, while the cathode was kept afloat on the water surface by cubic feet of foam. Four holes (10 cm in diameter) were made on the cathode, allowing rice transplantation and growth. Electrodes were connected to a circuit using copper cables and a 100 Ω external resistor, except in Run C as it had an open circuit. The voltage generated from the MFCs system was recorded every 10 min using a hand-type logger (Midi data logger GL220, Graphtec, Japan).

Samples collection and analysis

Samples of irrigated wastewater were collected from the influent and effluent tanks once a week. TN and total organic carbon (TOC) were analyzed in the samples by high-temperature catalytic oxidation using a TOC analyzer (TOC-VCSV, Shimadzu, Japan) attached to a total nitrogen measuring unit (TNM-1, Shimadzu, Japan). Mobile meters (OM-51 and D-54, HORIBA) were also used for on-site measurements of dissolved oxygen (DO), pH, electrical conductivity, temperature, and oxidation-reduction potential (ORP). In addition, components of nitrogen (i.e. nitrate, nitrite, and ammonium) were determined using a colorimeter (DR-890, HACH). Heavy nitrogen was determined in the water samples using the isotope ratio mass spectrometry (Flash EA1112-DELTA V PLUS, Thermo Fisher Scientific).

The yield and dry biomass of the harvested rice were examined in all runs using standard methods. The quality of rice as an animal feed was evaluated based on its protein content, which was derived from its nitrogen content measured using an automatic high-sensitivity NC analyzer (SUMIGRAPH NC-220F, SCAS, Japan). Nitrogen contents in other parts of rice plant and paddy soil were analyzed using the same NC analyzer and the ratio of heavy nitrogen in those samples was measured using an organic elemental analyzer (FLASH 2000, Thermo Scientific).

For heavy metals (Cr, Mn, Ni, Zn, Cu, Mo, Cd, and Pb) determination, water samples were treated with the standard wet-digestion method using nitric acid, while a mixture of nitric and hydrochloric acids was used for samples of brown rice, rice plant, and paddy soil (i.e. solid samples). For arsenic measurement, solid samples were digested using a mixture of nitric and sulfuric acid, whereas water samples were treated using the same method used for the others mentioned metals. The digested solutions were analyzed for the above elements with an inductively coupled plasma mass spectrometer (ICP-MS) (Elan DRC II, PerkinElmer, Japan).

RESULTS

Basic water quality parameters

Basic water quality parameters of the wastewater used for irrigation are illustrated in Figure 2. Influent and effluent water pH values varied from 6.0 to 8.0. As a result of nitrification, the pH in the influent tank gradually decreased with time, and rapidly increased when more TWW was added. A higher value of pH was observed in the effluent tank than in the influent tank in all the runs, probably due to denitrification in the paddy soil. DO of the influent water was around 4.0 mg/L, which was notably lower than the values in the effluent in all the runs. Similar to pH, the DO reached its highest value in run A, while it was the lowest in run F throughout the experiment. The effects of the flow rate and irrigation direction will be discussed in the following section. ORP in the inlet was always higher than those in the outlets in all the runs, which could be attributed to the presence of free chlorine residuals from disinfection process in WWTP. TOC concentrations (Figure 3) in the wastewater used for irrigation varied from 4.7 to 8.0 mg/L in the influent and effluent tanks, showing no dramatic difference.

Removal of nitrogen from treated wastewater

Figure 4(a) illustrates changes in the TN concentration of the irrigation water, which was measured in the influent

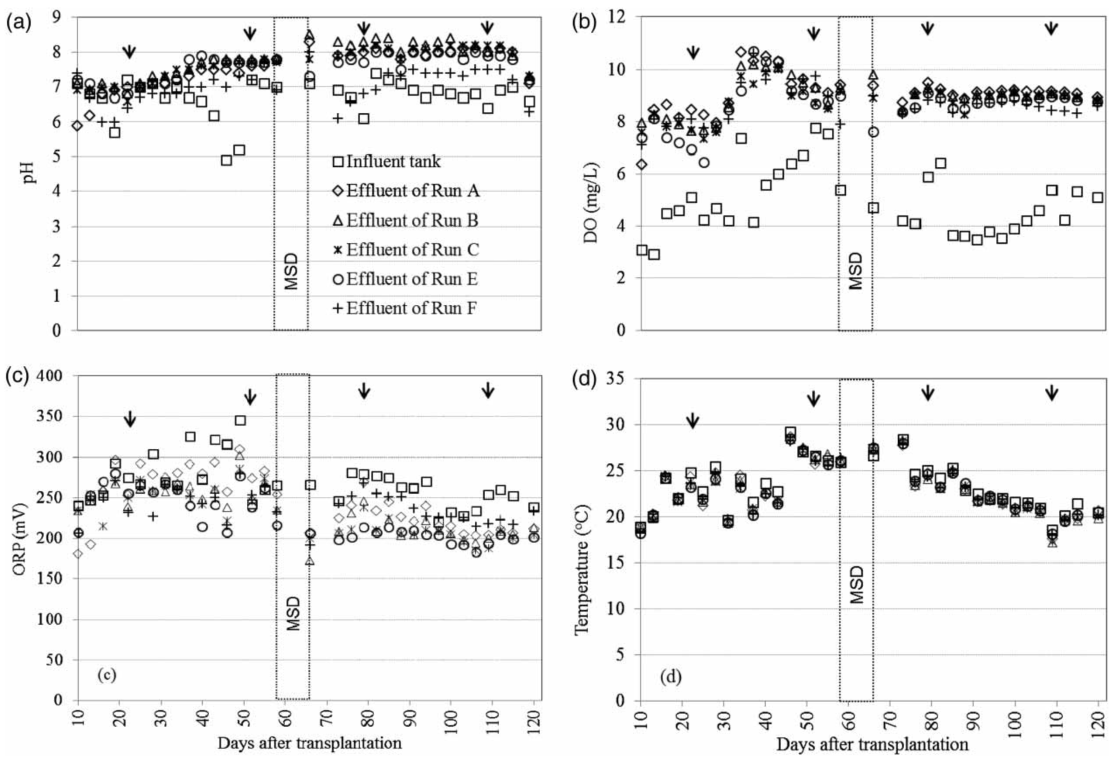

Figure 2 | pH (a), DO (b), ORP (c) and temperature (d) of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

and effluent tanks. The TN concentration in the irrigation water tended to decrease slightly in the influent tank throughout the experiment, except when the tank was refilled with new TWW from WWTP. In the initial stage, TN concentration in the effluents from bottom-to-top runs decreased slightly and reached 8.5–10.7 mg/L on June 8, that is 10 days after transplantation. TN concentrations in the effluents were then gradually decreased to around 3.5 mg/L on July 27, just before MSD practice, in Runs A, B, C, and E, as a result of the huge demand for nitrogen from rice plants for tillering. In Run F, the TN concentration fluctuated between 20.3 and 25.3 mg/L during the first few weeks but then decreased dramatically to a level comparable to that in other runs. After the MSD, as the paddy field was flooded again, the TN concentrations in Runs A, B, C, and E remained at a low level until September 6 – the end of the milk stage, in contrast to the rise to around 18.0 mg/L in Run F. Throughout the experiment, nitrogen removal efficiency ranged from 79 to 91%, and it was clearly higher in the bottom-to-top irrigation than that in the top-to-top irrigation (58%).

The fates of nitrogen removed from the irrigated wastewater, which was calculated by multiplying the mass of removed nitrogen from TWW by proportion of heavy nitrogen to rice plant, soil or atmosphere, are illustrated in Figure 5. In Runs E and F, the largest part of removed nitrogen was emitted into the atmosphere, followed by those was absorbed by rice plants and grains. In contrast, the amount of emitted nitrogen was much smaller in Run A. Bottom-to-top irrigation at a higher flow rate increased the nitrogen emission into the atmosphere, corresponding to a higher efficiency of nitrogen removal from TWW as described above. On the other hand, there was no significant difference in the amount of nitrogen absorbed by rice plants among the runs, and nitrogen remaining in the soil accounted for a very small portion of the total supplied amount, regardless of experimental conditions.

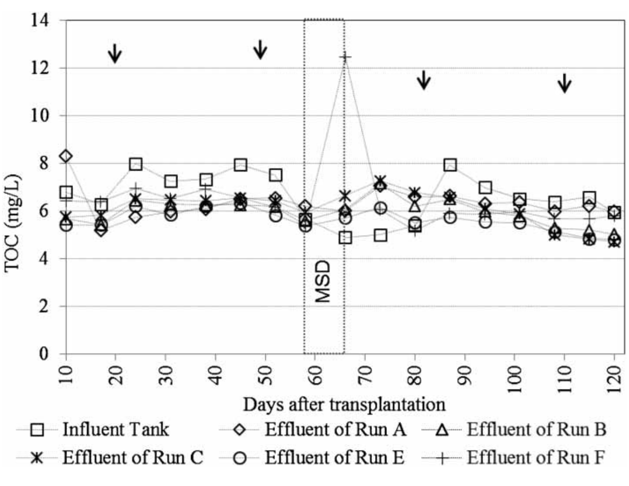

Figure 3 | TOC of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

Protein content, yield of the harvested rice, and amount of dry biomass

Tables 2 and 3 summarize the rice yield, amount of dry biomass, and protein content in the harvested brown rice of all the runs. In general, the application of TWW with bottom-to-top irrigation at a higher flow rate achieved better results in dry biomass, yield, and protein content of brown rice. In Run E, the number of the kernels (73.2 kernels/ear) was less than that in Run A (74.4 kernels/ear). The single-grain weight in Run E (29.5 mg) was also lower than those in Runs A, D and F (30.4, 29.9, and 31.1 mg, respectively). However, Run E had the highest yield of rice (9.0 ton/ha) among all the runs, possibly because it had the highest number of ears. Rice yields in Runs A, C, D and F were higher than in Run B (7.3 ton/ha), although the same irrigation and fertilizer conditions as Run C were applied.

The quality of the rice in terms of protein content was not considerably different among runs. Same as grain yield, the highest protein content belonged to Run E, followed by Runs B and C (12.2%). Runs A and F achieved the same protein content (11.6%), comparable to the control treatment (11.7%).

The cultivar used in this experiment 'the whole plant excluding grains' can be also used as animal feed. For this usage, the dry biomass of the rice plant excluding rice grains was also assessed. As in the cases of the yield and the protein content of the brown rice, Run E showed the highest dry mass (12.1 ton/ha). Runs C and F got the second highest dry mass, followed by Run B (10.7 ton/ha) and Run A (10.6 ton/ha). The lowest rice plant mass belonged to the tap water irrigation (10.4 ton/ha).

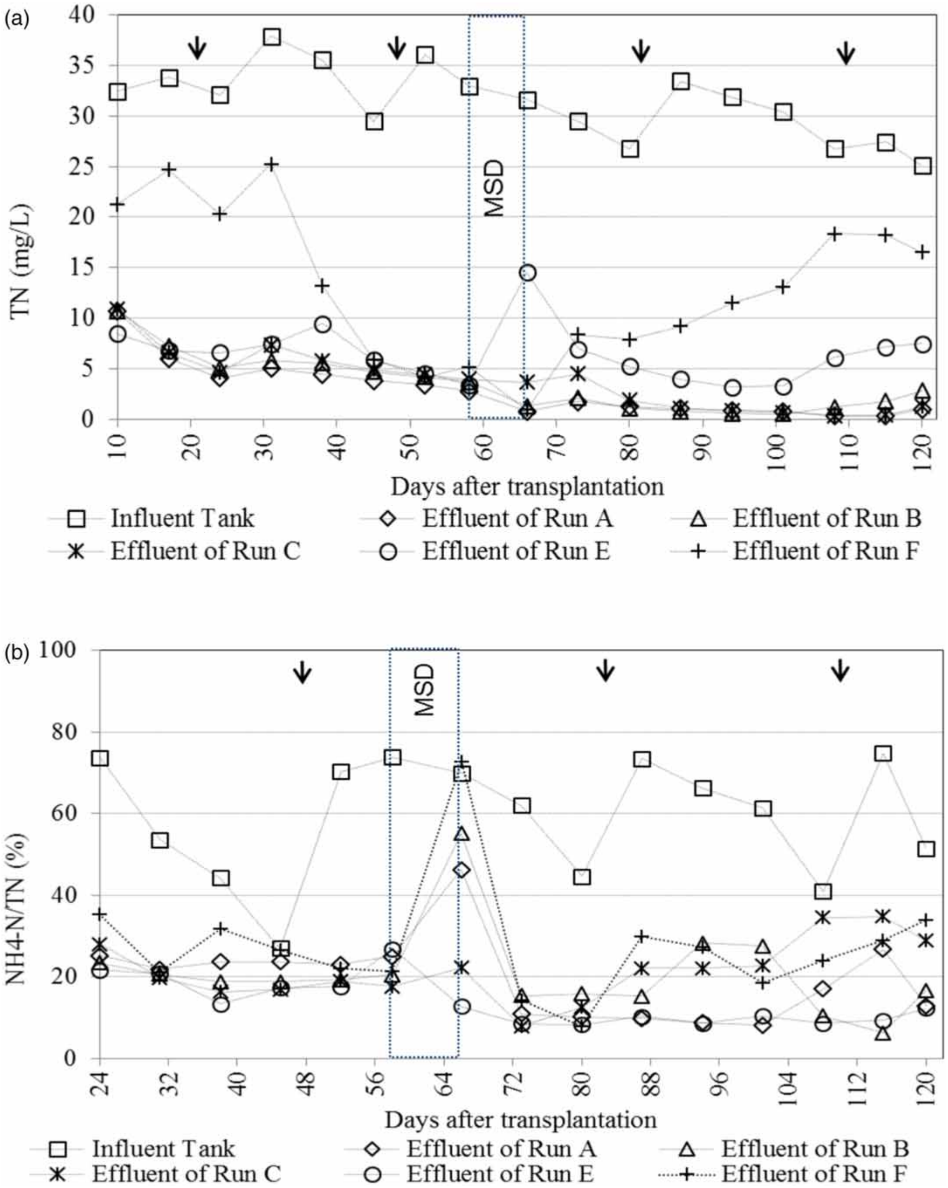

Figure 4 | TN (a) and proportion of ammonium (b) of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

Heavy metals in brown rice and soil

Along with the undeniable benefits, the use of wastewater in agriculture can seriously harm animal, human health, and the environment by transferring contaminants such as heavy metals and pathogens, especially helminths' eggs (Jiménez 2006; Qadir *et al.* 2010; Mateo-Sagasta *et al.* 2013). Rice and soil contamination by heavy metals resulting from municipal wastewater irrigation is a serious concern due to the potential health impacts (Chung *et al.* 2011). We compared heavy metal contents in the soil before and after the experiment (Table 4) and found no metal accumulation, except for copper, in the paddy soils. The significant increase in copper occurred even in the control run, indicating that the accumulation was not from the TWW, but rather from the oxidation of copper cable used in the MFC system. The contents of the heavy metals (Table 5) such as Cu, Cr, Zn, Cd, Pb and As in the harvested brown rice did not

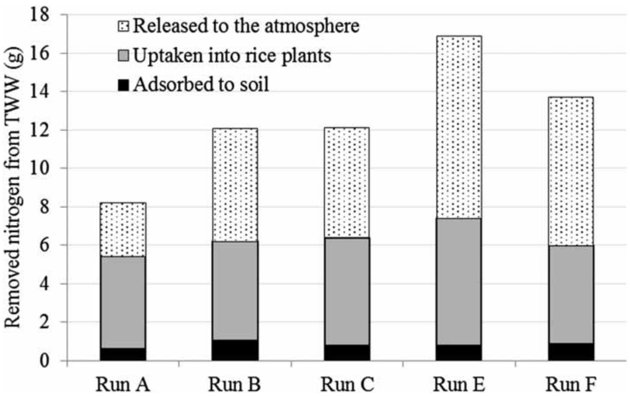

Figure 5 | Fate of nitrogen removed from irrigated wastewater.

show any significant differences between runs, implying no remarkable effects of flow rate, flow direction, or TWW irrigation on the accumulation of heavy metals in rice grains. Cadmium levels in the harvested rice varied from 0.05 to 0.10 mg/kg with the highest value in Runs D and F. Although lead is associated with several health issues even at low concentrations (Batista *et al.* 2012), its concentration in the brown rice was 0.02 mg/kg in Run A and 0.01 mg/kg in the other runs. The concentrations of Cd and Pb in the brown rice were much lower than the safe limits set by FAO/WHO (2004) and EU Communities (2006) in all the runs. However, continuous monitoring of these hazardous materials in brown rice and soil is needed to avoid potential long-term accumulation or accidental high contamination when the same paddy fields are repeatedly used for rice cultivation with TWW.

Electric output

Immediately after the MFC systems were set in the experimental apparatus, an electric output of around 100 mV

Table 2 | Yield components and grain yield

	Ears (/m^2)	Kernels (/ear)	Single-grain weight (mg)	Manured kernels (%)	Yield of rice (ton/ha)
Run A	411	74.4	30.4	88.8	8.3
Run B	428	68.0	28.8	88.2	7.3
Run C	450	68.8	28.9	92.3	8.4
Run D	400	66.1	29.9	90.6	8.3
Run E	472	73.2	29.5	88.7	9.0
Run F	428	71.2	31.1	90.9	8.6

Table 3 | Protein content in brown rice and dry biomass of whole plant

	Dry biomas (ton/ha)	Protein content (%)
Run A	10.6	11.6
Run B	10.7	12.2
Run C	11.2	12.2
Run D	10.4	11.7
Run E	12.4	13.1
Run F	11.2	11.6

was obtained and then it increased to nearly 196 mV within 5 days after transplantation, which is equivalent to 2.1 mW/m^2 of the used power density in Run E. This is comparable to the results reported at the same stage in another study examining MFC system in normal paddy fields (PF-MFC) (Kaku *et al.* 2008), and higher than that obtained in the same apparatus with circulated treated wastewater irrigation (Watanabe *et al.* 2017). However, after a period (from June 13 to 30) when we could not record the data of the electric output because of a trouble in the logger, the measured power density was lower than 1.0 mW/m^2 in all the runs except for a short time in Run A when it was 3.5 mW/m^2. The output almost stopped during the MSD in all the runs and we found that the poor connections between the electrodes and the copper cables which were apparently oxidized. After changing the cables on September 9, the electric outputs in all runs immediately increased to around 100 mV, which were similar to that recorded at the first stage. The electric output in this experiment was much less than those reported in normal PF-MFCs (Kaku *et al.* 2008; Takanezawa *et al.* 2010). In due course of time, poor connection between the cables and the electrodes resulted in a low density of the electric generating bacteria on the anode of the MFC as found in the open circuit system (De Schamphelaire *et al.* 2010). Figure 6 exhibits the highest electric output, which was generated in Run E (around 0.4 mW/m^2), whereas the lowest value (<0.1 mW/m^2) was generated in Run D. This is understandable since wastewater contained much more organic matter, some of which are probably available for power generation, than tap water, and its irrigation at a higher flow rate supplied a larger amount of organic matter. Nevertheless, further studies are necessary for deeper understanding of this phenomenon. As mentioned above, we expected to gain a

Table 4 | Concentrations of heavy metals in soils before and after experiment (mg/kg)

		Soil after experiment					
	Soil before experiment	Run A	Run B	Run C	Run D	Run E	Run F
Cu	22.6	294.2	435.3	142	272.6	203.1	146.8
Cr	20.3	19.8	22.6	21.3	21.8	21.4	21.2
Zn	103.5	113	107.7	119.7	101.8	114.7	106.9
Cd	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Pb	14.9	15.9	16.6	15.8	15.7	16.9	15.4
As	10.7	9.7	10.2	10.3	10.4	9.2	9.5

higher electric output by supplying more organic matter from TWW. However, after September 9 when the MFC circuits were connected again and the electric output stabilized, the TOC in the effluents did not decrease (Figure 3), implying that the electric generation bacteria on the anode of the MFC might not have used the organic matter in the TWW as effectively as those from soil and rice root exudates.

DISCUSSION

Effects of flow rate and irrigation direction on water quality improvement

At the beginning of the experiment, nitrogen removal efficiencies for all the runs were low. This is probably because the bacteria communities were not completely developed yet and the rice plants were not ready for nutrient absorption after the shock of transplantation (Li *et al.* 2016). After the development of the rice root system, the uptake of nitrogen from water was improved. Watanabe *et al.* (2017) reported that the direction of the circulated irrigation did not affect the removal efficiency of nitrogen. However, in the present study, Run F with top-to-top irrigation demonstrated much lower nitrogen removal efficiency than other runs, since the irrigated wastewater did not percolate through the soil layer and nitrogen was not absorbed. This implied that bottom-to-top irrigation enhanced nitrogen removal from irrigated TWW. Among the runs sharing bottom-to-top irrigation, the lower flow rate, which resulted in the longer water retention time, appeared to enhance the bacterial reactions such as nitrification and denitrification in the soil. Our system achieved much higher removal of nitrogen from the wastewater than those reported in normal constructed wetlands (40–50%) (Lee *et al.* 2009).

Rice yield, quality and plant mass improved by continuous bottom-to-top irrigation with TWW

Rice yields obtained in the present work are comparable to the results of Fukushima (2012), in which the same type of rice was cultivated in the same region of Japan. However, these yields were significantly higher than those reported for rice cultivation irrigated with wastewater for human consumption (5.2 to 5.4 ton/ha) (Jung *et al.* 2014; Nyomora

Table 5 | Concentrations of heavy metals (±SD) in brown rice (mg/kg)

	Run A	Run B	Run C	Run D	Run E	Run F	Allowable limit set by FAO/WHO
Cu	4.50 ± 0.23	5.70 ± 0.57	4.80 ± 0.14	7.90 ± 1.38	6.10 ± 0.53	6.50 ± 0.52	NA
Cr	0.04 ± 0.02	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.02	NA
Zn	14.5 ± 2.2	14.0 ± 0.7	13.1 ± 0.6	13.7 ± 0.6	13.8 ± 0.8	12.6 ± 0.9	NA
Cd	0.05 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.10 ± 0.05	0.07 ± 0.01	0.10 ± 0.03	0.40
Pb	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.20
As	0.13 ± 0.00	0.15 ± 0.01	0.19 ± 0.01	0.15 ± 0.05	0.10 ± 0.01	0.11 ± 0.00	NA

NA: Not available.

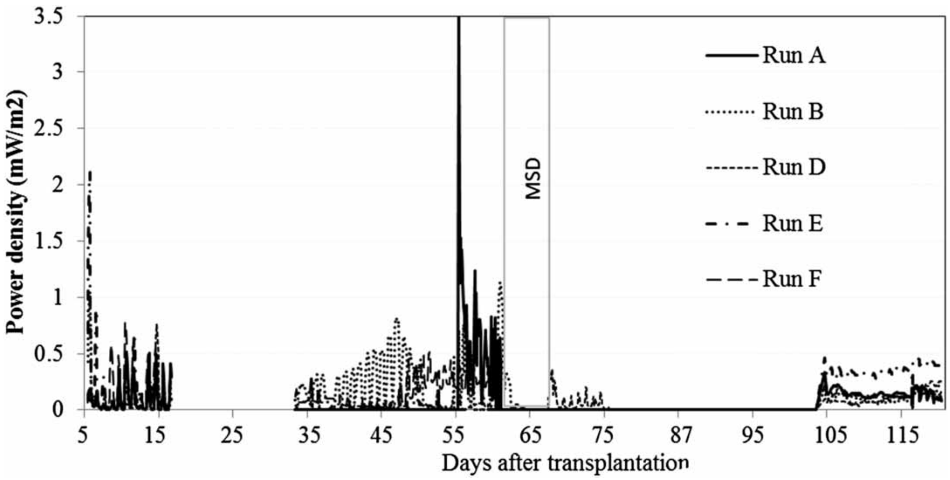

Figure 6 | Power density from the PF-MFCs. MSD means midsummer drainage to dry up the soil layers. F indicates a period from days 16 to 30 after transplantation, which data could not be obtained due to technical difficulties in the logger.

2015). The difference in rice yield between Runs B and C may be attributed not to the power generation in the MFC, but to the much higher content of copper in the soil in Run B (Table 4). Xu *et al.* (2006) reported that a high copper concentration in the soil resulted in a lower rice yield. The yield in Run A, which was irrigated with the smallest amount of TWW, was not lower than those in Runs B and C, because it could use solar energy more efficiently at the edge of the bench, called 'the border effect' (Wang *et al.* 2013).

The protein contents of rice harvested in this experiment were noticeably higher than those obtained in the previous studies (Muramatsu *et al.* 2015; Watanabe *et al.* 2017). These studies cultivated the same cultivar of rice using the same bench-scale apparatus with circulated irrigation of TWW. Therefore, the quality of rice could be significantly improved through continuous irrigation. The highest values of rice quality, rice yield and plant growth found in Run E are rarely reported in normal paddy fields supplied with chemical or organic fertilizers. The rice cultivated with continuous bottom-to-top irrigation at the highest flow rate here seems to have a potentially high market value as a new type of animal feed that can provide both protein and energy. Further improvements may be expected by the increase in the flow rate unless the TN concentration in the effluent reaches an alarming level and/or if lodging of the rice plants occurs.

CONCLUSIONS

Based on the successful results from our previous studies on developing a system to cultivate rice for animal feeding with circulated irrigation with TWW, we applied continuous irrigation to the developed system to improve its nitrogen removal from TWW, production of high-quality rice for animal feeding, and power generations with PF-MFC. The bench-scale experiment including six treatments with different cultivation conditions revealed some interesting findings:

- The continuous irrigation enabled us to supply a larger amount of TWW to the cultivation system and to achieve a higher yield and protein content of rice compared to that achieved with the circulated irrigation. Bottom-to-top irrigation at a higher flow rate contributed to increases in the yield and protein content as well as the amount of dry mass of the whole plant.
- The bottom-to-top irrigation at a lower flow rate enhanced the efficiency of the nitrogen removal from TWW used for irrigation. The TN concentration in the effluent from the paddy fields with bottom-to-top irrigation was less than 10 mg/L throughout the experiment, regardless of the flow rate.
- The electric output from MFC in our cultivation system was so low compared to those reported in normal paddy fields, because of the poor connection between

cables and electrodes in our case. The oxidation of copper cables accelerated by the TWW irrigation might have caused this trouble. To realize the electricity generation using organic matter in the TWW, adjustments to the MFC should be made to tolerate such a severe environment. This is a topic for investigation in future studies.

- A high copper concentration, which must have been released from the oxidized cables, was found in the paddy soil after the experiment. Except for this, no harmful metals were accumulated in the brown rice or the soil by the TWW irrigation. This ensures the safety of the rice harvested in our system using a large amount of TWW by continuous irrigation. Nevertheless, continuous monitoring of heavy metals in the soil and brown rice every season is highly recommended to avoid long-term accumulation or accidental contamination.

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