

Cultivation of rice for animal feed with circulated irrigation of treated municipal wastewater for enhanced nitrogen removal: comparison of cultivation systems feeding irrigation water upward and downward

A. Muramatsu, H. Ito, A. Sasaki, A. Kajihara and T. Watanabe

ABSTRACT

To achieve enhanced nitrogen removal, we modified a cultivation system with circulated irrigation of treated municipal wastewater by using rice for animal feed instead of human consumption. The performance of this modified system was evaluated through a bench-scale experiment by comparing the direction of circulated irrigation (i.e. passing through paddy soil upward and downward). The modified system achieved more than three times higher nitrogen removal (3.2 g) than the system in which rice for human consumption was cultivated. The removal efficiency was higher than 99.5%, regardless of the direction of circulated irrigation. Nitrogen in the treated municipal wastewater was adsorbed by the rice plant in this cultivation system as effectively as chemical fertilizer used in normal paddy fields. Circulated irrigation increased the nitrogen released to the atmosphere, probably due to enhanced denitrification. Neither the circulation of irrigation water nor its direction affected the growth of the rice plant and the yield and quality of harvested rice. The yield of rice harvested in this system did not reach the target value in normal paddy fields. To increase this yield, a larger amount of treated wastewater should be applied to the system, considering the significant amount of nitrogen released to the atmosphere.

Key words | circulated irrigation, nitrogen removal, rice cultivation, rice for animal feed, treated municipal wastewater

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INTRODUCTION

Treated municipal wastewater is recognized as a relatively stable water source alternative to natural sources such as surface water and groundwater. A large volume of reused wastewater is already applied for agricultural irrigation as it usually contains rich nutrients that are beneficial for plant growth (Qadir *et al.* 2010; Norton-Brandao *et al.* 2013). However, produced foods may be contaminated by harmful substances such as heavy metals (Singh *et al.* 2010) and pathogens (Mok & Hamilton 2014) in reused wastewater, thereby posing health risks to consumers. Paddy rice is one of the agricultural products that require considerable water; therefore, reuse of treated wastewater is suitable for its cultivation (Tanaka & Okamoto 2011). As rice plants grow by consuming nutrients in the treated wastewater used for irrigation, the removal of nutrients from the wastewater must be achieved simultaneously (Li

et al. 2009; Jang *et al.* 2012). In particular, nitrogen can be removed by the activities of bacteria (i.e. nitrification and denitrification) in paddy soils.

Although the reuse of untreated or treated wastewater for rice cultivation has been widely investigated (Yoon *et al.* 2001; Chiou 2008; Papadopoulos *et al.* 2009), we recently designed a new cultivation system with circulated irrigation for effective removal of nitrogen from treated wastewater (Muramatsu *et al.* 2014). Through a bench-scale experiment for two farming seasons, we successfully demonstrated the feasibility of the system to remove nitrogen from reused wastewater with efficiency higher than 95% without the accumulation of harmful metals in rice and paddy soil. However, the nitrogen supply based on standards of practice in normal paddy fields seemed too much for this system. This resulted in the overgrowth of the rice

plants, causing their lodging and reducing the eating quality of rice.

To avoid these problems and to improve the system performance for nitrogen removal without these troubles, we propose the use of rice for animal feed instead of human consumption in our cultivation system. Rice cultivars used for animal feed have several advantages, such as higher yield and resistance to lodging than those used for human consumption. Moreover, the high protein content in rice, which results from the adsorption of excess nitrogen, is preferable for animal feed; in contrast, it leads to low eating quality of rice for human consumption. Therefore, a larger amount of fertilizer is usually applied to normal paddy fields in which rice for animal feed is cultivated. This study aimed to assess the improvement in the performance of our system by cultivating rice for use in animal feed, through a bench-scale experiment as performed in our previous study (Muramatsu *et al.* 2014). Furthermore, the present study focuses on the direction of circulated irrigation and compares the performance of a system feeding irrigation water from the top to the bottom of paddy soil with that of a system working in the reverse direction. We expect that, although its scale-up is an additional challenge, our modified system will contribute not only to improving the quality of treated municipal wastewater, but also to promoting water resource and nitrogen circulations among urban dwellers who consume animal products and produce wastewater, rice farmers who produce rice for animal food by reusing treated wastewater, and livestock farmers who use the cultivated rice.

MATERIALS AND METHODS

Experimental apparatus

Figure 1 illustrates the experimental apparatus for rice cultivation with circulated irrigation. The experimental apparatus consisted of a simulated paddy field with area of 0.18 m^2 and a storage tank for irrigation water. The simulated paddy field had an underdrain at the bottom of a 15-cm-thick soil layer. In Run A, the irrigation water was continuously supplied from the surface of the paddy field at a constant rate of $\sim 20 \text{ L/d}$. The irrigation water infiltrated the soil layer and drained from the underdrain ($7\text{--}10 \text{ L/d}$). The drained water and overflowed surface water were stored in the storage tank and then continuously pumped into the paddy field. This is the same system as that used in the previous study (Muramatsu *et al.* 2014). In Run B, the irrigation water in the storage tank was supplied to the underdrain at the same flow rate as in Run A. The irrigation water passed through the soil layer upward, overflowed from the paddy field, and returned to the storage tank. In Run C, which was used as a control, irrigation water was not circulated, as in a normal paddy field, and the loss of surface water due to evapotranspiration was compensated by manually adding water in the storage tank. In all runs, the apparatus was covered by a transparent roof and a plastic sheet to avoid the effect of rainfall. The temperature in the system was continuously measured under the roof and it was almost the same as the ambient temperature.

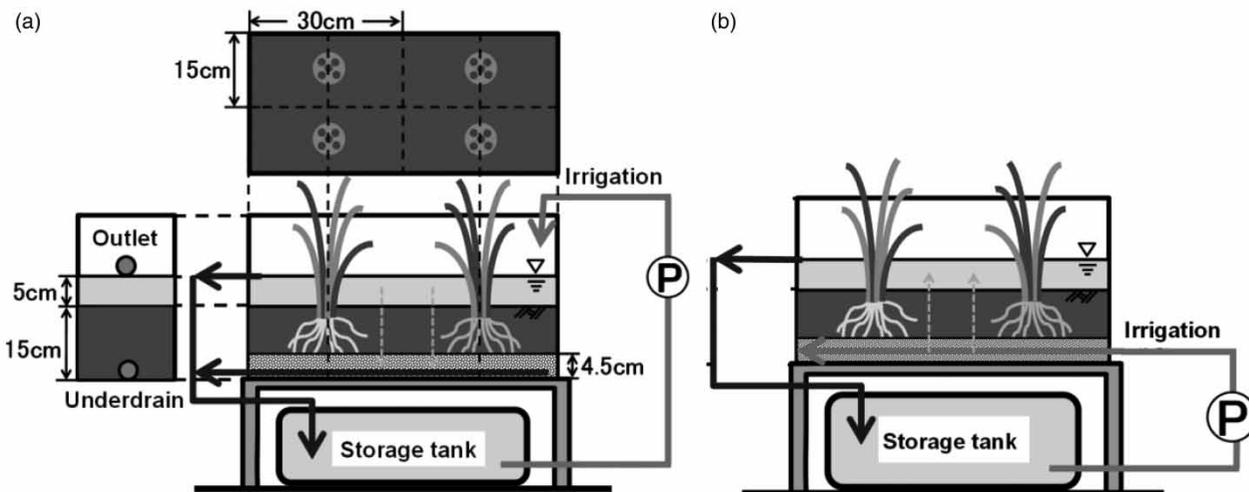


Figure 1 | Simulated paddy fields with different direction of circulated irrigation. Irrigation water is circulated and passes through the paddy soil downward and upward in Run A (a) and Run B (b), respectively, whereas it is not circulated in Run C (not illustrated).

Experimental conditions

The amount of basal fertilizer per unit area of paddy field (N, 120 kg/ha; P as P₂O₅, 160 kg/ha; K as K₂O, 140 kg/ha), which was equivalent to twice the maximum level for the cultivation of rice for human consumption, was determined based on the standards of practice in this region. In all runs, 51.4 L of treated wastewater obtained from a municipal wastewater treatment plant, which contained the above amount of nitrogen (2.0 g for 0.18 m² in our system), was collected in the storage tank at the beginning of the experiment together with 48.6 L of channel water obtained at the University Farm, Yamagata University, Tsuruoka, Yamagata, Japan. To trace the nitrogen removed by the cultivation system, 48.4 mg of heavy nitrogen (¹⁵N), which is equivalent to 2.23 atom% of total nitrogen (TN) supplied from the treated wastewater, was added to the irrigation water. The deficiencies of phosphorus (98.4% of required amount) and potassium (70% of required amount) in the treated wastewater were compensated by addition of chemical fertilizers. Soil samples were collected from the surface of paddy fields in the farm where channel water was taken and applied to the simulated paddy field.

Schedule of transplantation, cultivation, and harvesting

In all runs, the experiment started with the transplantation of young rice plant (*Oryza sativa* L. cultivar 'Bekoaoba') on 31 May 2013. This rice cultivar is popularly used as animal feed. During the experiment, the level of surface water in the simulated paddy field was maintained at 5 cm, except during the period from July 16 to 25, when the pump-up of stored water was intermittent and the water remaining in the paddy field was drained for drying up the paddy soil. This intermittence of irrigation, called midsummer drainage (MSD), is usually performed in this region to remove unused nitrogen in the soil and to enhance root growth. At the end of the MSD period, 48.3 L, 48.7 L, and 35.2 L of treated wastewater were added for top-dressing to the storage tanks in Runs A, B and C, respectively. As with basal fertilizer, heavy nitrogen equivalent to 2.23 atom% of nitrogen in the added water was supplied in all runs. At that time, the same amount of nitrogen (80 kg/ha) was present in the tanks in all runs, and then the circulation of irrigation water was restarted and continued until harvesting on 27 September without any more addition of water.

Analyses of water, rice and soil

From the beginning of the experiment, the water temperature, electric conductivity, pH and dissolved oxygen (DO) of irrigation water in the storage tank were routinely monitored using mobile meters (OM-51 and D-54, HORIBA, Japan). The TN concentration in the tank was also analyzed using a total nitrogen measuring unit (TNM-1, SHIMADZU, Japan) attached to the total organic carbon analyzer. In parallel, the growth of the rice plant was recorded by measuring its height, number of shoots, and Soil Plant Analysis Development (SPAD) value that indicates leaf chlorophyll concentration (Markwell *et al.* 1995).

After harvesting, the yield and dry weight of brown rice were measured following the standard method. To examine the quality of the harvested rice, we analyzed the content of protein, the main component of animal feed. The protein content was estimated from the nitrogen content in the harvested rice analyzed using the automatic high-sensitivity NC analyzer (SUMIGRAPH NC-220F, SCAS, Japan). The nitrogen contents in the other parts of the rice plant and paddy soil after the experiment were analyzed using the same NC analyzer. Moreover, heavy nitrogen in the plant and soil samples was analyzed using the organic elemental analyzer (FLASH 2000, Thermo Scientific).

Statistical analysis

We could not prepare replicates of the experiment in any runs. Instead, we cultivated four sets of rice plants in each run, as illustrated in Figure 1, and conducted statistical analyses on the assumption that a set of rice plants grew independently from the other sets. Student's *t*-test was used for comparing the results from the two runs, and Tukey's test was used for comparison among three or more runs.

RESULTS AND DISCUSSION

Removal of nitrogen from treated wastewater

Figure 2(a) illustrates the TN concentration in irrigation water in the storage tank. In Runs A and B, the nitrogen concentration decreased rapidly from 20 mg/L at the beginning of the experiment until the MSD period. The concentration increased to 15 mg/L upon the addition of treated wastewater at the end of MSD, and thereafter, it again decreased rapidly to less than 1 mg/L. Nitrogen was

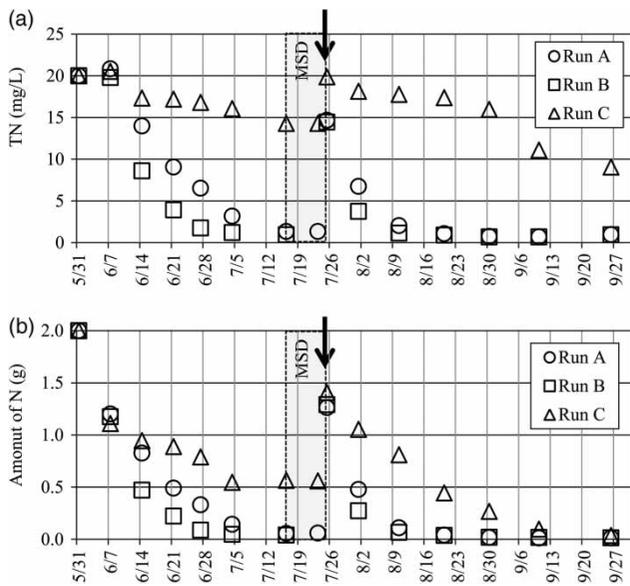


Figure 2 | Total nitrogen concentration (a) and total amount of nitrogen (b) in irrigation water in the storage tank. The total amount of nitrogen was estimated as a product of the nitrogen concentration and the volume of water existing in the tank. MSD means midsummer drainage. An arrow indicates addition of treated wastewater for top-dressing to the tank.

removed more rapidly in Run B than in Run A. As explained in the ‘Materials and methods’ section, nearly half of the irrigation water supplied to the surface in Run A was simply overflowed without infiltrating the paddy soil. Conversely, all irrigation water supplied from the underdrain in Run B should have passed through the paddy soil upward, resulting in the more rapid removal of nitrogen. In Run C, although the irrigation water was not circulated, the TN concentration was reduced from 20 mg/L to less than 10 mg/L following the addition of treated wastewater. This is probably due to dilution by overflowed surface water when we added irrigation water to the surface manually to maintain the water level. Denitrification and volatilization of nitrogen in the tank are also possible, but limited because of the slightly low pH (4.7–6.7) and moderate DO (2.9–7.0 mg/L).

Due to evapotranspiration, the volume of irrigation water in the storage tank gradually decreased. Figure 2(b) shows the total amount of nitrogen estimated as a product of the nitrogen concentration and the volume of water existing in the storage tank. The irrigation water applied at the beginning of the experiment contained 2.0 g (= 20 mg/L × 100 L) of nitrogen in all runs. The amount of nitrogen decreased to 0.056 g in Run A and 0.039 g in Run B before the MSD, whereas a larger amount of nitrogen (0.57 g) remained in Run C. It recovered to the same level (~1.3 g) upon the addition of treated wastewater on July 25 and then decreased continuously in all runs until the end of

the experiment. The removal efficiencies of nitrogen by this rice cultivation system, which were calculated based on the amounts of nitrogen applied at the beginning, added after the MSD, and left at the end, were 99.7%, 99.6%, and 98.4% in Run A, B, and C, respectively. This finding indicated that the direction of circulated irrigation (i.e., feeding irrigation water upward or downward) does not affect the removal efficiency of nitrogen in this system and that the rice cultivation system without circulated irrigation can achieve comparably effective removal of nitrogen from treated wastewater. The total amount of nitrogen (3.2 g) removed in Run A was over three times larger than that (0.99 g) removed by the same cultivation system using rice for human consumption in almost the same cultivation period (Muramatsu *et al.* 2014), indicating that rice for animal feed surely improved the performance of this system as the tertiary treatment of municipal wastewater. Abe & Ozaki (2012) compared a variety of plant species for nitrogen removal from domestic wastewater with a similar TN concentration. They reported higher nitrogen removal rates (0.44–1.65 g/m²/d) and poorer quality of treated wastewater (>5 mgN/L at lowest) than the present study (0.14 g/m²/d for four months as a whole cultivation period). This is because their irrigation system was not circulated and received wastewater continuously.

Fate of removed nitrogen

The nitrogen removed from treated wastewater in the cultivation system should have been taken up by the rice plant and other plants, adsorbed to soil particles or released into the atmosphere. Table 1 shows where the removed nitrogen in the three runs went based on the analysis of heavy nitrogen added as tracer to the treated wastewater at the beginning and midterm of the experiment. The percentage of nitrogen (35–39%) taken up by the rice plant was comparable in all runs. This is the same level as that (38%) in normal paddy fields cultivating rice for human consumption, which was

Table 1 | Percentages of nitrogen taken by rice plant and other plants, adsorbed to soil particle and released to the atmosphere, to its total amount in treated wastewater reused as irrigation water

	Run A	Run B	Run C
Uptake by rice plant	37	35	39
Uptake by other plants	14	6	16
Adsorption to soil particles	6	17	22
Release to the atmosphere	43	42	22

estimated based on the basal and additional fertilizer efficiencies (30% and 50%, respectively) (Shoji & Mae 1984). This fact revealed that nitrogen in the treated wastewater circulated in our cultivation system was available for the rice plant to the same extent as was nitrogen from the chemical fertilizer applied to normal paddy fields following standards of practice. The percentage of nitrogen released from irrigation water to the atmosphere in Run C was evidently lower than those in Runs A and B, indicating that circulated irrigation enhanced its release. The main mechanisms of nitrogen release from paddy fields are the denitrification of nitrates in the soil layer and volatilization of ammonium in surface water. The latter occurs under high pH resulting from photosynthesis. Although the pH of surface water was not measured, photosynthesis should have occurred comparably in all runs, or more effectively in Run C due to higher nitrogen concentration in the irrigation water (Figure 2). This implies that volatilization in surface water did not significantly contribute to the release of nitrogen from irrigation water. Thus, denitrification may have been enhanced by circulated irrigation. The redox potential (Eh) in paddy soil showed temporal and spatial variations in Runs A and B (data not shown) probably due to the circulated irrigation water and activity of plant roots; however, it occasionally became as low as in Run C. This fact indicates the existence of a local environment suitable for denitrification in Runs A and B, supporting the above hypothesis that circulated irrigation enhanced denitrification.

Growth of rice plant, yield, and quality of harvested rice

There was no significant difference in plant growth among the three runs in terms of plant height, number of shoots, and SPAD (data not shown). The dry mass of the whole plant at the end of the experiment was also comparable (Figure 3), indicating that neither circulated irrigation nor its direction affected the plant growth. These factors also showed no contribution to the yield and quality of harvested rice as evaluated by the protein content, as shown in Figure 3. The yield did not reach the target value (8 t/ha) corresponding to the cultivar used in every run. Upon its components, we identified a smaller number of grains per panicle (70.6, 62.2, and 72.7 in Runs A, B, and C, respectively) compared with the target value (100) as the main reason for the low yield. As the number of grains per panicle is usually dependent on the additional fertilizer in normal paddy fields, the amount and frequency of added treated wastewater seemed insufficient in the experiment. As mentioned above, circulated irrigation in our cultivation system

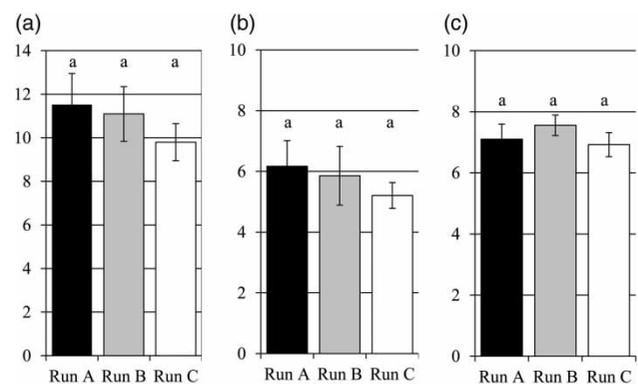


Figure 3 | Dry mass (a) of whole rice plant at the end of the experiment, yield (b) and protein content (c) of harvested rice. Error bars indicate standard errors ($n = 4$). Different letters indicate a statistically significant difference between runs ($p < 0.05$).

increased the nitrogen released to the atmosphere. Considering this fact, to achieve the target yield value a larger amount of treated wastewater should be applied to the system. For example, we could have added treated wastewater 1 month after the beginning of the experiment when the TN concentration in the irrigation water became lower than 5 mg/L (Figure 2(a)) in Runs A and B. In Run C, the number of panicles per area was also significantly lower (216/m²) than the target (300/m²). This is due to insufficient basal fertilizer, as one-quarter of the nitrogen in the treated wastewater supplied at the beginning was not used in this run without circulated irrigation (Figure 2(b)). Conversely, the kernel weight (34.5 mg/grain) was markedly higher in this run than in the other runs because an amount of nitrogen remained in the irrigation water for a longer time after the addition of treated wastewater (Figure 2(b)) and could be used for the enlargement of kernels. This finding also supports the necessity of increased supply of treated wastewater to obtain a higher yield of rice in this cultivation system.

CONCLUSIONS

We proposed the idea of using rice for animal feed in a cultivation system with the circulated irrigation of treated municipal wastewater to achieve enhanced nitrogen removal. The performance of the proposed system was evaluated in terms of the nitrogen removal as well as the yield and quality of harvested rice, and it was compared in terms of the direction of circulated irrigation. The main conclusions obtained in this study are listed below.

1. The system cultivating rice for animal feed achieved over three times larger amount of nitrogen removal from treated wastewater than that cultivating rice for human consumption. The removal efficiency was higher than 99.5%, regardless of the direction of circulated irrigation. Comparably efficient removal of nitrogen was found in the system without circulation.
2. The rapid reduction of nitrogen concentration in irrigation water was observed when the water was circulated. The reduction in the case of feeding irrigation water downward was slightly less rapid than in the case of feeding it upward, as a part of the irrigation water did not pass through the paddy soil.
3. Nitrogen in treated municipal wastewater was taken up by rice plant in this cultivation system as effectively as that in chemical fertilizer applied to normal paddy fields. This was observed in the cases of both circulated and normal irrigation. Conversely, circulated irrigation showed increased nitrogen release to the atmosphere, probably due to enhanced denitrification.
4. Neither the circulation of irrigation water nor its direction affected the growth of rice plant and the yield and quality of harvested rice.
5. The yield of rice harvested in this system did not reach the target value in normal paddy fields. To increase this yield, a larger amount of treated wastewater should be applied to the system, considering the significant amount of nitrogen released to the atmosphere.

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