DEVELOPMENT OF TREATED MUNICIPAL WASTEWATER REUSE SYSTEM TO CULTIVATE RICE FOR ANIMAL FEEDING TOWARD SUSTAINABLE WATER AND NUTRIENT CIRCULATION

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Abstract

One of the biggest challenges facing the world today is feeding the continuously growing population in the scene of climate change and water pollution. Serving as a stable food source for more than half of the world's population, rice is cultivated in at least 95 countries across the globe and consumes around 50% of the worldwide irrigation water. Recently, municipal wastewater for rice irrigation has been adopted as an effective measure in many countries for recycling nutrients and water resources and avoiding the discharge of pollutants from sewage effluents to surface water bodies.

The objective of this study was to develop a proper cultivating system of rice for animal feed with continuous irrigation of treated municipal wastewater (TWW). Firstly, the study has evaluated nitrogen (N) removal from TWW, rice yield and grain quality, and accumulation of heavy metals in paddy soil and rice grains. Secondly, the capacity of generating electricity from the paddy field irrigated with TWW has been assessed by installing a microbial fuel cell (MFC) system which utilized the organic matter source in TWW. Thirdly, the need of phosphorous (P)-fertilizer for the rice cultivation under TWW irrigation was also evaluated in two seasons. In addition, the emission fluxes of two major greenhouse gases, namely methane (CH_4) and nitrous oxide (N_2O), were also evaluated.

The experiments were conducted in three farming seasons from 2015 to 2017, using a bench-scale apparatus which consist of a simulated paddy field with an area of 0.18 m^2 and influent and effluent tanks. Bekoaoba, a large-grain-type high-yield rice variety was selected to transplant in six treatments (called runs) in 2015 and 2016 seasons, and in four treatments in 2017 season with different cultivation conditions. Among these, one run was used as the control, in which the paddy soil was supplemented with N-P-K composite fertilizers and irrigated with tap water as seen in normal paddy fields. The other runs were served continuously with TWW taken from a municipal wastewater treatment plant in Tsuruoka, Yamagata, Japan, which employs the standard activated sludge process followed by chlorine disinfection. Two types of TWW irrigation at different directions were applied. One was bottom-to-top irrigation, in which TWW was supplied from the underdrain pipe at the bottom of the field, infiltrated the paddy soil layer upward and then flowed into the effluent tank. The other was top-to-top irrigation, in which TWW was pumped to the surface of the rice field and discharged from the top at the other

side of the field. The MFC system was constructed using electrodes (0.6 m x 0.3 m) made of carbon graphite felt. The electrodes were connected to a circuit using copper cables and the voltage generated from the MFC system was recorded every 10 min using a logger.

During the experiments, the qualities of the irrigation water in the influent and effluent tanks, relevant to total nitrogen (TN) and N-components, total organic carbon (TOC), dissolved oxygen (DO), pH, electrical conductivity (EC), temperature, and oxidation-reduction potential (ORP), were monitored routinely. The growth of rice plants, the whole plant dry biomass, and grain yield were also examined using the standard methods. The quality of rice was evaluated based on the protein content of grains. In addition, the contents of TN and total phosphorous (TP) in the soil before and after the experiment were evaluated. As harmful substances primarily concerned in TWW irrigation, the concentrations of heavy metals (Cr, Mn, Ni, Zn, Cu, Mo, Cd, and Pb) in water, rice and soil were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS). Furthermore, CH_4 and N_2O gases samples were collected once a week with the manual static chamber and then analyzed using gas chromatography.

The results of the experiments indicated that bottom-to-top irrigation had improved the performance of rice cultivation with the grain yield of 14.1 t/ha, the dry mass of 16.2 t/ha, and the protein content in the brown rice of 14.6 %, which were markedly higher than those achieved in top-to-top irrigation. Throughout the 3-season experiments, N removal efficiencies in bottom-to-top irrigation (ranging from 79 to 93%) have been found to be much greater than those obtained in the treatments using top-to-top irrigation (42-63%). No accumulation of the harmful metals in the paddy soil was found after three growing seasons under TWW irrigation, except for an increase of Cu in the experimental soil in 2015 season. This was probably resulted from the oxidation of the copper wire used for MFC system rather than the effect of TWW irrigation. Those metals' content levels in the harvested rice grains were also lower than the permissible limits of the international standards. The electric output from the MFC system in 2015 season was much lower than that reported in normal paddy fields, probably due to the poor connection between the cables and the electrodes. However, it remained to be low in 2016 season even when the connection was modified using graphite rods instead of the copper cables. CH_4 emission was not found in 2015 season, probably due to the inhibitory effect of Cu in the experimental soil. This gas was detected in the following two seasons from all the runs but the fluxes were much lower than those observed in normal paddy fields. The first measurement of

N_2O in 2017 season revealed it was emitted in all runs, and that the emission fluxes from the runs applied with TWW irrigation were significantly higher than the run using tap water irrigation. The combined global warming potential (GWP) was found to be significantly increased in the treatments of TWW application using top-to-top irrigation, while decreased in the runs using bottom-to-top irrigation, as compared with that in the tap-water-irrigation treatment.

This study implied that bottom-to-top irrigation enhanced N removal efficiency from TWW. High yield and quality of brown rice could be achieved under continuous irrigation of TWW from bottom-to-top without application of any chemical fertilizer. TWW irrigation decreased CH_4 emission but increased N_2O emission from the paddy fields, resulting in increased combined GWP. No accumulation of the harmful metals was found in the harvested grains and the experimental soils after the three-cropping seasons under the continuous irrigation of TWW. Electric output from the MFC system under the continuous irrigation of TWW was lower than that previously reported from normal paddy fields as well as the paddy fields under circulated irrigation of TWW. From all mentioned results, the bottom-to-top irrigation of TWW could be recommended to be applied to the real paddy fields. Although there was no building up of the heavy metals in the experimental soils and brown rice through the three-farming seasons, continuous monitoring of heavy metals in the soil and brown rice in every season is highly recommended to avoid long-term accumulation or accidental contamination. Beside the cultivation of rice for animal feed, further studies should be conducted to cultivate rice for other beneficial purposes. The content of P in the soil would be decreased after a long-term TWW irrigation without P-fertilization, which consequently could decrease the rice yield and quality. Thus, P content in the soil should be evaluated after harvesting in each season. In addition, further studies on the efficiency of power generation of the MFC system utilizing C source in TWW are highly recommended. With a high removal efficiency of N from TWW revealed in this study, paddy fields would be considered a step in a wastewater treatment process. To avoid the adverse effect of hazardous materials in raw wastewater, paddy fields should be established as an advanced treatment after normal treatment processes such as activated sludge process.

【和訳】

今日の世界が直面している大きな課題の一つは，気候変動と水質汚濁の中で増え続ける人口に食料を供給することである。米は，世界人口の半分以上にとっての主食で

あり，世界の灌漑用水の 50%程度を消費しながら，少なくとも 95 ヵ国で栽培されている。近年，水田での都市下水灌漑が，栄養塩と水資源のリサイクルと，下水処理水から表流水への汚濁物質の流出の防止のための有効な手段として，多くの国々で導入されている。

本研究の目的は，都市下水処理水（TWW）の連続灌漑による適切な飼料用米栽培システムを構築することであった。この研究では第一に，TWW からの窒素除去，米の収量と品質，そして，土壌と米への重金属の蓄積を評価した。第二には，TWW を灌漑した水田での発電の可能性を，TWW に含まれる有機物を利用する微生物燃料電池（MFC）を導入することで調べた。第三には，TWW 灌漑のもとでの水稲栽培におけるリン酸肥料の必要性を２年間に渡って評価した。さらに，２つの主要な温室効果ガスであるメタンと亜酸化窒素の発生量も評価した。

栽培実験は，表面積 0.18m^2 の模擬水田，流入水と放流水のタンクからなるベンチスケールの実験装置を用いて，2015～2017 年の３年間に渡って行われた。粒が大きく高収量タイプの品種である「べこあおば」を，2015 年と 2016 年の実験では栽培条件の異なる６つ（2017 年の実験では４つ）の処理区（Run と呼ぶ）に移植するために選んだ。これらのうち，１つの Run は対照区として，通常の水田で見られるように土壌に窒素，リン酸，カリの化学肥料を与え，水道水で灌漑を行った。他の Run では，標準活性汚泥プロセスと塩素消毒を採用している山形県鶴岡市の下水処理場から採取した TWW を連続灌漑した。２つのタイプの TWW 灌漑を適用した。１つは「Bottom-to-top 灌漑」であり，TWW は水田土壌に設置した暗渠から供給され，土壌を上向きに透過した後，放流水タンクに流れ出る。もう一つは「Top-to-top 灌漑」であり，TWW は水田の表面に供給され，反対側の表面から放流される。MFC システムは，カーボングラファイトフェルト製の電極（0.6 m x 0.3 m）を用いて構築された。この電極は銅線を用いて電気回路に接続され，MFC システムで生み出された電圧を 10 分間隔でロガーを用いて記録した。

実験の期間を通して，灌漑用水の水質（全窒素と無機窒素成分，総有機炭素，溶存酸素，pH，電気伝導度，水温，酸化還元電位）を流入水と放流水のタンクで定期的にモニタリングした。水稲の生長もモニタリングし，収穫後には，総乾物重と収量を標準法によって調査した。収穫された米の品質については，粗タンパク含量にもとづいて評価した。施肥の必要性を明らかにするために，土壌中の全窒素および全リンの含有量も実験の前後で測定した。TWW 灌漑に対する主たる懸念として，灌漑用水，米，そして土壌の重金属（Cr, Mn, Ni, Zn, Cu, Mo, Cd, Pb）の濃度を誘導結合プラズマ質量分析計（ICP-MS）によって分析した。さらに，週に一度，静的チャンバーを用いてガス試料を収集し，その中のメタンと亜酸化窒素の濃度をガスクロマトグラフィーによって分析した。

実験の結果は，Bottom-to-top 灌漑が水稲栽培のパフォーマンスを向上させることを示した。収量，乾物重，粗タンパク質含量はそれぞれ最大で 14.1 t/ha，16.2 t/ha，14.6 %であり，それらは Top-to-top 灌漑で達成された値よりも著しく高かった。３年間

の実験を通して，Bottom-to-top 灌漑での窒素除去効率（79～93 %）は，Top-to-top 灌漑で得られる効率（42～63 %）よりもずっと高かった。TWW 灌漑のもとで３年間の実験で連続使用された水田土壌には，有害金属は蓄積していなかった。唯一，2015 年の実験では，土壌中の銅の含有量が増加した。これは，TWW 灌漑の影響ではなく，MFC システムに用いられていた銅線が腐食した結果であろう。収穫された米のそれらの金属レベルは，国際的な基準の許容上限値を下回っていた。2015 年の実験における MFC システムからの電気出力は，通常の水田で報告されている値よりずっと小さかった。これはおそらく，電極と銅線との接続不良による。ただし，銅線の代わりにカーボンロッドを用いて両者の接続を改善した 2016 年の実験でも，その出力は低いままであった。

2015 年の実験では，おそらく土壌中の銅による細菌活動の阻害のために，メタン放出は見られなかった。土壌中の銅含有量が低下した 2016 年と 2017 年の実験では，すべての Run でメタンが検出されたが，その放出フラックスは通常の水田での観測よりもずっと小さかった。2017 年の実験では初めて亜酸化窒素の測定を試み，すべての Run でその放出が確認された。TWW を灌漑した Run におけるその放出フラックスは，水道水を灌漑した Run のそれよりも明らかに大きかった。合計した地球温暖化ポテンシャル（GWP）は，TWW の Top-to-top 灌漑によって，水道水を灌漑する場合と比較して増加することが分かった。一方で，TWW の Bottom-to-top 灌漑では GWP は減少した。

本論文は，Bottom-to-top 灌漑が TWW からの窒素除去を促進することを示した。玄米の高い収量や高い品質は，TWW の Bottom-to-top 灌漑によって無施肥で達成された。TWW 灌漑は，水田からのメタンの放出を減らしたが，亜酸化窒素の放出を増やし，合計した GWP を増加させた。収穫された米や水田土壌には，TWW の連続灌漑による３年間の栽培の後でも，有害金属の蓄積が見られなかった。TWW の連続灌漑のもとでの MFC からの電気出力は，通常の水田や TWW を循環灌漑する水田からの報告値よりも小さかった。

ここで述べた結果から，実際の水田に TWW の Bottom-to-top 灌漑を適用することが推奨される。３年間の実験を通して，水田土壌や玄米への重金属の蓄積は無かったが，長期での蓄積や偶発的な汚染を回避するために，土壌や玄米中の重金属の継続的なモニタリングは不可欠である。本研究で構築した栽培システムを，飼料用米以外の用途の米の栽培に適用することは興味深い。土壌中のリン含有量は，長期に渡り無施肥で TWW 灌漑を続けることで低下し，結果として，米の収量や品質が低下するかもしれない。よって，毎年の栽培の後には，土壌中のリン含有量を評価すべきである。加えて，TWW 中の炭素源を利用する MFC システムの発電効率に関するさらなる研究も強く推奨される。TWW からの高い窒素除去効率を考慮すると，水田は下水処理プロセスの１つのステップとみなすことができるだろう。その際，生下水に含まれる有害物質の影響を避けるため，水田は活性汚泥プロセスのような通常処理に続く三次処理ステップに，硝化・脱窒処理のような高度な処理技術の代替として，位置づけられるべきである。

Contents

Chapter 1

Introduction

1.1. Introduction

Climate change and the global population explosion have been putting the scarcity of water resources in many corners of the world to the alarming status (Hussain et al. 2002; Arnell 2004), with around 1.1 billion people lacking access to fresh water in developing countries, and nearly 2.4 billion lacking adequate sanitation (Simonovic 2002). It is estimated that two-thirds of the world's population will suffer from moderate to high water stress, and about half of the population will face severe water supply constraints in 2025 (Lazarova et al. 2001). Agriculture is known as the largest consumer of fresh water resources accounting for over 70% of global water withdrawals at the beginning of the twenty-first century (UNESCO 2003; Gheewala et al. 2014). However, agricultural irrigation water does not usually require the same high grade of water quality as drinking water (Jang et al. 2013). Currently, it has been estimated that 190.4 million tons of N-P-K fertilizers were used in approximately 1.5 billion hectares of agricultural land all over the world (FAO 2003, FAO 2011). Wastewater is believed to be able to supply a significant amount of nutrients which can improve soil fertility, plant growth and crop production, reducing the consumption of required fertilizers (Hanjra et al. 2012). In this circumstance, municipal wastewater is evaluated as a new source of water, and the practice of using reclaimed wastewater for agricultural irrigation is likely to be adopted more commonly in many countries with a vast volume (UNEP 2005; Chung et al. 2011; Norton et al. 2013). Besides, though not being considered the main objective of the practice, the interruption of discharge of nutrients and organic matters from wastewater into the water environment is claimed to be achieved simultaneously (Jiménez 2006).

Being the staple food of more than half of the world's population, rice (*Oryza sativa*) is cultivated in at least 95 countries across the globe (Tsukaguchi et al. 2016). Around 9% of the entire arable land on Earth approximately has been covered by 150 million hectares of paddy fields, of which 55% is under irrigated rice cultivation and contributes to 75% of the global rice production (IRRI 2002). In general, rice cultivation is estimated to consume approximately 50% of the total irrigation water (Tuong & Bouman 2003; Muramatsu et al. 2014), and a reduction of

10% in the total water amount used for rice irrigation would save 150,000 million m^3 of water. However, rice is very sensitive to water regimes and attempts to reduce water use in rice cultivation may result in yield reduction that consequently threatens food security (Tuong & Bouman 2003). Furthermore, the reuse of municipal wastewater for rice irrigation can be considered an effective and sustainable way to save water resources only when rice yield is either maintained or increased.

1.2. Cultivation of rice for animal feeding with irrigation of municipal wastewater

In the next chapter, the benefits and downsides of municipal wastewater reuse for irrigation will be discussed, in particular for rice cultivation. Most of the drawbacks are from the contaminants in the irrigated wastewater, and therefore, one of the best ways to reduce its adverse effects is to use treated wastewater after the contaminants are removed to the suitable level. For this purpose, advanced treatments are not necessary and low-cost technologies are preferable to keep the total cost for cultivation acceptable. Such low-cost technologies, even standard activated sludge process, are difficult to remove nutrients from wastewater, and the application of treated wastewater may lead to overgrowth of rice plants, resulting in lodging.

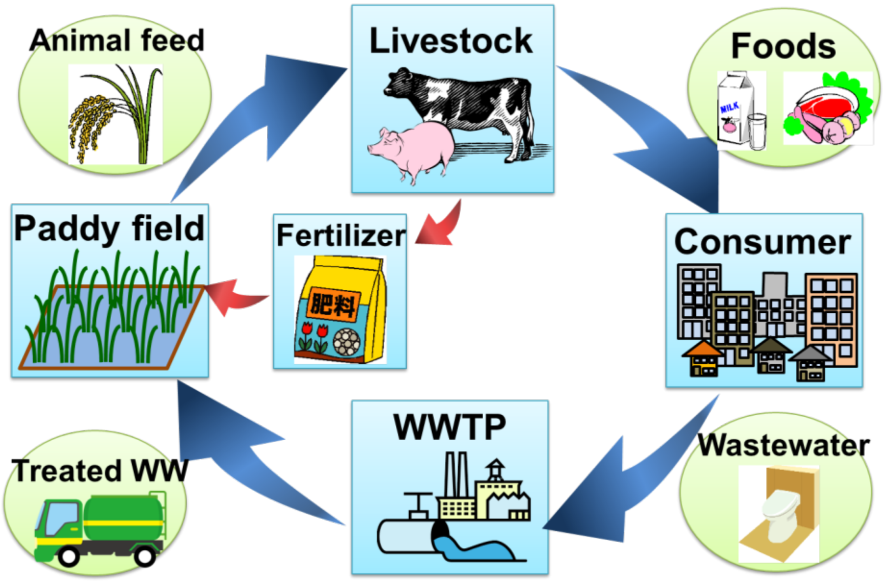


Figure 1.1. Resource circulation involving urban (consumers) and rural areas (rice and livestock famers) which is realized with cultivation of rice for animal feeding with irrigation of treated municipal wastewater.

Recently, Muramatsu et al. (2014) have designed a new rice cultivation system that used circulated irrigation to remove nitrogen (N) from TWW effectively. The experiments were conducted using a bench-scale experiment over two farming seasons. They have successfully implied the feasibility of the system to remove N from TWW with an efficiency of higher than 95% without accumulation of harmful metals in the rice grains and the paddy soil. However, overgrowth of the rice plants was recorded due to excessive supplication of nutrients, especially N, from TWW throughout the cultivation period, which could cause plant lodging, and reduction in eating quality due to a high content of protein of rice grains.

In the follow-up study, Muramatsu et al. (2015) have modified the system to cultivate rice for animal feed rather than for human consumption, since the rice cultivar used for animal feed has several advantages compared with that used for human consumption. These advantages include higher crop yield and plant resistance to lodging. Moreover, the high protein content in this rice cultivar resulted from the excessive adsorption of massively available N in TWW is preferable for animal feed. The modified system was expected to contribute to an improvement in the quality of TWW effluents and to promote water and N circulation among urban dwellers who consumed animal products and produced wastewater, farmers who produced rice for animal food by reusing TWW, and livestock farmers who used the cultivated rice as fodder for the animals. Results from the bench-scale experiment indicated that the modified system could remove N from TWW with the amount of three time higher than that the system of rice cultivation for human consumption could do. Moreover, the experiment showed that the circulated irrigation increased the amount of N released into the atmosphere, probably because of enhanced denitrification. However, the rice yield of this system was not comparable with the target value of normal paddy fields. In order to increase rice yield, and in view of the significant amount of N released into the atmosphere, a larger volume of TWW probably needs to be applied to the system.

Subsequently, with the aim of improving the yield and the quality of the harvested rice for animal feed, Watanabe et al. (2016) have modified the system of Muramatsu et al. (2015) by increasing the volume of TWW used for irrigation. In addition, based on our hypothesis that electricity could be generated more efficiently in the modified cultivation system than in normal paddy fields by supplying the organic matters contained in TWW to the PF-MFC, Watanabe et al. (2016) attempted to generate electricity in the system by applying microbial fuel cells (MFC).

The results have shown that the modified system for resource-saving rice cultivation with circulated irrigation of TWW achieved simultaneously an effective removal of N, a high yield and good quality of rice for animal feeding, and an electric output comparable to normal paddy fields. Based on the mentioned outcomes, it is expected that rice yield and quality could be significantly increased via applying continuous irrigation instead of circulated irrigation.

Figure 1.2. Bench-scale experiment to reveal the performance of TWW irrigation to cultivate rice for animal feeding.

1.3. Objective and outline of this study

This study performs as a follow-up research applying continuous irrigation of TWW to cultivate rice for animal feed. The main objective of this study is ***to develop a proper technology to reuse treated municipal wastewater through rice cultivation experiments using the bench-scale apparatus.***

This study consists of six chapters (Figure 1.3), in which, the overview of this study has just been discussed herein this chapter, while the other related researches will be reviewed within the next chapter. The experiments throughout three farming seasons will be discussed in Chapters 3, 4, and 5, while the conclusion and recommendation based on the research outcomes will be pointed out in Chapter 6. In Chapter 3, performance of continuous irrigation, effects of the direction and flow rate of TWW irrigation on N removal efficiency, yield and quality of harvested rice, and power generation, as well as accumulation of heavy metals in brown rice and soil, will be evaluated. An improved performance of rice cultivation with a high quality and quantity of rice for animal feeding achieved without P-fertilization will be illustrated in Chapter 4, in which, CH_4 emission from the paddy field, electricity generation, and the accumulation of harmful metals in rice and soil after two seasons irrigation of TWW will also be investigated. For a long-term evaluation of the cultivation system, Chapter 5 will demonstrate the performance of the 3rd cropping season of 2017, in which greenhouse gases (CH_4 and N_2O) emission from the paddy fields, and accumulation of harmful metals in rice and soil after three seasons irrigated with TWW will also be assessed. Chapter 6 will give a general conclusions and recommendations based on the main findings in Chapters 3 to 5.

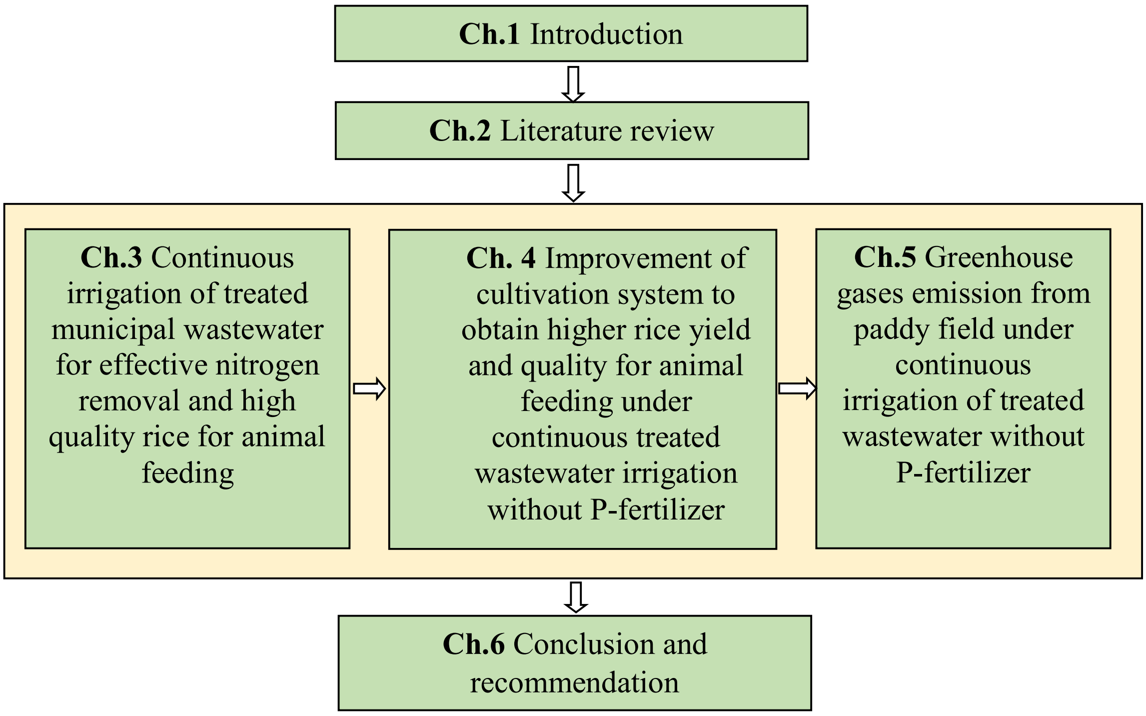


Figure 1.3. Outline of this study

Reference

Arnell, N.W., 2004. Climate change and global water resources: SRES emissions and socio-economic scenarios. *Global Environmental Change*, 14(1), pp.31–52.

Chung. B. Y, Song. C. H, Park. B. J, and Cho. J. Y. 2011. Heavy Metals in Brown Rice (Oryza

Sativa L.) and Soil After Long-Term Irrigation of Wastewater Discharged from Domestic Sewage Treatment Plants. *Pedosphere*, 21 (5), 621–627. doi:10.1016/S1002-0160(11)60164-1.

FAO, 2011. *Current world fertilizer trends and outlooks to 2015*, Available at: http://www.fao.org/3/a-av252e.pdf.

FAO, 2003. World agriculture: towards 2015/2030. An FAO perspective. , p.432.

Gheewala Shabbir H, Thapat Silalertruksa, Pariyapat Nilsalab, Rattanawan Mungkung, Sylvain R. Perret, and Nuttapon Chaiyawannakarn. 2014. Water Footprint and Impact of Water Consumption for Food, Feed, Fuel Crops Production in Thailand. *Water (Switzerland),* 6 (6), 1698–1718. doi:10.3390/w6061698.

Hanjra Munir A., John Blackwell, Gemma Carr, Fenghua Zhang, and Tamara M. Jackson. 2012. Wastewater Irrigation and Environmental Health: Implications for Water Governance and Public Policy. *International Journal of Hygiene and Environmental Health,* 215 (3),255–269. doi:10.1016/j.ijheh.2011.10.003.

Intizar Hussain, Liqa Raschid, Munir A. Hanjra, F.M. and W. van der H., 2002. *Wastewater Use in Agriculture: Review of Impacts and Methodological Issues in Valuing Impacts*,

Jang, Taeil, Jung Myungpyo, Lee Eunjeong, Park Seongwoo, Lee Joonho, and Jeong Hanseok. 2013. Assessing Environmental Impacts of Reclaimed Wastewater Irrigation in Paddy Fields Using Bioindicator. *Irrigation Science,* 31 (5), 1225–1236. doi:10.1007/s00271-013-0401-5.

Jiménez, B., 2006. Irrigation in Developing Countries Using Wastewater. *International Review for Environmental Strategies*, 6(2), pp.229–250.

Lazarova. V, Levine. B, Sack. J, Cirelli. G, Jeffrey. P, Muntau. H, Salgot. M, B.F., 2001. Role of water reuse for enhancing integrated water management in Europe and Mediterranean countries. *Water Science and Technology*, 43(10), pp.25–33.

Muramatsu, A, Ito. H, Sasaki. A, Kajihara. A, and Watanabe. T. 2015. 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. *Water Science and Technology,* 72 (4), 579–584. doi:10.2166/wst.2015.251.

Muramatsu Ayumi, Watanabe Toru, Sasaki Atsushi, Ito Hiroaki, and Kajihara Akihiko. 2014. Rice Production with Minimal Irrigation and No Nitrogen Fertilizer by Intensive Use of Treated Municipal wastewater. *Water Science and Technology,* 70 (3), 510–516. doi:10.2166/wst.2014.250.

Norton-Brandão, D., Scherrenberg, S.M. & van Lier, J.B., 2013. Reclamation of used urban waters for irrigation purposes - A review of treatment technologies. *Journal of Environmental Management*, 122, pp.85–98. Available at: http://dx.doi.org/10.1016/j.jenvman.2013.03.012.

Simonovic, S.P., 2002. World water dynamics: global modeling of water resources. *Journal of Environmental Management*, 66(3), pp.249–267. Available at: http://www.sciencedirect.com/science/article/pii/S0301479702905852.

Tsukaguchi, T., Nitta, S. & Matsuno, Y., 2016. Cultivar differences in the grain protein accumulation ability in rice (Oryza sativa L.). *Field Crops Research*, 192, pp.110–117. Available at: http://dx.doi.org/10.1016/j.fcr.2016.04.022.

Tuong, T.P. & Bouman, B.A.M., 2003. *Chapter 4: Rice Production in Water-scarce Environments* D. Kijne, J. W., Barker, R., Molden, ed., Cambridge, UK: CABI Publishing. Available at: http://www.iwmi.cgiar.org/publications/CABI_Publications/CA_CABI_Series/Water_Prouctivity/Unprotected/0851996698ch4.pdf.

UNEP, 2005. *Water and Wastewater reuse: An Environmentally Sound Approach for Sustainable Urban Water Management*, Osaka, Japan.

UNESCO, 2003. *Agriculture, food and water: A contribution to the World Water Development Report*, Available at: http://www.fao.org/catalog/bullettin/12_03.htm.

Watanabe. T, Mashiko. T, Maftukhah. R, Kaku. N, Pham. D. D and Ito. H. 2016. Nitrogen removal and power generation from treated municipal wastewater by its circulated irrigation for resource-saving rice cultivation. *Water Science and Technology,* 75 (4), 898-907. doi: 10.2166/wst.2016.572.

Chapter 2

Literature review

2.1. Municipal wastewater and its treatment

2.1.1. Characteristics of municipal wastewater

Municipal wastewater, which is usually conveyed in a combined sewer or sanitary sewer, consists of domestic wastewater, industrial wastewater, and storm water and groundwater seepage entering the municipal sewage network. Domestic wastewater includes effluent from households, institutions, commercial buildings and the like. Industrial wastewater is the effluent discharged from manufacturing units and food processing plants. In general, characteristics of domestic wastewater are not significantly different from one region to another, while there are many types of industrial wastewater based on industrial processes as its origin.

Municipal wastewater mainly consists of water (99.9%) together with relatively low concentrations of suspended and dissolved organic and inorganic solids. Parts of the organic substances present in wastewater are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products as well as various natural and synthetic organic chemicals from the process industries. Table 1 shows the levels of the major constituents in municipal wastewater.

Table 2.1. Typical composition of untreated domestic wastewater.

Contaminants	Unit	Range
Total solid (TS)	mg/L	390-1230
Total dissolved solid (TDS)	mg/L	270-860
Total suspended solid (TSS)	mg/L	120-400
Biochemical oxygen demand (BOD_5)	mg/L	110-350
Chemical oxygen demand (COD)	mg/L	250-800
Total organic carbon (TOC)	mg/L	80-260
Total nitrogen (TN)	mg/L	20-70
Total phosphorus (TP)	mg/L	4-12
Total coliform	no./100mL	10^6-10^9
Fecal coliform	no./100mL	10^3-10^7

Sources: (Metcalf & Eddy 2007)

2.1.2. Treatment of municipal wastewater

It is not recommended to reuse municipal wastewater directly for rice cultivation due to its drawbacks, which are described in the next section. Treatment of wastewater at any level is required to overcome the drawbacks. The principal objective of wastewater treatment is to remove contaminants such as solids, organic matter and nutrients before the treated wastewater is discharged into water bodies. The quality of treated wastewater depends on the treatment technology and operation.

Although wastewater treatment includes physical, chemical and biological processes, it normally has four basic steps: preliminary, primary, secondary and advanced treatments (Sonune & Ghate 2004). Preliminary treatment is designed to remove coarse solids and other large materials, which are often found in raw wastewater. These solids consist of pieces of wood, cloth, paper, plastics, sand, gravel, etc. The objective of primary treatment is to extract organic and inorganic solids from wastewater by the physical process of sedimentation and flotation. Approximately 25-50% of the BOD, 50-70% of the SS and 65% of the oil are removed throughout this treatment step (Pescod 1992).

Secondary treatment, in general, follows primary treatment to do the further treatment. Its objective is the removal of biodegradable dissolved and colloidal organic matters from effluent of primary treatment using many different types of microorganisms in a controlled environment. The principal secondary treatment techniques are the trickling filter and the activated sludge process. The latter one, which is used most commonly all over the world, can remove organic matters effectively but cannot do nutrients, especially nitrogen, from wastewater. Hence, the secondary effluent from wastewater treatment plants still has a high content of nutrients available for crop growth.

At most treatment plans, the secondary effluent is discharged into receiving water environment after disinfection with chlorine, ozone or ultraviolet radiation. To prevent eutrophication in the water environment, advanced treatment is sometimes applied to remove specific contaminations such as nutrients in the secondary effluent (EPA 1998).

2.1.3. Advantages and disadvantages of wastewater irrigation for rice production

These characteristics of municipal wastewater make us imagine advantages and drawbacks of its irrigation for rice production. Major advantages are:

- Higher crop yields with reduced use of synthetic fertilizers, resulting in saved cost for cultivation.
- Enhanced recycles of nutrients and organic matters, improving soil properties.
- Reduced discharges of pollutants to surface water bodies.
- Decreased freshwater withdraw during irrigation.

On the other hand, we should pay attentions to its drawbacks such as:

- Contamination of irrigated soil with salt, heavy metals and toxic compounds originated from wastewater, resulting in reduced soil productivity.
- Contamination of agricultural products (rice crop) with heavy metals and toxic compounds, posing health risks to consumers.

- Farmers' risk of health problems due to exposure to paddy water contaminated with pathogens, heavy metals and toxic compounds.

- Contamination of groundwater due to infiltration of wastewater used for irrigation.

The following sections describe more detail explanation about the above advantages and drawbacks. Most of them are common to irrigation of the treated wastewater, although its treatment may highlight the advantages and overcome the drawbacks.

2.2. History of studies on potential impacts of municipal wastewater reuse for rice production

2.2.1. Effects on crops

In general, wastewater irrigation can affect rice crops in terms of yields and crop quality such as appearance and flavour. Municipal wastewater is a rich source of nutrients necessary for crop growth, so it is expected that crops irrigated with municipal wastewater get higher yield than normal.

If nitrogen supplied to the crop exceeds its dose recommended for optimal yields, crop growth may be stimulated together with yield loss and delayed ripening (Jiménez 2006). This situation can happen accidentally. For example, urea plant effluents, as a rich source of liquid fertilizer in concentrated forms, have adverse effects on rice and corn yields (Hussain et al. 2002). Also oversupply of nitrogen may be resulted in overgrowth of rice plants, which triggers their lodging and reduces eating quality of rice due to increased content of proteins (Muramatsu et al. 2015).

Thu (2001) also reported that wastewater irrigation brought 10–15% higher yield of rice crops. Yoon et al. (2001) reported that treated sewage irrigation resulted in 10 - 50% greater yield than in the control that used clean water. Kang et al. (2007) reported that wastewater irrigation increased rice yield 35-55% compared to groundwater irrigation and no adverse effects were found on chemical concentrations, including the heavy metals in either the brown rice or the paddy soil. In the study of Li et al. (2009), a field experiment was conducted using irrigation of RW plus urea fertilization under equal nitrogen (N) rate, namely, black water (BW), domestic wastewater (DW), gray water (GW), SW, and SW without any N application as a control (CK), to elucidate N removal by the paddy wetland system during the rice growing season of 2007. The results showed that yield for the CK was significantly less than those of SW, GW, DW, and BW. Results of the study conducted by Jung et al. (2014) using experimental plots (5x5m) showed that wastewater irrigation plots were significantly higher than ground water in rice yield and protein content in harvested rice. The study by Nyomora (2015) illustrated that wastewater irrigation resulted in four times higher rice yield than tap water irrigation.

However, wastewater irrigation may cause a reduction in rice yield. Mukherjee et al. (2011) reported that wastewater irrigation produced less rice than groundwater irrigation, implied the negative effect of heavy metal toxicity outweighs the positive effects of organic nutrients. Alghobar & Suresha (2016) conducted filed experiment using treated wastewater (TWW), untreated wastewater (UTW) and groundwater (GW) irrigation and found that in comparison with GW, TWW and UTW increased heavy metals in the soil, and decreased rice yield by 16.8 and 10.1%, respectively. A greenhouse experiment using pot was conducted by (Carlos et al. 2016) revealed that irrigation with the treated industrial effluent decreased tiller number and grain yield compared with freshwater irrigation.

2.2.2. Effects on soil resources

Wastewater can affect paddy soil in two opposite ways: by providing benefits or causing problems. It is usually difficult to predict which effect appears in wastewater irrigation because soil is a very complicated structure involving inorganic and organic matters. One of the most recognizable effects of wastewater irrigation is a rise of yield due to nutrients supplied with wastewater as well as soil texture improved by organic matters in wastewater (Mara 2004). Supplying organic matter improves soil texture by enhancing soil humidity and microbial activity (Ortega-Larrocea et al. 2001).

Nitrogen in wastewater consists of several chemical forms such as nitrate, nitrite, ammonia and organic nitrogen. All of these forms are soluble and mobile in water and, when the wastewater is irrigated, all forms of nitrogen except ammonia are easily washed out and may cause pollution of groundwater and surface water receiving the runoff water. Only ammonia in wastewater can attach to soil particles and is retained in paddy fields but, at the surface of soil layer and rhizosphere with presence of oxygen, it is gradually converted to nitrite and finally nitrate with bacterial activities. By contrast, phosphorus, which can exist as a trivalent cation, is so stable in soil layer. In addition to this fact, since wastewater contains a smaller amount of phosphorus than that required by crops, its irrigation hardly gives an adverse impact on the water environment (Jiménez 2006). On the other hand, wastewater irrigation may make consequent adverse effects on soils. The most commonly reported impact is accumulation of metals that, depending on the level, may be harmful.

A field research in Thessaloniki, Greece during a 2-year period (Papadopoulos et al. 2009) reported no adverse effects on the physicochemical properties of soil, whereas macro and trace elements concentration showed discrepancies between the two years and the three treatments (river water with N-P fertilizer, treated wastewater with N fertilization and treated wastewater without fertilizer).

Wastewaters including industrial discharges with a high metal concentration are harmful to crops and eventually to consumers, as a result of metal accumulation in soil. The elements of major concern are heavy metals such as cadmium, copper, molybdenum, nickel and zinc.

Wastewater, particularly domestic wastewater, normally contains salts which may be accumulated in the root zone with possible harmful impacts on soil health. Increase rate of salinity depends on the salinity of irrigated water, soil transmissivity, organic matter concentration, land drainage, irrigation rate, depth to the groundwater level and the type of soils. Long-term use of wastewater with high salt contents is a potential hazard for the soil as it may erode the soil structure, resulting in less productivity. The problem of soil salinity can be settled by the application of natural or artificial solutions, although it is costly and leads to economic constraints.

Wastewater with a large amount of solids may cause soil clogging, depending on soil porosity, concentration (>100 mg/L can cause the problem) and chemical composition. The most concerning components are minerals that are not biodegraded. If soil is clogged, irrigation will become less effective due to dismissed water percolation (WHO 2006). To investigate the Cu contamination in rice and soil, (Cao & Hu 2000) used Cu-rich wastewater (12 mg/L) for rice cultivation. The results indicated that wastewater irrigation increased Cu 5 times in soil and 10 times in brown rice. Consequently, rice yield decreased by 25% compared with that under non-wastewater irrigation. Yang et al. (2006) reported that the paddy soil irrigated with untreated mining wastewater in Lechang lead/zinc mine area was heavily contaminated by Cd and would pose a human/animal health risk through Cd mobility in the food chain. A very high concentrations of As, Cd, Cu, Pb and Zn were found in the paddy soils irrigated by river water, which received wastewater from mining activity (Rogan et al. 2009). Chung et al. (2011) indicated that application of domestic wastewater to arable land for three years slightly increased the levels of Pb, Cd, Cu and Zn in the soil.

2.2.3. Effects on ground and surface water

The first effect of irrigated agriculture on groundwater resource is aquifer recharge. The recharge happens almost always non-intentionally and has the advantage of increasing the local availability of water (Stephen et al. 2005). Pescod (1992) estimated that 50–70 percent of the irrigation water could infiltrate to groundwater aquifer in some parts.

Due to this phenomenon, wastewater irrigation can cause adverse effects on groundwater resource. The most famous adverse effect is infiltration of nitrates in irrigated wastewater into groundwater. Groundwater contaminated with nitrates is known to cause methemoglobinemia in infants, so-called blue baby syndrome, if it is used as a source of drinking water (WHO 2006).

Not only nitrogen but also organic matters and metals may contaminate groundwater in municipal wastewater irrigation. If some of most toxic metals to humans—cadmium, lead, mercury and arsenic—are present in irrigated wastewater at a higher concentration than the acceptable level, groundwater is severely contaminated, posing risk of serious diseases like cancer to the groundwater users. Contamination of groundwater with organic matters brings another type of health risk to its users, through the formation of organochlorides when the groundwater is disinfected with chlorine (the most common method) for drinking purpose (Gallard & Von Gunten 2002).

Long-term irrigation of municipal wastewater may result in a significant increase of salt content in aquifers, although the quality of irrigated wastewater, soil characteristics and original quality of the receiving groundwater are all important factors to determine the extent to which the quality of groundwater is impacted. Even though groundwater has a low salt concentration, addition of salts originated from irrigated wastewater may not be considered too adverse if its movement is limited or if it is not used for any purposes. Thus, the impact of increased salts in groundwater by wastewater irrigation, which is sometimes inevitable, needs to be weighed up in consideration with all the risks and benefits from the irrigation (Toze 2006).

Surface water bodies are also affected due to drainage and runoff from the fields irrigated with municipal wastewater. The inevitable contamination in surface water is almost the same as that in groundwater, but the extent of the impact depends on the strength of wastewater and the type of water body (i.e., river, irrigation channel, lake or dam) as well as hydraulic retention time in the fields.

2.2.4. Effects on quality of irrigated wastewater

Although wastewater irrigation has a potential to contaminate fresh water sources, it is expected that the quality of the wastewater is improved by being used for irrigation. Suspended solids including pathogenic microorganisms are trapped and absorbed in upper soil layers and removed from the wastewater. The efficiency of solid removal depends on the sizes of soil pore and the solids (Stephen et al. 2005). Adsorption of microorganisms to soil particles is favored at low pH, high salt concentration in the sewage and high relative concentrations of calcium and magnesium over monovalent cations such as sodium and potassium in soil (Jiménez 2006).

Organic matters in wastewater can be rapidly converted in soils to stable and non-toxic ones such as humic and fulvic acids. In fact, we can find biodegradation of a wider variety and greater amount of organic matters in soils than in water bodies. So the organic matters in term of COD and BOD in the irrigated wastewater are significantly decreased after percolation through soil layers.

More significant reduction of nitrogen concentration is expected at paddy fileds with wastewater irrigation due to three main reasons: absorption by plants, release to the atmosphere as the result of nitrification and denitrification by nitrogen bacteria such as *Nitrobacter* and *Nitrosomonas*, and adsorption of ammonium to soil particles. Firstly, rice plants grow taking nutrients in wastewater used for irrigation, and nitrogen, one of the fundamental nutrients for plant development, is removed from the wastewater stored in soil layers (Muramatsu et al. 2014; Jang et al. 2012). Secondly, soil and rice rhizosphere microorganisms contribute to transformation of organic nitrogen or ammonium to nitrogen gas as well as nitrous oxide gas under a variety of redox conditions in soil layers (Li et al. 2009). Nitrogen removal is enhanced if flooding and drying periods are alternated for promoting nitrification/denitrification process, with 75% removal at the maximum (Jiménez 2006). Thirdly, ammonium as a cation has an affinity to the surface of soil particles normally with positive charge. However, a large amount of ammonium is supplied, and as mentioned above, excess nitrogen will be transported to groundwater with infiltrated irrigation water. Nitrites and nitrates, which are anions, are easily lost from paddy fields, resulted in groundwater contamination.

2.2.5. Effects on human health

As mentioned above, municipal wastewater includes pathogenic microorganisms such as bacteria, viruses and parasites. These microorganisms potentially pose human health risks when the wastewater is reused for some activities. Particularly, human parasites such as protozoa and helminth eggs are of special significance in this concern as they are known as being more difficult to remove by treatment processes (Hussain et al. 2002).

Paddy fields irrigated with municipal wastewater may have unfavourable health effects on farmers. It has been reported that the practice of reuse of raw or even treated wastewater for irrigation may cause epidemiological problems among nearby populations and consumers of uncooked agricultural products (Peasey et al. 2000). The degree of risk may vary among the various age groups (Hussain et al. 2002) and, in a study (An et al. 2007), children were found to have a greater risk of infection with *Escherichia coli*.

Municipal wastewater sometimes has harmful metals such as Zn, Cu, Pb, Mn, Ni, Cr and Cd, depending upon the type of activities in the associated area. Continuous irrigation of municipal wastewater may result in heavy metal accumulation in the soil and agricultural products (Singh et al. 2004). In case of rice plant, it is well known that Cd is the metal to which a special attention should be paid because it is accumulated so intensively in edible part of rice.

Most of heavy metals are normally removed well by wastewater treatment processes. Even so, we should take a case about heavy metal contamination in the paddy field considering the subsequent food chain involving agricultural products and consumers (Fytianos et al. 2001). Due to the non-biodegradable and persistent nature, heavy metals are accumulated in viscera and born, and are associated with numerous serious health disorders (Duruibe et al. 2007). Singh et al. (2010) indicated that rice and wheat grains contained less heavy metals than vegetables, but health risk was more significant due to higher contribution of cereals in the diet. (Trang et al. 2007) assessed the risk of skin disease among farmers occupationally exposed to wastewater, showing that exposure to wastewater is a major risk factor for skin disease, but it is not clear which chemical and biological agents might play the main role in causing the diseases. Rhee et al. (2011) examined the concentrations of *E. coli* in a paddy rice field irrigated with reclaimed wastewater and evaluated the risk of its infection among farmers using Beta-Poisson dose-response model. The results showed that the risk was lower in irrigation of groundwater and reclaimed wastewater irrigation than in irrigation of direct effluent from wastewater treatment plant.

2.2.6. Socioeconomic effect

Wastewater irrigation brings various economic benefits. Wastewater for irrigation does not require as high quality as the effluent which is discharged to water bodies. Indeed, thanks to the function of paddy fields to improve water quality as explained in the section 2.2.4, the discharge from the field has a better quality than the irrigation water. By using this function effectively, we can save the cost of wastewater treatment.

In addition, when wastewater containing rich nutrients is used for irrigation, we can reduce the amount of fertilizer applied to the field, resulted in cost saving or higher yield obtained. This must contribute to the improvement of the economic status of farmers. Papadopoulos et al. (2009) conducted an experiment using three treatments including (1) river irrigation water with N–P fertilization, (2) reclaimed wastewater irrigation with surface N fertilization, and (3) reclaimed wastewater irrigation without fertilization. The results indicated that (2) and (3) decreased the total production cost 8.8% and 11.9%, respectively, compared to the first treatment.

2.2.7. Effects on greenhouse gas emission

Global warming is caused by the emission of greenhouse gases (GHGs) such as methane (CH_4) and nitrous oxide (N_2O). On global scale, agricultural activities accounted for about 50% of CH_4 and 60% of N_2O in the total anthropogenic GHGs emissions in 2005 and nearly 17% increase of these emissions from 1990 to 2005 (IPPC 2007). In particular, paddy fields and irrigated lowland rice production systems are known to be significant sources of CH_4 and N_2O, which are two important trace gases contributing to an observed increase of approximately 0.6–0.7°C in global surface temperature during the last century.

GHGs emission from paddy fields may be affected by many factors such as water regime, organic matter and nitrogen resource including fertilizer. As introduced above, municipal wastewater is rich in organic matters and also contains an appreciable amount of macronutrients and micronutrients, and thus nutrient levels of soils are expected to increase with its irrigation. Several studies focused on the effects of water regime and fertilizer application on GHGs emission strength; however, to our knowledge, there was only one research examining the effect of wastewater irrigation on CH_4 and N_2O emissions from paddy field (Zou et al. 2009). Reports showed that CH_4 and N_2O emissions from rice paddies are closely associated with soil carbon and nitrogen availabilities and transformation processes, which are significantly dependent on soil properties, soil heavy metal contents and soil microbial communities (Jiao et al. 2005; Ali et al. 2008; Xu et al. 2015). Consequently, Zou et al. (2009) hypothesized that wastewater irrigation would significantly increase these gas emissions from rice paddies. The increments of CH_4 and N_2O emissions were 27% and 68%, respectively, compared to paddy fields irrigated with river water.

2.3. Detailed objectives of this study

In this study, I would like to assess the effect of continuous irrigation on rice yield and quality. As mentioned above, it is hypothesized that a higher rice yield and quality would be achieved when higher content of nutrients from TWW are supplied to rice plants with the practice of continuous irrigation. I also would like to illustrate the influence of two irrigation direction types on N removal efficiency as well as rice yield and quality, including bottom-to-top (in which irrigation water infiltrated the soil layer upward) and top-to-top irrigation (in which irrigation water is supplied to the soil surface and allowed to flow horizontally through the rice field). Muramatsu et al. (2015) and Watanabe et al. (2016) have reported that the direction of circulated irrigation did not affect N removal efficiency and the rice development. However, I expect that the direction of continuous irrigation will affect both N fate and rice growth. In addition, the previous studies of Muramatsu et al. (2015) and Watanabe et al. (2016) have achieved high rice yield and quality without exogenous application of N fertilizers. However, in this study, I await that a sufficient amount of N and P provides for plant growth by supplying a big amount of wastewater under continuous irrigation. Consequently, rice cultivation under continuous irrigation would also be applied without P-fertilizer. Besides, no accumulation of heavy metals in the soil and brown rice was found under circulated irrigation of TWW in one season. However, in the same behaviour as P and N, the more water is supplied, the more heavy metals would be accumulated in brown rice and soil, especially when the soil is used under a long-term TWW irrigation. Moreover, to my knowledge, there has been only one study about greenhouse gas emission from paddy fields under wastewater irrigation. Thus, I also would like to assess the impact of TWW irrigation on greenhouse gas emission from the paddy fields. Furthermore, this study tries to evaluate a capability of electricity generation with the expectation that the electric output from PF-MFC system could be increased by applying continuous irrigation instead of circulated irrigation.

Reference

Alghobar, M.A. & Suresha, S., 2016. Effect of Wastewater Irrigation on Growth and Yield of Rice Crop and Uptake and Accumulation of Nutrient and Heavy Metals in Soil. *Applied Ecology and Environmental Sciences*, 4(3), pp.53–60.

Ali, M.A., Oh, J.H. & Kim, P.J., 2008. Evaluation of silicate iron slag amendment on reducing methane emission from flood water rice farming. *Agriculture, Ecosystems and Environment*, 128(1–2), pp.21–26.

An Youn Joo, Chun G Yoon, Kwang Wook Jung, and Jong Hwa Ham. 2007. Estimating the Microbial Risk of E. Coli in Reclaimed Wastewater Irrigation on Paddy Field. Environmental *Monitoring and Assessment,* 129 (1-3), 53–60. doi:10.1007/s10661-006-9425-0.

Cao, Z.H. & Hu, Z.Y., 2000. Copper contamination in paddy soils irrigated with wastewater. *Chemosphere*, 41(1–2), pp.3–6.

Carlos Filipe Selau, Dos Santos Bruno Loss, Andreazza Robson, Tedesco, Marino José, Morris, Lawrence de Oliveira Camargo Flávio Anastácio. 2016. Irrigation of paddy soil with industrial landfill leachate: impacts in rice productivity, plant nutrition, and chemical characteristics of soil. *Paddy and Water Environment*, pp.1–12.

Chung. B. Y, Song. C. H, Park. B. J, and Cho. J. Y. 2011. Heavy Metals in Brown Rice (Oryza Sativa L.) and Soil After Long-Term Irrigation of Wastewater Discharged from Domestic Sewage Treatment Plants. *Pedosphere*, 21 (5), 621–627. doi:10.1016/S1002-0160(11)60164-1.

Duruibe, J.O., Ogwuegbu, M.O.C. & Egwurugwu, J.N., 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*, 2(5), pp.112–118.

EPA, 1998. Wastewater Treatment Works... The Basics. , (May), p.United States Environmental Protection Agency.

Gallard, H. & Von Gunten, U., 2002. Chlorination of natural organic matter: Kinetics of chlorination and of THM formation. *Water Research*, 36(1), pp.65–74.

Intizar Hussain, Liqa Raschid, Munir A. Hanjra, F.M. and W. van der H., 2002. *Wastewater Use in Agriculture: Review of Impacts and Methodological Issues in Valuing Impacts*,

IPPC, 2007. *Mitigation of climate change: Contribution of working group III to the fourth assessment report of the Intergovernmental Panel on Climate Change*,

Jang, T. I., Kim H. K., Seong. C. H., Lee. E. J., and Park. S. W. 2012. Assessing Nutrient Losses of Reclaimed Wastewater Irrigation in Paddy Fields for Sustainable Agriculture. *Agricultural Water Management,* 104, , 235–243. doi:10.1016/j.agwat.2011.12.022.

Jiao Yan, Huang Yao, Zong Lianggang, Zheng Xunhua, Sass Ronald L. 2005. Effects of copper concentration on methane emission from rice soils. *Chemosphere*, 58(2), pp.185–193.

Jiménez, B., 2006. Irrigation in Developing Countries Using Wastewater. *International Review for Environmental Strategies*, 6(2), pp.229–250.

Jung Kiwoong, Jang Taeil, Jeong Hanseok, and Park Seongwoo. 2014. Assessment of Growth and Yield Components of Rice Irrigated with Reclaimed Wastewater. *Agricultural Water Management*, 138, , 17–25. doi:10.1016/j.agwat.2014.02.017.

Kang Moon Seong, Kim Min Sang, Park Woo Seung, Lee Jae Jeong, and Yoo H Kyung. 2007. Assessment of Reclaimed Wastewater Irrigation Impacts on Water Quality, Soil, and Rice Cultivation in Paddy Fields. *Journal of Environmental Science and Health. Part A, Toxic/hazardous Substances & Environmental Engineering,* 42 (4), 439–445. doi:10.1080/10934520601187633.

K. Fytianos, G. Katsianis, P. Triantafyllou and G. Zachariadis. Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. *Bulletin of Environmental Contamination and Toxicology*. vol. 67, no. 3, pp. 423–430, 2001.

Li Song, Li Hua, Liang Xingqiang, Chen Yingxu, Cao Zhihong, and Xu Zhihong. 2009. Rural Wastewater Irrigation and Nitrogen Removal by the Paddy Wetland System in the Tai Lake Region of China. *Journal of Soils and Sediments,* 9 (5), 433–442. doi:10.1007/s11368-009-0095-8.

Mara, D., 2004. *Domestic WastewaterTreatment in Developing Countries*, London. England: Earthscan.

Metcalf & Eddy, 2007. *Water reuse: Issue, Technology and Application* T. Asano et al., eds., New York. US: AECOM Press. Mc Graw Hill.

Mukherjee, V., And & Gupta, G., 2011. *Toxicity and profitability of rice cultivation under wastewater irrigation: The case of the East Calcutta Wetlands*,

Muramatsu, A, Ito. H, Sasaki. A, Kajihara. A, and Watanabe. T. 2015. 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. *Water Science and Technology,* 72 (4), 579–584. doi:10.2166/wst.2015.251.

Muramatsu Ayumi, Watanabe Toru, Sasaki Atsushi, Ito Hiroaki, and Kajihara Akihiko. 2014. Rice Production with Minimal Irrigation and No Nitrogen Fertilizer by Intensive Use of Treated Municipal wastewater. *Water Science and Technology,* 70 (3), 510–516. doi:10.2166/wst.2014.250.

Nyomora, A.M., 2015. Effect of treated domestic wastewater as source of irrigation water and nutrients on rice performance in Morogoro, Tanzania. *Journal of Environment and Waste Management*, (2).

Ortega-Larroceaa M.P, Siebea. C, Bécardb. G, Méndezc. I, Webster. R. 2001. Impact of a century of wastewater irrigation on the abundance of arbuscular mycorrhizal spores in the soil of the Mezquital Valley of Mexico. *Applied Soil Ecology*, 16(2), pp.149–157.

Papadopoulos Frantzis, Parissopoulos George, Papadopoulos Aristotelis, Zdragas Antonios, Ntanos Dimitrios, Prochaska Chara, and Metaxa Irene. 2009. Assessment of Reclaimed

Municipal Wastewater Application on Rice Cultivation. *Environmental Management,* 43 (1), 135–143. doi:10.1007/s00267-008-9221-4.

Peasey Anne, Blumenthal Ursula, Mara Duncan, Ruiz-palacios Guillermo. 2000. A Review of Policy and Standards for Wastewater Reuse in Agriculture : A Latin American perspective. No. 68, Part II. *Water and Environmental Health at London and Loughborough (WELL), London, England. 74p.*

Pescod, M.B., 1992. Wastewater treatment and use in agriculture. *FAO Irrigation and Drainage Paper*. vol. 47, pp. 169. 1992.

Rhee, H. P, Yoon. C. G, Son. Y. K, and Jang. J. H. 2011. Quantitative Risk Assessment for Reclaimed Wastewater Irrigation on Paddy Rice Field in Korea. *Paddy and Water Environment,* 9 (2), 183–191. doi:10.1007/s10333-010-0212-8.

Rogan. N, Serafimovski. T, Dolenec. M, Tasev. G, Dolenec. T. 2009. Heavy metal contamination of paddy soils and rice (Oryza sativa L.) from Kocani Field (Macedonia). *Environmental Geochemistry and Health*, 31(4), pp.439–451.

Singh. A, Kumar Sharmaa. R, Agrawal. M, Marshall. F. M. 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Food and Chemical Toxicology*, 51(2 SUPPL.), pp.375–387. Available at: http://dx.doi.org/10.1016/j.fct.2009.11.041.

Singh. K. P, Mohan. D, Sinha. S, Dalwani. R. 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. *Chemosphere*, 55(2), pp.227–255.

Sonune, A. & Ghate, R., 2004. Developments in wastewater treatment methods. *Desalination*, 167(1–3), pp.55–63.

Stephen Foster, Héctor Garduño, Albert Tuinhof, K.K. and M.N., 2005. Sustainable Groundwater Groundwater Management : Management Urban Wastewater as Groundwater Recharge evaluating and managing the risks and benefits. *The World Bank*, Note 12(Table 1), pp.1–6.

Thu, N.N., 2001. Urbanization and wastewater reuse in peri-urban areas: a case study in Thanh Tri District, Hanoi City. *IWMI Working Paper*, (30), pp.16–17. Available at: http://www.iwmi.cgiar.org/Publications/Working_Papers/working/WOR30.pdf.

Toze, S., 2006. Reuse of effluent water - Benefits and risks. *Agricultural Water Management*, 80(1–3 SPEC. ISS.), pp.147–159.

Trang Do Thuy, Wim Van Der Hoek, Nguyen Dang Tuan, Phung Dac Cam, Vu Huu Viet, Do Duc Luu, Flemming Konradsen, and Anders Dalsgaard. 2007. Skin Disease among Farmers Using Wastewater in Rice Cultivation in Nam Dinh, Vietnam. *Tropical Medicine and International Health,* 12 (SUPPL. 2), 51–58. doi:10.1111/j.1365-3156.2007.01941.x.

Watanabe. T, Mashiko. T, Maftukhah. R, Kaku. N, Pham. D. D and Ito. H. 2016. Nitrogen removal and power generation from treated municipal wastewater by its circulated irrigation for resource-saving rice cultivation. *Water Science and Technology,* 75 (4), 898-907. doi: 10.2166/wst.2016.572.

WHO, 2006. WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater. Volume 2: Wastewater Use in Agriculture. *Wastewater Use in Agriculture*, II, p.222.

Xu. Y, Ge. J, Tian. S, Li. S, Nguy-Robertson. A. L, Zhan. M, Cao. C. 2015. Effects of water-saving irrigation practices and drought resistant rice variety on greenhouse gas emissions from a no-till paddy in the central lowlands of China. *Science of the Total Environment*, 505, pp.1043–1052. Available at: http://dx.doi.org/10.1016/j.scitotenv.2014.10.073.

Yang. Q. W, Lan. C. Y, Wang. H. B, Zhuang. P, and Shu. W. S. 2006. Cadmium in Soil-Rice System and Health Risk Associated with the Use of Untreated Mining Wastewater for Irrigation in Lechang, China. *Agricultural Water Management,* 84 (1-2), 147–152. doi:10.1016/j.agwat.2006.01.005.

Yoon, C.G., Kwun, S.K. & Ham, J.H., 2001. Effects of treated sewage irrigation on paddy rice culture and its soil. *Irrigation and Drainage*, 50(3), pp.227–236.

Zou. J, Liu. S, Qina. Y, Pana. G, Zhu,. D. 2009. Sewage irrigation increased methane and nitrous oxide emissions from rice paddies in southeast China. *Agriculture, Ecosystems and Environment*, 129(4), pp.516–522.

Chapter 3

Continuous irrigation of treated municipal wastewater for effective nitrogen removal and high quality rice for animal feeding

3.1. Introduction

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).

Beside 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 (MFC) to the rice cultivation system. MFC are bio-electrochemical 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). MFC 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; Jan et al. 2014). The application of the MFC 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.*, 2016).

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.*, 2016).

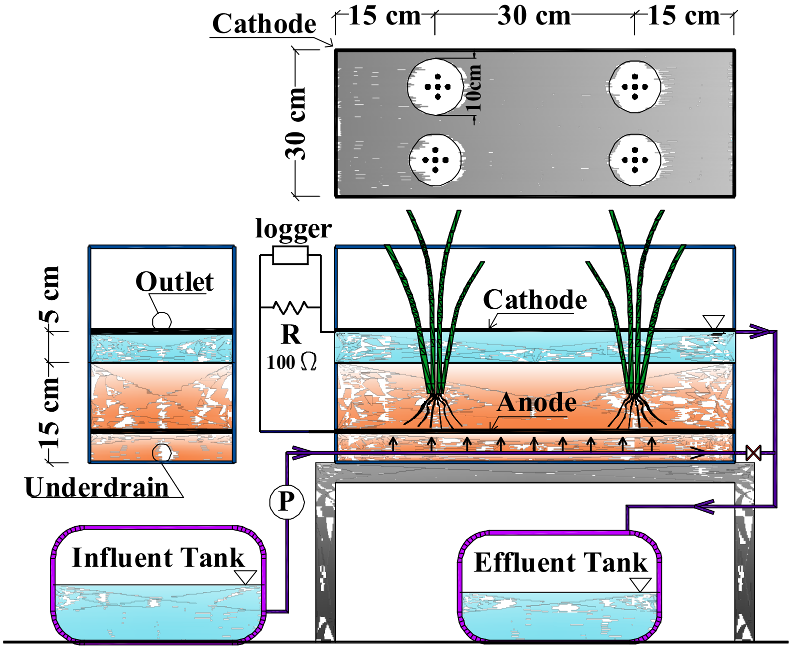
3.2. Objectives

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.

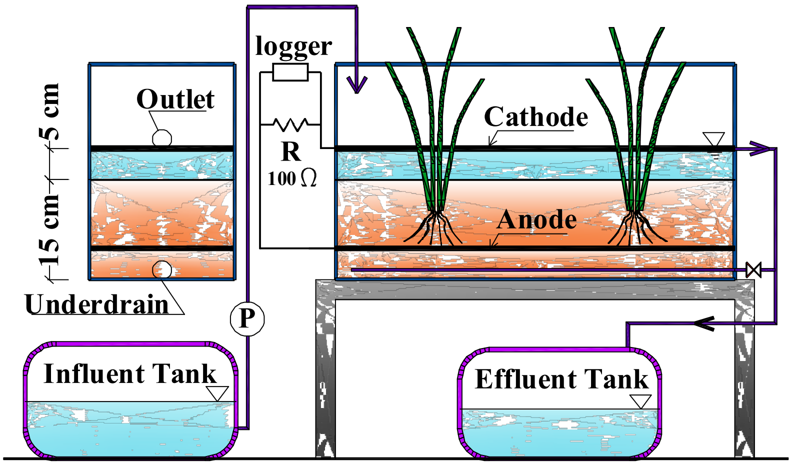
3.3. Materials and Methods

3.3.1. Experimental apparatus

The experiment was conducted using a bench-scale apparatus with a simulated 0.18 m^2 paddy field (Figure 3.1). This apparatus was used in our previous studies (Muramatsu *et al.*, 2014; Muramatsu *et al.*, 2015; Watanabe *et al.*, 2016). 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 3.1). 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 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.



(a) Bottom-to-top irrigation



(b) Top-to-top irrigation

Figure 3.1. Simulated paddy fields with different directions of continuous irrigation.

Table 3.1. Experimental conditions.

Cultivation conditions	Run A	Run B	Run C	Run D	Run E	Run 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		

3.3.2. 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. The soil used 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.

3.3.3. Microbial fuel cell (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 x 0.3 m) made of carbon graphite felt, was the same as the one used in the study of Watanabe et al. (2016). 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 MFC system was recorded every 10 min using a hand-type logger (Midi data logger GL220, Graphtec, Japan).

3.3.4. Samples collection and analysis

Samples of irrigated wastewater were collected from the influent and effluent tanks once a week. Total nitrogen (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 (EC), temperature, and oxidation-reduction potential (ORP). In addition, components of nitrogen (i.e. nitrate, nitrite, and ammonium) were determined using a colorimeter (DR-890, HATCH). 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).

3.4. Results

3.4.1. Basic water quality parameters

Basic water quality parameters of the wastewater used for irrigation are illustrated in Figure 3.2. 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 3.3) 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.

3.4.2. Removal of nitrogen from treated wastewater

Figure 3.4 (a) illustrates changes in the TN concentration of the irrigation water, which was measured in the influent 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 ten 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.

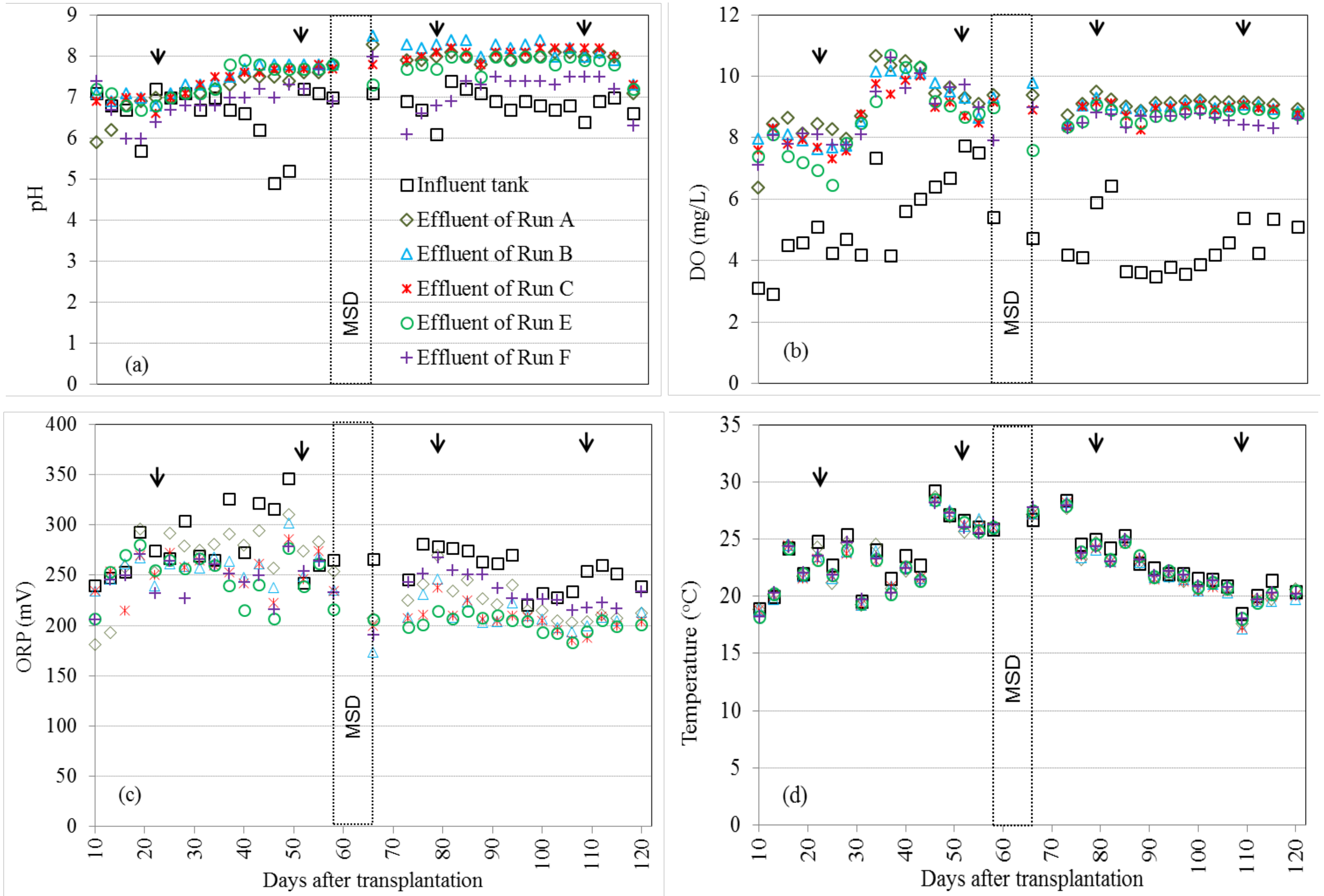


Figure 3.2. pH (a), DO (b), ORP (c) and Temperature (d) of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

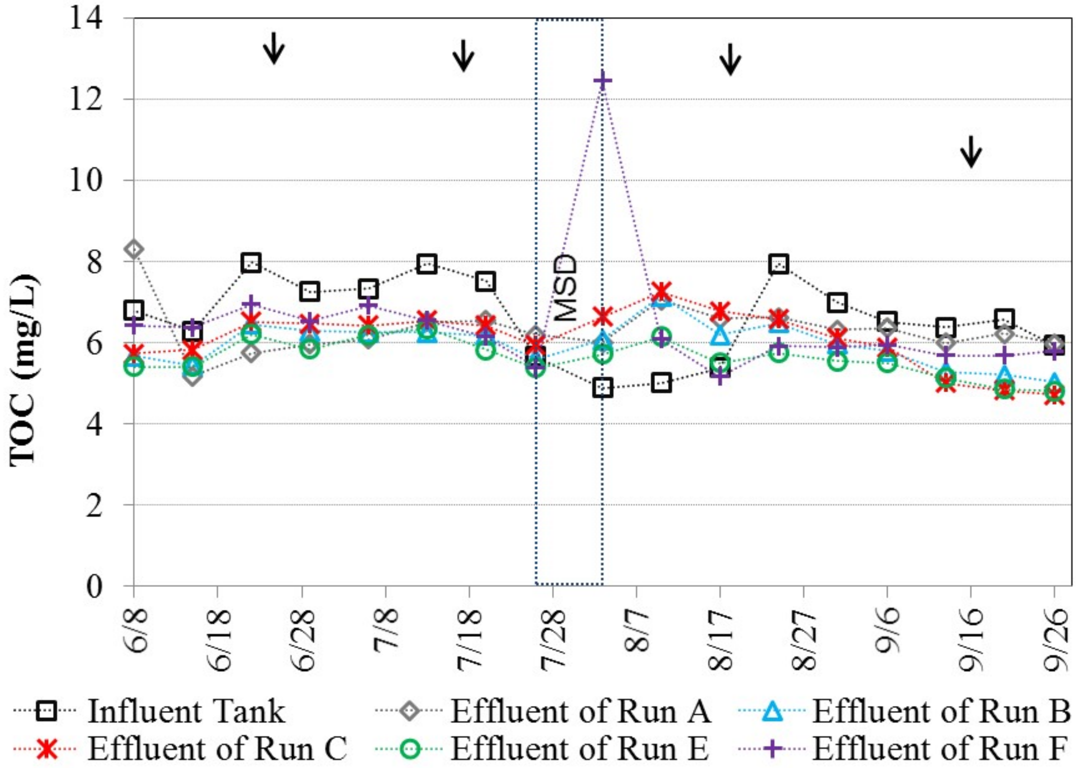
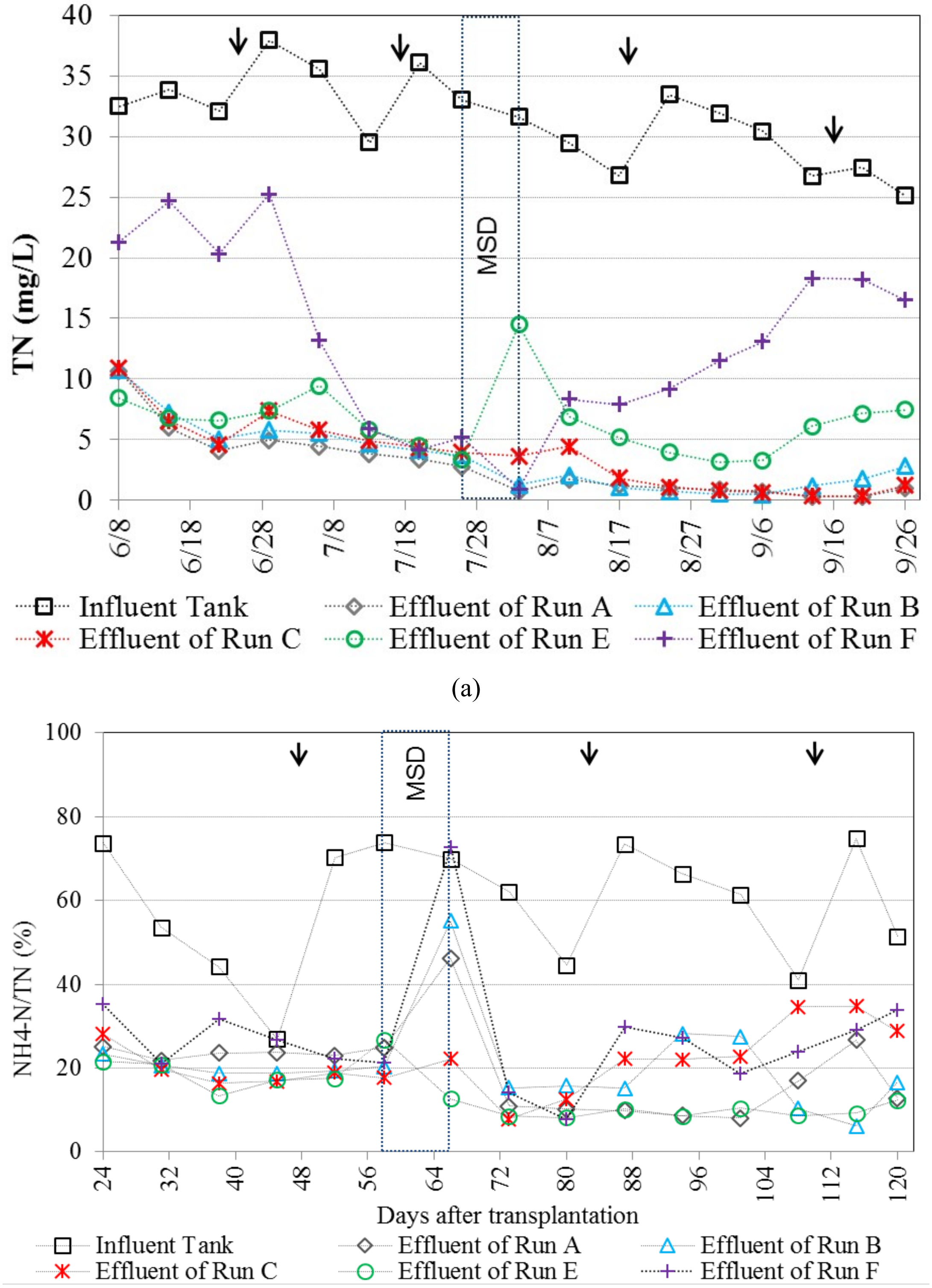


Figure 3.3. Total organic carbon of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.



(a)

(b)

Figure 3.4. Total nitrogen (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.

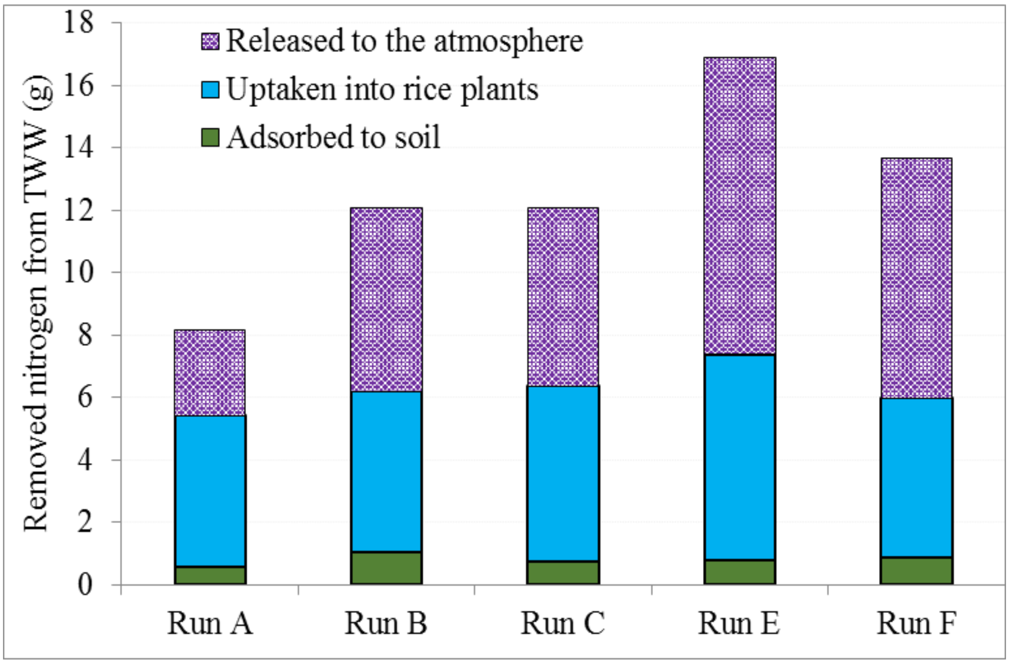


Figure 3.5. Fate of nitrogen removed from irrigated wastewater.

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

Tables 3.2 and 3.3 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 t/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 t/ha), although the same irrigation and fertilizer conditions as Run C were applied.

The quality of the rice in term of protein content, which 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 t/ha). Runs C and F got the second highest dry mass, followed by Run B (10.7 t/ha) and Run A (10.6 t/ha). The lowest rice plant mass belonged to the tap water irrigation (10.4 t/ha).

Table 3.2. Yield components and grain yield.

	Panicle density (panicles/ m^2)	Grain per panicle (grains/panicle)	Single-grain weight (mg)	Filled grain (%)	Yield of rice (t/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 3.3. Protein content in brown rice and dry biomass of whole plant.

	Dry Biomas (t/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

3.4.4. 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; Javier *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 3.4) 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 3.5) such as Cu, Cr, Zn, Cd, Pb and As in the harvested brown rice did not 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 (Bruno 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. Table 3.5 also showed the comparisons of the minerals such as K, Ca, Mg, Fe and Mn between the harvested rice and the standard compositions for animal feed of Japan (NARO. 2009). The concentrations of K and Mn in the brown rice were higher than the standard tables, while the concentrations of Ca and Fe were lower than the standard tables. The concentrations of Mg, Cu and Zn in the brown rice were comparable with the standard tables.

Table 3.4. Concentrations of heavy metals in soils before and after the experiment (mg/kg).

	Soil before experiment	Soil after 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

Table 3.5. 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	Standard tables edited by NARO
K	3665.26±519.05	3566.09±261.30	3610.57±205.89	3374.10±157.09	3338.10±203.84	3308.62±230.30	NA	2500.00
Ca	104.18±9.56	110.46±6.84	113.57±11.86	114.21±5.70	107.99±8.42	105.03±8.77	NA	300.00
Mg	1238.31±95.97	1182.30±48.62	1214.42±43.52	1256.39±49.76	1130.35±34.56	1090.67±56.95	NA	900.00
Fe	13.37±1.90	12.63±0.69	12.52±0.49	13.87±0.79	13.51±1.09	13.56±0.83	NA	36.00
Mn	41.44±2.64	43.88±3.40	38.98±3.65	44.30±3.43	39.04±2.74	51.11±3.13	Mn	21.00
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	3.30
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	17.00
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	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	NA
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	NA
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

NA: Not available.

3.4.5. Electric output

Immediately after the MFC systems were set in the experimental apparatus, an electric output of around 100 mV 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*, 2016). 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-MFC (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 3.6 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 to a deeper understanding of this phenomenon. As mentioned above, we expected to gain a 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 NAand rice root exudates.

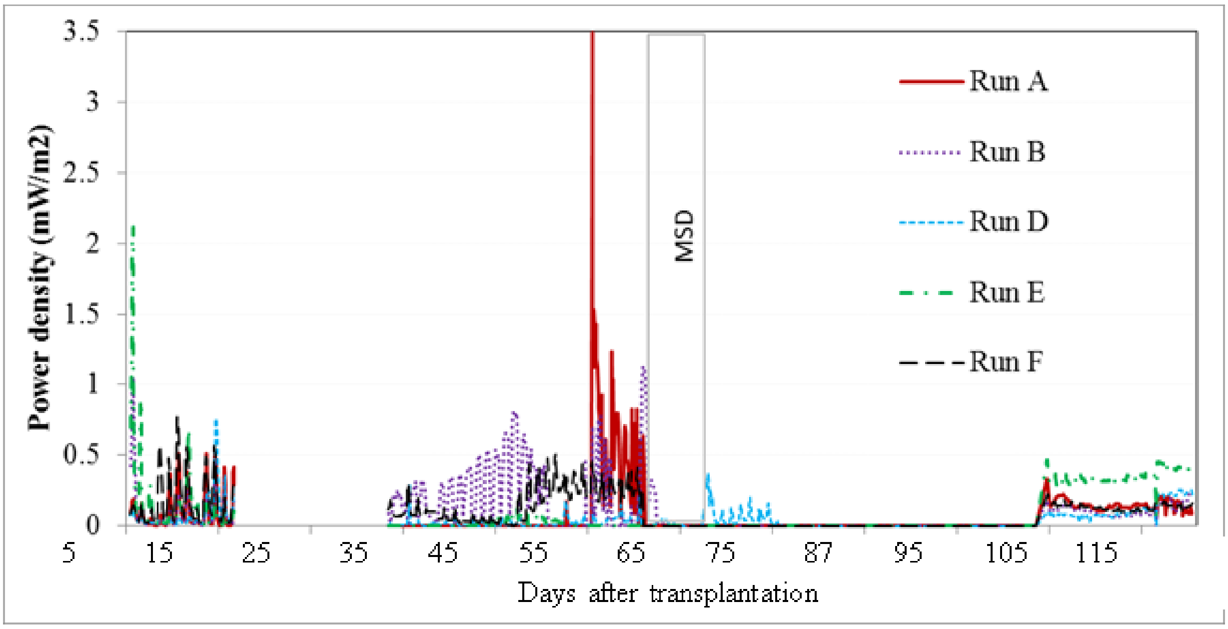


Figure 3.6**.** Power density from the PF-MFC. MSD means midsummer drainage to dry up the soil layers. F indicates a period from day 16 to 30 after transplantation, which data could not be obtained due to technical difficulties in the logger.

3.5. Discussion

3.5.1. 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. (2016) 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).

3.5.2. 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 t/ha) (Jung et al. 2014; Nyomora 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.* 2016). 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.

3.6. Summary

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.

Reference

An, Youn-Joo, Chun G Yoon, Kwang-Wook Jung, and Jong-Hwa Ham. 2007. Estimating the Microbial Risk of E. Coli in Reclaimed Wastewater Irrigation on Paddy Field. *Environmental Monitoring and Assessment* 129 (1–3): 53–60. doi:10.1007/s10661-006-9425-0.

Batista, Bruno Lemos, Letícia Ramos Nacano, Rodolfo de Freitas, Vanessa Cristina de Oliveira-Souza, and Fernando Barbosa. 2012. Determination of Essential (Ca, Fe, I, K, Mo) and

Toxic Elements (Hg, Pb) in Brazilian Rice Grains and Estimation of Reference Daily Intake. *Food and Nutrition Sciences* 3 (1): 129–34. doi:10.4236/fns.2012.31019.

Behera, Manaswini, Partha S. Jana, Tanaji T. More, and M. M. Ghangrekar. 2010. Rice Mill Wastewater Treatment in Microbial Fuel Cells Fabricated Using Proton Exchange Membrane and Earthen Pot at Different pH. *Bioelectrochemistry* 79 (2). Elsevier B.V.: 228–33. doi:10.1016/j.bioelechem.2010.06.002.

Chung, B. Y., C. H. Song, B. J. Park, and J. Y. Cho. 2011. Heavy Metals in Brown Rice (Oryza Sativa L.) and Soil After Long-Term Irrigation of Wastewater Discharged from Domestic Sewage Treatment Plants. *Pedosphere* 21 (5): 621–27. doi:10.1016/S1002-0160(11)60164-1.

Ciria, M. P., M. L. Solano, and P. Soriano. 2005. Role of Macrophyte Typha Latifolia in a Constructed Wetland for Wastewater Treatment and Assessment of Its Potential as a Biomass Fuel. *Biosystems Engineering* 92 (4): 535–44. doi:10.1016/j.biosystemseng.2005.08.007.

De Schamphelaire, L., A. Cabezas, M. Marzorati, M. W. Friedrich, N. Boon, and W. Verstraete. 2010. Microbial Community Analysis of Anodes from Sediment Microbial Fuel Cells Powered by Rhizodeposits of Living Rice Plants. *Applied and Environmental Microbiology* 76 (6): 2002–8. doi:10.1128/AEM.02432-09.

De Schamphelaire, Liesie, Leen Van Den Bossche, Son Dang Hai, Monica H??fte, Nico Boon, Korneel Rabaey, and Willy Verstraete. 2008. Microbial Fuel Cells Generating Electricity from Rhizodeposits of Rice Plants. *Environmental Science and Technology* 42 (8): 3053–58. doi:10.1021/es071938w.

De Schamphelaire, Liesje, Angela Cabezas, Massimo Marzorati, Michael W. Friedrich, Nico Boon, and Willy Verstraete. 2010. Microbial Community Analysis of Anodes from Sediment Microbial Fuel Cells Powered by Rhizodeposits of Living Rice Plants. *Applied and Environmental Microbiology* 76 (6): 2002–8. doi:10.1128/AEM.02432-09.

EU Communities. 2006. *Commission Regulation (EC) No 1881/2006 - Setting Maximum Levels for Certain Contaminants in Foodstuffs*. Vol. L364. doi:10.2203/dose-response.06-012.Hanekamp.

FAO/WHO. 2004. Evaluation of Certain Food Additives and Contaminants. Sixty-First Report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series 922; WHO: Geneva*, 127–32.

Fukushima, Akira. 2012. Yield Potential of High-Yield Rice Varieties in the Tohoku Region of Japan. *Japan Agricultural Research Quarterly* 46 (3): 199–204. doi:10.6090/jarq.46.199.

Jan B. A. Arends, Jonas Speeckaert, Evelyne Blondeel, Jo De Vrieze, Pascal Boeckx, Willy Verstraete, Korneel Rabaey, and Nico Boon. 2014. Greenhouse Gas Emissions from Rice Microcosms Amended with a Plant Microbial Fuel Cell. *Applied Microbiology and Biotechnology* 98 (7): 3205–17. doi:10.1007/s00253-013-5328-5.

Jang, T. I., H. K. Kim, C. H. Seong, E. J. Lee, and S. W. Park. 2012. Assessing Nutrient Losses of Reclaimed Wastewater Irrigation in Paddy Fields for Sustainable Agriculture. *Agricultural Water Management* 104. Elsevier B.V.: 235–43. doi:10.1016/j.agwat.2011.12.022.

Jiménez, Blanca. 2006. Irrigation in Developing Countries Using Wastewater. *International Review for Environmental Strategies* 6 (2): 229–50.

Jung, Kiwoong, Taeil Jang, Hanseok Jeong, and Seongwoo Park. 2014. Assessment of Growth and Yield Components of Rice Irrigated with Reclaimed Wastewater. *Agricultural Water Management* 138. Elsevier B.V.: 17–25. doi:10.1016/j.agwat.2014.02.017.

Kaku, Nobuo, Natsuki Yonezawa, Yumiko Kodama, and Kazuya Watanabe. 2008. Plant/microbe Cooperation for Electricity Generation in a Rice Paddy Field. *Applied Microbiology and Biotechnology* 79 (1): 43–49. doi:10.1007/s00253-008-1410-9.

Kang, Moon Seong, Sang Min Kim, Seung Woo Park, Jeong Jae Lee, and Kyung H Yoo. 2007. Assessment of Reclaimed Wastewater Irrigation Impacts on Watlds. *Journal of Environmental Science and Health. Part A, Toxic/hazardous Substances & Environmental Engineering* 42. doi:10.1080/10934520601187633.

Kouzuma, Atsushi, Nobuo Kaku, and Kazuya Watanabe. 2014. Microbial Electricity Generation in Rice Paddy Fields: Recent Advances and Perspectives in Rhizosphere Microbial Fuel Cells. *Applied Microbiology and Biotechnology* 98 (23): 9521–26. doi:10.1007/s00253-014-6138-0.

Lee, Chang Gyun, Tim D. Fletcher, and Guangzhi Sun. 2009. Nitrogen Removal in Constructed Wetland Systems. *Engineering in Life Sciences* 9 (1): 11–22. doi:10.1002/elsc.200800049.

Li, Song, Hua Li, Xingqiang Liang, Yingxu Chen, Zhihong Cao, and Zhihong Xu. 2009. Rural Wastewater Irrigation and Nitrogen Removal by the Paddy Wetland System in the Tai Lake Region of China. *Journal of Soils and Sediments* 9 (5): 433–42. doi:10.1007/s11368-009-0095-8.

Li, Xiaochun, Qiuyi Zhong, Yuxiang Li, Ganghua Li, Yanfeng Ding, Shaohua Wang, Zhenghui Liu, She Tang, Chengqiang Ding, and Lin Chen. 2016. Triacontanol Reduces Transplanting Shock in Machine-Transplanted Rice by Improving the Growth and Antioxidant Systems. *Frontiers in Plant Science* 7 (June 2016): 1–10. doi:10.3389/fpls.2016.00872.

Liu, Shentan, Hailiang Song, Xianning Li, and Fei Yang. 2013. Power Generation Enhancement by Utilizing Plant Photosynthate in Microbial Fuel Cell Coupled Constructed Wetland System. *International Journal of Photoenergy* 2013. doi:10.1155/2013/172010.

Logan, Bruce E., Cassandro Murano, Keith Scott, Neil D. Gray, and Ian M. Head. 2005. Electricity Generation from Cysteine in a Microbial Fuel Cell. *Water Research* 39 (5): 942–52. doi:10.1016/j.watres.2004.11.019.

Mateo-Sagasta, Javier, Kate Medlicott, Manzoor Qadir, Liqa Raschid-Sally, Pay Drechsel, and Jens Liebe. 2013. *Proceedings of the UN-Water Project on the Safe Use of Wastewater in Agriculture*.

Mukherjee, Vivekananda, Abhishek Das, Anirban Akhand, and Gautam Gupta. 2013. Toxicity and Profitability of Rice Cultivation under Wastewater Irrigation: The Case of the East Calcutta Wetlands. *Ecological Economics* 93. Elsevier B.V.: 292–300. doi:10.1016/j.ecolecon.2013.06.010.

NARO, National Agriculture and Food Research Organization. 2009. Standard Table of Feed Compositions in Japan. *Japan Livestock Industry Association.*

Nyomora, Agnes MS. 2015. Effect of Treated Domestic Wastewater as Source of Irrigation Water and Nutrients on Rice Performance in Morogoro, Tanzania. *Journal of Environment and Waste Management*, no. 2.

Oh, SangEun, and Bruce E. Logan. 2005. Hydrogen and Electricity Production from a Food Processing Wastewater Using Fermentation and Microbial Fuel Cell Technologies. *Water Research* 39 (19): 4673–82. doi:10.1016/j.watres.2005.09.019.

Papadopoulos, Frantzis, George Parissopoulos, Aristotelis Papadopoulos, Antonios Zdragas, Dimitrios Ntanos, Chara Prochaska, and Irene Metaxa. 2009. Assessment of Reclaimed Municipal Wastewater Application on Rice Cultivation. *Environmental Management* 43 (1): 135–43. doi:10.1007/s00267-008-9221-4.

Qadir, M., D. Wichelns, L. Raschid-Sally, P. G. McCornick, P. Drechsel, A. Bahri, and P. S. Minhas. 2010. The Challenges of Wastewater Irrigation in Developing Countries. *Agricultural Water Management* 97 (4): 561–68. doi:10.1016/j.agwat.2008.11.004.

Reimers, C. E, P Girguis, H.A Stecher, and L.M Tender. 2006. Microbial Fuel Cell Energy from an Ocean Cold Seep. *Geobiology* 4: 123–36.

Rhee, H. P., C. G. Yoon, Y. K. Son, and J. H. Jang. 2011. Quantitative Risk Assessment for Reclaimed Wastewater Irrigation on Paddy Rice Field in Korea. *Paddy and Water Environment* 9 (2): 183–91. doi:10.1007/s10333-010-0212-8.

Son, Y. K., C. G. Yoon, H. P. Rhee, and S. J. Lee. 2013. A Review on Microbial and Toxic Risk Analysis Procedure for Reclaimed Wastewater Irrigation on Paddy Rice Field Proposed for South Korea. *Paddy and Water Environment* 11 (1–4): 543–50. doi:10.1007/s10333-012-0347-x.

Takanezawa, Kazuko, Koichi Nishio, Souichiro Kato, Kazuhito Hashimoto, and Kazuya Watanabe. 2010. Factors Affecting Electric Output from Rice-Paddy Microbial Fuel Cells. *Bioscience, Biotechnology and Biochemistry* 74 (6): 1271–73. doi:10.1271/bbb.90852.

Tender, Leonard M, Clare E Reimers, Hilmar a Stecher, Dawn E Holmes, Daniel R Bond, Daniel a Lowy, Kanoelani Pilobello, Stephanie J Fertig, and Derek R Lovley. 2002. Harnessing Microbially Generated Power on the Seafloor. *Nature Biotechnology* 20 (8): 821–25. doi:10.1038/nbt716.

Trang, Do Thuy, Wim van der Hoek, Phung Dac Cam, Khuong Thanh Vinh, Nguyen Van Hoa, and Anders Dalsgaard. 2006. Low Risk for Helminth Infection in Wastewater-Fed Rice Cultivation in Vietnam. *Journal of Water and Health* 4 (3): 321–31. doi:10.2166/wh.2006.013.

Trang, Do Thuy, Wim Van Der Hoek, Nguyen Dang Tuan, Phung Dac Cam, Vu Huu Viet, Do Duc Luu, Flemming Konradsen, and Anders Dalsgaard. 2007. Skin Disease among Farmers Using Wastewater in Rice Cultivation in Nam Dinh, Vietnam. *Tropical Medicine* *and International Health* 12 (SUPPL. 2): 51–58. doi:10.1111/j.1365-3156.2007.01941.x.

Wang, Kai, Hongying Zhou, Bangju Wang, Zaiping Jian, Fei Wang, Jianliang Huang, Lixiao Nie, Kehui Cui, and Shaobing Peng. 2013. Quantification of Border Effect on Grain Yield Measurement of Hybrid Rice. *Field Crops Research* 141. Elsevier B.V.: 47–54. doi:10.1016/j.fcr.2012.11.012.

Wang, Runying, Virginie Baldy, Claude Périssol, and Nathalie Korboulewsky. 2012. Influence of Plants on Microbial Activity in a Vertical-Downflow Wetland System Treating Waste Activated Sludge with High Organic Matter Concentrations. *Journal of Environmental Management* 95 (SUPPL.). Elsevier Ltd: S158–64. doi:10.1016/j.jenvman.2011.03.021.

Wang, X., Y. J. Feng, and H. Lee. 2008. Electricity Production from Beer Brewery Wastewater Using Single Chamber Microbial Fuel Cell. *Water Science and Technology* 57 (7): 1117–21. doi:10.2166/wst.2008.064.

Watanabe, Toru, Takuma Mashiko, Rizki Maftukhah, Nobuo Kaku, Dong Duy Pham, and Hiroaki Ito. 2016. Nitrogen Removal and Power Generation from Treated Municipal Wastewater by Its Circulated Irrigation for Resource-Saving Rice Cultivation. *Water Science and Technology* 75 (4): 898–907. doi:10.2166/wst.2016.572.

Xu, Jiakuan, Lianxin Yang, Ziqiang Wang, Guichun Dong, Jianye Huang, and Yulong Wang. 2006. Toxicity of Copper on Rice Growth and Accumulation of Copper in Rice Grain in Copper Contaminated Soil. *Chemosphere* 62 (4): 602–7. doi:10.1016/j.chemosphere.2005.05.050.

Yang, Q. W., C. Y. Lan, H. B. Wang, P. Zhuang, and W. S. Shu. 2006. Cadmium in Soil-Rice System and Health Risk Associated with the Use of Untreated Mining Wastewater for Irrigation in Lechang, China. *Agricultural Water Management* 84 (1–2): 147–52. doi:10.1016/j.agwat.2006.01.005.

Yoon, Chun G., Soon K. Kwun, and Jong H. Ham. 2001. Effects of Treated Sewage Irrigation on Paddy Rice Culture and Its Soil. *Irrigation and Drainage* 50 (3): 227–36. doi:10.1002/ird.27.

Chapter 4

Improvement of the cultivation system to obtain higher rice yield and quality for animal feeding under continuous treated wastewater irrigation without P-fertilizer

4.1. Introduction

In the previously mentioned studies, high N removal efficiency from TWW and high rice yield were obtained in a rice cultivation system for animal feeding with circulated irrigation of TWW without N-fertilizer (Muramatsu et al. 2015; Watanabe et al. 2016). Following that, with the objective of increasing the rice quality and quantity by increasing the amount of irrigated TWW, the system was modified applying continuous irrigation instead of circulated irrigation. The modified system has achieved a higher rice yield (up to 9.0 t/ha) and a nutritional value with a protein content of up to 13.1% (Pham et al. 2017). However, a large amount of P-fertilizer was compensated to the paddy field (160 kg P/ha), which may decrease the benefit for farmers. Hence, in the current study, the performance of the cultivation system has been evaluated without any P-fertilizer. Though the concentration of P in TWW is low, it is expected that a high flow rate of irrigation can effectively supply enough P required for either remaining the rice yield and quality or improving the rice performance without any application of exogenous chemical P-fertilizer.

In the context of TWW irrigation, we should pay attention to the emission flux from paddy fields of methane (CH_4) - the second most important greenhouse gas (GHG) after carbon dioxide (CO_2), implicated in global warming. Because the CH_4 molecule has 25 times higher global warming potential than the CO_2 molecule, a small changes of CH_4 in the atmosphere significantly contribute to global warming (Scott D. Bridgham et al.2013). Paddy fields are considered one of the largest anthropogenic sources of CH_4 (Meijide et al. 2016). It was calculated that rice cultivation emitted roughly 63.8Tg of CH_4 (CO_2 equivalent) worldwide in 2014, which was responsible for approximately 19.5% of the total agricultural GHG emissions (FAO 2017). The emission of CH_4 from rice fields is a result of multiple simultaneous processes such as CH_4 formation, oxidation and transportation (Lee et al. 2014). It was well reported that CH_4 emissions from rice fields are closely associated with temperature, water regime, soil redox potential, pH and especially organic resources (Chen et al. 2013; Zou et al.

2009). Aside from the mentioned essential nutrients for plant growth, municipal wastewater is also rich in organic matters, which is the main resource for CH_4 formation. Therefore, we hypothesized that municipal wastewater irrigation would significantly increase CH_4 emission from paddy soil. Until present, to our knowledge, there has been only one study has examined the effect of wastewater irrigation on GHG emission from paddy fields (Zou et al. 2009), showing the increments of 27% of CH_4 emission in the fields irrigated by wastewater compared with those irrigated by river water.

Microbial fuel cells (MFC) are devices that utilize microorganisms to generate electricity from organic matter (Kaku et al. 2008). Some research groups have already addressed the implementation of MFC on marine sediments (Reimers et al. 2001; Tender et al. 2002; Reimers et al. 2006), planted systems (Strik et al. 2008; Venkata Mohan et al. 2011), constructed wetlands (Yadav et al. 2012; Fang et al. 2013; Villaseñor et al. 2013; Corbella et al. 2014) and recently also rice paddy fields (De Schamphelaire et al. 2008; Kaku et al. 2008; L. De Schamphelaire et al. 2010; TAKANEZAWA et al. 2010; Kazuya Watanabe and Koichi Nishio 2010; Ueoka et al. 2016). Rice paddy field microbial fuel cells (PF-MFC) are sediment-type MFC that generate electric power from the conversion of soil organic matter with the aid of rhizospheric microbes (Ueoka et al. 2016). In our previous studies, MFC systems were set up in the simulated paddy rice to examine the effects of TWW and irrigation direction on the performance of PF-MFC. The first trial achieved an electric output comparable to those were reported in normal paddy fields when circulated irrigation was deployed (Watanabe et al. 2016), higher than those were obtained in the experiment that applied TWW continuous irrigation in the following season (Pham et al. 2017). The low electric output in the MFC of (Pham et al. 2017) might be caused by the oxidation of copper cables. Thus, in the current work, we tried to improve the MFC system to generate higher electricity.

4.2. Objectives

For the development of a more attractive system to cultivate rice for animal feeding with continuous TWW irrigation based on our previous studies, the objectives of this study are to demonstrate a high quality and quantity of rice for animal feeding achieved without P-fertilization and to assess CH_4 emission from the paddy field as well as electricity generation.

The accumulation of harmful metals in rice and soil after two seasons irrigation of TWW was also evaluated.

4.3. Materials and Methods

4.3.1. Experimental apparatus

Paddy was grown in the same bench-scale apparatus that was used in 2015 season (Figure 3.1). Six treatments without replicates were implemented with different cultivation conditions (Table 4.1). In Runs A, B, C and E, TWW was irrigated at the similar hydraulic load (4.5 L/d) using a "bottom-to-top" irrigation, in which TWW from the influent tank was pumped to drainpipe continuously and infiltrated upward the paddy soil layer. In Run F, "top-to-top" irrigation was applied, that TWW was irrigated to the soil surface at the same flow rate as other runs. The effluents of the TWW irrigation runs were collected to the effluent tanks via overflow pipe. Run D was used as the control, by adding tap water to make up the water loss due to evapotranspiration with supplementation of N-P-K fertilizers.

4.3.2. Cultivation management

Water used for the experiment was got from the effluent of the same municipal wastewater treatment plant as 2015 season (section 3.3.2). To track down the fate of nitrogen in irrigated WWT, the stable isotope of nitrogen (^{15}N) was added to the TWW used for irrigation at 3 atm% of total nitrogen. The soil for Runs A, D and F was sampled on March 29, 2016, from the surface layer (0 to 20 cm) of a paddy field in the farm of Yamagata University (Tsuruoka, Yamagata, Japan); while the other runs repeatedly used the soil from our experiment in the previous season (Pham et al. 2017).

Seeds of the same rice variety as 2015 season (section 3.3.2) were sown in a plastic tray on April 20, 2016 and then seedlings were transplanted in the bench-scale apparatus on May 20, 2016, at the same rate as 2015 season. An approximately 5 cm depth of standing water was constantly maintained after transplantation. Midsummer drainage (MSD) was conducted from July 4 to 11, in which water supply was stopped and paddy soil was kept in dried in order to enhance rice root growth by serving oxygen to the rice root zone. The rice grains were finally harvested on September 28, 2016.

Basal fertilizer was applied before transplantation for Runs D and E to supply 160 kg/ha N-P-K and 160 kg/ha P, respectively. On July 11, just 14 days before the flowering stage, the top-dressing of N-K fertilizer (100 kg/ha) was applied to Run D.

Table 4.1. Experimental conditions.

Cultivation condition	Run A	Run B	Run C	Run D	Run E	Run F
Soil	New	Old		New	Old	New
Water	TWW			Tap water	TWW	
Flow rate (L/day)	4.5			Depend on evaporation	4.5	
Flow Direction	Bottom-to-top			No flow	Bottom-to-top	Top-to-top
Water supply	Continuous			As needed	Continuous	
Chemical Fertilizer	No			N, P, K (for basal); and N-K (14 days before flowering)	P (for basal)	No
MFC circuit	Close		Open	Close		

4.3.3. *Microbial Fuel Cells (MFC) System*

The configuration of the PF-MFC system was fundamentally the same as that described in Chapter 3. To avoid copper cable oxidation, graphite rod was used to connect with anodes then connect with the copper cable out of the water surface.

4.3.4. *Field measurement and sampling*

The vegetative growth parameters of rice plant during the growing season, the water quality, and soil were measured using the same methods as those were described in Chapter 3. The quality of brown rice was evaluated according to (AOAC 1990) via six nutritional components including crude protein, fat, fibre, nitrogen free extract (NFE) and organic matter (OM). Phosphorus (P) in soil samples was extracted using the same method as for As extraction from solid samples. The digested solutions and water samples were analyzed for P using the spectrophotometer same meter as for N-components measurement.

CH_4: Methane gas samples were collected with the manual static chamber. Chambers were made of acrylic with 20 cm x 22 cm footprint and two heights; 60 cm for early rice growing stage and 115 cm for middle and later rice growing stages. Each chamber covered two hills of rice and was installed with an air-circulating fan to ensure complete gas mixing during the sampling period. Chambers were placed on the frame of apparatus in each plot before the gas sampling. Sampling was conducted four times in interval of 20 min in the morning (10:30–12:00) once a week using a 60-ml-syringe through a silicon tube embedded at the flank of the chamber and then the samples were immediately transferred to a 40-ml-glass vial containing HCl at pH 2 for the measurement using a gas chromatography (Hitachi GC-163) with a flame ionization detector (FID). The flux of gas emission was calculated according to the equation proposed by Kazunori et al. (2015).

4.4. Results

4.4.1. Irrigation water characteristics

The monthly average of chemical characteristics of TWW during the experimental period are shown in Table 4.2. pH value was maintained from 7.0 to 7.3. ORP varied in a high range of 199.0-258.3 mV. This could be ascribed to the existence of free chlorine in the influent from disinfection process in WWTP (Pham et al. 2017). Average TN varied from 23.0 to 31.0 mg/L while TP was in the range of 0.1 to 0.2 mg/L.

Table 4.2. Monthly average quality of irrigation water.

	pH	ORP	EC	DO	TN	TP	Cu	Cr	Zn	Cd	Pb	As
		mV	mS/m	mg/L			µg/L					
May	7.3	199.0	60.8	4.5	28.8	0.2	15.4	0.6	58.0	NA	0.8	NA
June	7.0	239.2	72.5	4.2	31.0	0.2	11.0	0.6	50.4	NA	0.6	NA
July	7.3	210.0	63.5	3.9	26.8	0.1	10.8	0.6	45.0	NA	0.8	NA
August	7.2	258.3	64.5	3.1	28.6	0.2	8.4	0.6	44.0	NA	0.6	NA
September	7.0	241.4	56.7	3.3	23.0	0.2	10.6	0.8	40.2	NA	0.6	NA

NA: Not available.

4.4.2. Growth of rice plant

Table 4.3 shows the plant height, tiller number and chlorophyll content on flag leaf at the end of the vegetative stage. Plant height is used as a scale of crop growth. There were no significant differences of average plant height between runs. The average final plant height was in the order of Run F < Run D < Run A < Run E < Run B < Run C. The same range of plant height in Runs B, C and E implies that the great amount of nutrient supply from the treated sewage might have been sufficient to grow the plants without P-fertilizer. Comparison of the plant height between Runs E and F indicates the TWW irrigation upward could promote the growth of rice plant by supplying more nutrient to rice root. The plant height in this season much was higher than in the previous season (Watanabe et al. 2016b). This was commonly found in Runs D under the same cultivation conditions as in the previous season, indicating the possible attribution of different climate condition between two seasons.

Similarly, the numbers of tiller in all runs in this season were also greater than in the previous season (Watanabe et al. 2016b). The tiller numbers in all runs were in the same range (24.0-28.3/hill) except for Run F (21.0/hill). Despite the differences in supplied nitrogen, the same range of SPAD was observed in all runs, which were similar to those in the previous season.

Table 4.3. Comparison of crop growth characteristics.

	Plant height (cm)	number of tiller per hill	SPAD
Run A	101.7±3.1	24.0±1.4	46.3±2.2
Run B	104.7±3.7	26.5±1.7	46.3±2.5
Run C	105.7±7.8	28.3±3.9	47.4±0.4
Run D	99.7±0.3	25.8±1.7	46.0±1.1
Run E	104.5±3.3	28.0±3.7	47.2±1.1
Run F	99.4±4.8	21.0±0.8	44.9±1.3

4.4.3. Grain yield, yield components and biomass

The yield of rice grain and its components are shown in Table 4.4. Significantly higher panicle density was produced under TWW irrigation from bottom-to-top (Runs A, B, C and E, 528-556 panicles/m^2) as compared to the top-to-top irrigation of TWW (Run F, 433 panicles/m^2) and the control treatment (Run D, 461 panicle/m^2). Likewise, TWW irrigation created a notably greater number of grain per panicle (Runs A, B, C and E, 77.8-90.8 grains/panicle) than tap water irrigation with N-P-K fertilizer (Run D, 63.6 grains/panicle). On the contrary, Run D got the highest weight of 1000 grain (30.4 g). Alghobar & Suresha (2016) reported that TWW irrigation did not increase weight of rice grain. The highest rice yield was got in Run A (14.1 t/ha), followed by Runs B, C and E (from 12.3 to 12.8 t/ha). Run D achieved the lowest rice yield (9.0 t/ha). Fageria & Baligar (1999) reported that among different yield components, panicle density had the largest positive effect on rice yield. However, the results in the present work indicate that number of rice grain per panicle mainly influenced the grain yield. Compared to top-to-top irrigation (Run F), bottom-to-top irrigation (Runs A, B and C) increased rice yield by 19.5-37.5%.

Table 4.4. Yield components, grain yield and biomass.

	Panicle density (panicles /m^2)	Grain per panicle (grains/panicle)	Single-grain weight (mg)	Filled grain (%)	Yield of rice (t/ha)	Dry Biomass (t/ha)
Run A	528	90.8	29.2	90.3	14.1	15.5
Run B	556	77.8	28.3	89.4	12.3	15.0
Run C	550	79.8	28.7	89.6	12.5	16.2
Run D	461	63.6	30.4	91.3	9.0	10.4
Run E	550	80.7	28.8	90.1	12.8	15.5
Run F	433	80.5	29.3	90.9	10.3	11.8
Run D*	400	66.1	29.9	90.6	8.3	10.4
Run E*	472	73.2	29.5	88.7	9.0	12.4

*: Result of the experiment in 2015. Dry biomass means whole plant dry weight excluding grain.

Rice straw, a by-product of the rice grain production, can be utilized for animal feed as a part of forage or for new energy as a type of fuel. For these aims, we also evaluated the biomass of the whole plant excluding grain. TWW irrigation from bottom-to-top significantly increased the dry mass compared to tap water irrigation. The dry mass achieved in Run A and Run E were the same (15.5 t/ha), followed by Run B (15.0 t/ha) and Run F (11.8 t/ha). The highest biomass was belonged to Run C (16.2 t/ha), opposed to the biomass was observed in Run D (10.4 t/ha).

4.4.4. Nutritional compositions of brown rice

The main nutritional compositions of the harvested rice are shown in Table 4.5 with the values in the standard of feed compositions in Japan. Moisture was around 13% in all runs without significant difference. The quality of rice strongly depends on the concentration of protein-the second main nutritional composition of grain after starch. The protein levels in the current study were in the range of 12.9-14.2% for the runs were applied TWW irrigation, much higher than that got from the tap water irrigation (10.3%). Bottom-to-top irrigation of TWW (Runs A, B, C and E) got richer-protein rice (13.2-14.2%) than top-to-top irrigation (Run F, 12.9%) and the protein contents got from runs used old soil were higher than those achieved from runs used new soil. The highest protein content belonged to Run B, was the same as that was obtained from Run E.

Rice crude fat is a good source of linoleic and other vital fatty acids but does not include cholesterol (Devi et al. 2015). The fat content in this study ranged from 2.3 to 2.7%, same as the result in the previous study Watanabe et al. (2016 b), but slightly lower than the value of the standard.

The presence of fibre in food increases the bulk of faeces, improves bowel function and help prevent digestive disorders (De Jan et al. 2015). The crude fibre in rice observed from this study varied from 0.4 to 0.7%, slightly lower than the standard but comparable to the rice observed in the earlier study (Watanabe et al. 2016 b). For human, fibre-rich food helps to improve proper bowel function and diminish risk of developing intestinal disorders. However, fibre low food may promote the fattening period for animal. Besides, with many animals such as cow, horse, rice is not the main source of ingesting fibre, but forage hence a slight difference of fibre between rice and feed standard is negligible.

NFE (nitrogen-free extract) in this work varied from 80.9 to 84.7%. The decrease in NFE in this work may be due to the higher temperature in the apparatus after heading stage. Same as the report of Watanabe et al. (2016 b), the transparent roof that used to avoid the effect of rainfall tended to trap the heat caused a higher temperature in the apparatus zone, was the main reason for the reduction in NFE.

Ash content represents the total mineral content in foods. The values for percentage ash content obtained in this study ranged between 1.6-1.8%, slightly higher than the value of the standard. All of these contents in all runs were slightly lower than the standard values and there was no significant difference between runs. The negative correlation between protein and other nutritional compositions suggest that rice cultivar high in protein may likely be low in other nutritional composition contents. These results were well supported by the findings of Oko et al. (2012).

Table 4.5. Nutritional compositions of brown rice

	Moisture (%)	Dry mass (%)	Protein (%)	Fat (%)	NFE (%)	Fiber (%)	Ash (%)	OM (%)
Normal value of feed compositions in Japan			8.8	3.2	85.6	0.8	1.6	
Run A	13.1±0.4	86.9±0.4	13.2±0.8	2.5±0.3	81.9±0.9	0.6±0.3	1.7±0.0	98.3±0.0
Run B	12.8±0.3	87.2±0.3	14.2±0.3	2.7±0.4	80.9±0.5	0.4±0.3	1.8±0.1	98.2±0.1
Run C	13.2±0.1	86.8±0.1	13.9±1.1	2.3±0.1	81.4±1.3	0.6±0.4	1.7±0.0	98.3±0.0
Run D	12.7±0.2	87.3±0.2	10.3±0.7	2.7±0.1	84.7±0.6	0.6±0.1	1.8±0.1	98.2±0.1
Run E	13.0±0.3	87.0±0.3	14.0±0.1	2.6±0.1	81.1±1.0	0.7±0.3	1.7±0.1	98.3±0.1
Run F	12.9±0.1	87.1±0.1	12.9±0.5	2.6±0.1	82.2±0.6	0.6±0.4	1.6±0.0	98.4±0.0

4.4.5. N-P-K in soil

Figure 4.1 shows the change of N, P and K contents in the soil before and after the experiment. Overall, N content increased in Runs D (81 kg/ha) and F (34 kg/ha) while decreased in the other runs. While other runs used TWW with upward irrigation decreased P in the soil, an increase in P in Run E was found may be attributed to the addition of P-fertilizer to Run E. Both N and P was found to be increased in Run D but the yield and quality of rice obtained from Run D still lower than other runs used TWW implies that rice plant used N and P in fertilizer not effectively. The decrease of N and P in upward irrigation of TWW without fertilizer could make the soil poorer. Higher rice yield and quality could be obtained in one season, but for long-term use, it needs to be assessed in the further study. Regardless of the TWW irrigation or P-fertilizer application, K content in the experimental soil tended to decrease in new soil but increase in old soil.

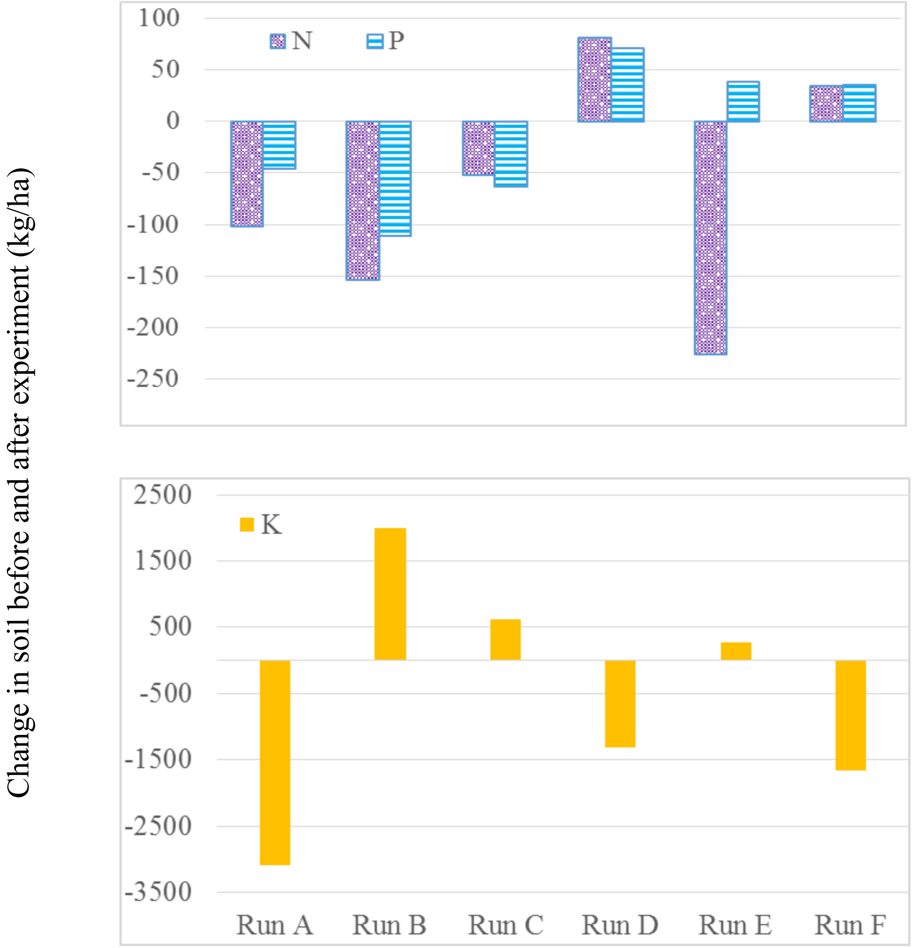


Figure 4.1. Change of nutrient content in soil before and after experiment (kg/ha). The positive value means increase while the negative value means decrease.

4.4.6. CH4 emission

In 2015, CH_4 emission was not found probably due to the inhibitory effect of the high copper levels in the soil (Mao et al. 2015) as described before (Pham et al. 2017). As shown in Figure 4.2, CH_4 was detected successfully in all runs in the present experiment. Fluxes of CH_4 emission from all runs varied from 0.06 to 0.64 mg/m^2.h, which was much lower than those reported from normal paddy fields (Yang et al. 2012; Win et al. 2013; Liu et al. 2015; Riya et al. 2015). CH_4 emission rates were low at the early vegetative growth stage (week 4 to 7) and gradually increased with the development of soil reductive conditions and plant growth. After MSD, since the paddy fields were re-flooded, CH_4 emission rates increased gradually again, gained peaks during the flowering period (week 13), and finally dropped to the same level as the initial stage during the grain maturation stage (week 15 to 19). Throughout the experiment, CH_4 emission fluxes from Runs D and F were higher than other runs. This was probably attributed to the bottom-to-top irrigation in Runs A, B, C and E since TWW fed from the bottom supply oxygen to the soil layer and negatively affects on methanogens bacteria as well as stimulates the oxidation of produced methane gas. On the contrary, CH_4 emission rate was usually lower in Run C with open circuit than the runs with a closed circuit. Zhong et al. (2017) reported that power generation reduced CH_4 emission in the limitation of available organic matter. In the present work, such a reduction of CH_4 emission was not found probably because the irrigated wastewater supplies much of organic matter to the rhizospheric zone. Since the irrigated TWW supply much nitrogen to the paddy field and high nitrogen removal efficiencies were achieved, the measurement of N_2O gas, another important greenhouse gas, is necessary to evaluate the net impacts this cultivation system on the climate.

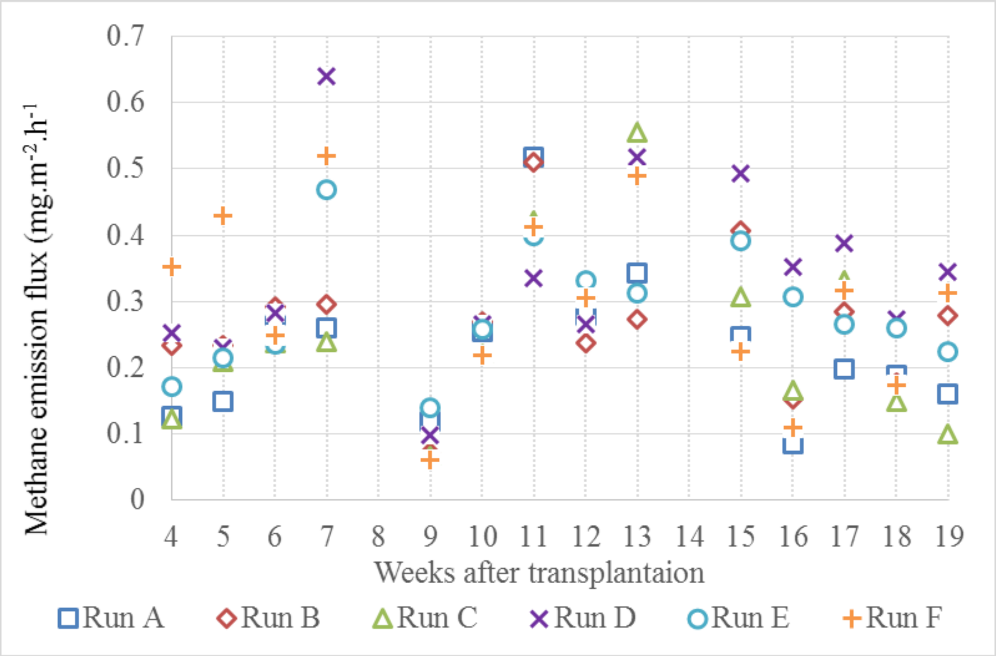
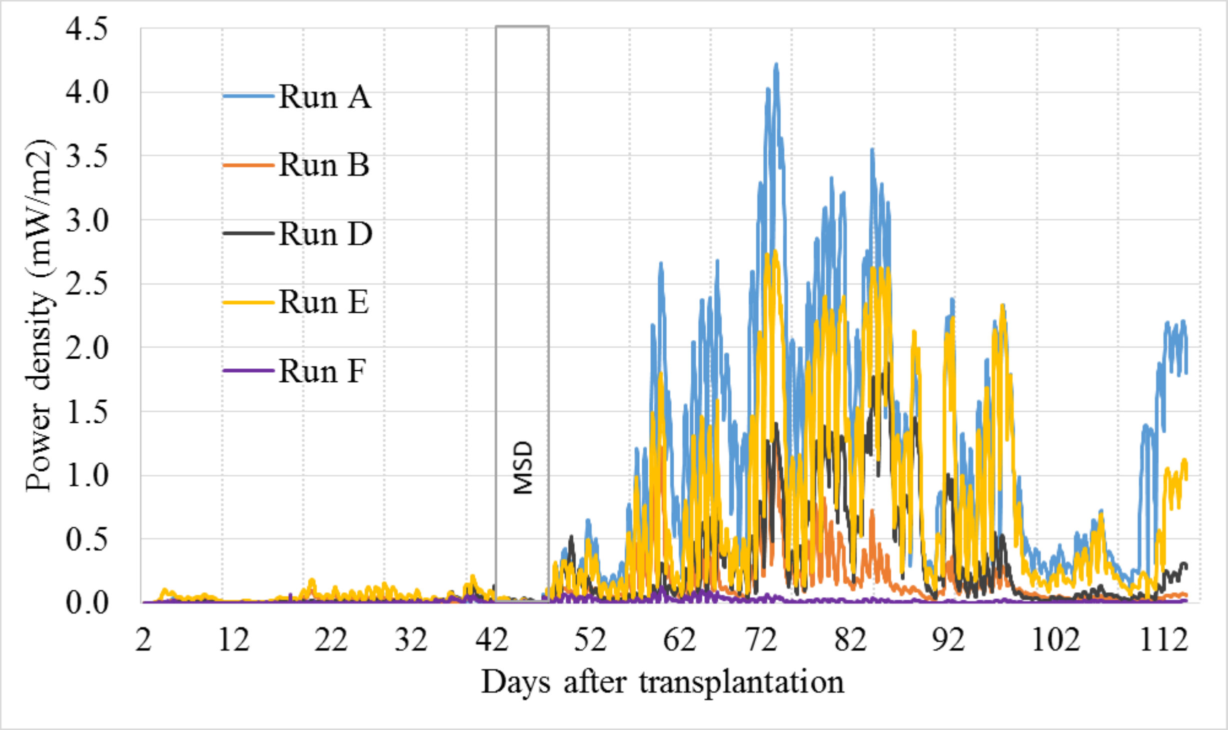


Figure 4.2. Seasonal variations in methane emission flux (mg/m^2.h) from paddy field.

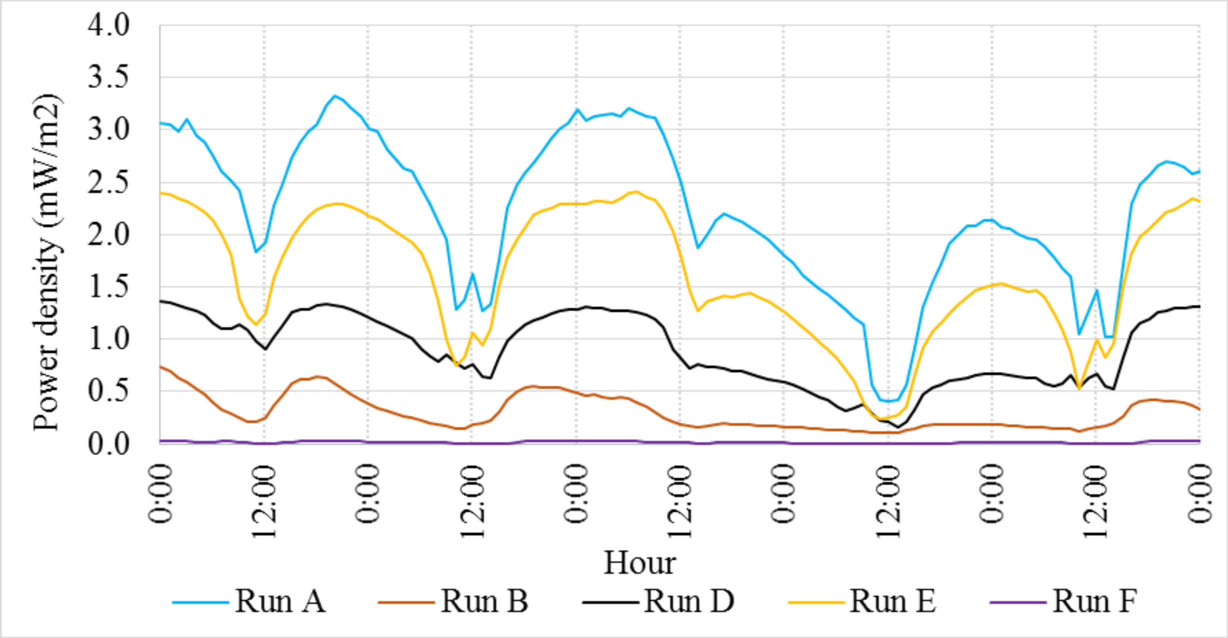
4.4.7. Electric output

Fig 4.3a shows the variation for electricity generated from PF-MFC system. Before the heading stage, the electric output obtained from the MFC systems was lower than 50 mV, which is equivalent to 0.14 mW/m^2 and much lower than the observations in the normal paddy field (Kaku et al. 2008). During the MSD, the MFC system nearly stopped since the soils were kept dried. After that, electric outputs increased rapidly and reached 4.2 mW/m^2 in Run A and 2.8 mW/m^2 in Run E. The lower output from Runs D and F may support our expectation that a higher electricity can be gained by supplying more organic matter from TWW.

Fig. 4.3b shows the day/night cycles of the output fluctuations during a 5-day period in August (flowering stage). Surprisingly, the electric output decreased in the daytime and increased at night. These fluctuations seemed in the opposite direction to those observed in the works of (De Schamphelaire et al. 2008; Kaku et al. 2008; TAKANEZAWA et al. 2010; Chiranjeevi et al. 2012; Watanabe et al. 2016) using plant-type MFC. Photosynthesis causes organic matter creation via root exudates in the anodic compartment, which would have the positive effect on the MFC performance. However, photosynthesis also causes oxygen generation through the rice roots, which would increase the redox potential in the root zone and thus have a negative effect on the electricity generation. The results obtained in the present work indicate that sunlight caused a power decrease, and so it is clear that the oxygen production effect outweighed the exudate generation.



(a)



(b)

Figure 4.3. (a) Power density from the PF-MFC. MSD means midsummer drainage to dry up the soil layers. (b) Day/night variations of power density during 5 days in the ripening stage.

4.4.8. Removal of nitrogen from treated wastewater

Figure 4.4 displays the influent and effluents quality in terms of TN. TN concentration in the influent tank varied from 19.6 to 33.5 mg/L. The TN concentration in the effluent of Run F, the top-to-top irrigation, varied from 2.4 to 18.7 mg/L, higher than those from the bottom-to-top irrigation runs. This observation was consistent with the results of the previous reports (Watanabe et al. 2016; Pham et al. 2017). The average nitrogen removal efficiencies in Runs A, B, C and E were 85, 90, 86 and 86%, respectively, much higher than that obtained from Run F (63%) implying that nitrogen removal was enhanced by infiltration of TWW through paddy soil.

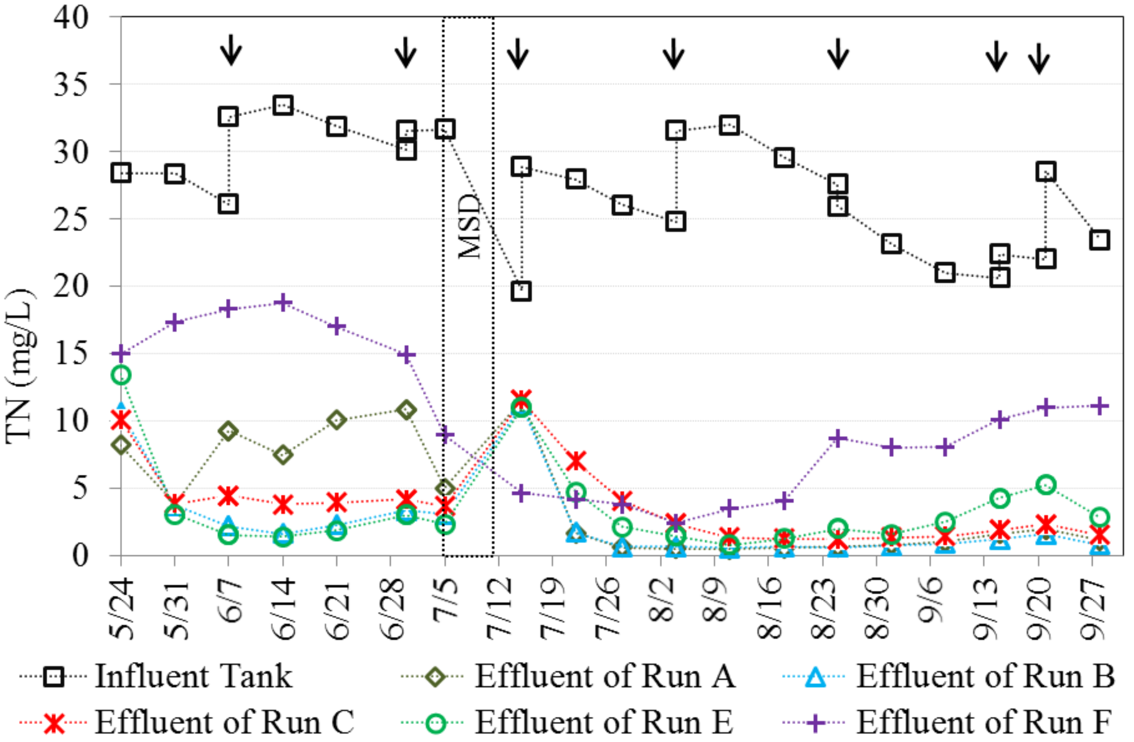


Figure 4.4. Total nitrogen of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to

4.5. Discussion

4.5.1. High rice yield and protein content could be achieved without any fertilizer

Yield was higher in bottom-to-top irrigation (Runs A, B, C and E) than top-to-top irrigation (Run F) by supplying more nutrients to the rice root as discussed above. The highest yield of rice was not obtained in Run E with P-fertilizer but in Run A without P-fertilizer, although these runs shared the common irrigation condition. The following explanations may be risen as the possible reason for this difference. First, since Runs A and F were placed at the edges of the bench-scale experimental apparatus, they may have received more solar energy than other runs, which is known as "the border effect" (Wang et al. 2013; Pham et al. 2017). Second, Run A used the new soil that contained much lower Cu concentration than the old soil used for Runs B, C and E (Table 4.6), and it has been well documented that a high copper content in soil may cause an inhibitory effect on grain yield (Yan et al. 2006; Xu et al. 2006; Pham et al. 2017). The yields obtained in Runs B, C and E were the same even Run E was applied P-fertilizer. The rice yield obtained in the control treatment (Run D) in this season was higher than in the previous season (8.3 t/ha) (Pham et al. 2017) probably due to a better climate condition in this season. The lower Cu content in the soil in Run D in this season than the previous season was also a reason.

Similar to grain yield and dry mass, the quality of brown rice via protein content in the runs received TWW was much higher than that in the control treatment with tap water irrigation. Bottom-to-top irrigation (Runs A, B, C and E) got richer-protein rice than top-to-top irrigation (Run F). These were in agreement with the observation of (Pham et al. 2017). The highest protein content in this work was higher than the top value in continuous irrigation of TWW in the previous season (13.1%) (Pham et al. 2017) and much greater than the top value in circulated irrigation (Watanabe et al. 2016) since the amount of nitrogen was supplied to the rice field increased from 6.7g (in 220L irrigation water) in circulated irrigation to 18.2g in this continuous irrigation (596L irrigation water). These protein contents also far higher than the values set in the Japanese standard of feed compositions (2009) (8.8%) as well as the grain protein in the same type of variety (Bekoaoba) was cultivated in Japan (Tsukaguchi et al. 2016) (6.2-7.0%). Protein is a key factor influencing the eating quality of rice. High protein content may reduce the eating quality of rice for human consumption, but it is preferable for animal feed. Hence, these levels of protein in rice is a great advantage of this study in case for animal feed.

4.5.2. No accumulation of heavy metals in brow rice and soil

Implementation of municipal wastewater may increase the great accumulation of harm matters in agricultural ecosystems, which may result in a potential risk to human health if these pollutants come into the food chain (Al-Lahham et al. 2007). Among the numerous hazardous matters, heavy metals are extremely persistent in the environment, and they are nonbiodegradable and nonthermodegradable, and thus, readily accumulate to toxic levels in irrigated soil then can affect human health directly through consumption of rice grown in the contaminated soils (Pham et al. 2017). Alghobar & Suresha (2016) reported that TWW irrigation significantly increased heavy metals such as Mn, Cu, Cd, Ni in the rice soil compared to the well water irrigation. A slight increase in Cd, Cu, Pb and Zn in soil was observed for domestic wastewater irrigation compared to ground water irrigation (Chung et al. 2011). The concentrations of heavy metals in the experiments soils in the present study are shown in Table 4.6. Relative to the initial soils, heavy metals concentrations in the soils after the experiment showed no considerable difference in treatments applied with TWW. However, a slight increase in Cu content was observed in the soil of the control treatment irrigated with tap water. This was attributed to the oxidation of a part of copper cable used for the MFC system. Runs B, C and E repeatedly used the soil from the previous season (Pham et al. 2017), in which Cu concentrations were much higher than that in the new soil used for Runs A, D and F.

Table 4.6. Concentrations of heavy metals in soils before and after the experiment (mg/kg).

	Before experiment		After experiment					
	Runs A, D, F	Runs B, C, E	Run A	Run B	Run C	Run D	Run E	Run F
Cu	17.7	97.1	15.0	113.4	98.7	45.1	94.3	22.4
Cr	20.5	28.5	18.2	32.0	30.4	21.9	28.7	22.7
Zn	98.0	119.2	86.9	127.4	120.1	103.3	117.0	101.3
Cd	0.06	0.07	0.07	0.05	0.05	0.05	0.04	0.04
Pb	12.8	16.1	11.4	16.6	16.9	13.3	15.7	13.4
As	1.5	3.3	5.6	2.9	1.7	2.0	1.3	2.0

The mean concentration of Cu, Cr, Zn, Cd, Pb and As in the harvested brown rice from all runs are presented in Table 4.7, did not indicate significant impacts of TWW and irrigation direction on the building-up of heavy metals in rice grain. Cadmium and lead are non-essential elements that may be phytotoxic to sensitive species at low concentrations (Chung et al. 2011). Levels of Cd, Cu and Zn in the rice from this study were varied from 0.03 to 0.09, 4.95 to 7.40 and 14.06 to 15.07 mg/kg, respectively, were lower than those in the normal Japanese rice (Herawati et al. 2000). 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 runs. The concentrations of some minerals such as K, Ca, Mg, Fe and Mn of the harvested rice are shown in Table 4.7. Compared with the Standard levels of NARO 2009, the harvested rice showed higher concentrations in Mn and Cu but lower contents in K and Fe. The concentrations of K, Mg and Zn in the harvested rice were the same range as the standard tables. The levels of all those metals in brown rice in this season also same as those were obtained in previous work (Chapter 3). However, ongoing monitoring of these harmful metals in brown rice and soil is necessary to avoid potential long-term accumulation or accidental contamination when the same paddy fields are repeatedly used for rice production with TWW irrigation.

Table 4.7. 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	Normal value of feed compositions in Japan
K	3140.71±333.82	2994.12±197.39	2859.04±104.30	2749.73±115.92	2808.88±265.80	2375.87±127.46	NA	2500.00
Ca	53.03±6.22	50.54±12.49	43.50±9.61	52.91±8.58	47.92±8.83	41.77±11.69	NA	300.00
Mg	1206.01±77.12	1160.45±72.44	1076.73±26.66	1059.99±37.85	1042.99±114.97	881.14±86.48	NA	900.00
Fe	16.88±4.94	14.24±2.07	13.26±1.70	11.91±0.84	13.04±1.23	12.35±1.99	NA	36.00
Mn	29.11±1.12	30.09±2.64	26.27±5.91	26.37±3.02	29.92±4.37	33.42±3.83	NA	21.00
Cu	4.95±0.51	7.40±0.63	6.31±1.24	5.20±0.54	6.58±0.92	6.88±0.88	NA	3.30
Zn	15.03±0.73	15.07±3.21	14.06±2.05	14.18±0.72	14.79±0.75	14.83±2.01	NA	17.00
Cr	0.04±0.02	0.05±0.02	0.04±0.01	0.05±0.02	0.04±0.01	0.03±0.01	NA	NA
Cd	0.03±0.01	0.04±0.00	0.03±0.01	0.04±0.01	0.04±0.02	0.09±0.03	0.40	NA
Pb	0.03±0.01	0.03±0.01	0.03±0.00	0.02±0.00	0.03±0.01	0.02±0.00	0.20	NA
As	0.16±0.01	0.13±0.02	0.17±0.01	0.25±0.02	0.16±0.02	0.14±0.01	NA	NA

NA: Not available

4.6. Summary

To develop a more attractive system to cultivate rice for animal feeding based on the findings from the previous season, six treatments with different conditions were conducted using the same bench-scale as the previous season and the main achievements are listed below.

- Despite our previous study achieved high quality and yield rice for animal feeding with continuous TWW irrigation using a bench-scale apparatus, we performed an experiment using the same apparatus with six different experimental conditions indicated following discoveries:A very high rice quality and yield could be achieved by continuous TWW irrigation without any P-fertilization. Soil P remained in the first season must have been used instead. Monitoring available P in the soil before cultivation is also recommended.
- CH4 emission in this season was detected since the copper content in soil was reduced. However, its emission fluxes were still low due to unknown reasons.
- No hazardous metals were built up in the soil and harvested rice with the continuous TWW irrigation when the soil was used repeatedly. However, monitoring of heavy metals in the soil and brown rice in every season is highly recommended to avoid long-term of accumulation or accidental contamination.
- The electric output from the MFC system was still low, compared to normal paddy fields, even when the connection was modified using graphite rods instead of copper cables. Further studies are necessary for a deeper understanding of this issue.

Reference

Al-Lahham, O., N. M. El Assi, and M. Fayyad. 2007. Translocation of Heavy Metals to Tomato (Solanum Lycopersicom L.) Fruit Irrigated with Treated Wastewater. *Scientia Horticulturae* 113 (3): 250–54. doi:10.1016/j.scienta.2007.03.017.

Alghobar, Mohammed Abdullah, and Sidduraiah Suresha. 2016. Effect of Wastewater Irrigation on Growth and Yield of Rice Crop and Uptake and Accumulation of Nutrient and Heavy Metals in Soil. *Applied Ecology and Environmental Sciences* 4 (3): 53–60. doi:10.12691/aees-4-3-1.

AOAC. 1990. Official Methods of Analysis. *Association of Official Agricultural Chemists. Washington, D.C.* 15th (Volume 1): 136–38.

Chen, Huai, Q I U A N Zhu, Changhui Peng, Ning Wu, and Yanfen Wang. 2013. Methane Emissions from Rice Paddies Natural Wetlands , Lakes in China : Synthesis New Estimate. *Global Change Biology* 19: 19–32. doi:10.1111/gcb.12034.

Chiranjeevi, P., G. Mohanakrishna, and S. Venkata Mohan. 2012. Rhizosphere Mediated Electrogenesis with the Function of Anode Placement for Harnessing Bioenergy through CO2 Sequestration. *Bioresource Technology* 124. Elsevier Ltd: 364–70. doi:10.1016/j.biortech.2012.08.020.

Chung, B. Y., C. H. Song, B. J. Park, and J. Y. Cho. 2011. Heavy Metals in Brown Rice (Oryza Sativa L.) and Soil After Long-Term Irrigation of Wastewater Discharged from Domestic Sewage Treatment Plants. *Pedosphere* 21 (5): 621–27. doi:10.1016/S1002-0160(11)60164-1.

Corbella, Clara, Marianna Garfí, and Jaume Puigagut. 2014. Vertical Redox Profiles in Treatment Wetlands as Function of Hydraulic Regime and Macrophytes Presence: Surveying the Optimal Scenario for Microbial Fuel Cell Implementation. *Science of the Total Environment* 470–471. Elsevier B.V.: 754–58. doi:10.1016/j.scitotenv.2013.09.068.

De Jan, Vries, E. Miller Paige, and Verbeke Kristin. 2015. Effects of Cereal Fiber on Bowel Function: A Systematic Review of Intervention Trials. *World Journal of Gastroenterology* 21 (29): 8868–77. doi:10.3748/wjg.v21.i29.8952.

De Schamphelaire, L., A. Cabezas, M. Marzorati, M. W. Friedrich, N. Boon, and W. Verstraete. 2010. Microbial Community Analysis of Anodes from Sediment Microbial Fuel Cells Powered by Rhizodeposits of Living Rice Plants. *Applied and Environmental Microbiology* 76 (6): 2002–8. doi:10.1128/AEM.02432-09.

De Schamphelaire, Liesie, Leen Van Den Bossche, Son Dang Hai, Monica H??fte, Nico Boon, Korneel Rabaey, and Willy Verstraete. 2008. Microbial Fuel Cells Generating Electricity from Rhizodeposits of Rice Plants. *Environmental Science and Technology* 42 (8): 3053–58. doi:10.1021/es071938w.

Devi, G Nirmala, G Padmavathi, and V Ravindra Babu. 2015. Proximate Nutritional Evaluation of Rice (Oryza Sativa L .). *Journal of Rice Research* 8 (1): 23–32.

Dong Duy Pham, Sumiko Kurashima, Nobuo Kaku, Atsushi Sasaki, Jian Pu and Toru Watanabe. 2017. Bottom-to-Top Continuous Irrigation of Treated Municipal Wastewater for Effective Nitrogen Removal and High Quality Rice for Animal Feeding. *Water Science and Technology: Water Supply* in press. doi:10.2166/ws.2017.190.

EU Communities. 2006. *Commission Regulation (EC) No 1881/2006 - Setting Maximum Levels for Certain Contaminants in Foodstuffs*. Vol. L364. doi:10.2203/dose-response.06-012.Hanekamp.

Fageria, N. K., and V. C. Baligar. 1999. Yield and Yield Components of Lowland Rice as Influenced by Timing of Nitrogen Fertilization. *Journal of Plant Nutrition* 22 (1): 23–32. doi:10.1080/01904169909365603.

Fang, Zhou, Hai Liang Song, Ning Cang, and Xian Ning Li. 2013. Performance of Microbial Fuel Cell Coupled Constructed Wetland System for Decolorization of Azo Dye and Bioelectricity Generation. *Bioresource Technology* 144. Elsevier Ltd: 165–71. doi:10.1016/j.biortech.2013.06.073.

FAO. 2017. FAOSTAT Emission Database. http://www.fao.org/faostat/en/#data. Accessed February 2017.

FAO/WHO. 2004. Evaluation of Certain Food Additives and Contaminants. Sixty-First Report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series 922; WHO: Geneva*, 127–32.

Herawati, N., S. Suzuki, K. Hayashi, I. F. Rivai, and H. Koyama. 2000. Cadmium, Copper, and Zinc Levels in Rice and Soil of Japan, Indonesia, and China by Soil Type. *Bulletin of Environmental Contamination and Toxicology* 64 (1): 33–39. doi:10.1007/s001289910006.

Kaku, Nobuo, Natsuki Yonezawa, Yumiko Kodama, and Kazuya Watanabe. 2008. Plant/microbe Cooperation for Electricity Generation in a Rice Paddy Field. *Applied Microbiology and Biotechnology* 79 (1): 43–49. doi:10.1007/s00253-008-1410-9.

Kazuya Watanabe and Koichi Nishio. 2010. *Electric Power from Rice Paddy Fields. Paths to Sustainable Energy*. doi:10.5772/12929.

Lee, Hyo Jung, Sang Yoon Kim, Pil Joo Kim, Eugene L Madsen, and Che Ok Jeon. 2014. Methane Emission and Dynamics of Methanotrophic and Methanogenic Communities in a Flooded Rice Field Ecosystem. *Federation of European Mcrobiological Scieties* 88: 195–

212. doi:10.1111/1574-6941.12282.

Liu, Gang, Haiyang Yu, Jing Ma, Hua Xu, Qinyan Wu, Jinghui Yang, and Yiqing Zhuang. 2015. Effects of Straw Incorporation along with Microbial Inoculant on Methane and Nitrous Oxide Emissions from Rice Fields. *Science of the Total Environment* 518–519. Elsevier B.V.: 209–16. doi:10.1016/j.scitotenv.2015.02.028.

Mao, Ting Ting, Rui Yin, and Huan Deng. 2015. Effects of Copper on Methane Emission, Methanogens and Methanotrophs in the Rhizosphere and Bulk Soil of Rice Paddy. *Catena* 133 (October). Elsevier B.V.: 233–40. doi:10.1016/j.catena.2015.05.024.

Meijide, A, C Gruening, I Goded, G Seufert, and A Cescatti. 2016. Water Management Reduces Greenhouse Gas Emissions in a Mediterranean Rice Paddy Fi Eld. *Agriculture, Ecosystems and Environment* 238. Elsevier B.V.: 168–78. doi:10.1016/j.agee.2016.08.017.

Muramatsu, A., H. Ito, A. Sasaki, A. Kajihara, and T. Watanabe. 2015. 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. *Water Science and Technology* 72 (4): 579–84. doi:10.2166/wst.2015.251.

Muramatsu, Ayumi, Toru Watanabe, Atsushi Sasaki, Hiroaki Ito, and Akihiko Kajihara. 2014. Rice Production with Minimal Irrigation and No Nitrogen Fertilizer by Intensive Use of Treated Municipa Lwastewater. *Water Science and Technology* 70 (3): 510–16. doi:10.2166/wst.2014.250.

NARO, National Agriculture and Food Research Organization. 2009. Standard Table of Feed Compositions in Japan. *Japan Livestock Industry Association.*

Oko, B. E., Ubi, A. A., Efisue, N., and Dambaba. 2012. *Comparative Analysis of the Chemical Nutrient Composition of Selected Local and Newly Introduced Rice Varieties Grown in Ebonyi State of Nigeria*. *International Journal of Agriculture and Forestry*. Vol. 2. doi:10.5923/j.ijaf.20120202.04.

Reimers, C. E, P Girguis, H.A Stecher, and L.M Tender. 2006. Microbial Fuel Cell Energy from an Ocean Cold Seep. *Geobiology* 4: 123–36.

Reimers, C E, L M Tender, S Fertig, and W Wang. 2001. Harvesting Energy from the Marine Sediment--Water Interface. *Environmental Science & Technology* 35 (1): 192–95. doi:10.1016/j.jpowsour.2008.06.079.

Riya, Shohei, Yurie Muroi, Miu Kamimura, Sheng Zhou, Akihiko Terada, Yuso Kobara, and Masaaki Hosomi. 2015. Mitigation of CH4 and N2O Emissions from a Forage Rice Field Fertilized with Aerated Liquid Fraction of Cattle Slurry by Optimizing Water Management and Topdressing. *Ecological Engineering* 75. Elsevier B.V.: 24–32. doi:10.1016/j.ecoleng.2014.11.034.

Scott D. Bridgham, Hinsby Cadillo-Quiroz, Jason K. Keller and Quianlai Zhuang. 2013. Methane Emissions from Wetlands : Biogeochemical , Microbial , and Modeling Perspectives from Local to Global Scales. *Global Change Biology*, 1–22. doi:10.1111/gcb.12131.

Strik, David P. B. T. B., H. V.M. Hamelers (Bert), Jan F. H. Snel, and and Cees J. N. Buisman. 2008. Green Electricity Production with Living Plants and Bacteria in a Fuel Cell. *International Journal of Energy Research* 32: 870–76. doi:10.1002/er.

Takanezawa, Kazuko, Koichi Nishio, Souichiro Kato, Kazuhito Hashimoto, and Kazuya Watanabe. 2010. Factors Affecting Electric Output from Rice-Paddy Microbial Fuel Cells. *Bioscience, Biotechnology and Biochemistry* 74 (6): 1271–73. doi:10.1271/bbb.90852.

Tender, Leonard M, Clare E Reimers, Hilmar a Stecher, Dawn E Holmes, Daniel R Bond, Daniel a Lowy, Kanoelani Pilobello, Stephanie J Fertig, and Derek R Lovley. 2002. Harnessing Microbially Generated Power on the Seafloor. *Nature Biotechnology* 20 (8): 821–25. doi:10.1038/nbt716.

Tsukaguchi, Tadashi, Sumiyo Nitta, and Yuri Matsuno. 2016. Cultivar Differences in the Grain Protein Accumulation Ability in Rice (Oryza Sativa L.). *Field Crops Research* 192. Elsevier B.V.: 110–17. doi:10.1016/j.fcr.2016.04.022.

Ueoka, Nagayoshi, Naoko Sese, Mayu Sue, Atsushi Kouzuma, and Kazuya Watanabe. 2016. Sizes of Anode and Cathode Affect Electricity Generation in Rice Paddy-Field Microbial Fuel Cells. *Journal of Sustainable Bioenergy Systems* 6: 10–15.

Venkata Mohan, S., G. Mohanakrishna, and P. Chiranjeevi. 2011. Sustainable Power Generation from Floating Macrophytes Based Ecological Microenvironment through Embedded Fuel Cells along with Simultaneous Wastewater Treatment. *Bioresource Technology* 102 (14). Elsevier Ltd: 7036–42. doi:10.1016/j.biortech.2011.04.033.

Villaseñor, J., P. Capilla, M. A. Rodrigo, P. Cañizares, and F. J. Fernández. 2013. Operation of a Horizontal Subsurface Flow Constructed Wetland - Microbial Fuel Cell Treating Wastewater under Different Organic Loading Rates. *Water Research* 47 (17): 6731–38. doi:10.1016/j.watres.2013.09.005.

Wang, Kai, Hongying Zhou, Bangju Wang, Zaiping Jian, Fei Wang, Jianliang Huang, Lixiao Nie, Kehui Cui, and Shaobing Peng. 2013. Quantification of Border Effect on Grain Yield Measurement of Hybrid Rice. *Field Crops Research* 141. Elsevier B.V.: 47–54. doi:10.1016/j.fcr.2012.11.012.

Watanabe, Toru, Takuma Mashiko, Rizki Maftukhah, Nobuo Kaku, Dong Duy Pham, and Hiroaki Ito. 2016. Nitrogen Removal and Power Generation from Treated Municipal Wastewater by Its Circulated Irrigation for Resource-Saving Rice Cultivation. *Water Science and Technology* 75 (4): 898–907. doi:10.2166/wst.2016.572.

Win, Khin Thawda, Ryoko Nonaka, Aye Thida Win, Yu Sasada, Koki Toyota, and Takashi Motobayashi. 2013. Effects of Water Saving Irrigation and Rice Variety on Greenhouse Gas Emissions and Water Use Efficiency in a Paddy Field Fertilized with Anaerobically Digested Pig Slurry. *Paddy and Water Environment* 13 (1): 51–60. doi:10.1007/s10333-013-0406-y.

Xu, Jiakuan, Lianxin Yang, Ziqiang Wang, Guichun Dong, Jianye Huang, and Yulong Wang. 2006. Toxicity of Copper on Rice Growth and Accumulation of Copper in Rice Grain in Copper Contaminated Soil. *Chemosphere* 62 (4): 602–7. doi:10.1016/j.chemosphere.2005.05.050.

Yadav, Asheesh Kumar, Purnanjali Dash, Ayusman Mohanty, Rouzbeh Abbassi, and Barada Kanta Mishra. 2012. Performance Assessment of Innovative Constructed Wetland-Microbial Fuel Cell for Electricity Production and Dye Removal. *Ecological Engineering* 47. Elsevier B.V.: 126–31. doi:10.1016/j.ecoleng.2012.06.029.

Yan, Yu Ping, Jun Yu He, Cheng Zhu, Chang Cheng, Xue Bo Pan, and Zhong Yang Sun. 2006. Accumulation of Copper in Brown Rice and Effect of Copper on Rice Growth and Grain Yield in Different Rice Cultivars. *Chemosphere* 65 (10): 1690–96. doi:10.1016/j.chemosphere.2006.05.022.

Yang, Shihong, Shizhang Peng, Junzeng Xu, Yufeng Luo, and Daoxi Li. 2012. Methane and Nitrous Oxide Emissions from Paddy Field as Affected by Water-Saving Irrigation.

Physics and Chemistry of the Earth 53–54. Elsevier Ltd: 30–37. doi:10.1016/j.pce.2011.08.020.

Zhong, Wen-hui, Lv-cheng Cai, Zheng-gui Wei, Hong-jing Xue, Cheng Han, and Huan Deng. 2017. The Effects of Closed Circuit Microbial Fuel Cells on Methane Emissions from Paddy Soil Vary with Straw Amount. *Catena* 154. Elsevier B.V.: 33–39. doi:10.1016/j.catena.2017.02.023.

Zou, Jianwen, Shuwei Liu, Yanmei Qin, Genxing Pan, and Dawei Zhu. 2009. Sewage Irrigation Increased Methane and Nitrous Oxide Emissions from Rice Paddies in Southeast China. *Agriculture, Ecosystems and Environment* 129 (4): 516–22. doi:10.1016/j.agee.2008.11.006.

Chapter 5

Greenhouse gases emission from paddy field under continuous irrigation of treated wastewater without P-fertilizer

5.1. Introduction

In 2015 and 2016 seasons, experiment on rice production for animal feed was conducted using bench-scale apparatus and found that compared with top-to-top irrigation, bottom-to-top irrigation increased nitrogen removal from irrigated wastewater, rice yield, dry mass and rice quality. Very high rice yield and quality were obtained under TWW irrigation without any P-fertilization. No building up of harmful metals was found in soil and harvested rice after two farming seasons. However, long-term irrigation of wastewater may increase the excessive accumulation of harmful metals in agricultural ecosystems, which may cause a potential risk to human health if these pollutants enter the food chain (Chung et al. 2011), and very few reported data related to heavy metals levels in rice and soil after long-term irrigation with TWW. N is abundant in wastewater and even in treated wastewater, however, P content in TWW is low. Thus, rice cultivation under TWW irrigation without P-fertilization would decrease in yield and quality, especially after long-term application.

CH_4 and N_2O are two important greenhouse gases emitted mostly from soil biotic sources and lead to chemical changes in the atmosphere (Majumdar 2003). CH_4 is produced in anaerobic environments by obligate anaerobic microorganisms through either CO_2 reduction or transmethylation processes (Hou et al. 2000). Most of the N_2O is produced through the biological processes of nitrification and denitrification (Signor et al. 2013). Rice cultivation is considered one of the most important sources of atmospheric CH_4 and possibly an important source of N_2O (Hou et al. 2000). As discussed in chapter 1, CH_4 and N_2O emission from paddy fields may be affected by many factors such as water regime, organic matter and nitrogen resource including fertilizer. Municipal wastewater is rich in organic matters and contains an appreciable amount of macronutrients and micronutrients, and thus nutrient and organic matter levels of soils are expected to increase with its irrigation. In 2015 season experiment (chapter 3), we tried to measure CH_4 emission from paddy field but the detection was not successful probably due to the inhibitory effect of high concentration of Cu in the experiment soil on CH_4

production. In the following year (chapter 4), we also tried to measure CH_4 emission from paddy filed when the Cu content in soil was reduced. CH_4 emission was detected but at the very low emission fluxes, compared with those were reported from normal paddy fields due to unknown reason and need to be assessed in further studies. Moreover, in the evaluation the effects of TWW irrigation on greenhouse gases emission and its impact on global warming, only CH_4 emission measurement is not enough since TWW irrigation may increase N_2O emission due to its high N concentration. Thus, in this season cultivation, we would determine both these gases to have a more meaningful evaluation the impact of TWW irrigation on global warming.

5.2. Objectives

For the development of a more attractive system to cultivate rice for animal feeding with continuous TWW irrigation based on our previous studies, the objectives of this study are still demonstrate a high rice yield and quality for animal feeding achieved without P-fertilization and to assess CH_4 and N_2O emission from the paddy field as well as electricity generation. The accumulation of harmful metals in rice and soil after three seasons with TWW irrigation was also evaluated.

5.3. Materials and Methods

5.3.1. Experimental apparatus

The experimental installation was exactly same as the bench-scale apparatus that was used in the 2015 and 2016 season. Four treatments without replicates were carried out with different growing conditions (Table 5.1). In Run A, TWW was delivered from bottom-to-top at the flow rate of 4.5 L/d. Run C was supplied 2.0 L/d of TWW with the same irrigation direction as in Run A. Run F was supplied from top-to-top at the same flow rate as Run A. Run D was implemented as the control, in which N-P-K fertilizers was supplied and tap water was added manually to maintain the water depth due to the water loss via evapotranspiration.

5.3.2. Cultivation management

Water used for the experiment was taken from the effluent of the same municipal wastewater treatment plant that was described in Chapter 3. The soil for Run D was taken on April 3, 2017, from the surface layer (0 to 20 cm) of a paddy field in the farm of Yamagata University (Tsuruoka, Yamagata, Japan); while the other runs repeatedly used the soil from our experiment in the 2016 season.

Same rice variety as 2015 and 2016 season was used. Rice seeds were sown in a plastic tray on April 17, 2017 and then seedlings were transplanted in the bench-scale apparatus on May 17, 2017, at the same density as that was described in Chapter 3. Water depth of approximately 5 cm was constantly maintained after transplantation. Midsummer drainage (MSD) was conducted from July 10 to 16, in which water supply was stopped and paddy soil was kept in dried in order to enhance rice root growth by serving oxygen to the rice root zone. The rice grains were finally harvested on October 1, 2017.

In Run D, a basal fertilization rate of 160 kg/ha N-P-K was applied before transplantation and a top-dressing of N-K fertilizer at the rate of 100 kg/ha was applied on July 16, 2017, just 22 days before the flowering.

Table 5.1. Experimental conditions.

Cultivation condition	Run A	Run C	Run D	Run F
Soil	Old		New	Old
Water	TWW		Tap water	TWW
Flow rate (L/day)	4.5	2.0	Depend on evapotranspiration	4.5
Flow Direction	Bottom-to-top		No flow	Top-to-top
Water supply	Continuous		As needed	Continuous
Chemical Fertilizer	No		N, P, K (for basal); and N-K (14 days before flowering)	No

TWW: Treated municipal wastewater.

5.3.3. Field measurement and sampling

Rice and Soil, water quality and CH_4 gas emission were determined using the same methods as 2016 season. N_2O gas was taken at the same time as CH_4 to a vacuumed 10-ml-glass vial then was analyzed by a Shimadzu GC-14B gas chromatography with an electron capture detector (ECD). When CH_4 and N_2O were sampled, the soil redox potentials (Eh) were simultaneously measured by using an Eh meter (Fujiwara PRN-41, Japan). For the measurements of soil Eh, the platinum Eh electrode (EP-201, Fujiwara, 24 cm) was installed permanently at around 10 cm soil depth throughout the rice cultivation period.

The platinum Eh electrode (EP-201, Fujiwara, 24 cm) was installed permanently at 10 cm soil depth during growing season. The soil redox potential (Eh) was measured during gas sampling using an Eh meter (PRN-41, Fujiwara, DKK-TOA Corporation).

5.4. Results

5.4.1. Irrigation water quality

The average qualities of irrigated wastewater during the experiment are displayed in Table 5.2. pH was in the range of 7.0-7.6. ORP varied from 196.5 to 278 mV. EC was maintained in the rage of 61.7 – 71.7 mS/m and DO was in the range of 2.9-4.8 mg/l.

Table 5.2. Monthly average quality of irrigation water.

	pH	ORP	EC	DO	TP	Cu	Cr	Zn	Cd	Pb	As
		mV	mS/m	mg/L				µg/L			
May	7.3	196.5	71.7	4.8	0.3	8.5	NA	25.4	0.2	3.2	NA
June	7.3	210.8	61.7	2.9	0.3	7.7	NA	14.1	NA	1.2	NA
July	7.6	219.3	67.4	3.2	0.4	7.8	NA	13.5	0.2	4.1	NA
August	7.2	234.0	67.7	2.9	0.2	7.4	NA	12.1	NA	0.9	0.2
September	7.0	278.5	64.2	3.0	0.2	6.5	NA	18.5	NA	1.6	0.3

NA: Not available

5.4.2. Growth of rice plant

The mean values for highest plant height, tiller number and chlorophyll content on flag leaf at the end of the vegetative stage are presented in Table 5.3. There were no significant differences in average plant height between runs. The plant height was the same in Run A and Run D. It was 100.5 and 101.4 cm in Run A and Run D, respectively. Comparisons of the plant height between Runs A, C and F indicate the TWW irrigation upward at higher flow rate could encourage the growth of the rice plant by supplying more nutrient to rice root. However, the effect of flow direction and flow rate in this case was not significant. The plant heights in Run A and Run D in this season were similar to those in the previous season (Table 4.3). The same range of SPAD value was obtained in all runs (45.9-47.2), which were similar to those in the previous season. Maximum shoots/hill (26.8 shoots/hill) was produced from the control (Run D), followed by Run A (23.8 shoots/hill) and Run C (19.5 shoots/hill), and minimum in the top-to-top irrigation of TWW (Run F, 16.5 shoots/hill), indicated the effect of flow rate or flow direction on rice shoot initiation was significant.

Table 5.3. Comparison of crop growth characteristics.

	Height of rice plant (cm)	Number of shoot per hill	SPAD
Run A	100.5±2.0	23.8±2.2	47.2±1.7
Run C	95.3±3.5	19.5±2.6	46.8±2.6
Run D	101.4±2.8	26.8±2.5	45.9±1.5
Run F	92.8±4.3	16.5±3.0	46.0±1.4
Run D*	99.7±0.3	25.8±1.7	46.0±1.1

*: results of the control in 2016 season which was conducted in the same condition as the control in this season (Run D).

5.4.3. Grain yield, yield components and biomass

Table 5.4 shows the yield components and grain yield from all runs. Rice cultivation with N-P-K fertilizer could produce more rice ear than TWW irrigation. The highest rice ear density was produced from the control (489 ear/m^2), higher than that was produced in the control in the previous season. This may thanks to the better climate condition in this season than the previous season. Same as the last two seasons, TWW irrigation from bottom-to-top created higher rice ear (400-472 ear/m^2) than the top-to-top irrigation. There was no significant influence of TWW irrigation or flow direction on the number of kernels per ear, but it was significantly affected by the flow rate of TWW. The mean number of kernels per ear was 89.2, much greater than 63.5 that in Run C. The single weight of the grain in all runs was similar, indicated that TWW, flow direction or flow rate of irrigation did not affect the weight of rice grain. Among all runs, Run D got the highest rate of manured kernels (93.9%), against the lowest rate in Run A since Run A received much nutrients and the rice plant had still created more young ear. As a consequence of the high ear density and number of kernels per ear, Run A achieved the yield of 10.4 t/ha. Run D got the same rice yield as in Run A (10.1 t/ha) thanks to highest ear density and manured kernels were produced. Run C and Run F achieved lower yields than two others, 9.1 and 9.4 t/ha, respectively. The difference in rice yield in this season from the previous season could be attributed to a better climate condition was in this season.

Table 5.4. Yield components and grain yield.

	Panicle density (panicles/m^2)	Grain per panicle (grains/panicle)	Single-grain weight (mg)	Filled grain (%)	Yield of rice (t/ha)
Run A	472	89.2	29.5	82.9	10.4
Run C	400	63.5	30.6	91.3	9.1
Run D	489	74.3	29.2	93.9	10.1
Run F	367	86.5	31.4	90.7	9.4
Run D*	461	63.6	30.4	91.3	9.0

*: results of the control in 2016 season which was conducted in the same condition as the control in this season (Run D).

Same as the previous seasons, this season we also evaluated the dry mass of the whole plant excluding rice grain. TWW irrigation from bottom-to-top at the highest flow rate significantly increased the dry mass compared with tap water irrigation or top-to-top irrigation of TWW. The control in this season achieved much greater plant dry mass (11.7 t/ha) than the previous season (10.4 t/ha). Besides, the quality of brown rice was also evaluated via protein content in rice – a very important parameter for the quality of animal feed. The highest protein content was obtained in Run A (12.8%), followed by Run C (11.8%). Run D got the same rice protein level (10.4%) as Run F (10.3%), and same as the value of the control in the previous season (10.3%).

Table 5.5. Protein content in brown rice and dry biomass of whole plant.

	Dry Biomass (t/ha)	Protein content (%)
Run A	13.2	12.8
Run C	11.1	11.8
Run D	11.7	10.4
Run F	10.4	10.3
Run D*	10.4	10.3

*: results of the control in 2016 season which was conducted in the same condition as the control in this season (Run D).

5.4.4. Greenhouse gases emission

Methane emission fluxes are presented in Figure 5.3. The emission flux of CH_4 under tap water irrigation was significantly greater than those under TWW irrigation. In contrast, in TWW irrigation runs, Run F emitted the highest CH_4 flux, while Run A produced the lowest CH_4. This may attribute to the bottom-to-top irrigation in Run A and C, since TWW was fed from the bottom of the field supplied oxygen to the anaerobic soil layer and negatively affected on methanogens bacteria activities as well as stimulates the oxidation of produced methane gas, and the higher flow rate was applied, the more severely effect was caused. Under TWW irrigation upward, CH_4 emission fluxes averaged 0.18 mg/m^2.h for the irrigation flow rate 4.5 L/day and 0.23 mg/m^2.h for the irrigation flow rate 2.0 L/day. In TWW irrigation from the paddy field surface, CH_4 emission fluxes averaged 0.28 mg/m^2.h, while it was 4.37 mg/m^2.h in the control used tap water. Before the week 12 of rice growing, CH_4 emission flux in the control was comparable to those in TWW irrigation, all the emission fluxes were lower than 0.5 mg/m^2.h. However, after it increased significantly and reached 16.23 mg/m^2.h in the week 17.

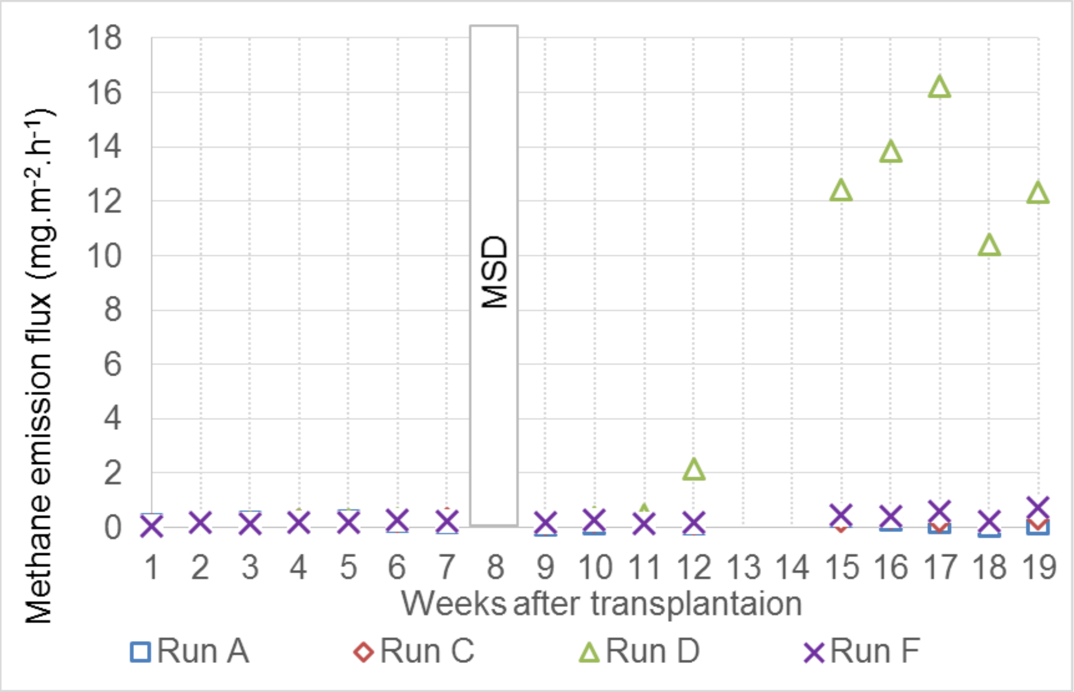


Figure 5.3. Seasonal variations in methane emission flux (mg/m^2.h) from the paddy field. Weeks 13 and 14 were the flowering stage, gas sampling had to be stopped.

Seasonal variations in N_2O emission flux are shown in Figure 5.4. N_2O emission was highest in top-to-top irrigation (Run F) with the average emission flux 505.3 µg/m^2.h. Average N_2O emission rate from tap water irrigation was the lowest value (46.2 µg/m^2.h). however, this emission rate was comparable with normal paddy fields (Yang et al. 2012). When bottom-to-top irrigation of TWW was applied, seasonal fluxes of N_2O averaged 180.0 µg/m^2.h for the higher irrigation flow rate (Run A) and 55.7 µg/m^2.h for the lower irrigation flow rate (Run C). Compared with the control (Run D), therefore, TWW irrigation increased N_2O emission by 242, 21 and 994% in upward TWW irrigation at the highest flow rate, lowest rate and top-to-top irrigation, respectively.

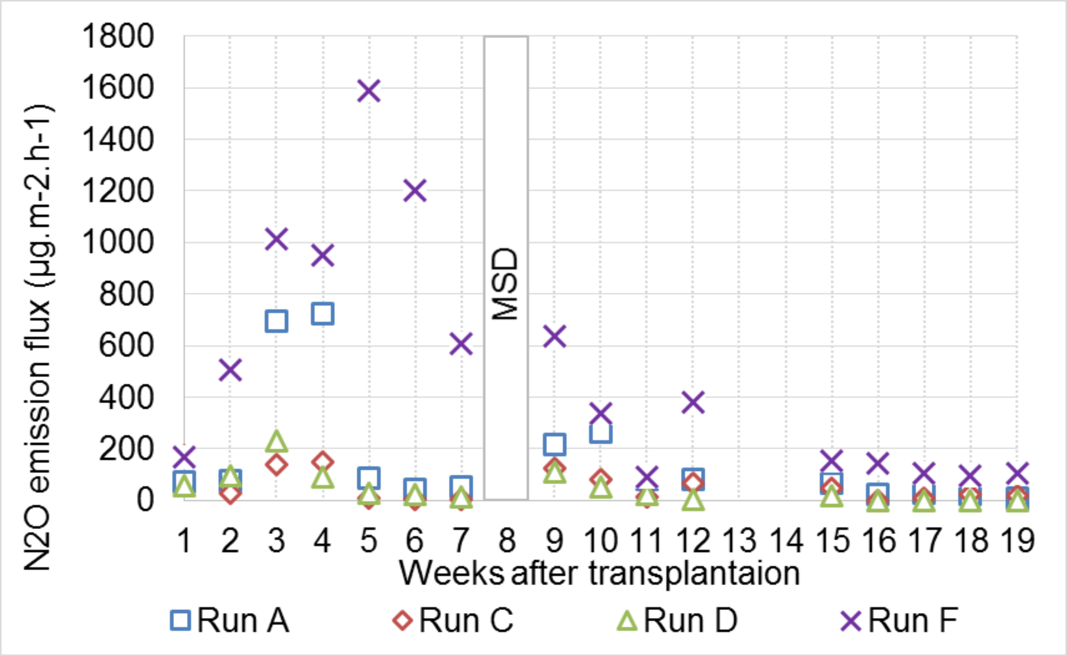


Figure 5.4. Seasonal variations in nitrous oxide emission flux (µg/m^2.h) from the paddy field.

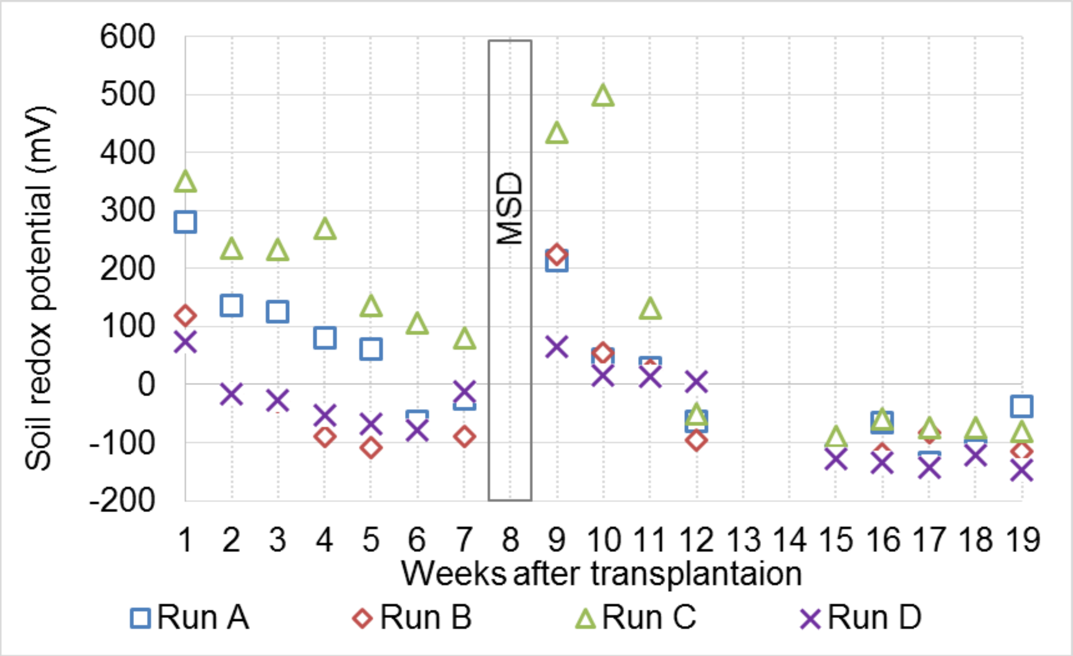


Figure 5.5. Seasonal variations in soil redox potential (mV) of the paddy soils.

Global warming potential (GWP) is an indicator that compares the contributions of GHGs to the atmospheric temperature. In GWP estimation, CO_2 is typically used as the reference gas, and an increase or reduction in emission of CH_4 and N_2O is converted into CO_2-equivalents by means of their GWP. (Zhang et al. 2014). In the present study, we used the IPCC factors to calculate the combined GWPs (CO2-equivalents, 100-year time horizon) from CH_4 and N_2O for all runs (IPPC 2007). As shown in Table 5.6, TWW irrigation from the surface of the paddy field significantly increased combined GWP while it was decreased when the TWW was supplied from the bottom of the rice field, compared with tap water irrigation.

Table 5.6. Average emission fluxes of CH_4 and N_2O from paddy fields and their net GWPs (CO_2-equivalents).

	Run A	Run C	Run D	Run F
CH_4 average emission flux (mg/m^2.h)	0.18	0.23	4.37	0.28
N_2O average emission flux (µg/m^2.h)	158.0	55.7	46.2	505.3
CH4 and N2O average net GWP (mg CO2-equip/m2.h) *	52	22	123	158

*: The GWPs factors (mass basis) for CH_4 and N_2O are 25 and 298 in the time horizon of 100 years, respectively (IPPC 2007).

5.4.5. Removal of nitrogen from treated wastewater

TN concentrations in the influent and effluent tanks are displayed in Figure 5.6. TN concentration in the influent tank was in the range of 23.6 mg/l and 40.8 mg/l. TN concentration in the effluent of Run F varied from 8.2 to 30.4 mg/l, much higher than those in the effluents of Run A and Run C (0.1 mg/l - 12.2 mg/l) as a consequence of the flow direction and flow rate that was explained in chapter 3. Average nitrogen removal efficiencies in Run A and Run C were 85% and 93%, respectively, same range as those in bottom-to-top irrigation in two previous seasons; while it was 42% in Run F, much lower than those in Run F in the last two seasons.

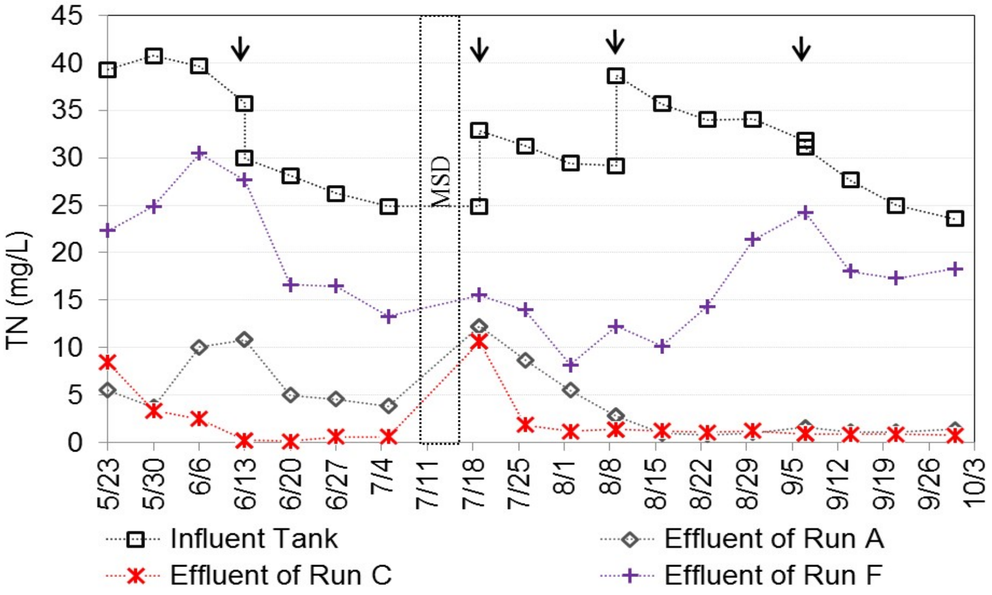


Figure 5.6. Total nitrogen of the irrigated water. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to.

5.5. Discussion

5.5.1. Bottom-to-top irrigation without any fertilization could get a high yield and a good quality of rice for animal feed

As mentioned above, rice yield in the control in this season was higher than that was obtained in the previous season, indicated that the climate condition for rice development in this season was better than the previous one. Therefore, the decrease of rice yield in Run A in this season compared with the previous season could not be attributed to the climate condition. Instead, Run A in this season used the old soil from the last two seasons with a higher Cu concentration than the new soil was used for Run A in the previous season, and this decrease may due to the adverse effect of Cu in the soil on rice yield as was discussed above. This explanation could also be applied to the decrease in rice yield in Run F in this season, compared with that in Run F in the previous season. Relative to top-to-top irrigation (Run F), bottom-to-top irrigation at the same flow rate (Run A) increased rice yield by 10.2 % and protein content in brown rice by 24.3%. while the comparison between Run A and Run C in which were applied the same flow direction of irrigation indicated that the highest flow rate increased rice yield by 14.3% and rice protein content by 8.5%. These implied that TWW irrigation upward at a high flow rate could produce high rice yield and quality without any fertilizer even when the soil was used repeatedly.

The control treatment could get the rice yield comparable with the bottom-to-top irrigation at the highest flow rate irrigation but much amount of fertilizers were applied to this treatment thus can reduce the benefit for farmers.

5.5.2. TWW irrigation decreased CH4 but increased N2O emission

Zou et al. (2009) reported that in comparison with river water irrigation, sewage irrigation significantly increase CH_4 emission form paddy field. Several explanations were given to this. First, sewage was rich in organic matters that could promote CH_4 production (Zou et al. 2005). Second, wastewater irrigation may change the condition of soil physio-chemical properties and bacterial communities that may encourage CH_4 production. However, the results of the current study did not consistent with the finding. Relative to tap water irrigation, TWW irrigation remarkably decreased CH_4 emission. It would be attributed to the inhibitory effect of nitrogen in TWW on CH_4 formation. Indeed, TWW was rich in nitrogen, and denitrification is generally believed to happen before methanogenesis, and the presence of denitrification intermediates such as NO_2^-, NO may inhibit methanogenic microorganisms thus reduce CH_4 formation (Chen and Lin 1992). In addition, during the phase of reduction of NO_3^-, NO_2^-, and N_2O, the partial pressure of H_2 may reduce to lower required concentrations to support CH_4 production (Bao et al. 2016). In normal paddy fields, a peak flux of CH_4 emission is normally obtained in the early stage of rice development (Zou et al. 2009; Yang et al. 2012). By contrast, in this study, Figure 5.3 shows that a peak of CH_4 emission was acquired in the ripening stage of the rice plant. The above inhibitory effect of nitrogen on CH_4 production would be taken to this phenomenon since in the final stage of rice growing, the concentration of nitrogen in soil was lower than the earlier stage, thus the negative effect on methanogenesis was reduced and more CH_4 could be produced.

Opposite to CH_4 emission, TWW irrigation significantly increased N_2O emission, compared with tap water irrigation, especially when top-to-top irrigation or bottom-to-top irrigation at higher flow rate was applied. This may due to the two following reasons. Firstly, high N contained in TWW increased N for nitrification and denitrification processes in soil and consequently increased N_2O emission. Secondly, C is supposed to be a key factor to control soil nitrification and denitrification processes. TWW rich in organic matter brought more C to the soil, enhanced nitrification and denitrification processes thus improved N_2O emission (Ndour et al. 2008). Among TWW irrigation, top-to-top irrigation emitted notably greater N_2O than bottom-to-top irrigation. Hou et al. (2000) reported that N_2O emission was strongly depended on soil redox potential. When soil redox potential decreased, less N_2O was emitted probably due to the reduction of N_2O to N_2 under low redox potential conditions. In this study, higher soil redox potential in Run F than other runs was recorded (Figure 5.5).

5.5.3. No accumulation of heavy metals in brown rice and soil except for Pb in brown rice

Long-term application of wastewater may increase the excessive accumulation of heavy metals in agricultural product and soil, which may cause a potential risk to human health (Chung et al. 2011). Same as the last two seasons, there was no accumulation of heavy metals in experimental soil under TWW irrigation. As shown in Table 5.7, there was no significant difference in these heavy metals concentrations between the soils before and after the experiment.

Concentrations of heavy metals and minerals in brown rice are shown in Table 5.8. Relative to the control, harmful metals concentrations in the treatments under TWW irrigation exhibited no notable difference. The concentrations of these metals in this season were also same as the concentrations in brown rice of 2015 and 2016 seasons except for a considerable increase in Pb. These concentrations of Pb were higher than the allowable level set by FAO/WHO (2004) and EU Communities (2006) for human consumption, and 10-25 times higher than those in the two previous seasons, even in the control due to unknown reason. This increase happened to the control treatment also, indicated the reason was not from TWW irrigation but due to unknown reason.

Table 5.7. Concentrations of heavy metals in soils before and after the experiment (mg/kg).

	Before experiment		After experiment			
	Runs D	Runs A, C and F	Run A	Run C	Run D	Run F
Cu	16.7	59.6	55.7	54.3	17.4	50.7
Cr	38.5	34.3	32.1	31.1	36.4	28.6
Zn	82.4	88.5	84.3	83.3	73.8	76.5
Cd	0.14	0.12	0.09	0.09	0.13	0.10
Pb	18.4	16.6	15.5	15.1	17.0	13.8
As	2.6	2.0	2.8	2.8	2.4	3.8

Table 5.8. Concentrations of heavy metals (±SD) in brown rice (mg/kg).

	Run A	Run C	Run D	Run F	Allowable limit set by FAO/WHO	Standard tables edited by NARO
K	2257.26±149.00	2310.24±168.92	2304.46±107.79	2541.28±176.63	NA	2500.00
Ca	85.97±10.56	81.95±5.46	85.55±4.15	84.75±5.13	NA	300.00
Mg	1067.92±80.88	1097.51±58.50	1140.24±54.90	1064.81±33.35	NA	900.00
Fe	14.08±1.46	13.91±0.58	18.26±6.71	16.53±1.02	NA	36.00
Mn	49.93±5.33	44.30±3.65	22.78±1.93	61.20±6.65	NA	21.00
Cu	4.27±0.44	3.56±0.27	3.19±0.28	5.17±0.46	NA	3.30
Zn	8.62±0.82	7.45±0.25	8.59±0.60	8.53±0.23	NA	17.00
Cr	0.17±0.08	0.09±0.03	0.25±0.39	0.14±0.02	NA	NA
Cd	0.04±0.01	0.05±0.02	0.02±0.01	0.14±0.04	0.40	NA
Pb	0.35±0.27	0.40±0.14	0.35±0.14	0.42±0.15	0.20	NA
As	0.11±0.02	0.12±0.03	0.06±0.01	0.11±0.01	NA	NA

NA: Not available.

5.6. Summary

Based on the results of the previous experiment, the system to cultivate rice for animal feeding was still developed in this farming season by carrying out an experiment in four treatments with difference cultivation conditions using the same bench-scale apparatus as two previous studies and found some findings.

- High rice yield and quality could be achieved in bottom-to-top irrigation of TWW at high flow rate without any fertilizer.
- TWW irrigation decreased CH_4 emission but increase N_2O emission, and thus caused higher combined global warming potential than tap water irrigation.
- Bottom-to-top irrigation produced less CH_4 and N_2O than top-to-top irrigation of TWW.
- No accumulation of heavy metals in soil and brown rice after 3-year irrigation of TWW, except for an increase in Pb contents in rice in all runs due to unknown reason, compared with the two previous seasons due to unknown reason. This rice can be used for animal feed but should not be used for human consumption.

Reference

Al-Lahham, O., N. M. El Assi, and M. Fayyad. 2007. Translocation of Heavy Metals to Tomato (Solanum Lycopersicom L.) Fruit Irrigated with Treated Wastewater. *Scientia Horticulturae* 113 (3): 250–54. doi:10.1016/j.scienta.2007.03.017.

Alghobar, Mohammed Abdullah, and Sidduraiah Suresha. 2016. Effect of Wastewater Irrigation on Growth and Yield of Rice Crop and Uptake and Accumulation of Nutrient and Heavy Metals in Soil. *Applied Ecology and Environmental Sciences* 4 (3): 53–60. doi:10.12691/aees-4-3-1.

AOAC. 1990. Official Methods of Analysis. *Association of Official Agricultural Chemists. Washington, D.C.* 15th (Volume 1): 136–38.

Bao, Qiongli, Yizong Huang, Fenghua Wang, Sanan Nie, Graeme W. Nicol, Huaiying Yao, and Longjun Ding. 2016. Effect of Nitrogen Fertilizer And/or Rice Straw Amendment on Methanogenic Archaeal Communities and Methane Production from a Rice Paddy Soil. *Applied Microbiology and Biotechnology* 100 (13). Applied Microbiology and

Biotechnology: 5989–98. doi:10.1007/s00253-016-7377-z.

Chen, Huai, Q I U A N Zhu, Changhui Peng, Ning Wu, and Yanfen Wang. 2013. Methane Emissions from Rice Paddies Natural Wetlands , Lakes in China : Synthesis New Estimate. *Global Change Biology* 19: 19–32. doi:10.1111/gcb.12034.

Chen Kuo-Cheng, Lin Ying-Feng. 1992. Inhibitory Effect of Ionic Nitrogen Oxides on Methanogenesis of Methanol in Methanogenic Sludge Authors and Affiliations. In *Proceedings of Asia-Pacific Biochemical Engineering*, 839–42. Tokyo.

Chiranjeevi, P., G. Mohanakrishna, and S. Venkata Mohan. 2012. Rhizosphere Mediated Electrogenesis with the Function of Anode Placement for Harnessing Bioenergy through CO2 Sequestration. *Bioresource Technology* 124. Elsevier Ltd: 364–70. doi:10.1016/j.biortech.2012.08.020.

Chung, B. Y., C. H. Song, B. J. Park, and J. Y. Cho. 2011. Heavy Metals in Brown Rice (Oryza Sativa L.) and Soil After Long-Term Irrigation of Wastewater Discharged from Domestic Sewage Treatment Plants. *Pedosphere* 21 (5): 621–27. doi:10.1016/S1002-0160(11)60164-1.

Corbella, Clara, Marianna Garfí, and Jaume Puigagut. 2014. Vertical Redox Profiles in Treatment Wetlands as Function of Hydraulic Regime and Macrophytes Presence: Surveying the Optimal Scenario for Microbial Fuel Cell Implementation. *Science of the Total Environment* 470–471. Elsevier B.V.: 754–58. doi:10.1016/j.scitotenv.2013.09.068.

De Jan, Vries, E. Miller Paige, and Verbeke Kristin. 2015. Effects of Cereal Fiber on Bowel Function: A Systematic Review of Intervention Trials. *World Journal of Gastroenterology* 21 (29): 8868–77. doi:10.3748/wjg.v21.i29.8952.

De Schamphelaire, L., A. Cabezas, M. Marzorati, M. W. Friedrich, N. Boon, and W. Verstraete. 2010. Microbial Community Analysis of Anodes from Sediment Microbial Fuel Cells Powered by Rhizodeposits of Living Rice Plants. *Applied and Environmental Microbiology* 76 (6): 2002–8. doi:10.1128/AEM.02432-09.

De Schamphelaire, Liesie, Leen Van Den Bossche, Son Dang Hai, Monica H??fte, Nico Boon, Korneel Rabaey, and Willy Verstraete. 2008. Microbial Fuel Cells Generating Electricity from Rhizodeposits of Rice Plants. *Environmental Science and Technology* 42 (8): 3053–58. doi:10.1021/es071938w.

Devi, G Nirmala, G Padmavathi, and V Ravindra Babu. 2015. Proximate Nutritional Evaluation of Rice (Oryza Sativa L .). *Journal of Rice Research* 8 (1): 23–32.

Dong Duy Pham, Sumiko Kurashima, Nobuo Kaku, Atsushi Sasaki, Jian Pu and Toru Watanabe. 2017. Bottom-to-Top Continuous Irrigation of Treated Municipal Wastewater for Effective Nitrogen Removal and High Quality Rice for Animal Feeding. *Water Science and Technology: Water Supply* in press. doi:10.2166/ws.2017.190.

EU Communities. 2006. *Commission Regulation (EC) No 1881/2006 - Setting Maximum Levels for Certain Contaminants in Foodstuffs*. Vol. L364. doi:10.2203/dose-response.06-012.Hanekamp.

Fageria, N. K., and V. C. Baligar. 1999. Yield and Yield Components of Lowland Rice as Influenced by Timing of Nitrogen Fertilization. *Journal of Plant Nutrition* 22 (1): 23–32. doi:10.1080/01904169909365603.

Fang, Zhou, Hai Liang Song, Ning Cang, and Xian Ning Li. 2013. Performance of Microbial Fuel Cell Coupled Constructed Wetland System for Decolorization of Azo Dye and Bioelectricity Generation. *Bioresource Technology* 144. Elsevier Ltd: 165–71. doi:10.1016/j.biortech.2013.06.073.

FAO. 2017. FAOSTAT Emission Database. http://www.fao.org/faostat/en/#data. Accessed February 2017.

FAO/WHO. 2004. Evaluation of Certain Food Additives and Contaminants. Sixty-First Report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series 922; WHO: Geneva*, 127–32.

Herawati, N., S. Suzuki, K. Hayashi, I. F. Rivai, and H. Koyama. 2000. Cadmium, Copper, and Zinc Levels in Rice and Soil of Japan, Indonesia, and China by Soil Type. *Bulletin of Environmental Contamination and Toxicology* 64 (1): 33–39. doi:10.1007/s001289910006.

Hou, A.X., G.X. Chen, Z.P. Wang, O. Van Cleemput, and W.H. Patrick. 2000. Methane and Nitrous Oxide Emissions from a Rice Field in Relation to Soil Redox and Microbiological Processes. *Soil Science Society of America Journal* 64 (6): 2180. doi:10.2136/sssaj2000.6462180x.

Kaku, Nobuo, Natsuki Yonezawa, Yumiko Kodama, and Kazuya Watanabe. 2008. Plant/microbe Cooperation for Electricity Generation in a Rice Paddy Field. *Applied*

Microbiology and Biotechnology 79 (1): 43–49. doi:10.1007/s00253-008-1410-9.

Kazuya Watanabe and Koichi Nishio. 2010. *Electric Power from Rice Paddy Fields*. *Paths to Sustainable Energy*. doi:10.5772/12929.

Lee, Hyo Jung, Sang Yoon Kim, Pil Joo Kim, Eugene L Madsen, and Che Ok Jeon. 2014. Methane Emission and Dynamics of Methanotrophic and Methanogenic Communities in a Flooded Rice Field Ecosystem. *Federation of European Mcrobiological Scieties* 88: 195–212. doi:10.1111/1574-6941.12282.

Liu, Gang, Haiyang Yu, Jing Ma, Hua Xu, Qinyan Wu, Jinghui Yang, and Yiqing Zhuang. 2015. Effects of Straw Incorporation along with Microbial Inoculant on Methane and Nitrous Oxide Emissions from Rice Fields. *Science of the Total Environment* 518–519. Elsevier B.V.: 209–16. doi:10.1016/j.scitotenv.2015.02.028.

Mao, Ting Ting, Rui Yin, and Huan Deng. 2015. Effects of Copper on Methane Emission, Methanogens and Methanotrophs in the Rhizosphere and Bulk Soil of Rice Paddy. *Catena* 133 (October). Elsevier B.V.: 233–40. doi:10.1016/j.catena.2015.05.024.

Meijide, A, C Gruening, I Goded, G Seufert, and A Cescatti. 2016. Water Management Reduces Greenhouse Gas Emissions in a Mediterranean Rice Paddy Fi Eld. *Agriculture, Ecosystems and Environment* 238. Elsevier B.V.: 168–78. doi:10.1016/j.agee.2016.08.017.

Muramatsu, A., H. Ito, A. Sasaki, A. Kajihara, and T. Watanabe. 2015. 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. *Water Science and Technology* 72 (4): 579–84. doi:10.2166/wst.2015.251.

Muramatsu, Ayumi, Toru Watanabe, Atsushi Sasaki, Hiroaki Ito, and Akihiko Kajihara. 2014. Rice Production with Minimal Irrigation and No Nitrogen Fertilizer by Intensive Use of Treated Municipa Lwastewater. *Water Science and Technology* 70 (3): 510–16. doi:10.2166/wst.2014.250.

NARO, National Agriculture and Food Research Organization. 2009. Standard Table of Feed Compositions in Japan. *Japan Livestock Industry Association.*

Ndour, Ndèye Yacine Badiane, Ezékiel Baudoin, Aliou Guissé, Mountakha Seck, Mamadou Khouma, and Alain Brauman. 2008. Impact of Irrigation Water Quality on Soil Nitrifying and Total Bacterial Communities. *Biology and Fertility of Soils* 44 (5): 797–803.

doi:10.1007/s00374-008-0285-3.

Oko, B. E., Ubi, A. A., Efisue, N., and Dambaba. 2012. *Comparative Analysis of the Chemical Nutrient Composition of Selected Local and Newly Introduced Rice Varieties Grown in Ebonyi State of Nigeria*. *International Journal of Agriculture and Forestry*. Vol. 2. doi:10.5923/j.ijaf.20120202.04.

Reimers, C. E, P Girguis, H.A Stecher, and L.M Tender. 2006. Microbial Fuel Cell Energy from an Ocean Cold Seep. *Geobiology* 4: 123–36.

Reimers, C E, L M Tender, S Fertig, and W Wang. 2001. Harvesting Energy from the Marine Sediment--Water Interface. *Environmental Science & Technology* 35 (1): 192–95. doi:10.1016/j.jpowsour.2008.06.079.

Riya, Shohei, Yurie Muroi, Miu Kamimura, Sheng Zhou, Akihiko Terada, Yuso Kobara, and Masaaki Hosomi. 2015. Mitigation of CH4 and N2O Emissions from a Forage Rice Field Fertilized with Aerated Liquid Fraction of Cattle Slurry by Optimizing Water Management and Topdressing. *Ecological Engineering* 75. Elsevier B.V.: 24–32. doi:10.1016/j.ecoleng.2014.11.034.

Scott D. Bridgham, Hinsby Cadillo-Quiroz, Jason K. Keller and Quianlai Zhuang. 2013. Methane Emissions from Wetlands : Biogeochemical , Microbial , and Modeling Perspectives from Local to Global Scales. *Global Change Biology*, 1–22. doi:10.1111/gcb.12131.

Strik, David P. B. T. B., H. V.M. Hamelers (Bert), Jan F. H. Snel, and and Cees J. N. Buisman. 2008. Green Electricity Production with Living Plants and Bacteria in a Fuel Cell. *International Journal of Energy Research* 32: 870–76. doi:10.1002/er.

Takanezawa, Kazuko, Koichi Nishio, Souichiro Kato, Kazuhito Hashimoto, and Kazuya Watanabe. 2010. Factors Affecting Electric Output from Rice-Paddy Microbial Fuel Cells. *Bioscience, Biotechnology and Biochemistry* 74 (6): 1271–73. doi:10.1271/bbb.90852.

Tender, Leonard M, Clare E Reimers, Hilmar a Stecher, Dawn E Holmes, Daniel R Bond, Daniel a Lowy, Kanoelani Pilobello, Stephanie J Fertig, and Derek R Lovley. 2002. Harnessing Microbially Generated Power on the Seafloor. *Nature Biotechnology* 20 (8): 821–25. doi:10.1038/nbt716.

Tsukaguchi, Tadashi, Sumiyo Nitta, and Yuri Matsuno. 2016. Cultivar Differences in the Grain

Protein Accumulation Ability in Rice (Oryza Sativa L.). *Field Crops Research* 192. Elsevier B.V.: 110–17. doi:10.1016/j.fcr.2016.04.022.

Ueoka, Nagayoshi, Naoko Sese, Mayu Sue, Atsushi Kouzuma, and Kazuya Watanabe. 2016. Sizes of Anode and Cathode Affect Electricity Generation in Rice Paddy-Field Microbial Fuel Cells. *Journal of Sustainable Bioenergy Systems* 6: 10–15.

Venkata Mohan, S., G. Mohanakrishna, and P. Chiranjeevi. 2011. Sustainable Power Generation from Floating Macrophytes Based Ecological Microenvironment through Embedded Fuel Cells along with Simultaneous Wastewater Treatment. *Bioresource Technology* 102 (14). Elsevier Ltd: 7036–42. doi:10.1016/j.biortech.2011.04.033.

Villaseñor, J., P. Capilla, M. A. Rodrigo, P. Cañizares, and F. J. Fernández. 2013. Operation of a Horizontal Subsurface Flow Constructed Wetland - Microbial Fuel Cell Treating Wastewater under Different Organic Loading Rates. *Water Research* 47 (17): 6731–38. doi:10.1016/j.watres.2013.09.005.

Wang, Kai, Hongying Zhou, Bangju Wang, Zaiping Jian, Fei Wang, Jianliang Huang, Lixiao Nie, Kehui Cui, and Shaobing Peng. 2013. Quantification of Border Effect on Grain Yield Measurement of Hybrid Rice. *Field Crops Research* 141. Elsevier B.V.: 47–54. doi:10.1016/j.fcr.2012.11.012.

Watanabe, Toru, Takuma Mashiko, Rizki Maftukhah, Nobuo Kaku, Dong Duy Pham, and Hiroaki Ito. 2016. Nitrogen Removal and Power Generation from Treated Municipal Wastewater by Its Circulated Irrigation for Resource-Saving Rice Cultivation. *Water Science and Technology* 75 (4): 898–907. doi:10.2166/wst.2016.572.

Win, Khin Thawda, Ryoko Nonaka, Aye Thida Win, Yu Sasada, Koki Toyota, and Takashi Motobayashi. 2013. Effects of Water Saving Irrigation and Rice Variety on Greenhouse Gas Emissions and Water Use Efficiency in a Paddy Field Fertilized with Anaerobically Digested Pig Slurry. *Paddy and Water Environment* 13 (1): 51–60. doi:10.1007/s10333-013-0406-y.

Xu, Jiakuan, Lianxin Yang, Ziqiang Wang, Guichun Dong, Jianye Huang, and Yulong Wang. 2006. Toxicity of Copper on Rice Growth and Accumulation of Copper in Rice Grain in Copper Contaminated Soil. *Chemosphere* 62 (4): 602–7. doi:10.1016/j.chemosphere.2005.05.050.

Yadav, Asheesh Kumar, Purnanjali Dash, Ayusman Mohanty, Rouzbeh Abbassi, and Barada Kanta Mishra. 2012. Performance Assessment of Innovative Constructed Wetland-Microbial Fuel Cell for Electricity Production and Dye Removal. *Ecological Engineering* 47. Elsevier B.V.: 126–31. doi:10.1016/j.ecoleng.2012.06.029.

Yan, Yu Ping, Jun Yu He, Cheng Zhu, Chang Cheng, Xue Bo Pan, and Zhong Yang Sun. 2006. Accumulation of Copper in Brown Rice and Effect of Copper on Rice Growth and Grain Yield in Different Rice Cultivars. *Chemosphere* 65 (10): 1690–96. doi:10.1016/j.chemosphere.2006.05.022.

Yang, Shihong, Shizhang Peng, Junzeng Xu, Yufeng Luo, and Daoxi Li. 2012. Methane and Nitrous Oxide Emissions from Paddy Field as Affected by Water-Saving Irrigation. *Physics and Chemistry of the Earth* 53–54. Elsevier Ltd: 30–37. doi:10.1016/j.pce.2011.08.020.

Zhong, Wen-hui, Lv-cheng Cai, Zheng-gui Wei, Hong-jing Xue, Cheng Han, and Huan Deng. 2017. The Effects of Closed Circuit Microbial Fuel Cells on Methane Emissions from Paddy Soil Vary with Straw Amount. *Catena* 154. Elsevier B.V.: 33–39. doi:10.1016/j.catena.2017.02.023.

Zou, Jianwen, Yao Huang, Jingyan Jiang, Xunhua Zheng, and Ronald L. Sass. 2005. A 3-Year Field Measurement of Methane and Nitrous Oxide Emissions from Rice Paddies in China: Effects of Water Regime, Crop Residue, and Fertilizer Application. *Global Biogeochemical Cycles* 19 (2): 1–9. doi:10.1029/2004GB002401.

Zou, Jianwen, Shuwei Liu, Yanmei Qin, Genxing Pan, and Dawei Zhu. 2009. Sewage Irrigation Increased Methane and Nitrous Oxide Emissions from Rice Paddies in Southeast China. *Agriculture, Ecosystems and Environment* 129 (4): 516–22. doi:10.1016/j.agee.2008.11.006.

Chapter 6

Conclusions and recommendations

6.1. Conclusions

Based on the outcomes 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 N removal from TWW and to cultivate high rice yield and quality rice for animal feeding. Besides, we tried to assess greenhouse gases emission from the paddy field and tried to generate electricity by installing PF-MFC in paddy field under TWW irrigation. The bench-scale experiments, including six treatments in 2015 and 2016 seasons, and four treatments in 2017 season with different cultivation conditions have revealed the following core findings.

6.1.1. Bottom-to-top irrigation enhanced nitrogen removal efficiency from TWW

In this study, top-to-top irrigation achieved a N removal efficiency from 42 to 63%, while it varied from 79 to 93% in bottom-to-top irrigation, demonstrating that much higher N removal efficiency was obtained in bottom-to-top irrigation than top-to-top irrigation, since the irrigated wastewater did not percolate through the soil layer in top-to-top irrigation and N was not absorbed as much as in bottom-to-top irrigation. 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, resulting in increased N removal efficiency.

6.1.2. High rice yield and quality could be achieved under continuous irrigation of TWW from bottom-to-top without any fertilization

Throughout three farming seasons, rice yield was always higher in bottom-to-top irrigation than top-to-top irrigation since more nutrients were supplied to the rice roots when TWW percolated through the paddy soil. Compared with top-to-top irrigation, bottom-to-top irrigation increased rice yield by 4.7 % - 37.5 %. In the 2016 season, the highest rice yields obtained in bottom-to-top irrigation was up to 14.1 t/ha, much higher than that was got in the control, but it was similar in the 2017 season. These rice yields were comparable to the results of the same variety 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. In addition, the rice yields obtained from continuous irrigation in the 3 farming seasons were also much higher than those were got in the same bench-scale apparatus applied circulated irrigation.

Similarly, the protein contents of rice harvested in these experiments were noticeably higher than those were obtained in the previous studies using circulated irrigation of TWW. The quality of brown rice via protein content in the runs receiving TWW was much higher than that in the control treatment irrigated with tap water. Bottom-to-top irrigation achieved richer-protein rice than top-to-top irrigation. These protein contents were also much higher than the values set in the Japanese standard of feed compositions as well as the grain protein in the same type of variety cultivated in Japan. Protein is a key factor influencing the eating quality of rice. High protein content may reduce the eating quality of rice for human consumption, but it is preferable for animal feed. Hence, these levels of protein in the rice are a great advantage of this study in case for animal feed.

6.1.3. TWW irrigation decreased CH4 emission, increased N2O emission and increased combine GWP

In 2015, CH_4 emission was not found probably due to the inhibitory effect of the high copper levels in the soil. However, it was found in 2016 and 2017 seasons. In 2016, average seasonal fluxes of CH_4 emission from all runs (0.23 to 0.30 $mg/m^2.h$) were much lower than those reported from normal paddy fields. In 2017, seasonal fluxes of CH_4 emission from runs using TWW were in the range of 0.18 to 0.28 $mg/m^2.h$. Irrigation of wastewater is believed to increase CH_4 emission due to its high organic matters that supplies C sources to the soil, and may change the soil's physio-chemical properties and bacterial communities that can encourage CH_4 production. However, in this study, TWW irrigation decreased CH_4 emission notably, compared to the control using tap water (0.34 and 4.37 $mg/m^2.h$ in 2016 and 2017, respectively). It would be attributed to the inhibitory effect of nitrogen in TWW on CH_4 formation. More than that, in runs applied bottom-to-top irrigation, TWW fed from the bottom may supplied oxygen to the soil layer, which can negatively affect on methanogens bacteria as well as stimulates the oxidation of producing CH4 gas. In normal paddy fields, a peak flux of CH_4 emission is normally obtained in the early stage of rice development. By contrast, our results showed that a peak of CH_4 emission in the 2017 season was acquired in the ripening stage of the rice plants. The above inhibitory effect of nitrogen on CH_4 production would be taken to this phenomenon. In the final stage of rice development, the concentration of N in soil was lower than the earlier stage, decreasing the negative effect on methanogenesis.

N_2O emission is strongly depended on the N ability in the soil. Sewage irrigation is proposed to increase N_2O emission from paddy field since high N contained in TWW increased N for nitrification and denitrification processes in soil and consequently increased N_2O emission. Consistent with that, in this study, TWW irrigation significantly increased N_2O emission, compared with tap water irrigation. Among runs under TWW irrigation, top-to-top irrigation emitted notably greater N_2O than bottom-to-top irrigation.

6.1.4. No accumulation of harmful metals in rice and soil after 3 years under continuous irrigation of TWW

One of the most common adverse impacts of wastewater reclamation is the accumulation of metals, which may result in a potential risk to human health if these pollutants come into the food chains. Even though wastewater was treated and part of heavy metals was removed, TWW may still cause heavy metal contamination in soil and crops. Although it has been well documented that wastewater irrigation could result in an accumulation of heavy metals in soil and brown rice, the results of this study indicated that no considerable difference in heavy metals concentrations was found between the initial soils and the soils after three seasons under TWW irrigation. However, an increase in Cu content was observed in the soil of all runs in 2015 and in the control treatment irrigated with tap water in 2016, which is not from TWW but rather than from the oxidation of copper cables used in the MFC system.

The concentrations of Cu, Cr, Zn, Cd, Pb and As in the harvested brown rice from all runs did not indicate significant impacts of TWW and irrigation direction on the building-up of heavy metals in rice grain except for an increase of Pb in the 2017 season due to unknown reason. However, ongoing monitoring of these harmful metals in brown rice and soil is necessary to avoid potential long-term accumulation or accidental contamination when the same paddy fields are repeatedly used for rice production with TWW irrigation.

6.1.5. Low electric output from MFC system under continuous irrigation of TWW

As mentioned in Chapter 2, our trial for generating power from the MFC system in the paddy field under continuous irrigation of TWW was based on the hypothesis that electric output can be enhanced when a large amount of organic matters is supplied to the soil. However, the results of the two farming seasons indicated that electric output from this study was lower than those obtained from normal paddy fields as well as from the previous study which used the same bench-scale apparatus under circulated irrigation of TWW.

6.2. Recommendations

In this study, a continuous irrigation system with TWW from bottom-to-top achieved a high N removal efficiency from TWW and high rice yield and quality for animal feeding.

Based on the results, it would be strongly recommended to apply bottom-to-top irrigation method to the real fields for removing more nutrients from wastewater, reducing commercial fertilizers and saving freshwater irrigation in rice production for animal feeding.

However, it may difficult to supply water equally to every point in the real field, so a large number of underdrain pipes are necessary which can increase the cost. Thus, before applying this system, farmers need to consider its cost and benefit.

Through three farming seasons, no harmful metals accumulation in brown rice and soil was found, but monitoring of heavy metals in the soil and brown rice in every season is highly recommended to avoid long-term of accumulation or accidental contamination.

Beside cultivation rice for animal feed, further study should be conducted to cultivate rice for other purposes. P content in soil would be decreased after long-term TWW irrigation without P-fertilization, resulting in decreasing rice yield and quality. Thus, P content in soil should be evaluated after each season.

To utilize C source in TWW effectively by installing MFC system in paddy filed under TWW irrigation, further studies are highly recommended.

Appendix

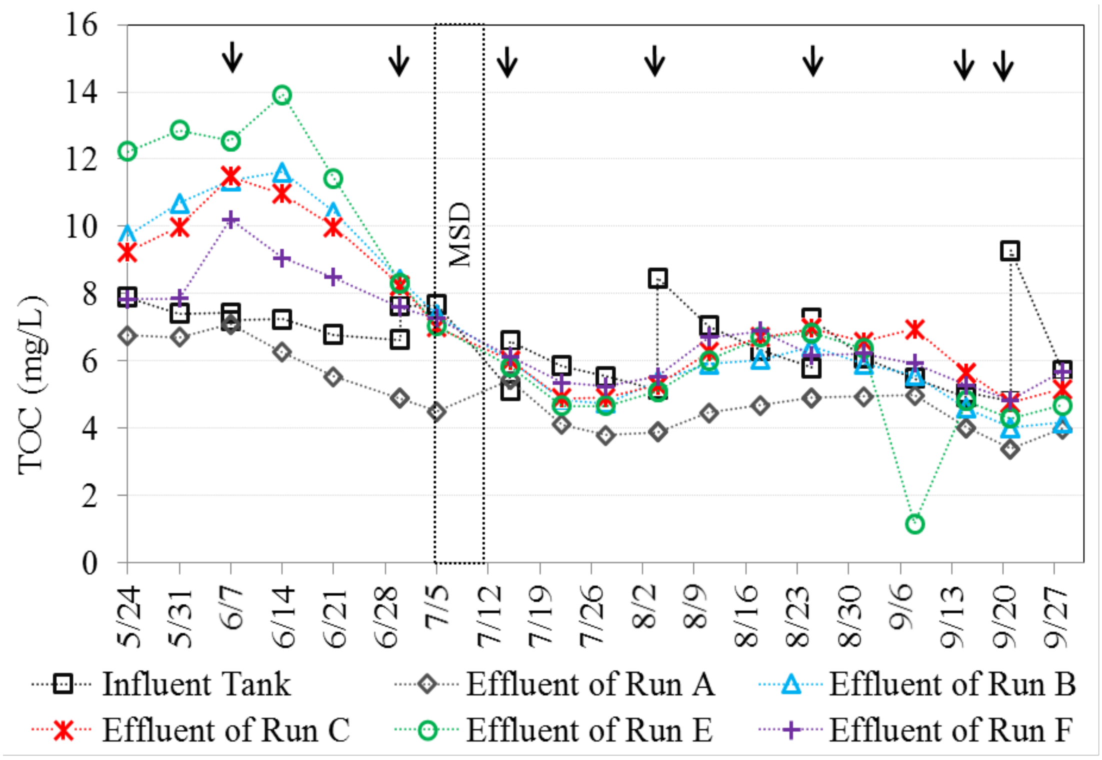


Figure A-1. Total organic carbon of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

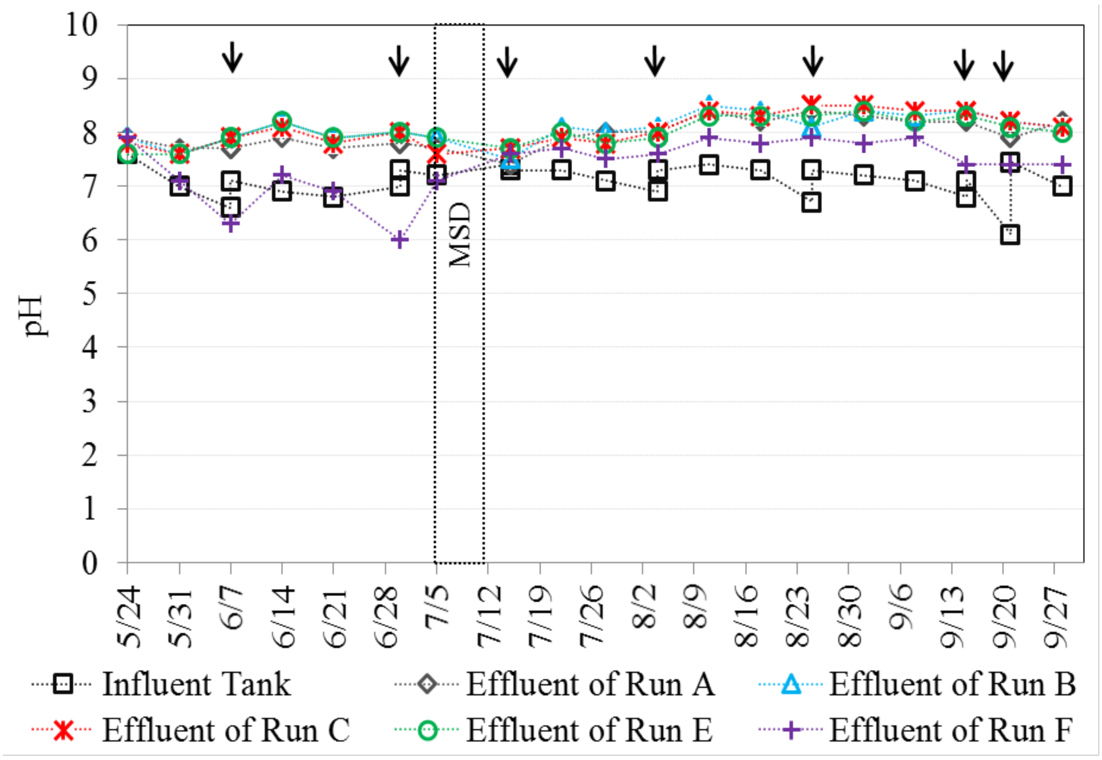


Figure A-2. pH of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

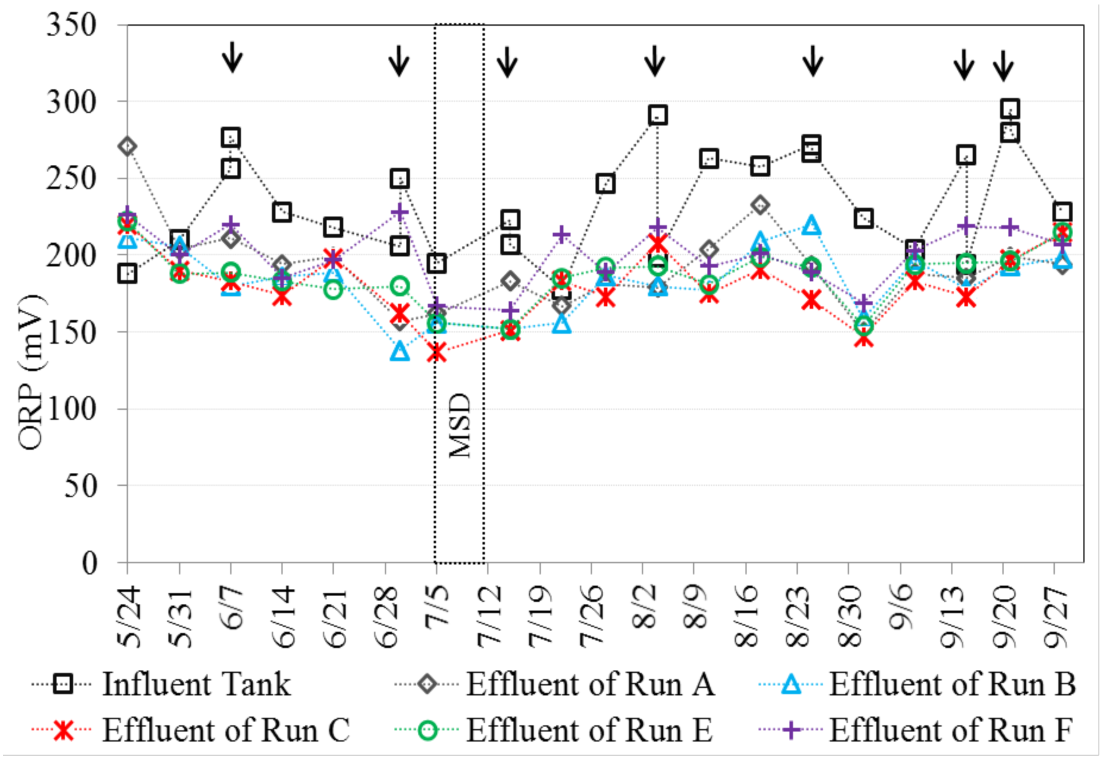


Figure A-3. Oxidation-reduction potential of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

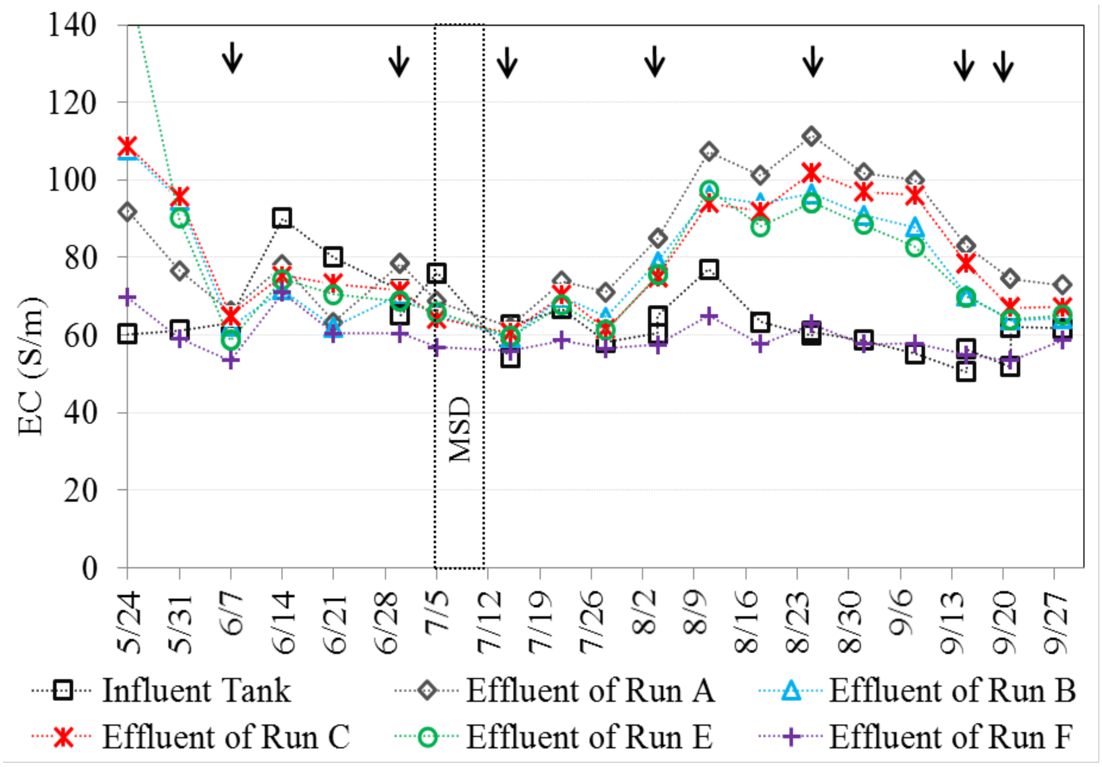


Figure A-4. Electric conductivity of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

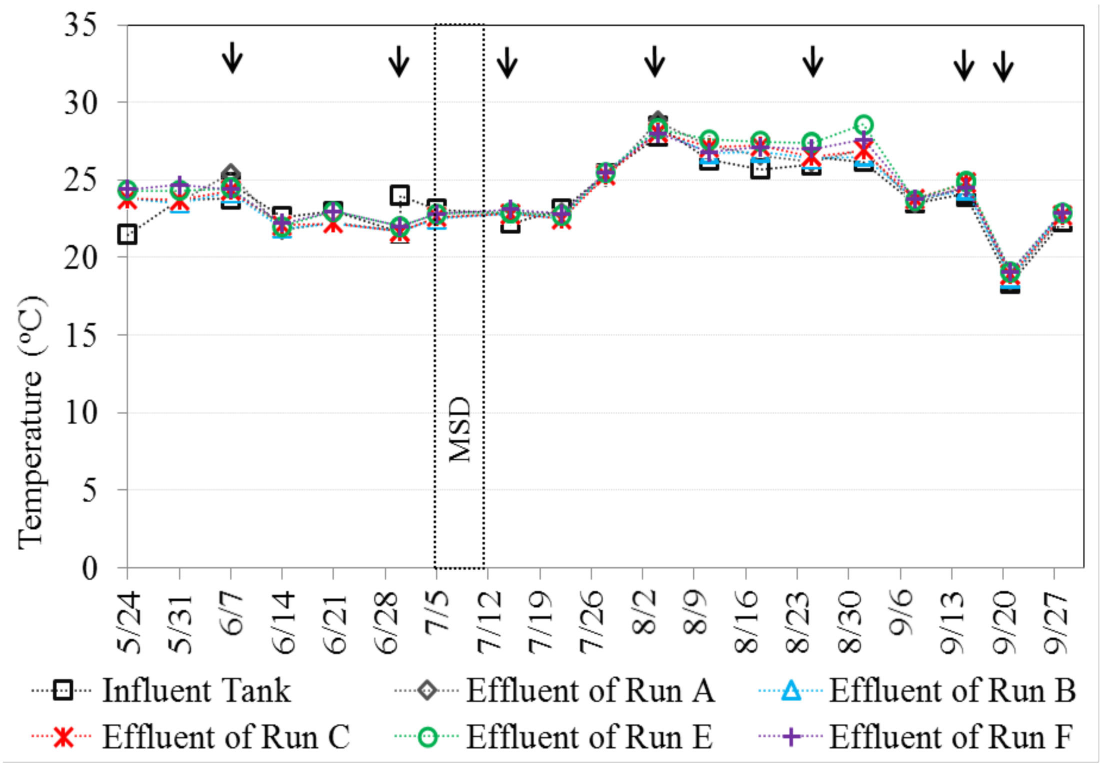


Figure A-5. Temperature of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

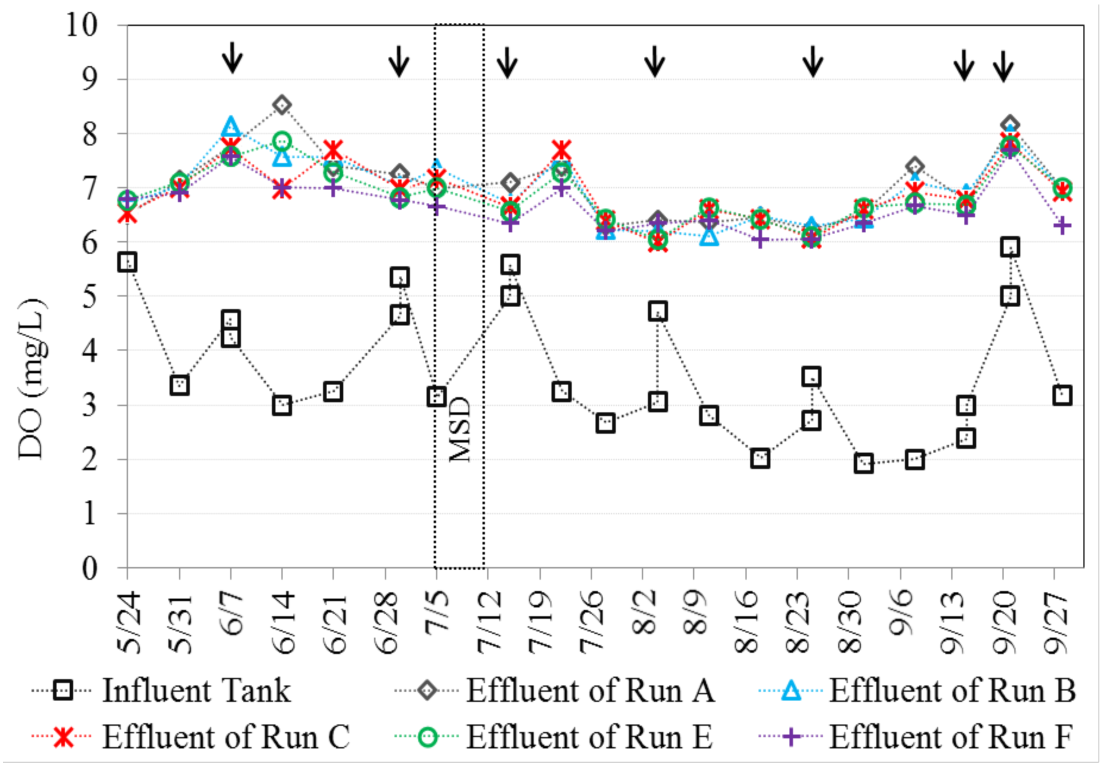


Figure A-6. Dissolved oxygen of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

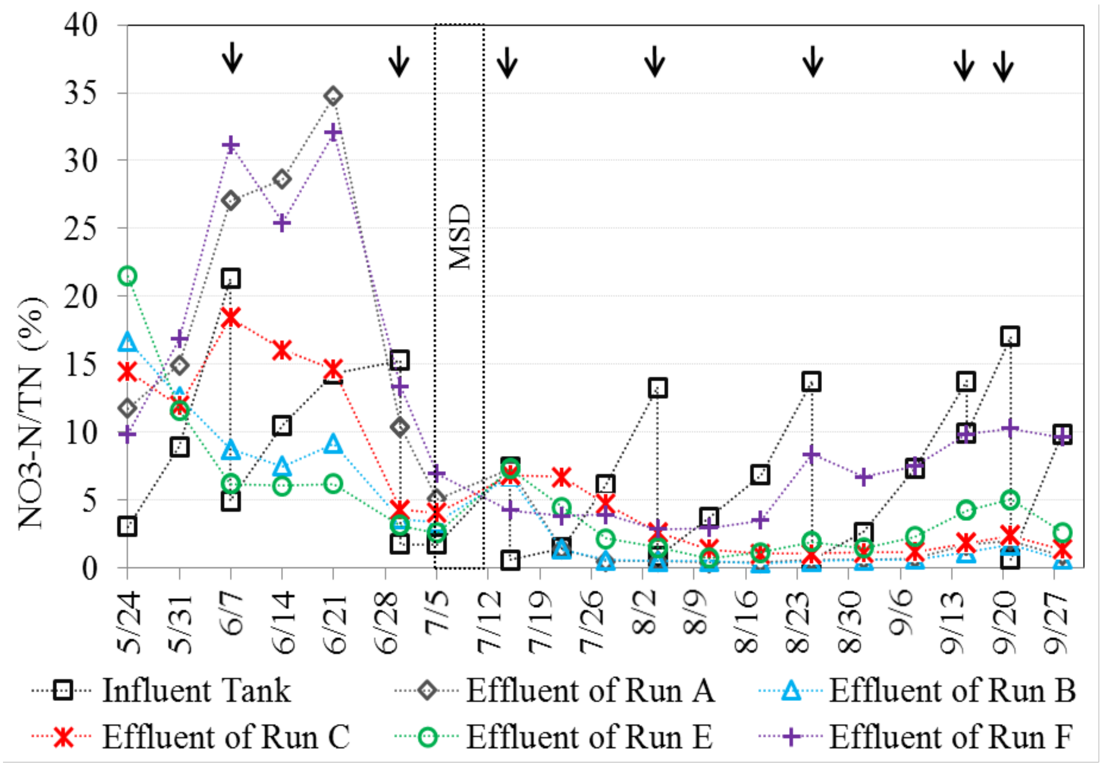


Figure A-7. Proportion of nitrate of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

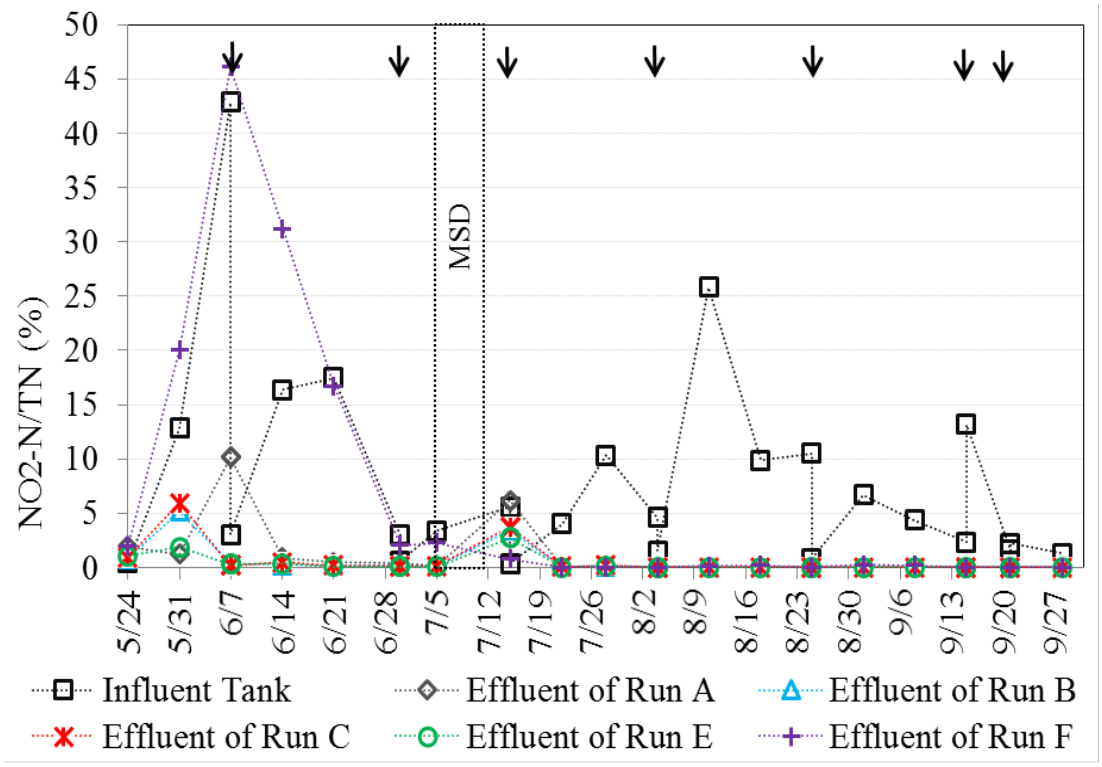


Figure A-8. Proportion of nitrite of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

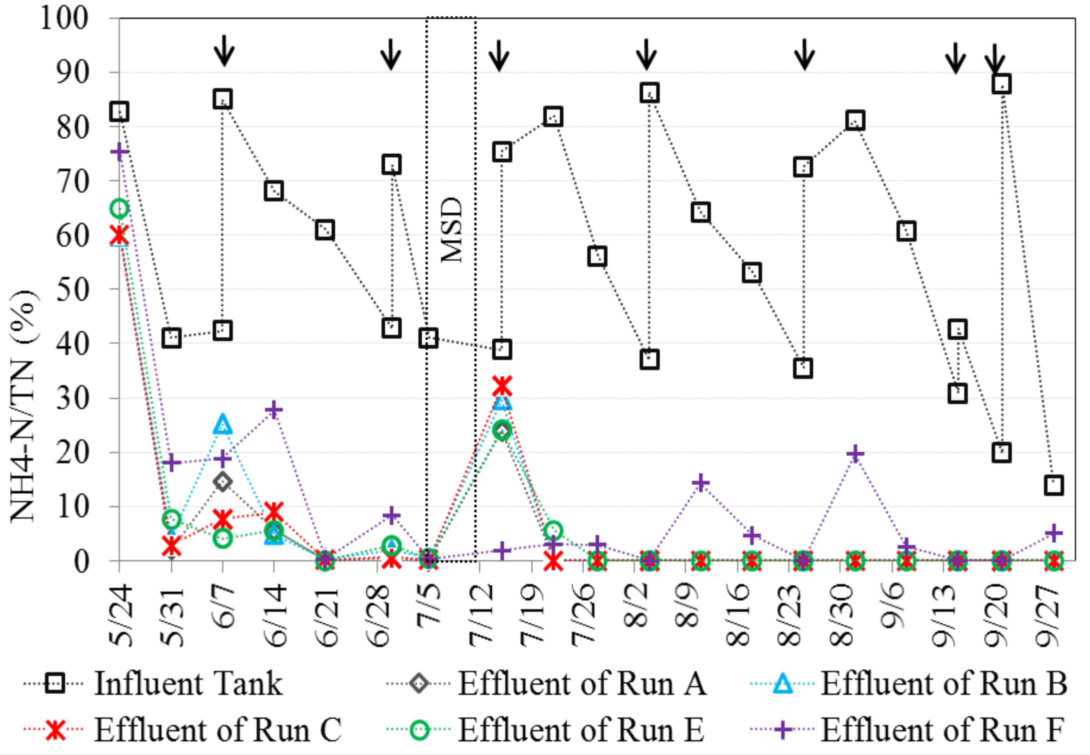


Figure A-9. Proportion of ammonium of the irrigated water in 2016 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

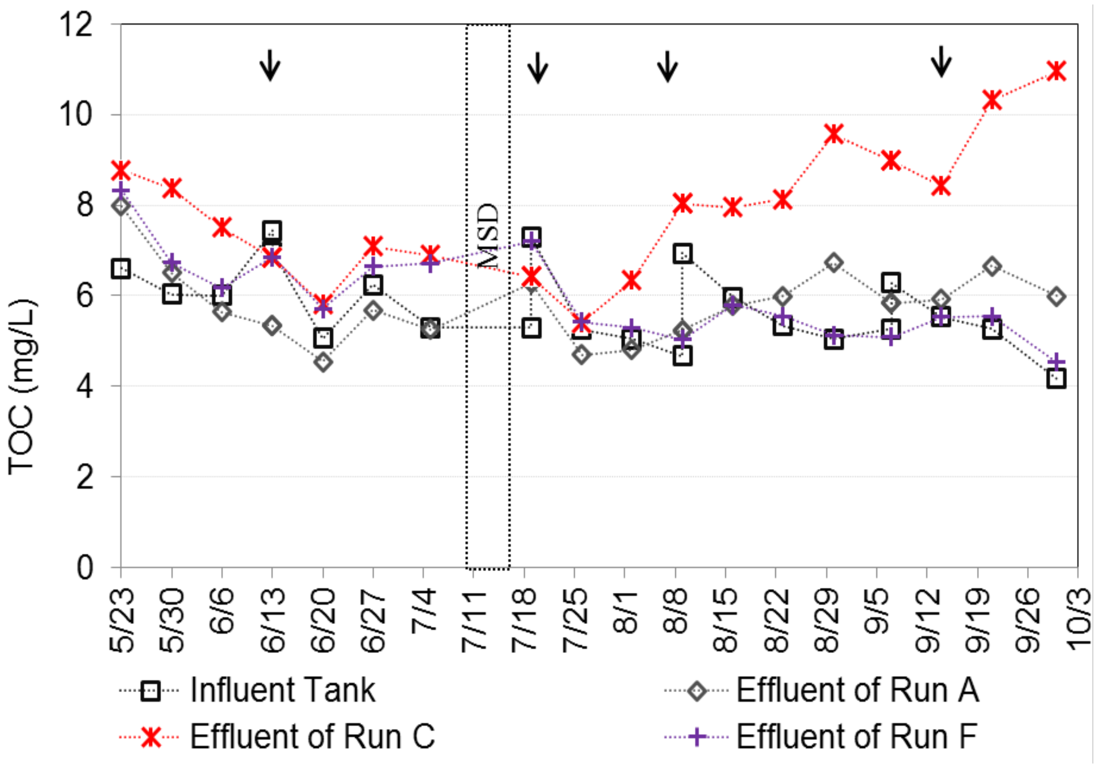


Figure A-10. Total organic carbon of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

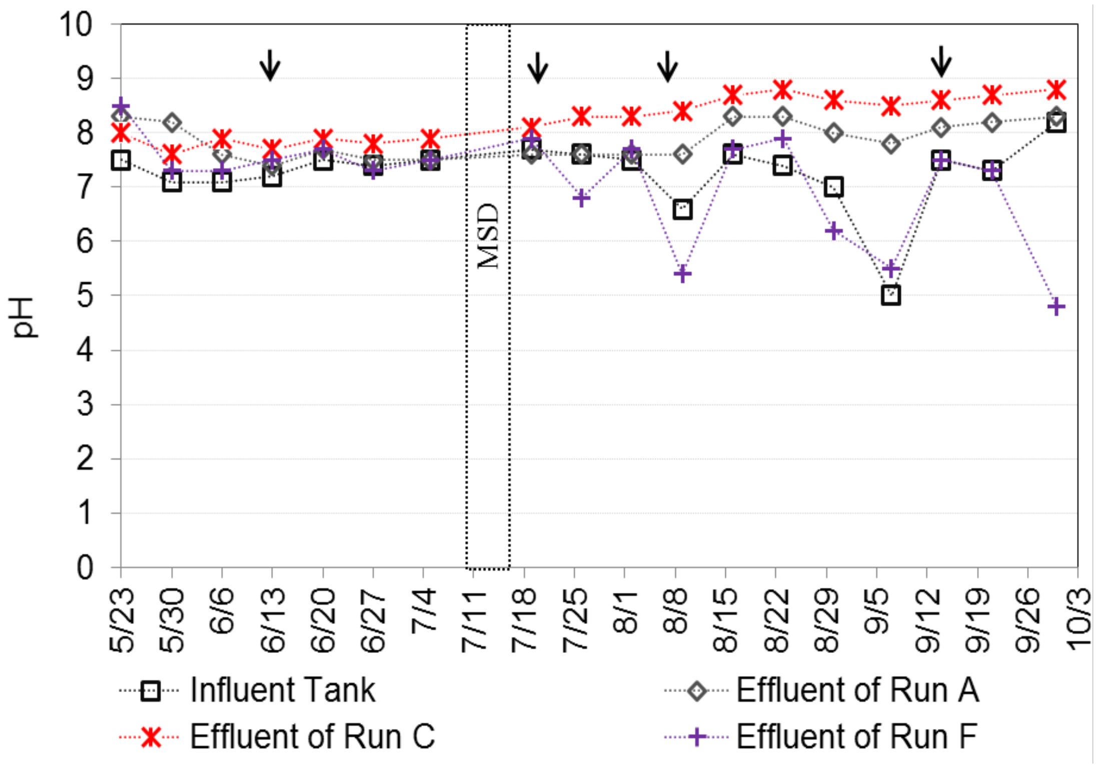


Figure A-11. pH of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

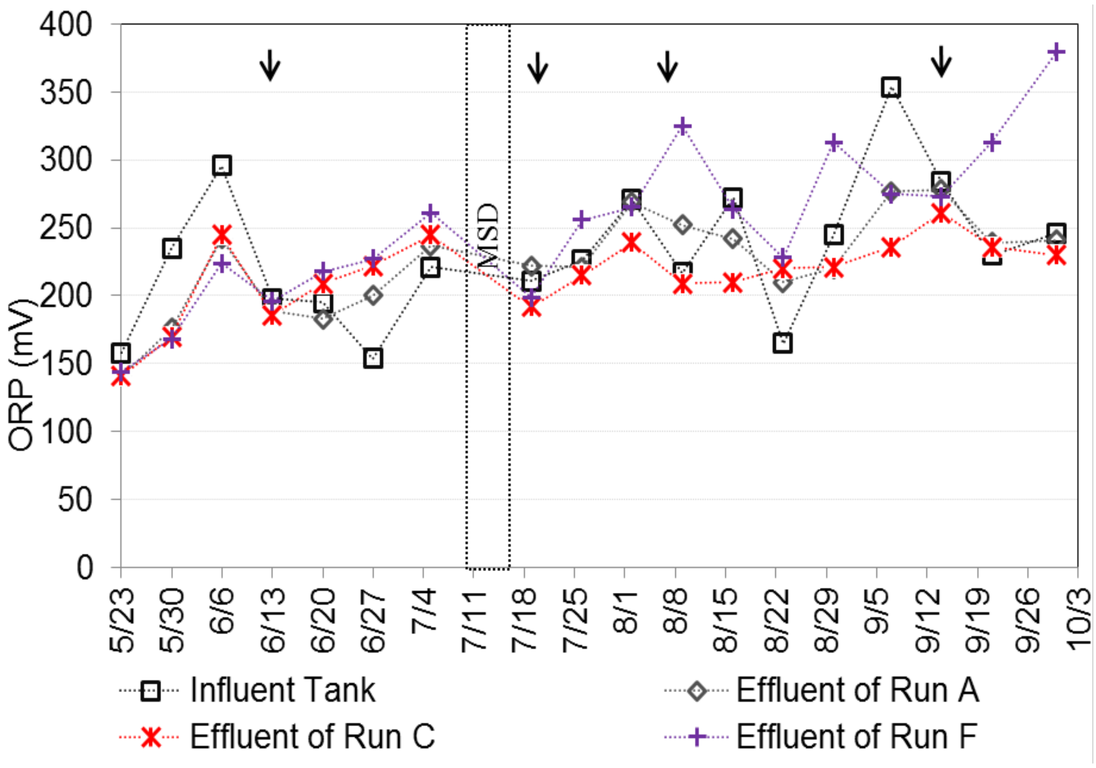


Figure A-12. Oxidation-reduction potential of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

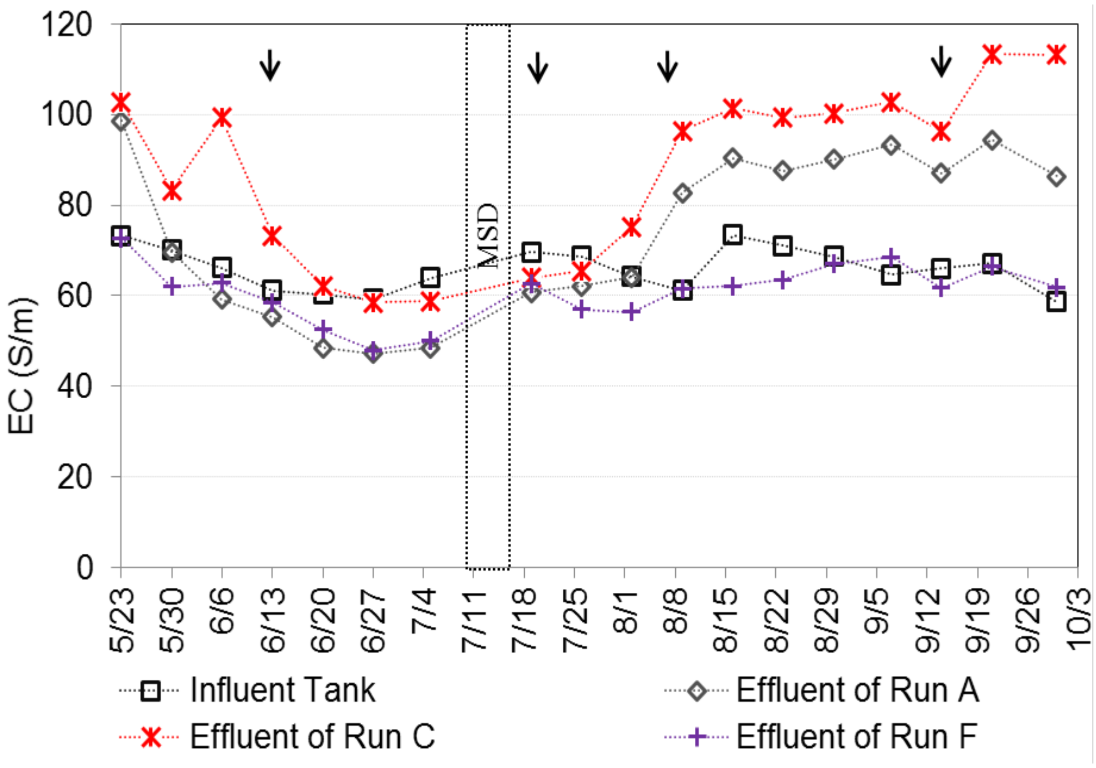


Figure A-13. Electric conductivity of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

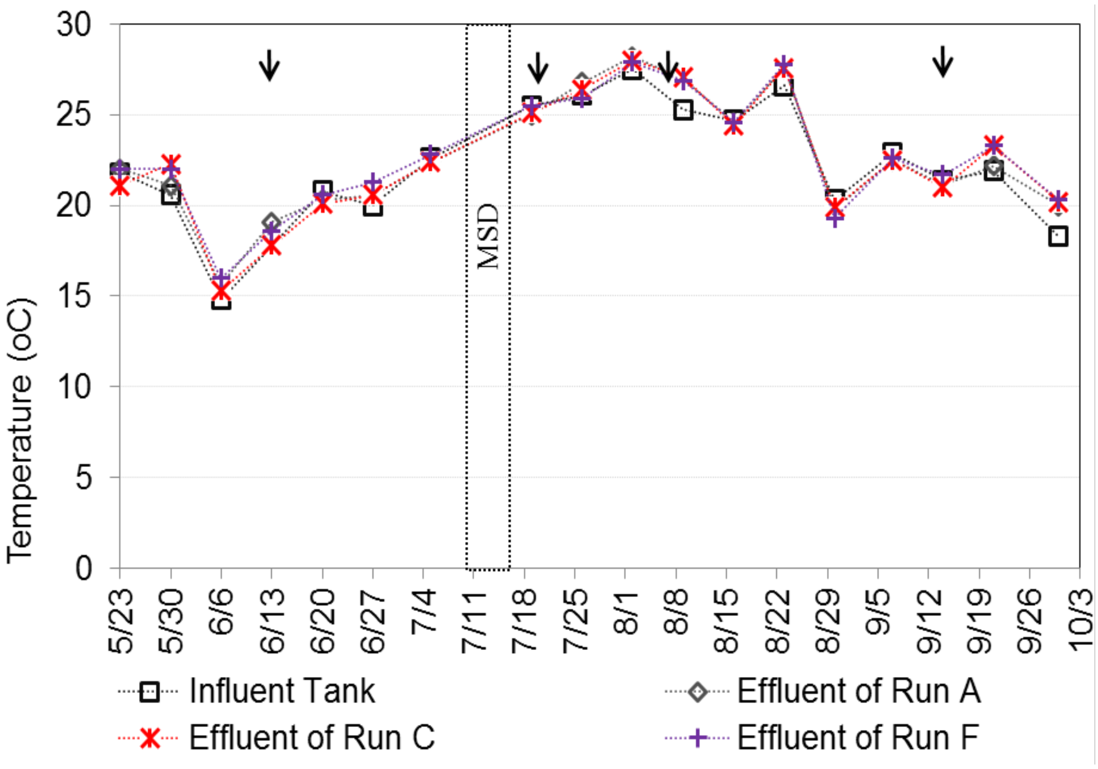


Figure A-14. Temperature of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

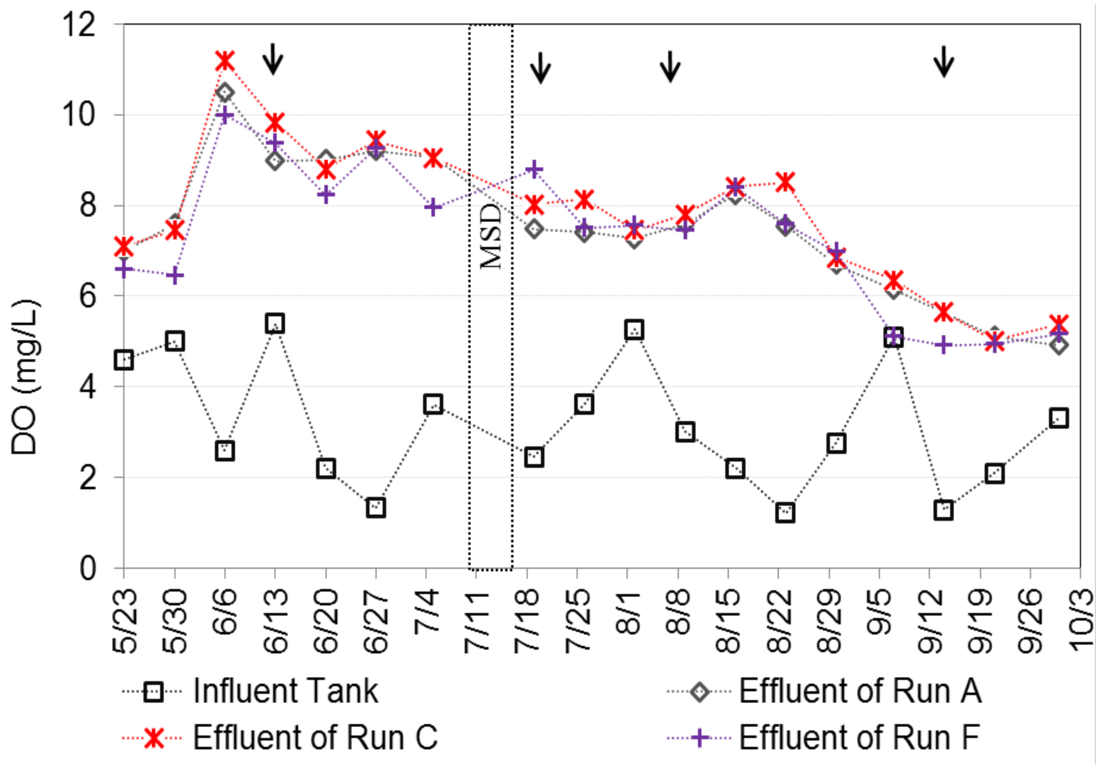


Figure A-15. Dissolved oxygen of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

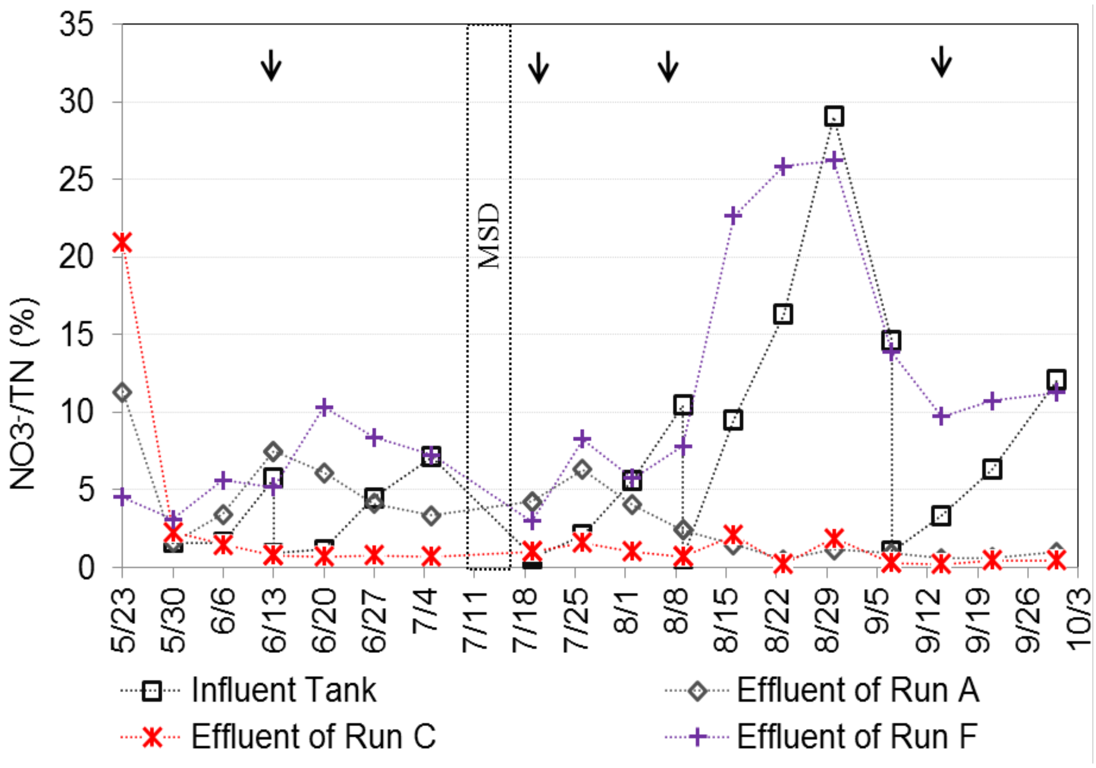


Figure A-16. Proportion of nitrate of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

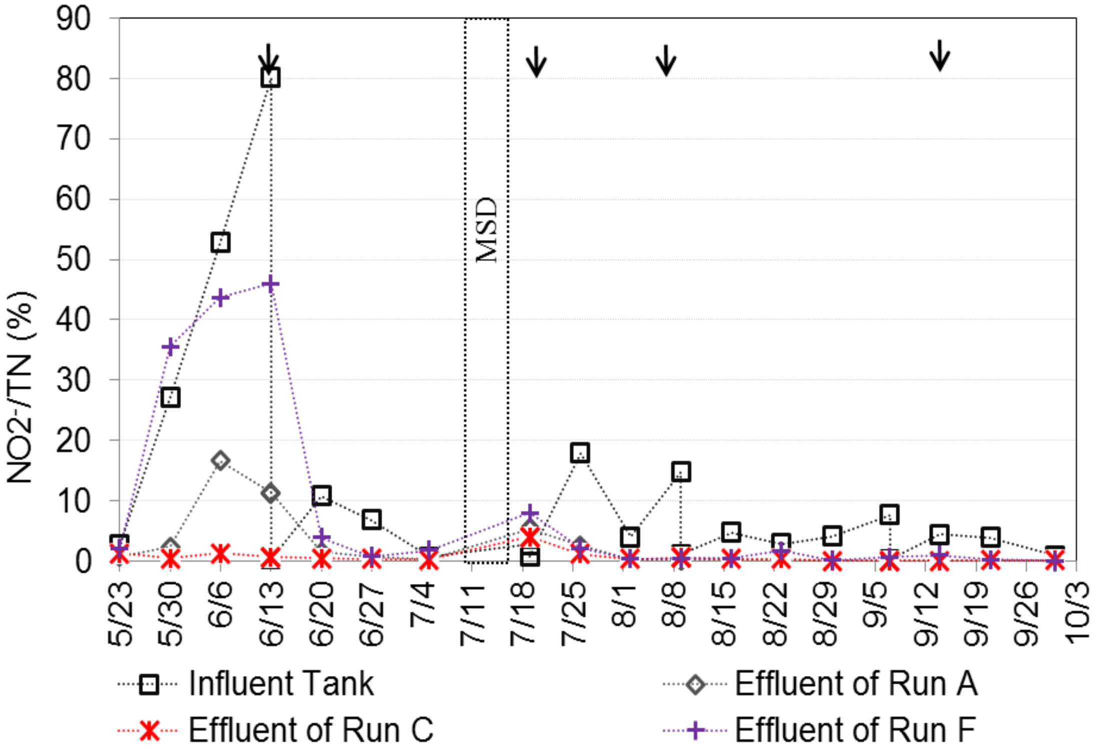


Figure A-17. Proportion of nitrite of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

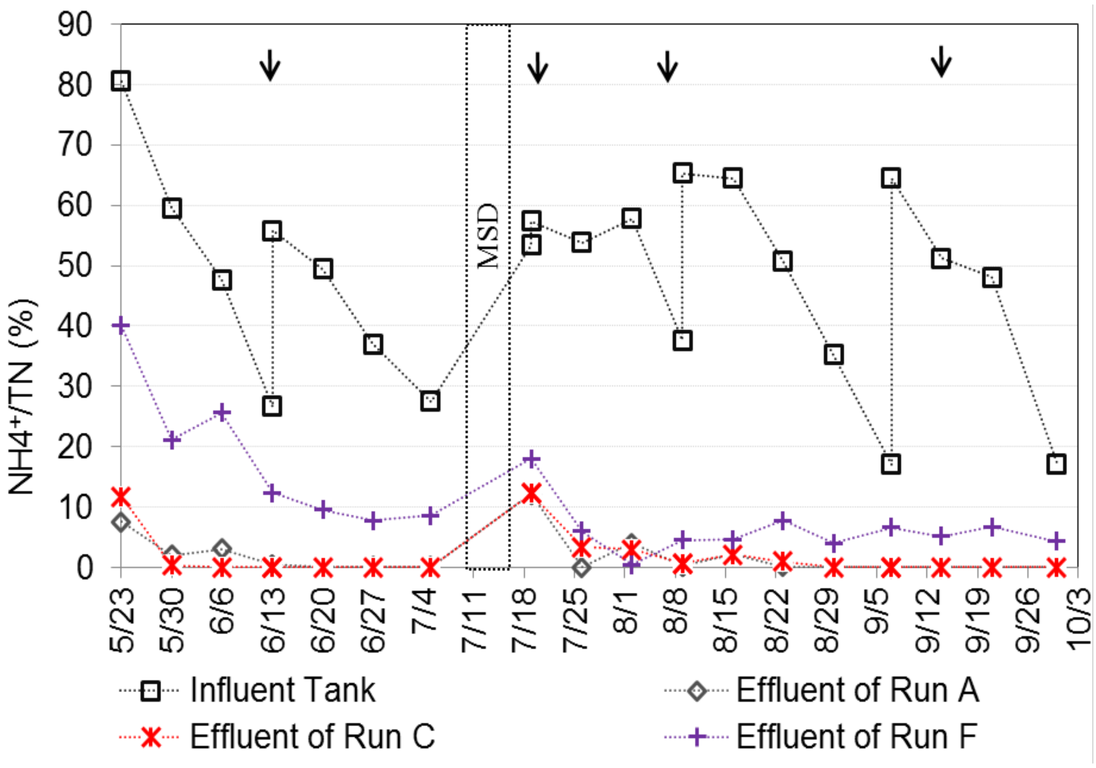


Figure A-18. Proportion of ammonium of the irrigated water in 2017 season. Solid arrows indicate dates when treated wastewater was added to the influent tank, and MSD means midsummer drainage to dry up the soil layers.

Academic Achievement

Publications

Book chapter

Dong Duy Pham, Toru Watanabe. 2017. "Municipal Wastewater Irrigation for Rice Cultivation." Current Perspective on Irrigation and Drainage, edited by Suren N. Kulshreshtha. Intech Publishing.

Academic journals

Watanabe. T, Mashiko. T, Maftukhah. R, Kaku. N, Pham. D. D and Ito. H. 2016. "Rice cultivation and power generation circulated irrigation of treated municipal wastewater." Water Science and Technology 74 (12).

Watanabe. T, Kurashima. S, Pham. D. D, Horiguchi. K, Sasaki. A and Pu. J. 2016. "Nitrient characteristics of rice for animal feed cultivated with continuous irrigation of treated municipal wastewater." Journal of Japan Society for Civil Engineers, Ser.G (Environmental Research), Vol. 72(7) (in Japanese).

Pham. D. D, Kurashima. S, Kaku. N, Sasaki. A, Pu. J and Watanabe. T. "Bottom-to-top continuous irrigation of treated municipal wastewater for effective nitrogen removal and high quality rice for animal feeding." Water Supply. Accepted.

Pham. D. D, Cai. K, Kaku. N, Sasaki. A, Pu. J, Sasaki. Y, Horiguchi. K and Watanabe. T. "Continuous irrigation of treated municipal wastewater from bottom-to-top for very high quality and yield rice for animal feeding without any fertilization." In preparation.

Presentations

International conferences

Pham. D. D, Kurashima. S, Pu. J and Watanabe. T. "Cultivation of rice for animal feeding with continuous irrigation of treated municipal wastewater." 12th SEAWE. Hanoi, Vietnam. Nov 28-30, 2016.

Pham. D. D, Kurashima. S, Horiguchi. K, Sasaki. A, Pu. J and Watanabe. T. "Impact of Treated Municipal Wastewater Irrigation on Growth of Rice Crop for Animal Feeding and Heavy Metals in Paddy Soil." 11th IWA. Long Beach, US. Jul 23-27, 2017.

Watanabe. T, Pham. D. D, Kurashima. S, Kaku.N and Pu. J. "Nitrogen Removal, Electricity Generation and Greenhouse Gas Emissions in Paddy Fields Irrigated with Treated Municipal Wastewater." 11th IWA. Long Beach, US. Jul 23-27, 2017.

Domestic conferences

Pham. D. D, Kurashima. S, Kaku. N, Ito. H and Watanabe. T. "Development of Municipal Wastewater Reuse System to Cultivate Rice for Animal Feeding toward Sustainable Water and Nutrient Circulation." JSCE. Tohoku. Jan 09. 2016.

Awards

Technology Development Award (Tohoku Branch, JSCE). "Cultivation of rice for animal feeding with continuous irrigation of treated municipal wastewater" (Presented at 71st Annual Conference of JSCE Tohoku Branch in March 2016).

Award for Excellent Poster Presentation. "Cultivation of rice for animal feeding with continuous irrigation of treated municipal wastewater." Presented at 12th SEAWE. Hanoi, Vietnam. Nov 28-30, 2016.

Curriculum Vitae

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