Investigation of Nitrogen Removal via CANON Process in a Single Stage Constructed Wetland System

M. Khajah[1,2*], A.O. Babatunde[1]

[1]Hydro-environmental Research Centre, Energy and Environment Theme, Cardiff University School of Engineering, Queen's Buildings, The Parade, CF24 3AA, Cardiff, Wales, UK
[2]Kuwait Institute for Scientific Research, Water Resources Department, Water Technology Division, Kuwait

ABSTRACT

This research study attempts to explore the new route of nitrogen removal called the Complete Autotrophic Nitrogen removal Over Nitrite process (CANON) to enhance the nitrogen removal in constructed wetlands. To achieve high-rate nitrogen removal via CANON, a single stage tidal flow constructed wetland system was adopted by implementing internal downflow recirculation, shorter unsaturated time, and long saturated time. It was found that the recirculation strategy with shorter unsaturated time would limit the oxygen supply, which favoured partial nitrification and the anammox process. High inorganic carbon could promote both partial nitrification and anammox activities, providing a strong selective method to maintain the CANON route. By combining the control of oxygen supply (internal recirculation, six cycles a day) and high influent inorganic carbon concentration of 150 mg/L, this study achieved ammonium and total nitrogen removal of 98% and 67%, respectively, from synthetic domestic wastewater.

Keywords: Anammox; CANON process; constructed wetlands; inorganic carbon; partial nitrification
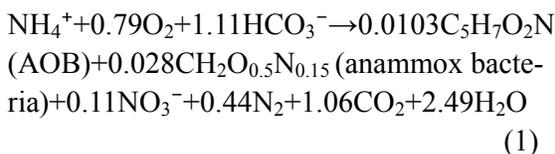
1. INTRODUCTION

High nitrogen content and low organic carbon often limit conventional nitrogen removal (i.e. nitrification-denitrification process) during wastewater treatment. In particular, lack of organic carbon is the main factor hindering the conventional nitrogen removal process. In contrast to the conventional nitrogen removal process, the anaerobic ammonium oxidization (anammox) process provides an efficient and cost-effective alternative to nitrogen removal from wastewater without organic carbon requirement (Mulder et al., 1995). In the anammox process, anammox bacteria convert ammonium together with nitrite, which is the

electron acceptor, directly to dinitrogen gas in the absence of oxygen (Wang and Li, 2011; Zhu et al., 2011). It is a precondition for the anammox process that the antecedent nitrification process stops at nitrite by ammonia-oxidizing bacteria (AOB) (partial nitrification, PN), i.e. the oxidation of nitrite to nitrate carried out by nitrite-oxidizing bacteria (NOB) has to be avoided (Hu et al., 2014; Wiesmann et al., 2007). This can be achieved by making selective conditions where AOB grow faster than NOB. Several operational parameters have been manipulated for this purpose, including temperature, dissolved oxygen (DO), free ammonia (FA) and free nitrous acid, etc. Among them, low DO (~1

mg/L) and especially elevated temperature (above 25°C) have been proven to be the key controlling factors for partial nitrification (Hu et al., 2014; Peng and Zhu, 2006).

Since different conditions are required for PN (aerobic) and anammox (anoxic), a two-reactor (aerobic/anaerobic) system was primarily adopted to assist and facilitate these autotrophic nitrogen conversion processes (Ahn, 2006). After that it was found possible to combine and integrate these two processes within a single aerobic reactor under oxygen-limiting conditions, referred to as Complete Autotrophic Nitrogen removal Over Nitrite (CANON) process as described in Eq. 1:

$$NH_4^+ + 0.79O_2 + 1.11HCO_3^- \rightarrow 0.0103C_5H_7O_2N$$
(AOB)$+0.028CH_2O_{0.5}N_{0.15}$ (anammox bacteria)$+0.11NO_3^- + 0.44N_2 + 1.06CO_2 + 2.49H_2O$

(1)

The CANON process relies on the interaction between two bacteria cooperating with each other in a single reactor: Nitrosomonas and Nitrospira aerobic bacteria and Planctomycete anaerobic ammonium oxidizing (anammox) bacteria under limited oxygen conditions (Liang et al., 2014; Sliekers et al., 2003). Regarding aerobic ammonium oxidizer bacteria, these bacteria oxidize ammonium to nitrite under oxygen limiting conditions, while anammox bacteria use nitrite produced from an aerobic process to oxidize ammonium as an electron acceptor to convert it to dinitrogen gas and trace amounts of nitrate (Third et al., 2001). The CANON is an autotrophic (self-feeding) process, which avoids an additional or external organic source. The entire nitrogen removal can be achieved in a single reactor with very low aeration (Bagchi et al., 2010; Hu et al., 2014), therefore reducing the space and energy requirements resulted in lower capital or operating costs (Khin and Annachhatre, 2004; Vazquez-Padın et al., 2009). The CANON process requires limited oxygen for the process

to take place; therefore, it requires shorter unsaturated time and longer saturation time in the bed. Moreover, the CANON process consumes 63% less oxygen and 100% less reducing chemicals than the conventional nitrification-denitrification process for nitrogen removal (Khin and Annachhatre, 2004).

As a passively aerated biological system, constructed wetlands (CWs) possess natural advantages, such as redox stratification, limited oxygen supply and high biomass retention, etc. to assist and facilitate the CANON process; although some studies have reported nitrogen removal by the CANON process with different types of CW.

However, it is still a challenge for some systems to achieve and reach stable and high-rate autotrophic nitrogen transformation (Dong and Sun, 2007; Hu et al., 2011; Peng and Zhu, 2006). One of the main challenges is that the CWs system operates under ambient temperature, which is kinetically unfavourable to maintaining PN (Peng and Zhu, 2006). Besides this, it is difficult to control oxygen supply and maintain the appropriate level of DO in the CWs system. In tidal flow constructed wetlands (TFCWs), oxygen supply is significantly improved by the tidal operation (periodic saturated/unsaturated conditions) (Sun et al., 2006) and can be controlled by manipulating the duration of the saturated/unsaturated phases and the number of tides. However, the oxygen supply is often fundamental to promoting PN and anammox in these systems.

Oxygen supply in TFCWs could be weakened by adopting a long saturated time and a short unsaturated time. Therefore, a single bed TFCW was adopted in this research study to achieve the CANON process by implementing internal downflow recirculation and a short unsaturated time period. Moreover, it is also expected that the influent inorganic carbon (IC) could be a significant controlling factor for the CANON route, since IC is the

carbon source for all the autotrophic microorganisms and may have an important influence on the nitrogen transformation pathway (Bagchi et al., 2010).

The aim of this study is to achieve a high rate of nitrogen removal through the CANON process route in a single reactor CW. The objective is to implement an internal recirculation and tide period (saturated and unsaturated period) to investigate the effectiveness of the recirculation number and create a suitable environment for PN and anammox for nitrogen removal.

2.　MATERIALS AND METHODS

It is known that the CANON process requires limited aeration and this can be achieved by using upflow instead of downflow recirculation (Hu et al., 2014). Otherwise, this experiment used a downflow stream with a very short unsaturated time to obtain the limited aeration. A long saturation time would limit the oxygen in the bed, therefore weakening the nitrification step as well as protecting the anammox bacteria (Dong and Sun, 2007).

The single reactor CW was constructed from a PVC pipe having a diameter of 10 cm and a length of 100 cm. The bottom layer of the reactor was filled with coarse gravel as support and a drainage layer (20-25 mm) to the height of 10 cm. Above that layer was 50 cm high of gravel comprising the main media layer (5-9 mm) followed by the distribution layer (11-19 mm) at a height of 10 cm.

Synthetic wastewater was used in this study to simulate domestic wastewater after the secondary treatment, because the CANON process would be hindered by high concentrations of the contaminants, particularly when NH_4^+-N is over 14.5 mg/L, COD>100 mg/L, TP>6 mg/L and sodium bicarbonate as IC>150 mg/L (Metcalf & Eddy, 2003). If a high COD is applied, anammox can be inhibited; this is because in such a case, nitrite will be utilized predominantly by heterotrophic denitrifiers, leaving very little or none at all to anammox bacteria. 2 L of the synthetic wastewater was pumped from the influent and holding tank (the tank that holds the synthetic wastewater after being pumped out and then pumped in again to the reactor CW for recirculation purposes) into the bed for 7 minutes, as shown in Fig. 1, to completely submerge the main media layer. Wastewater stayed in the bed for 3.5 hours before being pumped out (drained) for 7 minutes into the influent and holding tank and staying unsaturated for 30 minutes. The same procedure was repeated six times a day. The system was controlled by two peristaltic pumps with pre-programmed timers for feeding and decanting purposes. The single stage system was planted with a *Phragmites australis*. Redox potential (referred to as oxidation reduction potential, ORP) and DO probes were inserted into the column at certain depths of 20 cm (upper point) and 50 cm (lower point) from the surface of the distribution layer and were monitored for 24 hours a day. The hydraulic retention time (HRT) was 4 hours (3.5 hours saturated and 30 minutes unsaturated) and the hydraulic loading rate (HLR) was 0.255 m³/m²·d.

2.1　Wastewater

The synthetic wastewater was prepared in the laboratory of Cardiff University and is fed into the CW every two days. The characteristics of the synthetic wastewater to simulate domestic wastewater after the secondary treatment are: 0.1275 g/L (CH_3COONa), 0.0125 g/L (KH_2PO_4), 0.0125 g/L (K_2HPO_4), 0.1 g/L (KCl), 0.1 g/L (NaCl), 0.0575 g/L (NH_4Cl), 0.1 g/L ($MgSO_4 \cdot 7H_2O$), 0.1 g/L ($CaCl_2 \cdot 2H_2O$) and 0.15 g/L ($NaHCO_3$) as IC with 1 ml/L of the trace elements mixture (containing 0.5 L) consisting of: 0.5 g/L ($FeSO_4 \cdot 7H_2O$), 0.035 g/L ($ZnCl_2$), 0.05 g/L ($MnCl_2 \cdot 4H_2O$), 0.003 g/L

(A)   (B)

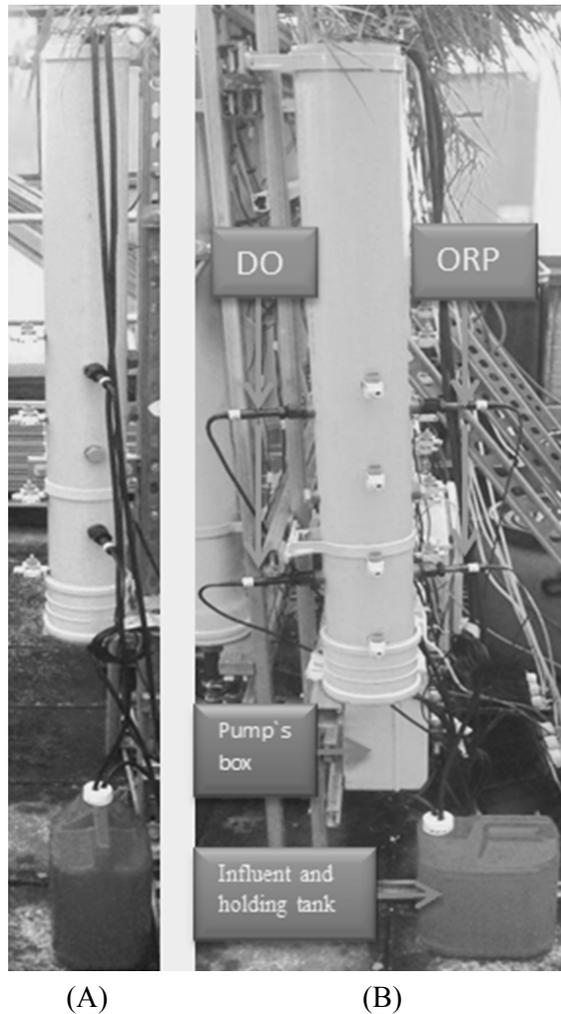**Figure 1**   Single constructed wetland from (A) side view and (B) front view

(H_3BO_3), 0.065 g/L ($CaCl_2·6H_2O$), 0.001 g/L ($CuCl_2·2H_2O$), 0.012 g/L ($NiCl_2·6H_2O$), 0.018 g/L ($Na_2MoO_4·2H_2O$) and 0.119 g/L ($CoCl_2·6H_2O$).

2.2   Water quality monitoring and performance evaluation

Grab samples were taken from the effluent twice a week and analyzed from the influent and effluent of the bed for chemical oxygen demand (COD), nitrite (NO_2^--N), nitrate (NO_3^--N), ammonium (NH_4^+-N), total nitrogen (TN), orthophosphate (PO_4^{3-}-P) and total phosphorous (TP). Temperature and pH were measured *in situ* by pH/ EC/ TDS meter (HANNA HI 991301). COD, NO_2^--N, NO_3^--N, NH_4^+-N, TN and PO_4^{3-}-P were determined using a Hach DR/3900 spectrophotometer according to its standard operating procedures. TP was measured by an ICP-OES machine (Optima 2100DV, Singapore).

The efficiency of nitrogen removal was determined from the decrease of total inorganic nitrogen (TN). In particular, the nitrate accumulating ratio (NAR) (Hu et al., 2014) was used to estimate the nitrogen transformation activities, as shown in Eq. 2: if aerobic ammonium oxidization (full nitrification) is the major conversion route of NH_4^+-N, most of

the influent NH_4^+-N will be converted to NO_3^--N, causing a high NAR close to 1; otherwise the transformation follows the CANON route, a low NAR of 0.11 can be expected according to Eq. 1. This method was justified in the current study because no significant nitrite build-up was detected throughout the experimental period.

$$NAR = \text{effluent } NO_3^-\text{-N/ } NH_4^+\text{-N removed} \quad (2)$$

3.   RESULTS AND DISCUSSION

3.1   Overall treatment performance

The overall treatment performance through the operational periods is summarized in Table 1. During this period, NH_4^+-N removal efficiency achieved at 98%. Nevertheless, most of the NH_4^+-N influent was converted to NO_3^--N in the systems; therefore, TN removal in the system reached 67%.

3.2   Effect of recirculation frequency (N_c) on nitrogen treatment performance

It is common and well-known that NH_4^+-N removal rate increases with the increase of recirculation number (N_c) or tide and vice versa (Hu et al., 2014) due to the aeration for NH_4^+-N conversion (nitrification step). The operating strategy was six cycles a day with 210 minutes of saturated stage and 30 minutes of unsaturated stage. The influence of the recirculation frequency on treatment performance is presented in Fig. 2. Moreover, Fig. 3 illustrates the time of filling and draining the CW, ensuring that oxygen was present in the CW and frequently replenished with every tide (selected random day combined 6 cycles).

The NH_4^+-N removal efficiency increased gradually to > 97% with N_c of 6 times/day, while TN reached ≈ 70%. As shown in Fig. 2, NAR values were close to 0.11, which demonstrates that the conversion of ammonium followed the CANON route according to Eq. 1.

However, the authors conducted this experiment over three phases with different recirculation numbers (unpublished data) and different performance across the three phases were observed. The results indicate the effectiveness of the recirculation technique, and this is also in agreement with similar published work by (Hu et al., 2014; Prost-Boucle and Molle, 2012; Wen et al., 2013).

Table 1   Influent and effluent wastewater characteristics and their percentage removal

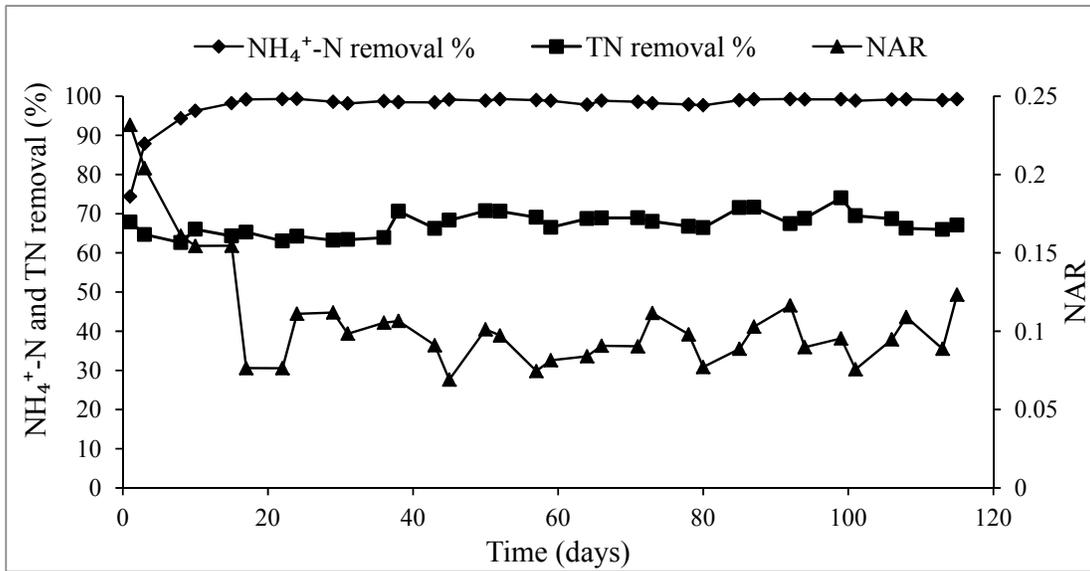| Parameters	Influent	Effluent	Removal (%)
pH	8.01 (±0.03)	7.6 (±0.08)	–
NH_4^+-N (mg/L)	14.63 (±0.16)	0.36 (±0.26)	98
NO_2^--N (mg/L)	0.003 (±0.001)	0.045 (±0.008)	–
NO_3^--N (mg/L)	0 (±0)	1.51 (±0.4)	–
TN (mg/L)	16.7 (±0.29)	5.45 (±0.44)	67
COD (mg/L)	102 (±1.61)	18.43 (±7.46)	82
TP (mg/L)	5.71 (±0.56)	6.43 (±1.2)	–
PO_4^{3-}-P (mg/L)	4.95 (±0.6)	5.57(±1.22)	–

Figure 2   Treatment performance under recirculation frequency (N_c=6)
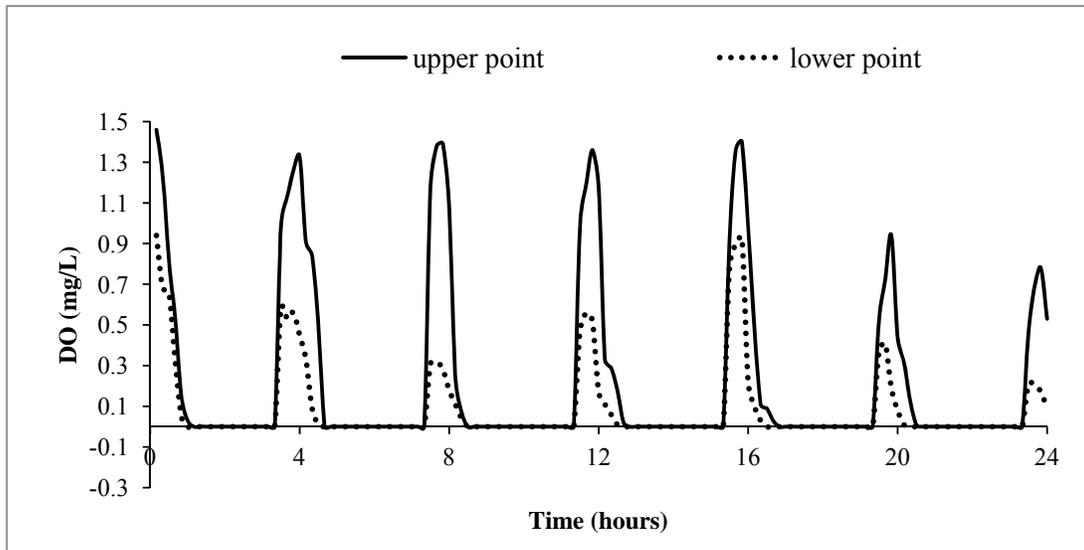

Figure 3   Oxygen diffusion into the CW during the filling and draining period

3.3   ORP and DO

ORP has been used for many years in facilities (i.e. municipal wastewater treatment plants). When used in wastewater treatment systems, ORP is a measurement of the ability or potential of wastewater to permit the occurrence of specific biological (oxidation-reduction) reactions such as nitrification, denitrification and phosphorus removal. ORP is measured in millivolts (mV). On the ORP scale, the existence of an oxidizing agent such as oxygen increases the ORP value, while the existence of a reducing agent such as substrate or carbon BOD decreases the ORP value. ORP has proven to be an effective technique of monitoring wastewater processes that can

determine what biological reaction is taking place in the system. Oxygen is a fundamental factor in the steady operation of the system. In the nitrification process, oxygen plays a role as a co-substrate with nitrogenous compounds, and its concentration significantly affects the metabolism of both AOB and NOB.

The relationship between ORP and DO is substantial to this study. As a matter of fact, ORP is heavily driven by DO; when the oxygen is present, DO and ORP increases and vice versa. Real-time data were collected over a four month period for monitoring and measuring purposes at every 10 minutes at two certain depth points, as shown in Fig. 1. The first point was called the upper point at 20 cm beneath the surface of the distribution layer, whereas the second point was named the lower point at 50 cm below the surface of the distribution layer.

ORP and DO data are shown in Figs. 4 and 5, respectively. The figures show a proportional relationship between ORP and DO. This relationship illustrates that DO in both points (upper and lower) was less than 0.5 mg/L which is considered as limited oxygen because almost all the time the unit is saturated. Therefore, ORP values were negative and remained negative at both points throughout the study period due to the microbial-mediated redox process, which has been shown to decrease the ORP level when the microbial processes are intense (Dušek et al., 2008). Negative ORP also indicates that anoxic or anaerobic conditions were taking place as well.

Fig. 4 represents ORP values at the upper and lower points in CW. ORP varied during the experimental period due to many factors that affect redox readings such as temperature (the range is between 12.3 to 23.7°C), the depth of the probes inserted into the CW (upper point 20 cm from the surface and lower point 50 cm from the surface), the concentrations of the

synthetic wastewater (the wastewater was recirculating 6 times a day), pH, DO and microbial activities. Regarding Fig. 5, which represents DO values at the upper and lower point in CW, DO varied during the experimental period due to the consuming of the DO in the synthetic wastewater by the microbial community and the depth of the probes inserted into the CW (upper point 20 cm from the surface and lower point 50 cm from the surface). The maximum reading for DO was 0.5 mg/L which is considered as limited oxygen condition suitable for the PN. Consequently, lower points have higher ORP and lower DO values than upper points; one of the reasons is due to the closeness of the upper point to the surface, where oxygen can penetrate into the matrices bed of CW for respiration.

In general, reducing aeration decreases the availability of the oxygen supply. Consequently, it decreases DO concentration. A corresponding decrease in ORP is expected as oxygen is a driving force of ORP. On the other hand, low DO and anoxic conditions reduce the nitrification rate, thus the concentrations of nitrite and nitrate in the effluents were small, as shown in Table 1. A small trace of nitrate production was detected during the operation of the experiment, which indicated that the activities of NOB were effectively suppressed. Therefore, NOB might be outcompeted during the contention for oxygen with AOB. It can also be said that an adequate oxygen supply induces the metabolic activity of AOB more than NOB.

The relationship of ORP and DO, and the relationship between DO and nitrogen as well, logically indicate a relationship between ORP and nitrogen. In this study, nitrogen concentration decreased with a decrease in ORP, which means microbial activities were intense to reduce/remove nitrogen.
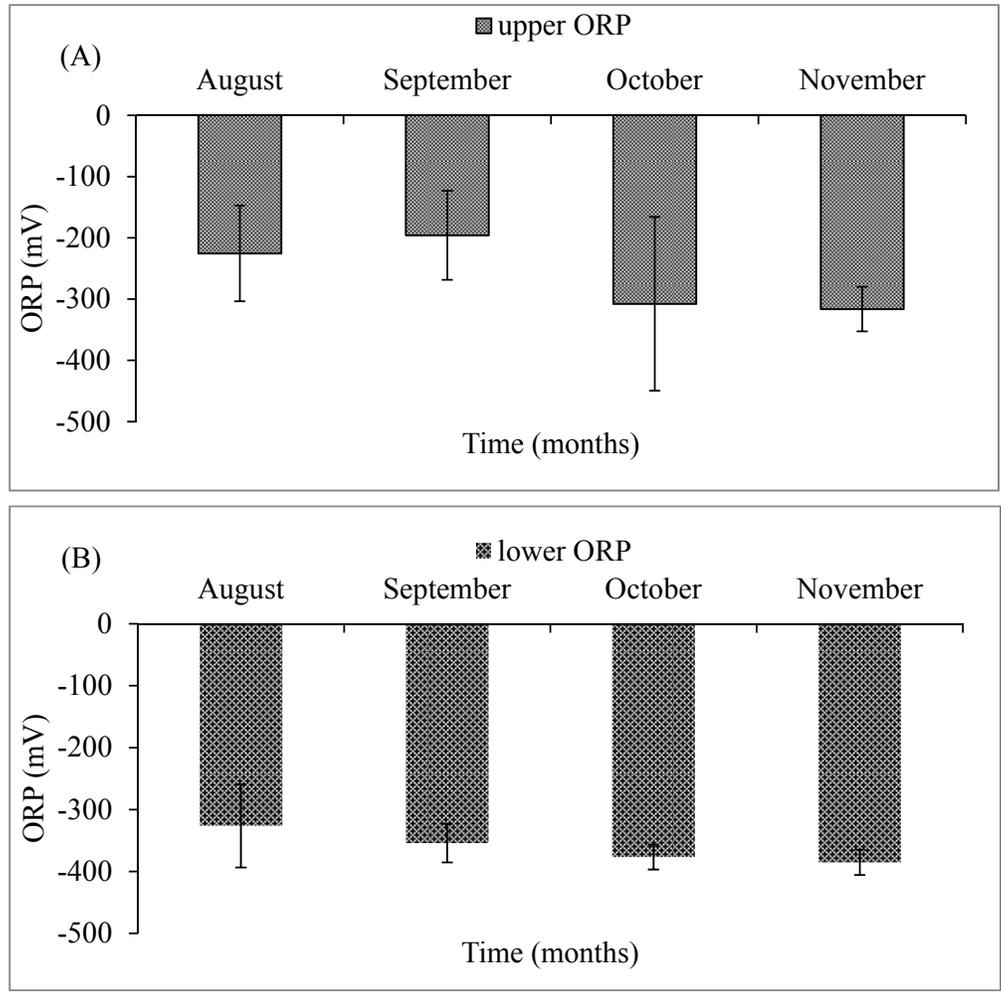
Figure 4   ORP values according to their depth from the surface of the distribution layer   (A) upper point at 20 cm and (B) lower point at 50 cm
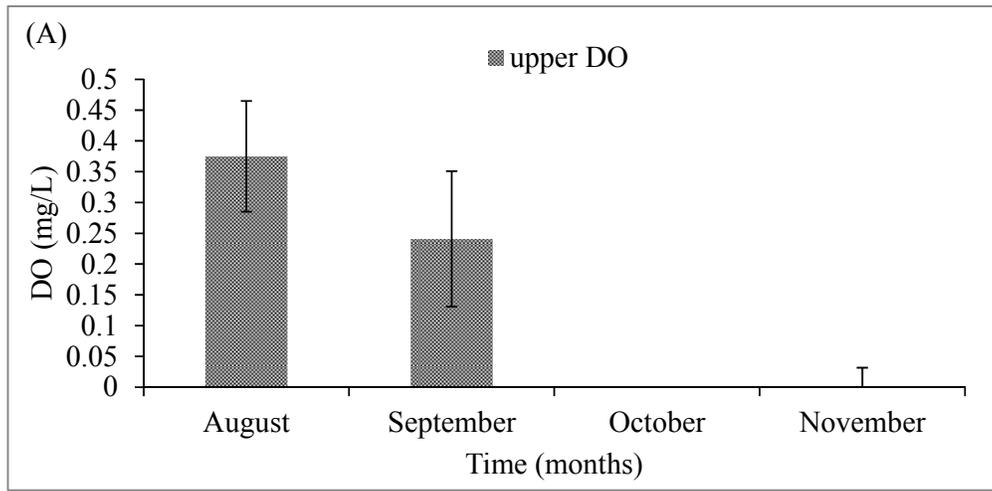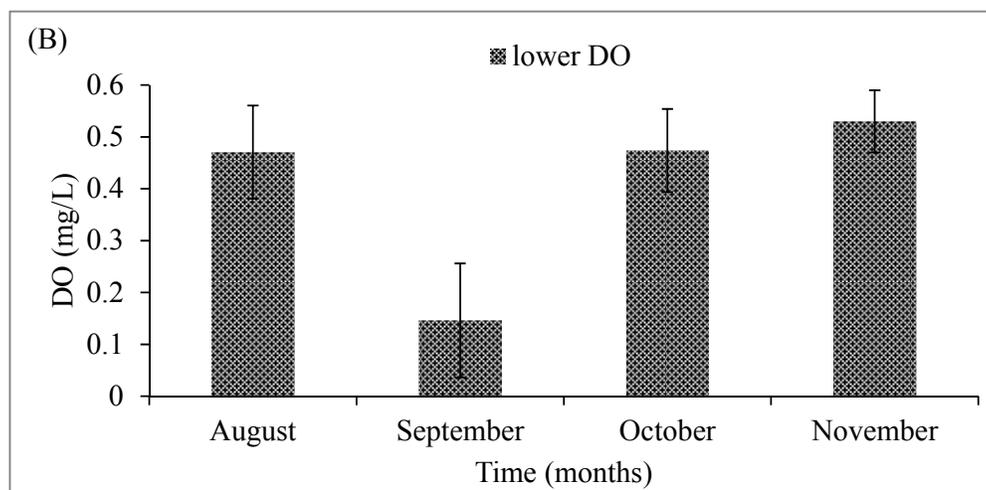

Figure 5A   (to be continued)

Figure 5 DO values according to their depth from the surface of the distribution layer (A) upper point at 20 cm and (B) lower point at 50 cm

CONCLUSIONS

The adoption of downflow tidal flow mode, a shorter bed resting time (30 minutes) and intermittent recirculation can create a persistent oxygen-limiting environment to facilitate the CANON pathway in TFCW. The recirculation strategy acts as the oxygen supply to the system. High influent IC concentration can simultaneously improve and enhance the activity of AOB and anammox bacteria, but have no effect on NOB, which provides a strong selective pressure to trigger the CANON route. Overall, combining the recirculation and high influent IC, the system achieved 67% of TN removal through the CANON process in this study.

REFERENCES

Ahn, Y.H. (2006). Sustainable nitrogen elimination biotechnologies: a review. *Process Biochemistry*, 41(8), 1709-1721.

Bagchi, S., Biswas, R. and Nandy, T. (2010). Alkalinity and dissolved oxygen as controlling parameters for ammonia removal through partial nitritation and ANAMMOX in a single-stage bioreactor. *Journal of Industrial Microbiology & Biotechnology*, 37(8), 871-876.

Dong, Z. and Sun, T. (2007). A potential new process for improving nitrogen removal in constructed wetlands-promoting coexistence of partial-nitrification and ANAMMOX. *Ecological Engineering*, 31(2), 69-78.

Dušek, J., Picek, T. and Čížková, H. (2008). Redox potential dynamics in a horizontal subsurface flow constructed wetland for wastewater treatment: Diel, seasonal and spatial fluctuations. *Ecological Engineering*, 34(3), 223-232.

Hu, Y., Kumar, J., Akintunde, A., Zhao, X. and Zhao, Y. (2011). Effects of livestock wastewater variety and disinfectants on the performance of constructed wetlands in organic matters and nitrogen removal. *Environmental Science and Pollution Research*, 18(8), 1414-1421.

Hu, Y., Zhao, X. and Zhao, Y. (2014). Achieving high-rate autotrophic nitrogen removal via Canon process in a modified single bed tidal flow constructed wetland. *Chemical Engineering Journal*, 237(2), 329-335.

Khin, T. and Annachhatre, A.P. (2004). Novel microbial nitrogen removal processes. *Biotechnology Advances*, 22(7), 519-532.

Liang, Y., Li, D., Zhang, X., Zeng, H., Yang, Z.,

Cui, S. and Zhang, J. (2014). Nitrogen removal and microbial characteristics in CANON biofilters fed with different ammonia levels. *Bioresource technology*, 171, 168-174.

Metcalf, E. & Eddy (2003). *Wastewater Engineering: Treatment, Disposal, Reuse*. McGraw-Hill, New York, USA.

Mulder, A., Van De Graaf, A.A., Robertson, L. and Kuenen, J. (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. *FEMS Microbiology Ecology*, 16(3), 177-184.

Peng, Y. and Zhu, G. (2006). Biological nitrogen removal with nitrification and denitrification via nitrite pathway. *Applied Microbiology and Biotechnology*, 73(1), 15-26.

Prost-Boucle, S. and Molle, P. (2012). Recirculation on a single stage of vertical flow constructed wetland: Treatment limits and operation modes. *Ecological Engineering*, 43(7), 81-84.

Sliekers, A.O., Third, K., Abma, W., Kuenen, J. and Jetten, M. (2003). CANON and Anammox in a gas-lift reactor. *FEMS Microbiology Letters*, 218(2), 339-344.

Sun, G., Zhao, Y., Allen, S. and Cooper, D. (2006). Generating "tide" in pilot-scale constructed wetlands to enhance agricultural wastewater treatment. *Engineering in Life Sciences*, 6(6), 560-565.

Third, K., Sliekers, A.O., Kuenen, J. and Jetten, M. (2001). The CANON system (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. *Systematic and Applied Microbiology*, 24(4), 588-596.

Vazquez-Padın, J., Figueroa, M., Fernandez, I., Mosquera-Corral, A., Campos, J. and Mendez, R. (2009). Post-treatment of effluents from anaerobic digesters by the Anammox process. *Water Science & Technology*, 60(5), 1135-43.

Wang, L. and Li, T. (2011). Anaerobic ammonium oxidation in constructed wetlands with bio-contact oxidation as pretreatment. *Ecological Engineering*, 37(8), 1225-1230.

Wen, J., Tao, W., Wang, Z. and Pei, Y. (2013). Enhancing simultaneous nitritation and anammox in recirculating biofilters: Effects of unsaturated zone depth and alkalinity dissolution of packing materials. *Journal of hazardous materials*, 244-245(2), 671-680.

Wiesmann, U., Choi, I.S. and Dombrowski, E.M. (2007). *Fundamentals of biological wastewater treatment*. John Wiley & Sons, New York, USA.

Zhu, G., Wang, S., Feng, X., Fan, G., Jetten, M.S. and Yin, C. (2011). Anammox bacterial abundance, biodiversity and activity in a constructed wetland. *Environmental science & technology*, 45(23), 9951-9958.