Pol. J. Environ. Stud. Vol. 22, No. 6 (2013), 1685-1690

Original Research

Using Passive Atmospheric Oxygenation to Increase Nitrification Potential in a non-Planted Vertical Flow Constructed Bed System

Xiaoli Du[1]*, Zuxin Xu[2], Junqi Li[1], Kunming Fu[1]

[1]Key Laboratory of Urban Stormwater System and Water Environment, Ministry of Education,
School of Environmental and Energy Engineering, Beijing University of Civil Engineering and Architecture,
Beijing, 100044, China
[2]School of Environmental Science and Engineering, Tongji University, Shanghai, 200092, China

Received: 28 March 2013
Accepted: 25 July 2013

Abstract

Nitrification potentials in micro-environment were examined in a non-planted vertical flow constructed bed system with an air pipe. The results showed that nitrification potential was significantly correlated with the dissolved oxygen inside the system. Enhanced nitrification of 10.15 $g/m^2 \cdot d$ was achieved, twice as much as the 4.71 $g/m^2 \cdot d$ obtained in the traditional one. Atmospheric oxygenation by the air pipe was a remarkable 23.7 $g/m^2 \cdot d$, which improved the aerobic micro-environment for extra nitrification. Passive atmospheric oxygenation, which was increased by the air pipe without energy consumption, stimulated the growth of nitrifying bacteria and provided favorable habitats of bacterial richness. This results in high nitrification potential in the constructed bed, which is good for high-concentration rural wastewater treatment.

Keywords: atmospheric oxygenation, bacteria population, nitrification

Introduction

As a type of constructed wetland, vertical flow constructed wetland has become an alternative method for domestic wastewater treatment in rural areas of China because of the enhanced pollutant removal capacity, low maintenance cost and energy consumption [1, 2]. However, unsatisfactory nitrogen removal performance has been reported in previous vertical flow constructed wetlands [3, 4]. The sustainable nitrogen removal processes in most vertical flow treatment wetlands are based on a principle of traditional biological nitrification/denitrification. Classic nitrification consists of two successive aerobic reactions: ammonia oxidation with the conversion of ammonium to nitrite by nitrite bacteria (*Nitroso*-) and nitrite oxidation with the conversion of nitrite to nitrate by nitrate bacteria (*Nitro*-). Classic denitrification is the reduction of nitrite/nitrate to $NO/N_2O/N_2$, which requires an anoxic condition with organic carbon as the electron donor and nitrite/nitrate as the electron acceptor by the heterotrophic denitrifying bacteria. Generally, ammonia nitrogen is dominant and the concentration of nitrite and nitrate is usually very low in sewage and other types of wastewater.

To achieve total nitrogen removal, denitrification should be coupled with nitrification. Therefore, in most cases the extent of nitrification influences nitrogen removal in constructed wetlands. Ammonia oxidation, the first step of ammonia-nitrogen conversion, is considered the limiting step of nitrogen removal. Sufficient ammonia nitrogen removal in the treatment wetlands is the precondition of total nitrogen removal [5, 6]. Oxygen, as one of the reactants of nitrification, is normally in great shortage in vertical flow constructed wetlands when treating high concentration rural wastewater, and thus plays a key role in ammo-

---

*e-mail: duxiaoli@bucea.edu.cn

nia oxidation [2, 6]. Therefore, typical oxygen supply improvement is necessary not only for ammonia oxidation, but also nitrification in vertical flow constructed wetland systems, where there are no additional areas occupied and energy consumption.

Most oxygen in constructed wetlands originates from macrophyte photosynthesis, the atmosphere, and influent. In most cases, atmosphere is considered the main contributor [7, 8]. Therefore, the more fresh air supplied, the better the ammonia nitrogen removal effect will be. A modified method is developed to enhance the atmospheric oxygenation in vertical flow constructed wetland [9]. An air pipe is introduced into the vertical flow constructed wetland to increase passive atmospheric oxygenation depth and intensity without additional energy consumption.

In order to investigate the influence of atmospheric oxygenation on nitrification potential and aerobic micro-environment variation by air pipe, a non-planted vertical flow constructed bed was established. Ammonia nitrogen removal performance, nitrification ability, atmospheric oxygenation potential, and microorganism distribution tendency were compared with the conventional constructed bed in this study.

Materials and Methods

Vertical Flow Constructed Bed Systems

Two pilot-scale vertical flow constructed beds, named beds A and B, respectively, were used in this study. Both steel containers (0.8 m long, 0.8 m wide, and 1.5 m high) were filled with coarse gravel (d_{10}=16 mm, C_u=2.40) 15 cm, fine gravel (d_{10}=2.40 mm, C_u=3.98) 30 cm, sand (d_{10}=0.41 mm, C_u=7.32) 40 cm, and fine gravel 45 cm from bottom to top (Fig. 1). Distribution pipe and drainage pipe were installed 5 cm below the gravel surface and 5 cm above the flat bottom, respectively. In order to improve atmospheric oxygen transfer, no aquatic plants were planted in both constructed beds. Additionally, an air pipe with a diameter of 10 cm was installed in bed B. The air pipe was distributed to four branches with three 5 mm diameter apertures each at bottom.

Rural wastewater was fed continuously and discharged intermittently in both constructed bed systems with a hydraulic loading of 0.2 $m^3/m^2 \cdot d$. When the water level reached 1,050 mm, electromagnetic valves of drainage pipe would be open for draining. When the water level went down to 50 mm, electromagnetic valves would be closed for flooding. The flooding time and drainage time were 35 hours and 2 hours, respectively. A new cycle started just after the end of the drainage of the previous one. Both constructed bed systems were in operation for 5 months to reach a stable status for microorganism population establishment. Then 30 days batch experiments were carried out to evaluate the efficiency of pollutant removal. Temperature ranged from 20.8 to 30.1ºC during the experiments.

Pollutant Sampling and Analysis

Rural wastewater with low C/N ratio, obtained on a daily basis from the municipal sewer located near the Bei Tang Xin Yuan Apartment building in Shanghai, China, was treated by the vertical flow constructed beds. Influent and effluent samples were collected and analyzed immediately to measure the concentrations of organic matter (BOD_5), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and total nitrogen (TN) using the standard methods [10]. Effluent samples were the composite samples collected every 30 min during one drainage time. When the water level reached 1050 mm in bed A and B, samples from valves of different height were extracted to obtain DO con-

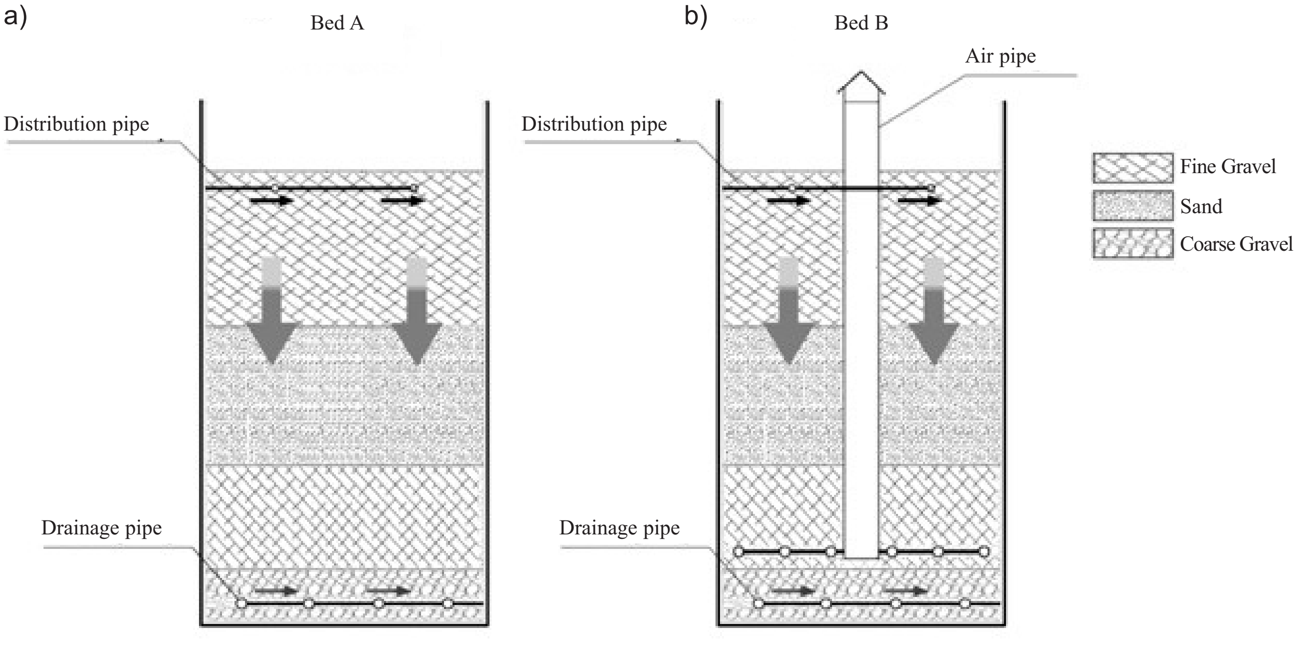

Fig. 1. Schematic diagram of the non-planted vertical flow constructed bed systems. (The arrows represent the flow direction of wastewater.)

centration and ORP values. ORP and DO measurements were carried out by JPBJ-608 detector produced in Shanghai, China, and the sensors were attached to a rod submerged to the middle of the water depth. A PHS-25 pH meter was used to measure pH.

Bacteria Analysis and Nitrification Potential Ability Analysis

After both constructed beds functioned, media samples at a depth of 15 cm (A1, B1), 65 cm (A2, B2), and 85 cm (A3, B3) below the distribution pipe were taken for bacteria population counts and nitrification potentials' measurement. Total bacteria, ammonium oxidizing bacteria, and nitrite oxidizing bacteria population counts were measured by the MPN method [11].

50 g substrate samples were taken from the sampling points, placed in five average conical flasks, and incubated with 100 ml nutrition liquid of NH_4^+ at 20ºC for 24 h. Nitrification potential could be obtained as the difference of NO_3^--N concentration of the nutrition liquid before and after incubation.

Results and Discussion

Nitrogen Removal

Influent and effluent samples drawn from both bed systems were investigated in terms of BOD_5, NH_4^+-N, NO_3^--N, and TN removal, as shown in Table 1. In general, high average removal rates of 67% and 72% for BOD_5 were achieved in bed A and B, respectively. Compared with the conventional constructed bed A, NH_4^+-N removal rate in the modified vertical flow constructed bed B was higher, in which the average removal rate was 84%. TN removal rates were about the same in both beds. More NO_3^--N was produced in constructed bed B because of the incomplete denitrification. Conversely, more NH_4^+-N was left and NO_3^--N was not accumulated in constructed bed A, suggesting good denitrification. Incomplete nitrification in bed A resulted in low total nitrogen removal rate, consistent with the theory that denitrification should be coupled with nitrification.

A possible pathway of NH_4^+-N removal involves ammonia volatilization, nitrification, media adsorption, biomass assimilation, and other biological processes [12, 13]. Because pH generally remained <7.63, maximum temperatures in the systems were always <30ºC, ammonia volatilization was not likely to be a principal removal mechanism in the systems. NH_4^+-N removal by media adsorption can be believed to be negligible because the inert substrate materials do not have significant cation exchange capacity [14]. Because of NO_3^--N concentration rising and pH decreasing in effluent, other NH_4^+-N removal processes only made a minor contribution and nitrification was the major contributor to remove NH_4^+-N in both constructed beds [15]. Because that 0.6 g biomass is generated as 1.0 g BOD_5 and 12.4% of $C_5H_7O_2N$ mass is constituted by nitrogen [16], mean amounts of NH_4^+-N immobilized by biomass assimilation in bed A and bed B were calculated at about 1.26 $g/m^2{\cdot}d$ and 1.36 $g/m^2{\cdot}d$, respectively. Consequently, mean amount of NH_4^+-N removal by nitrification was estimated to be about 4.71 $g/m^2{\cdot}d$ and 10.15 $g/m^2{\cdot}d$ in bed A and B, respectively. Enhanced nitrification was achieved in modified constructed bed B. Following a pipe system with a non-pipe system could achieve good total nitrogen removal.

Table 1. Concentrations of influent and effluent in the vertical flow constructed bed systems ($mg{\cdot}L^{-1}$, excluding pH).

Parameters	Influent Range	Effluent Range in bed A	Effluent Range in bed B
BOD_5	106.53~159.19	36.14~45.59	32.45~38.57
NH_4^+-N	64.88~70.17	35.44~40.45	7.89~14.53
TN	69.32~78.75	41.43~50.48	40.85-~-55.56
NO_3^--N	4.44~8.55	5.95~9.30	32.95~40.38
pH	7.39~7.63	6.09~6.53	6.12~6.32
DO	0.22~0.68	0.35~0.53	0.46~0.86

Physico-Chemical Conditions

With the conditions that DO in influent was 0.48 mg/L and redox potentials were -143 mV, DO and ORP vertical distribution in both beds were shown in Figs. 2 and 3, respectively. DO concentration in bed A was in the range 0.55~1.91 mg/L vertically, illustrating anoxic micro-environment inside. DO in bed B was higher than that in bed A (shown as 0.48~2.51 mg/L), indicating some aerobic micro-environments inside. DO reached maximum value at water surface level and decreased gradually with depth in both beds, slightly increased in the air pipe oxygenation position in bed B. With depth increasing, ORP presented a decrease trend from 287 mV to 128 mV in bed A and 432 mV to 198 mV in bed B, respectively. Reduction conditions existed in the entire bed A, and below the depth of 80 cm in bed B. From 0 to 80 cm below the distribution pipe in bed B, oxidation conditions existed. Noticeably, more aerobic micro-environment and oxidation zones were observed in bed B. The air pipe enhanced aerobic micro-environment inside the constructed bed B.

Additionally, NH_4^+-N concentration in bed A ranged from 35.12 to 41.72 mg/L vertically, much higher than 7.32-11.80 mg/L in bed B. NH_4^+-N concentration variation coincided with DO concentration variation in both systems, indicating the simple classic nitrification. Atmospheric oxygenation enhanced by the air pipe provided enough oxygen for nitrification without energy consumption in bed B. Conversely, nitrification was limited by low oxygen availability in the predominantly anoxic constructed bed A, resulting in low NH_4^+-N removal rate.

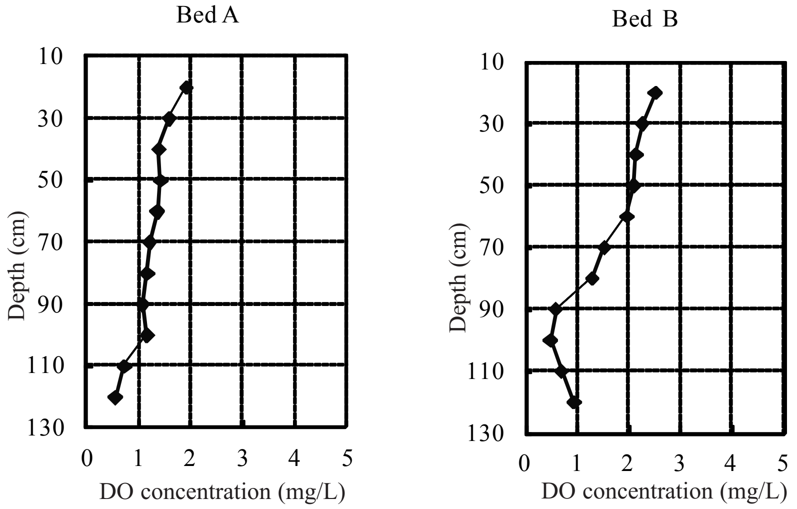

Fig. 2. DO vertical distribution in the vertical flow constructed bed systems.

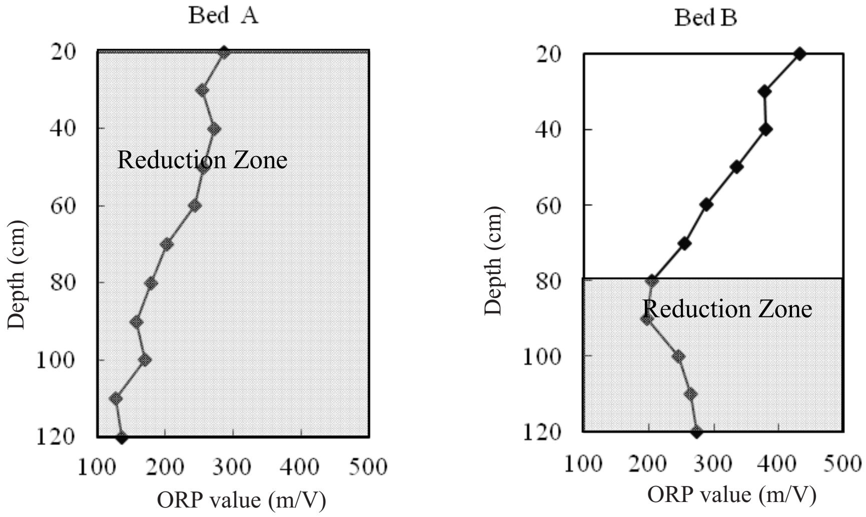

Fig. 3. ORP vertical distribution in the vertical flow constructed bed systems.

Oxygen Supply by Air Pipe

Regarding the possible pathways of oxygen transferred into and consumed inside the constructed beds in this study, the following formulation should be satisfied in bed A and B.

$$O_{air}+O_{in}=O_n+O_o+O_{out} \quad (1)$$

...where O_{air} – atmospheric oxygenation (g/m^2·d), the amount of O_2 supplied by air in bed A and the amount of O_2 supplied by air and the air pipe in bed B, respectively; O_{in} – oxygen in influent (g/m^2·d); O_n – oxygen consumed by nitrification (g/m^2·d); O_o – oxygen consumed by organic matter-s' degradation (g/m^2·d); O_{out} – oxygen in effluent (g/m^2·d).

Theoretically, 1 g oxygen is required to remove 1 g BOD_5 and 4.35 g oxygen is required to transform 1 g ammonia nitrogen into nitrate. Average oxygen supply and consumption rates each day in constructed bed A and B during the whole experiment period were estimated. According to Table 1, the consumption rate of oxygen was estimated, accounting for 35.7 g/m^2·d and 59.4 g/m^2·d in bed A and B, respectively. Then the amount of oxygen supplied by air could be calculated by oxygen mass balance calculation in bed A, which is equal to the difference between the amount of oxygen consumption and the amount of oxygen in influent. The mean amount of oxygen supplied by air in bed B is the value of that in bed A because of the same design. Therefore, mean amount of oxygen supplied by the air pipe can be calculated by the oxygen mass balance in bed B, accounting for 23.7 g/m^2·d. Consequently, remarkable oxygenation was provided by the air pipe in bed B, and total atmospheric oxygenation in bed B was higher than that obtained in the conventional bed A because of the pipe oxygenation.

Most organic matters and ammonia nitrogen were removed by aerobic microorganisms. Compared with bed A, no apparent difference was shown on BOD_5 removal in

Table 2. Bacteria population counts and nitrification potentials at different positions in both constructed bed systems (per 10 g media for bacteria population counts, μg/g·day for nitrification potentials, based on wet weight).

Samples	Total bacteria count	Ammonium oxidizing bacteria count	Nitrite oxidizing bacteria count	Nitrification potential
A1	1.8E+06	1.1E+04	1.3E+04	0.02
A2	5.7E+05	5.7E+04	1.8E+04	0.08
A3	8.3E+05	4.5E+04	1.4E+04	0.03
B1	7.6E+06	1.8E+04	8.2E+04	0.16
B2	2.3E+06	7.2E+04	1.1E+05	0.28
B3	5.2E+06	1.7E+05	4.5E+05	1.2

bed B, as shown in Table 1. However, NH_4^+-N removal was enhanced in the modified vertical flow constructed bed B. Oxygen supplied by the air pipe was supposed to increase the depth and intensity of oxygen transfer and promote ammonia oxidation in the constructed bed matrix, resulting in considerable nitrification. Considering the enhanced passive atmospheric oxygenation ability by the air pipe, it was predicated that optimal design of the air pipe would enhance nitrification potentials for high concentration rural wastewater treatment in modified vertical flow constructed wetland systems.

Bacteria Population Analysis

Total bacteria, ammonium oxidizing bacteria, nitrite oxidizing bacteria population counts and nitrification potentials in both systems are shown in Table 2. Both bacteria population counts and nitrification potentials in bed B were higher than those in the same position of bed A, consistent with the results reported previously [17]. With depth increasing, nitrifying bacteria (ammonium oxidizing bacteria and nitrite oxidizing bacteria) population counts increased in bed B, while in bed A the population first increased then decreased. Nitrification potentials at middle and bottom position (A2, A3, B2, B3) were higher than that of upper position (A1, B1) in the corresponding systems, consistent with the amount of nitrifying bacteria counted. Therefore, the air pipe oxygenation provided favorable habitats for microorganism growth, leading to a prolific microbial community in bed B.

Classic nitrification in wetlands is a two-step process of ammonia oxidation to nitrite by ammonium oxidizing bacteria, followed by nitrite oxidation to nitrate by nitrite oxidizing bacteria under aerobic conditions [18, 19]. Since nitrifying bacteria are autotrophic and use CO_2 and bicarbonate for cell synthesis, growth of nitrifying bacteria would be restrained by heterotrophic bacteria metabolism [18]. The concentration of organic matters was high and this stimulated heterotrophic bacteria growth on position A1 and B1 near the distribution pipe, restricting autotrophic nitrifying bacteria growth and resulting in low nitrification potentials. Conversely, the amount of autotrophic nitrifying bacteria increased with depth because organic matters were primarily removed at depth of 65 cm and 85 cm in both systems. Thereafter, higher nitrification potentials were achieved at the position of A2, A3, B2, and B3. Compared with bed A, nitrifying bacteria counts at a depth of 85 cm was higher because of the air pipe oxygenation in bed B. It is concluded that the penetration and intensity of oxygen transfer were improved by the air pipe, increasing nitrification potentials in the deep zone of constructed wetlands.

Additionally, ammonium oxidizing bacteria counts were higher than nitrite oxidizing bacteria counts in bed A, while nitrite oxidizing bacteria population counts were higher than ammonium oxidizing bacteria counts in bed B. Thereafter, extra oxygen supply by air pipe promoted nitrite oxidizing bacteria growth in bed B, resulting in complete nitrification and higher NH_4^+-N removal rate. Inadequate oxygen in bed A resulted in ammonium-oxidizing bacteria overgrowth and limited growth of nitrite-oxidizing bacteria, leading to incomplete nitrification and weakening nitrification potentials. Therefore, oxygen transferred by air pipe improved nitrite oxidizing bacteria growth for high nitrification potentials, further for high concentration rural wastewater treatment potential in vertical flow constructed wetlands.

Conclusion

Compared with a conventional constructed bed system, ammonia nitrogen removal was enhanced in the modified constructed bed with an air pipe when treating high concentration rural wastewater in the eastern rural area of China. Mean percentage removal of NH_4^+-N was 85.03%. Nitrification was considered as the predominant process, accounting for 10.15 $g/m^2 \cdot d$ under the hydraulic loading of 0.2 $m^3/m^2 \cdot d$. Atmospheric oxygenation by the air pipe was a remarkable 23.7 $g/m^2 \cdot d$, resulting in more aerobic microenvironments, which satisfied the need of estimated oxygen consumption for NH_4^+-N removal from the rural wastewater. Passive atmospheric oxygenation increased by air pipe stimulated nitrite oxidizing bacteria growth for high nitrification potentials in constructed wetland, providing favorable habitats of bacterial richness for high concentration rural wastewater treatment potential.

Acknowledgements

This work was supported by the Major Science and Technology Program for Water Pollution Control and Management of China (2010ZX07320-002) and the Research Founds of Beijing University of Civil Engineering and Architecture (101200902).

References

1. WU S., ZHANG D., AUSTIN D., DONG R., PANG C. Evaluation of a lab-scale tidal flow constructed wetland performance: Oxygen transfer capacity, organic matter and ammonium removal. Ecol. Eng., **37**, 1789, **2011**.
2. YE J., WANG L., LI D., HAN W., YE C. Vertical oxygen distribution trend and oxygen source analysis for vertical-flow constructed wetlands treating domestic wastewater, Ecol. Eng., **41**, 8, **2012**.
3. CLAUDIANE O.P., FLORENT Ch., YVES C., JACQUES B. Artificial aeration to increase pollutant removal efficiency of constructed wetlands in cold climate. Ecol. Eng., **27**, 258, **2006**.
4. LI H.Z., YE J.F., WANG S., FU W., XING S.W. Applicance and development of vertical-flow constructed wetland technology for rural wastewater treatment in Shanghai. Environ. Pollut. Control. **30**, (8), 84, **2008**.
5. LEE C.G., FLETCHER T.D., SUN G.Z. Nitrogen removal in constructed wetland systems. Eng. Life Sci. **9**, (1), 11, **2009**.
6. ALLEN J.G., BEUTEL M.W., CALL D.R., FISCHER A.M. Effects of oxygenation on ammonia oxidation potential and microbial diversity in sediment from surface flow wetland mesocosms. Bio. Technol. **101**, (4), 1389, **2010**.
7. VYMAZAL J. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ., **380**, (1-3), 48, **2007**.
8. KADLEC R., WALLACE S. Treatment Wetlands, 2nd ed.; CRC Press: Boca Raton, FL., **2008**.
9. XU Z.X., WANG S., LI H.Z. A Modified cyclic batch Constructed Wetland: 200510111535.7. Chinese Patent. **2006**.
10. CHINESE NEPA. Water and Wastewater Monitoring Methods, 4th ed.; Chinese Environmental Science Publishing: Beijing, China, **2002**.
11. YU Y.X., WU G.Q., MENG X.T. Booklet of microorganism measurement. Chinese Environmental Science Publishing: Beijing, China, **1990**.
12. XU Z., DU X., WANG S. Simultaneous nitrification and denitrification in non-planted pilot-scale modified vertical flow constructed wetland system. Can. J. Civil Eng. **36**, 1, **2009**.
13. SAEED T., SUN G. Kinetic modelling of nitrogen and organics removal in vertical and horizontal flow wetlands. Water Res., **45**, 3137, **2011**.
14. AUSTIN D. Influence of cation exchange capacity (CEC) in a tidal flow, flood and drain wastewater treatment wetland. Ecol. Eng., **28**, (1), 35, **2006**.
15. SUN G., ZHAO Y., ALLEN S. Enhanced removal of organic matter and ammoniacal-nitrogen in a column experiment of tidal flow constructed wetland system. J. Biotechnol., **115**, (2), 189, **2005**.
16. CANNON A.D., GRAY K.R., BIDDLESTONE A.J., THAYANITHY K. Pilot-scale development of bioreactor for the treatment of dairy dirty water. J. Agric. Eng. Res., **77**, (3), 327, **2000**.
17. CHAZARENC F., GAGNON V., COMEAU Y., BRISSON J. Effect of plant and artificial aeration on solids accumulation and biological activities in constructed wetlands. Ecol. Eng. **35**, 1005, **2009**.
18. HOOPER A.B., VANNELLI T., BERGMANN D.J., ARCIERO D. Enzymology of the oxidation of ammonia to nitrite by bacteria. Antonie van Leeuwenhoek. **71**, 59, **1997**.
19. KOOPS H.P., POMMERENING-ROSER A. Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiol. Ecol. **37**, 1, **2001**.