Bioresource Technology 193 (2015) 424–432

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Performance of system consisting of vertical flow trickling filter and horizontal flow multi-soil-layering reactor for treatment of rural wastewater

Yi Zhang [a,b], Yan Cheng [a,b], Chunping Yang [a,b,c,*], Wei Luo [a,b], Guangming Zeng [a,b], Li Lu [c]

[a] College of Environmental Science and Engineering, Hunan University, Changsha, Hunan 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha, Hunan 410082, PR China
[c] Zhejiang Provincial Key Laboratory of Solid Waste Treatment and Recycling, College of Environmental Science and Engineering, Zhejiang Gongshang University, Hangzhou, Zhejiang 310018, PR China

HIGHLIGHTS

- A novel two-stage hybrid system was developed and evaluated with better TN removal.
- MSL in horizontal flow mode provided ideal anoxic condition and adequate HRT.
- High C/N ratio improved TN removal.
- High feeding frequency benefited intermittent feeding system for pollutant removal.

ARTICLE INFO

Article history:
Received 6 May 2015
Received in revised form 24 June 2015
Accepted 28 June 2015
Available online 2 July 2015

Keywords:
Rural wastewater
Horizontal flow multi-soil-layering
Denitrifying
Intermittent feeding system
Carbon–nitrogen ratios

ABSTRACT

In order to improve nitrogen removal for rural wastewater, a novel two-stage hybrid system, consisting of a vertical flow trickling filter (VFTF) and a horizontal flow multi-soil-layering (HFMSL) bioreactor was developed. The performance of the apparatus was observed under various carbon–nitrogen ratios and water spraying frequencies separately. The maximum removal efficiency of total nitrogen (TN) for the hybrid system was 92.8% while the removal rates of COD_{Cr}, ammonium (NH_4^+-N), and total phosphorus (TP) were 94.1%, 96.1%, 92.0% respectively, and the corresponding effluent concentrations were 3.61, 21.20, 1.91, and 0.33 mg L^{-1}. The horizontal flow mode for MSL led the system to denitrifying satisfactorily as it ensured relatively long hydraulic retention time (HRT), ideal anoxic condition and adequate organic substrates supply. Also, higher water spraying frequency benefited intermittent feeding system for pollutants removal. Shock loading test indicated that the hybrid system could operate well even at hydraulic shock loadings.

1. Introduction

Rural water pollution has attracted greater attention over the past few decades (Wang et al., 2011). In developing countries like China, water pollution loads caused by rural activities are becoming cumulatively prominent because of lacking applicable sewage management (Chen et al., 2006). In recent years, the technology of decentralized wastewater treatment has been gaining popularity as a low-cost, low-maintenance and effective alternative in vast rural areas. A wide range of decentralized wastewater treatment formats and hybrid combinations have been developed, each with different performance attributes and area requirements (Vymazal, 2011).

Most decentralized wastewater treatment systems including conventional septic tank-soil trench systems, land treatment systems, sand filter systems, constructed wetland systems, etc., can provide advanced treatment for rural wastewater. Brix and Arias (2005) studied the vertical constructed wetland system which fulfilled requirements of 95.0% removal of biochemical oxygen demand (BOD), 90.0% removal of total phosphorus (TP), and 90.0% nitrification for single dwelling and house in rural regions of Danish. Luo et al. (2014) developed a two-stage vertical hybrid system including a trickling filter and a multi-soil-layering (MSL) biofilter for simulated septic tank effluent in China, and the system

* Corresponding author at: College of Environmental Science and Engineering, Hunan University, Changsha, Hunan 410082, PR China. Tel./fax: +86 731 88823987.
E-mail address: yangc@hnu.edu.cn (C. Yang).

http://dx.doi.org/10.1016/j.biortech.2015.06.140
0960-8524/

steadily achieved removal rates of 90.3–95.2% chemical oxygen demand (COD_{Cr}), 85.1–86.9% ammonium (NH_4^+-N), and 92.0–94.0% TP. However, few of them are optimized for total nitrogen (TN) removal owing to the lack of sufficient denitrification (Oakley et al., 2010). That is to say, the concentrations of effluent nitrate from such systems usually keep at a relatively high level, which still have potential impacts on groundwater.

The insufficient denitrification might result from less ideal anaerobic condition and shortage of enough retention time (Hao et al., 2013). Horizontal flow decentralized systems, such as the horizontal subsurface-flow constructed wetlands which are simple to operate and have relatively longer residence time, cope well with fluctuations in loading and usage. Oxygen transporting into the saturated media of the horizontal flow systems is limited, so they are predominantly anaerobic systems (Bezbaruah and Zhang, 2005). While this low level of oxygen availability largely restricts the nitrifying rates, such anoxic conditions can significantly facilitate denitrification achievable in these systems.

Microbial denitrification also requires adequate supply of organic carbon besides perfect anoxic condition and enough retention time. Recirculation, which is equivalent to the process of pre-anaerobic nitrification–denitrification, has been introduced to enhance the denitrification via repeated contact with inflowing carbon-rich wastewaters (Gross et al., 2007). The technology of post-anoxic nitrification–denitrification through uninterrupted dosing with external organic substrates in anoxic denitrifying reactor following the aeration tank is applied commonly in centralized plants. Both the approaches mentioned above are effective solution for TN removal, but not likely to be an affordable and practical option for on-site and decentralized wastewater treatments with economic and technical considerations.

High performances of multi-soil-layering (MSL) systems have been exhibited in many studies on decentralized wastewater treatments including polluted river water (Masunaga et al., 2003), cafeteria wastewater (Attanandana et al., 2000) and livestock wastewater (Chen et al., 2007). MSL system, a novel soil-based technology, enhances inherent ability of soil via improving the inside structure (Luanmanee et al., 2001; Sato et al., 2011). In the MSL, soil mixture block (SMB) layers, surrounded by permeable layers (PL), are arranged in a brick-like pattern. The PL usually consists of granular zeolite with features of advanced porous structure, high ion exchange capacity and low density. The SMB is mainly composed of soil mixed with 20–30% additional materials such as iron, sawdust, charcoal, etc. Organic materials added to SMB, such as rice straw, kenalf, corncob and sawdust, can significantly enhance microbial activity and promote denitrification process by providing adequate hydrogen suppliers. Wakatsuki et al. (1993) evaluated the life of the additional organic matter in SMB for denitrification would be 12.8 years, meanwhile metal iron for fixation of phosphorus (P) could last for 11.7 years. The system developed in Japan has been reported to operate at least nine years and the removal rates of BOD, TN and TP could still keep at 95.0%, 75.0% and 80.0% (Luanmanee et al., 2001). Consequently, the use of MSL as a denitrification bioreactor will be an option with considerable potential, if such system is reasonably designed.

In present study, a novel two-stage system, named as vertical flow trickling filter–horizontal flow multi-soil-layering (VFTF–HFMSL) system, was developed to strengthen the TN removal for rural wastewater treatment. The vertical flow trickling filter (VFTF), followed by the horizontal flow multi-soil-layering (HFMSL) bioreactor, was well designed to supply pre-nitrified wastewater considering that the vertical flow decentralized systems can promote more efficient nitrification through enhancing oxygen transfer rate from air to water (Alfiya et al., 2007; Wang and Yang, 2004). Zeolite, an aluminosilicate mineral with good ion exchange selectivity for NH_4^+, was utilized as the filter media in VFTF. This study mainly focused on the removal efficiencies for COD_{Cr}, NH_4^+-N, TP, and especially the TN in the hybrid system. The specific objectives were to examine performances and removal mechanisms of the system under conditions of different C/N ratios and water spraying frequencies, to figure out the optimal operating conditions, and then to evaluate the system stability at hydraulic shock loadings under the referred conditions.

2. Methods

2.1. Experimental apparatus and materials

The VFTF–HFMSL system microcosm is built, as shown in Fig. 1. Two lidless acrylic boxes filled with different media worked as VFTF and HFMSL respectively. VFTF (320 mm length × 160 mm width × 600 mm height), the vertical one with apertured bottom, was packed with gravel and zeolite. HFMSL (1200 mm length × 160 mm width × 320 mm height), the horizontal one divided into three parts by the grid, consisted of inlet pool, outlet pool, and MSL bioreactor. The MSL bioreactor in HFMSL included six soil mixture block (SMB) layers surrounded by permeable layers (PL) in brick-like pattern. Simulated sewage from the storage tank was pumped intermittently into the system using a submerged pump which was controlled by a time switch (DH48S-S), dispersed evenly onto the filter media in VFTF through a set of perforated pipes, and finally gravitated into HFMSL. The structure of VFTF and HFMSL is shown in Fig. 2. Table 1 lists the effective material compositions of their segments as well as properties of materials.

Natural zeolite (3–5 mm diameter in VFTF, 1–3 mm diameter in HFMSL) mixed with iron scraps at the ratio of 95:5 by dry weight was obtained from Jinyun, Zhejiang province of China. The SMB was the mixture of clayey soil, sawdust and iron scraps at the ratio of approximately 65:25:10 by dry weight, respectively (Luanmanee et al., 2001). The soil got from the Tianma Mountain in Changsha, China was sifted through meshed screen with 2 mm mesh size after being air dried and crushed. The lathe iron cutting scraps were washed in boiled 5% NaOH solution to remove adsorbed oil and activated in diluted HCl solution for 30 min, respectively before using.

2.2. Influent wastewater quality and operating conditions

Typically, on-site wastewater treatment for an individual home simply consists of a septic tank and an effluent dispersal system in rural regions (Leverenz et al., 2010). Wastewater in experiment was prepared through dissolving starch, glucose, peptone, $NaHCO_3$, NH_4Cl, KH_2PO_4, K_2HPO_4, $MgSO_4$, $CaCl_2$, $MnSO_4$ into 100 L tap water to simulate outflow of the septic tank in rural area. The simulated wastewater quality, varied in steps according to requirements in different experiment phases, is listed in Table 2.

The system mainly operated for three phases in which the effects of C/N ratio, water spraying frequency, and hydraulic shock loadings on the system removal efficiency were studied. In phase 1, the apparatus ran at C/N ratio of 5:1, 8:1, 2:1, referred as phase a, b, c, by stages with water spraying frequency at 16 s/60 min (water feeding for 16 s per 60 min) for 22 days. In phase 2, the apparatus ran at water spraying frequency of 8 s/30 min, 4 s/15 min, referred as phase d, e, step by step with C/N ratio at 5:1 for another 22 days. In phase 3, the apparatus firstly ran at the optimal operating conditions (C/N ratio of 7:1, water spraying frequency of 8 s/30 min), referred as phase f, for 22 days after analyzing the previous experimental data in this study and then the stability of apparatus under hydraulic shock loadings was tested, referred as phase g, for about 10 days. In phase g, the water spraying frequency was kept at

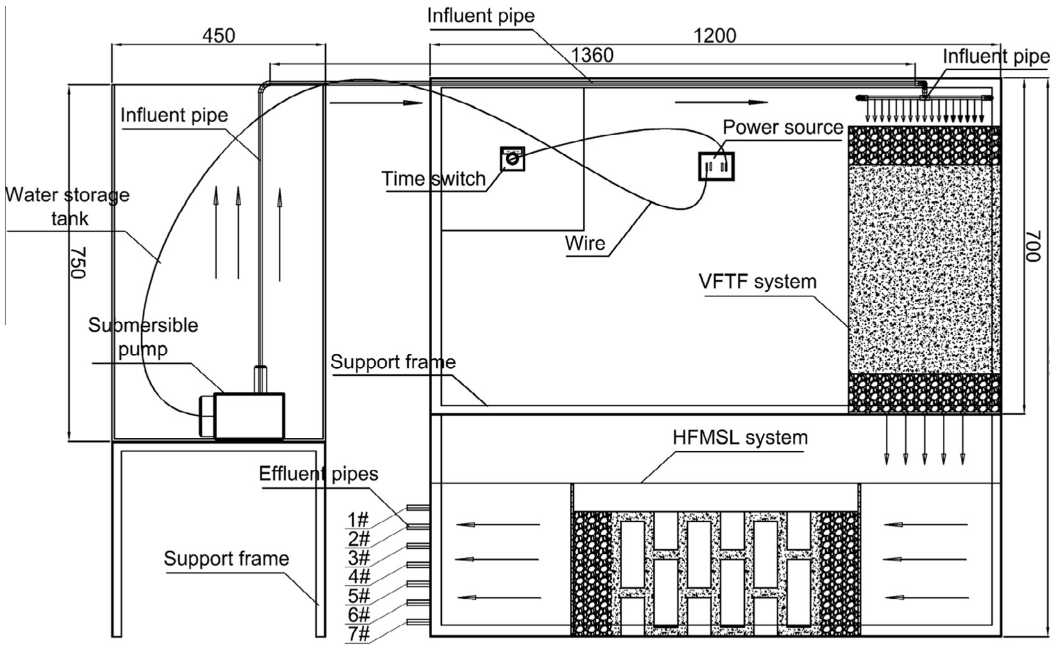

Fig. 1. Schematic showing of the experimental apparatus. Note: (i) the dimension unit is mm. (ii) effluent only discharged from pipe 1# when the apparatus normally operated and pipe 7# was used for draining the water in HFMSL system when necessary. (iii) pipes 2# to 6# designed for the further research were closed during the whole experiment.

8 s/15 min from 16:00 to 19:00 in the afternoon and was set at 8 s/30 min for the rest of the day with C/N ratio at 7:1. The hydraulic loading rate (HLR), measured via dividing water volume decrement in the storage tank per day by area of the apparatus inlet, was kept approximately at the same rate of 660 $L\,m^{-2}\,d^{-1}$ through adjusting the water feeding time in all experiment phases except in phase g when the HLR was set at about 1300 $L\,m^{-2}\,d^{-1}$ from 16:00 to 19:00 in the afternoon. The corresponding nominal hydraulic retention time (HRT) was about 1.8 days.

For microbial growth in the system, simulated wastewater was pumped into the apparatus under the same operating conditions with phase a, for almost 4 weeks before the startup of the formal experiment. Water and ambient temperature were continuously recorded during the whole study. The maximum, minimum and average ambient temperatures were 35.2 °C, 25.3 °C, 29.6 °C, respectively. The maximum, minimum and average water temperatures were 23.5 °C, 9.8 °C, 15.8 °C, respectively. To ensure a relatively constant operating condition, the inlet pipes, outlet pipes as well as the storage tank were cleaned up weekly.

2.3. Water sampling and analysis

Samples of influent, mid effluent and final effluent were taken from the storage tank, inlet pool in HFMSL and outlet pool in HFMSL, respectively. Water samples were taken and analyzed every three days except in phase g when samples were taken at 08, 14, 18, 20, 22 o'clock every day. Chemical analyses of every batch samples were accomplished in the day.

Analysis methods were summarized as follows: COD_{Cr} using the potassium dichromate method, TP using the potassium persulfate digestion colorimetric method, NH_4^+-N using the Nessler's reagent colorimetric method, NO_2^--N by the N-(1-naphthyl)-ethylenediamine spectrophotometric method, NO_3^--N by the ultraviolet spectrophotometry method, TN by the potassium persulfate oxidation-ultraviolet spectrophotometry method, and water pH was measured using pH meter (PHS-3C) all according to the standard methods (APHA, 1998).

3. Results and discussion

3.1. Performance of the VFTF–HFMSL system in carbon–nitrogen ratio and water spraying frequency test

In this part, series of data obtained in phase a, b, c, d, e as well as phase f were inspected in order to evaluate the performance of the VFTF–HFMSL system under conditions of different C/N ratios and water spraying frequencies. Tables 3a–3c showed the average value of parameters in each experiment phase and would be referred as Table 3 together when necessary in the article. The phase a, b, c were observed together as C/N ratio test group (Table 3a), while the phase a, d, e were assessed together as water spraying frequency test group (Table 3b). Data of the effluents from the upper VFTF section and the lower HFMSL section were both plotted and respectively marked with suffixes of "mid" and "final" in each figure below.

3.1.1. Chemical oxygen demand removal

Fig. 3a and Table 3 presented the COD removal effect. The final effluent COD concentration of the hybrid VFTF–HFMSL system steadily kept at 20.54 $mg\,L^{-1}$ on average, with the constant mean COD removal rate of 90.9% despite the varied operating conditions in phase 1 and phase 2. Even when the influent COD reached 417.47 $mg\,L^{-1}$ in days 29–50, corresponding to the maximum organic loading rate (OLR) of about 275.25 g $COD_{Cr}\cdot m^{-2}\,d^{-1}$, the final effluent COD still kept at fairly low level of 31.18 $mg\,L^{-1}$.

The results indicated that the running conditions including different C/N ratios and water spraying frequencies had greater influences on the mid effluent than the final effluent, which might result from the longer HRT in the lower HFMSL, ensuring the stable removal of organic matters through physical and chemical absorption and subsequent microbial degradation (Sato et al., 2011). In more details, both the primary COD removal rate in VFTF and the total COD removal rate in VFTF–HFMSL obviously decreased following the reduction of influent COD in days 57–78 corresponding to phase c, indicating that the hybrid system might hardly have a

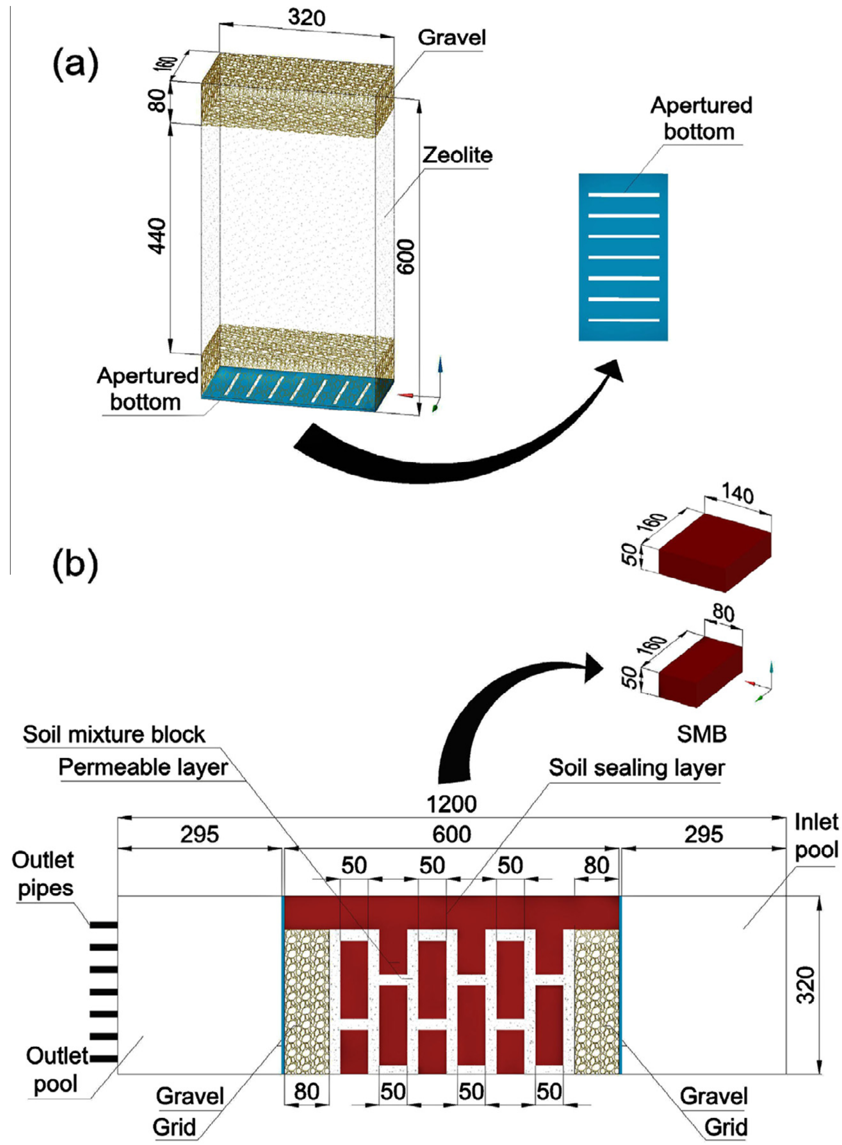

Fig. 2. Schematic of the VFTF–HFMSL system: (a) the structure of VFTF; (b) the structure of HFMSL. Note: the dimension unit is mm.

Table 1
Properties of materials and effective compositions of different system segments.

	Zeolite		Soil	Sawdust	Iron scraps
Granularity (mm)	1–3	3–5	<2	<1	1–5
Bulk density (g cm^{-3})	0.86	0.79	1.20	0.11	0.75
Mass percent by dry weight (%)					
VFTF (effective composition)	0	95	0	0	5
SMB	0	0	65	25	10
PL	95	0	0	0	5

satisfying performance with the continuous operation at low influent COD. With the water spraying frequency increasing by stages in days 1–22, 85–106, 113–134 corresponding to phase a, d, e, the primary COD removal rate enhanced from 74.1% to 80.3%, and then to 86.8%, which might because the HRT in the upper VFTF relatively increased (Wang et al., 2009). In phase f, the hybrid system also showed high COD removal efficiency under the conditions of C/N ratio at 7:1, water spraying frequency at 8 s/30 min. As a result, the hybrid system designed into such structure can effectively remove the organic matters in wastewater, disregarded of the changed operating conditions tested.

3.1.2. Phosphorus removal

Excellent system performance in TP removal was exhibited throughout the whole study (Fig. 3b and Table 3). The effluent TP always kept at the mean concentration of approximately 0.35 mg L^{-1}. Numerous studies previously reported that phosphorus can be chemically absorbed by the Al and Fe hydroxides in filter media such as the zeolite and soil filled in the current hybrid system. The added iron, for instance, will transform into ferrous iron (Fe^{2+}), and then be oxidized to ferric ion (Fe^{3+}) which aids in the phosphorus fixing through forming chemical precipitate (Wakatsuki et al., 1993). Thus, appropriate doses of iron scraps were added into the system.

Fig. 3b showed that TP removal rates including primary removal rate and total removal rate were extremely stable when the apparatus ran at a constant HLR of about 660 L m^{-2} d^{-1} in each experiment phase, which is in line with the conclusions of Sato et al.'s (2005) research that phosphorus removal is principally due to the chemical precipitation, a process mainly confined by the contact time between ferric ion and orthophosphate. The iron scraps paved in aerobic VFTF had much more effect in phosphorus fixing

Table 2
Simulated wastewater quality in different experiment phases.

		COD_{Cr} (mg L^{-1}) (n = 8)	TN (mg L^{-1}) (n = 8)	NH_4^+-N (mg L^{-1}) (n = 8)	TP (mg L^{-1}) (n = 8)	pH (n = 8)
Phase 1	a	255.20 ± 65.12[a]	49.31 ± 9.91	48.91 ± 7.93	3.91 ± 0.41	6.62 ± 0.31
	b	417.47 ± 89.01	49.02 ± 7.74	48.65 ± 6.41	4.22 ± 0.82	6.80 ± 0.32
	c	98.68 ± 35.22	49.58 ± 7.23	49.08 ± 6.72	3.93 ± 0.31	6.90 ± 0.44
Phase 2	d	254.31 ± 45.67	49.39 ± 12.95	49.11 ± 9.35	3.76 ± 0.45	7.02 ± 0.12
	e	253.63 ± 73.31	49.68 ± 9.21	49.52 ± 8.61	3.88 ± 0.51	6.73 ± 0.36
Phase 3	f	357.78 ± 57.65	50.00 ± 11.33	49.57 ± 10.11	4.11 ± 0.53	6.95 ± 0.21
	g	349.46 ± 66.63	50.92 ± 11.12	49.79 ± 9.23	3.93 ± 0.28	6.88 ± 0.26

[a] Mean value ± standard deviation.

Table 3a
Performance of the hybrid system in carbon–nitrogen ratio test corresponding to phase 1 including phase a, b, c.

			Influent (mg L^{-1}) (n = 8)	Mid effluent (mg L^{-1}) (n = 8)	Final effluent (mg L^{-1}) (n = 8)
Phase a	C/N of 5:1	COD_{Cr}	255.20 ± 65.12 [a]	66.22 ± 6.52 (74.1) [b]	19.76 ± 2.11 (92.3)
	Frequency of 16 s/60 min	TN	49.31 ± 9.91	22.14 ± 2.31 (55.1)	5.21 ± 1.05 (89.4)
		Nitrate	–	8.61 ± 0.51	1.74 ± 0.13
		Ammonium	48.91 ± 7.93	13.39 ± 1.12 (72.6)	3.03 ± 0.41 (93.8)
		TP	3.91 ± 0.41	1.01 ± 0.05 (74.2)	0.31 ± 0.03 (92.1)
Phase b	C/N of 8:1	COD_{Cr}	417.47 ± 89.01	125.04 ± 21.23 (70.0)	31.18 ± 3.12 (92.5)
	Frequency of 16 s/60 min	TN	49.02 ± 7.74	22.21 ± 2.35 (54.7)	5.17 ± 1.21 (89.5)
		Nitrate	–	5.75 ± 0.72	0.21 ± 0.01
		Ammonium	48.65 ± 6.41	16.19 ± 3.11 (66.7)	4.82 ± 1.02 (90.1)
		TP	4.22 ± 0.82	1.08 ± 0.21 (74.4)	0.38 ± 0.03 (91.0)
Phase c	C/N of 2:1	COD_{Cr}	98.68 ± 35.22	40.91 ± 5.23 (58.5)	17.92 ± 2.21 (81.8)
	Frequency of 16 s/60 min	TN	49.58 ± 7.23	29.67 ± 3.52 (40.2)	13.11 ± 1.53 (73.6)
		Nitrate	–	18.56 ± 2.83	7.09 ± 1.61
		Ammonium	49.08 ± 6.72	11.01 ± 1.01 (77.6)	2.91 ± 0.89 (94.1)
		TP	3.93 ± 0.31	1.11 ± 0.23 (71.8)	0.32 ± 0.02 (91.9)

[a] Mean value ± standard deviation.
[b] The values in the round brackets represent the mean removal rate (%) of the corresponding pollutants.

Table 3b
Performance of the hybrid system in water spraying frequency test corresponding to phase 2 including phase a, d, e.

			Influent (mg L^{-1}) (n = 8)	Mid effluent (mg L^{-1}) (n = 8)	Final effluent (mg L^{-1}) (n = 8)
Phase a	Frequency of 16 s/60 min	COD_{Cr}	255.20 ± 65.12 [a]	66.22 ± 6.52 (74.1) [b]	19.76 ± 2.11 (92.3)
	C/N of 5:1	TN	49.31 ± 9.91	22.14 ± 2.31 (55.1)	5.21 ± 1.05 (89.4)
		Nitrate	–	8.61 ± 0.51	1.74 ± 0.13
		Ammonium	48.91 ± 7.93	13.39 ± 1.12 (72.6)	3.03 ± 0.41 (93.8)
		TP	3.91 ± 0.41	1.01 ± 0.05 (74.2)	0.31 ± 0.03 (92.1)
Phase d	Frequency of 8 s/30 min	COD_{Cr}	254.31 ± 45.67	50.11 ± 5.23 (80.3)	17.92 ± 3.11 (93.0)
	C/N of 5:1	TN	49.39 ± 12.95	24.23 ± 3.23 (50.9)	6.33 ± 1.21 (87.2)
		Nitrate	–	15.67 ± 2.72	4.14 ± 0.78
		Ammonium	49.11 ± 9.35	8.67 ± 3.62 (82.3)	1.62 ± 0.51 (96.7)
		TP	3.76 ± 0.45	1.00 ± 0.21 (73.4)	0.38 ± 0.03 (89.9)
Phase e	Frequency of 4 s/15 min	COD_{Cr}	253.63 ± 73.31	33.41 ± 5.12 (86.8)	16.67 ± 4.68 (93.4)
	C/N of 5:1	TN	49.68 ± 9.21	25.02 ± 3.56 (49.6)	8.19 ± 1.21 (83.5)
		Nitrate	–	16.83 ± 2.63	6.01 ± 1.32
		Ammonium	49.52 ± 8.61	8.21 ± 0.89 (83.4)	1.73 ± 0.11 (96.5)
		TP	3.88 ± 0.51	0.91 ± 0.11 (76.5)	0.41 ± 0.02 (89.4)

[a] Mean value ± standard deviation.
[b] The values in the round brackets represent the mean removal rate (%) of the corresponding pollutants.

than those added into SMBs in HFMSL as nearly 74.1% of TP was removed in the upper stage (Table 3). This might because iron can more easily transform into ferric ion in the aerobic conditions according to Luo et al.'s (2014) study which also suggested TP removal in MSL was mainly restricted by the processes of iron ion production and diffusion. Furthermore, the structure of the upper VFTF system provided a relatively high Fe/P ratio at partial zones, enhancing the phosphorus removal (Fytianos et al., 1998). The precipitate would subsequently be adsorbed and/or intercepted by the filter media in the hybrid system.

3.1.3. Ammonium removal and nitrification

The influent nitrogen only contained ammonium with the concentration of about 50.00 mg L^{-1} (Table 2). In order to have a more intuitive demonstration about the effects of operating conditions on system performance, the histograms were drawn with the order of C/N ratio at 2:1, 5:1, 8:1 corresponding to phase c, a, b and water spraying frequency of 16 s/60 min, 8 s/30 min, 4 s/15 min, corresponding to phase a, d, e in Fig. 4a and b.

Judging from Fig. 3c and Table 3, the hybrid system presented noticeable removal rates for ammonium, especially in water

Table 3c
Performance of the hybrid system under the optimal operating conditions in phase f.

			Influent (mg L^{-1}) (n = 8)	Mid effluent (mg L^{-1}) (n = 8)	Final effluent (mg L^{-1}) (n = 8)
Phase f	C/N of 7:1	COD_{Cr}	357.78 ± 57.65 [a]	92.18 ± 10.11 (74.2) [b]	21.20 ± 3.14 (94.1)
	Frequency of 8 s/30 min	TN	50.00 ± 11.33	22.41 ± 4.21 (55.2)	3.61 ± 0.88 (92.8)
		Nitrate	–	13.02 ± 1.23	1.29 ± 0.31
		Ammonium	49.57 ± 10.11	9.29 ± 1.22 (81.3)	1.91 ± 0.44 (96.1)
		TP	4.11 ± 0.53	1.10 ± 0.21 (73.2)	0.33 ± 0.05 (92.0)

[a] Mean value ± standard deviation.
[b] The values in the round brackets represent the mean removal rate (%) of the corresponding pollutants.

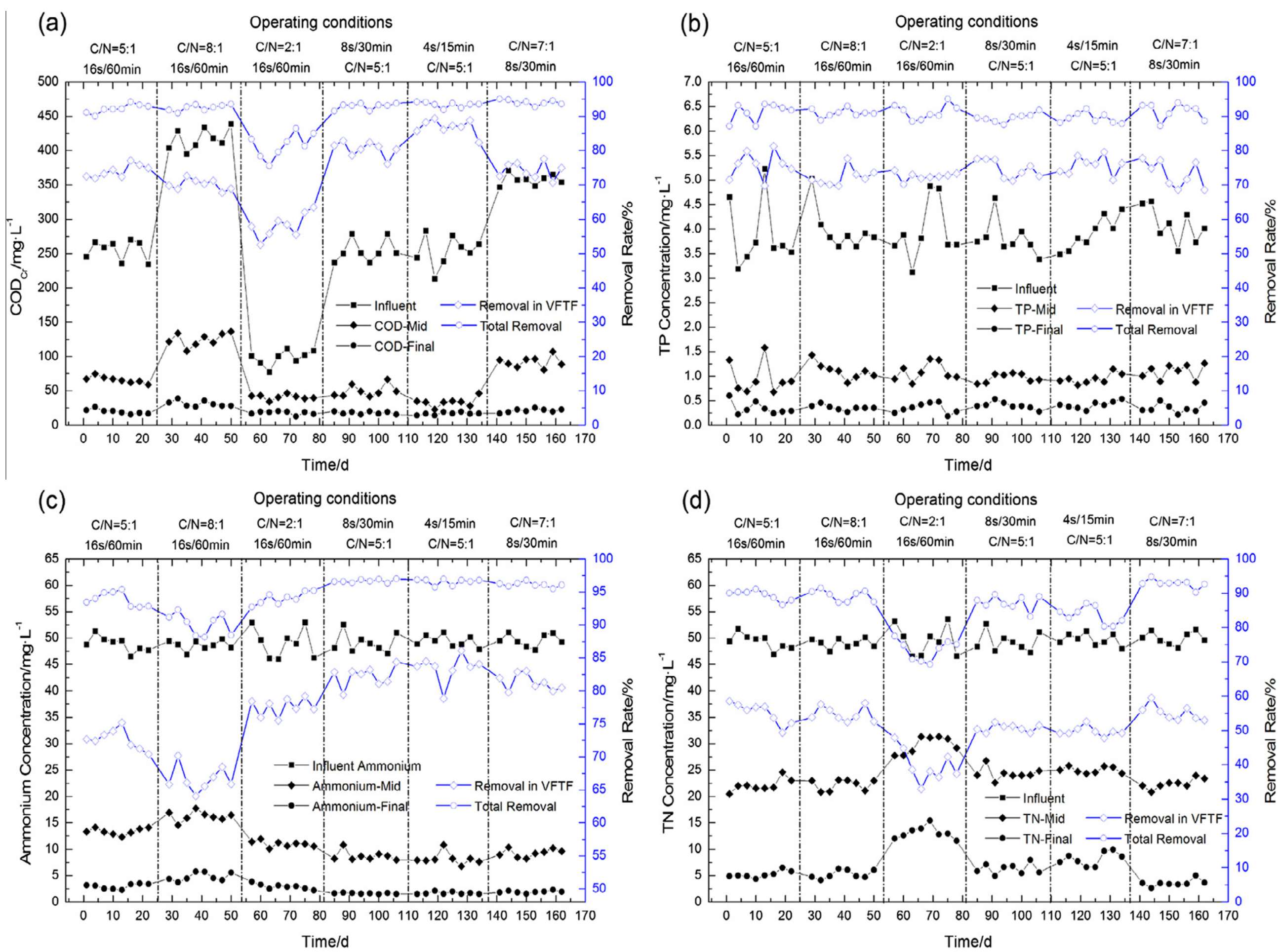

Fig. 3. Pollutant removal efficiency in the hybrid system in phase 1, phase 2 and phase f: (a) COD_{Cr} and its removal efficiency; (b) TP and its removal efficiency; (c) ammonium and its removal efficiency; (d) TN and its removal efficiency.

spraying frequency test, due to the high adsorption capacity of zeolite and the effective nitrification in VFTF which ensured zeolite regeneration in adsorption capacity. Fig. 3c also showed that the ammonium removal rate dramatically decreased merely when the C/N ratio was 8:1, which might because the heterotrophic bacteria had a dominant growth while the growth of autotrophic bacteria was inhibited under high C/N ratio, leading to the insufficient nitrification in the upper VFTF which mainly worked as a nitrification bioreactor in the current hybrid system. This phenomenon was quite in agreement with the previous studies on nitrification/-denitrification processes in decentralized systems (Van den Akker et al., 2011). Consequently, the final effluent ammonium would be relatively high owing to the visible increment of mid effluent ammonium concentration and the weak nitrification in the lower HFMSL which was mainly designed for denitrification. Table 3b indicated that the primary ammonium removal efficiency largely enhanced from 72.6% to 82.3%, almost 10 percent increments, when water spraying frequency increased from 16 s/60 min to 8 s/30 min, but only enhanced from 82.3% to 83.4% with the frequency increasing from 8 s/30 min to 4 s/15 min. This revealed that the optimal water spraying frequency, at least for the ammonium removal, was likely to be 8 s/30 min with the economic and technical considerations in this study. Moreover, the higher frequency might result in the lower level of COD in mid effluent, which would restrict the TN removal in the lower HFMSL.

It showed that the final effluent nitrate respectively kept at a relatively high level of 7.09 mg L^{-1}, 4.14 mg L^{-1}, 6.01 mg L^{-1} when C/N ratio was 2:1 in C/N ratio test, and frequency was 8 s/30 min and 4 s/15 min in water spraying frequency test according to Fig. 4b, indicating that either the low concentration of influent

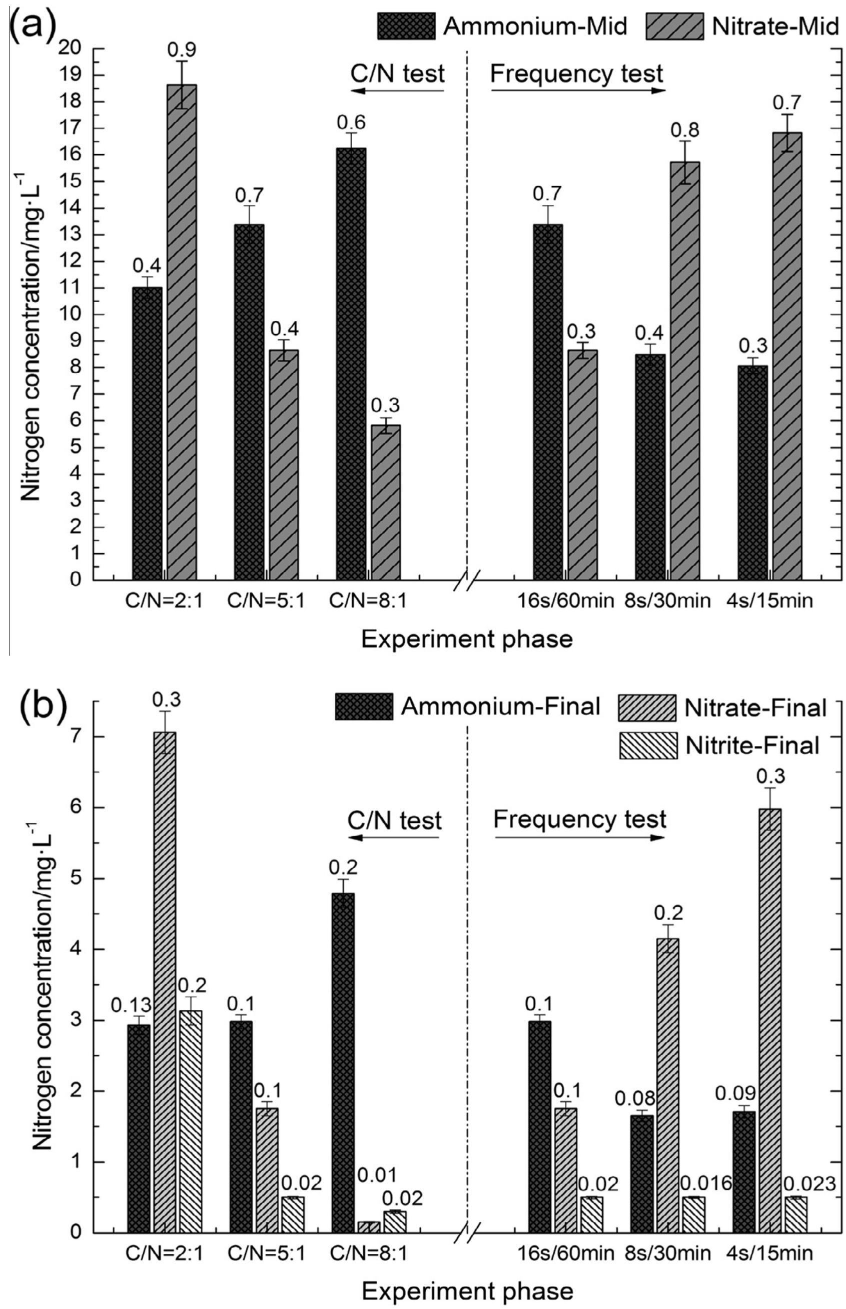

Fig. 4. Effluent nitrogen concentration in carbon–nitrogen (C/N) ratio test and water spraying frequency test: (a) mid effluent nitrogen; (b) final effluent nitrogen. Note: (i) the influent nitrogen only contained ammonium with the concentration of approximately 50 mg L^{-1}. (ii) The histograms were drawn with the order of C/N ratio at 2:1, 5:1, 8:1 corresponding to phase c, a, b and water spraying frequency of 16 s/60 min, 8 s/30 min, 4 s/15 min corresponding to phase a, d, e.

COD or the high frequency of water feeding would lead to the low level of mid effluent COD which accordingly had negative impacts on denitrification in the next stage. As shown in Fig. 4a and Fig. 4b, the mid effluent nitrite could be neglected throughout the whole study, and the final effluent nitrite concentration was usually quite low at about 0.50 mg L^{-1} except in phase c when the nitrite concentration was 3.11 mg L^{-1} suggesting that large amounts of nitrate could not be completely reduced to gaseous nitrogen with the inadequate carbon source (Oakley et al., 2010). Additionally, the amounts of ammonium removal were constantly higher than that of nitrate production (Fig. 4a), which proved the existence of remarkable adsorption and simultaneous denitrification in the upper VFTF. Krasnits et al. (2013) suggested that the polyhydroxyalkanoates (PHA) storage bacteria might be the primary contributor to the visible denitrification as high HLR shortened the HRT, supplied more carbon resource and consumed bulk of dissolved oxygen (DO).

3.1.4. Denitrification and TN removal

In most researches on denitrifying bioreactors, it was reported that conventional heterotrophic denitrification played a major role on nitrate (NO_3^-) removal, in which the denitrifiers transform nitrate to nitrogen gases utilizing degradable organic substrates as electron donor and for growth. Other possible fates for NO_3^- involved anammox, nitrogen immobilization into organic matter, and dissimilatory nitrate reduction to ammonium (DNRA)

(Behrendt et al., 2014; Pant and Adholeya, 2009). However, there was clear evidence that denitrification was the dominant mechanism of NO_3^- removal. For instance, Greenan et al. (2006) found that the immobilization and DNRA merely accounted for less than 4.0% nitrate removal through using the isotope labeling technique. Thereby, heterotrophic denitrification was mainly concerned about in this study.

As shown in Fig. 3d, considerable improvement was achievable in the hybrid system, compared with the previous studies on TN removal. The average total TN removal rate was about 85.4% with the nitrogen mass removal rate of 27.83 g N m^{-2} d^{-1} in phase 1 and phase 2 (Tables 3a and 3b), and the removal efficiency was even high at the rate of 92.8% corresponding to the maximum nitrogen mass removal rate of 30.62 g N m^{-2} d^{-1} in phase f (Table 3c). The key to achieve a better performance on TN removal in the current system is to ensure the adequate concentration of organic substrates and rationalize the distribution of ammonium and nitrate in mid effluent through adjusting influent C/N ratio and water spraying frequency. For example, it was reasonable to keep the concentration of mid effluent nitrate higher than mid effluent ammonium in phase d when water spraying frequency was set as 8 s/30 min, considering the major function of each stage in the hybrid system. Low frequency could result in the insufficient nitrification in the upper VFTF, while high frequency would require more influent COD, neither of which benefited for the long-term removal of TN (Carrera et al., 2004). On the other hand, as discussed in Section 3.1.3, the total TN removal rate kept at 89.5% in phase b when the C/N ratio was 8:1, but the mid effluent ammonium remained a high level which was beyond the removal capacity of the lower HFMSL. Meanwhile, the relatively low concentration of mid effluent nitrate revealed that most nitrogen might just be removed by the process of physical–chemical absorption in the form of NH_4^+-N, so the high performance in TN removal would not be sustainable after the added zeolite reached adsorption saturation (Fig. 4a) (Carrera et al., 2003). As a result, the excess influent COD, especially with a low water spraying frequency, was not suitable for this hybrid system in TN removal. According to the data gained from Table 3, mid effluent C/N ratios were partially figured out in order to have a more accurate understanding on denitrification. The mid effluent C/N ratio was 3:1 in phase a when the final effluent TN was 5.21 mg L^{-1} with the removal rate of 89.4%. Though the determined value was lower than the ideal C/N ratio for denitrification, the performance was satisfying due to the release of degradable carbon from carbonaceous media in SMBs (Schipper et al., 2010; Wu et al., 2013).

At the microbial level, the rate of denitrification was controlled by the concentrations of oxygen (O_2), nitrate and degradable organic matters (Seitzinger et al., 2006). Aerobic microorganisms obtain energy via the oxidation of organic compounds utilizing O_2 as the electron acceptor before the environment gradually becomes energetically favorable for the utilization of NO_3^- as the electron acceptor (Oakley et al., 2010). Fig. 4b showed that the final effluent nitrate could stay at less than 5.00 mg L^{-1}, when the operating conditions were moderate, indicating that the denitrifiers were able to remove nitrate effectively due to the adequate HRT, relatively anoxic condition and sufficient carbon resource in the lower HFMSL. So the influent C/N ratio of 7:1 and the water spraying frequency of 8 s/30 min were suggested as the optimal running conditions according to the performance of the hybrid system and considering the COD removal rates as well as the nitrate production rates in the upper VFTF. Data obtained in phase f subsequently confirmed the conclusion (Table 3c). However, the demand of higher C/N ratio in sewage might restrict the application of such system in rural wastewater treatment, so alternatives for sustainable denitrification should be adding more effective carbonaceous media into SMBs.

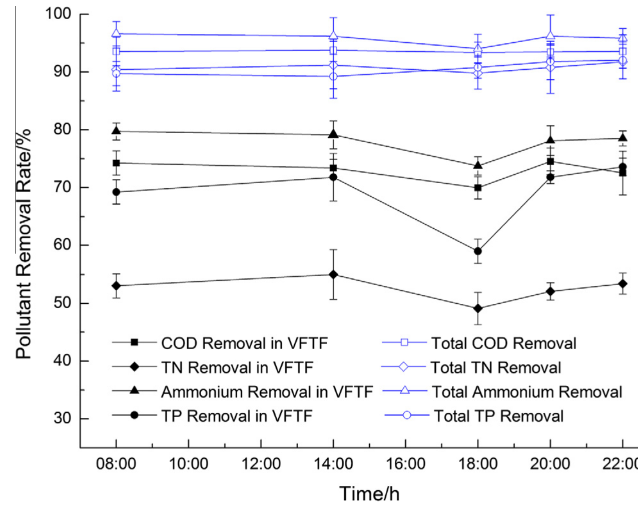

Fig. 5. The 24-h performance of the two-stage hybrid system under hydraulic shock loadings in phase g.

3.2. Reliability of the hybrid system against shock loadings

Table 3c showed the high removal efficiency of the apparatus during phase f. In this part, the stability of apparatus was tested under hydraulic shock loadings for 3 h from 16:00 to 19:00 in a 24-h cycle basing on phase f.

Fig. 5 presented the performance of the hybrid system against hydraulic shock loadings in phase g. The results showed that the average total pollutant removal efficiencies were 93.5% COD, 90.8% TN, 95.8% NH_4^+-N, 90.7% TP, respectively though the removal rates fluctuated when the shock loadings occurred. In more details, more remarkable fluctuations happened in the upper VFTF stage, which was in agreement with the performance of the whole system in the previous experiment phases. As for the TP, for instance, the primary removal rate had a sudden and obvious drop following the HLR increasing to 1300 L m^{-2} d^{-1}, yet the total removal rate showed even little correlation with the hydraulic shock loadings. On the other hand, though the primary pollutant removal efficiencies decreased when the shock loadings happened, the performance of the hybrid system was still satisfactory because the HFMSL bioreactor as the second stage showed effective denitrification as well as high removal efficiency in COD and TP (Guan et al., 2012).

Throughout this study, there was little clogging, channeling or ponding happening during the operation of the apparatus. Most pollutants were consumed in the upper VFTF stage which also provided sufficient nitrification, assuring the stable performance of the lower HFMSL stage. Therefore, the structure of the two-stage hybrid system showed great reliability in performance against shock loadings.

4. Conclusions

The HFMSL–VFTF system was developed and evaluated at various operation conditions. High C/N ratio in sewage improved TN removal for the system, while it is recommended that more carbonaceous media should be added in SMBs in practical application. For vertical intermittent feeding system, a high water spraying frequency could enhance the pollutants removal efficiencies except for TN removal due to the limited availability of carbon source. The hybrid system showed high TN removal as well as steady removal for COD_{Cr}, NH_4^+-N and TP, and could be cost-effective for rural wastewater treatment.

Acknowledgements

Financial support from the National Natural Science Foundation of China (Nos. 51278464 and 51478172) and the International S&T Cooperation Program of China (Project Contract No: 2015DFG92750), and the Department of Science and Technology of Hunan Province (Project Contract No: 2014GK1012) is greatly appreciated.

References

Alfiya, Y., Green, M., Lahav, O., 2007. Modeling the aeration efficiency of a passively aerated vertical-flow biological filter. J. Environ. Eng. 133 (10), 970–978.
APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.
Attanandana, T., Saitthiti, B., Thongpae, S., Kritapirom, S., Luanmanee, S., Wakatsuki, T., 2000. Multi-media-layering system for food service wastewater treatment. Ecol. Eng. 15 (1), 133–138.
Behrendt, A., Tarre, S., Beliavski, M., Green, M., Klatt, J., de Beer, D., Stief, P., 2014. Effect of high electron donor supply on dissimilatory nitrate reduction pathways in a bioreactor for nitrate removal. Bioresour. Technol. 171, 291–297.
Bezbaruah, A.N., Zhang, T.C., 2005. Quantification of oxygen release by bulrush (*Scirpus validus*) roots in a constructed treatment wetland. Biotechnol. Bioeng. 89, 308–318.
Brix, H., Arias, C.A., 2005. The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines. Ecol. Eng. 25 (5), 491–500.
Carrera, J., Baeza, J.A., Vicent, T., Lafuente, J., 2003. Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system. Water Res. 37 (17), 4211–4221.
Carrera, J., Vicent, T., Lafuente, J., 2004. Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater. Process Biochem. 39 (12), 2035–2041.
Chen, M., Chen, J., Du, P., 2006. An inventory analysis of rural pollution loads in China. Water Sci. Technol. 54 (11), 65–74.
Chen, X., Sato, K., Wakatsuki, T., Masunaga, T., 2007. Comparative study of soils and other adsorbents for decolorizing sewage and livestock wastewater. Soil Sci. Plant Nutr. 53 (2), 189–197.
Fytianos, K., Voudrias, E., Raikos, N., 1998. Modelling of phosphorus removal from aqueous and wastewater samples using ferric iron. Environ. Pollut. 101 (1), 123–130.
Greenan, C.M., Moorman, T.B., Kaspar, T.C., Parkin, T.B., Jaynes, D.B., 2006. Comparing carbon substrates for denitrification of subsurface drainage water. J. Environ. Qual. 35 (3), 824–829.
Gross, A., Shmueli, O., Ronen, Z., Raveh, E., 2007. Recycled vertical flow constructed wetland (RVFCW) – a novel method of recycling greywater for irrigation in small communities and households. Chemosphere 66 (5), 916–923.
Guan, Y.D., Chen, X., Zhang, S., Luo, A.C., 2012. Performance of multi-soil-layering system (MSL) treating leachate from rural unsanitary landfills. Sci. Total Environ. 420, 183–190.
Hao, R.X., Li, S.M., Li, J.B., Meng, C.C., 2013. Denitrification of simulated municipal wastewater treatment plant effluent using a three-dimensional biofilm-electrode reactor: operating performance and bacterial community. Bioresour. Technol. 143, 178–186.
Krasnits, E., Beliavsky, M., Tarre, S., Green, M., 2013. PHA based denitrification: municipal wastewater vs. acetate. Bioresour. Technol. 132, 28–37.
Leverenz, H.L., Haunschild, K., Hopes, G., Tchobanoglous, G., Darby, J.L., 2010. Anoxic treatment wetlands for denitrification. Ecol. Eng. 36 (11), 1544–1551.
Luanmanee, S., Attanandana, T., Masunaga, T., Wakatsuki, T., 2001. The efficiency of a multi-soil-layering system on domestic wastewater treatment during the ninth and tenth years of operation. Ecol. Eng. 18 (2), 185–199.
Luo, W., Yang, C.P., He, H.J., Zeng, G.M., Yan, S., Cheng, Y., 2014. Novel two-stage vertical flow biofilter system for efficient treatment of decentralized domestic wastewater. Ecol. Eng. 64, 415–423.
Masunaga, T., Sato, K., Zennami, T., Fujii, S., Wakatsuki, T., 2003. Direct treatment of polluted river water by the multi-soil-layering method. J. Water Environ. Technol. 1 (1), 97–104.
Oakley, S.M., Gold, A.J., Oczkowski, A.J., 2010. Nitrogen control through decentralized wastewater treatment: process performance and alternative management strategies. Ecol. Eng. 36 (11), 1520–1531.
Pant, D., Adholeya, A., 2009. Nitrogen removal from biomethanated spentwash using hydroponic treatment followed by fungal decolorization. Environ. Eng. Sci. 26 (3), 559–565.
Sato, K., Iwashima, N., Wakatsuki, T., Masunaga, T., 2011. Quantitative evaluation of treatment processes and mechanisms of organic matter, phosphorus, and nitrogen removal in a multi-soil-layering system. Soil Sci. Plant Nutr. 57 (3), 475–486.
Sato, K., Masunaga, T., Wakatsuki, T., 2005. Characterization of treatment processes and mechanisms of COD, phosphorus and nitrogen removal in a multi-soil-layering system. Soil Sci. Plant Nutr. 51 (2), 213–221.
Schipper, L.A., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.C., 2010. Denitrifying bioreactors – an approach for reducing nitrate loads to receiving waters. Ecol. Eng. 36 (11), 1532–1543.
Seitzinger, S., Harrison, J.A., Bohlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C., Van Drecht, G., 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol. Appl. 16 (6), 2064–2090.
Van den Akker, B., Holmes, M., Pearce, P., Cromar, N.J., Fallowfield, H.J., 2011. Structure of nitrifying biofilms in a high-rate trickling filter designed for potable water pre-treatment. Water Res. 45 (11), 3489–3498.
Vymazal, J., 2011. Constructed wetlands for wastewater treatment: five decades of experience. Environ. Sci. Technol. 45 (1), 61–69.
Wakatsuki, T., Esumi, H., Omura, S., 1993. High performance and N & P-removable on-site domestic waste water treatment system by multi-soil-layering method. Water Sci. Technol. 27 (1), 31–40.
Wang, J.L., Yang, N., 2004. Partial nitrification under limited dissolved oxygen conditions. Process Biochem. 39 (10), 1223–1229.
Wang, L.M., Zheng, Z., Luo, X.Z., Zhang, J.B., 2011. Performance and mechanisms of a microbial-earthworm ecofilter for removing organic matter and nitrogen from synthetic domestic wastewater. J. Hazard. Mater. 195, 245–253.
Wang, Y.Y., Peng, Y.Z., Stephenson, T., 2009. Effect of influent nutrient ratios and hydraulic retention time (HRT) on simultaneous phosphorus and nitrogen removal in a two-sludge sequencing batch reactor process. Bioresour. Technol. 100 (14), 3506–3512.
Wu, W.Z., Yang, L.H., Wang, J.L., 2013. Denitrification using PBS as carbon source and biofilm support in a packed-bed bioreactor. Environ. Sci. Pollut. Res. 20 (1), 333–339.