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THEMATIC ISSUE

Study of nitrogen removal performance in pilot-scale multi-stage vermi-biofilter: operating conditions impacts and nitrogen speciation transformation

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Abstract The present work investigates pollutant removal and the transformation of nitrogen from sewage wastewater using a pilot-scale multi-stage bio-vermifilter system. Over a study period of 48 weeks, the pollutant removal performance of the system was measured and the effects of hydraulic loading rate (HLR) and dry–wet ratio (D/W) were estimated. The relationship between oxygen transfer rate and load of oxygen necessity was calculated and analysed for system optimisation. The method for diluting the isotope δ^{15}N-NO_3^- was applied to study nitrogen transfer. Moreover, statistical correlations were analysed to determine the crucial factors which influence nitrogen transfer efficiency. The system removes pollutants efficiently; specifically, the average removal efficiencies are 94.2 % for chemical oxygen demand (COD), 93.3 % for NH_4^+-N, and 58.2 % for total nitrogen (T-N). Lowering HLR and D/W can enhance nitrogen removal. Nitrogen speciation and transformation were examined under an optimised condition with an HLR of 0.36 m day^{-1} and a D/W of 3. The results of isotope δ^{15}N-NO_3^- dilution showed that NO_3^--N was mainly produced in trickling bio-filter and vermibio-filter (VBF) I. By contrast, NO_3^--N was mainly reduced in VBF II. Under stable operating conditions and environmental factors, COD/T-N was verified as the crucial factor in nitrogen removal.

Keywords Multi-stage bio-vermifilter system · Nitrogen removal · Hydraulic loading rate · Dry-wet ratio · Isotope δ^{15}N-NO_3^-

Introduction

Nitrogen is a crucial factor in eutrophication and algal bloom. This element threatens the natural aquatic ecosystem and public health (Conley et al. 2009). Cases of severe nitrogen pollution include the Ain River in France (Frossard et al. 2014) and Bagsvaerd Lake in Denmark (Zhang and Angelidaki 2012). In China, Dianchi Lake suffers from extreme eutrophication caused by excessive nitrogen input (Zhou et al. 2014; Huo et al. 2014). Wang et al. (2009) reported that the total nitrogen (T-N) contents in Dianchi Lake increased by 9 times in the past 25 years. Most of the pollutants originated from 22 river estuaries around this lake (Wang et al. 2013a, b, c). The main pollution source is the wastewater discharged into the rivers and into Dianchi Lake. Hence, an effective nitrogen removal method must be developed for wastewater treatment to enhance water purification and to prevent eutrophication (Wendling et al. 2013). With the development of urban wastewater treatment plants (WWTPs), activated sludge processes and membrane bio-reactors are sample point sources of pollution which can be treated efficiently. As a result, non-point sources of pollution, mainly rural domestic water, gradually become dominant especially in relation to nitrogen emission (Schock et al. 2014). Therefore, novel WWTPs

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must be established for rural non-point sources of pollution.

Land treatment systems serve as natural, environmentally friendly systems. Such systems include constructed wetlands (CWs) (Saeed and Sun 2012), stabilisation ponds (Hosetti and Frost 1995) and soil infiltration systems (Lei et al. 2013). Based on these foundation researches, new materials made it more efficient treating sewage pollutants (Liu et al. 2013). Bio-vermifilter (BVF) is a land treatment process which has been widely developed and applied in the past decade. Pioneer bench-scale studies have confirmed the favourable performance of the system in relation to pollutants (Fang et al. 2010; Tomar and Suthar 2011; Wang et al. 2011a, b). Tolerant plants and soil media are used to treat wastewater efficiently at low-energy consumption. The corporate function of plants, soil media and microorganisms can produce effluent which removes more nitrogen than the effluent produced by conventional WWTPs (Vymazal 2007, 2011; Kim et al. 2011).

The performance of the nitrogen removal treatment is influenced by a variety of operating conditions. Significant conditions are hydraulic loading rate (HLR) and the system ratio of dry–wet strength (D/W). According to Cui et al. (2010), an increase in HLR from 0.07 to 0.21 m day^{-1} reduced nitrogen removal efficiency considerably. Bastviken et al. (2009) obtained the same result when HLR was increased from 0.08 to 0.17 m day^{-1}. D/W determines intermittent inflow mode and significantly affects nitrogen removal (Haberl et al. 1995; Osorio and García 2007). With respect to research on the nitrogen removal mechanism, microbial community and structure have been examined in relation to both ammonia oxidation (Wang et al. 2013a, b, c) and denitrification (Wang et al. 2011a, b). However, information on nitrogen transfer is limited.

A multi-stage BVF (MBVF) system in the pilot scale for sewage wastewater treatment is established in the current study. Pollutant removal performance is evaluated given different HLRs and D/Ws. This study aims to enhance nitrogen removal, to increase HLR and to determine an appropriate D/W. Load of oxygen necessity (LON) and oxygen transfer rate (OTR) (Cooper 2005) are obtained to interpret the relationship between oxygen consumption and operating conditions. The outcome for nitrogen is observed, and isotope δN^{15} is detected to elucidate the mechanisms of nitrification and denitrification. This study provides systematic information regarding the outcome and removal of nitrogen in pilot scale, fills in the gaps in the study of pollutant removal processes, optimises system design and operating conditions and transforms pollutants. This information serves to enhance the application of this project.

Method and system design

System design

As shown in Fig. 1, the MBVF system consisted of four polymethyl methacrylate filters in pilot scale, namely, anaerobic bio-filter (ABF, height, $H = 1$ m, length, $L = 1$ m, width, $W = 1$ m), trickling bio-filter (TBF, $H = 2$ m, $L = 1$ m, $W = 0.2$ m) and two BVFs (BVFs I and II, $H = 1$ m, $L = 1$ m, $W = 0.4$ m). The ABF was packed into guide plates and soft packing to generate an anaerobic condition. TBF was a down-flow trickling filter which was filled with bio-ceramic to a maximum of 1.5 m (20–30 mm in diameter). Each BVF contained the following layers from bottom to top: 20 cm gravel (20–40 mm in diameter), 30 cm bio-ceramsite (20–30 mm in diameter), 15 cm sand (0.2–1.0 mm in diameter) and 30 cm soil. The guide plates in the filters prevented the system from short-circuiting. Trickle irrigation was employed to facilitate inflow in the system. Polyvinyl chloride (PVC) pipes with holes (diameter = 2 mm) were designed and installed on the top of the filter to ensure uniform wastewater distribution. Similar PVC pipes without holes were placed at the bottom of the filter for collecting treated wastewater.

Magnetism forle pumps and rotameters were used to control flow rate. Earthworms (*Eisenia foetida*) were released in soil with a density of 12.5 g L^{-1} (Wang et al. 2011a, b). *Lolium perenne* L., which is a plant with strong tolerance for wastewater, was cultivated in the two BVFs.

The system was operated in semi-continuous mode under natural conditions in Fudan University, Shanghai, China for 48 weeks. HLR and D/W were investigated in this work as operation condition factors. The optimised system conditions were statistically evaluated, as were several other factors including temperature, pH and colloidal organic N/T-N. For a fixed bed area, HLR was determined by flow rate and inflow time, as indicated in Eq. (1). D/W was equal to the ratio of inflow and non-inflow times, as suggested in Eq. (2).

$$\mathrm{HLR} = v \times T/A, \tag{1}$$

$$\mathrm{D/W} = T/(24 - T) \tag{2}$$

where HLR, v, A, T and D/W refer to hydraulic loading rate, flow rate, bed area, inflow time and ratio of dry–wet strength, respectively.

All operating factors are provided in Table 1. P1, P2 and P3 compared the effects of different HLRs and D/Ws, namely, low HLR and low D/W, high HLR and low D/W and high HLR and high D/W. In another period (P4), nitrogen transformation was investigated in a stock condition.

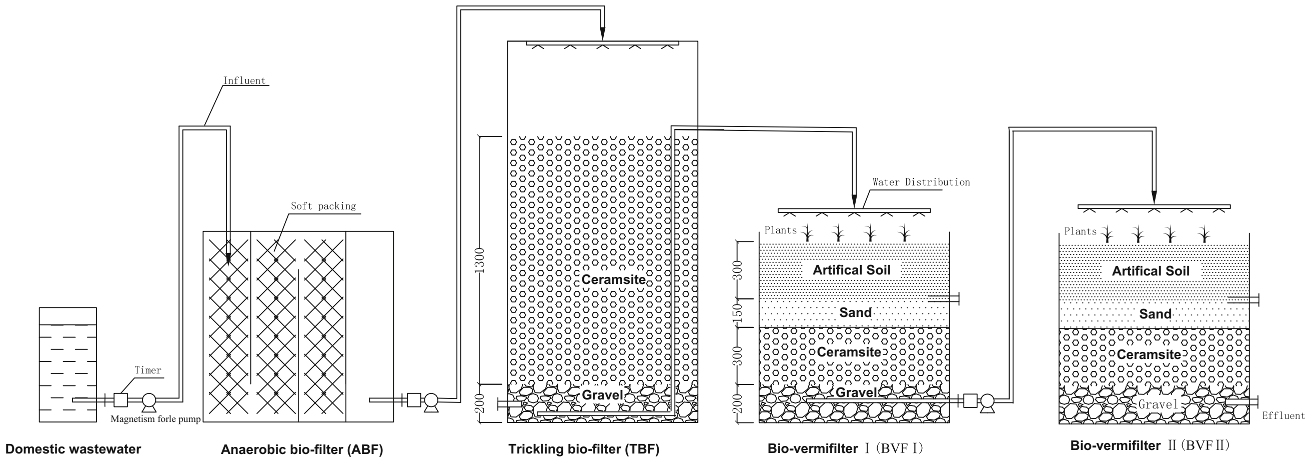

Fig. 1 Schematic section of the pilot-scale multi-stage bio-vermifilter

Table 1 Operation chart for the experiment system

Operating conditions	Unit	Weeks of operating				
		Period1 (P1) Week 1–9	Period2 (P2) Week 10–18	Period3 (P3) Week 19–27	Period4 (P4) Week 28–36	Period5 (P5) Week 37–45
Flow rate	L min^{-1}	1	3	2	1	1.5
Inflow time	h day^{-1}	6	6	3	6	6
Hydraulic loading rate	m day^{-1}	0.36	1.08	0.36	0.36	0.54
Ratio of dry–wet strength		3	3	7	3	3

Table 2 Properties of inflow wastewater

Parameters	pH	*T* (°C)	COD (mg L^{-1})	T-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	NO_3^--N (mg L^{-1})	NO_2^--N (mg L^{-1})	Org-N (mg L^{-1})
Inflow	6.9–7.6	14.8–26.3	178–504	28.9–46.0	20.7–39.1	2.3–6.2	0.01–0.20	6.2–13.2

Wastewater properties

The system was fed with domestic wastewater which contained a high concentration of NH_4^+-N and low chemical oxygen demand (COD). This wastewater composition was consistent with the pollutant characteristic of rural sewage. Dissolved oxygen (DO), pH and temperature (*T*) were measured in the process of sampling. The detailed properties are presented in Table 2.

Sampling and measurement

Water was sampled from all outlets of each cell once a week. Samples were immediately analysed for COD, NO_3^--N, NO_2^--N, NH_4^+-N and T-N. COD, NO_3^--N, NO_2^--N and NH_4^+-N were measured with a UV-759S spectrophotometer (Shanghai Precision & Scientific Instrument Co. Ltd., China), whereas T-N was measured with T-NM-L (Shimadzu Corporation, Japan). Organic nitrogen (Org-N) concentration was calculated according to the difference between T-N and inorganic nitrogen, which was the sum of all remaining nitrogen. The samples were delivered to the Fudan University Isotope Laboratory for isotope analysis after evaporation and lyophilisation.

Data analysis

Median and median absolute deviation described centralising tendency and data distribution. Correlations between water quality variables and T-N removal efficiencies were computed to elucidate the crucial factors in nitrogen removal. Differences were considered statistically significant if $p < 0.05$ and highly significant if $p < 0.01$.

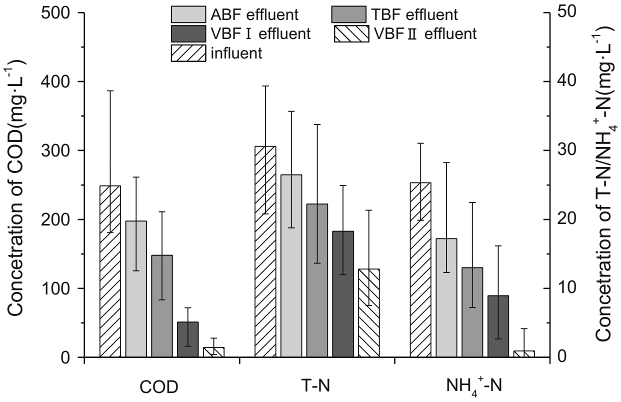

Fig. 2 Median pollutants concentration (and median absolutely deviation) by each stage of the experiment system during the P1, P2 and P3

Results and discussion

System performance and effect of operating conditions

COD, NH_4^+-N and T-N

The median values of COD, NH_4^+-N and T-N concentration for the entire experiment are presented in Fig. 2. Trickle irrigation generated access to adequate oxygenation (Gross et al. 2007), and the multi-stage design facilitated the efficient treatment of pollutants at minimal area and cost (Kato et al. 2013; Rivas et al. 2011). A significant portion of organic matters and ammonia was either consumed or transformed. At an inflow of 249 mg L^{-1} COD and 25.3 mg L^{-1} NH_4^+-N, the system removed much pollutant (94.2 and 93.3 % of COD and NH_4^+-N removed, respectively). The overall effluent was stable given low levels of COD (14 mg L^{-1}) and NH_4^+-N (0.9 mg L^{-1}) (Fig. 2).

Reduction in T-N is a corporate result of ammonification, nitrification and denitrification (Saeed and Sun 2012). In this study, mean removal rate was 58.2 %, and the median T-N concentration in effluent was 12.8 mg L^{-1} (Fig. 2). Oxygen was consumed in the upper section of the filter. Thus, this section is in an aerobic ambient state and the lower section is anaerobic. This process was repeated in multi-stage combination. The multi-stage system generated considerable aerobic/anaerobic ambient cycles and promoted the function of multiple nitrification and denitrification processes. Therefore, this system performed better than single-bed systems did, as reported by Cui et al. (2010) and Stefanakis and Tsihrintzis (2012).

Impact of HLR

The impact of HLR on pollutant removal is shown in Table 3. When HLR ranged from 0.36 to 1.08 m day^{-1}, the COD concentration in effluent generally remained stable. The system achieves a high HLR for nitrogen removal and for maintaining removal efficiency. Given HLRs of 0.36 and 0.54 m day^{-1}, the NH_4^+-N concentrations in effluent were 0.1–1.6 and 0.2–1.3 mg L^{-1}, whilst T-N concentrations were 6.0–9.8 and 5.4–10.4 mg L^{-1}, respectively. When HLR increased to 1.08 m day^{-1}, however, nitrogen removal efficiency decreased significantly.

The bottleneck in HLR is complicated in the operation of traditional artificial wetland technology because choosing between a small system and high hydraulic load is difficult as well. A high hydraulic load can also induce clogging and system breakdown, thereby reducing the efficiency of pollutant removal (Kato et al. 2013). Unlike the systems proposed by Cui et al. (2010), Prochanska et al. (2007) and Bastviken et al. (2009), the MBVF system presented in the current study effectively promoted operating HLR. According to Table 3, the multi-stage design weakened the influence of HLR on nitrogen removal. Furthermore, the modified porous soil and the plants overcame the clogging issue, which had not been observed in previous studies.

Impact of the ratio of dry–wet strength (D/W)

The intermittent inflow mode applied in the current work efficiently enhanced the removal of organic matters and nitrogen (Haberl et al. 1995; Osorio and García 2007). Dry–wet periods determined the intermittent inflow mode and influenced system performance. As indicated in Table 3, changes in D/W did not significantly impact COD and NH_4^+-N removal. However, the T-N concentration in effluent decreased from 9.3–17.7 to 6.0–9.8 mg L^{-1} when D/W decreased from 7 to 3.

The authors attributed this enhanced performance as a result of low D/W to the following reasons: (a) the reduced D/W diffused inflow, which in turn increased hydraulic efficiency and improved nitrogen removal (Koskiaho 2003; Su et al. 2009). (b) Setting a proper influent mode could eliminate the existence of 'dead zones' which weaken pollutant removal by maximising each part of the filter, according to Wang et al. (2013a, b, c). (c) The provision of a sufficient dry period propelled oxygen diffusion in packing media, which maintained the strong and steady removal of organic matter and of NH_4^+-N.

Table 3 Pollutants concentration of influent and effluent by each system stage

Parameters	HLR: 0.36 m day^{-1} D/W: 3			HLR: 1.08 m day^{-1} D/W: 3			HLR: 0.36 m day^{-1} D/W: 7			HLR: 0.54 m day^{-1} D/W: 3		
	COD (mg L^{-1})	T-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	COD (mg L^{-1})	T-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	COD (mg L^{-1})	T-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	COD (mg L^{-1})	T-N (mg L^{-1})	NH_4^+-N (mg L^{-1})
Inflow	144–504	22.6–40.9	16.7–37.5	177–400	29.3–46.0	25.6–39.1	163–340	29.2–39.6	18.0–33.9	175–385	24.5–39.8	20.3–35.2
ABF[a]	98–200	20.8–31.9	16.8–30.2	48–298	21.0–42.9	16.6–40.1	98–297	18.2–41.7	13.8–31.1	109–245	23.6–38.9	18.2–33.4
TBF[a]	54–148	17.8–25.7	15.8–24.5	26–234	25.5–40.5	10.5–39.2	105–219	15.4–44.0	7.4–21.2	64–149	19.8–29.1	11.9–28.5
BVFI[a]	23–135	8.5–16.4	2.8–9.0	8–79	18.0–32.3	3.8–22.5	32–58	14.8–24.7	0.7–17.3	23–98	9.3–17.5	3.2–10.5
BVFII[a]	8–32	6.0–9.8	0.1–1.6	7–33	19.6–29.5	0.5–8.6	8–18	9.3–17.7	0.6–1.8	7–35	5.4–10.4	0.2–1.3

[a] Effluent of each filter

OTR under different operating conditions

System performance was evaluated further by calculating OTR with reference to the work which analysed CW (Cooper 2005). LON was defined as the sum of influent COD and NH_4^+-N loads, and OTR as the decrement in COD and NH_4^+-N during each filter stage (Cooper 2005). These factors are calculated as follows:

$$\mathrm{OTR} = \frac{v\left[0.5(\mathrm{COD_{in}} - \mathrm{COD_{out}}) + 4.3\left(\mathrm{NH_4^+ - N_{in} - NH_4^+ - N_{out}}\right)\right]}{A} \tag{3}$$

$$\mathrm{LON} = \frac{v\left(0.5\mathrm{COD_{in}} + 4.3\mathrm{NH_4^+ - N_{in}}\right)}{A} \tag{4}$$

where v and A refer to flow rate and bed area, respectively. COD and NH_4^+-N are expressed in the unit of mg L^{-1}. The ratio of flow rate and bed area is defined as HLR, with a unit of m day^{-1}.

Figure 3 presents LONs and OTRs of each stage of the system in P1, P2 and P3. The OTRs were proportional to the LONs in the two BVFs. This result agreed with previous findings (Kato et al. 2013). Furthermore, the ratio of LONs and OTRs in the BVF system was higher than that obtained in existing studies (Cooper 2005; Kato et al. 2013), thereby indicating improved pollutant removal performance.

Given an HLR of 0.4 m^3 d^{-1} m^{-2} and a D/W of 3 (P1), the order of oxygen transfer and consumption capability was BVF I > BVF II > TBF > ABF. Nevertheless, the order was complicated when HLR increased to 1.2 m^3 d^{-1} m^{-2} (P2). The result shows that the two BVFs exhibited the same oxygen transfer capability, whereas the capabilities of TBF and ABF first increased and then decreased with an increase in LONs. This result can be explained by the fact that the high mass loading rates of pollutants exceeded filter capacity. Under another operating condition in which HLR was 0.4 m^3 d^{-1} m^{-2} and D/W was 7 (P3), OTRs varied with LONs given different characteristics. The OTRs of ABF stabilised at a high value in comparison with the results obtained under the other two conditions. LONs had almost no impact on these values. In the other three filters, the capabilities remained in the order of BVF I > BVF II > TBF.

OTR is a key parameter in bio-processes and is significant in microbial processes (Garcia-Ochoa and Gomez 2009). This rate estimates pollutant removal capability in WWTPs, especially in wetlands and ponds (Ro et al. 2010; Tyroller et al. 2010). Kato et al. (2013) discussed whether or not OTR can be a reference factor for system design; however, their work lacked statistical support. The data in Fig. 3 support the possibility of system design for different types of wastewater and a variety of oxygen loads under diverse operating conditions. A multi-stage bed system

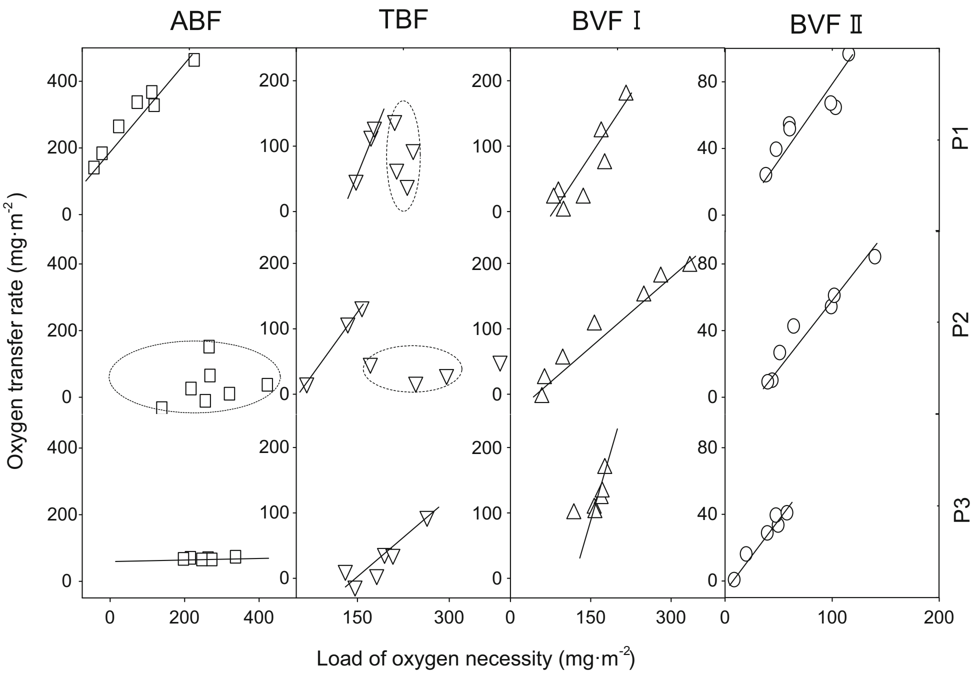

Fig. 3 OTRs and LONs under different operating conditions and different stages of the system

Table 4 Transformation of nitrogen by each system stage

Parameters	T (°C)	pH	COD (mg L^{-1})	T-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	NO_3^--N (mg L^{-1})	NO_2^--N (mg L^{-1})	Org-N (mg L^{-1})	$\delta^{15}N\text{-}NO_3^-$/‰
Inflow	22.3 ± 2.2	6.7 ± 0.2	236 ± 52	36.5 ± 3.7	24.5 ± 2.8	3.0 ± 1.3	0.03 ± 0.06	8.9 ± 2.3	5.14 ± 0.64
ABF	22.2 ± 2.1	6.4 ± 0.4	206 ± 55	24.9 ± 3.0	16.8 ± 1.6	1.7 ± 1.1	0.01 ± 0.01	6.4 ± 1.8	5.61 ± 0.51
TBF	22.2 ± 2.3	6.8 ± 0.2	140 ± 40	21.3 ± 3.3	13.5 ± 3.1	4.4 ± 2.1	0.12 ± 0.10	3.3 ± 1.1	8.81 ± 3.00
BVFI	22.1 ± 2.2	7.0 ± 0.2	54 ± 20	14.9 ± 1.8	2.3 ± 0.6	11.0 ± 1.8	0.01 ± 0.01	1.6 ± 0.5	10.87 ± 4.54
BVFII	22.1 ± 2.1	7.3 ± 0.2	14 ± 5	10.2 ± 0.9	0.7 ± 0.2	8.4 ± 0.6	0.18 ± 0.12	1.0 ± 0.6	8.72 ± 2.79

with minimal required area and cost can be designed when the relationship between LONs and OTRs is considered. In this study, however, disregarded factors include climate, sewage properties and system type. Thus, further research must be conducted on these factors in the future.

Nitrogen speciation and transformation

Nitrogen speciation

Nitrogen speciation was examined at a stable HLR of 0.36 m day^{-1} and a D/W of 6 (P4). Isotope $\delta^{15}N\text{-}NO_3^-$ was detected to analyse nitrification and denitrification (Table 4).

According to Hu et al. (2014), the key processes of NH_4^+-N conversion in vertical wetland were substance adsorption and microorganism nitrification. The latter regenerated the adsorption capacity of substances. In this study, the variation in NH_4^+-N was mainly concentrated in vermibio-filter (VBF) I (Fig. 4). This VBF consisted of a soil substance with SiO_3^{2-} and high DO values generated through trickle irrigation. The adsorption, nitrification and regeneration processes were smooth and removed much NH_4^+-N. Although the inflow concentration of NH_4^+-N was low in VBF II, NH_4^+-N was further converted. Its concentration in effluent ranges from 1.79 to 0.69 mg L^{-1} (Fig. 4).

A high NH_4^+-N conversion rate resulted in NO_3^--N accumulation. Nitrite concentration kept low in the system. This is different from the report of Im et al. (2014), in which study the ammonia-oxidising bacteria played a crucial role. DO values and carbon sources were two crucial factors in denitrification (Ingersoll and Baker 1998; Lu et al. 2009). The DO level of VBF inflow is satisfactory; however, the removal rate of carbon sources fails to reach the standard. This failure is strongly proven to be the main limitation in promoting nitrogen removal. Misiti et al. (2011) reported the severe impact of a COD/T-N value of below 3 on denitrification. In the current study, the mean COD/T-N values in the two VBFs were 6.6 and 3.6 (Table 4). The total NO_3^- produced can be calculated on

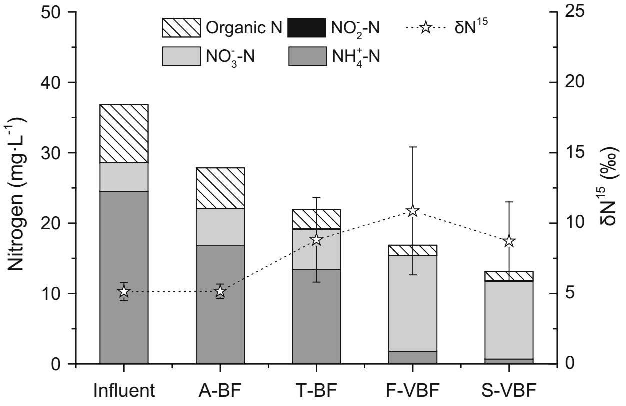

Fig. 4 Nitrogen compositions in influent and effluent of each stage of the system

Table 5 Mean values of NO_3^--N production ($Y_{\text{prodution}}$) and NO_3^--N reduction ($Y_{\text{reduction}}$) of each system stage calculated by isotope δ^{15}N-NO_3^- dilution method

System stage	NO_3^--N production ($Y_{\text{prodution}}$) (mg L^{-1})	NO_3^--N reduction ($Y_{\text{reduction}}$) (mg L^{-1})
ABF	−0.66	0.64
TBF	5.48	2.78
BVF I	9.18	2.58
BVF II	3.42	6.02

the basis of the reduction in Org-N and NH_4^+-N given the low concentration of NO_2^--N and the low volatilisation of NH_4^+-N. NO_3^--N consumption can be determined according to the difference in total production and apparent increment.

Actual nitrogen formation varied substantially from the outcome in the system. The presence of a significant amount of organic matters during a brief aeration process in TBF caused the majority of the filter to enter an anaerobic condition. The same was true in the case of ABF. The ammonolysis in anaerobic surroundings primarily induced the reduction of Org-N in ABF (Reddy et al. 1984); the concentration of Org-N in ABF effluent was 6.4 mg L^{-1} versus the value of 8.9 mg L^{-1} in inflow. Moreover, NH_4^+-N decreased in ABF. Denitrification facilitated nitrate removal given DO and carbon sources. Simultaneous nitrification and denitrification (SND) may occur in this section of the system to reduce NH_4^+-N and NO_3^--N at the same time (Zhang et al. 2011).

Isotope δ^{15}N-NO_3^- analysis

The T-N mass balance in the MBV system can be attributed to three main aspects, namely, microbial removal, including nitrification and denitrification; soil storage; and plant function. Nitrogen microbial removal can be reflected in nitrification rate. The isotope δ^{15}N-NO_3^- dilution method has been applied to analyse nitrification rates (Delaune et al. 1998). NO_3^--N production and NO_3^--N reduction can be calculated for each stage of the system by the following equations (Delaune et al. 1998; Zhou et al. 2012):

$$Y_{\text{production}} - Y_{\text{reduction}} = C^{\text{E}} - C^{\text{I}} \tag{5}$$

$$Y_{\text{production}}X - Y_{\text{reduction}}\overline{X} = C^{\text{E}}X^{\text{E}} - C^{\text{I}}X^{\text{I}} \tag{6}$$

where $Y_{\text{prodution}}$ and $Y_{\text{reduction}}$ are the production of and reduction in NO_3^--N; C^{E}, C^{I} and X^{E}, X^{I} are NO_3^--N concentration and δ^{15}N-NO_3^- values in the effluent and influent of each stage, respectively; $\overline{X}$ is the average isotopic δ^{15}N-NO_3^- content of the effluent and influent in each stage and X is the natural abundance of δ^{15}N-NO_3^- (3.70 %) (Zhou et al. 2012).

The calculated results for NO_3^--N production and reduction are presented in Table 5. These findings suggested that nitrification and denitrification occurred simultaneously. The dominant process differed across stages; NO_3^--N production was relatively high in TBF and BVF I, and the corresponding values were 5.48 and 9.18 mg L^{-1}, respectively. NO_3^--N was reduced more significantly in BVF II than in the others (to 6.02 mg L^{-1}).

When these findings are combined with the results for nitrogen formation variation (Fig. 4), the microbial function in nitrogen removal can be identified. In BVF I, NH_4^+-N loss was 11.67 mg L^{-1} and T-N loss was 4.63 mg L^{-1}. The isotope analysis results suggested that microbial function contributed 9.18 mg L^{-1} to NH_4^+-N removal and 2.58 mg L^{-1} to T-N removal. The remaining nitrogen loss was attributed to soil storage and plant uptake. In BVF II, nitrification and denitrification played a comprehensive role. NH_4^+-N loss was 1.10 mg L^{-1}, whilst T-N loss was 4.63 mg L^{-1}. NO_3^--N production was 3.42 mg L^{-1}, and NO_3^--N reduction was 6.02 mg L^{-1}. These results suggested a frequent exchange between NO_3^--N and NH_4^+-N.

Partial water logging established the coexistence of aerobic and anaerobic conditions at soil microscale (Zhou et al. 2012). This logging facilitated the SND process. Nevertheless, denitrification may be inhibited by the lack of strictly anaerobic surroundings (Pinay et al. 2007). Nitrification can produce NO_3^--N, which serves as an important nitrogen source for denitrification (Pina-Ochoa and Alvarez-Cobelas 2006).

Crucial factors in T-N removal

The results of correlation analysis are presented in Table 6. The removal efficiencies and mass rates of T-N were mutually and significantly correlated. The two parameters were also positively correlated with COD/T-N(I) and COD/NH_4^+-N(I). Temperature was identified to be an important

Table 6 Correlations between the water qualities variables and T-N removal efficiencies

Item	T (E)	pH (E)	COD/T-N (I)	COD/NH_4^+-N (I)	COD (E)	T-N (E)	T-N (RE)	T-N (MR)
T (E[a])	1							
pH (E[a])	−0.340	1						
COD/T-N (I[b])	0.127	0.066	1					
COD/NH_4^+-N (I[b])	0.195	0.042	0.930**	1				
COD (E[a])	−0.023	0.791*	−0.154	−0.229	1			
T-N (E[a])	−0.095	−0.248	−0.733*	−0.743*	−0.125	1		
T-N (RE[c])	0.517	0.089	0.734*	0.795*	0.008	−0.845*	1	
T-N (MR[d])	0.679*	−0.045	0.731*	0.648	−0.121	−0.652	0.953**	1

* Significant, ** highly significant

[a] Effluent

[b] Influent

[c] Removal efficiency

[d] Mass removal rate by each stage of the experiment system during the P1, P2 and P3 different stages of the system

factor which influences nitrogen removal in CWs (Chang et al. 2013; Huang et al. 2013).

COD/T-N(I) was strongly correlated with effluent concentration, mass rate and T-N removal efficiency (Table 6). The Pearson correlation coefficient (R^2) values were −0.733, 0.734 and 0.731. COD/T-N(I) was crucial in nitrogen removal; this finding was highly consistent with those of previous studies (Zhao et al. 2010, 2011). And the external carbon sources, which significantly determine COD/T-N(I) value, have significant effects on the removal of T-N (Zhou et al. 2013).

Conclusion

A pilot-scale, MBV system was constructed and operated continuously for the systematic study of nitrogen removal, including operating condition optimisation and nitrogen speciation transformation. The continuous monitoring of system performance presented several feasible suggestions for the design and operation of MBV systems.

- MBV efficiently removed many pollutants, especially nitrogen. Mean NH_4^+-N removal was 93.3 % (influent NH_4^+-N, 25.3 mg L^{-1}), and mean T-N removal could reach 58.2 % of influent concentration (30.6 mg L^{-1}).
- The MBV system achieved an HLR of 0.54 m day^{-1} and maintained a nitrogen removal efficiency of 60–65 %.
- We tracked the transformation of nitrogen speciation. The isotope δ^{15}N-NO_3^- dilution method interpreted the production of and reduction in NO_3^--N. This method also confirmed the occurrence of nitrification and denitrification.
- Aside from the impact of operating conditions on nitrogen removal, COD/T-N was also a crucial factor.

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