Intensified organics and nitrogen removal in the intermittent-aerated constructed wetland using a novel sludge-ceramsite as substrate

Haiming Wu [a, b, 1], *Jinlin Fan* [c, 1], *Jian Zhang* [b]*, *Huu Hao Ngo* [d], *Wenshan Guo* [d], *Shuang Liang* [b], *Jialong Lv* [a], *Shaoyong Lu* [b], *Weizhong Wu* [b], *Suqing Wu* [b]

[a] College of Natural Resources and Environment, Northwest A & F University, Yangling, Shaanxi 712100, PR China

[b] Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science & Engineering, Shandong University, Jinan 250100, PR China

[c] National Engineering Laboratory of Coal-Fired Pollutants Emission Reduction, Shandong University, Jinan 250061, PR China

[d] School of Civil and Environmental Engineering, University of Technology Sydney, Broadway, NSW 2007, Australia

[1] These authors contributed equally to this work.

**Corresponding author. Email address: zhangjian00@sdu.edu.cn*

Abstract

In this study, a novel sludge-ceramsite was applied as main substrate in intermittent-aerated subsurface flow constructed wetlands (SSF CWs) for treating decentralized domestic wastewater, and intensified organics and nitrogen removal in different SSF CWs (with and without intermittent aeration, with and without sludge-ceramsite substrate) were evaluated. High removal of 97.2% COD, 98.9% NH_4^+-N and 85.8% TN were obtained simultaneously in the intermittent-aerated CW system using sludge-ceramsite substrate compared with non-aerated CWs. Moreover, results from fluorescence in situ hybridization (FISH) analysis revealed that the growth of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in the intermittent-aerated CW system with sludge-ceramsite substrate was enhanced, thus indicating that the application of intermittent aeration and sludge-ceramsite plays an important role in nitrogen transformations. These results suggest that a combination of intermittent aeration and sludge-ceramsite substrate is reliable to enhance the treatment performance in SSF CWs.

Keywords: Subsurface flow constructed wetlands; Domestic wastewater; Aeration; Nitrogen removal

1. Introduction

With globally increasing world population, urbanization and industrialization, environmental problems such as water shortages and pollution have become a serious concern, and thus the pervasive issue of inadequate access to clean drinking water is expected to worsen in coming decades (Shannon et al., 2008). Due to less construction and management in wastewater treatment infrastructures, discharging directly large volumes of untreated wastewater into surface water bodies is a common practice in many cities and small towns especially in developing countries (Wu et al., 2015a). Consequently, unmanaged wastewater such as the decentralized domestic wastewaters can be a source of pollution (mainly organics and nitrogen), resulting in negative health and environmental consequences such as deterioration of water quality and eutrophication of the lake (Chen et al., 2011; Saeed and Sun, 2012; Wu et al., 2011a). Therefore, besides sewage treatment facilities, ecological treatment technologies have been attracted more attention in these years when facing the strict wastewater discharge standards and the growing environmental legislation (Rai et al., 2013; Li et al., 2014; Ju et al., 2014).

Constructed wetland (CW) as a typical and optimal ecological wastewater treatment, has low costs and can easily be operated and maintained. Thus, CWs have been extensively used to treat a variety of wastewaters such as domestic sewage, agricultural wastewater, industrial effluent, mine drainage, landfill leachate, urban runoff, and polluted river water (Wu et al., 2014; Coban et al., 2015; Doherty et al., 2015). Among the various types of CWs, free water surface (FWS) and subsurface-flow (SSF) CWs are the most commonly used types for wastewater treatment. According to the flow direction the SSF CWs can further be classified into vertical-flow (VF) and horizontal-flow (HF) types (Wu et al., 2015a). It was also reported in numerous studies that CWs could be efficient for removing various pollutants (organic matter, nutrients, heavy metals, pharmaceutical contaminants, etc.) from wastewater (Saeed and Sun, 2012; Tromp et al., 2012; Verlicchi and Zambello 2014). Among the different types of pollutants, the removal of organics and nitrogen is enormously important because the organics-rich wastewater often depletes dissolved oxygen (DO) concentration in water bodies, leading to the death of aquatic life in freshwater ecosystems.

A number of previous papers indicate SSF CWs can achieve higher organics removal performances compared with FWS CWs. Oxygen is a crucial environmental parameter that controls nitrification and organics biodegradation in CWs, and classical microbiological nitrogen removal reactions are also often restricted by lack of organic carbon in wetland systems (Saeed and Sun, 2012). However, SSF CWs often exhibit limited and fluctuating nitrogen removal efficiency because of insufficient oxygen supply and lack of biodegradable organics (Saeed and Sun, 2012; Wu et al., 2014). Therefore, in order to obtain effective nitrification or to increase the applied wastewater loads, artificial aeration wetland systems have been designed and operated as an alternative of supplement oxygen (Boog et al., 2014; Li et al., 2014). However, some common drawbacks such as increasingly energy inputs, excessive oxygenation and inefficient oxygen diffusion can limit the successful and sustainable application of aeration CWs (Fan et al., 2013a; Wu et al., 2015b). Currently, based on numerous studies on laboratory and pilot scale SSF CWs, intermittent aeration CWs have been proved to be a more cost-effective strategies (Fan et al., 2013b; Boog et al., 2014), because it not only saved operating cost but also greatly increased nitrogen removal efficiency by creating favourable conditions for nitrification and denitrification simultaneously (Foladori et al., 2013; Fan et al., 2013c; Meng et al., 2014). Additionally, compared with the commonly used gravel medium in CWs, researchers have paid considerable attention to other substrates mainly including natural material, artificial media and industrial by-product, and these optional substrates were frequently used for optimizing the removal of nitrogen and organics in CWs in recent years (Wu et al., 2015b). These mixed substrates, such as organic wood-mulch, alum sludge, bentonite, dolomite, wollastonite, activated carbon and light weight aggregates, not only have reactive surfaces for microbial attachment, but also could provide a high hydraulic conductivity and higher porosity associated with better aeration in CWs (Saeed and Sun, 2012). Recently, several investigations have successfully made the porous sludge-ceramsite from drinking-water treatment sludge and wastewater treatment sludge (Xu et al., 2008; Qi et al., 2010), and it has also been demonstrated that sludge-ceramsite can be used in biological wastewater treatment (Zou et al., 2012; Wu et al., 2015c). For example, a novel sludge ceramsite was prepared and employed in the up-flow biological aerated filter for soy protein secondary wastewater treatment, and the results showed that COD and NH_4^+-N removal could be 91% and 90%, respectively (Wu et al., 2015c). However, very little research focuses on the application of sludge-ceramsite substrate for enhancing treatment performance in intermittent-aerated SSF CWs treating decentralized domestic wastewater.

Therefore, the aim of this study was to evaluate the effectiveness of intermittent-aerated SSF CWs with a novel sludge-ceramsite (prepared from dehydrated sewage sludge and clay) for intensifying organics and nitrogen removal simultaneously. Specific objectives of this study are: (i) to evaluate the removal performance of organics and nitrogen in intermittent aeration SSF CWs with sludge-ceramsite substrate for treating decentralized domestic wastewater; (ii) to identify the contribution of intermittent aeration and sludge-ceramsite on enhancing the pollutants removal efficiency in SSF CWs by comparing with common SSF CWs; and (iii) to investigate the influence of intermittent aeration and sludge-ceramsite substrate on the growth of wetland microorganisms, as well as to analyze mechanisms of nitrogen removal in the SSF CWs.

2. Material and Methods

2.1 Characterization of microcosm wetlands

The experiment work was carried out under the transparent rain shelter in Baihua Park in Jinan, northern China (36°40′36″N, 117°03′42″E). Four parallel laboratory-scale SSF CWs designed in a vertical-flow (VF) style (System I: Non-aerated CW; System II: intermittent aeration CW; System III: intermittent aeration CW with sludge-ceramsite substrate; System IV: Non-aerated CW with sludge-ceramsite substrate) were developed (Fig. 1). CW systems, each with a height of 65 cm and an inner diameter of 20 cm, were constructed from a perspex tube with an outlet at the bottom. Multi-dimensional gradation of the substrate was adopted in this study: a 10 cm bottom layer of coarse gravel (3-4 cm in diameter) in each wetland was served as the supporting layer; the following medium gravel or sludge-ceramsite layer (2-3 cm in diameter) as the main substrate layer was filled in each wetland with a depth of 25 cm, above which was a 15 cm fine gravel layer (1-2 cm in diameter); a 15 cm top layer of washed river sand(1-2 mm in diameter) was added for facilitating the dispersion of wastewater and the growth of plants. Specifically, sludge-ceramsite employed in the system III and system IV was made from dried sewage sludge and clay obtained from wastewater treatment plant according to our previous study (Qi et al., 2010; Wu et al., 2011b). Preparation of sludge-ceramsite mainly included the following steps: crushing and screening of raw materials, mixing, dosage, pelletizing and drying, preheating and sintering treatment, and cooling treatment. This sludge-ceramsite had the good physical properties with low bulk, grain density (350 kg m^{-3} and 931 kg m^{-3}) and water absorption (8.2%), and had no potential environmental risks. In addition, the appearance and microstructure (numerous apertures of about 30-60 μm in diameter) of the sludge-ceramsite indicated that it was suitable for the attached growth of microorganisms (Wu et al., 2015c). In CW system II and system III, in order to supply oxygen, the porous air sparger was installed in the bottom supporting layer of each system. Each wetland tub had an average gravel bed porosity of 35 % with an average void volume of 6.5 L. In this study, *Phragmites australis* was selected and planted at a density of eight rhizomes per system. After transplanting, CW systems were feed with synthetic wastewater and stabilized for about two months, and then the experiment started.

2.2 Experimental procedure

In order to minimize variability in the experiment, all systems in this study were fed with synthetic wastewater, which was prepared by using 386 mg L^{-1} sucrose, 188 mg mg L^{-1} $(NH_4)_2SO_4$, 17.5 mg L^{-1} KH_2PO_4, 10 mg L^{-1} $MgSO_4$, 10 mg L^{-1} $FeSO_4$, and 10 mg L^{-1} $CaCl_2$ dissolved in tap water. All systems were operated by a sequencing fill-and-draw batch mode. The influent wastewater was pumped into the systems at the load influent flow rate of 0.21 m^3 m^{-2}-$batch^{-1}$ using a peristaltic pump, and the characteristics of the influent during the experimental period were periodically monitored. The hydraulic retention time (HRT) was 72 h according to previous experiments (Fan et al., 2013b). CW system II and system III were intermittently aerated at an airflow rate of 1.0 L min^{-1} for 4h (hours 0-1, 6-7, 12-13 and 18-19) each day while system I and system IV were operated without aeration. At the end of every cycle of batch operation, effluent was discharged from the outlets at the bottom of CWs. The whole experiment operated and lasted for eight months.

2.3 Sampling and analysis

Water samples for chemical analyses were collected from the influent tank and from the outlet of each system every 3 d, respectively. All water samples were transferred immediately to the lab and stored at 4℃ before analysis. Water samples were analyzed for nitrogen (NH_4^+-N, NO_3^--N, NO_2^--N and TN) and organic matter (COD). Water temperature and dissolved oxygen (DO) were measured in situ by a DO meter (HQ 30d 53LED^{TM} HACH USA). All of the analyses were performed according to standard methods (APHA, 2005).

2.4 Microbial analysis

Fluorescence in situ hybridization (FISH) was employed in this study to investigate microbial community composition in each VF CW system. At the end of the system operation, microbial samples were collected from the four VF CWs by collecting mixtures of substrate at a depth of about 30 cm (in the middle of the layer) and the interstitial water in the saturated zone. Then all samples were kept in ice, transported to the laboratory immediately and stored at 4℃. The samples were prepared according to the method described by Fan et al. (2013c). In brief, samples were immersed in freshly prepared 4% (w/v) paraformaldehyde solution for 2 h, and then washed twice with phosphate-buffered saline (PBS). Before hybridization, samples were air dried at room temperature and then dehydrated by successive passages through 50%, 80%, and 100% ethanol. Hybridization steps were performed following an established method (Sawaittayothin and Polprasert, 2007). Group-specific 16S rRNA gene probes EUB_{mix} (EUB 338, EUB 338-II and EUB 338-III) labeled with FITC, Nso 1225 labeled with Cy3, and Nstpa 662 and Nit 3 labeled with Cy3 (TAKARA, Japan) were used in this study to detect the dominant bacteria most bacteria, ammonia-oxidizing β- Proteobacteria, and all nitrite-oxidizing bacteria, respectively. FISH micrographs were captured digitally with an optical fluorescence microscope (BX53, Olympus Co., Ltd., Japan). Digital images were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Bethesda, MD).

2.5 Statistical analysis

All statistical analyses were performed through the statistical program SPSS 11.0 (SPSS Inc., Chicago, USA). Two-sample t-tests were used to evaluate the significance of differences between means. In all tests, differences and correlations were considered statistically significant when $P<0.05$.

3. Results and Discussion

3.1 Overall treatment performance in different CW systems

With the characteristics of the influent identified, the difference in treatment performance of different SSF CWs could be revealed from the varied effluent quality. The average influent concentrations of COD, NH_4^+-N, NO_3^--N and TN in the present study were 426.2 mg L^{-1}, 39.6 mg L^{-1}, 4.3 mg L^{-1} and 44.1 mg L^{-1}, respectively. Table 1 gives the average effluent concentrations of COD, NH_4^+-N, NO_3^--N, NO_2^--N, TN, and removal efficiencies COD, NH_4^+-N and TN for each SSF CW system in the long-term experiment. On the whole, System II and system III achieved a satisfactory removal of COD, NH_4^+-N and TN, when compare to System I and system IV. As summarized in Table 1, System II and system III achieved average COD removal efficiencies of 95.8% and 97.2%, respectively, while a lower COD removal in the System I (76.1%) and system IV (79.4%) was observed. Moreover, the intermittent aeration SSF CW with sludge-ceramsite substrate demonstrated higher COD removal efficiency than the results reported by other studies, such as 95% by Zhi et al. (2015), 90% by Li et al. (2014), 85-90% by Foladori et al. (2013) and 87-95% by Ding et al. (2014). This enhanced treatment performance in terms of COD removal efficiency was largely attributed to intensified oxygen supply generated by intermittent operation and sufficient oxygen diffusion provided by the porous sludge-ceramsite, which was in accordance with previous studies (Fan et al., 2013a; Zhong et al., 2015).

Furthermore, the intermittent aeration associated with sludge-ceramsite substrate also intensifying nitrogen removal significantly. Average removal efficiencies of NH_4^+-N and TN in the system III were 98.8% and 85.8%, respectively, and a slightly decrease of average removal efficiency for NH_4^+-N (97.4%) and TN (81.7%) was presented in the System II. This result indicates that simultaneous nitrification and denitrification occurred when intermittent aeration and sludge-ceramsite substrate were applied in CWs. However, due to the limited oxygen supply, a lower removal efficiency of NH_4^+-N (22.1%) and TN (27.6%) in the System I was achieved, and the system IV also obtained the negative removal for NH_4^+-N (27.1%) and TN (32.3%). This is consistent with other research which reports that artificial aeration could increase nitrogen removal (Maltais-Landry et al., 2009; Zhong et al., 2015). In addition, our results show a substantial improvement for the removal of NH_4^+-N and TN compared to other aerated CWs reported in previous studies, such as NH_4^+-N (88%) and TN (83%) by Ding et al. (2014) and NH_4^+-N (95%) and TN (80%) by Fan et al. (2013c). This could be explained by that sludge-ceramsite is propitious to develop microbial communities and to improve the permeable capacity of biofilm layers in CWs (Zou et al., 2012; Wu et al., 2015c).

3.2 Variation of organics and nitrogen removal in a typical cycle

3.2.1 COD removal

In order to understand the removal variation of organics in the typical cycle, the time-profile of COD concentration during the 72 h-treatment phase in different CW systems is shown in Fig. 2. The most part of the influent total COD was removed within 4 h after feeding in the System II and system III. The COD concentration dropped to under 50 mg L^{-1} in just 12 hours, which can comply with the Class I (A) of Wastewater Discharge Standard (GB18918-2002) in China, and then became mainly stable for the rest of the cycle. While in the System I and system IV, the influent COD concentration, which was 426 mg L^{-1} on average, decreased to around 75-100 mg L^{-1} after 12 h of treatment and remained approximately constant until the end of the cycle. These results show that COD was removed more rapidly in the intermittent aerated systems (DO 5-6 mg L^{-1}) than in the systems without aeration (DO 0.3-0.4 mg L^{-1}), suggesting that intermittent aeration had obvious impacts on enhancing the removal of COD in SSF CW. Organic matters could be degraded aerobically and anaerobically in subsurface flow constructed wetlands (Saeed and Sun, 2012). According to Saeed and Sun (2012), the substantial oxygenated conditions inside the media created by intermittent aeration foster aerobic bio-degradation pathways of organics but also can stimulate anaerobic organics degradation, and therefore allowed stable COD reduction (i.e. mean removal rate was above 95%). This positive impact of the intermittent aeration inside CW systems is also confirmed in the previous literature (Foladori et al., 2013; Fan et al., 2013a, c; Li et al., 2014). In addition, by comparison with CW systems without sludge-ceramsite substrate, the high COD removal rate was observed in CW systems with sludge-ceramsite substrate. These findings indicate the benefits of sludge-ceramsite substrate in the wetlands in allowing sufficient oxygen diffusion and promoting growth and reproduction of heterotrophic bacteria (Wu et al., 2011c).

3.2.2 NH_4^+-N removal

The time-profiles of the NH_4^+-N, NO_3^--N, NO_2^--N and TN concentrations during the 72 h-treatment phase in different CW systems are shown in Fig. 3. In the System II and system III, an immediate NH_4^+-N decrease was observed during the 4 h after feeding, and a further progressive reduction of NH_4^+-N (removal efficiency of around 85%) was obtained in just 12 h. At the end of the cycle, the effluent concentration of NH_4^+-N decreased to below 5 mg L^{-1}, which can meet the Class I (A) of Wastewater Discharge Standard (GB18918-2002) in China. On the contrary, because of the insufficient oxygen supply, only slightly removal of NH_4^+-N could be found in the System I and system IV, and the NH_4^+-N concentration stabilized at 25-30 mg L^{-1} by the end of the cycle. Moreover, NO_3^--N concentrations during the 72 h cycle indicated that significant difference of nitrification occurred among the different CWs with and without aeration. It is generally accepted that DO concentrations above 1.5 mg L^{-1} are essential for nitrification to occur (Saeed and Sun 2012). Oxygen supply via intermittent aeration in CWs enhanced DO concentrations (5-6 mg L^{-1} in this study), which boosted the growth of nitrifying bacteria of the wetland matrix, and therefore ensured the potential nitrification. However, in the non-aerated VFCWs the DO was rapidly consumed due to high levels of oxygen demand presents in influent wastewaters. The DO (below 0.4 mg L^{-1}) in most time of the cycle caused an anaerobic environment in CWs and thus may result in negligible nitrification (Li et al., 2014).

3.2.3 TN removal

In SSF CWs, the complete nitrogen removal could be mainly accomplished by nitrification-denitrification. Nitrogen retention in CWs is firstly dependent on complete nitrification, and the nitrified N must be permanently removed via denitrification, an anaerobic and heterotrophic microbial process, which could be limited by various factors such as insufficient organic carbon source, excess oxygen and lack of nitrate (Maltais-Landry et al., 2009; Fan et al., 2013b). As shown in Fig. 3, a TN removal trend similar to NH_4^+-N removal was observed in different CW systems during the whole 72 h cycle, and the TN concentration the systems applied with intermittent aeration decreased more rapidly than that in the non-aerated CWs. At the end of the cycle, the effluent concentration of TN reduced to 4-6 mg L^{-1} in the System II and system III, and the corresponding removal efficiency (82-86%) was slightly lower than NH_4^+-N removal rate. The accumulation of NO_3^--N (Fig. 3 and Fig. 4) starting at the later stage indicated that full denitrification could not be achieved due to carbon deficiency.This result is in accordance with other studies investigating aerated CWs (Maltais-Landry et al., 2009; Fan et al., 2013a, c). However, a weak reduction of TN was obtained in the System I and system IV, and the low TN removal efficiency in non-aerated CWs is mainly attributed to poor nitrification (Fig. 4) and low pH (Table 1). In the current study, intermittent aeration combined with sludge-ceramsite well developed alternate aerobic and anaerobic conditions for nitrification and denitrification, moreover, it could be benefit to available carbon supply for promoting denitrification. Fan et al. (2013b,c) and Foladori et al. (2013) reported that intermittent aeration could simultaneously enhance nitrification and denitrification. The high removal rates of NH_4^+-N and TN in this study also showed that intermittent aerated CWs using sludge-ceramsite substrate would be a potential choice to intensify nitrogen removal performance for the wastewater with high influent strength.

3.3 FISH results

The community composition, diversity and abundance of microbes in CWs, which play a significant role in pollutant removal from wastewater, are mainly dependent on various factors such as environmental parameters (i.e., pH, DO, redox potential, and temperature), wastewater properties, substrate types, plants, and operating conditions (Meng et al., 2014; Li et al., 2014). In this study, FISH analysis was conducted to investigate the influence of intermittent aeration and sludge-ceramsite substrate on microbial community and bacterial population (Table 2). According to the FISH results, negligible nitrifying bacteria (AOB and NOB) was detected in all CW systems at the beginning, but in the final phases of the experiment there was remarkable difference in microbial community composition of various CWs. Much more nitrifying bacteria and other viable bacteria were detected in intermittently aerated CWs, and nitrifying bacteria was also enhanced in SSF CWs with sludge-ceramsite substrate. As shown in Table 2, there was approximately 44.2-50.1% of AOB and 31.2-33.1% of NOB in aerated CWs, while fewer AOB (5.3-10.3%) and NOB (6.4-8.3%) were detected in non-aerated CWs. In addition, AOB and NOB in CWs with sludge-ceramsite substrate increased 5-6% and about 2% compared with conventional CWs. These results are consistent with the findings of Li et al. (2014) and Fan et al. (2013c). In those studies, the microbial abundance and diversity in wetland systems were stimulated by artificial aeration strategy, and the low DO in non-aerated CWs seriously limited the growth of nitrifying bacteria. Although FISH analysis is a semi-quantitative estimation to identify the relative abundances of AOB and NOB, our FISH results could explain the high removal of NH_4^+-N and TN achieved in present study.

4. Conclusions

Intermittent aeration and sludge-ceramsite substrate significantly intensified the removal of organic pollutants and nitrogen in SSF CWs. The best COD (97.2%), NH_4^+-N (98.9%), and TN (85.8%) removal was achieved in the intermittent-aerated CW using sludge-ceramsite substrate. More nitrifying bacteria (AOB and NOB) was detected in intermittently aerated CWs with sludge-ceramsite substrate, demonstrating that the application of intermittent aeration and sludge-ceramsite plays an important role in nitrogen transformations. The strategy of integrating intermittently aerated CWs with sludge-ceramsite substrate may be suitable for enhancing the removal performance in decentralized rural sewage treatment.

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Figure Captions:

Figure 1 Schematic diagram of experimental laboratory-scale CWs (System I: Non-aerated CW; System II: intermittent aeration CW; System III: intermittent aeration CW with sludge-ceramsite substrate; System IV: Non-aerated CW with sludge-ceramsite substrate).

Figure 2 COD profiles in different CW systems during the typical operating period.

Figure 3 Dynamic transformations of nitrogen (NH_4^+-N, NO_3^--N, NO_2^--N and TN) in the non-aerated CW (a), intermittent aeration CW (b), intermittent aeration CW with sludge-ceramsite substrate (c) and non-aerated CW with sludge-ceramsite substrate (d) during the typical operating period.

Figure 4 NH_4^+-N, NO_3^--N and NO_2^--N concentration variations of influent and effluent i in different CW systems throughout the experiment.

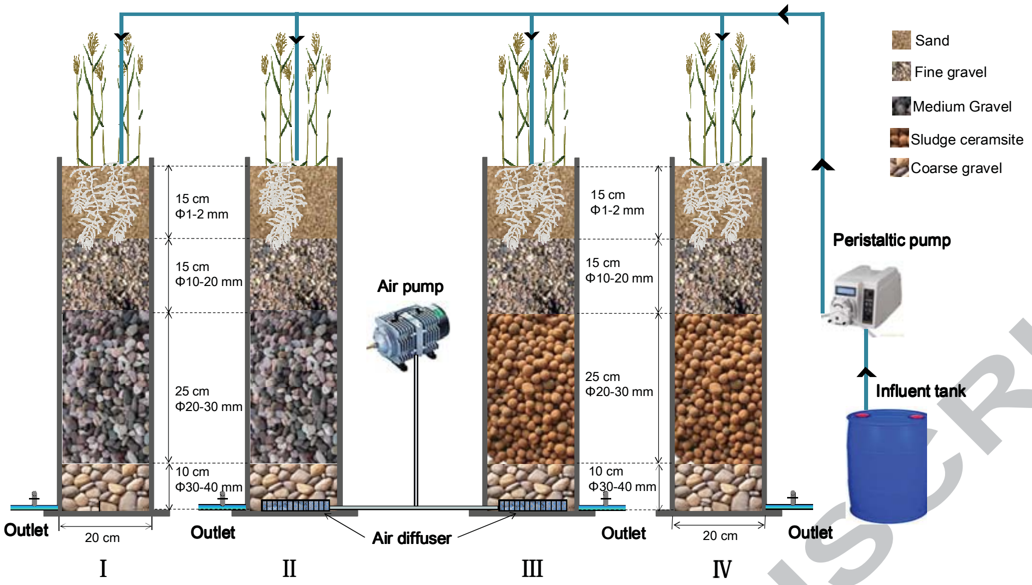


Figure 1 Schematic diagram of experimental laboratory-scale CWs (System I: Non-aerated CW; System II: intermittent aeration CW; System III: intermittent aeration CW with sludge-ceramsite substrate; System IV: Non-aerated CW with sludge-ceramsite substrate).

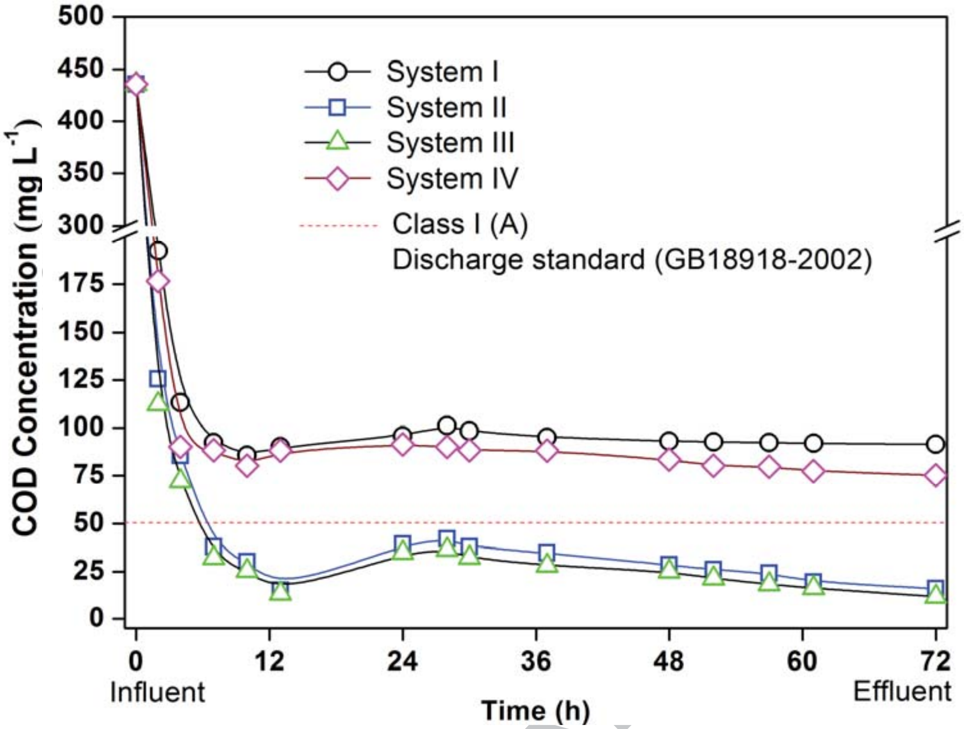


Figure 2 COD profiles in different CW systems during the typical operating period.

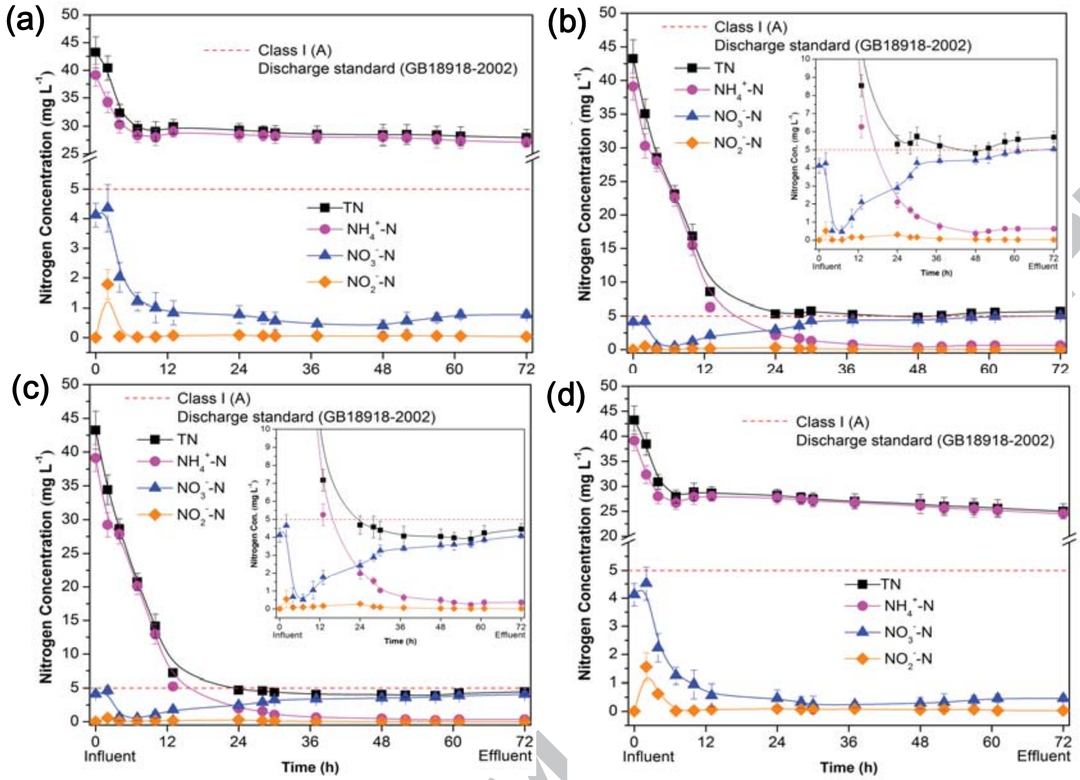


Figure 3 Dynamic transformations of nitrogen (NH_4^+-N, NO_3^--N, NO_2^--N and TN) in the non-aerated CW (a), intermittent aeration CW (b), intermittent aeration CW with sludge-ceramsite substrate (c) and non-aerated CW with sludge-ceramsite substrate (d) during the typical operating period.

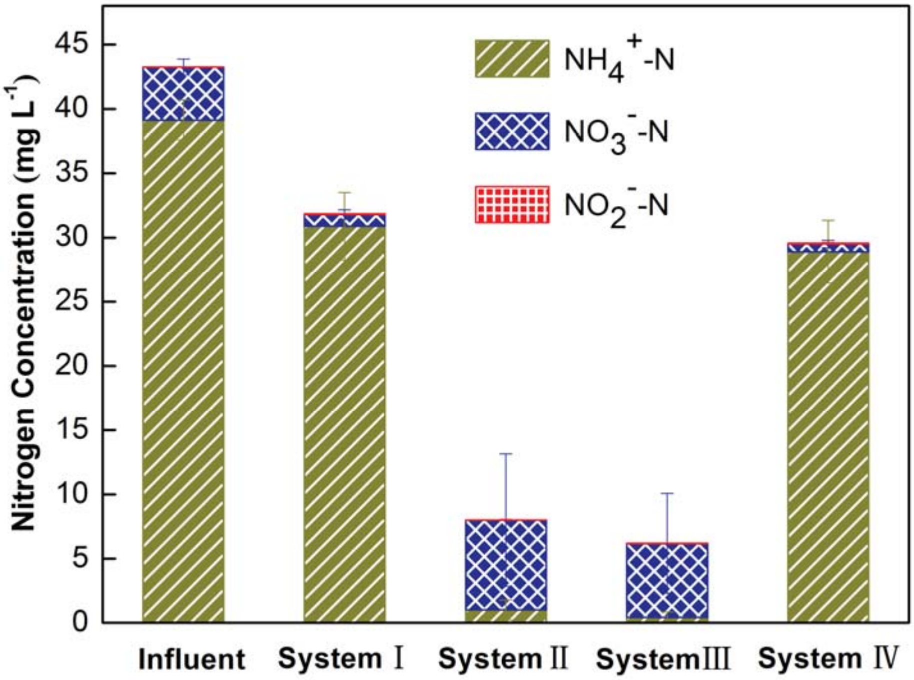


Figure 4 NH_4^+-N, NO_3^--N and NO_2^--N concentration variations of influent and effluent i in different CW systems throughout the experiment.

Table 1 Characteristics of effluent and respective removal efficiencies (Mean ± SD, n=20)

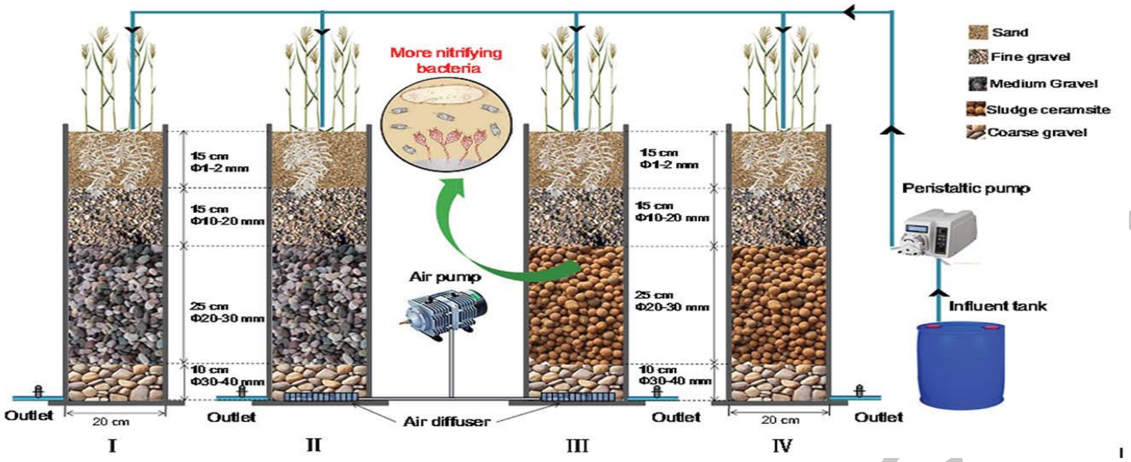
Parameters	Experimental systems			
	I	II	III	IV
COD (mg L^{-1})	101.9±13.11	17.6±7.0	11.9±5.0	87.5±11.8
(%)	76.1±3.0	95.8±1.6	97.2±1.1	79.4±2.7
NH_4^+-N (mg L^{-1})	30.8±2.6	1.01±0.7	0.4±0.4	28.8±2.4
(%)	22.1±6.6	97.4±1.9	98.8±1.0	27.1±6.1
NO_3^--N (mg L^{-1})	0.9±0.3	6.9±5.1	5.7±3.8	0.6±0.3
NO_2^--N(mg L^{-1})	0.06±0.03	0.02±0.05	0.02±0.02	0.06±0.04
TN (mg L^{-1})	31.8±2.6	8.0±5.6	6.2±4.1	29.7±2.5
(%)	27.6±5.9	81.7±12.8	85.8±9.2	32.3±5.7
pH	6.9±0.3	7.6±0.3	7.6±0.2	6.9±0.2
DO (mg L^{-1})	0.3±0.2	5.7±2.1	6.0±1.9	0.4±0.2

System I: Non-aerated CW; System II: intermittent aeration CW; System III: intermittent aeration CW with sludge-ceramsite substrate; System IV: Non-aerated CW with sludge-ceramsite substrate.

Table 2 Relative abundance of AOB and NOB in different SSF CWs based on FISH analysis.

Experimental systems	AOB: total bacteria (%)	NOB: total bacteria (%)
I	5.3±2.4	6.4±3.2
II	44.2±6.3	31.2±5.1
III	50.1±7.4	33.1±8.3
IV	10.3±3.1	8.3±4.2

System I: Non-aerated CW; System II: intermittent aeration CW; System III: intermittent aeration CW with sludge-ceramsite substrate; System IV: Non-aerated CW with sludge-ceramsite substrate.


▨ Sand
▨ Fine gravel
▨ Medium Gravel
▨ Sludge ceramsite
▨ Coarse gravel
More nitrifying bacteria
Peristaltic pump
Influent tank
Air pump
15 cm Φ1-2 mm
15 cm Φ10-20 mm
25 cm Φ20-30 mm
10 cm Φ30-40 mm
15 cm Φ1-2 mm
15 cm Φ10-20 mm
25 cm Φ20-30 mm
10 cm Φ30-40 mm
Outlet
20 cm
Outlet
Air diffuser
Outlet
20 cm
Outlet
I
II
III
IV

Research Highlights

1) A novel sludge-ceramsite was integrated with intermittently aerated SSF CWs.
2) Intermittent aeration and sludge-ceramsite enhanced organics and nitrogen removal.
3) High removal of COD (97.2%), NH_4^+-N (98.9%) and TN (85.8%) were achieved.