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Effect of multilayer substrate configuration in horizontal subsurface flow constructed wetlands: Assessment of treatment performance, biofilm development and solid accumulation

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Response to Reviewers:	Please see the attached file, named as "response to comments".
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Response to Comments

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Answers to Editors and Reviewers

The revised manuscript entitled "Effect of multilayer substrate configuration in horizontal subsurface flow constructed wetlands: Assessment of treatment performance, biofilm development and solid accumulation" was earlier submitted for publication in *Environmental Science and Pollution Research*.

We are obliged to the reviewers of this manuscript, which contributed to improve its quality. The response explaining how the authors have dealt with the specific referees' comments are integrated in the reviewer's text. Relevant changes (highlighted with yellow colour) were introduced throughout the manuscript text and marked with page and line number in the attached word file.

Reviewer #1

[1] This is a good manuscript, I do have only few questions and comments.

Answer: Thank you very much for the comments.

General comment:

[2] Three years of operation is not a long term performance. It is difficult to say exactly, however, in my opinion, it should be at least 10 years of operation.

Answer: Thank you very much for your comments. It is truly that the common life span of a well-designed/maintained CWs will be 10-15 years or even longer. However, the established CWs for basic research in the publications are always shorter than 3 years. By considering the reviewer's comment, in order to make the language more accurate in this scientific paper, the terms of "long term performance" were either deleted or changed to "performance after 3-year operation" or "after relatively long term (3-year) operation" in the whole paper. (Page 1, line 31 and 46; Page 3, line 26; Page 4, line 16; Page 15, line 46)

[3] Page 4, line 44: The reference is not correctly written.

Answer: The typo was modified. It should be the location of the experiment site (latitude and longitude) rather a reference. Thus, the wrong term "(N25cember 2012 at." was changed to "N25^{o} 28', E110^{o} 31'". (Page 4, line 47)

<u>[4] Why TVC was calculated using this weird approximation and not counted?</u>

Answer: In the present paper, total viable cells were quantified by extraction of phospholipids and analysis of phosphate (cleaved from phospholipids). Compare with plate count method, phospholipids have the advantages that their concentration remains fairly constant in relation to cell biomass and once a cell dies the phospholipids have a short half-life. Thus, the more accurate and precise method was selected to be used in the present study.

<u>[5] Page 9, line 4: what kind of improvement do authors speak?</u>

Answer: The sentence was re-written to improve the clarity. Thus the old sentence "Both maximum and minimum temperatures showed clearly improvement along with the season changes from April to September." was changed to "Along with the season changes from April to September, the maximum and minimum air temperatures showed clearly improvement from approximately 25 ^{o}C to 30 ^{o}C and 17 ^{o}C to 25 ^{o}C, respectively.". (Page 9 , line 4-10)

<u>[6] Did authors measure hydraulics in various layers?</u>

Answer: Thanks for raising this point. We did not measure the hydraulics in various layers in the present study. However, it is an interesting point which is worth to be investigated in the followed study.

Effect of multilayer substrate configuration in horizontal subsurface flow constructed wetlands: Assessment of treatment performance, biofilm development and solid accumulation

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Abstract

This study investigates the influence of multilayer substrate configuration in horizontal subsurface flow constructed wetlands (HSCWs) on their treatment performance, biofilm development, and solid accumulation. Three pilot-scale HSCWs were built to treat campus sewage and have been operational for three years. The HSCWs included mono-layer (CW1), three-layer (CW3), and six-layer (CW6) substrate configurations with hydraulic conductivity of the substrate increasing from the surface to bottom in the multilayer CWs. It was demonstrated the pollutants removal performance after 3-year operation improved in the multilayer HSCWs (49-80%) compared to the monolayer HSCW (29-41%). Simultaneously, the multilayer HSCWs exhibited significant features that prevented clogging compared to the mono-layer configuration. The amount of accumulated solids was notably higher in the mono-layer CW compared to

multilayer CWs. Further, multilayer HSCWs could delay clogging by providing higher biofilm development for organics removal and consequently, lesser solid accumulations. Principal component analysis strongly supported the visualization of the performance patterns in the present study and showed that multilayer substrate configuration, season, and sampling locations significantly influenced biofilm growth and solid accumulation. Finally, the present study provided important information to support the improved multilayer configured HSCW implication in the future.

Keywords: Biofilm growth; clogging; organics removal; spatial difference; total valid cell count

1. Introduction

Horizontal subsurface flow constructed wetlands (HSCWs) have been widely used for wastewater treatment as an environmental friendly technology (Kadlec & Wallace, 2008; Vymazal, 2009). However, clogging is the most common problem encountered in HSCWs, which can result in a large proportion of the influent water passing over the bed as surface-runoff. Clogging reduces the possibility of contact between pollutants and biofilm required for biodegradation in the HSCWs, which significantly weakens treatment performance and shortens their life span (Osorio et al., 2007). Thus, solutions that prevent clogging and optimize CW utilization have gained considerable attention in the recent decades.

Substrate configuration is an important factor that influences clogging as most clogging solids wrap around the substrate pores (Brovelli et al., 2011). Theoretically, substrate configuration can be improved by placing finer media with low hydraulic conductivity on the top and coarser media with high hydraulic conductivity at the bottom, which should prevent short-circuit of water flow on the surface and decrease dead zones at the bottom. It is believed that this substrate configuration enables a natural and even water flow, which prevents insalubrious clogging of the CWs (Knowles et al., 2011; Morales et al., 2013). Furthermore, the uniform water flow pattern would improve pollutants biodegradation due to high biofilm/pollutant contact possibility. The system following this concept was built and recorded higher pollutants removal efficiencies the year following construction (Bai et al., 2016). However, the performance after relatively long term (3-year) operation has not been evaluated after over three years of operation.

Clogging is a complex process, however, the exact mechanisms and consequences of clogging are not completely clear. Blazejewski and Murat-Blazejewska (1997) reported that biofilm growth and solid accumulation are important factors that cause clogging. An investigation of the development process of biofilm during changing seasons showed that temperature was an important factor in clogging (Platzer and Mauch, 1997). Higher temperatures result in higher biological activity, which decreases organic matter accumulation. However, it may also cause higher biofilm growth that promotes clogging. In horizontal flow CWs, solids accumulation has been recognized as a major factor that decreases CW longevity (Cooper et al., 2005). Accumulated solids in CWs include both organic and inorganic solids (Knowles et al., 2011). However, comprehensive investigation of biofilm growth and solid accumulation related to spatial and seasonal differences in HSCWs with multilayer substrate configuration has not been conducted.

The aim of this study was to determine the influence of multilayer substrate configuration on treatment performance, biofilm development, and solid accumulation after 3-year operation in HSCWs. Pollutant removal efficiencies were measured in a pilot HSCW treating campus sewage that had been operational for three years. Biofilm development and solid accumulation were analyzed in different seasons (April, July, and September) of 2016 to compare the spatial difference between monolayer and multilayer HSCWs. Total viable cell counts (TVC), extracellular polysaccharide (EPS), and extracellular protein (EP) were measured to represent biofilm development. Different solid components, including organic and inorganic matters, were investigated to evaluate solid accumulation.

2. Materials and methods

2.1 Experimental setup

Three parallel pilot-scale HSCWs were constructed in December 2012 at Guilin University of Technology, in south-west China (N25° 28', E110° 31'). A detailed description of the experimental setup can be found in Bai et al. (2016). Briefly, each system had distinct dimensions of 2 m length, 1.2 m width, 0.7 m height, and water depth of 0.6 m (Fig. 1a). Each HSCW was divided into influent distribution zone (0.2 m long), main reaction bed (1.6 m long), and effluent collection zone (0.2 m long). The main reaction beds of the three HSCWs had different configurations (Fig. 1b). CW1 was mono-layer substrate configured, filled with quartz sand (0-6 mm) with hydraulic conductivity (K) of 65 m/d. CW3 consisted of three equal layers with 0.2 m thickness. The K values of the layers were 26, 36, and 64 m/d from the surface to the bottom, and the corresponding sand diameters were 0-0.4, 0.4-0.6, and 1-3 mm, respectively. CW6 comprised of six equal layers with 0.1 m thickness. From the top to bottom, sand sizes were 0-0.4, 0.4-0.6, 0.6-0.9, 1-2, 2-4, and 4-6 mm, with K values of 26, 36, 43, 55, 75, and 176 m/d, respectively.

The laboratory facility was subject to natural temperature variations and light exposure but protected from rain by a glass roof. The influent of this experiment was original campus sewage pre-treated by a septic tank. The three HSCWs shared a 200 L influent tank with separate pump stations functioning at identical hydraulic loading rate (HLR) of 0.3 $m^3/m^2/d$, to ensure consistent operation mode. The systems had been continually operational for the past three years. The present study was conducted from April 1, 2016 to September 1, 2016. All HSCWs were planted with *Canna indica* at the density of 20 individuals per square meter. The average influent COD, NH_4^+-N, TN, and TP were approximately 145, 23, 32, and 5 mg/L during experimentation period (Table 1).

2.2 Water sampling and measurement

Triplicate water samples were collected from the influent tank and effluent pipes of the HSCWs once a week to monitor water quality and pollutants removal. The pH and ORP were measured in situ using a Hanna Hi9828 multi-parameter portable meter. Air and influent water temperatures were recorded everyday using an automatic temperature logger connected to the sensor. Individual water samples were stored in 100 mL sterile plastic bottles, cooled to 5 °C, and transported to the laboratory. The concentrations of chemical oxygen demand (COD), ammonium (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), total nitrogen (TN), and total phosphorous (TP) were analyzed within 24 h using standard methods (APHA, 2005).

2.3 Substrate sampling

Substrate samples of the attached biofilm and solid accumulation analyses were collected on April 1, July 1, and September 1, 2016 using a push core sampler (ø 5 cm, length 100 cm). Substrate samples were taken from the front and back parts of each HSCW at a distance of 0.3 m and 1.7 m from the inlet, respectively. Three 50 g samples were taken from each sampled core at depths of -10 cm (A1), -30 cm (A2), and -50 cm (A3) from the surface (Fig. 1a). Visible roots and debris were removed and samples were stored in sterile plastic bags, placed on ice, and transferred immediately to the lab for the following analyses**.**

2.4 Biofilm analysis

Total viable cell counts (TVC), extracellular polysaccharide (EPS), and extracellular protein (EP) were tested to evaluate biofilm development (Ragusa et al., 2004). TVC was measured using the method described by Findlay et al. (1989). Briefly, phospholipids were extracted from substrate samples and concentrations of phosphate released by phospholipids were determined using the method described by Shatton et al. (1983). TVC was calculated from the phosphate concentration using the conversion factor, 1 nmol phosphate is proportional to 3.4×10^{7} cells as determined by Findlay et al. (1989). Additionally, bovine serum albumin was used for construction of standard curves. The quantification of biofilm EPS was assayed in phenol sulfuric acid, as detailed by Frolund et al. (1996). The substrate samples were placed in glass reaction tubes. Subsequently, 1ml each of distilled water and 5% phenol were added to individual tubes. The tubes were mixed in a vortex mixer for 30 s. The volume of 5 mL concentrated sulfuric acid was added to each tube during the mixing process. Absorbance was measured at 485 nm after cooling. Glucose was used to construct standard curves for polysaccharide concentration. EP was extracted from the substrate samples by adding 1N NaOH and incubating at 55 °C for 2 h. Equal volumes of 1N HCl were added to each sample to neutralize the NaOH after cooling to room temperature (Ragusa et al., 2004). The solubilised protein was quantified using PIERCE (Rockford IL, USA) BCA Protein Assay Reagent Kit (Cat. No. 23227) according to the method specified by Lowry et al. (1951).

2.5 Solid analysis

Accumulated solids were detached from 5 g substrate samples using ultrasound equipment for 15 min with 100 mL phosphorus buffer (K_2HPO_4, 9.3 g/L; KH_2PO_4, 1.8 g/L) to form an accumulated solids solution (AS solution). Initially, the AS solution was manually divided into two 50 mL portions. The first 50 mL AS solution was dried at

105 °C for 24 h. Subsequently, the dry residue was burned at 550 °C for 15 min. The weight difference between the final burned crucible and original clear crucible was estimated as the total inorganic matter (TIM) content. The weight difference of the crucible before and after burning was estimated as the total organic matter content. The second 50 ml AS solution was filtrated using 0.45 μm filter membrane, and the filtrate solution was analyzed using the same method described above to indicate dissolved organic matter (DOM). The weight difference between total organic matter (the first 50 ml AS solution) and DOM (the second 50 ml AS solution) was calculated as insoluble organic matter (IOM). DOM and IOM were added to calculate the accumulated total organic matter. The accumulation of total solids was the sum of TIM, DOM, and IOM. Additionally, the total organic matter and total inorganic matter (TIM) were added to represent the accumulated total matter.

2.6 Statistical analyses

One-way analysis of variance (ANOVA) at 95% confidence level ($p<0.05$) was used to evaluate significant differences in pollutant removal efficiencies between the three HSCWs. The performance patterns of biofilm growth and solid accumulation for the HSCWs were analyzed using principal components analysis (PCA). XLStat Pro® (XLStat, Paris, France) was used for plotting and data analyses in the present study.

3. Results and discussion

3.1 Water quality and pollutants removal

The experiment site was located in a typical subtropical environment with maximum and minimum air temperature ranged of 22-36°C and 13-28°C, respectively, during the whole experiment (Fig. 2). Along with the season changes from April to September, the maximum and minimum air temperatures showed clearly improvement from approximately 25 °C to 30 °C and 17 °C to 25 °C, respectively. The influent water temperature also showed a similar tendency which increased from around 20°C to 26°C from April to June and then kept relative stable until September (Fig. 2). Additionally, the ORP values of effluent for the three HSCWs (CW1, CW3 and CW6) were not significantly different with an average of -100 mV. The 3 HSCWs also showed similar pH values in a range of 6.9-7.5 along the experiment (data are not shown).

Significantly higher pollutants removal abilities were continually observed in the multi-layer substrate configured CW6, followed by CW3, and the monolayer CW1 throughout the experiment (Fig. 3). The average removal efficiencies of COD, NH_4^+-N, TN, and TP were about 70%, 78%, 62%, and 80% for CW6; 58%, 60%, 49%, and 63% for CW3; and 29%, 40%, 30%, and 41% for CW1, respectively. Generally, pollutants removal performance before June fluctuated notably and recorded slightly lower than average values for all the HSCWs than the corresponding values between June and September.

Our previous study had reported that multilayer substrate configuration enhanced pollutants removal in the HSCW in the year following system construction (Bai et al., 2016). The higher removal efficiencies were attributed to the multilayer configuration with larger size substrate set at the bottom that improved hydraulic performance by reducing short circuit in the HSCWs. After three operational years, the same enhanced performance was observed in the present study, which indicated that the optimized multilayer configuration can promote stable and positive effect on pollutants removal. The corresponding values were slightly lower that values observed three years ago, especially for CW1, which may due to solid accumulation and clogging (Blazejewski & Murat-Blazejewska, 1997; Morales et al., 2013). Additionally, higher pollutants removal abilities after June may be due to higher temperature (Fig. 2). The microorganisms responsible for COD and nitrogen removal function optimally in relatively higher temperature conditions (Akratos & Tsihrintzis, 2007; Lv et al., 2016).

3.2 Biofilm development

Multilayer substrate configured HSCWs generally recorded higher concentrations of TVC, EPS, and EP than the monolayer HSCW in samples acquired both from the front and back parts (Fig. 4). The results indicated that multilayer configuration promoted biofilm growth under better hydraulic performance in the HSCWs. Regarding pollutants removal, organic matters and nitrogen were mostly degraded by substrate attached biofilms (Lee et al., 2009; Lv et al., 2017). TVC represents the absolute number of bacterial cells that contribute to pollutants degradation. Polymers are mainly composed of polysaccharide (EPS) and protein (EP) and extracellular polymers can trap, bind, and concentrate organic materials increasing their susceptibility to bacterial biodegradation (Zhao et al., 2009). Thus, the superior biofilm amount in CW3 and CW6 supported improved pollutants removal performance, as shown in Fig. 3.

Seasonal differences in the biofilm development were also clearly observed. The concentrations of TVC, EPS, and EP showed distinct increase from April to July and September (Fig. 4a-c). The total TVC values in CW1, CW3, and CW6 showed similar values in July and September with a range of 3.6-3.9 × 10^5 CFU/g DM. However, in April, CW1 exhibited a significantly lower total TVC value (1.5 × 10^5 CFU/g DM) compared to CW3 (2.4 × 10^5 CFU/g DM) and CW6 (2.7 × 10^5 CFU/g DM). Similar tendencies were also observed for EPS and EP concentrations, which was probably due to warmer temperature as well as active plant growth during July and September. Relatively higher temperatures are suitable for bacterial survival and biofilm growth, and presence of active plants provide more rhizosphere area for bacterial attachment (Stein & Hook, 2005; Wynn & Liehr, 2001).

Notably, the seasonal change in biofilm growth also presented a spatial difference, especially for the samples collected at different depths. In April, the values of TVC located in the top layer (A1 at depth of -10 cm) were clearly lower than the deeper layers. However, A1 TVC increased significantly in July and September, which may attributed to oxygen release from active plants and bacteria growth. The bacteria located in the top layer of the HSCW was mainly aerobic, such as heterotrophic and nitrifying bacteria (Samsó & García, 2013). These results are in agreement with Chazarenc et al. (2009) who measured higher bacterial cell count in aerated CWs with higher oxygen content. The TVC in the deeper positions of A2 and A3 were generally stable over the experimentation period, which may be mainly due to bacterial survival under anaerobic conditions were not dramatically change.

3.3 Solids accumulation

All the HSCWs exhibited significant increase in total solid contents from April to September for both front and back parts of the systems (Fig. 5). It is evident that the increase rate and accumulated amount was higher in CW1, followed by CW3 and CW6. This elevated accumulation could be a consequence of decrease in large void space availability with time. Additionally, the amount of solid accumulated probably increased due to biofilm development and solid trapping ability, which showed an increasing tendency (Fig. 4). A comparison of the total solid accumulation between the different HSCWs revealed that the multi-layer HSCWs had notably lower values than the mono-layer CW. Optimized hydraulics with even water flow path may distribute solid trapping possibility to a larger area in each HSCW. Moreover, the total solid accumulation was generally always higher in the front part (In) compared to the back part (Out) of the CWs. Chazarenc et al. (2009) also indicated that more solids would be trapped in the front part of CWs when the influent begins flowing into the CWs and majority of the solid would be trapped by the substrate.

Fig. 6 shows the accumulation of DOM, IOM, and TIM in each CW with time. TIM was the main component of the total solids in all three CWs (72-85%), and the amount increased slightly from April to September. Nevertheless, it was assumed that the TIM did not play a crucial role in clogging. As confirmed by previous studies, clogging matter typically consists of highly hydrated gels and sludge with inorganic and organic solids, and the porosity loss due to inorganic solid accumulation is very small (Llorens et al., 2009). This may be due to substrate micro-pore clogging by dead bacteria or growth of organic matter such as active biofilm inside.

Organic matter (OM) accumulations were predominantly composed of humic, humin, and fulvic acids, derived from lignocellulosic humic compounds and plant detritus. Nearly, 63-96 % of these organic matter fractions were relatively refractory (Nguyen, 2000). Humic compounds are highly colloidal and amorphous with high hydrophilic potential and physical binding properties. They can form complexes with small quantities of biological fraction to form low-density gelatinous sludge with very high water retention capacity. All these properties increase the organic matter‘s potential to block big pores, which consequently act as a sieve and restricts flow through of larger particulate solids (Hua et al., 2013). OM contents, including DOM and IOM, also exert crucial effect on clogging, even though their contribution is less than 20% of the total solid accumulation. As shown in Fig. 6, an increase in accumulated OM was found from April to September for all CWs, while multi-layer configuration CW exhibited lower OM content compared to the mono-layer CW. Lower OM contents in CW6 exhibited better characteristics to offset clogging.

For the contents of DOM and IOM, a higher proportion of the DOM was found in multi-layer configuration CWs compared to the mono-layer CW, while mono-layer CW had higher IOM proportion. IOM was composed of refractory matter, while DOM was mainly composed of adsorbed or entrapped residual pollutants or soluble microbial products. Thus, DOM accumulation was assumed to be better than IOM accumulation for clog prevention, because DOM could be flushed out at certain flow rates, while IOM stayed for longer periods. Therefore, multi-layer CWs with higher DOM and lower IOM contents seemed to be more beneficial than mono-layer CW to prevent clogging.

Regarding solid accumulation at different depths, most solids accumulated in the bottom layers (A3 and B3) in CW1. In the multi-layer CWs, solid accumulation was distributed more homogeneously among the three layers during all three months. In the mono-layer CW, preferential paths often existed in the surface layer, which led to higher solids accumulation in the middle and bottom layers, resulting in preferential water flow through the surface layer (Osorio et al., 2007). Thus, the results proved that a rational arrangement of substrates with increasing K values from the surface to bottom was more successful in achieving even distribution and mitigate clogging.

3.4 Patterns of solid accumulation and biofilm growth

Solid accumulation (TIM, DOM, and IOM) and biofilm growth (TVC, EPS, and EP) in different layers (A1, A2, and A3) of the three CWs (CW1, CW3, and CW6) under different sampling seasons (April, July, and September) were analyzed using PCA to assess performance patterns (Fig. 7). The first two principal components accounted for 64.94% of the variation. The PCA results were further marked to compare the systems (Fig. 7a), season (Fig. 7b), and spatial differences (Fig. 7c). The performance patterns for different CWs were generally separated, especially between the multilayer and monolayer CWs (Fig. 7a). CW1, located closer to the positive direction of PC2 (up direction), indicated high contribution of solid accumulation (IOM and TIM). Fig. 7b shows clearly separated groups in different sampling seasons. The samples from July and September were located closer to the positive direction of PC1 compared with April, which was highly contributed by the biofilm development (higher EP, TBC, and EPS concentrations). The performance patterns of the samples from different layers (A1, A2, and A3) generally overlapped and did not show clear group differences (Fig. 7c). However, the difference were not found may due to the mixing up of different HSCWs, and cause the neglect influence of the performance patterns difference. This is supported by Fig. S1, which shows that spatially different patterns were generally found for all the HSCWs when each CW was separately analyzed. Thus, the PCA plots helped visualize the changes in performance patterns under different influencing factors in the present study. It can be concluded that the multilayer substrate configuration, season and sampling locations could significantly influence the solid accumulation and biofilm growth performance.

4. Conclusions

- Multilayer substrate configuration HSCWs can promote pollutants removal efficiencies after relatively long-term (3-year) operation compared to monolayer HSCW due to optimized hydraulics of the water flow path.
- Lower solid accumulation was found in multi-layer HSCWs, was attributed to better use of its available bed space and even flow pattern, which prevented clogging in CWs.

- Multilayer configured HSCWs can delay clogging by providing higher biofilm development for organics removal and consequently lesser solid accumulations.
- Principal component analysis strongly supported the visualization of performance patterns and showed that the multi-layer substrate configuration, season, and sampling locations significantly influence solid accumulation and biofilm growth.

Acknowledgements

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Figure 1

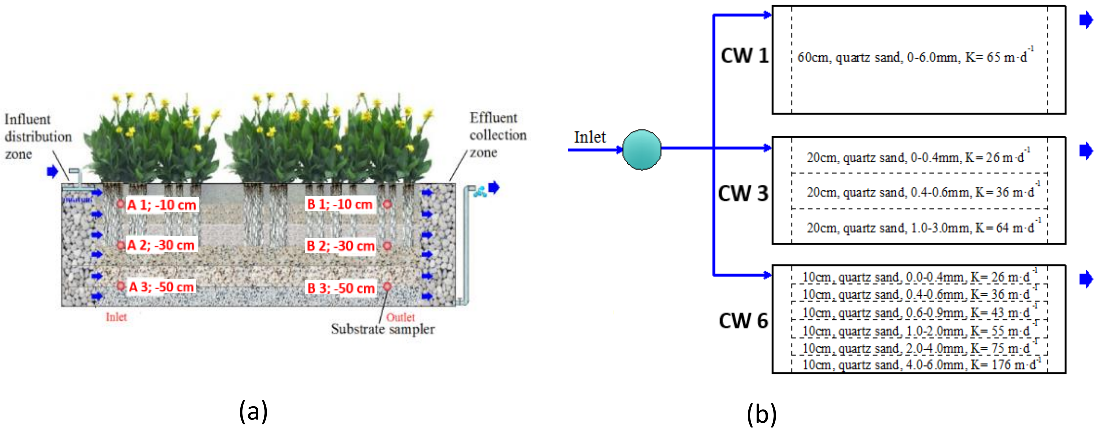

Fig. 1 (a) Schematic diagram of the HSCWs and (b) substrate configuration of the three parallel HSCWs.

Figure 2

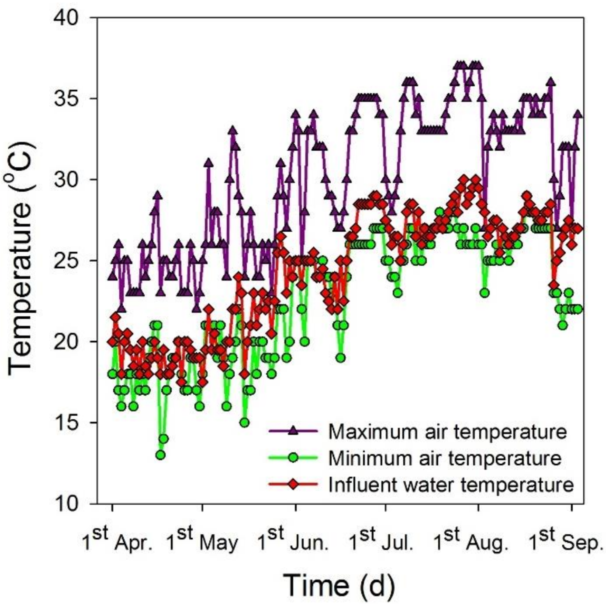

Fig. 2 The daily maximum, minimum air temperature and influent water temperature during the whole experiment.

Figure 3

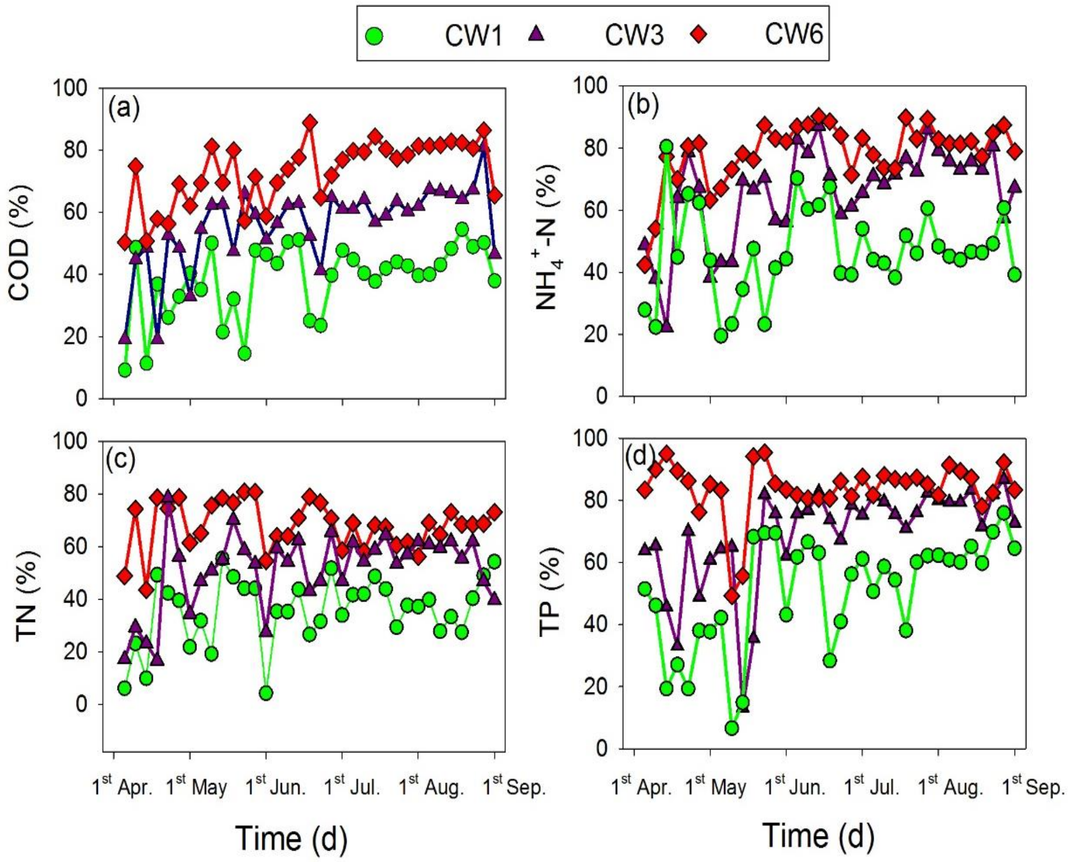

Fig. 3 Removal efficiencies of (a) COD, (b) NH_4^+-N, (c) TN, and (d) TP for monolayer (CW1) and multilayer (CW3, CW6) HSCWs during the experiment.

Figure 4

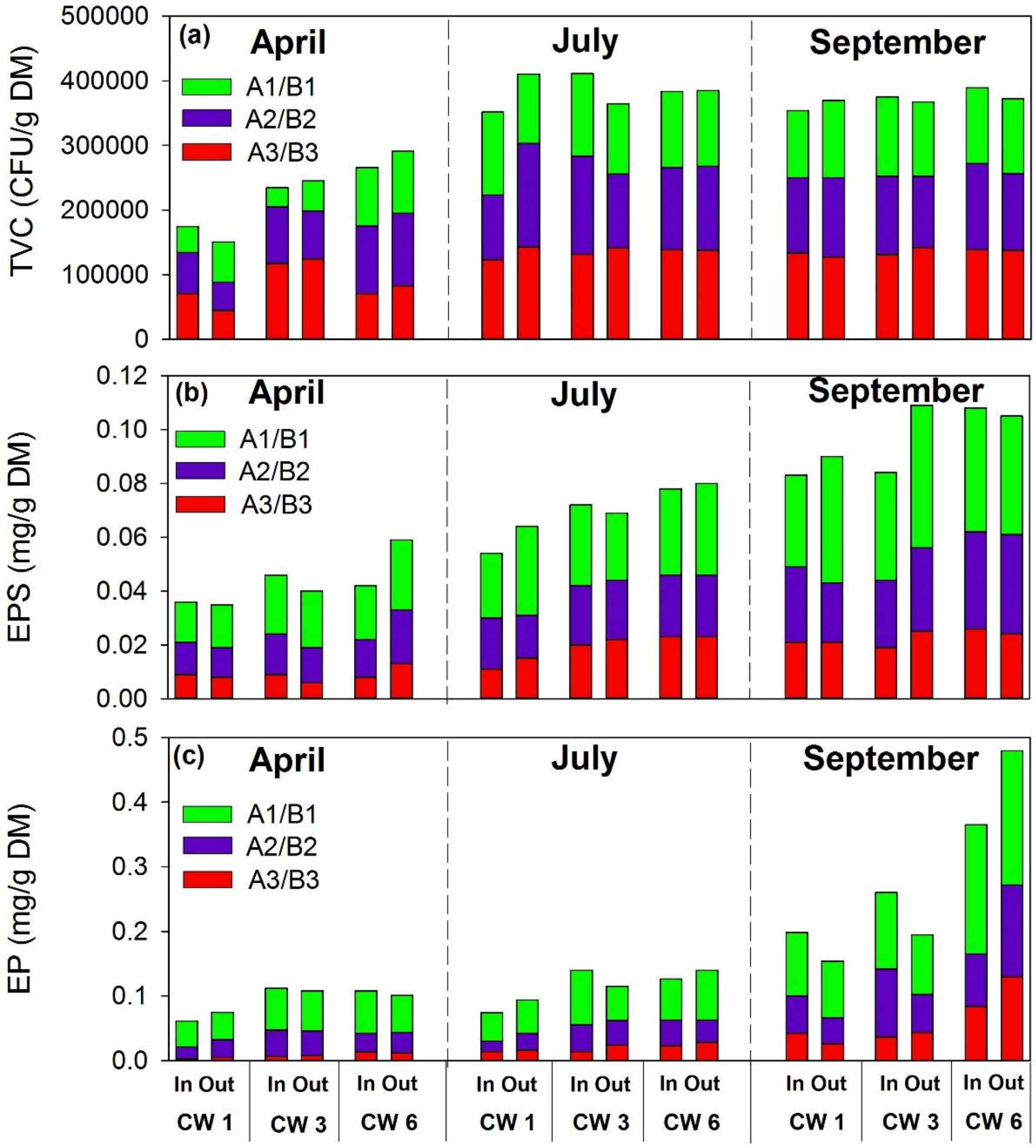

Fig. 4 Development of total viable cell counts (TVC), extracellular polysaccharide (EPS), and extracellular protein (EP) at each sampling point in the mono-layer (CW1) and multi-layer CWs (CW3, CW6) in April, July, and September, 2016. A1/B1, A2/B2, and A3/B3 represent the sampling height of -10 cm, -30 cm, and -50 cm, respectively. 'In' and 'Out' represent sampling positions at the front and back parts of each HFCW, respectively.

Figure 5

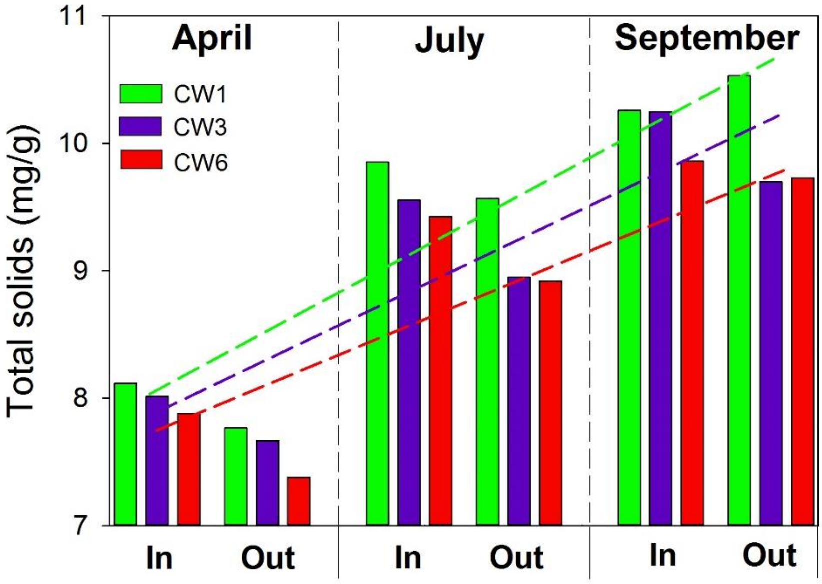

Fig. 5 Accumulation of total solid at each sampling point (summary for all layers) in monolayer (CW1) and multilayer CWs (CW3, CW6) in April, July, and September 2016. The dotted lines highlight the increasing tendency of total solid accumulation with time for each CW. 'In' and 'Out' represent the samples from front and back part of each CW, respectively.

Figure 6

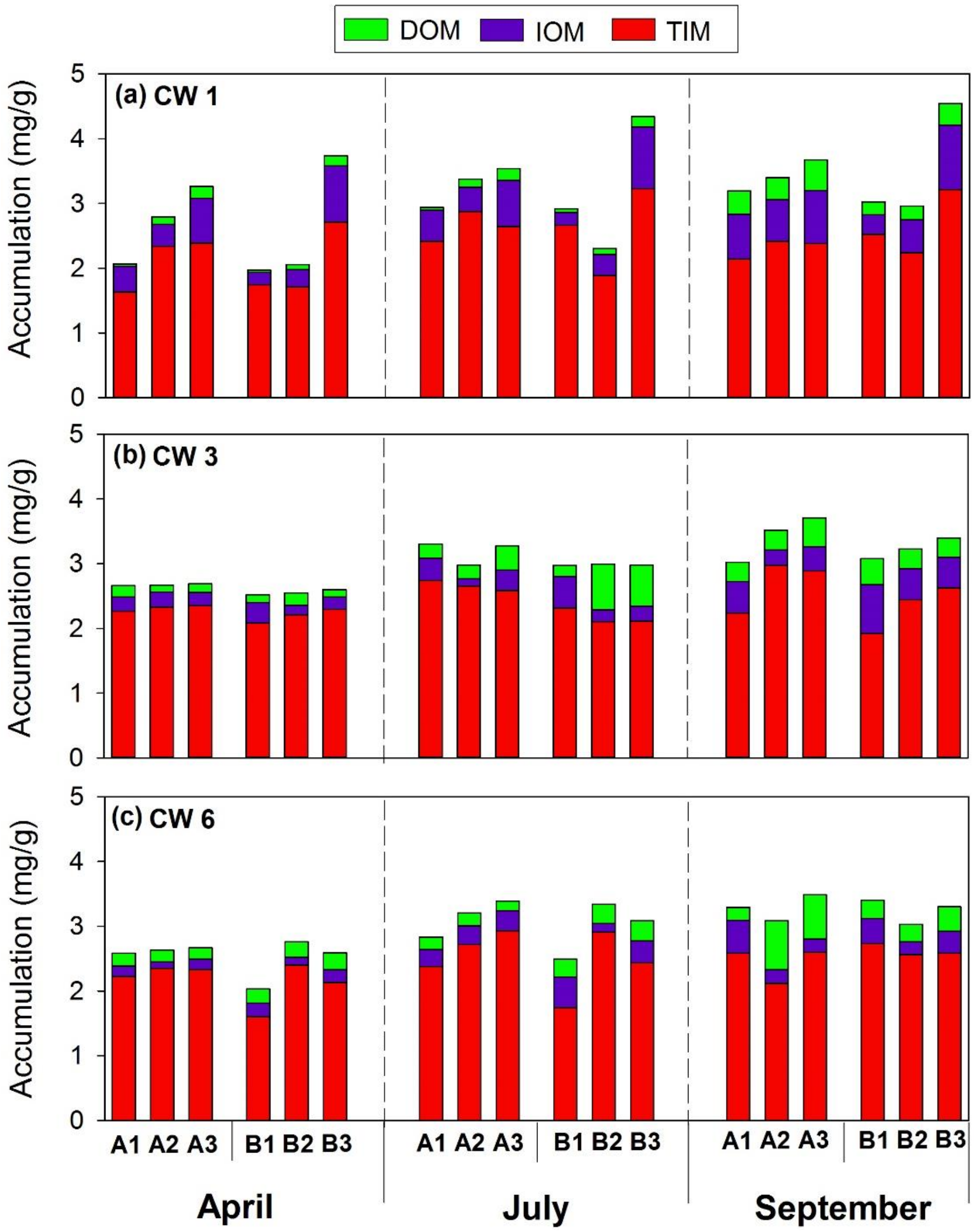

Fig. 6 Accumulated TIM, IOM, and DOM levels at each sampling point in (a) CW1, (b) CW3, and (c) CW6 during April, July, and September. A1, A2, and A3 represent the sampling height of -10 cm, -30 cm, and -50 cm in the front part, respectively. B1, B2, and B3 represent the sampling height of -10 cm, -30 cm, and -50 cm in the back part, respectively.

Figure 7

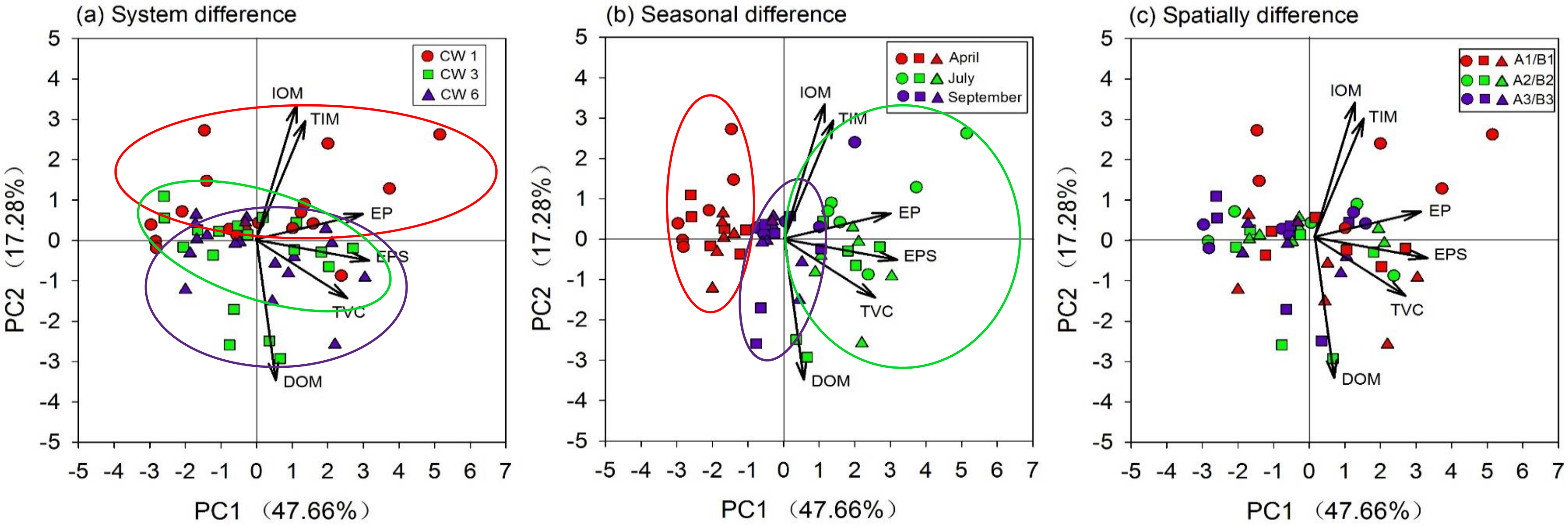

Fig. 7 Principal component analysis of the performance patterns of biofilm development (TBC, EPS, and EP) and solid accumulation (DOM, IOM, and TIM) in CW1, CW3, and CW6. The same analysis results with different symbols are shown to compare (a) systems difference, (b) seasonal difference, and (c) spatial difference. The arrows in each plot represent the loading factors in the principal component analysis.

Table 1
Physicochemical parameters of the influent for the three HSCWs in the experiment.

Parameters	Values	Unit
pH	7.3 ± 0.3	-
DO	0.3 ± 0.2	mg/L
ORP	-158 ± 22	mV
COD	145 ± 36	mg/L
NH_4^+-N	23 ± 8	mg/L
TN	32 ± 7	mg/L
NO_3^--N	0.9 ± 0.5	mg/L
TP	5 ± 3	mg/L