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Treatment of anaerobic digested effluent in biochar-packed vertical flow constructed wetland columns: Role of media and tidal operation

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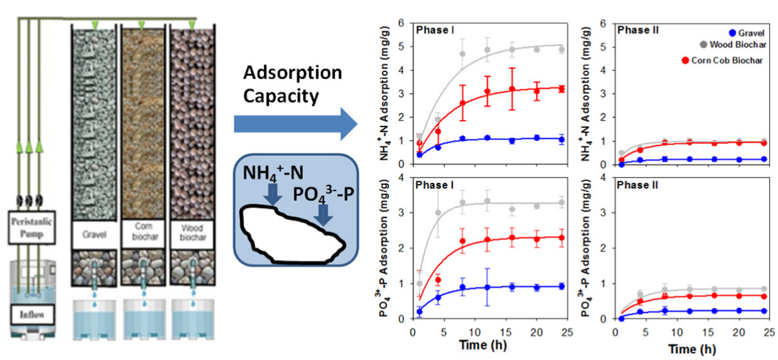
HIGHLIGHTS

- Biochar-packed VFCWs have better long-term treatment ability than gravel.
- The better performance of biochar is attributed to its high sorption capacity.
- Tidal operations enhance anaerobic digested effluent treatment lifespan of CWs.
- Biochar supports a diverse microbial community compared to gravel in VFCWs.

GRAPHICAL ABSTRACT



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ABSTRACT

Three types of vertical flow constructed wetland columns (VFCWs), packed with corn cob biochar (CB-CW), wood biochar (WB-CW) and gravel (G-CW) under tidal flow operations, were comparatively evaluated to investigate anaerobic digested effluent treatment performance and mechanisms. It was demonstrated that CB-CW and WB-CW provide significantly higher removal efficiencies for organic matter (>59%), NH_4^+-N (>76%), TN (>37%) and phosphorus (>71%), compared with G-CW (22%–49%). The higher pollutants removal ability of biochar-packed VFCWs was mainly attribute to the higher adsorption ability and microbial cultivation in the porous biochar media. Moreover, increasing the flooded/drained ratio from 4/8 h to 8/4 h of the tidal operation further improved around 10% of the removal of both organics and NH_4^+-N for biochar-packed VFCWs. The phosphorus removal was dependent on the media adsorption capacities through the whole experiment. However, the NH_4^+-N biodegradation by microbial communities was demonstrated to become the dominant removal mechanism in the long term treatment, which compensated the decreased adsorption capacities of the media. The study supported that the use of biochar would increase the treatment performance and elongate the lifespan of CWs under tidal operation.

1. Introduction

Globally, the expansion of industrialised-scale pig farms, without appropriate treatment systems for manure and wastewater, is causing severe water pollution (Mallin et al., 2015). China, as a leading pork

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production country of the world, is heavily experiencing this problem (Zhou et al., 2014). There is a boom in intensive pig production, about 465 million tonnes of pig manure is generated annually in China (Geng et al., 2013). To manage the increasing volume of pig manure, the Chinese government has intensified the promotion of anaerobic digestion (AD) technology as a strategy for both, manure stabilisation and bioenergy generation, in the intensive pig-farming areas (Wu et al., 2016b). Therefore, >90,000 AD plants, with a total treatment capacity of 14 million m^3 per year, have been built (Song et al., 2014). With the increase in AD digester installation, the problem of unprocessed manure has been reduced. However, large quantities of anaerobic digested effluents continue to be generated in different parts of China.

For the past few decades, the nutrient-rich AD effluent has been recognized as a valuable resource in fertilising croplands to increase agricultural yields (Tambone et al., 2010). Nowadays, as a result of the rural-urban migration, the consequent shrinkage of agricultural activities and the continued expansion of AD technology, the generated digestate nutrients are in excess of the carrying capacity of the available farmland (Wu et al., 2016a, 2016b). Moreover, the transport of nutrients from the point of surplus to distant croplands is not economically feasible. When anaerobic digested effluent is generated in excess of what can be utilised in agriculture, it ceases to be a resource. Instead, it becomes a potential source of pollution due to its high nutrient and organic content (Holm-Nielsen et al., 2009). Therefore, proper disposal of the surplus anaerobic digested effluent is required to avoid detrimental pollution.

Constructed wetlands (CWs) technology is recognized as a low-cost and operationally simple technology for treating various types of wastewater, including anaerobic digested effluent (Guo et al., 2016; He et al., 2016; Wu et al., 2016a). The treatment performance of CWs can be strongly affected by its construction and operation strategies, among which, the role of media has been reported as a major influencing factor (Arias and Brix, 2005; Liu et al., 2014; Lu et al., 2016). Considering the cost of construction, gravel, sand and rocks have been used as the "traditional" media in CW designs (Kadlec and Wallace, 2008). However, using the aforementioned non-reactive materials to treat high strength wastewater often requires a relatively large square footage to achieve sufficient degradation efficiency. To enhance the treatment efficiency of CWs, some new and highly reactive media, such as limestone, shale, pelleted clay, Opaka and pumice have been developed and successfully tested to achieve high treatment performance in CWs (Vohla et al., 2011). In addition, industrial wastes such as blast furnace slag and alum slag have been suggested for intensified phosphorus removal (Lu et al., 2016). The improved pollutant removal efficiencies are mainly due to the higher sorption capacity of these chemically-reactive materials, with a large surface area and cation exchange capacity. However, further disposal of these media after adsorption saturation will become another bottleneck, given that they may not be used in long-term operational CWs.

Biochar derived from waste biomass is increasingly being recognized as a multifunctional material with a wide range of environmental applications, such as the removal of organic and inorganic contaminants from wastewater and polluted soils (Mohan et al., 2014). Biochar is also renowned for its soil amendment properties, with numerous advantages such as crop growth enhancement, nutrient leaching reduction and CH_4 emission reduction (Lehmann and Joseph, 2015). For CWs, the potential use of biochar as an alternative medium could be based on its high porosity, large surface area and cation exchange capacity. These characteristics are benefit for the pollutants adsorption and biofilm attachment ability, which can improve the pollutants degradation (Dalahmeh et al., 2012). Another important aspect is that exhausted, but nutrient-rich biochar, could be used for soil amendment, thus minimizing the exhausted material disposal problems (Shepherd et al., 2016). Thus, the use of biochar was not only economically by reusing the waste biomass, but also can promote the environmental sustainability. Previous studies have demonstrated that the use of biochar in CWs can significantly improve its ability to remove BOD_5, TSS, and coliforms (De Rozari et al., 2015); TN and NH_4^+-N (Gupta et al., 2015; Lu et al., 2016). However, to the best of our knowledge, few studies have investigated the suitability of biochar packed CWs treating high strength wastewater, such as anaerobic digested effluent.

In this study, three types of laboratory-scale vertical flow constructed wetland columns (VFCWs), packed with two types of biochar (produced from corn cobs and wood) and common gravel were set up to evaluate and compare the long-term treatment performance of anaerobic digested effluent. The effect of tidal operations, with different flooded/drained (F/D) ratios on the treatment performance of anaerobic digestate has also been investigated. Moreover, to understand the pollutant removal mechanisms, the pollutant-adsorption capacities of different media, as well as the microbial community structures in different VFCWs, were compared.

2. Materials and methods

2.1. Biochar and gravel characterisation

Two types of biochar, produced from corn cobs and wood (Chinese Oak), were used as alternative CW media in the present study. The conversion of each raw materials to biochar was carried out under slow pyrolysis, at a temperature ramp of 10 °C/min to a maximum temperature of 600 °C, with a 10 h retention time. The resultant biochar materials were gently crushed using a bench scale hammer mill and sieved to a particle size range of 2–10 mm. The particles were subsequently washed with distilled water to remove ash, fine particles and dust. The other alternative CW media was gravel, which was collected from the local field. The collected gravel was washed with distilled water to remove soil and debris. Subsequently, it was sieved to a particle size range of 2–10 mm, which was similar to the biochar materials. The physical characteristics, including bulk density, surface area, pore volume, average pore diameter and pH, of both biochar and gravel were measured according to the method described by Kizito et al. (2015). The main chemical composition of the gravel was SiO_2. The chemical characteristics of both the biochar, including the content of fixed carbon, ash, volatile matter, carbon, hydrogen, nitrogen, sulphur and oxygen, were tested by following the ASTM D-1762-84 standard for the analysis of charcoal (ASTM, 2007).

2.2. Design and operation of constructed wetlands

Six laboratory-scale VFCWs were created in six Perspex columns, with identical dimensions of 0.9 m in height and 0.2 m in inner diameter. Of these, 4 VFCWs were packed with biochar (2 columns for each type of biochar), while the other 2 VFCWs were packed with gravel. The main treatment layer of all VFCWs had a depth of 0.6 m. In addition, a 0.1 m high layer of fine gravel (diameter of 50 mm) was placed at the top of each VFCW to prevent early clogging and allow even distribution of the influent. In addition, a 0.1 m high layer of fine gravel (diameter of 50 mm) was placed at the bottom to enable proper effluent drainage. The biochar-packed columns had a working volume of approximately 10.4 L (55% porosity), while the gravel columns had a working volume of approximately 9 L (48% porosity). The high strength influent wastewater was the anaerobic digested effluent from AD plants. The effluent was collected from a mesophilic biogas plant, treating pig manure, located in the Dong Hua Shan Village, Shunyi District (40°06′24.59″N, 116°54′30.68″E) in Beijing, China.

After each collection, the raw anaerobic digested effluent was diluted with tap water to keep the influent COD, NH_4^+-N and suspended solid (SS) concentrations at approximately 1500 mg/L, 500 mg/L and 0.1%, respectively, throughout the experiment. Detailed influent compositions are reported in Table 2. During feeding, the diluted effluent was supplied from the top of each VFCW, at a flow rate of 30 mL/min, with the draining effluent being collected at the bottom. In order to compare the treatment performance of the different selected CW media, all the systems were left unplanted during the entire experiment. All the VFCWs were operated under a tidal mode. Thus, the wetland matrix is rhythmically filled with wastewater in the flooded period and then flow out in the drained period. The draining water creates as a suction pressure that draws the oxygen from the atmosphere into the matrix in the drainage period to significantly enhance the oxygen condition in CWs (Lv et al., 2013; Wu et al., 2011). To determine the effect of flooded/drained (F/D) ratios on the performance of VFCWs during the tidal operation, the entire experimental period was divided into two phases (I and II). Phase I was from 0 d to 150 d, with an F/D ratio of 4/8 h. Phase II was from 150 d to 300 d, with an F/D ratio of 8/4 h. The F/D cycles were controlled by peristaltic pumps and drainage valves, which were connected to programmable timers. The entire experiment was conducted indoors for a period of 300 d under an ambient temperature range of 18–24 °C. The indoor temperature were controlled at 24 °C from 6 A.M. to 9 P.M. and 18 °C from 9 P.M. to 6 A.M. to simulate an average summer day in moderate climatic conditions.

2.3. Water sampling and analysis

For sampling, triplicate influent and effluent samples from each VFCW were collected 2–3 times a week for routine analyses. The pH and Oxidation Reduction Potential (ORP) were measured directly using a portable pH/ORP meter (Orion Model 115A, Thermal Fisher Scientific, USA). Dissolved Oxygen (DO) was measured with a portable DO meter (Seven2Go Pro, Mettler Toledo, USA). Chemical Oxygen Demand (COD) was measured (method 8000) using a Hach DR5000 Colorimeter, according to its standard calibration and operation. BOD_5 concentrations were determined using a manometric BOD_5 apparatus (BODTrak II; HACH, USA). The concentrations of ammonium (NH_4^+/4500-NH_3 F; phenate method), nitrite (NO_2^-/4500-NO_2 B; colorimetric method), phosphate ($PO_4{}^{3-}$/4500-P E; ascorbic acid method), TN (TN/4500-N C; Persulfate Method) and TP (Persulfate method; TP/4500-P) were determined based on the APHA standard (APHA, 2005) using ultraviolet and visible spectrophotometers (Gold S54T; Lengguang Tech, China). Nitrate (NO_3^--N) was analysed based on 4500-NO_3^- B method (APHA, 2005), using continuous flow colorimetry equipment (SEAL AutoAnalyzer 3, UK).

2.4. Media adsorption test

Both the biochar and gravel were tested through batch adsorption equilibrium experiments before and after their 300 days of use in VFCWs. Briefly, the adsorption capacity of the fresh and aged (after use in the VFCWs) media was determined through shaking experiments, using 5 g of each material and 100 mL of solution. The aged media was taken from the middle layer of each VFCW. The solutions contained 100 mg/L of either NH_4^+-N or PO_4^{3-}-P, at an optimum pH of 7.0 ± 0.2 and temperature of 25 °C. The N and P solutions were prepared from analytical grades of K_2HPO_4 and NH_4Cl that were dissolved in Milli-Q water. The vials containing both, the nutrients and tested materials, were placed in a water bath at 25 °C and shaken (120 *rmp*) for 24 h. The residual NH_4^+-N and PO_4^{3-}-P concentrations in the liquid phase were determined colorimetrically every 4 h, after which the adsorption capacity of each type of media was calculated along the 24 h shaking experiment. The adsorption capacity of the biochars and gravel were calculated as equilibrium-adsorbed amount of PO_4^{3-}-P or NH_4^+-N per unit mass of adsorbent, based on Eq. (1):

$$Q_e = \frac{[C_0 - C_e]^* V}{W_b} \quad (1)$$

where C_o and C_e (mg/L) are the initial and equilibrium PO_4^{3-}-P or NH_4^+-N concentrations in the solution, respectively. Q_e (mg/g) is the adsorbed amount of PO_4^{3-}-P or NH_4^+-N at equilibrium, V (L) is the volume of solution used, while W_b (g) is the mass of the adsorbent (biochar or gravel).

2.5. Microbial community analysis

In order to investigate the nitrogen transformation and removal mechanisms within the studied VFCWs, 16S rDNA characterisation was carried out to analyse the microbial community at the end of the experiment (300 d). A sample (20 g) of media taken from the middle layer of each VFCW was used to extract microbial DNA using the FastDNA® SPIN Kit (MP Biomedicals, Santa Ana, CA), in accordance with the manufacturer's instructions. The extracted soil DNA was initially detected using agarose gel electrophoresis (1.0% agarose in 0.5 × TAE) to examine its integrity and approximate concentration. Subsequently, the quality and quantity of DNA samples were determined using a NanoPhotometer® Spectrophotometer (IMPLEN, Carlsbad, California, USA) and a Qubit® RNA Assay Kit in a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, California, USA). The 16S rDNA gene amplification was carried out as suggested by Kozich et al. (2013). Briefly, the extracted DNA was amplified with a universal primer set (314F/805R) targeting the V3 + V4 hypervariable region of bacterial domains. Detailed PCR conditions were adopted as described by Kozich et al. (2013). The amplicons were purified using a Wizard® SV Gel and PCR Clean-up System (Promega, Madison, Wisconsin, USA) after gel extraction. The purified 16S rDNA amplicons were sequenced using the Illumina Miseq platform (Illumnina Inc., San Diegom, CA) at the Anoroad Bio. Tech. Inc. (Beijing, China). After trimming the low quality sequences, residual sequences were aligned using MOTHUR (Schloss et al., 2009). The aligned sequences were checked for chimera using USEARCH 6.1 in QIIME and classified into Operational taxonomic units (OTUs) within a 0.03 difference (97% similarity) by the de novo OTU picking workflow in QIIME (Caporaso et al., 2010).

2.6. Statistical analysis

All samples from the VFCWs with the same media type were considered as replicates to calculate a mean value and standard deviation throughout the experiment. Analysis of variance (ANOVA) at 95% confidence level ($p < 0.05$) was used to evaluate significant differences in pollutant removal efficiencies among the VFCWs packed with different media. Principal Component Analysis (PCA) was used to identify the treatment performance patterns among the VFCWs in different experimental phases. PCA was conducted with all the measured parameters, which included pH, DO, ORP, the removal efficiencies of COD, BOD_5, NH_4^+-N, TN, PO_4^{3-}-P, TP and the production efficiencies of NO_2^--N and NO_3^--N in each VFCW, through different experimental phases. Sigmaplot software (version 12.5, Sigma, InC) and XLStat Pro® (XLStat, Paris, France) were used for plotting and data analyses, respectively.

3. Results and discussion

3.1. Biochar and gravel characteristics

The physical and chemical characteristics of all media used in the present study were shown in Table 1. Both corn cob biochar (CB) and wood biochar (WB) had clearly higher surface area (123–147 m^2/g), pore volume (0.098–0.176 cm^3/g) and pH (8.9–9.8) than gravel. However, gravel had a higher bulk density (1.2 g/cm^3) than WB (0.5 g/cm^3) and CB (0.4 g/cm^3). The higher porosity and surface area exhibited by both biochar explain their NH_4^+-N and PO_4^{3-}-P sorption capacities, which were significantly higher than that of gravel (Fig. 2). Moreover, compared to gravel, which is mainly composed of SiO_2, the two biochar contained high amounts of organic matter and other elements (Table 1).

The two types of biochar were also fairly different in chemical composition due to the difference in their raw materials. Wood contains more lignocelluloses in its cell wall than corn cobs. The lignocellulose structure would be condensed into graphite sheets made of aromatic carbon rings during pyrolysis, leading to a higher content of fixed

Table 1
Physical and chemical characteristics of the media used in vertical flow constructed wetlands in this study.

Parameters	Corn cobs biochar	Wood biochar	Gravel
Physical characteristics			
Bulk density (g/cm^3)	0.4	0.7	1.2
Surface area (m^2/g)	123	147	0.1
Pore volume (cm^3/g)	0.098	0.176	0.001
Average pore diameter (nm)	6.2	5.3	-
pH	8.9	9.8	7.3
Chemical characteristics			
Fixed carbon (%)	53	81	-
Ash (%)	39	12	-
Volatile matter content (%)	8.3	6.9	-
Carbon (%)[a]	69	90	-
Hydrogen (%)[a]	3.4	1.5	-
Nitrogen (%)[a]	6.1	0.5	-
Sulphur (%)[a]	4.4	0.3	-
Oxygen (%)[b]	17.6	8.3	-

- Represents not determined.
[a] Determined on dry matter (ash free) basis.
[b] Determined as difference (C + H + N + S–Ash).

carbon in WB (81%) than CB (53%) (Liu et al., 2014). However, the contents of ash (39%), nitrogen (6.1%), sulphur (4.4%) and oxygen (17.6%) were more abundant in CB. The higher nutrient content in corn cobs could be attributed to high uptake and tissue accumulation during the corn growth period, which remained in the CB after pyrolysis (Hale et al., 2013).

3.2. Water quality and pollutant removal performance of VFCWs

3.2.1. Water quality

The pH, DO and ORP of the influent were relatively stable throughout the experimental period (phase I and II), with values of 8.3 ± 0.1, 1.7 ± 0.2 mg/L and 108 ± 10 mV, respectively (Table 2). After the first 50 days of stabilisation in phase I, the average pH values in the effluent of the three VFCWs, packed with corn cob biochar (CB-CW), wood biochar (WB-CW) and gravel (G-CW), were 7.5, 7.9 and 7.3, respectively. Generally, the biochar-packed CWs exhibited a significantly higher pH than the gravel-packed CWs, which may be attributed to the alkaline nature and high ash content of the medium (Table 1). Due to the tidal operation of the VFCWs (Lv et al., 2013; Wu et al., 2011), the effluent DO and ORP values in all the three VFCWs were significantly higher than the influent in phase I, oscillating within a range of 2.7–3.6 mg/L and 154–169 mV, respectively.

In traditional CWs (without tidal operation), the effluent DO concentration would be lower than the influent, due to the consumption of oxygen by the occurrence of simultaneous organics oxidation and nitrification (Fan et al., 2013; Vymazal, 2010). However, the effluent DO remained more than the influent DO in the present study, indicating the natural aeration of the CW beds by the tidal operation. In phase II, the pH, DO and ORP of the effluents from all VFCWs showed a slight decreased tendency (not significant) compared with phase I. The relatively stable values in these VFCWs between the two experimental phases, implies that during treatment of high strength wastewater such as anaerobic digested effluent used in the present study, the change in F/D ratios from 4/8 h to 8/4 h may not affect the pH, DO and ORP of the effluents.

3.2.2. Organic matter removal

The influent COD and BOD_5 for the entire experiment were 1588 ± 61 mg/L and 699 ± 28 mg/L, respectively (Table 2). The COD removal efficiency tended to be stable, reaching approximately 59%, 72% and 52% in the CB-CW, WB-CW and G-CW, respectively. The corresponding BOD_5 removal efficiencies of CB-CW, WB-CW and G-CW were 75%, 83% and 70%, respectively. The previous studies showed the COD removal in unplanted traditional CWs, such as horizontal surface flow CWs packed by common sand, when treating anaerobic digested effluent was always below 50% (Comino et al., 2013; Guo et al., 2016; He et al., 2016). The present results demonstrated that the VFCWs operating under tidal flow strategy could effectively remove organic matter from anaerobic digested effluent. The relatively higher organic removal level could be attributed to the sufficient oxygenation of the CW beds during the drained cycles, under tidal operation. This hypothesis is supported by

Table 2
Water quality, pollutant concentrations in the influent and effluent, along with the pollutant removal efficiency of the vertical flow constructed wetlands packed with corn cob biochar, wood biochar and gravel, during the two experimental phases I and II, after the 50-day stabilisation time in each phase.

Parameters	Influent	Phase I (n = 32)						Phase II (n = 31)					
		Effluent			Removal efficiency (%)			Effluent			Removal efficiency (%)		
		CB	WB	Gravel	CB	WB	Gravel	CB	WB	Gravel	CB	WB	Gravel
pH	8.3 ± 0.1	7.7 ± 0.2^{b}	7.9 ± 0.1^{a}	7.3 ± 0.2^{c}	–	–	–	7.5 ± 0.1^{b}	7.8 ± 0.1^{a}	7.2 ± 0.1^{c}	–	–	–
DO (mg/L)	1.7 ± 0.2	3.2 ± 0.2^{a}	2.7 ± 0.2^{b}	3.6 ± 0.2^{a}	–	–	–	$2.7 \pm 0.2^{a*}$	2.2 ± 0.1^{b}	3.1 ± 0.2^{a}	–	–	–
ORP (mV)	108 ± 10	169 ± 24	167 ± 24	154 ± 20	–	–	–	145 ± 30	130 ± 33	141 ± 19	–	–	–
COD (mg/L)	1588 ± 61	650 ± 150^{b}	438 ± 145^{b}	759 ± 151^{a}	59 ± 5^{b}	72 ± 15^{b}	52 ± 4^{a}	472 ± 60^{a}	221 ± 42^{b}	739 ± 61^{a}	67 ± 12^{a}	86 ± 6^{b}	53 ± 10^{a}
BOD_5 (mg/L)	699 ± 28	127 ± 23^{b}	108 ± 18^{c}	206 ± 62^{a}	75 ± 6^{b}	83 ± 8^{c}	70 ± 11^{a}	98 ± 29^{b}	89 ± 15^{c}	191 ± 24^{a}	86 ± 7	92 ± 9	72 ± 9
NH_4^+-N (mg/L)	496 ± 20	120 ± 13^{b}	84 ± 17^{c}	190 ± 23^{a}	76 ± 6^{b}	83 ± 12^{c}	62 ± 8^{a}	68 ± 21^{b}	48 ± 10^{c}	123 ± 22^{a}	86 ± 4	90 ± 7	75 ± 9
TN (mg/L)	581 ± 25	368 ± 21^{b}	308 ± 31^{b}	452 ± 22^{a}	37 ± 4^{b}	47 ± 5^{b}	22 ± 2^{a}	$248 \pm 64^{b*}$	$171 \pm 54^{b*}$	370 ± 45^{a}	$57 \pm 8^{b*}$	$71 \pm 11^{b*}$	$36 \pm 5^{a*}$
NO_3^--N (mg/L)	5 ± 1	136 ± 22^{b}	118 ± 24^{b}	162 ± 34^{a}	–	–	–	137 ± 21^{b}	$97 \pm 20^{b*}$	152 ± 14^{a}	–	–	–
NO_2^--N (mg/L)	5 ± 1	113 ± 18	106 ± 25	103 ± 12	–	–	–	$67 \pm 17^{*}$	$52 \pm 10^{*}$	$65 \pm 16^{*}$	–	–	–
PO_4^{3-}-P (mg/L)	25 ± 1	7 ± 1^{b}	4 ± 1^{c}	10 ± 1^{a}	71 ± 5^{b}	85 ± 4^{c}	59 ± 2^{a}	9 ± 1^{b}	8 ± 1^{c}	12 ± 1^{a}	64 ± 5^{b}	$68 \pm 8^{c*}$	52 ± 4^{a}
TP (mg/L)	37 ± 3	10 ± 1^{b}	6 ± 1^{c}	16 ± 2^{a}	71 ± 1^{b}	83 ± 4^{c}	56 ± 7^{a}	12 ± 1^{b}	8 ± 1^{c}	19 ± 1^{a}	68 ± 6^{b}	78 ± 5^{c}	49 ± 4^{a}

TN is the sum of NH_4^+-N, NO_3^--N and NO_2^-N. F/D ratios were 4/8 h and 8/4 h in experimental phases I and II, respectively. Different letters next to each parameter in the same experimental phase represent significant differences among VFCWs packed with different media. * represents the significantly different pollutant removal efficiencies between experimental phases I and II.

a previous study (Hu et al., 2014), which suggested that the presence of sufficient oxygen, entrapped within the CW beds, support microbial degradation of organics and ammonia adsorbed onto the medium during the flooded period, by organotrophic bacteria during the drained periods. The significantly higher COD and BOD_5 removal efficiencies in the biochar-packed CWs than the gravel-packed CWs could be attributed to the more reactive media surface with a strong presence of π bonds (Chen et al., 2008). Due to the π bonds on the biochar surface, organic molecules can be easily adsorbed via electrostatic attraction and intermolecular hydrogen bonding onto the medium (biochar), resulting in high organic matter removal. Zhao et al. (2004) has reported a lab-scale tidal flow reed bed system can produce the highest pollutants removal efficiencies, especially for organics, with a relatively short flooded period and long drained period when treating agricultural wastewater. It was attribute to the high oxygen transportation into reed bed using for the microbial oxidation. However, the organics removal efficiencies were not significantly different between the experiment phases with different F/D ratio in the present study (Table 2). It may due to the hard degradable organics remained after the anaerobic digestion process, which cause the limited organic biodegradation abilities under different tidal operations.

3.2.3. Nitrogen removal

The influent NH_4^+-N and TN concentrations throughout the experiment were 496 ± 20 and 581 ± 25 mg/L, respectively. In phase I, the average removal of NH_4^+-N in CB-CW (76%) and WB-CW (83%) was significantly higher ($p < 0.05$) than that in G-CW (62%). However, the values of TN removal in all the three CWs were relatively low, equalling approximately 37%, 47% and 22% for CB-CW, WB-CW and G-CW, respectively. The influent concentrations of NO_3^--N and NO_2^--N were both around 5 mg/L. After around day 65, the concentration of accumulated NO_3^--N and NO_2^--N ranged from 136 to 162 mg/L and 103–113 mg/L, respectively, in the effluents from all the studied VFCWs. Previous studies by Gupta et al. (2015) and Lu et al. (2016) also reported the clearly higher removal efficiencies of NH_4^+-N and TN in biochar packed CWs compare with the common sand packed CWs for domestic wastewater treatment. However, the accumulation of NO_3^--N and NO_2^--N were additionally investigated in the present study, which clearly indicated the occurrence of nitrification, converting NH_4^+-N to NO_x-N in all the studied CWs (Fitzgerald et al., 2015). This lower TN removal may because of the limited denitrification under oxygen condition of ≥2.2 mg/L under the tidal operation.

The NH_4^+-N removal efficiencies were slightly improved in phase II, which could be attribute to the enhanced NH_4^+-N sorption during the longer flooded period and its subsequent degradation during the drained periods. Moreover, the microbial communities after the long term running may also contribute to the higher NH_4^+-N removal. The NO_2^--N and NO_3^--N concentrations were generally reduced for all the VFCWs and the values were in a ranged from 52 to 152 mg/L in the effluents after the flooded period was prolonged. TN removal efficiencies became significantly higher for all VFCWs in phase II, compared with the corresponding removal by the system in phase I. The results may cause by the high potential for denitrification during the prolonged flooded period. Moreover, significantly higher TN removal was achieved in CB-CWs and WB-CWs than G-CWs. The higher TN removal could be attributed to two factors, 1) the presence of some liable organic carbon, either entrapped in the porous structure or directly released from the biochar through chemical metabolism by the bacteria (Gupta et al., 2015). As previously mentioned in Table 1, CB and WB had high fixed carbon contents, implying that some carbon could have facilitated heterotrophic denitrification (Saeed et al., 2012), resulting in higher TN removal. 2) the highly porous biochar may have created anoxic conditions and a large surface area for the formation of denitrifying bacteria microbial biofilm, leading to a larger reduction of TN in the biochar-packed CWs, as compared to gravel-packed CWs.

3.2.4. Phosphorous removal

For the entire experimental period of 300 days, the influent PO_4^{3-}-P and TP concentrations were 25 ± 1 mg/L and 37 ± 3 mg/L, respectively. In phase I, significantly higher removal efficiencies of PO_4^{3-}-P (71–85%) and TP (71–83%) were observed in CB-CW and WB-CW, compared with G-CW (56–59%). In phase II, the removal efficiencies were slightly decreased for all corresponding CWs, with a range of 52–68% for PO_4^{3-}-P removal and 49–78% for TP removal. It was also supported by previous study (Gupta et al., 2015), which reported high TP and PO_4^{3-}-P removal efficiencies of 79% and 68%, respectively, when treating artificial wastewater in biochar integrated horizontal subsurface flow CWs. The removal of phosphorus was mainly caused by media adsorption, with the removal being usually low, in the range of 45–60% in the traditional VFCWs (Vymazal, 2010). Therefore, the higher phosphorus removal efficiency achieved in the present biochar-packed VFCWs demonstrated that biochar might be a potential candidate to enhance the phosphorus removal. However, the contrast conclusion was also reported by De Rozari et al. (2016), which showed that biochar amended sand did not enhance TP removal when compare with pure gravel in VFCWs treating the secondary sewage. It may cause by the different TP removal mechanisms in the CWs with mixed medium, which need to be further studied.

Notably, the present study preliminarily investigate the feasibility of biochar medium used in CWs for high strengthen anaerobic digestate effluent treatment. It was demonstrated that the biochar packed CWs can provide significantly higher nitrogen and phosphorous removal compare with the common gravel packed CWs. However, the effluent was still not meeting the discharge permits. Thus, the next step study about the treatment enhancement, e.g. effluent recirculation and multi-stage configuration, should be further conducted.

3.3. Treatment performance patterns of VFCWs

The effluent water qualities (pH, DO and ORP), removal efficiencies of COD, BOD_5, NH_4^+-N, TN, PO_4^{3-}-P, TP and the production of NO_2^--N and NO_3^--N for all VFCWs were analysed using principal component analysis (PCA) to assess the treatment performance patterns (Fig. 1). Independent PCA analysis was conducted three times during the entire experiment (Fig. 1a), being conducted separately for experimental phases I (Fig. 1b) and II (Fig. 1c). The first two principal components accounted for a variation of 81.6%, 82.7% and 90.8% for the entire experiment, phases I and II, respectively. The treatment patterns for VFCWs packed with different media types clearly differentiated the various groups between experimental phases I and II (Fig. 1a). The removal efficiencies of COD, BOD_5, NH_4^+-N and TN had high positive loadings on direction of phase II data (Fig. 1d), which indicated that the higher removal efficiencies of such pollutants were achieved in phase II by increasing F/D ratios from 4/8 h to 8/4 h. Moreover, VFCWs packed with different media types clearly showed different groups for both, phase I (Fig. 1b) and phase II (Fig. 1c). The same tendency was also found in both phases, with biochar-packed CWs located in the left part (negative direction of PC1) of the plot, while gravel-packed CWs were in the right part. All the pollutant removal efficiencies had a high negative loading, while DO had a high positive loading on PC1 (Fig. 1e, f). The results indicated that biochar-packed CWs achieved clearly higher removal efficiencies with regard to the pollutants (NH_4^+-N, TN, TP, COD and BOD_5) compared with gravel-packed CWs. The results further confirmed that the use of porous media (biochar) can clearly improve pollutant removal in CWs due to the high adsorption abilities of the media.

3.4. NH_4^+-N and PO_4^{3-}-P removal mechanisms

3.4.1. Adsorption test of fresh and used media

The highest NH_4^+-N adsorption amount on fresh biochar were 3.2 ± 0.3 and 4.9 ± 0.3 mg/g for CB and WB, respectively (Fig. 2a), in 100 mg/L initial NH_4^+-N solutions. These values were significantly higher than that

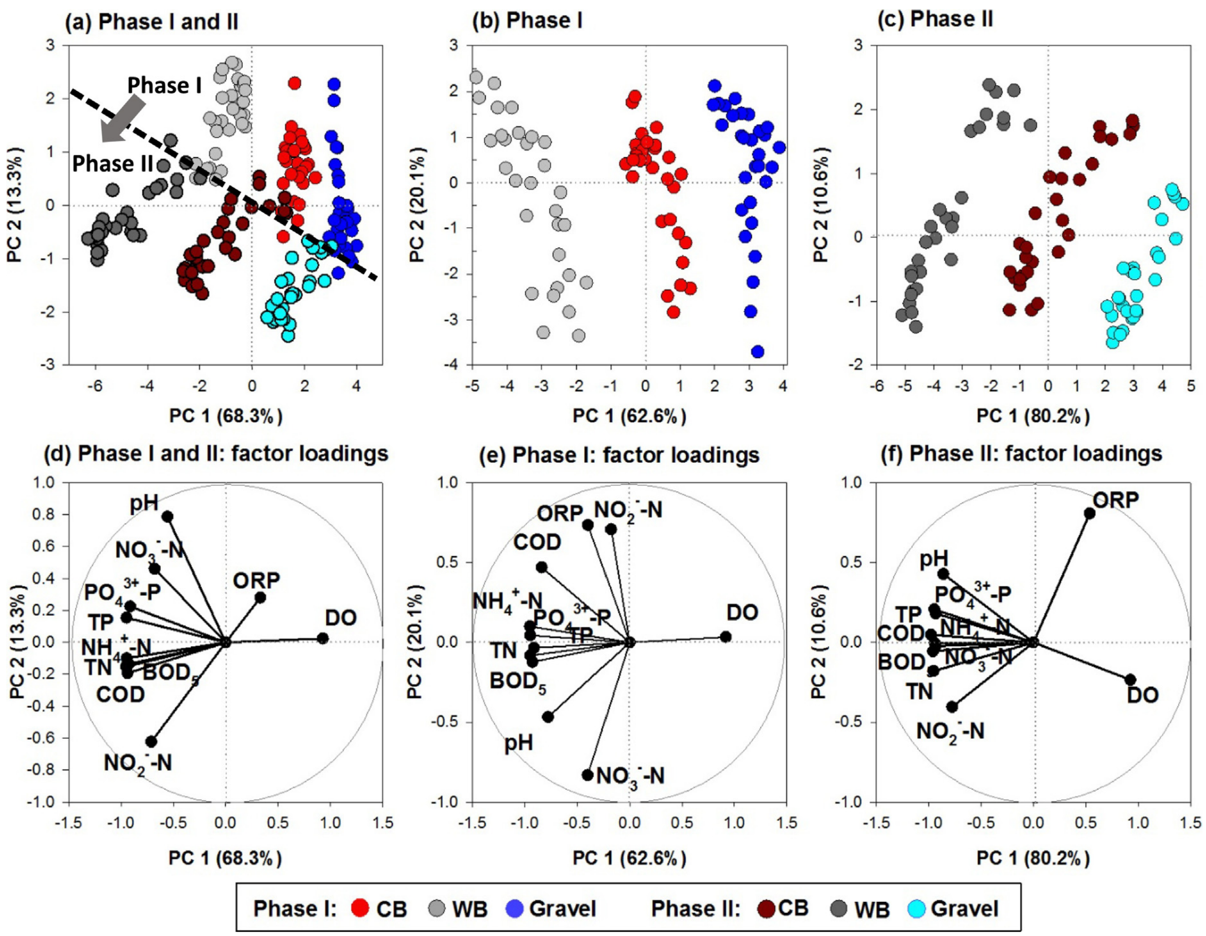

Fig. 1. Principal component analysis of treatment performance patterns in the three vertical flow constructed wetlands packed with corn cob biochar (CB), wood biochar (WB) and gravel during the entire experiment (a), experimental phases I (b) and II (c), respectively. The letters d, e, and f represent the factors loading plots for each principal component analysis in the entire experiment, experimental phases I and II, respectively.

for gravel (1.0 ± 0.4 mg/g). The highest PO_4^{3-}-P adsorption amount on fresh biochar for CB and WB were 2.2 ± 0.2 and 3.3 ± 0.6 mg/g, respectively (Fig. 2c), in 100 mg/L initial PO_4^{3-}-P solutions. While the PO_4^{3-}-P adsorption capacity for gravel was 0.9 ± 0.2 mg/g. From Fig. 2b and d, it can be seen that the adsorption capacities of both, NH_4^+-N and PO_4^{3-}-P (below 1 mg/g), were significantly lower for all types of aged media after 300 days in the VFCWs, compared with fresh media. However, the adsorption capacities were clearly higher for biochar (CB and WB) than gravel. Moreover, the strong positive correlations between both NH_4^+-N and PO_4^{3-}-P adsorption capacities and corresponding pollutant removal were found and shown in Fig. 3. In this correlation analysis, the adsorption capacities of fresh and aged media were considered to represent the media function in VFCWs at earlier (Phase I) and later (Phase II) stage, respectively.

Previous studies have indicated that NH_4^+-N degradation in CWs under tidal operation occurs in two steps (Sun et al., 2005; Wu et al., 2011). Initially, NH_4^+-N is adsorbed onto the media during the flooded period, while during the drained phase, oxygen is drawn into the empty voids of the bed, oxidizing the previously adsorbed NH_4^+-N into NO_3^--N via nitrification. Thus, the biochar with significantly higher surface area and pore volume (Table 1) demonstrated significantly higher NH_4^+-N removal efficiencies due to their higher adsorption ability. The theory of NH_4^+-N degradation highly supports the strong positive linear relationship ($R^2 \geq 0.90$) between NH_4^+-N adsorption capacity and removal for each experiment phases (Fig. 3a). Even through the adsorption capacity of the aged media (phase II) was significantly lower, the slope of linear regression for phase II was clearly higher than that for phase I (Fig. 3a). Moreover, the microbial analysis revealed that NH_4^+-N microbial biodegradation at the end of the experiment could have contributed to the higher NH_4^+-N removal efficiency (higher correlation slope). The residual higher adsorption capacities of biochar media compared with gravel at the end of the experiment further indicated that media with a long adsorptive lifespan are essential for NH_4^+-N removal in CWs.

3.4.2. Microbial community analysis

Microbial community composition in the VFCWs packed with three types of media were analysed and compared by 16S rDNA pyrosequencing. After quality trimming, 37,342, 31,988 and 26,410 valid pyrosequencing reads of the 16S rDNA gene were obtained for the media samples from CB-CWs, WB-CWs and G-CWs, respectively (Table S1). Rarefaction curves, based on the OTUs AT 3% dissimilarity (Fig. S1), indicated that the sequences were sufficient to reflect the diversity of the microbial communities. The higher microbial diversity (Shannon and Simpson index values) in both the biochar-packed VFCWs (Table S1) indicated that biochar could support a rich and diverse consortium for pollutant degradation, potentially resulting in the observed higher pollutant removal in biochar-packed VFCWs.

The microbial communities showed high diversity at the levels of phylum and family (Fig. 4). At phylum level, the bacterial DNA could be assigned to 3 major phyla (relative abundance >10%), namely, Firmicutes, Proteobacteria and Bacteroidetes (Fig. 4a). Ammonia oxidizing bacteria (*Nitrospira, Nitrosomonas*), *acidobacteria, actinobacteria* and other bacteria were found to belong to the Proteobacteria phyla (Wagner et al., 2002). Ammonia oxidizing bacteria works in the nitrification process by transforming NH_4^+-N to NO_2^--N and then to $NO3^-$-N

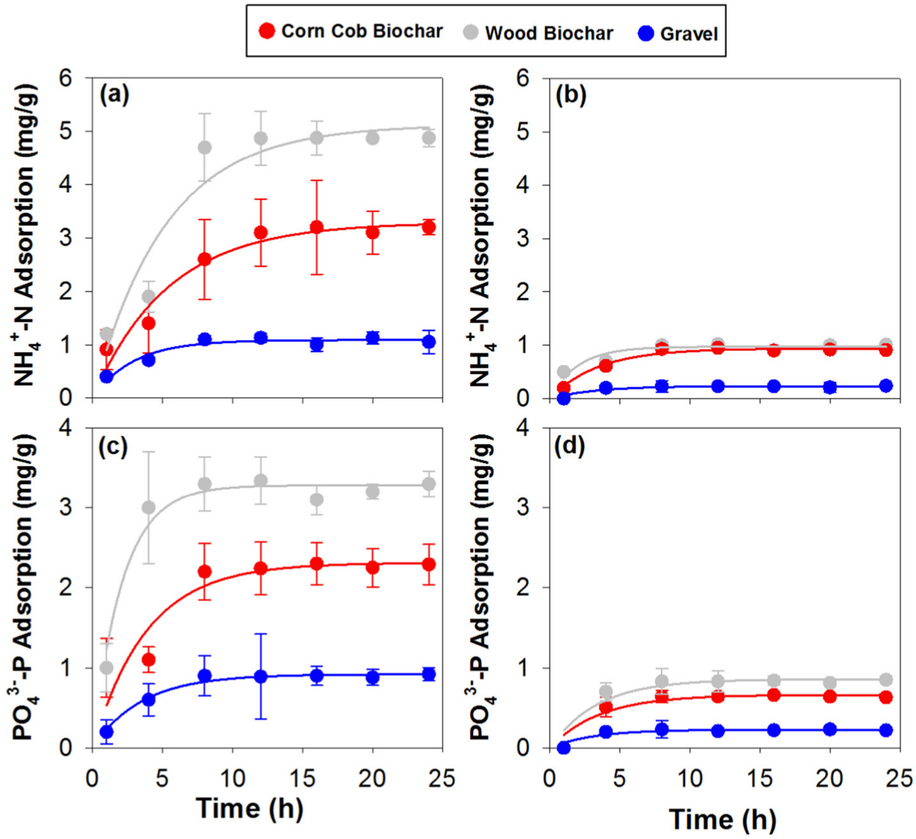

Fig. 2. The adsorption dynamics of NH_4^+-N and PO_4^{3-}-P on fresh media (a) for NH_4^+-N and (c) for PO_4^{3-}-P, and used media in VFCWs after 300 days (b) for NH_4^+-N and (d) for PO_4^{3-}-P.

to achieved removal. They were more abundant in WB-CWs and CB-CWs, compared with G-CWs, which could explain the higher removal efficiencies of NH_4^+-N in the biochar-packed VFCWs. At the family level (Fig. 4b), the bacteria from the *Clostridiaceae* and *Xanthomamonodaceae* families were identified as responsible for organic matter degradation, while the only ammonia oxidizing and nitrifying bacteria present, belonged to the family of *Nitrosomonadaceae* (Kelly et al., 2014; McCarthy and Williams, 1992). The relative abundance of these nitrogen and organic removal related microbes was clearly higher in biochar-packed columns, which might be the reason of higher pollutants removal in these biochar-packed columns.

It can be hypothesised that during the initial stages of treatment in all VFCWs, adsorption was the dominant mechanism for both, organic and nitrogen, removal. However, the sorption capacities of all the media significantly decreased along the experiment. It indicated that the physical adsorption of pollutants was only dominant in the early stages of VFCWs operation. Along with the adsorption capacity decrement, however, the removal of ammonia and organics kept still

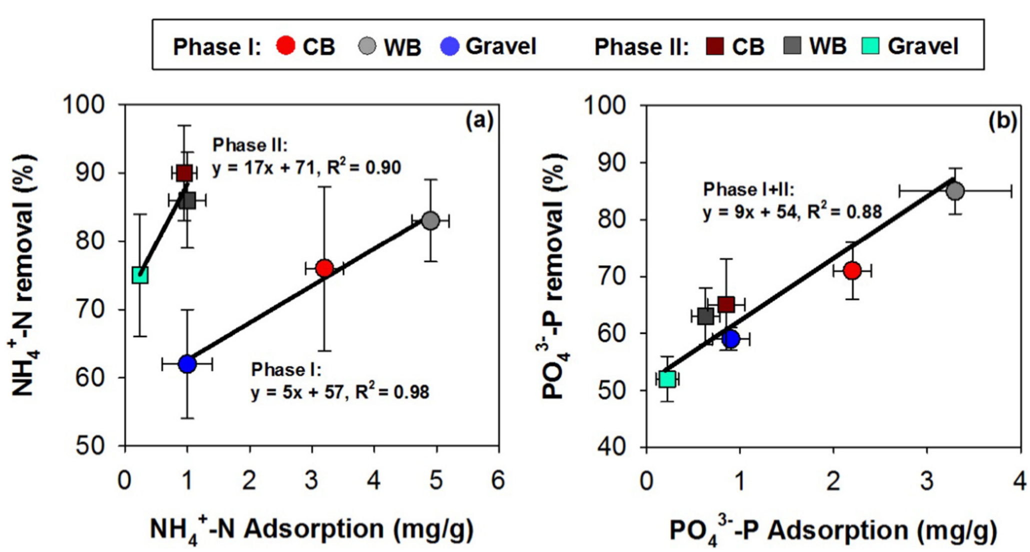

Fig. 3. The correlations between NH_4^+-N (a) and PO_4^{3-}-P (b) adsorption capacities and correspondence pollutants removal efficiencies in both experiment phases.

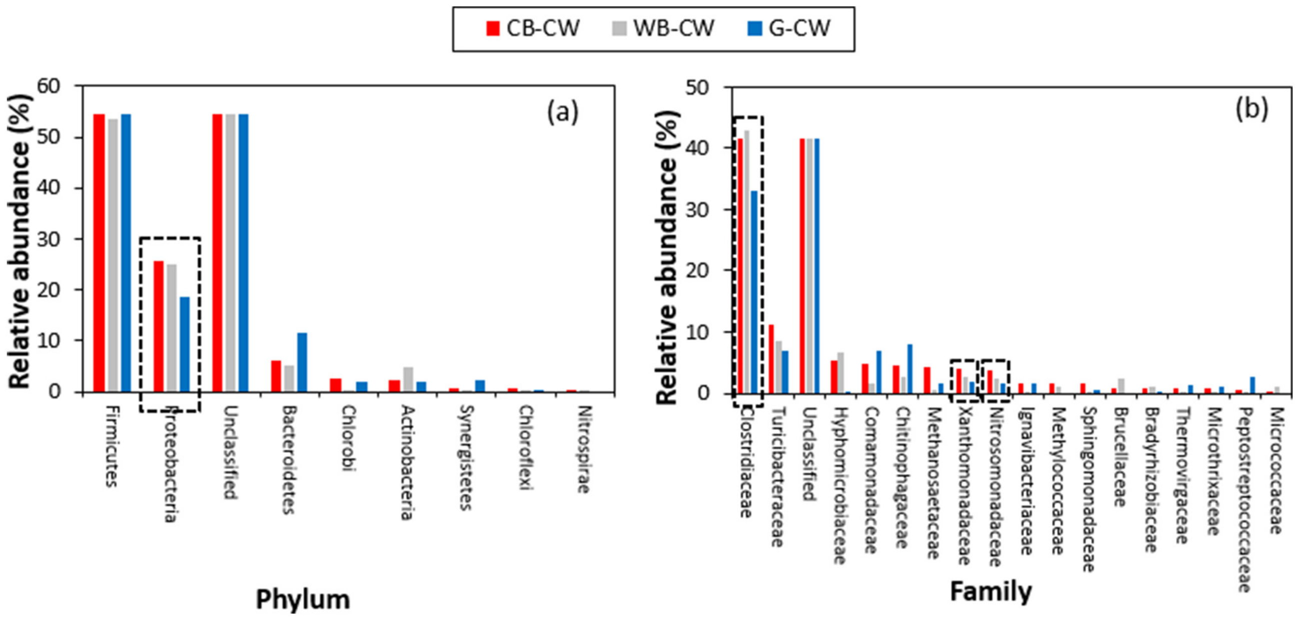

Fig. 4. Taxonomic classification of bacterial 16S rDNA gene reads of the media sample from the vertical flow constructed wetland packed with corn cob biochar (CB-CWs), wood biochar (WB-CWs) and gravel (G-CWs) at phylum (a) and family (b) levels (the relative abundances of bacterial 16S rDNA gene reads <0.1% are not shown).

relatively stable in both experiment phases. The stable ammonia and organics removal suggested that microbial biodegradation may become the main mechanism after long-term operation. It is, however, important to note that even though the adsorption capacity of media tended to decrease with the experimental time (Fig. 2), it continued to be persistent until the end, as the aged particles continued to adsorb both, NH_4^+-N and PO_4^{3-}-P (Fig. 2). The long-term persistence of adsorption observed in the biochar columns could be attributed to the tidal operation. It is strongly believed that the application of rhythmic cycles of flooded and drained periods created a series of repeated adsorption (flooded phase) and microbial oxidation (drained period). The microbial oxidation and metabolisation of the previously adsorbed pollutants on the media surface helped regenerate the active sites, leading to more adsorption. The cycle of adsorption-degradation-adsorption thus created during tidal operation is believed to have enhanced the long-term pollutant removal in the biochar VFCWs due to high porosity, surface area and sorption capacity of these media compared to gravel (Hu et al., 2014). This finding suggests that coupling tidal flow operation with biochar as the medium in VFCWs could be a good option for the long-term treatment of high strength anaerobic digested effluents.

4. Conclusions

Biochar-packed VFCWs could provide significantly higher removal of organics, nitrogen and phosphorus, compared to gravel-packed VFCWs, when treating anaerobic digested effluents. Better pollutant removal in biochar VFCWs could be attributed to larger biochar surface area and higher porosity, which enabled higher pollutant adsorption, higher microbial colonisation and consequently higher biological pollutant degradation. The relatively high removal of organics and nitrogen in both the experimental phases (I and II) suggests that biodegradation become the main mechanism in VFCWs after long-term operation, which can complement the decreased adsorption functions of all media. However, the phosphorus removal was more dependent on the media adsorption for the whole experiment. Furthermore, tidal flow cycles enhanced the treatment efficiency of biochar CWs by creating a synergy between adsorption and biological degradation, whereby the adsorbed pollutants during the flooded periods were readily oxidised during the drained periods, thus regenerating the biochar actives sites and elongating its lifespan for long-term treatment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.03.125.

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