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Biochar-amended constructed wetlands for eutrophication control and microcystin (MC-LR) removal

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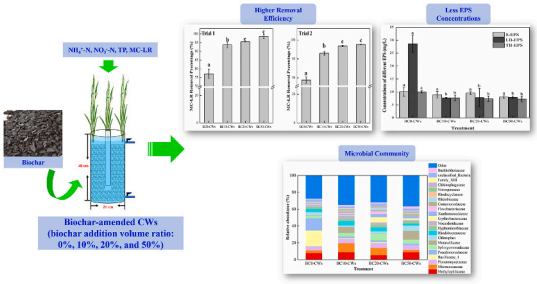
HIGHLIGHTS

- CWs with biochar addition ratios of 20% and 50% showed higher TP and MC-LR removal efficiency.
- Relative abundance of functional microorganisms was promoted by biochar addition.
- Content of extracellular polymeric substance was reduced in biochar-amended CWs.

GRAPHICAL ABSTRACT



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ABSTRACT

Microcystins (MCs) pollution caused by eutrophication and climate change has posed a serious threat to ecosystems and human health. Constructed wetlands (CWs) with biochar addition volume ratios of 0% (BC0-CWs), 10% (BC10-CWs), 20% (BC20-CWs) and 50% (BC50-CWs) were set up to evaluate the efficiency of biochar-amended CWs for eutrophication and MCs pollution control. The results illustrated that removal efficiencies of both NH_4^+-N and NO_3^--N were enhanced by biochar addition to varying degrees. The average TP and MC-LR removal efficiencies increased with increasing biochar addition ratios, and the average TP and MC-LR removal efficiencies in biochar-amended CWs were significantly ($p < 0.05$) improved by 5.64–9.58% and 10.74–14.52%, respectively, compared to that of BC0-CWs. Biochar addition changed the microbial community diversity and structure of CWs. The relative abundance of functional microorganisms such as *Burkholderiaceae*, *Nitrospiraceae*, *Micrococcaceae*, *Sphingomonadaceae* and *Xanthomonadaceae* was promoted by biochar addition regardless of addition ratios. The higher relative abundance of the above microorganisms in BC20-CWs and BC50-CWs may contribute to their better removal performance compared to other CWs. The concentrations of extracellular polymeric substance (EPS) in biochar-amended CWs were significantly ($p < 0.05$) lower than that in BC0-CWs, which can reduce the risk of system clogging. This study demonstrated that biochar addition may be a potential intensification strategy for eutrophication and MCs pollution control by CWs. Considering both the removal

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performance and economic cost, a biochar addition ratio of 20% was recommended as an optimal addition ratio in practical application.

1. Introduction

The eutrophication of freshwater has frequently occurred worldwide due to the excessive input of anthropogenic nutrients derived from intensified industrial, agricultural, and domestic wastewater (Machado et al., 2017). The increased level of eutrophication and climate change result in the frequent occurrence of cyanobacterial harmful blooms in freshwater (Paerl et al., 2016). Apart from their offensive odor and taste, the cyanobacterial harmful blooms have been considered as a threat to human and environmental health partly due to the production of potential cyanotoxins (Zhang et al., 2012). Microcystins (MCs), a family of monocyclic heptapeptide hepatotoxins, are mainly produced by various cyanobacteria (e.g., *Microcystis*, *Anabaena*, *Aphanizomenon*, *Oscillatoria* and *Planktothrix*, etc) (Wang et al., 2018a). They are recognized as one of the most widely encountered cyanotoxins due to their high detection frequency, wide distribution, high toxicity and resistance to degradation (Zhou et al., 2018a). As reported in previous studies, MCs pose a serious threat to biodiversity and the equilibrium of aquatic ecosystems through food chains/webs due to their bioaccumulation, and even elicit lethal effects on human and animals exposed to them, such as liver damage and tumor promotion, cardiotoxic and neurotoxic effects (Amado and Monserrat, 2010; Vichi, 2016; Wang et al., 2021). Among more than 200 variants of MCs, MC-LR is the most toxic and abundant variant because of its amino acid composition and structure (Merel et al., 2013). As preventive steps to reduce risks caused by MCs, the World Health Organization (WHO) recommends a MC-LR provisional guideline value of 1 μg/L for drinking water and tolerable daily intake (TDI) of 0.04 μg/kg body mass per day for human consumption (WHO 2017). Thus, the effective removal of MCs is imperative for water utilities.

Various conventional treatment technologies have been developed to eliminate MCs pollution (Zhou et al., 2018b). However, the large-scale applications of these technologies are usually limited by unsatisfactory removal efficiency and high maintenance cost. Furthermore, some of these technologies could often form unfavorable harmful by-products (Yang et al., 2020). For example, approximately 28% protein phosphatase-1 (PP-1) inhibition remained, which was associated with breakdown products, after MCs were completely degraded in the solar photo-Fenton systems (Karci et al., 2018). The occurrence of harmful cyanobacterial blooms globally is supported by excessive nutrient (i.e., nitrogen (N) and phosphorus (P)) from intensive anthropogenic activities (Conley et al., 2009). Excessive N and P in freshwater was also related to the MCs production process and the increased toxicity of cyanobacteria bloom (Chen et al., 2019). Hence, dual nutrient reduction of N and P in water bodies should also be considered as a vital step during control of MCs pollution. However, the above-mentioned treatment technologies cannot achieve the dual goal of eutrophication control and MCs risk reduction. During recent decades, constructed wetlands (CWs) have become popular for the treatment of various wastewater as a promising technology owing to their low energy consumption, plain maintenance requirements and simple operation (Liang et al., 2017; Wang et al., 2020). CWs are widely applied to eliminate various contaminants (e.g., organic matter, nutrients, heavy metals, micropollutants, etc) and have exhibited good removal performance (Liang et al., 2018; Yu et al., 2019; Chen et al., 2020a; Wen et al., 2021). Research studies of CWs have demonstrated their ability to simultaneously reduce the potential risk of eutrophication and MCs pollution (Wang et al., 2018b). However, our previous research showed that the effluent MC-LR concentration of traditional CWs exceeded the provisional guideline concentration (i.e., 1 μg/L) under high MC-LR loading influent, and the potential risks caused by MCs still exist in the effluent (Cheng et al., 2021). Therefore, it is necessary to develop intensification strategies for obtaining higher MCs removal efficiency in CWs.

Biochar is a pyrogenic carbon material formed by oxygen-limiting pyrolysis of biomass materials at low to moderate temperature (<700 °C) (Minh et al., 2020). In recent years, biochar is rapidly evolving for application in agriculture and environmental modification due to its carbon-rich, cost-effective, higher sorption capacity and ecological compatibility (Chen et al., 2020a; Kasak et al., 2018). Biochar is recognized as a simple and sustainable option to trap MCs considering its characteristics (Li et al., 2017a; Liu et al., 2018). During wastewater treatment process in CWs, the use of a substrate is an essential factor for pollutant removal and daily operation (Wang et al., 2020). The substrate can not only support the growth of wetland plants but also provide attachments for functional microorganisms (Wu et al., 2015). Many purification interactions (i.e., sorption, sedimentation, filtration, complexation and precipitation, etc) occur on the surface of substrate (Yang et al., 2018). Compared with other traditional substrates (e.g., gravel, red brick and sand) in CWs, biochar receives extensive attention as an innovative and promising amendment in the improvement of multiple pollutant removal performance (Table S1) and support of aquatic plant growth (Deng et al., 2021). Biochar not only provide an extra carbon source for heterotrophic denitrification process, but also reduce greenhouse gas emissions, especially for N_2O (Chen et al., 2020b). Biochar addition can change the microbial community diversity and composition in CWs by providing the suitable microbial colonization environment and essential nutrient (Deng et al., 2019). In addition, biochar is usually derived from waste biomass (e.g., agricultural solid wastes and municipal sludge) and can be used to solve the problem of disposal of exhausted materials (Li et al., 2017a). Thus, we can infer that biochar addition in CWs might be a sustainable and reasonable intensification strategy for eutrophication control and MCs removal.

However, to date, there is no research on estimating the influence of biochar addition on nutrients and MCs removal in CWs, and it is vital to determine the positive effects exerted by biochar addition and to explore the underlying microbial mechanism. In addition, the determination of the optimal addition ratio is urgently required considering both economic cost and various contaminant removal efficiencies. Therefore, the main objectives of this study were (1) to evaluate the effectiveness of biochar addition on nutrients and a typical MCs variant (i.e., MC-LR) removal, and to reveal the response of the microbial community to biochar addition in CWs; (2) to assess the influence of different biochar addition ratios on the removal efficiency of nutrients and MC-LR in CWs and select the optimal biochar addition ratio. This study can provide a more comprehensive basis for the design and application of biochar-amened CWs in eutrophication and MCs pollution control.

2. Materials and method

2.1. Materials and chemicals

Biochar-amended CW microcosms made of cylindrical polyethylene (40 cm in height × 20 cm in diameter) were established in this study (Fig. 1a). The packed substrate consisted of biochar (particle size: 1–2 cm) and gravel (particle size: 4–6 mm). Four types of biochar addition proportion (volume ratio) were 0%, 10%, 20%, and 50%, respectively. The biochar was purchased from Zhengzhou, Henan Province and its detailed physicochemical property is presented in Supplementary Materials (Table S2 and S3). All the CW microcosms were set up in the greenhouse situated in Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (CAS), Changchun, China (Fig. 1b). All the substrates were thoroughly rinsed with water before filling in the CWs. The giant reed (*Arundo donax*) was selected as wetland plant

species due to its great temperature tolerance and excellent purification efficiency (Idris et al., 2012). The seedling of *A. donax* was developed to maturity in 1/4 Hoagland nutrient solution. Mature *A. donax* plants with a height of 30 cm were transplanted into CW microcosms with a density of three plants per microcosms. All *A. donax* plants were then cultivated in 1/4 Hoagland solution for two months before the formal experiment in order to culture new roots and leaves, as well as biofilm on the rhizosphere and surface of the substrate.

MC-LR was selected as typical MCs model in this study. MC-LR standard (≥95% purity) applied in MC-LR analysis was purchased from Taiwan Algal Science Co., Ltd. (China). MC-LR used for the preparation of synthetic wastewater was extracted and purified from the algae liquid of cyanobacterium *Microcystis aeruginosa* FACHB-905 (obtained from Freshwater Algae Culture at the Institute of Hydrobiology, CAS). Before the extraction, *M. aeruginosa* was grown in BG-11 medium at 25 °C with 2000–3000 Lux illumination using a 12:12 h light-dark cycle. The detailed procedures of extraction and purification were followed according to the method of Morón-López et al. (2019) with some modification. Methanol and trifluoroacetic acid (TFA) used for the preparation of high-performance liquid chromatography (HPLC) mobile phase was HPLC-grade. All the other chemicals were of reagent grade.

2.2. Experimental design and operation

CWs amended with biochar at addition volume ratios of 0%, 10%, 20%, and 50% were named as BC0-CWs, BC10-CWs, BC20-CWs and BC50-CWs, respectively. Three replicates were set up for each treatment. Two batch tests were conducted with the operation period of 3 days for each batch. A total of 5 L synthetic wastewater was supplied into each CW microcosm at the start of each operation cycle. The composition of the synthetic wastewater was based on field freshwater eutrophication investigations in various regions of China (Wang et al., 2018a; Zhang et al., 2015). The influent concentrations of ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP) and MC-LR were 2.5 mg/L, 3.5 mg/L, 1 mg/L and 15 μg/L, respectively. Influent samples were taken from the feed tank at the beginning of each trial and effluent samples were collected at 12 h, 24 h, 48 h and 72 h from the drainage tap during each trial to evaluate the nutrient removal efficiency of each CW. A total of 1 L water sample was collected at 72 h for quantifying the MC-LR removal efficiency. At the end of the experiment, a 200 g substrate sample was collected from each CW for analysis of substrate characterization and microbial community diversity and structure.

2.3. Water sample measurement

Before MC-LR analysis, a modified solid phase extraction (SPE) was used for 1 L water sample pretreatment. The detailed pretreatment and analysis procedure were described in our previous study (Cheng et al., 2021). Concentrations of MC-LR were determined by HPLC (Waters, e2695, USA) equipped with an C18 column (250 mm × 4.6 mm) and UV-detector. The wavelength of the detector was set at 238 nm and the column temperature was maintained at 35 °C. The mobile phase consisted of menthol and Milli-Q water containing 0.01% TFA, using a linear gradient from 15% to 85%. The analysis was performed at a flow rate of 1 mL/min with an injection volume of 20 μL. The concentrations of NH_4^+-N, NO_3^--N and TP of the water samples were determined by automatic chemical analyzer (Mode Smartchem 200, Italy).

2.4. Substrate sample analysis

2.4.1. Biochar characterization analysis

To further explore the characteristics of biochar, Fourier transform infrared (FTIR) and scanning electron microscope (SEM) (XL-30 ESEM FEG) were used to scan the samples. FTIR was used to analyze functional groups of biochar with infrared absorbance spectra (0–4000 cm^{-1}) via potassium bromide-disk method. SEM was employed to characterize the surface morphology.

2.4.2. Extracellular polymeric substances (EPS) of substrate analysis

Approximately 150 g substrate sample was collected randomly from different positions (i.e., top, middle and bottom) of the CWs and then thoroughly mixed together. A heat extraction method was used to extract soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) from each sample. The detailed method was developed with some modification of the method in a previous study by Zhou et al. (2020). The dissolved organic carbon (DOC) of each extraction was considered as the indicator to reflect the concentration of S-EPS, LB-EPS and TB-EPS of each sample. The DOC concentrations were determined by using a total organic carbon analyzer (Shimadzu, Japan).

2.5. Microbial analysis

After two operation periods, the combined biochar and gravel samples were collected from each CW following a method described in our previous study (Chen et al., 2020a). Approximately 50 g of substrate sample was collected from each CW and sent to Sangon Biotech Co., Ltd.

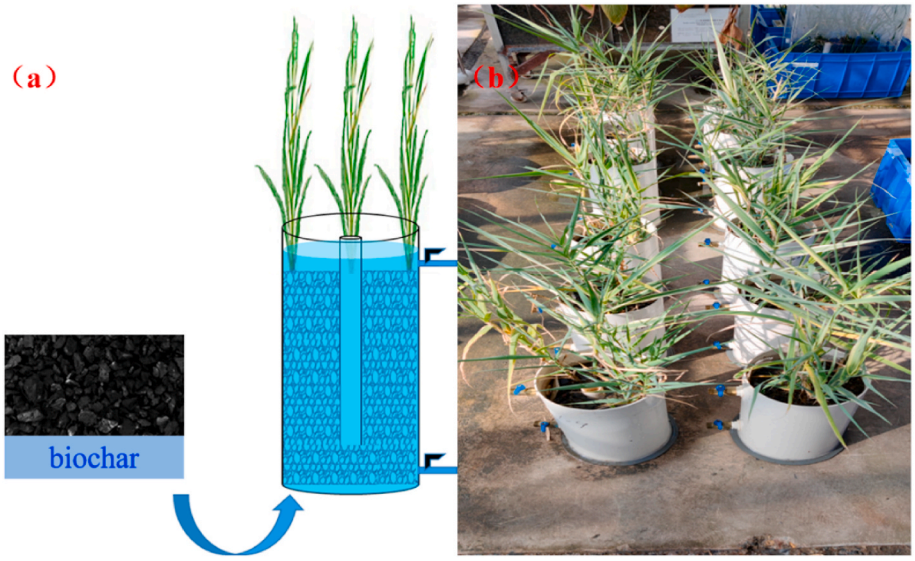

Fig. 1. The schematic diagram (a) and a photo of biochar-amended CWs (b).

(China). Illumina MisSeq sequencing was conducted to analyze the microbial community diversity and structure. The database used in microbial analysis is RDP (Ribosomal Database Project) database (http://rdp.cme.msu.edu/index.jsp).

2.6. Statistical analysis

The statistical analysis was performed with software SPSS 19.0 (SPSS Inc., Chicago, USA). All the data were presented as mean ± standard deviation (SD). Statistical significances among different treatments were compared by one-way analysis of variance (ANOVA) with Tukey HSD test at 95% confidence level ($p < 0.05$). All figures were designed and plotted by Origin 8.5 (OriginLab Inc., USA).

3. Results

3.1. Nutrient removal

The effluent NH_4^+-N concentrations in different CWs during two operation periods are presented in Fig. 2a. The average effluent NH_4^+-N concentrations of BC0-CWs, BC10-CWs, BC20-CWs and BC50-CWs were 0.12 ± 0.04 mg/L,0.10 ± 0.01 mg/L, 0.09 ± 0.01 mg/L and 0.09 ± 0.01 mg/L, respectively. The final removal efficiency of all biochar-amended CWs exceeded 95% in both trials. There was no significant difference in the final effluent NH_4^+-N concentration among different treatments. The main NH_4^+-N removal process occurred during the first 12 h, and all CWs exhibited a high removal efficiency (>90%) within 12 h.

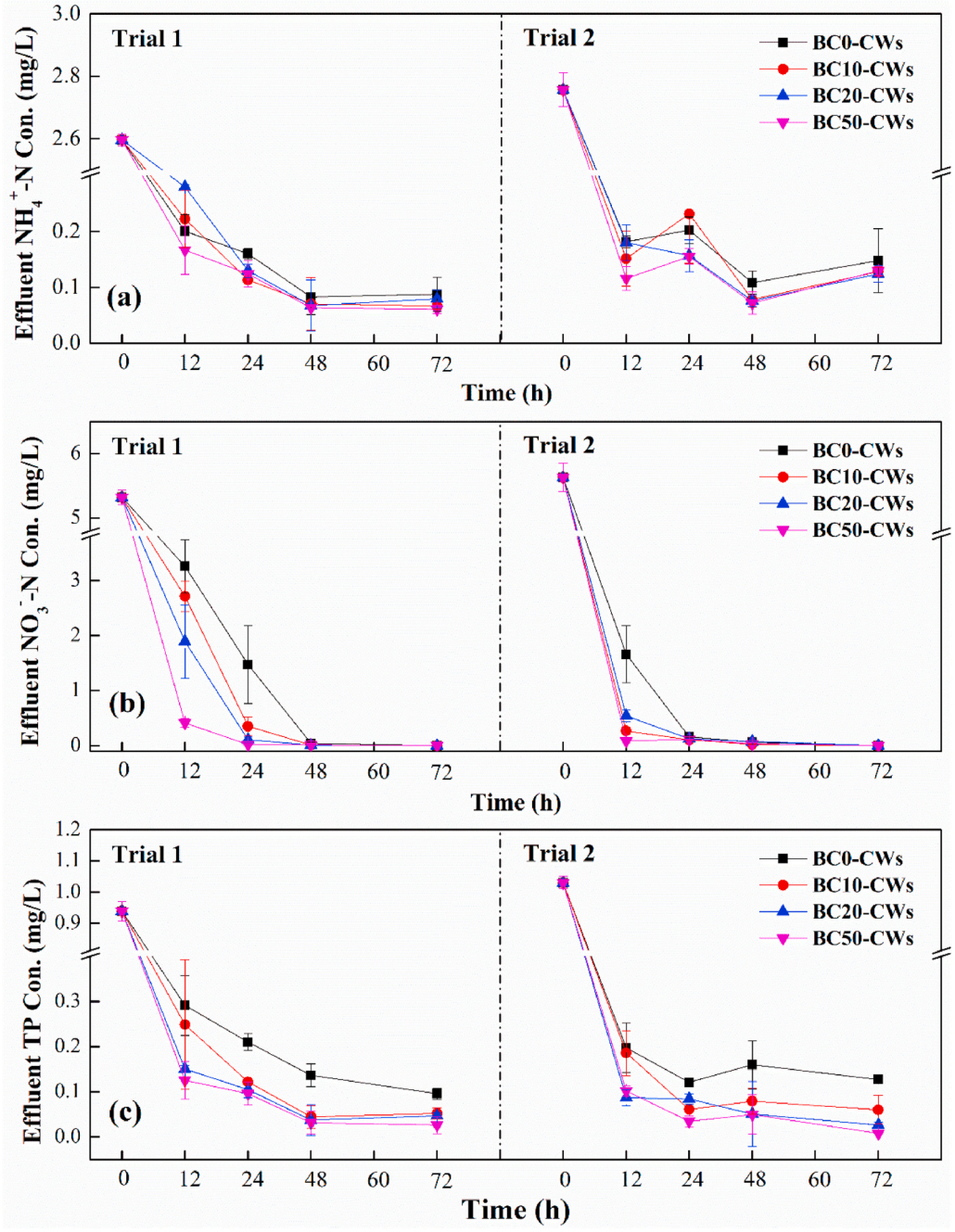

Fig. 2. The effluent NH_4^+-N (a), NO_3^--N (b) and TP (c) concentrations of traditional (BC0-CWs) and biochar-amended CWs (BC10-CWs, BC20-CWs, BC50-CWs) with increasing operation time (BC0-CWs: CWs without biochar addition; BC10-CWs: CWs with 10% volume ratio biochar addition; BC20-CWs: CWs with 20% volume ratio biochar addition; BC50-CWs: CWs with 50% volume ratio addition).

Meanwhile, in both trials, the average effluent NH_4^+-N concentration of BC50-CWs (0.13 ± 0.01 mg/L) after 12 h was significantly ($p < 0.05$) lower than the other CWs, with a removal efficiency of 95.21%.

The effluent NO_3^--N concentrations in all CWs decreased with increasing operation time during the experimental period (Fig. 2b). The final NO_3^--N removal percentages of all CWs achieved approximately 100%. During the first 48 h, the influent NO_3^--N concentration was reduced to near zero in both trials. The difference mainly occurred in the first 12 h. For both trials, the highest average effluent NO_3^--N concentration at 12 h was observed in BC0-CWs (2.46 ± 0.50 mg/L), which was significantly ($p < 0.05$) higher than for other CWs. In Trial 2, compared with BC0-CWs, lower effluent NO_3^--N concentration was observed in BC10-CWs (0.32 ± 0.01 mg/L), BC20-CWs (0.48 ± 0.05 mg/L) and BC50-CWs (0.08 ± 0.03 mg/L), with removal efficiency ranging from 91.20% to 98.46%.

As presented in Fig. 2c, the final effluent TP concentrations were lower than 0.15 mg/L with the removal percentages greater than 85% in all CWs in both trials. The effluent TP concentrations in all CWs significantly ($p < 0.05$) decreased during the first 12 h and then changed slightly with further operation time. The final average effluent TP concentrations of BC0-CWs, BC10-CWs, BC20-CWs and BC50-CWs were 0.11 ± 0.01 mg/L, 0.06 ± 0.02 mg/L, 0.04 ± 0.01 mg/L and 0.02 ± 0.01 mg/L, with the removal percentages of 88.70%, 94.34%, 96.27% and 98.28%, respectively. Therein, the final average effluent TP concentrations of BC0-CWs was significantly ($p < 0.05$) higher than that of other CWs. The greatest TP removal percentage was observed in BC50-CWs throughout the entire experiment. The average effluent TP concentrations of BC20-CWs and BC50-CWs were significantly ($p < 0.05$) lower at 12 h than those of BC0-CWs and BC10-CWs, which were all below 0.16 mg/L.

3.2. MC-LR removal

The MC-LR removal efficiency in all CWs is presented in Fig. 3. The removal efficiency of BC0-CWs was significantly ($p < 0.05$) lower than in all the biochar-amended CWs. The average final effluent MC-LR concentrations in BC0-CWs were 2.99 ± 0.34 mg/L and 1.36 ± 0.39 mg/L in Trial 1 and 2, respectively, which exceeded the provisional safety guideline of WHO (1 μg/L). While, for CWs with different biochar addition ratios, the effluent MC-LR concentrations were all below the provisional safety guideline (1 μg/L). During the entire experiment, the average MC-LR removal efficiency of biochar-amended CWs was BC50-CWs (98.62%) > BC20-CWs (96.79%) > BC10-CWs (94.84%). The relatively higher removal percentage was observed in BC20-CWs and BC50-CWs, but there was no statistically significant difference between BC20-CWs and BC50-CWs. In Trial 2, the removal efficiencies of MC-LR in both BC20-CWs and BC50-CWs were greater than 98%.

3.3. Concentrations of EPS in biochar-amended CWs

The concentrations of different EPS in biochar-amended CWs is presented in Fig. 4. The total EPS concentrations in BC0-CWs (48.71 ± 1.81 mg/L) was significantly ($p < 0.05$) higher than that in BC10-CWs (23.24 ± 1.68 mg/L), BC20-CWs (24.77 ± 0.69 mg/L) and BC50-CWs (24.29 ± 0.83 mg/L). As shown in Fig. 4, the largest EPS concentrations in BC0-CWs was LB-EPS, accounting for 58.86% of the total EPS concentration. While for biochar-amended CWs, the largest EPS concentrations was S-EPS (34.64–38.46% of the total EPS). The concentrations of LB-EPS (10.06 ± 1.67 mg/L) and TB-EPS (28.67 ± 3.29 mg/L)

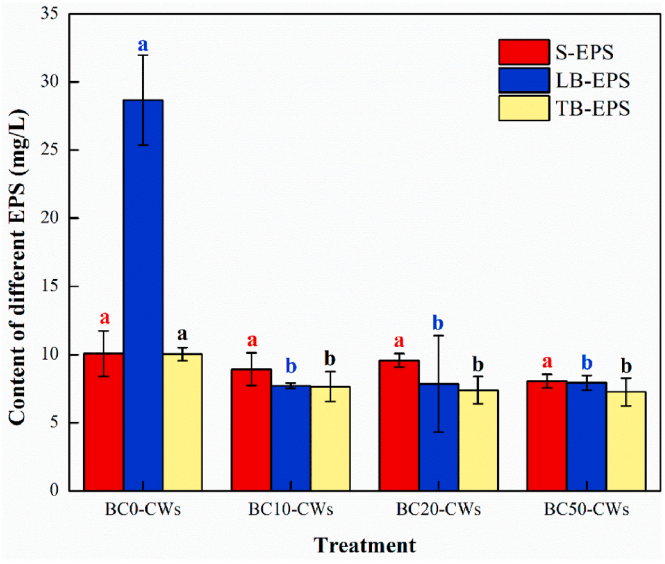

Fig. 4. Concentrations of different types of extracellular polymeric substances (EPS) (i.e., soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS)) in traditional (BC0-CWs) and biochar-amended CWs (BC10-CWs, BC20-CWs, BC50-CWs) (BC0-CWs: CWs without biochar addition; BC10-CWs: CWs with 10% volume ratio biochar addition; BC20-CWs: CWs with 20% volume ratio biochar addition; BC50-CWs: CWs with 50% volume ratio addition). Values represent the mean of three replicates and error bars represent the standard deviation. Different letters with different colors in columns represent a significant difference in the same type of EPS in different treatments, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

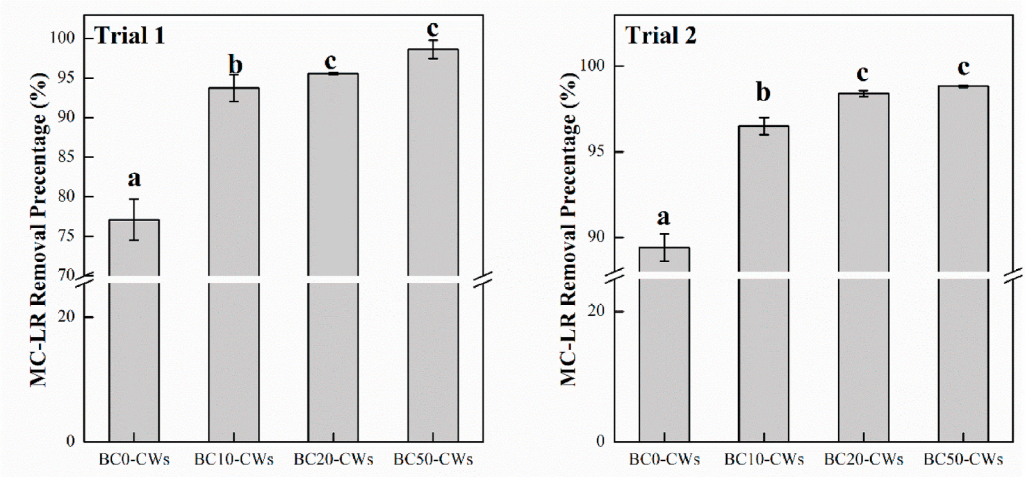

Fig. 3. MC-LR removal percentage in traditional (BC0-CWs) and biochar-amended CWs (BC10-CWs, BC20-CWs, BC50-CWs) (BC0-CWs: CWs without biochar addition; BC10-CWs: CWs with 10% volume ratio biochar addition; BC20-CWs: CWs with 20% volume ratio biochar addition; BC50-CWs: CWs with 50% volume ratio addition). Values represent the mean of three replicates and error bars represent the standard deviation. Columns containing different letters represent significant differences among different treatments.

in BC0-CWs was significantly ($p < 0.05$) higher than those in biochar-amended CWs. However, there was no significant difference among CWs with different biochar addition ratios.

3.4. Biochar characteristics

The SEM images and FTIR analysis were determined for investigating the surface property of biochar used in this study (Fig. 5). According to the SEM images (Fig. 5a), there was a high abundance of micropores on the surface of biochar, with BET surface area of more than 800 m^2/g (Table S2).

FTIR analysis of biochar before and after MC-LR adsorption was performed, as illustrated in Fig. 5b, there were characteristic peaks at 3450 cm^{-1}, 1600-1900 cm^{-1}, 1400-1600 cm^{-1} and 1000-1300 cm^{-1} in FTIR spectra (ranged from 0 to 4000 cm^{-1}). The corresponding functional groups were hydroxyl (O–H), carboxyl (C═O), aliphatic and aromatic carbon (C═C) and amino (C–N), respectively. According to the characteristic intensity of peaks, all the above functional groups apparently existed on the surface of biochar. After the MC-LR treatment process in CWs, some characteristic peaks intensity (i.e., O–H, C═O and C═C) of used biochar was reduced compared with that of the unused biochar. However, the characteristic peaks intensity of amino (C–N) increased in used biochar.

3.5. Microbial community analysis

The microbial community richness and α-diversity in each treatment is presented in Table 1. The coverage in all CWs were higher than 99%, suggesting that almost all the operational taxonomic units (OTUs) can ensure the comparability of α-diversity. The OTUs number ranged from 1766 to 2116. The biochar addition in CWs increased the OTUs number. The richness of microbial community was assessed by Chao estimator. The value of Chao estimator in biochar-amended CWs (2199.65 in BC10-CWs, 2292.37 in BC20-CWs and 2394.95 in BC50-CWs) was higher than that of control (2065.46). The value of Chao estimator in biochar-amended CWs increased with the increasing biochar addition ratio. The microbial community diversity can be represented by the Shannon index. Biochar addition in CWs enhanced the value of Shannon index. Similarly, the Shannon index also increased with the increasing biochar addition ratio. The higher value of the Chao and Shannon index in biochar-amended CWs also indicated that the addition of biochar can enhance the microbial richness and diversity of CWs.

The microbial community composition of each sample at the taxonomic levels of phylum, order and family are shown in Fig. 6. At the phylum level (Fig. 6a), *Proteobacteria* (47.44–61.95%) was the most predominant phylum in all CWs, followed by *Actinobacteria* (7.64–20.95%) and *Planctomycetes* (5.13–5.71%). The relative abundance of *Proteobacteria* and *Planctomycetes* of biochar-amended CWs was higher than that of BC0-CWs. The highest relative abundance of both *Proteobacteria* and *Planctomycetes* was observed in BC50-CWs. The relative abundance of *Proteobacteria* and *Actinobacteria* in BC10-CWs was comparable with that of BC20-CWs.

Table 1
Microbial community richness and diversity in traditional (BC0-CWs) and biochar-amended CWs (BC10-CWs, BC20-CWs, BC50-CWs).

	OTUs	Shannon	Chao	Coverage
BC0-CWs	1766 ± 172	4.79 ± 0.52	2065.46 ± 133.68	0.99 ± 0.001
BC10-CWs	1916 ± 90	5.42 ± 0.36	2199.65 ± 148.68	0.99 ± 0.002
BC20-CWs	1945 ± 94	5.45 ± 0.36	2292.37 ± 73.92	0.99 ± 0.002
BC50-CWs	2116 ± 97	5.51 ± 0.62	2394.95 ± 36.72	0.99 ± 0.001

As presented in Fig. 6b, the predominant microorganisms of in traditional and biochar-amended CWs were detected as *Actinomycetales* (5.3–18.3%), *Methylophilales* (5.3–8.9%), *Pseudomonadales* (2.1–14.8%), *Burkholderiales* (2.9–5.8%), *Sphingomonadales* (3.5–14.2%) and *Rhizobiales* (6.9–9.4%). Some functional microorganisms, including *Nitrospirales* (0.2–0.8%), *Rhodocyclales* (0.4–0.9%) and *Xanthomonadales* (0.9–3.8%), were also observed in all CWs. Biochar addition in CWs can enhance the relative abundance of *Sphingomonadales*, *Planctomycetales*, *Nitrospirales*, *Rhodocyclales* and *Xanthomonadales*. The relative abundance of *Sphingomonadales*, *Nitrospirales* and *Xanthomonadales* in BC10-CWs was lower than that of BC20-CWs and BC50-CWs.

At family level (Fig. 6c), the most dominant family in all CWs were *Methylophilaceae* (5.3–8.9%), followed by *Planctomycetaceae* (4.9–5.6%), *Micrococcaceae* (2.8–10.8%), *Sphingomonadaceae* (2.9–7.9%), *Burkholderiaceae* (1.7–2.7%), *Rhodobacteraceae* (1.8–5.0%), *Xanthomonadaceae* (0.9–3.8%), *Comamonadaceae* (0.9–1.7%) and *Nitrospiraceae* (0.16–0.88%). Compared with BC0-CWs, the biochar addition promoted the relative abundance of *Planctomycetaceae*, *Comamonadaceae*, *Nitrospiraceae*, *Burkholderiaceae* and *Sphingomonadaceae*. The higher relative abundance of *Comamonadaceae*, *Burkholderiaceae*, *Nitrospiraceae Planctomycetaceae*, *Sphingomonadaceae* and *Xanthomonadaceae* was observed in BC20-CWs and BC50-CWs compared with that of BC0-CWs and BC10-CWs.

4. Discussion

4.1. The effect of biochar addition on nutrient removal

In this study, a slightly improved NH_4^+-N removal efficiency was achieved in all biochar-amended CWs compared with that of BC0-CWs, which was consistent with previous studies (Chen et al., 2020b). This finding could be attributed to the following reasons: 1) High abundance of micro-mesoporous structure and large specific surface area of the biochar (Fig. 5a) utilized in this study are favorable for NH_4^+ adsorption

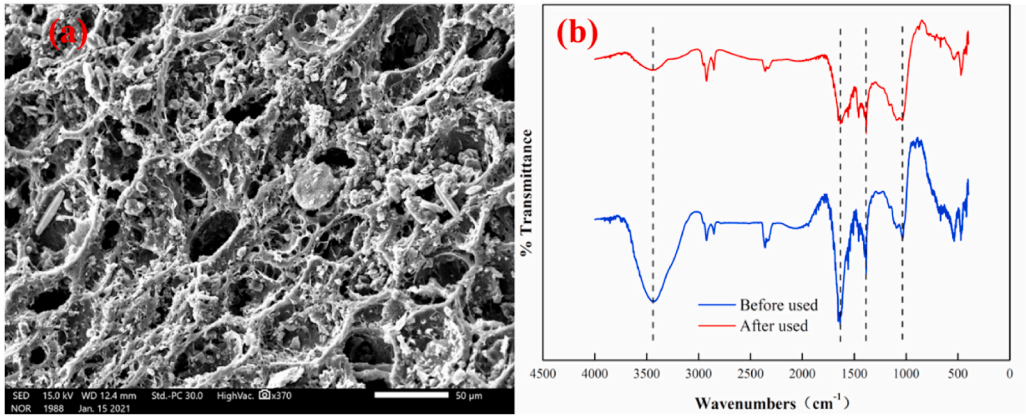

Fig. 5. SEM images (a) of biochar and FTIR analysis of biochar before and after MC-LR treatment process (b).

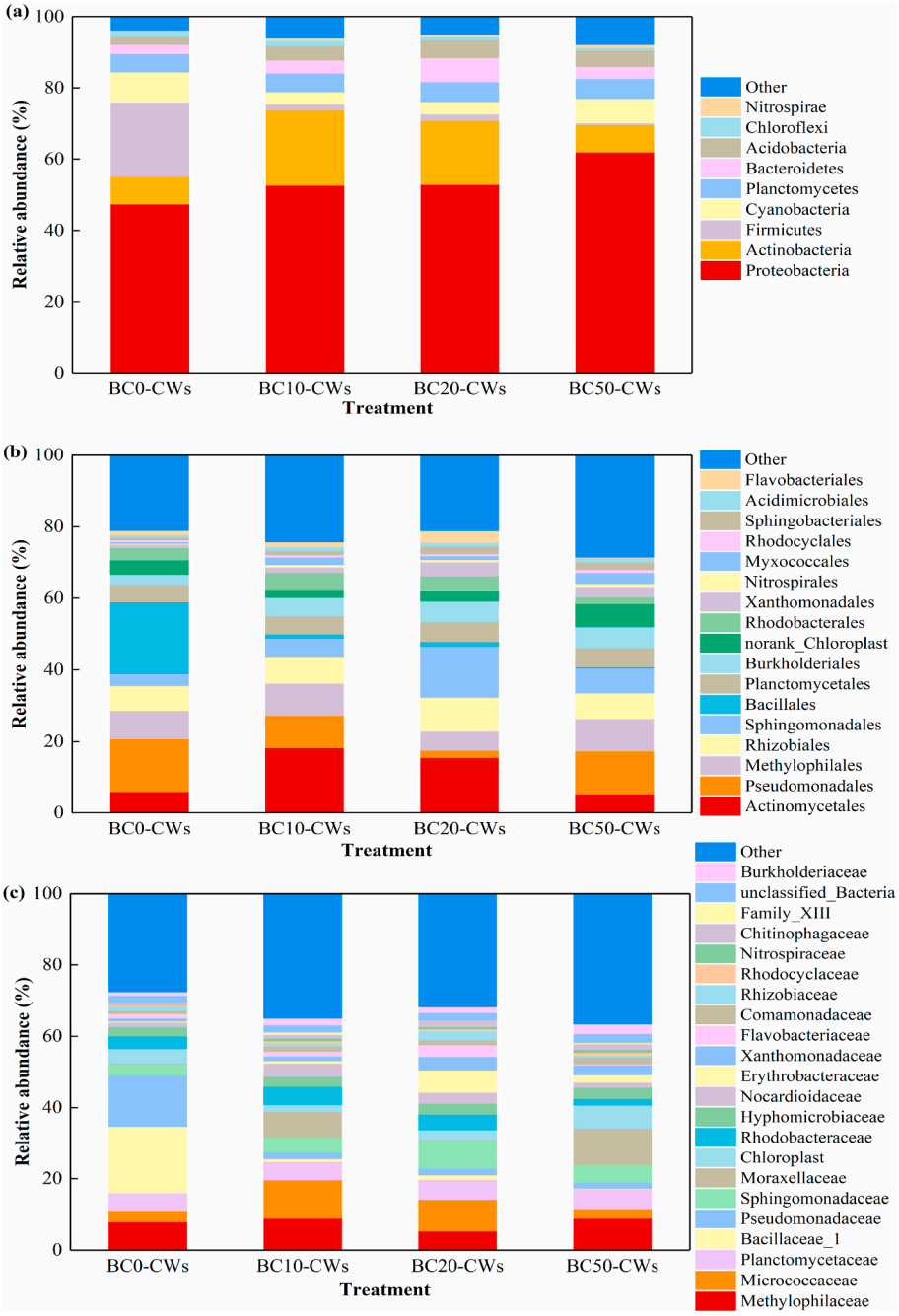

Fig. 6. Relative abundance of microbial communities of traditional (BC0-CWs) and biochar-amended CWs (BC10-CWs, BC20-CWs, BC50-CWs). ((a) Phylum, (b) Order, (c) Family).

(Hou et al., 2016). Various abundant oxygen-containing functional groups (e.g., carboxyl and carbonyl functional groups) on the surface of biochar are involved in the sorption of ammonium (Cui et al., 2016). Thus, the polarity and hydrophilic properties of biochar surfaces may contribute to the subsequent nitrification (Zhou et al., 2020); 2) Biochar can accelerate the air transfer and result in high DO levels, which can change the oxidation and reduction environment of CWs, and the increasing oxidation reduction potential (ORP) in biochar-amended CWs can enhance the nitrification process (Deng et al., 2019; Zhou et al., 2020); 3) The microenvironment in biochar-amended CWs can provide a porous environment for abundant microorganism attachment, which facilitates the enrichment of microorganism associated with NH_4^+-N removal. Microbial analysis indicated that the relative abundance of some bacteria related to nitrification was enhanced by biochar addition. For example, *Nitrospiraceae* (Fig. 6c), which are nitrite-oxidizing-bacteria (Park et al., 2021). *Nitrospiraceae* were recognized as key and predominant members in the nitrifying process, which converts nitrite into nitrate (Liang et al., 2020; Mehrani et al., 2020). The higher relative abundance of *Nitrospiraceae* in biochar-amended CWs was advantageous to complete nitrification compared with the control. Additionally, the higher relative abundance of *Planctomycetaceae* which are anammox bacteria, was observed in biochar-amended CWs. This results indicate that the internal environment of biochar-amended CWs can increase the enrichment of anammox bacteria, which may be another promoter to conduce higher NH_4^+-N removal performance in biochar-amended CWs (Jia et al., 2020). The biochar amendment may also promote plant uptake ability, root surface and root oxygen secretion and thus lead to the enhancement of aerobic

microbial metabolism including organic degradation and nitrification (Kasak et al., 2018; Shen et al., 2020). In this study, the different biochar addition ratio did not significantly ($p > 0.05$) affect the NH_4^+-N removal in CWs, although Deng et al. (2019) indicated that higher biochar addition ratio in CWs can enhance its NH_4^+-N removal efficiency. This observation may be caused by the lower influent NH_4^+-N concentration compared with previous research (Deng et al., 2019).

The influent NO_3^--N in all CWs was completely removed within 48 h (Fig. 2b). The main difference of the removal performance among different treatments was observed in the first 12 h. Improved NO_3^--N removal performance was observed in biochar-amended CWs, compared with BC0-CWs. This improvement may be attributed to the increased denitrification process in biochar-amended CWs (Zhang et al., 2019). Denitrification is the essential pathway for NO_3^--N removal in wastewater treatment (Yang et al., 2020b). The dissolved organic carbon released by biochar can provide a carbon source for heterotrophic denitrifying bacteria (i.e., *Comamonadaceae*, *Rhodobacteraceae* and *Xanthomonadaceae*), leading to the NO_3^--N removal enhancement in biochar-amended CWs. The micro-porous structure of biochar can provide anoxic conditions and various attachment sites for the formation of denitrifying bacteria microbial biofilm, altering the microbial community to enhance NO_3^--N removal (Zhou et al., 2020). These above explanations are evidenced by the increased relative abundance of *Comamonadaceae*, *Rhodobacteraceae* and *Xanthomonadaceae* by biochar addition (Fig. 6c). Some species in the above family have been verified as denitrifying bacteria. The increased proportions of some microbial groups verified as denitrifiers in the microbial community are responsible for the stable and high denitrification efficiency in biochar-amended CWs. In addition, biochar is also an efficient adsorbent of NO_3^--N, which can enhance the NO_3^--N trapping ability of systems (Yao et al., 2012). A higher biochar addition ratio in the BC-CWs (i.e., 20% and 50%) exhibited a greater removal performance in the first 12 h (Fig. 2b). This is because of the higher biochar addition ratios in the CWs and the more dissolved organic carbon, porous structure and surface area that they can provide. The highest relative abundance of all the above denitrification bacteria (i.e., *Comamonadaceae*, *Rhodobacteraceae* and *Xanthomonadaceae*) was observed in BC20-CWs or BC50-CWs, which enhanced the denitrification process and then contributed to the higher NO_3^--N removal in both treatments.

As the main microbial metabolite, EPS is verified to be closely related to nitrogen removal in CWs. The EPS adsorption of NH_4^+-N and NO_3^--N can improve nitrogen removal performance in wastewater (Yan et al., 2016a). However, a lower concentration of EPS in biochar-amended CWs also lead to an enhanced nitrogen removal performance in this study. This might have been caused by the positive effect of EPS on NH_4^+-N and NO_3^--N adsorption not being stronger than that of biochar. On the contrary, a high density and abundance of EPS in BC0-CWs might inhibit the mass transfer and limit the nitrogen removal (Vlaeminck et al., 2010).

TP removal in CWs was dependent on the combined effect of several pathways, including physicochemical effect of substrates, plant uptake and microbial degradation (Yang et al., 2018). Meanwhile, the adsorption and chemical precipitation by the substrate are the main mechanisms of TP removal (Wang et al., 2020). It has been reported that TP removal through substrate accumulation accounts for 36.2–87.5% of TP removal (Lan et al., 2018). Therefore, the application of appropriate substrates can enhance TP removal efficiency of CWs. As illustrated in Fig. 2c, the effluent TP concentration of biochar-amended CWs was significantly ($p < 0.05$) lower than that of BC0-CWs. The addition of biochar in CWs was beneficial to the TP removal, and can be attributed to the high adsorption capacity, high cation exchange capacity and high concentration of metal (e.g., Al, Ca and Mg) ion on the surface of biochar (Table. S2) (Shakoor et al., 2021). Based on the above features and structure of biochar, but not limited to, P was easily bound on the surface of biochar compared to gravel. In addition, the relative abundance of *Comamonadaceae* was promoted in biochar-amended CWs (Fig. 6c). Various microorganisms of *Comamonadaceae* were shown to have the function of P accumulation (Ge et al., 2015). For CWs with different biochar addition ratios, although there was no significant difference in the final effluent TP concentration, the significant difference on removal efficiency was observed in the first 12 h and the TP removal efficiency improved with increasing biochar addition ratio. The highest removal efficiency occurred at BC50-CWs. This finding was caused by the better adsorption ability and proliferation of phosphorus-accumulating microorganisms in BC50-CWs compared to other treatments.

4.2. The effect of biochar addition on MC-LR removal

Biochar addition in CWs can significantly ($p < 0.05$) enhance the MC-LR removal efficiency. A higher average MC-LR removal efficiency was observed in biochar-amended CWs throughout the entire experiment (Fig. 3). In contrast to the biochar-amended CWs, the final average effluent MC-LR concentration (1.37–2.99 mg/L) of BC0-CWs exceeded the WHO provisional safety guideline concentration (<1 μg/L), indicating that the effluent of BC0-CWs could still pose a threat to aquatic ecosystem and human health and this finding is consistent with our previous study (Cheng et al., 2021). The removal efficiency of MC-LR in the biochar-amended CWs increased with the increasing biochar addition ratio. The final removal efficiencies of MC-LR in BC20-CWs and BC50-CWs were significantly ($p < 0.05$) higher than those in BC10-CWs. The promoted MC-LR removal efficiency in biochar-amended CWs was caused by the improved adsorption and microbial activities. The possible mechanisms responsible for enhanced MC-LR removal efficiency in biochar-amended CWs are proposed as follows: (1) the multiporous structure facilitated their adsorption capacity and affinity toward MC-LR. MC-LR can be absorbed on biochar via pore-filling (Liu et al., 2018); (2) MC-LR was prone to interact with O-containing functional groups via intermolecular hydrogen bonds and electrostatic attraction due to the high abundance of hydroxyl and carbonyl on the surface of biochar (Fig. 5b) (Liu et al., 2018). This explanation was evidenced by the reduction of characteristic peaks intensity of hydroxyl and carbonyl on the surface of biochar after use (Fig. 5b); (3) the aromatic carbon in the biochar was also acknowledged to play a vital role in MC-LR adsorption by biochar (Yuan et al., 2020). Various interactions occurred between aromatic carbon and MC-LR include π+-π electron donor-acceptor interaction (π+-π EDAI) and π-π stacking interaction provide a specific attractive force for MC-LR adsorption (Liu et al., 2018; Teng et al., 2013). MC-LR can act as π-electron acceptor due to the existence of protonated guanidine groups, which can promote MC-LR removal via π+-π electron donor-acceptor interaction (Liu et al., 2018). The benzene ring of MCLR can be captured by aromatic units in the biochar via π-π stacking interaction (Teng et al., 2013). The adsorption and biodegradation in CWs are not two independent processes. As stated in reports of studies, The complete MC-LR degradation in nature mainly relies on the microbial community of the system (Li et al., 2017b). The stronger MC-LR trapping ability in biochar-amended CWs can improve the interception of MCs on the substrate matrix, which is beneficial to the subsequent microbial degradation (Yuan et al., 2020). Various MC-degrading bacteria from phylogenetically distinct branches have been identified in nature, which mainly includes *Sphingomonadaceae*, *Burkholderiaceae*, *Nocardiaceae*, *Micrococcaceae* and *Xanthomonadaceae* (Lezcano et al., 2017). Many of the above MC-degrading bacteria (e.g., *Sphingomonadaceae, Burkholderiaceae, Micrococcaceae* and *Xanthomonadaceae*) were observed in this study (Fig. 6c). Therein, *Sphingomonadaceae* and *Xanthomonadaceae* were recognized as MC-degrading bacteria using specific enzymes coded by a series of *mlr* genes (Li et al., 2017b). While *Burkholderiaceae* and *Micrococcaceae* also have a marked high ability to biodegrade MCs without *mlr* enzymes (Dziga et al., 2013). Such multiporous structure of biochar can enhance microbial biofilm formation and provide favorable condition for these MC-degrading bacteria (Zhou et al., 2020). The microbial community and activity in BC-CWs can be regulated to favor contaminant removal due to biochar

addition. According to microbial community analysis, the relative abundance of *Sphingomonadaceae*, *Burkholderiaceae*, *Micrococcaceae* and *Xanthomonadaceae* was all promoted by biochar addition. A greater abundance of functional microorganisms in biochar-amended CWs can improve MCs degradation, transformation and elimination. For different biochar-amended CWs, a higher adsorption ability and pore structure provided stronger electrostatic and pore-filling interaction for MC-LR (Yuan et al., 2020). In addition, the relative abundance of most of the above-mentioned MC-degrading bacteria in BC20-CWs and BC50-CWs was higher than in other treatments, which also contributed to the higher MC-LR removal efficiency in biochar-amended CWs with higher biochar addition ratio.

4.3. Practical application

Compared to traditional CWs (i.e., BC0-CWs), a promotive nutrient and MC-LR removal performance of biochar-amended CWs was observed in this study. During the operation process of CWs, microbes in CWs can release a complex mixture of high-molecular-weight polymer secreted (i.e., EPS). EPS exhibits dual roles in operations and maintenance. In this study, the significantly ($p < 0.05$) lower loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) concentrations were observed in biochar-amended CWs compared to those of BC0-CWs (Fig. 4), illustrating that biochar could reduce the LB-EPS and TB-EPS concentrations in the CWs, which was in agreement with the results of a previous study (Deng et al., 2019). A large specific surface area and abundant pores of biochar could support more microbial growth. Therefore, the increased heterotrophic bacteria could enhance utilization of organic compounds (Yan et al., 2016b). The change in the microbial communities might be another major factor for EPS reduction. At phylum level (Fig. 6c), the improved relative abundance of *Proteobacteria* and *Bacteroidetes* can enhance polymeric organic matter removal efficiency in biochar-amended CWs (Wang et al., 2009). Overall, more heterotrophic bacteria and greater oxygen transfer could decrease the total EPS concentration by accelerating LB-EPS and TB-EPS consumption (Yoon Kim et al., 2006). In practical applications, the lower density and deficiency EPS content in CWs systems can improve various pollutant removal performance due to the greater mass transfer and more microbial enzymes release (Vlaeminck et al., 2010). Of more importance, a lower content of EPS was recognized to be conducive to reducing the risk of clogging of systems and increase of service life (Du et al., 2016).

The selection of biochar addition ratio should follow the principles of high-efficiency and cost-effectiveness. In this study, the removal efficiencies of BC20-CWs and BC50-CWs, especially for TP and MC-LR removal efficiency, were better than those of other treatments. Therefore, the biochar addition ratio of 20% is suggested to be more suitable in CWs for eutrophication and MCs pollution control from the perspective of both removal efficiency and economic cost. Biochar addition in CWs could not only facilitate the sustainable operation of CWs but expand the application range of biochar. A large amount of solid waste (e.g., livestock manure and crop straw) is added to the environment without any correct treatment and disposal procedures each year in China, which cause various problems including land-take required for storage and environmental pollution from leaching and runoff (NDRC, 2014). A useful transition of these waste materials to biochar can provide an alternative method for solid waste disposal and alleviate environmental burdens and threats (Li et al., 2017a). Discarded biochar substrate in CWs can be collected and applied to agroecosystems to improve the crop yields and quality, which can solve their disposal issues (Deng et al., 2021).

5. Conclusions

In this study, the influence of biochar addition in CWs on eutrophication and MCs pollution control as well as the microbial community was evaluated. Major findings obtained are as follows: (1) biochar addition can enhance, to varying degrees, the nutrient and MC-LR removal performance in CWs. (2) All effluent MC-LR concentrations of biochar-amended CWs were below the WHO provisional limit concentration. Therein, BC20-CWs and BC50-CWs outperformed BC10-CWs in MC-LR removal due to the increased adsorption ability and microbial activities. (3) The change in the microbial community were derived by biochar addition. The higher relative abundance of functional microorganisms resulted in higher nutrient and MC-LR removal capacity in biochar-amended CWs. (4) Biochar addition in CWs can reduce the content of EPS, which might reduce the risk of system clogging and extend the service life. Therefore, biochar addition can be recognized as a potential intensification strategy for eutrophication and MCs pollution control. Overall, a biochar addition ratio of 20% is recommended as a priority addition ratio for CWs applied to eutrophication and MCs pollution control.

Credit author statement

Rui Cheng: Methodology, Investigation, Writing – original draft. **Shengnan Hou:** Investigation, Writing – review & editing. **Jingfu Wang:** Resources, Project administration, Funding acquisition. **Hui Zhu:** Resources, Writing – review & editing, Project administration, Supervision, Funding acquisition. **Brian Shutes:** Writing – review & editing. **Baixing Yan:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.133830.

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