Journal of Southeast University (English Edition) Vol. 39, No. 2, pp. 115 – 126 June 2023 ISSN 1003—7985

Enhancement of wastewater treatment performance in constructed wetlands by calcium-modified basalt fiber

Huang Juan Wei Zhihui Ji Xiaoyu Yan Chunni Ma Yixuan Qian Xiuwen

(School of Civil Engineering, Southeast University, Nanjing 211189, China)

Abstract: To investigate the effects of calcium-modified basalt fiber (Ca-MBF) and its filling mode on the wastewater treatment performance of constructed wetlands (CWs), two pilot-scale CWs with vertical-filling and layered-tiling Ca-MBF were developed and compared with a conventional CW for pollution removal effect, enzyme activity spatial distribution, extracellular polymeric substances (EPS) content, and microbial community structure. The results showed that Ca-MBF could improve the microbial diversity and richness of CWs, increase nitrifying and denitrifying bacteria in the upper and middle layers of the substrates, and promote cooperation between functional genera. Ca-MBF significantly increased the enzyme activities in the middle and lower layers of CWs, resulting in higher average enzyme activity (ammonia monooxygenase, nitrite oxidoreductase, nitrate reductase, nitrite reductase, and phosphatase) of the whole system. Additionally, Ca-MBF facilitated the secretion of EPS with an average increase of 29.02% to 52.90%. Overall, Ca-MBF enhanced the ability of CWs to remove pollutants, in which the CW with layered-tiling Ca-MBF achieved the best treatment effect, with 16.72%, 6.95%, and 6.30% increase in ammonia nitrogen, total nitrogen, and total phosphorus removal, respectively. These results could provide valuable references for the application of Ca-MBF CWs.

Key words: constructed wetlands (CWs); calcium-modified basalt fibers (Ca-MBF); filling mode; enzyme activity; extracellular polymeric substances(EPS); microbial community

DOI: 10.3969/j.issn.1003 – 7985.2023.02.002

Constructed wetlands (CWs) are ecological systems composed of plants, substrates, and microorganisms, which can utilize the synergistic physical, chemical, and biological effects to efficiently purify sewage[1]. In the past decades, CWs have been widely used in wastewater treatment due to stable treatment performance, low energy consumption, and low construction costs[2]. It has always been a research hotspot to explore the possibilities for improving the performance of CW. Previous studies have shown that cultivating suitable wetland plants[3], filling substrates with strong adsorption capacity[4], and equipping aeration facilities[5] can effectively promote the degradation of pollutants. However, the effects of plants on wetlands depend on their growth, and the application of aeration equipment will undoubtedly increase the costs. Therefore, it is crucial to develop novel substrates to enhance the performance of CWs.

Substrates in CWs play a critical role in sewage purification. Substrates can retain and adsorb pollutants and provide growth carriers for plants and microorganisms[6]. Previous studies have shown that the microbial community structure could be affected by the same substrate composition but different filling modes in CWs[7-8]. Therefore, choosing suitable substrates and filling modes are crucial for the efficient and stable operation of CWs. Basalt fiber (BF) is an inorganic fiber, mainly composed of SiO_2, Al_2O_3, Fe_2O_3, and CaO[9]. In recent years, BF has received considerable attention in the field of wastewater treatment because of its properties, such as ecofriendliness, large specific surface area, and high mechanical strength[10]. However, smooth surface, hydrophobicity, and negative surface charge are the disadvantages of BF as carriers, which would decelerate biofilm formation[11]. Therefore, BF should be modified on the surface before being used as biocarriers. BF modification by glutamate[12], ferric citrate[13], and chitosan[14] has been proven to be successful in increasing fiber surface roughness and hydrophilicity. Calcium is crucial for bacterial growth and beneficial for the secretion of extracellular polymeric substances (EPS)[15]. Calcium modification has been applied to increase the surface potential of carbon fibers without reducing their hydrophilicity[16]. Additionally, Ca-MFB used in the contact oxidation reactor exhibited higher and more stable removal efficiency than conventional BF[17]. These results demonstrate that surface modification with calcium was an effective method to enhance carrier performance. However, most studies mainly focused on the performance of MBF as biocarriers in a contact oxidation reactor. To our knowledge, only one study involved CW with BF addition[18], and Ca-MBF has not been studied as fillers in CW. Moreover,

Received 2022-11-12, **Revised** 2023-03-08.

Biography: Huang Juan (1980—), female, doctor, professor, 101010942@seu.edu.cn.

Foundation items: The National Natural Science Foundation of China (No. 52270152), the Key R&D Projects of Jiangsu Provincial Department of Science and Technology (No. BE2022831).

Citation: Huang Juan, Wei Zhihui, Ji Xiaoyu, et al. Enhancement of wastewater treatment performance in constructed wetlands by calcium-modified basalt fiber[J]. Journal of Southeast University (English Edition), 2023, 39(2): 115 – 126. DOI: 10.3969/j.issn.1003 – 7985.2023.02.002.

the effects of Ca-MBF with different filling modes on treatment performance, substrate-enzyme activity, EPS content, and microbial community were unclear.

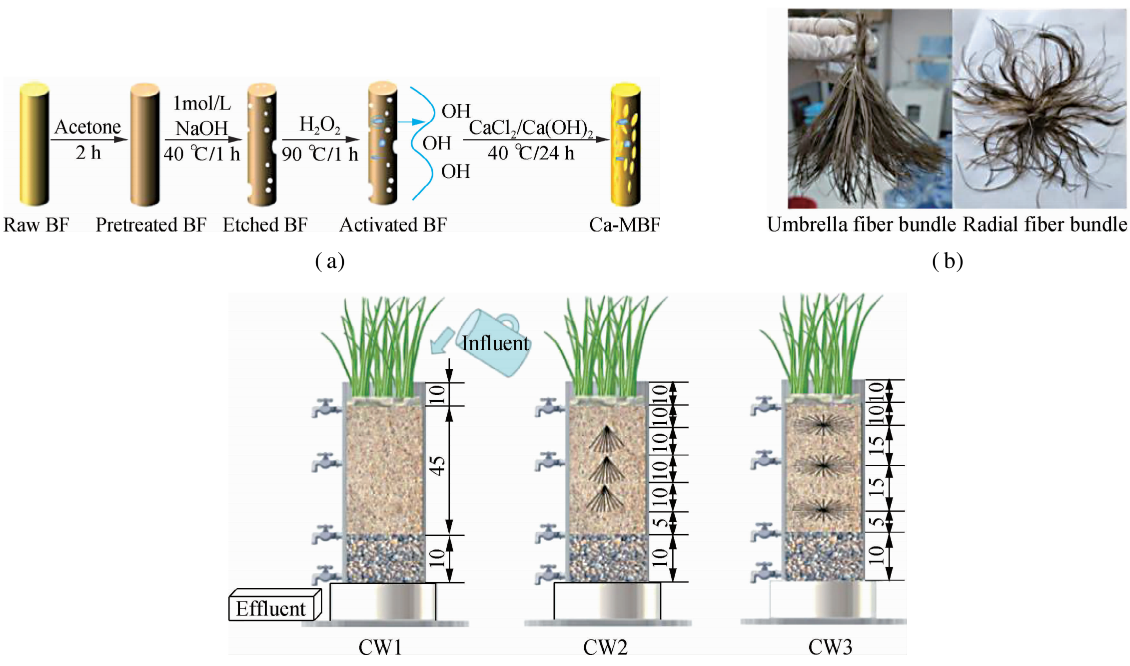
Thus, this study aimed to explore the effects of two filling modes of Ca-MBF on decontamination performance, the changes in enzyme activity and EPS content caused by Ca-MBF, and the effects of Ca-MBF on the microbial community. These studies were expected to determine a suitable filling mode for Ca-MBF in CWs.

1 Materials and Methods

1.1 Preparation of calcium-modified basalt fibers

BF (CBF13-1200; diameter 13 mm) used in this experiment was purchased from Jiangsu Green New Material Technology Development Co., Ltd., Jiangsu, China. Ca-MBF was prepared via the following steps[9, 17]. First, raw BF was soaked in acetone for 2 h to remove its surface wetting agent (see Fig. 1(a)). Further, the pretreated BF was etched using NaOH (1 mol/L, 40 ℃) for 1 h to increase its surface area and roughness. Then, the etched BF was immersed in 30% H_2O_2 solution (90 ℃) for 1 h to obtain active silanol groups (Si-OH). Finally, the same volume of $CaCl_2$ solution (1 mol/L) and saturated $Ca(OH)_2$ solution were added to the beaker containing activated BF. The activated BF was heated in a water bath for 24 h at 40 ℃. After completing the above process, Ca-MBF was washed and dried.

Two types of fiber bundles, 12 g in mass and 10 cm long, were produced (see Fig. 1(b)). The difference between the two fiber bundles lay in the location of the bound nodes, where one was with the node at the end of the fiber, and the other was in the middle. The former was called an umbrella fiber bundle, which was a common mode of fiber filling in contact oxidation reactors. The latter was called the radial fiber bundle, which enabled fibers to cover the entire filling plane.



(c)

Fig. 1 Building process of CWs with calcium-modified basalt fiber. (a) Schematic diagram for surface modification with calcium of BF; (b) Image of the umbrella fiber and radial fiber bundles; (c) Schematic diagram of conventional CW and calcium-modified basalt fiber CWs (unit: mm)

1.2 CWs setup and operation

Three CWs (CW1, CW2, CW3) made of plexiglass with an effective working volume of 4 L ($D \times H = 20$ cm $\times 65$ cm) were built in Southeast University, China. From bottom to top, each CW was divided into gravel layers ($\varphi = 5$-8 mm, $h = 10$ cm) and sand layers ($\varphi = 1$-2 mm, $h = 45$ cm). In detail, CW1 was the control without Ca-MBF. CW2 was filled with umbrella fiber bundles every 10 cm, namely vertical-filling group. CW3 was filled with radial fiber bundles every 15 cm, namely layered-tiling group (see Fig. 1(c)). Six rhizomes of *Iris pseudacorus* with similar heights were planted in each CW. The composition of the synthetic wastewater used in this experiment is shown in Tab. 1. Moreover, the theoretical concentrations of COD, TN, NH_4^+-N, and TP for the synthetic sewage obtained from the recipe were 300, 35, 16, and 4 mg/L, respectively. The hydraulic retention time and hydraulic loading of each CW were 72 h and 0.042 m/d. The synthetic wastewater of 4 L was introduced from the top of each CW within 15 min and kept in the bed for 71 h. Then, the effluent was drained within 15 min, and the bed was kept unsaturated for 30 min. To systematically investigate the effect of Ca-MBF on nutrient removal in CW, the whole experimental process was divided into three periods: periods Ⅰ (0-60 d), Ⅱ (60-120 d), and Ⅲ (120-180 d). Period Ⅰ can be considered the startup period of CWs, whereas periods Ⅱ and Ⅲ can be seen as the stabilization operation phases of CWs.

Tab. 1 Synthetic wastewater recipe

Composition	Concentration/(mg · L^{-1})
CH_3COONa	384.38
$CO(NH_2)_2$	19.29
$(NH_4)_2SO_4$	75.43
KNO_3	72.14
KH_2PO_4	17.55
$MgSO_4 \cdot 7H_2O$	50.00
$FeSO_4 \cdot 7H_2O$	3.50
$ZnSO_4 \cdot 7H_2O$	0.13
$Na_2MoO_4 \cdot 2H_2O$	0.03
H_3BO_3	0.025
$CuSO_4 \cdot 5H_2O$	0.03

1.3 Sampling and analysis

1.3.1 Properties characterization of carrier

After drying BF and Ca-MBF, the surface of the samples was sprayed with gold. The surface morphology and elemental composition of BF and Ca-MBF were observed and determined by Scanning electron microscopy (SEM)/energy dispersive spectroscopy (EDS) (FEI Inspect F50, USA).

1.3.2 Water quality determination

The influent and effluent were collected every 3 d and immediately assayed in the laboratory. COD was measured by the fast digestion spectrophotometric method with potassium dichromate oxidation. NH_4^+-N, TN, and TP concentrations were measured using standard methods[19].

1.3.3 Enzyme activity determination

Sand was sampled from three locations on each layer (upper layer (U), 10-15 cm; middle layer (M), 20-25 cm; lower layer (L), 35-40 cm) and evenly mixed to extract enzyme solution for enzyme activity analysis. Dehydrogenase (DHA) and phosphatase (PST) enzymes were measured following the procedure described by Hu et al[20]. Ammonia monooxygenase (AMO), nitrite oxidoreductase (NOR), nitrate reductase (NAR), and nitrite reductase (NIR) were determined using the procedure described by Zheng et al[21].

1.3.4 EPS content determination

At the end of the experiment, sand was collected from different depths (U, 10-15 cm; M, 20-25 cm; L, 35-40 cm) for EPS determination. The experiment of EPS consisted of an extraction and determination process. In this study, EPS was extracted by the heating method[22]. The specific components of EPS, such as polysaccharide (PS), were determined by the anthrone colorimetry method[23], whereas protein (PN) was determined by the modified Lowry method[24].

1.3.5 Microbial community composition determination

Sand at different depths (U, 10-15 cm; M, 20-25 cm; L, 35-40 cm) was collected for microbial community analysis at the end of the experiment. Genomic DNA samples were extracted using the kit and then checked for DNA integrity and purity using 1% agarose gel electrophoresis and Thermo NanoDrop One. The V4-V5 regions of the 16S rRNA gene were amplified by PCR with bacterial primers 515F (GTGCCAGCMGCCGCGGTAA) and 926R (CCGTCAATTCMTTTRAGTTT). Then, microbial high-throughput sequencing was conducted on the Illumina HiSeq platform by Guangdong Meige Gene Technology Co., Ltd.

1.4 Statistical analysis

Statistical analysis of the experimental data was performed using the SPSS 26.0 software. The results of the water quality among CWs were tested using ANOVA. A significant difference was considered when p value was smaller than 0.05. In this study, different letters on the histogram indicate significant difference ($p < 0.05$)

2 Results and Discussion

2.1 Microscopic morphology and elemental composition of BF and Ca-MBF

The surface morphology of BF and Ca-MBF was found to have distinct differences (see Fig. 2). The surface of BF was smooth without other particles attached (see Fig. 2(a)), whereas Ca-MBF had a certain thickness of solid particles deposited on its surface (see Fig. 2(b)). EDS analysis showed that the elemental composition of BF and

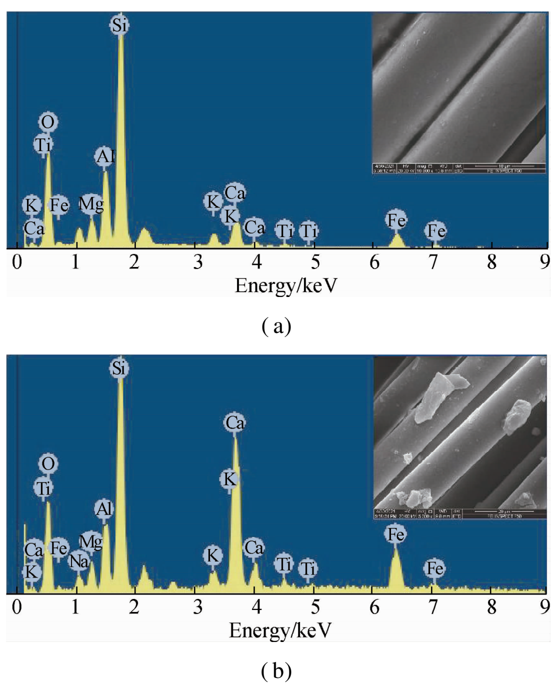


Fig. 2 SEM images and EDS spectra. (a) BF; (b) Ca-MBF

Ca-MBF was similar, mainly including O, Mg, Al, Si, K, Ca, and Fe (see Tab. 2). Remarkably, the relative calcium content in Ca-MBF was increased to 16.58%, which was higher than that of BF (4.87%), indicating the successful preparation of Ca-MBF.

Tab. 2 Elemental composition of BF and Ca-MBF %

Element	BF		Ca-MBF	
	Mass fraction	Atoms fraction	Mass fraction	Atoms fraction
O	47.90	63.88	41.37	60.25
Mg	3.31	2.90	2.37	2.27
Al	7.77	6.15	5.17	4.46
Si	27.30	20.74	18.14	15.05
K	1.92	1.05	1.55	0.92
Ca	4.87	2.59	16.58	9.64
Ti	0.61	0.27	0.95	0.46
Fe	6.33	2.42	11.95	4.98
Na	0	0	1.93	1.96

2.2 Overall removal performance

Nutrient removal is the main indicator to assess the performance of CWs. Figs. 3(a) and (b) show the COD effluent concentrations and average removal efficiencies of the three CWs at different periods. In period Ⅰ, the COD removal capacity of three CWs was comparable, with removal efficiencies of 75.53%-76.80%. With the operation time passing, Ca-MBF CWs exhibited superiority in COD removal. In period Ⅱ, the average COD effluent concentrations were (65.65 ± 16.13) mg/L in CW1, (49.30 ± 14.48) mg/L in CW2, and (51.00 ± 12.91) mg/L in CW3. The average COD removal efficiencies were (83.52 ± 4.70)% ($p < 0.05$) of CW2 and (82.92 ± 4.28)% ($p < 0.05$) of CW3, which were significantly higher than that of CW1 ((78.05 ± 5.09)%). In period Ⅲ, COD concentrations in the effluent were further reduced to (45.90 ± 13.22) mg/L in CW1, (42.15 ± 11.48) mg/L in CW2 and (36.30 ± 10.94) mg/L in CW3 on average, with removal efficiencies of (84.81 ± 4.56)%, (86.08 ± 3.82)% and (87.99 ± 3.71)%, respectively. These results indicated that filling Ca-MBF into CWs contributed to COD removal. It can be attributed to the fact that the fast-growing heterotrophs could colonize on Ca-MBF carriers and form big biological aggregates in the presence of abundant organic carbon, accounting for the high COD removal efficiency.

Excessive nitrogen and phosphorus discharge is crucial for eutrophication in water bodies; hence, it is relevant to improve the removal of nitrogen and phosphorus by CWs. As expected, Ca-MBF enhanced the capacity of CWs to remove nitrogen and phosphorus. As shown in Figs. 3(c) and (d), Ca-MBF boosted NH_4^+-N removal by 2.22% in CW2 and 8.17% in CW3 during period I, compared with the control (23.11%). Likewise, with an increase in time, significant differences in NH_4^+-N removal were shown between systems. The average NH_4^+-N removal efficiencies of CW2 and CW3 increased by 5.49% ($p < 0.05$) and 19.19% ($p < 0.05$) in period Ⅱ and 6.91% ($p < 0.05$) and 23.23% ($p < 0.05$) in period Ⅲ, respectively, compared with CW1 (with removal efficiencies of 21.81% and 17.81%, respective-

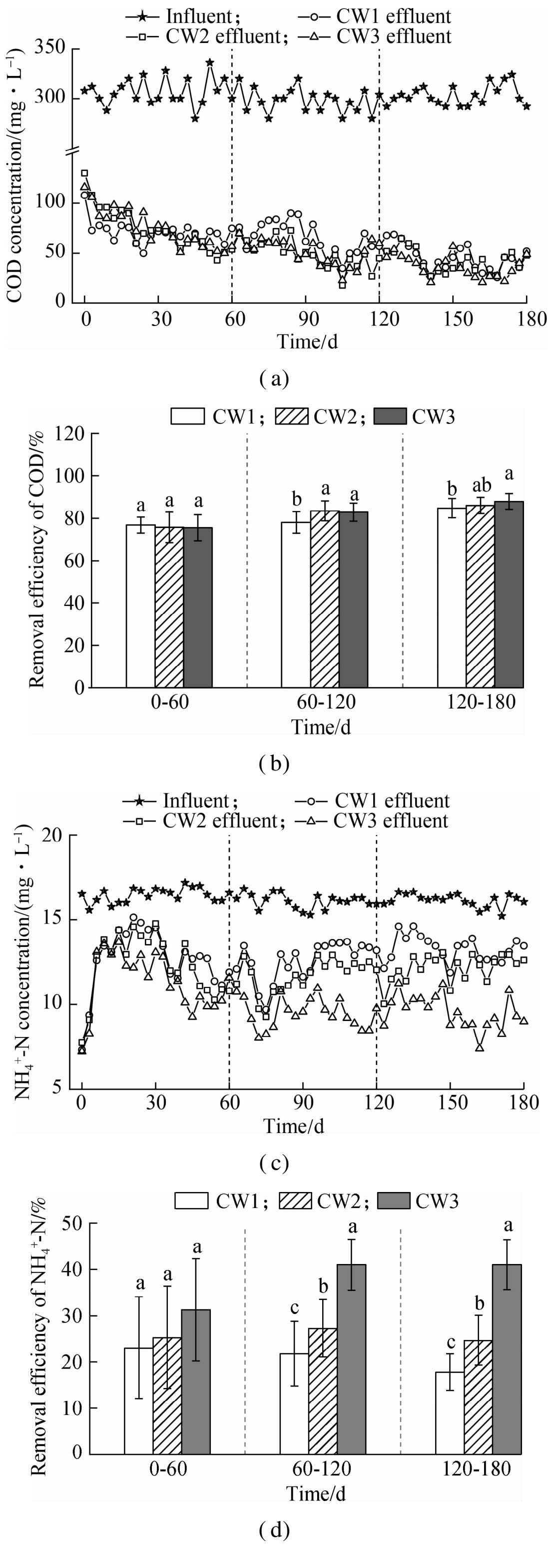


Fig. 3 COD and NH_4^+-N removal performance of three CWs. (a) COD concentrations; (b) Average removal efficiency of COD; (c) NH_4^+-N concentrations; (d) Average removal efficiency of NH_4^+-N

ly). The low NH_4^+-N removal efficiency in the three CWs may be due to the limited dissolved oxygen. The better NH_4^+-N removal in Ca-MBF CWs was probably due to the increased abundance of nitrifying bacteria. The inference was confirmed by the following discussion of the microbial community. In terms of the whole experimental period, the removal of NH_4^+-N in CW2 and CW3 increased by 4.82% ($p<0.05$) and 16.72% ($p<0.05$), respectively. Moreover, it indicated that layered-tiling Ca-MBF in CW was better for NH_4^+-N removal than vertical filling. Layered-tiling mode allowed Ca-MBF to be dispersed entirely, which increased their contact area with wastewater and facilitated NH_4^+-N adsorption. Moreover, Ca-MBF with layered tiling provided a larger attachment surface for microbial growth, thus enhancing the microbial degradation of pollutants.

The tendency of TN alterations was similar to NH_4^+-N (see Figs. 4(a) and (b)). In period Ⅰ, three CWs kept parallelism in TN removal, with removal efficiencies of 56.60%-59.60%. In periods Ⅱ and Ⅲ, more NH_4^+-N was reduced by CW3, resulting in better removal for TN than CW1 and CW2. Specifically, (65.38 ± 4.12)% and (64.70 ± 3.30)% of TN were removed by CW3 in periods Ⅱ and Ⅲ, respectively, which was higher than that in CW1 ((57.92 ± 3.28)% and (54.11 ± 3.48)%, respectively) ($p<0.05$) and CW2 ((59.33 ± 4.06)% and (58.48 ± 3.12) %, respectively) ($p<0.05$). Regarding the whole experimental period, CW3 achieved the best TN removal with (63.17 ± 4.90)%, which was 6.95% ($p<0.05$) and 4.95% ($p<0.05$) higher than CW1 and CW2, respectively. It revealed that adding Ca-MBF could enhance nitrogen removal in CWs, and layered tiling was a better filling mode.

Similar to nitrogen removal, the TP removal efficiency of three CWs was about 30% in period Ⅰ (see Figs. 4(c) and (d)), and no significant difference was noted between each other. Subsequently, Ca-MBF CWs showed advantages for phosphorus removal in period Ⅱ. TP removal was (32.12 ± 7.94)% in CW2 and (37.06 ± 7.23)% in CW3, which increased by 4.29% and 9.23% ($p<0.05$), respectively, compared to CW1. Thereafter, TP removal in all systems was decreased in period Ⅲ. However, two Ca-MBF CWs remained approximately 9% ($p<0.05$) higher than CW1 (21.63 ± 4.07)% in TP removal. Regarding the whole experimental period, CW3 achieved the best TP removal with (33.13 ± 10.13)%, which was 6.30% ($p<0.05$) and 2.29% higher than CW1 and CW2, respectively. These results revealed that Ca-MBF enhanced the removal of TP, and the layered tiling was slightly better than vertical filling. Generally, the pathways of phosphorus removal in CWs mainly include uptake by plants, physical adsorption and chemical precipitation by substrates, and degradation by microorganisms[25]. In the present study, calcium was used to modify BF, which could form more active phosphate

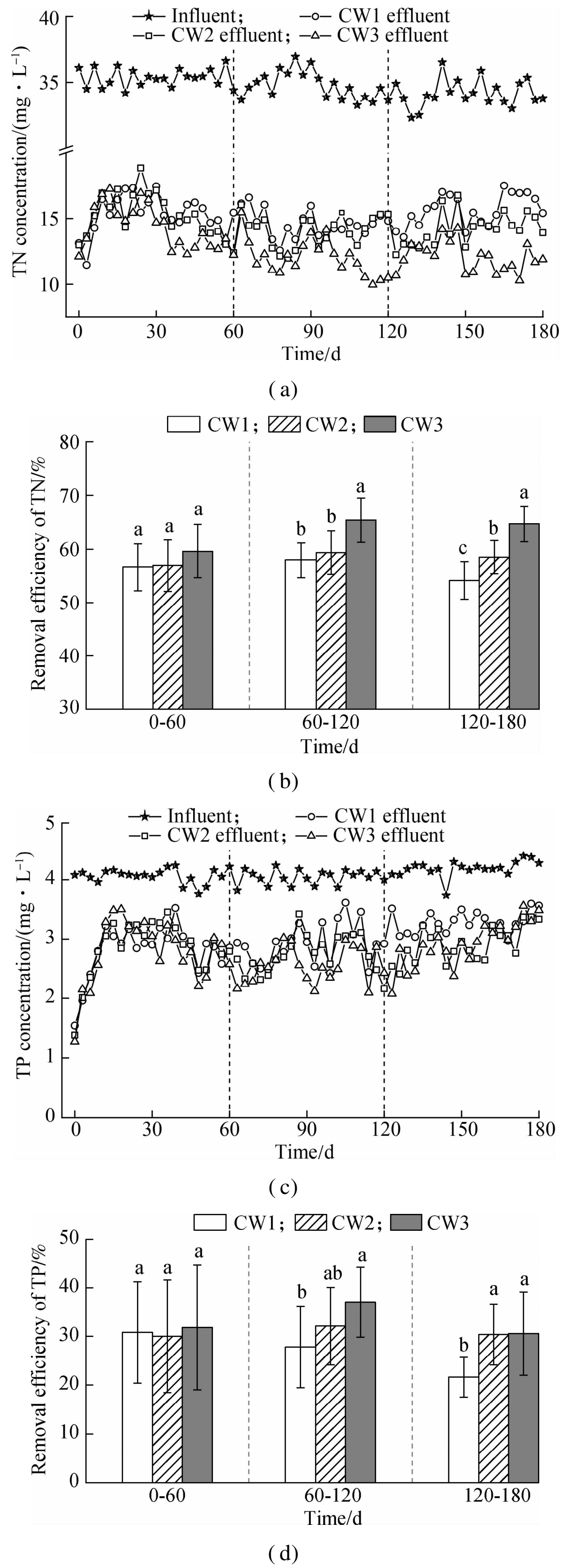


Fig. 4 TN and TP removal performance of three CWs. (a) TN concentrations; (b) Average removal efficiency of TN; (c) TP concentrations; (d) Average removal efficiency of TP

binding sites[26] on Ca-MBF and increase its maximum phosphorus adsorption capacity[27]. Therefore, Ca-MBF increased the amount of phosphorus adsorbed by the substrate, which was the reason for an increase in TP removal.

2.3 Substrate-enzyme activity

Substrate enzymes are crucial in the transformation and decomposition of contaminants; their activities can be used to assess the running status and microbial activity of CWs[28]. For instance, AMO and NOR are two enzymes in nitrification, whereas NAR and NIR are two enzymes related to denitrification. DHA is responsible for the removal of organic matter[29]. PST can catalyze the conversion of organic phosphorus to inorganic phosphorus[30].

It is widely known that wastewater purification is achieved by the whole CW system. The above six enzyme activities at different depths were measured to evaluate the CW performance. As shown in Fig. 5, enzyme activity decreased with increasing substrate depth in the three CWs. This was because plant root secretions were crucial sources of soil enzymes[31], whereas the middle and lower layers of CWs were far from the plant root. Ca-MBF dramatically enhanced the enzyme activity related to the nitrogen cycle in the middle and lower sand layers, which improved the mean enzyme activity on a depth scale. For instance, 14.16% and 59.00% increases in the average AMO activity of CW2 and CW3, respectively, were observed (see Fig. 5(a)), which can facilitate the conversion of NH_4^+-N to NO_2^--N. NOR was slightly enhanced by Ca-MBF, with CW2

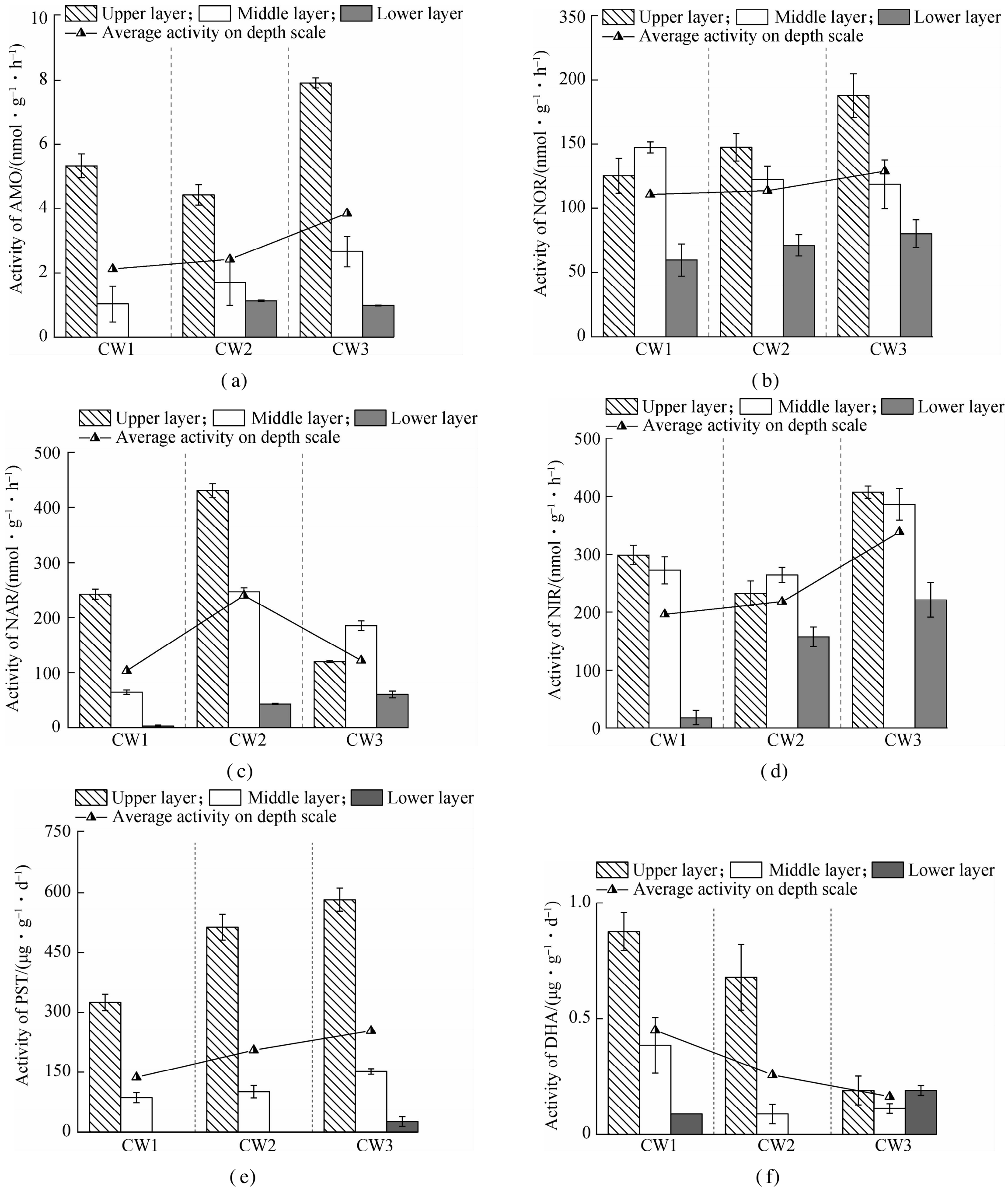


Fig. 5 Variations of enzyme activities in different substrate layers and average enzyme activities on a depth scale. (a) AMO; (b) NOR; (c) NAR; (d) NIR; (e) PST; (f) DHA

and CW3 improved by 2.62% and 16.29%, respectively (see Fig. 5(b)). NAR activity of CW2 and CW3 increased by 132.40% and 17.91%, respectively (see Fig 5(c)). NIR activity was increased by 10.65% in CW2 and 72.36% in CW3 (see Fig. 5(d)) owing to the increase of NIR activity in the lower layer, which was enhanced by 777.32% in CW2 and 1 133.59% in CW3 compared with the control. The increase in NAR and NIR activity supported better nitrogen removal in Ca-MBF CWs. The average PST activity of CW2 and CW3 increased by 49.45% and 84.82%, respectively (see Fig. 5(e)). It could explain the higher TP removal efficiencies of Ca-MBF CWs. However, Ca-MBF decreased the mean activity of DHA at different depths (see Fig. 5 (f)). The present study found that Ca-MBF with layered tiling was better than vertical filling in improving the enzyme activity of the whole system. The huge and rough surface of Ca-MBF favored microbial immobilization and biofilm formation. The biomass of microbes generally decreased with increasing soil depth[32]. Ca-MBF with layered-tiling horizontally affected the CWs microenvironment and enhanced microbial activity in Ca-MBF filled planes. However, Ca-MBF with vertical-filling affected the CWs microenvironment vertically and enhanced microbial activity in the space filled with Ca-MBF. Therefore, it meant that two filling modes had different effects on microbes, which led to differences in enzyme activity.

2.4 Impact of Ca-MBF on EPS content

EPS is the main component of biofilms, which can enhance the resistance of microbial cells to external environmental changes and influence biofilm stability[33]. PS and PN are the two major components of EPS[34]. In contrast to CW1, the content of PS + PN in CW2 and CW3 increased by 28.56% and 57.80% in the upper layer (see Fig. 6(a)), 35.84% and 26.99% in the middle layer (see Fig. 6(b)), and 22.29% and 74.60% in the lower layer (see Fig. 6(c)), respectively. EPS can enhance the removal of pollutants[35] and keep the internal environment more stable[36]. The average content of PS + PN improved by 29.02% in CW2 and 52.90% in CW3 compared to the control (see Fig. 6(d)), which were aligned with the removal of nutrients by the three CWs in the whole experiment period. Overall, Ca-MBF with layered tiling was better than that with vertical filling. EPS is secreted by microorganisms[37]. Two filling modes provided different attachment surfaces for microorganisms, which inevitably affected the content of EPS. Regarding specific components, Ca-MBF promoted PN secretion in EPS, particularly in the lower layer of CW2 and CW3, with an increase of 140.64% and 365.66%, respectively, because a higher concentration of calcium can increase the PN content in EPS[38].

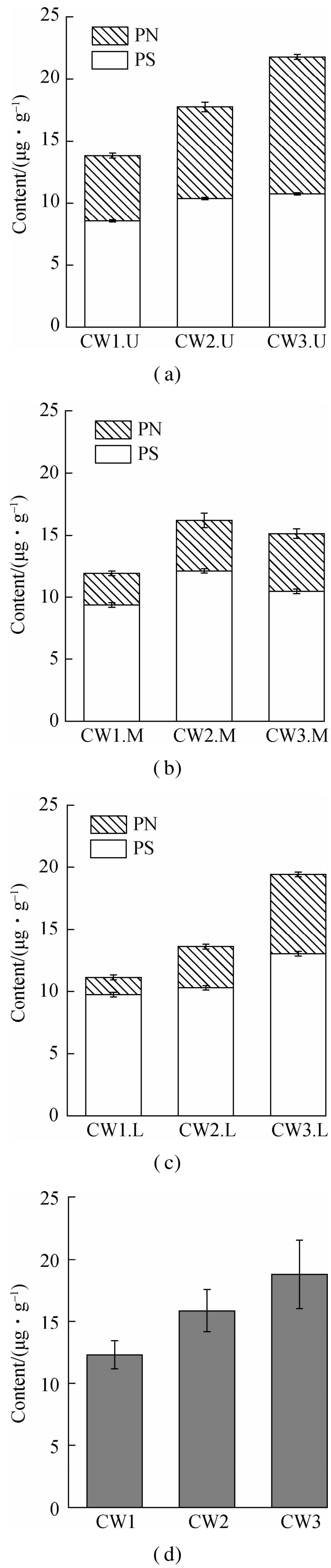


Fig. 6 Variations of PS and PN content in three CWs. (a) Upper layer; (b) Middle layer; (c) Lower layer; (d) Average content of PS + PN

2.5 Microbial community composition

2.5.1 Effect of Ca-MBF on alpha diversity

Alpha diversity is the diversity within a particular region or ecosystem. When evaluating the alpha diversity of the microbial community, the richness and diversity should be considered simultaneously. In this study, the Chao 1 index was used to evaluate species richness, and the Shannon and Simpson indexes were used to characterize species diversity. As shown in Tab. 3, Chao1 and Shannon and Simpson indexes of CW1 declined with increasing substrate depth, indicating that the richness and diversity of the microbial community diminished. However, the trend of the indexes in the two Ca-MBF CWs was different from those in the control. These indexes in the lower layers were greater than those in the middle layers. Additionally, the three indexes in the lower layer of two Ca-MBF CWs were higher than those of the control. Moreover, the average values of Chao1 and Shannon and Simpson in two Ca-MBF CWs were higher than that in the control. This indicated that Ca-MBF changed the spatial distribution of the microbial community and increased the alpha diversity in CWs.

Tab. 3 Alpha diversity analysis index at different depths in three CWs

System	Sample	Chao1 index	Shannon index	Simpson index
CW1	CW1. U	997.5	3.89	0.935 0
	CW1. M	658.6	3.29	0.906 1
	CW1. L	561.8	2.27	0.693 0
	CW1. average	739.3	3.15	0.844 7
CW2	CW2. U	936.9	3.38	0.900 7
	CW2. M	754.8	2.77	0.819 0
	CW2. L	758.7	3.31	0.898 0
	CW2. average	816.8	3.15	0.872 6
CW3	CW3. U	1 017.7	3.85	0.936 0
	CW3. M	630.4	2.75	0.810 0
	CW3. L	698.7	3.20	0.892 0
	CW3. average	782.3	3.27	0.879 3

2.5.2 Shift in microbial community composition

The distribution of microbial communities in different depths at the phylum level varied among CWs (see Fig. 7(a)). *Proteobacteria*, *Bacteroidetes*, *Cloacimonetes*, and *Chloroflexi* were the dominant phyla in all communities, accounting for 92.09%-98.10%. In the upper and middle layers, the relative abundance of *Proteobacteria* was 82.86% and 86.27%, respectively, in CW2 and 79.87% and 80.55%, respectively, in CW3, which were notably higher than 60.35% and 58.94%, respectively in CW1. *Proteobacteria* includes numerous bacteria associated with nitrogen and phosphorus removal[39], which may be the reason for Ca-MBF CWs achieving higher removal efficiencies of the pollutants. Conversely, compared with Ca-MBF CWs, *Bacteroidetes*, as the second largest phylum in the samples (7.65%-34.52%), was more abundant in the upper and middle layers of the control. Additionally, it is worth noting that *Cloacimonetes*, a phylum commonly found in anaerobic reactors[40], accounted for 19.27% at the lower layer in CW3, which was greater than that of the control. Not coincidentally, the relative abundance of *Chloroflexi* in the lower layer of CW2 (6.15%) and CW3 (4.70%) was higher than in the control (2.52%). It was clear that Ca-MBF caused a change in the distribution of microbial communities in CWs, especially in the distribution of *Proteobacteria* and *Bacteroidetes*.

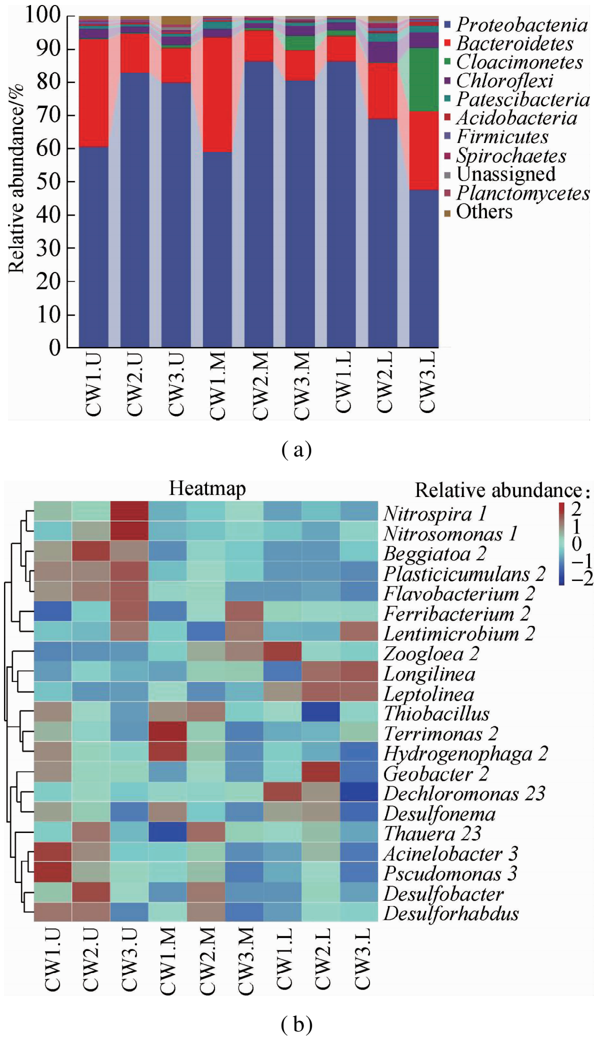


Fig. 7 Microbial community composition. (a) Relative abundance of the top 10 phyla; (b) Heatmap of major functional bacteria genera

Focusing on the function of bacteria at the genus level may help in understanding the reasons for enhanced decontamination in Ca-MBF CWs. In Fig. 7(b), the numbers 1, 2, and 3 marked on the bacteria represent the functions of nitrification, denitrification, and phosphorus removal, respectively. Ammonia-oxidizing bacteria (AOB) are known to play critical roles in NH_4^+-N removal[41], and nitrite-oxidizing bacteria can oxidize NO_2^--N to

NO_3^--N[42]. In the upper layer, *Nitrosomonas* increased to 0.029% in CW2 and 0.156% in CW3, from 0.003% in CW1. Interestingly, the abundance of *Nitrospira* in CW3 (0.621%) was over 10 times higher than that in CW1 (0.045%). This indicated that Ca-MBF was beneficial for the enrichment of nitrifying bacteria in the plant rhizosphere. The total relative abundance of denitrifying bacteria (DNB) in the upper layer of CW2 was 59.54%, followed by CW3 (53.92%) and CW1 (42.02%), suggesting that Ca-MBF was conducive to the colonization of DNB. Among them, *Zoogloea* (17.18%-18.59%), *Thauera* (5.21%-21.48%), *Dechloromonas* (6.95%-70.97%), *Ferribacterium* (0.80%-9.05%), and *Plasticicumulans* (3.03%-7.66%) were the main DNB. For polyphosphate-accumulating bacteria (PAO), *Acinetobacter* and *Pseudomonas*[43-44] in the upper layer of CW1 accounted for 0.533% and 0.356%, respectively, which were higher than those in Ca-MBF CWs. *Thauera* and *Dechloromonas* were denitrifying phosphorus-removing bacteria found in various wastewater treatment systems[45-46]. Compared with the control, *Thauera* was more abundant in the upper layer of CW2, with a relative abundance of 21.48%. However, *Dechloromonas* in the upper layers was close among the three systems, with an abundance of 6.95%-7.97%. In the middle layer, the abundance of nitrifying bacteria (0.001%-0.017%) decreased owing to limited oxygen. However, the abundance of DNB was further increased with 52.72% of CW1, 72.95% of CW2, and 71.27% of CW3, suggesting that Ca-MBF created a suitable environment for DNB growth. Among them, *Zoogloea* and *Thauera* were the most abundant bacteria in Ca-MBF CWs, which secrete EPS[47]. The abundance of *Zoogloea* and *Thauera* increased by 11.37% and 21.50% in CW2 and 17.68% and 8.15% in CW3, respectively, compared with the control, consistent with the increase of EPS content in the middle layer of Ca-MBF CWs. At the lower layer, DNB accounted for 39.26%-80.68% of the abundance in microbial communities. *Zoogloea* and *Dechloromonas* were more abundant in CW1. *Thauera* abundance in CW2 and CW3 decreased to 11.74% and 4.44%, respectively. Moreover, *Longilinea* and *Leptolinea* were more abundant in Ca-MBF CWs, which can facilitate the aggregation of microorganisms[48]. Thus, these suggested that Ca-MBF altered the distribution of microbial communities and promoted the abundance of bacteria associated with nitrogen removal in the upper and middle layers of CWs.

2.5.3 Correlation analysis between functional genera

To further investigate the effect of Ca-MBF on the interaction between microorganisms, a correlation analysis was conducted (see Fig. 8). *Nitrospira*, *Nitrosomonas*, *Acinetobacter*, and *Pseudomonas* showed positive correlations with more bacteria in all the three CWs. Compared with the control, *Thauera*, *Hydrogenophaga*, and *Lentimicrobium* in CW2 revealed a positive correlation with more bacteria. Furthermore, *Dechloromonas*, *Ferribacterium*, and *Hydrogenophaga* in CW3 were positively correlated with more bacteria. The above results indicated that the cooperative interaction of microorganisms was more prominent in Ca-MBF CWs.

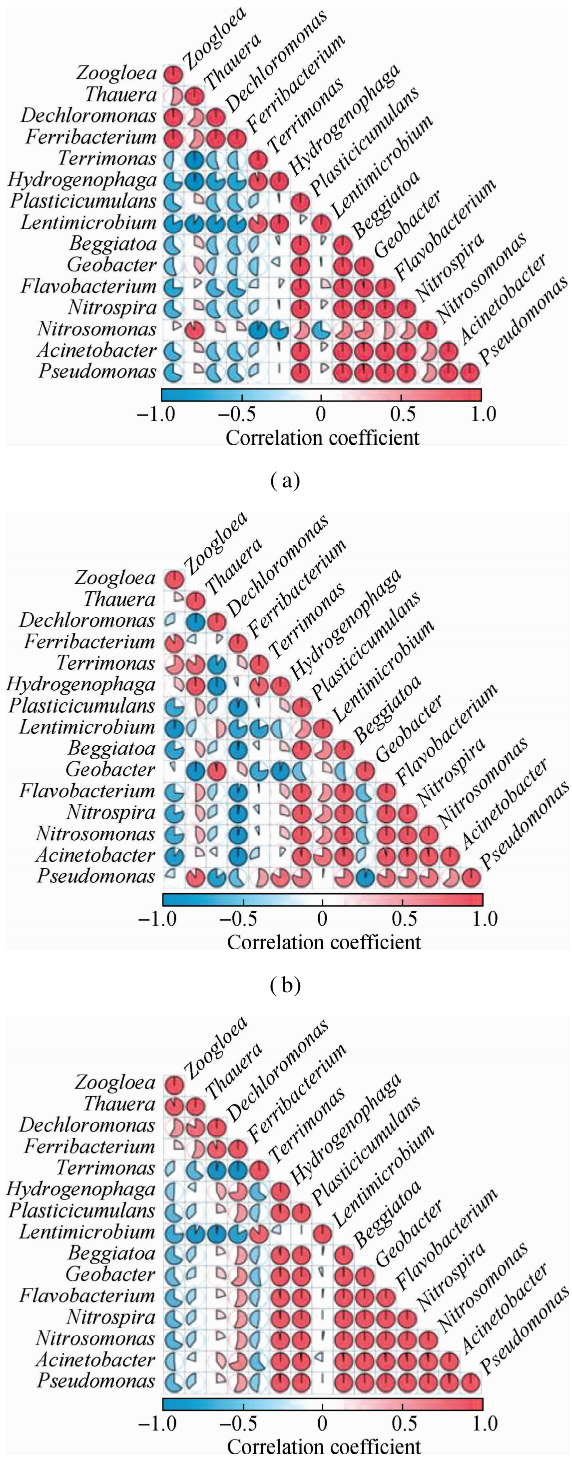


Fig. 8 Correlation analysis between functional genera in CWs. (a) CW1; (b) CW2; (c) CW3

3 Conclusions

1) Ca-MBF improved the mean activity of substrate enzymes at different depths, including ammonia monooxygenase, nitrite oxidoreductase, nitrate reductase, nitrite reductase, and phosphatase.

2) Ca-MBF provided a larger surface area for microbial attachment and stimulated EPS secretion.

3) Ca-MBF increased microbial richness and diversity, modified microbial community structure, increased the abundance of nitrifying and denitrifying bacteria in the upper and middle layers of CWs, and promoted cooperation between functional genera.

4) Ca-MBF with layered tiling was better than that with vertical filling in CWs. Compared with the conventional CW, the removal efficiencies of ammonia nitrogen, total nitrogen, and total phosphorus increased by 16.72%, 6.95%, and 6.30%, respectively, in CW with layered-tiling Ca-MBF.

References

[1] Wu H M, Zhang J, Ngo H H, et al. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation[J]. *Bioresource Technology*, 2015, **175**: 594 − 601. DOI: 10.1016/j.biortech.2014.10.068.

[2] Huang L, Wang N, Deng C R, et al. Interactive effect of carbon source with influent COD/N on nitrogen removal and microbial community structure in subsurface flow constructed wetlands[J]. *Journal of Environmental Management*, 2019, **250**: 109491. DOI: 10.1016/j.jenvman.2019.109491.

[3] Gu X S, Chen D Y, Wu F, et al. Function of aquatic plants on nitrogen removal and greenhouse gas emission in enhanced denitrification constructed wetlands: Iris pseudacorus for example[J]. *Journal of Cleaner Production*, 2022, **330**: 129842. DOI: 10.1016/j.jclepro.2021.129842.

[4] Blanco I, Molle P, de Miera L E S, et al. Basic oxygen furnace steel slag aggregates for phosphorus treatment. Evaluation of its potential use as a substrate in constructed wetlands [J]. *Water Research*, 2016, **89**: 355 − 365.

[5] Lai X S, Zhao Y Q, Pan F X, et al. Enhanced optimal removal of nitrogen and organics from intermittently aerated vertical flow constructed wetlands: Relative COD/N ratios and microbial responses[J]. *Chemosphere*, 2020, **244**: 125556. DOI: 10.1016/j.chemosphere.2019.125556.

[6] Ji Z H, Tang W Z, Pei Y S. Constructed wetland substrates: A review on development, function mechanisms, and application in contaminants removal [J]. *Chemosphere*, 2022, **286**: 131564. DOI: 10.1016/j.chemosphere.2021.131564.

[7] Li M, Duan R, Hao W, et al. Utilization of elemental sulfur in constructed wetlands amended with granular activated carbon for high-rate nitrogen removal[J]. *Water Research*, 2021, **195**: 116996. DOI: 10.1016/j.watres.2021.116996.

[8] Fu G P, Wu J F, Han J Y, et al. Effects of substrate type on denitrification efficiency and microbial community structure in constructed wetlands[J]. *Bioresource Technology*, 2020, **307**: 123222. DOI: 10.1016/j.biortech.2020.123222.

[9] Zhang X Y, Zhou X T, Xie Y J, et al. A sustainable biocarrier medium for wastewater treatment: Modified basalt fiber[J]. *Journal of Cleaner Production*, 2019, **225**: 472 − 480. DOI: 10.1016/j.jclepro.2019.03.333.

[10] Zhao Y, Zhang R, Jing L D, et al. Performance of basalt fiber-periphyton in deep-level nutrient removal: A study concerned periphyton cultivation, characterization and application[J]. *Chemosphere*, 2022, **291**: 133044. DOI: 10.1016/j.chemosphere.2021.133044.

[11] Guo K, Freguia S, Dennis P G, et al. Effects of surface charge and hydrophobicity on anodic biofilm formation, community composition, and current generation in bioelectrochemical systems [J]. *Environmental Science and Technology*, 2013, **47**(13): 7563 − 7570. DOI: 10.1021/es400901u.

[12] Ni H C, Wang C C, Arslan M, et al. Enhanced wastewater treatment by modified basalt fiber bio-carriers: Effect of etching and surface functionalization[J]. *Journal of Cleaner Production*, 2022, **343**: 130927. DOI: 10.1016/j.jclepro.2022.130927.

[13] Zhang X Y, Ding J Z, Gao F Y, et al. Enhancement of nitrogen removal in hybrid wastewater treatment system using ferric citrate modified basalt fiber biocarrier[J]. *Environmental Science and Pollution Research*, 2021, **28**(25): 33480 − 33490. DOI: 10.1007/s11356-021-12941-7.

[14] Zhang X Y, Rong X S, Xu J C, et al. Effect of surface modification of basalt fiber on biofilm attachment [J]. *Journal of Materials Engineering*, 2019, **47**(5): 129 − 136. DOI: 10.11868/j.issn.1001-4381.2018.000189. (in Chinese)

[15] Ou Q, Xu Y H, Li X L, et al. Interactions between activated sludge extracellular polymeric substances and model carrier surfaces in WWTPs: A combination of QCM-D, AFM and XDLVO prediction[J]. *Chemosphere*, 2020, **253**: 126720. DOI: 10.1016/j.chemosphere.2020.126720.

[16] Liu Q J, Zhang C, Bao Y L, et al. Optimizing carbonfibre supports for bioreactors by nitric acid oxidation and calcium ion coverage according to extended DLVO theory [J]. *Environmental Technology*, 2020, **41**(1): 86 − 99. DOI: 10.1080/09593330.2018.1491636.

[17] Gao F Y, Zhou X T, Ma Y T, et al. Calcium modified basalt fiber bio-carrier for wastewater treatment: Investigation on bacterial community and nitrogen removal enhancement of bio-nest[J]. *Bioresource Technology*, 2021, **335**: 125259. DOI: 10.1016/j.biortech.2021.125259.

[18] Xiao J, Huang J, Huang M J, et al. Application of basalt fiber in vertical flow constructed wetland for different pollution loads wastewater: Performance, substrate enzyme activity and microorganism community [J]. *Bioresource Technology*, 2020, **318**: 124229. DOI: 10.1016/j.biortech.2020.124229.

[19] Chinese N E P A. *Waterand wastewater monitoring meth*

ods[M]. 4th ed. Beijing: China Environmental Press, 2002: 422 - 426. (in Chinese)

[20] Hu X B, Liu X B, Yang X Y, et al. Acute and chronic responses of macrophyte and microorganisms in constructed wetlands to cerium dioxide nanoparticles: Implications for wastewater treatment[J]. *Chemical Engineering Journal*, 2018, **348**: 35 - 45. DOI: 10.1016/j.cej.2018.04.189.

[21] Zheng X, Wu R, Chen Y G. Effects of ZnO nanoparticles on wastewater biological nitrogen and phosphorus removal[J]. *Environmental Science and Technology*, 2011, **45**(7): 2826 - 2832. DOI: 10.1021/es2000744.

[22] Yin C Q, Meng F G, Chen G H. Spectroscopic characterization of extracellular polymeric substances from a mixed culture dominated by ammonia-oxidizing bacteria[J]. *Water Research*, 2015, **68**: 740 - 749. DOI: 10.1016/j.watres.2014.10.046.

[23] Pellicer-Nàcher C, Domingo-Félez C, Mutlu A G, et al. Critical assessment of extracellular polymeric substances extraction methods from mixed culture biomass[J]. *Water Research*, 2013, **47**(15): 5564 - 5574. DOI: 10.1016/j.watres.2013.06.026.

[24] Frølund B, Griebe T, Nielsen P H. Enzymatic activity in the activated-sludge floc matrix[J]. *Applied Microbiology and Biotechnology*, 1995, **43**(4): 755 - 761. DOI: 10.1007/BF00164784.

[25] Grace K A, Juston J M, Finn D, et al. Substrate manipulation near the outflow of a constructed wetland reduced internal phosphorus loading from sediments and macrophytes[J]. *Ecological Engineering*, 2019, **129**: 71 - 81. DOI: 10.1016/j.ecoleng.2018.11.006.

[26] Liu X N, Shen F, Smith R L Jr, et al. Black liquor-derived calcium-activated biochar for recovery of phosphate from aqueous solutions [J]. *Bioresource Technology*, 2019, **294**: 122198. DOI: 10.1016/j.biortech.2019.122198.

[27] Lü N, Li X F, Qi X G, et al. Calcium-modified granular attapulgite removed phosphorus from synthetic wastewater containing low-strength phosphorus [J]. *Chemosphere*, 2022, **296**: 133898. DOI: 10.1016/j.chemosphere.2022.133898.

[28] Yan Q, Xu Y F, Yu Y H, et al. Effects of pharmaceuticals on microbial communities and activity of soil enzymes in mesocosm-scale constructed wetlands [J]. *Chemosphere*, 2018, **212**: 245 - 253. DOI: 10.1016/j.chemosphere.2018.08.059.

[29] Huang L, Gao X, Liu M, et al. Correlation among soil microorganisms, soil enzyme activities, and removal rates of pollutants in three constructed wetlands purifying micro-polluted river water[J]. *Ecological Engineering*, 2012, **46**: 98 - 106. DOI: 10.1016/j.ecoleng.2012.06.004.

[30] Xiao J, Huang J, Wang M Y, et al. The fate and long-term toxic effects of NiO nanoparticles at environmental concentration in constructed wetland: Enzyme activity, microbial property, metabolic pathway and functional genes[J]. *Journal of Hazardous Materials*, 2021, **413**: 125295. DOI: 10.1016/j.jhazmat.2021.125295.

[31] Ai L, Wu F Z, Fan X B, et al. Different effects of litter and root inputs on soil enzyme activities in terrestrial ecosystems[J]. *Applied Soil Ecology*, 2023, **183**: 104764. DOI: 10.1016/j.apsoil.2022.104764.

[32] Liu R, Zhang Y, Hu X F, et al. Litter manipulation effects on microbial communities and enzymatic activities vary with soil depth in a subtropical Chinese fir plantation [J]. *Forest Ecology and Management*, 2021, **480**: 118641. DOI: 10.1016/j.foreco.2020.118641.

[33] Shi Y H, Huang J H, Zeng G M, et al. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: An overview[J]. *Chemosphere*, 2017, **180**: 396 - 411. DOI: 10.1016/j.chemosphere.2017.04.042.

[34] Dsane V F, Jeon H, Choi Y, et al. Characterization of magnetite assisted anammox granules based on in-depth analysis of extracellular polymeric substance (EPS)[J]. *Bioresource Technology*, 2023, **369**: 128372. DOI: 10.1016/j.biortech.2022.128372.

[35] Liu H Q, Hu Z, Jiang L P, et al. Roles of carbon source-derived extracellular polymeric substances in solids accumulation and nutrient removal in horizontal subsurface flow constructed wetlands[J]. *Chemical Engineering Journal*, 2019, **362**: 702 - 711. DOI: 10.1016/j.cej.2019.01.067.

[36] Xiong Y H, Liu Y. Importance of extracellular proteins in maintaining structural integrity of aerobic granules[J]. *Colloids and Surfaces B: Biointerfaces*, 2013, **112**: 435 - 440. DOI: 10.1016/j.colsurfb.2013.07.060.

[37] Siddharth T, Sridhar P, Vinila V, et al. Environmental applications of microbial extracellular polymeric substance (EPS): A review[J]. *Journal of Environmental Management*, 2021, **287**: 112307. DOI: 10.1016/j.jenvman.2021.112307.

[38] Higgins M, Novak J. The effect of cations on the settling and dewatering of activated sludges: Laboratory results [J]. *Water Environment Research*, 1997, **69**(2): 215 - 224.

[39] Petitjean A, Forquet N, Boutin C. Oxygen profile and clogging in vertical flow sand filters for on-site wastewater treatment [J]. *Journal of Environmental Management*, 2016, **170**: 15 - 20. DOI: 10.1016/j.jenvman.2015.12.033.

[40] Qin X C, Ji M M, Wu X G, et al. Response of treatment performance and microbial community structure to the temporary suspension of an industrial anaerobic bioreactor [J]. *Science of the Total Environment*, 2019, **646**: 229 - 237. DOI: 10.1016/j.scitotenv.2018.07.309.

[41] Su Y, Wang W D, Wu D, et al. Stimulating ammonia oxidizing bacteria (AOB) activity drives the ammonium oxidation rate in a constructed wetland (CW)[J]. *Science of the Total Environment*, 2018, **624**: 87 - 95. DOI: 10.1016/j.scitotenv.2017.12.084.

[42] Wang J T, Zhan G Q, Yang X, et al. Rapid detection of nitrite based on nitrite-oxidizing bacteria biosensor and its application in surface water monitoring[J]. *Biosensors and Bioelectronics*, 2022, **215**: 114573. DOI: 10.1016/j.bios.2022.114573.

[43] Li H K, Zhong Y M, Huang H, et al. Simultaneous nitrogen and phosphorus removal by interactions between phosphate accumulating organisms (PAOs) and denitrifying phosphate accumulating organisms (DPAOs) in a sequencing batch reactor[J]. *Science of the Total Environ-*

ment, 2020, **744**: 140852. DOI: 10.1016/j.scitotenv.2020.140852.

[44] Nie Z B, Huo M X, Li Q Z, et al. Sewage treatment effect of AOA-SBR under different C/P value and its mechanism of nitrogen and phosphorus removal[J]. *Journal of Water Process Engineering*, 2022, **47**: 102774. DOI: 10.1016/j.jwpe.2022.102774.

[45] Liu X Y, Yang H, Wang S L, et al. Study on the effectiveness of an independent biological phosphorus removal system based on immobilized biological fillers nitrogen removal system in municipal wastewater[J]. *Process Safety and Environmental Protection*, 2021, **156**: 17-28. DOI: 10.1016/j.psep.2021.09.017.

[46] Ren T, Chi Y L, Wang Y, et al. Diversified metabolism makes novel Thauera strain highly competitive in low carbon wastewater treatment[J]. *Water Research*, 2021, **206**: 117742. DOI: 10.1016/j.watres.2021.117742.

[47] Qian X W, Huang J, Yan C N, et al. Ecological restoration performance enhanced by nano zero valent iron treatment in constructed wetlands under perfluorooctanoic acid stress[J]. *Science of the Total Environment*, 2022, **846**: 157413. DOI: 10.1016/j.scitotenv.2022.157413.

[48] Chen C M, Liu Z Y, Wu B C, et al. Improving the anaerobic treatment of phenolic wastewater by using peat soil [J]. *Industrial Water Treatment*, 2019, **39**(12): 19-22, 54. DOI: 10.11894/iwt.2019-0377. (in Chinese)

钙改性玄武岩纤维对人工湿地污水处理性能的提升

黄 娟 魏智辉 季小雨 闫春妮 马溢轩 钱秀雯

(东南大学土木工程学院,南京211189)

摘要:为研究钙改性玄武岩纤维(Ca-MBF)及其填充方式对人工湿地污水处理性能的影响,构建中试规模的分层平铺和垂直填充Ca-MBF人工湿地,并与传统人工湿地的除污效果、酶活性空间分布、胞外聚合物含量及微生物群落结构进行对比.结果表明,Ca-MBF能提高人工湿地微生物多样性和丰富度,增加基质上层与中层硝化菌和反硝化菌丰度,促进功能属之间合作.Ca-MBF显著提高了湿地中层和下层的酶活性,使整个系统的平均酶活性升高(氨单加氧酶、亚硝酸氧化还原酶、硝酸还原酶、亚硝酸还原酶和磷酸酶).此外,Ca-MBF促进了胞外聚合物的分泌,平均增加了29.02%~52.90%.Ca-MBF能提升湿地去除污染物的能力,分层平铺的Ca-MBF湿地效果最好,其对氨氮、总氮和总磷去除率分别提高16.72%、6.95%和6.30%.这些结果为Ca-MBF人工湿地的应用提供了有价值的参考.

关键词:人工湿地;钙改性玄武岩纤维;填充方式;酶活性;胞外聚合物;微生物群落

中图分类号:X703