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Biofilm cultivated in SBR, together with hydroponic *Chlorophytum comosum* for eutrophic water remediation

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ABSTRACT

Recently, the eutrophication of water bodies has become increasingly severe in China. Considering the single method of phytoremediation, it still remains the issues including low removal efficiency, high cost, and secondary pollution. However, the combination approach of biofilm and plants to strengthen the remediation for eutrophic water bodies has advantages of cost-effectiveness without causing secondary pollution. Hence, microorganisms from biofilm developed in polymeric fibrous carriers from SBR, together with *Chlorophytum comosum* (*C. comosum*), for eutrophic water body recovery, were investigated in this study, to improve water quality, reduce engineering cost and ensure ecological safety. The results indicated that the microorganisms from the bottom sediment of the eutrophic water body were commendably acclimated and formed a mature biofilm in SBR. Each cycle period setting up aeration 10 h and idleness 2 h, the effluent water quality maintained a stable level after the 20th cycle period and the highest removal efficiency of ammonia nitrogen (NH_4^+–N), total phosphorus (TP), and chemical oxygen demand (COD_{Cr}) in SBR accounted for 86%, 74%, and 67%, respectively. The biofilm microbes combined with *C. comosum* were improvement of COD_{Cr} removal efficiency at least 8% and increased 10%–19% of permanganate index (PI) removal efficiency and 10%–18% of total nitrogen (TN) removal efficiency, as compared to separate biofilm microbes and single *C. comosum*. The reduction of TP was at 57% using the biofilm developed in polymeric fibrous carriers combined with *C. comosum*, which was 8% lower than that of separate *C. comosum* while 7% higher than that of single biofilm. The bench-scale combination of the biofilm developed in polymeric fibrous carriers and the hydroponic *C. comosum* could be capable of repairing eutrophic water originated from the Huajin River. This work may further provide implications for remediation of eutrophic Huajin River *in situ*.

Keywords: Biofilm; *Chlorophytum comosum*; Remediation; Eutrophication

1. Introduction

With the rapid development of urbanization and industrialization, anthropogenic activities produce a large amount of various wastewater, including domestic, industrial, and livestock wastewater. Due to the irrational discharge of wastewater, water bodies have been suffered serious eutrophication [1]. The input of excess nutrients, such as nitrogen

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and phosphorus, causes overgrowth of single species, a low oxygen condition, numerous creature death, leading to the deterioration of water quality, the inhibition or destruction of the ecosystem and aquatic functions [2,3]. It has been reported that the phenomenon of eutrophication frequently occurred in many municipal rivers and lakes owing to humanity activities [4–6]. The eutrophication of municipal rivers and lakes has not only reduced the life quality of residents but also posed a huge threat to human health [7,8]. Thus, it is essential to explore an energy-efficient method to inhibit eutrophication.

Currently, the treatment methods for eutrophic water bodies mainly include physical, chemical, or biological processes [9–11]. However, the physical approach has been known to require a long-term construction period and expensive construction cost, and the chemical pathway produces harmful by-products due to extra additives [10,12]. A financially practical, reliable, and efficient method is biological treatment technology [9,13]. In fact, activated sludge and biofilm are the traditional biological methods to degrade organic matter, nitrogen, and phosphorus loading in treating eutrophic water bodies [14–16]. It is in particular that the biological method using biofilm has the advantages of a relatively stable microenvironment for microbial aggregation and a low suspended solid production [17,18]. On one hand, biological carriers provide a longer solids retention time, as compared to the activated sludge process, to promote degradation efficiency of chemical oxygen demand (COD_{Cr}), permanganate index (PI), ammonia nitrogen (NH_4^+–N), total nitrogen (TN), and total phosphorus (TP) [19,20]. On the other hand, biofilm configured sequencing batch reactor (SBR) with intermittent operation mode is crucial for organic matter degradation [20,21].

Aside from activated sludge and biofilm method, phytoremediation has been increasingly used to clean up contaminated soil and water systems due to its lower costs and fewer negative effects than physical or chemical engineering approaches [22,23]. Many studies have documented the ability of hydrophytes such as free-floating, floating-leaved, emergent, and submerged plants to remediate eutrophication water bodies [24–26]. The hydrophytes showed several qualities that make them attractive for use in phytoremediation owing to their high biomass production, efficient nutrient uptake, and obvious organic degradation through their root exudates or rhizosphere microorganisms [27–30]. *C. comosum*, a liliaceous plant species, is a common ornamental plant [31,32]. To our best knowledge, *C. comosum* plays an important role in the phytoremediation of heavy metals such as Cd, Pb, and Zn contaminated soil due to its excellent tolerance and accumulation [31,33,34]. Although it can also grow well in water, little investigation focuses on *C. comosum* in response to N, P nutrients removal in eutrophication water bodies.

Most of the microorganisms appear in water as planktonic or pelagic organisms. If microorganisms were attached or fixed in the biological carriers to ensure a massive microbial population and stable reproduction and growth, the attachment of microorganisms would play an essential role in water purification relying on their decomposition, nitrification, or denitrification [16,20]. Currently, an ecological floating bed (EFB) is highly efficient in removing nitrogen and phosphorus in water and plays an important role in purifying water bodies, greening, and beautifying the environment [30,35,36]. The EFB combines the aquatic plants and microorganisms together, which makes the plant roots and water microorganisms form a steady mutualism system resulting in water purification and governance [36,37]. Although the EFB has attracted considerable attention in recent years, little is known about a strengthened EFB that is constructed on the combination of biofilms developed in polymeric fibrous carriers and plants to remediate eutrophic water bodies.

Thus, the objective of this paper was to utilize the biofilm developed in polymeric fibrous carriers combined with *C. comosum* to explore remediation efficiency for eutrophic water. Firstly, the bottom sediments originated from the eutrophic water body were cultured in SBR to form mature biofilm using polymeric fibrous carriers that could attach microorganisms. Then four experimental groups of the control group (CK), the biofilms developed in polymeric fibrous carriers (M), *C. comosum* (P) as well as the combination of biofilms developed in polymeric fibrous carriers and *C. comosum* (MP) were subsequently set to degrade COD_{Cr}, PI, TN, and TP in eutrophic water. Based upon the degradation, the restoration efficacy of eutrophic water was discussed through the biofilms developed in polymeric fibrous carriers combined with *C. comosum*.

2. Materials and methods

2.1. Biofilm cultivating and domesticating

2.1.1. Experiments apparatus

The bench-scale SBR with the dimensions of 20 cm × 20 cm × 40 cm (in length × in width × in height) and a working volume of 14 L was fabricated using transparent glass material. A schematic representation of the bioreactor is depicted in Fig. 1. The reactor was an open vessel with an installation of a set of the cross on the top of the container to hang biofilm carriers. The polymeric fibrous carriers consisted of several hollow discs in series. Each hollow disc with a diameter of

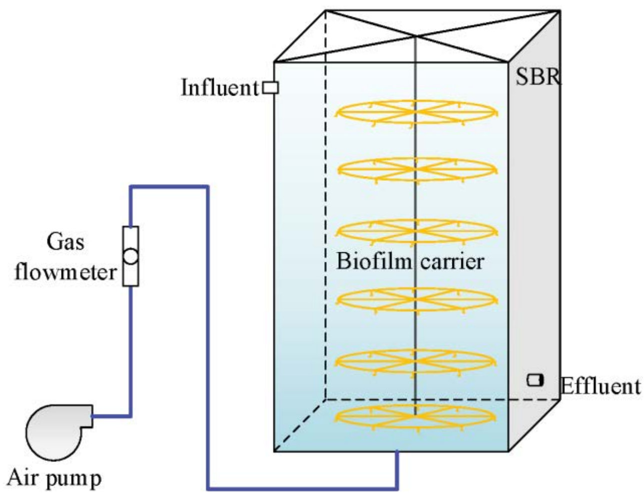

Fig. 1. Schematic diagram of biofilm cultivating in SBR.

15 cm was made of polyamide, polypropylene, and polyethylene. The influent and effluent vents with a diameter of 1 cm were installed in the reactor. The effluent port at 5 cm from the bottom of the reactor was connected with a rubber pipe and pinchcock. The reactor was aerated by fine bubble diffusers provided at the bottom of the vessel. Air was supplied to the reactor by an air pump (F3900) and the aeration rate was controlled by a flow meter (LZLZB-10).

2.1.2. Raw wastewater and seed sludge

Raw wastewater used in this study was obtained from Huajin River in Anhui Normal University in China because the river has become eutrophic water. The initial concentration of water quality in the Huajin River is shown in Table 1. The reactor was seeded with activated sludge collected from the bottom of sediments in the Huajin River, which was used to cultivate biofilm microorganisms.

The specific sampling process was detailed as follows. Field sampling points were set along the Huajin River. In each sampling point, the raw wastewater was taken at the depth of about 30 cm using a collection container with 2 L volume and then was poured into a plastic bucket with 5 L volume. The bottom sludge of the river was fetched using the mud sampler in sampling points. Those rotten biological tissues, stones, and other impurities were removed before putting sludge into the collecting bag.

2.1.3. Biofilm cultivating in SBR

The SBR was run at room temperature (22°C–25°C). The volume ratio of the raw wastewater to the seed sludge was 4 to 1. And the raw wastewater and the seed sludge were further mixed and stirred. The mixture of water and sludge was then flowed into SBR to cultivate the biofilm and the reactor was sequentially aerated 72 h with the objective of forming aerobic microorganisms. Subsequently, each cycle of the SBR was 10 h, which included 8 h of aeration reaction, 2 h of static time. The treated water in the reactor was replaced by the raw wastewater per 5 cycles and the volume exchange ratio was close to 100%. Air was pumped and diffused to the bottom of the reactor to provide oxygen to the reactor with an air-flow rate of 0.2–0.3 m^3/L during the aeration reaction phase.

2.1.4. Effectiveness analysis of cultured biofilms

Water samples of 100 mL were collected from reactors in the effluent stage of the 5th, 10th, 15th, 20th, 25th, and 30th cycle period to determinate COD_{Cr}, NH_4^+–N, and TP. Sludge samples were sampled from reactors when sludge and water were completely mixed in the aeration stage of the 5th, 15th, 25th, and 35th to measure MLSS, SV, and SVI.

Table 1
Initial concentration of water quality in Huajin River

Water quality parameters	Concentration (mg/L)
COD_{Cr}	30.98–51.08
NH_4^+–N	1.51–1.89
TN	1.25–1.34
TP	0.44–0.56

2.2. Source and treatment of C. comosum

Seedlings of *C. comosum* with aerial roots were separated from the fertile mother branch, which was from the ecology laboratory of Anhui Normal University. Those seedlings were cultivated using a 50% modified Hoagland's solution [23,38] and the concentration of the solution is shown in Table 2. After 7 weeks, the seedlings' roots have been stabilization and similar plants of the seedlings were selected to use the subsequent experiment from section Combination of the mature biofilm and *C. comosum*.

2.3. Combination of the mature biofilm and C. comosum

The laboratory-scale EFB (Fig. 2) was developed. The size of the EFB device was 20 cm × 20 cm × 20 cm with an effective depth of 15 cm and a total volume of 6 L. The EFB has consisted of the biofilm developed in polymeric fibrous carriers, styrofoam plate, and hydroponic *C. comosum*. The styrofoam plate with 1 cm thickness and 15 cm diameter was placed in the device as a floating bed. Below the styrofoam plate, the mature biofilm attached to the polymeric fibrous carrier was connected. There was a hole with a 3 cm diameter to be used to insert hydroponic *C. comosum* with a strong root system.

The combination of the mature biofilm and hydroponic *C. comosum* as EFB was used to treat the eutrophic water from the Huajin River. The combination group was regarded as MP in the experiment. The other three groups were considered as a contrast including the blank control group (CK), the mature biofilm (M), and the hydroponic *C. comosum* (P). The operation was at ambient temperature, and each group was provided dissolved oxygen intermittently from the bottom of the water tank using the air compressor. The properties of COD_{Cr}, COD_{Mn}, TN, and TP were evaluated on days 2, 3, 6, 9, 12, and 15 during the experiment.

2.4. Determination and data analysis

COD_{Cr}, PI, NH_4^+–N, TP, TN, mixed liquor suspended solids (MLSS), settling velocity (SV), and sludge volume index (SVI) were measured according to the Water and Wastewater Monitoring Analysis Method [40]. Analysis of

Table 2
Level of 50% modified Hoagland solution [39]

Composition	Concentration (g/L)
KNO_{3r}	0.253
$Ca(NO_3)_2$	0.472
NH_4NO_3	0.040
$NH_4H_2PO_4$	0.068
$MgSO_4$	0.247
$FeSO_4$	0.013
H_3BO_3	0.003
$C_{10}H_{14}N_2Na_2O_8·2H_2O$	0.019

experimental data was performed with Micro Office Excel 2010 and figures were plotted by Origin Pro 9.0.

3. Results and discussion

3.1. Effectiveness of biofilm cultivating

3.1.1. Biofilm for COD_{Cr} removal

Fig. 3 shows that biofilm was cultivated to remove COD_{Cr} from the eutrophic water of Huajin River in SBR. The inflow concentration of COD_{Cr} during the test was between 30.98 and 51.08 mg/L (Table 1). Based on China's National Environmental Quality Standards for Surface Water (GB3838-2002) [41], the Huajin River was worse than category V water quality standards. The concentration of COD_{Cr} in the effluent was decreased obviously during the first 20 cycles. The trend remained at a level of about 12.05 mg/L after the 20th cycle, which obtained the Standard of the Class I Status (GB3838-2002). The removal efficiency of COD_{Cr} was achieved to 67% in the 30th period. As a result, the degradation of COD_{Cr} in SBR was of significant increase. During cultivation, the biomass (MLSS) of biofilm started with a gradual increase tendency then remained a light decrease. While both sludge settling velocity (SV) and sludge volume index (SVI) from biofilm increased with cultivating period extension (Table 3). This was attributed to the formation of the mature biofilm with rich microbial aggregation attached to the polymeric fibrous carriers [17,19,21].

3.1.2. Biofilm for NH_4^+–N removal

As shown in Fig. 4, the contaminant of NH_4^+–N from the eutrophic Huajin River was removed through cultivating biofilm in SBR. The influent concentration of NH_4^+–N from Huajin River ranged from 1.51 to 1.89 mg/L (Table 1), without meeting the Standard of the Class V Status (GB3838-2002) [41]. The concentration of NH_4^+–N

Table 3
Performance of biofilm biomass in SBR during operational periods

Index Periods	5	15	25	35
SV (mL/L)	100	109	140	147
MLSS (g/L)	1.25	1.28	1.30	1.26
SVI (mL/g)	80.00	85.16	107.69	116.66

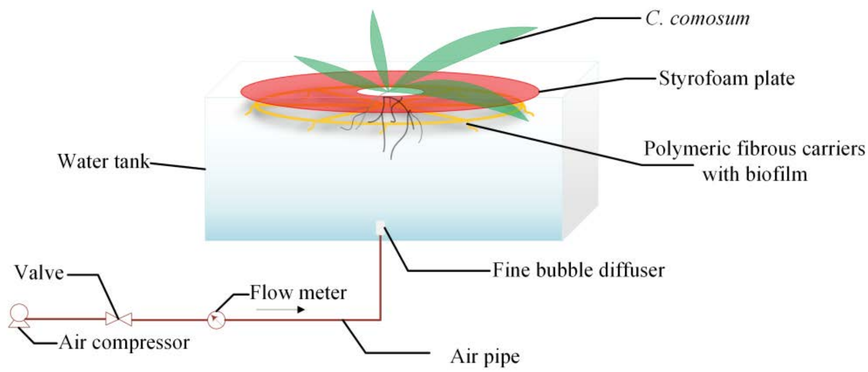

Fig. 2. Sketch of the pilot ecological floating bed (EFB) consisting of the mature biofilm and hydroponic *C. comosum.*

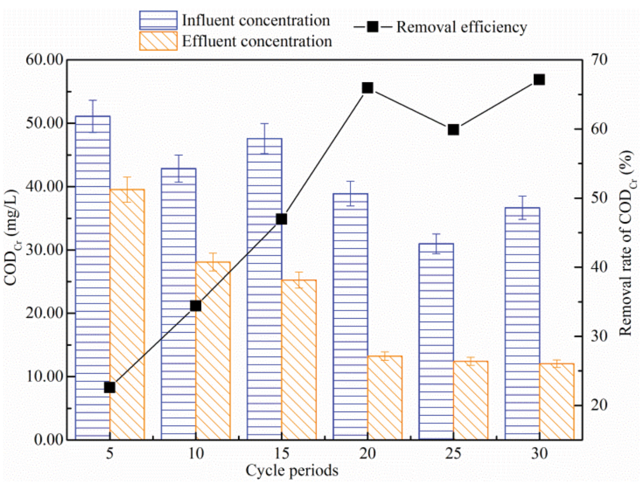

Fig. 3. COD_{Cr} removal of eutrophic water by cultivating biofilm in SBR.

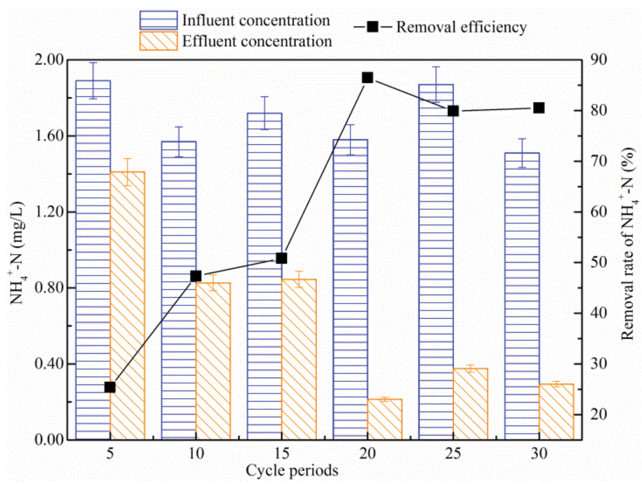

Fig. 4. NH_4^+–N removal of eutrophic water by cultivating biofilm in SBR.

in the effluent decreased significantly during the cultivation periods of biofilm. The effluent concentration of NH_4^+–N was reduced to the lowest value of 0.21 mg/L at the 20th period but increased to 0.38 and 0.29 mg/L at the 25th and 30th period, respectively. The removal efficiency of NH_4^+–N showed an increasing trend at the beginning. And the highest removal efficiency of NH_4^+–N was achieved to 86% at the 20th period. Then the NH_4^+–N removal efficiency showed a slight decrease, which was at 81% at the 30th period. The result indicated that the mature biofilm has been formed in the 30th period. That's contributed to the biofilm with an abundance of microbial aggregation adhered to the polymeric fibrous carrier for NH_4^+–N decomposition and degradation [15,18,20].

3.1.3. Biofilm for TP removal

The biofilm in SBR was cultured for the total phosphorus removal of eutrophic water from Huajin River as shown in Fig. 5. The influent concentration of TP during cultivation was between 0.44 and 0.56 mg/L, which was worse than the Standard of the Class V Status (GB3838-2002) [41]. The effluent concentration of TP decreased obviously with the extension of cultivated periods. The final effluent concentration of TP at the 30th period was closed to the Standard of the Class II Status (GB3838-2002) [41]. The removal efficiency of TP showed a remarkable increase tendency. The removal efficiency of TP was only at 22% and 40% at the 5th and 10th periods, respectively. Then the removal efficiency of TP was achieved to 51% at the 15th period and 69% at the 20th period. Ultimately, the removal efficiency of TP was achieved to 74% at the 25th period and maintained the value at the 30th period. The result indicated that the mature biofilms have been formed in the 30th period owning to the biofilm with abundant microorganism growth clinging to polymeric fibrous carriers [5].

According to the above analysis of COD, NH_4^+–N, and TP degradation, it was found that the removal efficiencies of COD, NH_4^+–N, and TP were increased with a period prolonged before forming the mature biofilm. The degradation for COD and NH_4^+–N was capable of achieving the highest point at the 20th cycle period, while the removal for TP was still going up. It is evident that there was a good performance of cultivating the mature biofilm within a short time in SBR. Simultaneously, the mature biofilm was beneficial to degrade COD, NH_4^+–N, and TP of the eutrophic Huajin River. The results might be mainly related to the microbial communities attached to polymeric fibrous carriers to form a biofilm, the mature biofilm biomass, and the complicated structure of biofilm thickness and viscosity. According to previous studies, there were luxuriant autotrophic and heterotrophic bacteria to form a multiple-species biofilm to achieve effective and simultaneous COD degradation, nitrification, and denitrification, as well as TP removal [42,43]. Besides, biomass in biofilm was more stable than that in activated sludge, due to the slower speed of detachment from the biofilm than the direct washout of biomass from the activated sludge [20]. Moreover, the thickness of the biofilm layer played an essential role in bacteria habitat and growth. Generally, some denitrifying bacteria and anaerobic bacteria would prefer to gather in the inner layer of biofilm while some aerobic bacteria and nitrifying bacteria stayed in the external layer of biofilm [20,42]. Those multiple species in biofilm secreted abundant extracellular polymeric substance (EPS) to improve the biofilm adhesion and stability to withstand ambient shock and disturbance [20,42,43].

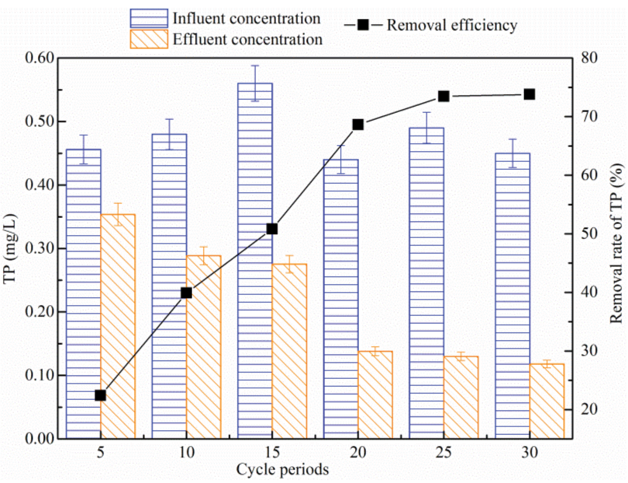

Fig. 5. TP removal of eutrophic water by cultivating biofilm in SBR.

3.2. Combination of biofilm and C. comosum for eutrophic water remediation

The mature and steady biofilm was obtained based on the analysis in section Effectiveness of biofilm cultivating. Hence, the biofilm developed in polymeric fibrous carriers was selected after the 20th cycle period to use the subsequent experiment of EFB. The initial concentration of COD_{Cr}, PI, TN, and TP was 41.03, 15.05, 1.34, and 0.50 mg/L from Huajin River which belonged to eutrophic water according to the water quality standard in China GB3838-2002) [41]. The percent of COD_{Cr}, PI, TN, and TP removal from eutrophic Huajin River was comprehensively assessed by a combination of the biofilm and *C. comosum*.

3.2.1. Combination of biofilm and C. comosum for COD_{Cr} and PI degradation

The combination of biofilm developed in polymeric fibrous carriers and hydroponic *C. comosum* (MP), compared with contrast group (CK), the biofilm (M), and the hydroponic *C. comosum* (P), was used to fabricate EFB to deal with COD_{Cr} and PI of the eutrophic water from Huajin River, as shown in Fig. 6. The raw concentration of COD_{Cr} in Huajin River was at 41.03 mg/L. The COD_{Cr} degradation was markedly decreased in MP and P, while the trend of decrease was fluctuated in CK and M, according to increasing with processing days (Fig. 6a). Relatively low COD_{Cr} utilization rate observed in CK and M during the initial phase on days 2, 6, and 9 might be attributed to the low dissolved oxygen leading to endogenous consumption and metabolite release for microorganisms [16,19]. But relatively high COD_{Cr} utilization rate was found in MP and P due to plant absorption of the mineralized nutrient products of microbial decomposition result in water purification and governance [35,36]. On day 15, the removal efficiency of COD_{Cr} in MP, P, M, and CK accounted for 70%, 63%, 62%, and 52%, respectively. The result indicated that the biofilm developed in polymeric fibrous carriers combined with the hydroponic *C. comosum* has more efficient on COD_{Cr} degradation, compared with the biofilm or the *C. comosum* and could enhance at about 8% of COD_{Cr} removal efficiency.

Fig. 6b shows that COD_{Mn} degradation for eutrophic Huajin River in MP was compared with that in CK, M, and P. PI was presented as a synthetic indicator to evaluate reduction organic or inorganic substances due to MnO_4^- was capable to oxidize some reduction matters such as sulfide, nitrite, and ferrous salt [11]. The initial concentration of PI from the eutrophic Huajin River was achieved to 15.05 mg/L. With the extent of processing time, the PI degradation has the most conspicuous decrease in MP, compared with that in P, M, and CK. On day 15, the best removal efficiency of PI was found in MP, followed by P, M, and CK. The removal efficiency of PI in MP, P, M, and CK was obtained at 57%, 47%, 38%, and 33%, respectively. Thus, the combination of the biofilm and the *C. comosum* could separately enhance by 19% and 10% of PI degradation in comparison to single-pattern cultured biofilm and single-pattern hydroponic *C. comosum*.

3.2.2. Combination of biofilm and C. comosum for TN degradation

Fig. 7 shows that the comparison of TN degradation for eutrophic Huajin River among the groups of MP, P, M, and CK. The initial concentration of TN in Huajin River achieved was 1.34 mg/L, which belongs to eutrophic water [39]. During the test, the trend of TN degradation decreased significantly with the extension of days in four groups experiment. The best TN degradation was MP, followed by M, P, and CK. The removal efficiency of TN in MP, M, P, and CK was 50%, 40%, 32%, and 25% on day 15, respectively. The results indicated that the biofilm developed in polymeric fibrous carriers combined with the hydroponic *C. comosum* for TN removal was higher 10% and 18% than that of the separate biofilm and the single *C. comosum*. Because the biofilm with abundant microorganisms aggregation attached to carriers could resist external shock and play an essential role in ammonification, nitrification, and denitrification [20,36]. Besides, *C. comosum* could adsorb the nitrogen nutrient through its well-developed root system [35,44]. And the strong root system of *C. comosum* could form mechanical protection to mitigate hydraulic push for the biofilm developed in polymeric fibrous carriers in eutrophic water. More importantly, in the rhizosphere of *C. comosum*, root exudates such as sugars, amino acids, and other secondary metabolites were produced that were considered to the major carbon sources for denitrifying bacteria [32,45]. That is to say, there was an excellent microenvironment in the rhizosphere of *C. comosum* that was beneficial for microorganism growth to decompose organic substances [31,32,35,44,45].

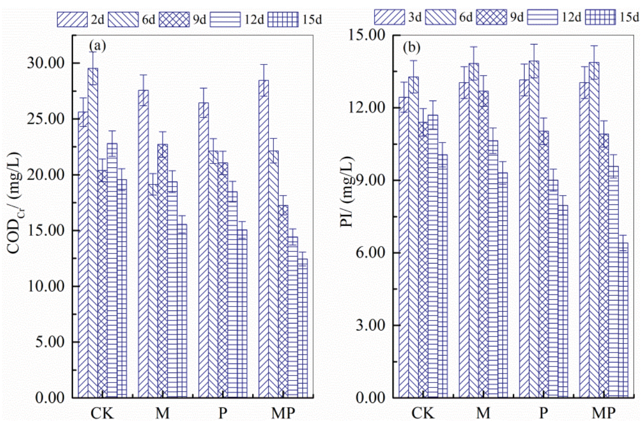

Fig. 6. Combination of the mature biofilm and hydroponic *C. comosum* for COD_{Cr} and PI degradation from eutrophic water of Huajin River.

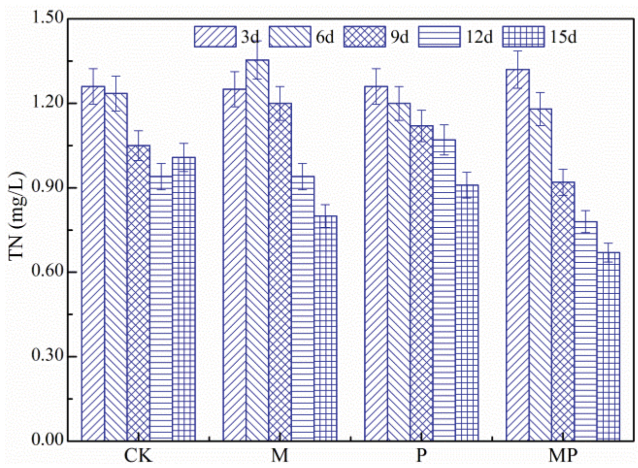

Fig. 7. Combination of the mature biofilm and hydroponic *C. comosum* for TN degradation from eutrophic water of Huajin River.

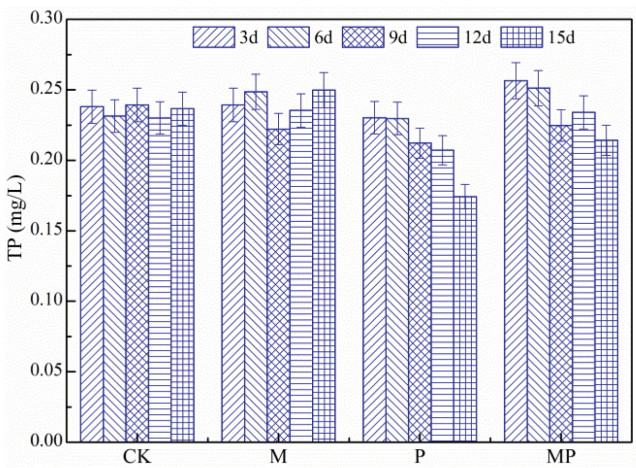

Fig. 8. Combination of the mature biofilm and hydroponic *C. comosum* for TP degradation from eutrophic water of Huajin River.

3.2.3. Combination of biofilm and C. comosum for TP degradation

The degradation of TP for eutrophic Huajin River in MP, P, M, and CK is shown in Fig. 8. The initial concentration of TP from Huajin River was at 0.50 mg/L, which has significantly exceeded the threshold value of eutrophication [39]. Among the four groups of MP, P, M, and CK, there was a decreased trend with the extension of days. On day 15, the removal efficiency of TP from the group of MP was achieved to 57%, while the TP removal efficiency in group P was capable to obtain 65%. TP removal efficiency in groups of M and CK was at 50% and 53%, respectively. Obviously, the best group for removal efficiency of TP was the group of P. That could be potentially linked to the anaerobic context from the biofilm which was not in favor of polyphosphate accumulating bacteria (PAO) [5,9]. In addition, the TP removal in MP was not as efficient as that in P. That might be attributed to those POAs from biofilm was competed with indigenous microorganisms from the root of *C. comosum* [22,23].

4. Conclusions

The mature biofilm was capable to be cultivated and formed in SBR using the eutrophic Huajin River. The effluent concentration was achieved to China's National Environmental Quality Standards for Surface Water (GB3838-2002) although the influent concentration was worse than the Standard of the Class V Status during the experiment. The removal efficiency of COD_{Cr}, NH_4^+–N, and TP accounted for 67%, 86%, and 74%, respectively. After the 20th cycle period, the mature and steady biofilm could be obtained in SBR. The biofilm developed in polymeric fibrous carriers combined with *C. comosum* was capable of improving COD_{Cr}, PI, and TN of eutrophic water from Huajin River. The experimental results showed that the improvement of 8% of COD_{Cr} degradation, 10%–19% of PI degradation, and 10%–18% of TN was from the combination of biofilm developed in polymeric fibrous carriers and hydroponic *C. comosum*, compared with the single biofilm and the separate hydroponic *C. comosum*. It was found that the biofilm combined with the hydroponic *C. comosum* for TP degradation was at 57%, 8% lower than that of the single *C. comosum*, and 7% higher than that of the separate biofilm. The results indicated that the eutrophic water originated from Huajin River was well repaired by the bench-scale combination of the biofilm developed in polymeric fibrous carriers and the hydroponic *C. comosum*, further providing a guideline for remediation of eutrophic Huajin River *in situ*.

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