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

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\textbf{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 \textit{Chlorophytum comosum} (\textit{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 (\(\text{NH}_4^+\)-N), total phosphorus (TP), and chemical oxygen demand (\(\text{COD}_\text{cr}\)) in SBR accounted for 86\%, 74\%, and 67\%, respectively. The biofilm microbes combined with \textit{C. comosum} were improvement of \(\text{COD}_\text{cr}\) 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 \textit{C. comosum}. The reduction of TP was at 57\% using the biofilm developed in polymeric fibrous carriers combined with \textit{C. comosum}, which was 8\% lower than that of separate \textit{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 \textit{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 \textit{in situ}.

\textit{Keywords:} Biofilm; \textit{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 \cite{1}. The input of excess nutrients, such as nitrogen...
floating bed (EFB) is highly efficient in removing nitrogen fication, or denitrification. Currently, an ecological purification relies on their decomposition, nitrification, 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 and governance. 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 CODCr, 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 domesticking

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...
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 flown 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 m³/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 determine COD Cr, NH₄–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.

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 det, 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, NH₄–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].

<table>
<thead>
<tr>
<th>Composition</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>0.253</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>0.472</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>0.400</td>
</tr>
<tr>
<td>NH₄H₂PO₄</td>
<td>0.068</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.247</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>0.013</td>
</tr>
<tr>
<td>H₂BO₃</td>
<td>0.003</td>
</tr>
<tr>
<td>C₆H₅N₂Na₂O₅·2H₂O</td>
<td>0.019</td>
</tr>
</tbody>
</table>
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
in the effluent decreased significantly during the cultivation periods of biofilm. The effluent concentration of \( \text{NH}_4^+ \)–\( \text{N} \) 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 \( \text{NH}_4^+ \)–\( \text{N} \) showed an increasing trend at the beginning. And the highest removal efficiency of \( \text{NH}_4^+ \)–\( \text{N} \) was achieved to 86% at the 20th period. Then the \( \text{NH}_4^+ \)–\( \text{N} \) 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 \( \text{NH}_4^+ \)–\( \text{N} \) 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 cultivation 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, \( \text{NH}_4^+ \)–\( \text{N} \), and TP degradation, it was found that the removal efficiencies of COD, \( \text{NH}_4^+ \)–\( \text{N} \), and TP were increased with a period prolonged before forming the mature biofilm. The degradation for COD and \( \text{NH}_4^+ \)–\( \text{N} \) 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, \( \text{NH}_4^+ \)–\( \text{N} \), 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].

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, \( \text{NH}_4^+ \)–\( \text{N} \), and TP degradation 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, \( \text{PI} \), 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, PI and TP 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, PI, and TP of the eutrophic water from Huajin River, as shown in Fig. 6. The raw concentration of COD in Huajin River was at 41.03 mg/L. The COD 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 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 degradation 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 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].
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].

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