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Effect of Combined Plant-Rope Ecological Floating Beds on Improvement of Eutrophic Water Quality*

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Abstract: Recently, lake eutrophication has become a serious concern and its causes and controls have attracted growing attention worldwide. In the experiments described here, we have used Ipomoea aquatica as an experimental plant for use with ecological floating beds. Three kinds of combined ecological floating bed systems were constructed by hanging two kinds of rope (hemp and PST) below the beds, and their effectiveness for improving water quality was investigated. The results showed that the plant ecological floating bed, plant-PST- rope combined bed, and plant-hemp-rope combined bed provided significant removal effects on TN, NH_4^+-N, TP and DIP. Removal efficiencies were 39.09%, 49.46% and 60.43%, 44.55%, 67.79% and 70.05%, 63.68%, 54.72% and 71.70%, and 53.70%, 50.62% and 64.20%, respectively. The plant-hemp-rope combined floating bed had especially high N and P removal rates. In the three kinds of ecological floating beds, the contributions of plant absorption to N and P removal were 36.13% and 71.85%, 32.15% and 87.07%, 27.11% and 74.34%, respectively. This suggested that for N removal in the system, plant uptake accounted for a relatively small portion of total absorption, and that denitrification by microorganisms might play a more important role. However, plant uptake took the leading role in P removal in all three kinds of floating beds. Therefore, the combined ecological floating bed having both ropes and plants was an effective way for improving the quality of eutrophic water.

Key words: ecological floating bed; *Ipomoea aquatica*; eutrophication; PST rope; hemp rope; N; P

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Introduction

With water pollution intensifying worldwide, there is an urgent need to reduce eutrophication in water columns. It is evident that nitrogen (N) and phosphorus (P) are the two major factors causing eutrophication. For example, 61% of water bodies in the United States of America fail to meet EPA standards because of excessive total N and total P discharges [1-2], and up to 66% and 22% of lakes in China are eutrophic or hypereutrophic, respectively [3]. Therefore, the removal of N and P is an effective way to reduce the level of eutrophication. Much research and practice on water restoration has been performed in many countries [4-8]. Traditional treatments include: constructed wetlands, oxidation ponds, storage ponds, spreading lake-derived biomass on land, and composting [9]. These measures operate with low running costs and low power consumption, but they occupy large areas of land and cause secondary pollution of water. Since the 1970s, macrophytes have been widely applied in restoration of freshwater ecosystems due to their high efficiency in taking up nutrients [10].

Artificial ecological floating bed (EAFB) technology consisting of aquatic or terrestrial plants growing in a hydroponic manner with a buoyant framework on the

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surface of the water column has been shown to be a feasible and effective *in situ* solution for addressing the global water eutrophication problem[11-14]. EAFB has been widely installed in many rivers, lakes and ponds[15]. EAFB plants can take up nutrients directly from the water body because they are not rooted in any substrate, and this may increase the nutrient absorption rates. EAFB provides the useful functions of water purification, improvement of landscape, and improvement of habitat for fish and birds. In addition, floating beds do not occupy land, and the plants cultured on them can be easily harvested. The by-products of these plants can be utilized as animal or even human food. The plants can also be processed into biogas, bio-fertilizer and other bio-materials. As a result of these benefits, EAFB has been widely applied all over the world in the treatment of water eutrophication because of its efficacy in assimilating nutrients and the potential economic returns[16]. Of course, EAFB also has some shortcomings. For example, low temperatures and hurricanes greatly affect the nutrient uptake efficiency of floating beds.

Many studies on floating beds have focused on selecting suitable herbaceous plant species, especially aquatic plants[17] such as water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and duckweed (*Lemna spp.*)[18]. More recently, terrestrial plants such as canna (*Canna indica*) have been the subjects of EAFB studies.

Previous studies of floating bed systems have shown that only a small amount of N is accumulated in plant tissues, while more is removed by microbial nitrification-denitrification[19-20]. The main objective of our study was therefore: (1) to compare plant growth and nutrient removal in three kinds of floating bed; (2) to evaluate the relative capacity for total nitrogen (TN) and total phosphorus (TP) removal by plant absorption and microbial transformation in floating beds.

1 Materials and Methods

1.1 Floating bed plants

Ipomoea aquatica, known as water spinach, was used. It is distributed worldwide. It grows fast and is widely used for water pollution control.

1.2 Design of ecological floating beds

The framework of the EAFB was made of four bamboo poles (100 mm in diameter and 3 m in length). This framework was covered with a nylon net which was used for attaching and growing plants during the experiments. *Ipomoea aquatica* was transplanted onto the nets. All the artificial floating beds so constructed were connected in a line and fixed onto the lake surface using wooden stakes. A number of hemp rope and PST rope were respectively fixed under the net as substrates for adherence of microorganisms, thereby increasing the potential for removal of nutrients.

The floating beds were placed on semi-closed waters near Datian village in Baiyang Lake for 40 days. The physico-chemical characteristics of the lake water are shown in Tab. 1.

Tab. 1 Characteristic of the water quality for experiment of EAFB

Components	Concentration/(mg · L^{-1})
Total nitrogen, TN	2.84
Ammonium nitrogen, NH_4^+-N	2.61
Total phosphorus, TP	0.217
Dissolved inorganic phosphorus, DIP	0.172
Chemical oxygen demand, COD	34.0

In order to assess the N and P removal effectiveness of the three kinds of EAFB, laboratory experiments were performed. Same-size vigorously growing plants were selected from the floating beds and brought back to the laboratory, along with samples of the two kinds of bio-ropes obtained from the floating bed bottom. Static studies on the plants and bio-ropes were carried out in aquaria (40 cm × 40 cm × 30 cm). Seven plants and 18 L water were placed in each tank. Water for the experiments was taken nearby the floating beds. In each tank the biomass was approximately similar (64.16 ± 6.89 g of each plant), the bio-ropes were equal in length (25 cm), and only one bio-rope was put in. A control group and five experimental groups were set (Tab. 2). All treatments had two replicates. The experiment was conducted in laboratory, with natural light and normal day length. The ambient temperature was 20 ~ 25 ℃, and the water temperature was 18 ~ 21 ℃.

Tab. 2 Setting of different experiment groups

group	treatment
control	Experiment water
Group 1	Experiment water+PST rope
Group 2	Experiment water+hemp rope
Group 3	Experiment water+plant
Group 4	Experiment water+plant+PST rope
Group 5	Experiment water+plant+hemp rope

1.3 Sampling and analyses

1.3.1 *Water samples* The study was initiated on October 28, 2010 and ended on November 14, 2010. Water samples were taken from each tank every 2 d for the analysis of water quality parameters. The sampling locations were in the centre of the aquarium and nearby its wall. In order to approach the natural state, water was not added to the tanks and corrections were made according to Ge at al[21]. Removal rate = $[(C_0V_0 - C_iV_i) \div C_0V_0] * 100\%$, where C_0 was the initial concentration of the parameter in the water samples; V_0 was the initial volume; and C_i was the concentration of the parameter in the water samples on the i-th day of the experimental period. TN, TP, NH_4^+-N and nitrate nitrogen (NO_3^--N) were determined according to the protocols described in Chinese Standard Methods[22].

1.3.2 *Microorganism samples* At the end of experiment, the number and configuration on the hemp rope and PST rope were recorded. Samples of length 1 cm were cut from the two kinds of bio-ropes and placed into 100 mL sterile saline, then shaken and cultured for 2 hours. The dilution plate method was used to calculate the number of microorganism, using the unit CFU/mL. The results were compared according to BLAST.

1.3.3 *Plant samples* At the beginning and the end of the experiment, plant heights, root lengths, and weights were measured. The plant samples were analyzed for TN and TP content, with TN determined by potassium sulphate oxidation[22], and TP determined by the ammonium molybdophosphoric colorimetric method.

1.3.4 *Statistics and analyses* Data were analyzed with the software Statistical Package for the Social Science (SPSS 13.0) and Sigmaplot (9.0). ANOVA was used to test the significance of difference between the treatments and control, using $p < 0.05$ as significant. Mean values and standard deviations were calculated from the different replicates ($n = 6$).

2 Results and Discussion

2.1 *Ipomoea* growth under different experimental conditions

The growth characteristics of plants between the beginning and end of the experiment were compared as shown in in Tab. 3. After eighteen days, the plant of groups 3, 4 and 5 grew well and their survival rates were 100%. In the early part of the test, the plants grew vigorously, but later some of the leaves appeared yellowish and wilted due to insufficient supply of nutrients. Over the eighteen days, the biomass of all increased significantly ($p < 0.05$). The plant dry weight of groups 3, 4 and 5 increased 4.38 g, 4.94 g and 5.09 g, respectively. These results showed that *Ipomoea* grew well in each test group. Hence it could grow in polluted water and absorb wastewater nutrients efficiently. These results are similar to those of other related reports[23-26]. However, the differences in plant height, root length and dry weight among the test groups were not significant ($p > 0.05$). The results also showed that the growth of *Ipomoea* was not influenced by the two kinds of bio-rope attached to their floating islands.

2.2 Nitrogen removal

The changes of concentrations of TN and NH_4^+-N in the aquarium water of each group are given in Tab. 4, and the corresponding removal rates are shown in Fig. 1.

Tab. 3 Biological characteristics of *Ipomoea* at the beginning and end of trial

	Initial test			End test		
	Plant length /cm	Root length/cm	Dry weight/g	Plant length /cm	Root length /cm	Dry weight /g
Group 3	38.6±4.1	7.3±1.8	63.59±2.09	66.0±4.5	8.6±2.0	67.97±4.83
Group 4	36.3±3.2	7.8±1.3	63.18±3.28	63.1±5.2	9.4±1.2	68.12±4.16
Group 5	36.4±3.8	6.7±1.6	62.25±5.21	68.3±3.9	8.4±1.0	67.34±5.89

Tab. 4 Change of nitrogen contents of water samples among different treatments at the beginning and end of trial

	Group	Concentrations of TN/($mg \cdot L^{-1}$)	concentrations of NH_4^+-N/($mg \cdot L^{-1}$)
Initial test	All groups	2.741±0.018a	2.164±0.021a
End test	control	2.459±0.027b	2.111±0.009b
	Group 1	1.787±0.015d	1.516±0.027c
	Group 2	1.922±0.022c	1.486±0.013c
	Group 3	1.670±0.018e	1.200±0.011d
	Group 4	1.385±0.036f	0.697±0.031e
	Group 5	1.085±0.041g	0.648±0.029e

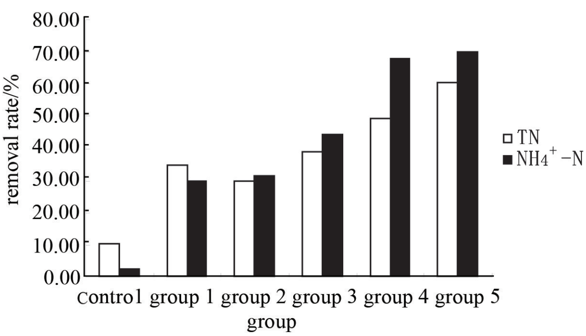

Fig. 1 Rate of removal of TN and NH_4^+-N in the various groups

At the end of the experiment, the concentrations of TN and NH_4^+-N in all test groups had decreased significantly. The results indicate that each test group purified the wastewater. The TN and NH_4^+-N removal rates were in the range of 34.81% ~60.43% and 29.94% ~70.05% respectively. The removal rates of group 5 were the highest in both cases at 60.43% (TN) and 70.05% (NH_4^+-N).

As revealed in Table 4, it was found that the TN and NH_4^+-N concentrations in wastewater of groups 1 and 2 decreased significantly compared to the control ($p < 0.05$) at the end of the test. The results showed that hemp rope and PST rope could both increase the efficiency of the removal. The TN and NH_4^+-N removal rates of group 3, 4 and 5 were 30.9%, 49.46% and 60.43%; and 44.55%, 67.79% and 70.05%, respectively. These data show that the floating beds could obviously remove nitrogen, and the presence of bio-rope could have an additive effect between plants and microorganisms, thereby increasing the nitrogen removal rate.

As an economically useful plant, *Ipomoea* had strong resistance to high temperatures and pollution. It can be planted once and harvested repeatedly, so it is an effective plant for purification purposes. *Ipomoea* can remove nutrients from the water without making secondary pollution. Hence, *Ipomoea* has been selected by many scholars as a plant to be cultured in floating beds. According to previous studies, *Ipomoea* floating beds could efficiently remove nitrogen from polluted water at removal rates that were similar our findings [23-24,26]. However, the results reported by Li [27] for *Ipomoea* floating beds in Suzhou River were different to ours. His results for TN and NH_4^+-N removal rates were 92.9% and 93.9%; which are much higher than ours. A reasonable explanation is the difference in experimental conditions. The nitrogen removal rate by *Ipomoea* in floating beds is limited by net growth and the nitrogen concentration in the plants, but the initial concentration of the test water is also an important factor. Furthermore, since the nitrogen concentration of the control decreased too, it is probable that the experiment water taken from Baiyang Lake contained microorganisms able to absorb nitrogen.

Tab. 5 shows nitrogen removal using three kinds of floating bed systems among the different treatment groups. Nitrogen removal rates in groups 3, 4 and 5 by the plants were only in the relatively low range of 27% ~37%. It appears that the nitrogen removal mechanism was microbial nitrification-denitrification in the floating bed system. This result is similar to those obtained by Fan et al. [28] and Li et al. [29]. According to previously reported research results, 40% ~92% of nitrogen in artificial wetlands was removed by nitrification-denitrification[30]. The total numbers of bacteria on hemp rope and PST rope are given in Tab. 6. The biomass of bacteria on hemp rope was higher than that on PST rope, which could presumably be the cause of the increased rate of nitrogen removal.

Tab. 5 Nitrogen removal and relative contributions of plants and microorganisms to this among floating-bed types

Group	N removal/($mg \cdot L^{-1}$)		Contribution rate/%	
	Floating bed	Plant	Plant	microorganism
Group 3	1.071	0.387	36.13	66.37
Group 4	1.356	0.436	32.15	67.85
Group 5	1.656	0.449	27.11	72.89

Tab. 6 Total numbers of bacteria on PST rope and hemp rope

	Hemp rope	PST rope
Total number of bacteria /(CFU · mL^{-1})	2.37×10^7	1.67×10^7

2.3 Phosphorus removal

The changes of TP and DIP concentrations in the treatments are given in Tab. 7, and the removal rates for TP and DIP are shown in Fig. 2.

Tab. 7 Differences among treatments of nutrient contents at the beginning and end of the trial

	Group	Concentrations of TP/(mg · L^{-1})	Concentrations of DIP/(mg · L^{-1})
Initial test	All groups	0.212±0.009a	0.162±0.007a
End test	control	0.107±0.011b	0.096±0.006b
	Group 1	0.103±0.007bc	0.095±0.006b
	Group 2	0.095±0.013bc	0.090±0.006bc
	Group 3	0.077±0.011cd	0.075±0.002cd
	Group 4	0.096±0.008bc	0.080bc±0.004
	Group 5	0.060±0.012d	0.058±0.007d

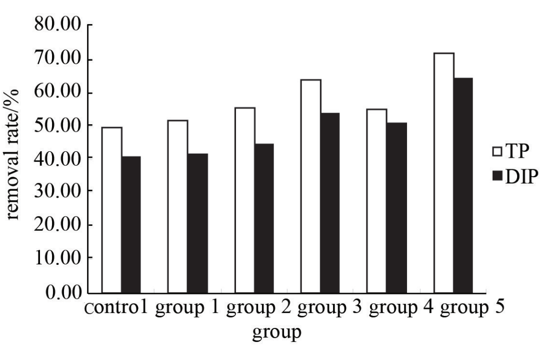

Fig. 2 Removal rates for TP and DIP in the experiment groups

At the end of the study, the TP and DIP concentrations of each group were significantly decreased ($p<0.05$), indicating that P could be removed efficiently by each test group. The TP and DIP removal rates of each test group were in the range of 51.42% ~71.70% and 41.36% ~64.20% respectively. Rates of removal of TP and DIP in group 5 were 71.70% and 64.20%, which is noticeably higher than in the other groups.

As shown by the data in Tab. 6, it was found that the TP and DIP concentrations of groups 1 and 2 were decreased significantly compared with the control ($p<0.05$) at the end of the test. This showed that the two kinds of bio-rope could both efficiently increase the TP and DIP removal rates. It is possible that the bacterial biomass was increased by the presence of the ropes, thereby increasing the P-removal capacity of the floating bed system. However, there was no significant difference between groups 4 and 5 ($p<0.05$) which differ with respect to the type of rope. Therefore, it appears that P can be removed efficiently by *Ipomoea* floating beds, and bio-ropes can increase the P-removal capacity.

By contrast to studies on N removal by *Ipomoea* floating beds, there has been less research on P removal. The removal pathway of P by floating beds has been shown to be by plant absorption, sedimentation, and microbial immobilization. Our findings on P removal were similar to those of Zhou et al. [31], but was lower than those of Li et al. [27]. A reasonable explanation is the difference of experimental conditions. On the other hand, or additionally, the initial concentration of P in the water could also affect the ability of the floating system to remove it.

Tab. 8 shows P removal and the contribution of floating bed components among the various groups. P removal rates by the plants of Groups 3, 4 and 5 were in the range 70% ~90%. This showed that plant absorption of P played an important role in P removal from the water. It was evident that the N removal rate by the floating beds was higher than the P removal rate. The results show that the demand for N by the aquatic plant-microbial system was much higher than the demand for P. This finding is similar to that of Tang et al [32-33].

Tab. 8 Nutrient removal and contribution of floating-bed components among different floating-bed types

Group	P removal/(mg · L^{-1})		Contribution /%	
	Floating bed	Plant	Plant	microorganism
Group 3	0.135	0.097	71.85	28.15
Group 4	0.116	0.101	87.07	12.93
Group 5	0.152	0.113	74.34	25.66

2.4 Bacteria on the two kinds of bio-rope

Total numbers of bacteria on the two kinds of bio-rope are shown in Tab. 6. We selected that the probability of occurrence of colonies on the plates were greater than 5%, and preliminarily identified them (Tab. 9). In this study, the N and P removal rates of groups 4 and 5 were found to be much higher than those of the other groups. We have concluded that this was due to changes in the quantity and composition of microorganisms associated with the floating beds. It appears that the number and variety of bacteria attached to the hemp rope in group 5 were higher than those on the PST rope in group 4. This may therefore be why the N and P removal rates are higher.

Tab. 9 The varieties of bacteria on PST rope and hemp rope

Hemp rope		PST rope	
The closest strain (accession number)	Population	The closest strain (accession number)	Population
Microbacterium laevaniformans (AB004726) (98.9%)(3)	Actinobacteria	*Hydrogenophaga flava* (AF078771)(99.6%)(3)	β- Epsibobacteria
Hydrogenophaga pseudoflava (AF078770) (99.1%)(3)	β-Epsibobacteria	*Rhodobacter sphaeroides* (CP000143)(99.2%)(2)	α- Epsibobacteria
Microbacterium profundi (EF623999)(99.1%)(3)	Actinobacteria	*Cloacibacterium rupense* (EU581834)(99.7%)(3)	Flavobacteria
Lysobacter brunescens (AB161360)(100%)(2)	γ- Epsibobacteria	*Porphyrobacter donghaensis* (AY559428)(99.7%)(3)	α- Epsibobacteria
Catellibacterium aquatile (EU313813)(100%)(3)	α- Epsibobacteria	*Sandaracinobacter sibiricus* (Y10678)(99.6%)(3)	α- Epsibobacteria
Malikia granosa (AJ627188)(98.8%)(3)	β- Epsibobacteria	*Novosphingobium lentum* (AJ303009)(99.7%)(3)	α- Epsibobacteria
Providencia vermicola (AM040495)(99.8%)(2)	γ- Epsibobacteria	*Ancylobacter dichloromethanicus* (EU589386)(99.6%)(3)	α- Epsibobacteria

3 Conclusions

1) In this study we have evaluated the efficiency of *Ipomoea* floating bed systems in treating eutrophic water. The presence of bio-ropes could increase the removal rates of N and P, and the efficiency of hemp rope was better than that of PST rope. The hemp-rope floating-bed system could remove 70.5% of N and 64.2% of P.

2) In the floating bed systems, the percent contribution of plants to N removal was in the range of 27% ~37%, while plants contributed 71% ~88% to the TP removal. This indicates that N was mostly removed by microbial nitrification and denitrification, while P was mostly absorbed by the plants.

3) There were differences among the bacterial numbers, diversity and dominant flora of the two kinds of bio-ropes. These differences might account for the fact that the removal rates of N and P by the hemp-rope floating-bed system was the better of the two rope-test groups.

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