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Synergistic Action of Plants and Microorganism in Integrated Floating Bed on Eutrophic Brackish Water Purification in Coastal Estuary Areas

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Eutrophic water bodies in coastal estuary areas usually show saline-alkaline characteristics influenced by tides. The purification performance of traditional planted floating beds in this water body is limited because of the poor growth of plants. A novel integrated floating bed with plants (*Iris pseudoacorus*), fillers (volcanic rocks and zeolites), and microbes named PFM was established, and the pollutant removal performance was studied. Results showed that the average ammonia nitrogen (NH_4^+-N), total nitrogen (TN), total phosphorus (TP), and permanganate index (COD_{Mn}) removal efficiencies of PFM were higher with the value of 81.9, 78.5, 53.7, and 72.4%, respectively, when compared with the other floating beds containing plants (P), fillers (F), microbes (M), and plants and fillers (PF) in this study. Therein, the most of NH_4^+-N (30.1%), TN (27.9%), TP (22.5%), and COD_{Mn} (43.6%) were removed by microbes, higher than those removed by plants and fillers. Analysis of the microbial community revealed that the establishment of PFM led to a higher microbial richness than M, and *Acinetobacter*, as the main microbes with the function of salt tolerance and denitrification, were dominated in PFM with a relative abundance of 6.8%. It was inferred that the plants and fillers might enrich more salt-tolerance microbes for pollutants removal, and microbes favored the growth of plants via degradation of macromolecular substrates. Synergistic actions in the process of eutrophic brackish water purification were established. This study provided an idea for the application of integrated floating bed in eutrophic and brackish water bodies purification in coastal estuary areas.

Keywords: integrated floating bed, brackish water, synergistic action, microorganism, *Iris pseudoacorus*

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INTRODUCTION

Coastal estuaries are the ecotone of terrestrial and marine ecosystem with low water exchange rate, and susceptible to human activities. Thus, eutrophication is prone to occur. It is generally accepted that excessive nutrient input is the main reason of water eutrophication (Bhagowati and Ahamad, 2019). The consequences are the outgrowth of harmful algae, decreased light intensity, water body anoxia, and extinction of submerged plants and aquatic animals (Ma et al., 2019). More

importantly, eutrophication may lead to serious health hazards to humans in various pathways. In addition to eutrophication, salt or brackish water bodies caused by NaCl or HCO_3^--CO_3^{2-} are also a challenge to the environment in coastal estuary areas. The growth of plants and microorganisms is inhibited under the influence of salt and alkali stress, and the self-purification ability of water bodies is restrained, resulting in a deterioration of waterfront ecological landscape (Zhao et al., 2005; Benzarti et al., 2014). Therefore, a suitable remediation method should be selected with the advantages of an effective water purification performance and the tolerance to salt and alkali when eutrophication occurs in the coastal estuary areas.

Integrated floating bed is an innovative water remediation technology developed from the conventional constructed wetlands. It is mainly composed of aquatic plants and microbial carrier packing. The plants in integrated floating beds grow in a hydroponic floating mass on the surface of water bodies, and the microbes are fixed mainly on the fiber fillers and plant roots (Li et al., 2010; Liu et al., 2016a). Integrated floating bed has been increasingly researched and applied to control the water eutrophication with the advantages of low investment, high efficiency, no additional land occupation, and flexible operation (Gottschall et al., 2007; Wang et al., 2020a). Wu et al. (2016) established the enhanced ecological floating beds with total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), and chemical oxygen demand (COD_{Cr}) removal efficiencies of 49.3, 49.2, 69.5, 48.7, and 70.6%, respectively. Olguín et al. (2017) assessed the performance of floating treatment wetlands for the water quality improvement of a eutrophic urban pond, and fecal coliforms and nitrate decreased by 86 and 76%, respectively, in 2 years. Nevertheless, most of the researches about integrated floating bed are related to fresh water purification according to previous studies (Huang et al., 2013; Abed et al., 2017), and the studies of integrated floating bed applied in coastal estuary areas for brackish water purification are limited. The stability of traditional floating bed is usually low in the actual application (Wang et al., 2020b), and it is a challenge to build a stable floating bed system.

Water purification would be achieved via the integrated floating bed with comprehensive effects of plant absorption, microbial degradation, and others (Wang et al., 2020a). Integrated floating beds have been applied in many field-scale river remediation projects, and the water purification processes indicated the synergistic effects of plants and microbes (Chang et al., 2012; Wang and Sample, 2014; Wang et al., 2015). Macromolecule pollutants would be decomposed into micromolecular substrates via enzymes secretion. On the other hand, some micromolecular substrates would be degraded by microbes, and others would be easily uptaken by plant roots (Wu et al., 2016; Urakawa et al., 2017). Meanwhile, the developed plant roots could provide the attachment sites for microbial growth, and oxygen released from plant roots would facilitate microbial growth and enhance biofilm formation (Keizer-Vlek et al., 2014; Saeed et al., 2016). The biofilm structure could resist lots of environmental stresses, such as salinity, pH, and toxic substance, and the interaction between microbes and plants in the integrated floating bed system would enhance the pollutants removal performance. There are diverse microbial communities in brackish aquatic ecosystems. In addition, lots of salinity-tolerance microbes could produce bioactive compounds and secondary metabolites to combat osmotic stress (Wang et al., 2020c). Nonetheless, the microbial community composition in brackish water ecosystems is poorly understood. Furthermore, *Iris pseudoacorus* is a common aquatic plant with a good landscape effect in the estuary area. It has been proved to have a high capacity to take up nutrients from the effluent water and retain N and P (Yousefi and Mohseni-Bandpei, 2010; Gacia et al., 2019).

The first objective of this study is to document the performance of the integrated floating bed for eutrophic brackish water purification in the coastal estuary areas. Six floating beds with different structures were established. The second objective is to evaluate the contribution of plants, fillers, and microbes for pollutants removal performance. The contribution rates of plants, fillers, and microbes to NH_4^+-N, TN, TP, and permanganate index (COD_{Mn}) removal were calculated. At last, the microbial community in integrated floating bed system were analyzed by 16S rRNA high-through sequencing, and the microbial diversity and structure were analyzed. Therefore, the third objective is to prove the synergistic action for water purification in the integrated floating bed in the aspect of microbial enrichment.

MATERIALS AND METHODS

Reactors and Operation

Reactors

Six identical reactors were established with a size of 80 cm (length) × 20 cm (width) × 50 cm (height). The reaction zone was 70 cm × 20 cm × 40 cm and an effective volume of 56 L. The reactor consisted of a floating bed system, an aeration system, a water inlet system, and an effluent system (**Figure 1**). The floating bed system consisted of four parts: plants, floating plate, planting baskets, and artificial bio-carriers. A polyethylene foam board was placed in each device as a floating plate, and the size was 50 cm × 19 cm × 2.5 cm. Six planting holes with a diameter of 5 cm were set on each plate. The planting baskets made of 30 mesh gauze with a diameter of 5 cm and a height of 10 cm were installed in the holes. Fillers (30 g of volcanic rock and 30 g of zeolite) were placed at the bottom of each planting basket. *Iris pseudoacorus* was selected as the floating bed plant. It was planted in the basket and the root was fully in contact with the fillers (**Figure 1**). Four strings of artificial bio-carrier (aldehyde fiber) with the capability of enhancing microbial adhesion were hung under the floating plate, and each length was 35 cm. The artificial bio-carriers were hung for 3 days by the smoldering method before the start of the operation, and installed on the floating bed after the yellowish viscous biofilm appeared on them. An aeration system was installed at the bottom of the reaction zone.

Materials

The *I. pseudoacorus* was collected from the estuary areas of the Licun River. Before the reactor operation, *I. pseudoacorus* was cultured in water for 7 days, and the plants with a similar

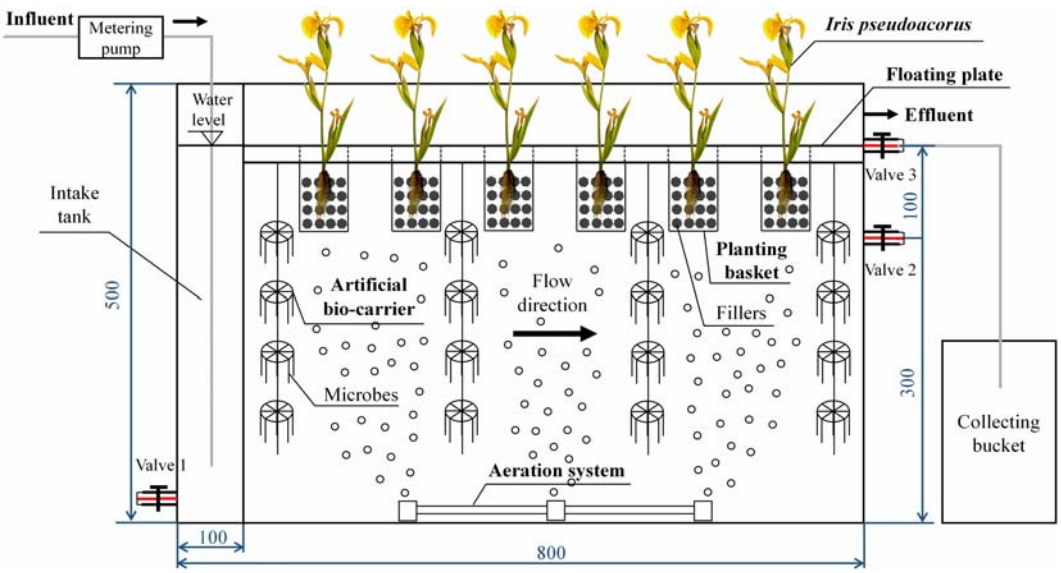

FIGURE 1 | Structure of the integrated floating bed. The integrated floating bed consisted of a floating bed system, an aeration system, a water inlet system, and an effluent system. The floating bed system consisted of four parts: plants, floating plate, planting baskets with fillers, and artificial bio-carriers.

height and fresh weight were selected as the testing plants. The membrane-hanging microorganism was a compound microbial agent and an original bacterium concentrated one-generation probiotic powder provided by the Ningu Country Bacteria Password Biological Co., Ltd. NH_4Cl, $NaNO_3$, KH_2PO_4, and glucose were used to simulate the water quality of the estuary areas of the Licun River. The concentrations of NH_4^+-N, NO_3^--N, TN, TP, and COD_{Mn} in the influent water were 3.6, 2.6, 6.2, 3.4, and 40.0 $m{\cdot}L^{-1}$, respectively. The salinity of the influent was 5‰, and pH was 8.0 (**Table 1**).

Experimental Design

Six floating beds with different structures were constructed: plant floating bed (P), filler floating bed (F), microbe floating bed (M), plant + filler floating bed (PF), plant + filler + microbe integrated floating bed (PFM), and blank floating bed (CK). The operation time of the reactors was 31 days, and the dynamic test was carried out by continuous water inflow and effluent. The hydraulic retention time was 4 days, and the aeration rate was 0.8 L/min.

The plant characteristics were analyzed at the beginning and end of the operation for fresh and dry weight, water content, root activity, proline content, leaf relative conductivity, and chlorophyll content. The effluent of the six floating beds were analyzed after the start of operation, and the average concentrations and removal efficiencies of NH_4^+-N, TN, TP, and COD_{Mn} were calculated.

Analytical Methods

Analysis of Plant Characteristics

Plant height and root length were measured by scale. The fresh weight and the dry weight were determined by gravimetric method with an electronic analytical balance. Chlorophyll content, root activity, leaf relative conductivity, and root proline content were measured by *N, N*-dimethylformamide extraction, triphenyltetrazolium chloride method, immersion method, and acid ninhydrin colorimetry, respectively (Puniran-Hartley et al., 2014; Abdelaziz et al., 2017).

Water Quality

NH_4^+-N, TN, TP, and COD_{Mn} were measured by the Nesslerizatin colorimetric method, alkaline potassium persulfate-ultraviolet spectrophotometry, ammonium molybdate–antimony potassium tartrate–ascorbic acid spectrophotometry, and potassium permanganate method, respectively, according to the protocols described in the Chinese Standard Methods (State Environmental Protection Administration of China, and Editorial Board of Monitoring and Analytical Method of Water and Wastewater, 2002).

Microbial Community Analysis

At the end of the operation, the fillers and artificial bio-carriers were crushed and mixed evenly with the effluent to prepare for DNA extraction. Total genome DNA from samples was extracted using the CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. 16S rRNA genes of distinct regions (16S V4) were amplified using a specific primer (341F: CCTAYGGGRBGCASCAG; 806R: GGACTACNNGGGTATCTAAT) with the barcode. All PCR reactions were carried out with the PhusionHigh-Fidelity PCR Master Mix (New England Biolabs). Mix same volume of 1X loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. Samples with a bright main strip between 400 and 450 bp were chosen for further experiments. PCR products was mixed in equidensity ratios. Then, the mixture PCR products was purified with

the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, United States) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an IlluminaHiSeq2500 platform and 250 bp paired-end reads were generated.

Data Processing

The relative growth rate RGR ($mg{\cdot}g^{-1}{\cdot}d^{-1}$) was calculated as follows:

$$RGR = \left[\ln(W_2) - \ln(W_1)\right] / (t_2 - t_1) \quad (1)$$

where W_1 and W_2 were the dry weight (g) of the plant at the beginning (t_1) and at the end (t_2) of the experiment, respectively.

In order to assess the role of plants and microbes in purifying eutrophic brackish water, the change of pollutant concentration in effluent was characterized by c/c_0 based on the determination of pollutant indexes. c and c_0 were the concentration of effluent pollutant ($mg{\cdot}L^{-1}$) and influent pollutant ($mg{\cdot}L^{-1}$), respectively. The integrated floating bed removal efficiency η_{PFM}, other effects (aeration, illumination, etc.) removal efficiency, i.e., CK removal efficiency η_{CK}, filler removal efficiency η_F, plant removal efficiency η_P, microbe removal efficiency η_M, and synergistic removal efficiency η_S were calculated, respectively (Wang et al., 2012).

$$\text{Removal efficiency}\,(\%) = (c_0 - c)\, c_0 \times 100\% \quad (2)$$

$$\text{Filler removal efficiency}\ \eta_F(\%) = \text{Filler floating bed F removal efficiency} - \eta_{CK} \quad (3)$$

$$\text{Plant removal efficiency}\ \eta_P(\%) = \text{Plant floating bed P removal efficiency} - \eta_{CK} \quad (4)$$

$$\text{Microbe removal efficiency}\ \eta_M(\%) = \text{Microbial floating bed M removal efficiency} - \eta_{CK} \quad (5)$$

$$\text{Synergy removal efficiency}\ \eta_S(\%) = \eta_{PFM} - \eta_{CK} - \eta_F - \eta_P - \eta_M \quad (6)$$

The average value, standard deviation, and analysis of variance (ANOVA) were determined by using the SPSS software (PASW Statistics 20.0). The means were compared by using paired sample t-tests, and the significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Pollutants Removal Performance

The concentrations of NH_4^+-N and TN in PFM decreased at the first 7 days, and gradually stabilized after Day 17 with effluent concentrations of 0.65 and 1.33 $mg{\cdot}L^{-1}$ and average removal efficiencies of 81.9 and 78.5%, respectively, higher than those of the other reactors. In addition, microbes and plants were the main reason for NH_4^+-N removal performance of PFM, η_M and η_P were 30.1 and 20.2%, respectively, at the end of the operation (**Figure 2A**). Similarly, the contribution of microbes and plants for TN removal performance was 27.9 and 14.9%, higher than the other factors (**Figure 2B**). The mechanisms of nitrogen removal in water mainly consist of physical adsorption, plant absorption, and microbial degradation. Zeolites are effective in the adsorption of ammonium as an ion exchanger. With the increase of other cations such as Ca^{2+}, Mg^{2+}, K^+, the adsorption of the fillers was weakened and gradually maintained at a stable state (Wang and Peng, 2010). Plants could directly absorb and utilize NH_4^+-N to synthesize a variety of amino acids in an aerobic environment, while most NH_4^+-N is mainly converted into NO_3^--N and NO_2^--N by nitrifying bacteria. Most of the NO_3^--N and NO_2^--N are reduced to nitrogen during denitrification process by microbes, and others are absorbed and utilized by plants (Bartucca et al., 2016; Liu et al., 2016a). In traditional plant floating bed systems, the growth rate and absorption capacity of plants limit the ability of pollutants purification, nevertheless, microbial nitrification-denitrification greatly improves nitrogen removal performance in the integrated floating bed (Sun et al., 2019). It was indicated that microbes may play a major role in the nitrogen removal process.

The effluent TP from P, M, PF, and PFM dropped sharply during the reactors' operation, then tended to be stable, while changed less in CK and F (**Figure 2C**). The effluent TP in F gradually decreased at the first 5 days. After Day 5, the phosphate might be precipitated with the Ca^{2+} exchanged from fillers to be continuously removed (Karapınar, 2009), and reached a steady state, indicating that the adsorption of TP by the filler tended to be saturated at the end of the operation. In floating bed PFM, the effluent TP decreased sharply after Day 5, and basically stabilized after Day 17 ($p < 0.05$). Considering the effluent TP of PFM was the least, followed by that of PF and M, it was speculated that TP removal in the early operation period of floating bed PFM mainly relied on the absorption of plants and microbes, adsorption of the filler further enhanced TP removal performance of PFM at the first 5 days of operation (Guo et al., 2014). The results demonstrated the importance of plants and microbes for TP removal in floating bed systems.

During the reactors' operation process, the effluent COD_{Mn} of F, P, M, PF, and PFM decreased continuously and tended to be stable on the 9th, 11th, 15th, 13th, and 19th day of operation, respectively (**Figure 2D**). The reactors of PFM and M obtained higher COD_{Mn} efficiency than others, and COD_{Mn} removal efficiency of PF was significantly higher than that of P and F. The results indicated the importance of microbes for COD_{Mn} removal in the floating bed system, and the obvious synergistic effect of fillers and plants for pollutants removal in the system. Based on the results above, it was indicated that the plants and microbes were the main factors for pollutants removal in the floating bed system, and the filler could enhance the pollutants removal performance with plants and microbes synergistically.

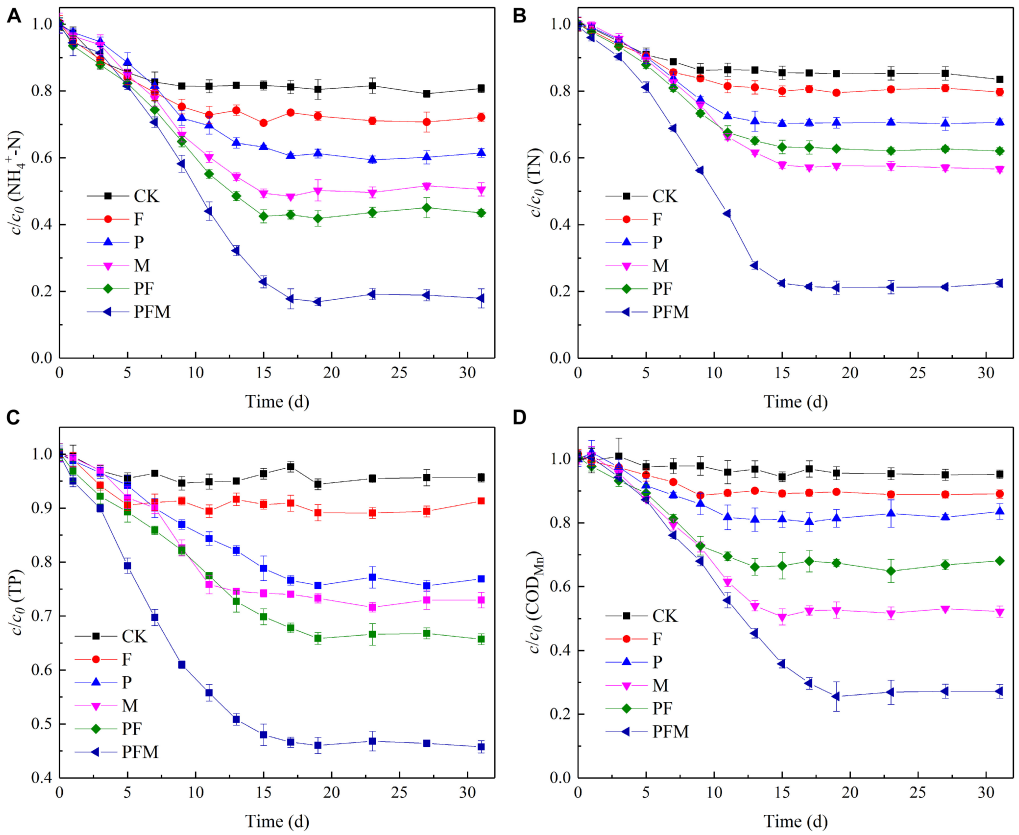

FIGURE 2 | Change of pollutants concentration in different floating beds. **(A)** NH_4^+-N. **(B)** TN. **(C)** TP. **(D)** COD_{Mn}.

Growth and Physiological Characteristics of Plants

Plant growth and physiology are important factors for pollutant removal performance of plants. With the reactors' operation, the roots of *I. pseudoacorus* penetrated the planting basket, plant height increased significantly with the growth of new shoots. In the three reactors planted with *I. pseudoacorus*, the plants in PFM obtained excellent growth characteristics with a relative growth rate (RGR), net increment of root length, and net increment of plant height of 11.59 $mg{\cdot}g^{-1}{\cdot}d^{-1}$, 9.31 cm, and 25.51 cm, respectively, at the end of the operation, significantly higher than those in PM and P ($p < 0.05$) (**Table 2**). The results indicated that fillers and microbes played important roles in enhancing the root and plant growth of *I. pseudoacorus*.

Saline-alkali could decrease root activity, increase proline content, and decrease chlorophyll content in plant cells (Puniran-Hartley et al., 2014). As shown in **Table 3**, the root activity and Chlorophyll content of *I. pseudoacorus* in PF and PFM were similar and higher than those in P. Especially the root activity of *I. pseudoacorus*, the value in PF and PFM was 1.13 and 1.18 $mg{\cdot}g^{-1}{\cdot}h^{-1}$, respectively, at the end of the operation, significantly ($p < 0.05$) higher than that in P. The results indicated that fillers and microorganisms could enhance the physiological characteristics of plants, particularly the root activity in the integrated floating bed. Accordingly, the salt tolerance of *I. pseudoacorus* in PFM was enhanced, and the proline content of *I. pseudoacorus* in PFM was 0.49 $\mu mol{\cdot}g^{-1}$ higher than the other two reactors. Also, the relative conductivity of the leaves in PFM was 11.96%, which is significantly lower than that in P and PF (**Table 3**). Microorganisms could colonize around the root zone through fillers and provide positive feedback for plant growth by discharging beneficial compounds in the rhizosphere (Ahemad and Kibret, 2014). In addition, salt-tolerance microorganisms could suppress the accumulation of reactive oxygen species and sodium accumulation in plants, and improve salt-tolerance characteristics of plants by stimulating the activities of antioxidant enzymes (Yasmeen et al., 2020). The results indicated that the integrated floating bed could facilitate the salt tolerance and root activity of *I. pseudoacorus* and increase the contribution of plants for pollutants removal.

Microbial Structure of Floating Bed M and PFM

The microbial structure was analyzed by 16S rRNA high throughput sequencing technology. The results showed that *Acinetobacter*, *Bdellovibrio*, *Hydrogenophaga*, and *Shewanella* were mainly enriched in PFM with a relative abundance of 6.8, 6.1, 4.7, and 3.1%, respectively, when compared with the microbial structure in M (**Figure 3**). According to previous studies, *Acinetobacter, Bdellovibrio,* and *Hydrogenophaga* showed

TABLE 1 | The influent components of floating bed in this study.

NH_4^+-N (mg·L^{-1})	NO_3^--N (mg·L^{-1})	TN (mg·L^{-1})	TP (mg·L^{-1})	COD_{Mn} (mg·L^{-1})	Salinity (‰)	pH
3.6	2.6	6.2	3.4	40.0	5	8.0

TABLE 2 | Growth characteristics of *I. pseudoacorus* in the floating beds P, PF, and PFM.

Floating bed	*RGR* (mg·g^{-1}·d^{-1})*	Net increment of root length (cm)*	Net increment of plant height (cm)*
P	5.18 ± 0.46 a	6.22 ± 0.66 a	15.25 ± 2.52 a
PF	7.57 ± 1.12 b	7.61 ± 0.82 ab	19.93 ± 1.50 b
PFM	11.59 ± 1.04 c	9.31 ± 0.91 b	25.51 ± 1.17 c

**Results were the mean of three replicates ± standard deviation. Different small letters behind the values indicated a significant difference among the different floating beds ($P < 0.05$).*

TABLE 3 | Physiological characteristics of *I. pseudoacorus* in the floating beds P, PF, and PFM.

Floating bed	Root activity (mg·g^{-1}·h^{-1})*	Root proline content (μ mol·g^{-1})*	Relative conductivity of leaves (%)*	Chlorophyll content (mg·g^{-1})*
P	1.05 ± 0.02 a	0.42 ± 0.04 a	21.87 ± 1.19 a	2.13 ± 0.04 a
PF	1.13 ± 0.03 b	0.47 ± 0.05 a	14.99 ± 2.53 a	2.33 ± 0.04 ab
PFM	1.18 ± 0.02 b	0.49 ± 0.04 a	11.96 ± 2.34 b	2.43 ± 0.05 b

**Results were the mean of three replicates ± standard deviation. Different small letters behind the values indicated a significant difference among the different floating beds ($P < 0.05$).*

the characteristic of salt tolerance and the function of nutrient removal performance in water (Hood et al., 2010; Ji et al., 2016; Zuo et al., 2016; Wang et al., 2017). Lots of species in *Shewanella* have been detected in hypersaline environments, and it has demonstrated its tolerance to a wide range of salt concentrations (Fu et al., 2014). In addition, *Shewanella* plays an important role in marine P transformation through producing a large amount of extracellular polymeric substances and showing a relatively high P removal performance under aerobic conditions (Jiang et al., 2018). *Acinetobacter* and *Pseudomonas* have great nitrogen removal ability, and could remove NH_4^+-N and NO_3^--N via heterotrophic nitrification and aerobic denitrification, respectively (Huang et al., 2021; Li et al., 2021). Thus, it was inferred that plants in PFM could enrich more microbes with the function of salt tolerance and pollutant removal performance. Plants improved the salt tolerance of biofilm while strengthening their own growth, and ultimately, promoted the stability of the system and the effective removal of pollutants.

Synergistic Action of Integrated Floating Bed PFM

The synergistic action of *I. pseudoacorus*, filler, and microbe on the purification of brackish water was characterized by the synergistic pollutant removal efficiency (η_S) of the floating bed PFM. As shown in **Table 4**, the synergistic pollutant removal efficiency of NH_4^+-N, TN, TP, and COD_{Mn} in PFM was 3.9, 16, 1.9, and 4.2%, respectively. In a single ecosystem with only plants, assimilation by plant root and the associated denitrification are the main mechanisms of nitrogen removal. In the microbial system, various biochemical reaction, including nitrification and denitrification, existed in the nitrogen removal process as well (Liu et al., 2016a). Nevertheless, there are some different interactions occurring in an integrated plant and microbe ecosystem. The relative growth rate, root activity, and root proline content of plants in the integrated floating bed PFM were higher than those in P and PF (**Tables 2**, **3**). It was indicated that the addition of microorganisms enhanced the salt tolerance of plant and promoted plant growth. On the one hand, the macromolecules could be degraded into micromolecules by microbes, the plants then directly absorbed and utilized these micromolecules (**Figure 4**). On the other hand, microorganisms could improve the survival ability of plants by remitting the toxicity of plant pathogens (Lu et al., 2015). Microorganisms can also alleviate water deficiency and increase antioxidant capacity of plants by establishing new ion balance (Kudoyarova et al., 2013).

Previous studies have shown that the removal mechanisms of pollutants in water mainly include microbial degradation, plant root retention, and filler adsorption, among them, microbial degradation played a major role (Yu et al., 2019). This is consistent with the results of this study (**Table 4**). The relative abundance of functional bacteria for nitrogen and phosphorus removal increased in PFM according to the results of microbial community analysis (**Figure 3**), leading to a higher contribution rate of pollutant removal than plants and fillers. In the PFM system, fillers and plant roots provided carriers for attachment and habitation of the functional bacteria (Ning et al., 2014b; Keizer-Vlek et al., 2014) because oxygen secreted by plant roots could form many anaerobic–anoxic–oxic micro areas, which were equivalent to many series–parallel A^2/O units. These units could enhance microbial nitrification and denitrification, and indirectly improve the denitrification efficiency (Cao and Zhang, 2014; Li et al., 2015). Furthermore, plant roots can also secrete

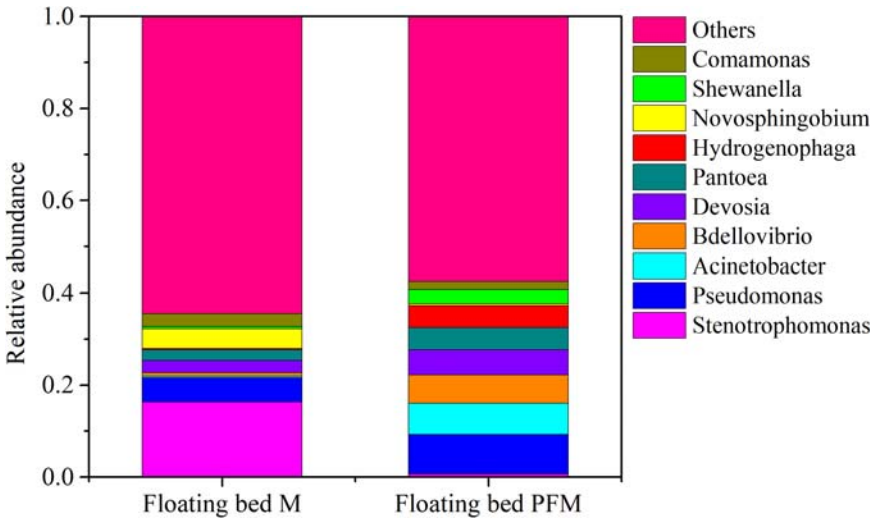

FIGURE 3 | Relative abundance of microbial community at the Genus level.

TABLE 4 | Removal efficiency of pollutants in the integrated floating bed PFM.

Removal efficiency	η_{PFM} (%)	η_{CK} (%)	η_F (%)	η_P (%)	η_M (%)	η_S (%)
NH_4^+-N	81.9	19.5	8.2	20.2	30.1	3.9
TN	78.5	14.9	4.8	14.9	27.9	16
TP	53.7	4.5	5.6	19.2	22.5	1.9
COD_{Mn}	72.4	4	7.1	13.5	43.6	4.2

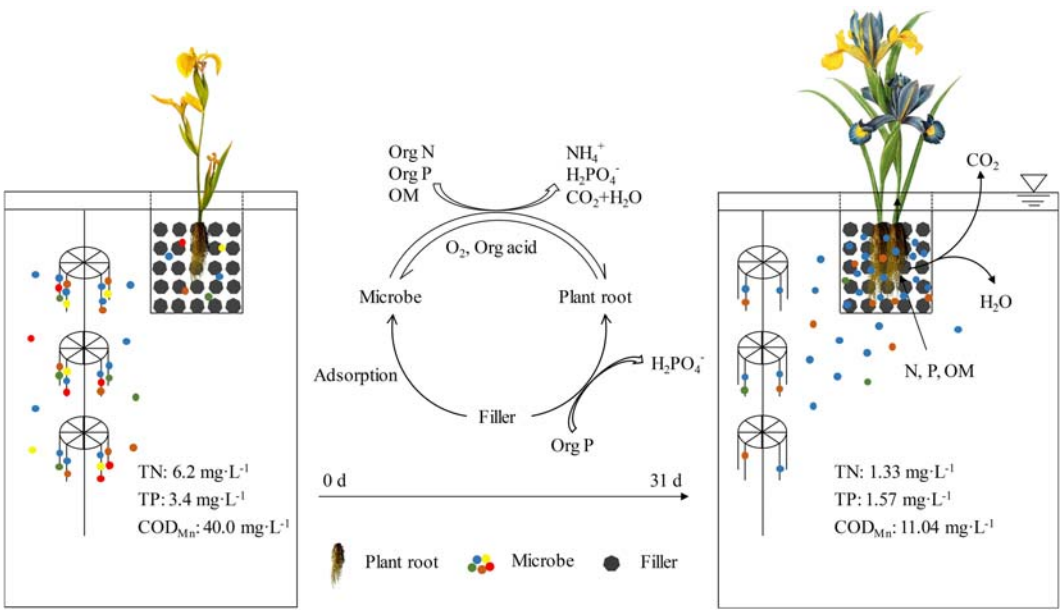

FIGURE 4 | Mechanism of synergistically purifying eutrophic brackish water by plants, microbes, and fillers. Microorganisms can degrade the macromolecules into micromolecules that can be directly absorbed and utilized by plants. Plant roots and fillers provide carriers for attachment and habitation of the functional bacteria. Plant roots can also secrete a variety of unstable carbon compounds, such as organic acids and amino acids that promote microbial metabolism and form biofilms on the root surface.

a variety of unstable carbon compounds, such as organic acids and amino acids that promote microbial metabolism and form biofilms on the root surface (**Figure 4**; Rocha et al., 2015). As a result, microbial population, quantity and metabolic rate were improved. On the other hand, the metabolic process of microorganisms in the system could be further studied in the aspect of metabonomics, and the metabolic mechanism of the microorganisms would be illustrated more clearly.

CONCLUSION

A novel integrated floating bed (PFM) with plants (*Iris pseudoacorus*), fillers (volcanic rocks and zeolites), and microbes achieved excellent NH_4^+-N, TN, TP, and COD_{Mn} removal performance, and most of the pollutants were removed by microbes. In addition, the enrichment of *Acinetobacter* in PFM enhanced the salt tolerance and nitrogen removal performance of the system. The analysis of the synergistic action of PFM indicated that the plants and fillers could enrich more salt-tolerant microbes for pollutant removal, which in turn promoted the growth and salt-tolerance characteristics of plant. Plants and fillers had synergistic actions with microbes in the process of eutrophic brackish water purification. Finally, the novel integrated floating bed system for high-efficiency pollutants removal performance of eutrophic and brackish water bodies purification was established.

DATA AVAILABILITY STATEMENT

NCBI Sequence Read Archive, and the accession number is PRJNA691644. To browse the data, use SRA Run Selector: https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA691644.

AUTHOR CONTRIBUTIONS

ML and YC planned the experiments. ML and YW performed the experiments and wrote the manuscript. JG and PS contributed to literature collection and data analysis. YC and ZZ participated in critically revising the manuscript for important intellectual content.

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