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The *in situ* remediation of aquaculture water and sediment by commercial probiotics immobilized on different carriers

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ABSTRACT

In the present study, we investigated the effect of probiotics immobilized by oyster shells (Os), vesuvianite (Ve) and walnut shells (Ws) on the remediation of aquaculture water and sediment by analyzing the variation of ammonia-nitrogen (NH_4–N), nitrate-nitrogen (NO_3–N), nitrite-nitrogen (NO_2–N), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD_{Cr}), as well as the microbiota of the water and sediment. The positive or negative effects of the treatment groups on the water quality parameters were both observed. Compared with their effects on water quality parameters, the treatment groups had better effects on sediment parameters. Group Ve had the best remediation effect of NH_4–N and NO_3–N in the sediment (decreased by 5.22 and 1.66 times, respectively). Group Os showed a lower relative concentration of TN and COD_{Cr} (decreased by 3.77 and 0.95 times, respectively). The high-throughput sequencing results revealed that the immobilized probiotics increased the relative abundances of functional bacteria in the treatment groups at the phylum and genus level. The above results showed that probiotics immobilized by oyster shells, vesuvianite and walnut shells positively affected the aquaculture environment's remediation, especially the sediment.

Key words: aquaculture water, immobilized probiotic, *in situ* remediation, microbial community

HIGHLIGHTS

- The effects of probiotics immobilized by oyster shells, vesuvianite and walnut shells on the remediation of aquaculture water and sediment were researched.
- The effect of immobilized probiotics on nutrient content and microbiota in the water and sediment was tested.
- Probiotics immobilized by oyster shells, vesuvianite and walnut shells affect the remediation of aquaculture water and sediment.

INTRODUCTION

Globally, 48 million tons of aquatic feed was produced in 2015, and the output is expected to reach 71 million tons by 2020 (FAO 2012, 2018), while approximately 50–80% of the nitrogen in feed is released into breeding ponds (Schneider *et al.* 2005). With the increases in aquaculture production and aquaculture area, the amount of eutrophic aquaculture wastewater to be treated dramatically increases. Physical, chemical and biological methods for remediation of nutrient-rich water and sediment have been applied and investigated for many years (Wang *et al.* 2017). As an eco-friendly and cost-effective biological method, probiotics for environmental remediation in aquaculture systems have gained more attention (Zhu *et al.* 2019). While due to the resulting low growth rate and unstable effect of probiotics, one of the main challenges for carrying out environmental remediation with probiotics is to ensure their retention inside aquaculture systems. Fortunately, this problem can be solved by utilizing immobilization. The immobilized biomass could increase the biodegradation rate, enhanced the control of the bioprocess, improved biocatalyst stability and increased the tolerance of harsh environmental conditions (Covarrubias *et al.* 2012). For these reasons, immobilized bacteria have received more attention in aquaculture environmental remediation to minimize the risk of washed-out bacterial cells from the systems and provide a stable effect. Therefore, selecting and producing the ideal carriers are essential for the immobilization of probiotics.

The ideal immobilized carriers should be chemically inert, cost-efficient, readily available and renewable and should have a favorable adsorptive capability. Oyster shells, composed of biogenic carbonate, are an efficient adsorbent for removing some pollutants. Many studies have used oyster shells in wastewater remediation (Shih & Chang 2015; Yen & Chou 2016). Oyster shells showed good performance in removing phosphate (Oladoja *et al.* 2015), hydrogen sulfide (Asaoka *et al.* 2009) and dissolved contaminant cations (Wu *et al.* 2014) from wastewater benefit from its porous structure and high calcium oxide content after being thermally treated. The rough surface and sufficient alkalinity of the oyster shell is conducive to microbe growth. These characteristics make it a promising media with higher NH_3-N removal efficiency in biological aerated filters (Liu *et al.* 2010). Oyster cultivation on the coast of China exceeded 5.14 million tons in 2018 (Ministry of Agriculture and Rural Affairs of the People's Republic of China 2019). However, the discarded oyster shells caused environmental and health problems in coastal regions (Khan *et al.* 2018). Therefore, the reuse of waste oyster shells as an immobilization carrier of probiotics is eco-friendly and economically viable. Similarly, approximately 67% of the walnut weight is accounted by shell, and walnut shells are a low-cost, abundant agricultural by-product (Pirayesh *et al.* 2012). Usually, walnut shells, a lignocellulosic material with good chemical stability and high mechanical strength, are discarded or burned. Recently, walnut shells with a high fixed carbon content are derived biochar which possesses high basicity, large pore volume and surface area (Qiu *et al.* 2018). Modified walnut shells biochar have been used as a catalyst to remove organic sulfur and arsenic (Song *et al.* 2017). Walnut shells have been successfully used in wastewater and sewage sludge remediation as well (Segovia-Sandoval *et al.* 2018; Wójcik 2020). The porosity of walnut shells forms the oxic and anoxic zones and provided enough space for bacteria growth to generate high cell density (Wójcik 2020). These characteristics make it possible for walnut shells to be applied as immobilization carriers. The vesuvianite is another promising immobilization carrier. Vesuvianite shows a superior capability to remove pollutants, such as nitrogen, phosphate organic matter and CH_4 in the water and sewage sludge, which might be attributed to their high total pore volume, high specific area and low density (Li *et al.* 2009; Jiang *et al.* 2019; Kang *et al.* 2019).

The reuse of oyster shells and walnut shells as immobilized carriers for probiotics might be a good way to add value to this aquaculture and agricultural waste and increase the aquaculture water and sediment remediation efficiency of commercial probiotics. Furthermore, there has been no research so far taking oyster shells, vesuvianite and walnut shells as immobilized carriers for probiotics. Therefore, oyster shells, vesuvianite and walnut shells were selected as immobilized carriers for probiotics in this research. Our research aimed to investigate the influence of probiotics immobilized by oyster shells, vesuvianite and walnut shells on the remediation of aquaculture water and sediment. We tested the effect of immobilized probiotics on nutrient content in the forms of ammonia-nitrogen (NH_4–N), nitrate-nitrogen (NO_3–N), nitrite-nitrogen (NO_2–N), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD_{Cr}) in the water and sediment. To further visualize the probiotics immobilized by carriers and carriers' surface characterization, the scanning electron microscope (SEM) was applied. High-throughput sequencing was used to reveal the influence of immobilized probiotics on the microflora of water and sediment. The results of this research will verify the feasibility of further research concerning the improvement of oyster shells, vesuvianite and walnut shells as immobilized carriers for probiotics.

METHOD

Commercial probiotic immobilization and application

Four treatment groups (the control group, C; probiotics immobilized by oyster shells, Os; probiotics immobilized by vesuvianite, Ve; and probiotics immobilized by walnut shells, Ws) were studied (in triplicate per treatment) with 12 freshwater ponds. Sediment (10 cm) from a genetic improvement of farmed tilapia (*Oreochromis niloticus*, GIFT strain) pond covered the bottoms of the cement ponds (1.60 m length, 2.0 m width and 0.75 m depth). A total of 2.4 kg of the carrier, oyster shells, vesuvianite and walnut shells was loaded with 3.5 L of commercial probiotics for 6 h at room temperature. After immobilization, we added the carrier-immobilized probiotics to cement ponds. Furthermore, we added equal amounts of probiotics without a carrier to the control group. Oxygen was provided by a bottom microporous aeration system (approximately 8 cm above the top sediment layer). Temperature, pH and dissolved oxygen (DO) were measured daily by a YSI 6600 data sonde (YSI, USA).

SEM observation

A single colony of *Bacillus cereus* (NY5 strain, ingredient of the commercial probiotics) was inoculated with an LB culture medium at 200 rpm, 30 °C for 8 h. 5 mL 10^8 CFU/mL of NY5 were immobilized by 1 g oyster shells, vesuvianite and walnut shells, 30 °C for 12 h, respectively. Subsequently, 30, 50, 70, 80, 90, 95 and 100% ethanol were used to successive dehydration of immobilized NY5 fixed by glutaraldehyde (2.5%). The immobilized NY5 samples were coated with gold and then measured by SEM (S4800, Hitachi).

Water and sediment analyses

Water and sediment samples were collected from four locations in each tank at 0 and 12 weeks, as shown in Figure 1. Then, the samples in each tank were mixed and stored in polyethene plastic bottles. The samples were kept under low-temperature conditions, transported to the laboratory and stored in a 4 °C fridge for analysis within 24 h. The concentrations of NH_4–N, NO_3–N, NO_2–N, TN, TP and COD_{Cr} of the water samples were determined following standard procedures of Chinese Environmental Quality Standards (Nessler's reagent spectrophotometric method (HJ 535-2009), phenol disulfonic acid spectrophotometric method (GB 748-87), *N*-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometric method (GB 7493-87), alkaline potassium persulfate digestion spectrophotometric method (HJ 636-2012), ammonium molybdate spectrophotometric method (GB11893-89) and potassium dichromate method (GB 11914-89), respectively). Ten grams of sediment sample was added to 100 mL of KCl (1 mol/L) and shaken at 120 rpm for 1 h at 20 °C. Then, the samples were centrifuged at 3,000 rpm for 10 min to separate the supernatant from the solid. The supernatant was used to determine the NH_4^+-N, NO_3^--N, NO_2^--N, TN, TP and COD_{Cr} using the same methods for the water samples and KCl (1 mol/L) was used as a blank control.

The relative concentrations of water and sediment parameters (Equation (1)) were calculated for each treatment using the following equations:

$$C = C_{12w} - C_0 \tag{1}$$

where C_0 is the initial concentration (0 week) of the water and sediment parameters and C_{12w} is the concentration of the water and sediment parameters in the 12th week.

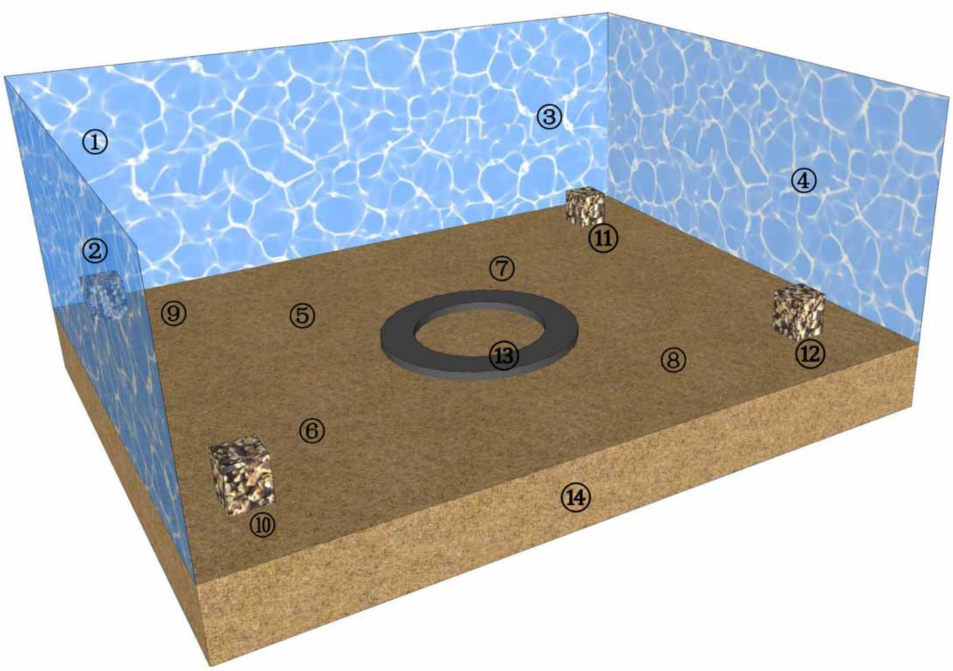

Figure 1 | The pond diagram, where ①, ②, ③, ④ were the water sampling location (20 cm underwater surface), ⑤, ⑥, ⑦, ⑧ were the sediment sampling location, ⑨, ⑩, ⑪, ⑫ were the carrier-immobilized probiotics, ⑬ was bottom microporous aeration system, and ⑭ was 10 cm sediment.

Water and sediment microbial DNA extraction, sequencing and analysis

Water samples (100 mL) were passed through a 0.22 μm polycarbonate membrane (GTTP04700, Merck Millipore, USA). Genomic DNA was extracted from filtrate and sediment samples by a DNeasy PowerSoil Kit (Qiagen, Carlsbad, CA). DNA quality was confirmed by 0.8% agarose gel electrophoresis and a spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). To investigate the bacterial community structure of the environmental samples, DNA was amplified according to a protocol described previously (Nicholaus *et al.* 2019). The V4 region of the 16S rRNA gene was amplified with the 515f/806r primer set. The reverse primer contains a 6-bp error-correcting barcode unique to each sample. The amplified products of each sample were mixed, purified, equilibrated and sequenced on the Illumina MiSeq high-throughput sequencing platform.

To investigate the bacterial community structure of water and sediment samples, paired sequence reads from the original DNA fragments were merged using FLASH. Sequence reads from the same sample were given similar barcodes. Microbial phylotypes were identified with Quantitative Insights into Microbial Ecology (QIIME) software and the UPARSE pipeline (Gregory Caporaso *et al.* 2010). The number of OTUs in each sample was used to represent species richness.

Statistical analysis

The data were analyzed by IBM SPSS 22.0. The results for all groups were compared using a one-way analysis of variance (one-way ANOVA). In all cases, the significance level of differences was set at $P < 0.05$.

RESULTS AND DISCUSSION

The immobilization efficiency of different carriers

Probiotics with good denitrification potential have been widely used in aquaculture remediation (Nimrat *et al.* 2012; Christianson *et al.* 2016). More microbes with good denitrification potential have been isolated and investigated (Kewcharoen & Srisapoome 2019; Zhu *et al.* 2019). Comparing with free-living microorganisms, immobilization protected microorganisms from reducing the impact of the environment on them and enhanced their activity and stability (Covarrubias *et al.* 2012). In the current study, oyster shells, vesuvianite and walnut shells were used as probiotics immobilized. To further visualize the probiotics immobilized by carriers, the SEM observation was carried out. SEM images of carrier structure and immobilized NY5 by oyster shells, vesuvianite and walnut shells were observed (Figure 2). SEM images showed that the surface of oyster shells (Figure 2(a)) and walnut shells (Figure 2(c)) was relatively smooth, but the smooth surface does not affect their immobilizing the bacterial. The perfect immobilization ability of oyster shells may be attributed to the electrostatic interactions and higher the external surface area (Hrenovic *et al.* 2009). The previous research also demonstrated walnut shells were an efficient carrier for bacterial immobilization (Zare *et al.* 2012; Hasanzadeh *et al.* 2020). Inversely, although the surface of vesuvianite is full of macropores, the probiotics were not immobilized (Figure 2(b)). The remediation effect of group Ve was due to the porosity and higher the external surface area. These characters of vesuvianite are beneficial to the absorption of contaminants (Li *et al.* 2009; Jiang *et al.* 2019). According to the good immobilization performance of oyster shells and walnut shells, and the large specific surface area of vesuvianite, probiotics immobilized by oyster shells, vesuvianite and walnut shells were applied to aquaculture water and sediment remediation in the follow-up experiment.

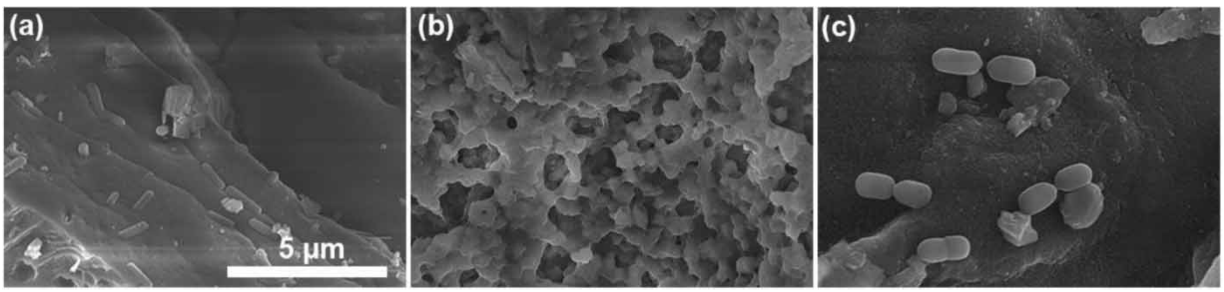

Figure 2 | SEM images of carrier and immobilized NY5. (a) NY5 immobilized by oyster shells. (b) NY5 immobilized by vesuvianite. (c) NY5 immobilized by walnut shells. Scale bar: 5 μm.

The aquaculture water and sediment remediation efficiency of commercial probiotics immobilized by different carriers

Reducing the nutrients entering aquaculture water is urgent for the sustainable development of pond aquaculture. Bioremediation has been widely employed to purify nutrient-rich aquaculture water due to its lower cost than physicochemical methods (Xia *et al.* 2020). In the current study, we investigated water and sediment quality improvement via probiotics immobilized by oyster shells, vesuvianite and walnut shells. Figure 3 shows the relative concentration of NH_4–N in the water and sediment. After the 12 weeks of treatment, the relative NH_4–N concentration in Ws was significantly higher than in the C and Os groups ($P < 0.05$). While in the sediment, the relative NH_4–N concentration in Ve remained at a lower level than those in the other groups (decreased approximately 5.22 times compared with that in group C, $P < 0.05$), and that in Ws was only slightly lower than that in the C (decreased approximately 2.72 times, $P < 0.05$). A reverse NH_4–N treatment effect of the same group for water and sediment was observed, such as Group C and Os had a lower relative concentration of NH_4–N in the water, but the relative concentration of NH_4–N in the sediment of the two groups were higher compared with groups Ve and Ws.

The relative concentrations of NO_3–N in the water and sediment are shown in Figure 4. The relative concentration of NO_3–N in the C was lower than that in the other groups ($P < 0.05$), but there were no significant differences between other groups ($P > 0.05$). In the sediment, the relative NO_3–N concentration in Ws was significantly higher than those in the other groups. On the other hand, Ve was significantly lower than that in group C (decreased approximately 1.66 times, $P < 0.05$). A reverse NO_3–N treatment effect of the same group for water and sediment was also observed. For example, group Ve had the highest relative concentration of NO_3–N in the water, but the relative concentration of NO_3–N in the sediment was the lowest compared with other groups.

As shown in Figure 5, the relative NO_2–N concentrations of the four groups (C, Os, Ve and Ws) had significant differences. In water, the relative NO_2–N concentrations in Ws and Ve were lower than those in C (decreased approximately 0.92 and 0.86 times, respectively) and Os and were significantly different from that in C ($P < 0.05$). But there were no statistical significances between the four groups in sediment ($P > 0.05$).

The relative TN concentrations in the four groups (C, Os, Ve and Ws) after the 12 weeks of treatment are shown in Figure 6. At the end of the experiment, the relative TN concentration of group C in water was significantly lower than those in Os, Ve

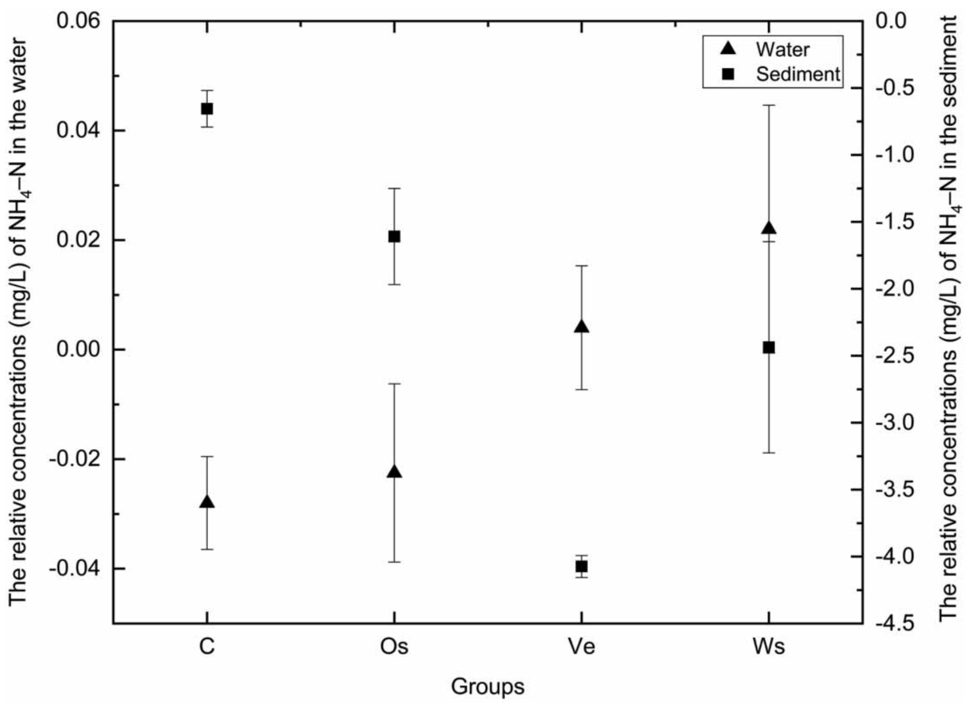

Figure 3 | The relative concentrations (mg/L) of NH_4–N in the water and sediment treated by commercial probiotics immobilized on different carriers.

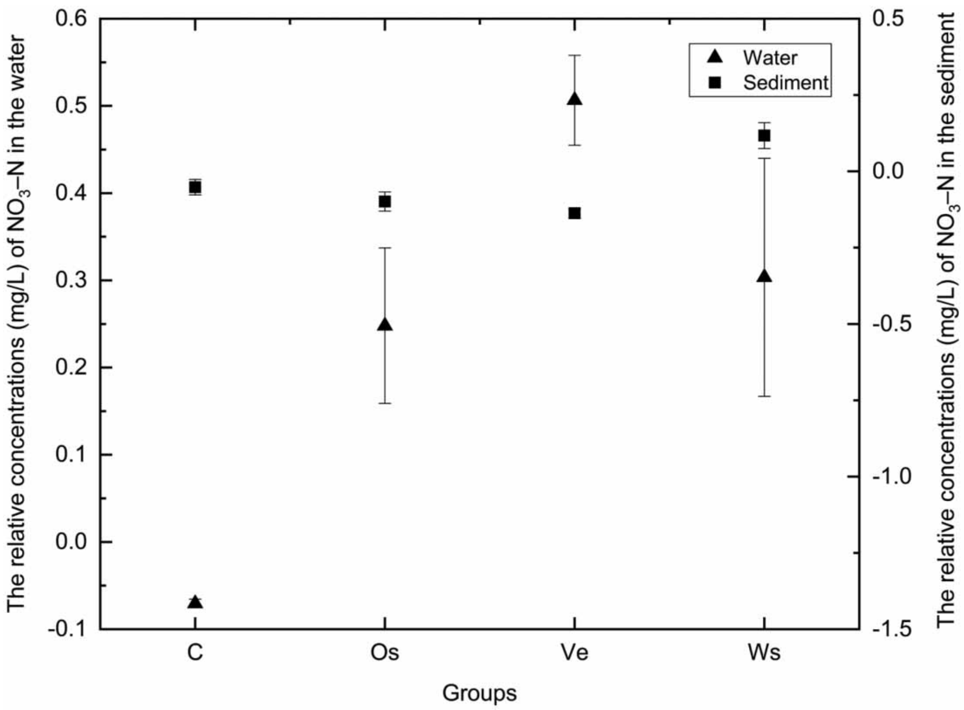

Figure 4 | The relative concentrations (mg/L) of NO_3–N in the water and sediment treated by commercial probiotics immobilized on different carriers.

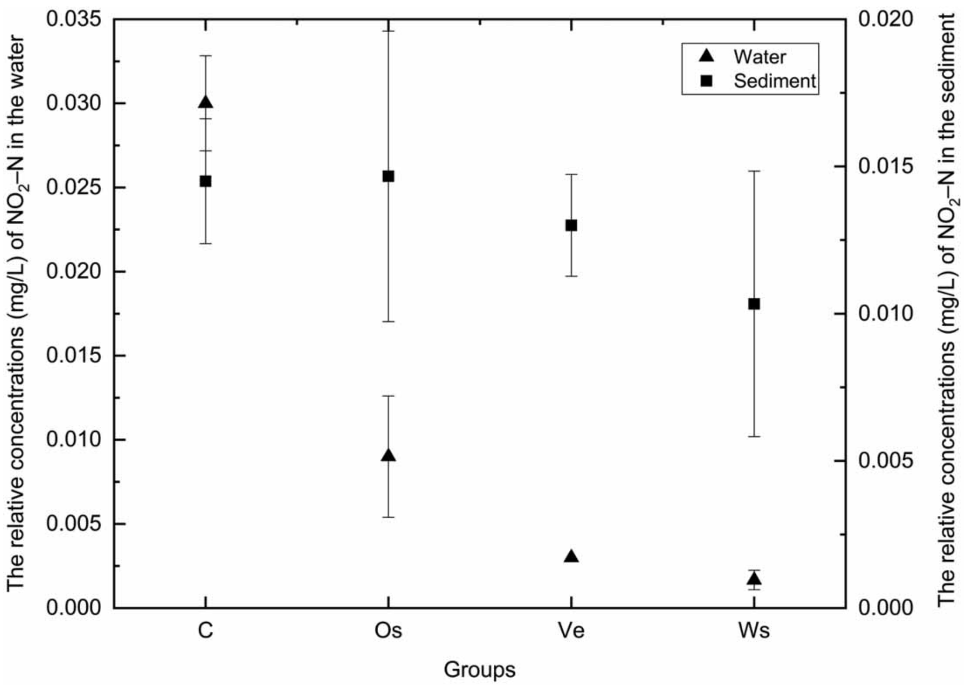

Figure 5 | The relative concentrations (mg/L) of NO_2–N in the water and sediment treated by commercial probiotics immobilized on different carriers.

and Ws ($P < 0.05$). In sediment, the concentration of group Os was significantly different from those in the other three groups (decreased approximately 3.77 times compared with that in group C, $P < 0.05$). A reverse TN treatment effect of the same group for water and sediment was also observed, such as Group Os had the highest relative concentration of TN in the

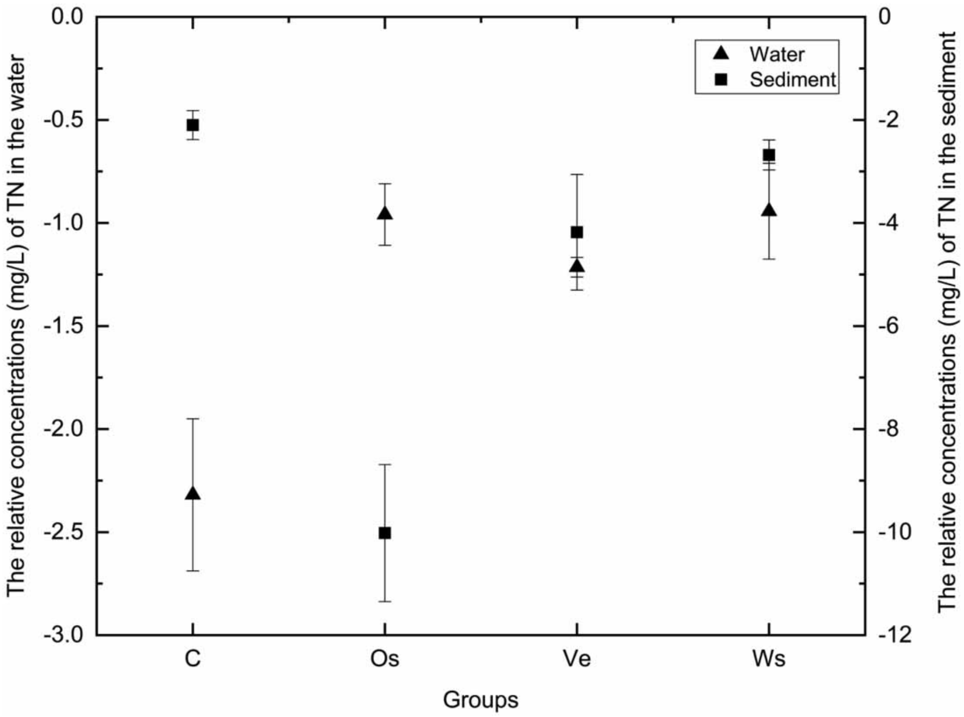

Figure 6 | The relative concentrations (mg/L) of TN in the water and sediment treated by commercial probiotics immobilized on different carriers.

water. Still, the relative TN concentration of group Os in the sediment was the lowest compared with other groups. Group C had the lowest relative concentration of TN in the water, but the relative TN concentration of group C in the sediment was the highest.

The relative concentrations of TP in the water and sediment are shown in Figure 7. At the end of the experiment, the relative concentrations in the C and Ws were significantly lower than those in Os and Ve ($P < 0.05$). In addition, the relative TP concentration of group C in sediment was lower than those in the other three groups and significantly lower than that of group Os and Ws ($P < 0.05$).

The relative concentrations of COD_{Cr} in the water and sediment are shown in Figure 8. There were no significant differences were observed between the four groups for COD_{Cr} treatment. However, in sediment, the highest relative concentration of COD_{Cr} was detected in the C, where it was significantly higher than in the other three groups (increased approximately 0.95 times compared with those in groups Os and Ve, $P < 0.05$).

Most of the residual feed, animal remains and excrement produced during aquaculture are deposited on the pond bottom and form parts of the sediment. And approximately 80–90% of the N and P input during aquaculture are deposited in the sediment (Schroeder 1987). The release of these nutrients into the water strongly influences the environmental quality of the aquaculture pond. Several strategies, such as bioremediation and physical treatment, have been used in the remediation of sediment. It was revealed that probiotic bacteria could enhance water quality (Nimrat *et al.* 2012). The addition of *Bacillus* directly to rearing water or biofloc could maintain the physicochemical parameters of water within the tolerable ranges for shrimp culture or elicit faster development of the biofloc and enhance water quality (Zokaeifar *et al.* 2014; Dash *et al.* 2018). Immobilized microbes were found to be more efficient than free cells (Fareed *et al.* 2019). Sodium carboxymethylcellulose anammox-immobilized sludge showed higher removal ratios of ammonium and nitrite (Zhu *et al.* 2009). Bacteria immobilized on wood chips, and maize straw exhibited more efficient biodegradation of oil in a marine environment than free bacteria (Xue *et al.* 2017). In our study, the relative concentrations of NH_4–N, NO_3–N, NO_2–N, TN and COD_{Cr} in the sediment were lower than those in water, indicating that the probiotic immobilized by vesuvianite, walnut shells and oyster shells decomposed the organic matter in the sediment. But part of the free N produced by the decomposition of the organic matter in the sediment was released into the aquaculture water. Accordingly compared with water quality parameters, a better effect on sediment parameters by immobilized probiotics was observed. Increased N content in the water of the immobilized

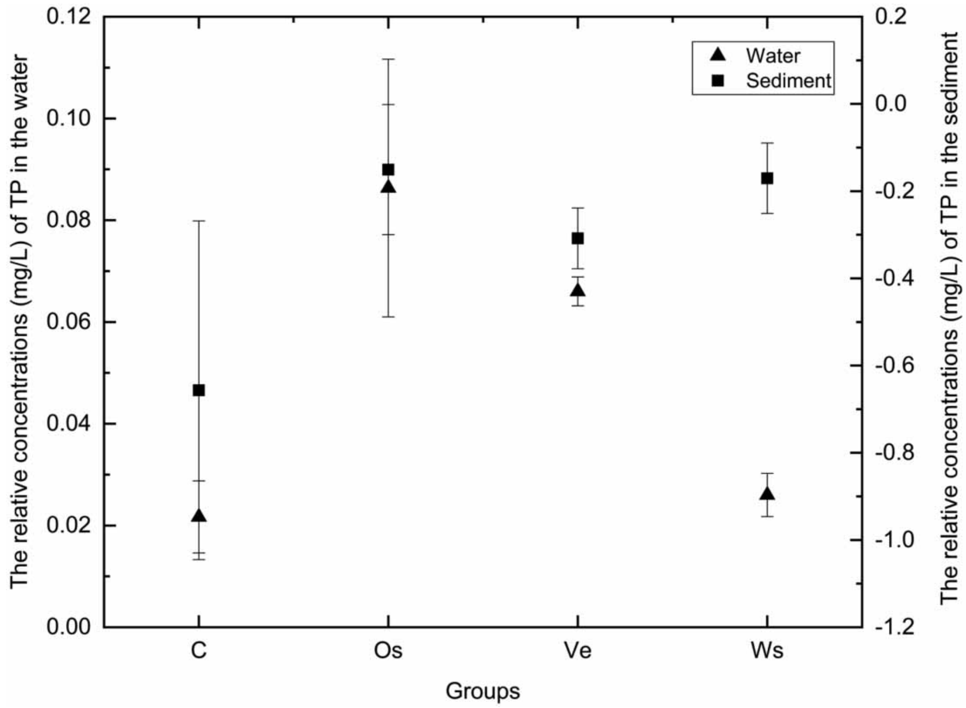

Figure 7 | The relative concentrations (mg/L) of TP in the water and sediment treated by commercial probiotics immobilized on different carriers.

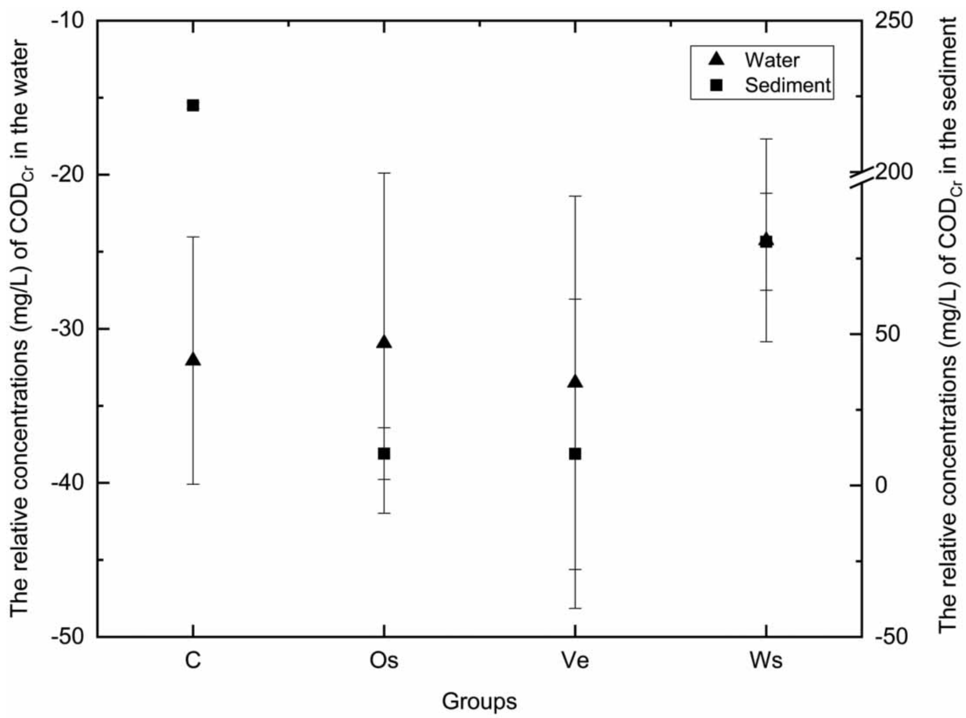

Figure 8 | The relative concentrations (mg/L) of COD_{Cr} in the water and sediment treated by commercial probiotics immobilized on different carriers.

probiotic groups was observed in the study. This is consistent with the finding of previous studies showing that organic nitrogen in the sediment is degraded during the initial days of investigation and the increase in nutrients in the overlying water was mainly due to ammonification by microbes (Fu *et al.* 2020). Similar results were found in which the release rate of nutrient fluxes from sediment was significantly increased due to bioturbation activities, in turn remediating the aquaculture effluents of blood clam (Nicholaus *et al.* 2019).

The remediation effects of immobilized probiotics were attributed to the activities of probiotics as well as the immobilization carriers. The results of SEM indicated that probiotics were not immobilized by vesuvianite, and group Ve showed lower relative concentrations of NH_4–N, TN and COD_{Cr} in sediment comparing with group C due to the absorption of vesuvianite. One study showed that the addition of vesuvianite accelerated organic matter degradation and decreased cumulative CH_4 emissions due to their special porosity microstructures (Jiang *et al.* 2019). Modified vesuvianite exhibited a high adsorption capacity for phosphate (Li *et al.* 2009). Other studies revealed that walnut shells are an efficient and cost-effective adsorbent for Cr (Banerjee *et al.* 2018), Cd (Qiu *et al.* 2018) and Zn (Segovia-Sandoval *et al.* 2018) removal, and the addition of ground walnut shells to sewage sludge reduce the total bacterial count and improved sewage sludge dewaterability (Wójcik 2020). Researches have also shown that oyster shells have a good water purification efficiency (Shih & Chang 2015; Jung *et al.* 2016). Therefore, the remediation efficiency of immobilized probiotics is a combined effect of probiotics and immobilization carriers attributed to their high mechanical strength and stability.

The effect of commercial probiotics immobilized by different carriers on the microbiota of the aquaculture environment

As the remediation effects of immobilized probiotics on sediment parameters were confirmed, high-throughput sequencing was used to investigate the structure and diversity of the bacterial community in water and sediment treated with probiotics immobilized by oyster shells, vesuvianite and walnut shells. The relative abundances of phyla detected with bacterial 16S rRNA sequences in the water and sediment samples in the different groups are shown in Figure 9. It is suggested that the presence and the activities exerted by immobilized probiotics are the reasons for the variation in microbial community composition. These results are supported by the finding of previous studies that reported considerable bacterial community change in water and sediment in response to probiotics (Nimrat *et al.* 2012; Chang *et al.* 2019; Nicholaus *et al.* 2019). Firmicutes was the most abundant phylum in the water of the C, with a relative abundance of 32.96% (Figure 9(a)). The

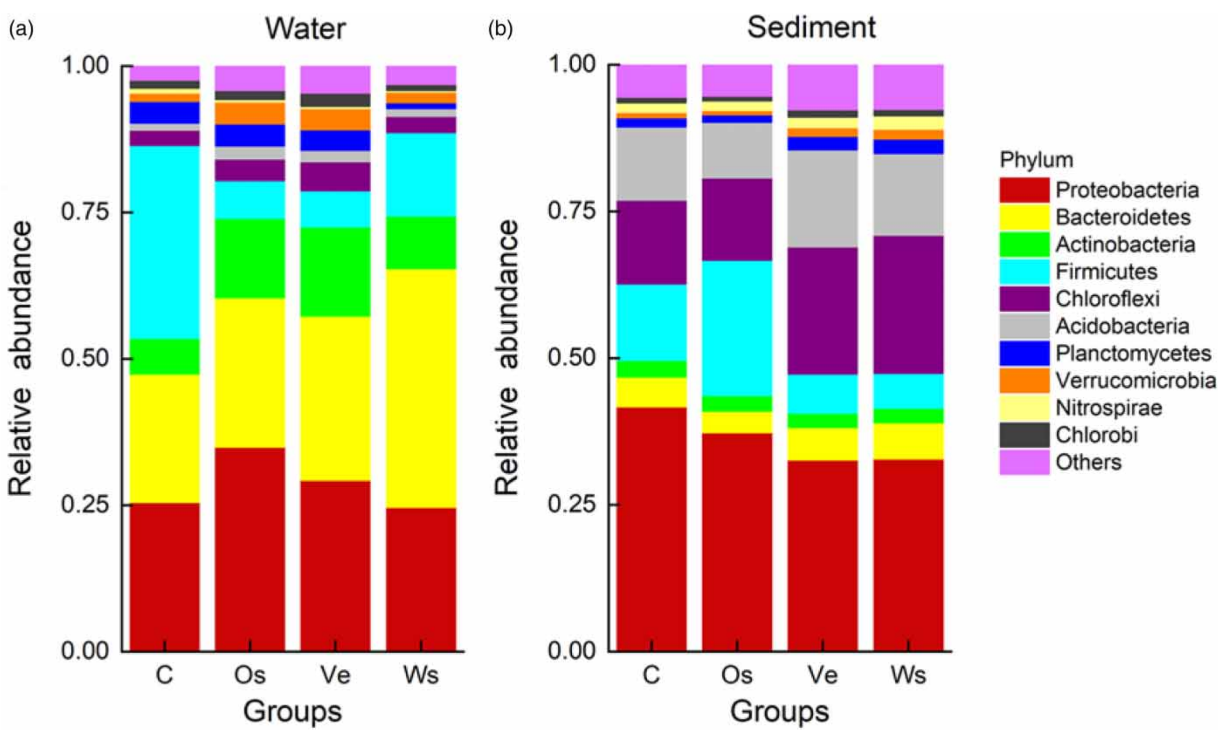

Figure 9 | The relative abundances of microbial community members (phylum level) of each group in the water (a) and sediment (b). 'Others' represents the phyla with a relative abundance lower than 0.01.

relative abundances of Proteobacteria in water from Os and Ve (34.91 and 29.29%, respectively) were higher than those of the other phyla (Figure 9(a)). Bacteroidetes was the most abundant phylum in Ws water, with a relative abundance of 40.76% (Figure 9(a)). All sediment samples in the different groups were mainly occupied by the phylum Proteobacteria, with relative abundance values ranging from 32.66 to 41.71% (Figure 9(b)). The second dominant phylum (relative abundance values >5%, the same as below) in the water of the C was Proteobacteria, followed by Bacteroidetes and Actinobacteria (25.47, 21.98 and 6.08%, respectively, Figure 9(a)). Group C's sediment was Chloroflexi, followed by Firmicutes, Acidobacteria and Bacteroidetes (14.31, 13.02, 12.45 and 5.13%, respectively, Figure 9(b)). The second dominant phylum in the water of Os (25.52, 13.60 and 6.46%, respectively) and Ve (28.03, 15.22 and 6.20%, respectively) was Bacteroidetes, followed by Actinobacteria and Firmicutes (Figure 9(a)). The second dominant phylum in the sediment of Os was Firmicutes, followed by Chloroflexi and Acidobacteria (23.05, 14.05 and 9.41%, respectively, Figure 9(b)). The second dominant phylum in the sediment of Ve was Chloroflexi, followed by Acidobacteria, Firmicutes and Bacteroidetes (21.67, 16.47, 6.71 and 5.56%, respectively, Figure 9(b)). The second dominant phylum in Ws water was Proteobacteria, followed by Firmicutes and Actinobacteria (24.67, 14.25 and 8.98%, respectively, Figure 9(a)). The second dominant phylum in the sediment of Ws was Chloroflexi, followed by Acidobacteria, Bacteroidetes and Firmicutes (23.51, 13.93, 6.16 and 5.96%, respectively, Figure 9(b)). Although Proteobacteria was the most abundant phylum in both water and sediment samples, except water samples from the C and Ws, the relative abundances of this phylum in the sediment from all groups were higher than those in the water. Bacteroidetes, Actinobacteria and Firmicutes were the second dominant phyla in most of the water samples, while Chloroflexi, Acidobacteria, Firmicutes and Bacteroidetes were the second dominant phyla in the sediment samples. Firmicutes, Proteobacteria, Bacteroidetes, Chloroflexi and Actinobacteria can degrade a wide range of substances via anaerobic digestion, which favors the hydrolysis of sewage sludge, and are dominant hydrolytic microbes involved in hydrolysis and anaerobic digestion of sewage sludge (Chouari *et al.* 2005; Chen *et al.* 2017; Peces *et al.* 2018; Ma *et al.* 2019). Consequently, they are always identified in the different types of anaerobic digestion reactors (Ma *et al.* 2019; Shin *et al.* 2019). Previous research revealed that the bacteria in tilapia ponds were dominated by Proteobacteria and Bacteroidetes (Grande Burgos *et al.* 2018; Zhou *et al.* 2018; Liu *et al.* 2020). Increasing the intensity of sewage sludge addition was found to increase the relative abundance of Chloroflexi, Firmicutes and Actinobacteria (Ma *et al.* 2019). The immobilized probiotics changed the structure and diversity of the bacterial community in water and sediment in our study. An enhanced abundance of Firmicutes contributed to better water parameters in group C than other groups. And consistent with lower relative concentrations of NH_4–N, NO_3–N, NO_2–N, TN and COD_{Cr} of immobilized probiotics groups than group C in the sediment, the relative abundance of Firmicutes and Chloroflexi in treated groups were increased. The remediation of the water and sediment might be attributed to the potential ability of these microbes to transfer electrons during the digestion of substances, such as N and organic matter in the water and sediment (Yang *et al.* 2019).

The relative abundances of genus detected with bacterial 16S rRNA sequences in the water and sediment samples in the different groups are shown in Figure 10. The immobilized probiotics changed the relative abundances of the genus in water. *Lactobacillus* was the most abundant genus in the water of the C (Figure 10(a)). The relative abundances of *Flavobacterium* in water from Os, Ve and Ws were higher than the other genus, and *hgcI clade* was the secondary dominant genus in the water of Os and Ve (Figure 10(a)). The relative abundances of *Lactobacillus*, *Escherichia–Shigella* and *Clostridium sensu stricto 1* in water from group C were increased. *Lactobacillus* showed positive impacts on both immunostimulatory and environmental remediation (Talpur *et al.* 2013; Son *et al.* 2018; Flores-Valenzuela *et al.* 2021). In our study, the better water parameters of group C in water than other groups were attributed to the enhanced abundance of *Lactobacillus*. In sediment, *Clostridium sensu stricto 1* and *Escherichia–Shigella* were the dominant genus in the C, Ve and Ws. The application of probiotics immobilized by Os, Ve and Ws changed the microbial community of sediment at the genus level as well. *Lactobacillus*, *Clostridium sensu stricto 1* and *Escherichia–Shigella* were dominant genus in the sediment of the Os (Figure 10(b)). *Escherichia–Shigella*, *Clostridium sensu stricto 1*, *Lactobacillus* and *Haemophilus* of group Os were increased compared with group C in sediment. And remediation effect of group Os on sediment was observed correspondingly. Although the relative abundances of *Escherichia–Shigella*, *Clostridium sensu stricto 1* and *Lactobacillus* of group Ve and Ws were decreased, *Flavobacterium* of group Ve and Ws were increased compared with group C in sediment (Figure 10(b)). Li *et al.* (2012) reported that *Flavobacterium* sp. showed perfect remediation ability of polluted surface water. Therefore, better water parameters of groups Ve and Ws in sediment than group C were attributed to the enhanced abundance of *Flavobacterium*.

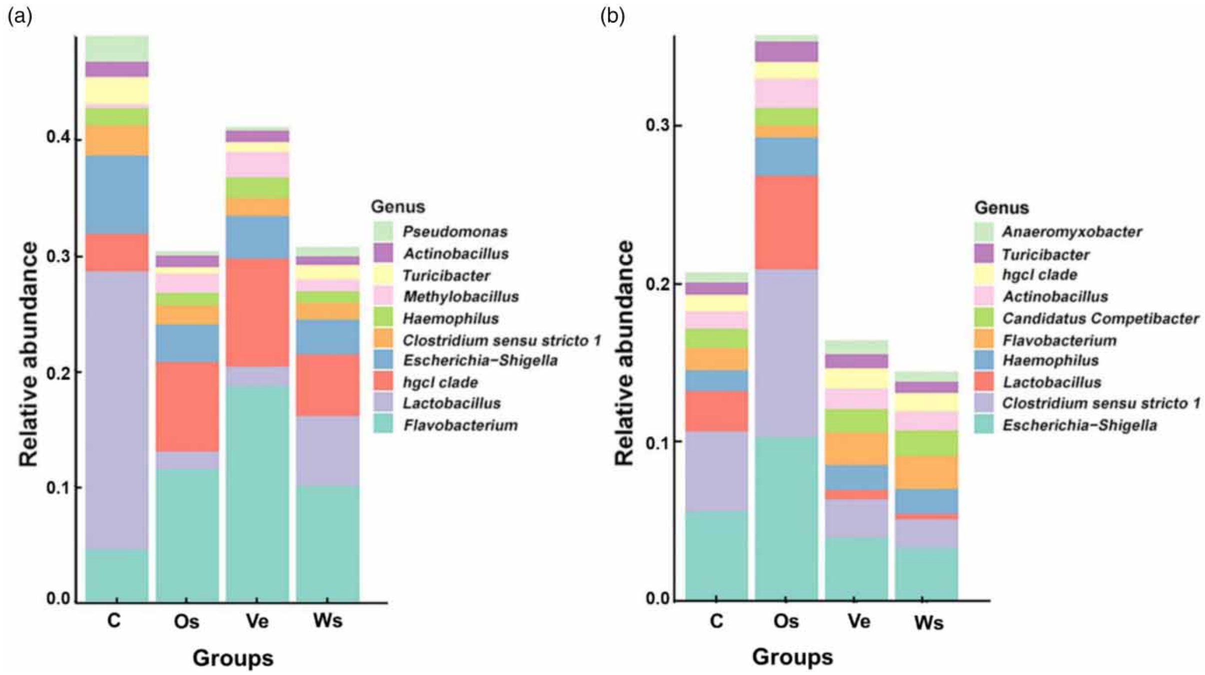

Figure 10 | The relative abundances of microbial community members (genus level) of each group in the water (a) and sediment (b).

CONCLUSION

In the present study, we determined that probiotics immobilized by oyster shells, vesuvianite and walnut shells could remediate aquaculture water and sediment. Oyster shells and walnut shells were effective materials for probiotics immobilization. In addition, the utilization of oyster shells and walnut shells as carriers of immobilized probiotics is a good approach for adding value to these aquaculture and agricultural wastes and increasing the aquaculture water and sediment remediation efficiency of commercial probiotics. It was also found that compared with the effects of immobilized probiotics on water quality parameters, they had better effects on sediment parameters attributed to the changed structure and diversity of the bacterial community. The current research applied immobilized probiotics in an aquaculture pond environment, providing a novel strategy for aquaculture environmental remediation.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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