IN-SITU BIOREMEDIATION OF POLLUTED AQUACULTURE WATER BY ECO-DAM IN YANGCHENG LAKE

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Abstract: Aquaculture is a fast growing food industry in China, but also a main course of eutrophication in shallow Chinese lakes. Unfortunately, despite various approaches to the control of lake eutrophication currently, direct and effective technologies for the treatment of aquaculture pollution in lakes are still missing. In the present study, the technology of ecological dam (Eco-dam) is proposed for the in-situ control of aquaculture pollution and biological remediation of polluted aquaculture water, as well as the recycle and reuse of residual breeding food due to the food chain of ecosystem. The unit device of Eco-dam consists of biological filler floating bed and
plant floating bed. Series of unit devices are connected with each other to form Eco-dam in either pollution control mode or biological remediation mode. The objective of the present study was to prove the biological remediation performance of Eco-dam established in polluted aquaculture water area from aspects of both water qualities and microbial mechanisms. A pilot scale test of Eco-dam was established in Yangcheng Lake and has been operated for more than one year. Regular water quality monitoring showed that the average turbidity, COD$_{Cr}$, NH$_4^+$-N, and TP in the remediation zone were lower than those in the breeding zone by 21.7%, 13.4%, 24.5%, and 29.4%, respectively. However, the strong water exchange between the remediation zone and the breeding zone driven by natural environmental factors made it insufficient to evaluate the water purification efficacy of Eco-dam depending only on the comparison of practical water qualities among three zones. Therefore, biofilm activity tests were conducted to quantify the specific activities of heterotrophs, nitrite oxidizing bacteria (NOB), ammonia oxidizing bacteria (AOB), and denitrifiers, with the value of 7.34, 6.12, 19.89 mg O$_2$/gVSS·h, and 1.35 mg NO$_3^-$-N/gVSS·h respectively. More deeply, the microbial community of biofilm were analyzed by illumine miseq sequencing platform. The results indicated that Eco-dam had enormous potentials of organic matter oxidation and nitrification, owing to abundant microbial populations including various kinds of functional bacteria.

**Key words:** Ecological dam (Eco-dam); Lake aquaculture pollution; Biological remediation; Biofilm activity; Microbial community

1. Introduction
1.1 Lake aquaculture accelerating lake eutrophication
Aquaculture industry in China has a very long history dating back to 2000 years ago (Boyd and Tucker 1998). For the past three decades, aquaculture has become the fastest growing sector within China’s agriculture and one of the pillar industries boosting its rural economic development (Li et al. 2011). In 2011, China remained as the largest producer of aquaculture products, accounting for 65.7% of the world total (Wang et al. 2014). With an increasing demand for natural, good quality and safe freshwater products, aquaculture is massively carried out in Chinese lakes, especially in shallow lakes densely distributed along the eastern coast or in the middle and lower reaches of the Yangtze River such as Yangcheng Lake, Taihu Lake, and Chaohu Lake (Jia et al. 2013). However, numerous studies have pointed out that, frequently-occurred eutrophication in shallow Chinese lakes in the past three decades is closely correlated with aquaculture activities inside (Tao et al. 2009, Peng 2010, Montanhini Neto and Ostrensky 2015). Pen culture is the uppermost form of aquaculture in shallow Chinese lakes, which experienced a shift from natural food based extensive culture of low-valued species such as grass carp in the 1980s to high-density feed-based intensive culture of relatively higher valued species like Chinese mitten crab from the 1990s. During intensive pen culture operations, excessive aquaculture feeds abundant in organic nutrients such as nitrogen and phosphorus are used to enhance the production through net retention of these nutrients as biomass of cultured species, generating a large quantity of aquaculture wastes made of solid uneaten feed, solid feces, and soluble excretion, with different mass percent of nutrients. Excessive settling of solid wastes to the sediment enclosed by pens may result in a serious oxygen depletion and the creation of anoxic conditions harmful to the benthic biota, through oxygen-consuming decompositon of these organic substances by bacteria and other
organisms. On the other hand, suspended solid wastes and dissolved wastes such as carbon dioxide, ammonia, and phosphate derived from soluble excretion and organic decomposition, can be flushed and dispersed from pens by natural water movement, leading to a series of negative impacts on the ambient lake ecosystems, of which the most severe is the eutrophication caused by excessive accumulation of nutrients (Li et al. 2011).

For example, Yangcheng Lake, located in northeast of Suzhou City in Jiangsu Province (31°35’~31°49’E, 120°66’~120°84’N) with a total area of 118.2 km², average depth of 1.61 m, and storage capacity of 1.7×10⁸ m³ (Song et al. 2010), is the third largest freshwater lake in the Taihu Lake Plain, and is world-famous for Chinese mitten crab culture. However, years of intensive pen crab culture on a large scale have led to the deterioration of water quality, extinction of submerged macrophytes and eutrophication of the lake. An investigation which is carried out in an aquafarm located in the middle part of Yangcheng Lake from 2008 to 2009 indicates that, the water quality is classified as inferior Class V with TN and TP acting as evaluation indexes and primary pollutants, and is close to the state of hyper eutrophic with a higher contribution of phosphorus (Song et al. 2010). Moreover, submerged macrophytes, acting as shelters for crab’s shellings, reduced greatly due to the lower transparent, having an adverse effect on the crab production in turn. Hence, the pen culture area for one household is restricted to 13, 340 m² by the local government to protect the lake from further degradation and eutrophication. Nowadays, the total culture area in Yangcheng Lake is reduced to 21.3 km².

At present, strategies of both management and technology are applied to alleviate the self-pollution and environmental impacts of lake aquaculture in China. From a management perspective, feed management is carried out to minimize uneaten feed and optimize feed convention ratios (FCR; weight of feed offered/weight of fish produced) by using high-quality diets and efficient feeding methods (Belle and Nash 2009). Besides, environmental management methods such as culture area compression and offshore culture are also commonly adopted. However, these management methods to some extent influence the development of both aquaculture and economy on one hand, and reflects a lack of effective control technologies of aquaculture pollution on the other hand. From a technology perspective, numerous physical, chemical and biological technologies have been adopted to fight against lake eutrophication, of which bioremediation is considered as the best in ecological restoration, including phytoremediation (Qin 2009), biomanipulation (Liu and Qiu 2007), and microbial remediation (Zheng and Zhou 2009), through planting aquatic or submerged macrophytes in eutrophic lakes, adjusting the food chain of lake water ecosystem, and microbial degradation by natural or domesticated microorganisms, respectively. Although the improvement in lake water quality by bioremediation helps to mitigate the environmental stress of lake aquaculture, there is a lack of direct control technology aiming at preventing aquaculture pollution from endangering the ambient lake ecosystems. In the present study, the technology of Ecological Dam (Eco-dam) was proposed for in-situ control of aquaculture pollution and bioremediation of polluted aquaculture water, as well as the recycle and reuse of residual breeding food due to the food chain of ecosystem in Eco-dam.

1.2 Concept of Eco-dam

*Unit device*
The unit device of Eco-dam (Figure 1A) consists of biological filler floating bed (BFB) and plant floating bed (PFB). The BFB includes floating bed frame, biological fillers and fixed net, which is fixed by the rope on the piling set at the bottom of lake. The biological filler is a kind of elastic filler made of Polypropylene ethylene plastics and is hanged down to the bottom. The PFB consists of floating bed unit and aquatic plants, which is placed in the rectangular water area formed by floating bed frame of BFB and can be taken out in non-plant seasons or in extreme weather such as typhoon.

**Application mode**

In practical applications, series of unit devices are connected with each other to form Eco-dam, which can be operated in two application modes, namely pollution control mode (PCM, Figure 1B) and biological remediation mode (BRM, Figure 1C). In the pollution control mode, a breeding zone is surrounded by Eco-dam established in a lake to prevent aquaculture pollutants from diffusion to the ambient lake zone. While in the biological remediation mode, Eco-dam is constructed in a relatively larger breeding zone. A remediation zone is formed and surrounded by Eco-dam by clearing away all aquatic animals including the cultured species and ceasing the aquaculture operations inside, with the purpose of restoring submerged macrophytes and further promoting the recovery of the entire ecosystem.

**Theoretical mechanism**

In either mode of operation, the mechanisms of Eco-dam can be elucidated from three levels. Firstly, Eco-dam has the ability to weaken the disturbance of wind waves and serve as the physical barrier against solid wastes and algae emerged in breeding zone, on account of the blockage of aquatic plants, floating beds, and biological fillers. Secondly, Eco-dam serves as the biological decomposer and transformer of aquaculture pollutants, owing to the aquatic plants and diverse microbial populations in rhizosphere and on biological fillers, including bacteria, fungi, protozoa, metazoan, and planktonic animals, in its ecosystem. As shown in Figure 1D, organic matter, nitrogen and phosphorus in the breeding zone mainly come from feces and residual breeding food, which firstly deposit and are decomposed by microorganisms in the sediment. Ammonia and phosphate are released in soluble excretion and from the organic decomposition of feces and residual breeding food. Under aerobic conditions, ammonia can be oxidized to nitrate by nitrifiers including ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). When passing through Eco-dam, organic matter is further decomposed and degraded by heterotrophic bacteria, while ammonia and nitrate are metabolized by bacteria based on nitrification and denitrification on biological fillers and plant roots. Phosphate removal mainly depends on the direct uptake of aquatic plants as nutrient, which is finally removed from water by plant harvesting. Thirdly, Eco-dam contributes to the recycle and reuse of aquaculture pollutants due to the food chain of the ecosystem. Accordingly, residual breeding food, generally considered as pollutants, can be further utilized as food of cultured species.
1.3 Objective of this study

A pilot scale test of Eco-dam in biological remediation pattern was established within a pen crab culture area in Yangcheng Lake. The main objective of this study was: (1) to study the actual water quality purification effect of Eco-dam on the remediation zone; (2) to investigate the specific activities of heterotrophic bacteria, nitrifiers and denitrifiers in biofilms; (3) to reveal the composition and diversity of microbial community formed in biofilms.

2. Materials and methods

2.1 Pilot scale test

The pilot scale test (Figure 2) was carried out in a breeding zone raising Chinese mitten crab in Yangcheng Lake, and has experienced the trial operation phase and accomplished the natural biofilm formation on the biological fillers in 2012. Thereafter, the structure and configuration of Eco-dam were optimized and remade. The unit device of Eco-dam is 2 m long and 1 m wide with the frame being made of high density polyethylene. 36 units connected together formed a remediation zone of 200 square meters including Eco-dam, and was surrounded by purse seine. Six kinds of aquatic plants, namely *Lythrum*, *Thalia dealbata*, *Pontederia*, *Water Iris*, *Iris wilsonii* and *Canna warscewiczii*, were planted on PFBs in May 2013. Moreover, a contrast zone with the same area was constructed only by purse seine, and was 10 m away from Eco-dam. Not a crab was cultured in these two zones.

Figure 1. Schematic diagrams of unit device (A), pollution control mode (B), biological remediation mode (C), and mechanisms (D) of Eco-dam.
2.2 Water sampling and analyses

Water samples were collected 30 cm below the water surface of the breeding zone, the remediation zone, and the contrast zone every about 15 d in the afternoon from March to October 2013, and were analyzed within 48 h. Turbidity, chlorophyll-a and water temperature were measured with a COMPACT-CLW type automatic monitor (JFE Advantech Co., Ltd) on the spot. The daily means of these continuously-monitored data were used as the corresponding values on regular sampling days. A portion of each water sample was filtered (Mixed cellulose esters membrane 0.45 μm pore size) and analyzed for ammonia nitrogen (NH$_4^+$-N) by the Nessler’s reagent colorimetric method, nitrate nitrogen (NO$_3^-$-N) by ultraviolet (UV) spectrophotometry, and nitrite nitrogen (NO$_2^-$-N) by N-ethylenediamine colorimetric method. Unfiltered subsamples were analyzed for total nitrogen (TN) by alkaline potassium persulfate digestion-UV spectrophotometry, total phosphorus (TP) by ammonium molybdate spectrophotometry and COD$_C$ by potassium dichromate method (Chinese 2002).

2.3 Biofilm sampling and analyses

2.3.1 Description of sampling sites

A total of 12 positions were selected for biofilm activity tests and microbial community analysis at August. Samples of biofilms attached on biological fillers were collected from 4 sites, namely 4 sides of Eco-dam, denoted by A, B, C, and D. Each site had three positions marked as 1, 2 and 3 which respectively represented the upper, middle, and lower portion of the same biological filler. For example, B2 referred to the biofilm sample collected at the middle portion of a biological
2.3.2 Biofilm activity tests

Biofilm samples from the upper position of each site where biofilms grew well were collected and mixed for activity tests. The specific activities, in terms of specific oxygen uptake rate (SOUR), of heterotrophic organic matter oxidizers and autotrophic nitrifiers including ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in biofilms were determined by closed respirometry. A certain amount of biofilm sample attached on filamentous fillers was carefully washed with deionized water and was put into a pre-cleaned 250 ml conical flask. Then, the flask was fully filled with pre-aerated nutrient solution (Table 1) and was stoppered with perforated silica gel plug, and the dissolved oxygen (DO) probe (HACH HQ30d) and 5 ml syringe were immediately inserted into the flask through the plug, ensuring that the flask was tightly sealed with no headspace. All tests were conducted in a water-bathing constant temperature vibrator at 25°C. Before and after injections of specific substrates or inhibitor into the flask, DO concentration was recorded at intervals of 30 s. Then OURs were determined by linear regression from the slope acquired from the plot of DO concentration versus time, and SOURs were obtained by OUR divided by the eventual amount of VSS determined by direct gravimetry and were finally expressed as milligrams of O₂ consumed per gram of volatile suspended solids per hour (mg O₂/g VSS·h).

For the determination of OURs of nitrifiers, namely (OUR)_{N}, the three-phase method described by Moussa (Moussa et al. 2003) was used for reference. Firstly, the endogenous OUR (OUR₁) of nitrifiers and heterotrophs was measured over a period of 12 min only with the nutrient solution. Then, OUR of NOB, namely (OUR)_{NO₂}, was measured with 1 ml NaNO₂ injected (resulting in 0.2 mg/L of NO₂⁻-N), and OUR₂ was recorded. Finally, OUR of AOB, namely (OUR)_{NH₄}, was measured with 1 ml NH₄Cl injected (resulting in 1 mg/L of NH₄Cl), and OUR₃ was recorded. In this way, (OUR)_{NO₂} was the difference between OUR₂ and OUR₁, while (OUR)_{NH₄} was the difference between OUR₃ and OUR₂.

For the determination of OUR of heterotrophs, namely (OUR)_{H}, allylthiourea (ATU) was used as one important component in the nutrient solution to inhibit ammonium oxidation by AOB. Similar respirometry test was conducted by differentiating endogenous and exogenous respiration. At first, the endogenous OUR of heterotrophs and NOB was measured for 12 min only with the nutrient solution. Then, (OUR)_{H} was measured with 1 ml NaAc injected (resulting in 20 mg/L of CODₐ) and was the difference between them as well.

The specific activity, in terms of specific denitrification rate (SDNR), of anaerobic denitrifiers in biofilms was determined by substrate utilization test, which was performed in a 500 ml conical flask arranged in the water-bathing constant temperature vibrator at 25°C. Nutrient solution containing NO₃⁻-N of 1 mg/L and CODₐ of 20 mg/L was poured into the flask, and nitrogen gas was flushed for several minutes to create anoxic condition, with DO concentration below 0.5 mg/L. Then, pre-washed biofilm sample was put into the flask, after which the flask was sealed by tinfoil. At a predetermined time (e.g., 30 min and 60 min), 5 ml supernatant samples were collected with a pipette for measuring concentrations of NO₃⁻-N, and nitrogen gas was flushed immediately for 30 s to maintain the anoxic condition. Finally, SDNR was calculated by the decreasing slope of NO₃⁻-N concentration with time and divided by the eventual amount of VSS (Gong et al. 2012).
Table 1. Composition of the nutrient solution for biofilm activity tests

<table>
<thead>
<tr>
<th>Categories</th>
<th>Compounds (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SOUR)$_H$</td>
<td>NH$_4$Cl 38.2</td>
</tr>
<tr>
<td></td>
<td>ATU 10</td>
</tr>
<tr>
<td>(SOUR)$_N$</td>
<td>NaHCO$_3$ 14</td>
</tr>
<tr>
<td>SDNR</td>
<td>NaAc 34.5</td>
</tr>
<tr>
<td></td>
<td>KNO$_3$ 7.2</td>
</tr>
<tr>
<td></td>
<td>KH$_2$PO$_4$ 4.4</td>
</tr>
<tr>
<td></td>
<td>CaCl$_2$ 5.5</td>
</tr>
<tr>
<td></td>
<td>MgSO$_4$·7H$_2$O 45</td>
</tr>
<tr>
<td></td>
<td>KCl 18</td>
</tr>
<tr>
<td>Common</td>
<td>FeCl$_3$·6H$_2$O 0.225</td>
</tr>
<tr>
<td></td>
<td>H$_3$BO$_3$ 0.0225</td>
</tr>
<tr>
<td></td>
<td>CuSO$_4$·5H$_2$O 0.0045</td>
</tr>
<tr>
<td></td>
<td>KI 0.027</td>
</tr>
<tr>
<td></td>
<td>MnCl$_2$·4H$_2$O 0.018</td>
</tr>
<tr>
<td></td>
<td>NaMoO$_4$·2H$_2$O 0.009</td>
</tr>
<tr>
<td></td>
<td>ZnSO$_4$·7H$_2$O 0.018</td>
</tr>
<tr>
<td></td>
<td>CoCl$_2$·6H$_2$O 0.0225</td>
</tr>
</tbody>
</table>

2.3.3 Microbial community analysis

Biofilm samples from all 12 positions were collected for analysis of microbial community structure by illumine miseq sequencing platform. The metagenomics DNA of samples used illumine MiSeq system for sequencing. DNA of each sample was extracted as described previously with minor modification (Griffiths et al. 2000, Paulin et al. 2013). The extracted metagenomic DNA was used as the template to amplify the V3-V4 region of 16S rRNA genes. PCR reactions, sequencing of the PCR amplicons and quality control of raw data were performed as described previously with minor modification according to direction and protocol of MiSeq system provide by Illumina. Sequencing library of V3-V4 regions in 16S rRNA gene was prepared as described in http://res.illumina.com/documents/products/appnotes/16s-metagenomic-library-prep-guide.pdf, with some modifications.

Both the forward and reverse ends of the same read were truncated at the fist base where Q value was no more than 2. If the pair of reads had a minimum of 50 bp-length overlap, they were then merged in to a complete read, which will not be kept unless it’s longer than 399 bp and the expected error was no more than 0.5 (Edgar 2010). Quality-filtered reads were dereplicated into unique sequences, then sorted by decreasing abundance and singletons were discarded. Non-chimeric OTU representative sequences were picked afterwards by Uparse’s default (Edgar 2013). Further reference-based chimera detection was performed using UCHIME (Edgar et al. 2011) against RDP classifier training database (v9) (Cole et al. 2014). OTU table was finalized by mapping quality-filtered reads to the remained OTUs with Usearch (Edgar 2010) global alignment algorithm at 97% cutoff.
All the followed analysis was performed based on QIIME platform (Caporaso et al. 2010). The alpha diversity of each sample was calculated with observed OTUs and Shannon index. Representative sequences for each OTU were built into a phylogenetic tree by FastTree, and subjected to RDP classifier to determine the phylogeny with a bootstrap cutoff of 80% (RDP database version 2.10).

3. Results and discussion
3.1 Water purification efficacy of Eco-dam
Variations of water quality indexes, including water temperature, turbidity, chl-a, COD\textsubscript{Cr}, TN, NO\textsubscript{3}-N, NH\textsubscript{4}+-N and TP, of the remediation zone, the contrast zone, and the breeding zone were shown in Figure 3. In addition, the average values of these indexes in each zone were calculated and listed in Table 2.
Figure 3. Variations of water temperature (A), turbidity (B), chl-a (C), COD
Cr (D), TN (E), NO3^-N (F), NH4^+-N (G), and TP (H) in remediation zone, contrast zone and breeding zone.

Table 2. Average values of water temperature, turbidity, chl-a, CODCr, TN, NO3^-N, NH4^+-N, and TP in the remediation zone, the contrast zone and the breeding zone.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Water temperature (°C)</th>
<th>Turbidity (NTU)</th>
<th>chl-a (μg/L)</th>
<th>CODCr (mg/L)</th>
<th>TN (mg/L)</th>
<th>NO3^-N (mg/L)</th>
<th>NH4^+-N (mg/L)</th>
<th>TP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remediation</td>
<td>22.98</td>
<td>7.08</td>
<td>6.64</td>
<td>33.15</td>
<td>0.97</td>
<td>0.73</td>
<td>0.081</td>
<td>0.084</td>
</tr>
<tr>
<td>Contrast</td>
<td>23.13</td>
<td>7.84</td>
<td>7.80</td>
<td>36.34</td>
<td>0.99</td>
<td>0.81</td>
<td>0.098</td>
<td>0.094</td>
</tr>
<tr>
<td>Breeding</td>
<td>23.06</td>
<td>9.04</td>
<td>7.06</td>
<td>38.30</td>
<td>0.97</td>
<td>0.79</td>
<td>0.107</td>
<td>0.119</td>
</tr>
</tbody>
</table>
As can be seen from Figure 3 and Table 2, there was hardly any difference in water temperature of three zones which changed regularly with the environmental temperature. Turbidity in each zone was relatively stable and had small differences before 19th May for lacking the participation of aquatic plants. After planting them on plant floating beds (PFBs), turbidity in the remediation zone was well controlled, although obvious fluctuations were observed in all three zones. The average of turbidity in the remediation zone was lower than that in the contrast zone and the breeding zone by 9.6% and 21.7%, respectively. Eco-dam protected the water body and sediment inside the remediation zone from severe disturbance of wind waves. The relatively slower water flow inside created better conditions for precipitation of solid wastes and weakened re-suspension and nutrient release from sediment, contributing to higher water transparency and lower turbidity in the remediation zone.

Similarly, chl-a in the remediation zone was the highest before 19th May for some reason, but showed a declined tendency and apparent control effect thereafter. The average of chl-a in the remediation zone was lower than that in the contrast zone and the breeding zone by 14.8% and 5.9%, respectively. It is worth noting that there is a positive correlation between chl-a and environmental factors such as water temperature and total phosphorus, and a negative correlation between chl-a and water transparency (RUAN et al. 2008). However, in the light of present water quality situation, chl-a in the remediation zone didn’t increase with the water temperature, conversely showed a decreasing tendency. It might be attributed to the aquatic plants observed, especially the submerged macrophytes, playing an important role in restraining algal growth, because they grew well under improved water transparency. On the other hand, the uptake of nutrients, especially phosphorus, by aquatic plants also contributed to restraining algal growth, resulting in the decrease of chl-a in the remediation zone (discussed later).

As for organic matter, COD$_{Cr}$ was used here to represent its concentration in water. COD$_{Cr}$ in each zone showed a slight upward tendency and reached the peaks in 15th August. Owing to the contribution of attached biofilm on biological fillers of Eco-dam, COD$_{Cr}$ in the remediation zone was lower than that in the contrast zone and the breeding zone by 8.8% and 13.4%, respectively.

As for nitrogen, NO$_3^-$-N in each zone accounted for the largest proportion of TN in comparison with NH$_4^+$-N (NO$_2^-$-N was much lower and could be overlooked). The main reason for this might be that aerobic nitrifiers including ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in both water bodies and Eco-dam played a significant role in converting NH$_4^+$-N into NO$_3^-$-N while anaerobic denitrifiers were inhibited due to always high concentrations of dissolved oxygen even at the bottom of water bodies. On the other hand, before June, variations of TN, NO$_3^-$-N, NH$_4^+$-N in each zone showed a declined tendency and values in the remediation zone were generally lower than those in other two zones. After June, TN and NO$_3^-$-N in each zone remained stable and had small differences, while NH$_4^+$-N showed a violent fluctuation. Even so, in terms of average, obvious decreases of these nitrogen nutrients could be seen in the remediation zone due to the role of Eco-dam. The averages of TN, NO$_3^-$-N, NH$_4^+$-N in the remediation zone were lower than those in the contrast zone by 2%, 10.2%, and 17.5%, and lower than those in the breeding zone by 0%, 8.6%, and 24.5%, respectively. As for phosphorus, TP in each zone showed an increasing tendency and the value in the remediation zone was lower than that in other two zones before June. After that, TP in the remediation zone and the contrast zone declined greatly in June and then rose and seemed to reach stable finally, while TP in the breeding zone were in fluctuation but higher than that in other two zones. The average of TP in the remediation zone was
lower than that in the contrast zone by 10.6% and lower than that in the breeding zone by 29.4%, respectively. As mentioned above, the variations of TP also reflected the influence of environmental factors, such as water temperature, hydrodynamic force and the ecosystem, on its release and conversion. There was a positive relationship between TP and chl-a. Therefore, the decrease of TP in the remediation zone became one of the reasons resulting in the decline of chl-a. Besides, aquatic plants played an important role in phosphorus uptake and restraining algal growth.

Eco-dam is an innovative water-permeable enclosure with biological fillers and aquatic plants serving as barriers and decomposers against pollutants from aquaculture breeding zone. However, such natural open system leads to the fact that the practical water quality situations in each zone are seriously affected by numerous environmental factors such as water temperature, tide, precipitation, and wind waves. In other words, the natural water exchange is fairly fierce around Eco-dam. Therefore, it is hard to give an effective evaluation of the water quality improvement ability of Eco-dam depending only on the comparison of practical water qualities among three zones. Biological fillers and aquatic plants were two of the most important parts of Eco-dam, while in this paper studies on aquatic plants were not included. Further studies on the biofilm activity and community of biological fillers were necessarily carried out to better understand Eco-dam from the mechanism level.

3.2 Biofilm activity
The specific activities of heterotrophs, NOB, and AOB in terms of SOUR, and denitrifiers in terms of SDNR were determined by corresponding methods under selected substrate concentrations. As shown in Figure 4, with respect to aerobic heterotrophs and nitrifiers, AOB showed the highest activity with the value of 19.89 mg O$_2$/gVSS·h of (SOUR)$_{NH_4}$, while NOB and heterotrophs showed close and obviously lower activities, with the value of 6.12 and 7.34 mg O$_2$/gVSS·h of (SOUR)$_{NO_2}$ and (SOUR)$_{H_2}$, respectively. For denitrifiers, SDNR was obtained during 540 min of substrate utilization test under anoxic condition, with the value of 1.35 mg NO$_3^-$/gVSS·h.

Apart from SDNR, the specific substrate utilization rate is also used as description for the activities of AOB and NOB, namely specific ammonium utilization rate (SAUR) and specific nitrite utilization rate (SNUR) respectively, which can be estimated from the already got (SOUR)$_{NO_2}$ and (SOUR)$_{NH_4}$ based on the stoichiometry of nitrification (0.95 mg NO$_2^-$/mg O$_2$ and 0.31 mg NH$_4^+$/mg O$_2$, respectively)(Bassin et al. 2012). As a result, SNUR and SAUR in this study were 5.81 mg NO$_2^-$/gVSS·h and 6.17 mg NH$_4^+$/gVSS·h. To better understand the biofilm activity of Eco-dam, test results of similar studies on the activities of heterotrophs, nitrifiers and denitrifiers by other authors were collected in Table 3 for comparison. Due to distinct test conditions, activities of these bacteria in different forms and systems differed to some degree. Unfortunately, relevant data from systems similar to Eco-dam such as ecological engineering systems were not found. Even so, the biofilm activity of Eco-dam was fairly good by contrast, especially the activities of heterotrophs, NOB, and AOB, indicating that Eco-dam possessed strong abilities of organic matter oxidation and nitrification which was reflected on the decrease of COD$_{Cr}$ and NH$_4^+$/N in the remediation zone. Moreover, the removal abilities of COD$_{Cr}$, NO$_2^-$/N, NH$_4^+$/N and NO$_3^-$/N by the whole Eco-dam were estimated roughly according to Table 4.
Figure 4. Specific activities of heterotrophs, NOB, and AOB in terms of SOUR, and denitrifiers in terms of SDNR

Table 3. Test results of similar studies on the activities of heterotrophs, nitrifiers and denitrifiers in biofilm or sludge of various systems

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>(SOUR)(_H)</th>
<th>(SOUR)(_{NO2})</th>
<th>(SOUR)(_{NH4})</th>
<th>SDNR</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm in MBBR</td>
<td>10.3 mg O(_2)/gVSS·h</td>
<td>/</td>
<td>29.0 mg O(_2)/gVSS·h</td>
<td>4.0–5.0 mg NO(_3^-)/gVSS·h/h</td>
<td>(Gong et al. 2012)</td>
</tr>
<tr>
<td>Biofilm in UBAF</td>
<td>/</td>
<td>4.5 mg O(_2)/gVSS·h</td>
<td>15.2 mg O(_2)/gVSS·h</td>
<td>/</td>
<td>(Fdz-Polanco et al. 2000)</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>6.4–10.6 mg O(_2)/gVSS·h</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>(Clouzot et al. 2011)</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>4.9 mg O(_2)/gVSS·h</td>
<td>4.0 mg O(_2)/gVSS·h</td>
<td>12.6 mg O(_2)/gVSS·h</td>
<td>/</td>
<td>(Raszka et al. 2011)</td>
</tr>
<tr>
<td>Granular sludge</td>
<td>/</td>
<td>1.2–10.5 mg O(_2)/gVSS·h</td>
<td>20.1–27.7 mg O(_2)/gVSS·h</td>
<td>/</td>
<td>(Winkler et al. 2011)</td>
</tr>
<tr>
<td>Biofilm in Eco-dam</td>
<td>7.34 mg O(_2)/gVSS·h</td>
<td>6.12 mg O(_2)/gVSS·h</td>
<td>19.89 mg O(_2)/gVSS·h</td>
<td>1.35 mg NO(_3^-)/gVSS·h/h</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Table 4. Rough estimation of removal abilities of COD\(_C\), NO\(_2^-\)–N, NH\(_4^+\)–N and NO\(_3^-\)–N by the whole Eco-dam

<table>
<thead>
<tr>
<th>Specific activity of relevant bacteria</th>
<th>VSS on one biological filler (g)</th>
<th>Number of biological fillers</th>
<th>Total VSS (g)</th>
<th>Removal ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD(_C)</td>
<td>7.34 mg O(_2)/gVSS·h</td>
<td>3.578</td>
<td>2962.6</td>
<td>21.75 g O(_2)/h</td>
</tr>
<tr>
<td>NO(_2^-)–N</td>
<td>5.81 mg NO(_2^-)/gVSS·h</td>
<td>828</td>
<td>2962.6</td>
<td>17.21 g NO(_2^-)/N/h</td>
</tr>
<tr>
<td>NH(_4^+)–N</td>
<td>6.17 mg NH(_4^+)/gVSS·h</td>
<td>828</td>
<td>2962.6</td>
<td>18.28 g NH(_4^+)/N/h</td>
</tr>
<tr>
<td>NO(_3^-)–N</td>
<td>1.35 mg NO(_3^-)/gVSS·h</td>
<td>828</td>
<td>2962.6</td>
<td>4.0 g NO(_3^-)/N/h</td>
</tr>
</tbody>
</table>

3.3 Microbial community of biological fillers
A total of 146064 16S rRNA sequences were selected for classification. Based on the 97% species similarity, a total of 2119 OTUs derived from sequences were detected in this study. The most abundant bacteria at phylum level among 12 samples were Proteobacteria, Actinobacteria, Cyanobacteria, Frimicutes, Planctomycetes, Bacteroidetes and Nitrospirae (Figure 5A). In order
to calculate microbial diversity of 12 samples, sequences of all the samples were downsized to 5000 to equal the difference in sequencing depth. The Shannon diversity result was shown in Fig3. The value of A3 is lower than other 11 sampling sites.

The illumina miseq sequencing analysis showed that there were large amount kinds of functional bacteria attached on the biological fillers at different sampling sites (Table 1). *Nitrosospira* and *Nitrosococcus* were the main ammonia oxidizing bacteria (AOB) at different positions around Eco-dam (Bock et al. 1989). A small quantity of *Methanobacterium* that is a taxon of methanogens was attached at several sampling sites. The main sulfate-reducing bacteria (SRB) which were attached on the fillers was *Desulfbacterium* (Hao et al. 1996), and the main nitrite oxidizing bacteria (NOB) was *Nitrospira* (Ehrich et al. 1995). The taxa of anaerobic ammonium oxidation (anammox) bacteria which exist in Eco-dam were *Candidatus Brocadia* and *Candidatus Anammoxoglobus* (Kartal et al. 2008). The main families of denitrifying bacteria were Neisseriaceae, Rhodospirillaceae, Bacillaceae 1, Cytophagaceae and Rhizobiaceae. The main genus aerobic denitrifying bacteria were *Bacillus*, *Pseudomonas*, *Paracoccus* and *Rhizobium*. Among 12 samples at different positions, *Cyanobacteria* was one of high-richness phylum which is correlated with degradation of organic metabolites, and *Steroidobacter* was one of high-richness genus which can specifically degrade steroids (Wang et al. 2013).
4. Conclusions
In this paper, the principle of Eco-dam was proposed for the in-situ control of aquaculture pollution and bioremediation of polluted aquaculture water. The biological remediation performance of Eco-dam established in polluted aquaculture water area was studied from aspects of both water purification efficacy and microbial mechanisms. The primary conclusions of the study were summarized as follows:

(1) Eco-dam showed good water purification performance in water transparency improvement, organic matter degradation, and nutrient removal, in spite of the strong water exchange between the breeding zone and the remediation zone driven by natural environmental factors. The average turbidity, COD\textsubscript{Cr}, NH\textsubscript{4}+-N, and TP in the remediation zone were lower than those in the breeding zone by 21.7%, 13.4%, 24.5%, and 29.4%, respectively.

(2) Eco-dam had enormous potentials of organic matter oxidation and nitrification. The specific activities of heterotrophs, NOB, AOB, and denitrifiers were 7.34, 6.12, 19.89 mg O\textsubscript{2}/gVSS·h, and 1.35 mg NO\textsubscript{3}–N/gVSS·h respectively. The removal abilities of Eco-dam for the corresponding pollutants were 21.75 g O\textsubscript{2}/h, 17.21 g NO\textsubscript{3}–N/h, 18.28 g NH\textsubscript{4}+-N/h, and 4.0 g NO\textsubscript{3}–N/h.

(3) In the biofilms attached on biological fillers of Eco-dam, the most abundant bacteria at phylum level were Proteobacteria, Actinobacteria, Cyanobacteria, Frimicutes, Planctomycetes, Bacteroidetes and Nitrospirae. Numerous kinds of functional bacteria were found, including AOB, NOB, SRB, denitrifiers, and organic decomposers, verifying the biological diversity of Eco-dam.

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References


