The study of recirculating aquaculture system in pond and its purification effect

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Abstract. In this paper, a recirculating aquaculture purification system (RAPS) was designed to solve the problems of aquaculture pollution and shortage of freshwater resource according to the characteristic of northern freshwater ponds of China. The system were arranged in series and composed of high density culture pond, deposit pond, floating and submerged plant pond, ecological floating bed pond and biofilm filtrate pond. At the fish density of 20~30kg/m³ in the high density culture pond, the water quality parameters were monitored seasonally. The results indicated that the removal rate of total nitrogen, total phosphorus, ammonia nitrogen and nitrite nitrogen in the recirculating aquaculture system were 69.59%, 77.89%, 72.54% and 68.68%, respectively. The floating and submerged plant pond and ecological floating bed pond can remove TN and TP obviously, and increase dissolved oxygen and transparency significantly. And the biofilm filtrate pond has good effect of removing ammonium nitrogen and nitrite nitrogen, meanwhile, the microbial communities in the recirculating aquaculture system regulate on the water quality. Therefore, the RAPS show significant effects on water saving and pollution emission reducing.

1. Introduction

Pond culture, as the dominant practice of freshwater aquaculture industry, its production and area is top of the whole world and provide communities with better incomes, stable jobs and safe food [1, 2, 3]. Although aquaculture industry development is positive to promote the social and economic progress, it also causes great pressure on the surrounding water environment [4, 5]. Especially in the north of China, the relative lack of water resource is seriously hampering aquaculture development. Therefore, the reuse and purification of wastewater from the pond has become one of the key technologies for the sustainable development of aquaculture.

In recent years, pond water purification and remediation technology such as physical remediation, chemical remediation and bioremediation technology have quickly developed in China [6, 7]. For achieving the main goal of energy saving, reduction discharge, ecological security and efficient breeding, our laboratory adopted multistage ecological and bioremediation technologies to regulate and maintain the balance and stability of pond ecological environment [8]. We designed a recirculating aquaculture purification system (RAPS) with high density culture according to the characteristic of northern farming pond.

The objective of this paper is (1) to access the purification efficiency in different remediation units of the health aquaculture recirculating system. (2) to understand the dynamic characteristics of microbial community structure in the aquaculture system.

2. Materials and methods
2.1. Test system
The RAPS is constructed in Beijing XinMiao fishery company field. The system was consisted of (1) High density culture pond: fish density is 20~30kg/m³. (2) Deposit pond: to ensure that most of the fish feces, bait and other suspended solid dissolved matter can be trapped. (3) Floating and submerged plant pond: some aquatic plants such as water hyacinth, Pistia stratiotes, Potamogeton crispus and Potamogetonaceae were used to purify water quality. (4) Ecological floating bed pond: to plant Iris wilsonii and Lythraceae on the floating bed to absorb pollutants. (5) Biofilm filtrate pond: use artificial substrates to immobilize biofilm microbial structure. (in series) (Figure 1).

![Diagram of recirculating aquaculture purification system](image)

**Figure 1.** The recirculating aquaculture purification system (sampling site and water flow direction is Y-Z-S-T).

2.2. Water quality monitoring
Water samples were collected from four sampling sites (Y, Z, S, and T) and stored at 500 ml sterile glass bottles at the middle of each quarter. The water quality parameters include water temperature (T), pH and dissolved oxygen (DO) were tested in the field with a water quality analysis machine (AP-7000, USA). The other parameters were tested in the lab according to the standard methods. All data were processed using Microsoft Excel 2007.

2.3. Microbial community monitoring
Water samples were collected, filtered and stored at -70°C. The DNA extraction, PCR amplification and sequencing were performed by Shanghai Major Bio-pharam Technology Co., Ltd. using Illumina Miseq PE 250 strategy.

3. Results
3.1. Water quality changes
The average values of water temperature, pH value and DO concentrations in recirculating aquaculture purification system were 15.79±6.5°C, 5.83±0.64, and 8.21±0.41 mg/L, respectively. As shown in Figure 2 (a-d), the water quality parameters of TN, TP, NH₄ and NO₂ in the high density culture pond were significantly decreased along the flow direction. The result indicates that wastewater pollutants have been effectively absorption and purification by the ecological engineering facilities. It is also suggested that the system can reduce the pollution to the outside environment, save water and maintain the balance of nutrients in the pond.
3.2. The purification efficiency
In total, the removal rate of total nitrogen, total phosphorus, ammonia nitrogen and nitrite nitrogen in the recirculating aquaculture system were 69.59%, 77.89%, 72.54% and 68.68%, respectively. The higher removal rate of total nitrogen (36.87%) and total phosphorus (45.44%) belong to the floating and submerged plants pond. Especially in summer and autumn, the removal efficiency of total nitrogen and total phosphorus in the floating and submerged plants pond were significantly higher than those of other water purification facilities. It means that the purification efficiency gradually increase with the growth of aquatic plants. The result also indicated that there was a significant purification effect of biofilm filtrate pond on ammonium nitrogen and nitrite nitrogen in aquaculture wastewater. In the high nutrient of summer and fall season, the removal rate of ammonium nitrogen and nitrite nitrogen has reached 71.58% and 52.52%, respectively. It is suggested that the biodegradation of biofilm filtrate have regulated on the water quality significantly (Table 1).

| season          | site | TN    | TP    | NH4   | NO2  |
|-----------------|------|-------|-------|-------|------|
| Spring (April)  | Z    | 12.00 | 24.00 | 20.00 | 31.11|
|                 | S    | 18.18 | 36.84 | 25.00 | 12.90|
|                 | T    | 33.33 | 33.33 | 11.11 | 33.33|
| total           |      | 52.00 | 68.00 | 46.67 | 60.00|
| Summer (June)   | Z    | 53.33 | 55.00 | 29.47 | 14.29|
|                 | S    | 28.57 | 33.33 | 23.88 | 38.89|
|                 | T    | 20.00 | 50.00 | 70.59 | 36.36|
| total           |      | 73.33 | 85.00 | 84.21 | 66.67|
| Autumn (August) | Z    | 50.00 | 76.67 | 17.58 | 43.48|
|                 | S    | 36.84 | 28.57 | 17.33 | 30.77|
|                 | T    | 41.67 | 60.00 | 72.58 | 66.67|
| total           |      | 81.58 | 93.33 | 81.32 | 86.96|

Table 1. Water Purify Efficiency in Recirculating Aquaculture System
3.3. Microbial community dynamics

The microbial community composition of the recirculating aquaculture system was assessed by Miseq PE250 platform. It is shown that there is a different composition of the microbial community in the purification sections (Figure 3). The main phyla were Proteobacteria (41%), Bacteroidetes (22%) and Firmicutes (20%) in the culture pond, and Lactococcus and Comamonadaceae were the dominate genera. In the floating and submerged plant pond, sequences belong to Proteobacteria (88%) or Rhodobacter was found in high abundances. Actinobacteria and Bacteroidetes were the most group in the ecological floating bed pond with 35% and 28%, respectively, as well as Alpinimonas and Runella were the main genera. At the biofilm filtrate pond, Proteobacteria (35%) and Fusobacteria (28%) were the dominate phyla, and Cetobacterium and Rhodobacte were the main species at the genus level. The result indicate that the composition of microbial communities in the recirculating aquaculture system regulate on the water quality, as well as the different purification sections contribute to the temporal variations.

| Winter (November) | Z  | 32.14 | 26.09 | 42.37 | 22.22 |
|-------------------|----|-------|-------|-------|-------|
|                   | S  | 36.84 | 29.41 | 38.24 | 21.43 |
|                   | T  | 33.33 | 33.33 | 38.10 | 36.36 |
|                   | total | 71.43 | 65.22 | 77.97 | 61.11 |

Figure 3. Microbial communities dynamic in the ponds.
Note: sp=spring, su=summer, au=autumn, wi=winter
4. Results
In order to explore the biodegradation efficiency of the whole system of recirculating aquaculture water, the water quality parameters such as the total nitrogen, total phosphorus, ammonia nitrogen, and nitrite nitrogen combined with the dynamic changes of microbial community were monitored seasonally. Our results show that the system purification effect is very significant, especially in summer and fall season. Many reports identified that the recirculating aquaculture system operated well and controlled over the water quality parameters and environments by utilize many different components, thus ensuring optimal conditions for fish culture [9, 10, 11].
In this study, water hyacinth and Pistia stratiotes were chosen to construct floating plant purification zone. Because of these two aquatic plants have developed root, nitrogen and phosphorus in water and other organic matters were removed effectively by rhizosphere adsorption and species competition mechanism. Previous studies have revealed that rhizoremediation is an environmentally friendly remediation technology which can remove and degrade pollutants and contaminants by microbial activity in the rhizosphere of aquatic plants [12, 13, 14]. The macrophyte allelopathy interact with phytoplankton is considered as one of the mechanisms that contribute to the water environments stabilization [15, 16, 17]. In the present study, the submerged macrophytes such as Potamogeton crispus and Potamogetonaceae can release allelochemicals can inhibit the growth of algae, decrease the algae biomass and improve the water quality and transparency. In the ecological floating bed sections, the aquatic plant roots were colonized to form the microorganism membrane, which increased the contact with surface water area and time, so as to enhance the effect of water purification and promote increased the content of dissolved oxygen in water. Luo et al (2011) also identified that submerged macrophytes combinations with ecological floating beds on in-situ purification and regulation of water quality is quite stable and water quality quite good [18]. Nowadays, Nitrification biofilters are widely used to remove ammonia and other metabolic waste products in recirculating aquaculture systems [19, 20]. Our results indicate that biofilms can significantly degrade ammonium nitrogen and nitrite nitrogen in the water, meanwhile, it also can change the structure of microbial commutates and diversity and strengthen the water self-purification function. Moreover, Lactococcus and Comamonadaceae were the dominate genera in the high density culture pond, which belong to beneficial bacterial benefit to promote the fish growth.
The recirculating aquaculture system is a water-saving, land-saving and high production yield culture mode, which have the advantage of resource demand, ecological protection and production capacity. With the continuous deterioration of the natural environment and more restrictive of environmentally policy frameworks, the recirculating aquaculture will become the future direction of aquaculture development. However, at present in our country, there are very few farmers utilize recirculating aquaculture technology in actual production, because of the technology is more specialized and need to train and improve their management. In order to apply the model to the actual production, it is necessary to explore in the experiment, exploration and practice.

5. Conclusions
During the investigation, the average concentrations of TN in floating and submerged plant pond, ecological floating bed pond and biofilm filtrate pond were 2.205, 1.425 and 0.975 mg/L, respectively. The average contents of TP in the different purification system sections were 0.153, 0.103 and 0.06 mg/L, respectively. According the national fishery wastewater discharge standards, TN and TP pollutants discharge all reached the first class discharge standard(TN≤2.0 mg/L, TP≤0.3mg/L). The results showed that the constructed recirculating aquaculture have good degradation effect on nitrogen, phosphorus and other pollutants.

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