Aquaculture Wastewater Treatment with Immobilized Microorganisms-aquatic Plants Strengthened Purification System

HY Cheng¹,², JX Long¹,², Z Liu¹,²
¹ School of environmental science and engineering, Xiamen University of Technology, 600 Ligong Road, Xiamen, 361024, China
² Key Laboratory of Environmental Biotechnology (XMUT), Fujian Province University, 600, Ligong Road, Xiamen, 361024, China

Abstract: Combined purification system of microorganism - aquatic plants were established by adding immobilized 3.0×10¹⁰ CFU/ m³ EM (Effective Microorganisms) to Eichhornia crassipes and Pistia stratiotes, to study the purification effect on prawn aquaculture water. The results showed that the purification effect of 2 groups of immobilized microorganism - aquatic plants (SEE and SEP) on ammonia nitrogen (NH₄⁺-N), total nitrogen (TN), total phosphorus (TP) and chemical oxygen consumption (COD) were significantly better than those of the immobilized microorganism SEM group (P<0. 05). The degradation efficiency of SEE and SEP system to ammonia nitrogen were 98.37% and 96.22%. The degradation rate of TP were 90.28% and 86.11% respectively. The removal efficiency of COD were both close to 86%. At the same time, Eichhornia crassipes was slightly better than Pistia stratiotes in microorganism - aquatic plants system.

1. Introduction
The zero discharge of ecological aquaculture and aquaculture water pollution is the direction and inevitable requirement of aquaculture development in China. Different experts and scholars adopt different methods to study the purification of aquaculture water. Effective microorganisms (EM) are composed of a variety of beneficial bacteria (Photosynthetic bacteria, Ni trobacteria, Lactobacill, Actinomy cetes, Yeast and Bacillus cohn), which play an important role in purifying water quality, reducing the concentration of ammonia nitrogen (NH₄⁺ -N) and nitrite nitrogen (NO₂⁻-N) and so on and realizing healthy culture [1], [2]. Wang added EM to source water in the ratio of 1 to 10000 into the experimental container, supplemented with low speed intermittent aeration treatment for 8 to 9 days, and the removal rates of TN, TP and COD in water samples were 45.25%, 55.48% and 82.37%, respectively [3]. Ma use the inoculation amount of 1 to 5 000 EM bacteria to treat nitrogen containing wastewater and this method has the characteristics of energy saving, sludge production less and deodorization [4]. Brasker and Han use immobilized algae bacteria to remove COD and ammonia nitrogen from aquaculture wastewater, and the effect is ideal [5], [6]. Mittler, Fraser, Badigannavar, Hunter and Hunter used aquatic plants to treat eutrophic aquaculture water, the results showed that aquatic plants such as shallot, Potamogeton crispus and other aquatic plants had better removal effect on N and P [7]-[10]. Tong used aquatic plants to control the eutrophic water body of the fish pond. The results show that 6 kinds of aquatic plants, such as golden fish and bitter grass, have good removal effect on total nitrogen and total phosphorus in water, but the effect on improving COD and DO is not obvious [11]. Therefore, under the indoor conditions, the immobilized effective bacteria group (EM) and the higher aquatic plant (Pistia stratiotes) and the water hyacinth (Eichhornia crassipes) were...
combined to purify the circulating aquaculture wastewater, and the effects of the combined action of immobilized microorganism and two aquatic plants and the immobilized microorganism on the purification of the aquaculture wastewater were emphatically analyzed. The purpose of this study is to provide a basis for constructing an efficient and stable purification mode of aquaculture wastewater.

2. Materials and methods

2.1 Reagents and instruments

EM original solution is provided by Zhengzhou Nong Fukang Biotechnology Co., Ltd. The effective microorganism content is $3 \times 10^{10}$ CFU/ml.

2.2 Experimental device

The experiment was carried out in the indoor aquarium (30 cm x 20 cm x 25 cm), and 2 treatment groups and 1 control group were set up. SEE was the strengthening group of the immobilized EM with Eichhornia crassipes, the SPE was the strengthening group of the immobilized EM with Pistia stratiotes, and the SEM was the control group of the immobilized effective bacteria group.

2.3 Sampling and analysis method

The experiment time was from April 5 to April 30, 2018. During this period, the water temperature was maintained at about 28°C with the heating rod. The aeration for 12 h and the static for 12 h were used alternately, and the distilled water was supplemented every day to keep the water volume in the aquarium constant. Sampling 1 time every 4 days. The analysis indexes include CODcr, NH$_4^+$-N, TN, TP.

2.3.1 Determination of water quality

Sampling time was 10:30 a.m. and water samples were collected at the same point. The collected water samples were immediately sent to the laboratory for testing. Determination method reference [12]: determination of COD with potassium dichromate method; determination of ammonia nitrogen (NH$_4^+$-N) with Nessler Reagent Spectrophotometry; determination of total nitrogen (TN) with potassium persulfate oxidation UV spectrophotometry; determination of total phosphorus (TP) with Mo-Sb-Vc spectrophotometry method.

2.4 Data analysis

The experimental data were analyzed by SPSS16. 0 software, and P < 0.05 was significant difference. Microsoft Excel 2007 is used for chart processing.

3. Results and discussion

3.1 Purification effect of immobilized EM and aquatic plants on ammonia nitrogen in water

![Figure 1. The changes of ammonia nitrogen (NH$_4^+$-N) concentration and removal rate](image)

From Figure 1, it can be seen that the fast removal of NH$_4^+$-N in the aquaculture water body of SEE, SEP and SEM mainly appeared in the former 4 days. With the prolongation of the purification time, the removal rate of NH$_4^+$-N was significantly decreased in three systems. After 4 days of degradation, the removal rates of NH$_4^+$-N were 69.02%, 56.76% and 35.36% respectively by SEE, SEP and SEM. Then after 28 days purification, the NH$_4^+$-N removal rates can be 98.37%, 96.22% and 67.96%, respectively. From the degradation effect of N NH$_4^+$-N, the removal rates of the two kinds of composite strengthening systems were all above 90%, and the ranking of the removal rate (purification effect) was SEE > SEP > SEM in turn.
## 3.2 Purification effect of immobilized EM and aquatic plants on total nitrogen in water

![Figure 2](image)

**Figure 2.** The changes of Total Nitrogen (TN) concentration and removal rate

The decrease of the total nitrogen mass concentration is faster in the first 16 days, and the decrease speed in the following 12 days is obviously slow down. It may be the soil matrix that adsorb and intercept part of the nitrogen. With the decrease of the mass concentration of TN in the water body, the nitrogen intercepted by the soil matrix may release back to the water body, making the TN mass concentration in the water body decreased slowly. The removal rates of SEE, SEP and SEM for shrimp aquaculture water TN were 90.27%, 86.11% and 59.17% respectively. The effect of combination of aquatic plants and immobilized EM to TN was better than that of Yuan, who using single aquatic plant Acorus calamus (TN removal rate was 77.77%) [13]. The microbiological enhancement effect of aquatic plant *Eichhornia crassipes* is close to that of Ma's experiment (TN removal rate of 90.4%), which is better than that of the *Pistia stratiotes* strengthening group [14].

Although the main degradation of organic nutrients in water is microorganism, the study of Gersberg [15] and Haberl [16] also showed that the main removal way of nitrogen is nitrification, denitrification and not plant absorption. However, the existence of aquatic plant community provides the substrate and habitat for microbes and microanimals. The microbe and aquatic plant form a good symbiotic relationship between microbes and aquatic plants by adapting to survival [17]. Microbes can degrade organic nitrogen and phosphorus and non soluble nitrogen and phosphorus in sewage into small soluble molecules, and continue to be absorbed and utilized by aquatic plants.

### 3.3 Purification effect of immobilized EM and aquatic plants on total phosphorous in water

![Figure 3](image)

**Figure 3.** The changes of Total Phosphorus (TP) concentration and removal rate

Figure 3 showed the purification effect of immobilized EM and aquatic plants on total phosphorous in the aquaculture water. It can be seen that the removal rate of TP in the initial 4 days is lower, and the removal rate is 19.4%, 22.2% and 13.89%, which may be related to a short adaptation period of the aquatic plants which were initially implanted. In the following 5 to 16 days, the removal rate of TP was greatly accelerated, and the removal rate of TP reached 66.67%, 72.22% and 58.33% after 16 days. Although the TP removal rate in the final 12 days of the experimental design had a small increase, the effect was not very obvious, and the TP removal rates in 28th day were 86.11%, 90.28% and 59.17%, respectively. The significant difference may be due to the fact that aquatic plants are the main factors to remove TP. There was no significant difference between SEE and SEP during the
whole degradation process, the enhanced microbial aquatic plant combination was stronger than that of the enhanced microorganism SEM system for the removal of TP.

3.4 Purification effect of immobilized EM and aquatic plants on chemical oxygen demand

![Figure 4. The changes of Chemical Oxygen Demand (COD) concentration and removal rate in System of SEE, SEP and SEM.](image)

From Figure 4, it can be seen that in the first 8 days of purification, the content of COD in the immobilized microorganism system SEM is lower than the combined strengthening system of microbiological aquatic plants, indicating that the removal rate of aquatic plants to COD is obviously lower than that of the immobilized EM during this period, which may also be related to an adaptation period after the implantation of aquatic plants. From the tenth day, the removal rate of COD in the aquatic plant strengthening system was improved rapidly. The removal rate of COD by SEE and SEP aquatic plants was higher than that of SEM system containing EM. In the subsequent 20 days of purification process, the concentration of COD in each system decreased gradually, and the COD removal rates of 3 systems in 28th day were 86.92%, 86.03% and 68.2%, respectively.

4. Conclusion

(1) Both the immobilized microorganism SEM and the combined strengthening system of microorganism and aquatic plant (SEE and SEP) have better purification effect on the removal of \(\text{NH}_4^+\)-N, TN, TP and COD in the wastewater of shrimp culture. The removal of \(\text{NH}_4^+\)-N by SEE and SEP is the best, the removal rate can reach 98.37% and 96.22%. The removal rate of SEE to TN is up to 90.27%, and the degradation rate of SEP to COD is higher than that of SEE.

(2) In most cases, the effect of the combined Strengthening System of microorganism and aquatic plants on the purification of shrimp culture wastewater is better than that of the immobilized effective bacteria group, and the addition of EM can greatly enhance the purification effect of combined purification system on aquaculture water.

(3) Considering the efficiency of comprehensive purification, two kinds of floating aquatic plants *Eichhornia crassipes* and *Pistia stratiotes*, Eichhornia crassipes are higher than those in the study.

5. References

[1] Shao Q 2001, China Water and Wastewater 17 74
[2] Tian GT, Liu F, Duan DX, Du XH, Zhang JL, CHEN SJ, Zhang ML and Wang H 2012, Journal of Shandong Agricultural University 43 381
[3] Wang P, Wu XF and Li KL 2004 Research of Environmental Sciences 17 39
[4] Ma ZQ, Lou BF 2000 Environmental Pollution and Control 22 6
[5] Braskerud B 2002 Ecological Engineering 18 351
[6] Han SQ 2006 Journal of Applied and Environmental Biology 12 251
[7] Mittler R, Rizhsky L 2000 Plant Molecular Biology 44 335
[8] Fraser L, Carty S and Steer D 2004 Bioresource Technology 94 185
[9] Badigannavar A, Kale D, Eapen S and Murty G 2002 The journal of Heredity 93 50
[10] Hunter R, Combs D and George D. 2001 Archives of Environmental Contamination and, Toxicology 41 274
[11] Tong CH, Yang X and Pu P 2005 Journal of Applied Ecology 15 1447
[12] Yu L 2002 Manual of standards for water quality monitoring and analysis China, Environmental Science Press Beijing 35
[13] Yuan DH, Ren QJ and Gao SX 2004 *Journal of Applied Ecology* **15** 2337
[14] Ma JQ, Zhou HD and Dong HR 2005 *Journal of China Institute of Water Resources and Hydropower Research* **3** 130
[15] Gersberg R, Elkins B and Lyon S 1986 *Water Res* **20** 363
[16] Haberl R, Perfler R 1991 *Water Sci Technol* **23** 729
[17] Cheng W, Cheng D, Li Q 2005 *Industrial Safety and Environmental Protection* **31** 6

**Acknowledgments**

This research was supported by funding from XMUT with Grant 0900200611; Key Laboratory of Environmental Biotechnology, Fujian Province University.