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Effects and Mechanisms of Microbial Agents and Carbon Sources on Aquaculture Environment

Quansheng Wang

Hebei Agricultural University, Baoding, Hebei, 071001, China

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Abstract: Improving aquaculture environment through microbial manipulation has been a hot topic in aquaculture research in recent ten years. The effectiveness of microbiological agents in improving aquaculture water quality, especially in the degradation of inorganic nitrogen, remains controversial. One of the reasons is the lack of understanding of the pathway through which microbiological agents affect the environment. The effects of carbon sources and two microbial agents on ammonia nitrogen conversion in a cultured environment were studied by two in-situ experiments. The results showed that the inorganic carbon and organic carbon increased significantly in the sterilized and non-sterilized groups. The conversion trend of inorganic nitrogen was the same. The ammonia nitrogen and nitrite did not decrease, and the nitrate decreased significantly. The results indicated that there was no significant change in the function and unextinguished effect of nitrifying bactericide after sterilization. The effect of adding microbial agents on the conversion of inorganic ammonia in the environment might be due to the role of carbon source itself.

1. Introduction

It has been 33 years since Boyd first explored the role of microbiotics in improving aquaculture environments in 1984. Although numerous publications have been published since then, the effectiveness of microbiotics in improving the environment remains controversial [1]. At present, there are two contradictions in the application of microbiological agents. The first is the contradiction between laboratory and outdoor effects. Another is the paradox of applying it differently in different contexts. The influence of foreign bacteria on the local environment depends on (1) whether foreign bacteria can colonize in the new environment; (2) whether foreign bacteria can compete with native bacteria [2]. In practical application, due to the differences in time and space between laboratory and outdoor laboratory, coupled with the complexity of aquaculture environment, the strains screened in laboratory often do not show corresponding functions in real application. In different aquaculture environments, the biological factors and abiotic factors are very different, resulting in different application effects.

At present, there are few literatures on adding carbon sources with microbial preparations in aquatic products [3]. Our previous in situ experiments in ponds showed that microbial preparations had no obvious effect on reducing ammonia nitrogen and nitrite, while adding carbon sources alone could effectively reduce ammonia nitrogen and nitrite [4]. Although the in situ medium scale experiment system was as consistent as possible, the differences between the groups before treatment were still large, and it was not possible to evaluate the pathways of action and the effects of interactions between microbial agents and carbon sources in a more detailed manner. In this paper, an in situ micro-experiment system was used to compare the effects of adding microbial agents alone, adding carbon sources alone and adding microbial agents and carbon sources together on improving the aquaculture environment, to explore whether microbial agents and carbon sources interact with each other and to further understand the ways of improving the environment with microbial agents.

2. Experimental methods and materials

2.1 Interaction between microbial preparations and carbon sources

A culture tank was used to construct an in-situ ammonia nitrogen conversion experiment to explore the interaction between microbial preparations and carbon sources. A culture bucket was selected and collected the water below the water surface (50 cm) with a 5 L water sampler and put into 18 glass bottles, each glass bottle was filled with 500 mL samples. At the beginning, 4 mg/L ammonia nitrogen source (NH4C1) was added to each water sample. The microbial preparations heterotrophic bacteria (HB) and nitrifying bacteria (NB) were purchased from Novozyme (Shenyang) Biotechnology Co., LTD., the added components in each experimental group are shown in Table 1. After the treatment, tighten the cap of the bottle, and then hang the bottle back into the bucket together, hanging 10 cm below the water, and mix the bottle twice a day. The experiment was carried out for 7 days, on the 1st to 7th day, 5 mL water was sampled every day for analysis of ammonia nitrogen, nitrous nitrogen and nitrous nitrogen. Samples were collected on days 0, 3 and 7 to analyze the bacterial biomass.

Table 1: The treatment in each group

Project	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Treatment	\	Glucose	\	\	Glucose	Glucose
	\	\	HB	NB	HB	NB

Note: The final concentration of glucose added was 0.5 mg L-1. The microbial products of HB or NB was added at a dosage of 0.5 g per bottle.

2.2 Potential carbon source effects of microbial agents

A mixed breeding bucket of California perch cultured for two months was selected, in which 60 california perch, 4 crucian carp and 2 silver carp were released. Commercial pellet feed was fed in the bucket at 4% of the total fish weight every day. Due to the high level of ammonia nitrogen in the selected culture buckets for two months, water was collected 50 cm below the water surface of each bucket with a 5L water sampler and filled into 9 glass bottles. Each glass was filled with 1000 mL sample. The experiment was divided into three groups. The control group added nothing (C), one group added 0.1 g nitrifying bacteria microbial preparation (SM) which was autoclaved at high temperature (120℃) for 30 minutes, and the other group added 0.1 g nitrifying bacteria microbial preparation (M). The experimental bottle was suspended 10 cm underwater, and the bottle was manually mixed upside down twice every morning and evening. The experiment was carried out for 7 days, and 10 mL water samples were collected every day to analyze ammonia nitrogen, nitrous nitrogen, nitrous nitrogen, total nitrogen, organic carbon and inorganic carbon.

3. Results

3.1 The interaction effect of adding microbial preparations and glucunxunose on the conversion of ammonia nitrogen

Except the control group, ammonia nitrogen in all groups decreased significantly on the second and third days, and it dropped below 1 mg/L by the end of the third day, and then continued to stay below 1 mg/L until the end of the experiment. In the control group, ammonia nitrogen decreased slowly until the end of the experiment, and remained above 2 mg/L. The change trend of nitrite was different in each group. The control group and the carbon source group had a rising trend during the whole experiment, and the rise trend of the control group was larger than that of the carbon source group. Heterotrophic bacteria group, carbon source and heterotrophic bacteria group, carbon source and nitrifying bacteria group has been on a downward trend since the beginning of the experiment, falling to the level below 0.1 mg/L on the second day, and then maintained below it. Nitrates in the nitrifiers showed a sudden increase from 0.15 mg/L to 2.52 mg/L on the second day, and then suddenly dropped below 0.1 mg/L on the third day, and then continued until the end of the experiment. The change of nitrate in each group was different, and the control group showed a slow rising trend. The carbon source group fluctuated very little throughout the period. The nitrobacterium and carbon source + nitrobacterium suddenly increased from 2.7 mg/L to more than 4.5 mg/L on the first day, but suddenly dropped to below 1 mg/L on the second day, and then to below 0.1 mg/L and remained there until the end of the experiment. The heterotrophic microflora and carbon source + heterotrophic microflora showed a downward trend from the beginning of the experiment, and dropped below 0.1 mg/L on the second day and remained until the end of the experiment.

3.2 Potential carbon source effect of microbial agents

After the addition of microbial agents and sterilized microbial agents, inorganic carbon in the two groups showed an upward trend, and the increasing degree was similar, while inorganic carbon in the control group basically remained stable, and slightly decreased on the 7th day. After the addition of microbial preparations and sterilized microbial preparations, the organic carbon showed an upward trend in the first two days, and then began to decline after the second day. From the fourth day to the seventh day, the organic carbon fluctuated slightly. During the whole process, the increase degree of organic carbon in the sterilized microbial preparations group was slightly higher than that in the microbial preparations group. The level of organic carbon in the control group had been decreasing throughout the experiment. The ammonia nitrogen in the control group was relatively stable during the whole experiment, and the trend of the microbiological preparation group was consistent with that of the sterilized microbiological preparation group, which decreased in the first two days and continued to rise from the second day. At the end of the experiment, it had risen to a level above 8 mg/L. At the end of the experiment, ammonia nitrogen in the microbial preparation group (8.09±0.58 mg/L) and the sterilized microbial preparation group (8.84±0.42 mg/L) was significantly higher than that in the control group (6.54±0.71 mg/L). At the end of the experiment, there was no significant difference in nitrite content among the groups. At the end of the experiment, nitrate in the control group (13.09±0.39 mg/L) was significantly higher than that in the microbial preparation group (0.04±0.02 mg/L) and the sterilized microbial preparation group (0.03±0.02 mg/L). At the end of the experiment, total nitrogen in the control group (23.310.42 mg/L) was significantly higher than that in the microbial preparation group (12.32±0.3 mg/L) and the sterilized microbial preparation group (12.68±0.47 mg/L).

4. Discussion

4.1 Conversion of inorganic nitrogen by microbial agents, carbon sources and their combined effects

The results of this experiment showed that ammonia nitrogen in both the experimental group and the control group showed a decreasing trend during the experiment, and the decreasing trend in each experimental group was greater than that in the control group. Both microbial agents and carbon sources could significantly reduce ammonia nitrogen, and there was an interaction effect between the two. The increasing trend of nitrite in the control group and the carbon adding group was less than that in the control group, while the decreasing trend of nitrite in the heterotrophic bacteria group, nitrifying bacteria group, carbon source + heterotrophic bacteria group, carbon source + nitrifying bacteria group, carbon source + nitrifying bacteria group, carbon source + nitrifying bacteria group, carbon source + nitrifying bacteria group, carbon source + nitrifying bacteria group. There was interaction between carbon source and microbial agents on the effect of nitrite. The nitrate in the control group increased during the whole experiment; the nitrate in the carbon source group did not change much during the whole experiment, and finally decreased at the end of the experiment; while the nitrate in the heterotrophic bacteria group, nitrobacteria group, carbon source + heterotrophic bacteria group, carbon source + nitrobacteria group, carbon source + nitrobacteria group all showed a downward trend, and there was an interaction between carbon source and microbial agents on nitrate. At the end of the experiment, nitrous nitrogen and nitrous nitrogen were increased in the control group, and nitrous nitrogen and nitrous nitrogen were also increased in the carbon source group, but the extent of the increase was not as good as the control group, and the heterotrophic bacteria group, nitrous nitrogen group, carbon source + heterotrophic bacteria group, carbon source + nitrous nitrogen group of nitrous nitrogen and nitrous nitrogen were decreased, and carbon source and microbial preparations on the influence of nitrous nitrogen and nitrous nitrogen interaction. In natural water, the conversion of ammonia nitrogen is generally transformed into nitrous nitrogen and nitrous nitrogen successively through nitrification. In the control group of this experiment, the nitrification pathway is the main route, which indicates that the cultured water body is capable of self-transforming ammonia nitrogen under the nitrification of autotrophic bacteria within a certain time, which is similar to the results reported in previous studies [5]. In this experiment, no matter the heterotrophic bacteria group and nitrobacteria group, or the heterotrophic bacteria + carbon source group and nitrobacteria + carbon source group, after the reduction of ammonia nitrogen, nitrite and nitrate did not increase, and nitrite and nitrate showed a decreasing trend, which indicates that these experimental groups showed a similar effect to the addition of carbon source to some extent, and its effect is more than the effect of simple addition of carbon source. Nitrite and nitrate can be used in addition to ammonia nitrogen. On the third day of the experiment, the effect of carbon source on bacteria was not significant, while microbial preparations significantly increased the number of bacteria, and there was no interaction effect between carbon source and microbial preparations on the number of bacteria. On the seventh day of the experiment, both carbon sources and microbial agents had an interaction effect on the number of bacteria. This further suggests that microbial agents activate the carbon source effect and that the combination of microbial agents and carbon sources can enhance this effect [6].

4.2 Potential carbon source effects of microbial preparations

The first miniature experimental system demonstrated the potential carbon source effect of microbial agents. In the second miniature experiment system, the preservation of inorganic carbon in the control group was relatively stable and began to decline on the fifth day, while the organic carbon showed a slow decline during the whole experiment. Both inorganic carbon and organic carbon increased significantly after the addition of microbial agents and sterilized microbial agents, and both trends remained consistent during the experiment. Organic carbon peaked on the second day and then began to decline. At the end of the experiment, the inorganic carbon and organic carbon in both treatment groups increased compared with before the experiment, while that in the control group decreased, indicating that the microbial agent has a carbon source effect. The ammonia nitrogen in the control group remained relatively stable, while the ammonia nitrogen in the microbiological preparation group and the sterilized microbiological preparation group first decreased, and maintained an upward trend after the second day. At the end of the experiment, the ammonia nitrogen in the two treatment groups increased, while the control group had no difference from before the experiment. In the control group, nitrite remained basically stable, while in the two treatment groups, it rose first in the first two days, and then began to decline. At the end of the experiment, there was no difference with the initial value. Nitrite and total nitrogen remained relatively stable in the control group, while kept decreasing in the two treatment groups, and were significantly lower than the initial value before the experiment at the end of the experiment. The above results showed that the effects of microbial preparations and sterilized microbial preparations on nitrogen conversion in water were the same, and the main effect was caused by carbon source effect.

In this experiment, under the carbon source effect, ammonia nitrogen showed an upward trend rather than a downward trend as shown in previous experiments, while nitrate showed a downward trend throughout the whole experiment. The utilization of ammonia nitrogen by heterotrophic bacteria is usually better than that of nitrate. However, in this experiment, the utilization of nitrate by bacteria is better than that of ammonia nitrogen, and the total nitrogen in the experiment also shows a downward trend, indicating that nitrogen conversion at this time is mainly through denitrification. In experiment 1, ammonia nitrogen is 4.2 mg/L, in which 4.0 mg/L is artificially added, nitrite and nitric acid are only 0.1 mg/L and 2.7 mg/L respectively, while in experiment 2, the initial value of ammonia nitrogen is 6.5 mg/L. Nitrite and nitrate were 0.1 mg/L and 12.0 mg/L, respectively. The environmental background of the two experiments was significantly different. The essence of carbon source was the strengthening of the original bacterial community, that is, the adjustment of the relative abundance of bacterial community species rather than the change of the species. The results of this experiment speculated that the role of carbon source effect in cultured water was related to the environmental physicochemical and bacterial community background.

5. Conclusion

The results of this experiment showed that there was a carbon source effect on the conversion of inorganic nitrogen in the culture environment, and there was an interaction effect when the microbial preparation was added cooperatively with exogenous carbon. Nitrifying bacteria in microbial preparations are at a disadvantage when competing with other bacteria in the environment under the carbon source effect, which will correspondingly affect the performance of nitrifying bacteria. The influence of potential carbon source effect of microbial preparations on inorganic nitrogen conversion in water environment is closely related to the background of water environment, including water chemistry and bacterial community information, and these background values will affect the action path of carbon source effect.

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