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Effect of Environmental Factors on Nitrite Nitrogen Absorption in Microalgae–Bacteria Consortia of *Oocystis borgei* and *Rhodopseudomonas palustris*

Yukun Ma [1], Zhishen Luo [1], Jiazhan Zhong [1], Hanyi Zhang [1], Xianghu Huang [1], Changling Li [1] and Yulei Zhang [1,2,*]

[1] Department of Aquaculture, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China; ma2865264239@163.com (Y.M.); luozhishen20@163.com (Z.L.); zhongjiazhan2002@163.com (J.Z.); zhanghanyi07120708@163.com (H.Z.); huangxh@gdou.edu.cn (X.H.); licl@gdou.edu.cn (C.L.)

[2] Guangdong South China Sea Key Laboratory of Aquaculture for Aquatic Economic Animals, Guangdong Ocean University, Zhanjiang 524088, China

* Correspondence: zhangyl@gdou.edu.cn

Abstract: The effects of temperature, salinity, and illumination on the nitrite uptake rate of the microalgae–bacteria consortia of *Oocystis borgei* and *Rhodopseudomonas palustris* were investigated. The absorption rates of nitrite and the contribution rate of each component in the consortia under different temperatures (15, 20, 25, 30, 35 °C), illuminations (0, 15, 25, 35, 45 μmol·m^{-2}·s^{-1}), and salinities (0, 5, 15, 25, 35‰) were determined by stable isotope labeling technique. The single and combined effects of three environmental factors on nitrite uptake by the microalgae–bacteria consortia were analyzed using single-factor and orthogonal experiments. The single-factor experiment showed that the microalgae–bacteria consortia could absorb nitrite efficiently when the temperature, salinity, and illumination were 20~30 °C, 0~15‰, and 25~45 μmol·m^{-2}·s^{-1}, respectively, with the highest absorption rates were 2.086, 3.058, and 2.319 μg·g^{-1}·h^{-1}, respectively. The orthogonal experiment showed that the most efficient environmental conditions for nitrite uptake were 30 °C, 5‰ salinity, 35 μmol·m^{-2}·s^{-1} illumination, and the rate of nitrite uptake by the microalgae–bacteria consortia was 3.204 μg·g^{-1}·h^{-1}. The results showed that the nitrite uptake rate of the *O. borgei*–*R. palustris* consortia was most affected by temperature, followed by salinity, and least by illumination. Under the same conditions, the nitrite absorption capacity of the microalgae–bacteria consortia was greater than that of single bacteria or algae, and *R. palustris* played a major role in the nitrite absorption of the consortia. The *O. borgei* and *R. palustris* consortia still maintain high nitrite absorption efficiency when the environment changes greatly, which has broad application prospects in the regulation and improvement of water quality in shrimp culture.

Keywords: algae–bacterial consortium; *Oocystis borgei*; *Rhodopseudomonas palustris*; nitrite absorption; environmental factor

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1. Introduction

Nitrite is one of the crucial important water quality indicators in aquaculture, and is easy to accumulate in the intensive culture of *Litopenaeus vannamei*, often reaching extremely high levels of more than 20 mg/L in the middle and late stages of culture [1]. Excessive nitrite is a major cause of stress in shrimp, which greatly reduces the survival rate of *L. vannamei*, and improper treatment can easily lead to heavy losses in culture [2–4]. What is more, nitrite is the main source of N_2O emissions from aquaculture systems. N_2O is one of the main greenhouse gases responsible for global warming and is proven to have 310 times higher global warming potential than carbon dioxide. At present, the global N_2O emissions caused by aquaculture have reached 3.83×10^{11} g [5]. Under the trend of green environmental protection and sustainable development concepts, it is imperative to reduce nitrite in aquaculture.

Oocystis borgei is a common dominant green alga in subtropical shrimp ponds with good water quality regulation. It can effectively reduce the concentration of ammonia and nitrite nitrogen in the water column [6], inhibit the growth of Vibrio, adsorb heavy metals [7], and has a stable population growth rate and strong resistance characteristics to maintain the ecological balance of the aquaculture system and promote shrimp growth [8]. It can also be rapidly concentrated by natural sedimentation and made into microecological preparations for aquaculture water quality control [9].

Rhodopseudomonas palustris, a purple non-sulfur photosynthetic bacterium with diverse metabolic mechanisms, survives in both light anaerobic and dark aerobic environments and is widely present in soil and water bodies [10], has genes encoding assimilatory and anisotropic nitrite reductases [11], and is capable of eliminating nitrite [12]. It is mainly used in aquaculture to control chemical oxygen demand, ammonia nitrogen, and nitrite in water bodies [13–15].

Microorganisms such as algae and bacteria have high efficiency for nitrite removal [16–18], and compared with the traditional physical and chemical methods, will not produce pollution and harmful by-products [19]. The joint use of microalgae and bacteria can greatly improve the efficiency of nitrogen conversion of bacteria and microalgae [20]. Microalgae and bacteria are the strongest partners of each other, bacteria can decompose macromolecules to facilitate absorption and use by microalgae and improve the efficiency of energy conversion, carbon dioxide produced by bacterial metabolism is also a necessary carbon source for microalgae to smoothly photosynthesis, and oxygen produced by microalgae photosynthesis can also guarantee the removal of nitrite by bacteria [21]. Moreover, larger microalgae adhering to each other can also be used by bacteria to climb and improve the conversion of nitrite by bacteria. It has been reported [20,22] that the use of specific algal–bacterial combinations can effectively reduce nitrite levels in cultured waters. Related studies have shown that the construction of microalgae–bacterial granular sludge (MBGS) using *Coelastrella* and *Rhodobacteraceaceae* is an effective environmental solution for treating aquaculture wastewater and improving nitrite nitrogen removal efficiency. Under non-aerated conditions, the MBGS process was able to remove 50.0% of nitrite nitrogen within 8 h, verifying that the microalgae–bacteria consortia can effectively remove nitrite content under synergistic action [23]. In addition, the addition of microalgae to the nitrifying bacterial community greatly improved the efficiency of nitrification. It was found that the addition of *Chlorella* to the biogas fermentation broth containing active sludge produced 2.7 times more nitrate than without *Chlorella*, and the co-culture of active sludge with *Chlorella* alone did not monitor the production of nitrate, concluding that the oxygen produced by *Chlorella* can facilitate nitrification and thus improve the conversion and absorption of nitrite [24]. Therefore, the selection of suitable microalgae and bacteria and the scientific construction of the microalgae–bacteria consortia have significant potential for the development of ecologically healthy shrimp culture and the prevention of global warming. Due to the variable environment of the pond, in order to construct the appropriate microalgae–bacteria consortia and screen the most suitable environmental conditions, the microalgae–bacteria consortia were constructed by using *O. borgei* and *R. palustris*, which are widely used in shrimp ponds. The stable isotope labeling technique was used to determine the uptake rate of nitrite by the microalgae–bacteria consortia and the contribution rate of each component in the consortia at different temperatures (15, 20, 25, 30, 35 °C), illuminations (0, 15, 25, 35, 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and salinities (0, 5, 15, 25, 35‰), and the single and combined effects of the three environmental factors on the absorption of nitrite by the microalgae–bacteria consortia were analyzed. This study provides a reference for the application of the microalgae–bacteria consortia in the regulation and improvement of water quality in shrimp culture.

2. Materials and Methods

2.1. Microalgae and Bacteria

O. borgei was isolated by Guangdong Ocean University, and the experimental medium was a "Zhanshui 107" medium prepared with artificial seawater [25] (nitrogen sources, vitamins, and biotins were removed from the medium). The algal solution was taken during the exponential growth period and centrifuged at 25 °C and 4500 r/min for 10 min, the supernatant was removed, and de-nitrogenized artificial seawater was added, washed, resuspended, and set aside. *R. palustris* DSM5859 was purchased from Beijing Biobw Biotechnology Co., Ltd. (Beijing, China) and purified and expanded with Vanier's yeast medium. The bacterial solution was taken when the bacteria were in the exponential growth phase, centrifuged at 700 r/min for 5 min, and the supernatant was taken. The supernatant was centrifuged at 25 °C and 8000 r/min for 10 min. The supernatant was removed, and the artificial seawater with nitrogen removal was added for suspension for further use.

2.2. Single-Factor Test Method

At 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ illumination and 15‰ salinity, the temperature gradient was set at 15, 20, 25, 30, and 35 °C, and intelligent light incubators were used to control temperature changes. At 30 °C and 15‰ salinity, the illumination gradient was 0, 15, 25, 35, and 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When the temperature is 30 °C and illumination is 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the salinity gradient was 0, 5, 15, 25, and 35‰. The distilled water was directly used as the salinity 0‰ group, and an artificial seawater without sodium chloride (salinity value 8.85‰) was prepared, then part of it was diluted with distilled water to salinity 5‰, and part was added with sodium chloride to salinities of 15‰, 25‰ and 35‰, respectively. The experiments were conducted in 250 mL conical flasks with a volume of 100 mL and three parallel groups for each environmental factor.

According to the growth curves of *O. borgei* and *R. palustris* obtained from the preliminary experiment, the algal and bacterial suspensions were washed and resuspended in nitrogen-free, sterile artificial seawater for a one-day nitrogen starvation treatment. The optical density values of algae and bacteria were analyzed by UV spectrophotometer at 680 and 660 nm, and the concentrations of both algae and bacteria were adjusted to 5×10^6 individuals/mL. The algae–bacteria consortia were constructed in a ratio of 2:1, for its higher removal rate of nitrite nitrogen compared with the other two ratios (1:1 and 1:2) in the previous test. Nitrogen absorption experiments under different environmental factors were performed using 250 mL conical flasks containing 7 mL of algae and 3.5 mL of bacteria suspensions and added to 100 mL with nitrogen-free artificial seawater. The samples under each condition were divided into two groups, one group measured the uptake rate of bacteria with actinomycin added (100 mg/L), which acts as an inhibitor of eukaryotic replication; another group measured the uptake rate of algae with penicillin-streptomycin added (100 mg/L), which inhibit bacterial growth [26]. After adding actinomycin or penicillin-streptomycin for 1 h, sodium nitrite was added to determine the nitrogen absorption rate. Sodium nitrite was prepared as a 1000 times concentration solution (N elemental mass concentration of ^{14}N-$NaNO_2$ and ^{15}N-$NaNO_2$ were 90 mg/L and 10 mg/L, respectively) and added to each treated group according to the ratio of 1‰. The bottles were immediately incubated in the PRX-350C light incubator (Plantronics) for 4 h, and shaken every 1 h to prevent the algae and bacteria from settling and attaching to the wall. At the end of the incubation process, 20 mL of the water sample was added to the Whatman GF/F glass fiber membrane, which was calcined to constant weight in advance and filtered. At the end of the filtration, 5 mL of deionized water was added immediately to continue the filtration, which was used to remove the attached ^{15}N isotope and reduce the experimental error. At the same time, three blank control groups without inhibitors were set up at each environmental gradient to determine the nitrogen absorption by the algae–bacteria consortia.

2.3. Orthogonal Experimental Design

Based on the results of the single-factor experiment, three environmental indicators including temperature, salinity, and illumination were selected to construct a three-factor, three-gradient orthogonal experiment with nine groups (Table 1), and three parallel groups were designed for each group. The specific operation steps were the same as in Section 2.2.

Table 1. Factor and level of orthogonal experiments.

Level	Temperature/(°C)	Salinity/(‰)	Illumination/(μmol·m^{-2}·s^{-1})
1	25	5	25
2	30	15	35
3	35	25	45

2.4. Sample ^{15}N Measurement and Data Analysis

The filter membranes with algal and bacterial bodies were dried in an oven at 60 °C for 24 h to constant weight. About 1 mg of sample powder was scraped and weighed. The sample was packed with Pressed Tin Capsules 5 mm × 9 mm tin capsules. The mass fraction (%) of total nitrogen was determined by an EA Isolink Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), and the $\delta^{15}N$ of the sample was determined by a 253 Plus Isotope Mass Spectrometer (Thermo Fisher Scientific). According to the following formula, the ^{15}N abundance [$A(^{15}N)$], nitrite uptake rate ρ (μg·g^{-1}·h^{-1}), and the contribution rate of algae/bacteria to nitrite absorption C (%) in the samples were calculated.

$$A(^{15}N) = (\delta^{15}N/1000 + 1) \times R_{sta} \quad (1)$$

In the formula, $\delta^{15}N$ is a thousand deviations between the abundance ratio of ^{15}N and ^{14}N in the sample and the abundance ratio of ^{15}N and ^{14}N in the standard substance under standard atmospheric pressure, and R_{sta} is the abundance ratio of ^{15}N and ^{14}N in the natural environment under standard atmospheric pressure (0.00365).

$$\rho = \frac{A(^{15}N)_S - A(^{15}N)_n}{[A(^{15}N)_{enr} - A(^{15}N)_n] \times t} \times w(N_{PON}) \quad (2)$$

In the formula, $A(^{15}N)_n$ is the natural abundance of ^{15}N in the sample under standard atmospheric pressure, $A(^{15}N)_S$ is the abundance of ^{15}N in the algae or bacteria at the end of the incubation, $A(^{15}N)_{enr}$ is the initial abundance of ^{15}N in the medium after the addition of the isotopic tracer, $w(N_{PON})$ is the total nitrogen mass fraction (μg/g) in the sample, and t is the culture time (h).

$$C(\%) = \rho_x/\rho_u \quad (3)$$

In the formula, ρ_x is the absorption rate of nitrite by algae or bacteria and ρ_u is the absorption rate of nitrite by the microalgae–bacteria consortia as a whole.

Excel 2019 was used to process the data, and the chart was drawn. SPASS 26 was used to perform a single-factor analysis of variance and Duncan's multiple comparisons on the nitrite absorption rate of the microalgae–bacteria consortia, and the difference between the data of each group was tested, $p = 0.05$.

3. Results

3.1. Effect of Temperature on Nitrite Nitrogen Uptake Rate and Its Contribution Rate in the Microalgae–Bacteria Consortia

The temperature had a significant effect on nitrite uptake by the microalgae–bacteria consortia, with an average uptake rate of 2.086 μg·g^{-1}·h^{-1} at 30 °C, significantly higher than the other gradients ($p < 0.05$) (Figure 1A). It was shown that *O. borgei* was significantly affected by temperature, with an average uptake rate of 0.726 μg·g^{-1}·h^{-1} at 30 °C, significantly higher than the other gradients ($p < 0.05$); however, too high or too low temperature

will inhibit its absorption of nitrite (Figure 1B). The temperature had a significant effect on the rate of nitrite uptake by photosynthetic bacteria ($p < 0.05$), which showed a trend of increasing and then decreasing with increasing temperature, with an average uptake rate of 1.429 $\mu g \cdot g^{-1} \cdot h^{-1}$ at 30 °C, followed by a decreasing trend. The uptake rates at 15 °C and 35 °C were 0.840 and 0.618 $\mu g \cdot g^{-1} \cdot h^{-1}$ on average, respectively, indicating that the appropriate temperature is suitable for the uptake of nitrite by photosynthetic bacteria, which is inhibited by too high or too low temperatures (Figure 1C). At 30 °C, the contribution rate of *R. palustris* was the highest (65.2%) by the microalgae–bacteria consortia of *O. borgei* and photosynthetic bacteria, and it reached the highest (75.7%) at 35 °C (Figure 1D).

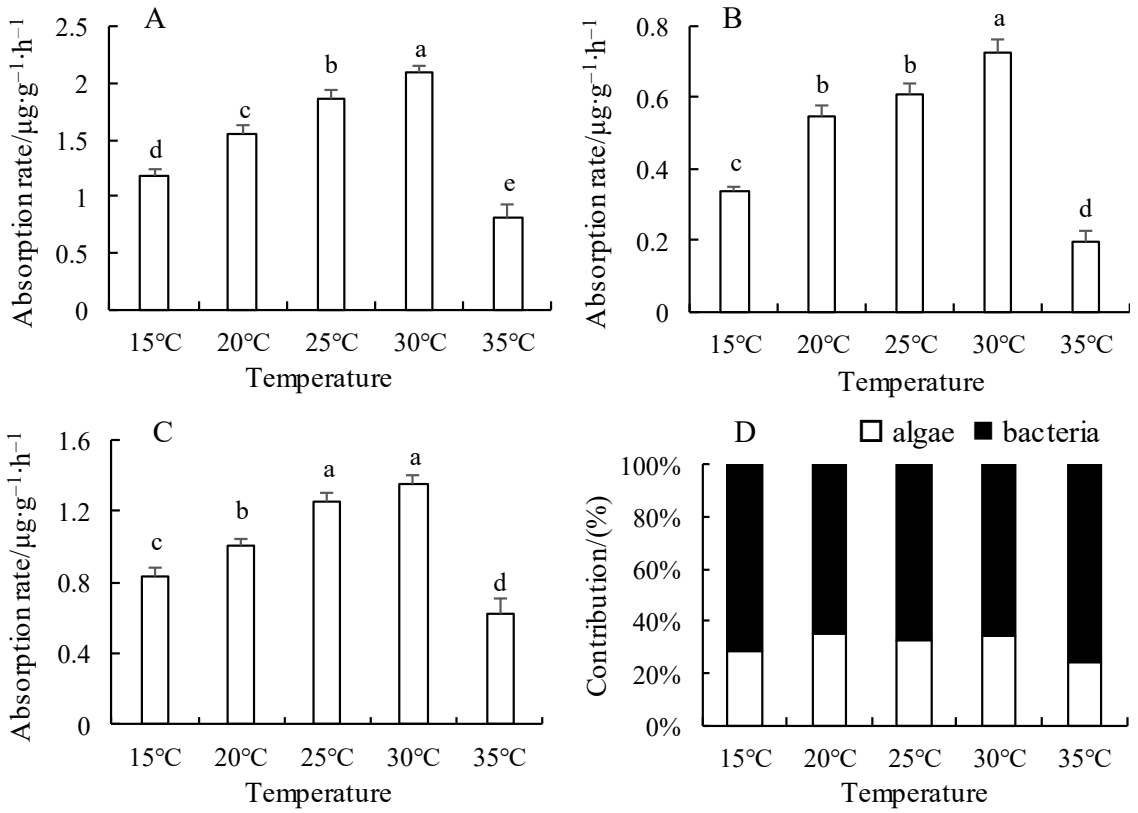

Figure 1. Effect of temperature on the uptake rate of nitrite nitrogen by the microalgae–bacteria consortia ((**A**): the microalgae–bacteria consortia; (**B**): algae; (**C**): bacteria) and its components and the contribution rate of nitrite nitrogen uptake by algae and bacteria (**D**). Significant differences ($p < 0.05$) between treatments are indicated by different lowercase letters (a−e).

3.2. Effect of Salinity on Nitrite Nitrogen Uptake Rate and Its Contribution Rate in the Microalgae–Bacteria Consortia

Salinity had a significant effect on the nitrite uptake by the microalgae–bacteria consortia, with an average uptake rate of 3.058 $\mu g \cdot g^{-1} \cdot h^{-1}$ in the group with 5‰ salinity, which was significantly higher than the other groups ($p < 0.01$) (Figure 2A). Salinity had a significant effect on the rate of nitrite uptake by *O. borgei*, with an average uptake rate of 0.726 $\mu g \cdot g^{-1} \cdot h^{-1}$ in the 15‰ salinity group, which was significantly higher than the other salinity groups ($p < 0.05$). Semi-saline water was suitable for nitrite uptake by *O. borgei*, while too high or low salinity was not conducive to uptake (Figure 2B). Photosynthetic bacteria were significantly affected by salinity, with the highest uptake rate of 2.610 $\mu g \cdot g^{-1} \cdot h^{-1}$ at a salinity of 5‰ and the lowest rate of 0.061 $\mu g \cdot g^{-1} \cdot h^{-1}$ at a salinity of 35‰, all with significant differences ($p < 0.05$). The lower salinity was suitable for nitrite uptake by photosynthetic bacteria, while the higher salinity inhibited the absorption (Figure 2C). At a salinity of 5‰, the contribution of nitrite uptake by *O. borgei* and photosynthetic bacteria in the microalgae–bacteria consortia was 14.6% and 85.4%, respectively, and photosyn-

thetic bacteria were the main absorbers of nitrite. The contribution rate of *O. borgei* in the salinity 25‰ group was the highest, which was 85.0%. The maximum contribution of photosynthetic bacteria was 91.5% in the 0‰ salinity group (Figure 2D).

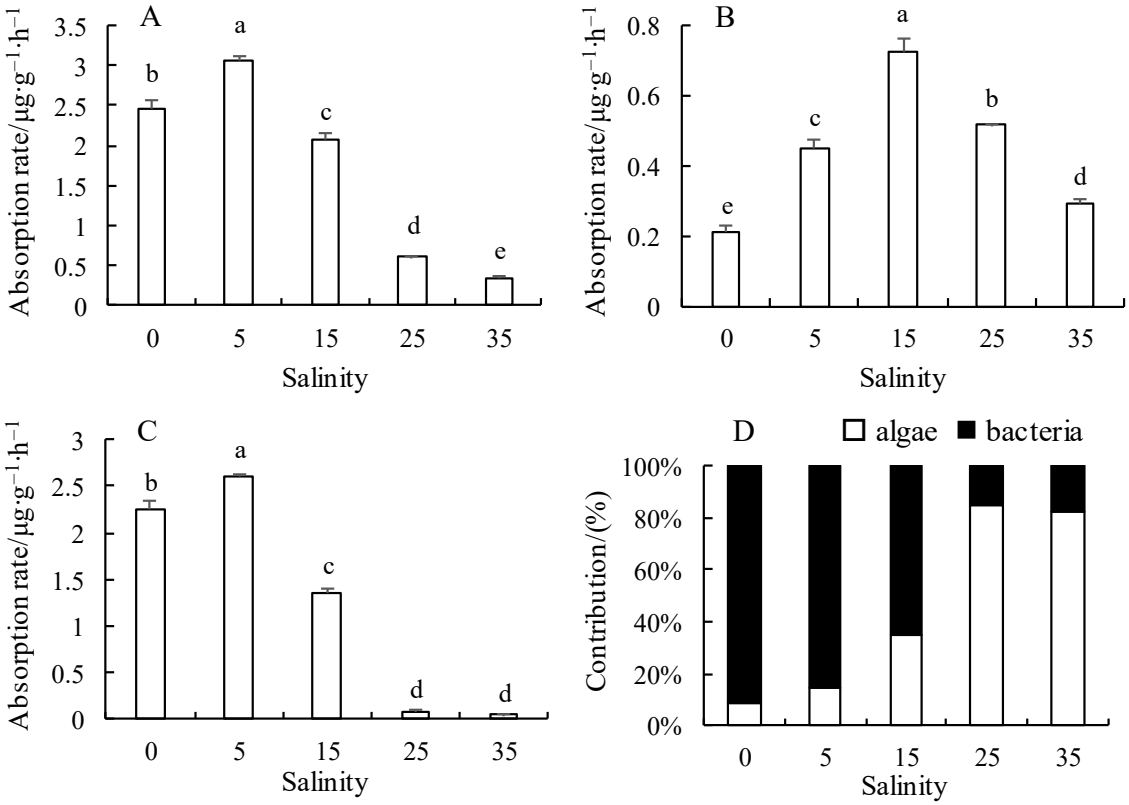

Figure 2. Effect of salinity on the uptake rate of nitrite nitrogen by the microalgae–bacteria consortia ((**A**): the microalgae–bacteria consortia; (**B**): algae; (**C**): bacteria) and its components and the contribution rate of nitrite nitrogen uptake by algae and bacteria (**D**). Significant differences ($p < 0.05$) between treatments are indicated by different lowercase letters (a−e).

3.3. Effect of Illumination on Nitrite Nitrogen Uptake Rate and Its Contribution Rate in the Microalgae–Bacteria Consortia

Illumination had a significant effect on nitrite uptake in the microalgae–bacteria consortia, with an average of 2.319 $\mu g \cdot g^{-1} \cdot h^{-1}$ at an illumination level of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$, significantly higher than the other gradients ($p < 0.05$) (Figure 3A). The nitrite uptake by *O. borgei* was significantly affected by the illumination level, and the uptake rate was significantly better in the illumination group of 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than in the other gradients ($p < 0.05$), with a mean value of 0.726 $\mu g \cdot g^{-1} \cdot h^{-1}$. The darker environment was not conducive to the nitrite uptake by *O. borgei* (Figure 3B). Illumination significantly affected the rate of nitrite uptake by photosynthetic bacteria ($p < 0.05$), with the highest uptake rate of 1.691 $\mu g \cdot g^{-1} \cdot h^{-1}$ at an illumination level of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the lowest uptake rate of 0.583 $\mu g \cdot g^{-1} \cdot h^{-1}$ at dark conditions, with too high or too low illumination inhibiting the uptake of nitrite by photosynthetic bacteria (Figure 3C). At an illumination level of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the contribution rates of *O. borgei* and photosynthetic bacteria in the microalgae–bacteria consortia were 27.0% and 73.0%, respectively, and the contribution rate of photosynthetic bacteria was the highest at this illumination (Figure 3D).

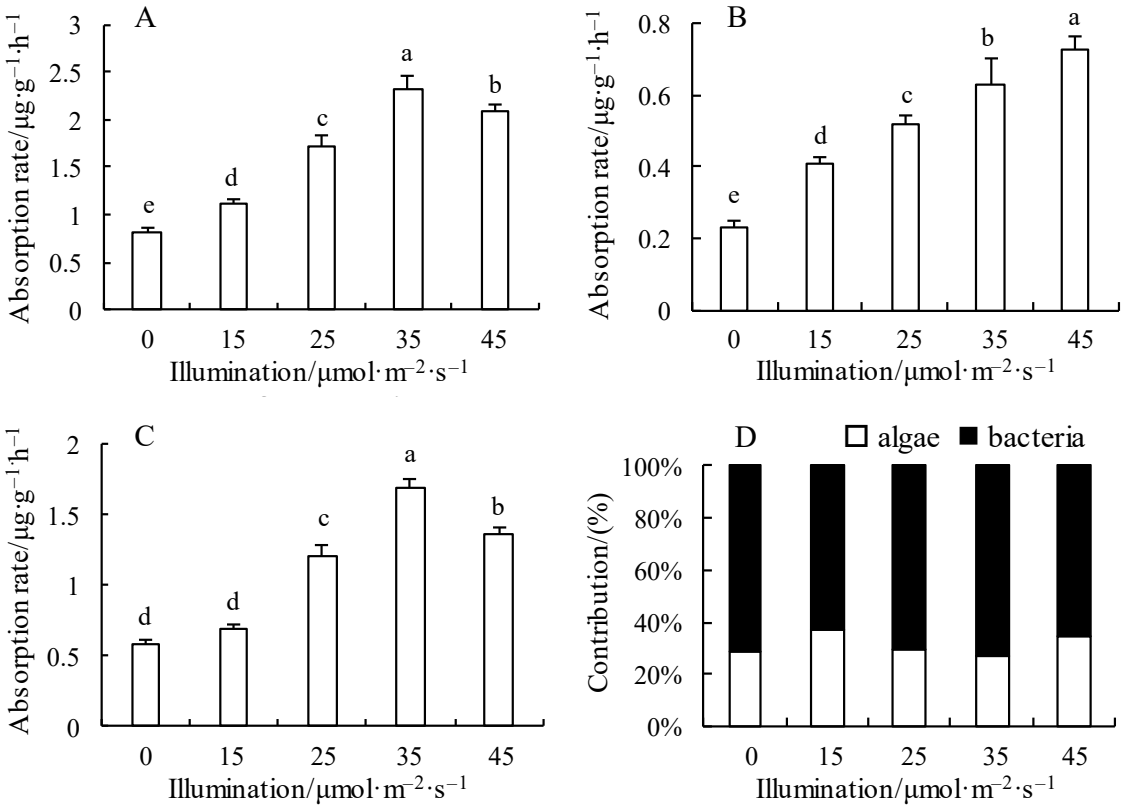

Figure 3. Effect of illumination on the uptake rate of nitrite nitrogen by the microalgae–bacteria consortia ((**A**): the microalgae–bacteria consortia; (**B**): algae; (**C**): bacteria) and its components and the contribution rate of nitrite nitrogen uptake by algae and bacteria (**D**). Significant differences ($p < 0.05$) between treatments are indicated by different lowercase letters (a–e).

3.4. Optimal Culture Conditions for Nitrite Nitrogen Uptake by the Microalgae–Bacteria Consortia

The extreme difference analysis method is often used to analyze the results of orthogonal tests, that is, the average extreme difference of each factor is used to obtain the extreme difference value, so as to obtain the primary and secondary order of the influencing factors and the best combination of factor levels of the indexes under investigation, the greater the extreme difference value, the greater the influence of the factor on the test indexes. The results of the orthogonal experimental analysis of the microalgae–bacteria consortia are shown in Tables 2 and 3. Figures 1A, 2A, 3A and Table 2 show that the average absorption rates for temperature, salinity, and illumination were 1.869, 2.452, and 1.728 $\mu g \cdot g^{-1} \cdot h^{-1}$ at level 1; 2.086, 3.058, and 2.319 $\mu g \cdot g^{-1} \cdot h^{-1}$ at level 2; 0.817, 2.086, and 2.086 $\mu g \cdot g^{-1} \cdot h^{-1}$ at level 3, respectively; the extreme differences were 1.052, 0.972, and 0.591 $\mu g \cdot g^{-1} \cdot h^{-1}$, respectively. Based on the magnitude of the extreme differences in the results of the experimental analysis, it can be seen that the uptake of nitrite by the microalgae–bacteria consortia was most affected by temperature, followed by salinity, and least by illumination. The optimum environmental conditions for the uptake of nitrite by the microalgae–bacteria consortia were a temperature of 30 °C, salinity of 5‰, and illumination of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Under these environmental conditions, the uptake rate of the microalgae–bacteria consortia (3.204 $\mu g \cdot g^{-1} \cdot h^{-1}$) was greater than that of the single algae (0.206 $\mu g \cdot g^{-1} \cdot h^{-1}$) and bacteria (0.780 $\mu g \cdot g^{-1} \cdot h^{-1}$).

Table 2. Result of orthogonal design test.

Level	Temperature (°C)	Salinity (‰)	Illumination ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Absorption ($\mu g \cdot g^{-1} \cdot h^{-1}$)
1	25 (1)	0 (1)	35 (2)	2.446
2	25 (1)	5 (2)	45 (3)	2.936
3	25 (1)	15 (3)	25 (1)	1.119
4	30 (2)	0 (1)	25 (1)	2.439
5	30 (2)	5 (2)	35 (2)	3.204
6	30 (2)	15 (3)	45 (3)	2.086
7	35 (3)	0 (1)	45 (3)	1.383
8	35 (3)	5 (2)	25 (1)	1.625
9	35 (3)	15 (3)	35 (2)	1.104

Note: Line with a grey background represents the optimum environmental conditions.

Table 3. Orthogonal test analysis of variance.

Resource of Gap	Quadratic Sum	Degree of Freedom	Mean Square	F	P
calibration model	42.091	7	6.013	119.582	0.008
temperature	2.257	2	1.128	22.442	0.043
salinity	2.003	2	1.001	19.915	0.048
illumination	0.453	2	0.227	4.508	0.182
error	0.101	2	0.05		
grand total	42.192	9			

4. Discussion

4.1. Temperature on Nitrite Nitrogen Uptake by the Microalgae–Bacteria Consortia

The effect of temperature on microalgae and bacteria was very direct, with significant effects on nitrite uptake by *O. borgei* and *R. palustris*. In the suitable temperature interval, the rate of nitrite uptake by the microalgae–bacteria consortia was positively correlated with temperature as the temperature increased, but when a certain temperature was exceeded, the uptake rate decreased steeply. Temperature can affect material metabolism by influencing ammonia and nitrite oxidative decoupling in greenhouse vegetable soils. When the temperature exceeds the appropriate range, it can affect the oxidative decoupling of ammonia (NH_3) and nitrite (NO_2^-), resulting in nitrite (NO_2^-) accumulation, thereby stimulating N_2O emissions [27]. A study using 16S rRNA high-throughput sequencing to analyze the effect of temperature on microbial community structure during nitrite oxidation showed that the diversity of the microbial community was positively correlated with temperature in the appropriate temperature range, with the greatest diversity at 35 °C. When the temperature was beyond the appropriate range, the richness and diversity of the flora in the system decreased, which affected the absorption efficiency of nitrifying bacteria for nitrite and other substances [28]. In this study, the nitrite absorption rate of the microalgae–bacteria consortia increased continuously at 20~30 °C. When the temperature exceeded 30 °C, the nitrite absorption efficiency of the microalgae–bacteria consortia decreased significantly. This is consistent with the temperature variation range of shrimp aquaculture ponds in southern China from 20 °C to 34 °C, indicating that the microalgae–bacteria consortia can play a good role in removing nitrite during most of the shrimp culture cycle and is suitable to act as a "buffer" to stabilize water quality in ponds. At the same time, the group with the highest nitrite absorption efficiency in the microalgae–bacteria consortia was the experimental group at 30 °C. The reason is that the *O. borgei* used in the experiment was isolated from the shrimp culture ponds in the subtropical region, and *R. palustris* had certain absorption and resistance to ultraviolet rays and can protect the important components of its cells such as cell membrane and DNA from damage. Both of them were adapted to the higher temperature environment.

4.2. Salinity on Nitrite Nitrogen Uptake by the Microalgae–Bacteria Consortia

Salinity is one of the important environmental conditions that affect the growth and metabolism of *O. borgei* and photosynthetic bacteria. Different salinities had different effects on nitrogen removal, and it was found that when the salinity was 0.8%, the abundance of nitrite-oxidizing bacteria was the highest, and almost all nitrite in the water was oxidized to nitrate. It was also found that the higher the salinity, the more obvious the inhibitory effect on nitrite-oxidizing bacteria, and it ultimately reduced the conversion efficiency of bacteria to nitrite [29]. Salt accumulation will affect denitrifying granular sludge performance and microbial community changes in the fraction with high nitrite accumulation. It was found that the denitrification activity of denitrifying sludge bacteria remained above 50% of the maximum value under the influence of transient high salinity, which steadily achieved high nitrite production, thus significantly reducing its nitrite removal efficiency [30], which was generally consistent with the results of the microalgae–bacteria consortia in this study. In this study, *O. borgei* has a wide range of adaptations to salinity and can grow in the salinity range of 0~45‰. However, too high or too low salinity may have a stressful effect on its physiology and biochemistry aspects and inhibit its absorption of nitrite; while photosynthetic bacteria have a small range of adaptation to salinity and only have a higher ability to remove nitrite at a salinity of 5‰ and below, high salinity may inhibit the growth and uptake of photosynthetic bacteria due to the absorption capacity of photosynthetic bacteria to nitrite in the algae–bacteria consortia being significantly higher than that of *O. borgei*, and the uptake rate of the algae–bacteria consortia group with a salinity of 5‰ is much higher than that of other groups. Therefore, in this study, 5‰ salinity is the best condition for the absorption of nitrite in the algae–bacteria consortia, and the nitrite absorption rate is also higher at lower salinity, which is consistent with the current model of shrimp culture mostly in freshwater and semi-saltwater.

4.3. Illumination on Nitrite Nitrogen Uptake by the Microalgae–Bacteria Consortia

Illumination is one of the most common environmental indicators affecting algal growth [31]. Studies have shown that light sources have a very important effect on changes in spirulina growth and nitrite removal, and it was found that the growth of algae and the removal rate of nitrite level are improved under the condition of continuous illumination within a suitable range [32], which is basically consistent with the results of the algae–bacteria consortia in this study. In this study, the nitrite absorption rate of the algae–bacteria consortia increased first and then decreased with the increase in illumination, even in the absence of light, the uptake rate of the algae–bacteria consortia was still high, maintaining above 0.815 $\mu g \cdot g^{-1} \cdot h^{-1}$. In view of the difficulty in cleaning the bottom of the pond in shrimp culture, the algae–bacteria consortia is suitable for maintaining the cleanliness of the bottom of the pond, which is beneficial to the healthy shrimp culture.

4.4. Advantages of the Algae–Bacteria Consortia over Separate Algae and Bacteria

Microalgae and bacteria are widely distributed in various water bodies and are the main absorbers of nitrite in ponds. The use of the algae–bacteria consortia can significantly reduce the content of nitrite in water. Due to the mutualism of microalgae and bacteria, bacteria can decompose macromolecular organic matter into small molecules to facilitate the absorption and utilization of microalgae, and the carbon dioxide produced by bacterial metabolism is the optimal carbon source for microalgae photosynthesis; therefore, the oxygen produced by microalgae photosynthesis can guarantee bacterial respiration required. The study showed that the removal efficiency of total dissolved nitrogen in water was 88.95% with the use of *Chlorella* and *Bacillus licheniformis*, which demonstrated a synergistic effect between algae and bacteria [33]. In addition, it was found that the immobilization of microalgae and bacteria by granulation was an effective strategy to achieve efficient biomass recovery and increase nitrogen removal efficiency [34].

The removal efficiency of nitrogen sources by the algae–bacteria consortia can also be greatly affected by changing the ratio of bacteria to microalgae in the consortia. By

regulating the ratio of *Chlorella* and *Bacilli*, it was found that the highest efficiency of ammonia nitrogen removal from sludge was achieved when the inoculum was 10:90, which guaranteed the nitrification effect of *Bacilli* [35]. Changing the ratio of algae to bacteria will also affect the nitrogen removal mechanism of the algae–bacteria consortia. It was found that when the algae/bacteria inoculation ratio was 5:1 and 1:1, nitrogen assimilation by the algae–bacteria consortia was the main mode of denitrification, and when the algae/bacteria inoculation ratio was 1:5, nitrification was the main mode of nitrogen conversion [36]. Therefore, the scientific construction of the beneficial algae–bacteria consortia in this study into the breeding ponds can quickly reduce the nitrite nitrogen content in the ponds, and microalgae and bacteria have a synergistic and complementary role in the ecosystem, which is important to maintain the stability of pond water quality. The nitrite uptake rate of the algae–bacteria consortia is faster than that of single algae and bacteria, and the consortia can survive more easily. Moreover, the adaptation range of algae and bacteria to the environment is different, which broadens the application range of the algae–bacteria consortia and makes the production process more stable. The results of preliminary experiments in this study showed that the absorption rate of nitrite by the microalgae–bacteria consortia constructed with different proportions of algae and bacteria was higher than that of single algae and bacteria [37–39], and the algae–bacteria consortia could efficiently uptake nitrite under the conditions of temperature 20–30 °C, salinity 0~15‰, and illumination 25~45 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and compared with single *O. borgei* [40] and photosynthetic bacteria [41], the environmental conditions interval for efficient uptake of nitrite was wider, indicating that the adaptability of the microalgae–bacteria consortia to the environment was greater than that of single algae and bacteria. Therefore, it has more potential for application than single algae or bacteria for environmental regulation using the algae–bacteria consortia in practical production.

4.5. Suggestions for the Application of the Microalgae–Bacteria Consortia in Shrimp Ponds to Control Nitrite Nitrogen

Both *O. borgei* and photosynthetic bacteria are common dominant species in shrimp culture ponds, and by artificially constructing the microalgae–bacteria consortia in the culture pond, the biological community in culture ponds can be kept relatively stable [42], and the purification capacity of the microalgae–bacteria consortia for the water body can be optimized by adjusting the composition ratio of algal–bacteria and various environmental factors. Studies have shown that both photosynthetic bacteria and *O. borgei* have better efficiency for nitrite removal [40,43]. It has been noted that the contribution of both bacteria and planktonic microalgae to nitrite uptake in the early and middle stages of shrimp culture is not negligible [44], and the contribution rate of bacteria to dissolved nitrogen uptake in the natural environment ranges from 11% to 61% [45]. Microalgae can directly use nitrite as one of the nutrient sources. The study showed that the use of a biofilter filled with the marine microalga *Picochlorum maculatum* to treat aquaculture wastewater resulted in the effective removal of 89.6% of nitrite in 10 cycles, thus proving the conclusion that marine microalgae can effectively utilize nitrite [46]. In addition, the uptake rates of ammonium and nitrite by *O. borgei* under different environmental conditions were evaluated by using the 15 N isotope labeling technique, providing valuable insights into the removal of dissolved nitrogen by microalgae by improving the nitrogen uptake rates of planktonic algae in shrimp ponds [8]. Excessive nitrite levels in the middle and late stages of aquatic water have a strong toxic effect on aquatic animals. In order to remove excess nitrite from the aquatic water and to address the deficiency of nitrite nitrogen utilization by the algae, bacteria were added to establish the algae–bacteria consortia, in which bacteria play a major role in nitrite uptake. The effect of algae–bacteria consortia on nitrite uptake is greater than that of single bacteria and single algae, which provides theoretical support to achieve the purpose of green and healthy regulation of water quality.

In the present study, the average absorption contribution rates of *O. borgei* and *R. palustris* to nitrite in the microalgae–bacteria consortia were 32.2% and 67.8%, respec-

tively, in the experimental temperature gradient range; 25.6% and 74.4%, respectively, in the experimental salinity gradient range; and 31.2% and 68.8%, respectively, in the experimental lightness gradient range. *R. palustris* was the main contributor to the absorption of nitrite in the microalgae–bacteria consortia. The contribution rate of photosynthetic bacteria to nitrite uptake ranged from 17.3% to 91.5%, with a wide range of variation. This study showed that a temperature of 30 °C, salinity of 5‰, and illumination of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were the environmental conditions with the highest efficiency of nitrite nitrogen uptake by the microalgae–bacteria consortia, and nitrite uptake by the microalgae–bacteria consortia was most influenced by temperature, followed by salinity, and least by illumination. In shrimp aquaculture, the illumination can be controlled by artificially building a canopy or removing it, lowering pond temperature by extracting lower-temperature groundwater, and adjusting salinity by introducing seawater, adding artificial sea salt, or adding freshwater, thus regulating the absorption efficiency of the microalgae–bacteria consortia for nitrite. The microalgae–bacteria consortia of *O. borgei* and photosynthetic bacteria selected in this study have a high absorption efficiency of nitrite and can efficiently absorb nitrite under the conditions of the temperature of 20~30 °C, the salinity of 0~15‰, and the illumination of 25~45 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The environmental conditions are basically consistent with the natural conditions in shrimp culture production and can be applied to the whole process of shrimp culture, so these microalgae–bacteria consortia can be used to regulate and improve the water quality environment of shrimp culture production [47].

5. Conclusions

Temperature, salinity, and illumination had significant effects ($p < 0.05$) on nitrite uptake by the microalgae–bacteria consortia, and the results of the orthogonal experiments showed that temperature was the main factor affecting nitrite uptake by the microalgae–bacteria consortia, followed by salinity and illumination. The optimum combination of the microalgae–bacteria consortia to absorb nitrite was temperature 30 °C, salinity 5‰, and illumination 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In the microalgae–bacteria consortia, *R. palustris* was the main contributor to absorbing nitrite. The microalgae–bacteria consortia of *O. borgei* and *R. palustris* still maintain high nitrite absorption efficiency when the environment changes greatly and has broad application prospects in the regulation and improvement of water quality in shrimp culture.

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