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The Effect of Algicidal and Denitrifying Bacteria on the Vertical Distribution of Cyanobacteria and Nutrients

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Abstract: Algicidal bacteria combined with the ability of aerobic denitrification is considered to be a promising way to control harmful cyanobacterial bloom and remove nitrogen. However, the effect of these bacteria on the vertical distribution of colonial cyanobacteria and nutrients remained unknown. In this study, two algicidal and denitrifying bacteria were respectively co-cultured with the colonial *Microcystis aeruginosa* to construct the microcosm systems, and then the cyanobacteria number, the ratio of bacterial to cyanobacterial abundance, the content of dissolved nitrogen, phosphorus and organic carbon in different water layers were investigated. The results showed that the distribution difference of *Microcystis* among the vertical water layers was further enlarged due to the short-term influence of algicidal bacteria *Brevundimonas diminuta* and *Pseudomonas stutzeri*. The number of *Microcystis* in the lower layer was further reduced by the inhibitory effect of the algicidal bacteria. However, there was a dramatic increase in the number of *Microcystis* in the upper layer, even when the ratio of algicidal bacteria to cyanobacteria increased significantly. *B. diminuta* and *P. stutzeri* both greatly promoted the removal of dissolved total nitrogen in the upper and middle layers of cyanobacteria blooming water, but they also boosted the release of dissolved phosphorus in all layers. These results enable us to better understand the possible limitations of algicidal bacteria in their application to control cyanobacteria blooms.

Keywords: aerobic denitrification; algicidal bacteria; cyanobacteria; colonial *Microcystis*; vertical distribution

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1. Introduction

Cyanobacterial blooms have been known to be a serious threat to public health and aquatic ecosystem functioning [1]. Toxic *Microcystis aeruginosa* is the most primary bloom-forming cyanobacteria in eutrophic freshwaters [2]. It can produce hepatotoxic and hepatocarcinogenic microcystins. Some water crises, such as the Wuxi drinking water crisis in China and the recreational water risk in Portugal, further increased public health concerns [3,4]. The control and mitigation measures of *Microcystis* bloom have been developed and highlighted by researchers. Many algicidal bacteria have been isolated [5,6] and constitute the major group of microorganisms showing inhibitory effects on cyanobacteria [7]. Algicidal bacteria, as a biological control method, are expected to be environmentally friendly and feasible for bloom control [8]. The research about algicidal bacteria mostly focuses on cyanobacteria species specificity [9], their attacking mode [10], the relationship between their concentration threshold and cyanobacteria decay [11], definite cyanobactericidal substances [12] and their cyanobactericidal mechanism [13]. Most *Microcystis* strains tested in the laboratory are not in a colonial state [6]. During coexistence with algicidal bacteria, vertical stratification of these cyanobacteria will not happen in glass Erlenmeyer flasks. However, *Microcystis* in the natural water environment tends to migrate vertically

through changing cell density and regulating buoyancy. The size of colonies and wind speed have significant effects on the migration, which leads to the stratified distribution of *Microcystis* [14]. Then, the physical and chemical environments in different water layers, such as dissolved oxygen (DO) and pH, are affected differently [15]. It is not clear whether the vertically stratified distribution of cyanobacteria will interfere with the inhibitory activity of algicidal bacteria. In addition, algicidal bacteria combined with the ability of denitrification are considered to have the dual effects of nitrogen and cyanobacteria bloom control [16]. However, it remains poorly defined how these bacteria further affect the vertical distribution of cyanobacteria and nutrients in the corresponding water layer.

The aims of this study were to explore the response of algicidal and denitrifying bacteria to the vertical distribution of cyanobacteria, and their effects on the distribution of cyanobacteria and nutrients in different water layers. For this, two algicidal bacteria were respectively co-cultured with the colonial *Microcystis*; then, the cyanobacteria number, the ratio of bacterial to cyanobacterial abundance, the content of dissolved nitrogen, phosphorus and organic carbon in different water layers were examined. The results of this study enable us to better understand the possible limitations of algicidal bacteria in their application to control cyanobacterial blooms as well as help to seek strategies for more effective use of them.

2. Materials and Methods

2.1. Strains and Culture Conditions

M. aeruginosa (FACHB 912) was purchased from Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). This strain was cultivated in 10% modified Hoagland's medium (pH 7.0) supplemented with Ca^{2+} and Fe^{2+} at the final concentration of 36 mg/L and 2.8 mg/L to maintain colony status. The incubator was maintained at 28 °C and illuminated with a light intensity of 2800 lux by cool-white, fluorescent lamps in 12 h diurnal cycles.

Brevundimonas diminuta Z1 (CGMCC 20909) and *Pseudomonas stutzeri* ZJ4 (CGMCC 20910) were isolated from a water bloom sample in the aquaculture ponds of the Taihu Lake Basin and deposited to the China General Microbiological Culture Collection Center (Beijing, China). They both exhibited algicidal activity (Table 1). These strains were grown aerobically in Luria-Bertani (LB) medium at 28 °C, respectively. All media were sterilized at 121 °C for 20 min before use.

Table 1. Algicidal effects of *B. diminuta* Z1 and *P. stutzeri* ZJ4 washed cells against unicellular *M. aeruginosa*.

Algicidal Bacteria	Concentration (Cells/mL)	Inhibitory Rate after 3 Days (%)
B. diminuta Z1	1.36×10^8	$24.29 \pm 3.29\%$
P. stutzeri ZJ4	1.02×10^8	$10.07 \pm 0.94\%$

Note: Initial concentration of unicellular *M. aeruginosa* (FACHB 912) was 7.18×10^6 cells/mL.

2.2. Co-Existence Experiments

M. aeruginosa was amplified in 10% modified Hoagland's medium (pH 6.8) supplemented with Ca^{2+} and Fe^{2+} in aseptic conical flasks (2 L) for 15 days with a thorough shake once a day. When it grew exponentially and reached the density of 1.07×10^7 cells/mL, it was transferred into cylindrical plastic containers (2.8 L, 50 cm high). These containers were pre-disinfected with 75% alcohol. The upper one-third part of the container was transparent, and the lower two-thirds part was shielded with aluminum foil. *B. diminuta* Z1 and *P. stutzeri* ZJ4 were individually collected during the exponential growth period and washed twice with 10% modified Hoagland's medium supplemented with Ca^{2+} and Fe^{2+}. Then, *B. diminuta* Z1 and *P. stutzeri* ZJ4 were respectively added into *M. aeruginosa* cultures and mixed well in cylindrical containers at a final density of 2.93×10^8 cells/mL (B treatment) and 2.75×10^8 cells/mL (P treatment) in the microcosm systems, while the *M. aeruginosa* cultures without *B. diminuta* Z1 or *P. stutzeri* ZJ4 were mixed and used as

controls. All microcosm experiments were conducted with three independent replicates. All treatments were maintained under the culturing conditions described above. The cool-white, fluorescent lamps were located above the culture containers.

At day 0, initial mixed samples were collected. After 3 days of static cultures, DO and pH were measured in three vertical stratified sampling sites with pre-fixed multi-parameter water quality monitoring sondes (HQ40d, HACH, Loveland, USA). The upper sampling site was located in the near-surface layer of the fluid column (0–2 cm). The middle site was in the middle of the fluid column (23–25 cm). The lower site was at the bottom of the fluid column (46–48 cm). Vertically stratified water samples were conducted by the slow siphon method without breaking the stratification.

2.3. Measures of Cyanobacteria Cell Density

M. aeruginosa cells were fixed by formaldehyde (2% final conc.) and counted in a hemocytometer using a microscope (CX41, Olympus, Tokyo, Japan), after dispersion by ultrasonication (5 s).

2.4. The Ratio of Bacterial to Cyanobacterial Abundance

Water samples were filtered through a 0.22 μm Millipore membrane. The filtered membrane was used for subsequent DNA extraction. Total genomic DNA was extracted by using a PowerWater® Sterivex™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The concentration of DNA was determined via spectrophotometer analysis. Amplification and sequencing of the 16S rRNA gene V4 region were performed at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). PCR was carried out using Q5™ High-Fidelity DNA Polymerase (NEB, Ipswich, MA, USA) with forward primer 520F (5′-AYTGGGYDTAAAGNG-3′) and reverse primer 802R (5′-TACNVGGGTATCTAATCC-3′). The amplicon libraries were generated using TruSeq Nano DNA LT Library Prep Kit (Illumina, San Diego, CA, USA) and then sequenced on the Illumina MiSeq, using MiSeq Reagent Kit V3 (600 cycles) for a 2 × 250 bp paired-end reads. The sequences with 97% similarity were assigned into the same operational taxonomic unit (OTU) by using the Qiime software platform. The relative abundances were calculated by the abundance of each OTU at specific taxonomic ranks, and then the ratio of bacterial to cyanobacterial abundance was obtained.

2.5. Chemical Analyses

Water samples were filtered through a 0.45 μm Millipore membrane. Nitrate-N (NO_3^--N) concentration was analyzed using the UV spectrophotometric screening method. Ammonium-N (NH_4^+-N) concentration was determined using Nessler's reagent spectrophotometry method. Nitrite-N (NO_2^--N) concentration was determined using N-(1-naphthyl)-1, 2-diaminoethane dihydrochloride method. Dissolved total nitrogen (DTN) was measured using alkaline potassium persulfate digestion-UV spectrophotometric method. Phosphate-phosphorous (PO_4^{3-}-P) was measured using ammonium molybdate spectrophotometric method. Dissolved total phosphorous (DTP) was measured using alkaline potassium persulfate digestion-ammonium molybdate spectrophotometric method. Dissolved organic carbon (DOC) was determined using a TOC analyzer (Vario TOC, Langenselbold, Germany).

2.6. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Differences between controls and treatments were determined by one-way ANOVA. Post hoc test was used to perform statistical multiple comparisons. LSD adjustment was adopted when the assumption of equal variances was met, and Games-Howell adjustment was adopted when the assumption of equal variances was not met. Results were expressed as letters. Different letters were marked in histograms to indicate significant differences ($p < 0.05$). Student's *t*-test was used to assess the correlation between groups of parameters and the significance level

(two-tailed; $p < 0.05$ was considered significant). All statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Spatial Distribution of Colonial Microcystis

Based on the different cyanobacteria concentrations in the vertical layers of water column under calm conditions for 3 days, colonial *M. aeruginosa* could migrate to the near-surface region and form surface scum even after thorough mixing. As shown in Figure 1, the density of cyanobacteria cells in the upper layer was up to 12.98 times that of the middle or lower layer in controls after 3 days of static cultures, unlike the initial homogeneous state. Compared to the controls, the density of cyanobacteria cells in the upper layer of the B treatments and P treatments at day 3 increased significantly by an average of 62.33% and 54.79%, respectively. On the contrary, the density of cyanobacteria cells in the lower layer of the B treatments and P treatments were on average 70.09% and 63.55% lower than controls, respectively. There was no significant difference between the cyanobacteria density in the middle layer of treatments and controls.

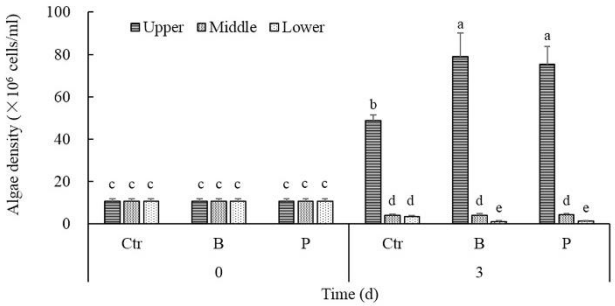

Figure 1. The effect of algicidal and denitrifying bacteria on the vertical distribution of *M. aeruginosa*.

3.2. Characteristics of Environmental Parameters in Vertical Space

Colonial *M. aeruginosa* was positively buoyant. After 3 days of static cultures, DO concentrations varied between 0.02 mg/L and 18.43 mg/L, depending on the vertical position and cyanobacteria density in water column (Figure 2a). There was no significant difference among DO concentrations of the same water layers in controls and treatments. Correlation analysis revealed that there existed significantly positive correlation between DO and the density of cyanobacteria cells, no matter in controls or treatments ($r_{ctr} = 0.989$, $p < 0.05$; $r_B = 0.977$, $p < 0.05$; $r_P = 0.984$, $p < 0.05$). However, along with the significant vertical gradients of oxygen at day 3, the difference of pH among water layers was not remarkable (Figure 2b). It might be due to the use of modified Hoagland's medium.

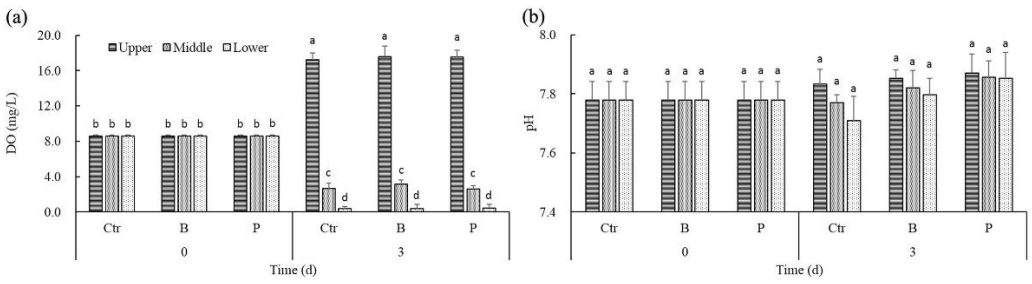

Figure 2. The effect of algicidal and denitrifying bacteria on DO (**a**) and pH (**b**) in the different layers of water.

In the medium, the main form of nitrogen was NO_3^--N. After 3 days, NO_3^--N concentrations of the upper water layer were significantly higher than that of the middle and lower layer in controls (Figure 3a). However, there was no obvious difference among the NO_3^--N concentrations in different water layers of B treatments and P treatments.

Significant positive correlations were found between NO_3^--N concentrations and the density of cyanobacteria cells only in controls ($r_{ctr} = 0.920$, $p < 0.05$).

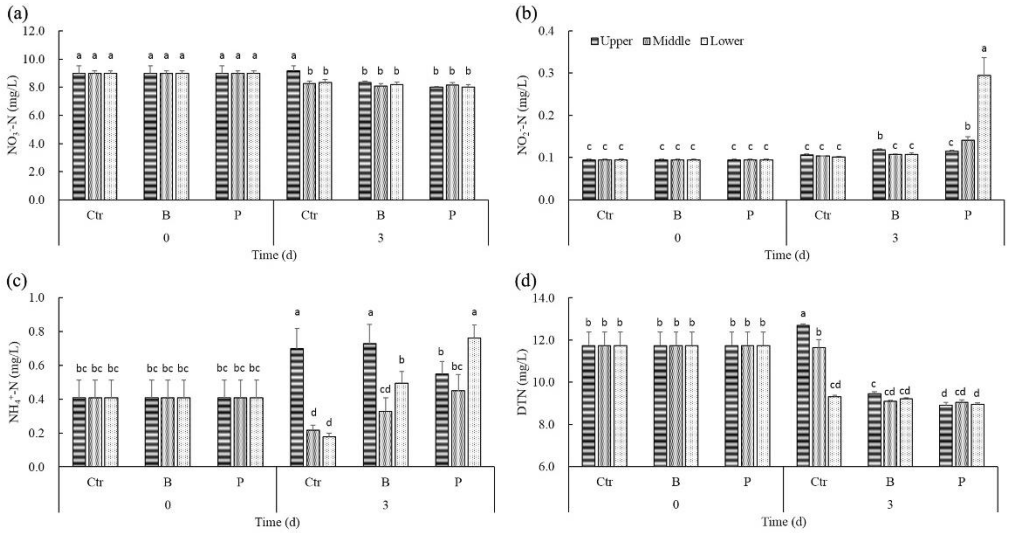

Figure 3. The effect of algicidal and denitrifying bacteria on NO_3^--N (**a**), NO_2^--N (**b**), NH_4^+-N (**c**) and DTN (**d**) concentrations in the different layers of water.

NO_2^--N distribution in controls after 3 days was the same as the initial homogeneous state (Figure 3b). However, NO_2^--N concentrations of the middle and lower water layers were significantly higher than that of the upper layer in P treatments. Significant negative correlations were found between NO_2^--N concentrations and DO in P treatments ($r_P = -0.675$, $p < 0.05$), while significant positive correlations were found between NO_2^--N concentrations and DO in B treatments ($r_B = 0.920$, $p < 0.05$).

NH_4^+-N concentrations of the upper layer amounted to about three to four times that of the middle or lower layer in controls, unlike the initial homogeneous state. NH_4^+-N distribution in B treatments was similar to that in controls after 3 days, except that NH_4^+-N concentrations of the lower layer in B treatments were enhanced as compared to controls (Figure 3c). However, NH_4^+-N concentrations of the lower layer were significantly higher than that of the upper and middle layers in P treatments, while its NH_4^+-N concentrations of upper layer were cut down as compared to controls at day 3. Correlation analysis indicated that NH_4^+-N concentrations had a significantly positive correlation with the density of cyanobacteria cells only in controls ($r_{ctr} = 0.954$, $p < 0.05$).

The trends of DTN distribution after 3 days in controls and treatments were similar to NO_3^--N. Unlike the significant vertical gradients of DTN in controls, there was no marked difference among the DTN concentrations in different water layers of B treatments or P treatments (Figure 3d). DTN concentrations in the upper layer of the B treatments were on average 23.76% lower than controls, while DTN concentrations in the upper layer of the P treatments were significantly lower than B treatments. Significant positive correlations were found between DTN concentrations and the density of cyanobacteria cells only in controls ($r_{ctr} = 0.738$, $p < 0.05$).

Different from the initial homogeneous state, PO_4^{3-}-P concentrations in the upper layer of controls and treatments were higher than that of the middle and lower layers after 3 days (Figure 4a). The PO_4^{3-}-P concentrations of the different water layers both in B treatments and P treatments were significantly higher than the corresponding concentrations in controls. Significant positive correlations were found between PO_4^{3-}-P concentrations and the density of cyanobacteria cells only in controls ($r_{ctr} = 0.965$, $p < 0.05$).

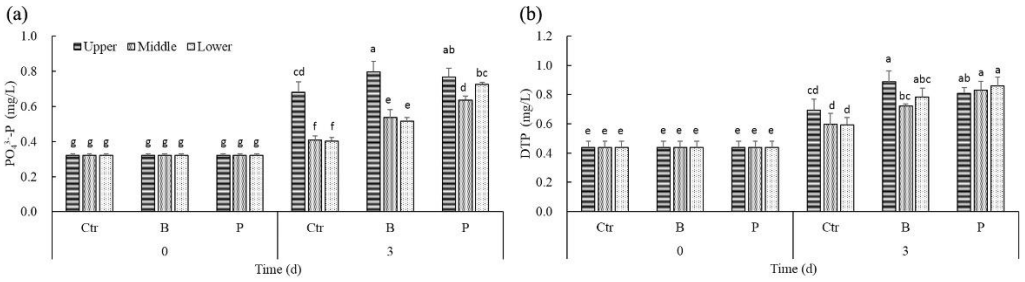

Figure 4. The effect of algicidal and denitrifying bacteria on PO_4^{3-}-P (**a**) and DTP (**b**) concentrations in the different layers of water.

Just like PO_4^{3-}-P, the DTP concentrations of the different water layers both in B treatments and P treatments were also significantly higher than the corresponding concentrations in controls (Figure 4b). Significant positive correlations were found between DTP concentrations and the density of cyanobacteria cells only in controls ($r_{ctr} = 0.661$, $p < 0.05$).

The trends of DOC gradients in controls and treatments after 3 days were similar to PO_4^{3-}-P. The DOC concentrations in the middle or lower layer of both B treatments and P treatments were significantly higher than that of controls (Figure 5). Correlation analysis revealed that there existed significantly positive correlation between DOC and the density of cyanobacteria cells only in controls ($r_{ctr} = 0.990$, $p < 0.05$).

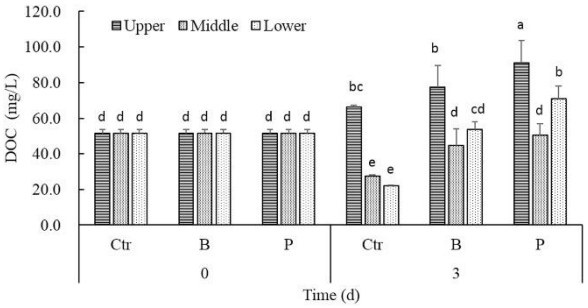

Figure 5. The effect of algicidal and denitrifying bacteria on DOC concentrations in the different layers of water.

3.3. Shifts in Ratios of Bacteria to Microcystis

As shown in Figure 6, the average ratio of bacterial to cyanobacterial abundance in the upper layer of B treatments and P treatments at day 3 remarkably increased to 6.95 times and 4.02 times of the initial, respectively. The average ratio of bacterial to cyanobacterial abundance in the middle layer of B treatments and P treatments at day 3 respectively decreased by 42.06% and 61.29% compared with their initial value. Moreover, the ratio of bacterial to cyanobacterial abundance of the middle layer was significantly lower than that of the upper and lower layers both in B treatments and P treatments. It was different from B treatments that the ratio of bacterial to cyanobacterial abundance in the lower layer of P treatments was significantly higher than that in the upper and middle layers. Correlation analysis indicated that there was no significant correlation between the ratio of bacterial to cyanobacterial abundance and the density of cyanobacteria cells in B treatments and P treatments. Moreover, the ratio of bacterial to cyanobacterial abundance showed a significant correlation with NO_3^--N concentrations ($r_B = 0.896$, $p < 0.05$; $r_P = -0.694$, $p < 0.05$), NH_4^+-N concentrations ($r_B = 0.850$, $p < 0.05$; $r_P = 0.828$, $p < 0.05$) and PO_4^{3-}-P concentrations ($r_B = 944$, $p < 0.05$; $r_P = 0.693$, $p < 0.05$) both in B and P treatments.

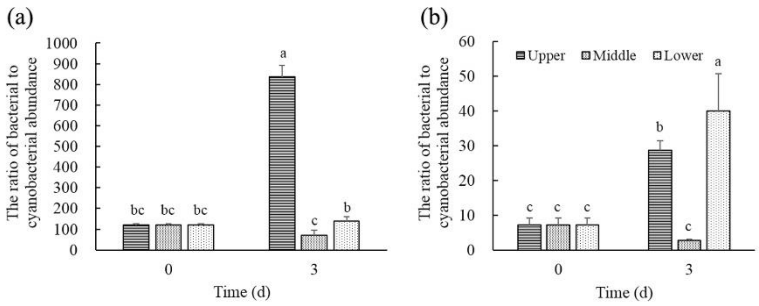

Figure 6. The ratio of bacteria to cyanobacteria in the different layers of water. (**a**) *B. diminuta* and (**b**) *P. stutzeri*.

4. Discussion

Under the influence of low wind speed and low wave height, the vertical distribution of cyanobacteria was uneven in many inland waters and cyanobacterial surface scum was often detected [17,18]. In this experiment, the vertical gradient distribution of colonial cyanobacteria was successfully simulated by the induction of high calcium ions in the water column. The data showed that the heterogeneous distribution of cyanobacteria also affected the physical and chemical properties of different water layers.

The biological control of cyanobacteria by using algicidal bacteria is a promising approach in an aquatic environment [11,19]. The two bacteria we used have been verified to be able to have algicidal effects on the unicellular *Microcystis* of the same strain in a short time, which was in accordance with previous reports on other *Microcystis* strains [20–22]. *P. stutzeri* ZJ4 belongs to one of the top three genera in cyanobactericidal bacteria [6]. Interestingly, however, they were found to have different effects on the populations of colonial *Microcystis* distributed in the upper, middle and lower layers. The biomass of colonial *Microcystis* in the upper layer was significantly elevated in B treatments and P treatments, while the number of the corresponding bacteria in the same layer was also increased significantly. The density of colonial *Microcystis* in the middle layer was not influenced in B treatments and P treatments. The lysis of the colonial *Microcystis* was only detected in the lower layer of B treatments and P treatments during the short coexisting period. The dissolved oxygen of the lower layer was much lower than that of the middle layer and the upper layer due to the vertical gradient distribution of colonial *Microcystis*, but *Microcystis* was proved to be able to tolerate the dark and anoxic environment [23]. Based on the nitrogen and phosphorus concentration in our experiment, nutrients' limitation for *Microcystis* were not realized by the algicidal bacteria. Therefore, it was speculated that the inhibition of *Microcystis* in the lower layer and the release of ammonia nitrogen and phosphorus were mainly due to the algicidal effect of the two bacteria.

Many researches indicated that the ratio of algicidal bacteria to cyanobacteria was set at least 1:1 for the effective suppression or lysis of cyanobacteria [22,24,25]. The cell ratio of each bacteria to cyanobacteria initially added in this experiment has exceeded 25:1. After three days of coexistence experiments, the ratio of bacteria to cyanobacteria in the upper layer was further elevated both in B treatments and P treatments. However, the algicidal effect of the two bacteria did not appear in the upper layer. It was reported that the *Microcystis* colonies larger than 180 μm made a major contribution to the formation of surface bloom scum, while the colonies smaller than 100 μm made a rather limited contribution to the surface scum [26]. Colony size was proved to be correlated with extracellular polysaccharides (EPS) levels. The presence of higher EPS in the population was also found to protect *Microcystis* cells against chemical stress [27,28]. Our preliminary experiments showed that the cell-free filtrates from these two bacteria had algicidal activity on *M. aeruginosa* (Table S1, Supplementary Materials). It indicated that the two bacteria we used could secrete algicidal substances. 1-methyl-β-carboline have been identified as a algicidal compound in *Pseudomonas* [29]. However, the amount of algicidal substances usually needed to be large enough to meet the effective threshold for bloom control [6].

Therefore, the large-sized *Microcystis* colonies in the upper layer were likely to be less susceptible to algicidal bacteria. Moreover, some studies suggested that the production of algicidal substances was stimulated under the condition of limited growth, instead of in optimum growth conditions [30,31]. Excessive photosynthetic products could be released into the water by cyanobacteria as an overflow mechanism, which were also important carbon sources and energy sources for heterotrophic bacteria [32,33]. Our data showed that high concentrations of cyanobacteria in the upper layer indeed created a high DOC environment which was conducive to bacterial growth. Furthermore, bacterial quorum-sensing system was found to control algicide production [34]. More interestingly, bacteria could switch from algicidal mode to symbiotic mode based on nutrient acquisition and bacterial density [35]. Just like the bacteria we used, it was reported for its algicidal effect [21,36], but it was also reported that it has the effect to promote nutrient exchange with cyanobacteria and further promote cyanobacteria growth [37]. These opposite phenomena were somewhat similar to the different growth of cyanobacteria in different water layers during the coexistence with algicidal bacteria in our results. However, the key points of the transformation between algicidal mode and symbiotic mode were still unclear. It was likely to increase the uncertainty of the large-scale application of algicidal bacteria.

For a long time, the research on the relationship between algicidal bacteria and cyanobacteria has mainly focused on the one-sided algicidal effect of bacteria on cyanobacteria [11,38]. However, it was also found in some reports that the growth of algicidal bacteria was inhibited by cyanobacteria [39,40]. Further data on the differential gene expression confirmed that algicidal bacteria also suffered from cell damage and oxidative damage caused by cyanobacteria [24,41]. Our results showed that the number of *Microcystis* in the middle layer was not affected by the two algicidal bacteria, but the ratios of each bacteria to cyanobacteria in the middle layer were significantly lower than that in the upper and lower layers. Moreover, the release of ammonia nitrogen and phosphorus in the middle layer was elevated. It was speculated that the bacteria in the middle layer of the water column might be under certain stress. Whether this stress was caused by carbon limitation or allelopathic stress needs to be further studied.

Aerobic denitrifying bacteria was considered to have application potential for bioremediation of eutrophic water bodies due to their nitrogen removal ability under aerobic conditions [42]. The two bacteria we used have the ability of aerobic denitrification, which was consistent with the previous reports [43,44]. Our results showed that *P. stutzeri* and *B. diminuta* both greatly promoted the removal of dissolved nitrogen in the cyanobacteria blooming waters rich in dissolved oxygen. *P. stutzeri* was good at remove NO_3^--N, NH_4^+-N and DTN. *B. diminuta* had good removal performance at NO_3^--N and DTN. Nitrite reductase was found sensitive to oxygen [45] and high oxygen environment was detected to easily lead to the accumulation of nitrite [46]. Our data showed that *B. diminuta* exhibited nitrite accumulation under high oxygen conditions, while *P. stutzeri* exhibited nitrite accumulation under anoxic conditions. Moreover, some potential risks should be paid attention to. Firstly, the number of cyanobacteria in surface water could further be increased under the influence of these bacteria. Once the cyanobacteria population declined, the release of nutrients and microcystins from cyanobacteria would not be ignored. Secondly, according to the previous report [47], the concentrations of TP showed an increase at algae-rich stations. In our experiment, the soluble phosphorus, especially in the upper layer of cyanobacteria blooming water increased under the coexistence with these bacteria, which might be primarily related to the high density of cyanobacteria. Cyanobacteria could release phosphorus in the whole life cycle, especially in the stationary and declining phase [37]. The coexistence of low concentrations of cyanobacteria and bacteria was shown to contribute to the removal of phosphorus from water [48]. Therefore, it was recommended that in order to better use denitrifying bacteria to improve water quality in cyanobacteria blooming waters, the application time of bacteria should be paid attention to, and the combination with mechanized salvage measures of cyanobacteria should also be considered.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14132129/s1, Table S1: Algicidal effects of bacterial LB cultures and cell-free filtrates against unicellular *M. aeruginosa*.

Author Contributions: Conceptualization, Q.Z. and Z.Z.; methodology, Q.Z. and Y.Z.; formal analysis, Q.Z. and Y.W.; investigation, Q.Z. and Y.W.; resources, X.W.; data curation, Q.Z. and Y.W.; writing—original draft preparation, Q.Z.; writing—review and editing, Q.Z. and Z.Z.; visualization, Q.Z. and X.W.; supervision, Z.Z.; project administration, H.L.; funding acquisition, Q.Z. All authors have read and agreed to the published version of the manuscript.

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