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Application of $BiVO_4$–Microalgae Combined Treatment to Remove High Concentration Mixture of Sulfamethazine and Sulfadiazine

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Abstract: Sulfonamides (SAs) are the most common and bio-refractory antibiotics detected in surface water systems, which cause long-term toxic effects on aquatic organisms. This study used the combination of a $BiVO_4$ photocatalyst and freshwater micro-green alga (*Dictyosphaerium* sp.) to remove sulfadiazine (SD) and sulfamethazine (SM2) at an initial concentration of 5 mg/L (1:1 *v*/*v*) for 7 days. We set up three gradient concentrations of $BiVO_4$ (0.5, 1 and 2 g/L) combined with the same concentration (80 mg/L) of *Dictyosphaerium* sp. and then prepared corresponding concentrations of pure $BiVO_4$ and pure microalgae as controls. We evaluated the ability of $BiVO_4$ and *Dictyosphaerium* sp. combined technology to remove SAs by observing the removal efficiency of antibiotics and explained the degradation mechanism of antibiotics and the key role of microalgae by studying the changes of reactive oxygen species (ROS) and inorganic ions (nitrogen, sulfur). The results showed that the degradation rate of these two SAs in the 0.5 g/L $BiVO_4$–algae group could reach >96% within 7 d, which was higher than that in the 2 g/L $BiVO_4$ group (93%) and the algae group (28%). The increased degradation efficiency of SAs in $BiVO_4$ and microalgae systems was mainly due to the increased amount of ROS. Meanwhile, more SAs were degraded to inorganic compounds such as NH_4^+-N, NO_3^--N and SO_4^{2-}-S under ROS stress. It was found that microalgae can absorb the degradation products of antibiotics such as NH_4^+-N for their own growth, thereby reducing the toxicity of antibiotic by-products. In addition, $BiVO_4$ had no damaging effect on the autofluorescence intensity of the microalgae. Our study provides an efficient and eco-economic approach to remove antibiotics using visible-light irradiation in aquatic environments and provides new insights into the biological removal of other antibiotic contaminants in aquatic environments.

Keywords: *Dictyosphaerium* sp.; $BiVO_4$; sulfamethazine; sulfadiazine; reactive oxygen species; NH_4^+-N

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1. Introduction

Since they were first discovered in 1929, antibiotics have been frequently detected in surface water, ground water, sediments, and even effluent from wastewater treatment plants [1,2]. Because antibiotics can inhibit the growth of bacteria, fungi, and other microorganisms [3], the widespread use of antibiotics in humans and veterinary medicine has had a major impact on the aquatic environment through domestic excretion, hospital waste, and overuse in agriculture and aquaculture [4]. Sulfonamides (SAs), antibacterial

agents that inhibit dihydrofolic acid synthesis, are a major class of antibiotics detected in the aquatic environment [5]. In China, the total concentration of SAs in the Fuzhou section of the Minjiang river basin was 60.9 ng/L [6], and water samples collected from feedlot wastewater pools and irrigation ditches of Shanghai city have shown that the total concentration of SAs was 198–323 μg/L [7]. Although concentrations of contaminated SAs range from ng/L to μg/L, this may increase bacterial resistance and magnify their toxicity via bioaccumulation in the food chain [8]. In light of the non-degradability of SAs and their continuous discharge into surface water, current wastewater treatment plants may not be sufficient to completely remove these compounds [9]. Therefore, we need to develop more effective methods to remove SAs.

Several methods including adsorption, coagulation, and membrane filtration have recently been used to remove antibiotic contaminants [10–12]. When compared with conventional removal methods, photocatalytic oxidation has been considered as an environmentally friendly, promising, and efficient alternative to remove antibiotic pollutants [13,14]. Recently, $BiVO_4$ has come into the focus of researchers for its unique properties, such as ferroelasticity [15], ionic conductivity [16], photocatalytic activity for water splitting [17], and ability to degrade harmful pollutants [18]. For instance, pure $BiVO_4$ has been shown to remove 65% of methylene blue (MB) and 90% of ibuprofen under visible-light irradiation [18,19]. $BiVO_4$ is considered an excellent visible-light photocatalyst because it is composed of inexpensive and non-toxic elements [20]. There are three crystal forms of $BiVO_4$: tetragonal zircon, monoclinic scheelite, and tetragonal scheelite. Monoclinic scheelite $BiVO_4$ exhibits a moderate band gap (2.40 eV) and good photocatalytic activity under visible-light irradiation [21]. Despite the advantages of $BiVO_4$, several drawbacks are responsible for the low usage rate of $BiVO_4$ as a photocatalyst. For instance, the photocatalytic activity of $BiVO_4$ depends strongly on its crystalline phase [22] and needs to be improved due to its poor adsorptive performance and high electron-hole recombination rate [23]. Moreover, the preparation method is crucial in terms of the performance of $BiVO_4$. Parameters such as the morphology, crystal plane and surface area of the crystal form are directly dependent on the preparation process [23]. Many methods have been explored, such as doping with metal or non-metal ions, and its combination with metal oxides [24], which may add impurities and increase the overall cost of production [25]. Thus, we need to develop a more cost-effective treatment technique than using pure $BiVO_4$.

Microalgae-mediated bioremediation is attracting a great deal of attention in the scientific community due to its dual role of nutrient uptake and biodegradation/detoxification of hazardous organic pollutants from water bodies, such as phenolics, polyaromatic hydrocarbons and pharmaceuticals [26]. Previous studies have shown that the removal of antibiotics directly caused by microalgae mainly includes bioadsorption, bioaccumulation, and biodegradation [8,27,28]. Microalgae remove antibiotics mainly by breaking down complex toxic compounds into simpler and less toxic products [29]. According to the highest reported degradation rate by other researchers, 32% of sulfamethoxazole can be removed using *Scenedesmus obliquus* [30] and 29.3% of sulfamethazine (SM2) can be removed using *Chlorella pyrenoidosa* [31]. Our previous study found that *Dictyosphaerium* sp. (FACHB-1902) can effectively improve the degradation efficiency of sulfadiazine (SD) and SM2 to 45% and 51%, respectively [32]. Bioremediation has the advantage of low cost; thus, the combination of a photocatalyst and microalgae may be a potential technology for degrading antibiotics.

Our previous studies [32,33] have mainly focused on the removal rate of a single antibiotic using microalgae alone or a combination of $BiVO_4$ and microalgae. However, in this study, the monoclinic scheelite $BiVO_4$ and microalgae *Dictyosphaerium* sp. were selected, and SD and SM2 mixed SAs were explored for the first time. In our previous study, different antibiotic classes had significantly different removal efficiencies even at the same concentration level [32]. Microalgae have different sensitivities to different antibiotics, and generally many antibiotics exist in the water environment at the same time. Therefore, we chose the mixture of SD and SM2 as the experimental object to observe whether the combined technology of catalyst and microalgae can improve the degradation efficiency

of different antibiotics. The main objectives are to (1) evaluate the removal ability of the photocatalyst and microalgae combination technology toward antibiotics based on the removal kinetics of a SD and SM2 mixture and the change in the amount of reactive oxygen species (ROS), and (2) illustrate the degradation mechanism of antibiotics and the key role of microalgae by investigating the changes in the inorganic ion (nitrogen and sulfur) contents in the environment. This study provides a cost-effective way to remove antibiotic mixtures using visible light in an aquatic environment, thereby providing theoretical and technical support for the treatment of environmental pollution caused by multi-antibiotics. Moreover, it may provide new insights in terms of the bio-removal of other antibiotic contaminants in aquatic systems.

2. Materials and Methods

2.1. Chemicals

All the chemicals used in this study were of analytical grade, and special grade chemicals are stated otherwise in the text. SD and SM2 were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) with >98% purity (Table S1). $Bi(NO_3)_3{\cdot}5H_2O$ and NH_4VO_3 were purchased from Aladdin (Shanghai, China).

2.2. Algal Culture

The freshwater micro-green algae *Dictyosphaerium* sp. (FACHB-1902) were purchased from the Institute of the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-Collection), Wuhan City, China, and was cultured in BG-11 medium [34] within a homeothermic incubator at 25 ± 1 °C under 4000 lux illuminations with a light–dark period of 12:12 h. To retain the exponential growth phase, the algae were aseptically transferred to fresh media every 3–4 d and shaken three times per day [35].

2.3. Experimental Design

All experiments were performed in a homeothermic incubator at 25 ± 1 °C under 4000 lux illuminations with a light–dark period of 12:12 h. The $BiVO_4$ photocatalyst was prepared as per the method described in our previous study [33] (Figure S2). The stock solution of SAs was obtained by dissolving 25 mg of SD and 25 mg of SM2 into 500 mL of BG-11 medium. We used NaOH solution to dissolve SAs, and after the SAs were completely dissolved, we used HCl to adjust the pH of the test medium to 7. The $BiVO_4$ stock suspension was prepared using SAs stock solution. Prior to illumination, the suspension was magnetically stirred in the dark for 1 h to ensure the adsorption/desorption equilibrium of the SAs with the photocatalyst [18]. The final concentrations of $BiVO_4$ prepared in 100 mL of 5 mg/L SAs were 0.5, 1, and 2 g/L. It is necessary to dilute a certain volume of the algae culture with BG-11 medium to achieve an initial algae cell biomass of 80 mg/L. In short, the experiment was conducted in 250 mL Erlenmeyer flasks containing 100 mL of the test solution comprised of SAs, $BiVO_4$, and algae or no algae for 7 d under the same conditions used for the inoculum culture. Three treatment groups (0.5 g/L $BiVO_4$–algae, 1 g/L $BiVO_4$–algae, and 2 g/L $BiVO_4$–algae) and four control groups (algae, 0.5 g/L $BiVO_4$, 1 g/L $BiVO_4$, and 2 g/L $BiVO_4$) were tested in triplicate (n = 3). The flasks were gently shaken, and their positions were randomized and changed every 24 h to ensure the uniform distribution of light [36]. Before the start of the experiment, we carried out algae removal experiments of the two antibiotics under the same experimental conditions, taking the natural degradation of the antibiotics as a control, and found that *Dictyosphaerium* sp. could improve the removal rates of the two antibiotics [37] (Figure S1).

2.4. Determination of the Antibiotic Concentration

The concentration of antibiotics in the environmental solution was measured every 24 h. The concentrations of SD and SM2 were measured using ultra performance liquid chromatography mass spectrometry (UPLC-MS/MS). Before being used for UPLC-MS/MS analysis, the supernatants of the samples were decanted and filtered through a 0.22 μm

polytetrafluoroethylene filter within 7 d. A CORTES UPLC C_{18} column (100 × 2.1 mm, 1.6 μm) was used for the separation at a constant flow rate of 0.3 mL/min. The auto-sampler draws 2 μL of each sample. The mobile phase was composed of methanol (LCMS-grade) and an aqueous solution containing 0.1% formic acid (LCMS-grade). All experiments used Waters Xevo TQ-S triple-quadrupole mass spectrometry (MS) in electrospray-positive (ESI^+) mode and multiple reactive ion monitoring mode for qualitative analysis.

The removal rate (η) of antibiotics in different treatment groups was calculated using the following equation:

$$\eta(\%) = \frac{(C_0 - C_t)}{C_0} \times 100\%$$

where C_0 (mg/L) is the initial concentration of antibiotics at time 0, and C_t (mg/L) is the concentration of antibiotics at time t.

2.5. Determination of the Algal Autofluorescence Intensity

On days 3 and 7 of the experiment, a drop of the algae suspension was taken from the sample with algae and placed on a glass slide, after which the slide was immediately observed and photographed under a confocal laser scanning microscope (CLSM, Leica SP8, Shanghai, China), and the confocal system was controlled using Lasersharp software [38]. The samples were placed on an CLSM platform to focus on the most luminous scanning plane of the chloroplast autofluorescence. The excitation wavelength was 588 nm, the receiving range of the emitted light was set at 411–695 nm, and the pinhole size was 1 AiryUnit. The scanning resolution (frame size) was 1024 × 1024, the scanning speed was 3.15 μs/pixel and the averaging number was 2. The algal autofluorescence intensity was analyzed digitally using Lasersharp software (Bio-Rad Laboratories, Hercules, CA, USA).

2.6. Biochemical Indicators Measurements

2.6.1. Determination of the Superoxide ($\bullet O_2^-$) and Hydroxyl ($\bullet$OH)

The concentrations of the $\bullet O_2^-$ and $\bullet$OH radicals in the environmental solution were measured every 24 h using an ultraviolet–visible spectrophotometer (Unico UV-2800, Shanghai, China). The contents of $\bullet O_2^-$ were determined by measuring the nitrite formed from hydroxylamine in the presence of $\bullet O_2^-$ using the method described in a previous study by our group [39] with some modification. A total of 1.5 mL filtered sample was mixed with 0.5 mL of hydroxylamine hydrochloride (10 mmol/L) and incubated at 25 °C for 20 min. The supernatant (2 mL) was harvested by centrifugation at 3500 rpm for 10 min (Eppendorf, Centrifuge 5804R). Then, p-aminobenzene sulfonic acid (17 mmol/L) and α-naphthylamine (7 mmol/L) were added to the supernatant, which was incubated at 25 °C for 20 min. The absorbance of the aqueous solution at 530 nm was measured. A total of 1.5 mL of the filtered sample was mixed with 1.5 mL of MB solution (40 mol/L) in a centrifuge tube to react for 5 min in order to test the $\bullet$OH content [40]. The optical density was measured at 660 nm. The concentration of MB consumed in the reaction was the $\bullet$OH concentration captured by MB.

2.6.2. Determination of the Nitrogen and Sulfur Contents

In order to explore changes in the inorganic ions in the environmental solution, the contents of dissolved inorganic nitrogen and sulfur in the culture medium were measured on days 3 and 7 of the experiment according to the Chinese Water Analysis Methods Standard [41]. Briefly, ammoniacal–nitrogen (NH_4^+-N) was determined using Nessler's reagent spectrophotometry, nitrate–nitrogen (NO_3^--N) was determined using the zinc-cadmium reduction method, and sulfate (SO_4^{2-}-S) was determined using the barium chromate method.

2.7. Statistical Analysis

The differences in the SAs removal rate, algal autofluorescence intensity, ROS ($\bullet O_2^-$ and $\bullet$OH) contents, and inorganic ion (NH_4^+-N, NO_3^--N and SO_4^{2-}-S) contents between

the different groups were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics for Windows (version 18, Chicago, IL, USA). Prior to the one-way ANOVA test, normal distribution of data and homogeneity of variance were checked using Shapiro–Wilk tests and Levene's test, respectively. Pairwise comparisons were made using the post hoc analysis of Fisher's least significant difference (LSD) test. The difference was considered significant at $p < 0.05$. All figures were drawn using Origin 2017 software (OriginLab, Northampton, MA, USA), and the data shown in the figures are mean ± standard deviation.

3. Results

3.1. Synergistic Effect of $BiVO_4$ and Microalgae on the Degradation of SAs

The removal rate of SD and SM2 by the $BiVO_4$ and microalgae systems was investigated for 7 d (Figure 1). For SD, all treatment groups exhibited a relatively high removal rate on day 1, and then slowly increased (Figure 1a). The removal rates of the $BiVO_4$–algae group were significantly higher than those of the algae group by >70% ($p < 0.05$); when the $BiVO_4$–algae and $BiVO_4$ groups were compared, at the same $BiVO_4$ concentration, the addition of algae can significantly increase the removal rate ($p < 0.05$); namely, the maximum SD removal rates were from 66% (0.5 g/L $BiVO_4$) to 96.5% (0.5 g/L $BiVO_4$–algae), from 82% (1 g/L $BiVO_4$) to 99.2% (1 g/L $BiVO_4$–algae), and from 97% (2 g/L $BiVO_4$) to 99.2% (2 g/L $BiVO_4$–algae). Evidently, the combined effect of low concentrations (0.5 g/L and 1 g/L) of $BiVO_4$ and microalgae can reach or exceed the effect of a high concentration (2 g/L) of pure $BiVO_4$.

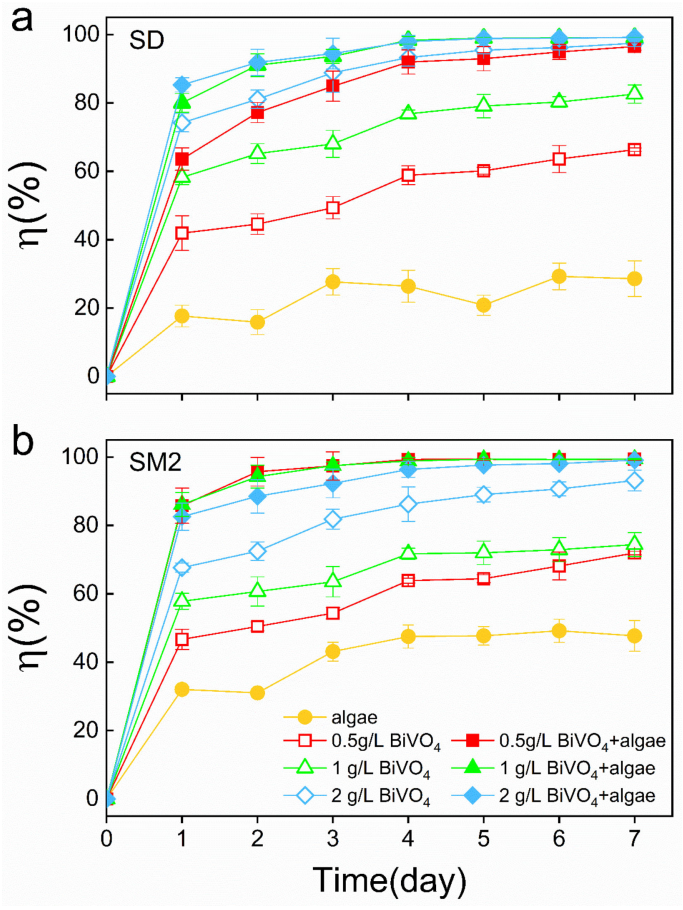

Figure 1. Effect of the treatment group ($BiVO_4$–algae) and control groups (algae and $BiVO_4$) on the removal of SD (**a**) and SM2 (**b**) during the experiment.

For SM2, the overall removal trend within 7 d was similar to SD (Figure 1b). Unlike SD, the 0.5 g/L $BiVO_4$–algae group exhibited the best SM2 removal effect, whose maximum η value was 99.4%. Moreover, the removal rates observed in the $BiVO_4$–algae group were all higher than those in the $BiVO_4$ group ($p < 0.05$). In addition, the combined removal effect of SM2 by $BiVO_4$ and microalgae was better than that of SD (Figure 1).

3.2. Effects of $BiVO_4$ on the Algal Photosynthetic System

When compared with the control group (algae), the autofluorescence intensity of chloroplast in *Dictyosphaerium* sp. increased when exposed to 0.5, 1, and 2 g/L $BiVO_4$ on days 3 and 7 (Figure 2). With the extension of the culture time, the chloroplast autofluorescence intensity of the microalgae was observed to be higher on day 7 when compared to day 3. In the three treatment groups, the autofluorescence intensity of chloroplasts was the lowest at 2 g/L $BiVO_4$.

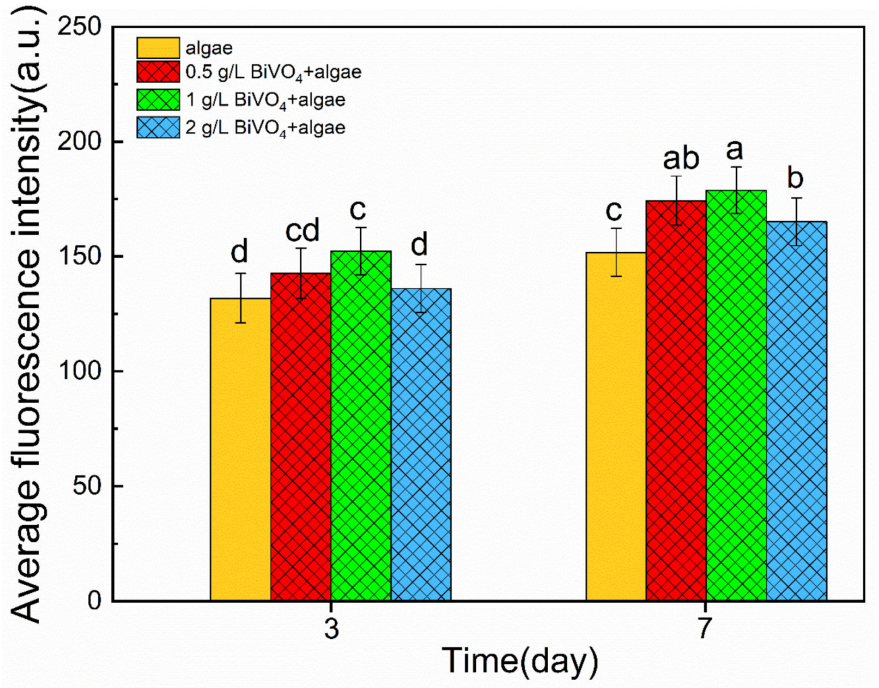

Figure 2. Effect of the treatment group ($BiVO_4$–algae) and control group (algae) on average autofluorescence intensity of *Dictyosphaerium* sp. on days 3 and 7 of the experiment. Identical letters denote no significant difference ($p < 0.05$).

3.3. Changes in the $\bullet O_2^-$ and $\bullet$OH Radical Contents

The $\bullet O_2^-$ contents in all of the treatment groups ($BiVO_4$–algae) were lower when compared with the control group (algae) and significantly higher than those in the other control group ($BiVO_4$) ($p < 0.05$) (Figure 3a). In the $BiVO_4$–algae group, the $\bullet O_2^-$ concentration first decreased and then increased, and as the concentration of $BiVO_4$ increased, the $\bullet O_2^-$ concentration basically does not change. Similarly, in the $BiVO_4$ group, while increasing the concentration of $BiVO_4$, the concentration of $\bullet O_2^-$ remained essentially unchanged over 7 d.

When compared to the algae group, the $\bullet$OH contents in all of the $BiVO_4$–algae groups and $BiVO_4$ group increased (Figure 3b). There was no significant difference in the $\bullet$OH concentration between these two groups with different concentrations of $BiVO_4$. In addition, two peaks were observed during the experiment, one peak appeared from day 2 to 3, and the other appeared from day 5 to 7. In other words, the $\bullet$OH concentrations at day 4 were relatively low.

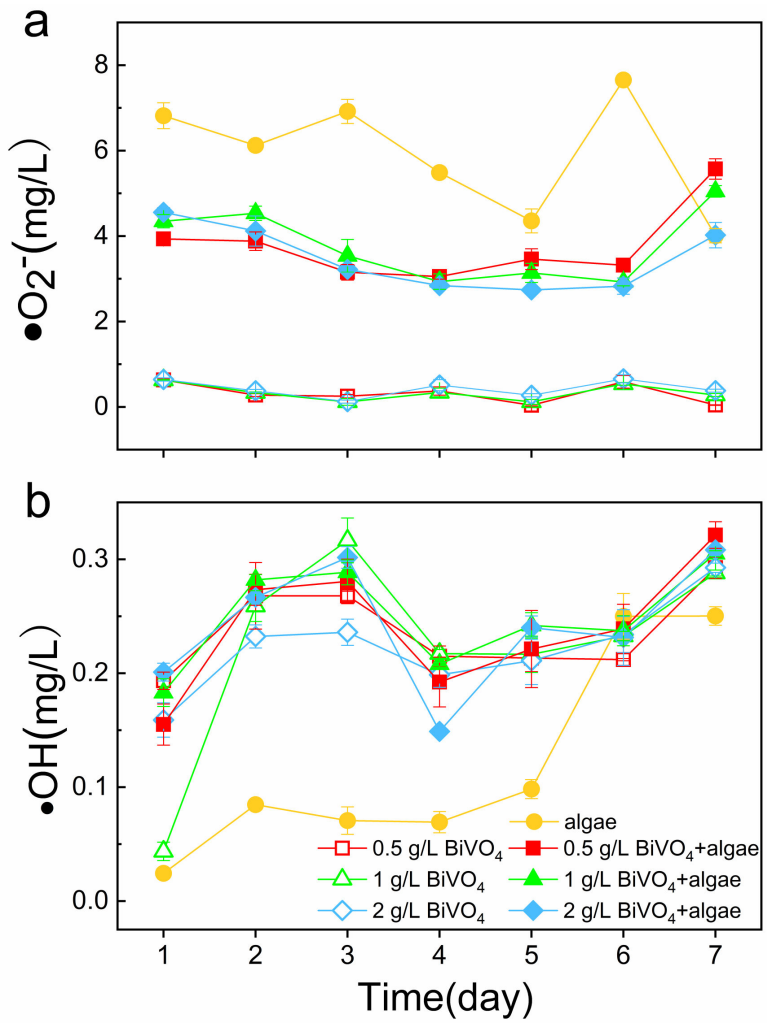

Figure 3. Changes of $\bullet O_2^-$ (**a**) and •OH (**b**) contents in the treatment group ($BiVO_4$-algae) and control groups (algae and $BiVO_4$) during the experiment.

3.4. Changes in the Nitrogen and Sulfur Contents

On days 3 and 7, when compared with the algae group, the NH_4^+-N concentrations of the $BiVO_4$–algae group increased; when compared with the $BiVO_4$ group, the NH_4^+-N concentrations of the $BiVO_4$–algae group decreased (Figure 4a). The NH_4^+-N concentrations of the $BiVO_4$–algae group were significantly lower than those of the $BiVO_4$ group and significantly higher than those of the algae group on day 7. The NH_4^+-N concentrations of the algae group decreased slightly on day 7 when compared to day 3. However, the concentrations of NH_4^+-N in the $BiVO_4$ group and $BiVO_4$–algae group were significantly higher on day 7 than on day 3.

The NO_3^--N contents in all of the $BiVO_4$–algae group increased significantly on days 3 and 7 when compared with the algae group, and the contents on day 7 were significantly higher than those on day 3 (Figure 4b). In addition, on day 7, the highest NO_3^--N content was observed in the 0.5 g/L $BiVO_4$–algae group. On day 3 or day 7, there was no significant difference in NO_3^--N contents between the $BiVO_4$–algae group and $BiVO_4$ group. However, the NO_3^--N contents in the same group were significantly different between these two days.

Figure 4c shows that the SO_4^{2-}-S contents in solution hardly change in the algae and $BiVO_4$–algae groups on days 3 and 7. However, the SO_4^{2-}-S contents of the $BiVO_4$ group on day 7 were significantly higher than those on day 3. In addition, from day 3 to 7, the SO_4^{2-}-S contents changed from a difference to no difference between the $BiVO_4$–algae and $BiVO_4$ groups.

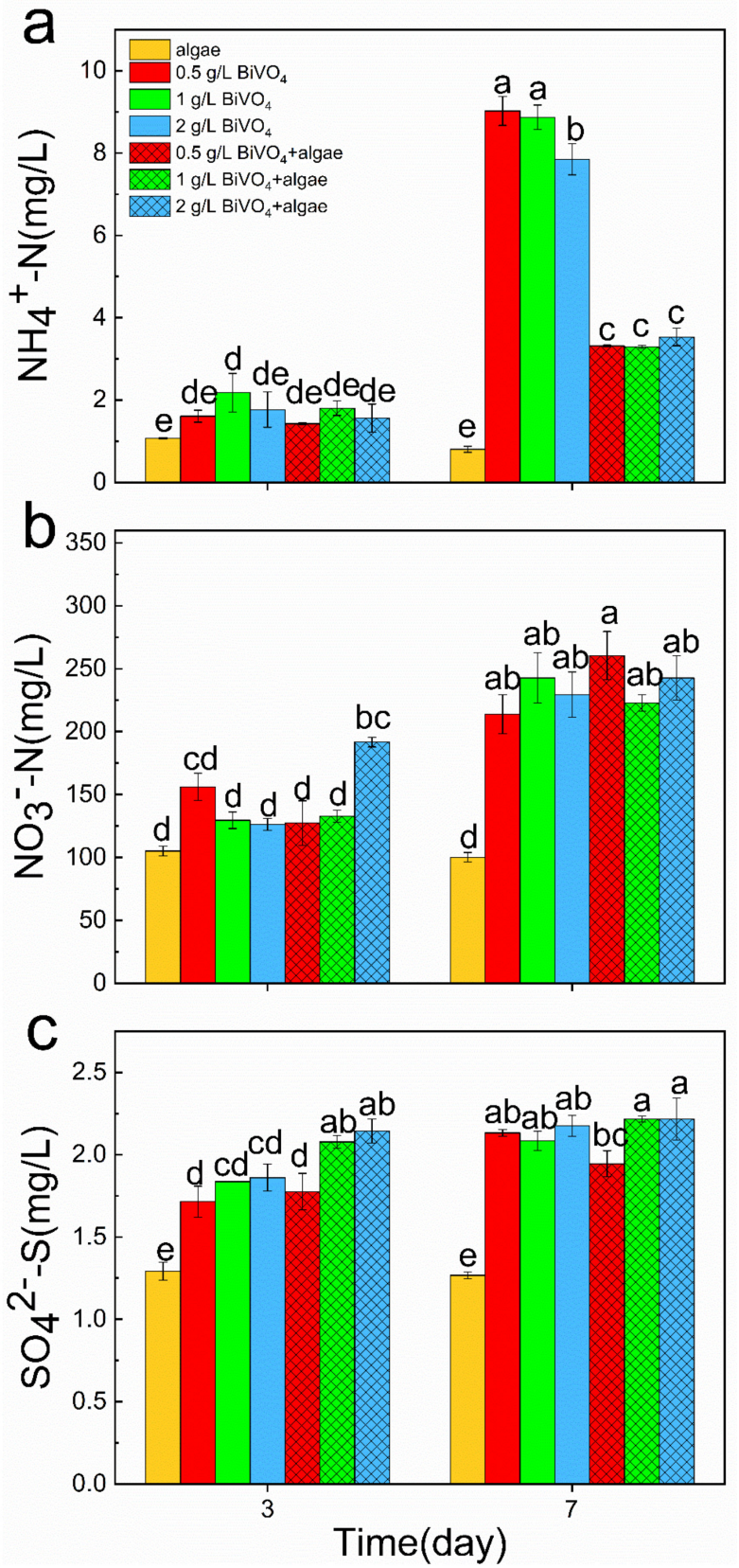

Figure 4. Changes of NH_4^+-N (**a**), NO_3^--N (**b**), and SO_4^{2-}-S (**c**) contents in the treatment group ($BiVO_4$-algae) and control groups (algae and $BiVO_4$) during the experiment. Identical letters denote no significant difference (p 0.05).

4. Discussion

Generally, the degradation of organic pollutants catalyzed by a semiconductor is an oxidative process using either the direct oxidizing power of photogenerated holes or indirectly via oxidation by ROS [42]. ROS can convert chemically stable organic pollutants into simpler non-toxic products [43]. A photocatalyst can use light as energy to generate strong oxidizing ROS to degrade organic pollutants. For example, $BiVO_4$ mainly triggers the photocatalytic degradation of ibuprofen by generating $\bullet O_2^-$ and •OH [18]. In this study, the $BiVO_4$ groups exhibit high concentrations of •OH; thus, SAs degradation was achieved through their reaction with •OH, which has also been confirmed by Yan et al. [44] and Wang et al. [45]. However, excessive use of $BiVO_4$ will cause catalyst accumulation [18]. It may be that aggregation reduces the contact area between $BiVO_4$ and light; thus, the ROS contents of the high-concentration (2 g/L) $BiVO_4$ group were lower than those of the low-concentration (0.5 g/L) $BiVO_4$ group (Figure 3).

The $BiVO_4$–algae group can efficiently remove antibiotics not only because $BiVO_4$ can produce ROS, but because the algae can also produce ROS [46]. There are studies showing that microalgae can produce a large amount of ROS under stress, which can oxidatively degrade antibiotics and other pollutants [47,48]. For example, Xue et al. [49] reported that the main oxidation species observed in the degradation of rhodamine B is $\bullet O_2^-$. In addition, Zhang et al. [50] showed that the main oxidative species observed during the degradation of phenol are holes and $\bullet O_2^-$. The addition of algae increased the $\bullet O_2^-$ content and the total amount of ROS in the water column, thereby greatly improving the SAs removal rate. In addition, the main components of the algae extracellular polymer are protein and polysaccharide [51]. Microalgae can absorb pollutants via glycoproteins formed from a combination of protein and polysaccharide in the cell wall to produce the effect of removing antibiotics in water [52]. The removal of antibiotics using algae is an indirect photodegradation process that relies on active oxidants produced by photosensitizers, such as extracellular organic matter (EOM) [53]. Therefore, we speculate that the exposure of antibiotics can stimulate algal cells to continuously secrete ROS, extracellular polymer, and EOM in order to degrade pollutants.

With the addition of $BiVO_4$ and *Dictyosphaerium* sp., more SAs are degraded into inorganic compounds such as NH_4^+-N, NO_3^--N, and SO_4^{2-}-S under the stress of more ROS. However, NH_4^+-N is also one of the most important pollutants found in water bodies, which has a significant impact on the community structure of zooplankton [54]; concentrations of >1.2 mg/L will cause *Daphnia magna* and *Daphnia similoides* to delay sexual maturation, reduce reproduction rate, and even die [55–57]. In this study, the NH_4^+-N content of the $BiVO_4$ group on day 7 could reach >8 mg/L (Figure 4). It can be seen that the use of photocatalytic materials alone to remove antibiotics may bring secondary hazards. However, when algae were added, the NH_4^+-N contents in the $BiVO_4$–algae group were significantly lower than those in the $BiVO_4$ group. This may be because algae use NH_4^+-N as their own nutrient source [58], which can reduce the secondary harm caused by the degradation of SAs. Simultaneously, the NO_3^--N contents in the treatment group on day 7 were significantly higher than those on day 3, indicating that more antibiotics were degraded into inorganic ions. In addition, the SO_4^{2-}-S contents in the treatment group showed no significant difference between days 3 and 7, indicating that the microalgae also slowly utilize the SO_4^{2-}-S degraded by SAs after using the SO_4^{2-}-S in the BG-11 medium.

In order to explore whether $BiVO_4$ has an adverse effect on algae cells, we measured the changes in the autofluorescence intensity of algae. The autofluorescence intensities of the $BiVO_4$–algae group were higher than those of the algae group (Figure 2), indicating that the growth state of microalgae was not inhibited but improved after adding $BiVO_4$. This may be because when $BiVO_4$ was added, more SAs were degraded into algal nutrients, which accelerated the growth of algae and increased the autofluorescence intensity. Moreover, in our previous studies, it was also demonstrated that glucose metabolism in microalgae can efficiently biodegrade antibiotics in the $BiVO_4$–microalgae system [30]. In addition, we found that the degradation efficiencies of SAs by 0.5 g/L $BiVO_4$–algae were better than

those using 2 g/L $BiVO_4$. The preparation cost of $BiVO_4$ is higher than the cultivation cost of microalgae. In this situation, using microalgae to replace parts of the $BiVO_4$ used to remove antibiotics cannot only surpass the effect achieved by high-concentration $BiVO_4$, but it is also economical and environmentally friendly.

5. Conclusions

This study showed that a combined 0.5 g/L $BiVO_4$ and *Dictyosphaerium* sp. treatment can remove 96% of SD and 99% of SM2, and the removal effect was higher than that of 2 g/L pure $BiVO_4$ and significantly higher than that of pure algae. The increase in the photocatalytic degradation rate of SAs was due to the increase in the contents of $\bullet O_2^-$ and •OH radicals after the combination of the photocatalyst and microalgae. Moreover, the degradation products of antibiotics (i.e., inorganic ions) can also be absorbed by microalgae to promote their own growth, thereby reducing the toxicity of antibiotic by-products. The economic cost of the low-concentration photocatalyst and microalgae was lower than that of the high-concentration photocatalyst, and the detoxification ability was stronger than that of the algae or high-concentration photocatalyst alone. Therefore, this study has illustrated that under visible light irradiation, the use of a low-concentration photocatalyst and freshwater microalgae for the photocatalytic degradation of SD and SM2 mixtures has application prospects. However, the subsequent recovery process of the photocatalyst and the reuse efficiency of the combination of the photocatalyst and microalgae still require further investigation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14050718/s1, Figure S1: Effect of algae on the removal of SAs under vis-light irradiation; Figure S2: TEM image of BiVO4; Table S1: Physicochemical characteristics of the test pharmaceuticals.

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