



# **The Effect of Light on Nitrogen Removal by Microalgae-Bacteria Symbiosis System (MBS)**

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**Abstract:** The littering of nitrogen into water bodies has led to several adverse effects on the environment. "Nitrification-denitrification" is still a prevalent method for removing nitrogen from water bodies, which demands high energy consumption and complex operational conditions. In recent years, MBS has attracted much attention because of its advantages in recovering nitrogen, emitting oxygen, and capturing CO<sub>2</sub>. It has been proven that light is the top factor influencing the performance of MBS. This paper will critically review the effects of light parameters on nitrogen removal by MBS, and the nitrogen-removal mechanisms of MBS driven by artificial illumination.

Keywords: bacterial-algal symbiosis systems; light; nitrogen removal; water treatment



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## 1. Introduction

Nitrogen is not only one of the main elements causing eutrophication in water bodies but also a major component of proteins [1]. Microalgae are considered an ideal method for nitrogen pollution control in water bodies due to their high efficiency in nitrogen absorption and high protein content [2]. However, the process of harvesting and separating microalgae from water bodies is challenging. Due to its efficient sedimentation, MBS has become a potential solution for nitrogen removal and protein recovery [3].

The applications of MBS in water treatment can be traced back to 1952, with highefficiency algal-bacterial ponds as the most popular application [4]. Because of the poor settling performance of bacterial-algal flocculant and the difficulty of separating it from the water, MBS technology fell into a period of low development, and it was not until 2012 that it began to develop rapidly after it was discovered that bacterial-algal symbiotic floc could achieve high settling ability by controlling the bacterial-algal ratio [5,6]. In the past few years, MBS had made remarkable advancements in improving nitrogen-removal efficiency for various water bodies, including but not limited to domestic sewage [7], pig farm wastewater [8,9], and aquaculture water [10,11].

Light serves as an ecological element that is crucial for the growth and development of algae and fungi, acting as a source of energy for photosynthesis. Using sunlight as an energy source, microalgae make use of  $NH_4^+$ ,  $PO_4^{3-}$ , and  $CO_3^{2-}$  as nutrients to synthesize algae cell matter, releasing oxygen for bacteria to continue to oxidize organic matter [12]. MBS not only removes nutrients from water but also reduces the footprint, which is of great significance for environmental improvement [13]. The influence of light wavelength and intensity on photosynthesis is particularly prominent [14]. For example, Akizuki et al. (2020) studied ammonium removal efficiency by a consortium of microalgae (*Chlorella sorokiniana*) and partial nitrifying granules under different light intensities, and found that the removal efficiency of ammonia nitrogen decreased from 66% to -10% when the light intensity increased from 0 to 1600 mmol photons m<sup>-2</sup> s<sup>-1</sup> [15]. However, there are also research cases that use LED lighting to enhance the growth rate of microalgae [16]. Under suitable light conditions, higher algal biomass will be produced, but overly fast biomass growth and condensation of microalgae may hinder photon penetration, resulting in inefficient use of light. Furthermore, higher algal biomass produces more dissolved oxygen (DO), which affects nitrogen metabolism. Presently, most research is focused on finding suitable operating conditions for MBS to remove pollutants [11,17], with few studies focusing on the effect of light on MBS. In this paper, the influence of light on the MBS is discussed, including the effects of light on nitrogen-removal efficiency, nitrogen-removal pathways, nitrogen-removal mechanisms and interactions, and prospects, to provide new ideas for water environmental governance and sustainable development.

## 2. The Effect of Light on Nitrogen-Removal Efficiency in MBS

## 2.1. Effect of Light Wavelength on Nitrogen-Removal Efficiency of MBS

Microalgae are capable of utilizing spectra that are predominantly located in the visible spectrum, which can be categorized into seven distinct colors based on their respective wavelengths, i.e., violet (380–450 nm), blue (450–485 nm), cyan (485–500 nm), green (500–565 nm), yellow (565–590 nm), orange (590–625 nm), and red (625–740 nm). The absorption of different spectrums of light varies greatly between microalgae of different categories due to the formation of their unique photosynthetic pigments and complementary pigment complexes [18]. In Table 1, Zhang et al. (2022) summarized the types of photosynthetic pigments and their spectral absorption characteristics in different microalgae.

Table 1. Absorption characteristics of photosynthetic pigments in algae [18].

Pigment	Algae	Absorption Spectra/nm
Chlorophyll a	All algae	436, 670~690
Chlorophyll b	Chlamydomonas, green algae, Euglena, and diatoms	455, 650~660
Chlorophyll c	Heterokonts, excluding diatoms	442~444,630
Chlorophyll d	Cyanobacteria (blue-green algae) and red algae	380, 440, 700~720
Chlorophyll f	Cyanobacteria (blue-green algae)	700~760
Carotenoids	All algae	420~470
Lutein	All algae	410~500 (540)
Dhucogugania	Dinoflacellates Distance Red alcos	610~635 (PC)
Рпусосуатт	Dinonagenaies, Diatoms, Ked algae	495~560 (PE)

Note: PC means Phycocyanin, and PE means Phycoerythrin.

The pigment composition varies between microalgae, indicating the ability of algae to utilize the light of different spectral compositions for photosynthesis. This, in turn, results in variation in algal biomass, lipids, pigment (Carotenoid), and in the removal efficiency of nutrients from wastewater by MBS. Various studies have also compared the effects of red, yellow, green, blue, and white light on microalgae and the effects of monochromatic and mixed light on microalgae growth, lipid accumulation, and Carotenoid composition [19]. For example, compared to white, blue, purple, and green light, the nitrogen-removal efficiency of Chlorella supplemented with red light was significantly increased [20]. Table 2 summarizes some of the studies on the effects of light color on nitrogen-removal efficiency in the MBS.

Inoculum	Inoculum Sewage Source		Light Quality	Initial Ammonia Nitrogen Concentration (mg-N/L)	Total Ammonia Nitrogen-Removal Rate (%)	Reference
Algae, bacteria	Poultry processing wastewater	LED, Fluorescent lamp	Red light White light	200.00	$\begin{array}{c} 45.70 \pm 2.00 \\ 36.20 \pm 0.70 \end{array}$	[21]
Cyanobacteria, green algae, Activated sludge	High-strength synthetic wastewater containing ammonia	LED	Blue light Red light Cold white light Natural white light	800.00	$53.00 \pm 2.00$ $41.00 \pm 3.71$ $51.00 \pm 3.00$ $50.00 \pm 3.00$	[22]
C. vulgaris, bacteria	Synthetic domestic sewage	LED	Red light white light Yellow light Purple light Blue light Green light	184.00	$\begin{array}{c} 75.08 \pm 3.65 \\ 71.36 \pm 2.63 \\ 67.59 \pm 1.45 \\ 49.42 \pm 1.78 \\ 47.37 \pm 2.64 \\ 29.63 \pm 1.72 \end{array}$	[23]
Spirulina platensis	Artificial urban wastewater	Artificial light source	Blue light Red light Purple light White light	37.00	18.00 56.00 68.00 60.00	[24]
C. vulgaris	Synthetic high-carbon wastewater	LED	Red light White light Yellow light Blue light	$53.82\pm7.21$	$\begin{array}{c} 76.04 \pm 8.39 \\ 61.31 \pm 5.79 \\ 35.72 \pm 4.06 \\ 17.35 \pm 3.92 \end{array}$	[25]
Chlorella sp.	Biogas slurry	LED	Red light White light Yellow light	$51.34 \pm 1.85$	$54.94 \pm 2.09$ $49.32 \pm 3.61$ $46.64 \pm 3.57$ $41.52 \pm 4.05$	[26]
Microalgal mixture, Active sludge	Synthetic wastewater	LED	Blue light Green light	$15.10\pm3.10$	$41.55 \pm 4.05$ $68.00 \pm 1.00$ 60.00	[27]
Algal-Bacterial Consortia	Real domestic sewage	LED	Blue light Green light Red light White light	$31.80\pm3.40$	$56.90 \pm 2.50$ $60.40 \pm 1.60$ $88.30 \pm 0.70$ $79.00 \pm 2.00$	[28]

	Table 2.	Effect of light	color on nitrogen	-removal efficiency	of the MBS.
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As can be seen from Table 2, the removal efficiency of ammonia nitrogen varies with light wavelength, and the efficiency of ammonia nitrogen removal varies widely between experiments. For example, studies on the nitrogen-removal efficiency of *C. vulgaris* with monochromatic and white lights found that the nitrogen-removal efficiency was higher with blue light compared to white, red, and green light [29]. Kang et al. [28] found that with different wavelengths of light-emitting diodes (LEDs) in culturing MBS in domestic wastewater, TKN removal rates were 93.9  $\pm$  0.5%, 91.8  $\pm$  2.3%, 58.8  $\pm$  2.7%, and 61.0  $\pm$  1.8% under blue, green, red, and white light irradiation, respectively. Another study noted that microalgae consumed more nitrate under blue light, which may be because nitrate and phosphorus reductases of microalgae are activated under blue light irradiation, increasing rates of nutrient uptake [30]. However, a different study also found that there was no significant difference in nitrogen removal under cool white light, natural white light, and blue light [31]. Additionally, the use of a specific ratio of mixed lights had better nutrient removal than white light [22].

Based on existing studies, it can be concluded that light wavelength affects the nitrogenremoval efficiency of MBS. Red and blue light is more conducive to nitrogen removal by microalgae because of the diversity of photosynthetic pigments contained in microalgae. The absorption spectra of these pigments and their complexes differ among different microalgae, resulting in varying ranges of light absorption and utilization. However, all microalgae contain chlorophyll a, with red and blue light being the main wavelengths that support photosynthesis effectively [32].

#### 2.2. Effect of Light Intensity on Nitrogen-Removal Efficiency of MBS

Light intensity is another factor affecting the productivity of microalgae [33,34], and changes in light intensity have a direct impact on algal photosynthesis, the organic removal of bacterial cells, and nitrification [35]. It is generally accepted that the optimal light intensity provides high photosynthetic photon fluxes for the photosynthetic pigments of microalgae, which in turn affects the uptake of carbon and nitrogen by cells [36]. In order

to evaluate the effect of light intensity on  $NH_4^+$ -N removal, Table 3 summarizes the effect of different light intensities on ammonia nitrogen removal by the MBS.

Inoculum	Sewage Source	Light Source	Light Intensity (µmol/m²/s)	The Initial Concentration of Ammonia Nitrogen (mg-N/L)	Total Ammonia Nitrogen-Removal Rate (%)	Reference
Chlorella sp., Chlamydomonas, Stichococcus	Digested pig manure	White fluorescent lamp	74.5 105.0	$301.00 \pm 16.00$	$\begin{array}{c} 65.00 \pm 6.00 \\ 93.00 \pm 2.00 \end{array}$	[37]
Chlorella sorokiniana, Activated sludge	Synthetic wastewater containing ammonia	LED	0.0 100.0 450.0 1600.0	43.00	66.40 61.60 5.20 -10.00	[15]
C. vulgaris	Synthetic high-carbon wastewater	LED	500.0 1000.0 1500.0 2000.0 2500.0	53.82 ± 7.21	$\begin{array}{c} 12.00\pm 1.00\\ 54.00\pm 1.00\\ 64.00\pm 1.00\\ 75.00\pm 1.00\\ 80.00\pm 1.00\\ 100\\ 100\\ 100\\ 100\\ 100\\ 100\\ 100$	[25]
<i>Microalgae, bacteria</i> (including Cyanobacteria)	The effluent of an AnMBR pilot plant	Fluorescent lamps	45.0 85.0 125.0	57.40 ± 2.20	$14.00 \pm 1.00 97.20 \pm 2.30 99.90 \pm 0.20 99.50 \pm 0.20 \\90.50 \pm 0.20 \\$	[38]
Chlorella sorokiniana, nitrifying bacterial culture	Synthetic wastewater	Warm white lamp	$\begin{array}{c} 0.0 \\ 150.0 \\ 500.0 \\ 1500.0 \\ 2000.0 \end{array}$	100.00	$7.20 \\ 100.00 \\ 100.00 \\ 6.20 \\ -2.20$	[39]
C. vulgaris	Simulating domestic sewage	Fluorescent light	36.0 60.0 120.0 180.0	250.00	$\begin{array}{c} 42.30 \pm 1.60 \\ 53.60 \pm 1.00 \\ 76.40 \pm 4.00 \\ 86.20 \pm 1.70 \end{array}$	[40]
Chlorella sp.	Biogas slurry	LED	800 1200 1600 2000	$51.34 \pm 1.85$	$\begin{array}{c} 44.12 \pm 2.40 \\ 51.03 \pm 2.23 \\ 53.81 \pm 1.96 \\ 54.94 \pm 2.09 \end{array}$	[26]

 Table 3. Nitrogen removal in bacteria–algae symbiotic system under different light intensities.

From Table 3, it can be found that the removal efficiency of ammonia nitrogen is higher with higher light intensity in a certain range. Compared with low light intensity, higher light intensity is beneficial to the growth of algae. Under different light intensities (36, 60, 120, and 180 µmol/m<sup>2</sup>/s), the nitrogen-removal efficiency was found to increase with increasing light intensity, using *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina*, and *Microcystis aeruginosa* as the test objects [40]. Although higher light intensities are beneficial to algal growth, nitrification inhibition should not be overlooked. Nitrifying bacteria are sensitive to extreme light, which can lead to nitrification failure [39]. Generally, low-intensity light is beneficial for the secretion of extracellular polymers, promoting the formation of algal particles, but high light intensity can cause photoinhibition of microalgae and nitrifying bacteria, leading to the accumulation of ammonia nitrogen and nitrite [41]. The research indicated that MBS was 40% more effective at removing ammonia nitrogen at a light intensity of 1000 Lx compared to 2500 Lx [22]; however, some studies had also found that low light intensity was more advantageous for the removal of ammonia nitrogen [42].

#### 2.3. Effect of Photoperiod on the Efficiency of Nitrogen Removal in MBS

Eukaryotic microalgae in nature have evolved circadian rhythms to regulate metabolic and physiological activities during optimal phases of the light–dark cycle. Continuous light has been reported to cause photoinhibition, while dark periods (including short-term dark periods) can help restore damaged photosystems [43]. Table 4 compares the effects of different photoperiods on the nitrogen-removal efficiency of the MBS in selected studies.

Inoculum	Sewage Source	Light Source	Photoperiod	Initial Ammonia Nitrogen Concentration (mg-N/L)	Total Ammonia Nitrogen-Removal Rate (%)	Reference
<i>Scenedesmus</i> sp. activated sludge	urban sewage	LED	12:12 12:60	$40.6\pm1.3$	$\begin{array}{c} 75.40 \pm 3.80 \\ 54.50 \pm 1.80 \end{array}$	[44]
P. subcapitata	Simulated municipal wastewater	fluorescent light	10:14 14:10 24:0	250.0	$43.50 \pm 0.70$ $74.40 \pm 2.90$ $88.00 \pm 2.70$	[40]
Cyanobacteria, green algae, Activated sludge	Concentrated wastewater with high ammonium content	LED	24:0 16:8 2:1	800.0	$65.00 \pm 5.00$	[22]
Microalgae, Activated sludge	Municipal wastewater	Fluorescent lamp	12:12 12:36 12:60	39.5	87.85 64.56 35.19	[33]
Microbial aggregates (microalgae, bacteria, and other microorganisms)	Municipal wastewater	Fluorescent lamp	12:12 18:6	$41.6\pm11.7$	99.74 99.66	[45]
Chlorophyta, Trebouxiophyceae, Bacillariophyceae, activated sludge	Synthetic wastewater	warm white light	0:24 12:12	200.0	96.90 99.00	[46]

#### Table 4. Effect of photoperiod on the nitrogen-removal efficiency of MBS.

In Table 4, most experimental results show that the total ammonia nitrogen-removal rate of the MBS increased with longer light exposure under suitable light intensity. Typically, a photoperiod of 12 h and above is very beneficial to the growth of microalgae by providing sufficient light for biomass production and nitrogen-removal efficiency. However, as stated previously, longer photoperiods may trigger photoinhibition effects, while shorter photoperiods can lead to light-limiting effects, both of which can cause a decrease in ammonia nitrogen-removal efficiency.

Because algae and bacteria are the most important subjects in MBS, light affecting the nitrogen-removal ability of these subjects will indirectly affect the nitrogen removal of the whole system. It has been demonstrated that dark periods between short flashes of light can enhance the photosynthetic efficiency of algae, particularly at high light intensities. One study that evaluated the effects of four different microalgal strains, including *Chlorella vulgaris, Pseudokirchneriella subcapitata, Synechocystis salina,* and *Microcystis aeruginosa,* on nutrient removal under culture conditions with different light–dark cycles (10:14, 14:10, and 24:0 h) [40] showed that nitrogen-removal efficiency improved with longer light cycles. Similarly, one study also found that  $NH_3^+$ -N removal by photosynthetic bacteria increased with the duration of the light–dark cycle, from 20% in the 6 h cycle to 48% in the 24 h cycle [47].

#### 3. The Effect of Light on the Nitrogen-Removal Pathway

The interactions between bacteria and algae in the MBS result in multiple nutrientremoval pathways in the system. Throughout MBS, oxygen produced by microalgae exposed to light is beneficial in nitrification by nitrifying bacteria [37]. The anoxic zone created by the depletion of oxygen in the reactor under dark conditions contributes to nitrogen removal [48]. Nitrogen removal in MBS can be carried out through nitrificationnitrogen removal by bacteria and assimilation by algae. A summary of various ammonia nitrogen-removal pathways under different light conditions is provided in Table 5.

Inoculum		Initial Ammonia wage Source Nitrogen Concentration	Light Source -	Light Intensity			Ammonia Nitrogen-Removal Rate Pathway		
	Sewage Source			(Lx)	(µmol/m <sup>2</sup> /s)	Photoperiod	Nitrification	Microalgae Assimilation	Reference
Chlorella sorokiniana, Mixed bacterial cultures	Pretreated pigmanure	60 150 290 650	Fluorescent lamps	10,000.0	180.0	24	0.00 7.00 23.00 8.00	100.0 93.00 77.00 92.00	[49]
Chlorella sorokiniana Activated sludge	Synthetic wastewater	100	LED	-	135.0	-	61.00	39.00	[15]
S. quadricauda Activated sludge	BG-11 medium improves artificial wastewater	50	Warm white light	-	60.0	-	80.00	20.00	[50]
Microalgae Aerobic sludge	Synthetic wastewater	1400	Artificial light source	-	67.5	-	60.00	40.00	[51]
Microbiota (microalgae, bacteria, and other microorganisms)	Municipal wastewater	$41.6\pm17.1$	Fluorescent lamps	-	45.0	12:12 18:6	72.00 83.00	28.00 17.00	[45]
Chlorella spp. Chlamydomonas, Stichococcus,	Treated pig manure	$301\pm16$	Fluorescent lamps	-	$74.0\pm5.0$	-	85.05	14.95	[37]

Table 5. Removal	l pathways	of nitrogen und	er different light	conditions.
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Note: "-" indicates that the data are not available.

In MBS, ammonia removal usually occurs through two different mechanisms: nitrification/nitrogen removal by microorganisms and assimilation by microalgae [52]. As shown in Table 5, at high light intensities, ammonia nitrogen is mainly removed through assimilation by microalgae, while at low light intensities or at longer light times, ammonia nitrogen removal is dominated by nitrification. In most experiments with synthetic wastewater as the substrate, the main pathway for nitrogen-pollutant removal using the MBS was nitrification, with algal assimilation playing a smaller role [53]. However, in experiments with treated pig manure as substrate a different situation emerged in which nitrification or algal assimilation predominated, respectively. High concentrations of microalgal biomass lead to excessive production of  $O_2$  by microalgae, which may affect the nitrification of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) because of the build-up of free ammonia and reactive oxygen species; on the contrary, a low concentration of microalgal biomass may lead to an insufficient supply of  $O_2$ , which also limits the nitrification efficiency of AOB and NOB [15].

On the one hand, under certain conditions, microbial nitrification/nitrogen removal is the main pathway for nitrogen removal in MBS, and only a small fraction of nitrogen is removed by algal assimilation [54]. On the other hand, algae can remove nitrogen from water through photosynthesis by assimilating inorganic ions such as  $NH_4^+$ ,  $NO_3^-$ , etc. from wastewater. Algae can also assimilate organic matter such as urea to a carbon skeleton to form algal cells. If nitrification by nitrifying bacteria is inhibited, algae play a dominant role in the removal of  $NH_4^+$ -N [55]. For example, Gao et al. [14] found that  $NH_4^+$ -N is readily assimilated by C. vulgaris because  $NH_4^+$ -N can be directly transported into algal cells for compound synthesis (e.g., proteins, nucleic acids, and phospholipids) [56]. The main pathways of nitrogen removal by MBS differ under light conditions, and the mechanisms involved in the removal of  $NH_4^+$ -N from the MBS by controlling light conditions are still not clear in available studies.

### 4. The Effect of Light on the Mechanism of Nitrogen Removal in the MBS

Under certain conditions, light intensity is the key to controlling the mechanism of  $NH_4^+$ -N removal by MBS [57]. Bacteria and algae are the two main constituents of MBS. Aiming to understand the role of light in the nitrogen-removal processes of MBS, we first need to investigate the effects of light on algae and bacteria. Figure 1 depicts the effect of light on the physiological processes of the symbiotic system.



Figure 1. Effect of light on the mechanism of nitrogen removal in an MBS [42].

#### 4.1. Effect of Light on the Physiological Mechanism of Microalgae

As photosynthetic organisms, algae cannot live without light. In recent years, the emergence of light-emitting diodes (LEDs) has pushed the research on the effects of light on the photosynthesis of microalgae to the forefront. The light irradiation of specific wavelengths of LEDs not only changes the photosystem ratio and bile body arrangement of algal cells, which results in changes in photosynthetic efficiency, but also affects the growth rate of cells, which in turn affects population dynamics and community composition. For example, blue LED light could stimulate the protein synthesis of the microalga *Isochrysis*, red LED light was beneficial to higher productivity of fatty acids, and green LED light was conducive to the highest relative growth rates in *G. lemaneiformis* [58]. This shows that the effects of light quality on algae are far-reaching [16].

The equilibrium of photosynthetic electron production and utilization between PSII and PSI in algal cells is influenced by light intensity. Compared to low light intensity, high light intensity has a more prominent inhibitory effect on PSII [59]. Light intensity also affects the photosynthesis and metabolism of algae by influencing the activity of related enzymes. Rubisco plays a pivotal role in the Calvin cycle of photosynthesis [60], while citrate synthase is identified as the primary enzyme of the TCA cycle in organic carbon metabolism [61]. Gao et al. [14] investigated the activities of Rubisco and citrate synthase under different light intensities. The results showed that appropriate light intensity improved the fixation of carbon monoxide in photosynthesis, while excessive light supply led to a decrease in citrate synthase activity [62].

#### 4.2. Effect on Nitrogen-Removal Functional Microorganisms

Photosynthetic bacteria (PSB) and nitrifying bacteria are two functional groups of bacteria that play a crucial role in nitrogen cycling in MBS. First, PSB contains photosynthetic pigments, bacteriochlorophyll (BChl) and carotenoids (CD), and grows only with CO<sub>2</sub> as a carbon source. Enhanced growth of photosynthetic *Pseudomonas Syringae* was found under blue light-emitting diodes (LED-Blue) at approximately 470 nm wavelengths [63]. Similarly, Govarthanan et al. [64] studied the growth of *Erythrobacter* spp. BT18 at different wavelengths of light, demonstrating that the growth of *Erythrobacter* spp. was optimized under blue light at 470 nm, and the order of bacterial growth was blue light > white light > green light > red light > yellow light > no light.

Second, light wavelength and intensity affect the performance of NOB due to the photosensitivity characteristics of their synthesis of cytochrome c [22]. In contrast, AOB

can synthesize oxidative stress enzymes to mitigate the damage of light on cells [65]. NOB is more susceptible to the effects of light than AOB [66]. In experiments investigating the differences in photoinhibition of ammonium oxidation in oceanic bacteria and archaea, it was noted that under continuous illumination at light intensities of 500  $\mu$ mol/m<sup>2</sup>/s, photoinhibition occurred in AOB and (ammonium-oxidizing archaea) AOA, with the inhibition of NOB by light radiation significantly higher than that of AOB [15].

Regarding the causes of photoinhibition in nitrifying bacteria, Hyman and Arp [67] suggest photodamage of key intracellular proteins, such as disruption of polypeptides in ammonia monooxygenase metabolism and cytochrome-c associated with the electron transport chain. Zhang et al. [61] reported that the nitrification activity of the MBS was enhanced at a light intensity of 1600  $\mu$ mol/m<sup>2</sup>/s due to the presence of AOB in the form of partially nitrifying particles.

#### 5. The Effect of Light on the Interaction of the MBS

Currently, few studies have focused on understanding the underlying mechanisms associated with algal–bacterial interactions, particularly those related to the formation and interaction of mycorrhizal symbioses, such as light wavelength, light intensity, and light–dark cycles [68,69]. The interactions in MBS regulate a variety of physiological processes and ecological functions, and an in-depth understanding of the interactions between bacteria and algae can help gain insight into water treatment mechanisms of MBS.

#### 5.1. Extracellular Polymeric Substances

Extracellular Polymeric Substances (EPS) can be divided into bound EPS (B-EPS) and soluble EPS (S-EPS). S-EPS are produced by microbial metabolism and autolysis and are uniformly distributed in the aqueous phase, while B-EPS often appear as capsules [70]. Figure 2 depicts the relationship between the MBS and extracellular polymers.



Figure 2. Relationship between the bacteria-algae symbiotic system and EPS [42].

Light affects EPS production in MBS. Many reports have indicated that EPS production in MBS improves with increasing light intensity [71]. It was found that LB-EPS and TB-EPS in granular sludge enhanced significantly with increasing light intensity at low, medium, and high light intensities (0  $\mu$ mol/m<sup>2</sup>/s, 142 (±10)  $\mu$ mol/m<sup>2</sup>/s, and 316 (±12)  $\mu$ mol/m<sup>2</sup>/s) [72]. The composition of C-Phycocyanin(C-PC), EPS, and biomass was also affected by light [73]. Low light favors the production of C-PC, while high light

favors the production of EPS, and blue light at 380–470 nm is more favorable for enhanced cell growth activity [74].

## 5.2. Physical Effects

The physical effect of light on MBS is mainly reflected in the size of bacteria and algae particles and their settling properties. Dahalan et al. [75] verified the binding mechanisms of mycorrhizal algal particles including the characteristics of the algal cell surface and the number of cations that contribute to the formation of aggregates with good settling ability; environmental factors such as light also affect the colony formation and buoyancy of *Microcystis aeruginosa* [76]. The physicochemical characteristics of MBS particles developed under various light intensities [72]. Biomass concentrations were higher at high and low light intensities compared to no light, i.e., the biomass of the mycorrhizal algal symbiotic particles grew with increasing light intensity. Similarly, He et al. [77] found that a light intensity of 200  $\mu$ mol/m<sup>2</sup>/s could efficiently promote protein formation in the MBS, forming a mutually beneficial MBS particle, and improving sludge settling performance. However, it was also found that the acceleration of biomass production in high light intensities may lead to mutual shading within the community, reducing the photosynthetic efficiency and leading to the lysis of some algal cells inside the granules, decreasing the settling performance of the bacteria–algae granules [68].

## 6. Summary and Prospects

#### 6.1. Eco-Friendly Lighting Fixtures and Supplementary Lighting Techniques

Firstly, precise light supplementation should be carried out according to the light absorption characteristics of algae species, and it is worthwhile to develop lighting fixtures that are more energy-efficient. Secondly, it is important to focus on the basic research of using LED lighting to improve MBS nitrogen-removal efficiency and consider greener methods of supplementation, such as utilizing solar energy. Thirdly, we should pay more attention to new water treatment technologies based on MBS to enhance  $CO_2$  fixation and nitrogen recovery in water bodies, supporting global carbon neutrality.

#### 6.2. Nitrogen-Removal Kinetics of MBS Driven by LED Light

First, although has MBS gained rapid development in recent years, its kinetic foundation is still quite weak compared with traditional activated sludge technology, and further systematic experiments are needed to consolidate kinetic foundation data. Second, the nitrogen-removal pathway of MBS has yet to be resolved. The nitrogen-removal principles of algae symbiosis are different from those of the traditional activated sludge method, and it is not clear what kind of changes in the nitrogen-removal pathway will occur under different LED light conditions. Third, due to insufficient kinetic data, the research on MBS-based water treatment models is still limited at present. Developing effective MBS water-treatment models and using them to simulate and predict water-treatment performance would be of great significance for advancing water-treatment technology.

### 6.3. Interaction Mechanism of MBS under Targeted Light Irradiation

Firstly, for microalgal photoreceptors, research on targeted light supplementation is relatively limited, especially regarding the synergistic effect between photoreceptors, and targeted research on the molecular regulation mechanism and photosynthetic physiological function of microalgal photoreceptors is yet to be conducted. Secondly, the degree of inhibition and inhibition mechanisms of functional bacteria under targeted light supplementation need to be studied in depth, especially to understand the inhibition mechanisms of functional bacteria by single or multiple light wavelengths. Thirdly, the response of the algal–bacterial interaction mechanism under targeted light supplementation needs further study, including algal community sensing, electron transfer, and physicochemical interactions of the algal–bacterial interface. **Author Contributions:** Conceptualization, S.W., Y.Z. and G.F.; methodology, S.L.; software, X.D. and L.L.; investigation, Z.J. and C.Z.; writing—original draft preparation, Z.G.; writing—review and editing, S.W., Y.Z. and G.F. All authors have read and agreed to the published version of the manuscript.

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