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A Comparative Study of *Scenedesmus dimorphus* Cultured with Synthetic and Actual Wastewater

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Abstract: This study compared the growth of the microalgae *Scenedesmus dimorphus* in synthetic wastewater and actual wastewater under different cultivation conditions, in terms of nitrogen and phosphorus availability, wastewater quality, light condition and CO₂ addition. The results show that the form of nitrogen source had a significant effect on the growth of microalgae. Urea as a nitrogen source increased the growth rate of *S. dimorphus* significantly, while the high concentration of inorganic nitrogen inhibited the growth. When phosphate was 4 mg/L and pH was 7, the growth of *S. dimorphus* was the greatest. The bacteria in actual wastewater not only promote the growth of microalgae but also facilitate the formation of flocs, which is conducive to biomass harvest. With the increase in light intensity and light duration, *S. dimorphus* showed primarily an increasing and then a decreasing trend. Higher light intensity was required in actual wastewater than in synthetic wastewater, which may be due to the barrier effect of wastewater turbidity. *S. dimorphus* grew well in both kinds of wastewater with the addition of 2% CO₂.

Keywords: *Scenedesmus dimorphus*; synthetic wastewater; actual wastewater; nutrient source; light condition

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1. Introduction

Water scarcity and environmental degradation have caused widespread concern about wastewater treatment and disposal worldwide. Urban wastewater treatment technologies can effectively prevent aquatic pollutants produced by human activities from entering natural water bodies [1]. Common treatment processes can effectively remove organic matter and suspended solids (SS), but the removal effect of nitrogen and phosphorus is limited [1,2]. In addition, conventional technologies have several problems such as high operating costs, large floor space, and difficulty in sludge disposal, etc. [2,3]. Therefore, it is necessary to explore an efficient and environmentally friendly wastewater treatment technology to replace traditional technologies.

Microalgae are a kind of single-celled microorganism that exist widely in aquatic environments. Microalgae can utilize carbon, nitrogen, and phosphorus as the main nutrients to maintain their growth [4]. Compared with the traditional biological treatment process, the microalgae treatment process can reduce the concentration of organic matter, nitrogen, phosphorus, heavy metals, and other substances in wastewater [5–7]. Microalgae also capture and utilize carbon dioxide through photosynthesis [8]. Meanwhile, the microalgae biomass cultured in wastewater can be converted into animal feed [9], biodiesel [10,11], biological fertilizer [12], and other substances [13,14] for resource utilization. The application of microalgae in wastewater treatment can not only achieve the removal of nutrients but also recover biomass for resource utilization, which has promising application prospects in the future.

The growth of microalgae in wastewater is influenced by various biological and abiotic factors, such as nutrients, original microorganisms, light condition, and CO₂ concentration, etc. [15]. Previous studies have shown that there is a symbiotic relationship between

microalgae and bacteria, effectively facilitating the growth rate and nutrient removal efficiency of microalgae [16]. In the microalgae–bacteria symbiotic system of wastewater culture, the aerobic microorganisms can remove more than 60% COD, while the microalgae can provide O_2 (6 mg/L) through photosynthesis, and remove 40% nitrogen (NH_4^+-N) and more than 90% phosphorus [17]. The concentration of nitrogen and phosphorus and other nutrients in wastewater can affect the purification efficiency of microalgae and the recovery effect of microalgae biomass. Hulya and Ilgi [18] reported that the removal efficiency of nitrogen and phosphorus was mainly affected by the concentration of influent ammonia nitrogen. Arumugam et al. [19] found that *S. bijugatus* grew better under the concentration of 5–10 mM nitrogen than that of 15–20 mM. CO_2 and light intensity are also important factors to promote the efficiency of photosynthesis, so as to improve the uptake of nutrients by microalgae, and then increase the biomass accumulation [20]. Goncalves et al. [21] found that the growth and CO_2 fixation rates of four microalgae strains (i.e., *C. vulgaris*, *P. subcapitata*, *S. salina*, and *M. aeruginosa*) all showed a trend of first increasing and then decreasing under different concentrations of CO_2 . The optimum CO_2 concentration ranges between 4.87% and 5.62%, and the maximum biomass can reach 0.126 g dw/L·D. Shih-hsin et al. [22] found that with the increase in light intensity, the specific growth rate of *S. obliquus* primarily went up and then gradually stabilized, while biomass productivity and CO_2 fixation rate first increased to the maximum value and then decreased significantly. Under 420 $\mu mol/m^2 \cdot s$ light intensity, the maximum biomass yield and CO_2 fixation rate were 840.56 mg/L·d and 1435.90 mg/L·d, respectively. Lee, C. S. et al. [23] reported that the efficiency of nitrogen and phosphorus removal in a microalgae–heterotrophic bacteria system was negatively correlated with the dark duration. The chlorophyll a content and cell dry weight were the highest under a 12 h: 12 h light–dark photoperiod. Under the condition of a long period of light, the removal efficiency of nitrogen and phosphorus by microalgae and bacteria was high, and the biomass of microalgae reached the maximum. Therefore, it is necessary to investigate the effects of different cultural conditions on the growth of microalgae.

The objective of this study was to compare the effects of different culture conditions on the growth of *Scenedesmus dimorphus* in synthetic and actual wastewater. Using single-factor and uniform-design experiments, the growth of *S. dimorphus* in synthetic wastewater under different forms and concentrations of nitrogen and phosphorus sources was first investigated, and then the interaction between wastewater quality and bacteria on the growth of *S. dimorphus* in actual wastewater was investigated. Finally, the effects of light conditions and CO_2 on the cultivation of *S. dimorphus* were studied.

2. Materials and Methods

2.1. Microalgae Strain and Culture

The microalgae strain *Scenedesmus dimorphus* (FACHB-959) used in this study was purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (Wuhan, China, FACHB). *S. dimorphus* was cultured in a 3N-BBM + V medium (Bold Basal Medium with 3-fold Nitrogen and Vitamins; modified). Each liter of medium consists of the following: $NaNO_3$ 0.75 g, $CaCl_2 \cdot 2H_2O$ 0.025 g, $MgSO_4 \cdot 7H_2O$ 0.075 g, $K_2HPO_4 \cdot 3H_2O$ 0.075 g, KH_2PO_4 0.175 g, NaCl 0.025 g, trace element mixed liquor 6 mL, Vitamin B_1 (1.2 g/L) 1 mL, Vitamin B_{12} (1.0 g/L) 1 mL, and distilled water. The trace element mixed liquor contained: Na_2EDTA 0.75 g, $FeCl_3 \cdot 6H_2O$ 97 mg, $MnCl_2 \cdot 4H_2O$ 41 mg, $ZnCl_2 \cdot 6H_2O$ 5.0 mg, $CoCl_2 \cdot 6H_2O$ 2.0 mg, $Na_2MoO_4 \cdot 2H_2O$ 4.0 mg, and distilled water 1000 mL. The strain was incubated in a 250 mL conical flask in a light incubator at 20 °C and exposed to a weak light condition (~1000 lux, 12 h light/12 h dark). The strain was transferred every 2–3 months in a sterile environment. The enlargement conditions were at 25 ± 1 °C and a continuous light of 2000–4000 lux for a transfer cycle of 7–14 d. The flasks were shaken regularly 2–3 times a day to replenish the carbon dioxide needed for growth.

The formula of synthetic wastewater used for this study is shown in Table 1. The water quality parameters of the synthetic wastewater are shown in Table 2.

Table 1. The formula of synthetic wastewater.

Medium Composition	Consumption
soluble starch (g)	0.10
urea (g)	0.15
MgSO ₄ ·7H ₂ O (g)	0.075
(NH ₄) ₂ SO ₄ (g)	0.075
NaHCO ₃ (g)	0.30
K ₂ HPO ₄ ·2H ₂ O (mg/L)	0.05
FeCl ₃ (1%) (mL)	0.15
milk powder (g)	0.2
tap water (mL)	1000

Table 2. The water quality parameters of the synthetic wastewater.

Water Quality Index	Concentration
pH	7.4–8.0
SS (mg/L)	98
COD (mg/L)	340–400
NH ₄ ⁺ -N (mg/L)	10–15
TN (mg/L)	70–80
TP (mg/L)	5–7

The actual municipal wastewater was obtained from the Shenzhen Buji wastewater treatment plant in Guangdong, China. The primary sedimentation tank (PT) wastewater was taken from the grit chamber and settled for 30 min; the secondary sedimentation tank (ST) wastewater was taken directly from the outlet of the secondary sedimentation tank. The main characteristics of PT wastewater and ST wastewater are shown in Table 3.

Table 3. The main characteristics of PT wastewater and ST wastewater.

Water Quality Index	Concentration in PT Wastewater	Concentration in ST Wastewater
pH	7.8	7.9
SS (mg/L)	122	34
COD (mg/L)	188	45.6
NH ₄ ⁺ -N (mg/L)	46.5	23.2
NO ₃ ⁻ -N (mg/L)	0.8	7.3
NO ₂ ⁻ -N (mg/L)	0.375	0
TP (mg/L)	2.77	2

2.2. Analytical Methods

The growth of microalgae was expressed in terms of optical density and chlorophyll a content, depending on the effluent quality used in this study. The optical density was measured at 680 nm by the spectrophotometer (OD₆₈₀), and the growth curve of microalgae was expressed as its optical density versus time. Microalgal growth could also be estimated using the specific growth rate, as shown in Equation (1).

$$\mu = \ln(N_2/N_1)/t \quad (1)$$

where μ is the specific growth rate, N_1 and N_2 are the initial and final OD₆₈₀ value, and t is the cultivation time (d).

Chlorophyll a content was determined by the hot ethanol extraction method [24]. Briefly, approximately 10 mL of the sample was extracted through a 0.45 μ m microporous membrane and then frozen at -20 °C for 12 h. Subsequently, 7 mL of pre-warmed 90% ethanol was added and extracted for 2 min in a water bath at 80 °C. The extract was shaken with ultrasound for 10 min and set in the dark for 4–6 h, filtered and then diluted to 25 mL. The absorbance values at 665 nm and 750 nm were measured with 90% ethanol

as a reference. After acidification with 1 mol/L hydrochloric acid, the extract was shaken adequately and stood for 15 min, and the absorbance values at 665 nm and 750 nm were read again. The chlorophyll a content was calculated as shown in Equation (2).

$$C = 27.9 \times V_m [(E_{665} - E_{750}) - (A_{665} - A_{750})]/V_s \quad (2)$$

where C is the chlorophyll a content (mg/m³), V_m is the ethanol volume (mL), V_s is the sample volume (L), E₆₆₅ and E₇₅₀ are the absorbance values at 665 nm and 750 nm after extraction, and A₆₆₅ and A₇₅₀ are the absorbance value at 665 nm and 750 nm after acidification.

2.3. Experimental Design

2.3.1. The Effect of Nutrients in Synthetic Wastewater

A series of batch tests were conducted to explore the effects of different nitrogen sources and concentrations on the growth of *S. dimorphus*. The sources of NH₄⁺-N, NO₃⁻-N, urea (CH₄N₂O), and mixed nitrogen were (NH₄)₂SO₄, NaNO₃, urea, and synthetic wastewater, respectively. Each was set at three nitrogen concentrations of 20 mg/L, 40 mg/L, and 85 mg/L. The initial pH of inoculation was 7, and the subsequent pH was no longer adjusted. The growth curve of *S. dimorphus* was measured for 8 days. In addition, OD₆₈₀ on day 4 and chlorophyll a content on day 3 were measured. Additionally, then a repeatable two-factor ANOVA was performed.

Since pH affects the phosphorus forms in the aquatic environment [25], the interaction between pH and phosphorus concentration needs to be explored. The effects of phosphorus concentration and pH on the growth of *S. dimorphus* were investigated by uniform experimental design. The experimental scheme is shown in Table 4. Phosphorus concentration was set at 2 mg/L, 4 mg/L, 6 mg/L, and 8 mg/L. The pH value was set to 4–11. 5% KOH and 10% CH₃COOH solution was used to adjust the pH value to the set value every 12 h. The growth curve of *S. dimorphus* was measured for 7 days. The cubic polynomial stepwise regression equation was fitted to the OD₆₈₀ of microalgae cultured for 4 days.

Table 4. The uniform design of the effects of phosphorus concentration and pH on *Scenedesmus dimorphus* growth.

Group	Phosphorus Concentration (mg/L)	pH
1	2.0	5.0
2	2.0	9.0
3	4.0	7.0
4	4.0	11.0
5	6.0	8.0
6	6.0	4.0
7	8.0	6.0
8	8.0	10.0

In the nutrient effects experiments, the light intensity was approximately 4000 lux for 24 h a day, and the culture temperature was 23.2 ± 2 °C. Each set of samples was performed in triplicate.

2.3.2. The Effect of Wastewater Quality and Bacteria in Actual Wastewater

To investigate the effect of actual wastewater quality and original bacteria in wastewater on the growth of *S. dimorphus*, the effluent from the primary sedimentation tank and the secondary sedimentation tank (i.e., PT and ST) were selected for microalgae culture. Additionally, the sterilized and unsterilized groups were set, respectively. The experimental groups were PTS (PT sterilized), PTU (PT, unsterilized), STS (ST, sterilized), and STU (ST, unsterilized). The light intensity was about 4000 lux, the daily light was 24 h, and the culture temperature was 23.2 ± 2 °C. Three replicates were set for each experimental

group. The growth curve of *S. dimorphus* culture for 9 days and the chlorophyll a contents of the microalgae at day 4 and day 7 were determined. A repeatable two-factor ANOVA was performed.

2.3.3. The Effect of Illumination Conditions

The effects of light intensity and the light–dark cycle on the growth of *S. dimorphus* in synthetic wastewater and actual wastewater were investigated through the uniform experimental design, respectively. The experimental scheme is shown in Table 5. Different lighting intensifiers were set via the number and installation height of fluorescent lamps, at 2500 lux, 5000 lux, 7500 lux, 10,000 lux, 12,500 lux, and 15,000 lux. The daily illumination period was automatically controlled by the timing controller and set to six levels: 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h. The culture temperature was 23.2 ± 2 °C. Each set of samples was performed in triplicate. The growth curve of *S. dimorphus* cultured for 8 days was measured. Considering the different growth conditions of microalgae in different wastewater qualities, the cubic polynomial stepwise regression equation fitting of OD₆₈₀ of microalgae cultured at day 3 in synthetic wastewater and of chlorophyll a content at day 4 in actual wastewater were conducted, respectively.

Table 5. The uniform design of the effect of light intensity and daily light period on *Scenedesmus dimorphus* growth.

Group	Light Intensity (lux)	Time
1	15,000	12
2	15,000	24
3	12,500	4
4	12,500	16
5	10,000	8
6	10,000	20
7	7500	20
8	7500	4
9	5000	16
10	5000	12
11	2500	24
12	2500	8

2.3.4. The Effect of CO₂ Conditions

In order to investigate the effect of CO₂ on microalgae growth, three groups of experiments were set, including air, air enriched with 2% CO₂ (0.004 v/v·min), and air enriched with 5% CO₂ (0.01 v/v·min). The air flow rate under various conditions was controlled at 300 mL/min, and the light intensity was about 6000 lux, with a daily illumination of 24 h. The culture temperature was set as 23.2 ± 2 °C. The growth curves of synthetic wastewater and actual wastewater cultured for five days were measured, respectively.

3. Results and Discussion

3.1. The Effect of Nutrients in Synthetic Wastewater on the Growth of *S. dimorphus*

The growth curves of *S. dimorphus* under different forms and concentrations of nitrogen in the synthetic wastewater are shown in Figure 1. After 3–4 days, the growth of most experimental groups of microalgae gradually slowed down and began to differentiate. Figure 2 displays the OD₆₈₀ on day 4 and chlorophyll a content on day 3. Different forms of nitrogen source had a significant effect on the growth of microalgae according to OD₆₈₀ ($p = 9.59 \times 10^{-6}$) and chlorophyll a content ($p = 1.31 \times 10^{-8}$). The highest specific growth rate was 0.432 d^{-1} at 20 mg/L of ammonia. Compared to ammonia and nitrate nitrogen, urea was more beneficial to the growth of *S. dimorphus*. The growth rate of *S. dimorphus* increased significantly when urea was used as a nitrogen source, while there was no significant difference in microalgal growth between the two inorganic nitrogen sources. This was similar to the findings of Campos et al. [26], who reported that *Nannochloropsis*

salina achieved the maximum cell density and maximum sustainable yield in urea as the single nitrogen source compared to NH_4Cl , NH_4OH , NaNO_3 , and mixed nitrogen medium. However, Li et al. [19] found that the specific growth rates of *Scenedesmus* sp. LX1 cultivated with different nitrogen sources were, in descending order, $\text{NH}_4^+\text{-N} > \text{urea} > \text{NO}_3^-\text{-N}$ when cultured with different substances as the nitrogen source. According to Arumugam et al., nitrate was the preferred nitrogen source form of *S. bijugatus*. This may be due to different microalgae strains using nitrogen forms with varying utilization efficiency. *S. dimorphus* grew best in the original synthetic wastewater, indicating that microalgae are better adapted to a mixed nitrogen source. This was similar to the findings of Mandal et al. [27], who found that the mean biomass yield of five microalgal strains cultivated in a mixed nitrogen source was 88% higher than a single species of microalgae.

Figure 1. Growth curves of *Scenedesmus dimorphus* under different forms and concentrations of nitrogen sources in synthetic wastewater ($n = 3$).

Figure 2. Growth of *Scenedesmus dimorphus* under different nitrogen sources and nitrogen concentrations: (a) OD_{680} ; (b) chlorophyll a content ($n = 3$).

The concentration of the nitrogen source also had a significant effect on microalgal growth according to OD_{680} ($p = 0.0015$) and chlorophyll a content ($p = 3.88 \times 10^{-5}$). The results show that different concentrations of urea had little effect on the growth of *S. dimorphus*. For groups that utilize inorganic nitrogen, the growth rate of microalgae slowed with the concentration of ammonia nitrogen and nitrate nitrogen increasing, in-

dicating that a high concentration of inorganic nitrogen can inhibit the growth. This may be due to the release of H^+ from NH_4^+ in the process of microalgae culture, which reduces the pH value of the aquatic environment and affects the growth of microalgae [28]. Arumugam et al. found that there was an interaction between the species and concentration of nitrogen sources on the growth of microalgae. When using urea, nitrate, and ammonium as nitrogen sources, the growth of microalgae showed no differences under low-concentration conditions [19].

The uniform experimental design results of *S. dimorphus* cultivation with different phosphorus sources and pH values are shown in Figure 3a. The results illustrate that the optimum growth of *S. dimorphus* was obtained when phosphate was 4 mg/L and pH was 7. The corresponding specific growth rate could reach 0.469 d^{-1} . Cubic polynomial stepwise regression equation fitting was performed for OD_{680} on day 4 (see Equation (3)), and the contour line fitting diagram is shown in Figure 3b.

$$Z = -4.990 + 1.197X + 1.067Y - 0.219X^2 - 0.0691Y^2 - 0.0079XY + 0.0128X^3 \quad (R^2 = 0.9910), \quad (3)$$

where Z is the OD_{680} , X is the phosphorus concentration (mg/L), Y is the pH, and R^2 is the determinate coefficient. The results show that the growth of *S. dimorphus* was significantly affected by pH. pH can change the permeability of cell membranes [29]. It can also change the morphology of phosphates, possibly forming brown deposits that inhibit microalgae photosynthesis [30]. The growth of *S. dimorphus* was inhibited under both acid and base conditions. The optimum pH for the growth of *S. dimorphus* is about 7.5. In the typical domestic wastewater phosphorus concentration range (2 mg/L–8 mg/L), the phosphorus concentration of about 4 mg/L is appropriate for microalgae growth. The increase in phosphorus concentration can inhibit the growth of algae. Sharma and Kumair reported that the dry weight of microalgae increased with a phosphate concentration increase from 0 to 250 mg/L, while the concentration of phosphate increased to 300 mg/L when the dry biomass decreased [31]. In addition, there was no significant interaction between phosphorus concentration and pH value.

Figure 3. (a) The uniform design results under different phosphorus sources and pH of the effect on the growth of *Scenedesmus dimorphus*. (b) The contour line fitting diagram of the effect on the growth of *Scenedesmus dimorphus* ($n = 3$).

3.2. The Effect of Wastewater Quality and Bacteria in Actual Wastewater on the Growth of *S. dimorphus*

Figure 4a shows the curves of OD_{680} over time of *S. dimorphus* cultivated in wastewater of different qualities (i.e., PTS, PTU, STS, and STU), respectively. The growth trends of microalgae were similar under four wastewater quality conditions. It should be noted that the OD_{680} of microalgae cultured in PTU was negative on day 2. During the experiment, it

was observed that the initial wastewater was turbid, which may be due to the presence of a large number of suspended organic substances in the primary sedimentation tank effluent. After a day of bacterial degradation, water quality became clearer, resulting in a decline in absorbance value. The phenomenon was repeated in subsequent experiments. Due to the different qualities of wastewater, the increase in absorbance in this experiment cannot fully reflect the growth of algae in the actual wastewater. Therefore, the chlorophyll a contents of *S. dimorphus* on day 4 and day 7 were determined (Figure 4b).

Figure 4. (a) Growth curve of *Scenedesmus dimorphus* under different wastewater qualities (i.e., PTS, PTU, STS, and STU) characterized by OD₆₈₀. (b) The chlorophyll a content of *Scenedesmus dimorphus* under different wastewater qualities at day 4 and day 7 ($n = 3$).

On day 4, the wastewater quality had a very significant impact on the growth of *S. dimorphus* ($p = 4.75 \times 10^{-7}$). The effluent from the PT was more suitable for the growth of microalgae than that from the ST, possibly because the nutrients in the PT were more abundant. This is consistent with the previous experimental results of nutrients in synthetic wastewater. Bacteria also had a very significant effect on the growth of algae ($p = 1.97 \times 10^{-5}$). This may be due to the interaction between the growth of bacteria and microalgae; bacteria can decompose organic nutrients into small molecular organic substances or inorganic substances that are conducive to the absorption of microalgae. Meanwhile, bacteria can degrade organic carbon in water and release CO₂, which can promote the photosynthesis of microalgae [32]. The synergistic cooperation between native bacteria and microalgae increased the biomass and improved the efficiency of nutrient removal [33,34]. Compared with the secondary sedimentation tank, the PTS and PTU were significantly different ($p = 5.18 \times 10^{-4}$), which indicated that the effluent from the primary sedimentation tank contained more bacteria and had a more obvious promoting effect on the growth of microalgae. On day 7, there was no significant effect on chlorophyll a content under four conditions. It may be that the nutrients in the effluent from the primary sedimentation tank have already been decomposed and absorbed, and the effects of other environmental factors on *S. dimorphus* have replaced the effects of water quality and sterilization as the limiting factors for the growth of algae. The decrease in the number of bacteria also had a reduced effect on the growth of *S. dimorphus*. In addition, we observed that *S. dimorphus* cultured with PTU could easily form flocculants due to the influence of bacteria, which is more conducive to biomass harvest and recovery. This is because bacteria can induce microalgae to precipitate by adhesion or secretion of extracellular secretions [35]. Therefore, the effluent from the primary sedimentation tank was selected for the cultivation of *S. dimorphus*, pointing out that the microalgae treatment of wastewater has some limitations: it is affected by operating conditions and wastewater type (types of pollutants).

3.3. The Effect of Illumination Conditions in Synthetic and Actual Wastewater on the Growth of *S. dimorphus*

The effects of light intensity and the light–dark cycle on the growth of *S. dimorphus* in synthetic and actual wastewater are shown in Figure 5, respectively. In the synthetic wastewater, *S. dimorphus* gradually reached the stable phase from day 2, and the growth slowed down from day 3. The time to reach the stable period of each experimental group was affected by the illumination intensity and light–dark cycle. Cubic polynomial stepwise regression equation fitting was performed for OD₆₈₀ of microalgae solution cultured for day 3 (see Equation (4)). The contour line fitting diagram was shown in Figure 6a.

$$Z = -1.069 + 0.216 + 0.155Y - 0.0175X^2 - 0.00859Y^2 - 0.00583XY + 0.000582X^3 + 0.000157Y^3 + 0.000145XY^2 \quad (4)$$

(R² = 0.9147)

where Z is the OD₆₈₀, X is the light intensity (10³ lux), Y is the daily light period (h), and R² is the determinate coefficient. As shown in Figure 6a, there is a complex interaction between light intensity and illumination period on the growth of *S. dimorphus*. Under the condition of low light intensity and low light duration, *S. dimorphus* grew slowly. With the increase in light intensity and daily light time, the growth of *S. dimorphus* showed an increasing trend. However, when the light time increased to 24 h at the light intensity of 15,000 lux, it was not conducive to the growth of microalgae compared with the 12 h group. A similar situation has been widely reported in previous studies. According to Li et al. [36], the biomass of *C. kessleri* increased gradually when the light intensity increased in the range of 0–120 μmol/m²·s. However, when the light intensity was further increased by 200 μmol/m²·s, the accumulation of biomass decreased, possibly due to photoinhibition.

Figure 5. The uniform design results of the effect under different light intensity and daily light time on the growth of *Scenedesmus dimorphus* in (a) Synthetic wastewater, (b) Actual wastewater. (*n* = 3).

The growth curve of *S. dimorphus* characterized by OD₆₈₀ displayed a continuous increasing trend in actual wastewater and began to differentiate on about day 4 (Figure 5b). Due to the influence of bacteria, suspended organic substances, etc., we chose the chlorophyll a content to characterize the microalgae biomass in actual wastewater. Cubic polynomial stepwise regression equation fitting was performed for chlorophyll a content for day 4 (see Equation (5)), and the contour line fitting diagram is shown in Figure 6b.

$$Z = -9686 + 1255X + 1513Y - 42.69Y^2 - 124.9XY - 2.221X^3 + 2.078XY^2 + 3.197X^2Y \quad (R^2 = 0.9875) \quad (5)$$

where Z is the chlorophyll a content (mg/m^3), X is the light intensity (10^3 lux), Y is the daily light period (h), and R^2 is the determinate coefficient. It can be seen from Figure 6b that in actual wastewater, there is a complex interaction between light intensity and the light cycle. Compared with synthetic wastewater, the optimal daily light period extended to about 15 h under the illumination intensity condition of 15,000 lux. Due to the complex composition and high turbidity in actual wastewater, the light exposure of microalgae is correspondingly reduced. Therefore, the optimum growth condition of *S. dimorphus* can be achieved by properly extending the daily light period in the actual wastewater condition.

Figure 6. The contour line fitting diagram of the effect of light intensity and daily light time on the growth of *Scenedesmus dimorphus*: (a) characterized by OD₆₈₀ in synthetic wastewater; (b) characterized by chlorophyll a content in actual wastewater ($n = 3$).

3.4. The Effect of CO₂ in Synthetic and Actual Wastewater on the Growth of *S. dimorphus*

The effects of CO₂ on the growth of *S. dimorphus* in synthetic wastewater and actual wastewater are shown in Figure 7. In synthetic wastewater, the growth of *S. dimorphus* was significantly promoted in comparison with the 2% CO₂ group, while the 5% CO₂ group showed no difference from the air group. The average specific growth rate was 0.683 in the 2% CO₂ group. As shown in Figure 7b, the growth of *S. dimorphus* in the CO₂-enriched group was much greater than that in the air group cultivated in actual wastewater. After 4–7 days of culture, the growth of microalgae in the 2% CO₂ group was the fastest. There was no significant difference between the growth rate of the 2% and 5% CO₂ groups ($p = 0.9206$). The specific growth rate was around 0.279–0.309 in these three groups. It can be found that the growth curve of *S. dimorphus* reached its peak when the air was enriched with 2% CO₂ in both kinds of wastewater. The final biomass characterized by OD₆₈₀ in actual wastewater was less than that in synthetic wastewater. Goncalves et al. [21] reported four microalgae strains that achieved the highest biomass yield (0.126 g dw/L·d) and carbon fixation rate (0.101 g c/L·d) with a range up to 10% CO₂. The optimum CO₂ concentration was calculated as 5.35%. Razzak et al. [37] also reported that the 4% CO₂ can recover the maximum biomass production in the cultivation of *Chlorella* sp. among 2–12% CO₂. This indicated that adding CO₂ of low concentration can greatly promote the growth of microalgae. The different optimum CO₂ concentrations may be due to the different microalgae species in the literature compared with this study.

Figure 7. Growth curve of *Scenedesmus dimorphus* under different CO₂ concentrations in (a) synthetic wastewater and (b) actual wastewater ($n = 3$).

4. Conclusions

From this study, the following conclusions can be made:

- (1) When urea was used as a nitrogen source, the growth of *S. dimorphus* was increased. The high concentration of inorganic nitrogen (ammonia nitrogen and nitrate nitrogen) could inhibit the growth of *S. dimorphus*.
- (2) The growth of *S. dimorphus* reached its highest when phosphate was 4 mg/L and the pH was 7.
- (3) The bacteria in actual sewage can promote the growth of microalgae and facilitate biomass harvest.
- (4) Under the condition of an illumination intensity of 15,000 Lux, when the optimal daily illumination time was about 15 h, the growth of *S. dimorphus* in actual sewage was optimum.
- (5) When adding 2% (0.004 v/v·min) CO₂, the growth of *S. dimorphus* was the best in both synthetic and actual wastewater.

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