Research Article

Campus Sewage Treatment by Golenkinia SDEC-16 and Biofuel Production under Monochromic Light

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The integration of microalgal cultivation in wastewater can fulfill the dual roles of pollutant degradation and biomass output. Meanwhile, the LED lights with different wavelengths have a great effect on the growth and metabolism of microalgae. In this study, Golenkinia SDEC-16, a strain isolated for biofuel production, was evaluated to verify its potentials for campus sewage treatment and lipid accumulation under the red, green, and blue lights. The results indicated that the treated campus sewage met the first grade level in the Chinese pollutant discharge standards for municipal wastewater treatment plants within seven days under both red and blue light. The green light failed to exhibit excellent performance in nutrient removal, but facilitated the lipid synthesis as high as 42.99 ± 3.48%. The increased lipid content was achieved along with low biomass accumulation owing to low effective light utilization, indicating that the green light could be merely used as a stimulus strategy. The red light benefited the photosynthesis of Golenkinia SDEC-16, with the maximal biomass concentration of 0.80 ± 0.03 g/L and lipid content of 36.90 ± 3.62%, which can attain the optimal balance between biomass production and lipid synthesis.

1. Introduction

Microalgae act as a powerful biotechnology platform to synthesize valuable products by recovering nutrients from the culture medium [1–4]. Although the idea of biofuel production by using microalgae is not new, it is still being taken seriously, owing to the probability to alleviate global warming and inhibit the escalating price of petroleum [5]. Moreover, the algal biodiesel is an eco-friendly option where microalgae can survive in wastewater and make use of the nutrients [1, 6]. The consumption of nutrients can reduce environmental impacts and fulfill the water reclamation. In addition, the introduced wastewater can lower the requirements of microalgae on additional nutrients and freshwater compared with the conventional biofuel feedstock [7]. The dual roles of wastewater treatment and microalgal cultivation are economical.

To date, a variety of wastewater types, such as brewery wastewater [4], livestock wastewater [8–10], aquaculture sewage [11], and municipal wastewater [12] have been successfully utilized for the microalgal production. Campus sewage is one type of municipal wastewater, and the volume of discharge is vast. Campus sewage contains sufficient nitrogen, phosphorus, and organic substances, which can support microalgae growth and exerts few adverse toxic effects [13]. Given that the diversity of microalgal species, the selection of appropriate species can promote nutrient removal. In addition, the responses to the changes in the culture conditions are dependent on the specific species [1]. There remain the contemporary challenges to effective wastewater treatment and high biomass output.

Previously, we discovered that the Golenkinia SDEC-16, under the general culture conditions, exhibited highly
effective nutrient removal, favorable biomass output, and easily harvesting [14]. What is more, there are few relevant studies concerning varying culture conditions in the field of wastewater treatment in comparison with the widely used strains. The follow-up work needs to be carried out to enhance lipid output and maintain the concurrent high nutrient removal efficiency by Golenkinia SDEC-16.

Regarding the culture conditions, the monochromic LED lights emerged as an effective strategy to regulate the microalgae, and an appropriate selection of light wavelengths can facilitate the rate of nutrient remediation in response to the different monochromatic lights. For instance, the red light was found to give the highest growth and nutrient removal in Chlorella vulgaris [15]. In contrast, the blue LED light plays an outstanding role in high biomass productivity and favorable fatty acid composition for biofuel production of the green microalgae [16].

Therefore, this motivated us to evaluate the potentials of Golenkinia SDEC-16 for campus sewage treatment and biofuel production under the red, green, and blue lights. Our study will shed light on the effects of the monochromatic light on the wastewaster treatment, biomass, lipids, and fatty acids composition distinctively.

2. Method

2.1. Microalgal Strain. The microalgal strain used in the present work was Golenkinia SDEC-16 (GeneBank accession number: KT180320) isolated from a lake in the Quancheng Park, Jinan, Shandong Province, China. Previous cultivations of Golenkinia SDEC-16 were performed in the photobioreactors with BG11 liquid medium at 25 ± 2°C to attain healthy seed cells for further studies.

2.2. Experimental Design. Flasks with 1 L of campus sewage filtered through six layers of gauze were employed to evaluate the characteristics of wastewater treatment under red, green, and blue lights. The flasks were placed under LED lights providing red (660 nm), green (520 nm), and blue (465 nm) with intensities of approximately 1,300 lux with a photoperiod (24:0 h of light: dark period) at 25 ± 2°C for seven days. Continuous air aeration (1.0 L/min) was provided from an aerator through glass tubes in flasks to prevent cell sedimentation. All of the experiments were conducted in triplicate.

2.3. Nutrient Measurements. Samples were collected every day and filtered by a 0.45 μm cellulose nitrate membrane for determining the wastewater nutrients by a UV/Vis spectrophotometer (U-3010, Hitachi Co., Japan) according to the Chinese state standard testing methods [17]. Regarding the total nitrogen (TN), 1 mL of the filtrated sample was digested for 30 mins by alkaline potassium persulfate solution under 121°C and the absorptions at wavelengths of 220 and 275 nm were measured for calculating nitrogen concentration. As for the ammonium, 1 mL of filtered samples was added with a potassium sodium tartrate solution and Nessler’s reagent, and then absorption at 420 nm was read. Regarding the total phosphorus (TP), 5 mL of the filtrated sample was digested by a potassium persulfate solution (5%) under 121°C, then added 1 mL ascorbic acid solution (10%) and 2 mL ammonium molybdate solution following by determining the absorptions at 700 nm. All the concentrations were calculated according to the calibration curve and the tested sample volume. The total organic carbon (TOC) was analyzed by a TOC/TN analyzer (TOC-VPCH, Shimadzu, Japan) with 10 mL of the filtrated sample.

The initial campus wastewater characteristics were as follows: TN 61.23 ± 4.77 mg/L, ammonium 56.38 ± 2.91 mg/L, TP 4.91 ± 0.08 mg/L, and TOC 187.47 ± 6.54 mg/L. The nutrient removal efficiency (NRE) (%) could be expressed as

\[ \text{NRE} = \left( \frac{X_t - X_0}{X_0} \right) \times 100\% \]  

where \( X_t \) and \( X_0 \) are the nutrient concentrations (mg/L) at the end and beginning of a batch run, respectively, and \( \Delta t \) (d) is the duration of the run.

Fluorescence excitation-emission matrices (EEMs) were acquired using a fluorescence spectrometry (5J2-004, Hitachi, Japan) with a 1 cm quartz cell to indicate organic composition. The spectra of filtrated samples were obtained by scanning the sample based on excitation wavelengths from 220 to 450 nm with 5 nm steps and emission wavelengths from 250 to 550 with 2 nm steps. Moreover, the scanning speed was set at 1,000 nm/min for all of the measurements.

2.4. Biomass Concentration Measurements. The microalgal growth was monitored daily by weighing the dry biomass. A 15 mL volume of microalgae solution was filtered by 0.45 μm membrane in order to remove the supernatant and then transferred to a clean and weighed aluminium foil pan, which was then dried to constant mass in a thermostatically controlled oven at 60°C for 24 h. The biomass productivity \( P_b \) (mg/L/day) and the specific growth rate \( \mu \) (d\(^{-1}\)) were calculated according to dry biomass by equations (2) and (3):

\[ P_b = \frac{X_m - X_0}{\Delta t}, \]  

\[ \mu = \frac{1}{\Delta t} \ln \left( \frac{X_m}{X_0} \right), \]

where \( X_m \) and \( X_0 \) are the concentrations of biomass (g/L) at the end and beginning of a batch run, respectively, and \( \Delta t \) (d) is the duration of the run.

2.5. Morphology Observation and Cell Size Measurements. Morphology of the algae strains that survived in campus sewage was observed under an optical microscope (CX31, OLYMPUS, Japan), and the microalgal diameters were
2.6. Lipid Content. The lipid content was measured by solvent extraction and gravimetric method based on a reported method [18]. The harvested microalgae were freeze-dried and then ground to an approximately uniform powder for lipid extraction. Approximately 0.1 g of dry biomass powder was added with 10 mL of chloroform-methanol solution (2:1, v/v) in a centrifuge tube. The mixture was treated by ultrasonication (Ultrasonic Cell Crusher SCIENTZ-IID, China) for 10 min and subsequently centrifuged at 2,000 g for 10 min at 4°C. The supernatant was transferred to a clean and dry 60 mL separatory funnel. The entire extraction process was repeated twice. After the extraction, 4 mL of sodium chloride solution (0.9%) was added into the separatory funnel to separate organic facies from hydrofacies. The mixture was shaken well for 1 min and then allowed to stand for 15 min to stratify. The volume of the lower organic phase solution was measured, and 5 mL of the solution was then transferred to a clean and weighed 10 mL glass tube. The solution in the glass tube was then evaporated under a nitrogen stream and dried in an oven at 60°C to constant mass. The lipid content (LC) was expressed as a fraction of the biomass’s dry mass and calculated based on equation (4):

\[
LC = \frac{(m_2 - m_1) \times V}{(5 \times m_0)} \tag{4}
\]

where \(m_0\) (g) is the dry biomass’s mass, \(m_1\) (g) and \(m_2\) (g) are the masses of the clean glass tube and the tube containing lipid, and \(V\) (mL) is the volume of the lower-phase.

Lipid productivity (LP, mg/L/d) was determined according to the following formula:

\[
LP = P_b \times LC, \tag{5}
\]

where \(P_b\) (mg/L/day) is the biomass productivity, LC (%) is the corresponding lipid content.

2.7. Fatty Acid Profile Analysis. The fatty acid composition was analyzed through the GC-MS method as described by [18]. The degree of unsaturation was calculated by fatty acid compositions using

\[
ADU = \sum P_i \times N_i \tag{6}
\]

where ADU is the average degree of unsaturation of microalgaie lipid, \(P_i\) is the mass fraction of each fatty acid component, and \(N_i\) is the associated number of carbon-carbon double bonds in each fatty acid component. Furthermore, the predicted properties of biodiesel could be calculated based on the ADU [18].

Kinematic viscosity = \(-0.6316\ \text{ADU} + 5.2065\), \(R^2 = 0.67\),

specific gravity = \(0.0055\ \text{ADU} + 0.8726\), \(R^2 = 0.66\),

cloud point = \(-13.356\ \text{ADU} + 19.994\), \(R^2 = 0.68\),
cetane number = \(-6.6684\ \text{ADU} + 62.876\), \(R^2 = 0.80\),
iiodine value = \(74.373\ \text{ADU} + 12.71\), \(R^2 = 0.94\),
higher heating value = \(1.7601\ \text{ADU} + 38.534\), \(R^2 = 0.38\). \tag{7}

3. Results

3.1. Effects of Specific Lights on Wastewater Treatment. Campus sewage is rich in nutrients (e.g., organic matter, nitrogen, and phosphorus), which would pose a threat to the environment if it was not well treated before discharging to the environment. Figure 1 quantitatively depicts the remediation of campus sewage by Golenkinia SDEC-16 under the different monochromic LED lights, in which the red and blue lights gave productive removal efficiencies of total nitrogen, ammonium, and total phosphorus. In particular, the red light exhibited the highest nutrient removal efficiency, with the lowest residues of \(7.77 \pm 0.98\) and \(3.31 \pm 0.85\) mg/L in total nitrogen and ammonium. Inferior to the red light, the total nitrogen and ammonium reduced to \(9.17 \pm 1.65\) and \(5.41 \pm 0.66\) mg/L under the blue light. In contrast, the green light exhibited a poor behavior of nitrogen removal, with the high residue values of \(19.13 \pm 1.35\) and \(16.73 \pm 0.4\) mg/L in total nitrogen and ammonium. Similar to the case of nitrogen degradation, the most effective total phosphorus removal was found upon exposure to the red and blue lights, with the values of \(0.39 \pm 0.06\) and \(0.11 \pm 0.01\) mg/L. The residue value of total phosphorus was \(1.93 \pm 0.13\) mg/L under the green light.

As depicted in Figure 2, the initial total organic concentration in campus sewage was \(187.47 \pm 6.54\) mg/L. The total organic carbon concentration fast decreased within the first two days, and the decreasing trend began to slow down and stabilized as time went on. The removal efficiencies achieved 43.46%, 40.71%, and 47.43%, corresponding to the red, green, and blue lights, respectively.

The present work also evaluated their makeup based on EEMs. The fluorescence EEMs of campus wastewater under the different monochromic LED lights manifests in Figure 2. The raw campus sewage has a peak in fluorescence at an Ex/Em pairing of around 280/340 nm, indicating the existence of protein-like substances [19]. As time went by, the fluorescence intensity of the substances weakened and moved its position on the EEMs, indicating that the degradation of organic substances.
3.2. Effects of the Different Monochromatic Lights on Biomass and Lipid Production. Golenkinia SDEC-16 manifested the promising characteristics as an oleaginous candidate for bioenergy production in previous work [14]. To achieve the goal of economic production, the application into wastewater treatment coupling with biofuel production is a favorable choice. Given that the achievement of the favorable wastewater treatment, seven days was determined as the cultivation period for Golenkinia SDEC-16.

As shown in Figure 3, the biomass concentration in Golenkinia SDEC-16 vigorously grew upon exposure to the red and blue lights than the green light. The highest biomass concentration and productivity occurred under the red light, with the values of $0.80 \pm 0.03$ g/L and 101.27 mg/L/d. The blue light leads Golenkinia SDEC-16 to attain a maximal biomass concentration of $0.75 \pm 0.04$ g/L, average biomass productivity of 93.95 mg/L/d, and a specific growth rate of 0.29 d$^{-1}$. The low biomass concentration in Golenkinia SDEC-16 occurred under the green light, with a maximal biomass concentration of $0.55 \pm 0.09$ g/L, average biomass productivity of 65.60 mg/L/d, and a specific growth rate of 0.25 d$^{-1}$.

The lipid contents in Golenkinia SDEC-16 are depicted in Figure 3. The highest lipid content was attained under the green light, with a value of 42.99 ± 3.48%, followed by 36.90 ± 3.62% under the red light, and 27.93 ± 1.95% under the blue light. However, the highest lipid productivity was observed under the red light illumination (37.37 mg/L/d), benefiting from rapid biomass growth. Upon exposure to the blue light, Golenkinia SDEC-16 grew slower than that under the red light and accumulated less lipid in comparison to another two monochromatic LED lights, resulting in low lipid productivity. Moreover, the red light achieved superior performance about nutrient removal. With that in mind, it would be advisable to apply red light to wastewater treatment as well as biofuel production.

3.3. Effects of Specific Lights on Fatty Acids Profile. The fatty acid composition of the lipid in Golenkinia SDEC-16 in response to the monochromatic light is shown in Figure 4. The palmitic acid (C 16:0), oleic acid (C 18:1), and linoleic acid (C 18:2) dominated the compositions and were affected by the changes in light wavelengths. The palmitic acid (C 16:0) compositions were weakly affected by changes in light wavelengths, with values of 26.55%, 24.26%, and 25.77%, corresponding to the blue, red, and green lights, respectively. The red light promoted oleic acid (C 18:1) accumulation, with the highest value of 25.67%, followed by 20.54% under the green light and 18.76% under the blue light. The linoleic

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**Figure 1:** Degradation of campus sewage by Golenkinia SDEC-16 cultured under red, green, and blue lights. TN represents the total nitrogen concentration; TP represents the total phosphorus concentration.

| Time (days) | Red light | Green light | Blue light |
|-------------|-----------|-------------|------------|
| Start       |           |             |            |
| 1           | 56.38     | 56.31       | 56.36      |
| 2           | 56.49     | 56.4       | 56.41      |
| 3           | 56.56     | 56.5       | 56.49      |
| 4           | 56.63     | 56.57      | 56.5       |
| 5           | 56.69     | 56.62      | 56.59      |
| 6           | 56.76     | 56.69      | 56.59      |
| 7           | 56.83     | 56.75      | 56.61      |
| End         | 56.90     | 56.77      | 56.66      |

| Time (days) | Red light | Green light | Blue light |
|-------------|-----------|-------------|------------|
| Start       |           |             |            |
| 1           | 0.04      | 0.04        | 0.03       |
| 2           | -0.01     | -0.01       | -0.01      |
| 3           | -0.03     | -0.03       | -0.03      |
| 4           | -0.05     | -0.05       | -0.05      |
| 5           | -0.07     | -0.07       | -0.07      |
| 6           | -0.09     | -0.09       | -0.09      |
| 7           | -0.11     | -0.11       | -0.11      |
| End         | -0.13     | -0.13       | -0.13      |

| Time (days) | Red light | Green light | Blue light |
|-------------|-----------|-------------|------------|
| Start       |           |             |            |
| 1           | 1.95      | 1.95        | 1.95       |
| 2           | 1.91      | 1.91        | 1.91       |
| 3           | 1.86      | 1.86        | 1.86       |
| 4           | 1.81      | 1.81        | 1.81       |
| 5           | 1.76      | 1.76        | 1.76       |
| 6           | 1.70      | 1.70        | 1.70       |
| 7           | 1.65      | 1.65        | 1.65       |
| End         | 1.61      | 1.61        | 1.61       |
acid (C 18:2) achieved 29.45%, 33.04%, and 32.18%, corresponding to the blue, red, and green lights, respectively. Linolenic acid (C 18:3) was only accumulated by the blue light, which accounted for 10.45%, and no observation under the green and red lights.

The low contents of palmitic acid gave rise to less than 30% of saturated fatty acids in Golenkinia SDEC-16 upon the exposure to the monochromic lights. Similar to the contents of saturated fatty acids, the monounsaturated contents took up less than 30%. The polyunsaturated fatty acids take up 40.26%, 51.28%, and 32.18% of the fatty acid compositions upon the exposure to the red, blue, and green lights, respectively. The changes in the unsaturated fatty acids would affect the property of biodiesel.

3.4. Morphology of Golenkinia SDEC−16. The diameters were recorded at the end of the cultivation to characterize the morphology of Golenkinia SDEC-16 under three monochromatic LED lights. The results manifested that larger cell sizes occurred under the blue light than red and green lights, according to Figure 5. Upon the exposure to the blue light, the mean, maximum, and minimum values of the diameters were 17.65 μm, 29.8 μm, and 9.76 μm, respectively. Smaller cell sizes occurred under the red light, with a mean of the diameter of 13.64 μm and the green light, with a mean of the diameter of 13.95 μm.

4. Discussion

In terms of nitrogen, ammonium and nitrate were the primary nitrogen sources for most plants, and both could be effectively used by microalgae. In general, the nitrate and ammonium are assimilated via different metabolic processes in microalgal cells [20]. The ammonium utilization is given priority to be taken up by microalgae on account of consuming less energy compared with other nitrogen sources [21]. The ammonium would be employed to synthesize glutamine directly with the help of ATP and enzyme. Elevated photosynthetic activity under the red and blue light would generate more electrons and ATP that would facilitate ammonium consumption.

The residual total nitrogen concentrations upon exposure to the red and blue lights met the first grade level (less than 15 mg/L) in the pollutant discharge standard for municipal wastewater treatment plants (GB T18918-2002, China). Similar to the case of the total nitrogen, the residue of ammonium concentration also met the first grade level.
lessthan5mg/L in the discharge standard of pollutants for municipal wastewater treatment plants (GB T18918-2002, China) when the algae grew under the red and blue lights. In contrast, the residual total nitrogen concentration under the green light failed to achieve a good performance, which is higher than the standard value of the first grade level in the pollutant discharge standard for municipal wastewater treatment plants (GB T18918-2002, China).

Phosphorus is another vital element in microalgal metabolism and cell growth by generating ATP through phosphorylation along with adenosine diphosphate (ADP) \[22\]. It is commonly known that phosphorus removal efficiencies (>90%) were reported in other studies using municipal wastewater \[23, 24\], suggesting that Golenkinia SDEC-16 has a high phosphorus uptake capacity with the assistance of the red and blue lights. It is reasonable to conclude that the red and blue lights validated favorable total phosphorus removal efficiency and met the first grade level (0.5 mg/L) in the discharge standard of pollutants for municipal wastewater treatment plants.

The remaining total organic carbon concentration was still high, owing to part of the biodegradable constituents of

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**Figure 3:** Biomass concentration and lipid contents of Golenkinia SDEC-16 on day 7 under red, green and blue lights.

**Figure 4:** Fatty acid compositions of Golenkinia SDEC-16 cultivated under red, green and blue lights.
the effluent that could be consumed by microalgae [25]. To satisfy the ultimate discharge standard for organic waste, further intensive treatment through other wastewater treatment units would be well advised. Microalgae were able to exert organic matters, or the natural materials outflow from the dead cells, which lead to the appearance of humic or fulvic-like substances. No differences in EEMs were observed for growth under the three respective light sources, demonstrating that they had little effects on the degradation of organic substances.

In terms of the biomass concentration, it is acknowledged that the chlorophylls possess two major absorption bands at (600–700 nm) and (400–500 nm) [26]. That is the reason to explain microalgal flourishing occurred upon exposure to the red and blue light. In the present work, the red light plays an active role in promoting biomass concentration in Golenkinia SDEC-16. Despite, some studies concluded strains, such as Nannochloropsis oceanica, Nannochloropsis salina, and Nannochloropsis oculata, preferred blue light, indicating the light utilization is species-dependent. The concurrent conclusion displayed that microalgae failed to thrive under the green light, owing to ineffective energy utilization [27].

The effects on lipid synthesis by different monochromic lights were interesting as there is not a clear general rule on the microalgal lipid synthesis. For example, Chlorella spp. showed the most desirable preference of lipid accumulation under blue light other than red or white light source [28]. In contrast, Ra et al. found that the green light was able to increase the lipid content of Nannochloropsis sp [27]. There is another study reported on the same genus of Nannochloropsis sp that attained the highest lipid content under blue light [29]. The most top lipid content in Golenkinia SDEC-16 was found under the green light. It can be ascribed to a slow growth rate, which is in favor of lipid accumulation [30]. For the rewarding lipid output, we still need to consider the biomass. Retrospectively, the red light would be well advised to be applied to wastewater treatment as well as biofuel production owing to the highest lipid productivity.

The fatty acid compositions responded to the changes in wavelengths, which exerted effects on their biofuel property. Table 1 provides parameters of biodiesel based on the fatty acid unsaturated degree of the present species. There are no significant changes in biodiesel properties, except for iodine number and cloud point. The iodine number is 78.15 under the green light, which is lower than 93.69 and 89.93 under the red and blue lights, respectively. The iodine value is directly affected by the unsaturation degree of fatty acids, and the value increases with the increase in the number of double bonds [33]. The oil tends to polymerize and results in engine deposition if the iodine value increased [33].

In contrast, the cloud point is 8.19 under the green light, which is higher than 5.40 and 6.07 under the red and blue lights, respectively. Cloud point value is closely affected by the solid phase consisting mainly of the saturated methyl esters at the equilibrium point, and there are no strict specifications of the cloud point [34]. Fortunately, the standard of biodiesel still satisfied the level made by the U.S.A and Europe, indicating the application of biodiesel.

The present work also evaluated the effects of monochromic LED lights on the morphology of Golenkinia SDEC-16 under red light, green light, and blue light. Figure 5: Cell sizes of Golenkinia SDEC-16 grown under three lights.
SDEC-16. Similar to the observations in the reported articles, the red light tends to make microalgal cells smaller [35], while the blue light would cause the microalgal cells larger [36]. That would be explained by the differential rate of cell divisions or cell cycle progression [37]. Smaller cells were produced under the red light, owing to the early autospore release. Whereas blue light delayed the division in microalgae, resulting in producing larger size and fewer numbers of autospores [37].

5. Conclusion

Microalgal biofuel production coupling with wastewater is a promising strategy. Results revealed that campus sewage treatment under both red and blue lights was able to meet the first grade level in the Chinese pollutant discharge standards for municipal wastewater treatment plants within seven days. Regarding biofuel production, the green light facilitated lipid synthesis as high as 42.99 ± 3.48% but with low biomass concentration (0.55 ± 0.09 g/L). The Golenkinia SDEC-16 achieved the maximal biomass concentration, with a value of 0.80 ± 0.03 g/L as well as a lipid content of 36.90 ± 3.62%, which is the promising light spectrum to attain a balance between biomass production and lipid synthesis.

Data Availability

The data used to support the findings of this study are included in the article

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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