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Effects of various LED light wavelengths and intensities on microalgae-based simultaneous biogas upgrading and digestate nutrient reduction process

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HIGHLIGHTS

- Biogas upgrading and digestate nutrient reduction could be simultaneously achieved.
- Red was the optimal light for biogas upgrading and digestate nutrient reduction.
- The optimal light intensity range was between 1200 and 1600 μmol m^{-2} s^{-1}.
- The CH_4 content attained the highest value of 92.74 ± 3.56% (v/v).

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ABSTRACT

Biogas is a well-known, primary renewable energy source, but its utilizations are possible only after upgrading. The microalgae-based bag photo-bioreactor utilized in this research could effectively upgrade biogas and simultaneously reduce the nutrient content in digestate. Red light was determined as the optimal light wavelength for microalgae growth, biogas upgrading, and digestate nutrient reduction. In the range of moderate light intensities (i.e., 800, 1200, 1600, and 2000 μmol m^{-2} s^{-1}), higher light intensities achieved higher biogas upgrade and larger digestate nutrient reduction. Methane content attained the highest value of 92.74 ± 3.56% (v/v). The highest chemical oxygen demand, total nitrogen, and total phosphorus reduction efficiency of digestate were 85.35 ± 1.04%, 77.98 ± 1.84%, and 73.03 ± 2.14%, respectively. Considering the reduction and economic efficiencies of the carbon dioxide content of biogas and digestate nutrient as well as the biogas upgrading standard, the optimal light intensity range was determined to be from 1200 to 1600 μmol m^{-2} s^{-1}.

1. Introduction

Biogas production not only avoids the deforestation of natural land, but also does not compete for agricultural production materials (arable land, irrigation, and fertilizers) with grain crop, given that feedstock of anaerobic digestion usually comes from biomass waste (Crutzen et al., 2008). Raw biogas consists mainly of methane (CH_4) (40–75%, v/v) and carbon dioxide (CO_2) (15–60%, v/v). The trace amounts of other components are mainly vapor (H_2O, 5–10%, v/v), oxygen (O_2, 0–1%, v/v), and hydrogen sulfide (H_2S, 0.005–2%, v/v) (Ryckebosch et al., 2011). Biogas can be directly and efficiently combust as fuel only after increasing the CH_4 content to higher than 90% (v/v) to increase its calorific value and decrease the relative density (Ryckebosch et al., 2011). Therefore CO_2 removal is essential for biogas upgrading.

Numerous methods have been used to remove CO_2 from biogas, including physical or chemical absorption, pressure swing adsorption, membrane separation, and cryogenic separation (Kapdi et al., 2005; Ryckebosch et al., 2011). In general, the disadvantages of such methods are the requirement for a large amount of energy, auxiliary materials and chemicals, as well as the generation of wastes and wastewater needs treatment (Kapdi et al., 2005; Ryckebosch et al., 2011). Photosynthesis has long been recognized as the most cost-effective means to sequester anthropogenic CO_2, and microalgae have been identified as a fast growing species whose carbon fixing rates are much higher than those of terrestrial plants (Jeong et al., in press). Therefore, the biological method of the microalgae CO_2 fixation by photosynthesis is a highly potential method for biogas upgrading. The continuous cultivation of a number of specific microalgae species (e.g., *Chlorella* sp.) likewise yields value-added products from biomass (Jeong et al., in press). Moreover, Abatzoglou and Boivin (2009) suggested that biogas upgrading in a photo-bioreactor should be applied to wastewater

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treatment. Hence, the digestate can be utilized as a perfect nutrient source in microalgae-based biogas upgrading processes as it is freely obtained and possesses nutrients required for microalgae growth (Chinnasamy et al., 2010).

Light intensities and wavelengths are essential parameters for microalgae growth (Ugwu et al., 2007). However, the varying illumination intensities in outdoor conditions are likely to inhibit microalgae growth because of the shortage in light energy, e.g., very low light intensities during rainy days or the photoinhibition caused by excessive irradiance, or very high light intensities at noontimes during summer (Ugwu et al., 2007). Culturing microalgae with artificial light sources is a solution to this concern. Using specific narrow bands of light, e.g., light-emitting diode (LED), is more economical in microalgae photosynthesis maintenance compared with using ordinary fluorescent lamps, because ordinary light wavelengths may not contain the absorption bands of chlorophyll pigments or comprise only a combination of the growth efficient and inefficient light spectra (Cheirsilp and Torpee, 2012). Microalgae requires optimum illumination conditions to economically achieve maximum photosynthetic rates.

Few reports exist on the microalgae-based simultaneous biogas upgrading and digestate nutrient reduction process, especially on its performance under various LED light wavelengths and intensities. Therefore, this study focuses on the responses of microalgae growth, biogas upgrading, and digestate nutrient reduction to various LED light wavelengths and intensities. Furthermore, the intrinsic connections among the above factors are analyzed to explain the performance of the microalgae-based photo-bioreactor.

2. Methods

A total of 96 bag photo-bioreactors (24 types of treatments and quadruplicate) were used in this study. Each bag photo-bioreactor treatment was filled with raw biogas (96 L) and microalgae digestate culture suspension (12 L). All treatments were incubated at 25.0 ± 0.5 °C for 168 h, while imposing a range of LED light wavelengths and intensities, measuring the biogas component and the cell dry weight (CDW) of microalgae, as well as determining the characteristics of the digestate culture.

2.1. Materials

The microalgae *Chlorella* sp. was used in this research. It was obtained from the stock cultures in our laboratory and proven to be a highly biogas-tolerant and fast-growing microalgae (Li, 2012). The CDW of *Chlorella* sp. in the stock cultures was 80.25 ± 10.45 mg L^{-1}. The stock culture was BG-11 medium, which contained $NaNO_3$ 1.5 g, $K_2HPO_4{\cdot}3H_2O$ 0.04 g, $KH_2PO_4{\cdot}3H_2O$ 0.2 g, EDTA 0.0005 g, Fe ammonium citrate 0.005 g, citric acid 0.005 g, Na_2CO_3 0.02 g and 1 mL of trace metal solution per liter with pH 7.0. The trace metal solution contained H_3BO_3 2.85 g, $MnCl_2{\cdot}4H_2O$ 1.8 g, $ZnSO_4{\cdot}7H_2O$ 0.02 g, $CuSO_4{\cdot}5H_2O$ 0.08 g, $CoCl_2{\cdot}6H_2O$ 0.08 g and $Na_2MoO_4{\cdot}2H_2O$ 0.05 g per liter (Tansakul et al., 2005). The cultivating conditions of the stock culture were as follows: cool-white fluorescent light with a surface light intensity of 300 μmol m^{-2} s^{-1}, 25 ± 0.5 °C, light–dark cycle 12 h:12 h (8:00 AM–8:00 PM is light, and the rest is dark), and artificial intermittent shaking three times a day (8:00 AM, 2:00 PM, and 8:00 PM) at approximately 60 rpm.

The digestate and raw biogas were both obtained from an anaerobic digestion equipment in Hongmao Hacienda, Kunshan City, Jiangsu Province, PR China. The digestate was filtered by using a glass microfiber filter (GF/C, 1.2 μm pore size, Whatman, USA) and then treated using an ultraviolet sterilizer (KCJ-10W, Konche Water Treatment Co., Ltd) to prevent interference from other sediments and microorganisms. The digestate characteristics before and after these pretreatments were examined. Before the pretreatment, the chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) of digestate were 1227.61 ± 54.98, 492.02 ± 21.53, and 53.28 ± 2.74 mg L^{-1}, respectively, whereas after the pretreatment, the values were 1203.61 ± 49.58, 481.37 ± 16.42, and 51.34 ± 1.85 mg L^{-1}, respectively. Before the pretreatment, the pH, dissolved oxygen (DO), and dissolved inorganic carbon (DIC) were 6.55 ± 0.05, 7.04 ± 0.31, and 998.24 ± 16.57 mg L^{-1}, respectively, whereas after the pretreatment, the values were 6.50 ± 0.05, 7.01 ± 0.34, and 997.32 ± 15.86 mg L^{-1}, respectively. After filtration and sterilization, only a fraction of COD, TN, and TP was reduced. Meanwhile, pH, DO, and DIC were nearly unchanged. Therefore, the pretreatment process did not present any inappropriate characterization of the digestate attributes. The filtrate could effectively represent the digestate characteristics. After the desulfurization by chemical absorption to reduce the H_2S concentration to less than 50 ppm (Chung et al., 2006), the composition of the raw biogas was determined as CH_4 67.35 ± 4.32% (v/v), CO_2 28.41 ± 2.29% (v/v), O_2 0.73 ± 0.05% (v/v), and H_2O 3.48 ± 0.36% (v/v).

The LEDs (CN-PT10-15) utilized in this research were 25 mm wide and 600 mm long, purchased from the Canal Optoelectronic Technology Co., Ltd., PR China. The LEDs had a narrow output spectrum at the peak wavelength. The wavelength of the white LED was 380 nm to 760 nm. Table 1 indicated the characteristics of the LEDs, including their full width at half maximum (FWHM) and power consumptions.

A transparent polyethylene bag (90 cm × 80 cm × 15 cm) equipped with double zipper closure and sampling valve was used in this research as photo-bioreactor (Fig. 1). With only a 0.03 mm thickness of the polyethylene film, this bag did not alter the light imposed. The sampling valve comprised a plug and a rubber gasket. The bag photo-bioreactor was washed with deionized water and sterilized using an ultraviolet sterilizer (KCJ-10W, Konche Water Treatment Co., Ltd) before usage.

2.2. Experimental procedure

Air was removed from the bag photo-bioreactor by squashing with hands after adding 12 L of *Chlorella* sp. digestate culture suspension (initial cell density of 150.84 ± 7.25 mg L^{-1}). Raw biogas was then added, with a total gas volume of 96 L. The microalgae digestate culture suspension (12 L) was prepared through the following procedures. First, about 22.5 L of microalgae stock culture was filtered using a glass microfiber filter (GF/C, 1.2 μm pore size, Whatman, USA) to obtain CDW of 1805.76 ± 87.00 mg of microalgae. Second, the microalgae attached in the filter were rinsed with the digestate, and the washing fluid was placed in a 20 L measuring cylinder. Third, the rinsing process ended when the volume of the washing fluid was about 11 L, and then the digestate was added to reach 12 L. The digestate and the inoculum (i.e., microalgae stock culture) were integrally mixed in the microalgae digestate culture suspension. All bag photo-bioreactor treatments were preserved in an illuminating incubator (SPX-400I-G, Boxun Industry & Commerce Co., Ltd, PR China). The LEDs were installed in the illuminating incubator as light sources. During the 7 day/168 h-experimental period, temperature was maintained at 25.0 ± 0.5 °C and the light–dark cycle was maintained at 12 h:12 h (light was from 8:00 AM to 8:00 PM, and the rest was dark). Artificial intermittent shaking was performed for the bag photo-bioreactors three times a day (8:00 AM, 2:00 PM, and 8:00 PM), with approximately 60 rpm lasting for 5 min. The optimal light wavelengths for efficient CO_2 removal from biogas, digestate nutrient reduction, and microalgae growth were determined by exposing the bag photo-bioreactor treatments to various light wavelengths (red, white, yellow, and blue) under various light intensities (i.e., 400, 800, 1200,

Table 1
LED characteristics under various light wavelengths and intensities.

Light	Peak wavelength (nm)	FWHM (nm)	Energy consumption (W)					
			400 (μmol m^{-2} s^{-1})	800 (μmol m^{-2} s^{-1})	1200 (μmol m^{-2} s^{-1})	1600 (μmol m^{-2} s^{-1})	2000 (μmol m^{-2} s^{-1})	2400 (μmol m^{-2} s^{-1})
Red	660	18.4	1.1	2.7	3.2	4.1	6.9	7.8
White	–	–	0.8	2.5	4.4	5.4	7.2	8.3
Yellow	590	15.3	4.4	7.2	9.3	11.5	11.9	14.5
Blue	460	20.9	2.3	3.4	6.3	9.2	14.4	17.1

FWHM stands for full width at half maximum.

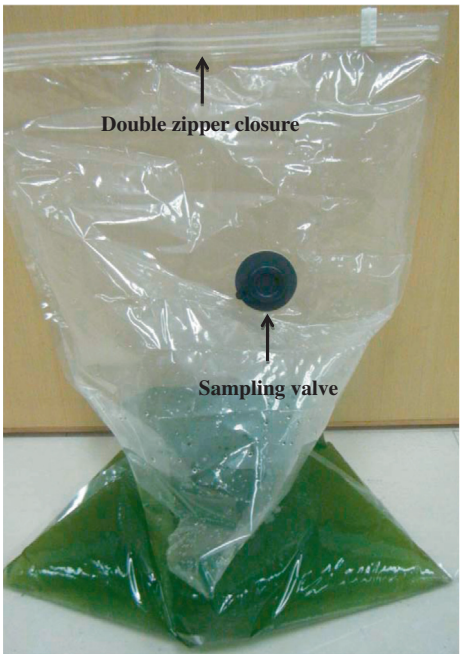

Fig. 1. Experimental device of bag photo-bioreactor.

1600, 2000, and 2400 μmol m^{-2} s^{-1}). Thus, 24 types of bag photo-bioreactor treatments were obtained. The optimal light intensity range was determined by analyzing the economic efficiencies of the CO_2 removal from biogas and the digestate nutrient reduction. The required light intensity was achieved by increasing the number of LEDs. All treatments were performed in quadruplicates, and the total number of bag photo-bioreactors was 96. The cultures were sampled and analyzed daily at 8:00 AM, and then the mean values were calculated.

2.3. Analysis

The components of biogas (CH_4, CO_2, O_2, and H_2O) in the bag photo-bioreactors were measured by using a gas analyzer (GA94, ONUEE Co., Ltd., PR China) through the sampling valve in the bag (Fig. 1). The analyzer was equipped inside with an air circulation pump, so that the measured biogas returned to the bag. The digestate culture suspension was sampled through the following procedures. First, the bag photo-bioreactor was tilted to allow the culture suspension to flow into the sampling valve. Second, the plug of the sampling valve was turned on while the rubber gasket was still sealed to prevent the culture suspension from leaking. Third, the rubber gasket was pierced using a 50 mL syringe, and 25 mL of the culture suspension was sampled. Fourth, the syringe was pulled out while the sampling valve was plugged in. The culture suspension in the syringe was used to measure the CDW of microalgae and to determine the characteristics of the digestate culture suspension. The CDW of microalgae was measured through the following procedures. First, 20 mL of culture suspensions was filtered using a glass microfiber filter (GF/C, 1.2 μm pore size, Whatman, USA). Second, the filter with attached microalgae cells were dried at 100 °C for 12 h and then cooled to room temperature in the desiccator. Finally, the CDW was determined by measuring the difference between the filter weights before and after filtration. The filtrates of the cultures were analyzed for COD, TN, and TP using standard methods (APHA, 1995). The pH, DO, and DIC of the culture suspension were measured using pH meter (Orion 250 Aplus ORP Field Kit, USA), DO meter (TP350, Beijing Timepower Measurement and Control Equipment Co., Ltd., PR China), and DIC analyzer (Shimazu TOC 5000A, Japan), respectively. Light intensity was measured using a waterproof light meter (CEM, DT-1308, Shenzhen Everbest Machinery Industry Co., Ltd., PR China) on the inner surface of the culture suspension. In this study, the biogas CO_2 removal efficiency indicated the biogas upgrading effect.

The biogas CO_2 or the digestate nutrient reduction efficiency was calculated as follows:

$$R = \left(1 - \frac{C_i}{C_o}\right) \times 100 \quad (1)$$

where R is the biogas CO_2 or the digestate nutrient reduction efficiency (%), C_0 is the inflow CO_2 content in biogas (%, v/v) or the nutrient concentration in the initial culture suspension (mg L^{-1}), and C_i is the outflow CO_2 content in biogas (%, v/v) or the nutrient concentration in the culture filtrates (mg L^{-1}).

The economic efficiency of the biogas CO_2 (or the digestate nutrient) reduction efficiency was calculated as follows:

$$E = \frac{R}{kTP} \quad (2)$$

where E is the economic efficiency of the biogas CO_2 (or the digestate nutrient) reduction (USD^{-1}), R is the biogas CO_2 (or the digestate nutrient) reduction efficiency (%) in Eq. (1), k is the electric charge per unit of power consumption (USD kW^{-1} h^{-1}), T is the actual illumination time (h), and P is the LED power consumption during the actual illumination time (W). Table 1 demonstrated the relationship between the power consumption and the light intensity of various LED light wavelengths.

2.4. Statistical analyses

All statistical analyses were performed using SPSS software (SPSS, 2003). One-way analysis of variance (ANOVA) was used to evaluate the differences among the 24 types of bag photo-bioreactor treatments, which were illuminated in four kinds of light wavelengths (i.e., red, white, yellow, and blue) under six various light intensities (i.e., 400, 800, 1200, 1600, 2000, and 2400 μmol m^{-2} s^{-1}). The light wavelength and intensity were included in the same statistical analysis, but no interaction was

assessed between them. The factors in the applied model included the mean values of the reduction and economic efficiencies of biogas CO_2, COD, TN, and TP. Duncan's multiple range tests were utilized to further assess the differences among the light wavelengths that were significant in ANOVA. A $P = 0.05$ level of significance was used as the threshold.

3. Results

3.1. Physico-chemical parameters

Table 2 showed the variations of pH, DO, and DIC in the culture suspension under 2000 μmol m^{-2}. The three physico-chemical parameters for all light wavelengths were similar. The pH and DIC values varied a little from approximately 6.35–6.83 and 980.31–1008.26 mg L^{-1}, respectively. The DO only slightly increased from 7.01 ± 0.34 mg L^{-1}–9.54 ± 0.82, 9.21 ± 0.46, 9.38 ± 0.51, and 9.03 ± 0.72 mg L^{-1} for red, white, yellow, and blue light wavelengths, respectively.

3.2. Microalgae growth

Fig. 2 showed the DW of microalgae under four different wavelengths, i.e., red, white, yellow, and blue, with various light intensities, i.e., 400, 800, 1200, 1600, 2000, and 2400 μmol m^{-2} s^{-1}. The treatments exposed to light intensities of 400 and 2400 μmol m^{-2} s^{-1} (Fig. 2a and f, respectively) were both much lower compared with the other light intensity treatments. Meanwhile, the light intensity of 400 μmol m^{-2} s^{-1} was too low to maintain the growth of microalgae, whereas 2400 μmol m^{-2} s^{-1} was too high to avoid photoinhibition. Therefore, only the moderate light intensities, i.e., 800, 1200, 1600, and 2000 μmol m^{-2} s^{-1}, were appropriate for *Chlorella* sp. culture, and the DW values increased over the time course. Furthermore, under moderate light intensities (Fig. 2b–e),

Table 2
Variation tendencies of the physico-chemical parameters in culture suspension under 2000 μmol m^{-2} with various light wavelengths.

Light wavelength	Physico-chemical parameters	Time (h)							
		0	24	48	72	96	120	144	168
Red	pH	6.50 ± 0.05	6.63 ± 0.14	6.61 ± 0.25	6.82 ± 0.19	6.73 ± 0.39	6.45 ± 0.25	6.38 ± 0.21	6.42 ± 0.33
	DO (mg L^{-1})	7.01 ± 0.34	7.22 ± 0.59	7.48 ± 0.56	7.95 ± 0.31	8.53 ± 0.69	8.84 ± 0.26	9.31 ± 0.75	9.54 ± 0.82
	DIC (mg L^{-1})	997.32 ± 15.86	983.11 ± 17.35	992.53 ± 20.45	1004.36 ± 11.47	991.47 ± 18.32	986.02 ± 17.96	1002.59 ± 13.52	993.50 ± 16.43
White	pH	6.50 ± 0.05	6.51 ± 0.23	6.44 ± 0.18	6.37 ± 0.38	6.42 ± 0.25	6.83 ± 0.19	6.62 ± 0.35	6.39 ± 0.17
	DO (mg L^{-1})	7.01 ± 0.34	7.14 ± 0.36	7.33 ± 0.62	7.80 ± 0.59	8.40 ± 0.57	8.72 ± 0.61	9.05 ± 0.68	9.21 ± 0.46
	DIC (mg L^{-1})	997.32 ± 15.86	994.05 ± 23.56	1008.26 ± 17.23	985.27 ± 16.32	994.38 ± 16.46	987.25 ± 24.71	991.34 ± 16.02	987.46 ± 20.75
Yellow	pH	6.50 ± 0.05	6.44 ± 0.27	6.35 ± 0.31	6.49 ± 0.35	6.52 ± 0.17	6.67 ± 0.38	6.41 ± 0.19	6.75 ± 0.28
	DO (mg L^{-1})	7.01 ± 0.34	7.28 ± 0.42	7.51 ± 0.39	7.96 ± 0.53	8.32 ± 0.71	8.69 ± 0.42	9.11 ± 0.83	9.38 ± 0.51
	DIC (mg L^{-1})	997.32 ± 15.86	980.31 ± 23.47	987.42 ± 16.94	992.43 ± 13.50	1003.24 ± 17.28	989.47 ± 19.03	994.65 ± 12.87	1002.32 ± 17.26
Blue	pH	6.50 ± 0.05	6.63 ± 0.39	6.71 ± 0.13	6.52 ± 0.47	6.24 ± 0.22	6.29 ± 0.34	6.70 ± 0.52	6.52 ± 0.46
	DO (mg L^{-1})	7.01 ± 0.34	7.26 ± 0.58	7.31 ± 0.42	7.76 ± 0.49	8.12 ± 0.69	8.49 ± 0.71	8.82 ± 0.79	9.03 ± 0.72
	DIC (mg L^{-1})	997.32 ± 15.86	1003.28 ± 14.39	997.26 ± 19.03	986.45 ± 13.24	999.35 ± 24.62	983.56 ± 24.30	984.38 ± 25.32	998.23 ± 25.54

DO stands for dissolved oxygen, DIC stands for dissolved inorganic carbon.

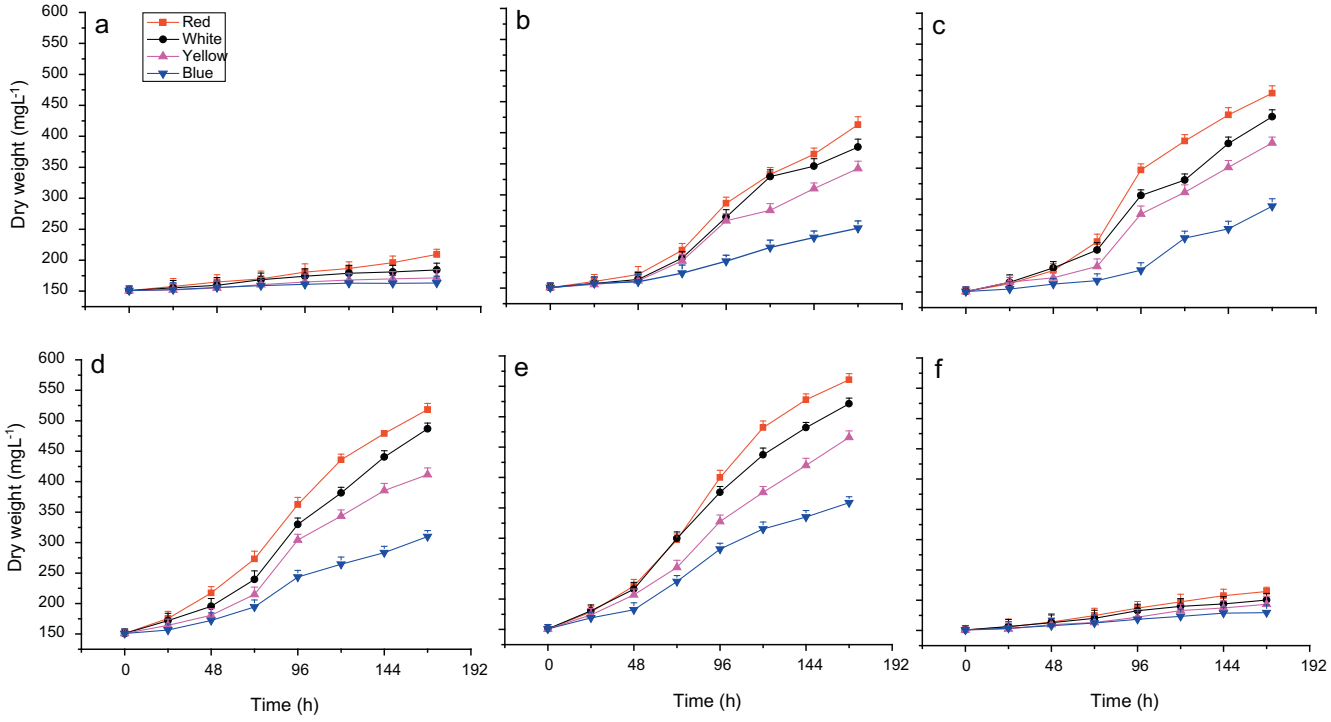

Fig. 2. Microalgae dry weight over time at various light wavelengths and intensities: (a) 400, (b) 800, (c) 1200, (d) 1600, (e) 2000, and (f) 2400 μmol m^{-2} s^{-1}.

the light wavelengths of red, white, and yellow treatments achieved similarly higher DW than that of the blue treatment. Red light was the optimal light wavelength for *Chlorella* sp. growth because it achieved the highest DW values of 412.93 ± 12.83, 470.74 ± 12.03, 518.43 ± 10.05, and 560.79 ± 10.05 mg L^{-1} under the light intensities of 800, 1200, 1600, and 2000 μmol m^{-2} s^{-1}, respectively. Therefore, the moderate light intensities and red light wavelength were the optimal conditions for microalgae growth.

3.3. Biogas upgrading effect

Table 3 demonstrated that the biogas CO_2 removal efficiency of red light wavelength treatment was significantly higher ($P < 0.05$) than the other light wavelengths. This result was similar with that of microalgae growth in Fig. 2. Therefore, red light was determined as the optimal light wavelength for biogas upgrading. Table 4 showed that no significant difference ($P > 0.05$) existed among the treatments of moderate light intensities for biogas CO_2 removal efficiencies. By contrast, Fig. 3 demonstrates that higher light intensity achieved higher CH_4 content (v/v), lower CO_2 content (v/v), and better biogas upgrading effect. This result was consistent with the variation trends of the DW of microalgae (Fig. 2b–e). Over the time courses, the CO_2 content decreased, whereas the CH_4 content increased. The CH_4 content (v/v) attained the highest values of 87.54 ± 5.43%, 90.52 ± 5.37%, 91.96 ± 4.09%, and 92.74 ± 3.56%, under light intensity of 800, 1200, 1600, and 2000 μmol m^{-2} s^{-1}, respectively. However, the O_2 and H_2O contents (v/v) were both nearly invariable. O_2 content (v/v) remained between 0.97 ± 0.03% and 0.73 ± 0.05% under moderate light intensities. H_2O content (v/v) varied between 3.31 ± 0.26% and 3.65 ± 0.41%. The coefficient of the electric charge per unit of power consumption, k, in Eq. (2) was calculated as 0.09921 USD kW^{-1} h^{-1} based on the prices in Shanghai City, PR China. The power consumptions of the red LED for moderate light intensity treatments were demonstrated in Table 1. Regarding the economic efficiency of the biogas CO_2 removal, no significant difference ($P > 0.05$) existed among the light intensity treatments of 800, 1200, and 1600 μmol m^{-2} s^{-1}, but they were all significantly higher ($P < 0.05$) than that of the light intensity of 2000 μmol m^{-2} s^{-1} (Table 4). Therefore, the optimal light intensity range for biogas upgrading was determined to be from 1200–1600 μmol m^{-2} s^{-1}.

3.4. Digestate nutrient reduction effect

Table 3 showed that the digestate COD, TN, and TP reduction efficiencies of red light wavelength treatment was significantly higher ($P < 0.05$) than those of other light wavelengths. This result was consistent with those of biogas CO_2 removal efficiency and microalgae growth. Therefore, red light was identified as the optimal light wavelength for digestate nutrient reduction. Fig. 4 demonstrated the digestate nutrient reduction efficiency over time under red light wavelength for various moderate light intensities (i.e., 800, 1200, 1600, and 2000 μmol m^{-2} s^{-1}). The variation trends of COD, TN, and TP were similar. They all increased during the experimental period, but a higher light intensity treatment achieved higher nutrient reduction efficiency. Therefore, under the light intensity of 2000 μmol m^{-2} s^{-1} (Fig. 4), the highest COD, TN, and TP reduction efficiencies were 85.35 ± 1.04%, 77.98 ± 1.84%, and 73.03 ± 2.14%, respectively. Furthermore, Table 3 indicated that no significant difference ($P > 0.05$) existed among the treatments of light intensities 1200, 1600, and 2000 μmol m^{-2} s^{-1} for the digestate nutrient reduction efficiency, but they were significantly higher ($P < 0.05$) than the treatment of light intensity 800 μmol m^{-2} s^{-1}. Considering the economic efficiency of the nutrient reduction, the light intensity treatments of 800, 1200, and 1600 μmol m^{-2} s^{-1} were all significantly higher ($P < 0.05$) than that of light intensity 2000 μmol m^{-2} s^{-1}. However, no significant difference ($P > 0.05$) was observed among them (Table 4). As a result, the optimal light intensity range for digestate

Table 3
Mean values ± SD and SEM of biogas CO_2 and digestate nutrient reduction efficiency under 2000 μmol m^{-2} with various light wavelengths. Values with different superscript letters in the same column indicate significant differences at $P = 0.05$, according to Duncan's multiple range tests.

Light wavelength	Statistical analyses	Reduction efficiency (%)			
		CO_2	COD	TN	TP
Red	Mean values ± SD	51.28 ± 9.68	63.70 ± 2.35	54.94 ± 2.09	51.14 ± 2.09
	SEM	13.24[a]	16.45[a]	14.19[a]	13.20[a]
White	Mean values ± SD	48.02 ± 8.76	57.26 ± 3.41	49.32 ± 3.61	46.31 ± 4.73
	SEM	12.40[b]	14.78[b]	12.73[b]	11.96[b]
Yellow	Mean values ± SD	44.36 ± 8.19	54.38 ± 4.26	46.64 ± 3.57	42.08 ± 4.21
	SEM	11.45[c]	14.04[c]	12.04[c]	10.87[c]
Blue	Mean values ± SD	40.73 ± 6.85	46.92 ± 3.86	41.53 ± 4.05	37.92 ± 3.15
	SEM	10.52[d]	12.11[d]	10.72[d]	9.79[d]

SEM stands for standard error of the mean, COD stands for chemical oxygen demand, TN stands for total nitrogen, and TP stands for total phosphorus.

Table 4
Mean values ± SD and SEM of reduction and economic efficiencies of biogas CO_2 and digestate nutrient reduction under moderate light intensities of red light wavelength. Values with different superscript letters in the same column indicate significant differences at $P = 0.05$, according to Duncan's multiple range tests.

Light intensity (μmol m^{-2} s^{-1})	Statistical analyses	Reduction efficiency (%)				Economic efficiency (USD^{-1})			
		CO_2	COD	TN	TP	CO_2	COD	TN	TP
800	Mean values ± SD	43.09 ± 12.94	49.91 ± 2.20	44.12 ± 2.40	42.60 ± 2.57	19.59 ± 1.74	22.18 ± 1.12	19.61 ± 1.82	18.93 ± 1.33
	SEM	11.13[a]	12.89[b]	11.39[b]	11.00[b]	5.06[b]	5.73[b]	5.06[b]	4.89[b]
1200	Mean values ± SD	43.39 ± 10.83	56.46 ± 2.62	51.03 ± 2.23	47.08 ± 2.36	16.27 ± 1.16	21.17 ± 1.03	19.14 ± 1.14	17.65 ± 1.05
	SEM	11.20[a]	14.58[a]	13.18[a]	12.16[a]	4.20[b]	5.47[b]	4.94[b]	4.56[b]
1600	Mean values ± SD	47.77 ± 11.72	59.14 ± 2.39	53.81 ± 1.96	48.69 ± 2.24	13.98 ± 1.03	17.31 ± 1.25	15.75 ± 1.69	14.25 ± 1.74
	SEM	12.33[a]	15.27[a]	13.89[a]	12.57[a]	3.61[b]	4.47[b]	4.07[b]	3.68[b]
2000	Mean values ± SD	51.28 ± 9.68	63.70 ± 2.35	54.94 ± 2.09	51.14 ± 2.09	8.92 ± 1.57	11.08 ± 1.58	9.55 ± 1.73	8.89 ± 1.12
	SEM	16.45[a]	14.19[a]	13.20[a]	2.30[a]	2.86[a]	2.47[a]	2.30[a]	16.45[a]

SEM stands for standard error of the mean, COD stands for chemical oxygen demand, TN stands for total nitrogen, and TP stands for total phosphorus.

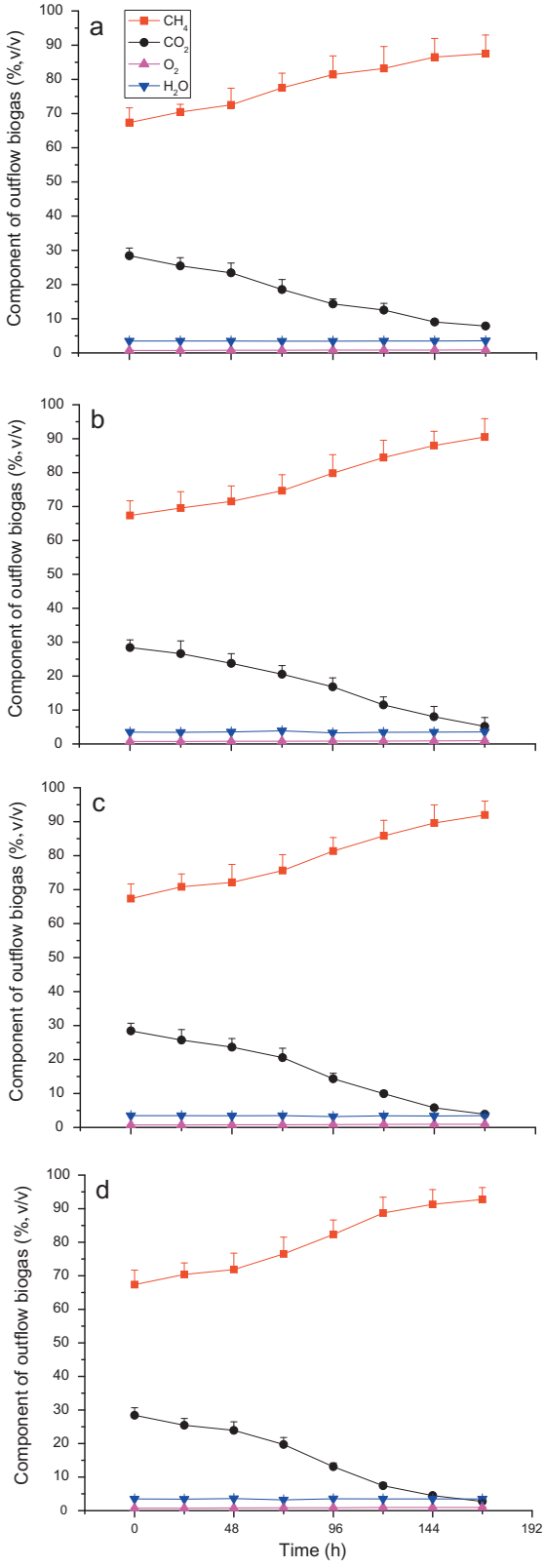

Fig. 3. Component of outflow biogas over time under red light wavelength at various moderate light intensities: (a) 800, (b) 1200, (c) 1600, and (d) 2000 μmol m^{-2} s^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nutrient reduction was determined to be from 1200–1600 μmol m^{-2} s^{-1}.

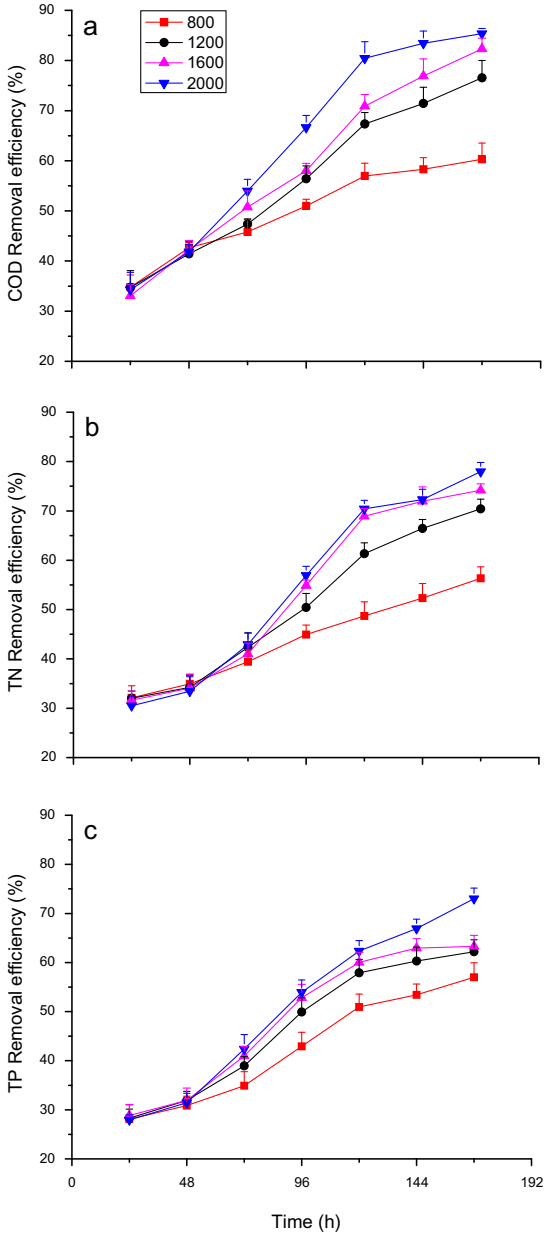

Fig. 4. Digestate nutrient reduction efficiency over time under red light wavelength for various moderate light intensities: (a) chemical oxygen demand, (b) total nitrogen, and (c) total phosphorus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The pH results obtained in the this study were consistent with those of Papazi et al. (2008), who reported that pH values slightly varied between 6.00 and 7.00 under CO_2 30% (v/v) with *Chlorella minutissima*. By contrast, Oswald (1988) showed that relatively higher CO_2 concentration could lead to a sharp change of the pH value and then destroy the microalgae physiology. This phenomenon could be explained by the theory that the biogas CO_2 content in this research could meet the requirement for microalgae growth, and sufficient CO_2 were always to be supplied to dissolve in the digestate suspension culture. Therefore, the CO_2 consumption of the suspension culture almost did not affect the pH value (Papazi et al., 2008). In fact, the DIC and pH values of the digestate suspension culture both only slightly changed during the experimental

period (Table 2), because a large amount of CO_2 was produced and dissolved in the digestate during the anaerobic digestion process. Thus, the digestate suspension culture had a high concentrated DIC (Table 2), given that CO2 was its main component (Lei et al., 2007). The dissolved CO_2 in the digestate suspension culture was utilized by microalgae cells for growth through photosynthesis. Then, the consumed CO_2 in the culture could be immediately replaced by the CO_2 in the biogas, maintaining the DIC and pH values (Carvalho et al., 2006). The DO value in this research was always below the excessive limitation (35 mg L^{-1}), preventing microalgae inhibition (Table 2). de Godos et al. (2010) reported a similar result that high CO_2 content gas had no significant effect on the DO of a microalgae photo-bioreactor. O_2 produced by microalgae may become a self-inflicted poison, especially in closed reactors, e.g., the bag photo-bioreactor in this research. When the DO concentration in the culture was above its counterpart in equilibrium with its partial pressure in the surroundings, the photosynthesis and metabolic processes of microalgae would be inhibited (Carvalho et al., 2006). Furthermore, the O_2 accumulation in the culture could cause photorespiration, resulting in a decrease in biomass growth and CO_2 assimilation (de Godos et al., 2010). In this research, such adverse impacts were avoided by the existence of organic carbon in the digestate suspension culture and relatively higher CO_2 content (28.41 ± 2.29%, v/v) in the bag photo-bioreactor surroundings (i.e., biogas). van Den Hende et al. (2011) suggested that the increase of CO_2:O_2 ratio by adding more inorganic carbon to the reactor and the addition of organic carbon to the culture could overcome the potential photosynthesis inhibition. Moreover, the photosynthesis likewise consumed O_2. Therefore, the DO in the digestate culture suspension in this research remained below the air saturation (Table 2), and no inhibition occurs.

Insufficient light intensity, i.e., 400 μmol m^{-2} s^{-1} (Fig. 2a), resulted in reduced microalgae biomass and slow growth rates because the microalgae consumed carbohydrates during photorespiration, although they were unlikely to cause fatal damage (Jeong et al., in press). This result agreed with those of Wang et al. (2007), who reported that the microalgae *Spirulina platensis* biomass produced via exposure to a light intensity of 300 μmol m^{-2} s^{-1} was similar to the results observed in a dark condition. Meanwhile, excessive light intensity, i.e., 2400 μmol m^{-2} s^{-1} (Fig. 2a), damaged microalgae because of the overloaded photosystems, bleached pigments, and broken photosystem II (Jeong et al., in press). Cheirsilp and Torpee (2012) found a similar result that microalgae could not grow under the light intensity of 3000 μmol m^{-2} s^{-1}. For moderate light intensities (Fig. 2b–e), higher light intensity achieved greater DW biomass values because adequate illumination is an important factor for microalgae growth (Carvalho et al., 2006). Higher light intensities had deeper light penetration capacities and higher photosynthetic activity in the microalgae culture (Ugwu et al., 2007). The microalgae reproduction capacity was largely dependent on the light wavelengths. The results of this research (Fig. 2) were consistent with those of Matthijs et al. (1996), who reported that red light wavelength was the best light source for the growth of *Chlorella pyrenoidosa*. The green pigment chlorophyll in microalgae could efficiently absorb the red rather than the blue light wavelength (Matthijs et al., 1996). On the one hand, the photons of a shorter wavelength, i.e., blue with a wavelength of 460 nm (Table 1), had a much higher probability of striking the light harvesting complex at its peak electrical energy, resulting in significant energy for photosynthesis that inevitably causes photoinhibition (Das et al., 2011). On the other hand, longer wavelengths, i.e., red, yellow, and white (a combination of red and other growth inefficient light wavelengths) (Table 1), could avoid photoinhibition (Matthijs et al., 1996).

The microalgae-based biogas upgrading processes utilized in this research efficiently removed CO_2 from biogas. The CH_4 content (v/v) in the treatment of higher light intensities (1200, 1600, and 2000 μmol m^{-2} s^{-1}) (Fig. 3) reached the standard of fuel requirements (i.e., higher than 90%, v/v) (Ryckebosch et al., 2011). O_2 content (v/v) was also outside the explosive range of methane [0–4% (v/v)] (Ryckebosch et al., 2011). This phenomenon was attributed to the microalgae developed CO_2-concentrating mechanism (CCM) for acclimating and adapting to changes in both CO_2 and O_2 concentrations. Two main factors were important for CCM, such as the inorganic carbon transporters that facilitate the DIC membrane transport of CO_2 or bicarbonate through the plasmalemma, as well as the chloroplast envelope and carbonic anhydrases that facilitate diffusion by stimulating the indirect supply of CO_2 from outside the cells to Rubisco (Baba and Shiraiwa, 2012). Relatively high O_2 and H_2O (Fig. 3) contents in biogas were not expected to have any significant effect toward microalgae growth, given that raw biogas was always saturated with water. Water and O_2 are also among the compounds used in microalgae photosynthesis (Kubler et al., 1999).

The microalgae-based biogas upgrading process used in this research could efficiently reduce the nutrient in the digestate. Higher light intensities achieved higher nutrient reduction efficiencies (Fig. 4), because adequate illumination intensity was essential for the microalgae cultures, and the nutrient reduction was mainly achieved by the assimilation of microalgae reproduction. The reproduction of microalgae cells required abundant carbon, nitrogen, and phosphorous sources, and the synthesis of nucleic acids, phospholipids, and proteins required luminous energy (Munoz and Guieysse, 2006; Kumar et al., 2010). The high CO_2 concentration in supplied biogas (28.41 ± 2.29%, v/v) and the high initial microalgae cell density (150.84 ± 7.25 mg L^{-1}) could both promote the photosynthetic efficiency of microalgae to reproduce within a shorter time. Thus, more quantity of microalgae biomass could be attained and better nutrient reduction effects from the digestate suspension culture could be achieved (Chiu et al., 2008).

In this research, the effects of biogas upgrading and digestate nutrient reduction were both remarkable. This result was consistent with those of Mallick (2002) and Ho et al. (2011), who suggested that microalgae CO_2 fixation was environmentally sustainable when combined with wastewater treatment process. However, the organic carbon in the culture has been reported to inhibit the autotrophic metabolism of microalgae (Rittenberg, 1969; Hu et al., 2011). This phenomenon could be explained by the theory that the microalgae could use the cumulative ATP from the metabolism of organics in the digestate during the autotrophic metabolism as an energy source and enzyme activator (Moore and Cohen, 1967). Therefore, the CO_2 fixation efficiency was enhanced by the existence of digestate.

5. Conclusions

Red light was the optimal light wavelength for microalgae growth, biogas upgrading, and digestate nutrient reduction. Higher values were attained when exposed to higher light intensity in the range of moderate light intensities (i.e., 800, 1200, 1600, and 2000 μmol m^{-2} s^{-1}). Considering the reduction and economic efficiencies of biogas CO_2 and digestate nutrient reduction as well as the biogas upgrading standard, the optimal light intensity range was determined to be from 1200–1600 μmol m^{-2} s^{-1}. The microalgae-based bag photo-bioreactor utilized in this research could effectively upgrade biogas and simultaneously reduce nutrient content in digestate.

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