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Short Communication

Enhancing the light utilization efficiency of microalgae using organic dyes

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HIGHLIGHTS

- Organic dye can modify the light spectrum differently depending on its property.
- Cell growth and lipid accumulation were increased by the organic dye converter.
- Rates of growth and lipid accumulation were found to differ for each dye.
- The different light spectrum was easily combined by the simple mixing process.

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ABSTRACT

Solar radiation is composed of wide light spectrum including the range which cannot be utilized for microalgae. To enhance the light utilization efficiency, organic dye solutions of rhodamine101 and 9,10-diphenylanthracene were used as wavelength converters. Each dye affected cell growth and lipid accumulation differently, based on the response of each to different light spectrum. Under a light intensity of 50 W/m^2, maximum cell growth (1.5 g/L) was obtained with the red organic dye rhodamine101, whereas best lipid content (30%) with the blue type 9,10-diphenylanthracene. These two separate and complementary traits could be combined by simple mixing, and in so doing optimal growth (1.5 g/L) as well as lipid accumulation (30%) was achieved: lipid productivity was 2.3 times greater than without the organic dye. This study proved that certain organic dye solutions could convert useless wavelengths to be useful for algae cultivation, thereby increasing the productivity of biomass and lipids.

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1. Introduction

Biodiesel is viewed as a green fuel of choice, and even more so when produced with oil from microalgae. Microalgae can consume global warming gas CO_2, and accumulate oil to a high degree through photosynthesis. For example, the most well-known example of oleaginous microalgae *Chlorella vulgaris* contained 14–30% lipid content (Illman et al., 2000).

The growth of these phototrophic microbes is decisively affected by light, in terms of intensity, wavelength, and illumination time. Since their optimal range of light intensity falls between 100 and 200 W/m^2, to be adequate, locations for algae cultivation must be regions with solar radiation of more than 5 kWh/m^2/day, and this consequently limits cultivation to certain low latitude areas (i.e., latitudes below 30) (Williams and Laurens, 2010). To increase the range of cultivatable areas, which is required if algae-based biodiesel is to be produced at a commercial scale, the effective utilization of light, especially dim sources, is essential.

Solar radiation is composed of a wide spectrum of wavelengths, most of which cannot be used by microalgae and are therefore wasted: though variation does exist, typical algal photosynthesis occurs only in blue (400–500 nm) and red regions (600–700 nm) (Seo et al., 2014). These regions are called photosynthesis active radiation (PAR), and they account for less than 15% of total solar radiation. In theory, if the currently unused regions, i.e., wavelengths outside PAR, were made available for photosynthetic activity by the microalgae, the overall productivity of biomass and bio-oil would be greatly improved. It would also make algal cultivation possible in more regions, including those with suboptimal light.

Fluorophores, such as organic dyes, rare earth materials and quantum dots, are capable of converting high energy photons (short wavelength) to lower energy ones (long wavelength), and thus enabling the efficient utilization of solar radiation

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(Mohesenpour et al., 2012). This useful trait has been actively tested and exploited in the areas of physics and chemistry, but only to a limited extent for microalgae-cultivation. Mohesenpour and co-workers (2012) reported an increase of 20% in dry weight when microalgae were cultivated using a polymethyl methacrylate (PMMA) filter coated with Lumogen F dyes. This pioneering work, though meaningful, have the critical limitation of using solid types of fluorescent materials rather than more applicable solution types; what is more, they monitored biomass as a whole, instead of lipid content.

In this study, two organic dye solutions, 9,10-diphenylanthracene (DPA) and Rhodamine 101 (R 101) were chosen as model flourophores to shift wavelengths. DPA and R 101 are able to convert UV light into the blue region, and 500–600 nm wavelengths into the red region, respectively. This study utilized the solution form because it is known to have selective advantages over solid-type converters, including better quantum yield, lower cost, and less toxicity (Zou et al., 2008). Besides, the solution form makes it easy to handle the dyes and prepare different dye-mixtures. The efficacy of the dyes was evaluated in terms of both microalgae growth and lipid accumulation, and the optimal condition for the maximum utilization of the organic dye solution was sought.

2. Methods

2.1. Microalgae strain, media and reactor

C. vulgaris was obtained from the Korea Collection for Type Culture (KCTC), South Korea. BG 11 medium was used for algae cultivation, and it contained the following ingredients in 1 L of deionized water: $NaNO_3$ 1.5 g, $MgSO_4{\cdot}7H_2O$ 75 mg, Na_2CO_3 20 mg, $CaCl_2{\cdot}2H_2O$ 36 mg, Citric acid 6 mg, Na_2EDTA 1 mg, K_2HPO_4 40 mg, Ammonium ferric citrate 6 mg, and trace element solution (Oilgae) 1 mL. The pH of the medium was initially adjusted to 6.5, and then not controlled during the cultivation. CO_2 enriched air (5%, v/v) was flushed into the algae cultivation reactors at an air flow rate of 50 mL/min, and cultivation was done at 27 °C.

An algae cultivation reactor was designed to have two layers (Seo et al., 2014): one for algae cultivation and the other with the organic dye solution. The reactor had a cross sectional area of 10 cm × 10 cm and volume of 250 mL. DPA and R101 were purchased from Sigma, USA. The dyes were dissolved in ethanol and diluted to have an absorbance value of 1.0 at peak wavelength. A dye mixture was made in a volume ratio of 1:1. A control experiment, in which the microalgae cells received light directly from a solar simulator, was prepared by filling the second layer with ethanol instead of an organic dye solution.

2.2. Light source

A solar simulator equipped UV light (Sankyo, Japan) and a visible light (Osram, Germany) was used as a light source. Various light intensities from 50 W/m^2 to 200 W/m^2 were employed for microalgae cultivation. The light intensity and spectrum composition were measured using a high resolution spectrometer (Horiba Jobin Yvon, France).

2.3. Fluorescence properties of organic dyes

Absorbance spectra of organic dyes were measured using a UV–VIS spectrophotometer (Scinco, Korea), and ethanol was used as a blank. A high-resolution micro photoluminescence (PL) system (Horiba Jobin Yvon, France) equipped with He-Cd CW laser (330 nm and 530 nm) was used to observe the fluorescence intensities. Relative quantum yield, which is a parameter of the fluorescent efficiency, was calculated based on the known quantum yield of reference samples. The quantum yield of DPA was measured using quinine sulfate, and R101 was measured using cresyl violet (Zargoosh et al., 2013; Magde et al., 1979).

$$Ø_s = Ø_r(A_rF_s/A_sF_r)(n_s^2/n_r^2) \tag{1}$$

A_s and A_r are the absorbance of the sample and reference solutions, respectively, and F_s and F_r are the fluorescence intensities, and n is the refractive index; the refractive indices of pure ethanol (n_s = 1.3642) and chloroform (n_r = 1.4858) were used.

2.4. Growth rate and photosynthesis efficiency of microalgae

The growth of the microalgae was monitored by measuring absorbance at 680 nm. Dry cell weight was calculated using a calibration curve, which was plotted with the absorbance versus cell dry density. Dry cells were obtained using the following process: (1) Five milliliters of culture was filtered through a pre-dried and pre-weighed filter (0.45 μm, Whatman, USA), (2) each loaded filter was dried at 100 °C until the weight no longer changed, and (3) the change of weight was finally measured.

The photosynthetic efficiency (PE) of microalgae was calculated according to the method described by Gonzalez-Fernandez et al. (2010).

$$\text{PE }(\%) = \frac{\text{Energy recover as biomass}}{\text{Energy supplied as light}} \times 100 \tag{2}$$

$$\text{Energy recovered as biomass} = \text{Biomass (g)} \times \text{carbon content} \times \text{conversion factor} \tag{3}$$

In Eq. (3), carbon content was measured using an element analyzer (Flash 2000 series, Thermo Scientific), and conversion factor was defined as 47.7 kJ per 1 g carbon in dry cell.

2.5. Lipid extraction and analysis

Lipid content and composition were analyzed according to the procedure described by Seo et al., 2012. Cultivated microalgae cells were harvested by centrifugation at 2000 rpm for 10 min, and lyophilized at −52 °C for 2 days. Chloroform: methanol (2 mL, 2:1, v/v) was added to the 10 mg samples of freeze-dried cells, and then vortexed for 20 min. Extracted lipid was converted to Fatty acid methyl esters (FAMEs) through transesterification using methanol (1 mL) as a reactant and sulfuric acid (300 μL) as a catalyst at 100 °C for 20 min. After the reaction, samples were cooled down to room temperature and then centrifuged at 2000 rpm for 10 min. FAMEs in organic phase were analyzed by gas chromatography (HP5890, Agilent, USA) with a flame ionized detector (FID) and INNOWAX capillary column (Agilent, USA, 30 m × 0.32 mm × 0.5 μm). Each fatty acid was identified and quantified by comparing the retention times and peak areas with a FAME Mix C8-C24 (Sigma Aldrich, 18918-1 AMP, USA).

3. Results and discussion

3.1. Light properties of organic dyes

Normalized UV–VIS and PL spectra of the organic dyes are shown in Fig. S1. Compared to the UV–VIS graph, the PL spectrum was shifted more to the right, because of the Stokes shift phenomenon, which is produced by the energy difference between an absorbed photon and re-emitted photon. DPA absorbed light ranging from 300 nm to 400 nm and emitted it at 400–500 nm, whereas R101 absorbed light between 500 nm and 600 nm and emitted it at 550–700 nm.

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