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Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N

Shih-Hsin Ho [a], Chun-Yen Chen [b], Jo-Shu Chang [a,b,c,*]

[a] Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
[b] University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan
[c] Research Center for Energy Technology and Strategy Center, National Cheng Kung University, Tainan, Taiwan

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ABSTRACT

Engineering strategies were applied to improve the CO_2 fixation rate and carbohydrate/lipid production of a *Scenedesmus obliquus* CNW-N isolate. The light intensity that promotes cell growth, carbohydrate/lipid productivity, and CO_2 fixation efficiency was identified. Nitrogen starvation was also employed to trigger the accumulation of lipid and carbohydrate. The highest productivity of biomass, lipid, and carbohydrate was 840.57 mg L^{-1} d^{-1}, 140.35 mg L^{-1} d^{-1}. The highest lipid and carbohydrate content was 22.4% (5-day N-starvation) and 46.65% (1-day N-starvation), respectively. The optimal CO_2 consumption rate was 1420.6 mg L^{-1} d^{-1}. This performance is better than that reported in most other studies. Under nitrogen starvation, the microalgal lipid was mainly composed of C16/C18 fatty acid (around 90%), which is suitable for biodiesel synthesis. The carbohydrate present in the biomass was mainly glucose, accounting for 77–80% of total carbohydrates. This carbohydrate composition is also suitable for fermentative biofuels production (e.g., bioethanol and biobutanol).

1. Introduction

The global environment is currently threatened by a sharp rise in CO_2 emissions, which have a significant impact on climate change (Lopez et al., 2009). In light of this, more attention is being paid to efforts to reduce such emissions, and various physical, chemical, and biological methods have been applied to capture CO_2 (Chen et al., 2011). Among these, the biological method using microalgae is considered one of the most effective and environmental-friendly approaches to fixing CO_2 (de Morais and Costa, 2007a). Microalgae are the fastest growing plants on earth, can grow about 10–50 times faster than terrestrial plants, with a very high CO_2 fixation rate (Chen et al., 2011). Moreover, the organic biomass produced by photosynthetic microalgae and cyanobacteria can be transformed into a wide range valuable products, such as biofuels, food additives, health-care products, and so on, giving additional benefits from the microalgal CO_2 reduction process (Ho et al., 2011).

Recently, liquid biofuels have received wide attention since they are made from non-toxic, biodegradable, and renewable resources, and their use can lead to a decrease in the emission of harmful air pollutants. The most common liquid biofuels to date are biodiesel and bioethanol, which have been mostly produced from food crops (e.g., rapeseed, soybean, and sugarcane). However, producing enough liquid biofuels from these sources to satisfy the existing demand would have a serious impact on food supplies, rainforests, or arable land, and thus there is an urgent need to find new feedstocks (Siaut et al., 2011). Today, many researches are looking to microalgae, which are considered as "third generation biofuels" with several favorable characteristics, such as high biomass and biofuel productivity. In addition to their extremely high cell growth rate and seasonal tolerance, microalgae have relatively higher photon conversion efficiency (photosynthesis efficiency) to fix CO_2, and can effectively accumulate large quantities of lipids (for biodiesel) and carbohydrates (for bioethanol), along with other valuable end-products (Subhadra and Edwards, 2010). Therefore, microalgae could be a promising alternative feedstock for the next generation of biofuels, because they have a relatively high content of energy-rich compounds (carbohydrate/lipids), as well as a high growth rate via cultivation on non-arable land and with non-potable water. In addition, there are no seasonal culture limitations, as they can be harvested daily (Siaut et al., 2011).

To enhance the economic feasibility of microalgal-based biofuel production, it is necessary to improve the microalgal biomass productivity, lipid/carbohydrate content, and overall

* Corresponding author at: Department of Chemical Engineering, National Cheng Kung University, No. 1 University Road, Tainan, Taiwan. Tel.: +886 6 2757575x62651; fax: +886 6 2357146.
E-mail address: changjs@mail.ncku.edu.tw (J.-S. Chang).

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lipid/carbohydrate productivity. The ideal process is that the microalgae are able to produce biofuels (biodiesel/bioethanol) at the highest productivity with the highest lipid/carbohydrate content. Unfortunately, this is not always achievable, because high lipid/carbohydrate contents usually occur under environmental stress (typically nutrient deficiency), which is often associated with relatively low biomass productivity, and thus low overall lipid/carbohydrate productivity (Dragone et al., 2011). As a result, strategies that could achieve the best combination of lipid/carbohydrate content and biomass production rate should be applied, leading to optimization of overall lipid/carbohydrate productivity.

In this study, a microalgal isolate (i.e., *Scenedesmus obliquus* CNW-N) was used to fix CO_2 and also to produce lipid/carbohydrate suitable for making liquid biofuels (Ho et al., 2010). Irradiance intensity and nitrogen starvation were applied to optimize CO_2 fixation and lipid/carbohydrate productivity. Combining CO_2 mitigation and biofuel production systems by using microalgae may provide an innovative alternative to current carbon-reduction and biofuel-production strategies (Subhadra and Edwards, 2010).

2. Methods

2.1. Microalga strain and growth medium

The microalgal species used in this work were obtained from freshwater located in southern Taiwan. The microalgae were identified as *S. obliquus* by their morphology as well as by 23S rDNA sequence matching (details described in previous study). A modified version of Detmer's Medium (DM) was used to grow the pure culture of *S. obliquus*. The medium consisted of (g L^{-1}): $Ca(NO_3)_2{\cdot}4H_2O$, 1.00; KH_2PO_4, 0.26; $MgSO_4{\cdot}7H_2O$, 0.55; KCl, 0.25; $FeSO_4{\cdot}7H_2O$, 0.02; EDTA·2Na, 0.2; H_3BO_3, 0.0029; $ZnCl_2$ 1.1×10^{-4}; $MnCl_2{\cdot}4H_2O$, 1.81×10^{-3}; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1.8×10^{-5}; $CuSO_4{\cdot}5H_2O$, 8.0×10^{-5}. The *S. obliquus* strains were grown under a light intensity of approximately 60–540 μmol m^{-2} s^{-1} (illuminated by TL5 lamp). The light intensity was measured by a Li-250 Light Meter with a Li-190SA pyranometer sensor (Li-COR Inc., Lincoln, Nebraska, USA).

2.2. Operation of photobioreactor

The photobioreactor (PBR) used to perform the microalgal growth and CO_2 fixation experiments was a 1-L glass-made vessel illuminated with an external light source (14 W TL5 tungsten filament lamps; Philips Co., Taipei, Taiwan) mounted on both sides of the PBR. The *S. obliquus* CNW-N strain was pre-cultured and inoculated into the photobioreactor with an inoculum size of 40–45 mg L^{-1}. The PBR was operated at 28 °C, pH 6.2, and an agitation rate of 300 rpm. Serving as the sole carbon source, 2.5% CO_2 with 0.4 vvm was fed into the microalgal culture continuously during cultivation. The liquid sample was collected from the sealed glass vessel with respect to time to determine microalgae cell concentration, pH, lipid/carbohydrate content, and residual nitrate concentration.

2.3. Determination of microalgal cell concentration

The cell concentration of the culture in the PBR was determined regularly by measuring optical density at wavelength 685 nm (denoted as OD_{685}) using a UV/Vis spectrophotometer (model U-2001, Hitachi, Tokyo, Japan) after proper dilution with deionized water to give an absorbance range of 0.05–0.9. The dry cell weight (DCW) of the microalgae biomass was estimated by filtering 50 mL aliquots of culture through a cellulose acetate membrane filter (0.45 μm pore size, 47 mm in diameter). Each loaded filter was dried at 105 °C until the weight was invariant. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight. The OD_{685} values were converted to biomass concentration via appropriate calibration between OD_{685} and dry cell weight and the conversion factor was determined (i.e., 1.0 OD_{685} equals approximately 0.23–0.42 g DCW L^{-1}).

2.4. Determination of growth kinetic parameters and CO_2 fixation rate

Time-course profile of the biomass concentration (X; g L^{-1}) was used to calculate the specific growth rate (d^{-1}) by plotting the dry cell weight on a logarithmic scale versus time. The biomass productivity (P, mg L^{-1} d^{-1}) was calculated according to Eq. (1):

$$P = \frac{\Delta X}{\Delta t} \tag{1}$$

where ΔX is the variation of biomass concentration (mg L^{-1}) within a cultivation time of Δt (d).

Moreover, according to the mass balance of microalgae, the fixation rate of CO_2 (mg L^{-1} d^{-1}) in each PBR was calculated from the relationship between the carbon content and volumetric growth rate of the microalgal cells, as indicated in Eq. (2):

$$\text{CO}_2 \text{ fixation rate } (\text{mg L}^{-1}\ \text{d}^{-1}) = P \times C_{\text{carbon}} \times (M_{\text{CO}_2}/M_{\text{C}}) \tag{2}$$

where P is the biomass productivity (mg L^{-1} d^{-1}); C_{carbon} is the content of carbon in the biomass (g g^{-1}) which was determined by elemental analyzer (Elementar vario EL III); M_{CO_2} is the molar mass of CO_2; and M_C is the molar mass of carbon.

2.5. Determination of the lipid composition

After appropriate cell growth, the microalgae cells were harvested from the culture broth by centrifugation (9000 rpm for 10 min). The cells were washed twice with deionized water, lyophilized, and weighed. The lipid composition was determined as fatty acid methyl esters (FAMEs) through the direct transesterification method proposed by Lepage and Roy (Su et al., 2007). The sample was analyzed using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). Samples were injected into a 30 m long capillary column (Type No. 260M143P, Thermo Fisher Scientific, Waltham, MA, USA) with an internal diameter of 0.32 mm. Helium was used as the carrier gas with a flow rate of 1.3 mL/min. The temperature of the injector and detector were set at 250 and 280 °C, respectively. The oven temperature was initially set at 110 °C, increased from 150 to 180 °C at a rate of 10 °C/min, 180 to 220 °C at a rate of 1.5 °C/min, 220 to 260 °C at a rate of 30 °C/min, and held at 260 °C for 5 min.

2.6. Determination of residual nitrate content

Nitrate concentration was determined according to the modified method reported by Collos et al. (1999). A liquid sample collected from the photobioreactor was filtered using a 0.22 μm pore size filter and then diluted 20-fold with DI water for each sample. The sample was collected and residual nitrate content was determined according to optical density at wavelength of 220 nm (i.e., OD_{220}) using a UV/Vis spectrophotometer (model U-2001, Hitachi, Tokyo, Japan).

2.7. Determination of the carbohydrate content and profile

The carbohydrate content and profile in microalgae were determined using the modified quantitative saccharification (QS) method reported by the National Renewable Energy Laboratory (NREL), USA (Moxley and Zhang, 2007). A small amount of dry algal