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Sufficient utilization of natural fluctuating light intensity is an effective approach of promoting lipid productivity in oleaginous microalgal cultivation outdoors

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HIGHLIGHTS

- Six microalgae were identified and analyzed for growth and lipid accumulation.
- 0.25 g L^{-1} urea was optimal concentration for culture outdoors.
- Lipid accumulation under natural fluctuating light intensities was investigated.
- Lipid yield and neutral lipid content were enhanced by high fluctuating light intensity.
- The reduction in glycolipids contributed to NL accumulation under HFI.

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ABSTRACT

The effects of fluctuating intensity of solar radiation on biomass and lipid in oleaginous microalgae are important. However, this topic has not been the subject of studies for a long time. In this study, four oleaginous microalgae from semi-arid areas were screened and cultivated outdoors under different fluctuating intensities. Results showed that the highest lipid productivities and neutral lipid (NL) contents occurred under high fluctuating intensity (HFI), in which 13–20% of the increased NL came from glycolipid transformation without phospholipid conversion. *Chlorella* sp. L1 and *Monoraphidium dybowskii* Y2 obtained from biological soil crusts in desert had the largest biomass (137.13, 106.61 mg L^{-1} d^{-1}) and lipid yields (35.06, 32.45 mg L^{-1} d^{-1}) under HFI. The highest areal lipid productivities of 9.06 and 8.95 g m^{-2} d^{-1} and better biodiesel quality were observed under HFI. Accordingly, sufficiently adopting fluctuating light intensity outdoors to culture microalgae was an economic and effective approach.

1. Introduction

Given the rapid depletion of fossil fuels and serious environmental problems caused by the extensive use of fossil fuels resources, exploring and employing alternative and environmentally friendly energy resources are receiving considerable attention (William and Laurens, 2010). Alternative energy resources have also become an important strategic direction of global energy structure. Microalgae with various advantages are currently regarded as one of the effective and exceedingly optimistic alternative energy resources (Hu et al., 2008; Griffiths and Harrison, 2009; Siaut et al., 2011). However, cultivating microalgae at large-scale remains to be a problem (Rodolfi et al., 2009), particularly the lipid content decreases with increasing cultivation (Yang et al., 2014). Lipid is biologically considered an energy storage of microalgae; its production generally increases as a result of exposure to stressful environment, such as nitrogen, phosphorus, and iron deficiency, high salinity, and high pH etc. (Courchesne et al., 2009). Although the lipid content is enhanced, biomass is usually low and lipid productivity is constrained under such condition.

Light is the fundamental driving force in the growth of photoautrophic microalgae. In particular, light significantly affects microalgal growth and lipid accumulation (Solovchenko et al., 2008; Liu et al., 2012). Among the effects of light, fluctuations in light intensity are one of the primary factors that influence the generation of high biomass and lipid content in microalgae (Wahidin et al., 2013). Therefore, the sufficient and effective utilization of sunlight during outdoor cultivation deemed extremely potential. However, previous studies presented conflicting results. Some observations

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noted that low light intensity results in the accumulation of lipids and/or triacylglycerols (TAG) (Breuer et al., 2013). By contrast, other studies specified that lipid content can be enhanced by high light intensity (Li et al., 2012; Liu et al., 2012), particularly neutral lipids (NLs) (Sharma et al., 2012; Gwak et al., 2014), and low light essentially increases polar lipids (Hu et al., 2008). Outdoor cultures are subjected daily to cyclic changes in dramatic fluctuations of light intensity. In addition to weather conditions and diurnal cycle, the capability of harvesting light and self-shading in microalgae and the reactor translucent influence lipid accumulation in mass cultivation (Pulz et al., 2001; Simionato et al., 2013). Culture productivity is invariably controlled by the availability of light, particularly when the scale of photobioreactor increases. In fact, more people realize the challenge between cost and productivity in microalgae biodiesel. Thus, according to the local natural light resource, maximizing areal lipid productivity should be a parameter that is better associated for industrializing microalgae biofuels. Investigating the influence of natural light fluctuating intensity on the cultivation of oleaginous microalgae is rarely tested outdoors with photobioreactors; thus, it is still exceedingly relevant to be thoroughly explored.

Given the above conditions, this research intended to realize the following objectives: (1) identify the strains of microalgae isolated from the topsoil of semi-arid areas through morphological features and 18S rDNA sequences; (2) examine their potential in oil-producing; (3) optimize nitrogen concentrations in the form of low-cost urea outdoors; and (4) determine the effect of natural fluctuating light intensity on growth, lipid content, and lipid classes at both 5 L and 140 L scales to obtain the highest lipid productivity with better biodiesel quality in unit area by reasonable arrangement of photobioreactors.

2. Methods

2.1. Strains

The six microalgae used in this study were isolated from the topsoil of semi-arid areas in the northwest of China. Among them, *Chlorella* sp. L1 and *Monoraphidium dybowskii* Y2 were obtained from the biological soil crusts of Tengger desert in Ningxia (37°28′N, 105°00′E); *M. dybowskii* Y1, *M. dybowskii* D, *Chlorella* sp. X, and *Nannochloris* sp. L2 were obtained from the Loess Plateau in Lanzhou City of Gansu (36°02′N, 103°49′E). All microalgae were stored with BG11 medium in FACHB-Collection (Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China).

2.2. Experimental design

2.2.1. Identification

Six strains of microalgae were initially identified by morphology under a microscope and were further determined with 18S rDNA sequences. The total genomic DNA was extracted from algal cells with a DNA isolation kit (TianGen Biotech (Bei Jing) Co., Ltd, China). Polymerase chain reaction (PCR) was performed with general primers 18S-1 (5′-tggttgatcctgccagtagtc–3′ and 18S-2 (5′-tgatccttctgcaggttcacc-3′) to amplify 18S rDNA gene. The PCR products were excised from agarose gel, recovered with the DNA isolation kit, and were then sequenced. Finally phylogenetic analyses were conducted using the NCBI Gene Bank database.

2.2.2. Preliminary screen indoors

Six strains of microalgae were cultivated with 400 mL of BG-11 medium in 500 mL Erlenmeyer flasks. The initial OD was 0.3. Cool white fluorescent tubes acted as light source to provide the light intensity of 100 μmol photons m^{-2} s^{-1}, and the temperature was maintained at 25 ± 1 °C with an air conditioner. Filtered air was supplied to flasks by using an air compressor.

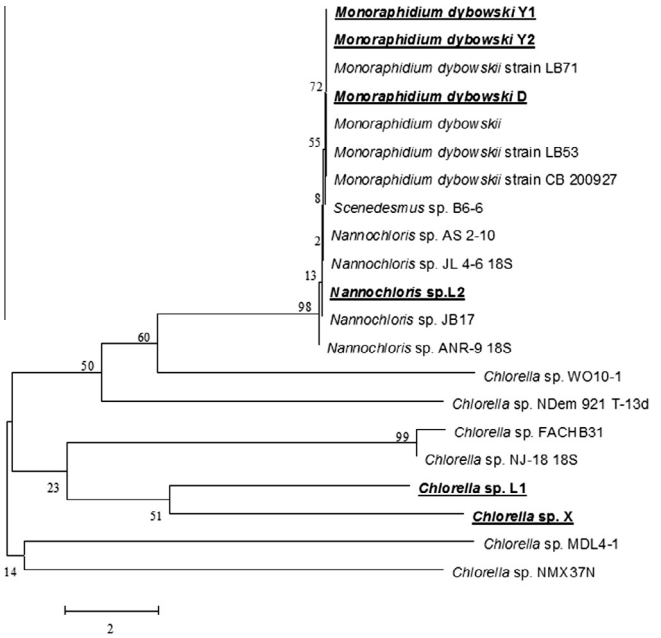

Fig. 1. Maximum-likelihood tree of six microalgae inferred from 18S rDNA gene sequence (strains obtained during this study are underlined). Bootstrap values are shown at the internal nodes for neighbor joining (5000 replications), maximum parsimony (1000 replications) and maximum likelihood (100 replications), respectively, if the node is supported by at least two bootstrap values of 50% or above. Branch lengths correspond to evolutionary distances. A distance of 2 is indicated by the scale.

2.2.3. Cultivation outdoors

The candidate strains were selected based on their lipid productivity. Scale-up experiments were performed during summer in the bioreactors (5-L flask and 140-L photobioreactor), which were placed inside a green house in Beijing, China (40°22′N, 116°20′E). Aeration in 5-L flasks was at a flow rate of 4 L min/L, and the reactor was stirred with a 5 cm magnetic stir bar (mixing at 150 rpm) at the middle of the reactor. The 140-L photobioreactor was composed of two connected 70-L polyvinylchloride hanging bags (22 cm diameter, 180 cm height) (Dalian Huixin Titanium Equipment Development, Co., Ltd) (Xia et al., 2013). The spatial layout was equipped with 80 photobioreactors in an area of 42 m^2. Aeration with 1.8% CO_2 was provided to the photobioreactors by using an air compressor as carbon source at daytime and as pure air at night. The temperature in the greenhouse was controlled by an air conditioner at a constant range between 25 °C and 28 °C.

2.2.3.1. Optimization of urea concentration in 5-L flasks outdoors. The BG-11 medium was used outdoors, but the form of nitrogen source in the culture was substituted by urea, which was optimized in 5-L flasks outdoors. The urea concentrations were set at 0, 0.1, 0.25, and 0.5 g/L. The screened four oleaginous microalgae were cultivated with 3.5 L of modified BG11 medium in 5-L flasks at different urea concentrations to induce lipid accumulation. The microalgae were harvested when the cultures reached the late exponential phase.

2.2.3.2. Experiments on natural fluctuating light intensity. Four oleaginous microalgae were operated in 5-L flasks and 140-L photobioreactors with the optimal 0.25 g/L urea concentration. Three different natural fluctuating light intensity levels were provided to examine the effects of natural fluctuating light intensity. In