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The influence of cultivation period on growth and biodiesel properties of microalga *Nannochloropsis gaditana* 1049

Hu Qunju [a,b], Xiang Wenzhou [a,*], Dai Shikun [a], Li Tao [a], Yang Fangfang [a,b], Jia Qikun [a,b], Wang Guanghua [a], Wu Hualian [a]

[a] Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

HIGHLIGHTS

- A common newly isolated strain of *Nannochloropsis gaditana* was investigated.
- The influence of cultivation period on growth and lipid properties were examined.
- Most of the examined items linear changed with the cultivation period extension.
- Lipid productivity revealed a growth cessation in 16 days.
- All of the biofuel properties satisfied the specifications of biodiesel standard.

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ABSTRACT

This work reported for the first time the detailed impacts of cultivation period on growth dynamics and biochemical composition of a microalga strain *Nannochloropsis gaditana* 1049. The results shown either the biomass accumulation, lipid content, neutral lipid content, monounsaturated fatty acids composition or the favorable fatty acid profile of C16–C18 increased along with the cultivation period extension, but the lipid productivity displayed a decrease since cultured for 16 days, with the highest value reached 289.51 ± 16.34 mg L^{-1} d^{-1}. Biodiesel properties of this microalga also changed with the cultivation period extension, with average unsaturated degree decreased from 1.24 ± 0.03 to 0.59 ± 0.02, cloud point increased from 3.39 ± 0.40 °C to 12.14 ± 0.32 °C, cetane number increased from 54.59 ± 0.20 to 58.96 ± 0.16 and iodine number reduced sharply from 105.15 ± 2.24 gI_2/100 g to 56.44 ± 1.76 gI_2/100 g, which all satisfied the specifications of biodiesel standard.

1. Introduction

Continuously usage of petroleum sourced fuels is now widely recognized as unsustainable and environmental unfriendly because of the depleting supplies and the emission of carbon dioxide into the atmosphere contributing to the worldwide greenhouse effect and global climate change (Wijffels and Barbosa, 2010; Hu et al., 2008). Biodiesel currently derived from oil crops, waste cooking oil or animal fat are of candidates to reduce the consume of fossil fuels, but they cannot realistically satisfy even a small fraction of the existing demand for transport fuels, unfortunately (Chisti, 2007; Matos et al., 2013). Microalga are considered as the only promising feedstock for biodiesel production, greatly exceed that of agricultural oleaginous crops, without competing for arable land (Wijffels and Barbosa, 2010). Biofuels from microalga are indicated to be sustainable, renewable, carbon neutral and transformation cost effectively because there is only a step or two away from lipid to biodiesel (Chisti, 2007). Among all research aspects that the researchers hold their focuses on, isolation of autochthonic appropriate microalga species and optimization of cultivation conditions are of critical importance in enhancing biomass and lipid production, and are the most direct and effective approaches to achieve higher biomass density, contributing to improvement of efficiency and economic benefits of biodiesel production by microalga cultivation (Wijffels and Barbosa, 2010; Roleda et al., 2013; Song et al., 2013).

Selection of appropriate microalga strains is the key consideration in microalgal biodiesel production pipeline (Scott et al., 2010). To evaluate whether a microalga strain is suitable for biodiesel

* Corresponding author. Tel.: +86 020 89023223.
E-mail addresses: huqunju2008@163.com (Q. Hu), xwz@scsio.ac.cn (W. Xiang), deepseabio@163.com (S. Dai), taoli@scsio.ac.cn (T. Li), ycuyang@163.com (F. Yang), wdyywxh@163.com (Q. Jia), wgh@scsio.ac.cn (G. Wang), hlwu@scsio.ac.cn (H. Wu).

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production or not, the key criteria are lipid content, lipid productivity, TAG content in total lipid and suitable fatty acid composition (Griffiths and Harrison, 2009; Talebi et al., 2013). Typically, lipid content is reported as percentage dry weight (% DCW). Although it is in some cases discarded recently, the data are readily available and consistently reported in the literature. Higher lipid productivity was the base of better biodiesel production efficiency in microalga outdoor large-scale cultivation, corresponding with that in pursuing of commercializing feasible biodiesel production, higher lipid productivity was one of the key criteria. Though almost all types of microalgal lipids can be extracted successfully, only triacylglycerol (TAGs) could be easily transesterified into biodiesel using traditional methods (Gong and Jiang, 2011). And when been put under unfavorable environmental or stress conditions for growth, many microalga cells alter their lipid biosynthetic pathways toward the formation and accumulation of neutral lipids (20–50% DCW), mainly in the form of TAGs (Hu et al., 2008; Bougaran et al., 2010). Thus neutral lipid percentage of total lipid could significantly influence the efficiency and quality of biodiesel produced by microalga. Differences in chemical and physical properties among biodiesel fuels can be explained largely by the fuels' fatty acid (FAs) profile. FAs structures show crucial acts on the size distribution and average degree of unsaturation (ADU) of a kind of microalga oil, which could predominantly influence the fuel properties – including kinematic viscosity (KV), specific gravity (SG), cloud point (CP), cetane number (CN), iodine value (IV), and higher heating value (HHV) of the biodiesel transformed from the examined microalga oil (Knothe, 2005, 2009; Hoekman et al., 2012).

Lipid content and FAs composition are always subjected to variability during the growth cycle of many examined microalga species, and the increase of TAGs are often observed during the stationary phase of microalga cultivation (Hu et al., 2008). With the extension of the cultivation period, the nutrients of the culture medium consumed, inhibitors accumulated, overshadow increased, or only the cells turn aged. The aging or senescence of the culture could affect the growth phase of the microalga cells in the cultivation medium (Han et al., 2015), resulting in lipid biosynthesize pathways changed from chloroplast or other cellular membranes to neutral storage lipids, mainly TAGs. For example, analysis of FAs composition in the diatoms *Phaeodactylum tricornutum* and *Chaetoceros muelleri* revealed significant increases in the levels of saturated and monounsaturated fatty acids (SFAs and MUFAs) (e.g. 16: 0, 16:1 and 18:1) and concomitant decreases in the levels of polyunsaturated fatty acids (PUFAs) (e.g. 16:3 and 20:5 (EPA)) with the increasing of cultivation time (Liang et al., 2006). *Nannochloropsis oculata* therefore shown an increase of the EPA proportion in TAGs from 8% during the exponential phase to 68% at the end of stationary phase of growth (Tonon et al., 2002).

Nannochloropsis have attracted sustainable interests from algal biodiesel researchers due to their high biomass accumulation rate, high lipid content, high EPA content (Rodolfi et al., 2009; Doan et al., 2011; Ma et al., 2014; Irina et al., 2006), and their been successfully cultured in large scale using natural sunlight (Radakovits et al., 2012). But much less information is available regarding the FAs profile of their lipids that could serve as biodiesel feedstock. And the influence of cultivation period on the FAs profile of these microalga for biodiesel production is also lack of information.

In this study, a strain of *Nannochloropsis gaditana* 1049, isolated from the coastal waters of South China Sea, was chosen to evaluate the influence of cultivation period on its biodiesel production characteristics by assessing the biomass accumulation, lipid content, lipid production, lipid productivity, lipid distribution and FAs profile. Furthermore, biodiesel properties which were estimated by FAs profile, such as KV, SG, CP, CN, IV, and HHV were also analyzed. The objective of this study was to determine how the cultivation period influence the growth and lipid yield properties of *N. gaditana* 1049, and the ultimate aim was to identify the best cultivation period which as suitable for biodiesel production of this microalga.

2. Methods

2.1. Microalgal strain and cultivation

The marine microalga strain *N. gaditana* 1049 was provided by Key Laboratory of Tropical Marine Bio-resources and Ecology (SCSIO, Guangzhou), isolated from South Chinasea's costal waters (Daya Bay). The *N. gaditana* 1049 cells were cultured in sterilized seawater enriched with modified F medium which contains: 1.5 g $NaNO_3$; 50 mg $NaH_2PO_4{\cdot}2H_2O$; 6.30 mg $FeCl_3{\cdot}6H_2O$; 8.72 mg $Na_2{\cdot}EDTA$; 0.36 mg $MnCl_2{\cdot}4H_2O$; 0.042 mg $ZnSO_4{\cdot}7H_2O$; 0.02 mg $CoCl_2{\cdot}6H_2O$; 0.013 mg $NaMoO_4{\cdot}2H_2O$; 0.013 mg $CuSO_4{\cdot}5H_2O$; 0.001 mg Vitamin B12; 0.001 mg thiamine and 0.2 mg Biotin per liter seawater, and 24 columns of 0.4-L glass bubbled tubes (3.0 cm in diameter) were used for cultivation of this microalga in laboratory scale. The 0.4-L tubes, which contained 0.35 L culture medium, were bubbled with a sterilized air/CO_2 mixture (95/5, v/v) to support growth and maintain pH of the culture medium within the desired range (7.8 ± 0.2). Culture temperature was controlled at 25 ± 1 °C by air-condition. Continuous artificial illumination (250–300 μmol photons m^{-2} s^{-1}) was provided by daylight fluorescent tubes on one side of the tubes.

2.2. Algal growth and biomass determination

Parameters determined to monitor the microalga cell concentration were optical density (OD) using a Thermo UV–vis spectrophotometer at the absorbance of 750 nm (three replicates) (Makridis and Vadstein, 1999). The initial cell density of *N. gaditana* 1049 in all the columns were about 0.5 at OD_{750} with initial pH 8.0, and cell density was monitored by OD_{750} after cultured for 1, 3, 5, 7, 9, 11, 13, 16, 19, and 21 days to measure the growth rate of the microalga. To investigate the induction of lipid, carbohydrates and protein synthesis characteristics changing with the cultivation period, the microalgal cells cultivated in the tubes were designed to harvest since day 7. At the same time, dry cell weight (DCW) was measured in the harvested columns to estimate the influence of cultivation period on biomass accumulation (Yang et al., 2014; Ma et al., 2014). Each designed harvesting experiment was performed in three replicates, and then harvested by centrifugation (5000g × 5 min) and freeze-dried the wet algal pellet immediately, the dry biomass was analyzed immediately or stored at −20 °C for up to 10-days prior to analysis.

The specific growth rate (k) of each period was characterized based on the OD_{750} using the equation reported by Wood et al. (2005). Biomass productivity (BP) was also determined by the biomass accumulation and cultivation time and calculated.

$$k = \frac{\ln^{N} - \ln^{N_0}}{t - t_0} \quad (1)$$

where k (d^{-1}) was the specific growth rate during the designed cultivation period. N was the cell density (OD_{750}) in the culture medium when the cells were to be harvested at t. N_0 represented the OD_{750} value at the beginning of inoculation time (t_0).

2.3. Determination of lipid content

To extract the intracellular lipids from the microalga, the method previously optimized by Khozin-Goldberg et al. (2005) was adopted. With which a mixture of methanol-dimethyl sulphoxide (9:1, v/v), diethyl ether and hexane (1:1:1, v/v/v) were