Bioresource Technology 184 (2015) 100–107

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition

Tse-Shih Lin, Jane-Yii Wu *

Department of Bioindustry Technology, Da-Yeh University, No. 168, University Rd., Dacun, Changhua 51591, Taiwan

HIGHLIGHTS

- Reports a novel microalgal isolate *Chlorella* sp. Y8-1.
- The high lipid content was obtained under mixotrophic condition.
- Fatty acid compositions of microalgae Y8-1 are appropriate for biodiesel production.

ARTICLE INFO

Article history:
Received 2 September 2014
Received in revised form 2 November 2014
Accepted 3 November 2014
Available online 18 November 2014

Keywords:
Chlorella sp.
Mixotrophic
Lipid
Fatty acid
Biodiesel

ABSTRACT

In order to produce microalgal lipids that can be transformed to biodiesel fuel, one isolate with high lipid content was identified as *Chlorella* sp. Y8-1. The growth and lipid productivity of an isolated microalga *Chlorella* sp. Y8-1 were investigated under different cultivation conditions, including autotrophic growth (CO_2, with light), heterotrophic growth (sucrose, without light) and mixotrophic growth (organic carbon sources and CO_2, with light). Mixotrophic *Chlorella* sp. Y8-1 showed higher lipid content (35.5 ± 4.2%) and higher lipid productivity (0.01 g/L/d) than *Chlorella* sp. Y8-1 cultivated under autotrophic and heterotrophic conditions on modified Walne medium. Fatty acid analysis of *Chlorella* sp. Y8-1 showed the major presence of palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acids (C18:3). The main fatty acid compositions of the *Chlorella* sp. Y8-1 are appropriate for biodiesel production.

1. Introduction

The rapid development of human activity and the precipitous consumption of fossil fuels have caused an energy crisis, constituting a major problem in the twenty-first century. Vasudevan and Briggs (2008) predicted that the crude oil and natural gas reserves on earth will be depleted in 40 and 64 years, respectively (Vasudevan and Briggs, 2008). Moreover, the over-consumption of fossil fuels releases substantial amounts of greenhouse gases, inducing climate change and global warming. Therefore, sustainable, renewable, and carbon–neutral energy sources must be exploited to replace fossil fuels.

Biodiesel is a nontoxic, renewable, and environmentally friendly energy source. Compared with conventional oil crops, microalgae are promoted as an ideal bioenergy feedstock because they exhibit a more rapid growth rate, demonstrate higher photosynthetic efficiency, can potentially produce considerably higher areal oil yields, do not compete with food or feed crops, and can be grown on barren land. Therefore, microalgae-based biodiesel has attracted increasing attention worldwide.

However, the relatively high cost of microalgae-based biodiesel production is a major obstacle to the commercial application of this biotechnology. Increasing biomass concentration, lipid content per microalgal biomass, and lipid productivity are necessary for the economic feasibility of microalgae-based biodiesel production (Ho et al., 2012). Today, photoautotrophic culture is the most common strategy for cultivating microalgae. But the photoautotrophic culture exists severely limiting biomass production because of cellular self-shading that hinders light availability towards the end of growth. The low cell concentration gained in the photoautotrophic culture increases the biomass harvesting cost. A feasible alternative strategy to improve the efficient use of light or eliminate its requirement by cells and so reduce the costs of microalgal biomass production would be a mixotrophic culture of microalgae. Mixotrophic growth requires relatively low light intensities and, consequently, can reduce energy costs (Fernández Sevilla et al., 2004). The mixo-

* Corresponding author. Fax: +886 4 8511323.
E-mail address: jywu@mail.dyu.edu.tw (J.-Y. Wu).

http://dx.doi.org/10.1016/j.biortech.2014.11.005
0960-8524/

trophic culture regime is a variant of the heterotrophic culture, where CO_2 and organic carbons are simultaneously assimilated and both respiratory and photosynthetic metabolism operates concurrently (Lee, 2004).

Many studies have been published in this research field. Previous studies have confirmed that the lipid content in some microalgae can be enhanced by applying various cultivation conditions such as nitrogen or phosphate starvation (Khozin-Goldberg and Cohen, 2006), and high light intensity (Cheirsilp and Torpee, 2012). Among these methods, using carbon and nitrogen sources are known to affect the metabolism of lipids and fatty acids in various microalgae. In addition, applying carbon and nitrogen conditions is simple and more cost-effective than using other methods. Therefore, the types and concentrations of carbon and nitrogen sources are critical in enhancing the lipid productivity of biodiesel production (Jeon et al., 2006). Some microalgal species, including the *Chlorella* (Zheng et al., 2012), *Chlamydomonas* (Chen and Johns, 1994), and *Nannochloropsis* species (Hu and Gao, 2003), have been reported to accumulate amounts of lipid in cells under various culture conditions. However, there are few studies on the effects of carbon resources, especially, the effects of carbon sources on the biomass production and lipid component of algae under mixotrophic cultivation (Andrade and Costa, 2007; Bouarab et al., 2004). Therefore, marine microalgae in seawater around Taiwan that exhibit high lipid productivity were isolated in this study to observe the effects of carbon and nitrogen sources on the growth and lipid productivity of the isolated microalgae under mixotrophic conditions.

2. Methods

2.1. Collection of samples, establishment and identification of algal strains

The microalgae samples were collected from seawater around Taiwan, stored in sterile centrifugal tubes, and sent to the laboratory within 3 d for algal cell isolation. Walne medium plates were prepared using full-strength seawater, containing 18 g/L of agar and 1 g/L of glucose. After inoculation, the plates were cultured at 30 °C for 2–7 d. Single colonies composed of spherical cells atypical of either yeast, fungi, or bacteria were extracted and carefully transferred to a new plate.

After becoming established, these algal strains were identified according to their 18S rRNA gene sequences, as well as some morphological characteristics. For morphological observation, cells from each strain were observed using a light microscope (ESPA, Taiwan).

For obtaining the DNA sequences of the 18S rRNA gene from one strain, a single colony of the strain grown on an agar plate was carefully transferred to a 50-mL tube containing 1 g/L of glucose and 10 mL of a Walne liquid medium prepared using seawater. The culture was then cultivated at 30 °C for 1 wk with continuous aeration (10% CO_2, 0.5 vvm). The algal cells were collected using centrifugation (5000 rpm × 5 min), rinsed with 5 mL of deionized water, and lyophilized prior to performing DNA sequencing.

The amplified 18S rRNA gene in the genomic DNA of algal cells was obtained and sent to Mission Biotech (Taipei, Taiwan) for DNA sequencing. The resulting 18S rRNA gene sequences were aligned and compared to the nucleotide sequences of known microorganisms in the GenBank database of the National Center for Biotechnology Information by using a Basic Local Alignment Search Tool (BLAST). The samples were also analyzed using MEGA 4.1 software (Tamura et al., 2007) and by employing the multiple alignment program CLUSTAL W to construct a neighbor-jointing (NJ) tree. The bootstrap values were obtained from 1000 replications of NJ analyses (Burja et al., 2006).

2.2. Serum bottle cultivation of isolated microalgae Y8-1

The microalga Y8-1 was cultivated in a 1 L serum bottle with a working volume of 0.8 L at 30 °C by using an exponentially growing seed culture, and 4300 lux of light intensity was adopted. Aeration was achieved by sparging air enriched with 10% CO_2 at 2 vvm. During microalgal growth, the liquid sample was collected from the serum bottle with respect to time to determine microalgal biomass concentration, pH, residual sugar concentration and lipid content of the microalgal biomass.

The microalga Y8-1 was grown in the serum bottle on modified Walne media. The modified Walne media was composed (per liter) of 30 g of malt salt, 2 mg of $NaH_2PO_4{\cdot}2H_2O$, 4.5 mg of Na2EDTA, 3.36 mg of H_3BO_3, 0.036 mg of $MnCl_2{\cdot}4H_2O$, 0.13 mg of $FeCl_3{\cdot}6H_2O$, and nitrogen sources (urea). At the same time, different carbon sources were also used for the growth of microalga Y8-1. Inorganic carbon (CO_2) was used as the carbon source in photoautotrophic cultivation, while organic carbon (sucrose) was used in heterotrophic cultivation. Mixotrophic cultivation, which means the microalgae could undergo photosynthesis and simultaneously use both organic (fructose, glucose, glycerol, sucrose, and xylose) and inorganic carbon (CO_2) as carbon sources, was also investigated in this study. The effects of these cultivation conditions on microalgae growth and lipid production were investigated.

2.3. Analytical method

Biomass was determined by measuring the OD of each sample at 680 nm (OD_{680}). The dry cell weights of the diluted samples were then detected and measured for plotting the standard curve. The amounts of total sugar were estimated by the phenol–sulfuric acid assay method of Dubois et al. (1956) using fructose, glucose, sucrose, and xylose standard calibration curves, respectively (Dubois et al., 1956). The glycerol concentration was determined using high-performance liquid chromatography (HPLC; Young Lin Acme 9000 HPLC).

2.4. Total lipid extraction

The total lipid content (dry weight) was measured by employing a modified version of the method used by Bligh and Dyer (1959) (Bligh and Dyer, 1959). After the cultivation was complete, the culture medium was centrifuged at 9000 rpm and 4 °C for 2 min; the cell pellets were then collected for freeze drying. The samples were pulverized after drying by using a homogenizer, and were extracted using a chloroform–methanol mixture (1:2 v/v). Approximately 15 mL of solvents was used for 50 mg of dried samples in each extraction step. After the samples were mixed using a vortex mixer for 1 min, they were ultrasonicated for 3 h and centrifuged at 3000 rpm for 10 min. The solid phases were separated carefully using Whatman No. 1 filter paper, and the solids were washed using 5 mL of chloroform. After this process, 9 mL of sterilized water was added to a solvent phase, and the solvent was mixed using a vortex mixer. The solvent phase was centrifuged at 3000 rpm for 10 min, and the chloroform layer was collected. The weight of the lipids was measured after removing the solvent by using a nitrogen blowing concentrator; the lipid content was then calculated.

2.5. Fatty methyl esters and fatty acid analysis

To observe the saponification/esterification reactions, each of the samples were mixed with 2 mL of NaOH–methanol solution and disrupted using a sonicator, heated in a 100 °C water bath for 10 min, and cooled to room temperature. The samples were