Journal of the Taiwan Institute of Chemical Engineers 000 (2016) 1–5

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Short Communication

Enhancing biomass and oil accumulation of *Monoraphidium sp.* FXY-10 by combined fulvic acid and two-step cultivation

Raoqiong Che[a,1], Ke Ding[a,1], Li Huang[b], Peng Zhao[a], Jun-Wei Xu[a], Tao Li[a], Huixian Ma[c], Xuya Yu[a,*]

[a] *Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, China*
[b] *Institute of Chemical Industry, Kunming Metallurgy College, Kunming, China*
[c] *School of Foreign Languages, Kunming University, Kunming 650200, China*

ARTICLE INFO

Article history:
Received 3 March 2016
Revised 14 June 2016
Accepted 25 June 2016
Available online xxx

Keywords:
Monoraphidium sp. FXY-10
Fulvic acid
Biomass
Lipid content
Heterotrophic–photoautotrophic two-stage

ABSTRACT

The combination of combined fulvic acid (FA) and two-step cultivation was proposed as a novel strategy to enhance biomass and oil accumulation of *Monoraphidium sp.* FXY-10 compared with the traditional monoculture of microalgae. In this study, the effect of FA on the growth and lipid accumulation of *Monoraphidium sp.* FXY-10 was investigated using one- and two-stage cultivation. In the heterotrophic one-stage cultivation, 1.5-fold biomass was enhanced with 80 mg L^{-1} FA (6.41 g L^{-1}) compared with the control biomass (4.21 g L^{-1}). In the heterotrophic–photoautotrophic two-stage cultivation, different FA concentrations were used to stimulate lipid accumulation. The lipid content increased from 30.78% to 54.65% when 25 mg L^{-1} FA was added to the culture. The main fatty acids in the FA-triggered *Monoraphidium sp.* FXY-10 were C16/C18 fatty acids (97%), which are appropriate for biodiesel production. Results showed that the proposed strategy is useful for biomass and oil production of *Monoraphidium sp.* FXY-10.

1. Introduction

Microalgal biomass has been considered as a promising feedstock for alternative fuel production because of its high lipid content, short growth cycles, and renewable and clean characteristics [14,16]. However, microalgal biofuels are economically unfeasible as fossil fuel substitute because of their low biomass, poor lipid yield, and expensive lipid production [8]. Hence, biomass and lipid productivity should be increased, and biodiesel production costs should be reduced [4]. The biomass and lipid contents of algae can be increased by changing the cultivation conditions, such as CO_2 sparging, illumination, temperature, and nutrient concentration [5].

Moreover, manipulating microalgal cultivation is necessary to enhance the biomass and lipid contents. Photoautotrophic, heterotrophic, and mixotrophic cultivation strategies are major microalgal cultivation methods to produce biodiesel. However, the optimization of cultivation methods for both biomass yield and lipid production of microalgae is a contradictory problem. Therefore, different cultivation methods should be combined to improve the biomass yield and oil content. Two-stage photoautotrophic cultivation strategies can improve the cell biomass yield and oil content [15,18]. Nevertheless, the cell yield of two-stage photoautotrophic cultivation strategies is less than that of heterotrophic culture strategies. In this case, two-stage heterotrophic–photoautotrophic cultivation strategies are very promising approaches [8] that can increase the biomass density in stage I (heterotrophic) and induce lipid accumulation in stage II (photoautotrophic). In stage I, microalgae usually grow under suitable conditions, such as adequate nutrition and illumination. Microalgal growth is also induced by plant growth regulators. In stage II, microalgae are usually subjected to stressful conditions, such as nitrogen and phosphorus deficiency, high salinity, excessive light, and inducer additions (Fe, NaAC, phytohormones, and humic substances) [11].

Fulvic acid (FA) is a plant growth regulator involved in increasing the cell membrane permeability, oxygen uptake, photosynthesis, respiration, and phosphate uptake, as well as in controlling the hormone levels and enhancing the secondary metabolites [3,12]. Zhao et al. [19] reported that FA can promote biomass and astaxanthin accumulation in *Haematococcus pluvialis*. However, limited information is available regarding the effect of FA on the growth and oil production of oleaginous microalgae, particularly *Monoraphidium sp.*

Monoraphidium sp. FXY-10, as a prominent biofuel feedstock, was proposed because of its adaptability to various culture

* Corresponding author.
E-mail address: xuya_yu@163.com (X. Yu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jtice.2016.06.035
1876-1070/

conditions and high biomass (heterotrophic) and lipid content (photoautotrophic). However, the optimization of cultivation conditions for both biomass yield and lipid production of *Monoraphidium sp*. FXY-10 is conflicting [17]. Thus, the present study investigated the effects of FA on the growth, total oil content, and fatty acid profiles of *Monoraphidium sp*. FXY-10 and then evaluated the usefulness of FA addition-based two-stage cultivation technology.

2. Materials and methods

2.1. FA, microalgal strain and cultivation condition

The FA in this study was the same as that used by Zhao et al. [19]; this FA was purchased from Shang Cheng Biotechnology Company (Yunnan, China). *Monoraphidium sp*. FXY-10 was provided by the Biorefinery Laboratory of the Kunming University of Science and Technology [17]. Our previous research has shown that Kuh1 medium with FA was more advantageous than BG11 to the growth and oil accumulation of algal cells. Therefore, Kuh1 medium was selected for the autotrophic and heterotrophic culture of *Monoraphidium sp*. FXY-10. The pH of the medium was adjusted to 6.8–7.0 before the addition of FA. For the heterotrophic culture, 10 g L^{-1} glucose was added in the Kuh1 liquid medium. *Monoraphidium sp*. FXY-10 was primarily activated by inoculating 30 mL (6×10^6 cells mL^{-1}) of the heterotrophic grown cells in a 500-mL Erlenmeyer flask with 300 mL of the heterotrophic medium and then cultivated in the dark. The flasks were incubated on a rotary shaker at 150 r min^{-1} at 25 °C. For the photoautotrophic culture, the cells were grown under continuous fluorescent illumination at 3500 lx. A 200 mL Kuh1 medium in a 500-mL flask was inoculated in the heterotrophic algal cell culture with an initial cell concentration of approximately 7.06×10^7 cells mL^{-1}. The flasks were incubated on a rotary shaker, and the rotational speed and culture temperature were the same as those in the abovementioned methods.

2.2. Experimental conditions

For the FA concentration optimization in the one-stage cultivation, cells were grown in Kuh1 medium with different initial FA concentrations of 0, 40, 80, 120, and 160 mg L^{-1} throughout 12 days of growth period. For the heterotrophic cultivation in stage I of the two-stage cultivation experiment, cells were first grown in Kuh1 medium, and 80 mg L^{-1} FA was subsequently added. Cells were harvested via centrifugation and then washed thrice with distilled water as seeds for the second stage. Supernatants were removed when the cultures reached the late exponential phase at a biomass concentration of approximately 6.40 g L^{-1}. In stage II of the photoautotrophic cultivation, cells were grown in Kuh1 medium at different initial FA concentrations of 0, 5, 25, 125, and 625 mg L^{-1} and then harvested within a 2-day interval.

2.3. Analytical procedures

2.3.1. Measurement of cell dry weight

Generally, microalgae dry cell weight (DCW) is correlated with optical density (OD) at a certain wavelength from 450 nm to 900 nm. In this study, the biomass concentration of *Monoraphidium sp*. FXY-10 was determined by measuring the OD of 750 nm (OD_{750}) by using an ultraviolet photo-spectrometer. The result was converted to DCW concentration by using the calibration curve of OD750 nm, as expressed in Eq. (1):

$$Y = 0.3226\times;\ R^2 = 0.9997 \quad (1)$$

where Y represents the dry weight, and X represents the absorbance of OD750 nm.

2.3.2. Lipid analysis

Total lipid extraction from dry biomass was performed as described by Bligh and Dyer [2]. Specific operational steps were recommended by Yu and Zhao [17]. Lipid content was calculated as follows:

$$\text{Lipid content } (\%) = W_L/W_A \times 100\% \quad (2)$$

where W_L (g) is the weight of the extracted lipids, and W_A (g) is the dry algae biomass.

Lipid productivity was calculated as follows:

$$P_{\text{Lipid}}\left(\text{mg L}^{-1}\text{d}^{-1}\right) = W_g \times C_{\text{lipid}}(\%)/V_L \times T_d \quad (3)$$

where P_{Lipid} is the lipid productivity, C_{Lipid} is the lipid content, W_g is the dry cell weight, V is the working volume, and T is the cultivation time.

2.3.3. Analysis of fatty acid methyl ester

The lipids extracted for fatty acid analysis were obtained from the maximal lipid content of the cultures (*i.e*., FA supplementation in stage II of 5 mg L^{-1} for 8 days, 25 mg L^{-1} for 6 days, 125 mg L^{-1} for 6 days, 625 mg L^{-1} for 2 days, and 80 mgL^{-1} FA in stage I for 8 days). Esterification and analysis of fatty acids were conducted using the modified method of Yu and Zhao [17].

2.3.4. Statistical analysis

All data were generated from three independent fermentations (samples were taken from three independent flasks). Data were recorded as means ± standard error of mean (SEM), which were statistically analyzed by one-way ANOVA (SPSS 19.0). Significant differences were identified by post-hoc Tukey's honestly significant difference (HSD) test. The level of significance for all comparisons was $p < 0.05$.

3. Results and discussion

3.1. Influence of initial FA concentration on biomass and lipid content in one stage

In order to standardize the optimum concentration of FA for *Monoraphidium sp*. FXY-10 growth, five different concentrations of FA (0, 40, 80, 120, and 160 mg L^{-1}) were tested. After 8 days of cultivation (Fig. 1), an increase in FA concentration from 0 mg L^{-1} to 80 mg L^{-1} led to a marked increase in the biomass concentration from 4.21 g L^{-1} to 6.41 g L^{-1} and biomass productivity from 250.66 mg L^{-1} day^{-1} to 422.83 mg L^{-1} day^{-1} (Table 1). The biomass concentration slightly decreased to 6.18 g L^{-1} when the FA concentration further increased to 120 mg L^{-1}. Cell growth was inhibited when the FA concentration reached 160 mg L^{-1}. The results suggested that an appropriate FA concentration in the culture medium can enhance the biomass production of microalgae, and high FA concentrations can inhibit the growth of *Monoraphidium sp*. FXY-10. After 8 days, the results showed that FA significantly ($p < 0.05$) promoted cell growth by approximately 1.52-fold compared with the control treatment. Zhao et al. [19] also observed a 7.13% and 10.58% increase in the biomass concentration of *H. pluvialis* with the supplementation of 5 and 10 mg L^{-1} FA in the medium, respectively. As a plant growth regulator, FA can promote cell growth and secondary metabolite accumulation [6]. FA can be active on surfaces and can promote nutrient uptake and cell membrane permeability. Additionally, FA can serve as an active structure functioning similar to plant hormones [1].

The lipid content of *Monoraphidium sp*. FXY-10 varied with the FA concentration in the culture and showed an evident trend (Table 1). The highest oil content was obtained by the exogenous addition of 120 mg L^{-1} FA in the culture, which increased by 1.8-fold compared with the control treatment. This finding showed

Please cite this article as: R. Che et al., Enhancing biomass and oil accumulation of *Monoraphidium sp*. FXY-10 by combined fulvic acid and two-step cultivation, Journal of the Taiwan Institute of Chemical Engineers (2016), http://dx.doi.org/10.1016/j.jtice.2016.06.035