Biomass and Bioenergy 92 (2016) 55–60

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Assessment of triacylglycerol content in *Chlorella vulgaris* cultivated in a two-stage process

Raquel Rezende dos Santos [a, *], Claudete Norie Kunigami [b], Donato Alexandre Gomes Aranda [c], Cláudia Maria Luz Lapa Teixeira [a]

[a] Laboratório de Biotecnologia de Microalgas, Divisão de Energia, Instituto Nacional de Tecnologia, CEP: 20081-312, Rio de Janeiro, RJ, Brazil
[b] Laboratório de Análise Orgânica Instrumental, Divisão de Química Analítica, Instituto Nacional de Tecnologia, CEP: 20081-312, Rio de Janeiro, RJ, Brazil
[c] Laboratório de Tecnologia Verde, Departamento de Engenharia Química, Escola de Química, Centro de Tecnologia, Universidade Federal do Rio de Janeiro, CEP: 21941-590, Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
22 January 2016
Accepted 13 May 2016

Keywords:
Chlorella vulgaris
Biomass productivity
Nitrogen starvation
Triacylglycerol (TAG) content
Two-stage process

ABSTRACT

Chlorella vulgaris cultivation in two-stage process was applied to increase the lipid productivity without compromising the biomass productivity. At the first stage, microalgae was cultivated under nutrient sufficient conditions to obtain a maximized cell density; at the second stage, nitrate conditions are changed to trigger the accumulation of TAG. During first stage, the maximum biomass productivity (32 mg L^{-1} d^{-1}) was observed after 13 days under nutrient sufficient conditions with 1.21 g L^{-1} $NaNO_3$ and 0.00449 g L^{-1} K_2HPO_4. Maximum lipid content (25.4% DW), lipid productivity (7.5 mg L^{-1} d^{-1}) and TAG content (41.3% in total lipids) were favored by the nitrogen starvation conditions for more 4 days, at the second stage. Oil extracted at the second stage contained lower percentage of PUFAs being more suitable for the biodiesel production when compared with the oil extracted at the first stage. This two-stage phototrophic process is promising to provide a more efficient way for on a large-scale production of algal biomass and biodiesel production.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that grow at an exceptional fast rate in open oceans, rocky shores, and freshwater habitats including rivers, lakes, ditches and ponds [1]. Microalgae biodiversity is enormous and represents an almost untapped resource [2]. This biodiversity permits the use of microalgae in biotechnology applications as pharmaceutical and nutraceutical products as well as raw material for biofuels production [1].

Microalgae lipids have attracted attention as future raw materials for biodiesel synthesis, among other factors, due to the microalgae potential of attaining higher lipid productivity in relation to the oilseed crops and modulation of the biochemical composition of the microalgae biomass by varying growth conditions [3]. Accordingly, the choice of the most appropriate condition for the microalgae cultivation is of paramount importance to the viability of the process.

Usually, biochemical composition of microalgae in exponential phase and growth in batch respects to the following order: protein > carbohydrate = lipid > nucleic acid. However, under conditions of nutritional stress and aging culture, this ratio can change with the increase of lipids and decrease of the protein concentration [4].

Being the nitrogen essential for the synthesis of amino acids, limiting conditions of this nutrient makes it impossible to the synthesis of other proteins involved in the cell division. When cells are no longer able to divide, lipids are synthesized and stored within cells as an energy source [5]. For this reason, it is easy to realize that the amount of nitrogen is an important intracellular factor involved in lipid biosynthesis. On one side nitrogen starvation favors biosynthesis of intracellular lipids in cells already developed, on the other, it can affect the cell growth of new cells. In this sense, increasing the lipid content and getting biomass simultaneously become a challenge for researchers.

A two-stage process strategy can be an option for enhancing lipid productivity [6]. In order to increase the biomass productivity,

* Corresponding author.
E-mail addresses: raquel.c.rezende@gmail.com (R.R. dos Santos), claudete.kunigami@int.gov.br (C.N. Kunigami), donato@eq.ufrj.br (D.A. Gomes Aranda), claudia.teixeira@int.gov.br (C.M. Luz Lapa Teixeira).

http://dx.doi.org/10.1016/j.biombioe.2016.05.014
0961-9534/© 2016 Elsevier Ltd. All rights reserved.

a high density culture of microalgae can be obtained under favorable conditions of temperature [7], light intensity [8], CO_2 concentration [9] and other nutrients with high mass transfer rates. In relation to the increase of the lipid content in cells, other cultivation conditions such as nitrogen deprivation [8–10], phosphate limitation [11] and iron supplementation [11] have been tested. In some works, two-stage process strategy involves the application of heterotrophic cultivation [12–14] at the second stage, being used glycerol, glucose, sucrose or wastewater derived from industrial and agricultural as organic carbon.

Microalgae produce a large variety of lipid like compounds, such as waxes, sterols, hydrocarbons, and glycerolipids. Glycerolipids are characterized when carboxylate end of the fatty acid molecule is bonded to an uncharged head group (i.e. glycerol) and can be divided into two large subclasses based on their specific function (i.e. energy storage or cell membrane lipids). Cell membrane lipids consists of fatty acids ester bonded to a glycerol backbone and is categorized according to its number of fatty acids. Lipids with three fatty acyl groups attached to glycerol backbone are known as triacylglycerols (TAG) [15].

TAG is the major compound of the oils used for biodiesel production and therefore the main raw material for its production. Other lipid molecules, which have fatty acid in its structure, can also be a raw material for biodiesel production in principle. Lipids as hydrocarbons and sterols can not be used for biodiesel production (fatty acid methyl esters). In this case, hydrocarbon it is possible to produce a bio-oil with fuel properties but it is not appropriately a biodiesel. Thus, it is very important to quantify this compound in total lipids to predict the potential of microalgae biodiesel. In fact, when there is a total lipid value and comparisons among different results from different cultivation conditions might sub or super estimate the potential for biodiesel production since in these total lipids can have different percentages of TAG [16]. Several investigations focus on the total lipids determination to indicate biodiesel production potential in studies where two-stage process was carried out but in many cases, TAG content in total lipids has not been considered.

In the present study, two-stage process strategy was applied in order to enhance lipid productivity. At the first stage, microalgae was cultivated under nutrient sufficient conditions to obtain a maximized cell density. At the second stage, nitrate conditions are changed to trigger the accumulation of TAG. Keeping in mind the importance of TAG determination for a real potential for biodiesel production, evaluation of this compound was quantified in total lipids extracted from algal biomass.

2. Materials and methods

2.1. Strain and maintenance

Microalgae *Chlorella vulgaris* was kindly donated by Dr. Armando A. H. Vieira from UFCar, Brazil. Strain was preserved in tubes containing 8 mL of WC medium [17] sterilized by autoclaving at 121 °C for 20 min. Tubes were kept in germination chamber under photon flux density of 20 μmol photons m^{-2} s^{-1} provided by fluorescent lamps and 21 ± 1 °C manually shaken every 48 h.

WC medium was composed of TRIS buffer (0.5 g L^{-1}), $NaNO_3$ (0.085 g L^{-1}), $NaHCO_3$ (0.0126 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.03676 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.03697 g L^{-1}), K_2HPO_4 (0.00871 g L^{-1}), 1% H_3BO_3 (0.1 mL L^{-1}), vitamin solution (1 mL L^{-1}) and trace metals solution (1 mL L^{-1}). Initial pH was adjusted to 8.5 with HCl 1 M. Vitamin solution was composed of thiamine (0.1 g L^{-1}), cyanocobalamin (0.0005 g L^{-1}) and biotin (0.0005 g L^{-1}) being filtered through 0.22 μm membrane (SARTORIUS®). Trace metals solution was composed of $CuSO_4{\cdot}5H_2O$ (0.0098 g), $ZnSO_4{\cdot}7H_2O$ (0.022 g), $CoCl_2{\cdot}6H_2O$ (0.01 g), $MnCl_2{\cdot}4H_2O$ (0.18 g), $NaMoO_4{\cdot}2H_2O$ (0.0063 g) and chelated iron (1 L). Chelated iron was composed of Na_2EDTA (4.36 g L^{-1}) and $FeCl_3{\cdot}6H_2O$ (3.5 g L^{-1}).

2.2. Microalgae cultivation process

2.2.1. First stage cultivation: nutrient sufficient conditions

To obtain the inoculum, cells were grown in 500-mL Erlenmeyer flasks containing 300 mL of WC medium. Flasks were kept under constant agitation of 180 rpm (MARCONI 140 CFT – 25 mm orbital motion), photon flux density of 100 μmol photons m^{-2} s^{-1} provided by fluorescent lamps and 25 ± 2 °C. Culture was cultivated until it achieved cell concentration of 0.3 g L^{-1} (exponential phase). Photon flux density measurements were carried out using a quantum meter (BIOSPHERICAL INSTRUMENT, QSL 2100). Sensor was located at the external surface of culture bottles.

The obtained inoculum was transferred to 6-L transparent bottles containing 5 L of WC medium. At this stage, WC medium was modified in relation to K_2HPO_4 (0.00449 g L^{-1}) and $NaNO_3$ (1.21 g L^{-1}) concentrations. This change was made to obtain a maximized cell density, what was observed in previous experiments. Before transfer, cells were centrifuged and washed with medium modified. Final cell concentration in the bottles with 5 L of medium was 0.05 g L^{-1}.

Cultivation was carried out in batch and kept under pneumatic stirring with airflow of 800 cm^3 min^{-1}, photon flux density of 100 μmol photons m^{-2} s^{-1} and room temperature of 25 ± 2 °C until they achieved cell concentration of 0.5 g L^{-1} (early stationary phase). The obtained biomass was harvested by centrifugation at 2607*g* and 25 °C for 10 min (NOVATECNICA 825), being a part lyophilized and stored at 4 °C until lipid content analysis (total lipid and TAG) and the other part was resuspended in the bottles containing WC medium without nitrate.

2.2.2. Second stage cultivation: nitrate starvation conditions

Cultivations were carried out in 6-L bottles containing 5 L of WC medium without nitrate. WC medium used at this stage contained K_2HPO_4 (0.00449 g L^{-1}) and other nutrients (as described in item 2.1) except $NaNO_3$. This medium was named stress medium. This nitrate deficient condition was carried out to trigger the accumulation of TAG in the obtained biomass at the first stage. Before transfer, cells were washed with the medium used in the cultivation. Conditions were the same and cultivation lasted more 4 days (late stationary phase). Stressed biomass was harvested by centrifugation at 2607*g* and 25 °C for 10 min, being lyophilized and stored at 4 °C until lipid content analysis.

2.2.3. Monitoring of biomass in culture medium

Microalgae grown in culture medium was monitored by measuring the optical density at 730 nm (BIOSPECTRO SP-22). Growth curve was constructed with dry weight values plotted logarithmically. Optical density was correlated with dry weight X (g L^{-1}) by the following equations:

$$X = (OD_{730nm}) / 3.5275 \left(R^2 = 0.992\right) \text{ Exponential phase}$$

$$X = (OD_{730nm}) / 2.331 \left(R^2 = 0.857\right) \text{ Stationary phases}$$

Dry weight (DW) was obtained by vacuum filtering in glass fiber membrane (0.45 μm nominal porosity, SARTORIUS®) previously weighed. After filtering, membranes were dried in oven at 105 °C overnight. Dry membranes were cooled in desiccator and weighed again. Dry biomass weight was calculated from the subtraction between the final weight and the initial weight of membrane.

Download English Version:

https://daneshyari.com/en/article/7063186

Download Persian Version:

https://daneshyari.com/article/7063186

Daneshyari.com