Bioresource Technology 169 (2014) 667–676

Contents lists available at ScienceDirect

Bioresource Technology

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

Outdoor pilot-scale production of *Nannochloropsis gaditana*: Influence of culture parameters and lipid production rates in tubular photobioreactors

A. San Pedro, C.V. González-López *, F.G. Acién, E. Molina-Grima

Chemical Engineering Area, Department of Engineering, University of Almería, E04120 Almería, Spain

HIGHLIGHTS

- Long-term outdoor cultures of *N. gaditana* in pilot tubular reactors are performed.
- *N. gaditana* growth and composition under various culture strategies are studied.
- Models predicting growth, lipid and fatty acids production rates are developed.
- The influence of nitrate limitation on lipids productivity is modeled.
- Continuous operation is compared to two-stage culture to improve lipid accumulation.

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 12 July 2014
Accepted 14 July 2014
Available online 19 July 2014

Keywords:
Tubular photobioreactors
Outdoor culture
Nitrate limitation
Growth model
Microalgae

ABSTRACT

This work studied outdoor pilot scale production of *Nannochloropsis gaditana* in tubular photobioreactors. The growth and biomass composition of the strain were studied under different culture strategies: continuous-mode (varying nutrient supply and dilution rate) and two-stage cultures aiming lipid enhancement. Besides, parameters such as irradiance, specific nitrate input and dilution rate were used to obtain models predicting growth, lipid and fatty acids production rates. The range of optimum dilution rate was 0.31–0.35 1/day with maximum biomass, lipid and fatty acids productivities of 590, 110 and 66.8 mg/l day, respectively. Nitrate limitation led to an increase in lipid and fatty acids contents (from 20.5% to 38.0% and from 16.9% to 23.5%, respectively). Two-stage culture strategy provided similar fatty acids productivities (56.4 mg/l day) but the neutral lipids content was doubled.

1. Introduction

In recent years, microalgae have been given considerable attention for their potential as a renewable fossil fuel alternative which may help replace the first generation of biofuels produced from food feedstock. Features such as high biomass productivity and high lipid content of some species make microalgal biodiesel an attractive option. Nevertheless, a cost reduction in biomass production has to be achieved and, at the same time, current technology must be scaled-up in order to make a significant contribution to world biofuel demand (Acién et al., 2012). Taking the leap into large-scale microalgal cultivation for biodiesel production still requires extensive pilot-scale research.

Factors affecting the viability of commercial microalgae production for biodiesel purposes should be investigated at the pilot scale: the effect of environmental conditions, the cultivation technology (closed or open systems), the selection of a robust and lipid-rich strain, the nutrient and carbon dioxide supply, along with the use of a water source that ensures low environmental impact (Rawat et al., 2013; Scott et al., 2010). In this respect, the use of marine microalgae species is regarded as imperative for the sustainable biofuel production of these microorganisms (San Pedro et al., 2013). Microalgae production at pilot scale has been conducted worldwide, with raceway ponds and flat-panel photobioreactors being the most commonly-used technologies (Cheng-Wu et al., 2001; Rodolfi et al., 2009; Silva Benavides et al., 2013). Conversely, although tubular photobioreactors involve higher investment costs, they have also been used for outdoor microalgae cultivation as they permit greater control of culture conditions, reducing the risk of contamination and offering high light utilization efficiency. Several species, such as *Phaeodactylum* and

* Corresponding author. Address: Chemical Engineering Area, Department of Engineering, University of Almería, Carretera Sacramento s/n, E04120 Almería, Spain. Tel.: +34 950 214456; fax: +34 950 015484.
E-mail address: cynthiagonzalez@ual.es (C.V. González-López).

http://dx.doi.org/10.1016/j.biortech.2014.07.052
0960-8524/

Haematococcus have been cultivated in tubular photobioreactors (Acién Fernández et al., 1998; García-Malea et al., 2009; Silva Benavides et al., 2013) and, more specifically, cultures of species in the genus *Nannochloropsis* have been carried out in flat-panel and tubular photobioreactors at pilot scale (Cheng-Wu et al., 2001; Zou et al., 2000). This microalga has been proposed as a promising candidate for outdoor cultivation, given its robustness and capability to accumulate lipids under nutrient-deprived conditions (Bondioli et al., 2012; Rodolfi et al., 2009). Concerning the adaptation of microalgae to changes in the culture conditions and their ability to modify their metabolism and enhance the synthesis of compounds such as lipids, an extensive number of studies have been conducted under laboratory conditions. Factors including nutrient limitation, temperature, salinity and irradiance have been highlighted as elements affecting the cellular composition of microalgae strains, and, in particular, of the genus *Nannochloropsis* (Camacho-Rodríguez et al., 2013; Hu and Gao, 2006; James et al., 1989; Rodolfi et al., 2009; San Pedro et al., 2013). On a pilot scale, the overproduction of lipids by the microalgae *Nannochloropsis* subjected to stressful conditions has been also investigated (Biondi et al., 2013; Bondioli et al., 2012; Rodolfi et al., 2009).

In the present work, a step forward from laboratory to pilot scale has been made, taking into account information extracted from a previous study conducted under laboratory conditions, in which *Nannochloropsis gaditana* was selected as a suitable candidate for biodiesel production (San Pedro et al., 2013). Thus, experiments conducted in continuous mode in three tubular photobioreactors (340 l) allowed investigation into several parameters affecting the growth and biomass composition of the strain, such as the dilution rate and the nutrient supply. Continuous cultures under nitrogen-sufficient conditions and at several dilution rates were carried out in order to determine the optimum range. Additionally, several nutrient input rates (at a fixed dilution rate) were tested so as to study the influence of this parameter on *N. gaditana* performance. Furthermore, a two-stage cultivation process (nutrient-sufficient continuous culture followed by a nitrogen-deprived phase) was performed in order to boost the synthesis of fatty acids in *N. gaditana*. Finally, the main parameters influencing the growth of the strain (irradiance, specific nitrate input and dilution rate) were used to obtain models predicting the growth and also the lipid and fatty acid production rates. The information obtained will assist in designing and operating closed systems for the production of lipid-rich cells of *N. gaditana* under outdoor pilot-scale conditions.

2. Methods

2.1. Microalgal strain and culture medium

The marine microalga *Nannochloropsis gaditana*, Lubián CCMP 527, was cultivated outdoors at the University of Almería, Spain (+36° 49′ 43.13″, −2° 24′ 9.39″). This strain was previously selected after an indoor study on several microalgal strains which are potentially suitable for high lipid accumulation, in which *N. gaditana* provided the best results (San Pedro et al., 2013). The culture medium was prepared by supplementing natural seawater with agricultural fertilizers: $NaNO_3$ (SQM Europe N.V., Belgium), KH_2PO_4 (Agro Mayor, Fuentes Fertilizantes S.A., Spain) and essential micronutrients (Welgro Hidroponic, Welgro, The Netherlands). The seawater used for medium preparation was pumped directly from the sea to a 500 l storage tank. The culture medium was prepared using a greenhouse irrigation system (Nutritec 9000, Ritec, Riegos y Tecnología S.L., Spain) and filtered through a set of three filters: 10 μm, 5 μm and 1 μm (CUNO C16, CUNO B16 and CUNO Y16, 3M, France).

2.2. Photobioreactors and operation mode

The experiments were carried out outdoors in a set of three fence-type tubular photobioreactors built as previously described (Acién et al., 2001; Molina et al., 2001). Each photobioreactor had a working volume of 340 l and consisted of a vertical tubular solar receiver (125 m length and 0.05 m diameter) and a bubble column for heat exchange and O_2 degassing (1.92 m high and 0.25 m diameter). A centrifugal pump (SE-150-M, Espa, Spain) was used to recirculate the culture through the reactor at 0.5 m/s (Molina Grima et al., 1999). The arrangement of the tubes was optimized for maximizing solar radiation capture (0.05 m inter-tube distance). The diameter of the tube was set to minimize biomass yield losses due to an excessive light path. The reactors were oriented east–west and the distance between them was 1.6 m so as to minimize shadowing. The temperature during the day was kept under 30.0 °C by circulating seawater through a heat exchanger, and the pH was controlled at 8.0 by on-demand injection of pure CO_2 into the inlet air stream. The air flow rate entering each photobioreactor was 0.1 v/v/min (FR4L72BVBN flow meters, Key Instruments, USA), while the CO_2 was injected when required at a constant flow rate of 0.01 v/v/min in all reactors (FR4A41BVBN flow meters, Key Instruments, USA). Dissolved oxygen, pH and temperature values were measured with OD and pH probes (5342 pH electrode and 5120 OD electrode, Crison Instruments S.A., Spain) connected to a MM44 control-transmitter unit (Crison Instruments, Spain). The data were logged in a PC control unit, which allowed the monitoring and control of the culture parameters. The culture medium volume entering the reactors was regulated by flow meters (FCIV0201D, 10100, FIP, Italy). The solar radiation received by the facility was measured with a thermoelectric pyranometer connected to an AC-420 adapter (LP-02, Geónica S.A., Spain). The reactors, the data logging system and the control software (DaqFactory 5.0, Azeotech Inc., USA) were designed and built by our research group.

The set of experiments in this study were carried out from February to October. The photobioreactors were inoculated with a culture at exponential phase grown outdoors in 100 l bubble columns. In the continuous-mode experiments, once the steady state was reached, the culture was maintained for at least 3 days. In the two-stage experiments, a continuous culture, at 0.21 1/day with a nutrient-replete medium, was followed by a batch mode for 12 days after nutrient removal. In the two-stage experiments, the entire culture in each reactor was collected, centrifuged (Gea Separator, SSD2, Westfalia, Germany), resuspended in nitrate-free culture medium and reinoculated into the reactors.

2.3. Analytical procedures

The biomass concentration was determined daily by measuring absorbance at 750 nm with a spectrophotometer (DR/4000 UV/Vis Spectrophotometer, HACH, USA). Spectrophotometric measurements were verified by dry weight determinations twice a week. The status of the cells was checked daily by measuring the variable to maximum chlorophyll fluorescence ratio (F_v/F_m ratio) using a fluorometer (AquaPen AP 100, Photon Systems Instruments, The Czech Republic). The biochemical composition of the biomass was determined after collecting, centrifuging (Sigma Sartorius 4-15, Sartorius A.G., Germany) and freeze-drying (Telstar Cryodos 50, Telstar, Spain) culture samples at the steady state of each experiment. The protein content was determined following a modification of the Lowry method (González López et al., 2010). The total lipid content was quantified using the method proposed by Kochert (1978). Regarding the fatty acid (FA) content and profile, these were obtained by direct transesterification and gas chromatography (6890N Series Gas Chromatograph, Agilent Technologies,