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

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ABSTRACT

This work is the last of a series of three papers. The previous ones were performed in tubular photobioreactors and raceways. In the present work, the outdoor continuous production of *Nannochloropsis gaditana* in flat-panel reactors is studied over a two-year period, analysing the influence of the dilution rate imposed in addition to the nitrate and phosphate supplied. Maximum biomass and lipid yields of 0.19 g/l day and 38.0 mg/l day were obtained when operating at a dilution rate of 0.35 1/day and concentrations of 10.0 mM NO_3^- and 0.8 mM PO_4^{3-} in the culture medium. By decreasing the specific nitrate input (below 2 mmol N/g day), and exposing the culture to moderate light availability and temperature, the volumetric productivity of saturated and monounsaturated fatty acids increased to 10.0 mg/l day. The specific nitrate input, together with other important parameters such as the average irradiance, temperature and dilution rate were used to develop a model describing this species' behaviour. The model is a powerful tool for further improvement and scaling-up of this technology.

1. Introduction

Ever-increasing global energy demand and environmental challenges resulting from greenhouse gas emissions have motivated increased research during the last years into microalgal production processes to produce biofuel and/or capture CO_2. Whilst an extensive range of studies investigating competitive strains and optimal culture conditions are available, the development of engineering strategies for microalgae cultivation and information on culture reliability and stability over a long period is still lacking [1,2].

In addition to selecting a suitable operating strategy for microalgae production, the use of an appropriate cultivation technology is imperative. Although a wide variety of closed photobioreactors have been proposed to fit the requirements of different microalgal strains, vertical flat-panel photobioreactors have emerged as an attractive alternative. This type of systems offers a substantial reduction in operating costs due to the lower power supply requirements when compared to other closed systems [3].

Among the species of microalgae that have been tested for biodiesel production purposes, those in the genus *Nannochloropsis* have attracted sustained interest given their high biomass productivity, high lipid content and the feasibility of growing them outdoors [4,5,6]. Even though several studies regarding the cultivation of *Nannochloropsis* sp. in flat-panel photobioreactors were conducted in the past, only a few looked at the outdoor production of this species. Furthermore, those studies were performed either over short periods, with no nitrogen limitation, or using a fixed dilution rate [7,4,8–10]. In this regard, as far as we know, research into the effects of dilution rate and nitrogen limitation on continuous, year-round outdoor production of this species is similarly lacking.

Recently, concern has arisen about the modelling of microalgae growth and lipid productivity, and the development of mathematical models has proven an effective method to predict the microalgal culture dynamics under various culture conditions. Therefore, microalgae performance modelling could assist in process optimization, culture conditions and scaling-up of the cultivation systems for a commercial microalgal-based technology [11–14]. Nonetheless, previously proposed models comprise a large number of parameters, and their application in outdoor scaled-up systems is limited, since the validation of these parameters is mostly conducted with data from short-term experiments and/or batch mode operation.

In the present study, outdoor continuous cultures of *Nannochloropsis gaditana* over a two-year period were performed. The microalgae were cultivated in flat-panel photobioreactors (400 l), with east-west and north-south orientations, so as to investigate the influence of the reactor's orientation on biomass and lipid yield of *N. gaditana*. A series of experiments were conducted to establish the optimal operating conditions for maximizing biomass and lipid output. In this way, experiments

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in continuous mode allowed to establish the optimal dilution rate under nutrient-sufficient conditions and the effect of the nitrate input in experiments with decreasing nitrate values. Finally, the modelling of the biomass, fatty acid (FA) and lipid productivities was performed taking into account parameters such as irradiance, temperature, dilution rate and specific nitrate input. The proposed models were validated using all the data collected during this work, providing a valuable tool for scaling up flat-panel photobioreactor technology in microalgae mass production.

2. Methods

2.1. Microalgal strain and culture medium

Nannochloropsis gaditana Lubián CCMP 527 was cultivated outdoors at the pilot plant located at the University of Almería, Spain (+36° 49′ 43.13″, −2° 24′ 9.39″). The culture medium consisted of natural seawater supplemented 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). Pure CO_2 was injected into the inlet air stream on demand to maintain a pH value of 8.0. The inoculum for the flat-panel photobioreactors was produced outdoors in 100 l bubble columns.

2.2. Photobioreactors and operation mode

N. gaditana was cultivated in two sets of three flat-panel photobioreactors (Fig. 1). Each reactor consisted of a disposable plastic bag placed between two steel frames separated by a mean distance of 0.09 m. Both plastic bag and frames had the same dimensions, 1.7 m high and 2.5 m long. The plastic bag was made of free-dispersant 0.75 μm polyethylene with a transparency index of 0.95 in the photosynthetically active spectrum; this could be replaced when necessary. Two different orientations were tested - the first set of three reactors, named East-West (E-W), were positioned facing East-West (+36° 49′ 43.07″, −2° 24′ 8.93″); whilst the second set of three reactors, North-South, were positioned facing North-South (N-S) (+36° 49′ 42.90″, −2° 24′ 8.88″). For each set, shadow nets, which simulated reactors (one-side metal frame covered by a black net, with 90% solar irradiance attenuation), were used in order to mimic the distance between flat-panel photobioreactors on a large scale. Three distances were tested in each set: 0.5, 1.0 and 1.5 m corresponding to reactors 1, 2 and 3 in both sets. The photobioreactors' volume to occupied land area ratios were calculated as the culture volume (400 l) divided by the land occupied area, it is, the reactor length (2.5 m) multiplied by the corresponding distance between reactors (0.5, 1.0 and 1.5 m) plus its width (0.1 m). Areal productivity was henceforth calculated as volumetric productivity multiplied by those V/S ratios. Aeration was provided by a gas sparger placed at the bottom of the reactor with a flow rate of 0.09 v/v/min (FR4L72BVBN flow meters, Key Instruments, USA) and CO_2 was injected when required at 0.008 v/v/min (FR4A41BVBN flow meters, Key Instruments, USA). Temperature-pH probes (5342 pH electrode, Crison Instruments S.A., Spain) were located at the top of the reactor and connected to transmitter units MM44 (Crison Instruments, Spain) and data acquisition software (DaqFactory 5.0, Azeotech Inc., USA). The design and construction of the reactors, the data logging system and the control software was made by our research group. A thermoelectric pyranometer connected to an AC-420 adapter (LP-02, Geónica S.A., Spain) was used to measure the impinging solar radiation in the facility (I_s).

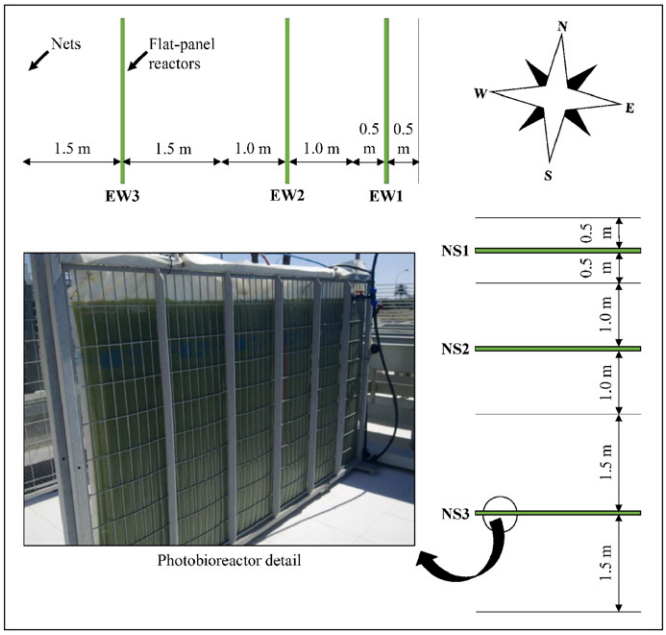

Fig. 1. Photobioreactors distribution.

The culture medium was prepared using a greenhouse irrigation system (Nutritec 9000, Ritec, Riegos y Tecnología S.L., Spain) and was filtered before entering the reactors (10, 5 and 1 μm filters, CUNO C16, CUNO B16 and CUNO Y16, 3M, France). The volume of culture medium entering the reactors was regulated by flow meters (FCIV0201D, 10100, FIP, Italy). The inlet for the medium was situated in the middle of the reactor above the culture level and the harvesting valve was located on one side of the reactor at the top of the culture. The reactors were operated for several months uninterruptedly in continuous-mode; in order to ensure a steady state, each experiment has to be performed for at least a period of time that guarantee that the medium supplied to the culture makes twice the whole volume of the reactor. After reaching steady state, the culture was maintained for at least three more days before collecting samples for biomass composition analyses, making sure that biomass concentration was stable around the same value. The experiments were conducted over two years and data were analysed and grouped to take similar daily culture temperature (light and dark periods) conditions throughout the seasons into account. In order to make a more appropriate analysis of the experimental data obtained for the determination of the optimal dilution rate, four temperature ranges were established depending on the season. These ranges were labelled by average temperature during the light period. For experiments conducted from December to February, the average daylight culture temperature was 13.0 °C; from March to April and from October to November, it was 20.0 °C; for May, June and September, it was 27.0 °C and from July to August, it was 33.0 °C (standard deviation 1.0–1.7 °C). No temperature control was used for the cultures. With regard to the irradiance impinging on the reactors, I_0, it is described as the average radiation of the light period measured with a pyranometer, I_s, by a distribution factor, α (ranging from 0.30 to 0.35) that depends on the photobioreactor design and represents the fraction of solar irradiance impinging on the reactor. It takes into account both walls of each reactor. Mean values for I_0 were 438, 560, 673 and 697 μE/m^2 s, respectively (with standard deviations of 115–164 μE/m^2 s).

2.3. Analytical procedures

Daily samples were taken from all the reactors in order to spectrophotometrically determine the biomass concentration (750 nm, DR/4000 UV/Vis Spectrophotometer, HACH, USA) and to evaluate the photosynthetic capacity of the cells (F_v/F_m ratio) with a fluorometer (AquaPen AP 100, Photon Systems Instruments, The Czech Republic) as an indicator of the good performance of the culture (PSII was working correctly). F_v/F_m fluctuated between 0.5 and 0.6 (data not shown). The absorbance measurements were verified by dry weight twice a week. When each experiment was at steady state, samples were collected, then centrifuged at 9000 × g (Sigma Sartorius 4-15, Sartorius A.G., Germany) and freeze-dried over 48 h (Telstar Cryodos 50, Telstar, Spain) for