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Comparative analysis of the outdoor culture of *Haematococcus pluvialis* in tubular and bubble column photobioreactors

M.C. García-Malea López [a], E. Del Río Sánchez [b], J.L. Casas López [a], F.G. Acién Fernández [a], J.M. Fernández Sevilla [a], J. Rivas [b], M.G. Guerrero [b], E. Molina Grima [a,*]

[a] *Department of Chemical Engineering, University of Almería, Cañada San Urbano S/N, Almería 04071, Spain*
[b] *Inst. Bioquímica Vegetal y Fotosíntesis, University Sevilla-CSIC, E-41092-Sevilla, Spain*

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Abstract

The present paper makes a comparative analysis of the outdoor culture of *H. pluvialis* in a tubular photobioreactor and a bubble column. Both reactors had the same volume and were operated in the same way, thus allowing the influence of the reactor design to be analyzed. Due to the large changes in cell morphology and biochemical composition of *H. pluvialis* during outdoor culture, a new, faster methodology has been developed for their evaluation. Characterisation of the cultures is carried out on a macroscopic scale using a colorimetric method that allows the simultaneous determination of biomass concentration, and the chlorophyll, carotenoid and astaxanthin content of the biomass. On the microscopic scale, a method was developed based on the computer analysis of digital microscopic images. This method allows the quantification of cell population, average cell size and population homogeneity. The accuracy of the methods was verified during the operation of outdoor photobioreactors on a pilot plant scale. Data from the reactors showed tubular reactors to be more suitable for the production of *H. pluvialis* biomass and/or astaxanthin, due to their higher light availability. In the tubular photobioreactor biomass concentrations of 7.0 g/L (d.wt.) were reached after 16 days, with an overall biomass productivity of 0.41 g/L day. In the bubble column photobioreactor, on the other hand, the maximum biomass concentration reached was 1.4 g/L, with an overall biomass productivity of 0.06 g/L day. The maximum daily biomass productivity, 0.55 g/L day, was reached in the tubular photobioreactor for an average irradiance inside the culture of 130 $\mu E/m^2 s$. In addition, the carotenoid content of biomass from tubular photobioreactor increased up to 2.0% d.wt., whereas that of the biomass from the bubble column remained roughly constant at values of 0.5% d.wt. It should be noted that in the tubular photobioreactor under conditions of nitrate saturation, there was an accumulation of carotenoids due to the high irradiance in this reactor, their content in the biomass increasing from 0.5 to 1.0% d.wt. However, carotenoid accumulation mainly took place when nitrate concentration in the medium was below 5.0 mM, conditions which were only observed in the tubular photobioreactor. A similar behaviour was observed for astaxanthin, with maximum values of 1.1 and

* Corresponding author. Tel.: +34 950 015032; fax: +34 950 015484.
E-mail address: emolina@ual.es (E.M. Grima).

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0.2% d.wt. measured in the tubular and bubble column photobioreactors, respectively. From these data astaxanthin productivities of 4.4 and 0.12 mg/L day were calculated for the tubular and the bubble column photobioreactors. Accumulation of carotenoids was also accompanied by an increase in cell size from 20 to 35 μm, which was only observed in the tubular photobioreactors. Thus it may be concluded that the methodology developed in the present study allows the monitoring of *H. pluvialis* cultures characterized by fast variations of cell morphology and biochemical composition, especially in outdoor conditions, and that tubular photobioreactors are preferable to bubble columns for the production of biomass and/or astaxanthin.
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1. Introduction

Microalgae are a potential source of biomass or specific products such as lipids, pigments, antioxidants, etc. One of the most recent processes based on microalgae is the production of astaxanthin from *Haematococcus pluvialis*. Astaxanthin is a high-value carotenoid pigment with important applications in the nutraceutical, cosmetics, food and feed industries (Guerin et al., 2003). The major market for astaxanthin is as a pigmentation source in aquaculture, primarily in salmon and trout (Guerin et al., 2003). In this sense, the microalgae *Haematococcus pluvialis* is the richest source of natural astaxanthin, and it is now cultivated on an industrial scale (Olaizola and Huntley, 2003).

Astaxanthin sells for US $2500 kg with an annual worldwide market estimated at US $200 million (Lorenz and Cysewski, 2000). Although 95% of this market consumes synthetically derived astaxanthin, consumer demand for natural products makes the synthetic pigments much less desirable and provides an opportunity for the production of natural astaxanthin by *H. pluvialis*. This strain contains 1.5–3.0% astaxanthin and has gained acceptance in aquaculture and other markets as a "concentrated" form of natural astaxanthin (Lorenz and Cysewski, 2000; Olaizola and Huntley, 2003). In this sense, natural astaxanthin from *H. pluvialis* is currently produced in a two-step process. In the first step green vegetative cells are produced under controlled culture conditions, frequently indoors, using stirred tank or bubble columns. In the second step, green cells are exposed to stress conditions (high irradiance, nitrate and/or phosphate deprivation, high temperature) to induce accumulation of astaxanthin, using open raceways or tubular photobioreactors. Flat panel, bubble columns and tubular photobioreactors have been extensively proposed as outdoor closed photobioreactors for the industrial production of microalgae (Tredici and Materassi, 1992; Richmond et al., 1993; Molina et al., 1994; Acién et al., 1998; García et al., 1999). However, only bubble columns and tubular photobioreactors have proven capable of scaling up to high volumes. Although the outdoor production of *H. pluvialis* in tubular photobioreactors has been referenced (Olaizola, 2000; Chaumont and Thepenier, 1995), no data from bubble column has been referenced in spite of the highly adequate light profile inside this type of reactor (García et al., 1999). In addition, Chaumont and Thepenier (1995) have reported the rapid variation of biomass concentration and pigment content in daylight using tubular photobioreactors, with carotenoid content increasing from 0.6 to 1.4% d.wt. in 4 h, from 7:00 to 11:00 h.

Due to the fast and great variation of *Haematococcus* cells during outdoor culture, a fast methodology to characterize the cultures at macroscopic and microscopic scale is necessary. Microscopic characterisation, i.e. cell population and size distribution, is usually performed either by direct observation, or using automated devices such as haemocytometers or more sophisticated cell counters (Harker et al., 1996; Tripathi et al., 1999). However, apart from cell counters, the other methods do not quantify cell size and homogeneity, and the use of cell counters for *Haematococcus* cells is very problematic due to variations in cell size between different cell morphotypes, frequently more than 10-fold. Macroscopic culture characterisation is usually performed as the dry weight measurement of biomass concentration and the determination of pigment content by spectrophotometry–HPLC (Del Campo et al., 2000). Dry weight measurements are tedious and results are not obtained until at least 12 h after sampling. The spectrophotometric measurement of pigment content requires a time-consuming extraction process of the pigments using adequate solvents, which must also ensure effective cell wall breakage. In the case of

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