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Variation of fatty acid profile with solar cycle in outdoor chemostat culture of *Isochrysis galbana* ALII-4

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Abstract

Outdoor chemostat cultures of the marine microalga *Isochrysis galbana* at constant dilution rate (0.034 h^{-1}) have been carried out under different weather conditions. Steady-state biomass concentrations were 1.61±0.03 kg m^{-3} in May and 0.95±0.04 kg m^{-3} in July, resulting in biomass output rates of 0.54 kg m^{-3} d^{-1} and 0.32 kg m^{-3} d^{-1} in May and July, respectively. Two patterns of daily variation with the solar cycle were observed in the fatty acid content. Saturated and mono-unsaturated fatty acids (16:0 and 16:1*n*7) show significant variation with the solar cycle, associated with short-term changes in environmental factors. Palmitic and palmitoleic acids are generated during daylight and consumed during the dark period. However, polyunsaturated fatty acids do not show a significant response to the solar cycle and their changes are associated with long-term variation in environmental factors. The maximum EPA productivity was obtained in May, 14.1 g m^{-3} d^{-1}, which is close to that found in the literature for indoor continuous cultures. Nonetheless, the outdoor EPA content (up to 2.61% d.wt) was lower than the indoor EPA content from a previous study (5% d.wt).

Introduction

Oceanic phytoplankton represent the major source of long-chain polyunsaturated fatty acids (LC-PUFAs) in the biosphere (Kyle, 1992). These *n*-3 (omega-3) class LC-PUFAs are being considered for use in medicine instead of fish oils (Yongmanitchai & Ward, 1989; Radwan, 1991).

To obtain high fatty acid production from microalgae, the most favourable environmental conditions and operating variables in the culture system have to be defined. These depend on the microalga strain selected and, factors affecting its growth and the synthesis of such products. Nonetheless, information about culture conditions for improving the productivity of polyunsaturated fatty acids is scarce, and in most cases presents only results from batch cultures of a few millilitres without considering the growth kinetics and biochemical composition under mass culture conditions.

In a previous work (Molina Grima *et al.*, 1994b), growth and fatty acid composition of *Isochrysis galbana* ALII-4 in batch and semicontinuous outdoor culture were reported. The eicosapentaenoic acid (EPA) generation rate during linear growth, with an average content of 2.56% dry weight, was 8.2 g m^{-3} d^{-1} and it was predicted that this productivity could be enhanced by improving culture conditions.

In the present paper, outdoor chemostat cultures of *Isochrysis galbana* ALII-4 in a 50-L closed tubular photobioreactor under different weather conditions are reported with special attention to the effect of solar cycle on fatty acid synthesis as well as the increase in polyunsaturated fatty acid productivity attained in continuous culture in comparison to semicontinuous mode.

Materials and methods

The microalga used was one of 42 isolates of a single strain of *Isochrysis galbana* obtained from a phenotypic selection programme for an EPA-rich strain of microalgae carried out in our laboratory (Sánchez Pérez, 1994). This isolate, labeled ALII-4, is registered with the Culture Collection of Algae and Protozoa (CCAP, 927/15). The inoculum for the photobioreactor was grown under laboratory conditions in a 5-L stirred-tank reactor at 20 °C. The culture medium was: $NaNO_3$, 10 mol m^{-3}; $NaH_2PO_42H_2O$, 0.1 mol m^{-3}; ferric citrate, 20 mmol m^{-3}; $ZnCl_2$, 1 mmol m^{-3}; $CuSO_45H_2O$, 0.1 mmol m^{-3}; $CoCl_26H_2O$, 0.1 mmol m^{-3}; $Na_2MoO_42H_2O$, 1 mmol m^{-3}; EDTA-Na_2, 29 mmol m^{-3}; $MnCl_24H_2O$, 5 mmol m^{-3}. Vitamin concentrations were: thiamine, 35 μg m^{-3}; biotin, 5 μg m^{-3}; cyanocobalamin, 3 μg m^{-3}. This composition was determined by a macronutrient optimization method based on a multifactorial response surface technique (García Sánchez *et al.*, 1994). Medium and culture vessel were autoclaved at 120 °C at 3×10^5 Pa. The culture was constantly illuminated by four Phillips (Eindhoven) TLD w/54 fluorescent lamps, providing a light intensity of 200 μmol photon m^{-2} s^{-1} on the vessel surface. The photon-flux density of photosynthetically active radiation (PAR) at the culture surface was determined with a quantum scalar irradiance meter (QSL-100 Biospherical Instruments Inc., San Diego, CA, USA).

The outdoor culture system was a 50 litre tubular photobioreactor based on a 2.6 cm inner diameter external loop airlift (Molina Grima *et al.*, 1995). The culture medium was the same as that used in the inoculum, but was sterilized by filtration through 1 μm and 0.22-μm porosity Millipore filters. The pH was set at 7.65 controlled by automatic addition of pure CO_2. Culture temperature, pH and dissolved oxygen (O_2) were measured on-line and biomass concentration was measured several times a day. Dilution rate was set at 0.034 h^{-1} by addition of 17 litres of fresh medium at a contant rate during the daylight period (0900–1900) and samples for determination of fatty acid profile were collected when steady-state was reached. The present study was conducted between May and July, 1994, in Almería (S-E. Spain: 36 ° 50′ N, 2 ° 27′ W).

Fresh centrifuged wet biomass was used for fatty acid analysis. Methylation was done by direct transesterification following the Lepage and Roy method (1984) with modifications by García Sánchez *et al.* (1993). The analysis of methyl esters was carried out by GLC, using a 30 m capillary column of fused silica (SP2330, Supelco, Bellefonte, Pa, USA), with an internal diameter of 0.25 mm, 0.20 μm standard film, split ratio of 100:1, and a flame ionization detector. Supelco PUFA-1, PUFA-2 and PUFA-3 patterns were used for the determination of retention times. Sigma patterns of the main fatty acids for this microalga were used for response factor determination. Nonadecanoic acid was used as an internal standard to quantify fatty acid content in biomass. Four replicates were taken at each sample time for ANOVA statistical analysis performed by the package software STATGRAPHICS ver. 5 (Fry, 1993).

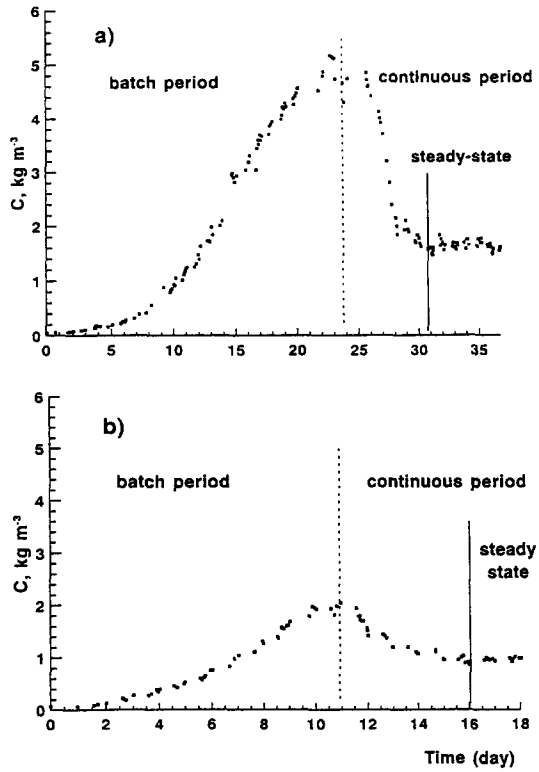

Fig. 1. Development of biomass concentration, C, in cultures in May (a) and July (b).

Results

Steady-state biomass concentration at constant dilution rate (0.034 h^{-1}) varied with weather conditions, 1.61 ± 0.03 kg m^{-3} being reached in May and 0.95 ± 0.04 kg m^{-3} in July (Fig. 1). Figure 2 shows the diurnal variation of dissolved oxygen concentration

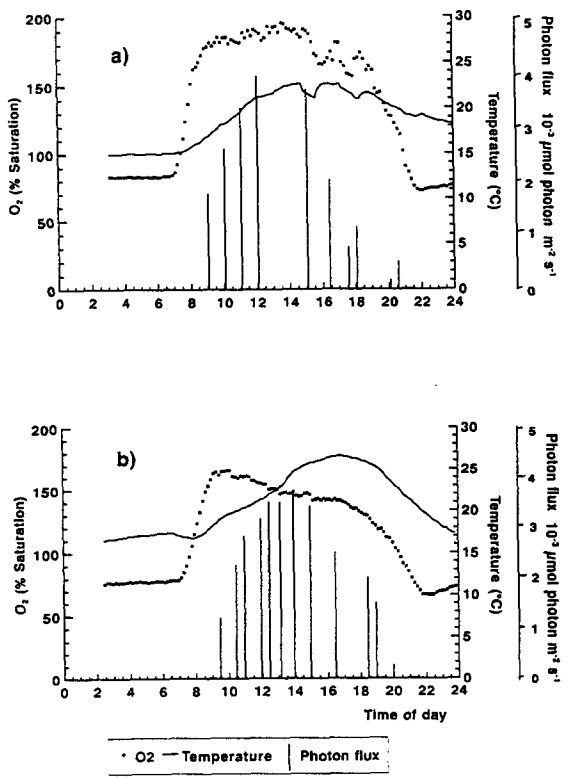

Fig. 2. Diel patterns of irradiance level, dissolved oxygen and temperature in continuous culture on a day in May (a) and July (b).

(O_2) and culture temperature as well as discrete measurements of solar irradiance for two days of continuous culture in May and July. Photosynthetically active radiation (PAR) per day at culture surface ranged in the same interval in May and July from 6.4×10^7 µmol photon m^{-2} d^{-1} to 1.16×10^8 µmol photon m^{-2} d^{-1} for cloudy and sunny days respectively, with averages of 9.6×10^7 µmol photon m^{-2} d^{-1} in May and 9.9×10^7 µmol photon m^{-2} d^{-1} in July. Nonetheless, culture temperature was higher in July, with maximum around 27 °C at noon, than in May, when it was around 22 °C. Furthermore, an inverse relationship between O_2 and temperature was observed regardless of solar irradiance.

During both batch periods prior to continuous culture, the biomass generation rate in the linear growth phase was 0.27 kg m^{-3} d^{-1}. This is lower than that reported for a semicontinuous culture of *Isochrysis galbana* ALII-4 carried out in March (Molina Grima *et al.*, 1994b). On the other hand, when steady-state was reached in the continuous culture procedure, the biomass output rate increased in comparison to biomass generation rate in batch, 0.54 kg m^{-3} d^{-1} being attained in May and 0.32 kg m^{-3} d^{-1} in July.

For analysis of daily cyclic variation of the fatty acid profile, four replicates were taken from early morning to late evening at both steady-states (Tables 1, 2). Seven samplings at two-hour intervals were tested in May, but only three in July, as this was considered enough to verify fatty acid variation. An increase in 14:0, 16:0 and 16:1*n*7 content and no variation in polyunsaturated fatty acid content were observed. Furthermore, the same fatty acid profiles were found at the same sampling time for three consecutive days. The decrease in short-chain fatty acid content, especially 16:1*n*7, during the dark period, is also noteworthy.

Discussion

The outdoor chemostat is a useful tool for obtaining steady-state conditions for the study of microalgal growth. The same dilution rate (0.034 h^{-1}) was set under the different weather conditions of May and July in order to study the response of the cells to uncontrolled environmental factors.

Generally, light availability determines the steady-state biomass concentration achievable in chemostat cultures. Nonetheless, culture temperature is another limiting factor usually related to solar radiation. In the present study, there was no great variation in photosynthetically active radiation between May and July, but air temperature was much higher in the latter. The average air temperature was 27.5 °C with a maximum of 39.4 °C during the culture period in July versus 19.3 °C average and 30 °C maximum in May. This gave rise to a greater increase in culture temperature during the solar cycle in July than in May as the cooling system was strongly affected by air temperature, and this temperature difference (Fig. 2) could justify the lower productivity attained in July. In fact, the specific growth rate of *Isochrysis galbana* has been reported to be maximum at 20 °C with a significative decrease at 25 °C (Ewart & Pruder, 1981; Molina Grima *et al.*, 1992). Furthermore, photosynthetic activity was also affected by culture temperature. As can be seen in Fig. 2a, at daybreak in May, O_2 sharply increased to remain constant at around 200% of saturation in early morning, while solar irradiance continued to increase up to noon. At the same time, culture temperature slowly increased up to 22.7 °C. On the other hand, in early morning in July, the same pattern of culture parame-

Table 1. Daily variation with solar cycle of the dry weight fatty acid content in steady-state for May. (Group means were calculated for a 95% confidence level). Daylight period: 0700–2100

Sampling day	Sampling time	14:0	16:0	16:1n7
Day before	2000	1.15 ± 0.03	1.72 ± 0.02	1.55 ± 0.02
	2100	1.21 ± 0.03	1.80 ± 0.02	1.64 ± 0.02
Day of experiment	0800	1.03 ± 0.03	1.30 ± 0.02	1.13 ± 0.02
	1000	1.07 ± 0.03	1.38 ± 0.02	1.21 ± 0.02
	1200	1.15 ± 0.03	1.54 ± 0.02	1.35 ± 0.02
	1400	1.07 ± 0.03	1.52 ± 0.02	1.35 ± 0.02
	1600	1.19 ± 0.03	1.63 ± 0.02	1.48 ± 0.02
	1800	1.26 ± 0.03	1.72 ± 0.02	1.59 ± 0.02
	2000	1.16 ± 0.03	1.66 ± 0.02	1.54 ± 0.02
Day after	0800	0.98 ± 0.03	1.17 ± 0.02	0.96 ± 0.02
	1000	0.99 ± 0.03	1.24 ± 0.02	0.99 ± 0.02
Sampling day	Sampling time	18:4n3	20:5n3	22:6n3
Day before	2000	0.63 ± 0.02	2.38 ± 0.04	1.29 ± 0.02
	2100	0.68 ± 0.02	2.61 ± 0.04	1.40 ± 0.02
Day of experiment	0800	0.69 ± 0.02	2.55 ± 0.04	1.41 ± 0.02
	1000	0.69 ± 0.02	2.57 ± 0.04	1.40 ± 0.02
	1200	0.72 ± 0.02	2.69 ± 0.04	1.41 ± 0.02
	1400	0.69 ± 0.02	2.50 ± 0.04	1.33 ± 0.02
	1600	0.74 ± 0.02	2.74 ± 0.04	1.36 ± 0.02
	1800	0.78 ± 0.02	2.74 ± 0.04	1.42 ± 0.02
	2000	0.74 ± 0.02	2.65 ± 0.04	1.40 ± 0.02
Day after	0800	0.79 ± 0.02	2.68 ± 0.04	1.41 ± 0.02
	1000	0.77 ± 0.02	2.61 ± 0.04	1.40 ± 0.02

Table 2. Daily variation with solar cycle of the dry weight fatty acid content in steady-state for July. (Group means were calculated for a 95% confidence level.) Daylight period: 0700–2100

Sampling day	Sampling time	14:0	16:0	16:1n7
Day before	1900	1.36 ± 0.02	1.66 ± 0.02	1.50 ± 0.02
Day of experiment	0900	1.00 ± 0.02	1.12 ± 0.02	0.86 ± 0.02
	1400	1.21 ± 0.02	1.55 ± 0.02	1.22 ± 0.02
	1900	1.31 ± 0.02	1.90 ± 0.02	1.53 ± 0.02
Day after	0900	1.00 ± 0.02	1.15 ± 0.02	0.84 ± 0.02
Sampling day	Sampling time	18:4n3	20:5n3	22:6n3
Day before	1900	0.32 ± 0.05	2.52 ± 0.04	1.10 ± 0.02
Day of experiment	0900	0.31 ± 0.02	2.39 ± 0.04	1.02 ± 0.02
	1400	0.29 ± 0.03	2.32 ± 0.04	1.05 ± 0.02
	1900	0.29 ± 0.04	2.42 ± 0.04	1.12 ± 0.02
Day after	0900	0.31 ± 0.03	2.55 ± 0.04	1.18 ± 0.02

ter variation was observed, although in the afternoon culture temperature rose to 26.7 °C while O_2 continuously decreased (Fig. 2b). Therefore both growth rate and photosynthetic activity seem to be limited at high temperature. This stresses the necessity for tight control of culture temperature which could be expensive and technically difficult on a large scale, or the need to use high-temperature tolerant algae in summer, as air temperature as well as solar radiation are a determining factors for culture management.

Fatty acid synthesis may be qualitatively and quantitatively affected by environmental conditions, such as media composition, temperature, light intensity and age of the culture (Alvarez Cobelas, 1989; Bajpai & Bajpai, 1993).

Tables 1 and 2 present the variation in fatty acid profile over the time of day for a complete solar cycle in May and July, respectively. The statistical analysis of this variation is given in Tables 3 and 4. Palmitic and palmitoleic acids (16:0 and 16:1*n*7) present the highest F-ratio, their variation being related to the different time of day. This pattern of variation could be explained by the different roles of the fatty acids in cell metabolism. 16:0 and 16:1*n*7 were found to be the main components of neutral lipids (storage lipids) in *Isochrysis galbana* ALII-4 (Molina Grima *et al.*, 1994a), and the initial step in the path of fatty acid synthesis (Hodgson *et al.*, 1991). Thus, the amounts of these fatty acids are subject to the daily cyclic variation of environmental conditions, that is, solar radiation, as when photosynthesis is active, storage biomolecules are accumulated for use as an energy source during darkness to maintain cell metabolism (Tables 1, 2). The proportion of these fatty acids synthesized during daylight is then consumed at night. On the other hand, polyunsaturated fatty acids are structural lipids, mainly found in glycolipids and phospholipids (Molina Grima *et al.*, 1994a), and therefore, their contents are more related to state of growth than short-term environmental variation, with averages of 0.72% d.wt, for 18:4*n*3,

Table 3. Variance analysis for the fatty acid content (based on dry weight) measured at different sampling times in the continuous culture in May.

Fatty acid	14:0	16:0	16:1n7	18:4n3	20:5n3	22:6n3
Source of variation (DF)	Sum of squares					
Sampling time (10)	0.297	1.682	2.139	0.090	0.445	0.069
Error (31)	0.102	0.039	0.037	0.022	0.168	0.040
Total (41)	0.398	1.721	2.176	0.112	0.613	0.109
F-ratio	9.04	132.89	177.45	12.79	8.24	5.37
(DF) degrees of freedom						

Table 4. Variance analysis for the fatty acid content (based on dry weight) measured at different sampling times in the continuous culture in July.

Fatty acid	14:0	16:0	16:1n7	18:4n3	20:5n3	22:6n3
Source of variation (DF)	Sum of squares					
Sampling time (4)	0.375	1.481	1.449	0.002	0.119	0.052
Error (11)	0.012	0.010	0.009	0.001	0.040	0.006
Total (15)	0.386	1.491	1.458	0.002	0.160	0.058
F-ratio	88.54	415.91	447.66	10.08	8.11	24.21
(DF) degrees of freedom						

2.61% d.wt for EPA and 1.38% d.wt for DHA in May against 0.32% d.wt, 2.43% d.w and 1.10% d.wt for 18:4*n*3, EPA and DHA, respectively, in July. Their variation with daily solar cycle presents less F-ratio than saturated ones (Tables 3, 4). The sum of squares for sampling time is near to the total for 16:0 and 16:1*n*7 and much higher than for the error, while for polyunsaturated fatty acids the difference between sum of squares for sampling time and error is low, so that the levels of polyunsaturated fatty acids are not related to sampling time.

In a previous work, (Molina Grima *et al.*, 1994b), EPA generation rate during linear growth, with an average content of 2.56%, was reported to be 8.2 g m^{-3} d^{-1}. In the present study, EPA productivity was maximum in May, 14.1 g m^{-3} d^{-1}, which is close to that obtained in indoor continuous cultures. Nonetheless, the indoor EPA content, which reached as high as 5% d.wt (Molina Grima *et al.*, 1993), was not reached in the outdoor culture.

A practical question arises as to how far it is feasible modulate the fatty acid profile of a microalga strain. In light-dark cycles, by harvesting at the beginning of the light period, saturated and monounsaturated fatty acid contents may be lowered; around 40% of the weight of 16:0 and 16:1 is consumed during the dark. Furthermore, at increasing dilution rates, storage lipid content decreases faster than structural lipid content (Molina Grima *et al.*, 1993, 1994a). Thus a combination of both factors, fast growth rate and early morning harvest, could enhance the PUFA proportion in microalgal biomass.

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