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Biomass nutrient profiles of the microalga *Porphyridium cruentum*

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Abstract

Nutritional composition is analysed for the red microalga *Porphyridium cruentum* cultured under various conditions in an external tubular photobioreactor. The data include the proximate composition (moisture, ash, crude protein, available carbohydrates, fiber, lipids and energy), nitrate, RNA, mineral elements (Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Cr, Mn, Co, S, As, Pb, Cd), C/N ratio, fatty acids and pigments (carotenoids, phycobiliproteins, chlorophylls and degradation products). On average, the biomass contains 32.1% (w/w) available carbohydrates and 34.1% crude protein. The mineral contents in 100 g dry biomass were: Ca (4960 mg), K (1190 mg), Na (1130 mg), Mg (629 mg) and Zn (373 mg). Toxic heavy metal contents were negligible. The fatty acid contents were (in percent dry wt): 1.6% of 16:0; 0.4% of 18:2ω6; 1.3% of 20:4ω6 and 1.3% of 20:5ω3. Nutrient composition of biomass was highly influenced by residence time in the bioreactor and the external irradiance. The biomass collected for short residence times was richer in protein and eicosapentaenoic acid.

Keywords: *Porphyridium cruentum*; Microalgae; Proximate composition; Mineral element; Fatty acid; Eicosapentaenoic acid; Arachidonic acid; Pigment; Carotenoids; Phycoerythrin; Nutrient composition

1. Introduction

The physiology and biochemistry of algae have been studied extensively, and microalga cultures have been developed for feeding marine molluscans used for human consumption (Bold, 1942; Cañizares, Molina & Domínguez, 1994). In some countries, such as Japan and Chile, microalgal biomass is added to foods, such as noodles, in order to improve nutrient profile (Markovits, López, Costa & Lutz, 1991; Schwartz, Jiménez, Markovits, Conejeros, López & Lutz, 1991). Microalgal biomass is a rich source of some nutrients, such as ω3 and ω6 fatty acids, essential amino acids (leucine, isoleucine, valine, etc.) and carotene (Becker, 1994).

Porphyridium cruentum is a red microalga (Rodophyta), with spherical cells that lack a cell wall (Adda, Merchuk & Arad, 1986; Arad, Adda & Cohen, 1985; Arad, Friedman & Rotem, 1988; Ramus, Kenney & Shaughnessy, 1989). Phycoerythrin, an accessory pigment, provides red colour characteristic of the cells (Gantt, 1969, 1981). Cells excrete a sulphurized polysaccharide which causes the cultures to become viscous, especially under limiting conditions. The polysaccharide is used commercially (Adda et al.; Arad, et al., 1985, 1988; Ramus et al.). The alga accumulates large amounts of lipids (Yongmanitchai & Ward, 1992), especially arachidonic acid (AA, 20:4ω6) (accumulated to 36% of total fatty acids) and noticeable amounts of eicosapentaenoic acid (EPA, 20:5ω3) (Ahern, Katoh & Sada, 1983; Cohen, Vonshak & Richmond, 1988; Lee & Tan, 1988; Lee, Tan & Low, 1989; Nichols & Appleby, 1969; Servel, Claire, Derrien, Coifford & Ďeroeckhdtzhaver, 1994). The protein content ranges from 28 to 39%, and the available carbohydrates vary between 40 and 57%. The total lipids may reach 9–14% of dry weight (Becker, 1994). The biomass contains tocopherol, vitamin K and a large amount of carotenes (Antia, Desai & Romilly, 1970).

This paper reports on the nutrient composition of the microalgal biomass of *Porphyridium cruentum*. Data are provided on proximate composition, RNA, fatty acids, mineral elements and several pigments. Biomass was produced in a external tubular photobioreactor, at different dilution rates and conditions of solar irradiances. The objective was to identity variations in nutrient profile of the microalgal biomass as a function

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of the two noted variables, and, hence, to determine the most appropriate conditions for producing biomass for use in human nutrition.

2. Materials and methods

2.1. Microorganism

A starter culture of *Porphyridium cruentum* was obtained from the collection of cultures of microalgae, the University of Texas in Austin, USA (UTEX 161). The alga was grown in continuous mode at a constant dilution rate for 10 h in daylight. The culture medium was a modification of Hemericks (1973) medium prepared from filter-sterilized seawater. The pH of the culture was controlled at 7.6 by automatic injection of carbon dioxide. The temperature was controlled at 20°C.

The photobioreactor consisted of a airlift pump that drove the culture fluid through a horizontal tubular solar receiver (Camacho Rubio, Acién Fernández, Sánchez Pérez, Garciá Camacho & Moina Grima, 1998). The total culture volume in the bioreactor was 0.220 m^3. Air was continuously supplied at a flow rate of 0.0124 mol s^{-1}. Carbon was added as pure CO_2 directly injected under pH requirement at a flow rate of 0.0014 mol s^{-1}. Solar irradiance on the reactor surface was measured on-line using a quantum scalar irradiance meter (LI-190 SA, Licor Instruments, Lincoln, NE, USA) connected to a data acquisition card. Biomass was collected at different growth stages and reactor residence times (Rt) as noted in Table 1.

Biomass was collected directly from the photobioreactor in a Pyrex glass container, centrifuged in a batch Selecta centrifuge, at 3500 rpm for 5 min. The harvested biomass had 80% moisture. The biomass cake was washed with 0.5 M NaCl and distilled water to remove non-biological material such as mineral salt precipitates. The biomass was freeze-dried (Edwards Modulyo-4K freeze drier) for storage. The dry biomass was stored in Ependorf vials at −18°C.

Cultures obtained at different reactor residence times and external irradiances were analysed.

Table 1
Cultivation variables

Steady state[a]	Date	Rt (days)	Biomass concentration (gl^{-1})	Eω ($\mu E\ m^{-2}\ day\ s^{-1}$)
A1	11/03/97	1.04	0.52	3.66×10^7
A2	07/23/98	1.03	1.69	1.18×10^8
B2a	10/02/97	1.43	0.64	4.89×10^7
B2b	11/04/98	1.43	1.47	5.40×10^7
B4	08/03/98	1.32	2.11	1.43×10^8
C1	12/02/97	2.04	0.93	3.98×10^7
C2	11/14/97	2.22	1.32	5.86×10^7
C3a	04/15/98	2.00	2.10	8.97×10^7
C3b	03/04/97	2.04	2.47	1.01×10^8
C3c	06/08/98	2.06	2.21	1.03×10^8
C4a	04/28/97	1.82	3.20	1.19×10^8
C4b	05/05/97	2.13	2.61	1.26×10^8
C4c	29/07/98	1.92	2.74	1.44×10^8
D1a	01/07/98	4.55	2.01	2.61×10^7
D1b	02/16/98	4.55	2.33	3.61×10^7
E1	12/30/97	9.09	3.07	2.45×10^7

[a] States coincident for residence time and external irradiance code are distinguished by means of a serial small letter.

2.2. Moisture

Determined by drying a representative 2 g sample in a oven with air circulation at 100–105°C for 40 h.

2.3. Crude protein

Total N was determined in a elemental analyzer Leco CHNS-932. The carrier gas was He, and burning gas, O_2. Results were compared with others obtained by means of a semi-micro Kjeldahl apparatus. Both methods gave similar results. Total protein was calculated from the evaluated nitrogen, multiplying by 6.25, after allowing for N from nucleic acids and nitrate (Becker, 1994).

2.4. Total lipids

Lipids were determined as the extract obtained with chloroform:methanol (2:1) (v/v) (Kochert, 1978).

2.5. Available carbohydrates

These were estimated by the anthrone spectrophomtometric method (Osborne, 1986).

2.6. Energy

The energy content of the biomass was determined by multiplying the values obtained for protein, available carbohydrates and fat by 4.00, 3.75 and 9.00, respectively, and summing the results.

2.7. Dietary fiber

This was determined by the neutral detergent fiber method (Goering & Van Soest, 1970).

2.8. Ashes

Total ash was determined by incineration of a representative 0.5 g sample in an oven at 450°C for 48 h.

2.9. RNA

RNA extraction was accomplished by the method of Shibko, Koivistoinen, Tratnyek, Newhall and Friedman

(1967). Ribose was determined in the supernatants by the orcinol spectrophotometric method (Ogur & Rosen, 1950).

2.10. Minerals

For determining metals, the ash obtained by incineration of the biomass was dissolved in a mixture of HNO_3 and HCl, diluted with water, and analysed for Na, K, Ca, Mg and Zn in a ionic charge chromatograph apparatus (Dionex DX-100). Fe, Cu, Cr and Mn were determined by an atomic absorption spectrophotometer (Perkin-Elmer ASS-1100B), equipped with a graphite chamber (HGA-700) (method 3.006–3.016, AOAC, 1984; Torija-Isasa, 1981).

2.11. Sulphur and carbon

These were determined by using the elemental analyzer.

2.12. Fatty acids

Methyl esters were prepared by treatment of the lipidic fraction with acetyl chloride and methanol (Lepage & Roy, 1984). The fatty acid methyl esters of the mixture were analysed by gas chromatography, the (FAME) were identified by comparing their retention times with those for standards ("Rapeseed oil mix" and "PUFAS-1", from Sigma®), in a Hewlett-Packard HP5890 series II chromatograph provided with a flame ionization detector and HP3394 integrator. A capillary column of high polarity fused silica was used (Supelco SP2330; length: 30 m; internal diameter: 0.25 mm; thickness of the film: 0.2 μm). The flow of carrier gas (N_2) was 0.75 l/min, and the split ratio of the injector was 100:1. The Injector temperature was 240°C and the detector temperature was 260°C. The starting temperature of the oven was 205°C and it was increased at a rate of 6°C/min until 240°C (5.83 min). The injection volume was 5 μl and a blank was run after every two analyses. Peaks were identified by using standard FAME and quantitated by using methyl heptadecanoate (17:0) as an internal standard.

2.13. Total carotenoids

These were evaluated spectrophotometrically (Whyte, 1987).

2.14. Phycobiliproteins

Phycocyanins, allophycocyanins and phycoerythrin were computed spectrophotometrically (Packer & Glazer, 1988).

2.15. Chlorophylls and degradation products

These were determined spectrophotometrically (White, Jones & Gibbs, 1963).

2.16. Statistical analysis

Principal components and cluster analyses were performed with the software package Statgraphics v. 3.0.

All analyses were performed in triplicate and variation on any one sample was routinely less than 5%. Mean values and standard deviations based on these results are shown in the tables.

3. Results and discussion

Steady state culture characteristics are shown in the Table 1. As the biomass was cultivated and harvested throughout the year, the external irradiance level varied widely. Biomass was also cultivated so that residence time varied. For statistical analysis, in order to provide a good global comprehension of the influence of the work variables on the values obtained for analysed nutrients, the data for residence time and external irradiance were grouped using codes, shown in Tables 2 and 3, respectively. Residence time ranged from 1.02 to 9.57 days, and external irradiance ranged between 2.38×10^7 and 1.26×10^8 μE m^{-2} day^{-1}.

All results of analysis of the biomass are expressed on a 100 g dry wt basis. Data referring to proximate composition, nitrate and RNA are provided in Table 4. Moisture content was low, with a mean of 3.24 g, a value in the range of general recommendations for a quality less than 10% (Becker, 1994). Available carbohydrates were lower than in other reports, probably because the biomass was washed to eliminate the extracellular polysaccharide. The main functions of the capsular polysaccharide are to protect the cells from desiccation (Ramus, 1981) and to enable the algae to grow in a marine environment (Kloareg & Quatrano,

Table 2
Residence time groups

Code	Days
A	$1.03 \leqslant Rt \leqslant 1.04$
B	$1.32 \leqslant Rt \leqslant 1.43$
C	$1.82 \leqslant Rt \leqslant 2.2$
D	$Rt = 4.55$
E	$Rt = 9.09$

Table 3
Irradiance groups

Code	Eω (μE m^{-2} $days^{-1}$)
1	$2.45\times10^7 < E\omega < 4.80\times10^7$
2	$4.80\times10^7 < E\omega < 8.40\times10^7$
3	$8.40\times10^7 < E\omega < 1.14\times10^8$
4	$1.14\times10^8 < E\omega < 1.44\times10^8$

Table 4
Proximate composition, RNA and nitrate (g/100 g dry biomass)

Steady state[a]	Moisture	Available carbohydrates	Lipid	Crude protein	Ash	Fiber	RNA	Nitrate	Energy (kJ)
A1	8.10 ± 0.81	22.8 ± 2.0	6.45 ± 0.41	40.7 ± 2.1	23.6 ± 2.0	0.24 ± 0.02	2.03 ± 0.14	0.14 ± 0.01	1280 ± 55
A4	4.74 ± 0.42	25.6 ± 1.9	5.78 ± 0.50	39.2 ± 3.0	22.4 ± 2.0	0.13 ± 0.01	2.10 ± 0.20	0.16 ± 0.02	1280 ± 88
B2a	6.70 ± 0.58	24.5 ± 1.3	6.75 ± 0.44	40.8 ± 2.3	23.4 ± 1.8	0.49 ± 0.02	1.50 ± 0.14	0.16 ± 0.01	1320 ± 67
B2b	3.22 ± 0.19	24.0 ± 2.0	6.65 ± 0.55	39.4 ± 2.7	23.0 ± 1.4	0.43 ± 0.03	1.60 ± 0.15	0.13 ± 0.02	1290 ± 87
B4	3.66 ± 0.29	26.8 ± 2.7	6.10 ± 0.43	38.3 ± 2.8	22.2 ± 2.1	0.51 ± 0.03	1.80 ± 0.22	0.15 ± 0.01	1290 ± 107
C1	7.93 ± 0.57	31.0 ± 2.5	6.36 ± 0.60	35.5 ± 2.4	20.9 ± 1.8	0.37 ± 0.04	1.74 ± 0.19	0.14 ± 0.02	1320 ± 88
C2	5.34 ± 0.45	36.8 ± 2.8	6.80 ± 0.58	36.3 ± 2.0	21.3 ± 2.0	0.51 ± 0.04	1.63 ± 0.16	0.10 ± 0.02	1440 ± 113
C3a	2.83 ± 0.24	35.8 ± 3.4	6.36 ± 0.61	33.0 ± 2.8	19.7 ± 1.1	0.35 ± 0.02	1.54 ± 0.20	0.05 ± 0.01	1350 ± 89
C3b	5.92 ± 0.50	39.3 ± 3.6	6.86 ± 0.57	27.7 ± 2.0	17.3 ± 1.4	0.32 ± 0.02	2.01 ± 0.18	0.20 ± 0.01	1340 ± 102
C3c	3.12 ± 0.15	35.1 ± .9	6.13 ± 0.53	32.1 ± 2.8	19.1 ± 1.3	0.31 ± 0.05	1.68 ± 0.15	0.06 ± 0.01	1320 ± 110
C4a	8.35 ± 0.77	33.5 ± 3.4	6.48 ± 0.65	29.8 ± 1.9	18.1 ± 1.4	0.26 ± 0.03	1.89 ± 0.13	0.19 ± 0.01	1270 ± 101
C4b	8.83 ± 0.80	37.4 ± 3.6	5.78 ± 0.57	31.2 ± 3.2	18.5 ± 1.3	0.35 ± 0.02	1.70 ± 0.12	0.07 ± 0.01	1330 ± 78
C4c	2.58 ± 0.12	37.6 ± 3.4	6.50 ± 0.45	31.4 ± 3.0	18.4 ± 1.5	0.24 ± 0.01	1.85 ± 0.21	0.17 ± 0.03	1330 ± 96
D1a	2.10 ± 0.15	33.9 ± 2.6	7.06 ± 0.65	31.5 ± 2.1	17.7 ± 1.8	0.54 ± 0.05	1.87 ± 0.19	0.12 ± 0.01	1320 ± 100
D1b	1.25 ± 0.09	32.3 ± 3.1	6.86 ± 0.63	28.9 ± 2.6	16.9 ± 1.3	0.51 ± 0.04	1.73 ± 0.17	0.07 ± 0.01	903 ± 110
E1	3.24 ± 0.22	37.5 ± 3.5	7.55 ± 0.66	30.4 ± 1.8	16.8 ± 1.4	0.61 ± 0.04	1.70 ± 0.21	0.07 ± 0.01	1380 ± 110
Mean	4.87 ± 2.48	32.1 ± 5.6	6.53 ± 0.46	34.1 ± 4.4	20.0 ± 2.4	0.39 ± 0.13	1.77 ± 0.18	0.12 ± 0.05	1300 ± 114

[a] States coincident for residence time and external irradiance code are distinguished by means of a serial small letter.

1988). Vonshak, Cohen and Richmond (1985) estimate that exopolysaccharide concentration may reach 0.24 g l^{-1} under adverse conditions such as nitrogen limitation. Fiber amounts were very low, ranging from 0.13 to 0.61 g. Low amounts of fiber could be due to the fact that, in this alga, the exopolysaccharide replaces the functions of the cell wall. Low values suggest an easily digestible biomass for human use. Other microalgae have high fiber content and this has been an argument against use of microalgae in human nutrition (Becker).

Protein content was high, as is the norm for most microalgae. Lipid contents (6.53 g mean), were lower than other referenced data, possibly because the cultures were harvested with a short residence time. The biomass had a moderate amount of RNA, slightly higher than 1 g for the majority of cases. These values are in the range of the cited quality criteria, since the ratio RNA:DNA is 3:1 for microalgae (Becker, 1994). The total nucleic acids may reach 2.36 g, wich is a lower value than the recommended 6 g. Consequently, the biomass provides few purines, which are similar to levels in the commonly-consumed vegetables (1–2 g). Nitrate values were also moderate (mean 0.12 g). For a daily biomass consumption of 15 g, nitrate may reach 0.018 g, a figure lower than the established 0.5 g considered to cause adverse effects to organisms (Lindner, 1995).

Ash contents were high in most cases, ranging from 16.8 to 23.6 g. Similar values have been reported for other marine microalgae used in human nutrition. For example, 20.2 g ash was reported in *Phaeodactylum tricornutum* (Markovits et al., 1991) and 21 g in *Tetraselmis chui* (Cañizares et al., 1994). Freshwater algae show lower figures, e.g. 6–15 g in *Scnedesmus* and *Spirulina* (Becker & Venkataraman, 1982).

Fatty acid profiles are recorded in Table 5. The major fatty acids were palmitic acid (PA, 16:0), 1.58 g, followed by arachidonic acid (AA, 20:4ù6), 1.29 g, eicosapentaenoic acid (EPA, 20:5ù3) 1.27 g and linoleic acid (LA, 18:2ω6) 0.37 g. This profile was widely affected by culture conditions. These results are similar to those reported earlier for EPA (Cohen et al., 1988) in external photobioreactors. The fatty acids EPA and AA play an important role in modulating human metabolism, since AA is an essential component of membrane phospholipids and a prostaglandin precursor; EPA is also an eicosanoid precursor (Dyeberg, 1986).

Pigment amounts are shown in Table 6. Carotenoids are a minor pigment in this microalga, with a mean value of only 102 mg. Phycobiliproteins constitute the main pigments present, with phycoerithrin being the dominant one. This pigment has been proposed for commercial use as a natural dye (Becker, 1994). Chlorophylls and degradation products were analysed because phaeophorbides can cause possible poisoning, causing inflammation in sensitive skins on exposure to sunlight. One case of this is documented as a consequence of *Chlorella* consumption in Japan in 1977 (Becker). Pheophorbides are present in amounts lower than the recommended upper limit (120 mg/100 g).

Mineral data are given in Table 7. The biomass was rich in Ca (1240 mg), K (1190 mg), Na (1130 mg) and Mg (628 mg). Figures for other mineral elements were: Zn 373 mg, Fe 661 mg, Cu 7.48mg, Mn 47.1 mg, Cr 0.02 mg and S 1410 mg. Toxic elements were lower than the recommended values. Thus, for all culture conditions, Pb < 0.25 mg, As < 0.1 mg and Cd < 0.025 mg. The sea water we used had no toxic elements.

Mineral element contents were sufficient for the recommended daily intakes for an adult, if they were bioavailable. Thus, a daily consumption of 65 g of microalga meets the calcium needs (800 mg), 56 g of this microalga fulfills the recommended daily allowance for magnesium (350 mg), and 4 g of algae meets the daily need for zinc (15 mg).

The correlation coefficients among variables were high and, generally significant, indicating that the variations could be due to a few related causes. In order to establish relationships among the variables, a multivariable data analysis was performed for data obtained at various states. Multivariable data analysis is a suitable approach to find underlying structures in complicated biological systems. One of the most powerful and widely used methods is principal component analysis (PCA), which reduces the number of variables to a limited number of principal components (PC) (Joliffe, 1986; Wold, Eseben & Geladi, 1987). PCA was initially applied to selected variables after the Pearson correlation study. In the present analysis, the two first PC explained 39.1 and 23.2%, respectively, of the total variance, and all variables had a strong influence on the model. A plot for the two first component weights (Fig. 1a) shows that there are groupings of variables. Thus, residence time has a great influence on component 1, and it is positively correlated with other variables: Lipid ($r = 0.677$; $P < 0.001$), palmitic acid ($r = 0.844$; $P < 0.0001$), arachidonic acid ($r = 0.813$; $P < 0.0001$) and sulfur ($r = 0.5726$; $P < 0.05$). These variables with others, such as fiber and chlorophyll b form a group which increases jointly, for large residence time values. This is explained by the fact that, for high residence times, the energy is accumulated in reserve lipid form. Furthermore, the variable ash is found in opposite form to these other variables, which implies that a low residence time induces high salt accumulation in the cellular interior, in order to offset the high osmotic pressure from the culture medium, which is rich in mineral nutrients. Grouping with the variable ash, there are other variables: protein, nitrogen and EPA, located oppositely to the previously mentioned variables and opposed also to the three variables, available carbohydrates, C/N ratio and biomass concentration (Bc). This can be interpreted by considering that low residence times imply younger cells with increasing proteins needs for the cell growth and cell reproduction (Fernández Sevilla, 1993; Kaixian & Borowitzka, 1992; Piorreck & Pohl, 1984).

Table 5
Fatty acid content (g/100 g dry biomass) (mean ± S.D.)

Steady state[a]	16:0	18:2ω6	20:4ω6	20:5ω3
A1	1.52 ± 0.09	0.28 ± 0.02	1.16 ± 0.10	1.63 ± 0.14
A4	1.36 ± 0.12	0.20 ± 0.01	0.94 ± 0.08	1.41 ± 0.12
B2a	1.57 ± 0.13	0.34 ± 0.02	1.11 ± 0.09	1.39 ± 0.12
B2b	1.42 ± 0.14	0.29 ± 0.04	1.26 ± 0.08	1.47 ± 0.10
B4	1.43 ± 0.13	0.27 ± 0.03	0.92 ± 0.09	1.32 ± 0.11
C1	1.59 ± 0.13	0.40 ± 0.02	1.38 ± 0.12	1.29 ± 0.13
C2	1.52 ± 0.13	0.38 ± 0.03	1.35 ± 0.11	1.40 ± 0.15
C3a	1.57 ± 0.08	0.35 ± 0.02	1.27 ± 0.10	1.22 ± 0.11
C3b	1.63 ± 0.12	0.40 ± 0.04	1.34 ± 0.11	0.94 ± 0.08
C3c	1.61 ± 0.13	0.40 ± 0.03	1.23 ± 0.07	1.23 ± 0.11
C4a	1.63 ± 0.14	0.36 ± 0.02	1.32 ± 0.11	1.04 ± 0.11
C4b	1.47 ± 0.12	0.35 ± 0.01	1.21 ± 0.09	1.12 ± 0.07
C4c	1.41 ± 0.13	0.37 ± 0.03	1.13 ± 0.09	1.20 ± 0.13
D1a	1.87 ± 0.16	0.49 ± 0.03	1.69 ± 0.12	1.30 ± 0.08
D1b	1.81 ± 0.18	0.51 ± 0.02	1.59 ± 0.07	1.21 ± 0.14
E1	1.93 ± 0.08	0.55 ± 0.02	1.72 ± 0.11	1.11 ± 0.08
Mean	1.58 ± 0.17	0.37 ± 0.09	1.29 ± 0.23	1.27 ± 0.17

[a] States coincident for residence time and external irradiance code are distinguished by means of a serial small letter.

As shown in Fig. 1a, the external irradiance (Eω) variable was placed opposite to variables such as chlorophyll a, ($r = -0.560$; $P < 0.05$), LIN ($r = -0.506$; $P < 0.05$), AA ($r = -0.675$; $P < 0.005$) and PAL ($r = -0.623$; $P < 0.01$). From this it is concluded that low irradiance induces the cells to increase chlorophyll content; cells exposed to high irradiance will use fewer resources for clorophyll biosynthesis than for synthesising ribulose 5-diphosphate and other enzyme implicated in the obscure stage of the phototosynthesis (Darley, 1982). Furthermore, low irradiance induces a longer cellular cycle and, consequently, a greater lipid accumulation. These observations agree with those of Cohen, Norman and Heimer (1995), and indicate that when growth is slowed by any limiting factor, such as light restriction, lipid and carbohydrate synthesis may be enhanced at the expense of protein synthesis. On the other hand, external irradiance is placed next to RNA and nitrate variables, which confirms that high irradiance induces protein biosynthesis. RNA amounts, as a function of nitrate source ($r = 0.559$; $P < 0.05$), have been cited elsewhere (Fábregas, Herrero, Abalde, Liaño & Cabezas, 1986).

The group of variables C/N, available carbohydrates and biomass concentration were located in the same area (Fig. 1a) suggesting that the cell concentration increases with available carbohydrates as the main component, and the C/N ratio depends mainly on the available carbohydrate contents.

The resulting scatterplot (Fig. 1b) provides a conceptual overview of the samples by showing a total of 62.1% of the variance. The relationships between those variables and the PC are defined as loadings. The pattern

Table 6
Pigment content in *P. cruentum* in mg/100 g dry biomass (mean ± S.D.)

		Phycobiliproteins			Chlorophylls and degradation products						
Steady state[a]	Carotenoid	Phycoerythrin	Phycocyanin	Alophycocyanin	Chlorophyll a	Chlorophyll b	Chlorophylide a	Chlorophyllide b	Pheophytin a	Pheophytin b	Pheophorbide a
A1	102 ± 5	250 ± 121	236 ± 32	208 ± 23	171 ± 11	22 ± 2	26 ± 2	0 ± 0	538 ± 32	0 ± 0	0 ± 0
A4	108 ± 7	2100 ± 112	257 ± 17	258 ± 24	191 ± 13	16 ± 2	34 ± 2	0 ± 0	410 ± 25	0 ± 0	41 ± 3
B2a	110 ± 10	2850 ± 189	426 ± 28	357 ± 21	334 ± 21	3 ± 0	96 ± 8	12 ± 1	381 ± 43	0 ± 0	0 ± 0
B2b	105 ± 8	1840 ± 130	233 ± 17	251 ± 14	0 ± 0	34 ± 3	23 ± 1	272 ± 14	22 ± 2	0 ± 0	0 ± 0
B4	111 ± 9	2270 ± 80	248 ± 16	268 ± 15	236 ± 14	29 ± 2	0 ± 0	0 ± 0	395 ± 24	0 ± 0	0 ± 0
C1	118 ± 6	2180 ± 119	250 ± 14	177 ± 13	263 ± 15	47 ± 5	89 ± 7	0 ± 0	230 ± 15	0 ± 0	0 ± 0
C2	125 ± 7	2310 ± 112	300 ± 21	251 ± 17	467 ± 33	0 ± 0	6 ± 0	3 ± 0	232 ± 16	0 ± 0	4 ± 0
C3a	84 ± 6	1610 ± 101	189 ± 14	178 ± 11	323 ± 22	63 ± 4	47 ± 3	9 ± 1	122 ± 10	0 ± 0	0 ± 0
C3b	66 ± 4	1360 ± 104	159 ± 9	134 ± 9	106 ± 7	22 ± 2	44 ± 4	22 ± 2	420 ± 31	0 ± 0	0 ± 0
C3c	88 ± 5	1480 ± 89	198 ± 11	149 ± 8	116 ± 6	99 ± 7	19 ± 2	0 ± 0	246 ± 13	30 ± 2	19 ± 1
C4a	100 ± 5	1760 ± 100	403 ± 15	246 ± 7	11 ± 2	15 ± 1	0 ± 0	0 ± 0	341 ± 24	0 ± 0	154 ± 11
C4b	94 ± 6	1880 ± 87	234 ± 20	207 ± 14	0 ± 0	19 ± 2	16 ± 2	136 ± 1	529 ± 45	3 ± 0	12 ± 1
C4c	106 ± 8	1940 ± 80	242 ± 18	198 ± 11	187 ± 12	5 ± 0	0 ± 0	64 ± 3	302 ± 21	0 ± 0	48 ± 1
D1a	101 ± 3	1880 ± 34	254 ± 10	169 ± 9	235 ± 14	121 ± 8	13 ± 2	28 ± 2	297 ± 23	0 ± 0	0 ± 0
D1b	106 ± 5	1990 ± 67	265 ± 8	222 ± 10	211 ± 21	62 ± 3	47 ± 3	0 ± 0	263 ± 21	0 ± 0	0 ± 0
E1	118 ± 3	2410 ± 45	307 ± 6	219 ± 8	308 ± 15	83 ± 4	0 ± 0	34 ± 1	317 ± 12	0 ± 0	79 ± 4
Mean	102 ± 14	2020 ± 391	262 ± 70	216 ± 54	213 ± 120	38 ± 34	29 ± 29	13 ± 18	331 ± 111	3 ± 9	22 ± 42

[a] States coincident for residence time and external irradiance code are distinguished by means of a serial small letter.

of covariation between the stationary phases can be seen in this Figure. For instance, the present scatterplot indicates that steady stages can be grouped, depending on their nutrient contents. The component plot and scatterplot can be interpreted together because objects with high scores for a specific PC also have high values for the variables, with high loading plots, and low values for those with low loadings. The scatterplot shows that states can be distinguished according to residence time and external irradiance values. Thus, states with low residence times are placed to the left of the plain opposed to those with high residence time. High external irradiance places the stages below in the loading plot and opposed to stages with low external irradiance values. This confirms the strong influence of the operational variables on the nutrient composition of the biomass.

We believe that, if minerals are bioavailable and no toxic or antinutritional factors are detectable, as in this work, *P. cruentum* biomass could be used for nutritional purposes, because of the amount and diversity of nutrients it contains. Also, the biomass composition can be modified by means of operational variables, principally by using short residence times to obtain EPA and protein enrichment.

Table 7
Mineral element content in *P. cruentum* in mg/100 g dry biomass (mean ± S.D.)

Steady state[a]	Na	K	Ca	Mg	Fe	Cu	Zn	Mn	Cr	S
A1	2480 ± 132	1490 ± 98	2220 ± 138	821 ± 66	530 ± 39	9.85 ± 35	427 ± 35	137.0 ± 6.5	1.31 ± 0.08	1490 ± 29
A4	1170 ± 98	1290 ± 110	1700 ± 187	495 ± 21	1130 ± 37	5.91 ± 0.39	317 ± 16	26.0 ± 2.5	1.77 ± 0.12	1300 ± 42
B2a	615 ± 49	970 ± 76	1650 ± 80	488 ± 23	1190 ± 56	10.90 ± 0.87	345 ± 25	95.1 ± 4.7	1.34 ± 0.96	1230 ± 35
B2b	1130 ± 70	1280 ± 88	732 ± 89	595 ± 34	707 ± 43	2.75 ± 0.08	314 ± 23	49.7 ± 3.0	1.05 ± 0.06	1220 ± 41
B4	780 ± 76	868 ± 73	867 ± 61	750 ± 43	912 ± 60	5.95 ± 0.34	346 ± 23	45.9 ± 2.8	1.10 ± 0.09	1160 ± 93
C1	1780 ± 88	1400 ± 81	1140 ± 57	765 ± 53	440 ± 38	6.85 ± 0.51	391 ± 14	35.2 ± 3.32	0.77 ± 0.05	1670 ± 71
C2	357 ± 29	1410 ± 134	466 ± 31	921 ± 42	695 ± 40	6.65 ± 0.48	550 ± 26	64.3 ± 4.8	0.51 ± 0.03	1620 ± 49
C3a	483 ± 24	1020 ± 64	2950 ± 48	545 ± 20	605 ± 31	13.60 ± 0.93	795 ± 67	79.6 ± 3.8	0.92 ± 0.07	1440 ± 42
C3b	585 ± 34	1400 ± 58	1660 ± 90	610 ± 98	424 ± 26	3.41 ± 0.21	463 ± 31	14.4 ± 1.1	0.59 ± 0.03	1320 ± 64
C3c	564 ± 34	940 ± 49	1650 ± 91	592 ± 23	1280 ± 89	10.10 ± 0.84	309 ± 21	20.5 ± 1.7	0.56 ± 0.03	1420 ± 48
C4a	2260 ± 127	1200 ± 110	448 ± 49	621 ± 19	257 ± 13	0.94 ± 0.04	176 ± 15	13.9 ± 0.9	0.41 ± 0.03	1480 ± 58
C4b	940 ± 57	955 ± 86	770 ± 16	425 ± 30	437 ± 32	0.15 ± 0.01	181 ± 11	17.1 ± 1.3	0.65 ± 0.04	1400 ± 76
C4c	952 ± 86	1070 ± 77	1390 ± 110	640 ± 38	699 ± 53	9.59 ± 0.76	467 ± 27	44.3 ± 2.8	1.72 ± 0.8	1250 ± 37
D1a	1240 ± 90	1440 ± 91	773 ± 66	559 ± 39	348 ± 15	10.00 ± 0.81	262 ± 17	30.9 ± 2.6	0.59 ± 0.04	1540 ± 43
D1b	624 ± 43	595 ± 49	720 ± 59	498 ± 35	349 ± 14	8.69 ± 0.72	266 ± 14	43.2 ± 1.9	0.69 ± 0.02	1370 ± 50
E1	2110 ± 116	1680 ± 139	785 ± 27	719 ± 48	570 ± 28	14.30 ± 1.03	322 ± 21	35.8 ± 2.2	0.79 ± 0.05	1680 ± 84
Mean	1130 ± 675	1190 ± 89	1240 ± 691	628 ± 135	661 ± 316	7.48 ± 4.17	373 ± 153	47.1 ± 33	0.92 ± 0.42	1410 ± 161

[a] States coincident for residence time and external irradiance code are distinguished by means of a serial small letter.

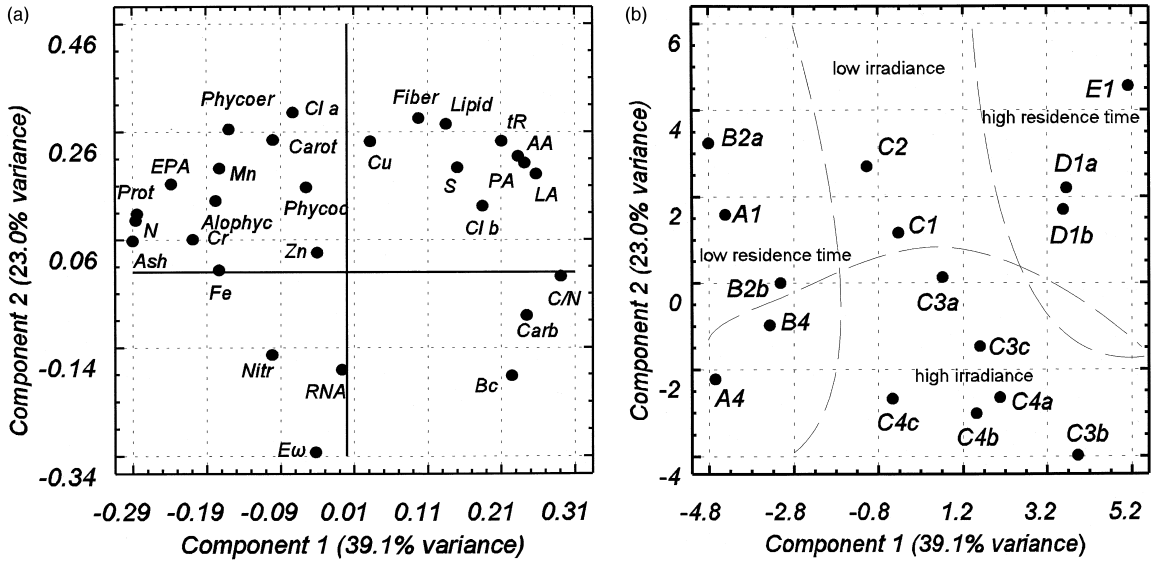

Fig. 1. (a) Alophyc, alophycocyanin; Carot, carotenoid; Phycoer, phycoerythrin; Phycoc, phycocianin; Alophyc, alophycocianin; Cl a, Chlorophyll a; Cl b, chlorophyll b; Eω, External irradiance; Bc, Biomass concentration; Carb, available carbohydrates; Nitr, nitrate:.

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