On-line control of light intensity in a microalgal bioreactor using a novel automatic system

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Abstract

The influence of light intensity upon biomass and fatty acid productivity by the microalga *Pavlova lutheri* was experimentally studied using a novel device. This device was designed to automatically adjust light intensity in a photobioreactor: it takes on-line measurements of biomass concentration, and was successfully tested to implement a feedback control of light based on the growth rate variation. Using said device, batch and semicontinuous cultures of *P. lutheri* were maintained at maximum growth rates and biomass productivities – hence avoiding photoinhibition, and consequent waste of radiant energy. Several cultures were run with said device, and their performances were compared with those of control cultures submitted to constant light intensity; the biomass levels attained, as well as the yields of eicosapentaenoic and docosahexaenoic acids were calculated – and were consistently higher than those of their uncontrolled counterpart.

Introduction

Microalgae are currently cultivated to produce a vast number of high added-value products, e.g. pigments and polyunsaturated fatty acids [1]. Production on the industrial level is usually performed in open ponds or raceways; however, they often lead to low biomass productivity, so they are restricted to only a few species [2]. Several closed systems – usually of the tubular or flat-panel types, have been developed [3]; however, they present difficulties for effective control, require a large area of land and are expensive to operate. Therefore, compact and sterilizable photobioreactors are urged [4].

One of the major parameters that affect microalga growth is light [2]; hence, a light control system is desirable for closed photobioreactors. It is well known that, in a batch culture run under constant light intensity provided externally, the amount of light actually available to cells is affected by mutual shading [5,6]; this affects negatively both their growth rate and biochemical composition. On the other hand, excess light can cause photoinhibition, thus wasting energy and promoting cell death. Therefore, assessment of the light available for photosynthesis throughout culture time is an important step toward accurate and continuous control of light intensity.

A model that describes light-limited growth of microalgae in steady-state, continuous cultures was proposed by Evers [7] – and later applied by Grima et al. [6]; this model takes into account the average light intensity inside a photobioreactor, as a function of incident light and biomass concentration. Use of this type of models allows calculation of the average light intensity associated with the maximum growth rate, hence avoiding photoinhibition; this feature is of major importance in what concerns light control. In this work, such a model was (for the first time) successfully applied to semicontinuous cultures under *quasi-steady-state* conditions.

Recall that *Pavlova lutheri* is widely employed to feed fish, bivalves and crustaceans, owing to its high content of polyunsaturated fatty acids [8–10] – mainly eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are claimed to be beneficial for human health [11,12]. Our research effort encompassed growth of *P. lutheri* in a closed photobioreactor, operated either

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batch- or semicontinuous-wise. An automatic feedback system for control of light intensity – that uses information pertaining to growth rate, was thus designed and tested. The production rates of both EPA and DHA by that microalga were assayed under various conditions of light and growth rate, so as to elucidate the features of said controlled process in a model system.

Experimental

Culture conditions

A genetically improved strain (II#2) of *P. lutheri* [13] was employed, using artificial sea water, ASW [14], as cultivation medium. Cultures of 1.5 L were performed in a 2-L bioreactor (Braun, Germany), with height and internal diameter of 240 and 130 mm, respectively – under both batch and semicontinuous modes. The temperature was maintained at $20 \pm 0.5\,^{\circ}C$ via a refrigeration jacket, the stirring rate was set to 50 rpm, and the pH was kept at 8.0 ± 0.2 by addition of 1 M NaOH or HCl, as appropriate. All these parameters were controlled with a Biostat B unit (Braun). Pure air, enriched with 0.3% (v/v) CO_2, was bubbled at the bottom of the bioreactor, at a volumetric flow rate of $0.54\,L\,L_{culture}^{-1}\,min^{-1}$.

Culture performance

Quasi-steady-state (QSS), semicontinuous cultures were obtained with daily dilutions, and adjusted every day so as to maintain the same initial biomass concentration at each new stage. For every light intensity level considered, several stages (3–7, depending on the actual culture conditions at stake) were performed – until three consecutive (equal) dilutions were made, in order to maintain the initial biomass concentration; the culture was considered to have achieved QSS immediately afterwards. Two different initial biomass concentrations were tested, and submitted to several incident light intensities (I_0) – so as to obtain desired average light intensities, regardless of I_0 and biomass concentration.

Processing setup

A biomass monitoring system – reported elsewhere in detail by Meireles et al. [15], was coupled to an electronic device for light control. Light was provided by a set of 16 fluorescent lamps (OSRAM 18W/21-840) placed vertically, and uniformly distributed around the reactor walls at ca. 10 cm from its surface. Assurance of a good spatial distribution of light is crucial, so lamps were used in pairs of opposite lamps; each pair accounted for $8\,W\,m^{-2}$. An electronic device, connected to the computer via an RS232 serial port, was built and installed; this device was aimed at controlling the number of lamps switched on at each time. Periodical measurements of biomass concentration in the bioreactor were performed by said device, according to a pre-defined schedule (i.e. 4 h); the specific growth rate (μ), calculated from each pair of two consecutive samples, was calculated and compared with the previous value – and a decision was automatically taken, according to the flowchart depicted in Fig. 1.

The threshold (in Fig. 1) was pre-defined, and depended on the culture being studied; said figure is the minimum variation of μ that can be taken as significant (and not merely caused by background noise, of the biomass monitoring system or the culture). The initial light intensity was manually set to slightly above light limitation; when a sample was taken for which μ was not above the aforementioned threshold, the system assumed that there was no variation in biomass concentration – so light intensity was maintained (this step is crucial when there is a lag phase, characterized by a low growth rate); when a sample was taken for which μ was above said threshold, the system assumed that biomass concentration had undergone an increase – so one of two actions was taken: when the current value of μ was not below the previous one, the system assumed that light is not limiting, so light intensity was maintained; conversely, when the current value of μ was lower than the previous one, the system assumed that the culture is light-limited, so light intensity was increased by one increment (i.e. $8\,W\,m^{-2}$).

The software developed previously for the biomass monitoring system [15] was essentially used as such, but a new *Control* menu was added. In this improved version, it was possible to define whether light would be automatically controlled or not, to include calibration curves for biomass, and to input growth rate thresholds. A new field was also included in the *Status* menu – where the current light intensity was recorded, so that it could also be deliberately changed.

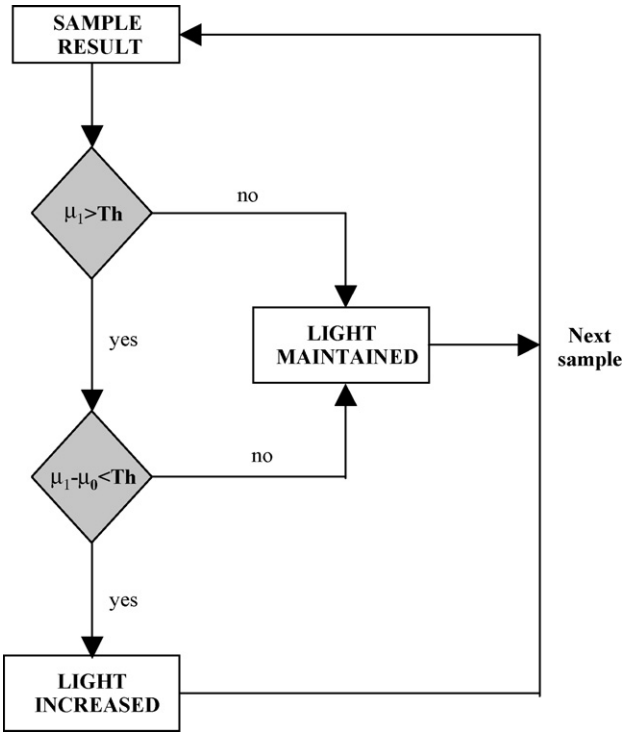

Fig. 1. Schematic diagram of the feedback protocol used to control light intensity. The previous and current values for biomass growth rate are denoted as μ_0 and μ_1, respectively. The predefined threshold for μ is denoted as *Th*.

Analytical assays

The incident light intensity (I_0) was measured using a luxmeter (LI-1000 Data Logger, from LI-COR, USA).

The average light intensity (I_{av}) inside the reactor was calculated following the model by Evers [7]. This model is able to describe the light distribution within a cylindrical transparent vessel, assuming that the attenuation of I_0, caused by mutual-shading, obeys Lambert–Beer's law; the final equation reads

$$I_{av}(C) = \frac{I_0}{\pi R}\int_0^R\int_0^\pi \exp(-K_a C[(R-S)\cos\varphi + \sqrt{R^2-(R-S)^2\sin^2\phi}])\,d\varphi\,dS \quad (1)$$

where C is the biomass concentration; R is the radius of the vessel; S is the distance of a given point to the reactor wall; ϕ is the angle of the light path with the axis of symmetry of the reactor; and K_a is the absorption coefficient of the microalga. In the case of *P. lutheri*, the value taken for K_a was $0.428\,m^2\,g^{-1}$ – following the method described by Grima et al. [6].

The ash-free dry weight (AFDW) was determined by filtering 10 ml of culture through pre-conditioned GF/C glass fiber filters (Whatman, UK), drying at 100 °C to constant weight, and finally heating to 550 °C for 1 h.

Fatty acid analysis took place on freeze-dried samples, and used gas chromatography after direct trans-methylation – according to the acidic method described by Lepage and Roy [8], after the modifications introduced by Cohen et al. [16]. Heptadecanoic acid was used as internal standard, and acetyl chloride was used as catalyst. The resulting esters were analyzed in a gas chromatograph (Perkin Elmer, USA), using detection by flame ionization; resolution was via a 60-m fused silica, capillary column Supelcowax-10 (Supelco, USA), using helium as carrier gas in splitless mode; sample injection and detection occurred at 250 and 270 °C, respectively; and the oven heating program included an increase of the column temperature linearly from 170 to 250 °C, at a rate of

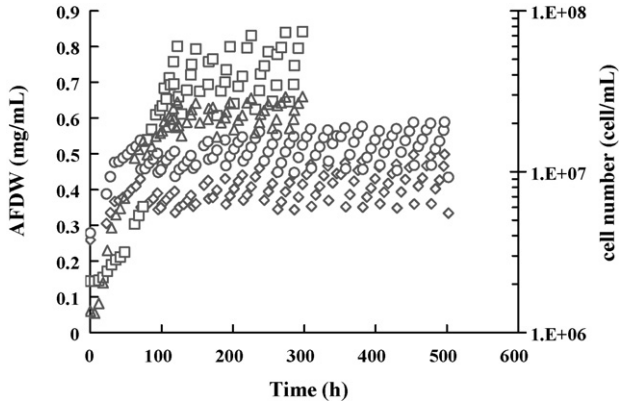

Fig. 2. Evolution of ash-free dry weight (AFDW) with time, for various initial biomass concentrations ((◊) 0.341 mg L^{-1}; (□) 0.592 mg L^{-1}) and cell number ((◯) 0.341 mg L^{-1}; (△) 0.592 mg L^{-1}), in the semicontinuous QSS stages.

1 °C min^{-1}. Pure standards of free fatty acids (Sigma, USA) were used for tentative identification and quantification, based on comparison of retention times.

Statistical analyses

Statistical analyses of variance (ANOVA) and Tukey's tests (when ANOVA indicated at least one significantly different result) were applied to the data generated in the semicontinuous cultures, both under constant and controlled light intensities, taking advantage of the three replicates available – so as to identify differences in growth rate.

Results

To study the influence of I_{av} on the growth rate of *P. lutheri*, several QSS stages – characterized by as many values of I_{av}, were achieved, so as to correspond to distinct biomass concentrations and incident lights. Two biomass concentrations were accordingly chosen, coupled with a wide range of I_{av} – in attempts to reduce mutual correlation of the data. The biomass concentrations in the various QSS stages are depicted in Fig. 2, in terms of cell number and ash-free dry wheight.

The model by Evers [7] and Grima et al. [6] assumes that uptake of light depends on light intensity: said functionality is characterized by a hyperbolic curve containing an exponential parameter (n), and is able to fit growth kinetics to the sigmoidal effect observed at low light intensities. The Hill-type rate expression finally takes the form

$$\mu = \frac{\mu_{max} {I_0}^n}{{I_0}^n + I_k^n} - m \qquad (2)$$

where I_k is a constant that represents the affinity of cells to light; and m is the specific maintenance rate.

For each QSS stage, μ was duly calculated based on the slope of the log (C) vs. time curve. The variation of μ as a function of I_{av} is plotted in Fig. 3. Non-linear regression analysis was performed – and the best estimates found for the parameters in Eq. (2) were: $\mu_{max} = 0.0148\ h^{-1}$; $I_k = 0.3\ W\ m^{-2}$; $m = -0.0018\ h^{-1}$; $n = 1.96$; and $r^2 = 0.999$.

Several batch and semicontinuous cultures were performed using the proposed light control device, and compared with

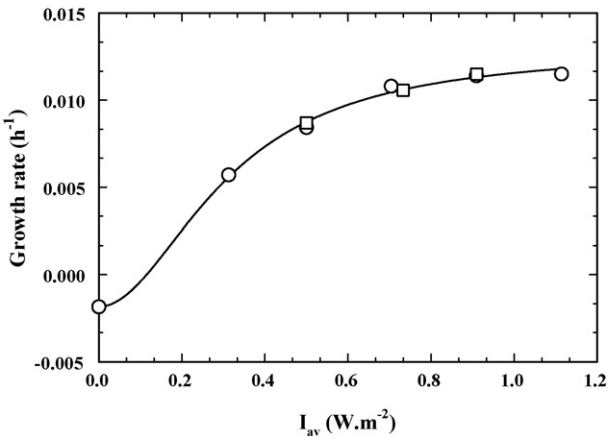

Fig. 3. Variation of growth rate with average light intensity (I_{av}) for various initial biomass concentrations ((◯) 0.341 mg L^{-1}; (□) 0.592 mg L^{-1}) – as obtained from Eq. (2), in the semicontinuous QSS stages.

runs carried out under constant light intensity. The initial light intensity in the controlled cultures was set at ca. 0.7 W m^{-2}, whereas three constant values for I_0 (i.e. 10, 25 and 40 W m^{-2}) were tested (except for 25 W m^{-2} in batch mode). Comparison between the controlled and the constant light cultures – in both batch and semicontinuous modes, is presented in Figs. 4 and 5, respectively; the kinetic parameters are in turn tabulated in Table 1. The results of Tukey's tests, applied to homogeneous subsets – in terms of differences in μ_{max} of semicontinous cultures, are included in Table 2. Significant differences ($P < 0.01$) do exist between the growth rates – except for cultures grown at 40 W m^{-2} and under light control.

The typical variations of I_{av}, throughout batch and semicontinuous culture time under controlled light, are shown in Fig. 6; the overall average light intensity was 0.622 and 0.661 W m^{-2}, in the batch and semicontinuous modes of operation, respectively. Each increment corresponds to the increase in I_0 introduced by switching on an extra set of lamps; the value for I_{av} then decreases, until a new light increment is triggered.

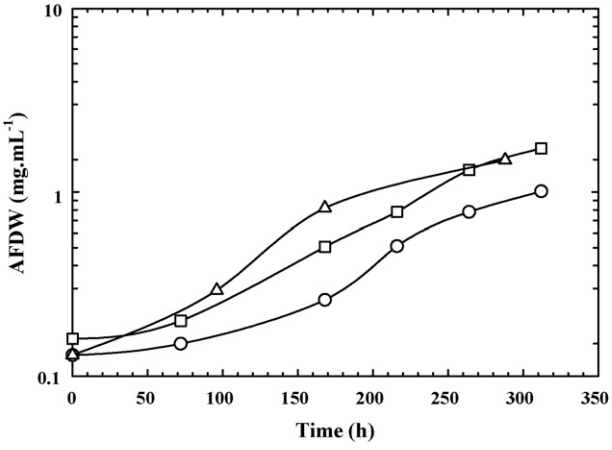

Fig. 4. Evolution of ash-free dry weight (AFDW) with time, under constant light intensity ((◯) 10 W m^{-2}; (□) 40 W m^{-2}) and light control (△), in batch cultures.

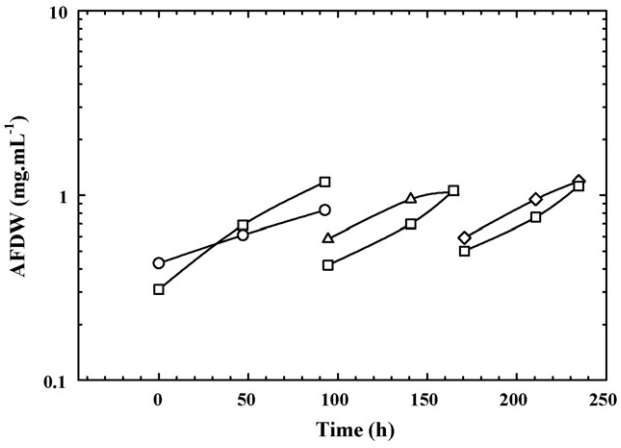

Fig. 5. Evolution of ash-free dry weight (AFDW) with time, under constant light intensity ((◯) 10 W m^{-2}; (◊) 25 W m^{-2}; (□) 40 W m^{-2}) and light control (△), in semicontinuous cultures.

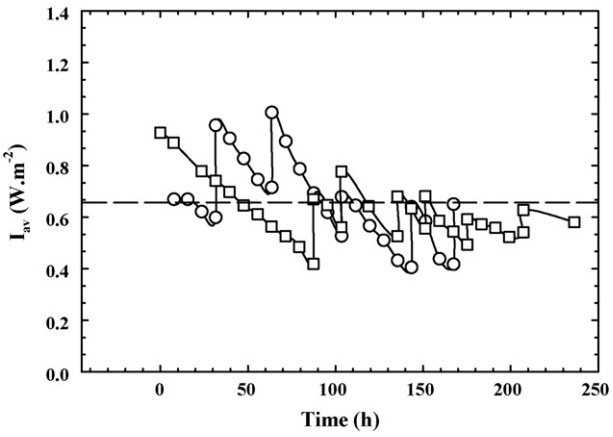

Fig. 6. Evolution of average light intensity (I_{av}) with time, under light control, in batch (□) and semicontinuous cultures (◯).

The results pertaining to the fatty acid profile of the various cultures, performed with and without light control, are presented in Table 3 – for the batch and semicontinuous modes. The overall EPA and DHA yields of these cultures are summarized in Table 4. Owing to the higher biomass productivity – associated with the higher EPA and DHA yields, light-controlled cultures eventually exhibited the higher EPA and DHA productivities.

Discussion

Inspection of the results depicted in Figs. 2 and 3, coupled with Eq. (2), one concludes that it is possible to anticipate when cultures will be light-limited or not; in order to attain growth rates close to the maximum (hence avoiding excess I_0, that would lead to both photoinhibition and energy waste), it was assumed that I_{av} should lie in the vicinity of 0.65 W m^{-2}.

The first datum in Fig. 3 was obtained when I_0 was set equal to zero (i.e. no light was provided to the reactor) – and thus represents the maintenance rate (m) obtained from Eq. (2). The data in Table 1 indicate that, under constant light intensity, both the growth rate and the productivity increase with increasing I_0 – as expected, and as previously confirmed [5,17]. However, there was no statistically significant difference between the maximum growth rate and the productivity of cultures run at the highest I_0 tested (i.e. 40 W m^{-2}) and of cultures with on-line control of light intensity. Finally, it is apparent in Fig. 4 that the batch cultures using on-line control of light experienced no lag-phase – unlike what happened with cultures run under 40 W m^{-2}; this result is easily accounted for by photoinhibition, which comes into play when excess light is provided at the startup of experiments.

In view of the above, it was possible to achieve the maximum productivity with lower consumption of radiant energy, and with essentially no photoinhibition – provided that the light control system kept the light intensity below limitation at all times. (Note that it was considered that the actual value of I_{av} should be ca. 0.65 W m^{-2}; and that the light intensity, in batch and semicontinuous modes of operation under light control, was accordingly maintained in the vicinity of that value – with an actual average light intensity of 0.622 and 0.661 W m^{-2}, respectively.)

The maximum growth rate achieved (i.e. 0.0129 h^{-1}, see Table 1) is close to the maximum one predicted by Eq. (2); the slight gap between them is probably due to limitations arising from non uniform nutrient distribution.

The major fatty acid residue constituents of the microalgal lipids were C16:0, C16:1(n-7), C18:4(n-3), C20:5(n-3) – EPA and C22:6(n-3) – DHA, which accounted for more than 75% of the total fatty acid inventory; similar results were reported for *P. lutheri* by Tatsuzawa et al. [18]. It is important to emphasize

Table 1
Specific growth rate (μ_{max} and $\mu_{average}$) and productivity (Ψ) of batch and semicontinuous cultures, under constant and controlled light intensity

I_0 (W m^{-2})	$\mu_{average}$ (h^{-1})	μ_{max} (h^{-1})	Ψ (mg L^{-1} d^{-1})
Batch			
10	0.0085	0.0104	67.69
40	0.0091	0.0112	111.5
Controlled	0.0103	0.0109	113.6
Semicontinuous			
10	–	0.0071 ± 0.0004	103.6 ± 16.5
25	–	0.0085 ± 0.0002	151.1 ± 5.8
40	–	0.0115 ± 0.0013	223.3 ± 9.5
Controlled	–	0.0129 ± 0.0005	229.3 ± 5.1

Table 2
Tukey's test of homogeneous subsets for growth of semicontinuous cultures, under constant and controlled light intensity

I_0 (W m^{-2})	Number of replicates	Subset for $\alpha = 0.01$		
		1	2	3
10	3	0.00707		
25	3		0.00847	
40	3			0.01188
Controlled	3			0.01293
Significance		1.000	1.000	0.037

Table 3
Fatty acid composition of batch and semicontinuous cultures, under constant and controlled light intensity

Fatty acid content (%, w/w)								
I_0 (W m^{-2})	Controlled		10		25		40	
	Semicontinuous	Batch	Semicontinuous	Batch	Semicontinuous	Batch	Semicontinuous	Batch
C14:0	8.92	8.08	9.56	9.50	8.89	–	10.04	8.61
C16:0	24.67	29.47	22.56	26.01	23.47	–	23.07	30.13
C16:1(n-7)	25.20	18.36	26.05	24.84	26.10	–	28.16	27.07
C18:0	0.47	2.98	0.42	0.50	0.43	–	0.57	0.70
C18:1(n-9)	1.91	6.92	1.87	2.03	2.77	–	2.38	2.96
C18:1(n-7)	1.48	2.92	1.89	2.24	1.32	–	1.16	1.37
C18:2(n-6)	2.60	2.83	2.65	1.59	3.35	–	3.31	3.39
C18:3(n-3)	1.49	1.00	1.49	1.72	1.44	–	0.80	0.79
C18:4(n-3)	4.54	3.01	6.39	5.56	5.57	–	5.37	3.65
C20:3(n-3)	0.67	0.65	0.49	0.98	0.18	–	0.10	0.92
C20:5(n-3)	19.19	16.79	17.85	18.12	18.27	–	16.06	12.68
C22:6(n-3)	8.85	6.99	8.78	6.90	8.21	–	8.98	7.73

that, for the experimental light-controlled cultures, the fractions of EPA and DHA, referred to total fatty acid content, were rather high; EPA accounted for ca. 17 and 19% of the total fatty acids in batch and semicontinuous cultures, respectively, whereas DHA accounted for 7 and 9%, respectively.

The amount of saturated fatty acids increased with increasing light intensity – as reported previously by Grima et al. [6]. Among the physiologically (and hence commercially) most important PUFA, EPA presented an atypical variation – with a slight increase in content at medium light intensity (25 W m^{-2}), followed by a decrease at the highest light intensity; DHA content was, in turn, highest at the highest light intensity. In light-controlled cultures, the content of C14:0, C16:0 and C16:1(n-7) was always lower than in their counterparts run at a constant light intensity 40 W m^{-2} (except for C16:0 in semicontinuous mode). Moreover, both EPA and DHA contents lied near the maximum observed, under all conditions tested.

Although the final concentration of individual fatty acids is an important feature, the fatty acid and the biomass yields are more important features towards eventually wider applicability; the results in Table 3, pertaining to semicontinuous cultures make it apparent that – despite a slightly smaller concentration, EPA and DHA yields were considerably higher in controlled cultures (likely because of the higher biomass productivity attained).

Table 4
Overall yield (mg L^{-1} d^{-1}) of EPA and DHA of batch and semicontinuous cultures, under constant and controlled light intensity

I_0 (W m^{-2})	EPA	DHA
Batch		
10	0.403	0.153
40	0.756	0.460
Controlled	0.820	0.452
Semicontinuous		
10	0.556	0.291
25	0.933	0.419
40	0.763	0.427
Controlled	1.105	0.560

Conclusions

Microalgae are currently used as polyunsaturated fatty acid-rich biomass in the aquaculture industry, so systems that automatically monitor and control algal culture parameters are urged. Since light is a crucial parameter in microalgal growth and metabolite production, its control is required if the highest production yields are sought. In this research effort, a simple system aimed at controlling light intensity in a microalgal bioreactor was designed and tested; said system permitted maximum biomass and PUFA yields to be achieved at the minimum expense of light energy – and performed better than resorting to the classical, constant light processing approach.

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