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Effects of light intensity and dilution rate on the semicontinuous cultivation of *Arthrospira* (*Spirulina*) *platensis*. A kinetic Monod-type approach

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ABSTRACT

Semicontinuous cultures were carried out at different dilution rates (D) and light intensities (I) to determine the maximum productivity of *Arthrospira platensis* cultivated in helicoidal photobioreactor up to the achievement of pseudo-steady-state conditions. At I = 108 μmol photons m^{-2} s^{-1}, the semicontinuous regime ensured the highest values of maximum cell concentration (X_m = 5772 ± 113 mg L^{-1}) and productivity (P_{XS} = 1319 ± 25 mg L^{-1} d^{-1}) at the lowest (D = 0.1 day^{-1}) and the highest (D = 0.3 day^{-1}) dilution rates, respectively. A kinetic model derived from that of Monod was proposed to determine the relationship between the product of light intensity to dilution rate (ID) and the cell productivity, which were shown to exert a combined influence on this parameter. This result put into evidence that pseudo-steady-state conditions could be modified according to circumstances, conveniently varying one or other of the two independent variables.

1. Introduction

Photosynthetic microorganisms are one of the most promising sources of energy since they are renewable and neutral with respect to CO_2 emissions. Among the photosynthetic microorganisms with commercial importance, there are species belonging to the genus *Spirulina*, now named *Arthrospira*, that are filamentous cyanobacteria (blue-green algae) and have a long history of use as food. *Arthrospira* species and strains can achieve 60–70% protein by dry weight and are a rich source of vitamins, especially vitamin B_{12}, provitamin A (β-carotene) and minerals, especially iron. In addition, they are one of the few sources of dietary γ-linolenic acid (GLA) and also contain other phytochemicals that have potential health benefits (Belay, 2002).

In order to increase productivity and reduce costs of production, different types of processes have been developed. An interesting alternative is the semicontinuous operation that consists in periodically replacing a part of the exhaust medium with fresh medium so as to keep the culture volume constant (Huang et al., 2010). Such a mode of operation offers in general many advantages over batch and continuous cultures. Batch culture systems, which are widely applied because of their simplicity and flexibility, are often considered the most reliable, but not necessarily the most efficient ones. On the other hand, large scale continuous cultures were not applied extensively up to now, due to the several difficulties in their control, among which higher risk of contamination, use of feeding pumps, lower yields, etc.

The semicontinuous cultures did not require any special equipment and can be implemented in almost any kind of mass culture, and if properly applied, can be as effective as the chemostat ones (Brown et al., 1993). Moreover, when operating under pseudo-steady-state conditions, the levels of components required for growth or for product formation can be always maintained at non-limiting or non-inhibitory levels along the run (Fábregas et al., 1985, 1998).

Light is the energy source driving photosynthesis and it is one of the most important factor determining the growth of photosynthetic microorganisms. These use light energy to convert CO_2 into carbohydrates, lipids and proteins, with higher areal efficiency than land plants (Jorquera et al., 2010).

In closed photobioreactors, high volumetric productivities are desired in order to reduce the photobioreactor size (Janssen et al., 2003). In a tubular photobioreactor, the light intensity is very high at the reactor wall and decreases with increasing the radial depth, owing to the absorption of light by the photosynthetic microorganism. As a consequence, the cells are exposed to light/dark cycles at high light intensities close to the reactor surface

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and meet a darkness zone in the interior of the photobioreactor (Molina Grima et al., 1999).

In this study, a helicoidal tubular photobioreactor was used for the cultivation of the cyanobacterium *Arthrospira platensis* using different light intensities and dilutions rates with the aim of obtaining high cell concentration, once the batch cultures had reached early stationary growth phase, and high cell productivity under semicontinuous pseudo-steady-state conditions.

2. Methods

2.1. Microorganism and culture conditions

A. platensis was maintained and cultivated in the culture medium of Schlösser (1982) having the following composition (per L): 13.6 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$. All the nutrients were dissolved in distilled water containing (per L): 6.0 mL of metal solution (97.0 mg $FeCl_3{\cdot}6H_2O$, 41.0 mg $MnCl_2{\cdot}4H_2O$, 5.0 mg $ZnCl_2$, 2.0 mg $CoCl_2{\cdot}6H_2O$, 4.0 mg $Na_2MoO_4{\cdot}2H_2O$), 1.0 mL of micronutrient solution (50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4{\cdot}5H_2O$, 44.0 mg $ZnSO_4{\cdot}7H_2O$, 20.0 mg $CoCl_2{\cdot}6H_2O$, 12.6 mg $MnCl_2{\cdot}4H_2O$, 12.6 mg $Na_2MoO_4{\cdot}2H_2O$) and 1.0 mL of a 1.5 g L^{-1} B_{12} vitamin solution.

A schematic diagram of the helical tubular reactor is shown in Fig. 1. Such a configuration was simple to build up and maintain, being a cylindrical shaped helical photostage made of glass, with slight inclination to make the fluid flow easier, linked by PVC tubes to a 1.0 L cylinder bottle, closed with cotton cap. Based on previous results (Converti et al., 2006), circulation was granted by an airlift mechanism, in which air, mixed with the broth, uplifted the liquid. Ambient air was injected through an air pump (model AC-1000, Resun, Shenzhen, China) at the bottom of the tubes to drive the liquid from the bottom to the top of the reactor. The glass helical cylinder had a diameter of 13 cm and was 0.70 cm long, the internal diameter of the photostage's tube being 1 cm, while the PVC tube had the same diameter and was 1.0 m long. The overall starting volume was 1.1 L, being the photostage volume about 0.6 L.

Cultivations were carried out at initial pH of 9.5 ± 0.2 (Sánchez-Luna et al., 2007; Tredici and Zittelli, 1998) and temperature of 30 ± 2 °C. An initial biomass concentration of 400 mg L^{-1} (Soletto et al., 2005) was always used at the start of batch cultures preceding the semicontinuous ones. Semicontinuous cultures were started, once the batch cultures had reached early stationary growth phase, by feeding the fresh medium at different dilution rates (D), specifically 0.1, 0.2 and 0.3 day^{-1}, corresponding to 10%, 20% and 30% of volume renewal per day. It was assumed that pseudo-steady-state conditions were achieved when cell concentration, which was determined daily, kept almost constant along a time period at least twice the residence time. The liquid volume was kept constant at 1.1 L during all the culture by replacing water evaporation when necessary. Three different values of the average irradiance on the surface of the tubular helicoidal photobioreactor were tested, specifically 36, 72 and 108 μmol photons m^{-2} s^{-1}. After each experimental run at different light intensity, the reactor was emptied, cleaned, and inoculated for a new run. Every run was operated non-aseptically for approximately 7 weeks without any contamination problem. Under these conditions cell concentration and biomass productivity were determined.

2.2. Analytical determinations

Biomass concentration was estimated by optical density measurements using a calibration curve relating the absorbance at 560 nm to dry biomass weight (Leduy and Therien, 1977). Light

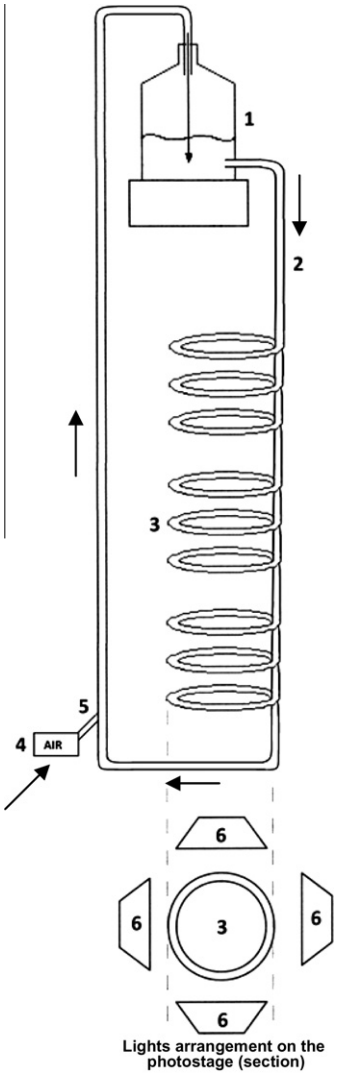

Fig. 1. Schematic diagram of the helical photobioreactor. (1) Cylinder bottle; (2) PVC tube; (3) glass photostage; (4) air pump; (5) air lift system; (6) variable number of 40 W-fluorescent lamps.

was furnished by means of a variable number of 40 W-fluorescent lamps, and its intensity periodically determined by an illuminance meter, model TL-1 (Minolta, Osaka, Japan). Initially, light intensity was expressed in klux, and then converted to photosynthetic photon flux density (PPFD), expressed in μmol photons m^{-2} s^{-1}, using the conversion factor (12 μmol photons m^{-2} s^{-1} $klux^{-1}$) proposed by McCree (1981) for white fluorescent light.

2.3. Parameters determination

The cell productivity of batch runs (P_{XB}), expressed in mg L^{-1} d^{-1}, was calculated as:

$$P_{XB} = \frac{X_m - X_i}{T} \quad (1)$$

where, X_m and X_i are the final and the initial cell concentrations, respectively, and T is the cultivation time.

On the other hand, the cell productivity in semicontinuous cultures (P_{XS}; mg L^{-1} d^{-1}) was calculated as the product of the dilution rate (D; day^{-1}) and the cell concentration under pseudo steady-state conditions (X_S; mg L^{-1}):

$$P_{XS} = DX_S \quad (2)$$

3. Results and discussion

Figs. 2–4 show the results of *A. platensis* batch and semicontinuous cultivations carried out at different light intensities and dilution rates. At the end of batch cultures (after about 8 days), the maximum cell concentration (X_m) increased from 5200 to 5800 mg L^{-1} when the light intensity was increased from 36 to 72 μmol photons m^{-2} s^{-1}, highlighting growth limitation by light intensity within this irradiance level. On the other hand, an additional increase in light intensity up to 108 μmol photons m^{-2} s^{-1} led only to a reduction in the cultivation time from 8 to 6 days, whereas X_m kept almost the same, thus resulting in a cell produc-

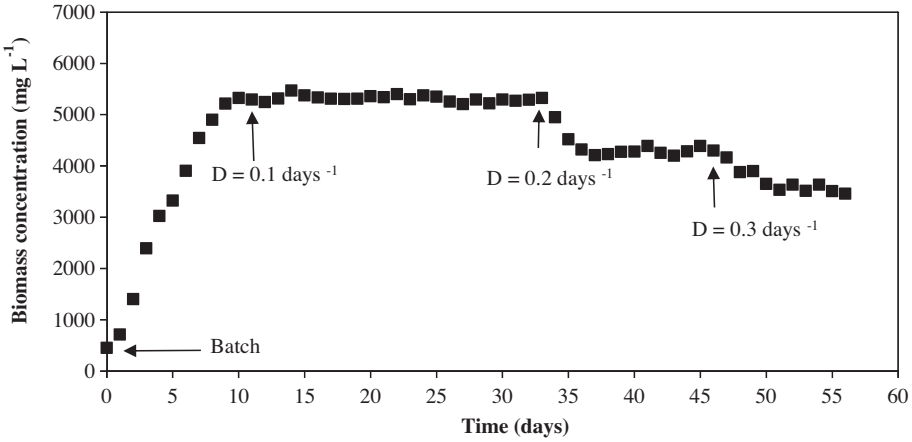

Fig. 2. Cell concentration of *A. platensis* during batch and semicontinuous cultures carried out at 36 μmol photons m^{-2} s^{-1} and different dilution rates (*D*): 0.1, 0.2 and 0.3 day^{-1}.

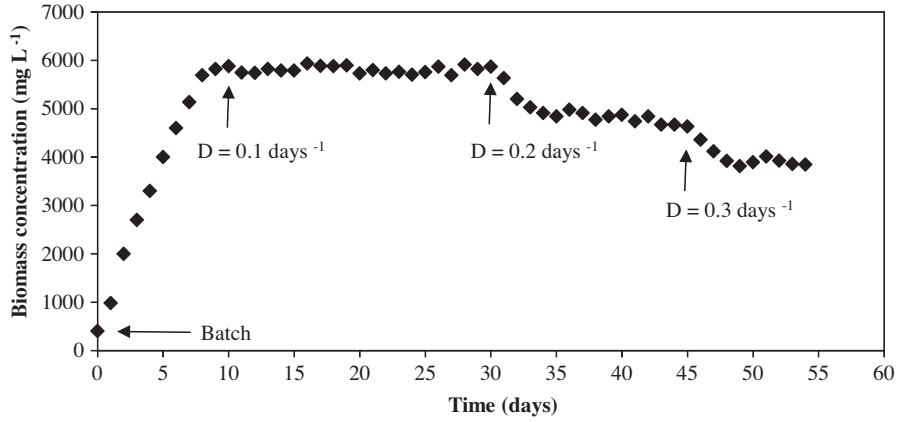

Fig. 3. Cell concentration of *A. platensis* during batch and semicontinuous cultures carried out at 72 μmol photons m^{-2} s^{-1} and different dilution rates (*D*): 0.1, 0.2 and 0.3 day^{-1}.

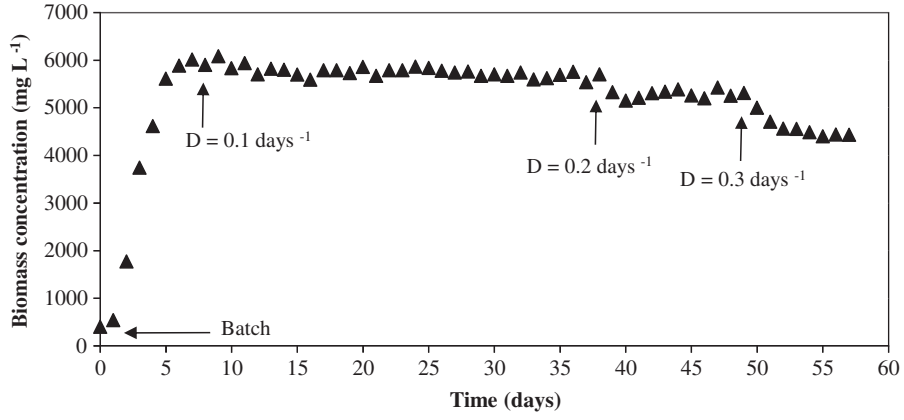

Fig. 4. Cell concentration of *A. platensis* during batch and semicontinuous cultures carried out at 108 μmol photons m^{-2} s^{-1} and different dilution rates (*D*): 0.1, 0.2 and 0.3 day^{-1}.

Table 1
Results of cell concentration and productivity obtained from semicontinuous cultivations of *A. platensis* carried out at different dilution rates and light intensities.

Run	Light intensity (μmol photons m^{-2} s^{-1})	Dilution rate (day^{-1})	X_m or X_S ($mg\ L^{-1}$)	Biomass productivity (P_x, $mg\ L^{-1}\ d^{-1}$)
Batch culture				
1	36	–	5200	562
2	72	–	5800	661
3	108	–	5900	913
Semicontinuous culture				
4	36	0.1	5313 ± 59	531 ± 5.9
5	72	0.1	5806 ± 74	581 ± 7.5
6	108	0.1	5772 ± 113	577 ± 11
7	36	0.2	4284 ± 64	857 ± 13
8	72	0.2	4839 ± 112	968 ± 22
9	108	0.2	5286 ± 82	1057 ± 16
10	36	0.3	3562 ± 75	1068 ± 23
11	72	0.3	3922 ± 100	1176 ± 30
12	108	0.3	4392 ± 83	1319 ± 25

tivity increase from 661 to 913 $mg\ L^{-1}\ d^{-1}$ (Table 1). Similar results were obtained by Danesi et al. (2004) using urea as a nitrogen source in the light intensity range of 2–5 klux. This behavior suggests that, at relatively high light intensity (108 μmol photons $m^{-2}\ s^{-1}$), cell growth was accelerated by the faster photosynthetic production of ATP and NADPH; but, when cell concentration reached 5800 $mg\ L^{-1}$, the growth stopped likely due to photosaturation or shadowing (Chojnacka and Noworyta, 2004; Hirata et al., 1998; Vonshak et al., 2000). Since the cell concentration in the saturation region depends on the light ability to penetrate the culture, the value of X_m obtained in this work is quite different from those reported for different reactor configurations such as long minitanks (1700 ± 41 $mg\ L^{-1}$) (Bezerra et al., 2008) and airlift-tubular photobioreactor (10,900 $mg\ L^{-1}$) (Converti et al., 2006).

The values of cell productivity either in batch (P_{XB}) or in semicontinuous operation (P_{XS}) are summarized in Table 1. Except for the cultures performed at the lowest dilution rate ($D = 0.1\ day^{-1}$), P_{XS} was higher than P_{XB}, because not only of the well-known elimination of any dead time typical of the batch process (Aiba et al., 1973), but also of the accumulation of any toxic metabolite and/or the depletion of some limiting micronutrient during batch growth (Fábregas et al., 1998). Additionally, in the semicontinuous cultures, a portion of cells is continuously removed, and the related increase in light availability to cell likely increased cell productivity.

As can be seen in Figs. 2–4, the semicontinuous process started at $D = 0.1\ day^{-1}$ and reached pseudo-steady-state conditions after about 6–8 days, at which cell concentration (X_S) was strongly dependent on the light intensity; after 20 days, i.e. a time equivalent to twice the residence time, the dilution rate was increased to $0.2\ day^{-1}$, and the cell concentration decreased until a new steady-state was attained. After additional 10 days, D was further increased up to $0.30\ day^{-1}$ following the same criterion. Pseudo-steady-state conditions were obtained at all dilution rates and light intensities, thus demonstrating an excellent ability of *A. platensis* to maintain stable conditions during at least two residence times. This result is quite promising taking into account that large productions of this cyanobacterium are usually performed by semicontinuous operation under pseudo-steady-state conditions.

In cultures performed at $D = 0.1\ day^{-1}$, an increase in light intensity from 36 to 72 μmol photons $m^{-2}\ s^{-1}$ led to a 9% raise in X_S, while an additional increase up to 108 μmol photons $m^{-2}\ s^{-1}$ did not influence this response, which reached an average value of 5789 $mg\ L^{-1}$ (Table 1). As expected by the well-known mass balances in continuous bioprocesses (Aiba et al., 1973), the highest biomass concentration's were obtained at the lowest dilution rate ($D = 0.1\ day^{-1}$) at all light intensities studied. Nevertheless, under these conditions, lower values of productivity (P_{XS}) were obtained.

In cultures performed at $D = 0.2$ and $0.3\ day^{-1}$, X_S increased by around 23% when the incident irradiance was raised from 36 to 108 μmol photons $m^{-2}\ s^{-1}$. This result demonstrates that the cultures carried out at 36 μmol photons $m^{-2}\ s^{-1}$ were light limited and suggests that every culture could have a cell density which is characteristic of a given illumination and a given dilution rate. Similarly, Molina Grima et al. (1996) observed in chemostat that high dilution rates must be supported by fast-growing cells, whose illumination requirements can only be met at low biomass concentrations, whereas low dilution rates give rise to cultures severely limited by self shading. In such a situation, if the irradiance supplied to the system is increased at a given dilution rate, the light availability inside the culture increases.

The values of X_S decreased with increasing D, while those of P_{XS} increased. Over the whole D range considered, the increase in light intensity from 36 to 108 μmol photons $m^{-2}\ s^{-1}$ led to a 20% increase in P_{XS}, confirming the influence of light intensity on cell growth. The highest productivity (1319 ± 25 $mg\ L^{-1}\ d^{-1}$) was obtained in the culture carried out at $D = 0.3\ day^{-1}$ and 108 μmol photons $m^{-2}\ s^{-1}$ (Table 1). This value was comparable to that (1590 $mg\ L^{-1}\ d^{-1}$) reported by Carlozzi (2003) for semicontinuous culture of *A. platensis* in tubular undulating row photobioreactor at $D = 0.318\ day^{-1}$ and higher than the one (400 $mg\ L^{-1}\ d^{-1}$) reported by Travieso et al. (2001) for the same microorganism in helical tubular photobioreactor at $D = 0.18\ day^{-1}$. This comparison demonstrates the potential of *A. platensis* to grow quickly in helicoidal photobioreactor in semicontinuous cultivation.

Taking into account that there are conditions under which the cell growth can be limited by a nutrient or light intensity (Healey, 1985), the following Monod-type empirical model was applied to the experimental data of productivity with the aim of demonstrating the relationship existing between this response and the product of light intensity and dilution rate (ID):

$$P_X = \frac{P_{X\,MAX}\,ID}{K_{ID} + ID} \tag{3}$$

where, $P_{X\ MAX}$ is the maximum cell productivity and K_{ID} the half-saturation constant related to ID.

As can be seen in Fig. 5, the linear Lineweaver–Burk approach applied to the results of Table 1 provided a good fit between $1/P_X$ versus 1/ID, being the best regression ($r^2 = 0.81$) obtained without taking into account the run 6. The lack of adjustment when considering the run 6 was likely due to the fact that, at very low dilution rate ($D = 0.1\ day^{-1}$), the growth was strongly limited by

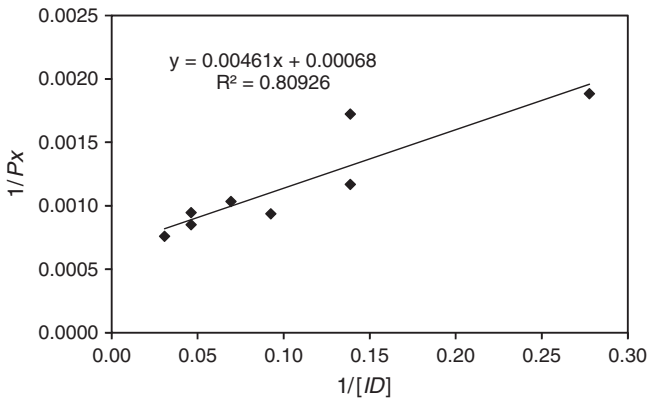

Fig. 5. Cell productivity (P_X) of the semicontinuous culture of *A. platensis* versus the product of light intensity and dilution rate (ID).

insufficient supply of any nutrient, and then no increase in cell productivity took place when I was increased from 72 to 108 μmol photons m^{-2} s^{-1} (Table 1). The kinetic parameters estimated by Eq. (3) were K_{ID} = 6.8 μmol photons m^{-2} s^{-1} day^{-1} and $P_{X\ MAX}$ = 1471 mg L^{-1} d^{-1}. Because K_{ID} expresses the value of ID at which 50% of maximum cell productivity is obtained, it may become a guideline to regulate the values of the selected independent variables (I and D) according to the desired value of productivity. Any further productivity increase beyond the 1471 mg L^{-1} d^{-1} threshold would require excessively high increases in I or D, which could make the process unstable (washout in the case of excess D), or photoinhibited (in the case of excess I), and certainly unfeasible from the economic viewpoint.

These results put into evidence a peculiar feature of cyanobacterial cultures, whose pseudo-steady-state conditions could be modified, according to circumstances, by conveniently varying these two independent variables.

4. Conclusion

The semicontinuous mode of operation under pseudo-steady-state conditions was shown to be a feasible alternative way to perform *A. platensis* cultivation. The highest productivity (1319 ± 25 mg L^{-1} d^{-1}) was obtained with the semicontinuous run carried out at dilution rate of 0.3 day^{-1} and light intensity of 108 μmol photons m^{-2} s^{-1}. The cell productivity increased with the product of light intensity and dilution rate following a Monod-type equation, which suggests that pseudo-steady-state conditions could be modified varying one or other of the two independent variables to improve the industrial production of this cyanobacterium by semicontinuous operation.

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