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Influence of light intensity and photoperiod on energy efficiency of biomass and pigment production of *Spirulina* (*Arthrospira platensis*)☆

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Abstract – Biomass and photosynthetic pigments production of cyanobacteria, in particular *Spirulina* (*Arthrospira platensis*), depend on light intensity. Production cost of *Spirulina* cultivation in photobioreactor illuminated with LED is strongly related to light cost. This work is aimed to evaluate the effects of light intensity and photoperiod on the energy efficiency of biomass and pigment production of *Spirulina platensis* UTEX 1926. Cultures illuminated with white light-emitting diodes (LED) were carried out in batch mode cultivation. Three lighting scenarios with different light intensities were tested: two with a constant light intensity at 80 and 160 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} and one with modulated light intensity. Concerning photoperiod, three cycles of light:dark (h:h) (24:00, 20:04 and 16:08) were studied under light intensity at 160 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}. Concerning *Spirulina* biomass and pigment production (phycocyanin, chlorophyll and carotenoids), it is found that the lower intensity tested allows a substantial increase of the process energy efficiency. Concerning the influence of the photoperiod, it has been found that the longer the off time is (up to 08 h 00 a day), the higher is the energy efficiency for *Spirulina* biomass as well as for pigmentary production.

Keywords: LED lighting / photoperiod / *Spirulina* cultivation / phycocyanin / carotenoids

Résumé – Influence de l'intensité lumineuse et de la photopériode sur l'efficacité énergétique de la biomasse et la production de pigments de *Spiruline* (*Arthrospira platensis*). La production de biomasse et de pigments photosynthétiques à partir des cyanobactéries, en particulier la *Spiruline*, dépend de l'intensité lumineuse. Le coût de production de la culture de la *Spiruline* dans un photobioréacteur éclairé par des LED est fortement lié au coût de la lumière. Ce travail vise à évaluer les effets de l'intensité de la lumière et de la photopériode sur l'efficacité énergétique de la biomasse et de la production de pigments de *Spiruline platensis*. Les cultures éclairées avec des diodes électroluminescentes blanches (LED) ont été réalisées en mode batch. Trois scénarios d'éclairage avec des intensités lumineuses différentes ont été testés : deux avec une intensité lumineuse constante à 80 et 160 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} et un avec une intensité lumineuse modulée. Concernant la photopériode, trois cycles de lumière : obscurité (h:h) (24:00, 20:04 et 16:08) ont été étudiés sous une intensité lumineuse à 160 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}. Concernant la biomasse de *Spiruline* et la production de pigments (phycocyanine, chlorophylle et caroténoïdes), on constate que la plus faible intensité lumineuse testée permet d'augmenter considérablement l'efficacité énergétique du procédé. Concernant l'influence de la photopériode, il a été constaté que plus le temps de non-éclairage est long (jusqu'à 08 h 00 par jour), plus l'efficacité énergétique est élevée pour la biomasse de *Spiruline* ainsi que pour la production de pigments.

Mots clés : éclairage LED / photopériode / Culture *Spiruline* / phycocyanine / caroténoïdes

1 Introduction

Microalgae are potential sources of biomass production. They have many and various applications including food and feed, cosmetic and pharmaceutical industry. In addition, these microorganisms are considered as a promising source of biofuels (Becker, 2007; Carvalho *et al.*, 2011; Blanken *et al.*, 2013). Among the valuable microalgae, we can mention *Spirulina*. Indeed, *Spirulina* can contain 60 to 70% of proteins in dry weight. It is rich in vitamins, especially vitamins B12, provitamin A and minerals, in particular iron (Bezerra *et al.*, 2011). Incorporating *Spirulina* biomass into food helps to increase its nutritional value (Koru, 2012). It is an important source of valuable pigments like chlorophyll a (Chl-a), carotenoids and phycobiliproteins (especially phycocyanin (Phy)) (Boussiba and Richmond, 1980). Phycocyanin is a very important pigment because it may be used in many applications. In the food industry, it is used as a natural blue food colouring. In biotechnology field, it serves as a fluorescent marker. It has neuroprotective, anti-inflammatory and antioxidant properties (Bhat and Madyastha, 2000; Romay *et al.*, 2003). As for chlorophyll, in addition to its health benefits (antioxidant, anti-inflammatory, antimutagenic, and antimicrobial properties) (Galasso *et al.*, 2019), it is also used as a colouring. Among the sources of chlorophyll production (plants, microalgae, etc.), *Spirulina* presents one of the highest chlorophyll content (Koru, 2012). Finally, *Spirulina* also contains carotenoids (known for its antioxidant effects) with a prominent amount of β-carotene (67 to 79% in carotenoids pigment group). Consuming carotenoids in form of provitamin A or β-carotene improves the immune system and reduces the risk of developing chronic degenerative diseases, cardiovascular diseases and certain types of cancer (Rodríguez-Concepcíon *et al.*, 2018).

Microalgae can be cultured by different methods of nutrient supply for their growth: autotrophic, heterotrophic, mixotrophic and photoautotrophic (Chojnacka and Noworyta, 2004). The photoautotrophic or mixotrophic growth of microalgae requires CO_2 and light for the realization of photosynthesis. Light energy can be provided by natural lighting (sun) or artificial lighting (lamps). In controlled growing environments such as indoor bioreactors, the use of artificial light is obligatory. Artificial lighting generally gives the possibility of controlling light intensity and the lighting time. Although artificial lighting can increase productivity, it also generates additional costs. Thus, optimal use in terms of photosynthesis, energy efficiency and spectral composition

can provide a wider and cheaper range of high benefit products obtained from microalgae biomass. In this respect, LED lighting technologies are in vogue compared to conventional technologies (halogen lamp, fluorescent lamp lamps, high-pressure sodium lamp, etc.). The main reasons are its monochromatic spectrum and dimmable intensity. These characteristics of LEDs are their main assets compared to other light sources. Indeed, these make it possible to evaluate efficiency of dimmed lighting for microalgae cultivation. In addition, cultures of microalgae cultivation are generally carried out under constant light intensity. Modulation of light intensity according to the cell density of *Spirulina* could be promising. Thus, this lighting technique could make it possible to save electrical energy. Moreover, a long lifetime and a high electrical efficiency of LED minimize heat generation. Their low heat production makes it possible to bring them closer to the crop, thus increasing the path of light. Finally, their small sizes allow them to be integrated practically in all growth systems, especially photobioreactors.

Several research works show the efficiency of LED lighting in microalgae culture including *Spirulina* (Schulze *et al.*, 2014; Glemser *et al.*, 2016). They also indicate that light intensity, spectrum and photoperiod have considerable effects on growth and biochemical composition (in particular pigment contents) of *Spirulina*. Light intensity and spectrum are thus key factors that can be tuned to match the whole or a particular absorption band to act on quantity and/or quality production of biomass and pigments. It is commonly accepted that red and white lights promote high quantities of biomass while high intensity light and blue light enhance phycocyanin production. Literature also mentions that green light is harmful for pigments production except phycocyanin. Moreover, for light intensities lower than the saturation and photo-inhibition lights, biomass quantity increases with light quantity (Pareek and Srivastava, 2001; Madhyastha and Vatsala, 2007; Madhyastha *et al.*, 2009; Chen *et al.*, 2010; Kumari *et al.*, 2015; Tian *et al.*, 2018). However, process energy efficiency is still an issue and has not been extensively discussed yet. The aim of this work is to investigate, in terms of energy efficiency and productivity, biomass production and photosynthetic pigment contents in *Spirulina platensis* under different light intensities, including dimmed light conditions and different photoperiods.

2 Materials and methods

2.1 Microorganism and culture conditions

Spirulina species used in this study was *Arthrospira platensis* (UTEX 1926) and was grown in *Zarrouk* medium (Zarrouk, 1966). The strain culture was grown in cylindrical tanks of 60 L at 28 °C with continuous illumination at 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by warm white (3000 K) LED tube lamp (MAS LEDtube HF 600 mm HE 8W 830 T5 from Philips). The light intensity corresponds to the photosynthetic photon flux density (PPFD). PPFD is a light measuring system that best responds to the study of light action in photosynthetic activity because it takes into account relative quantum efficiency (RQE) (McCree, 1971). Photometry is not suitable because it is based on the sensitivity to light of human eye

which differs from that of plants (Biggs, 1986). This lamp has been modified to be dimmable. Strain culture is agitated by a circulating pump. For inoculation, we took a certain amount of the strain culture, and filtered it by a 30 μm strainer, then diluted the *S. platensis* with *Zarrouk*. Photoautotrophic cultures were made in cylindrical tanks of 25 L in batch mode. In addition, they were illuminated with LED lamps (Maxline70 Warm white LED strip from lumitronix). The value of photosynthetic photon flux density (PPFD) was measured by the spectroradiometer Specbos 1201. Cultures were agitated with circular pumps (LAGUNA pump with a flow velocity of 5000 L/h). Thermostats (NICREW aquarium heater 100 W) were used to keep the cultures at 28 °C.

Regarding the influence of light intensity modulation on *Spirulina* growth and pigment contents, three lighting scenarios were tested:
- scenario 1: constant light intensity at $80 \, \mu mol \cdot m^{-2} \cdot s^{-1}$;
- scenario 2: constant light intensity at $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$;
- scenario 3: modulated light intensity illumination ($80 \, \mu mol \cdot m^{-2} \cdot s^{-1}$ during two first days and $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$ afterwards).

Regarding the photoperiods, we examined three light:dark (h:h) cycles: 24:00, 20:04 and 16:08 under light intensity at $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$.

Measurements were performed in triplicate and results were expressed as mean ± 95% confidence interval, *i.e.*: m ± ts/sqrt(n) where *m* is the mean of the *n* values ($n = 3$), *s* is the estimated standard deviation and t is given by the Student–Fisher distribution for 95% of confidence interval and a degree of freedom of $n - 1$.

2.2 Biomass analysis

Spirulina platensis Biomass was determined by measuring the optical density (OD) at 600 nm using UV/Visible/IR spectrophotometer (Spectronic 20 GeneisSYS, Spectronic Instruments, USA). Then a linear equation was established between biomass dry weight (DW, g·L-1) and the optical density at 600 nm. It follows the same protocol as Lee *et al.* (2016).

2.2.1 Kinetic and others parameters

Arthrospira platensis specific growth rate μ (day^{-1}) is calculated during the 8 days of cultivation according to equation (1) (Wang *et al.*, 2007):

$$\mu = \frac{\ln(DW_2) - \ln(DW_1)}{t_2 - t_1}, \qquad (1)$$

where DW_2 and DW_1 are the dry weights of biomass on days t_2 and t_1, respectively.

We calculated biomass production P $(mg \cdot L^{-1} \cdot day^{-1})$ (Eq. (2)) (Wang *et al.*, 2007) and energy efficiency η $(g \cdot day^{-1} \cdot kWh^{-1})$ of electrical energy to biomass production (Eq. (3)):

$$P = 1000 \cdot \frac{DW_n - DW_{w_0}}{T}, \qquad (2)$$

$$\eta = \frac{DW_n - DW_w}{T \cdot E_e} \cdot V, \qquad (3)$$

where DW_0 and DW_n are respectively the dry weights of biomass on initial and *n*th day of cultivation, V (L) is volume of culture and E_e (kWh) is electrical energy consumed by LEDs during cultivation time T(days).

2.2.2 Pigments quantification

Chlorophyll a and carotenoids were assayed using the method of Lichtenthaler (1987). The 1.8 mL of microalgae solution was centrifuged at $10\,000 \times g$ for 15 min. After discarding the supernatant, 1.8 mL of 99.8% methanol solution was added to fresh biomass, mixed well and incubated at 4 °C for 24 h in darkness. Then, sample was exposed at room temperature for 4 h in darkness. Finally, it was homogenized during 2 min by ZX3 Vortex Agitator at 2000 tr/min. Then, it was centrifuged at 20 °C, $4000 \times g$ for 10 min before spectrophotometric measuring. The Chl-a and carotenoids concentrations were calculated according to equations (4) and (5):

$$Chl - a \left(mg \cdot L^{-1}\right) = 16.72 * A_{665} - 9.16 * A_{652}, \qquad (4)$$

$$Carotenoid \left(mg \cdot L^{-1}\right) = (1000 * A_{470} - 1.63 * Chl - a)/221, \qquad (5)$$

where A_{665}, A_{652} and A_{470} are the absorbance readings at 665, 652 and 470 nm.

Phycocyanin was determined according to Bennett and Bogorad (1973). As for Chl-a and carotenoids, 1.8 mL of microalgae cells was centrifuged at $10\,000 \times g$ for 15 min. After discarding the supernatant, 1.8 mL of 1.5% CaCl$_2$ (W/V) solution was added to fresh biomass. Four freeze/thawing cycles were performed on fresh biomass (incubation at -20 °C for 24 h in darkness followed by 4 h in darkness at room temperature). Finally, it was homogenized during 2 min by ZX3 Vortex Agitator at 2000 tr/min and centrifuged at 20 °C, $4000 \times g$ for 10 min before spectrophotometric measuring. The Phy concentration was calculated according to equation (6):

$$Phy \left(g \cdot L^{-1}\right) = (A_{615} - 0.474 * A_{652})/5.34, \qquad (6)$$

where A_{615} is the optical density at 615 nm.

The calculated pigment contents are those of the eighth day.

3 Results and discussion

3.1 Effect of modulated light intensity on *Spirulina* growth and pigment content

3.1.1 Biomass production

Light is the basic source of energy and an important factor of photosynthesis for the cellular multiplication of microalgae. In photoautotrophic regime, light is essential for microalgae growth (Wahidin *et al.*, 2013). In the present work, no light inhibition was found for the range of intensities tested.
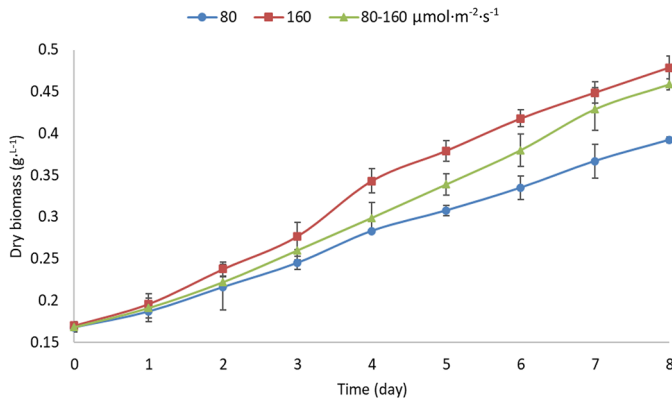
Fig. 1. Biomass produced of *Arthrospira platensis* growth according to the three lighting scenarios.
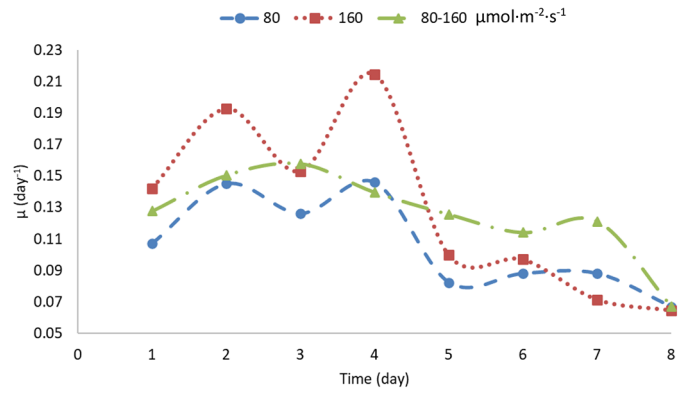

Fig. 2. Growth rates of *Arthrospira platensis* growth according to the three lighting scenarios.

It should also be noted that for each scenario, no lag phase has been observed. This is consistent with the fact that the lag phase duration depends essentially on culture medium and not on light conditions. According to the differences of the culture medium with the inoculum culture medium, this phase may be observed.

Figures 1 and 2 illustrate observations of the cell growth of *Spirulina platensis* for the three scenarios described above for 8 days of cultivation. The lowest biomass production was obtained with scenario 1 (constant light intensity at $80 \, \mu mol \cdot m^{-2} \cdot s^{-1}$), while the highest was obtained in runs with scenario 2 (constant light intensity at $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$) and scenario 3 (modulated light intensity $80–160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$) (Fig. 1). This is in line with previous works where it was demonstrated that specific growth rate of microalgae is proportional to light intensity, up to saturation levels (Qiang *et al.*, 1998; Wang *et al.*, 2007; Dubinsky, 2013; Rizzo *et al.*, 2015). For scenario 3, light intensity was changed from 80 to $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$ after two days of cultivation to evaluate how dynamic light intensity adjustment affects the *Spirulina* growth in terms of productivity and energy efficiencies. Indeed, up to days 2 a small growth gap between scenarios 1 and 2 can be observed and shows that it is not necessary to illuminate cultures at high light intensities at the beginning of cultivation.

It can be seen that the increase of light intensity does not have an immediate effect on *Spirulina* growth as there is no significant biomass increase up to day 4. These results suggest that there is a period of adaptation of cells to a change (especially an increase) in light intensity (Danesi *et al.*, 2004; Patel *et al.*, 2019).

It can also be observed that the growth rate in scenarios 1 and 2 drops drastically down after four days of cultivation while it decreases slowly and monotonically in scenario 3 (Fig. 2). In scenarios 1 and 2, average growth rate between days 1 and 4 passes respectively from 0.1311 and 0.1757 day^{-1} to 0.0814 and 0.0833 day^{-1} between days 5 to 8 (drop of 38% and 52% respectively). For comparison in scenario 3 average growth rate passes from 0.1439 day^{-1} to 0.1069 day^{-1} (drop of 26%). The higher average growth rate between days 5 and 8 explains that final dry biomass obtained with scenario 3 is only 4.18% lower than the one obtained with scenario 2.

These observations suggest that dynamically increasing light intensity as cell concentration increases may be a promising option for energy savings. Indeed, this type of lighting makes it possible to illuminate culture during first days with low light intensities without negatively impacting *Spirulina* growth. Also, it could make it possible to avoid using saturation light. With intensities of saturation lights, lighting is not optimal because their photosynthetic yields are identical (Qiang *et al.*, 1998).

Table 1 presents the average growth rate values, biomass productivities and energy efficiencies for different lighting scenarios. Productivity and specific growth rate were maximal for scenarios 2 and 3. Biomass productivities with scenarios 2 and 3 are 35% higher than with scenario 1. However, the highest energy efficiency is obtained for scenario 1. Actually, there is a light intensity range (low PPFD range) for which biomass productivity is proportional to light intensity (Chojnacka and Noworyta, 2004; Xue *et al.*, 2011). We can assume here that at $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$, the light intensity is near to or is a saturation intensity. Indeed, within the saturation light range, photosynthetic activity is almost constant and a significant part of absorbed photons are not used (Qiang *et al.*, 1998). Moreover, even if no photo-inhibition has been observed at $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$, it is possible that this light intensity is high enough to create light stress at the start of culture according our inoculation concentration. This may cause a lower process energy efficiency. Indeed, an excess light can occur when the ratio of PPFD to photosynthesis is high. Thus, at a constant PPFD, this ratio is high at the beginning of the cultivation (low photosynthetic cell concentration) and decreases gradually for increasing cell concentration (Demmig-Adams and Adams, 1992). Compared to scenario 2, the productivity obtained with scenario 3 is 5.93% lower but energy efficiency is 7.92% higher. This result shows that dynamically adjusting light intensity may be a good way to improve culture process efficiency while preserving high productivities. This principle has to be investigated in more details and can be done for instance by continuously adjusting the light intensity according to the optical density of the culture medium.

Table 1. Average growth rates, biomass productivities and energy efficiencies of three lighting scenarios.

Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	μ_{av} (day^{-1})	P ($mg \cdot L^{-1} \cdot day^{-1}$)	η ($g \cdot day^{-1} \cdot kWh^{-1}$)
80	0.1070 ± 0.0038	28.38 ± 0.82	1.48 ± 0.05
160	0.1295 ± 0.0008	38.63 ± 1.12	1.01 ± 0.03
80–160	0.1254 ± 0.0030	36.34 ± 0.20	1.09 ± 0.03
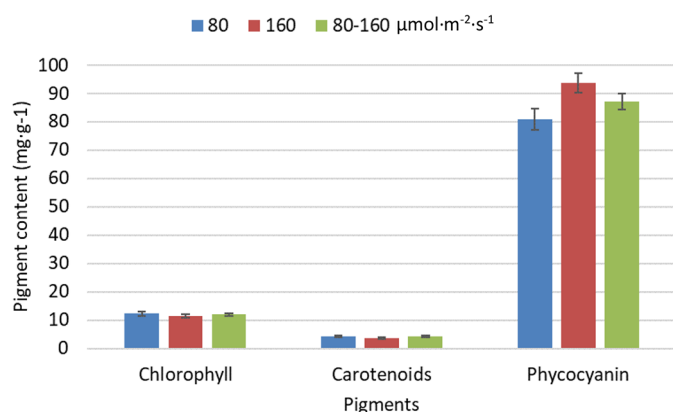
Fig. 3. *Arthrospira platensis* pigment content for three lighting scenarios.
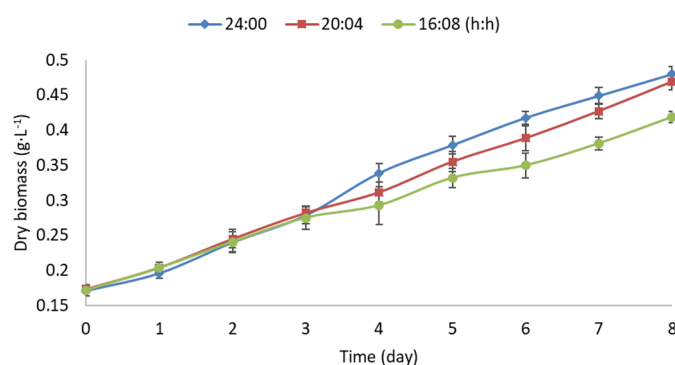
Fig. 4. *Arthrospira platensis growth* curves as a function of different photoperiods.

Table 2. Energy efficiencies of pigmentary production and light intensities.

Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Energy efficiencies η ($mg \cdot day^{-1} \cdot kWh^{-1}$)		
	Chl-a	Carotenoids	Phy
80	1.27 ± 0.08	0.44 ± 0.03	8.254 ± 0.17
160	0.73 ± 0.04	0.24 ± 0.02	5.92 ± 0.32
80–160	0.83 ± 0.04	0.28 ± 0.03	5.86 ± 0.14

3.1.2 Pigment production

To understand the relationships between pigment concentrations under different light intensities, chlorophyll, carotenoids and phycocyanin contents were measured and recorded, as shown in Figure 2. We observed that light intensities have a more or less pronounced influence on the concentrations of the different pigments.

For carotenoids and chlorophyll, the light intensity has a limited effect. The lighting scenarios 1, 2 and 3 give respectively 12.29, 11.48 and 12.09 $mg \cdot g^{-1}$ of Chl content per dry matter (Fig. 3). Concerning carotenoids concentration, the lighting scenario 1 provides slightly higher rates compared to scenarios 2 and 3 (respectively 13.52% and 9.02% more). It is consistent with the results of Kumar *et al.* (2011). Other authors, such as Ravelonandro *et al.* (2008), Chen *et al.* (2010) and Danesi *et al.* (2011), mentioned that they observed a decrease of chlorophyll and carotenoids concentration with an increasing light intensity. Indeed, the increase of light intensity induces a decrease in the photosystem concentration of the thylakoid membrane and a decrease in the size of the photosystem II (PSII). However, despite a decrease in the chlorophyll content, we can observe an increase in

photosynthetic activity because the capacity of absorption and conversion of photons into chemical energy by chlorophyll remains, or even greatly increases. In addition, in the case of too strong and prolonged light intensity, photo-inhibition causes destruction of photosynthetic pigments by photo-oxidation (Hendry *et al.*, 1987; Alves *et al.*, 2002). Finally, in some cases, the initiation of the xanthophylls cycle (pigment belonging to the carotenoids family) is then the main protection which allows to dissipate the energy absorbed by the chlorophylls at the PSII antenna in the form of heat.

Unlike the two previous pigments, effect of light intensity on phycocyanin concentration is more significant. Scenario 2 (constant light intensity at 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$) promotes a higher production of phycocyanin. The difference of phycocyanin content between scenarios 2 and 3 is 7.86 $mg \cdot g^{-1}$ (8.39%). This result is consistent with literature (Chen'v *et al.*, 1996; Chen *et al.*, 2013) where it was observed that phycocyanin content increases with increasing light intensity.

Energy efficiencies of the different scenarios are presented in Table 2. We can observe that scenario 1 (*i.e.* constant lighting intensity at 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$), gives the highest energy efficiencies for pigments production. Overall, in terms

Table 3. Growth rates, biomass productivities and energy efficiencies following photoperiod.

| Photoperiod (h:h)	μ_{max} (day^{-1})	P (g·L^{-1}·day^{-1})	η (g·day^{-1}·kWh^{-1})
24:00	0.1290 ± 0.0020	38.63 ± 1.13	1.01 ± 0.03
20:04	0.1244 ± 0.0025	36.96 ± 1.29	1.16 ± 0.04
16:08	0.1117 ± 0.0063	30.88 ± 1.47	1.21 ± 0.05

of chlorophyll a and carotenoids contents, the energy efficiency is almost twice higher compared to other scenarios. For phycocyanin scenario 1 is 1.4 times more efficient than scenarios 2 and 3. There is no significant difference between scenarios 2 and 3 in terms of energy efficiency for pigment production.

3.2 Effect of photoperiod on *Spirulina* growth and pigment content

3.2.1 Biomass production

Biomass concentration of *S. platensis* was evaluated for different photoperiods during 8 days of cultivation for constant light intensity at $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 4). Maximum biomass productivity *i.e.* $38.63 \pm 1.13 \, g \cdot L^{-1} \cdot day^{-1}$ and minimum *i.e.* $30.88 \pm 1.47 \, g \cdot L^{-1} \cdot day^{-1}$ were respectively observed at light/dark 24:00 and 16:08 (Tab. 3). We observed that the higher the lighting time, the higher is the biomass production (Fig. 4). However, photoperiod 24:00 gives the lowest energy efficiency. Photoperiod 16:08 exhibits the highest energy efficiency. As in this experiment light intensity is the same, energy efficiency improvement comes from duration of dark phase. Moreover, high photosynthetic efficiencies are achieved when light/dark cycle period approaches turnover time of the photosynthetic unit (Richmond *et al.*, 2003; Amini Khoeyi *et al.*, 2012; Liao *et al.*, 2014). Photosynthesis is carried out in two stages: light reactions and dark reactions. In light reactions, cyanobacteria use light to break down water molecule. This reaction produces chemical energy providing NADPH (nicotinamide adenine dinucleotide phosphate) and a highly energetic compound ATP (adenine tri-phosphate). NADPH2 and TP are necessary, in particular, for the assimilation of inorganic nutrients. In dark reactions or enzymatic reactions, cyanobacteria assimilate CO_2 and produce carbohydrates, lipids (Wahidin *et al.*, 2013). Thus, the dark reactions also occur during the lighting time especially when the culture is constantly illuminated. Part of the energy absorbed by the microalgae is therefore not used.

The dry biomass quantities after 8 days using photoperiods 24:00, 20:04 and 16:08 are 0.4800 ± 0.0110, 0.4690 ± 0.0115 and $0.4194 \pm 0.0095 \, g \cdot L^{-1}$ respectively. It can be seen that biomass production from 24 and 20 h of lighting is almost identical unlike a lighting time of 16 h. Similar results were obtained in work on growth of *Spirulina* and other microalgae (Pareek and Srivastava, 2001; Wahidin *et al.*, 2013). In addition, photoperiod 16:08 is respectively 1.20 and 1.04 times more efficient than photoperiod 24:00 and 20:04. Besides, when the exposure time of *Spirulina* cultures is less than 16 h, the energy efficiency decreases and becomes almost constant after 16 h of lighting (Niangoran *et al.*, 2018). Obtained results show that the best compromise between biomass productivity and energy efficiency for *Spirulina* growth is 20:04 h.
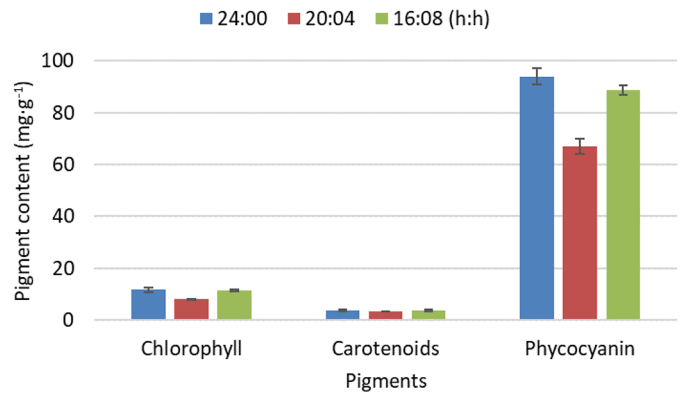
Fig. 5. Arthrospira platensis pigment content for different photoperiod treatment.

3.2.2 Pigment production

The effect of three different photoperiods (24:0, 20:00 and 16:08 light/dark) at light intensity $160 \, \mu mol \cdot m^{-2} \cdot s^{-1}$ on pigment content of *S. platensis* for eight days of cultivation period is presented in Figure 5. Minimum concentration $(67.15 \pm 2.91 \, mg \cdot g^{-1}$ of biomass dry weight) of phycocyanin was obtained for 20:04. Phycocyanin concentration is improved by 39.95% with cycle 24:00 and by 32.12% with cycle 16:08. Lowest chlorophyll and carotenoids contents are also obtained with the photoperiod 20:04. Maximum content of these two pigments are obtained with 16:08 light/dark cycles. These results are consistent with study of Pareek and Srivastava (2001) who observed the same behaviour. However, differences between chlorophyll and carotenoids contents for the photoperiods 24:0 and 16:08 light/dark are very small: $0.13 \, mg \cdot g^{-1}$ for chlorophyll and $0.04 \, mg \cdot g^{-1}$ for carotenoids. Overall, a lighting time of 16 h is optimal for obtaining high concentrations of photosynthetic pigments at high efficiency (Tab. 4).

Table 4 shows energy efficiencies of pigment production as a function of lighting time. We notice that lighting time of 16 h allows to have the highest energy efficiency for all pigments.

4 Conclusion

The effects of light intensity and photoperiod on biomass and pigment production of *Spirulina platensis* have been investigated. Absolute growth rates as well as energy efficiencies have been evaluated. While the highest productivity and specific growth rate is obtained for the highest light intensity $(160 \, \mu mol \cdot m^{-2} \cdot s^{-1})$, the best energy efficiency is obtained for the lowest light intensity $(80 \, \mu mol \cdot m^{-2} \cdot s^{-1})$: this is true for biomass (energy efficiency is improved by 47%

Table 4. Energetic efficiencies of pigment production and photoperiod.

Photoperiod (h:h)	Energy efficiencies η (mg · day^{-1} · kWh^{-1})		
	Chl-a	Carotenoids	Phy
24:00	0.73 ± 0.05	0.23 ± 0.03	5.88 ± 0.10
20:04	0.59 ± 0.03	0.24 ± 0.03	4.93 ± 0.13
16:08	0.94 ± 0.03	0.31 ± 0.03	7.27 ± 0.20

while productivity is lowered by 28%) as well as for pigment production and more particularly for phycocyanin (energy efficiency is increased by 39% while productivity is lowered by 16%).

It has also been shown that applying a light intensity step after two days of cultivation improves the process energy efficiency while preserving high productivities for biomass. The positive effect of light intensity change on process energy efficiency for pigments production is not as significant than for biomass production. Nevertheless the principle of dynamically adjusting light intensity according for instance the optical density of the culture medium is promising and has to be investigated in more details.

Concerning photoperiod, results show that while biomass productivity and growth rate decrease with the illumination time, the process energy efficiency increases. As light intensity was constant during these experiments, energy efficiency improvement is attributed to biomass created during the dark phase. The best compromise between energy efficiency and productivity for biomass is obtained for a cycle of 20:04. For pigment production and more particularly phycocyanin, 16:08 cycle provides the best energy efficiency.

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