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# Magnetic field action on outdoor and indoor cultures of *Spirulina*: Evaluation of growth, medium consumption and protein profile

Kricelle Mosquera Deamici[a], Lucielen Oliveira Santos[b], Jorge Alberto Vieira Costa[a,*]

[a] Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, 96203-900 Rio Grande, RS, Brazil
[b] Laboratory of Biotechnology, College of Chemistry and Food Engineering, Federal University of Rio Grande, 96203-900 Rio Grande, RS, Brazil

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ABSTRACT

This study aimed at evaluating whether a magnetic field (MF) affects the growth of *Spirulina* sp. when applied to it at different exposure times in indoor and outdoor culture systems. The effects of MF on chlorophyll content, medium consumption and protein profile were also investigated. In raceway tanks, a 25 mT MF was applied for 24 h or for 1 h $d^{-1}$. MF for 24 h to outdoor assays increased biomass concentration and chlorophyll-*a* content besides altering the protein profile. Outdoor *Spirulina* growth was higher (~3.65 g $L^{-1}$) than the growth found in indoor assays (~1.80 g $L^{-1}$), while nitrogen and phosphorus consumption was not enhanced by the application of MF. This is the first study that investigated the influence of MF on outdoor microalga assays, and the results showed that MF affected the metabolism of *Spirulina* cultured in raceways, especially when it was grown outdoors in uncontrolled environmental conditions.

## 1. Introduction

Microalgae have attracted considerable attention because their biomass is a great source of lipids, carbohydrates, proteins, antioxidants and pigments (natural food colorants) (Yimin and Seetharaman, 2013; Bauer et al., 2017). The optimal growth conditions, productivity, biomass composition and growth profile of the different microalgae species generally vary widely since its growth is influenced by several factors, such as temperature, luminous intensity, pH, salinity and the composition of the culture medium (Zhou et al., 2015).

*Spirulina*, one of the most popular microalgae, has been described by the World Health Organization as one of the greatest superfoods on Earth, which is one example of the potential of microalgae (Chacón-Lee and González-Mariño, 2010).

Different cultivation systems are used for *Spirulina* cultivation in laboratories and at an industrial scale. Open raceway tanks are generally used for large-scale *Spirulina* production (Morais et al., 2009). Raceway tanks are shallow ponds that have low-energy-consuming paddlewheels for gas/liquid mixing and circulation; they are made of less expensive materials, their construction involves lower costs, and they require less energy for agitation (Jorquera et al., 2010). It is necessary to select species that can grow under extreme conditions (e.g., high temperature, alkaline or acidic conditions, high salt concentrations, etc.) to avoid growth of contaminants in outdoor cultures. *Spirulina* is one of the most widely cultured commercial photosynthetic microorganisms in traditional open systems because of its preference for alkaline conditions (Lu et al., 2011).

Currently, magnetic fields (MF) are being studied as potential sources to increase and change the production of microalgae biomass and/or certain compounds of interest (Hunt et al., 2009; Deamici et al., 2016a,b; Bauer et al., 2017). Magnetic treatments have many advantages of convenient use, such as being low cost, non-toxic, non-polluting, safe, as well as having a wide range of applications (Tu et al., 2015). MF may act on the metabolism of microorganisms, and their effect on cell growth and the evaluated responses can be classified as inhibitory, stimulating or null depending on the application form, intensity and time of application (Rai, 1997).

There are some studies on the influence of MF during microalgae cultivation and microalgal biomass composition; however, the action of MF has not yet been considered in environments with non-ideal conditions for microalgae growth (outdoor cultures). Reports about magnetic force action on microalgal metabolism are scarce and additional studies are required. MF application in outdoor cultivation that uses sunlight as a light source reduces the costs involved in the process and constitutes a more efficient system for biomass production. In this way, the aim was to evaluate whether MF act on *Spirulina* sp. growth when cultured in outdoor and indoor systems, as well as whether chlorophyll content, protein profile and nitrate and phosphorus consumption are altered by magnetic action applied in different exposure times.

* Corresponding author.
*E-mail address:* jorgealbertovc@terra.com.br (J.A.V. Costa).

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## 2. Material and methods

### 2.1. Microorganisms, culture medium and cultivation conditions

*Spirulina* sp. LEB 18 (Morais et al., 2008) was used in the assays. The outdoor and indoor cultivation was carried out in raceway tanks (0.7 m long, 0.18 m wide 0.075 m deep) containing 4.5 L of *Spirulina* sp. culture with an initial biomass concentration of 0.2 g $L^{-1}$. The cultures were mixed using paddle wheels turning at 24 revs $min^{-1}$. *Spirulina* sp. was maintained and cultivated in Zarrouk medium (as described by Costa et al., 2004), which contains (g $L^{-1}$): $NaHCO_3$ (16.8), $K_2HPO_4$ (0.5), $NaNO_3$ (2.5), $K_2SO_4$ (1.0), NaCl (1.0), $MgSO_4{\cdot}7H_2O$ (0.22), $CaCl_2$ (0.04), $FeSO_4{\cdot}7H_2O$ (0.01), EDTA (0.08), A5 solution (1 mL $L^{-1}$) and B6 solution (1 mL $L^{-1}$). The A5 solution contains (g $L^{-1}$): $H_3BO_3$ (2.86), $MnCl_2{\cdot}4H_2O$ (1.81), $ZnSO_4{\cdot}7H_2O$ (0.22), $NaMoO_4{\cdot}2H_2O$ (0.015) and $CuSO_4{\cdot}5H_2O$ (0.079) whereas the B6 solution contains (g $L^{-1}$): $NH_4VO_3$ (0.023), $KCr(SO_4)_2{\cdot}12H_2O$ (0.048), $Na_2WO_4{\cdot}2H_2O$ (0.018), $TiO_2$ (0.0084) and $Co(NO_3)_2{\cdot}6H_2O$ (0.044).

Discontinuous assays were performed for 15 days. The outdoor assays were carried out in a greenhouse, in open PVC raceways with daytime sunlight (~300 $\mu_{molphotons}$ $m^{-2}$ $s^{-1}$) and ambient temperature (uncontrolled environmental conditions). The outdoor experiments were carried out from March to April, at the end of summer and beginning of fall (Southern Hemisphere). The maximum and minimum temperatures were monitored by thermometer.

The indoor assays were carried out in a growth chamber at 30 °C under a 12 h light/dark photoperiod. The illumination was supplied by four 32 W daylight-type fluorescent tubular lamps, which produced illuminance of 81.3 $\mu_{molphotons}$ $m^{-2}$ $s^{-1}$.

Evaporation of water from the culture was controlled by daily addition of distilled water in both outdoor and indoor cultures.

### 2.2. Application of magnetic fields in culture

The MF in assays was applied for 15 d with ferrite magnets (150 × 50 × 10 mm) with an average intensity of 25 mT. Six magnets were adopted in each raceway. Two application times were tested as follows: MF for 1 h $d^{-1}$ (in the light photoperiod) and throughout all the cultivation (MF 24 h, for 15 d). The magnetic intensity was measured in the center of the raceway by a Globalmag MF measuring device (model TLMP-HALL-05 k-T0, Brazil). The MF effect was evaluated and compared to the control culture, which was only exposed to the Earth's MF (0.005 mT) and in the same conditions of temperature, illumination, aeration and nutrients. The outdoor assays were performed in different periods; since the environmental conditions were different, it was necessary to do a control experiment for each condition. The assays performed were with MF application for 24 h (control and MF 24 h) and for 1 h $d^{-1}$ (control and MF 1 h $d^{-1}$). In indoor assays, the control assays (without MF) were performed; MF application for 24 h and for 1 h $d^{-1}$.

### 2.3. Analytical determinations

#### 2.3.1. Biomass concentration and pH

Biomass concentration (X, g $L^{-1}$) was monitored daily by optical density measurements at 670 nm with a UV–vis spectrophotometer (QUIMIS Q998U, Brazil) and related to the optical density by using the standard of *Spirulina* sp. LEB 18 inoculum (Costa et al. 2002) (data not shown). The pH was also directly measured daily with a digital pH meter (QUIMIS Q400MT, Brazil) in agreement with the official method (APHA, 1998).

#### 2.3.2. Biomass chlorophyll content

Chlorophyll-*a* extractions were performed with methanol 99.8% (v $v^{-1}$) in agreement with methodology proposed by Lichtenthaler (1987). Every three days, 2 mL of culture was taken and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and 1 mL methanol was added to the pellet, mixed well and incubated at 4 °C for 24 h in dark. The chlorophyll-*a* content was calculated according to Eq. (1).

$$\text{Chl}-a\,(\mu g\ mL^{-1}) = 16.72\ A_{665.2} - 9.16\ A_{652.4} \quad (1)$$

#### 2.3.3. Nitrogen and phosphorus removal analysis

Nitrogen-$NO_3$ form and phosphorus were quantified in the culture medium every 3 days. Nitrogen was measured using the method proposed by Cataldo et al. (1975) by a standard curve (0–250 mgN-$NO_3$ $L^{-1}$). The concentration was determined with a spectrophotometer (410 nm). The phosphorus content was quantified by colorimetric analysis with the Phosphate Kit (PhosVer 3 Hach, USA). Thus, absorbance was measured (810 nm), and a standard curve (0–2 mg $L^{-1}$) was used.

#### 2.3.4. Extraction and protein profile

The proteins were extracted every 72 h by adding sample buffer (80 mM of Tris-HCl (pH 6.8), 0.1 M of 2-mercaptoethanol, 2% (w $v^{-1}$) sodium dodecyl sulfate-SDS, 15% (v $v^{-1}$) glycerol and 0.006% (w $v^{-1}$) m-purple cresol), and the lysates were then heated for 5 min at 100 °C. After centrifugation at 10,000*g* for 1 min, the samples were subjected to discontinuous SDS polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970) using a 5% acrylamide stacking gel and 12.5% acrylamide resolving gel.

### 2.4. Evaluation of growth parameters

Biomass concentration values (X, g $L^{-1}$) were used for determining the maximum biomass productivity ($P_{max}$, g $L^{-1}$ $d^{-1}$), maximum specific growth rate ($\mu_{max}$, $d^{-1}$), maximum biomass concentration ($X_{max}$, g $L^{-1}$) and doubling time ($D_t$, d).

The $P_{max}$ was calculated applying equation $P = (X_t - X_0)/(t - t_0)$, where $X_t$ is the biomass concentration (g $L^{-1}$) at time t (d) and $X_0$ is the biomass concentration (g $L^{-1}$) at time $t_0$ (d). The $\mu_{max}$ was obtained by linear regression applied to the logarithmic growth rate of each assay obtained from a plot of ln X (g $L^{-1}$) versus t (d). The doubling time ($D_t$) was determined in the growth exponential phase for each culture, using equation $D_t = \ln 2/\mu_{max}$.

### 2.5. Statistical analysis

The influence of MFs was assessed by analysis of variance (ANOVA) and Tukey's test at 95.0%.

## 3. Results and discussion

### 3.1. Growth kinetics and pH

The microalga *Spirulina* sp. LEB 18 grew in all experimental conditions. Growth of control cultures was similar to that of cultures with application of MF (Fig. 1). However, in the outdoor assays, biomass concentration was 48.4% higher than that in the indoor cultivation. In indoor assays, there was no significant difference ($p \geq 0.05$) between assays with the application of MF and their control (Fig. 1B).

In outdoor assays (Fig. 1A), growth was exponential until the 7th d, and the biomass concentration remained constant from the 8th to the 12th d (~2.50–2.80 g $L^{-1}$) without any difference ($p \geq 0.05$) between values obtained on these days, except the condition with MF for 24 h. During this period (8–12 d) the application of MF for 24 h increased biomass concentration ~16.3% (11th d). Therefore, in this condition, a larger amount of biomass could be reached with the application of MF in comparison with the assay with no MF after 11 days. Thus, more biomass should be obtained in a shorter period.

The "window" effect, wherein the application of MF to biological