Bioresource Technology 220 (2016) 62–67

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Magnetic fields as triggers of microalga growth: evaluation of its effect on *Spirulina* sp.

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HIGHLIGHTS

- Magnetic fields increased the growth of *Spirulina* sp.
- Intensity and time of magnetic field influenced the productivity and carbohydrate.
- Twice as much biomass may be got when 30 and 60 mT are applied for 1 h d^{-1}.

ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form 7 August 2016
Accepted 10 August 2016
Available online 12 August 2016

Keywords:
Growth
Magnetic fields
Biomass composition
Stimulating effect

ABSTRACT

This study aimed at evaluating the influence of magnetic field on the growth and biomass composition of *Spirulina* sp., cultivated in vertical tubular photobioreactors. Magnetic fields of 5, 30 and 60 mT generated by electric current and ferrite magnets were applied at different lengths of time. The magnetic field of 30 and 60 mT for 1 h d^{-1} stimulated the growth, thus leading to higher biomass concentration by comparison with the control culture. Increase in productivity, protein and carbohydrate contents were 105.1% (60 mT for 1 h d^{-1}), 16.6% (60 mT for 24 h d^{-1}) and 133.2% (30 mT for 24 h d^{-1}), respectively. These values were higher than the ones of the control. Results showed that magnetic field may influence the growth of *Spirulina* sp., since it triggers a stimulating effect and can leads to twofold biomass concentration in equal cultivation time periods.

1. Introduction

Cultivating microalgae has attracted global attention in the past two decades for converting biological components of cells into organic matter, such as green, blue, red and brown pigments, commercially valuable natural products, as well as into lipids, proteins, carbohydrates and polyunsaturated fatty acids which may be used in several fields (Rangel-Yagui et al., 2004; Leema et al., 2010; Mohsenpour and Willoughby, 2013; Xia et al., 2016). The carbohydrates are an appropriate feedstock for microbial growth and the production of bioethanol. Moreover, the high lipid content in the biomass of some algal species is promising for biodiesel production (Yen et al., 2013). Though the commercialized production of microalgal biomass, as healthy food and valued additives is profitable, more efficient cultivation technique in pursuit of lower cost is also currently an important direction (Zhang et al., 2015). To increase microalga growth, the magnetic treatments have been considered. Magnetic field (MF) treatments may affect the metabolism of microorganisms, such as their photosynthetic efficiency (Hirano et al., 1998), synthesis of carbohydrates, lipids and pigments, accumulation of essential amino acids (Li et al., 2007; Small et al., 2012) and antioxidant defense system (Wang et al., 2008; Santos et al., 2010). All procedures of MF application to bioprocesses can be stimulated under specific conditions used in assays. Magnetic treatments have many advantages of convenient use as non-toxic, non-polluting, wide application range, inexpensive and safe (Tu et al., 2015).

However there are gaps in studies of the growth of this microalga to maximize biomass production. In addition, there are few studies of the effects of magnetic treatments with static magnetic fields (SMF) and electric current algal systems. This study aimed at evaluating the effect of MF application by magnets and electric current in a solenoid coil on the growth of *Spirulina* sp. LEB 18 and its biomass composition.

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http://dx.doi.org/10.1016/j.biortech.2016.08.038
0960-8524/

2. Material and methods

2.1. Culture conditions

Spirulina sp. LEB 18 was isolated from the Mangueira lagoon located in Santa Vitória do Palmar – RS, Brazil (latitude 33°31′08″S and longitude 53°22′05″W) (Morais et al., 2008). It belongs to the Culture Collection of the Laboratory of Biochemical Engineering at the Federal University of Rio Grande, located in Rio Grande, RS, Brazil. Assays were performed 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), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2$ (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), $ZnSO_4 \cdot 7H_2O$ (0.222), $NaMoO_4$ $2H_2O$ (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). Assays were performed in duplicate in an acrylic vertical tubular photobioreactor (VTP) (1.8 L working volume) in a growth chamber at 30 °C in 12 h dark/light photoperiod with illumination of 60 $\mu mol_{photons} m^{-2} s^{-1}$ provided by four 40 w daylight type fluorescent lamps in the light period. The initial biomass concentration in all assays was 0.3 g L^{-1}. Assays were performed for 15 days with constant aeration (0.2 vvm) by injection of compressed air filtered through sterile glass wool. Evaporation of water along the culture was controlled by daily addition of distilled water.

2.2. Application of magnetic fields in culture

A study of SMF in assays with *Spirulina* sp. LEB 18 was carried out with ferrite magnets and electric current (in solenoid) that were applied around VTP. Each magnet was at 180°, 15 cm above the base of the VTP (Fig. 1A). Two different models of ferrite magnets, with 30 mT and 60 mT, were used. Both intensities (30 and 60 mT) were measured in the center of the VTP by a Globalmag MF measuring device (model TLMP-HALL-05 k-T0, Brazil).

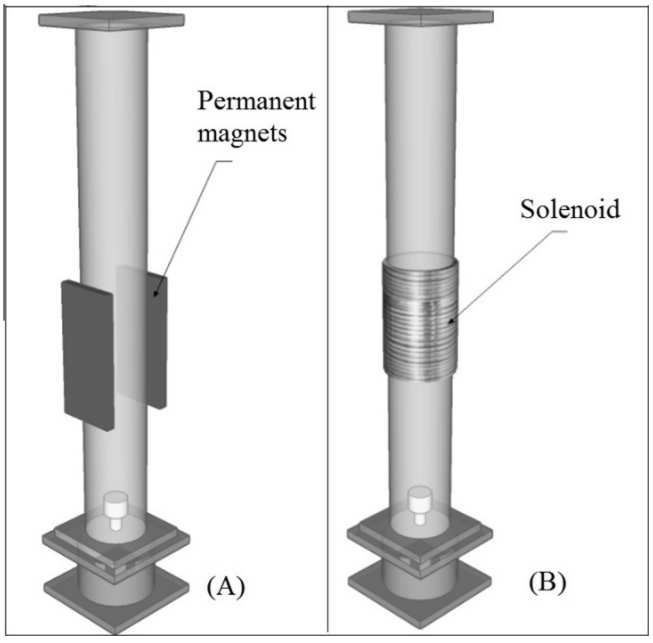

Fig. 1. Vertical tubular photobioreactor (VTP) with MF application through the magnets (A) and with solenoid (B).

To generate MF by electric current an enameled copper wire solenoid with 100 turns was built: it was attached to the outer surface of the VTP, as shown in Fig. 1B. The solenoid was connected to power sources EA Elektro-automatik (EA-PS 2032-050, Germany) and Dual Tracking DC Power Supply Kikusui (CC-3032, Japan). These sources provide electrical current of 4.5 A, thus generating the 5 mT uniform MF perpendicular to the straight section in the solenoid. Therefore, MF concentrated inside the VTP where microalgae were grown. In this step, assays were exposed to MF for 1 h d^{-1}. The effect of MF was evaluated and compared to the control, which was only exposed to the Earth's MF and in the same conditions of temperature, illumination, aeration and nutrients.

2.3. Analytical determinations

Biomass concentration (X, g L^{-1}) was monitored daily and determined by optical density measurements at 670 nm by an UV–vis spectrophotometer (QUIMIS Q998U, Brazil) and related to the optical density by the standard of *Spirulina* sp. LEB 18 (Costa et al., 2002). The pH was also directly measured by a digital pH meter (QUIMIS Q400MT, Brazil) daily, in agreement with the official method (APHA, 1998). At the end of the culture, biomass was separated from the Zarrouk medium by centrifugation (Hitachi Himac CR-GIII, Japan) at 15,000*g* for 15 min, resuspended in distilled water and centrifuged again under the same conditions to improve nutrient removal. The centrifuged biomass was frozen for 48 h at −80 °C; afterwards, it was lyophilized by a lyophilizer (LABCONCO, USA) to be used in the biomass analysis.

2.4. Chemical composition of biomass

The biomass resulting from the assays was analyzed for protein, carbohydrates and lipids. Non-clarified extracts not clarified of biomass were prepared for the analysis of protein and carbohydrate contents. This procedure was performed in order to release the intracellular material of microalgae into the liquid medium. Extracts were obtained from 5 mg lyophilized microalgal biomass and 10 mL distilled water which were sonicated by an ultrasonic probe (Cole Parmer, CPX 130, USA) for 10 min, in 59-s cycles. Analyses of lipids were performed directly on the lyophilized biomass.

The protein content of the biomass was determined by the colorimetric method described by Lowry et al. (1951) with a standard bovine serum albumin curve. The carbohydrate content was determined by the phenol-sulfuric method developed by Dubois et al. (1956) with a standard glucose curve. The lipid content was extracted by the method proposed by Folch et al. (1957) which is based on the extraction of nonpolar and polar lipids (at room temperature) with chloroform:methanol (2:1) and methanol:water (2:1) solvents, respectively. The moisture content of the biomass was determined by the official method of AOAC (2000).

2.5. Extraction and quantification of phycocyanin

The extraction of phycocyanin from *Spirulina* sp. LEB 18 biomass was carried out with water as solvent, at a concentration of 0.08 g mL^{-1} with lyophilized biomass (Silveira et al., 2007). The extract was stirred at 10 rpm for 1 h at 25 °C in a shaker (New Brunswick Scientific, Innova 44, Germany) and then was centrifuged at 6000*g* for 10 min (Hitachi Himac CT 6EL, Japan). After centrifugation, the optical density of the supernatant was measured at 615 and 652 nm. The concentration of phycocyanin (mg mL^{-1}) was obtained by the spectrophotometric method, applying Eq. (1) described by Bennet and Bogorad (1973), where CF is the concentration of phycocyanin (mg mL^{-1}) and A_{615} and A_{652} are the optical densities of the sample.