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Growth stimulation and synthesis of lipids, pigments and antioxidants with magnetic fields in *Chlorella kessleri* cultivations

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HIGHLIGHTS

- Magnetic fields (MF) stimulate cell growth and biocompounds synthesis in *C. kessleri.*
- The effect of MF in *C. kessleri* are dependent on specific intensities and exposure times.
- MF of 60 mT is economical tool ($ 7), making it viable biocompounds production of microalgal origin.
- The stimulation in cell growth indicates effect hormetic of MF on cells.

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ABSTRACT

This study aimed at applying different intensities (30 and 60 mT) and exposure times (24 and 1 h d^{-1}) of MF to cultures of *Chlorella kessleri* and evaluated their effects on cell growth, proximate composition of biomass, pigment production and antioxidant activity. The condition of 60 mT for 1 h d^{-1} stimulated biomass concentration of 83.2% by comparison with control culture (CC). Besides stimulated 13.7% lipid synthesis, 38.9% chlorophyll *a* and 59.1% chlorophyll *b*, 25.0% total carotenoids and antioxidants up to 185.7%. Thus MF application is an excellent alternative to stimulate cell growth and high biotechnological interest biocompounds.

1. Introduction

Biotechnological applications of microalgae have been growing in recent years due to the potential of being a source of a great variety of molecules with high-prized added and diversified uses. The biomass is rich in biologically active compounds and macromolecules, such as fatty acids, carotenoids (Maadane et al., 2015), amino acids (Waghmare et al., 2016), peptides (Kang and Kim, 2013) and polysaccharides (Geun Goo et al., 2013). Therefore, microalgal biomass has been used in food supplementation (Draaisma et al., 2013), pharmaceutical industry (Maki et al., 2014), aquaculture (Taelman et al., 2013) and biofuels production (Piemonte et al., 2015).

However, to obtain products from microalgae may be very costly. When microalgae biorefinery is not used, it is only feasible to produce compounds from microalgae when these products are of high added value (Koller et al., 2014). Studies have been done in order to stimulate growth and to modify metabolic behavior of these micro-organisms to make economically viable microalgal production. Thus productivity and biocompounds synthesis increased (Herrero et al., 2015; Hidalgo et al., 2015; Singh et al., 2016).

The chlorophyta *Chlorella kessleri* is a large producer of fatty acids which can be used in biodiesel production and for nutritional enrichment (Ambrozova et al., 2014). *Chlorella* is considered GRAS (Generally Recognized As Safe) by the FDA (Food and Drug Administration) and can be used as food with no risk to human health (Henrard et al., 2014).

Magnetic fields (MF) are a viable alternative to stimulate biotechnological processes. Some studies were performed with microalgal cultures and found that is possible to stimulate both cell growth and biocompounds synthesis, such as fatty acids and pigments (Deamici et al., 2016a,b; Li et al., 2007; Small et al., 2012; Wang et al., 2008). Thus, this study aimed at applying MF of different intensities and exposure time on the *Chlorella kessleri* LEB 113

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cultivation and assesses the effects on cell growth, proximal composition of biomass, pigments production and antioxidant activity.

2. Material and methods

2.1. Microorganism and culture conditions

Microalga used was *Chlorella kessleri* LEB 113, which belongs to the Culture Collection of the Laboratory of Biochemical Engineering of the Federal University of Rio Grande (FURG). This strain was isolated from ponds of ash, near The President Médici Thermoelectricity Plant, Candiota-RS, Brazil. The microalga was maintained and cultured in BG-11 (Rippka et al., 1979), which contains (g L^{-1}): $NaNO_3$ (1.5); $K_2HPO_4{\cdot}3H_2O$ (0.04); $MgSO_4{\cdot}7H_2O$ (0.075); $CaCl_2{\cdot}2H_2O$ (0.036); $C_6H_{11}FeNO_7$ (0.006); $C_{10}H_{14}N_2Na_2O_8.2H$ (0.001); Na_2CO_3 (0.02); $C_6H_8O_7$ (0.006) and A5 + Co solution (1 mL L^{-1}). The A5 + Co solution contains (g L^{-1}): H_3BO_3 (2.86), $MnCl_2{\cdot}4H_2O$ (1.81), $ZnSO_4{\cdot}7H_2O$ (0.222), $NaMoO_4$ (0.015), $CuSO_4{\cdot}5H_2O$ (0.079) and $Co(NO_3){\cdot}6H_2O$ (0.0494). The cultivations were carried out in 2 L tubular vertical photobioreactors (TVP) (1.8 L working volume) in batch at 30 °C, 12 h light/dark photoperiod, illuminance 41.6 μmol m^{-2} s^{-1} for 10 d. The initial biomass concentration was 0.2 g L^{-1}. Compressed air (0.3 vvm) was filtered through sterile glass wool and was used to stir the cultures with a sintered stone as diffuser (Morais and Costa, 2007). Water evaporation in cultivation was controlled by maintaining the cultures volume with daily replacement of sterile distilled water.

2.2. Magnetic field application on cultivation

MF application in TVP was performed by ferrite magnets adaptation with mean intensity of 30 mT (150 × 50 × 10 mm) or 60 mT (50 × 50 × 25 mm). It was disposed at 180° apart from each other and 15 cm above the base of the TVP (Deamici et al., 2016a,b). Thus, the MF generated was concentrated in the inner part of the TVP and the MF intensities were measured by MF meter (Global Mag, TLMP-HALL 05 k, Brazil). The control cultures (CC) were performed under the same conditions of the cultivation with MF, but with no magnets exposition, only MF of 0.005 mT (Earth's MF). In order to evaluate the exposure time of MF application in cultures, the magnets were applied in two ways: throughout all cultivation period (24 h d^{-1}) and for 1 h d^{-1} at light cultivation period.

2.3. Analytical determinations

2.3.1. Determination of the biomass concentration and recovery of biomass

Biomass concentration was determined daily by measured optical density of cultures in spectrophotometer (SHIMADZU UV MINI 1240, Japan) at 670 nm. Biomass concentration was determined with a standard curve, which relates optical density and biomass dry weight.

Biomass was recovered from the culture medium at the end of cultures by centrifugation (Hitachi CR-HIMAC GIII, Japan), washed with distilled water and centrifuged again for removal the culture medium salts. The biomass centrifuged was frozen for Ultrafreezer (Eppendorf, New Brunswick Scientific Innova U535, Brazil) for 48 h at −80 °C and thereafter lyophilized (Labconco, USA). Lyophilized samples were kept in a freezer at −20 °C for further analysis.

2.3.2. Pigments determination

Pigments extraction with acetone 90% (v v^{-1}) was performed according to methodology developed by SCOR-UNESCO (1966). Chlorophyll *a* and chlorophyll *b* were calculated applying Eqs. (1) and (2) (SCOR-UNESCO, 1966) and carotenoids applying Eq. (3) (Parsons and Strickland, 1963).

$$\text{Chlorophyll } a\,(\text{mg L}^{-1}) = 11.64\,(\text{Abs}_{663} - \text{Abs}_{750}) - 2.16\,(\text{Abs}_{645} - \text{Abs}_{750}) \quad (1)$$

$$\text{Chlorophyll } b\,(\text{mg L}^{-1}) = 20.97\,(\text{Abs}_{645} - \text{Abs}_{750}) - 3.94\,(\text{Abs}_{663} - \text{Abs}_{750}) \quad (2)$$

$$\text{Carotenoids}\,(\text{mg L}^{-1}) = 4\,(\text{Abs}_{480} - \text{Abs}_{750}) \quad (3)$$

The absorbance results were subtracted from the absorbance at 750 nm for turbidity correction. The concentration of each pigment in cultures was determined by Eq. (4) (SCOR-UNESCO, 1966), where CP is the pigments concentration obtained in Eqs. (1)–(3).

$$\text{Pigment}\,(\text{mg L}^{-1}) = \text{CP}\,(\text{mg L}^{-1}).\,\text{Extract volume}\,(\text{L})/\text{Sample volume}\,(\text{L}) \quad (4)$$

2.3.3. Biomass characterization

Biomass was analyzed for protein, carbohydrates and lipids contents. It is necessary prepare biomass extracts to perform proteins and carbohydrates analysis. Ten mg lyophilized biomass and 20 mL distilled water which were sonicated in ultrasonic probe (COLE PARMER, CPX 130, EUA). The protein content 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 of Dubois et al. (1956), with a glucose standard curve. The lipid content of the lyophilized samples were determined by the spectrophotometric method described by Marsh and Weinstein (1966), a tripalmitin standard curve was used.

2.3.4. Antioxidant activity

Methanol extracts of biomass were obtained to determine the antioxidant activity with the methodology proposed by Lichtenthaler (1987). Three methodologies were used to determine antioxidant activity: reducing power, DPPH assay and ABTS assay.

The reducing power was determined according to the method of Oyaizu (1986), for this, 0.25 mL methanol extract (2.5 mg mL^{-1}) was mixed with 2 mL sodium phosphate buffer 0.2 mol L^{-1} (pH 6.6) and 2 mL 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After that, it was added 2 mL 10% trichloroacetic acid. Aliquots of 2 mL (incubated samples) were mixed with 2 mL distilled water and 0.4 mL ferric chloride (0.1%). After 10 min, absorbance was determined at 700 nm in a spectrophotometer. The absorbance increase indicates an increase of reducing power.

The scavenger effect of free radical 2,2-diphenyl-1-picrylhidrazol (DPPH) was measured as described by Rufino et al. (2007a) with modifications. Samples of 0.1 mL methanol extract (2.5 mg mL^{-1}) were added in 3.9 mL DPPH (0.06 mmol L^{-1}) in methyl alcohol in the dark. After 60 min at ambient temperature, absorbance was measured at 517 nm in a spectrophotometer (Shimadzu UV MINI 1240, Japan). The lowest absorbance represents greater scavenging capacity of the DPPH radical which was calculated applying Eq. (5).

$$\text{Inhibition}\,(\%) = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \cdot 100 \quad (5)$$

The free radical capture capability ABTS was determined according to Rufino et al. (2007b), with modifications. The $ABTS^{\cdot+}$ was prepared from the reaction of 7 mmol L^{-1} ABTS and 140 mM potassium persulfate, leaving the mixture to stand in the dark at room temperature for 16 h. The $ABTS^{\cdot+}$ solution was diluted with