Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Short communication

Static magnetic fields in culture of *Chlorella fusca*: Bioeffects on growth and biomass composition

Kricelle Mosquera Deamici, Bruna Barcelos Cardias, Jorge Alberto Vieira Costa, Lucielen Oliveira Santos *

Laboratory of Biochemical Engineering, School of Chemistry and Food, Federal University of Rio Grande, 96203-900 Rio Grande, RS, Brazil

ARTICLE INFO

Article history:
Received 20 January 2016
Received in revised form 5 April 2016
Accepted 5 April 2016
Available online xxx

Keywords:
Microalgae
Magnetic field
Kinetic parameters
Growth kinetics
Bioreactors
Chlorella

Chemical compounds studied in this article:
Sodium nitrate (PubChem CID: 24268)
Citric acid (PubChem CID: 311)
Sodium carbonate (PubChem CID: 10340)
Dipotassium hydrogen phosphate trihydrate (PubChem CID: 16217523)
Calcium chloride dehydrate (PubChem CID: 6093260)
Ferric ammonium citrate (PubChem CID: 14457)
Sulfuric acid (PubChem CID: 1118)
Methanol (PubChem CID: 887)
Chloroform (PubChem CID: 6212)
Sodium hydroxide (PubChem CID: 14798)

ABSTRACT

Static magnetic fields (SMF) are being studied in microorganism cultures and there are reports that it could accelerate growth or cause changes in metabolism. Thus, this study aims to evaluating the influence of SMF on growth and biomass composition obtained in culture of *Chlorella fusca* LEB 111 in vertical tubular photobioreactor (VTP). SMF of 30 and 60 mT was generated by ferrite magnets and applied for 24 h d^{-1} and 1 h d^{-1} for 15 days. The growth of *C. fusca* was evaluated by kinetic parameters and the biomass obtained was evaluated by protein, carbohydrate, and lipid content. SMF of 60 mT for 24 h d^{-1} increased the biomass concentration and the carbohydrate content, it was 20.5% and 24.8% higher than control culture, respectively. Magnetic treatment is a new method to culture microalgae that can accelerate growth and increase compounds of interest, which can be used as food or biofuel.

1. Introduction

Microalgae are unicellular photosynthetic microorganisms that use light energy and carbon dioxide (CO_2) in the production of organic compounds. The microalgae are able to fix CO_2 in the atmosphere, which reduces atmospheric CO_2 levels [1]. These microorganisms are considered the most promising candidates for multi-purpose biomass production because they can grow rapidly, produce large quantities of lipids, carbohydrates and proteins, sequester and recycle CO_2 from industrial flue gases and remove pollutants from industrial, agricultural and wastewaters [2]. The biomass of microalgae may be utilized by several applications such as biofuel production [3], as an ingredient in food preparations [4], and pharmaceutical industries [5]. *Chlorella* is a unicellular microalga that grows easily, rapidly and therefore it is a good material for biotechnology research [6]. Commercial use of *Chlorella* occurs in various sectors such as a food additive in human, animal nutrition and in biofuels [7].

With the modification of some culture conditions, it is possible to improve the growth of the microorganism and increase the production of compounds of commercial interest such as fats, fatty acids [8], oil, sugars, vitamins, pigments, antioxidants, and high-value bioactive compounds [9]. Static magnetic field (SMF) could affect organisms in both a negative and positive manner, which

* Corresponding author.
E-mail address: santoslucielen@gmail.com (L.O. Santos).

http://dx.doi.org/10.1016/j.procbio.2016.04.005
1359-5113/

includes the acceleration of growth and changes in metabolism [2]. The electric field also could affect biological systems, such as cell culture growth and metabolism [10]. The effects on organisms due to the magnetic field action can be correlated the same reason of the effects from the electric field, and it still needs to be evaluated. SMF could cause a variety of effects in microorganisms and plants such as increased seed germination, growth and pigment content [11], increased glutathione content by *Saccharomyces cerevisiae* [12], increased oxidative stress in plant cells [13]. However, the bioeffects of SMF in microalgae are a field that needs to be explored because there are minimal reports currently available. The aim of this study was the analysis of the bioeffects of SMF (30 and 60 mT) using different times of exposure on the *Chlorella fusca* LEB 111 cultivation. Particularly, the impact of microalgae growth rate and the composition of biomass in relation to the protein, carbohydrate, and lipid content were analyzed.

2. Materials and methods

2.1. Microalgae strains and culture conditions

The microalga strain used was *C. fusca* LEB 111 that was isolated from ponds next to the President Médici Thermoelectrcity Plant, Candiota-RS, Brazil. This microalga was grown on BG 11 medium [14], 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), ferric ammonium citrate (0.006), EDTA (disodium magnesium salt) (0.001), Na_2CO_3 (0.02), citric acid (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), $NaMoO_4{\cdot}2H_2O$ (0.390), $CuSO_4{\cdot}5H_2O$ (0.079) and $Co(NO_3){\cdot}6H_2O$ (0.0494). *C. fusca* LEB 111 was cultivated in a 2 L tubular vertical photobioreactor (1.8 L working volume) [15]. *C. fusca* LEB 111 was grown at 30 °C for 15 days under 12 h dark/light photoperiod with illumination of 60 μmol photons $m^{-2}\,s^{-1}$ provided by four 40 w daylight-type fluorescent lamps during the light period. The initial biomass concentration was $0.3\,g\,L^{-1}$ and the cultures were aerated continuously by injection of compressed air, with 0.3 vvm and 0.03% CO_2 concentration. The evaporation of water during the culture was controlled by the daily addition of distilled water.

2.2. Application of static magnetic fields

The control culture (C_C) was only exposed to the Earth's magnetic field and it was performed under the same conditions as the cultures with the SMF as temperature, luminosity, aeration and nutrients. In cultures with SMF, was applied ferrite magnets around the VTP and it was performed for $24\,h\,d^{-1}$ (during 15 d) and for $1\,h\,d^{-1}$ (in the light photoperiod, 15 d). Two models of ferrite magnets were utilized with intensity of 30 mT and other with 60 mT. Each ferrite magnetic was at 180° to each other and 15 cm above the base of the VTP (Fig. 1). Intensities of SMF were measured in the center of VTP by a magnetic field measuring device Globalmag (model TLMP-HALL-05k-T0, Brazil).

2.3. Analytical determinations

The biomass concentration (X, $g\,L^{-1}$) was monitored daily and determined by optical density measurements at 670 nm by a UV–vis spectrophotometer Quimis (model Q998U, Brazil). The biomass concentration was related to the optical density by the standard curve for this microalga. The pH measurements were also monitored daily by a digital pH meter Quimis (model Q400MT, Brazil). After 15 days, the biomass was separated from the culture medium by centrifugation at 9690*g* at 20 °C for 20 min using a centrifuge (model CR22GIII, Hitachi, Japan). The centrifuged biomass

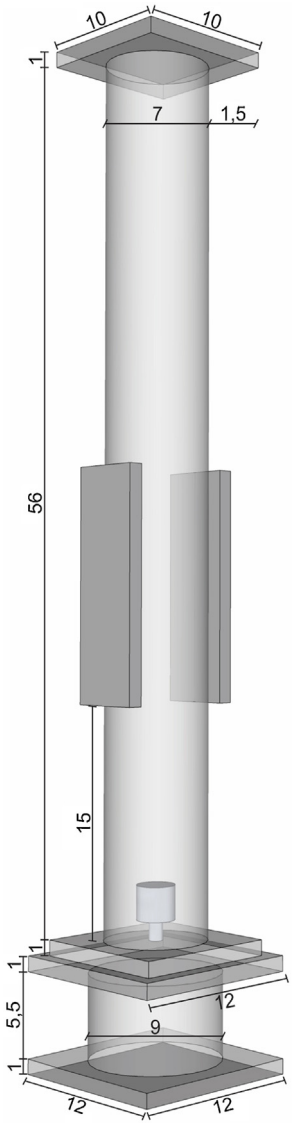

Fig. 1. Vertical tubular photobioreactor with application of a SMF through the magnets. All of the measurements are in centimeters.

was frozen for 48 h at −80 °C and lyophilized in a lyophilizer (Labconco, EUA) for use in the biomass analysis.

2.4. Kinetic parameters

The maximum biomass concentration (X_{max}, $g\,L^{-1}$) was obtained on the last day of the culture. The productivity (P, $g\,L^{-1}\,d^{-1}$) was calculated for each day as $P = (X_t - X_0)\,.t^{-1}$ where X_t and X_0 are the biomass concentration at time *t* and at the beginning of the culture, respectively; *t* is the final time of culture. The maximum productivity during cultivation was designated as P_{max}. The biomass concentration (X, $g\,L^{-1}$) and exponential regression were used to calculate the maximum specific growth rate (μ_{max}, d^{-1}) during the logarithmic phase [16]. The doubling time (t_b, d) is the necessary time for doubling the biomass concentration and was calculated as $t_b = ln2(\mu_{max})^{-1}$.

2.5. Biomass characterization

The biomass obtained was evaluated for protein, carbohydrate, and lipid content. To perform the analysis for protein and carbohydrate contents, it was necessary to prepare a biomass extract from

Please cite this article in press as: K.M. Deamici, et al., Static magnetic fields in culture of *Chlorella fusca*: Bioeffects on growth and biomass composition, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.04.005