Bioresource Technology 207 (2016) 220–228

Contents lists available at ScienceDirect

Bioresource Technology

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

A new bioenergetic and thermodynamic approach to batch photoautotrophic growth of *Arthrospira (Spirulina) platensis* in different photobioreactors and under different light conditions

Milena Fernandes da Silva [a,b], Alessandro Alberto Casazza [b], Pier Francesco Ferrari [b], Patrizia Perego [b], Raquel Pedrosa Bezerra [c], Attilio Converti [b,*], Ana Lucia Figueiredo Porto [c]

[a] Biological Science Center, Federal University of Pernambuco, Av. Prof. Moraes Rego 1235, Cidade Universitária, 50670-420 Recife, PE, Brazil
[b] Department of Civil, Chemical and Environmental Engineering (DICCA), University of Genoa, Via Opera Pia 15, 16145 Genoa, Italy
[c] Department of Morphology and Animal Physiology, Federal Rural University of Pernambuco, Av. Dom Manoel de Medeiros s/n, 52171-900 Recife, PE, Brazil

HIGHLIGHTS

- Batch *A. platensis* culture is studied in different photobioreactor configurations.
- Kinetics, bioenergetics and thermodynamics of *A. platensis* growth are evaluated.
- An increase in surface/volume ratio up to 1.94 cm^{-1} enhanced growth parameters.
- The influence of light intensity is investigated in the horizontal photobioreactor.
- The horizontal photobioreactor has the best configuration to perform batch culture.

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 29 January 2016
Accepted 30 January 2016
Available online 6 February 2016

Keywords:
Photobioreactor
Bioenergetics
Arthrospira platensis
Thermodynamics
Light intensity

ABSTRACT

Photobioreactor configuration, mode of operation and light intensity are known to strongly impact on cyanobacteria growth. To shed light on these issues, kinetic, bioenergetic and thermodynamic parameters of batch *Arthrospira platensis* cultures were estimated along the time at photosynthetic photon flux density (PPFD) of 70 μmol m^{-2} s^{-1} in different photobioreactors with different surface/volume ratio (*S*/*V*), namely open pond (0.25 cm^{-1}), shaken flask (0.48 cm^{-1}), horizontal photobioreactor (HoP) (1.94 cm^{-1}) and helicoidal photobioreactor (HeP) (3.88 cm^{-1}). Maximum biomass concentration and productivity remarkably increased with *S*/*V* up to 1.94 cm^{-1}. HoP was shown to be the best-performing system throughout the whole runs, while HeP behaved better only at the start. Runs carried out in HoP increasing PPFD from 40 to 100 μmol m^{-2} s^{-1} revealed a progressive enhancement of bioenergetics and thermodynamics likely because of favorable light distribution. HoP appeared to be a promising configuration to perform high-yield indoor cyanobacterial cultures.

1. Introduction

The energy management in a living cell is called bioenergetics. When combined with the fundamental principles of thermodynamics, it has proven to be a highly useful tool for the analysis of biosystems (Küçük et al., 2015). Photosynthetic microorganisms have recently gained huge attention worldwide (Cheah et al., 2015), since they are one of the most promising renewable and neutral energy sources, i.e., by consuming carbon dioxide, their cultivation has the additional benefit of combining valuable biomass production with CO_2 emissions reduction (Belay, 2002; Rodrigues et al., 2011). Among the photosynthetic microorganisms with commercial importance, the filamentous cyanobacterium (blue-green alga) *Arthrospira (Spirulina) platensis* has widely been studied, because its biomass has a large number of industrial applications besides being considered a high-value food (Belay, 2002; Benelhadj et al., 2016). *A. platensis* production is in fact increasing worldwide owing to its high contents of highly-valuable proteins, amino acids, essential fatty acids (i.e., γ-linolenic acid, GLA), polysaccharides, vitamins and pigments (β-carotene, chlorophyll a and phycocyanin) (Pulz and Gross, 2004); in addition, it contains other phytochemicals that find application in several industrial

* Corresponding author. Tel.: +39 0 10 3532593; fax: +39 0 10 3532586.
E-mail address: converti@unige.it (A. Converti).

http://dx.doi.org/10.1016/j.biortech.2016.01.128
0960-8524/

segments like those of health foods and therapeutics (Belay, 2002; Pulz and Gross, 2004; Raposo and Morais, 2015).

A lot of parameters can be tuned to obtain high biomass yield, among which are the mode of operation, light intensity and reactor configuration, that can strongly impact on the performance of *A. platensis* cultivation. Different types of processes have been developed to get an optimal compromise between high productivity and low production costs. Among these are: (a) the fed-batch process that consists in periodically replacing a part of the exhaust medium with fresh medium to keep the culture volume constant; (b) the batch culture systems, which are the most widely applied because of their simplicity and flexibility, even though not necessarily the most efficient ones; and (c) large-scale continuous cultures, which have not been applied extensively up to now, due to several difficulties in their control, among which are higher risk of contamination, use of feeding pumps, lower yields, etc.

Light irradiance should be provided with care to indoor systems performed using artificial light, since excess light leads to a phenomenon called "photooxidation" or "photoinhibition". That is, cell concentration increases with light intensity until reaching a maximum threshold value at the so-called "saturation level", beyond which a further increase in light intensity provokes damage of cell photosynthetic apparatus (Bezerra et al., 2012).

The reactor configuration is an additional factor greatly influencing cell growth. Photobioreactors can reduce the cultivation area by a vertical distribution of the photosynthetic organism and enlarge the surface exposed to light, thereby ensuring high surface/volume ratios and increasing cell concentration. Light is better captured by cells in tubular photobioreactors when compared to the conventional open ponds, where, owing to a relatively high depth of culture medium, it has to go through thick layers to reach the inner cells (Converti et al., 2006; Rodrigues et al., 2010). Although the open-channel raceway ponds is the most widely used configuration for *A. platensis* commercial production, tubular photobioreactors have been deeply studied, not only because of their high cell productivity, but also of many other advantages, such as low levels of contamination and better CO_2 solubilization in the medium, better light distribution and then higher photosynthetic efficiency (Converti et al., 2006).

Bioenergetic studies based on the Gibbs energy dissipation may be applied to describe or predict the microbial growth yield, the energy flow to ATP production, the increase in enthalpic content and the heat released by living organisms (Bezerra et al., 2012; Sassano et al., 2004; Torre et al., 2003). All of them are quite important to optimize any bioprocess and even to design the most suitable bioreactor to perform it; but, unfortunately, only a few studies dealt with the bioenergetic aspects of the growth of photosynthetic microorganisms based on Gibbs energy balances. In particular, biomass yield constitutes one of the key parameters in any bioprocess or experiment involving microbial cultures, since it determines the final biomass concentration, which must be maximized to obtain high productivities (Von Stockar et al., 2006). In addition, the Gibbs energy dissipation per C-mol of biomass can be regarded as a simple thermodynamic measure of the amount of biochemical "work" required to convert the carbon source into biomass (Bezerra et al., 2012; Heijnen and Van Dijken, 1991, 1993; Liu et al., 2007).

Based on this background, if from one hand previous works demonstrated that the use of the fed-batch mode of operation is able to promote the growth of photosynthetic microorganisms minimizing a number of well-known adverse phenomena (shading, inhibitions related to excess salt level or osmotic pressure, and so on), from the other it masks their effects. Therefore, to shed light on these issues and because of a certain lack of detailed and recent theoretical studies on the mechanisms ruling *A. platensis* cultures, the main bioenergetic and thermodynamic parameters of the photoautotrophic batch growth of such a cyanobacterium were investigated in this study based on the model proposed by Torre et al. (2003). For this purpose, we investigated four different photobioreactor configurations with different surface/volume ratios, namely shaken flask, open pond, helicoidal photobioreactor and horizontal photobioreactor, and varying the light irradiance in the last, best-performing configuration.

2. Methods

2.1. Microorganism and culture conditions

A. (Spirulina) platensis UTEX 1926 was obtained from the Culture Collection of Algae of the University of Texas (Austin, TX, USA). To allow a large growth of biomass, the microorganism was maintained and cultivated in the culture medium suggested by Schlösser (1982) modified so as to have a nitrogen concentration equal to about 4-fold that of the original medium. The resulting medium had the following composition (per liter): 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 10.0 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$. All the nutrients were dissolved in distilled water containing (per liter): 6.0 mL of metal solution (97 mg L^{-1} $FeCl_3{\cdot}6H_2O$, 41 mg L^{-1} $MnCl_2{\cdot}4H_2O$, 5 mg L^{-1} $ZnCl_2$, 2 mg L^{-1} $CoCl_2{\cdot}6H_2O$, 4 mg L^{-1} $Na_2MoO_4{\cdot}2H_2O$, 750 mg L^{-1} $Na_2EDTA{\cdot}2H_2O$), 1.0 mL of micronutrient solution (50.0 mg L^{-1} Na_2EDTA, 618 mg L^{-1} H_3BO_3, 19.6 mg L^{-1} $CuSO_4{\cdot}5H_2O$, 44.0 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 20.0 mg L^{-1} $CoCl_2{\cdot}6H_2O$, 12.6 mg L^{-1} $MnCl_2{\cdot}4H_2O$, 12.6 mg L^{-1} $Na_2MoO_4{\cdot}2H_2O$) and 1.0 mL of B12 vitamin solution (0.15 mg L^{-1}).

Cells were grown batch-wise either at photosynthetic photon flux density (PPFD) of 70 μmol m^{-2} s^{-1} in different photobioreactor configurations with different surface/volume ratios (S/V), namely open pond (Sassano et al., 2004) (S/V = 0.25 cm^{-1}), shaken flask (Frumento et al., 2016) (S/V = 0.48 cm^{-1}), horizontal photobioreactor (Ferreira et al., 2010) (S/V = 1.94 cm^{-1}) and helicoidal photobioreactor (Bezerra et al., 2011; Frumento et al., 2016) (S/V = 3.88 cm^{-1}), or progressively increasing PPFD from 40 to 100 μmol m^{-2} s^{-1} in the horizontal photobioreactor that proved the best-performing configuration. For this purpose, fluorescent artificial light was ensured by a variable number of 36 W-lamps. Schematics of horizontal and helicoidal photobioreactors were illustrated in a previous study (Frumento et al., 2013).

Cultivations were carried out at temperature of 30 ± 2 °C, by incubating the equipment in a thermostated chamber, using an initial biomass concentration of 0.40 g L^{-1}. The pH was controlled daily at 9.5 ± 0.2 through the addition of pure CO_2 from a cylinder. After growth, once the stationary phase had been reached after about 9 days of cultivation, biomass was separated from the culture medium by centrifugation at 7500 rpm for 10 min using a centrifuge, model 42426 (ALC, Milan, Italy). Recovered cells were washed twice with distilled water, dried at 105 °C for 24 h, pulverized in a mortar and stored at −20 °C for subsequent analysis of its elemental composition.

2.2. Analytical procedures

Cell concentration of *A. platensis* was determined daily by measuring the optical density (OD) at 560 nm by a UV-Visible spectrophotometer, model Lambda 25 (PerkinElmer, Milan, Italy), and expressed in grams of dried biomass per liter of medium (g L^{-1}) through a calibration curve relating OD to dry biomass weight. All measurements were done in triplicate.

The elemental composition of dried biomass stored at the end of culture was determined by an elemental analyzer Flash EA1112 series (CE Instruments, Wigan, UK). Since biomass composition varied very little among the different photobioreactor configurations