Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Carbohydrate productivity in continuous reactor under nitrogen limitation: Effect of light and residence time on nutrient uptake in *Chlorella vulgaris*

Carlos Eduardo de Farias Silva, Eleonora Sforza*

Department of Industrial Engineering DII, University of Padova, Via Marzolo 9, 35131 Padova, Italy

ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form 6 September 2016
Accepted 17 September 2016
Available online xxx

Keywords:
Bioethanol
Steady state
Photoinhibition
Photosaturation
Phosphorus uptake

ABSTRACT

Chlorella vulgaris is commonly recognized as an interesting species for bioethanol production due to its carbohydrate content. Carbohydrates accumulation is often obtained under nitrogen starvation, which on the other hand may lead to a reduced biomass production. In this work, the effect of nitrogen limitation in a continuous system was assessed, with the aim of finding an optimal value where biomass productivity and carbohydrate content are well balanced. The effect of light intensity was also investigated, and it was highlighted that in a continuous system light stress is the main variable affecting the carbohydrate content and productivity. It was also evidenced that increasing the residence time is a way to boost nitrogen starvation: the biomass yield on nitrogen noticeably changes with the residence time, thus modifying the elemental composition of the microalgal biomass, and resulting in an accumulation of carbohydrates.

1. Introduction

Fossil carbon sources account for about 85% of the global energetic consumption, with about 33% of oil, 29% of coal and 24% of natural gas. Renewable energy accounts for only 10% of the total. The growing need to expand the use of renewable energy sources in a sustainable manner has boosted, in particular, the production of biofuels worldwide [1].

In this scenario, bioethanol is one of the main biofuels produced. The global ethanol production corresponds to approximately 25 billion gallons per year (over 100 million m^{-3} y^{-1}). It is perceived that the US and Brazil hold hegemony in production accounting for almost 85%, using corn and sugarcane, respectively [2]. On the other hand, the production of bioethanol is expected to largely increase, leading to an unsustainable competition for arable land. In this perspective, microalgae and cyanobacteria are a source of biomass that can complement the agricultural raw materials and help to increase the global demand for food, biofuels and chemical production [3,4]. The biochemical composition of microalgae mainly includes proteins (30–50%), carbohydrates (20–40%) and lipids (8–15%) [5], but several studies have shown that lipids and carbohydrates can be accumulated under stress conditions thus decreasing the protein content [6,7]. It is important to mention that carbohydrates and lipids are energy rich molecules, which constitute reserves in microalgae, and they are an indispensable buffer against varying external growth conditions, allowing to survive for periods of light-energy absence [8].

In view of bioethanol production, an efficient accumulation of carbohydrates is the key factor: carbohydrates in microalgae are mainly composed of starch or glycogen, depending on the species, which can be hydrolyzed and easily fermented to produce bioethanol. Several studies report an increase of carbohydrate content up to 50%, for many microalgal and cyanobacterial species, such as *Chlorella vulgaris* [9], *Dunaliella tertiolecta* [10], *Scenedesmus* spp. [11,12], *Tribonema* sp. [7], *Arthrospira platensis* [13] and *Synechococcus* sp. [14], mainly cultivated under nitrogen starvation or limitation occurring at the stationary phase in batch cultivation. In fact, nitrogen plays a key role in the redirection of algal metabolism, for both carbohydrate and lipid accumulation. Both carbohydrate and lipid metabolism starts with a common initial pool of molecules consisting of three carbons and, even though some species produce preferentially one of the two macromulecules as reserve, other organisms can produce both, with ratios that differ depending on growth conditions. If nitrogen starvation is a commonly recognized method to trigger lipid accumulation, its effect on carbohydrates is still under investigation, in particular because it usually results in lower biomass productivity, thus affecting the efficiency of the whole process. Carbohydrates and lipid accumu-

* Corresponding author.
E-mail address: eleonora.sforza@unipd.it (E. Sforza).

http://dx.doi.org/10.1016/j.procbio.2016.09.015
1359-5113/

lation also depends on many other variables (or their interactions), such as light intensity, temperature, carbon source, growing time, nutrients availability, salinity and pH, as recently reviewed by Vitova et al. [8].

In addition, carbohydrates accumulation in batch system is not stable, and under extreme N starvation carbohydrates content may actually decrease [15], while N limitation seems a viable alternative [8].

Moreover, from an industrial perspective, batch systems are not suitable, while continuous processes could noticeably improve the productivity: steady state production is generally more efficient, has lower costs and is easier to operate [16]. The possibility of cultivating microalgae in continuous industrial system is still challenging, particularly due to the variability of environmental parameters in outdoor cultivation. On the other hand, at lab scale, cultivation of microalgae in continuous systems is quite established and studied for several species [17–20], but less information are available on the carbohydrate accumulation under continuous mode [21], in particular for *Chlorella vulgaris*. In addition, continuous systems working at steady-state are a viable tool to study physiological response to environmental condition since light intensity, residence time and nitrogen concentration can be managed for an efficient carbohydrate accumulation [11,22].

In this work *Chlorella vulgaris* was grown in a flat panel continuous photobioreactor (PBR), under different light intensities. The residence time and nitrogen inlet concentrations were changed, in order to assess their effect on biomass steady state concentration, carbohydrate productivity and photosynthetic efficiency. A N limitation approach in continuous system was used, to boost carbohydrate accumulation without strongly affecting the biomass productivity.

2. Materials and methods

2.1. Microalgae and media composition

The microalgal species *Chlorella vulgaris* was maintained and cultured in modified BG11 medium (buffered with 10 mM HEPES pH 8), sterilized in an autoclave for 20 min at 121 °C. The P and N content of the medium, in the form of K_2HPO_4 and $NaNO_3$, were optimized to study the nutrient starvation, and concentrations for each condition are reported in the corresponding sections.

2.2. Equipment

Batch experiments were performed in 250 mL working-volume glass vertical cylinders (5 cm diameter), continuously mixed by a stirring magnet placed at the bottom of the bottle.

Continuous experiments were performed in vertical flat-plate polycarbonate CSTR (continuously stirred tank reactor) PBR (see Refs. [23,24] for the schematic of the experimental setup), with a working volume of 300 mL, a depth of 1.2 cm, and a surface exposed to light of 250 cm^2. CO_2 in excess is provided by a CO_2–air (5% v/v) bubbling at the reactor bottom (1 L h^{-1} of total gas flow rate), which also provided mixing. A magnetic stirrer was also used to prevent any deposition of biomass and thus ensuring a good mixing of the reactor [24]. The fresh medium was fed at a constant rate by a peristaltic pump (Watson-Marlow sci400, flow rate range: 25–250 mL d^{-1}). Light was provided by a LED lamp (Photon System Instruments, SN-SL 3500-22) both for continuous and batch experiments. Photon Flux Density (PFD) was measured on both the front and back panels of the reactor using a photoradiometer (HD 2101.1 from Delta OHM), which quantifies the photosynthetically active radiation (PAR).

2.3. Experimental procedures

In steady state continuous experiments, *C. vulgaris* was inoculated into the reactor with the culture medium. At the beginning, batch operation mode was set to prevent the occurring of washout. Once a significant concentration (10^8 cells mL^{-1}) was reached, the operation was switched to continuous, feeding the fresh medium from a tank, through the peristaltic pump. The working volume (V_{PBR}) was controlled by an overflow tube, and the outlet flow rate Q (mL d^{-1}) was collected in another tank. The hydraulic residence time (τ) in the reactor was directly controlled by the peristaltic pump, according to:

$$\tau = \frac{V_{PBR}}{Q} \quad (1)$$

When steady-state operation was achieved, biomass was sampled and analyzed at least for 3–7 days. For each steady state the biomass density C_x, the carbohydrates content, as well as the N and P consumptions were measured. The volumetric biomass productivity P_X (g L^{-1} d^{-1}) was calculated as:

$$P_X = \frac{C_X}{\tau} \quad (2)$$

where C_X is the biomass concentration (DW) at steady state.

To assess the effect of nutrients ratios on biomass and carbohydrates concentrations, the inlet medium was changed and transient conditions were observed, until a new steady state was reached.

The photon flux density absorbed by the algae (PFD_{abs}) was also measured at steady state. This was calculated as:

$$PFD_{abs} = I_{in} - B_I - I_0 \quad (3)$$

where I_{in} is the incident light (mmol m^{-2} d^{-1}), B_I the back irradiance (mmol m^{-2} d^{-1}), and I_0 the light absorbed by the medium and the panel walls (mmol m^{-2} d^{-1}).

The photosynthetic efficiency (PE), i.e. the fraction of PAR converted to biomass, was calculated as:

$$\%PAR = \frac{C_x * Q * LHW}{PFD_{abs} * E_p * A_{PBR}} \quad (4)$$

where *LHV* is the Lower Heating Value (assumed equal to 20 kJ g^{-1}), E_p the energy of photons (kJ μmol^{-1}), and A_{PBR} is the irradiated surface of the reactor (m^2).

In addition, at steady-state a stable specific light supply per unit mass of cell r_{Ex} (mmol g^{-1} d^{-1}) [25,26] was calculated as:

$$r_{Ex} = \frac{PFD_{abs} * A_{pbr}}{c_x * V_{pbr}} \quad (5)$$

2.4. Analytical methods

The biomass concentration was monitored daily by spectrophotometric analysis of the optical density (OD_{750}) using a UV–vis spectrophotometer (UV 500, Spectronic Unicam, UK) correlated to cell concentration, measured with a Bürker Counting Chamber (HBG, Germany). The concentration of biomass was also gravimetrically measured as dry weight (DW) in terms of g L^{-1} in cells previously harvested with a 0.22 μm filter, and then dried for 4 h at 80 °C in a laboratory oven. The nutrients analyzed were nitrate ($N\text{-}NO_3$) and phosphate ($P\text{-}PO_4$), assessed at least three different times for each steady state. Culture samples were filtered in order to measure only dissolved nutrients (0.2 μm): $N\text{-}NO_3$ concentration was measured by an analytical test kits provided by St. Carlo Erba Reagenti, Italy, (code 0800.05482) and orthophosphates were measured by the ascorbic acid method described in APHA-AWWA-WEF, 1992. Nutrient concentrations were measured at the reactor inlet

Please cite this article in press as: C.E. de Farias Silva, E. Sforza, Carbohydrate productivity in continuous reactor under nitrogen limitation: Effect of light and residence time on nutrient uptake in *Chlorella vulgaris*, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.09.015