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Mass balance analysis of carbon and nitrogen in industrial scale mixotrophic microalgae cultures

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ABSTRACT

Large-scale cultivation of *Chlorella vulgaris* is of great interest given the extent of products and potential applications that can derive from its biomass. From an industrial point of view it is imperative to consistently obtain high productivities and high quality biomass at the lowest production costs. The mass balance of critical nutrients such as carbon and nitrogen is therefore necessary to quantify its recovery and consumption yields, the efficiency of the biomass production system and to identify operational optimization opportunities.

The mass balance of *C. vulgaris* mixotrophic growth throughout scale-up from 10 m^3 to 100 m^3 on acetate and urea as carbon and nitrogen sources was calculated using a black-box model developed to illustrate the inputs and outputs of the system in *quasi-real time* and resulted on recovery factors of 0.99 ± 0.08 and 0.99 ± 0.25, respectively. Under these conditions *C. vulgaris* cultivation yielded a maximal productivity of 0.14 g L^{-1} d^{-1} and maximal growth rate of 0.38 d^{-1}. Both parameters decreased throughout scale up reaching an average productivity of 0.09 g L^{-1} d^{-1} with an average growth rate of 0.13 d^{-1} for the whole process. Global carbon and nitrogen yields measured were 0.76 $mol_{C\text{-}X}$ mol_C^{-1} and 0.72 $mol_{N\text{-}X}$ mol_N^{-1}. The mass balance determination indicates the incorporation of both acetate and urea carbon atoms into the biomass. Therefore, external inorganic carbon from CO_2 was concluded to have little influence on microalgae growth in the conditions studied apart from pH control. Urea and ammonium were found to be effectively used by *C. vulgaris* cells. However, despite the satisfactory yield obtained for nitrogen, the metabolism of urea resulted in ammonium build-up in the culture medium.

To our knowledge this is the first report of growth parameters and mass balance analysis of a *Chlorella* sp. culture in industrial scale closed tubular photobioreactors.

1. Introduction

Large-scale microalgae production has been tested since the 1950's given their high potential as cell-factories [4,31]. Prospective and actual products such as carbohydrates, lipids, pigments, minerals, vitamins, enzymes and polymers can be obtained by the cultivation of different microalgae strains in different mineral media, with organic substrates or even in wastewater [27,29,31]. However, achieving highly productive dense cultures at industrial scale is still challenging [1,5]. Presently microalgae mass production has been achieved mainly in open ponds and tubular or flat plate photobioreactors (PBR) with optimization of photoautotrophy [2,4,11,13,19,29] and mixotrophy [21]. In photoautotrophy microalgae grow using carbon dioxide as the carbon source and light as the energy source. Thus, the presence of sufficient light is a major limitation by imposing high surface to volume ratios on the culturing vessels for maximum light exposure, resulting in a weather-dependent, seasonal production [4,27–29,34]. In order to overcome these limitations and to increase productivity, a mixotrophic growth regime can be implemented [27]. In mixotrophic cultures light energy is the main source of ATP for *C. vulgaris* in the exponential growth phase and as the culture ages and cell concentration increases, creating a mutual shading effect, nearly all ATP produced has been shown to originate from the catabolism of the organic substrate [34]. Therefore it is possible to improve biomass productivity through mixotrophic cultivation under low light intensities or when the culture is in the linear growth phase [6,26]. In fact, productivity increases up to 5.5 fold have been reported at laboratory scale [27]. Additionally the biomass production process becomes less dependent on external factors such as light intensity – typically associated to the heterotrophic metabolism – while the cells maintain the expression of photosynthetic pigments – characteristic of photoautotrophy. Notwithstanding, the use of organic substrates coupled with the difficulty of photobioreactor sterilization results in a culture more susceptible to bacterial contamination [3] and increase production costs. Hence, dose management of carbon feed is a critical operation factor. The continuous small quantity addition

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Nomenclature

a	afternoon (19:00 h)
Am	ammonium ion concentration $mol\ L^{-1}$
DW	dry weight $g\ L^{-1}$
feed	feeding medium stream
HAc	acetate/acetic acid concentration $mol\ L^{-1}$
harv	harvesting stream
i	indicator of time (day)
m	morning (9:00 h)
Nit	nitrate concentration $mol\ L^{-1}$
sup	supplementation stream
Turb	turbidity NTU
Ur	urea concentration $mol\ L^{-1}$
V	volume m^3
X	biomass $g\ L^{-1}$

of the organic substrate as opposed to less frequent high quantity additions is a proposed solution to favor higher microalgae biomass productivity and prevent bacterial proliferation [27]. Additionally, mixotrophic growth of microalgae is limited to a small number of species capable of metabolizing organic substrates in conventional bioreactors [27].

Studies have been developed to enlighten the microalgal mixotrophic metabolism, specifically the influence that light and inorganic CO_2 pose on the metabolic pathways of incorporation of organic carbon sources. Ogawa and Aiba (14) have shown that the maximum growth rate of a mixotrophic culture equals the sum of the growth rates of a photoautotrophic and a heterotrophic culture. This was supported by the addition of the photosynthesis inhibitor DCMU (1-(3,4-dichlorophenyl)-3,3-dimethylurea) to the growth media which resulted in a decrease of the mixotrophic growth rate of a *C. vulgaris* culture back to the heterotrophic level. Furthermore, while in heterotrophy all the carbon consumed for energy production is lost as CO_2, in mixotrophic cultures some of this carbon could be rechanneled across the mitochondrion and chloroplast membranes to form biomass via Calvin-Benson-Bassham (CBB) cycle using light energy. Consequently external CO_2 supply would be less critical for growth. In the same perspective, O_2 from photosynthesis could be consumed in the catabolism of organic substrates [3,6,14,20,25].

Understanding nutrient utilization by microalgae cultures is crucial to develop and manage the high cellular density culture systems necessary for the economic feasibility of industrial biomass production [30]. The mass balance of key nutrients is thus important to optimize microalgae cultivation as it enables the assessment of individual and global substrate yields. Nevertheless, laboratory systems often do not reproduce large-scale industrial conditions. Here, for the first time we analyzed biomass growth and nutrient consumption of an industrially relevant, large-scale mixotrophic *Chlorella vulgaris* culture, during the course of scale-up from a 10 m^3 to a 100 m^3 closed tubular PBR. Carbon and nitrogen balances were calculated, which allowed to gain insight into the interplay between the carbon and nitrogen metabolisms as well as the mixotrophic metabolism. This knowledge is directly applicable to the optimization of the industrial production of *Chlorella vulgaris* in closed photobioreactors.

2. Materials and methods

2.1. Microalgae strain and culture conditions

A 1 m^3 photoautotrophic culture of an industrial strain *Chlorella vulgaris* grown in a flat-panel PBR (C source: CO_2; N source: NO_3^-) was used to inoculate a 10 m^3 tubular PBR (Fig. 1). From this stage on the culture was grown mixotrophically in fed-batch with acetate and urea as the carbon and nitrogen sources, respectively. The remaining inorganic macro and micronutrients required were added as a concentrated basal medium solution when necessary. The basal medium is composed by the macronutrients to grow microalgae (nitrate, phosphate, sulfur, magnesium, potassium and calcium) and also trace elements, adapted to the local water composition. This adaptation was made to obtain a culture media with a composition similar to Guillard's f solution. When sufficiently concentrated, the culture was scaled-up to a 35 m^3 PBR in the same culture conditions and later to a 100 m^3 PBR where it was periodically harvested in a semi-continuous mode maintaining a concentration of approximately 1 $g\ L^{-1}$. All the reactors follow the structure presented on Fig. 1. The experiments took place between April 7th and June 18th of 2014 in a temperate climate zone (Portugal). All photobioreactors were located outdoors implying a natural circadian light:dark cycle with an average of 13 h of light per day and daily temperature fluctuations where the culture was always kept under 30 °C. During operation, cultures were kept in homogeneous suspension throughout the reactor and the culture flow comprised a tank where feeding media or other supplements could be added as well as an illuminated zone where cells circulated through transparent tubes (Fig. 1). Acetate and urea were fed together in a concentrated solution with a composition of 7.5C:1N during the photoperiod and according to biomass growth rate. Further nitrogen was given as needed in the basal medium. The stepwise addition of acetate was coupled to the incident radiation throughout the day in order to ensure that excess organic carbon substrate did not accumulate in the culture medium.

2.2. Quantification of operational parameters

Biomass growth was monitored online by a Hach Lange Solitax SC turbidity sensor. Dry weight ($g\ L^{-1}$) values were obtained by applying a turbidity (NTU) correlation:

$$Turb^{1/3} = (4.89 \pm 0.20)DW + (3.5 \pm 0.20); n = 485, P = 95\% \quad (1)$$

Specific growth rate was calculated according to:

$$\mu = \frac{\frac{DW_f - DW_i}{DW_i}}{\Delta t} \quad (2)$$

Urea concentration was determined by spectrophotometry according to [33]. Ammonium concentration was determined using an Ammonium-Ammonia test (Sera, Heinsberg, Germany) by comparison to a calibration curve and acetate concentration using the K-ACETRM 07/12 kit (Megazyme, Co. Wicklow, Ireland) method. Biomass elemental composition, $C_{5.84}N_{1.00}O_{2.85}H_{10.39}S_{0.001}$, was estimated as described by [12] by extrapolation of the biochemical analysis performed externally on the produced dried biomass obtained for the later stage of scale-up – the 100 m^3 PBR. Dietary fiber was determined by the PAFQ.230.1 method that combines a selective gravimetric technique after the digestion of the sample. Total protein was obtained by the PAFQ.360.1 method according to the Dumas method for nitrogen quantification. The fat content was determined by acidic hydrolysis as described by the PAFQ 069.0 method. Finally the carbohydrate content is obtained by subtracting the percentage of the remaining compounds from 100% [18].