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Continuous cultivation of *Chlorella minutissima 26a* in a tube-cylinder internal-loop airlift photobioreactor to support 3G biorefineries

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ABSTRACT

Microalgae *Chlorella minutissima 26a* was cultivated in a tube-cylinder internal-loop airlift photobioreactor under continuous cultivation conditions. The goal was to investigate the influence of different nitrate levels on the growth and composition of microalgae. Three nitrate concentrations (75, 150 and 225 mg L^{-1}) were assessed under a fixed flow rate and the outlet flow was analyzed for concentration of biomass, lipid, carbohydrate and protein. Nitrate concentration at higher level (225 mg L^{-1}) in the medium promoted biomass growth (188.6 mg L^{-1} d^{-1}) and lipid production (92.8 mg L^{-1} d^{-1}), and decreased carbohydrate amount (29.1 mg L^{-1} d^{-1}) without any change in protein content (37.7 mg L^{-1} d^{-1}). Use of tube-cylinder internal-loop airlift photobioreactor in continuous mode could be a promising approach in algal biorefineries so called 3G biorefineries, resulting in high biomass productivity in a simple cultivation system.

1. Introduction

Emission of carbon dioxide and other gases into the atmosphere by excessive burning of fossil fuels has considerably impacted on the greenhouse effect, thus promoting global warming and undesirable climate changes [1]. Strategic actions to reduce the greenhouse gas emissions and their harmful effect on the environment are urgent and mandatory. Gas emissions caused by burning of renewable fuels can be sequestered through photosynthesis during the biomass growth [2,3]. In this context, microalgae have been considered as one of the most promising alternative sources for biofuel production because of their fast growth rate and high biomass productivity thriving on cheap and renewable carbon sources. Microalgae biomass can be employed as a raw material for fuels production as it comprised of lipids and carbohydrates which are considered as promising feedstock for biodiesel, bioethanol and biochemicals production. Cultivation of microalgae require small physical spaces as compared to the terrestrial crops, and significantly contribute to the greenhouse gases reduction by CO_2 capture [4–6]. In addition, microalgae can also be harnessed for the production of large number of bioactive compounds for different applications in food supplements, cosmetics, pharmaceuticals, among others [7]. Thus, a flexible multiproduct industry can be envisioned through integrated installations of 3G biorefinery by harnessing the potential of microalgae [8–10].

However, there are several obstacles that must be overcome in order to take maximum advantage from microalgae in large-scale processes such as production cost reduction, biomass yield and productivity maximization, increase in the productivity of lipids, carbohydrates and other interesting compounds [11]. In order to increase biomass production and to modify its composition, appropriate strategies for example - evaluation of the effect of nitrogen source concentration in the medium [12,13], promotion of intense interaction of microalgae with the cultivation medium, supply of optimized light and carbon source during cultivation, and the choice of an alternative photobioreactor and operation mode are necessary to adopt [14,15].

For the appropriate yields from bench-scale microalgal cultivation, more studies are requires that allow to measure, evaluate and control environmental and nutritional factors affecting growth and composition of biomass production [16]. These benchmark studies

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will pave the outcome for acquisition of necessary information for large-scale microalgae propagation. In such a case, photobioreactors enable cellular photosynthetic metabolism and offer several unique advantages, such as low possibility of contamination, better control of gas-liquid mass transfer rate, higher biomass density, greater exposure to light, high productivity per area and low cost of biomass harvesting. However, the main disadvantage of photobioreactors is the low penetration of light due to the increased cell density in the medium during the cultivation time [15]. The later needs careful evaluation of reactor designing, internal geometry of reactor, appropriate air flow rate and other operating conditions, enabling ideal amount of oxygen transfer and removal, mixing, recirculation time and light exposure frequency for adequate microalgae growth [15,17–19]. In continuous cultivation of microalgae, high biomass productivity can be achieved by reducing non-productive time, employing a uniform and optimized culture medium which facilitates the design of downstream steps [20].

Despite their advantages, airlift photobioreactors have been scarcely reported in literature for microalgae cultivation under continuous mode [16,21,22], with no previous report on *Chlorella minutissima* cultivation in a tube-cylinder internal-loop airlift photobioreactor. *Chlorella* sp. have been reported as a promising candidate, as they have a fast and easy growth [23,24], with lipid content ranging from 10.0 to 48.0% (wt) [25,26], carbohydrate content from 12.0 to 17.0% (wt) and protein content from 40.0 to 60.0% (wt) [27,28] depending on cultivation conditions.

This study reports the effect of different nitrogen concentration on growth and composition of *C. minutissima 26a* in an autotrophic culture by using a tube-cylinder internal-loop airlift photobioreactor. The cultivation was operated in a continuous mode, and biomass production and chemical composition was assessed in terms of lipids, carbohydrates, protein and ash content.

2. Methods

2.1. Microalgae strain, storage and seed culture

The microalgae *C. minutissima 26a* was provided by the Seaweed Culture Collection of the Oceanographic Institute at the University of São Paulo (São Paulo, SP, Brazil). Microalgae were stored and kept in artificial seawater f/2 medium [29], composed of: 33.3 g L^{-1} NaCl, 75.0 mg L^{-1} $NaNO_3$, 5.0 mg L^{-1} $NaH_2PO_4 \cdot H_2O$, 3.15 mg L^{-1} $FeCl_3 \cdot 6H_2O$, 22.2 μg L^{-1} $ZnSO_4 \cdot 7H_2O$, 180 μg L^{-1} $MnCl_2$, 6.3 μg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 10 μg L^{-1} $CoCl_2 \cdot 6H_2O$, 9.8 μg L^{-1} $CuSO_2 \cdot 5H_2O$, 100 μg L^{-1} Thiamine (B_1), 0.5 μg L^{-1} Cyanocobalamin (B_{12}) and 0.5 μg L^{-1} Biotin (B_7), in 250 mL Erlenmeyer flasks kept at 20 °C under illuminating conditions (photon flux of 80–90 μmol m^{-2} s^{-1}), and sub-cultured every six weeks. Seed cultures were prepared in the same f/2 medium (900 mL) with the addition of a stock culture (100 mL). Thus, autotrophic cultivation was carried out during 10 days by using a batch tube photobioreactor which was consisted of a transparent plastic bottle cylinder of 12.0 mm of diameter and 28.0 mm of height, and aerated at 0.24 vvm. Temperature was kept at 30 ± 2 °C with continuous light supply at photon flux of 130 ± 2 μmol m^{-2} s^{-1}.

2.2. Tube-cylinder internal-loop airlift photobioreactor

The tube-cylinder airlift photobioreactor used in this study was having following dimensions: 550 mm in height and 120 mm in diameter with the concentric tube (one tube is placed inside another) of 340 mm by 60 mm, with a working volume of 3.8 L. The bioreactor lay out is shown in Fig. 1. The tubes were made of transparent acrylic polymer for the visual observation of effective light penetration. The cylindrical tube was located concentrically towards the outer column with 30 mm space between them, aiming to promote an upstream flow towards the central tube and downstream flow towards the outer tube. Compressed air was pumped through a sterile filter and supplied to the reactor via porous stones placed centrally at the base of the draft tube at the flow rate of 0.24 vvm. For all experiments, temperature was kept at 30 ± 2 °C and continuous light supply was provided via fluorescent lamps with photon flux density of 125–130 μmol m^{-2} s^{-1}. A peristaltic pump was used to feed the photobioreactor during the continuous mode operation.

2.3. Batch cultivation in airlift photobioreactor

Batch experiments were carried out to determine the maximum specific growth rate (μ_{max}), with the estimated feed flow rate during the continuous cultivation. Culture medium was consisted of synthetic seawater f/2 with 150 mg L^{-1} $NaNO_3$, and the seed culture (as described in section 2.1) was added at a volumetric fraction of 10%. Samples were taken periodically taken for dry cell weight (DCW) measurements. Specific growth rate was estimated by the slope of regression in the exponential phase (linear region) obtained from the curve of natural logarithm of dry cell weight as a function of time. The hydraulic residence time (HRT) was calculated as a function of the dilution ratio ("D") the by following equation:

$HRT = 1/D$ where $D = 0.8 \times \mu_{max}$.

2.4. Continuous cultivation in the airlift photobioreactor

In all experiments, seed culture was prepared as described in section 2.1, was added into the medium at a volumetric fraction of 10%. For continuous cultivation, firstly, the airlift photobioreactor was operated in batch mode until the microalgae growth reached to the exponential phase. Thereafter, the airlift photobioreactor was fed with the culture medium at a flow rate of 0.4 mL min^{-1}, corresponding to a hydraulic residence time (HRT) of 155 h. The harvesting was performed daily (approximately after 24 h), and the culture solution was centrifuged at 1800 × g and dried in an oven at 60 °C for 24 h.

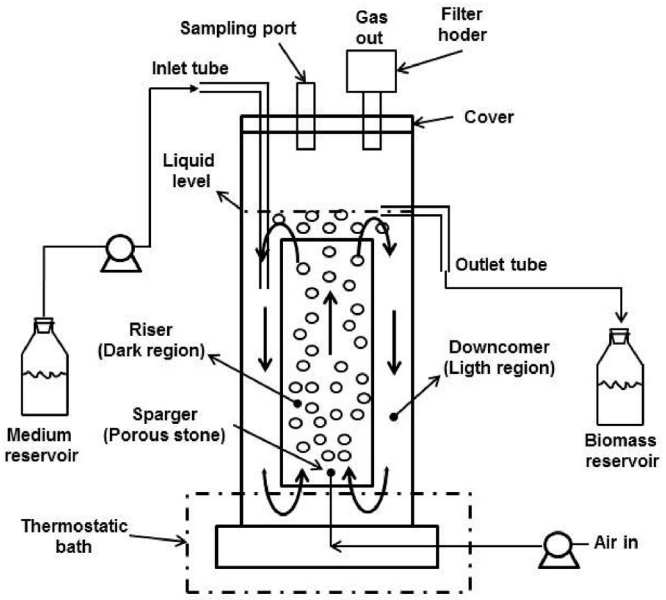

Fig. 1. Schematic diagram of continuous cultivation system used for the cultivation of microalga *C. minutissima*.