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Potential of algal biofuel production in a hybrid photobioreactor

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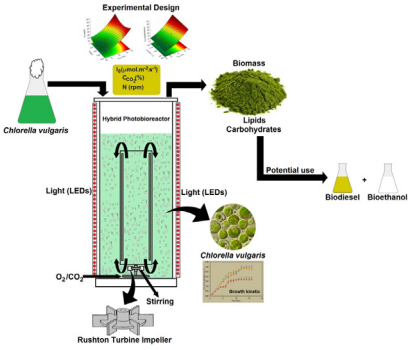
HIGHLIGHTS

- Hybrid photobioreactor was used to optimize biofuel production.
- Increasing CO_2 concentration and light intensity increased cell concentration.
- At a rotational speed of 800 rpm growth was negatively affected.
- Higher percentages of lipids and carbohydrates were obtained with low CO_2 concentrations.
- Rotational speed above 800 rpm favors lipids and carbohydrates production.

GRAPHICAL ABSTRACT



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ABSTRACT

Hybrid bioreactors are equipment featuring characteristics of pneumatic bioreactors and stirred tanks. The production of lipids and carbohydrates was evaluated in a concentric draft tube stirred airlift photobioreactor. Mixotrophic cultivation was carried out using the microalgae *Chlorella vulgaris* grown in vegetable waste. The response surface methodology was used for the experiment optimization. A central composite rotatable design was used to evaluate the effects of light intensity, CO_2 concentration and stirring (independent variables) on the lipid and carbohydrate production. The exponential phase of growth occurred mainly in the first seven days of cultivation, and the stationary phase was achieved under all experimental conditions (occurring between the 9th and 14th day). The increase in cellular concentration was obtained mainly in experiments performed with high light intensity and CO_2 concentration. At a rotational speed of 800 rpm, growth was affected due to excessive shearing forces caused by increased turbulence. On the other hand, in the absence or at low CO_2 concentrations there was a considerable increase in lipid and carbohydrate production. Through response surfaces, we observed that higher percentages of these metabolites may be obtained without adding CO_2 and operating the bioreactor with high light intensity and with rotation speed higher than 800 rpm or at low rotational speeds as well as conventional airlift. From an economic point of view, stirring can cause an increase in power consumption; however, if the bioreactor is operated at low rotational speeds, the difference in power consumption with airlift bioreactors is minimal, while the productivity of lipids and carbohydrates increases, which makes the use of this equipment very attractive.

1. Introduction

Increased global fuel demand, global warming, and the possibility of depletion of fossil fuels have put biofuels – among them biodiesel and bioethanol – in a prominent position as alter-

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native fuels. Studies conducted around the world pointed microalgae as an alternative potential source for biofuel production.

One of the challenges to be faced is the correct choice of equipment, operating variables, in addition to choice of a culture medium that is economically viable and has high cellular yield. Production of biofuel from microalgae biomass has in general higher costs and greater technical challenges than growing vegetable cultures (Alam et al., 2012).

Currently, 1.3 billion tons of foods are wasted, causing serious environmental damages. Besides the environmental impact caused by food waste, its economic cost may reach 750 billion dollars annually (Mohan et al., 2010; de Jesus et al., 2017a). These residues are suitable for growth of microalgae that produce lipids, carbohydrates, and other high value-added products such as carotenoids and proteins (de Jesus et al., 2017a). On the other hand, there is also the challenge of improving equipment and operating conditions, pondering advantages and disadvantages of each production system and process variables, *e.g.*, incidence of light, CO_2 concentration, stirring, pH, among others.

Open-culture systems are generally simple, with low operation and energy costs, and do not require much maintenance. However, they are more susceptive to weather conditions, not allowing control of water temperature, evaporation, lighting and low-efficiency use of CO_2 leads to low productivity and low biomass concentrations. Moreover, as they are exposed to the atmosphere, there is high possibility of culture contamination by other microalgae, bacteria, and even fungi depending on the medium used (Xu et al., 2009). In closed-culture systems cultivation of microalgae is carried out in photobioreactors (PBR), in which it is possible to control the process conditions (amount of nutrients, temperature, light intensity, pH, etc.). This leads to high productivity, enabling commercial production of a series of high value-added compounds (Tan et al., 2015).

Large-scale microalgae cultivation should be conducted so as to provide the best possible conditions for cell growth. PBRs design and configuration should be conceived to provide uniform light flux absorbed by microalgae as homogeneously as possible (Chen et al., 2013). The main factors to consider in new PBRs projects besides light supply would be tank depth and agitation. Depth has considerable effect on light penetration and availability, while agitation is vital to improve light distribution and medium homogeneity, thus avoiding cell sedimentation (Chen et al., 2013).

The difficulty in providing homogeneous light intensity and sufficient for microalgae led to new researches focusing on bioreactor design and optimization, mainly the airlift type, having as their main objective the improvement of mixture, light penetration, and gas injection (Xu et al., 2012). The use of hybrid bioreactors – *e.g.*, stirred airlift photobioreactors – may be a promising alternative. Studies have proved that stirring promotes mass transfer, gas holdup, and, consequently, mixture, which may increase medium homogenization and, as a result, higher incidence of light upon cell culture (de Jesus et al., 2015).

This study aimed at optimizing lipid and carbohydrate production in a stirred airlift photobioreactor (hybrid photobioreactor) using response surface methodology, in which three independent variables were used: light intensity, CO_2 concentration, and rotational speed of the impeller. We used mixotrophic cultures of *Chlorella vulgaris* grown in vegetable waste medium with constant pH.

2. Materials and methods

2.1. Materials

2.1.1. Microalgae

Chlorella vulgaris (CPCC 90) purchased from Canadian Phycological Culture Centre (Waterloo, Canada). Stock cultures of *C. vulgaris* were maintained routinely on agar slants of BG-11 medium (Imamoglu et al., 2007).

2.1.2. Culture medium

Culture medium was prepared using vegetables wasted by the Central Food Supply of Campinas (Campinas, Brazil).

Vegetable waste was washed and crushed with tap water. The material obtained was filtered, centrifuged, and subsequently diluted and sterilized in autoclave at 121 °C and 1 atm for 20 min.

Carbon concentration was determined by chemical oxygen demand (COD) and nitrogen concentration by Kjeldahl method (Kirk, 1950).

2.1.3. Photobioreactor

A concentric draft tube stirred airlift photobioreactor with working volume up to 4.0 L, made of borosilicate glass, described in de Jesus et al. (2015, 2017b) (Fig. 1) was used for the experiments. CO_2-enriched air was sparged into the internal zone through a circular perforated plate with 0.05 m diameter, with 90 equidistant holes with 0.001 m diameter, located on the bottom of the bioreactor, concentric in relation to the area comprised by the riser. Flow rates of CO_2-enriched air were determined using rotameters (RMA-13-SSV and RMA-11-SSV model, Dwyer, USA). Flow rates ranged from 0 to 0.24 $L.min^{-1}$.

To avoid vortex formation, four baffles were added inside the draft tube, in the region comprised by the riser. A pH probe (405-DPAS-SC-K8S/225 Mettler Toledo, Switzerland), a CO_2 electrode (CO_2-sensor InPro5000/12/220 Mettler Toledo, Switzerland), and a O_2 electrode (O2-sensor InPro6800/12/220 Mettler Toledo, Switzerland) were positioned in the center of the vessel, probes clearance was 0.15 m. Stirring was provided with a Rushton turbine with 6 blades, 0.04 m in diameter. Impeller clearance was 0.01 m. The effect of stirring rate, expressed as rotational speed of the impeller, ranged from 0 to 800 rpm. Light intensity was provided by a system of red LED (C503B-RAN-CY0B0AA1-ND, Cree, USA) with 624 nm wavelength and blue LED (365-1173-ND, Cree, USA) (approximately 2%) with 470 nm wavelength, with maximal light intensity up to 1400 ± 36 $\mu mol.m^{-2}.s^{-1}$.

Light intensity used in the experiments was measured using a photometer (LI-250A model and quantum sensor Li-192S, Li-Cor Biosciences, USA).

The photobioreactor was sterilized with a hydrogen peroxide solution 6% for 20 min. All tests were carried out at ambient pressure, constant temperature of 20 ± 2 °C, pH was kept constant at 6.00 ± 0.25, and adjusted with 0.1 N HCl or 0.1 N NaOH. Medium volume was 3.0 L.

2.2. Methods

2.2.1. Experimental design

Response surface methodology (RSM) was used for optimization (Montgomery, 2009). A five-level central composite rotatable design (CCRD) was used to evaluate the relation between light intensity (I_0), CO_2 concentration (C_{CO2}), and rotational speed of impeller (N) (independent variables) and lipid and carbohydrate production (dependent variable).

The number of experiments was given by the sum of a complete 2^n design, where n is the number of independent variables, and a fractional part given by: $\alpha = (2^n)^{1/4}$. In this study, we used three independent variables, which generated a complete 2^3 factorial design (eight experiments), plus two axial points $\alpha = \pm 1.68$ (six experiments), and three replicates at the central point were used to estimate experimental error, resulting in a total of 17 experiments, as shown in Table 1 (Montgomery, 2009).

Data from quality studies were subjected to analysis of variance (ANOVA) and the significance or nonsignificance of the variables