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## Lipid and carbohydrate profile of a microalga isolated from wastewater

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### Abstract

Native species of *Asterarcys quadricellulare* was isolated from wastewater from the International Centre for Reference and Water Reuse at the São Paulo University campus. Microalga was cultivated at  $24.2 \pm 1.2$  °C under mixotrophic conditions in Bold Basal medium supplemented with 0.1 g/L glucose, under 12 h:12 h light:dark photoperiod of artificial light, in 2 L photobioreactors. *A. quadricellulare* achieved 0.463-0.567 g/L (dw) concentration, with 20.0 % lipid and 36.6 % carbohydrate. Yields of 0.0198 g oil and 0.0362 g sugar /L<sup>-1</sup>.day<sup>-1</sup> show this microalga potential for biofuels production and wastewater treatment.

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**Keywords:** *Asterarcys quadricellulare*; carbohydrate composition; lipid profile and productivity; wastewater treatment

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### 1. Introduction

Microalgae are primitive plant organisms with no roots, stems or leaves, that can be found in all terrestrial ecosystems, usually in water (fresh, brackish and marine water), as well as in other media such as the wet surface of rocks, hot springs, air, snow or even in the desert soil [1]. There is a wide variety of microalgae species that live

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in a wide range of environmental conditions and have Chlorophyll as the main photosynthetic group of pigments [2]. In natural conditions, microalgae are preferably autotrophic and use carbon dioxide, soluble carbonate ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) and nutrients such as nitrogen, phosphorus and potassium, from the water environment [3]. Microalgae can be cultivated under autotrophic, mixotrophic or heterotrophic conditions. Under the autotrophic mode, photosynthesis occurs using inorganic carbon whereas under heterotrophic mode organic carbon sources, such as glucose and glycerol, are used in the absence of light. The mixotrophic mode is a combination of the two previous ones [4,5]. Light is essential for microalgae growth under autotrophic and mixotrophic modes of culture. Excess light can cause photo-oxidative stress and death of cells due to formation of hydrogen peroxide (a toxic substance for microalgae) in the presence of oxygen. Culture agitation in liquid media, keeps the cells in suspension and prevents their settling at the bottom of the photobioreactor (PBR), favouring light availability to all suspended cells and promoting the elimination of excess oxygen dissolved in the medium, which decreases the risk of cell death by photo-oxidation [6]. Currently, the microalgal biomass applications include human and animal nutrition and the production of high commercial value molecules for the pharmaceutical and cosmetic industry [7]. Certain species are today cultivated on a large scale, such as *Dunaliella sp.*, *Arthrospira sp.* and *Chlorella sp.*, and the resulting products are usually sold as nutritional supplements [8,9]. The biochemical structure of microalgae depends on, not only the nature of each species, but also on factors such as the lighting conditions (light intensity and photoperiod), temperature, pH, nutrients and medium agitation [10]. Microalgal biomass is frequently rich in fatty acids, of which polyunsaturated fatty acids, carbohydrates, proteins, antioxidants, minerals, such as sodium, potassium, calcium, magnesium, iron and zinc, and vitamins such as riboflavin, thiamine, carotene and folic acid, among others are of high value [11-13].

Production of microalgae as the source of lipids and carbohydrates is nowadays non-economical, if the only purpose is biofuel production [14]. However, their processing in a microalgal biorefinery has the potential to compete with the petrochemical industry, where production is targeted mainly for fuels for the transportation and energy sectors. Biorefineries can be sustainable, by maximizing microalgae conversion into a variety of higher value products targeted to the fine chemicals industry, biomaterials and energy, and minimizing waste production [15-18]. Besides the products that can be extracted from microalgae biomass, microalgae cultivation has the potential of high carbon sequestration and oxygen production and has an active role in bioremediation. Microalgae cultivation can be used both for the fixation of carbon dioxide from industrial gases [19] and for the removal of contaminants from wastewaters, such as ammonia, nitrate and phosphate [20]. Therefore, it is of the utmost importance to characterize microalgae that grow in wastewater medium, for their potential as raw material for biofuels as well as for other high value products.

In our work, a native species of microalga was isolated from a campus wastewater and identified as *Asterarcys quadricellulare*. The *A. quadricellulare* was cultivated under mixotrophic conditions and the biomass contents in lipids and carbohydrates was evaluated, aiming to estimate its potential for biofuel production, upon wastewater treatment.

## 2. Materials and methods

### 2.1. Microalgae cultivation

Stock culture of microalgae inoculum was grown in modified Bold Basal Medium (BBM), in an incubation chamber (Eletrolab, model EL 202) with light intensity of  $132.80 \mu\text{E}/\text{m}^2 \cdot \text{s}^{-1}$  in 12:12 h Light:Dark photoperiod, at  $21.0 \pm 0.5 \text{ }^\circ\text{C}$ . The modified BBM was prepared with (g/L) 0.750  $\text{NaNO}_3$ , 0.075  $\text{K}_2\text{HPO}_4$ , 0.175  $\text{KH}_2\text{PO}_4$ , 0.075  $\text{NaHCO}_3$ , 0.013  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.050  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.050 EDTA- $\text{Na}_2$ , 0.004  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.00400  $\text{H}_3\text{BO}_3$ , 0.00150  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.00024  $\text{Cl}_2\text{Mn} \cdot 4\text{H}_2\text{O}$ , 0.00024  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.00006  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 0.00020  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ . All reactants were of analytical grade.

Cultures of microalgae in modified BBM under mixotrophic conditions, 0.1 g glucose/L were prepared in triplicate, at room temperature ( $25 \pm 2 \text{ }^\circ\text{C}$ ). Lighting conditions were 12:12h L:D photoperiod, with daylight lamps, with a luminance of 17000 to 19000 lux, or light intensity of  $244 \mu\text{E}/\text{m}^2 \cdot \text{s}$ . Photobioreactors had useful growth volume of 2 L and filtered air ( $0.45 \mu\text{m}$  microfilters) was provided at  $6.25 \text{ mLair}/\text{s}^{-1} \cdot \text{L}^{-1}$  or 0.375 v.v.m. (volume of air per volume of culture per minute) (Fig. 1-a).

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