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Microalgae cultivation in sugarcane vinasse: Selection, growth and biochemical characterization

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HIGHLIGHTS

- A biorefinery strategy for microalgae cultivation using sugarcane vinasse is proposed.
- Screening of 40 microalgae allowed the selection of highly productive strains.
- The feedstock obtained can be used to provide proteins, carbohydrates and energy.
- The culture supernatant can be recycled for sugarcane crops fertilization.

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ABSTRACT

Sugarcane ethanol is produced at large scale generating wastes that could be used for microalgae biomass production in a biorefinery strategy. In this study, forty microalgae strains were screened for growth in sugarcane vinasse at different concentrations. Two microalgae strains, *Micractinium* sp. Embrapa|LBA32 and *C. biconvexa* Embrapa|LBA40, presented vigorous growth in a light-dependent manner even in undiluted vinasse under non-axenic conditions. Microalgae strains presented higher biomass productivity in vinasse-based media compared to standard Bold's Basal Medium in cultures performed using 15 L airlift flat plate photobioreactors. Chemical composition analyses showed that proteins and carbohydrates comprise the major fractions of algal biomass. Glucose was the main monosaccharide detected, ranging from 46% to 76% of the total carbohydrates content according to the strain and culture media used. This research highlights the potential of using residues derived from ethanol plants to cultivate microalgae for the production of energy and bioproducts.

1. Introduction

Microalgae-derived biomass is recognized as an alternative source for an wide variety of bioproducts, such as biofuels, especial oils, pigments and polymers (Perez-Garcia et al., 2011). These photosynthetic microrganisms present higher growth rates and lower land area requirements compared to terrestrial crops commonly used for biofuels production. However, the production of microalgal biomass is still not economically viable due to high costs of cultivation, harvesting and processing (Quinn and Davis, 2015).

Significant cost reductions can be achieved if CO_2, nutrients and water for microalgae cultivation are obtained at low cost (Brasil et al., 2016). Accordingly, the use of several types of waste streams derived from industrial processes, as well as rural/domestic wastewater, have been proposed for microalgae cultivation as a strategy for cost-reduction in microalgae cultivation (Kang et al., 2015).

A potential wastewater for microalgae cultivation is sugarcane vinasse. It is an acid, dark brown liquid, rich in organic compounds (e.g. glycerol, lactic acid, sugars), nitrogen, phosphorus and ions (e.g. K^+, Ca^{2+}, Mg^{2+}) (Ortegón et al., 2016; Parnaudeau et al., 2008). It comprises the main by-product of sugarcane ethanol plants, being generated at 12–14 L per liter of ethanol produced. Although this wastewater is commonly applied in the fertirrigation

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of sugarcane crops (Dias et al., 2015), its continuous application leads to changes in soil composition and reduces crop productivity (Christofoletti et al., 2013). The use of vinasse for microalgal biomass production has been proposed previously, however these studies showed that the use of sugarcane vinasse, even at low concentrations, can inhibit the growth of microalgae strains (dos Santos et al., 2016; Ramirez et al., 2014).

It is well known that not all strains are able to grow in adverse conditions such as that found in waste streams (Barrocal et al., 2010; Brasil et al., 2016). Therefore, the objectives of this study were to select highly productive microalgae strains capable of growing in sugarcane vinasse and to characterize algal growth requirements and nutrient uptake during cultivation. Additionally, the composition of algal biomass produced in 15 L capacity flat-plate photobioreactors supplemented with CO_2 was analyzed in order to evaluate the strains potential for the production of energy and bioproducts.

2. Materials and methods

2.1. Microalgae strains and inoculum preparation

Axenic microalgae cultures of Embrapa|LBA1 to Embrapa|LBA40 strains (S1 Table) derived from the Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries at Embrapa (Brasília/DF – Brazil) were used. Strains were kept in liquid cultures of Bold's Basal Medium – BBM medium (Nichols and Bold, 1965) containing ampicillin (100 μg/mL), chloramphenicol (25 μg/mL) and amphotericin B (2,5 μg/ml), at 26 °C ± 1 °C, light intensity of 50 $\mu Em^{-2}\,s^{-1}$ and 12/12 h light/dark regimen.

For inoculum preparation, microalgae strains were axenic cultured in BBM using Erlenmeyer flasks under 12/12 h light/dark regimen (light intensity of 100 $\mu\,Em^{-2}\,s^{-1}$), at 26 °C ± 1 °C and aeration with 5 $L{\cdot}h^{-1}$ of atmospheric air. During log phase of growth, these starter cultures were used to inoculate experimental units (i.e.; Erlenmeyer flasks or flat-plate photobioreactors) at an initial absorbance of 0.01 at 680 nm. Inoculum volumes ⩽5% of the working load were used.

2.2. Sugarcane vinasse and medium preparation

Crude sugarcane vinasse was obtained from Jalles Machado (Goianésia/Brazil) ethanol plant. Crude vinasse samples, referred hereafter only as "crude vinasse", were centrifuged at 4800 RCF during 10 min to remove suspended solids and debris. Diluted vinasse formulations, referred hereafter only as "diluted vinasse", were prepared by addition of distilled water to crude vinasse at the proportions indicated (i.e.: 25%, 50% or 75%). Clarified vinasse formulation, referred hereafter only as "clarified vinasse", was prepared as follows: Hydrated lime ($Ca(OH)_2$) was added to crude vinasse (3 $g{\cdot}L^{-1}$). The solution was maintained at rest for 40 min, then centrifuged at 4800 RCF during 10 min and the supernatant was collected. All vinasse-based media formulations were sterilized by autoclaving at 121 °C for 15 min and stored at 4 °C until use. Transmittance of vinasse-based media formulations was measured by spectrophotometry using Spectramax M3 plate analyzer.

2.3. Selection of microalgae for growth in vinasse

Embrapa|LBA1 to Embrapa|LBA40 microalgae strains (N = 40) were screened for growth in sugarcane vinasse. Starter cultures of each strain were inoculated in 250 mL of either diluted vinasse formulations at 25%, 50% and 75% concentration in distilled water or in 100% crude vinasse. Culturing was performed in sterile 500 mL Erlenmeyer flasks aerated with 5 $L{\cdot}h^{-1}$ of atmospheric air, at 26 °C ± 1 °C, light intensity of 100 $\mu Em^{-2}\,s^{-1}$ in a 16/8 h light/dark regimen. Microalgae growth was daily monitored through microscopic inspection during 30 days of culturing.

2.4. Microalgae cultivation

Selected microalgae strains were submitted to cultivation in 250 mL of crude vinasse or BBM (control) under different conditions: I) Axenic culturing using crude vinasse at 12/12 h light/dark regimen; II) Axenic culturing using crude vinasse without light (dark condition); III) Non-axenic culturing using crude vinasse (pH adjusted to 8.0) at 12/12 h light/dark regimen; IV) Axenic culturing using BBM at 12/12 h light/dark regimen. Culturing was performed in 500 mL Erlenmeyer flasks aerated with 5 $L{\cdot}h^{-1}$ of atmospheric air, at 26 °C ± 1 °C, light intensity of 100 $\mu Em^{-2}\,s^{-1}$ in a 12/12 h light/dark regimen.

2.5. Biomass dry weight determination

For biomass dry weight determination, 10 mL samples of the algal culture were collected, centrifuged during 10 min at 10700 RCF and the supernatant discarded. The pellet was washed through three cycles of resuspension in distilled water followed by 10 min centrifugation at 10700 RCF. The washed pellet was dried overnight using a dry oven at 105 °C and weighted.

2.6. Determination of organic compounds concentration in vinasse-based media

The concentration of total reducing sugars (glucose + fructose + sucrose), glycerol, lactic acid and acetic acid in vinasse-based media at the beginning (day 0) and the end (day 8) of microalgae cultivation (Section 2.4) was determined. One milliliter (1 mL) culture samples were collected, centrifuged for 10 min. at 10,700 RCF and the supernatant was analyzed through High Performance Liquid Cromatography analysis (Agilent 1260 Infinity Binary LC System) using Biorad Aminex HPX-87H column (H2SO4 0.005 M, 0.6 mL/min, 45 °C).

2.7. Microalgae cultivation in airlift flat-plate photobioreactors

Selected microalgae strains were cultivated in either diluted vinasse (50%), clarified vinasse or BBM (control) under non-axenic conditions using airlift flat-plate photobioreactors at 13 L of working load (Supplementary Fig. 2). Culturing was conducted for 3 days at 12 h/12 h light/dark regimen (light intensity of 400 $\mu Em^{-2}\,s^{-1}$) and a temperature of 37 °C ± 1 °C during light period and 24 ± 1 °C during dark period. Aeration with 64 $L{\cdot}h^{-1}$ of atmospheric air supplemented with 5% CO_2 was provided.

2.8. Analysis of biomass biochemical composition

After cultivation, algal biomass was harvested by centrifugation at 4800 RCF during 10 min. The biomass was washed three times with distilled water followed by 10 min centrifugation (4800 RCF) and freeze-dried prior biochemical analysis. The following analysis were performed: Total ash content (Van Wychen and Laurens, 2013c); total protein by the Kjeldahl method (AOAC, 1990), using the nitrogen-protein conversion factor proposed for microalgae (Lourenço et al., 2004); total carbohydrates (Van Wychen and Laurens, 2013a); total carotenoids (Huang and Cheung, 2011); and fatty acid (Van Wychen and Laurens, 2013b). For the determination of the calorific value, the microalgal biomass was analyzed using the protocol described by ASTM (2013).