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Biochemical compositions and fatty acid profiles in four species of microalgae cultivated on household sewage and agro-industrial residues

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HIGHLIGHTS

- The growth of four regional microalgae was evaluated in five residual media.
- Variations in yield and biomass composition were analyzed in different ways.
- The MDS and HB media showed promising results for microalgae cultivation.
- High levels of esters were obtained from biomass grown in HB.

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ABSTRACT

The potential of four regional microalgae species was evaluated in relation to their cell growth and biomass production when cultured in the following alternative media: bio-composts of fruit/horticultural wastes (HB), sugarcane waste and vinasse (VB) chicken excrements (BCE), raw chicken manure (RCM), and municipal domestic sewage (MDS). The cultures were maintained under controlled conditions and their growth responses, productivities, biochemical compositions, and the ester profiles of their biomasses were compared to the results obtained in the synthetic media. The MDS and HB media demonstrated promising results for cultivation, especially of *Chlorella* sp., *Chlamydomonas* sp., and *Lagerheimia longiseta*, which demonstrated productivities superior to those seen when grown on the control media. The highest lipid levels were obtained with the HB medium. The data obtained demonstrated the viability of cultivating microalgae and producing biomass in alternative media prepared from MDS and HB effluents to produce biodiesel.

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1. Introduction

There has been an increasing concern in recent decades about future scarcity of fossil fuels and the necessity of developing renewable and sustainable energy sources for the energetic demands of modern society (Drira et al., 2016). Bioenergy, which includes bio-methane, bio-ethanol, and biodiesel produced by photosynthetic organisms, offers promising renewable, biodegradable, and less toxic sources of energy when compared to fossil fuels (Cho et al., 2011).

Microalgae are potential candidates for biofuel production as many species demonstrate high photosynthetic efficiencies and high production levels of biomass and triglycerides. The commercial production of microalgae, however, must still overcome critical problems related to economic viability and the high operational costs associated with their cultivation (Jebali et al., 2015).

The cultivation of microalgae requires large quantities of nutrients, which will affect the final value of the fuels produced (Levine et al., 2011). Microalgae can grow under a wide variety of conditions, however, and many species are capable of using wastewaters as growth substrates (Brennan and Owende, 2010; Hu et al., 2008). As such, effluents offer the potential to generate microalgae bio-

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mass in an economically viable manner and to produce biofuels and other valuable biotechnological products.

One of the largest problems facing modern society is the enormous production of both domestic residues and effluents derived from industrial processes. If simply discharged into the natural environment, these residues produce innumerous undesirable effects – and, therefore, require adequate treatments to minimize their impacts. The presence of toxic compounds such as heavy metals and pesticides in these effluents (and the eutrophication of bodies of water receiving heavy discharges of nitrogen and phosphorus) threaten the environment and the human health and well-being, and must be dealt with urgently (Cai et al., 2013).

The cultivation of microalgae offers the possibility of an extremely efficient manner of dealing with these effluents due to the capacities of these microorganisms to remove many toxic substances and capture nutrients from aqueous environments to produce useful biomasses (Kothari et. al., 2013). These qualities suggest that the cultivation of microalgae in media prepared from different types of residual waters could minimize the production costs of algal biomass – with the perspective of multiple ecological and biotechnological benefits, including energy production.

Numerous types of effluents have been tested for microalgae biomass production, including: dairy farm wastes (Hena et al., 2015), pig farm wastes (Zhu et al., 2013), agro - industrial sugarcane wastes and effluents (Ramirez et al., 2014), domestic sewage (Kligerman and Bouwer, 2015), industrial paper and cellulose production wastes (Gentili, 2014), commercial captive fish production effluents (Mandal and Mallick, 2011), beer production (Farooq et al., 2013), carpet manufacturing (Chinnasamy et al., 2010), and palm oil processing (Lam and Lee, 2011).

Although a great deal of research related to this theme has already been undertaken, there are still substantial limitations concerning the diversity of residue sources that could be used and the pretreatment processes that might be necessary (Gentili, 2014). Additionally, not all microalgae species grow adequately in media prepared from these residues; consequently, the selection of species tolerant to certain effluents will be essential to guarantee maximum biomass production (Jebali et al., 2015).

The present study, therefore, evaluated the growth of four regional species of microalgae (*Chlorella* sp., *Chlamydomonas* sp., *Lagerheimia longiseta,* and *Pediastrum tetras*) in five different types of alternative media (derived from municipal sewage and various agro-industrial residues) in order to determine their biomass productions and chemical compositions.

2. Materials and methods

2.1. Species selection and cultivation under controlled conditions

Four species of microalgae isolated from different freshwater environments in northeastern Brazil were examined in the present study: *Chlorella* sp. (Strain D101Z), isolated from the Sewage Treatment Plant (ETE) in Mangabeira, João Pessoa – PB; *Chlamydomonas* sp. (D132WC), isolated from the Purificação Waterfall in the Chapada Diamantina Mountains, Vale do Capão - BA; *Lagerheimia longiseta* (D133WC), isolated from the Malhada Limpa reservoir – RN; and *Pediastrum tetras* (D121WC), isolated from the Prainha reservoir, in the municipality of Frei Martinho – PB. These cultures are now maintained in synthetic culture media in the microalgae culture collection of the Laboratório de Ambientes Recifais e Biotecnologia de Microalgae at the Federal University of Paraíba (LARBIM/UFPB) and were cultivated in alternative media prepared from municipal domestic sewage (MDS), fruit/horticultural biocompost (HB), bio-compost derived from sugarcane and vinasse (VB), raw chicken manure (RCM), and bio-compost of chicken excrement (BCE).

Zarrouk medium (Zarrouk, 1966) was used as the control medium for *Chlorella* sp., and WC medium (Guillard and Lorenzen, 1972) was used for the other species. These four species were selected for their capacity to produce fatty acids at levels superior to those of soybeans (previous studies).

The algae were cultured in triplicate in flasks containing 5 L of culture medium with aeration, in a temperature-controlled germination chamber (25 ± 1 °C, photoperiod of 12 h, under 150 μmol. photons.cm^{-2}.s^{-1} illumination). The cell growth was analyzed by cell counts using a Fuchs Rosenthal chamber and a Leica binocular microscope.

The experiments were interrupted at the beginning of the stationary phase and the biomass produced was concentrated by centrifuging at 18 °C; the algal biomass was then frozen (−30 °C) and subsequently lyophilized to determine the yield into biomass (YB) (in grams per liter).

The following parameters related to algal growth were analyzed: cultivation time; growth rate (k), which represents the daily duplication rate of the cells as calculated by the formula described by Fogg and Thake (1987); and maximum population growth rate (Rmax), which corresponds to the maximum number of cells obtained at the end of each experiment subtracting the initial cell density of the innoculum.

Biomass productivity (BP) is the dry biomass produced (in grams per liter per day) in the exponential growth phase (Griffiths and Harrison, 2009). For BP determination, samples were collected at the end of the exponential phase and calculated according to the equation: $BP = YB \times k$.

2.2. Preparation of the culture media

The bio-compost extracts utilized as alternative culture media were prepared from the following agro-industrial and food residues: fruit/vegetable biocompost (HB), prepared from composting fruit and vegetable residues left over from central distribution markets and acquired from EMPASA (Empresa Paraibana de Abastecimento e Serviços Agrícolas) located in the municipality of João Pessoa – PB; biocompost prepared from solid and liquid residues of the industrial processing of sugarcane into sucrose and alcohol (VB), acquired from the Japungu factory, Santa Rita, PB, through the Sindicato dos Produtores de Açúcar e Álcool da Paraíba (SINDALCOOL); and bio-compost prepared from chicken excrement (BCE), supplied by the Empresa Guaraves Alimentos, located in the municipality of Guarabira – PB. Raw chicken manure (RCM), obtained from a chicken farm in the municipality of Conde – PB, and municipal domestic sewage (MDS), collected from the treatment station of the Companhia de Água e Esgoto da Paraíba (CAGEPA), were also tested as alternative media.

The bio-compost extracts and chicken excrement were processed according to the procedures described by Erd-Schreiber (Gross, 1937) (modified), in which one liter of distilled water is added to 1 kg of the solid residue and then heated for 30 min in an autoclave and subsequently filtered; the filtrate liquid was then sterilized and maintained refrigerated under sterile conditions. The domestic sewage was filtered and autoclaved immediately after collection.

The final preparation of the media consisted of adding 10 mL L^{-1} and 5 mL L^{-1} of sterile distilled water to the bio-compost extracts and to the raw chicken manure, respectively, followed by the addition of nitrate and phosphate solutions (1 mL L^{-1}) to each medium and the pH adjustment to 7 (Gross, 1937). The municipal domestic sewage medium was prepared by diluting it to 25% (v/v) of its original concentration with sterile distilled water

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