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Productivity and fuel quality parameters of lipids obtained from 12 species of microalgae from the northeastern region of Brazil

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ABSTRACT

The present work evaluated the growth potential, lipid productivities and the fatty acids methyl esters profiles of 6 chlorophytes and 6 cyanobacteria isolated from the northeast of Brazil, aiming to predict the quality of the biodiesel that could be produced from these microalgae. Among the chlorophytes, *Chlorella* sp. (D101Z) stood out as having the greatest daily cell division rate (2.42 d^{-1}). Among the cyanobacteria examined, the species *Synechocystis* sp. (M3C) and *Synechococcus nidulans* (D109WC) showed elevated lipid (54.2 and 93.8 mg L^{-1} d^{-1} respectively) and biomass productivities (0.39 and 0.69 g L^{-1} d^{-1} respectively). The lipids produced by the chlorophytes were mainly saturated, monounsaturated, and triunsaturated esters, while the cyanobacteria produced high levels of saturated esters and fatty acids with different degrees of unsaturation. Statistical analyses indicated D101Z and D109WC as promising species, as they showed high lipid productivity and the biodiesel produced from their lipids demonstrated low cold filter clogging point values, with a low iodine index for D101Z; the rather high iodine index of D109WC, however, disqualified it for biodiesel production purposes. Among the species studied, only the chlorophyte *Monoraphidium contortum* (D173WC) and the cyanobacteria D109WC did not meet all of the quality specifications.

1. Introduction

Increases in global energy demands and the projected long-term scarcity of fossil fuels have stimulated searches for alternative energy sources. Within this context, biofuels represent a promising alternative for future economic and socio-environmental sustainability in terms of human energy needs [1].

Microalgae represent a viable option for future energy generation as those organisms can produce high levels of lipids suitable for biofuels [2,3]. Microalgae demonstrate high growth rates and lipid productivities as compared to conventional silviculture, require smaller areas for commercial production, and will not compete with human food crops or other economically important plantings for areas with productive soils [4].

Microalgae have numerous biotechnological applications and can be used for feeding animals and as sources of natural dyes, antioxidants, organic fertilizers [5,6]. Other applications include their use in bio-remediation programs, the production of medicines, pharmaceuticals, and diverse bio-active compounds, as nutrients for human consumption, as raw materials for the petrochemical sector, and for producing bio-plastics [7,8].

Zhu (2015) [9] noted that photosynthetic microorganisms can also be used in aquaculture and in the production of other types of biofuels in addition to biodiesel, such as bio-hydrogen, bio-ethanol, bio-methane, and bio-oil. Biofuels made from microalgae still face a number of technological barriers before their full

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expansion to commercial scales becomes feasible, including resolving: (a) logistical problems related to large-scale production; (b) difficulties related to the use of genetically modified organisms in open systems; (c) the high costs of culture media; (d) the complexity of industrial scale photo-bioreactors; (e) high energy demands for drying biomass and extracting lipids; and, (f) the high acidity of the isolated lipidic material [10,11]. As such, for biodiesel production from microalgae to reach its full potential it will be necessary to develop practical biotechnological research programs focusing on all of the production steps to optimize production and reduce costs, starting from the selection, cultivation, and management of microalgae, through the transesterification of the extracted oils. This will involve the development of integrated processes for microalgae biomass generation for biodiesel production as well as the commercialization of other high-value co-products (such as nutraceuticals) that can aggregate value to the productive chain and make biofuel production economically viable.

Various species of microalgae have been considered for biodiesel production, although numerous other factors must be taken into account in addition to their photosynthetic efficiency and productivity - such as their developmental capacity using available nutrients (under both controlled and variable environmental conditions), resistance to contamination, and their fatty acid compositions – as these parameters will have significant effects on the characteristics of the biodiesel produced [12].

Depending on the composition of the lipidic matrix utilized, biodiesel fuels may demonstrate low quality in terms of important attributes such as their cetane numbers, oxidative stability, lubricity, viscosity, and flow properties. Two factors are crucial to determining these chemical and physical properties: the distributions and the numbers of unsaturated bonds in the fatty acid chains [13,14]. *Nannochloropsis* sp., for example, has demonstrated high potential for producing biodiesel due to its elevated lipid and biomass productivity, but it also produces high concentrations of polyunsaturated fatty acids that give its biodiesel low oxidative stability [15]. The viability of microalgae as sources of raw materials for biodiesel production need to be evaluated starting with their growth characteristics, and lipidic production, all the way through to the final quality of the biodiesel produced.

Numerous processes of microalgae biodiesel synthesis have been examined, most of them involving either acidic or basic transesterification [16]. A number of authors have suggested enzymatic transesterification and the use of heterogeneous catalyzers as possible alternatives [17]. Additionally, the use of integrated processes of esterification followed by transesterification, hydroesterification, *in situ* transesterification, and transesterification conducted in the absence of a catalyzer using supercritical conditions have been suggested [18]. As the synthesis processes are generally affected by differences in the fatty matrix, maximum conversion to biodiesel must always be considered, and studies to develop models that can optimize conversion to biodiesel and consider crucial aspects such as mass transfer, reaction kinetics, and chemical equilibrium are extremely important [19,20].

The processes of biodiesel production from microalgae have advanced significantly, although evaluations of the quality of that fuel (in terms of physical measurements of its properties) still present some difficulties, such as the quantities of material needed for some analyses, costs in terms of time and resources, and the necessity of specific apparatuses [15]. Alternatively, the development of models that can predict some of the critical properties of the biodiesel to be produced (based on ester compositions) have been used, and show great promise as tools for evaluating the effective viability of microalgae species as sources of lipid raw material for biodiesel production [14,21–23]. As such, the present study was designed to examine the potentials of 12 strains of microalgae encountered in northeastern Brazil for the production of biodiesel by examining their standardized fuel quality parameters as predicted by mathematical equations based on the compositions of their fatty acid methyl esters.

2. Materials and methods

2.1. Cultivation and kinetic parameters of microalgae growth

Twelve species of microalgae were collected in various localities in northeastern Brazil and subsequently maintained in the culture collection of the Federal University of Paraíba/Laboratory of Reef Environments and Microalgae Biotechnology (LARBIM/UFPB). Of these species, six were freshwater chlorophytes: *Chlorella* sp. (D101Z), *Scenedesmus acuminatus* (D115WC), *Pediastrum tetras* (D121WC), *Chlamydomonas* sp. (D132WC), *Lagerheimia longiseta* (D133WC) and *Monoraphidium contortum* (D173WC); and six were cyanobacteria, of which three were marine species: *Sinechocystis* sp. (M3C), *Romeria gracilis* (M6C), *Aphanothece* sp. (M27C) and two were freshwater species: *Planktothrix isothrix* (D39Z) and *Synechococcus nidulans* (D82Z and D109WC).

The isolation and purification of these cultures were performed using traditional separation techniques employing a capillary micropipette and a binocular microscope. The identifications of the cultivated species were made based on their morphological characteristics using diacritical criteria [24] using traditional phycological keys and data published in scientific articles, including the works of Geitler (1932) [25], Komarék and Anagnostidis (2005) [26], Bicudo and Menezes (2006) [27], Sant'anna et al. (2012) [28], Bellinger and Sigee (2010) [29], and Franceschini et al. (2009) [30], and specific sites such as www.algaebase.com.

The single-algae cultures were maintained in a culture chamber under a 12:12 h light cycle and a constant temperature of 25 °C ± 1° C. The culture media used were those of Conway (C) [31], Zarrouk (Z) [32] and WC [33], prepared with fresh water or seawater filtered through glass fibers, as appropriate.

The microalgae were cultivated in triplicates in flasks containing 5 L of culture medium with agitation provided by the continuous bubbling of air (2.0 mL min^{-1}) using a Resun AOC2 membrane compressor. The cultures chamber was maintained at 25 °C with a 12 h photoperiod, with a light system of 4.5 ± 0.3 kLux. Culture growth was accompanied by cell counts in Fuchs Rosenthal or Sedgewick Rafter chambers (the latter for filamentous algae), using a Leica binocular microscope. These analyzes allowed to characterize the growth curves of each cultivated species and to determine some kinetic parameters, such as:

2.1.1. Cell division rate (k) - representing the numbers of cell divisions in the study population per unit time (day), determined using the equation [34]:

$$k = \frac{3.322}{T_2 - T_1} x \log \frac{N_2}{N_1} \quad (1)$$

In which T_1 and T_2 represent the initial and final phases of exponential growth; and N_1 and N_2 represent the initial and final cell densities of the exponential growth phase respectively.