Algal Research 9 (2015) 312–321

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Analysis of some chemical elements in marine microalgae for biodiesel production and other uses

Bruna Ferreira Silva [a], Eliéser Viégas Wendt [a], Juliana Cristina Castro [a], Anselmo Elcana de Oliveira [b], Aysha Jussara Ivonilde Carrim [c], José Daniel Gonçalves Vieira [c], Roberto Sassi [d], Cristiane Francisca da Costa Sassi [d], Aline Lourenço Vieira da Silva [d], Giuseppe Fernandes de Oliveira Barboza [d], Nelson Roberto Antoniosi Filho [a,*]

[a] Laboratory of Extraction and Separation Methods, Chemistry Institute, Federal University of Goiás, Goiânia, Brazil
[b] Tropical Pathologies and Public Health Institute, Federal University of Goiás, Goiânia, Brazil
[c] Chemistry Institute, Federal University of Goiás, Goiânia, Brazil
[d] Department of Systematics and Ecology, Federal University of Paraíba, João Pessoa, Brazil

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 15 March 2015
Accepted 3 April 2015
Available online 14 April 2015

Keywords:
Marine microalgae
Diatoms
Bioindicators
Biodiesel
Inorganic content

ABSTRACT

Due to the currently abundant supply of marine microalgae, which can be found in seawater, as well as microalgae's ability to uptake different chemicals, it appears as a promising raw material with potential for many commercial uses. Despite having a high amount of metal in their biomass, the lipids within marine microalgae can be converted into biodiesel. Analyses of 26 chemical elements (Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sn, Sr, Ti, Tl, V, and Zn) were performed by ICP-OES with the goal of quantifying the inorganic content of marine microalgae's biomass. Regardless of the cultivation media used, microalgae presented differences in their chemical element profile. Strains showed a 12.9% to 36.3% mass of analyzed elements per dry biomass, which represent a relatively high percentage for a feedstock used in biofuels. Among the 36 assayed microalgae, *Biddulphia* sp., *Planktolyngbya limnetica*, *Amphora* sp. (1), *Navicula* sp. *(3)* and *Synechococcus* sp. are most indicated for this purpose as they contain a lower concentration of chemical elements when compared to other samples. However, their profile warns that water quality control is needed for toxic metals such as Ba, Cd, and Pb.

1. Introduction

Algae are the object of the study of phycology, which places them at the base of the food chain due to their capacity to produce life's essential organic compounds from water, nutrients, and sunlight. Recently, microalgae aroused researchers' attention due to their special characteristics and suitability for use in variable commercial and industrial branches [1]. Microalgae are unicellular microscopic organisms that share many similarities with plants in terms of how they store compounds and their preservation strategies. Microalgae can be found and cultivated in a variety of aquatic environments, such as freshwater, marine, brackish, wastewater, or terrestrial places with a high degree of moisture [2,3]. Thus, the extent and variation of culture media provide different purposes for the biomass, but always take into account their biochemical and mineral compositions.

Because the cells of microalgae are in permanent contact with cultivation media, they are able to uptake the necessary elements for their growth, and, to adsorb them into their cellular membrane compartments, since these compartments contain negatively charged functional groups such as hydroxyl, carbonyl, and sulfate [4,5]. The aforementioned processes are known as bioaccumulation and biosorption respectively, and they lead to a raise in the amount of chemical elements in microalgae biomass [6]. Since, a majority of the time, the observed metal profile is a reflection of the environment in which the cultivation occurred [4], metal analysis of microorganisms is preferred instead of water analysis [7]. However, microalgae's mineral composition can be highly variable between different species [8], including the ones belonging to the same taxon.

According to their lipid producer potential which can be converted into fatty acid esters, microalgae seem promising for the biofuels' branch. This is the case because optimizing culture conditions allow an increase in lipid, fatty acid and other compound productivity, and, consequently, biodiesel amounts [9,10]. In addition the environment becomes the greatest beneficiary from this production as biodiesel creates less pollution than diesel, as well as being biodegradable [11].

* Corresponding author at: Laboratory of Extraction and Separation Methods, Chemistry Institute, Federal University of Goiás, Campus Samambaia, Goiânia, Brazil.
E-mail address: nelson@quimica.ufg.br (N.R. Antoniosi Filho).

http://dx.doi.org/10.1016/j.algal.2015.04.010
2211-9264/

Partial or total replacement of diesel with biodiesel would help to minimize society's dependence on petroleum based products, and, further encourage the development of technologies which are environmentally friendly and clean.

Also, marine strains showed more resistance to environmental changes in cultivation as they are adapted to survive in high levels of salinity. For this reason, and due to the large seawater availability which can be used in microalgae culture, it is expected that these microorganisms may supply the growing biofuel demand; shifting it from petroleum, natural gas, and coal derived fuels, to biofuels produced from marine microalgae [12,13]. Thus, in addition, microalgae would not compete with existing crops intended for human consumption and animal feed, such as soybean, palm, corn, and sugarcane. It would even be possible to use industrial wastes in the maintenance of microalgae cultivation, thus putting this waste to use in a beneficial manner [3,14]. Hence, all alternatives provide an end product which is cheaper, enhancing the economic feasibility for the use of this raw material [2].

It is necessary to monitor some elements in biodiesel, as it is important due to their influence in storage durability. Since biofuel is more polar than diesel, it is able to solubilize a greater amount of metals and water, and this can lead to corrosion of the metallic containers in which it is stored; in addition, it accelerates the process of fuel oxidation, resulting in lower overall product quality [15,16]. Moreover, a high phosphorus content may bring about poisoning of the catalysts used for engine emission control, as a large sodium concentration induces undesirable reactions between biodiesel and the engine, damaging it [17,18]. Another issue remains that some chemical elements found in biodiesel may be responsible for corrosion of the hosing and rubber tubes used on vehicles; in addition to leaving deposits of metallic oxides that promote gum formation in the engine, and clogging of the filters and fuel lines. Lastly, there is concern regarding the emission of metal traces by burning biodiesel, which can increase atmospheric pollution [19,20]. It is also important to consider that the chemical element analysis of microalgae can also be useful to understand the biofuel composition obtained by other processes, such as the production of alkanes and biocrude by hydrothermal liquefaction [21,22], as well to the quality control of these other biofuels.

This paper has as its aim to determine, by ICP-OES analysis, 26 chemical elements found in the biomass of marine microalgae, which could serve as potential raw material for the production of biofuels, mainly by direct conversion of the biomass into biofuel. Moreover, the inorganic content of the samples were quantified, trying, in this manner, to identify a promising species in relation to the content of chemical elements to be used in biofuels' industry, and, also to find relations between the content of chemical elements and the cultivation medium used as well as the taxonomic group of the analyzed species and elements profile.

2. Materials and methods

2.1. Equipment and reagents

Every material was initially cleaned by submerging it in an acid solution (HNO_3 10%), for 48 h. Next the material was washed with purified water (18.2 MΩ cm, Millipore). This water was also used in the sample dilution. Nitric acid (HNO_3) 70% ultrapure (VETEC) was applied during microalgae digestion. To prepare the standard solution, a 100 ppm multi-element standard was used, (SpecSol Multi G2V) containing 26 chemical elements (Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sn, Sr, Ti, Tl, V and Zn).

Microalgae biomass digestion proceeded in a microwave oven DGT100 Plus (Provecto Analitica), with power programming. TFM digestion flasks were washed daily at the beginning of the day with a 5 mL HNO_3 cleaning program: first step at 500 W for 5 min and second at 0 W for 5 min.

Analyses of elements were performed in an Inductively Coupled Plasma Optical Emission Spectrometer iCAP 6300 (Thermo Fisher Scientific). In addition, all ICP glassware parts were cleaned by the same process previously described.

2.2. Microalgae cultivation and sampling

In this study the dry biomass of twelve cyanobacteria were analyzed, including *Aphanothece* sp., *Planktolyngbya limnetica*, *Planktothrix isothrix*, *Romeria gracilis*, *Synechococcus* sp.*(1)*, *(2)*, and *(3)*, *Synechocystis aquatilis (1)*, *(2)*, and *(3)*, *Synechocystis* sp. *(1)* and *(2)* strains; three different chlorophyceae without identification, named *Chlorophyceae N. I. (1)*, *(2)*, and *(3)*; twenty diatoms, *Achnanthes* sp. *(1)* and *(2)*, *Amphora* sp. *(1)* and *(2)*, *Biddulphia* sp., *Biddulphia* sp. cf *longicruris*, *Biddulphia* sp. cf. *aurita*, *Chaetoceros simplex*, *Cylindrotheca closterium (1)* and *(2)*, *Entomoneis alata*, *Navicula* sp. *(1)*, *(2)*, and *(3)*, *Phaeodactylum tricornutum*, *Thalassiosira* sp. *(1)*, *(2)*, *(3)*, and *(4)*, *diatom not identified (Diatom N. I.)*; and lastly, one dynoflagellate, *Amphidinium carterae*.

These strains were collected in Brazilian northeast coast seawater and enriched with Conway medium [23] until cells began to grow. This step was performed in an acclimatized (25 °C ± 1 °C) culture chamber with a lighting system supplied by fluorescent lamps for a 12 hour photoperiod. Isolation of strains occurred using a microscope with microcapillaries. Each isolated strain passed through a codification process, and were incorporated into the Microalgae Collection of Department of Systematics and Ecology, Federal University of Paraíba. Biomass production experiments were carried out in 6 L flasks which contained 5 L of the culture medium. Cultivation was monitored continuously through cell count and in vivo fluorescent measurement. Microalgae growth curves were then determined. Experiments were discontinued when the stationary phase began. Then, the biomass was concentrated in a refrigerated centrifuge at 18 °C, frozen (−40 °C), and lyophilized. Dried biomass was sent for chemical analyses.

2.3. Light microscopy

Some marine microalgae samples, selected at random, were subjected to optical microscopy in a Zeiss Optic Microscope, Axioskop 2 (100×, 400×, and 10,000×), with glass lamina and a photographic camera having MPEGMovieEX 3.3 megapixels. This was completed to obtain microalgae biomass images. After dry biomass watching, NaCl 0.85% was added in order to rehydrate them and to check for possible changes.

2.4. Biomass digestion

A 100 mg sample was weighed in microwave digestion flasks for duplicate biomass metal analysis. Afterwards, 3 mL of HNO_3 70% ultrapure was added and flasks were closed. Samples were digested using a three step process in which equipment power was maintained at 330 W for 5 min in step 1, increased to 800 W for 8 min in step 2, and reduced to 0 W for 7 min in step 3, relative to sample cooling.

After the digestion procedure samples were placed in a 25 mL volumetric flask, having their volume completed using purified water. It was observed that few samples presented a white precipitate residue, which is supposed to be silica. The solution was transferred carefully to a 20 mL plastic bottle.

2.5. ICP-OES analysis

Digested biomasses samples were analyzed by ICP-OES with operational parameters and emission lines for Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sn, Sr, Ti, Tl, V, and Zn as described in Table 1. The calibration curve was made through 8 standard solutions (0, 0.05, 0.1, 0.3, 0.5, 1.0, 3.0, and 5.0 mg/L) prepared from a 100 ppm multielement standard in HNO_3 5% solution. For macroelement