Algal Research 33 (2018) 28–35

Contents lists available at ScienceDirect

Algal Research

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

Choricystis minor var. *minor* lipids: Extraction using conventional and pressurized solvents and assessment of their potential to produce fatty acid methyl esters

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ARTICLE INFO

Keywords:
Choricystis minor var. *minor*
Extraction
Carbon dioxide
Propane
Fatty acid methyl esters

ABSTRACT

Microalgae are potential raw materials for biofuel applications. In this work, microbial lipids from *Choricystis minor* var. *minor* were extracted with conventional solvents in a Soxhlet apparatus and with compressed fluids with or without the use of a co-solvent (ethanol or hexane). Also, the total achievable yield of fatty acid methyl esters (FAME) was determined in both unextracted microalga, extraction residue and extracted lipids. Ethanol was the best solvent for conventional extraction but hexane was more selective for saponifiable lipids. Compressed propane was better than supercritical carbon dioxide ($scCO_2$) for the extraction of microbial lipids and the use of ethanol as co-solvent improved both extraction rates and yields. The best FAME yield was achieved with $scCO_2$ plus ethanol at 80 °C and 150 bar (FAME yield of 16.7 g 100 g^{-1} dry biomass for a mass extraction yield of 31.6%). Hexane was not advantageous as an extraction co-solvent.

1. Introduction

Innumerous studies have been carried out to screen for optimal microalgae species and to determine the most appropriate cultivation conditions for optimal growth and lipid productivity [1–3]. Factors influencing lipid accumulation and productivity are the process design, the cultivation temperature, the incidence of light and the composition of the culture medium, including pH and the easy availability of nitrogen and phosphorus. Among several microalgae with good potential for biodiesel production, the green microalga *Choricystis minor* var. *minor* stands out mainly due to its high lipid content and good fatty acid profile for fuel applications [4,5]. However, lipid extraction of this microalgae is scarce and reports available so far have mostly focused on the optimization of growth conditions to improve its total lipid content.

Menezes et al. [4] reported a comparison among potential microalgae species for biodiesel production. *Choricystis* sp. showed a FAME yield of 422.9 mg g^{-1} while other species were not able to exceed 250 mg g^{-1}, with soybeans corresponding to only 196.9 mg g^{-1}. Sobczuk and Chisti [6] evaluated the influence of nutrient availability on the lipid production and fatty acid profile of *C. minor*. When the microalga biomass that had been grown at optimal conditions was deprived of phosphates and nitrates for 10 days, its lipid content increased nearly 55% in relation to the control from 260.0 to 595.0 mg g^{-1} dry mass. Also, after this post-harvest nutrient deprivation, the microbial lipids contained a better fatty acid profile for biodiesel production, with 55.6% saturated, 32.2% monounsaturated and only 11% polyunsaturated fatty acids against 44% of this latter component in the freshly harvested biomass. Menezes et al. [5] showed that the Wright's cryptophyte (WC) medium was the best nutrient source for *C. minor* var. *minor* to produce fatty acids that could be directly converted to fatty acid methyl esters (FAME). These results demonstrate the good potential of *Choricystis* sp. for biodiesel applications.

Microalgae lipids are normally composed of neutral lipids (such as triacylglycerols), phospholipids, glycolipids and other complex lipophilic materials [7]. Free fatty acids are usually found in large amounts and are partially related to the downstream processing of the microalgae biomass [8,9]. In addition, other classes of organic compounds are found in microalgae extracts, such as sterols, sterol glucosides, carotenoids, flavonoids, phenolic compounds and chlorophylls [7,10,11]. Therefore, microalgae extracts normally present a highly

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https://doi.org/10.1016/j.algal.2018.04.017
Received 9 November 2017; Received in revised form 24 March 2018; Accepted 23 April 2018

heterogeneous composition and the optimal conversion of such lipids into FAME is the ultimate challenge for the development of microalgae-based biodiesel production technologies.

The use of a Soxhlet apparatus [12–14] allows the exhaustive removal of biomass lipids by continuous of the extraction solvent. Hence, lipids are continuously extracted by contact with fresh solvent, which is easily recovered afterwards by evaporation [15]. Among the conventional solvents usually tested for extraction of microalgae oil are hexane, ethanol and binary mixtures such as chloroform:methanol (2:1 v/v) [7,12,16]. Baumgardt et al. [12] and Liau et al. [14] demonstrated that hexane provides lower extraction yields compared to polar solvents such as ethanol. However, hexane is selective for the extraction of lipids that can be converted to fatty acid alkyl esters by esterification and/or transesterification, while polar solvents provide higher extractions yields by removing polar molecules as well. About 50% of the hexane extract from *Nannochloropsis oculata* was readily converted to alkyl esters, against only 23% of the corresponding ethanol extract. However, the total ester yield in relation to the microalgae dry mass was higher for the extractions with ethanol (11.33 g 100 g^{-1}) compared to hexane (6.55 g 100 g^{-1}) [12]. Ethanol is useful for lipid extraction of different microalgae species [12,14,16,17] because of its low toxicity, high extraction yields and the possibility of its use in reactive extraction systems where fatty acid ethyl esters can be obtained by *in situ* esterification and/or transesterification. In addition, ethanol is a renewable fuel, differently from other solvents such as methanol, hexane and chloroform that are normally derived from petroleum. Despite the obtainment of high ester yields in relation to the amount of biomass used for extraction [12], the use of polar solvents may accumulate impurities such as phospholipids in the resulting fatty acid alkyl esters and these would have to be removed in the downstream by chemical processes such as degumming [18].

Lipid yields may vary according to the technology used for extraction, the physicochemical properties of the extraction solvent and the permeability of the microalgae biomass to the extraction solvent, as well as to the conditions used for cultivation and to the microalgae downstream processing. Baumgardt et al. [12] obtained extraction yields of 20% using ethanol and 5% using hexane from *N. oculata* in a Soxhlet apparatus, while Liau et al. [14] obtained yields of 40.9 and 5.8% from the same species using the same solvents, respectively. Tang et al. [19] obtained yields of 45% from *Schizochytrium limacinum* using hexane. D'Oca et al. [16] reported yields of 19.0 and 1.5% from *Chlorella pyrenoidosa* using ethanol and hexane, respectively. A search in the open literature shows that the extraction data from *C. minor* var. *minor* are scarce and to the best of our knowledge, only Sobczuk and Chisti [6] reported preliminary extraction yields 29.7% using hot isopropanol as the extraction solvent.

Pressurized fluids such as supercritical CO_2 ($scCO_2$) and compressed propane have also been used to extract lipophilic materials from microalgae and oil crops [7,13,20,21]. These pressurized fluids can be combined with co-solvents that are able to improve both extraction efficiency and selectivity [12,20]. Fabrowska et al. [11] used supercritical CO_2 ($scCO_2$) with ethanol as co-solvent for extraction of phenolic compounds and carotenoids from the microalgae *Cladophora glomerata*, *Ulva flexuosa* and *Chara fragilis*. The presence of ethanol provided an increase in extraction yield compared to extraction using only $scCO_2$, with the best results being obtained at 40 °C and 300 bar with 11% ethanol. Co-solvents such as methanol, ethanol and hexane at low pressure conditions have also been used in studies related to biodiesel production from microalgae [12,20,22]. However, there is no reference in the literature about lipid extraction with $scCO_2$ and compressed propane in co-solvent assisted processes for *C. minor* var. *minor*. This work demonstrates the potential of extracting lipophilic materials from *C. minor* var. *minor* using $scCO_2$ and compressed propane without and with ethanol and hexane as co-solvents. The results obtained from $scCO_2$ and compressed propane were compared to the conventional extraction using a Soxhlet apparatus. Also, the potential convertibility of the extracted lipids to FAME was assessed and the resulting materials were characterized by a range of analytical methods.

2. Materials and methods

2.1. Material

Different batches of *C. minor* var. *minor* were cultivated and supplied by the Aquaculture Department of the Federal University of Santa Catarina (UFSC, Brazil) in the form of frozen tablets of biomass slurries containing 20% total solids in average. These batches were homogenized into a single sample using a Waring blender and immediately subjected to drying in an air circulating oven at 60 °C until constant weight. Afterwards, the whole sample was sieved through a 48-mesh screen to eliminate fine particles. The retained material was sealed in a plastic bag and kept frozen at −10 °C until use. Anhydrous ethanol (99.5% purity), hexane (95.0% purity) and methanol (99.9% purity) were purchased from Neon (São Paulo, SP, Brazil) while chloroform was obtained from Dinâmica (São Paulo, SP, Brazil), all of these in analytical grade. Carbon dioxide and propane, both with a 99.5% purity in the liquid phase, were purchased from White Martins (Curitiba, PR, Brazil).

The following reagents were used as received to perform the conversion of the microalga lipids to FAME [4]: sodium hydroxide (Vetec, 97.0%), ammonium chloride (Vetec, 99.5% purity), heptane (Neon, 99.5% purity) and sulfuric acid (Merck, 95.0% purity). The FAME Mix C4-C24 from Supelco (Sigma, Analytical Standard) was used as reference for chromatographic analyses.

2.2. Methods

2.2.1. Sample preparation

Particle size distribution of the milled microalgae material was obtained in duplicate from a mechanical stirrer (Bertel, SP, Brazil) using a set of 6 sieves. After agitation for 5 min, the following fractionation in particle sizes was obtained: 8 (6.92 ± 0.48%), 12 (20.06 ± 0.23%), 20 (20.96 ± 0.31%), 24 (12.21 ± 0.22%), 32 (12.51 ± 0.26%) and 48 (15.04 ± 0.34%) mesh. An average particle diameter of 8.16×10^{-4} m was estimated using the method presented by Gomide [23], which considers the mass fraction of the milled materials that were recovered in different sieves. The fine particles that were not retained by the sieves (12.3%) were discarded and the retained fractions were homogenized again to compose a single sample.

2.2.2. Conventional extraction (Soxhlet)

The extractions were performed using hexane, ethanol and chloroform:methanol (2:1, vol/vol) in a Soxhlet apparatus. Experiments were performed in duplicate for each of these extraction solvents. Approximately 5 g of microalgae were transferred to a filter paper cartridge and allocated into the Soxhlet extraction chamber. Nearly 200 mL of the extraction solvent was added to a tared round bottom flask and the extraction was carried out for 12 h. After this period, the solvent was removed by evaporation in an air-circulating oven at 60 °C and the extraction yield (%) was measured gravimetrically to be expressed in relation to the microalgae dry mass.

2.2.3. Extraction with pressurized fluids

The experiments were organized in a 2^2 experimental design (two factors – pressure and temperature – in two levels) in which the center point was performed in duplicate (total of six extraction conditions). A home-made bench scale batch extractor (7.98×10^{-5} m^3 inner volume, L of 0.16 m and Φ of 2.52×10^{-2} m) was used, which consisted of a jacketed steel vessel connected to an ultrathermostatic bath to control the temperature during the whole extraction procedure. The vessel was connected to a high-pressure syringe pump (Teledyne Isco 500 D) that was kept at 10 °C during the entire extraction procedure, which was