Algal Research 14 (2016) 9–16

Contents lists available at ScienceDirect

Algal Research

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

Effect of low frequency ultrasound on microalgae solvent extraction: Analysis of products, energy consumption and emissions

Ana F. Ferreira *, Ana Paula Soares Dias, Carla M. Silva, Mário Costa

IDMEC, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 3 December 2015
Accepted 28 December 2015
Available online xxxx

Keywords:
Microalgae
Solvent extraction
Energy requirements
Fourier transformed infrared spectroscopy
Thermogravimetric analysis
Scanning electron microscopy

ABSTRACT

In this study, the effect of low frequency ultrasound assisted solvent extraction was assessed for three species of microalgae (*Chlorella vulgaris*, *Nannochloropsis oculata* and *Scenedesmus obliquus*). The microalgae contain a wide range of compounds (carbohydrates, proteins, lipids and pigments), which after extraction, can have several benefits, such as conversion of lipids into biofuel. Pure and binary (2:1 v/v) mixtures of high volatile solvents and relative low toxicity (*n*-hexane, chloroform, 2-butanol, isopropanol, ethanol and methanol) were used. Low concentration suspensions microalgal/solvent (0.03 g/mL) was sonicated for 180 min at 50/60 Hz at 60 °C using a reflux column to avoid solvent evaporation. The extent of the extraction procedure was assessed by weighing of extracts, after solvents evaporation, and by comparing the thermal degradation (under air by thermogravimetric analysis) and Fourier transformed infrared spectroscopy (FTIR) profiles of raw and leftover microalgae. Additionally, the raw and leftover microalgae and the liquid extract were characterized by FTIR. A scanning electron microscopy (SEM) was used to evaluate the effect of ultrasound on the microalgae morphology. Energy requirements were measured for the solvent extraction procedure and compared with literature data using other methods. The results reveals that (i) for all the analyzed microalgae the best extraction was achieved using the binary solvent *n*-hexane:isopropanol; (ii) the *C. vulgaris* showed the lowest yield of liquid extract; (iii) the absence of cell disruption, but a slight swelling, was observed by SEM; and (iv) the energy requirements of this method are roughly 0.15 MJ/$g_{dry\ microalgal}$, which is lower than previous soxhlet extraction, using *n*-hexane as a solvent, and supercritical fluid extraction.

1. Introduction

Microalgae are currently receiving a considerable attention due to their ability to synthetize valuable compounds (e.g., pigments), accumulate high energy compounds (e.g., lipids, carbohydrates) and sequester carbon. They are therefore considered as a third generation feedstock for biofuel production and have a great potential as renewable feedstock [1,2]. Exhaustive studies have been undertaken on the production of biofuels through biological and chemical methods using microalgae [2–6]. As biofuel-based microalgae remains expensive, the biorefinery is a way to overcome this bottleneck, taking advantage of all biomass components (e.g., Nobre et al. [7]; Ferreira et al. [8]). Table 1 shows some microalgae species previously highlighted for their high sugar and lipids content [9,10].

The variations on culture conditions (e.g., light intensity, pH, salinity, temperature, concentration of nitrogen, and other nutrients) during the growth of microalgae influence the contents distribution [11]. Microalgae produce a large variety of lipids that can be extracted before their conversion into biofuels or be used directly in pyrolysis processes without lipid extraction. A number of research works were undertaken in this field [11–20]. The lipid fractionation procedure and the selection of solvent depend on the particular classes of lipids content [12]. The extraction of lipids from microalgae is usually carried out using classical extraction methods, such as using organic solvents and Soxhlet extraction [11,13]. The *n*-hexane is the solvent most used in lipid extraction; however, in terms of pre-treatment, propanol has a positive effect on oil extraction [11]. To increase the lipid extraction efficiency, it is fundamental an appropriate cell disruption method. Extraction techniques have been developed for microalgae cell disruption, namely ultrasound assisted extraction and intracellular heating from microwave assisted extraction [14,15,20]. Araujo et al. [19] studied the ultrasound assisted extraction and observed that sonication increased the efficiency of the extraction since it was partially responsible for cell disruption. Prommuak et al. [16] examined various methods for microalgal lipid extraction of *Haematococcus pluvialis* and *Chlorella vulgaris* aiming to study the effect of different solvents and extraction methods on the extracted lipid yield. These authors showed that a highest amount of total lipid from microalgae could be extracted from a mixture of chloroform and methanol at the ratio of 2:1 (v/v) using a microwave extraction method, while hexane was found to be a good solvent in terms of providing more

* Corresponding author at: IDMEC, Instituto Superior Técnico, Dept. of Mechanical Engineering, Av. Rovisco Pais, Pav. Mecânica I, 2° Andar, 1049-001 Lisbon, Portugal.
E-mail address: filipa.ferreira@tecnico.ulisboa.pt (A.F. Ferreira).

http://dx.doi.org/10.1016/j.algal.2015.12.015
2211-9264/

Table 1
Sugars and lipids contents of different microalgae biomass [9,10].

Species	Sugars (wt.% biomass)	Lipids (wt.% biomass)
Anabaena cylindrica	25–30	4–7
Chlamydomonas rheinhardii	17	21
Chlorella vulgaris	12–17	14–22
Dunaliella salina	32	6
Porphyridium cruentum	40–57	9–14
Scenedesmus obliquus	10–17	12–14
Spirulina máxima	13–16	6–7
Synechococcus sp.	15	11
Chlorella pyrenoidosa	26	20
Spirogyra sp	33–64	11–21
Nannochloropsis sp.	17	31–68

desirable content of glycerides. Chloroform is, however, highly toxic and its usage is undesirable. Hexane/isopropanol mixtures have been suggested as a low-toxicity substitute to chloroform/methanol mixtures [17]. In microalgae lipid extraction processes, *n*-hexane:isopropanol mixtures were found to be more selective towards neutral lipids compared to chloroform/methanol mixtures [18].

Extraction methods have considerable energy consumption. Therefore, a number of recent studies concentrated on energy consumption and carbon footprint assessment as part of choosing the greenest process. According to [21] "*Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product*".

Ferreira et al. [8] examined two different extraction methods to obtain lipids of *Nannochloropsis* sp. microalgal: soxhlet extraction (SE) using *n*-hexane as the solvent, and supercritical fluid extraction (SFE). In the SE the total oil obtained was 40.7% of the dry microalgal mass and the final energy consumption was 0.87 MJ/g of dry microalgal. The SFE extraction consumed 1.79 MJ/g of dry microalgal and a total of 0.42 $g_{oil}/g_{dry\ microalgal}$ was produced. Khoo et al. [4] reported an energy consumption value of 114 $MJ/kg_{lipid\ depleted}$ of the dry biomass for *Nannochloropsis sp.* extracted by the solvents hexane and methanol in the ratio of 3:1 (by volume), using a high shear homogenizer, obtaining lipid extraction yield of 25%. Lee et al. [22] provide an overview of microalgal cell disruption processes, which are potentially suitable for large-scale lipid extractions. The energy requirements of these processes were compared between them and then compared with estimates of the theoretical minimum energy required for disruption. A range of 9.6 to 529 $MJ/kg_{dry\ microalgal}$ is reported for the final energy consumption, covering different microalgae and different methods [14,23–25]. Most of the researchers report ultrasound assisted extraction as a cost-effective technology for natural products extraction due to improved yields and the consequent opportunity to use alternative solvents (environmental benign) which cannot be used in conventional extraction processes [26–28]. Previous studies reported that ultrasound increases degradations of fat and oils [29,30]. These studies have shown by chemical analysis that there is degradation when ultrasound is used

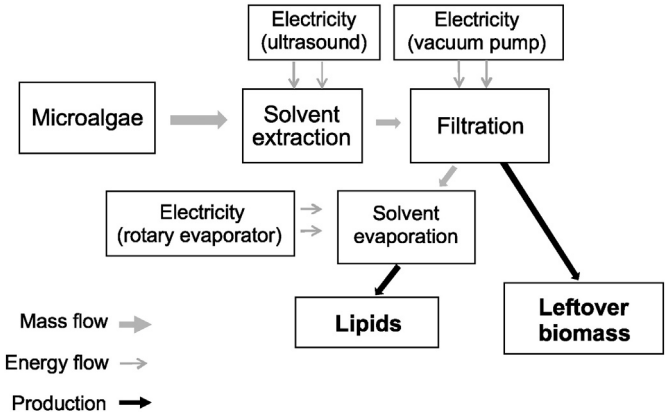

Fig. 2. Scheme of the ultrasound assisted extraction boundaries considered in the analysis.

for treatment of natural products. Additionally Michalak and Chojnacka [31] pointed out the ultrasound assisted extraction as a simple technique that can be scaled up to industrial production.

The present work evaluates the solvent extraction of microalgae, through an ultrasound assisted technique and characterizes the raw and after extraction biomass. Three raw and extracted microalgae species, *C. vulgaris*, *Nannochloropsis oculata* and *Scenedesmus obliquus*, were characterized by Fourier transformed infrared spectroscopy (FTIR, reflectance mode), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). The solvent mixture proportion and ultrasound frequency were kept constant (as of 2:1 and 60 Hz, respectively). The evaluation of the energy consumption and greenhouse gas (GHG) emissions per kg of dry microalgae, and kg of liquid extract were also considered for comparisons with other extraction techniques. A brief feasibility of this technique to be implemented from laboratory to pilot scale and its industrialization is presented.

2. Materials and methods

The *C. vulgaris*, *N. oculata* and *S. obliquus* microalgae used in this study were grown and obtained by the company A4F-Algae for Future located in Pataias, Portugal.

2.1. Ultrasound assisted solvent extraction

A solvent ultrasound extraction method was used to extract oil from the microalgae. The ultrasound extraction system was composed by an ultrasound bath and a condenser. In this extraction 5 g of microalgae powder was used along with pure and binary solvents for 3 h at 60 °C in a P-select ultrasounds-H using 50/60 Hz and 230 V (720 W) [32]. The extraction of the lipids and other components of the raw microalgae was tested for *C. vulgaris* using 150 mL of pure solvent (*n*-hexane and ethanol) and 150 mL of mix solvents (2:1 v/v) (chloroform:ethanol; chloroform:isopropanol; chloroform:methanol; *n*-hexane:methanol;

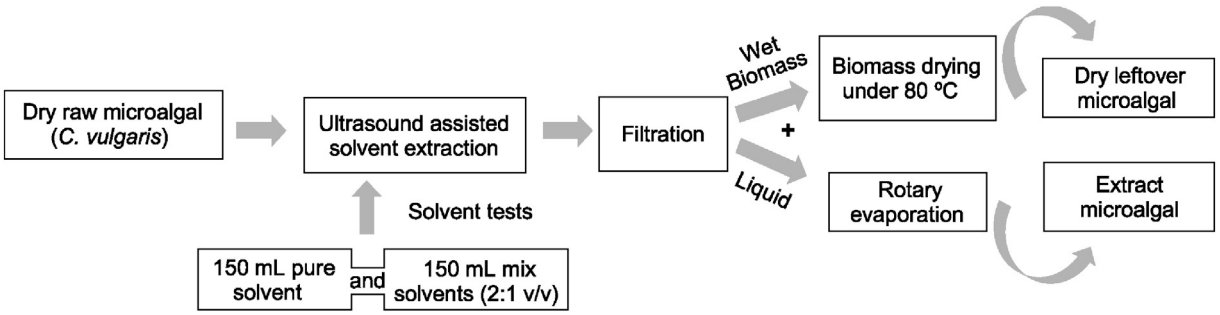

Fig. 1. Ultrasound assisted solvent extraction procedure.