Biomass and Bioenergy xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biomass and Bioenergy

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

Research paper

Extraction of *Acutodesmus obliquus* lipids using a mixture of ethanol and hexane as solvent

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ARTICLE INFO

Keywords:
Microalgae
Lipid extraction
Soxhlet
Assisted ultrasound

ABSTRACT

One of the difficulties of using microalgae for the production of biofuels is the development of an efficient and economically feasible process for oil extraction from these unicellular organisms. Current methodologies involve toxic solvents (such as chloroform, benzene and methanol, among others), high energy requirements and high capital cost. The extraction processes using organic solvents in a Soxhlet apparatus with or without the assistance of ultrasound irradiation have shown promising results. In this context, the main objective of this study was to improve the oil yield of *Acutodesmus obliquus* using different mixtures of ethanol and hexane. Ethanol to hexane volume ratios of 1:1, 2:1 and 1:2 (vol/vol) were used at 60 °C. The best extraction yield in relation to the total lipid content of the microalgae biomass was 92% for Soxhlet and 59% for ultrasonic irradiation using 1:2 (vol/vol) ethanol:hexane for extraction times of 12 and 2 h, respectively. These extraction yields were significantly better than those of the pure solvents, i.e., 24% and 217% higher than ethanol and hexane via Soxhlet and 55% and 68% higher than the ultrasound-assisted extraction procedure. The ethanol to hexane volume ratio of 1:2 (vol/vol) presented a superior performance in both Soxhlet and ultrasound-assisted extraction procedures by extracting a large amount of polar, non-polar and neutral lipids. Similar yields to the Soxhlet (12 h) with the ethanol:hexane 1:2 (vol/vol) solvent mixture were obtained with ultrasound (for 40 min) followed by Soxhlet (4 h) extraction leading to a reduction of 164% in energy consumption.

1. Introduction

Effects of climate changes caused by fossil fuels usage and the possible depletion of crude oil reserves have raised the demand for renewable energy sources worldwide. Biodiesel produced from vegetable oils (palm, soybean, cotton and rapeseed, among others) and animal fats (tallow, chicken and fish) [1–3] has been considered a sustainable alternative for the partial or total replacement of petrodiesel. However, the amount of biodiesel produced from oils and fats is not enough to support the increasing demand for this biofuel. Furthermore, it is noteworthy that some raw materials used to produce biofuels are also used extensively in the food industry [3–5].

To avoid the competition between the energy and the edible oil markets, microalgae have been investigated as an alternative lipid source for biodiesel production. Microalgae are organisms that can be found in aquatic and terrestrial ecosystems and their advantages over seed crop oils include rapid growth, high lipid content, high biomass production rates, presence of valuable co-products and small area requirements for cultivation using ponds, raceways or other types of photobioreactors [6,7].

The microalgae lipid content varies from 5 to 77% in mass (dry basis) depending on the species and its cultivation, harvesting and processing. These lipids, defined as any biological molecule soluble in organic solvent, are classified in two categories: (1) neutral lipids (mostly acylglycerides) and free fatty acids used by microalgae for energy storage, and (2) polar lipids as such phospholipids and glycolipids that are used to form the bilayer structure of cell membranes [8]. According to Scott et al. [3], fatty acids extracted from microalgae lipids have chain lengths varying within 8–24 carbon atoms in which there are variable proportions of polyunsaturated fatty acids and acids having an odd numbers of carbon atoms such as C11:0, C13:0, C15:0, C15:1, C17:0 and C17:1. Such occurrence normally varies among

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https://doi.org/10.1016/j.biombioe.2017.10.035
Received 3 January 2017; Received in revised form 20 October 2017; Accepted 24 October 2017

different microalgae species.

After microalgae cultivation, the biodiesel production process includes harvesting, drying, cell disruption, lipid extraction and (trans) esterification [9,10]. One of the most critical steps of this process is the lipid extraction, which requires specific extraction systems that present high affinity for lipids such as organic solvents, supercritical fluids and ionic liquids [7,10–12]. According to Mercer & Armenta [7], the main bottleneck of using microalgae for lipid production is attributed to the lipid extraction procedure from the cells. These authors report different extraction methods with effective lipid recovery ranging from 2 to 59% in relation to dry mass of different microalgae.

The most common extraction processes are mechanical pressing, supercritical fluid extraction, enzymatic extraction, extraction by osmotic shock, Soxhlet (solvent) extraction and both microwave-assisted and ultrasound-assisted extraction procedures [6,8]. All of these methods rely on cell wall disruption and the subsequent release of lipids to the extraction medium [10].

Solvent extraction methods are based on solvent-solute affinity in which different types of interactions are needed to achieve high extraction yields. Non-polar organic solvents disrupt hydrophobic interactions (van der Waals forces) between non-polar/neutral lipids while polar organic solvents break down hydrogen bonds between polar lipids [13]. The most applied organic solvents for microalgae liquid extractions are: benzene, which presents high selectivity for non-polar lipids and the disadvantage of being flammable and toxic; petroleum ether, which has low melting point (35–38 °C) and is extremely flammable and toxic; ethyl ether, which has a low boiling point (34.6 °C) and a polar character if compared to petroleum ether but it is also extremely flammable and toxic; ethanol, though flammable, allows the extraction of both polar and non-polar lipids; and hexane, which although extremely flammable solubilizes non-polar lipids selectively. These last two are used mainly due to their low costs and low toxicity compared to the other solvents [7,9]. In general, a good solvent for lipid extraction must present the following characteristics: high affinity for lipids, low boiling point, low toxicity and good susceptibility to recovery and reuse [7].

Solvent mixtures that are used for the fast removal of polar and non-polar lipids include chloroform/methanol/water at volume ratios of 1:2:0.8 (vol/vol/vol) (method proposed by Bligh and Dyer [14]) and of 8:4:3 (vol/vol/vol) (method proposed by Folch [15]). Both of these methods are used to determine the total lipid content by cold extraction [16]. In laboratory scale, extractions are usually performed via Soxhlet and the most widely used solvent is hexane, which extracts mainly only non-polar/neutral lipids under long extraction times and high solvent consumption [17].

Ultrasound-assisted methods significantly improve oil extraction yields from microalgae compared to mechanical pressing [8]. In the ultrasound-assisted method, microalgae recovery is induced by cavitation. This phenomenon occurs when the propagation of ultrasonic waves in the extraction environment creates a pressure variation, leading to the formation of microbubbles in the liquid. As these microbubbles expand (negative pressure) and compress (positive pressure), a compression-expansion cycle is constituted (cavitation). When these microbubbles expand and compress with the pressure variation, they collapse violently close to the cell wall promoting rupture and the subsequent release of their intracellular content [7,8,18]. According to Cravotto et al. [19], this is a low-cost, highly efficient and fast extraction process that improves the extraction performance when compared to lipid extraction via Soxhlet with hexane.

A. obliquus (previously known as *Scenedesmus obliquus*) has been identified as a potential source of biomass for biodiesel production due to its robustness, tolerance to pH variation, easy cultivation in open ponds using wastewater and resistance to several associated bio-pollutants, all of these due to its robust and rigid cell wall. The composition of this microalga presents a protein content around 38 wt%, 27 wt% carbohydrates and 7–27 wt% lipids, depending on the conditions used for cultivation [20–23]. However, the solvent extraction process and its optimization for this type of microalga has been little explored in the scientific literature.

The main subject of this study was to investigate the lipid extraction of *Acutodesmus obliquus* using a mixture of non-polar and polar solvents (ethanol:hexane) at different volume ratios, in a Soxhlet apparatus and in ultrasound-assisted extraction procedures. Additionally, the results were compared to the performance of conventional methods (Bligh and Dyer) for the extraction of total microalgae lipids [14].

2. Materials and methods

Acutodesmus oblíquus was kindly provided by the *Núcleo de Pesquisa e Desenvolvimento de Energia Autossustentável* (Center for Research and Development of Self Supporting Energy) (NPDEAS/UFPR/Curitiba/PR). Ethanol 99.5% (Neon®) and hexane P.A. (Synth®) as well as other reagents were used in analytical grade. Statistical analysis was performed using Assistat 7.7 beta.

2.1. Biomass production using A. obliquus

Cultivation of *A. obliquus* was held in a compact photo-bioreactor (12.0 m^3) by photoautotrophic route using CO_2, water and 2.5% (vol/vol) of porcine bio-digested effluent waste as culture medium. The CO_2 source (0.04% vol/vol) was provided by the compressed air injected into the system through a column with 8.0 m of height and 0.11 m of diameter. After 15 days of inoculation, 1 m^3 of culture medium was harvested and the microalgae biomass was separated by flocculation using a tannin-based product (Tanfloc SG 200 mg L^{-1}, 7.5 pH) followed by centrifugation at 3000 rpm. The biomass was dried at 60 °C to decrease its moisture content to a constant value in the range of 5–8 wt%.

2.2. Lipids extraction

2.2.1. Soxhlet extractions

Approximately 20 g of microalgae were transferred to a filter paper thimble and allocated in the extraction chamber of a Soxhlet apparatus of 250 mL. Extraction was held for 1, 2, 4, 8 and 12 h. The solvents used for extraction were ethanol (EtOH), hexane (Hex) and a mixture of EtOH:Hex at volume ratios of 1:1, 2:1 e 1:2 (vol/vol), which corresponded to molar ratios of 71:0.76, 2.40:0.53 e 1.20:1.06, respectively. Initially, tests were conducted by a period of 12 h with an average cycle time of 30 min. After these extraction periods, the solvent was removed by rotaevaporation and the lipid extract was transferred to a tared bottle, which was kept in an air-circulating oven at 60 °C to remove any residual amount of solvent. After cooling to room temperature, the sample was weighed to determine the extraction yield and stored under refrigeration for chemical analysis. The extraction yield was calculated in relation to the dry mass of the original material.

2.2.2. Ultrasound-assisted extractions

Extractions assisted by ultrasound were carried out in an ECOSONICS ultrasound bath, model Q 59/36, with 5.7 L capacity (dimensions 27 × 12 × 12 cm), 37 kHz frequency and 165 W power. The ultrasonic bath was operated at 37 kHz and its power was set to 165 W.

Samples of 5 g were placed in a wide-mouth Erlenmeyer containing the extraction solvent. The mixture was homogenized, capped with a silicon stopper and submitted to extraction for 5, 10, 20, 40, 80 and 120 min at 30 and 60 °C. Solvents used for the ultrasound-assisted extraction procedure were ethanol (EtOH), hexane (Hex) and a mixture of EtOH: Hex at volume ratios of 1:1, 2:1 and 1:2 (vol/vol). After extraction, the samples were filtered through qualitative filter paper and the filtrate was transferred to a flask and evaporated in a rotary evaporator to remove the excess of solvent. The sample was kept in an air-circulating oven at 60 °C for a complete solvent removal. Then, it was weighed to determine the extraction yield and stored under