Renewable Energy 75 (2015) 366–373

Contents lists available at ScienceDirect

Renewable Energy

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

Extraction of free fatty acids from wet *Nannochloropsis gaditana* biomass for biodiesel production

Estrella Hita Peña, Alfonso Robles Medina*, María J. Jiménez Callejón, María D. Macías Sánchez, Luis Esteban Cerdán, Pedro A. González Moreno, Emilio Molina Grima

Area of Chemical Engineering, University of Almería, 04120 Almería, Spain

ARTICLE INFO

Article history:
Received 21 January 2014
Accepted 7 October 2014
Available online

Keywords:
Biodiesel
Microalga
Nannochloropsis gaditana
Fatty acid
Esterification

ABSTRACT

The objective of this work is to develop a process for producing biodiesel from the saponifiable lipid (SL) fraction of the wet microalgal biomass *Nannochloropsis gaditana*. The method consists of five steps. Firstly, crude fatty acid salt extraction was carried out using a KOH-ethanol (96%) solution, which allows one to extract the SLs as potassium salts. This transformation permits better separation of the unsaponifiable lipids (the second step) and finally produces purer biodiesel. The unsaponifiable lipids were then separated with hexane, after establishing the ethanol-water solution water content at 30% w/w. Some unsaponifiable lipids (carotenoids and phytosterols) are products of interest that might be purified from this fraction thus helping to improve the process's profitability. Thirdly, free fatty acids (FFAs) were purified by acidification of the ethanol-water solution to pH 5 and were then extracted with hexane. Fourthly, the FFAs were transformed to biodiesel by esterification with excess of methanol catalyzed using sulphuric acid, removing the excess by washing with hot water. Under these conditions the biodiesel purity and yield were 74.8% and 82% w/w, respectively. Finally, the biodiesel was clarified/purified up to 96.5% purity by adsorption with bentonite. The final biodiesel yield was 80.9%.

1. Introduction

The search for renewable transportation biofuels has been stimulated by the negative environmental impact of fossil fuels and concerns about petroleum supplies. Microalgae have recently been considered one of the best alternative sources of biodiesel [1]. The lipid composition of microalgae is qualitatively different from that of common vegetable oilseeds and the conventional technologies for processing them may be unsuitable for microalgae. Furthermore, the particular microalgae species and the time of harvesting greatly affect lipid composition. Microalgae biomass harvested in the exponential growth phase will contain more polar lipids than biomass harvested in the late stationary growth phase. Only saponifiable lipids (SLs) are transformable into methyl esters (biodiesel) but algae contain a high content of other lipids, as well as non-lipid components. Thus, only around 24–27 % of total lipids in *Isochrysis galbana* and *Phaeodactylum tricornutum* microalgae are neutral lipids (mainly acylglycerols, although neutral lipids also contain unsaponifiable wax ester components, hydrocarbons and sterols), while the remainder are polar lipids (phospholipids and glycolipids) [2]. All the unsaponifiable constituents create difficulties in the crude lipid refining process necessary for biodiesel production.

The extraction of lipids from microalgae has been repeatedly attempted using both physical and chemical methods, as well as a combination of both. A comprehensive review of lipid and fatty acid extraction procedures can be seen elsewhere [3]. Once the lipids have been extracted, the crude extract obtained must be purified before commencing the conversion of lipids to fatty acid methyl esters. Processes for refining crude algal lipid extracts, similar to those existing for terrestrial plants, have not been developed to date.

An alternative to lipid extraction for biodiesel is the direct extraction of fatty acids from biomass by means of direct saponification, which enables fatty acids to be obtained as potassium or sodium salts instead of as crude lipids [4–6]. Direct saponification is faster and cheaper than lipid extraction although more intensive operating conditions are necessary [7]. In a previous paper, a

* Corresponding author. Area of Chemical Engineering, Department of Engineering, University of Almería, 04120 Almería, Spain. Tel.: +34 950 015065; fax: +34 950 015484.
E-mail address: arobles@ual.es (A. Robles Medina).

http://dx.doi.org/10.1016/j.renene.2014.10.016
0960-1481/

comprehensive study was carried out for optimizing fatty acid extraction from wet *I. galbana* biomass followed by its purification [6]. Fatty acid extraction was performed using a three-step method: direct saponification of the wet biomass, followed by the extraction of unsaponifiable constituents, and finally the extraction of purified fatty acids. Even though the high water content of the wet microalgal biomass (about 80% w/w) affects the saponification yield, it is preferable using wet rather than dry biomass because biomass drying involves high costs both in terms of equipment and energy.

The goal of this work was to optimize the variables involved in the above-mentioned three-step process and to adapt it for the extraction and purification of fatty acids from the wet biomass of the microalga *Nannochloropsis gaditana*. The free fatty acids extracted were transformed into methyl esters (biodiesel) by acid-catalyzed esterification. Finally, these methyl esters were washed with hot water and decoloured/purified by adsorption with bentonite.

2. Materials and methods

2.1. Microalgal biomass and chemicals

Wet paste biomass from the marine microalga *N. gaditana* was used as an oil-rich substrate. Cells were grown in an outdoor tubular photobioreactor, centrifuged at 7000 rpm for 10 min, and then stored at −20 °C until the time of use. This wet biomass contained 20.4 ± 0.2% w/w of dry biomass and 23.1 ± 0.2% w/w of total lipid to biomass dry weight. The total fatty acid content (or saponifiable lipids as equivalent fatty acids) in the biomass was 10.8 ± 0.5% w/w of dry biomass. Table 1 shows the fatty acid composition of the wet paste biomass from *N. gaditana* used in this study. The chemicals used were analytical grade hexane (95% purity), ethanol (96% v/v) (aqueous ethanol), HCl (37%), H_2SO_4 (96% purity) and diethyl ether from Panreac S.A. (Barcelona, Spain), methanol (99.9% purity, Carlo Erba Reagents, Rodano, Italy), KOH (85% purity, J.T. Baker, Deventer, Holland), bentonite (Guinama, Alboraya, Spain) and distilled water. All reagents used in the analytical determinations were also of analytical grade. These reagents were acetone, ethyl ether (both from Panreac S.A., Barcelona, Spain), nonadecanoic acid (19:0) and its methyl ester (used as internal standards for gas chromatography (GC) analyses (Fluka Analytical, Sigma–Aldrich, St. Louis, MO, USA) and acetyl chloride, used as the sample methylation catalyst for analysis by GC (Fluka Analytical). For the determination of carotenoids by HPLC and spectrophotometry, β-carotene and lutein standards were provided by Sigma Chemical Co. (St. Louis, MO); violaxanthin and zeaxanthin were obtained from DHI LAB (Horsholm, Denmark) and neoxanthin was provided by ChromaDex LGC Standards (Barcelona, Spain).

Table 1
Fatty acid composition (percentage with respect to the total fatty acids of the biomass) of the wet paste biomass used from *Nannochloropsis gaditana*.

Fatty acids	wt %
14:0	3.0 ± 0.0
16:0	16.0 ± 0.1
16:1n7	17.1 ± 0.1
16:3n4	4.8 ± 0.0
18:1n9	3.4 ± 0.0
18:1n7	0.5 ± 0.0
18:2n6	8.0 ± 0.1
18:3n3	9.0 ± 0.0
20:4n6	4.7 ± 0.0
20:5n3	20.5 ± 0.1
Others	13.0 ± 0.1
∑ Saturated	19.4 ± 0.0
∑ Monounsaturated	21.0 ± 0.0
∑ PUFAs	47.3 ± 0.0
Total fatty acids (*)	10.8 ± 0.5
Total lipids[a]	23.1 ± 0.2

[a] Percentage of biomass dry weight.

2.2. Fatty acid extraction from wet biomass

2.2.1. Direct saponification of wet biomass

Fatty acid extraction was performed using the three-step method shown in Fig. 1. In the first step, potassium salts were formed from the fatty acids contained in the saponifiable lipid (SL) fraction of the microalgal biomass. In a typical experiment (Fig. 1), 24.5 g of wet biomass (equivalent to 5 g of dry biomass) were treated with 150 mL of aqueous ethanol, containing 1.0 g of KOH, in a 1 L reactor that was jacketed for temperature control. Saponification was carried out at 60 °C for 1 h with constant magnetic agitation in an argon atmosphere. The mixture obtained was then filtered through a 100–160 μm microporous glass filter (Pobel, Madrid, Spain) and the biomass residue was washed with 70 mL aqueous ethanol. In this step, the KOH to biomass ratio ranged from 0.2 to 1.6 g KOH per gram of dry biomass, and the aqueous ethanol to biomass ratio ranged from 5 to 76 mL aqueous ethanol (96%) per gram of dry biomass. This ratio was modified in the biomass residue washing step. Up to three washings were tested with 70 mL of aqueous ethanol each (i.e. 14 mL aqueous ethanol/g of dry biomass).

2.2.2. Extraction of unsaponifiable lipids

The fatty acids dissolved in the ethanol-water solution were purified extracting the unsaponifiable lipids with hexane. In a typical experiment, 36 mL of water was added to 200 mL of the fatty acid salt solution (14.5% w/w water and 27.4 mg/L of carotenoids) to obtain a solution with 30% w/w water; and then the unsaponifiable lipids were extracted at 20 °C by adding hexane and shaking (Fig. 1). The two phases were subsequently separated by decantation and aliquots of each phase were taken to determine carotenoids (hexane phase) and fatty acids (ethanol-water phase). Several experiments were carried out with different percentages of water in the ethanol-water phase (14.5–70 % w/w) with a view to maximizing the recovery of unsaponifiable lipids. A hexane to ethanol-water solution ratio of 1:1 (v/v) was used in this extraction. The optimal water content of the ethanol-water phase proved to be 30% w/w. At this ethanol-water solution, and on this small scale, up to three extractions of unsaponifiable lipids from the ethanol-water phase were tested, with a hexane/ethanol-water solution ratio of 1:1 v/v per step, in order to reduce the amount of carotenoids in the ethanol-water phase.

2.2.3. Extraction of purified fatty acids

In this third step, the pH of the 70:30 w/w ethanol-water solution was adjusted to values of between 1 and 6.5 using 37% HCl. Extractions of fatty acids were then performed in an argon atmosphere, at 20 °C, shaking for 10 min and using a hexane/ethanol-water solution ratio of 1:1 v/v. The phases were then separated and an aliquot of the hexane phase was taken for fatty acid determination.

2.3. Scaling-up of fatty acid extraction from wet microalgal biomass

The scheme shown in Fig. 1 was followed. 500 g of wet biomass was treated with 3075 mL of aqueous ethanol, containing 20.6 g of KOH in a 5 L reactor, which was jacketed for temperature control