Fuel 202 (2017) 512–519

Contents lists available at ScienceDirect

Fuel

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

Full Length Article

Obtaining biodiesel from microalgae oil using ultrasound-assisted *in-situ* alkaline transesterification

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HIGHLIGHTS

- Efficiency of in-situ alkaline transesterification of dry biomass.
- Ultrasound-assisted process and addition of different co-solvents to the reaction medium.
- When process was carried out with ultrasound-assisted (80 W), ester content increased to 97.6%.
- Ultrasound increased yield of *in-situ* alkaline transesterification of *Spirulina* sp. biomass.

ARTICLE INFO

Article history:
Received 13 February 2017
Received in revised form 6 April 2017
Accepted 8 April 2017
Available online 26 April 2017

Keywords:
Microalgae
Spirulina sp.
In-situ transesterification
Biodiesel
Ultrasound
Co-solvents

ABSTRACT

Efficiency of *in-situ* alkaline transesterification of dry biomass from a crop of *Spirulina* sp., using ultrasound-assisted and/or the addition of different co-solvents to the reaction medium was studied.

The starting biomass had a total lipid content of 7.0% (Folch method), comprised mainly by the following fatty acids: 16:0 (44.2%), 18:3 (n–6) (23.3%), and 18: 2 (n–6) (11.1%).

When the transesterification reaction was carried out without ultrasound, it only extracted 12.8% of lipids (0.9% of the biomass) after 2 h of reaction, achieving only a 63.6% ester content. When the process was carried out under the same conditions except for the ultrasound-assisted (80 W), the weight yield remained mostly unchanged, but the ester content increased to 97.6%. Additionally, when chloroform was added to methanol as a co-solvent in a 2:1 ratio it was possible to extract 43% of lipids. However, the ester content was reduced to 69.7%. Moreover, with maximum ultrasound power (180 W) a high ester content (96.9%) and a relatively high extraction yield (26%) were obtained, even without the co-solvent.

These results show that the ultrasound significant increased the yield of the *in-situ* alkaline transesterification of *Spirulina* sp. biomass, promoting a higher percent recovery of lipids as well as ester content from the starting material.

1. Introduction

Increasing energy consumption and the adverse effects of the indiscriminate use of fossil fuels are growing concerns. This problem has been a driving force for developing an array of alternative renewable energy sources [1].

Unlike other sources of renewable energy, that require long-term assessment, biodiesel technology is already known and only requires minor adjustments to adapt to local raw materials and production capacities. For this reason, adoption of biodiesel has gradually increased and is regarded as a viable alternative for curbing fossil-fuel use and complying with international agreements to reduce emissions of greenhouse gases [1].

The cost of raw materials accounts for about 75% of total costs of biodiesel production [2]. Therefore, the choice of suitable raw materials is crucial to minimizing production costs. Transesterification is the key reaction in biodiesel production, and triglycerides are the fundamental reagent. Traditionally, the main source of triglyceride has been vegetable oil, such as soybean, rapeseed, palm, sunflower oils. However, whether these materials should be used for biodiesel production or not is under debate, since they are predominantly used as edible oils.

Liquid biofuels can be categorized into different generations according to the type of raw material they are made from [1]. First generation biofuels come from vegetable oils (edible raw

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http://dx.doi.org/10.1016/j.fuel.2017.04.040
0016-2361/

materials), while the second generation ones come from non-edible raw materials, such as oils coming from *Jatropha curcas* L. [3–5], *Croton megalocarpus* [6], *Cerbera odollam* [7], castor-oil plant, macauba, mahua [8] and jojoba [9].

However, growing demand of these raw materials for the food industry, coupled with limited availability of land for these crops, render first- and second-generation biofuels unsustainable. Third generation biofuels, on the other hand, are derived from micro- and macroalgae, which prooves to be advantageous over the two preceding categories [10]. Microalgae are photosynthetic, microscopic organisms that are found in seawater and freshwater [11,12], and are able to convert 1.83 tons of atmospheric CO_2 into 1 ton of biomass [13]. Microalgae are considered attractive for biofuel production since they have greater photosynthetic efficiency, increased biomass production and faster growth, compared to traditional crops [14]. Furthermore, growing microalgae can be exceedingly simple. For instance, wastewater and land that is unsuitable for conventional agriculture can be used for this purpose [15,16]. Lipid accumulation inside cells is quite appealing, being around 25–75% on the dry basis for these type of microalgae [17]. Reported yields [15] are at least 10 times higher in microalgae compared to palm trees, the highest-yielding oil crop.

Interest in microalgae culture has grown considerably in recent years. Research in this area, particularly in efficient conversion of microalgae oils into biofuels has become a new frontier in the field of renewable energy. Microalgae culture is quite promising, since it can be done in ponds, open lakes or photobioreactors, has a high yield per square mile, and does not compete with traditional food crops [18].

Spirulina sp. is a filamentous, spiral-shaped, photosynthetic, and multicellular plankton. It belongs to the filum *Cyanobacteriae*, formerly known as blue-green microalgae due to the presence of green (chlorophyll) and blue (phytocyanins) pigments. This microflora thrives in alkaline saline waters, up to a pH of 11.0 and can exist in a variety of habitats, such as soils, freshwater, brackish and sea waters, hot springs, as well as in industrial and household wastewaters. It has a high amount of phytonutrients and pigments with applications in foodstuffs, therapies and diagnostics [19,20]. Although, *Spirulina* sp. is used mostly for food as supplement because it has small amount of lipids (which are mostly structural lipid) there are several references of it use as feedstock for biodiesel production [18,21]. For example, Nautiyal et al., 2014 [18] studied a single stage extraction–transesterification from *Spirulina* algae biomass with a total lipid content of 8.6%. In the optimum conditions was achieved a maximum biodiesel yield of 75%. However, there are not too many references to the ester content achieved.

Key processes in biodiesel production from microalgae include culturing, harvesting, biomass processing, extraction of lipids, and subsequent transesterification. Lipid extraction from the microalgae is the most complex and expensive of these processes [22]. Extraction of the microalgae oil requires a mechanical, chemical, or biological, cell disruption followed by some oil collection method by means of solvents. The main difficulty in oil extraction comes from the cell walls of some microalgae species, which are strong and thick, considerably hindering the oil extraction process. The water content of the microalgae further reduce the overall yield [22].

Oils from microalgae are usually converted into biodiesel by transesterification using methanol and different types of catalysts (alkaline or acidic catalysis, depending on the oil characteristics). However, recent studies have shown that *in situ* transesterification (direct method) may be a more efficient process. In this case, oil extraction and conversion into biodiesel occurs simultaneously [22].

In situ transesterification happens in a single stage, including both free fatty acid esterification and triglyceride transesterification from the biomass-derived oil. This simplifies the production process and improves the biodiesel yield in compared to the conventional extraction, since the fewer stages the less oil is lost [18].

The *in situ* transesterification reaction can be carried out both in presence as well as in absence of co-solvents [23,24]. However, the use of a co-solvent may be crucial for a successful *in situ* transesterification. On the one hand, it acts as an extraction agent, and on the other hand, forms a homogeneous system with the microalgae oil, methanol and the catalyst. Usually, stirring and heating are required to promote the reaction of transesterification. However, co-solvent-assisted *in situ* transesterification should require less energy in the form of heating or stirring [25].

Releasing the lipids contained inside the cells of the microalgae could be difficult during *in situ* transesterification, therefore large volumes of solvent are generally required. This takes a lot of energy and substantially increases costs. Another factor to be considered is that oil must be released and extracted without contamination from other cellular components, such as DNA or chlorophyll [26].

Several approaches have been proposed for the selectively disrupting the cell wall, for instance, using ultrasound, microwaves, enzymes and pressurized fluids [27–29]. Although the mechanisms behind each of these techniques are different, most involve rupturing the cells to release the lipids within the cytoplasm.

The principle behind the ultrasound method is the generation of sound waves that propagate through the fluid causing alternate cycles of high and low pressures. During the high pressure cycle, small bubbles, which were generated during the low pressure cycle, violently collapse and result in the phenomenon called cavitation [1]. High pressure and liquid velocities create shear forces upon the microalgae during cavitation, mechanically breaking the cellular structures and enhancing the transfer of extracted lipids. This methodology improves the lipid yield (output) between 50 to 500% and produces up to a 10-fold reduction in extraction times [1].

Martinez-Guerra et al., 2014 [30] studied the effect of ultrasound on the *in situ* transesterification of lipids from *Chlorella* sp. using ethanol as co-solvent for lipid extraction and as a reagent in the transesterification reaction. Optimal conditions were: a 1:6 to 1:9 ratio of microalgae:ethanol (wtv), 2% w/w of catalyst (sodium hydroxide) and a 6-min treatment time at 490 W of ultrasound. The weight yield was 18.5% and the conversion to ethyl esters was 95.0%.

On the other hand, Keris-Sen et al., 2014 [31] tested different ultrasound intensities (0.1–0.5 W/mL), at a 30 kHz frequency for 5 to 60-min cycles. They also studied the effect of ultrasound on the lipid extraction efficiency in presence of co-solvents (hexane or a chloroform/methanol mixture). The results showed that in the sample with the mixture of co-solvents and no ultrasound, 13.6% of lipids could be extracted from the dry biomass. However, the biomass still contained a significant amount of lipids that had not been removed (43.4%). When the extraction process was performed by ultrasound, the use of the co-solvents mixture managed to extract 26.8% of lipids from the dry biomass. Consequently, ultrasound significantly increased the extraction of lipids [31].

Therefore, additional information is required to evaluate this type of biomass used for biodiesel conversion, which needs a process that is much more complex than that traditionally used to obtain this biofuel from vegetable oils or animal fats.

In this study, there were shown results for *in situ* transesterification of dried biomass of *Spirulina* sp. Several variables related to the reaction system, as well as the components of the reaction mixture were studied. Addition of different co-solvents (chloroform and hexane), and the use of ultrasound in *in situ* transesterification method were evaluated. The ultrasound power and treatment