Bioresource Technology 238 (2017) 609–615

Contents lists available at ScienceDirect

Bioresource Technology

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

Continuous production of biodiesel from microalgae by extraction coupling with transesterification under supercritical conditions

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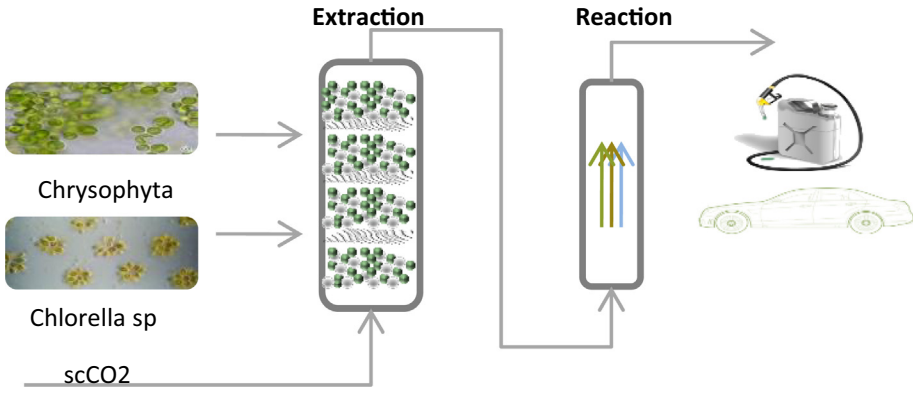
HIGHLIGHTS

- Two kinds of microalgae *Chrysophyta* and *Chlorella* sp. were selected as feedstock.
- A continuous process of transesterification coupling $scCO_2$ extraction was conducted.
- No catalyst loaded and residue after extraction can be reused for health products.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 February 2017
Received in revised form 22 April 2017
Accepted 24 April 2017
Available online 27 April 2017

Keywords:
Biodiesel
Microalgae
Supercritical fluid
Extraction coupling with reaction
Continuous

ABSTRACT

Raw material for biodiesel has been expanded from edible oil to non-edible oil. In this study, biodiesel continuous production for two kinds of microalgae *Chrysophyta* and *Chlorella* sp. was conducted. Coupling with the supercritical carbon dioxide extraction, the oil of microalgae was extracted firstly, and then sent to the downstream production of biodiesel. The residue after decompression can be reused as the material for pharmaceuticals and nutraceuticals. Results showed that the particle size of microalgae, temperature, pressure, molar ration of methanol to oil, flow of CO_2 and n-hexane all have effects on the yield of biodiesel. With the optimal operation conditions: 40 mesh algae, extraction temperature 60 °C, flow of n-hexane 0.4 ml/min, reaction temperature: 340 °C, pressure: 18–20 MPa, CO_2 flow of 0.5 L/min, molar ration of methanol to oil 84:1, a yield of 56.31% was obtained for *Chrysophyta*, and 63.78% for *Chlorella* sp. due to the higher lipid content.

1. Introduction

To avoid the crisis for the competition between human's food and feed stock of biodiesel, traditional biodiesel has a great rely on the edible oil, some researchers have attempted to make nonedible lipids as the feed for biodiesel, which include non-edible seeds from plants, waste oil and microalgae. That is Jatropha, chinaberry seeds, yellow fruit, *Sterculia foetida* and rubber tree, Mahua oil, castor oil, tobacco, etc. (Karmakar et al., 2010). It realizes the process of waste recovery and reuse that turn waste oil to biodiesel, and reduce the pollution for environment. However, most of them have high acid value and contain too many impurities which are kinds of polymers as well as degradation products, and the supply has a

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http://dx.doi.org/10.1016/j.biortech.2017.04.097
0960-8524/

great fluctuation, which is hard to use as the stable raw oil for large-scale industrialization. Microalgae cultivation has low requirement for space and high efficiency of photosynthesis. They have been considered as a bright prospect for biodiesel, as can be produced using saline and waste water with a remarkable growing rate and product yield. Compared with other material, microalgae has obvious advantages which are easy to cultivate, using non drinking water, transition solar to chemicals (Galadima and Muraza, 2014), and short growth period. Moreover, sulfur free feed also reduce the sulfide in the tail gas. Algae have much application value in Pharmaceuticals, Nutraceuticals, Cosmetics and Aquaculture purpose. Besides biodiesel, methane, ethanol and hydrogen can also be generated from microalgae. Due to the high content of polyunsaturated fatty acids such as linolenic acid, EPA and DHA, abundance of vitamins, minerals, and trace elements, they can be made as the feed of health products (Chauton et al., 2015). Recent research works on microalgae have identified this new bio-material as a promising technology for bioenergy production, wastewater treatment, the development of high value added products and CO_2 capture. Although the oil content of microalgae is similar to other feeds, the global annual output is larger.

One of the first issues is extracting oil from microalgae in order to transfer the microalgae oil to biodiesel. The most common extraction processes are solvent extraction, ionic liquid extraction, and subcritical water extraction and so on, in which solvent extraction is the most mature technology and have general use in industrial. However, the toxicity of organic solvent and the high energy consumption for recovery make it harmful for the environment. In particular, the residue can be used as the material for pharmaceuticals and nutraceuticals. $ScCO_2$ as a clean and green solvent, easy to separate with extracts and no residue in product, all these characters made it a suitable solvent for the extraction of microalgae (Halim et al., 2012).

Andrich using $scCO_2$ to extract oil from microalgae and obtained an extraction curve (Andrich et al., 2005). As the extraction time prolong, the extraction yield decreased. Within 5000 s, the extraction amount was over 80%, while exceeding 10,000 s, the extraction amount was not increasing a lot, and the trend of increase become slower. That's because the driving force of extraction is the oil concentration between bulk $scCO_2$ and the inner cell. The initial concentration difference is large, so the extraction speed was fast, extend the extraction time, the concentration difference become smaller and the driving force reduced, so the tendency become flat. Taher studied the extraction conditions for microalgae (Taher et al., 2014), and found that the temperature and pressure has strong impact on the extraction yield, but less effect on the extraction yield. The optimal extraction conditions are temperature at 53 °C, 50 MPa pressure and the flow of CO_2 was 1.9 g/min, and the extraction yield reached 7.41 wt% (dry algae). Solana utilized the ethanol as the entrainer to improve the extraction yield (Solana et al., 2014). The results showed that the extraction yield reached the highest at the temperature 60 °C, 30 MPa pressure, and CO_2 flow 0.4 kg/h and 5% ethanol. Furthermore, there is a crossover pressure 25 MPa for extraction algae oil. That is when the pressure above the crossover pressure, the key factor for extraction is the vapor pressure of solute rather than the density of CO_2. Cheng et al. compare the extraction with organic solvent and $scCO_2$, ethyl acetate and methanol mixed solvent has an extraction yield of 98.7%, much more than the extraction for $scCO_2$, which is 61.6% (Cheng et al., 2011). But ball-milling pretreatment can enhance the $scCO_2$ extraction to 98.7%. Millao et al. investigated the extraction of oil and carotenoids from *Nannochloropsis gaditana* using supercritical carbon dioxide (Millao and Uquiche, 2016), by response surface methodology, the maximum oil yield 152.2 g/kg dry substrate was gained at 64 °C and 59.3 MPa. They found that temperature had a greater effect than CO_2 density.

The extracted oil can be used to prepare biodiesel by supercritical methanol transesterification method, and its yield was affected by the operation conditions, such as temperature, reaction time, ratio of alcohol to oil (ratio of methanol to algae) and pressure. Reddy conducted the transesterification of algae (*Nannochloropsis salina*) in the condition temperature 265 °C, 20 min, dry algae to ethanol 1:9 (wt/v), and a maximum yield 67% was obtained (Reddy et al., 2014). Patil also used this one-step process for direct liquefaction and conversion of wet biomass containing about 90% of water to biodiesel under supercritical methanol conditions (Patil et al., 2011), in the optimal condition: wet algae to methanol (wt/vol) ratio of around 1:9, reaction temperature and time of about 255 °C, and 25 min lead to yield above 80%. They used the *Nannochloropsis salina*, too. Due to the deviation of species and culture environment, the ingredients of microalgae are different, but the free fat acid and water content have a great impact on the production process. When algae changed to *Nannochloropsis gaditana* as feed (Jazzar et al., 2015), only 48% yield was received in the optimized condition at 255–265 °C, 50 min reaction time, and using a methanol to dry algae ratio of 10:1 (vol/wt)

Batch reactions were employed in the above work. Continuous flow process (He et al., 2007; ZHOU et al., 2010) can be brought so as to provide much bigger manufacturing scale, and cut the cost. Nan et al. studied the continued production of biodiesel through non-catalytic transesterification of microalgae oil with methanol and ethanol (Nan et al., 2015), and optimization of continuous process by RSM showed that the best condition were 320 °C, 15.2 MPa, 19:1 M ratio, 31 min, 7.5 wt% of water content for methanol transesterification, for ethanol, that is 340 °C, 17 MPa, 33:1 M ratio, 35 min, and also 7.5 wt% of water content. The corresponding yields of fatty acid methyl ester (FAME) and fatty acid ethyl ester (FAEE) were 90.8% and 87.8%, respectively.

To our knowledge, little has been reported in regard to coupling supercritical CO_2 ($scCO_2$) extraction and continuous transesterification for the microalgae. Most works are focusing the in-situ transesterification, although the in-situ process can utilize the wet microalgae directly, the residue after the reaction can't be used again as the raw material of health products. That is a waste for the other ingredients of algae. Therefore, in this work, the continuous supercritical methanol transesterification coupling with $scCO_2$ extraction without catalyst for the production of biodiesel was investigated, and two kinds of microalgae *Chrysophyta* and *Chlorella* sp. were adopted as the feed stock. This work aims to expand the feed of biodiesel, and utilize the green solvent CO_2 so as to recover and reuse the residue. Through the investigation of the influence parameters of supercritical extraction and continuous transesterification for microalgae, the optimal operation conditions were obtained. We expected to expand and comprehensively utilize the raw material of biodiesel, and realize the continuous production in industry.

2. Materials and methods

2.1. Materials and instruments

Chrysophyta and *Chlorella* sp. were provided by Dalian institutions of Chemical Physics, with fatty acid contents of 10.5 wt% and 19.8 wt% respectively (analyzed by in-situ transesterification method due to the reason that the lipids extracted by organic solvents for microalgal case are very complicated, including the components without the fatty acid chains such as lipid-soluble pigments, sterol and so on, which cannot be converted to FAME, and the lipid content obtained by extraction method is usually higher than the actual value of the lipid components that could be converted to FAME) (Liu et al., 2015). Their GC profiles and