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Production of biodiesel from macroalgae by supercritical CO₂ extraction and thermochemical liquefaction

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Abstract We compare two different techniques for the extraction of biodiesel from macroalgae: the thermochemical liquefaction and the extraction using supercritical carbon dioxide (sc-CO₂). The first allows to use wet material, while sc-CO₂ requires dry material and uses moderate temperature and pressure so that it can be useful for the extraction of thermolabile compounds which may decompose at the temperature at which thermal methods are carried out. In both cases the extracted oil was characterized quantitatively and qualitatively. The novelty of the work is that in the literature the use of macroalgae for the production of biodiesel has not so far been described, while they are used mainly as food or other purposes.

Keywords Biodiesel · CO₂ · Biomass · Macroalgae · Thermochemical liquefaction · Supercritical carbon dioxide

Introduction

The interest towards alternative energy sources pushes the scientific community to implement technologies that use aquatic biomass for the production of biofuel. Biomass is considered a renewable source at zero emission as it fixes CO₂ from the atmosphere through photosynthesis. So, the replacement of fossil fuel with biomass can contribute to the reduction of the emission of CO₂ reducing the burning of the fossil carbon. In order to make possible the application of the new technology at industrial level several problems, such as the optimization of the technology efficiency and quality of the oil produced, must be solved. Relevant to marine biomass, several papers report on the use of mi-

croalgae for the production of biodiesel, but no information was available so far about the use of macroalgae.

One of the main advantages of biodiesel is that it can be used in the existing diesel engines without modification differently from hydrogen, another alternative energy source that requires specific engines. Chemically, biofuel comprises a mixture of mono-alkyl esters of long chain fatty acids. To convert all fatty acid components into the monoesters a transesterification process is used. The relative biodiesel obtained has combustion properties very similar to those of petroleum diesel, and can replace it in most current uses. At the present it can be used as additive to petroleum diesel, to improve the low lubricity of pure ultra low sulphur petrodiesel fuel. Considering the biodiesel from the environmental point of view, it includes several benefits as the reduction of carbon monoxide (50%) and carbon dioxide (78%) emissions (Sheehan et al. 1998), the elimination of SO₂ emission as biodiesel does not include sulphur, the reduction of particulate. As biodiesel is non-toxic and biodegradable its use and production is rapidly increasing, especially in Europe, United States, and Asia. A growing number of fuel stations are making biodiesel available to consumers, and a growing number of large transport fleets use some proportion of biodiesel in their fuel. Companies, including FIAT, produce tractors fuelled with biodiesel. Several large size refineries (10 ML/y) (Bender 1999) are already operated in Austria, Germany and France; smaller plants are under construction in France, Germany, Czech Republic, Denmark, Austria and Italy.

In this paper macroalgae from the Mediterranean Sea were used for the extraction of fuel similar to that obtained from terrestrial plants (fast growing) or seeds. Indeed, the use of algae is of great interest for their higher photosynthetic activity with respect to terrestrial plants, the easy adaptability to different growing conditions, the possibility of growing either in fresh-waters or marine-waters, avoiding the use of land. Two technologies (liquefaction and extraction) have been used. Liquefaction of algae uses moderate temperature and pressure and, most interesting, wet material can be used. The second technology that has been tested is the supercritical fluids

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extraction, in particular sc-CO₂. The reason why such technologies were selected is to compare the influence of using wet or dry material on the energetic balance. One of the most important issues with algae utilisation for energy purposes is their high content of water (up to 80–90%).

Experimental

Materials

All reagents and solvents were commercial products. Qualitative analyses were made by using a gas chromatograph (capillary column: 30 m; MDN-5S; Ø 0.25 mm, 0.25 µm film) coupled to a mass spectrometer. Quantitative determinations on the reaction solutions were recorded using a GC-FID (capillary column: 30 m; MDN-5S; Ø 0.25 mm, 0.25 µm film).

Gas analyses were carried out by using a GC-TCD (packed column Chromosorb SII).

For thermochemical liquefaction a stainless steel autoclave (70 ml) equipped with a pressure gauge connected to an electronic device to control the temperature was used.

The sc-CO₂ extraction was carried out using an equipment fully computer controlled for temperature and pressure. The range of the working conditions was: temperature up to 500 K, pressure up to 33 MPa.

Chaetomorpha linum (O.F. Muller) Kutzing (Cladophorales, Chlorophyta) was used. This green alga, a cosmopolitan species, was taken from Mar Piccolo of Taranto, near the Galeso river (southern Italy, Mediterranean Sea). The thalli of *C. linum* have a siphonocladous level of organization, i.e., with thick unbranched filaments made of multinucleate cells. The cell wall has an outer lamellar part mainly made of a highly crystalline cellulose and an inner amorphous matrix made of a complex branched arabino-galactan, a polymer of arabinose and galactose, together with some xylose (Hoek van den et al. 1995). It lives as unattached form in both the estuarine systems and coastal lagoons subject to eutrophication.

The fresh alga was washed with fresh water to remove the salty sea water prior to use.

Description of the liquefaction methodology

Almost 20 g (fw) of fresh, washed thalli were placed into the glass reactor which was then put into the autoclave. The latter was closed under N₂ atmosphere to purge the residual air and 3.0 MPa of N₂ was charged to control the evaporation of water during the liquefaction process.

The autoclave was then heated to the desired temperature (250–300–350 and 395°C) for 1 h. After that time the autoclave was cooled to room temperature and depressurized. The gas fraction was transferred into a cylinder and analyzed by GC-TCD. The autoclave was then opened, and the reaction mixture recovered and treated with CH₂Cl₂ (3×5 ml). A double phase was formed, an organic and an aqueous/solid suspension that were separated. From the organic solution, after evaporation of the solvent under

controlled conditions, an amber-yellow oily liquid was obtained. The aqueous solution was separated and a solid was recovered by centrifugation.

Description of the sc-CO₂ extraction methodology

For the sc-CO₂ extraction the thalli were dried for 5–8 days at room temperature, far from direct sun-light. 3–5 g of dried algae were milled in liquid nitrogen (5 ml) to break the cellular wall in order to increase the extraction yield. The fine powder obtained was then used for the sc-CO₂ extraction tests.

Three grams of fine powder of algae were placed into the glass reactor that was placed into the stainless steel tube and introduced in the sc-CO₂ apparatus. 0.5–1 ml of methanol were used as co-solvent to improve the efficiency of the extraction. After several tests were carried out varying temperature and pressure the optimal operative conditions were fixed at 50°C and 2.60 MPa for 7 h. After that time the apparatus was cooled to room temperature and depressurized condensing the gas phase in methanol. The residue, after the opening of the system, was recovered and washed with methanol to extract the oil phase. After the evaporation of methanol under reduced pressure an amber-yellow liquid (biodiesel) was obtained.

Transesterification process

In both cases the oil obtained was treated as described in literature (Carreau and Dubacq 1978) to convert all fatty acid components into the mono-methyl-esters for an easier determination.

Characterization of the oil

The extracted material was analyzed quantitatively by GC and qualitatively by GC-MS. The identification of the species extracted was carried out comparing the relevant retention time and the mass fragmentation with that of pure species used as standards. As internal standard methyl ep-tanoate was used.

Analyses were done in duplicate and data are reported as means of three measurements.

Results and discussion

Quality of biofuel

A variety of biomass can be used to produce biodiesel. The most commonly used are rapeseed and soybean, but also other crops such as mustard, palm oil, hemp, waste vegetable oil, and animal fats. Even algae are promising source of biodiesel.

As far as the biofuel quality is concerned, it is worth to note that biofuel extracted from plant seeds is affected

Table 1 Most abundant species identified by using thermochemical liquefaction

| Fatty acid methyl esters | Liquefaction temperature (°C) | | | |
|------------------------------|-------------------------------|-----------|-----------|----------|
| | 250 | 300 | 350 | 395 |
| methyl miristate (14:0) | 7.4±0.02 | 4.3±0.1 | 3.7±0.05 | 3.5±0.06 |
| methyl palmitate (16:0) | 21.5±0.01 | 13.4±0.14 | 10.9±0.62 | 9.8±0.4 |
| methyl palmitol. (16:1) | 3.2±0.15 | 2.4±0.02 | 2.3±0.12 | 2.0±0.03 |
| methyl stearate (18:0) | 2.4±0.08 | 2.3±0.12 | 1.5±0.2 | 1.4±0.2 |
| methyl oleate (18:1) | 14.9±0.48 | 11.2±0.58 | 8.3±0.13 | 8.2±0.01 |
| methyl linoleate (18:2) | 14.4±0.55 | 6.1±0.18 | 2.0±0.29 | 1.2±0.2 |
| methyl eicosan. (20:0) | 0.7±0.04 | 0.6±0.10 | 0.4±0.08 | 0.3±0.08 |
| methyl arachid. (20:4) | 1.2±0.21 | 0.9±0.10 | 0.3±0.1 | 0.1±0.03 |
| methyl eicosapentaen. (20:5) | 1.6±0.29 | 1.4±0.25 | 0.6±0.28 | 0.3±0.02 |
| methyl tetracosanoate (24:0) | 0.7±0.23 | 0.3±0.01 | 0.2±0.01 | 0.1±0.01 |
| Others ^a | 15.6±0.16 | 10.6±0.23 | 7.5±0.62 | 4.8±0.38 |
| Eptadecane | 0.2±0.02 | 0.3±0.06 | 0.3±0.01 | 0.3±0.01 |
| Phytols | 5.9±0.16 | 4.8±0.1 | 3.6±0.30 | 2.9±0.05 |

^aOther FA identified (15:0, 17:0, 14:1, 16:1, 16:2, 16:3, 18:3, 18:4, 20:2, 22:5)

by a quite high content of natural gums (phosphatides) that causes plugging and gumming of filters and injectors in engines. Conversely, free fatty acids can be directly extracted from several algal species (Cryptophyceae, Prasinophyceae, Prymnesiophyceae) obtaining a good quality oil (low P and negligible S content) with yield ranging from 16 to 24 wt% of dry matter (Renaud et al. 1999).

Oil can be obtained from *Dunaliella tertiolecta* (a microalga growing in sea water) with 37% yield (average value). It has a calorific value of 36 kJ/g (Minowa et al. 1995), comparable to that of fossil fuel oil (45 kJ/g) or coal (28 kJ/g) and significantly higher than the average value (18 kJ/g) of oil produced by dogbane, kudzu, and eucalyptus tree (Kumar and Pratt 1996).

Liquefaction of algae

Within the thermochemical technologies for biofuel production from algae, liquefaction was selected because of its lower energy input requirements (the heating temperature is usually 200–300°C versus 500°C required for pyrolysis and 2000°C for gasification). Another advantage of liquefaction with respect to other technologies is the possibility of using biomass with high moisture content like are algae (>80–90 wt%).

As in the liquefaction process a very important factor is the temperature, in order to ascertain its role on the yield, different reaction temperatures (250, 300, 350, and 395°C) were tested. As is shown in Fig. 1, increasing the temperature the total amount of oil extracted from the algae increases reaching a plateau at 350–395°C.

The composition of the oil was also studied and the most abundant species were identified by comparison with authentic samples. Table 1 gives a list of most abundant fatty acids as their methyl esters.

The Table 1 data show that increasing the temperature affects the amount of FA extracted. As a matter of fact, at higher temperature some oil was decomposed resulting in a decrease of the recovered amount of extracted FA (Table 1).

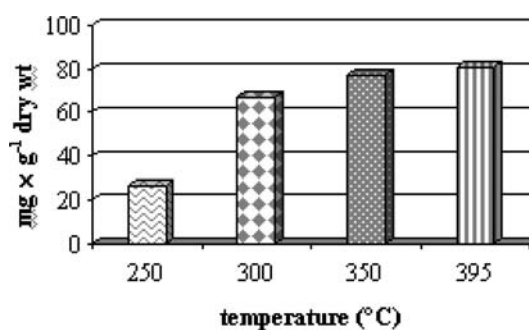


Fig. 1 Effect of temperature on the yield of oil extracted

A detailed study is being carried out on the oil in order to understand the effect of temperature and to characterize compounds formed upon FA conversion.

Extraction with sc-CO₂

Particular emphasis has been put on setting-up a procedure based on the use of sc-CO₂ as extractant as it can be easily recycled. Moreover sc-CO₂ combines an environmentally benign character with favourable physico-chemical properties. The sc-CO₂ extraction shows several advantages as it reduces the environmental impact and represents an alternative route for the recycling of carbon dioxide. The sc-CO₂ is not toxic and does not require high temperature and pressure (critical temperature and pressure are 31.1°C and 7.38 MPa, respectively). So it is possible to extract thermolabile compounds which decompose at higher temperature.

The benefits derived from its use are well known today, so that its utilisation is spreading in various industrial sectors. It is used in many chemical applications, such as the decaffeination of coffee beans (Anastas et al. 1996), the extraction of fragrances and essences from vegetals, or proteins (Wahbeh 1997) or fatty acids and hydrocarbons from algae (Aresta et al. 2003).

Because of the low permeability and resistance of the cell membrane of the algae, the efficacy of the sc-CO₂

Table 2 Most abundant species identified by using sc-CO₂

| Fatty acid methyl esters | % |
|---------------------------------|-----------|
| methyl miristate (14:0) | 9.8±0.1 |
| methyl palmitate (16:0) | 32±0.22 |
| methyl palmitoleate (16:1) | 3.3±0.18 |
| methyl stearate (18:0) | 1.5±0.08 |
| methyl oleate (18:1) | 14.2±0.38 |
| methyl linoleate (18:2) | 21.4±0.46 |
| methyl eicosanoate (20:0) | 0.3±0.07 |
| methyl arachidonate (20:4) | 1.6±0.15 |
| methyl eicosapentaenoate (20:5) | 2.6±0.26 |
| methyl tetracosanoate (24:0) | 0.6±0.22 |
| Others ^a | 18.3±0.27 |
| Phytols | 8.8±0.19 |

^aOther FA identified (15:0, 17:0, 14:1, 16:1, 16:2, 16:3, 18:3, 18:4, 20:2, 22:5)

extraction depends on the pre-treatment of the algae (liquid N₂ crashing of cells). Moreover, this methodology does not allow to use directly fresh-wet biomass, but a soft preliminary dry treatment (using solar energy) is required.

By using sc-CO₂ the total amount of the extracted oil is around 45 mg × g dry wt which was determined using the same procedure as above. Table 2 shows the amount of each identified FA-ME.

Comparison

We have shown that in *C. Linum* unsaturated and saturated fatty acids are present with a number of C atoms within a range between 14 and 24, in addition to hydrocarbons and phytols. Considering both the technologies two aspects have to be taken into consideration such as the amount of extracted oil and its FA composition.

In fact, if one compares the amount of oil extracted with both the technologies it comes out that the temperature affects the quantitative characterisation as the sc-CO₂ extraction is less effective than the thermochemical liquefaction. Moreover, within the range of temperatures used for thermochemical liquefaction the highest seems to be more efficient as the total yield of extracted oil is higher. Nevertheless, we have shown that increasing the temperature, the conversion of long chain FA into short chain acids may occur, and in fact the amount of characterised FAs is lower at higher temperature.

The two technologies show a different extraction capacity in the sense that, as shown in Tables 1 and 2, the extraction with sc-CO₂ allows to obtain a higher amount of long chain FA, while the liquefaction gives a higher amount of oily material. Also, the isolated yield of poly-unsaturated species (18:2, 20:4, 20:5) is higher with the sc-CO₂ extrac-

tion with respect to the thermochemical liquefaction. This can be due to the fact that long chain FA are cleaved at high temperature under the liquefaction conditions.

Conclusion

The extraction of biodiesel from a macroalga, *C. Linum*, has been carried out comparing two technologies. In both cases the oil obtained has been analysed and its composition determined. Thermochemical liquefaction requires temperature around 350 and 395°C in order to have the optimal amount of extracted oil. Nevertheless, its composition depends on the working temperature and the content of long chain FA is higher at lower temperature as decomposition may occur at higher temperatures. Between the technologies used, the thermochemical liquefaction seems to be more efficient than the extraction with sc-CO₂ from the quantitative point of view but decomposition of the FA may occur under the operative conditions.

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