Energies **2012**, *5*, 243-257; doi:10.3390/en5020243

OPEN ACCESS

energies

ISSN 1996-1073

www.mdpi.com/journal/energies

Article

Influence of *n*-Hexane on *in Situ* Transesterification of Marine Macroalgae

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Received: 20 December 2011; in revised form: 13 January 2012 / Accepted: 2 February 2012 / Published: 6 February 2012

Abstract: The purpose of this work is to investigate the influence of *n*-hexane addition on *in situ* transesterification of a solid raw material for biodiesel production. Extraction and reaction of macroalgae oil has been performed simultaneously in a batch reactor adding *n*-hexane with the reactants. In order to analyze the influence of *n*-hexane on the transesterification, the reaction was also carried out with sunflower oil. The results show that the presence of *n*-hexane does not have an important effect on the transesterification. It was also observed that this method requires large quantities of methanol to carry out the reaction. The best reaction conditions for *in situ* transesterification of marine macroalgae were 300:1 methanol-to-oil molar ratio, 1% catalyst concentration, 60 °C reaction temperature and 11 h reaction time, resulting in a methyl esters yield of 17.1%. Thus, biodiesel production from macroalgae by transesterification *in situ* could be feasible, using hexane for the extraction and eliminating the previous extraction. This integrated method is thus effective and technically attractive.

Keywords: macroalgae; sunflower; *in situ* transesterification; biodiesel; *n*-hexane

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1. Introduction

Biodiesel is a new energy source that has grown in importance over recent years [1]. Nowadays, used vegetable oils are potential renewable sources for the production of biodiesel as an alternative to petroleum based diesel fuel, which is derived from diminishing petroleum reserves and which has environmental consequences caused by the exhaust gases from diesel engines [2]. Biodiesel has several benefits such as a reduction in greenhouse gas emissions: it reduces emissions of carbon monoxide by about 50% and emissions of carbon dioxide by about 78% [3]. Moreover biodiesel is nontoxic and biodegradable, so using biodiesel instead of diesel reduces fossil fuel consumption. Furthermore, biodiesel is produced from a range of vegetable oils (such as soybean, rapeseed and sunflower) and animal fats [4,5], and can be used in diesel engines blended with petroleum diesel or on its own.

Biodiesel is obtained from a renewable source, so it is an important tool for combating environmental degradation [6,7]. However, a global debate has now emerged because this fuel is currently derived primarily from soybean oil or other cereals, and using food to produce fuel is considered unreasonable when the increasing world population is taken into account [8]. For this reason, several authors have studied the production of biodiesel from other sources (Table 1), such as used frying oil [2,9,10], coffee grounds [11], animal tallow [12–14] or microalgae [15].

Table 1. Composition of Biodiesel Obtained from Different Sources.

Source Material	14:0	16:0	18:0	18:1	18:2	18:3	20:5	20:0
Olive [16–18]	1.30	7.00–18.30	1.40–3.30	55.50–84.50	4.00–19.00			
Beef Tallow [16–18]	3.00–6.00	25.00–37.00	14.00–29.00	26.00–50.00	0.60–1.80	1.00–2.50		
Sunflower [16–18]		3.50–6.50	1.30–5.60	14.00–43.00	44.00–68.70			
Cottonseed [16–18]	0.80–1.50	22.00–24.00	2.60–50.00	19.00	50.00–52.50			
Spent Coffee Grounds [11]		7.23	9.39	9.71	10.38		11.38	12.56

In this work a solid raw material, marine macroalgae, is used for biodiesel production. This would offer a possible alternative to current biodiesel production methods that mainly use foodstuffs as raw materials. The new material sourced from the sea could avoid economic conflicts caused by the current situation. In turn, it is already known that production of biodiesel would lead to a decrease in dependence on petroleum in the automobile sector, and also to a decrease in atmospheric contamination produced by pollutant gas emissions proceeding from the consumption of fuels derived from the oil which the new material would replace.

The problem of using this kind of raw material is that the industrial process involves the isolation of oil by extrusion or solvent extraction and refining of the oil before its alkali-catalyzed transesterification. The most typical method is extraction with hexane. This previous step adds a significant increment in time and cost to the process, which makes reduction or elimination of oil extraction an interesting option.

In situ transesterification differs from the conventional reaction in that the original material is used directly instead of purified oil to produce biodiesel [19–22]. That is, extraction and transesterification proceed in one step. This could reduce the long production sequence associated with pre-extracted oil and maximize alkyl ester yield. The use of reagents and solvents is reduced, and the concerns about waste disposal are avoided [23].

In situ transesterification has been used for different raw materials: in Harrington and D'Arcy-Evans [24], sunflower seeds were transesterified under reflux conditions with an excess of methanol relative to the number of moles of triacylglycerols (TAG) present using sulfuric acid as catalyst. Qian *et al.* [20] reported the *in situ* alkali-catalyzed transesterification of cottonseed oil for biodiesel production. Georgogianni *et al.* [19] applied *in situ* alkali-catalyzed transesterification to sunflower seeds, using methanol and ethanol as reactants and NaOH as catalyst. However, the conversion of macroalgae to FAME by alkali-catalyzed *in situ* transesterification has not been reported. For that reason, in this work the transesterification of macroalgae has been carried out directly with *n*-hexane addition, and its effectiveness will be also investigated. In a previous work [8], fourteen species were analyzed to determine their oil content. Based on obtained results, a mix of two macroalgae (*Pelvetia canaliculata* and *Fucus spiralis*) in the same proportion has been used in this research.

2. Materials and Methods

2.1. Oil Characterization

In this work two types of Galician marine algae (*Fucus Spiralis* and *Pelvetia Canaliculata*) were collected from Galician beaches, washed with water and sun-dried for three days, since water inhibits transesterification. After that, the dried algae were crushed in two steps in order to obtain a fine powder. After this pretreatment, the small solid particles of macroalgae were introduced in a Soxhlet apparatus with the purpose of determining the algae oil content.

2.1.1. Oil Extraction

To determine the algae oil content, *n*-hexane (300 mL) supplied by Panreac (Barcelona, Spain), was used for 50 g of dried algae (25 g of each algae), for the oil extraction. The extraction was carried out in a Soxhlet apparatus for 4 h according to UNE-EN ISO 734-1 [25]. All the experiments were carried out using a 0.5 L round-bottomed glass flask. The *n*-hexane was separated by distillation. The solvent was reused in the next extraction batch. Finally, the sample was dried in an oven a (100 °C) until constant weight was achieved.

2.1.2. Analysis of Fatty Acids

The determination of the fatty acids was carried out using a gas chromatograph via injection of the previously transesterified oil. Biodiesel is composed of monoalkyl esters of long chain fatty acids derived from renewable lipids such as oils vegetables or animal fats, but all the oils do not contain the same type of fatty acids. For that reason, it is very important to determine the fatty acids in the oil. Moreover, starting from the fatty acid methyl esters (FAME), the molecular weight and the iodine index of oil can be calculated. The transesterification and gas chromatography procedures are explained in the following sections.

2.1.3. Preparation of Fatty Acid Methyl Esters

This method can only be applied when the chromatography analysis is performed at higher temperatures according to UNE-EN ISO 5509:2000 [26]. The method is based on the test sample dissolution in methyl *tert*-butyl ether and the preparation of the methyl esters by transesterification with trimethylsulphonium hydroxide (TMSH). The process is carried out in the following way: 10 mg ± 2 mg of the oil sample are weighed directly into a test tube with a precise analytical balance. Then, in the same tube 500 µL of methyl *tert*-butyl ether of 99% purity are added with a micro syringe (0–500 µL) dissolving the sample. Next, 250 µL of methanolic solution of 0.2 mol/L of trimethylsulphonium hydroxide are added and the mixture shaken vigorously during approximately 30 seconds. The obtained solution is ready for injection in the gas chromatograph.

2.1.4. Analysis of Fatty Acids Methyl Esters

Different parameters, such as molecular weight and iodine index, were calculated from the fatty acid methyl ester content of the oil. The fatty acid methyl ester content was quantified using a gas chromatograph Trace GC-Ultra [27,28] connected to an Innowax capillary column (60 m × 0.25 mm × 0.25 µm), from Agilent Technologies.

The temperature program was as follows: 50 °C for 2 min and raised to 240 °C at a rate of 10 °C/min and maintained for 27 min. The injector was set up for 240 °C and the FID detector at 220 °C. Helium was used as carrier gas, at constant flow of 1 mL/min. The analysis was carried out by diluting the biodiesel (diluted to 1 µL by adding 1000 µL of hexane), and 0.5 µL of this solution was injected through the column. Nonanoic acid methyl ester was used as an internal standard. A typical chromatogram is shown in Figure 1. Retention times for fatty acid methyl esters are given in Table 2.

Figure 1. Sample of cromatogram of macroalgae oil.

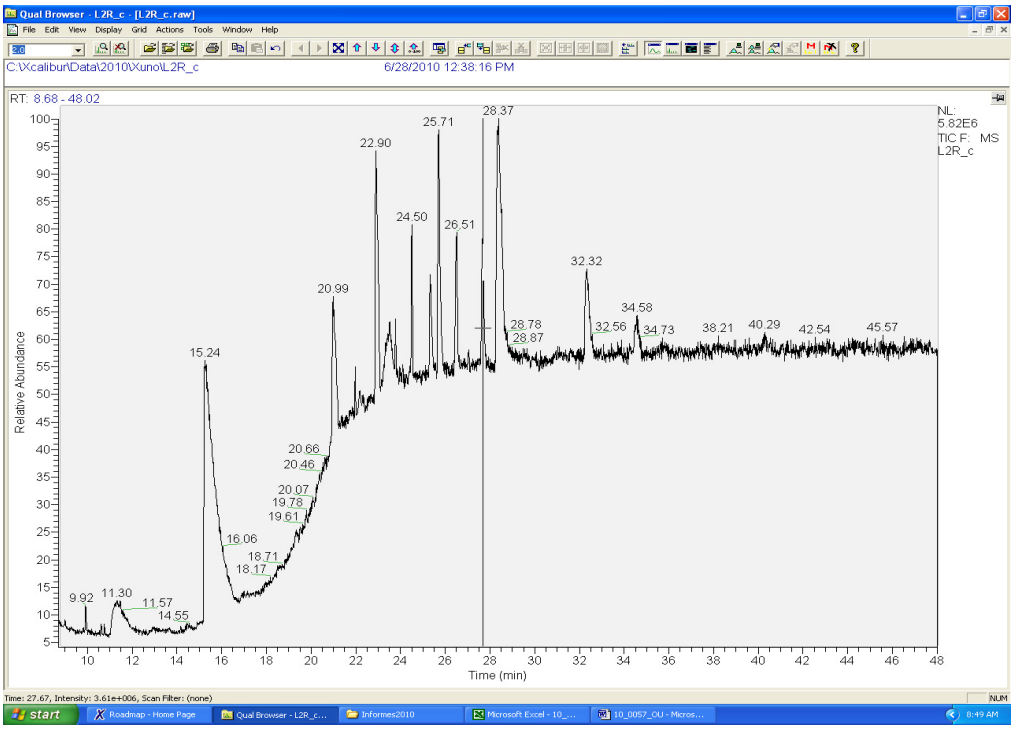


Table 2. Retention times of the FAMEs.

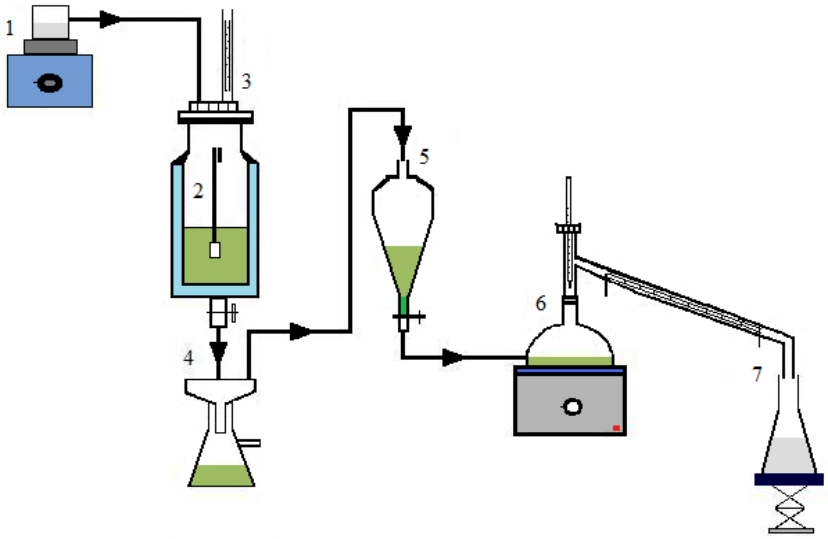
Triglycerides	Retention times (min)
Myristic	20.23
Palmitic	22.3
Stearic	24.82
Oleic	25.17
Linoleic	25.96
Linolenic	27.16
Arachidonic	31.64

2.2. Transesterification Process

Once the different marine species of macroalgae found on the Galician coast were characterized and their oil content has been calculated, the triglyceride transesterification from macroalgae oil can be carried out. The process selected for the transesterification is the alkaline catalysis transesterification, since it is the most used by industry due to its advantages.

The novelty of this work is that the oil extraction step before the reaction is eliminated, since both extraction and reaction of the macroalgae oil are performed simultaneously in the reactor by means of *in situ* transesterification. For that reason, it is necessary to add n-hexane to the reactor. In order to check the influence of adding *n*-hexane on the transesterification process, experiments were carried out using sunflower oil and macroalgae as raw materials, since biodiesel production from sunflower oil is well known. The schematic diagram of the process is shown in Figure 2.

Figure 2. Scheme of the global process: 1. solution of sodium hydroxide in methanol; 2. reactor; 3. refrigeration column; 4. Buckner funnel under vacuum; 5. decantation funnel; 6. distillation column; 7. Erlenmeyer flask.



2.2.1. Transesterification Process from Sunflower Oil

Three tests, two with *n*-hexane and one without *n*-hexane, were carried out with the purpose of comparing the results and to know how the *n*-hexane affects the reaction. In order to carry out the reaction, 250 g of sunflower oil and 300 mL of *n*-hexane (only in test 3) were introduced in a thermostated reactor. The mixture was heated at 60 °C and after that methanol in which sodium hydroxide had been previously dissolved was added to the reactor. The methanol to oil molar ratio was 6:1 for all tests. The quantity of catalyst is 1% by weight of methanolic solution for test 1 and 1% by weight of oil for test 2. Test 3 has the same parameters as test 2, but *n*-hexane was also added. Reactions were conducted at the same temperature and time with constant stirring. The reaction mixture, after the reaction, was cooled to room temperature. Then, the reaction mixture was introduced in a decantation funnel over 9 h, so the bottom layer, glycerin, was separated from the biodiesel mixture. After that, biodiesel was washed with water to remove the excess methanol and the traces of catalyst [29,30]. In order to obtain the crude biodiesel, it was necessary to remove the excess of methanol by distillation. In the test where *n*-hexane was introduced, this solvent was removed by distillation and reused in the next batch reaction.

2.2.2. Transesterification Process from Macroalgae

In order to carry out the reaction, 1000 g of dry algae (500 g of each algae) was mixed with 2.5 L of *n*-hexane and introduced in the thermostated reactor. One run test without *n*-hexane was also carried out. The mixture was heated at 60 °C and after that, methanol in which sodium hydroxide had been previously dissolved was added to the reactor. Different methanol to oil molar ratios were used (6:1 and 300:1) and the quantity of catalyst was 1% by weight of macroalgae. In this case, the reaction time was higher than 4 h (4 and 11 h), since a time of 4 h is necessary to extract all the oil.

A refrigeration column is coupled to the upper part of the reactor, where the evaporated reagents are condensed again. Reactions were conducted at the same temperature for different times with constant stirring. The reaction mixtures, after the reaction, were cooled to room temperature, then the solid phase was separated by filtration using a Buckner funnel under vacuum. After the reaction, the mixture was introduced in a decantation funnel over 9 h, so the bottom layer, glycerin, was separated from the biodiesel mixture and the *n*-hexane (top) layer. Finally, the latter was washed with about 500 mL of water to remove the excess methanol and the traces of catalyst [31,32]. In order to obtain the crude biodiesel, it was necessary to remove the *n*-hexane by distillation. The solvent was reused in the next batch reaction.

2.2.3. Analysis of Fatty Acid Methyl Esters

The analysis of the fatty acid methyl esters from obtained biodiesel was carried out by gas chromatography. Nonanoic acid methyl ester was used as an internal standard according to UNE-EN ISO 14103:2003 [27] and UNE-EN ISO 5508:1990 [28] in the same way that fatty acid methyl esters were determined for the oil.

3. Results and Discussion

This work has been divided into two main sections. The first step in this research consisted of oil characterization in order to determine the content of macroalgae oil and to compare some characteristic macroalgae oil with characteristic sunflower oil. The second step consisted of characterization of the biodiesel obtained after *in situ* transesterification from macroalgae and to compare it with biodiesel obtained from sunflower oil, checking the influence of *n*-hexane in the process.

3.1. Oil Characterization

In this section, the experimental values of the oil characterization corresponding to the oil ester content, molecular weight and iodine index are shown and discussed. The macroalgae oil was extracted before its characterization. This allows us to determine a value of 2% in weight for the oil content of the mix of macroalgae used in this work. The obtained results (Table 3) show that the obtained composition of sunflower oil is near to that obtained by Felizardo *et al.* [33]. However, the results obtained from algae oil are different from those of Aresta *et al.* [34]. The differences can be due to the fact that the species of studied macroalgae are different and, therefore, the oil composition.

Table 3. Oil composition.

FAME	Sunflower Oil (%)	Sunflower Oil [6] (%)	Macroalgae Oil (%)	Macroalgae Oil [25] (%)
Tetradecanoic A.M.E.	-	-	13.5	3.5–9.8
Palmitic A.M.E.	8.7	8.7	14.0	9.8–32
Stearic A.M.E.	4.8	4.7	2.6	1.5–2.4
Oleic A.M.E.	35.9	30.6	41.3	8.2–14.9
Linoleic A.M.E.	50.6	55.1	8.8	14.4–21.4
Linolenic A.M.E.	-	-	4.0	-
Arachidonic A.M.E.	-	-	15.8	0.1–1.6

Molecular weight is derived from the molecular weights of the triglycerides corresponding to the fatty acids methyl esters from biodiesel following Equation (1):

$$P_m = \sum P_{mi} \times X_i \quad (1)$$

where, P_m is the oil molecular weight, P_{mi} is the molecular weight of triglycerides from fatty acid methyl esters and X_i is the percent of fatty acid methyl esters.

Table 4 shows that the molecular weight of sunflower oil is 876.16 g/mol, which is near to the values obtained by other authors. Encinar *et al.* [32] obtained values of molecular weight for used oils of 882 g/mol, indicative of oils made up of mixtures of olive oil and sunflower. Whereas, Lapuerta *et al.* [35] obtained molecular weights of around 875 g/mol for mixtures of olive, sunflower and palm oil. Leung and Guo [36] obtained a molecular weight values of 856 g/mol, for a waste oil and 882 g/mol, for a vegetable oil composed mainly of C18:1 (oleic), C16:0 (palmitic) and C18:2 (linoleic) acids.

Table 4. Percentage of methyl esters, molecular weight and iodine index of algae oil and sunflower oil.

Triglycerides	P_{mi} (g/mol)	[a] M.E.A. X_i (%)	[b] M.E.A. X_i (%)	[a] $P_{mi} \times X_i$ (g/mol)	[b] $P_{mi} \times X_i$ (g/mol)	[a] Iodine Index	[b] Iodine Index
Myristic	723.16	0.0	13.5	0.00	97.63	0.00	0.00
Palmitic	807.32	8.7	14.0	70.24	113.02	0.00	0.00
Stearic	897.48	4.8	2.6	43.08	23.33	0.00	0.00
Oleic	885.43	35.9	41.3	317.87	365.68	30.87	35.52
Linoleic	879.39	50.6	8.8	444.97	77.39	87.64	15.24
Linolenic	873.37	0.0	4.0	0.00	34.93	0.00	10.46
Arachidonic	951.45	0.0	15.8	0.00	150.33	0.00	0.00
				876.16	**862.32**	**118.51**	**61.22**

[a] Sunflower oil; [b] Algae oil.

The molecular weight obtained for the oil algae is of 862.32 g/mol. Other data are not available to carry out comparisons, but this value is near to the molecular weight of sunflower oil. For that reason, sunflower oil was used to verify the effectiveness of the *in situ* transesterification.

Another parameter studied in this work is the iodine index. This value indicates the level of oil saturation. While saturation and fatty acid profile do not seem to have much impact on the transesterification process, they do affect the properties of biodiesel [35]. Iodine index was determined according to UNE-EN 14214 [37]. The degree of unsaturation of the oil determines its stability against oxidation and therefore its storage safety. The tendency to oxidation is greater when the iodine index is high. According to UNE-EN 14214 [37], the limit value of iodine index for biodiesel is 120 mg/100 g FAME.

In Table 4, the iodine indexes of sunflower oil (118.51 g/mol) and algae oil (61.22 g/mol), are reported. The value of the iodine index from algae oil is lower than that of sunflower oil, because algae oil is more stable than sunflower oil. This means that the algae oil contains a greater percentage of saturated or monounsaturated fatty acids, like palmitic and oleic, with smaller proportions of linoleic and linolenic acid.

3.2. Hexane Influence on Transesterification

The quantity of catalyst that needs to be added to the reactor is between 0.5% and 1% by weight of oil [12,38]. The interval of temperature to carry out the reaction with this type of catalysts is between 50 and 70 °C and the stoichiometric molar ratio of alcohol to oil is around 3:1, however, a 6:1 ratio is the one most used in the literature [9,36] since it gives an large conversion for the alkaline catalyst without using too much alcohol.

First, the optimum methanol to oil molar ratio and the quantity of catalyst will be studied and then the influence of hexane will be analyzed. Three different tests with sunflower oil were carried out to do this. Different parameters, such as molecular weight, iodine index and biodiesel conversion, were calculated from the acid methyl ester content (FAME) of biodiesel, to check if the results are among the requirements cited in UNE-EN 14214 [37]. Moreover, this norm sets out the minimum content in FAME (96.5%) that the biodiesel must contain to be used in diesel engines. If the biodiesel does not

reach this minimum value, it could not be used alone, but it could be mixed with petroleum diesel or another biodiesel obtained using another raw material. The results are displayed in Table 4.

The methyl esters of the biodiesel from test 1 (250 g sunflower oil, 6:1 methanol to oil molar ratio, NaOH 1% by weight of methanolic solution) are 91.7% and for test 2 (250 g sunflower oil, 6:1 methanol to oil molar ratio, NaOH 1% by weight oil), 97.4% (Table 5). Since, according to the UNE-EN 14214 [37], the minimum content in FAME should be greater than 96.5%, it can be said that the oil-methanol ratio and the quantity of catalyst used in the test 2 are the ideal parameters to meet the minimum methyl ester content requirement. For this reason in test 3, identical conditions are used as in test 2, except for the *n*-hexane addition. The percentage of FAME in test 3 is 86%, so there is a reduction of 9%. Thus, it could be seen that the presence of *n*-hexane diminishes the percentage of FAME; however, the reason could be that a small amount of methyl esters is dissolved by the *n*-hexane. One of the reasons for this decrease is the solvency power of the *n*-hexane, for that reason part of the reagents can be dissolved in the *n*-hexane without reacting. Nevertheless, this reaction could be solved by increasing the reaction time, or the quantity of alcohol and catalyst, to favor the displacement of the reaction toward the formation of methyl esters.

Table 5. Percentage of methyl esters, molecular weight, iodine index and biodiesel conversion in the all tests.

	Raw material	Hexane (mL)	Methanol -oil molar ratio	% NaOH	Time (h)	% FAME	P_m	Iodine Index	% Conversion
TEST 1	250 g sunflower oil	0	6:1	1 (weight of methylic solution)	2	91.7	293.43	124.07	91.97
TEST 2	250 g sunflower oil	0	6:1	1 (weight of oil)	2	97.4	293.20	124.67	86.94
TEST 3	250 g sunflower oil	300	6:1	1 (weight of oil)	2	86.0	293.46	120.42	79.75
TEST 4	1000 g dried algae	2500	6:1	1 (weight of macroalgae)	4	2.8	291.80	103.19	1.65
TEST 5	1000 g dried algae	2500	300:1	1 (weight of macroalgae)	11	17.10	286.49	33.23	11.42
TEST 6	1000 g dried algae	0	300:1	1 (weight of macroalgae)	11	0.1	282,95	33,3	6.08

On the other hand, molecular weight is derived from the molecular weights of the corresponding fatty acid methyl esters of biodiesel following the Equation (1), taking into account that, P_m is the biodiesel molecular weight, P_{mi} is the molecular weight of biodiesel methyl esters and x_i is the biodiesel methyl esters percent.

The molecular weight of obtained biodiesel in the three samples is very similar and its value is about 293 g/mol (Table 5). According to Van Gerpen [38], the approximate biodiesel molecular weight is calculated as a third of the molecular weight corresponding to the triglyceride from which it proceeds. The molecular weight of used oil in these tests is 876.16 g/mol (Table 4) and its third is

292.05 g/mol, similar to the results obtained in this work. Then, the molecular weight of biodiesel in these tests is in agreement with the results of other authors [39].

The iodine index offers a measure of the degree of unsaturation of the oil components. According to UNE-EN 14214 [37], the limit value of iodine index for biodiesel is 120 mg/100 g so the biodiesel from test 1 and test 2 do not meet this norm (Table 5). However, this value in test 3 is 120 (Table 5) so the *n*-hexane dismisses the iodine index, although the iodine index value is still very high. This value indicates a high degree of unsaturation and oxidation and causes problems in storing the biodiesel.

Finally, the conversion of triglycerides to methyl esters was determined from the methyl ester content of biodiesel which was analyzed using a gas chromatograph. The equation of molar conversion from oil to biodiesel is:

$$\% \text{ Conversion} = \frac{\frac{p_b}{p_{mb}} \times \text{biodiesel content}\%}{\frac{p_a}{p_{ma}} \times 3} \quad (2)$$

where, P_b is the weight of biodiesel, P_{mb} is the molecular weight of biodiesel, *biodiesel content %* is the methyl ester content of biodiesel, P_a is the weight of oil used in the transesterification and P_{ma} is the oil molecular weight of oil.

A high degree of conversion is obtained for tests 1 and 2 (Table 5), both more than 85%, but even the third test has a value of 79.75%. Then, it could be concluded that the *n*-hexane addition diminishes the conversion, since hexane dissolves the methyl esters. These results could be increased by increasing the reaction time or the quantity of methanol and catalyst. One test without *n*-hexane was carried out to verify this finding. It can be observed comparing the tests 5 and 6 that the amount of FAME when *n*-hexane was not added to the reaction is almost negligible. Then, under the conditions used in this research is not possible to obtain biodiesel without adding *n*-hexane, since the *n*-hexane extracts the oil from the macroalgae and in its absence the extraction is not possible. Other conditions, for example a higher amount of methanol could be necessary to carry out the reaction with a solid raw material [40].

3.3. Comparison of Biodiesel from Sunflower Oil and Macroalgae

Once the optimization of the transesterification reaction and influence of the *n*-hexane on the FAME yield were carried out, the final process, *in situ* transesterification from macroalgae, was carried out under the same conditions (test 4): the temperature is 60 °C, the methanol to oil molar ratio is 6:1 and the quantity of catalyst was 1% by weight of macroalgae. In this case, the reaction time was 4 h instead of 2 h, since a time of 4 h is necessary to extract all the oil. The results are displayed in Table 5.

The methyl ester content, in test 4, does not reach the minimum value given in UNE-EN 14214 [37]. This result could indicate that the reaction time is not enough to carry out the *in situ* transesterification and not all the extracted oil is reacting. Previous research was carried out to determine the reaction time with higher conversion and it was found that a time of 11 h is necessary [41]. On the other hand, the transesterification reaction from macroalgae is developed in a methanol-oil molar proportion of 3:1, but an excess of methanol is needed to favor the reaction. A maximum conversion [42] is obtained

with a molar relation of 6:1, but the relation could vary depending on the raw material. According to Hass [40], the best yields in FAME were obtained with a methanol to oil ratio of 543:1 for an *in situ* transesterification. For that reason, another experiment (test 5) was carried out increasing the reaction time (11 h) and the methanol to oil molar ratio (300:1).

In Table 5, it can be observed that test 5 gave better results than test 4. Therefore, increasing the reaction time and methanol to oil molar ratio gives better conversion and methyl ester content. Although the methanol to oil molar ratio is very high, it is in agreement with the results obtained by other authors that also used solid raw materials [11] This finding was expected considering that a greater amount of methanol is needed in order to allow good contact between the reactants.

However, the methyl ester content in test 5, still does not reach the minimum value given in norm UNE-EN 14214 [37], so it could be used mixed with petroleum diesel or other biodiesel obtained using another raw material. In this way, Kondamudi [11] has obtained a methyl esters content lower than 96.5%. Moreover, in future research into biodiesel production from macroalgae, the influence of others parameters that increase the methyl ester content could be analyzed.

Comparing tests 3 and 5, the molecular weight of biodiesel obtained in the two samples is very similar, with a value of about 286–293 g/mol (Table 4). In both samples, the biodiesel molecular weight is roughly a third of the molecular weight corresponding to the triglyceride from which it proceeds. The molecular weight of macroalgae oil used in these tests is 862.32 g/mol (Table 3) and one third of this is 288.44 g/mol similar to the obtained results.

The iodine index of biodiesel from macroalgae is less than 120 mg/100 g (Table 5) according to UNE-EN 14214 [37], while the iodine index of biodiesel from sunflower oil is 120 mg/100 g, the maximum allowed. The value of the iodine index from algae biodiesel is lower than that of sunflower biodiesel, indicating that algae oil is more stable than sunflower oil. Thus biodiesel storage from macroalgae should be easier than that of biodiesel from sunflower oil.

Biodiesel is composed of monoalkyl esters of long chain fatty acids derived from renewable lipids such as oils vegetables or animal fats, but all the oils do not contain the same type of fatty acids. The oils with high proportions of unsaturated fatty acids improve the operability of the biodiesel at low temperatures and diminish their stability to oxidation, which is translated into a high iodine index; sunflower or *Camelina sativa* oil are examples of this. For this reason, the possibility of producing biodiesel from genetically modified oil has been considered to reduce to proportion of unsaturated fatty acids, as happens with sunflower oil with high content in oleic acid [31]. The iodine value depends on the feedstock origin and greatly influences the oxidation tendency of the fuel. Consequently, in order to avoid oxidation, special precautions must be taken during the storage of biodiesel from sunflower oil with high iodine values, especially in the case of used oils [43]. For example, biodiesel obtained from waste frying oil will have a high iodine index (119 mg/100 g) [44], since it is a compound of mostly unsaturated fatty acids and it will be more difficult to store; whereas palm oil and beef tallow are formed mainly from saturated fatty acids, and thus biodiesel obtained from them will have a lower iodine index, 57.5 mg/100 g [45] and 35–48 mg/100 g [44] respectively, and will be easier to store.

Biodiesel conversion from sunflower oil was higher than biodiesel conversion from macroalgae oil (Table 5), but in future research into biodiesel production from macroalgae the influence of other parameters could be analyzed to increase the methyl ester content and conversion. The conditions used

here were chosen to achieve FAME production while improving the process with *in situ* transesterification, minimizing the use of *n*-hexane. However, other better conditions could be found, and could increase FAME yields with higher temperatures or higher reaction times. Moreover, biodiesel from macroalgae could be ideal for mixing with biodiesel with a high iodine index like biodiesel from sunflower oil.

4. Conclusions

Biodiesel production from macroalgae by *in situ* transesterification could be feasible, using hexane for the extraction and eliminating the previous extraction, so production costs could be lower. The authors of this work consider that algae biodiesel stocks may become a very attractive investment in the future due to the technique's positive points as regards technology.

Most of the residue from macroalgae is not used by industry. Residues are discarded, unused, in dumps. In Galicia alone, 100,000 Tn/year of biodiesel could be produced from macroalgae, also many species of macroalgae were not studied, which means more macroalgae with greater oil content could be found and biodiesel production improved. Moreover, biodiesel from macroalgae could be ideal for mixing with biodiesels with high iodine index like biodiesel from sunflower oil, since biodiesel from macroalgae has a low iodine index. The result of this mixing would be easier to store.

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