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Total Use of Microalgae as Feedstock for Biodiesel and Pellet Production

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ABSTRACT:Microalgae are one of the most promising feedstock for the biofuels production. However, high cost associated with the different stages involved in the transformation process is one cause of uncompetitiveness price of biofuels, compared with fossil fuels like diesel oil or gasoline. In this research, the potential of Chlorella Vulgaris microalgae for the production of two fuels, biodiesel and stove pellets, has been analyzed. Results showed that an efficient harvesting of algal biomass was achieved using inorganic flocculants, especially Aluminium chloride (AlCl3) which achieved more than 95% biomass recovery. Moreover, data obtained demonstrated that simultaneous application of microwaves and ultrasounds extracted more than twice lipids than only ultrasounds. Biodiesel conversions between 12 and 27% based on dry weight, were reached. Additionally, the algal residue generated in biodiesel production process has been used for making stove pellets. The analysis of their properties corroborated that algal pellets can be used as fuel in biomass stoves.

Key words: Biofuel, Pellet, Biomass, Microalgae, Harvesting

INTRODUCTION

In recent years, energy and environmental issues have encouraged the search for alternative energy sources to fossil fuels. Biofuels are presented as a promising solution at short and medium term. In this context, microalgae can be used as potential feedstock for biodieselproduction, bioethanol, high-value added products, health supplements and wastewater treatment (Brennan and Owende, 2010; Das et al. 2011).These microorganisms offer a range of advantages over traditional energy crops: fast and short time growth, high lipid content, low energy consumption and high photosynthetic production. Furthermore, microalgae culture can be adapted to different water sources (fresh, brackish, marine and wastewater) and grow on areas not suitable for traditional crops (Florentino et al. 2014; Gong and Jiang, 2011). These factors can heavily reduce the cost of biofuel production and increase its attractive as an alternative crop. Common stages in biodiesel production from microalgae are cultivation, harvesting, dewatering, lipid extraction and transesterification of lipids to form biodiesel, in the presence of an alcohol (methanol or ethanol) and a catalyst (acid or base) under certain conditions (Chisti, 2007).However, due to their high water content, harvesting step can account 20% to 30% percent of total production cost (Mata et al. 2010; Molina Grima et al. 2003). A reduction of the overall costs of biodiesel production is necessary to achieve a competitive price of this biofuel.

This research aims to evaluate the potential of Chlorella Vulgaris as raw material for biodiesel and pellet production so that valuable aspects such its high growth rate and easy cultivation make it a promising candidate (Lv et al. 2010). Part of the importance of this work lies in reducing biodiesel production costs. Therefore, after optimizing the specific conditions of harvesting technique, extraction and characterization of algal oil obtained were conducted. Besides, direct transesterification of the biomass was also carried out and the resulting residue was used as feedstock for pellet manufacturing. The designed process allowed a better use of raw material and obtaining two valuable fuels, biodiesel and pellets.

MATERIAL & METHODS

Microalgal strain and culture conditions: Strains of Chlorella Vulgaris green microalga were supplied by the Algae Collection of the University of Vigo Marine Science Station (ECIMAT) at Toralla (Vigo, Spain). The microalgae strains were cultured in a nutrient media

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(constituents: NaNO3, KH2PO4, MgSO4·7H2O, Na2CO3, MgCl2 6H2O, CaCl2.2H2O, H3BO3, MnCl2 4H2O, ZnCl2, FeCl3 6H2O, CoSO4 7H2O, Na2MoO4 2H2O, CuSO4 5 H2O and Na2EDTA 2H2O). The culture system used was a photobioreactor of polyethylene with a working volume of 45 L under artificial illumination of 14/10 light/dark cycle at 20±10C. After eight days of culture, the measured concentration of cells was 9.350 cells/microliter and the pH of the microalgae cultures was 8.63. All experiments were carried out with cells from a single harvest.

Harvesting and dehydration of biomass: Microalgae recovery techniques are dependent on the characteristics of the microalgae such as size and density. The most common harvesting methods are centrifugation, filtration, sedimentation, flotation and flocculation as well as a combination of them. Harvesting processes can be divided into two stages. Stage I depicts the separation of the biomass fractions from growing medium. In this research, gravity sedimentation and chemical flocculation process have been used in this stage. Whereas stage II consists in the concentration the suspension via techniques such as centrifugation to generate a wet paste. Taking into account that centrifugation is a very effective technique but with a high-energy consumption, this method is only recommended as second stage dewatering.

Harvesting methods: gravity sedimentation is a common method used in the solid-liquid separation under gravitational force. Microalga cells are deposited according to their density, radio and sedimentation velocity. The success of the gravity sedimentation is highly dependent on the density of cells of microalgae (Brennan and Owende, 2010). The advantages of this method are: low design cost, low energy requirement and low manpower requirement.Sedimentation experiments were performed in laboratory at two temperatures, 4°C and 25 °C, in order to analyze the influence of this factor in sedimentation process. The efficiency of gravity sedimentation method was calculated through measures of optical density at regular intervals of time. On the other hand, flocculation is a technique used to separate microalgal cells from medium by the addition of one or more flocculants. Through the interaction between flocculant and the surface charge of microalgae cells, the negative charge of the walls is reduced or neutralized, allowing the agglomeration into bigger flocs that settle down (Demirbas and Demirbas, 2010; Molina Grima et al. 2003).The effect of two organic flocculants, agar-agar and gum arabic, and four inorganic flocculants, Cu2SO4, KI, CaCl2 and AlCl3.6H2O, were evaluated at different doses: 50, 100 and 200 mg/L. Flocculation experiments were carried out using 500 mL of harvest volume at laboratory temperature of 20 - 25°C. The flocculant dose was added to the harvest volume and then the mixture was subjected to 1 minute of agitation at 200 rpm on a magnetic stirrer (HANNA HI 190M Magnetic Mini Stirrer). After that, the stirring was reduced to 50 rpm for 3 minutes to ensure the solubility of the flocculant. The optical density of the samples was measured to evaluate the efficiency of flocculation process.

Thus, harvesting efficiency was determinated in term of optical density (OD) using a spectrophotometer (LabomedSpectro 22) at 680 nm. Samples were collected in the middle of the clarified zone and harvesting efficiency was calculated using the following equation:

$$\text{Harvesting Efficiency} = (ODi - ODs)/ ODi \times 100 \quad (1)$$

Where ODiis the initial optical density of the culture medium at 680 nm and ODsis the optical density of the sample at 680 nm.

Dehydration of biomass: centrifugation is a method of dehydration that uses centrifugal acceleration to improve the concentration of solids. Biomass recovery depends on the particle size of microalgal cells and density difference between solid and liquid (Molina Grima et al. 2003).In this investigation, biomass coming from gravity sedimentation or chemical flocculation processes was concentrated by centrifugation through Mixtasel Selecta centrifuge at 4000 rpm for 20 minutes. An algal paste and a supernatant were obtained. After centrifugation, this paste was dried at 105°C to constant weight in J. P. Selecta Conterm 200208 drying oven with the aim of determine the humidity percentage of samples. The algal paste was used in later stages of biodiesel production process and the supernatant was discarded.

Lipid Extraction: in recent years, several research groups have investigated different extraction non-conventional methods in order to improve their performance. In this study, ultrasound (US)-assisted extraction has been used to extraction lipids from Chlorella Vulgaris microalgae. This technology has the ability to crack microalgal cell walls due to cavitation effect (Lee et al. 2010). Moreover, to improve the efficiency of lipid extraction, the effect of pretreatment with microwave technology was evaluated. This pretreatment could increase the lipid extraction thanks to its capacity to deeply penetrate through the cell wall structure (Dejoye et al. 2011; Passos et al. 2013). In the two processes studied, a mixture of methanol-chloroform (2:1 v/v) was used as solvent and methanol was added to a rate of 5 mL per gram of dry biomass.

Microwave pretreatment: a mixture of 24.5 g of microalgal biomass (80% humidity), methanol and chlo-

roform was irradiated with microwaves for 10 min with a power applied of 140 W. The microwave oven device used for pretreatment was BeckenEasycook Digital 2 with a rated irradiation power of 700 W and 2450 MHz frequency. After completing the pretreatment, the mixture was subjected to ultrasound-assisted extraction as is described below.

US-assisted extraction: In all experiments carried out, 10 g of microalgae were used. Lipids of microalgae were extracted in flask of 500 mL of capacity. The mixture was submitted to ultrasonic bath (Model S 300H from Elmasonic) working at 37 kHz for 60 min. After, the sample was separated from the residue by vacuum filtration and the solvent was evaporates at 60°C. The lipid fraction was dried to constant weight in an oven. Analysis of lipid: After lipid extraction, the algal oil obtained was characterized. Gas chromatography was used with the aim of analyze the Fatty Acids of Methyl Esters (FAME) composition coming from algal oil extracted of Chlorella Vulgaris microalga. The conversion of free fatty acids in FAME was performed according to the UNE-EN ISO 12966-2:2011.

Direct transesterification: biodiesel from microalgae biomass was produced by direct "in situ" transesterification. By this method, lipids extraction and transesterification were carried out in a single step by homogeneous alkaline catalysis. Thus, cell disruption and oil extraction are avoided which strongly impacts on time saveing and biodiesel production cost reducing (Velasquez-Orta et al. 2012; Yang et al. 2015). In addition, the effect of incubation was evaluated in order to improve the performance of the transesterification reaction. Microalgae biomass was transesterified to biodiesel by sodium methoxide (2% NaOH/Methanol w/v). The relationship between the algal mass and methoxide solution was 1:12 (w/v).

In the experiments conducted without previous incubation, 5 g of biomass (80% moisture) were mixed with 60 mL of methoxide solution and direct transesterificated at a constant temperature of 60 °C and a stirring rate of 160 rpm, using an orbital shaker OVAN Maxi MD OL30-ME, for 4 hours. The effect of previous incubation, prior to "in situ" transesterification, was studied. In these experiments, 10 g of algal biomass (80% moisture) and half of methanol necessary, according to the 1:12 ratio, were mixed in a reactor and kept incubating for 18 hours at 160 rpm and 40 °C in an OVAN OPCW I10-0E incubator. Then, the remaining methanol, and the basic catalyst, NaOH, were added, leaving the transesterification reaction taken place in the same way that in the predecing experiments, without incubation. Once transesterification reaction was complete, the algae residues were separated from the liquid by centrifugation. Later, the liquid was left to settle in a separating funnel to reach both phases separation, glycerin and biodiesel. Finally, the biodiesel layer (top) was separated from glycerin and washed with water to eliminate methanol excess and catalyst traces (Ahmad et al. 2013).

Pellet production: during the biodiesel synthesis an algal biomass residue was generated. In order to reach the maximum performance, this residue was used as raw material for stove pellets manufacturing. Due to the high humidity content, algal biomass was dried for 24 hours at 105 °C in an oven. Then, solid biomass was loaded in a manual pelletizer press to make 12 mm in diameter, 15 mm length pellets. They were kept stored for a week to constant properties. In order to determine if the algae waste pellets can be used in pellet boilers, different parameters were analyzed and compared with European standards EN ISO 17225-1 and EN ISO-17225-6. The percentages of ash content, volatile matter, moisture content and calorific value were determined according to UNE 14775:2010, UNE 15148:2010, UNE 14774-3:2010 and UNE14918:2011, respectively.

RESULTS & DISCUSSION

Biomass recovery efficiency of Chlorella Vulgaris cells at different harvesting method was analyzed. In the first place, the influence of two different temperatures, 4 °C and 25 °C, on sedimentation was monitored for 6 hours and at 24, 48 and 72 hours. Variations on percentage of recovered biomass over time are shown in Fig. 1a and 1b. The results obtained showed that the settling rate is hardly affected by the temperature. Low biomass recovery percentage was achieved during the first 6 hours of the process. However, high microalgal recovery rates were observed at 24, 48 and 72 hours, reaching almost 99%. These results are much better than those obtained by Harith et al. (2009) who managed about a 35% of biomass recovery at 27 °C and 72 h (3 days) while the recovery for the same time and 4 °C was only 20%. In their case, the temperature had a greater influence than in the experiments carried out in the present work.

Where:

In order to reduce the time necessary for an efficient harvesting of algal biomass, chemical flocculation experiments with organic and inorganic flocculants were conducted. Fig.2 shows the effect of settling time on harvesting efficiency for the four inorganic flocculants studied.

As can be observed, high biomass recovery, between 72 % and 99 %, was achieved by all of the flocculant agents used. The best results were with aluminum chloride, which reached biomass recovery between 70 and 80 % after 5 minutes. After 220 min, the

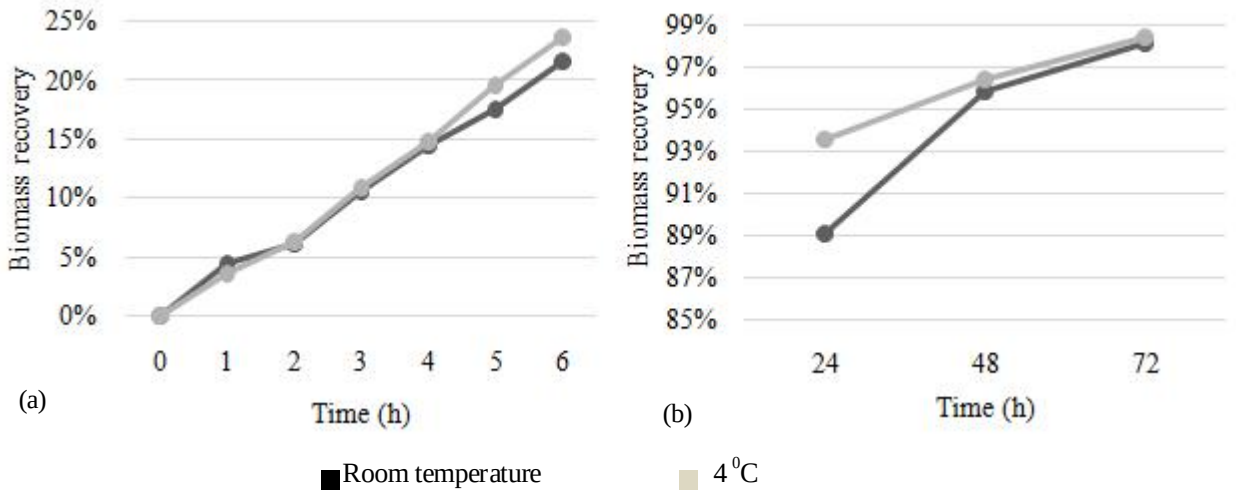

Fig. 1. Percentage of recovered biomass by sedimentation: (a) for the first 6 hours, (b) at 24, 48 and 72 hours

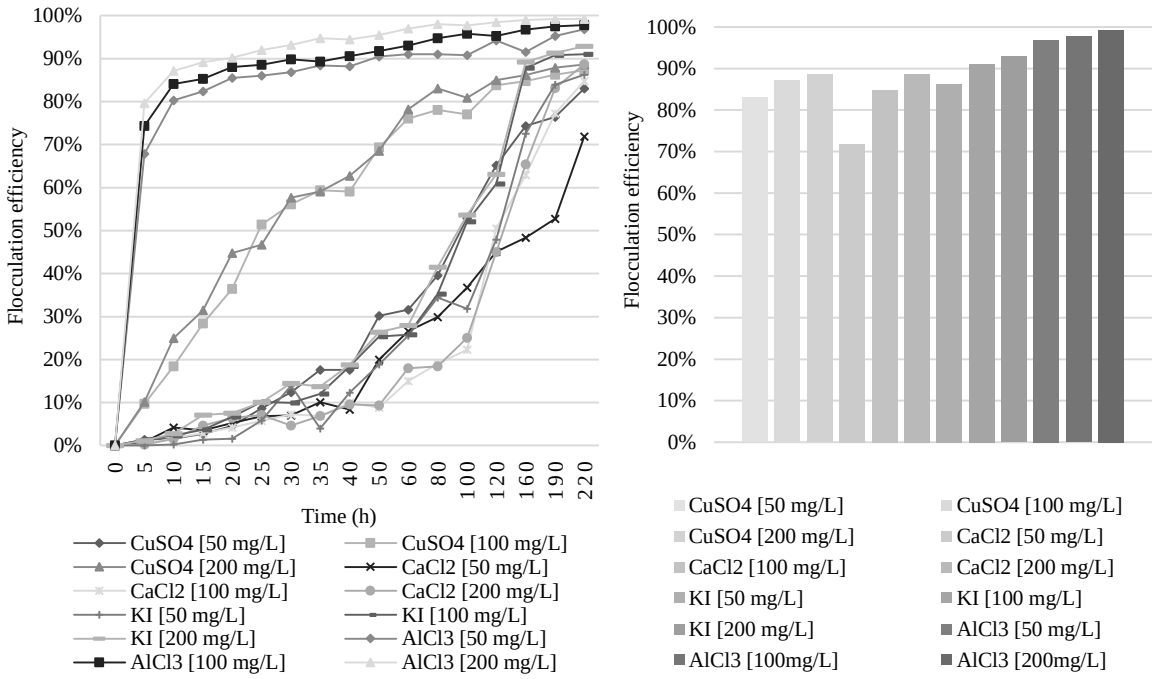

Fig. 2.Flocculation efficiency for different inorganic flocculants: (a) variation over time (b) after 220minutes

total biomass had settled. High recovery rates were also observed for copper sulfate, calcium chloride and potassium iodide. However, Surendhiran and Vijay (2013) only achieved a 65 % recovery efficiency of Chlorella Salina biomass to 210 min using AlCl3 as a flocculant in a concentration of 0.2 g/L. Meanwhile Papazi et al. (2010) had a 60 % biomass removal of Chlorella Minutissima with a dose of 0.25 g/L of aluminum chloride. For its part, Fig. 3 shows that a successful microalga recovery, between 64 and 85 %, was reached by adding the two organic flocculants: agar-agar and gumarabic. The highest collection efficiency was obtained at 220 minutes using 200 mg/L of agar-agar. However, time is longer than in the case of inorganic flocculants (220 min).In all cases, both organic and inorganic flocculation, biomass recovery (BR) increased with flocculant dose (BR50 mg/L <BR100 mg/L <BR200 mg/L).

Finally, the biomass obtained was dehydrated by applying two cycles of 10 min centrifugation at 4000 rpm. By this technique, 81 % of algal biomass was recovered. The resulting algal paste was dried. The sample moisture plot is in Fig. 4. A total moisture content of 80% was obtained for Chlorella Vulgaris microalgae.

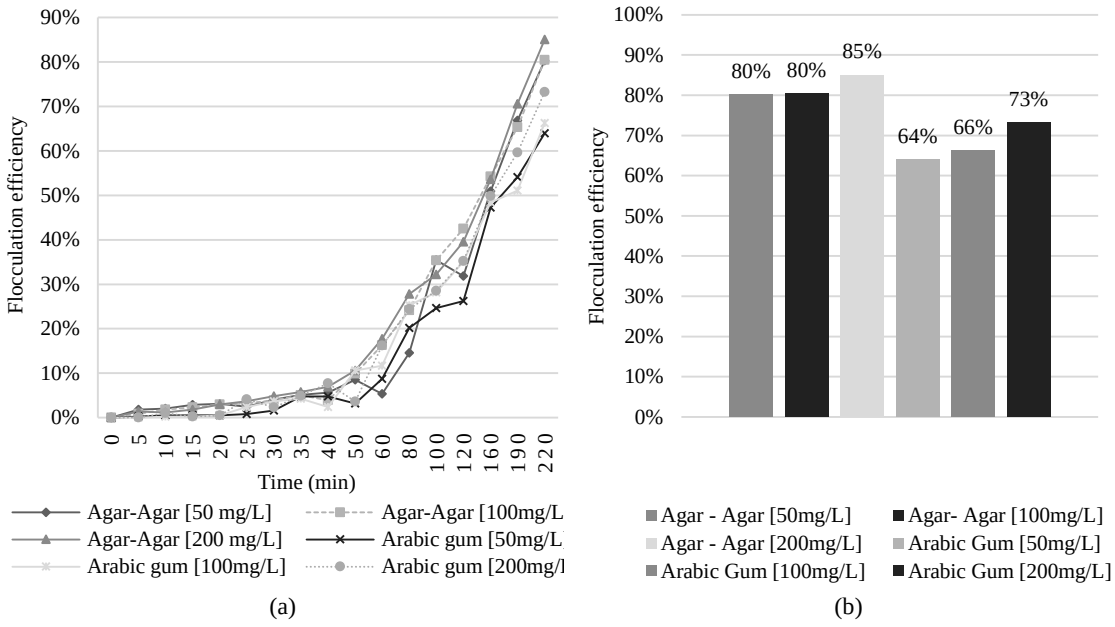

Fig. 3.Flocculation efficiency for two organic flocculants: (a) variation over time (b) after 220 minutes

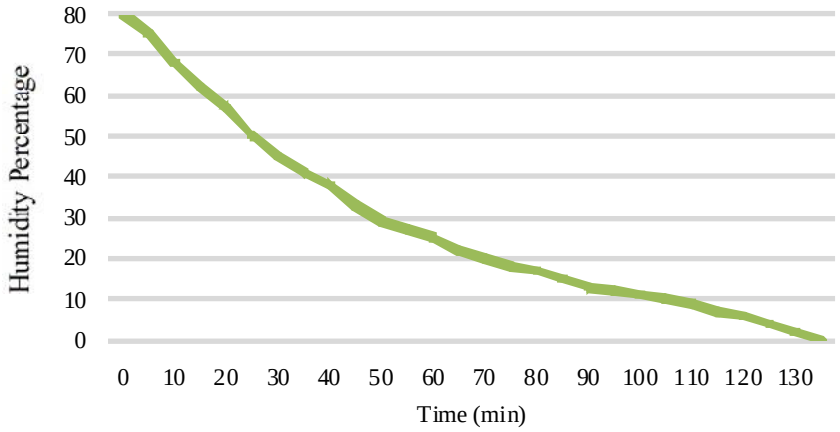

Fig. 4. Humidity plot for Chlorella Vulgaris microalgae.

Oil extraction: to discover the efficiency of oil extraction, two different types of experiments with novelty extraction techniques, microwave and ultrasound, were applied. As illustrated in Table 1, the application of microwave pretreatment, increases the oil extraction yield to 17% while only 7.5% yield was achieved when the ultrasonic extraction was used. Simultaneous application of ultrasound and microwave irradiation improves the quantity of microalgal oil extracted. This improvement cans be explain because the microwave energy, absorbed by microalgae, considerablely rises cell temperature and pressure. High pressure, higher than the tolerable pressure, causes breakages of microalgal cell walls and facilitates the release of oil retained inside. These effects combined with those produced by cavitation, intensive mixing caused by microturbulence and disruption of cell walls given by shock waves, which occurs when the ultrasound technique is applied, favors significantly the oil extraction (Ranjan et al. 2010).Different values of oil extraction were obtained by Yong-Ming Dai et al. (2014), who achieved only 5% of crude oil when ultrasonic extraction was explored, while higher yields, 18%, were reached for microwave energy application. However, data attained in this research are according with those showed by Pragya N. et al. (2013) for Chlorella Vulgaris microalgae, where oil extraction yields between 10 and 28.6% were obtained by microwave technique and values between 6.1 and 8.8% were reached through ultrasound.

Algal oil obtained in the extraction process was analyzed by gas chromatography. Its chemical composition is shown in Table 2. Fatty acids profile for Chlorella Vulgaris microalgae, showed that palmitic acid

(C16:0) was the predominant (55.22 ± 0.40%). Stearic acid (C18:0), oleic and elaidic acid (C18:1) formed also part of the composition of algal oil but to a lesser extent (13.55 ± 0.17% and 17.16 ± 0.17% respectively). Finally, the presence of acids such as lauric (C12:0), myristic (C14:0), linolelaidic (C18:2) or linoleic (C18:3) was detected but in very small concentrations. In the research carried out by Guldhe et al. (2014) for Scenedesmus sp. microalgae, palmitic acid was also found in a higher proportion (31.67 ± 0.32%) and other different acids than those obtained in the present study were localized (C16:1 and C20:1). By other hand, Axelsson M. and Gentili F. (2014) found that linoleic acid (C18:3) was the major component in SelenastrumMinutum oil with 40.06 ± 0.25% followed by palmitic acid with a 23.92 ± 0.19%.

Biodiesel production: biodiesel conversion of "in situ" transesterification experiments with or without incubation was tested. According to the data shown in Table 3, direct transesterification of harvested biomass exhibited a 12.12% conversion to biodiesel while slightly more than double conversion, 27.27%, was achieved by applying a previous incubation of biomass in methanol for 18 hours. Values obtained showed that a prolonged contact of algal biomass with solvent, significantly facilitates the lipid to fatty acid methyl esters conversion. Even so, low biodiesel conversions were obtained in the experiments performed. However, higher conversions (77.6 ± 2.3%) were reached by Velasquez-Orta S.B. et al. (2012) when dry biomass of Chlorella Vulgaris was used as feedstock. These data revealed that the presence of water in the biomass used as raw material to biodiesel production causes adverse effects on in situ transesterification(Cao et al. 2013; Kumar et al. 2014).

Finally, and in order to determine if the biofuel produced is in concordance with the European Standard UNE EN 14214 for its use as fuel in internal combustion engines, density of biodiesel was measured at 15 °C after transesterification. Values between 860-900 kg/m3are required for a correct combustion, nevertheless values below 860 kg/m3 (820-855 kg/m3) were obtained in this investigation. These data indicate that biodiesel obtained can not be used alone but it can serve as an additive to other fuels.

Pellet production: different properties of algae waste pellets were analyzed and the results obtained were compared with "European Standards for Graded non-Woody Pellets" in order to evaluate their possible use as fuel in boilers. Physical and chemical parameters analyzed, as heating power, ash content or humidity percentage are shown in Table 4 and compared with the values tagged on the UNE-EN ISO 17225-6:2014. It can be checked that Higher Heating Value (HHV) of algae pellets, 20681 kJ/kg, is higher than the minimum value required by the regulations, 14500 kJ/kg. Ash content, which should be less than 10% according to the standard, result 9.78%. Furthermore, also zero moisture content was achieved, verifying the legal limits. Based on this data, it can be concluded that algae waste pellets verified the requirements established to be considered a commercial biofuel, and can be used as fuel in biomass boilers.

Table 1. Percentage ultrasound assisted extracted oil with and without previous microwave disruption

Pretreatment	Dry weight (g)	Oil extracted (g)	Oil extracted (%)
No	2.00	0.15	7.50
Microwave	4.90	0.83	16.94

Table 2.Fatty acids analysis (in total lipids) extracted from Chlorella Vulgaris microalgae

Fatty acids	Common name	%
Dodecanoic acid, C12:0	Lauric acid	3.32 ± 0.10
Tetradecanoic acid, C14:0	Myristic acid	3.53 ± 0.05
Hexadecanoic acid, C16:0	Palmitic acid	55.22 ± 0.40
Octadecanoic acid, C18:0	Stearic acid	13.55 ± 0.17
Octadecanoic acid, C18:1	Oleic and Elaidic acid	17.16 ± 0.17
Octadecanoicacid, C18:2	Linolelaidicacid	4.60 ± 0.12
Octadecanoicacid, C18:3	Linoleicacid	5.93 ± 0.02

Table 3.Percentage of biodiesel conversion

Pretreatment	FAME Oil (%)	FAME Biodiesel (%)	BiodieselConversion (%)
Incubation	33	9	27.27
No Incubation	33	4	12.12

Table 4. Parameters analysed for Chlorella Vulgaris pellets

Chlorella Vulgaris pellet			
Parameter	**Unit**	**Regulation value**	**Sample value**
HHV	kJ/kg	=14500	20681
Ash	%	=10	9.78
Humidity	%	<15	0.0
LHV	kJ/kg	-	19358
Fixed carbon	%	-	16.87
Volatile matter	%	-	73.35

*LHV: lower heating value

CONCLUSIONS

Integral use of Chlorella Vulgaris microalgae for biodiesel and stove pellet production was studied. In addition, algal oil was extracted by highly efficient techniques such as microwave and/or ultrasounds, and results showed that 17% (MW+US) and 7.5% (US) of algal oil was extracted. Palmitic acid was the major component. Biodiesel conversions less than 30% were obtained by direct transesterification. On other hand, algal biomass residue resulting of biodiesel synthesis was used as raw material for making pellets. The properties of this fuel, which were tested, verified all standards for its use as fuel in biomass boilers. Therefore, a single raw material was used, microalgae, two biofuels were obtained, biodiesel and stove pellets, and no residues were left behind.

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