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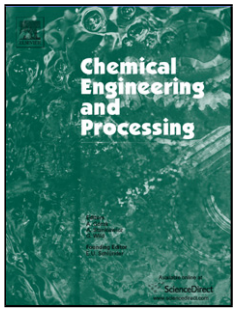

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Biodiesel production from *Neochloris oleoabundans* by supercritical technology

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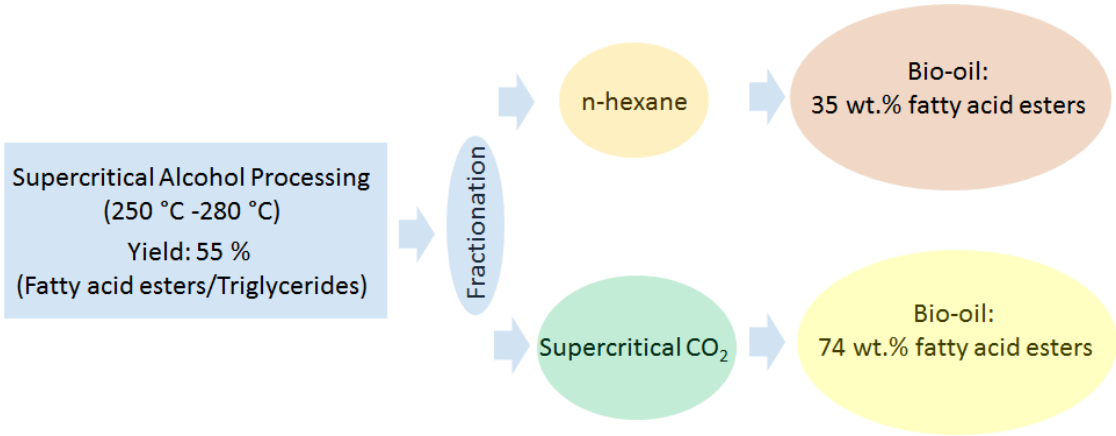
Graphical Abstract



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Highlights

- Fatty acid esters were obtained by supercritical alcohol processing of microalgae
- Products were sensitive to reaction conditions and solvent used in the fractionation
- Bio-oils with high fatty acid esters contents were obtained by supercritical CO_2.

Abstract. Oleaginous microalgae have been proposed as a sustainable alternative biomass to produce biodiesel in order to substitute conventional vegetable oils derived from oilseed crops. Particularly, recent studies pointed out the potential of *N. oleoabundans,* cultured in seawater or in anaerobically digested dairy manure, to produce triglycerides with high content of monounsaturated fatty acids. The supercritical technology has been recognized as a green

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sustainable alternative to transform biomass into valuable products. Thus, the aim of the present wok was to study the direct supercritical alcohol processing of partially dried *N. oleoabundans* biomass and later reaction products fractionation by supercritical CO_2 or liquid n-hexane. A direct alcoholysis of microalgae biomass was carried out at different temperatures (250°C and 280°C) and increasing reaction times in order to evaluate the fatty acid ester production. Bio-oils from microalgae with up to 35 wt.% fatty acid esters were obtained by two fold extraction with n-hexane. Conversely, supercritical CO_2 fractionation produced upgraded bio-oils with up to 68 wt.% of fatty acid esters content.

Keywords: Supercritical methanol; ; ; ; , supercritical CO_2, microalgae, bio-oil, fatty acid esters

1. Introduction

Microalgae can significantly contribute to replacing petroleum derived liquid fuels because they grow extremely rapidly and high oil contents have been reported in several species. Also, the fuels produced from microalgae oils do not compromise the production of food, as it is the case of oil crops [1, 2]. Popovich et al. [3] carried out a lipid analysis in *Neochloris oleoabundans* cultured in enriched seawater to evaluate its potential use as biodiesel source. The authors concluded that this microalga is a good alternative to produce high quality biodiesel due to its capacity to accumulate neutral lipids (20 wt.%) with a major concentration of monounsaturated fatty acids [3]. Similarly, cultures of *N. oleoabundans* in both anaerobically digested dairy manure [4] and mixotrophic conditions [5] produced lipids suited for biodiesel production.

Mayor problems related to the microalgae biodiesel production process are relative to high drying costs, lipids extraction with organic solvents, followed by transesterification [6]. The direct alcohol transesterification of algal biomass lipids has been the subject of many research works in the last decades because it can be a sustainable source of chemicals and liquid biofuel with the potential for the reduction of capital and processing costs [6, 7]. Basically, the methods reported in the literature for direct or in-situ lipid transesterification from microalgae can be classified in two broad categories as low and high pressure processes. The direct transesterification can be carried out at low temperatures (60 ºC – 80 ºC) and nearly atmospheric pressures by adding wet or dry microalgae to excess alcohol in the presence of a basic (KOH, NaOH) or acid (H_2SO_4) catalyst and results in the direct production of fatty acid esters [6,7]. It has the advantage of eliminates the need to completely dry the microalgae biomass to later extract and refine the lipids before converting it to biodiesel [7]. However, these methods in general require a considerable reaction time, and catalysts are difficult to recover in these processes which produce large wastewater and other solid wastes [6-10].

The direct transesterification of biomass lipids has been carried out at conditions where the alcohol is in near/supercritical state [8-10]. Patil et al. [8] worked in the simultaneous extraction and transesterification of the lipids contained in wet biomass from *Nannochloropsis* sp. by supercritical methanol. Levine et al. [9] proposed a two-step, catalyzed free biodiesel production process involving lipid hydrolysis from *Chlorella vulgaris´* wet biomass and subsequent supercritical ethanol *in-situ* esterification. Later, Levine et al. [10] also studied a two-step process involving the hydrolysis of *Chlorella protothecoides´* wet biomass at 220-250

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°C and the esterification of the fatty acids using subcritical ethanol (215 °C) in the presence of rare-earth metal triflate catalyst to obtain fatty acids ethyl esters. Zhou et al. [11] studied the production of bio-oil from the liquefaction of the macroalgae *E. prolifera* by supercritical alcohols. The authors fractionated the raw reaction products with dichloromethane and reported a bio-oil yield of ≈31 % in the methanol liquefaction at 280 °C [11]. The supercritical alcohol processing of biomass has similarities with the thermo-chemical liquefaction of biomass, a medium temperature (250–350 ºC) and pressure (10-20 MPa) process that can be aided by a catalyst in the presence of excess water to produce bio-oils [6]. Different compounds were identified in bio-oils obtained from the alcohol liquefaction of microalgaes such as fatty acid esters, N-containing components (indole, indolizine, pyrazines, pyridines), sugars (xylopyranosides and glucopyranosides), fatty alcohols, and hydrocarbons [11, 12]. Besides the elevated operating conditions of temperature and pressure that increased fixed capital costs of industrial plants, the high pressure alcohol processing of biomass is an interesting alternative to obtain bio-fuels because this technology notably reduces both the costs of drying the microalgae biomass, cost of refining the microalgae oil, as well as the reaction time required to obtain bio-oils with high fatty esters contents [8-12]. Regarding environmental concerns, the direct supercritical alcohol processing of biomass can be carried out without catalysts which allow reducing wastewater and solids wastes [6,7].

The supercritical extraction and fractionation process of vegetable oils and derivatives has been widely studied in the last decades [13, 14]. Particularly, CO_2 is an interesting green solvent with tunable properties according to process temperature and pressure that has been proposed to replace conventional organic solvents like n-hexane [14-16]. Regarding the application of supercritical CO_2 technology to microalgaes, for instance, Mouahid et al. [17] evaluated the supercritical CO_2 extraction at 60 °C and 400 bar of neutral lipids from four different microalgae submitted to different pretreatments (*Nannochloropsis oculata, Cylindrotheca closterium, Chlorella vulgaris* and *Spirulina platensis*). Taher et al. [18] evaluated the extraction of lipid from *Scenedesmus* sp. for biodiesel production with supercritical CO_2 and compared to conventional extraction methods. As proposed in this work, this technology can also be an interesting alternative to carry out the separation and concentration of fatty acid esters produced in the in-situ direct alcohol processing of microalgae.

Despite *Neochloris oleoabundans* has shown both to grow in sustainable cultures [3, 5, 19] and to be an interesting alternative to produce oils to bio-fuel [20-23], few studies have been carried out in order to generate innovative technologies to produce biodiesel from its biomass [24, 25]. BD. Wahlen et al. [24] reported the direct in-situ transesterification of *N. oleoabundans* biomass using alcohol and sulfuric acid as catalyst. S.Y. Yoon et al [25] carried out the lipids extraction from dried *N. oleoabundans* with chloroform/methanol and subsequent conversion of the lipids to methyl esters by methanolisis with sulfuric acid. To the best of our knowledge non-studies report the direct supercritical alcohol processing of *N. oleoabundans* to produce fatty acid esters. Thus, the main goal of the present work was to study the transesterification of the lipids present in *N. oleoabundans* by a single-step supercritical methanol transesterification. The direct supercritical processing of the microalgae biomass produced a complex multicomponent oily-solid mixture diluted in alcohol. Thus, after methanol evaporation the reaction products were extracted with liquid hexane or supercritical CO_2 in order to isolate bio-oils reach in the fatty acid esters produced in the thermochemical reaction process.

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2. Materials and methods

2.1 Materials

Biomass of *Neochloris oleoabundans* UTEX 1185 was used for this study. Cells were cultivated and acclimated to marine conditions, through successive transfers in modified SWES (seawater + soil extract + salts) according to Popovich et al. [3]. To increase the biomass necessary for this study, the following experiment was carried-out: an inoculum of 40 x 10^6 cells mL^{-1} was re-suspended in 18 L of complete SWES medium, harvested by centrifugation (10 min at 3600g) at the end of log-phase culture and transferred to 18 L of nitrogen-free medium SWES for 6 days, and finally harvested for supercritical alcohol transesterification. The biomass contained an initial humidity content of 80 wt.%. The cell pellet was rinsed with distilled water and dried in a convection oven at 60 °C during 6 h. Final water content in the biomass of 25 wt.% was determined by a gravimetric analysis (Sartorious moisture analyser MA 35). The neutral lipid content of the algal biomass was found to be 20 wt.% on a dry weight basis. Table 1 shows main fatty acids composition of the neutral lipids in the biomass according to GC/MS analysis. Methanol (99.6 wt.%) used in the experimental reactions was purchased from Ciccarelli SA. For GC analysis Methyl heptadecanoate and tetradecane standards were purchased from Sigma-Aldrich. Hexane (99.9%) and pyridine (99.9%) were used as solvents for separations and chromatography solutions, respectively.

2.2 Supercritical alcohol processing of microalgae and bio-oil recovery

Figure 1 describes the different experimental steps carried out in this work to study the direct supercritical alcohol processing of *N. oleoabundas* and the fractionation of the reaction products by supercritical CO_2 or liquid n-hexane. Supercritical alcohol reactions were carried out in stainless steel batch reactors of 12 mL and 41 mL capacity, both equipped with a thermocouple (± 1.5 °C) and a pressure gauge (± 2.5 bar) as shown schematically in Figure 2. The reactor of 12 mL was assembled with a Swagelok 316 SS ½ in. OD tube, a male Branch Tee, 1/2 in. Tube OD x 1/2 in. Tube OD x 1/4 in. male NPT, and tube fitting reducing adapters for the pressure gauge and thermocouple connections. The reactor of 41 ml capacity was assembled with a 316 SS Swagelok IPT series 1 in. nominal OD tube (0.56 in. ID) with specially machined 316 SS end caps for pressure and temperature sensing.

Operating conditions studied in this work were selected according to previous works [8-12, 26, 27]. The reactor (12 mL capacity) was loaded with methanol (≈2.94 g) and microalgae biomass (≈1.28 g) in a 2.3 methanol/algae mass ratio (3 g/g, methanol/dry algae), which according to the neutral lipid content (20 wt.%) and mean molar mass of the lipids indicates a molar ratio of 423 methanol to oil. It was shaken to mix the reactants and then submerged in a tin pre-heated bath. The reaction temperatures, 250 °C and 280 °C, were reached in about 3 and 5 min, respectively. Final pressure in the reaction system varied according to the reaction temperature from 105 bar to 130 bar. After the reaction time was completed the system was cooled at room temperature in a water bath.

After cooling down the reactor up to room temperature, the reactor content was transferred into a flask using methanol to clean the reactor. Volatiles and excess methanol were removed under vacuum in a rotary evaporator operated at 70 °C with a nitrogen stream. Reaction products were fractionated using n-hexane and supercritical CO_2. In the case of n-hexane, oily-

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hexane soluble products (bio-oil) were recovered by twofold extraction with 25 mL of hexane and centrifuged (3200 g). In the case of supercritical CO_2, reaction products were carefully transferred into a 10 mL high pressure column loaded with glass spheres and extracted with CO_2 at 40 °C and 140 bar using a CO_2 mass flow rate of 0.36 g min^{-1}. Operating conditions used in supercritical fractionations were selected to obtain good selectivity through the fatty acid esters extraction [27]. The extraction was performed in all cases during 60 min. following the experimental procedure explained in a previous work [28].

2.3 GC and GC/MS analysis

The fatty acid esters concentration in the non-volatile bio-oils was determined by gas chromatography in a GC Varian Star 3400 CX equipped with a flame ionization detector (FID) set at 370 °C, and a split/splitless injector temperature set at 320 °C with a split ratio of 25:1. A high temperature capillary column (J&W Scientific, model DB-5HT, 15 m length, 0.32 mm inner diameter, and 0.10□m film thickness) was selected to perform the GC analysis according to the temperature program reported elsewhere [27]. Tetradecane was used as internal standard, and methyl heptadecanoate was use as reference for fatty acid esters calibration. A stock solution of pyridine with a known amount of internal standard was prepared (~10 mg mL-1). The bio-oil sample solution was prepared diluting 50 mg of oily phase in 5 mL of pyridine. The sample injected to the chromatograph consisted of 1 μL of a solution prepared with 0.1 ml of the internal standard stock solution, 0.1 ml of bio-oil sample solution, 0.1 ml of silylating agent (MSTFA) and 0.1 ml of pyridine.

Fatty acid esters, free fatty acids, mono and diglycerides were identified by a GC/MS analysis. Standard calibration with perfluorotributylamine was performed following the protocol of Turbo-Mass Software. On the other hand, the NIST MS Search Software [29] was used to identify compounds from their mass spectra by comparison with mass spectral libraries. The samples were prepared according to the GC-analysis protocol reported in [27].

The yield of bio-oil was evaluated on a dry biomass basis. Replicated supercritical methanolysis of microalgae powder were reproduced with a gravimetric yields deviation of ca. 1.8 wt.% in the bio-oil production. Fatty acid esters content was evaluated in weight fraction. GC analysis of fatty acid esters in the bio-oil exhibit a deviation of ca. 1.5 wt.% in their concentration.

3. Results and discussion

3.1 bio-oil yields

Figure 3 shows the bio-oil yields obtained from the reaction products with both solvents according to the operating conditions tested in the supercritical alcoholysis. In general, lower net extraction yields were achieved with CO_2 in comparison to n-hexane solvent. Barely higher n-hexane soluble compounds were determined in reaction products obtained at 280 ºC (31±1.8 wt.%) with respect to 250 °C (27±1.8 wt.%). It is worth noting the fraction of hexane soluble products was significantly higher than the neutral lipid content in the original biomass (20 wt.%). These results point out that besides fatty acid esters and unconverted acylglycerides, also other products derived from the conversion of proteins, phospholipids, glycolipids and its derivatives could be present in hexane bio-oils. Zhou et al [11], in the bio-oil obtained from supercritical ethanol liquefaction of *Enteromorpha prolifera*, reported the presence of N-containing compounds, carbohydrates, hydrocarbons and fatty

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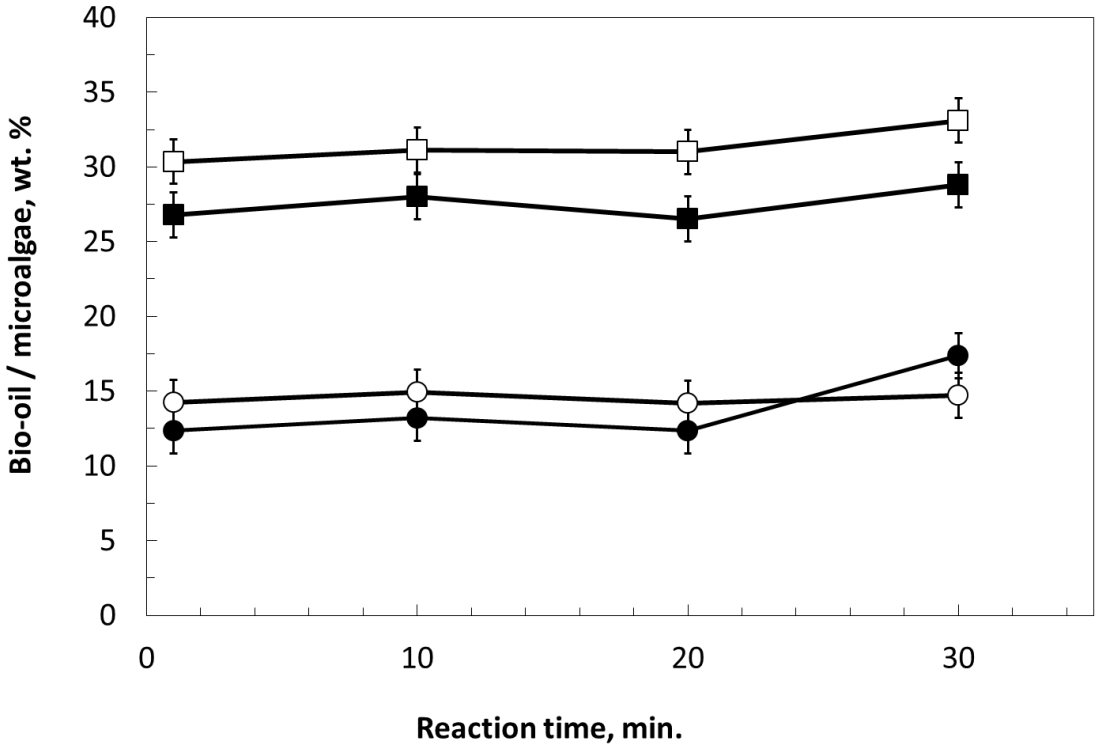
alcohols/ketones. However, the authors used dichloromethane to fractionate the reaction products obtained in the liquefaction.

As can be seen in figure 3, bio-oils yields obtained in the supercritical CO_2 fractionation of the reaction products varied between 12 wt.% and 17 wt.% according to reaction product sample extracted. A slightly increment in the CO_2 extraction yield was observed for the reaction products obtained in the supercritical alcoholysis at 250 ºC and 30 min. respect to the other samples processed (17 wt.%). The global bio-oil extract concentration in the solvent phase, according to the total CO_2 mass used in the extraction (≈12.5 g), was between 7 and 9 mg/g (extract/CO_2).

Up to our knowledge, supercritical CO_2 and hexane soluble fractions has not been reported in the literature for the non-catalytic direct supercritical alcoholysis of microalgae biomass. According to Dote et al. [30], hexane soluble products can be regarded also as "hydrocarbons" fraction. These authors studied the liquefaction of *Botryococcus braunii* at high temperatures (200-340 °C). They fractionated the reaction products with dichloromethane and successively hexane and found that the hydrocarbon fraction after the liquefaction was also greater than the neutral lipids initially present in the material. More recently, Valdez et al. [31] defined the hexane soluble products obtained in the liquefaction of *Nannochloropsis* sp. with supercritical water as light "bio-crude". These authors reported a light bio-crude yield in the order of 20 wt.% processing at 300 °C, while the original biomass exhibited a 14 wt.% of initial lipid content [31].



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<InlineShape8><InlineShape7><InlineShape6><InlineShape5>Figure 3. Bio-oil yields obtained with hexane and supercritical CO_2 from the different supercritical alcoholysis reaction products. Hexane soluble product from reactions samples at 250 °C (■

<InlineShape16>
) and 280 °C (□

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<InlineShape15>
). Supercritical CO_2 soluble products from reaction samples at 250 ºC (

<InlineShape14>
) and 280 °C

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).
GC/MS analyses of the bio-oils samples allowed the identification of the main fatty acid esters present in the system, also the minor quantities of free fatty acids and mono/diglycerides. Table 2 shows the components identified in the hexane bio-oil sample obtained from the supercritical alcoholysis at 250 °C and 20 min of reaction time. Similar components were detected in the different bio-oil samples obtained in this work. In general, the main fatty acid esters determined in the reaction products agreed well with the fatty acid profile of the neutral lipids reported for the original substrate [3]. CO_2 bio-oil samples showed a lower composition (Area%) of free fatty acids and acylglycerides.

3.2 Fatty acid esters content in bio-oils

Figure 4 reports the fatty acid esters content of n-hexane and supercritical CO_2 bio-oil samples against the operating conditions of the supercritical alcohol transesterification studies. As can be seen greater fatty acid ester contents were determined in supercritical CO_2 bio-oils indicating a higher selectivity of CO_2 to extract fatty acid esters in comparison with n-hexane. The results show the direct supercritical alcoholysis produced different amounts of fatty acid esters according to the operating reaction conditions. For instance, a fatty acid ester content of 35 wt.% was analyzed in the hexane bio-oil obtained from reaction products processed at 280 °C in the initial heating time. Then, the fatty acid ester content after 30 min. decreased notably to 28 wt.% at this temperature. On the other hand, n-hexane soluble products obtained from the supercritical alcohol processing of *N. oleoabundans* at 250 °C showed increasing fatty acid esters contents with reaction time. A fatty ester content of 22 wt.% was determined in n-hexane bio-oils corresponding to reaction products samples obtained during the heating period and it increase to 32 wt.% after 20 min. of reaction time.

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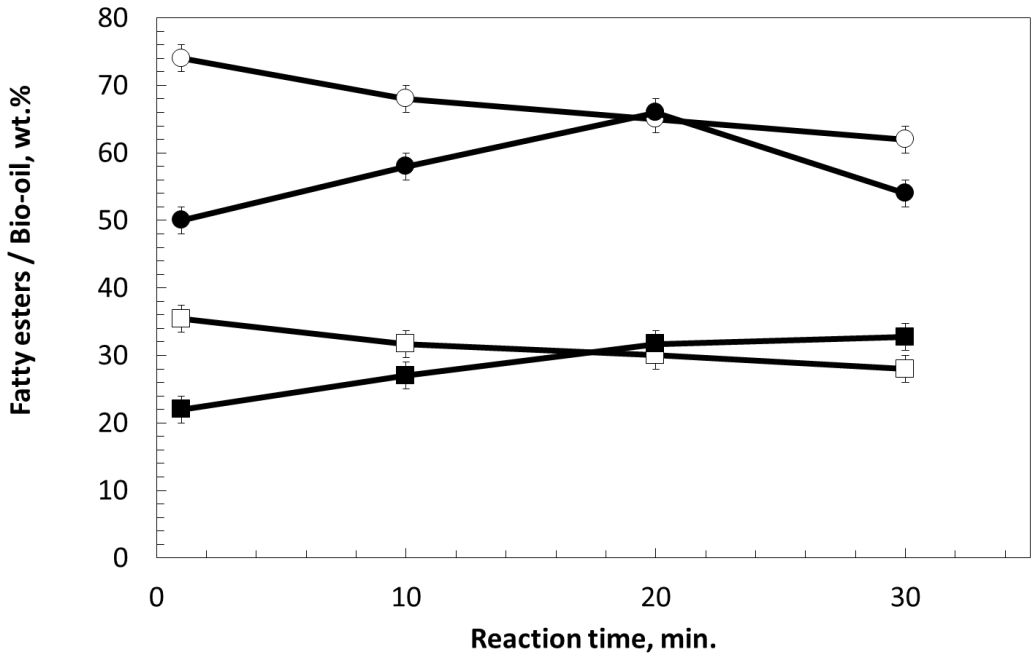

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<InlineShape4><InlineShape3><InlineShape2><InlineShape1>Figure 4. Fatty ester content in the bio-oils samples. Hexane bio-oils from reaction products obtained at at 250 °C (■

<InlineShape12>
) and 280 °C (□

<InlineShape11>
). Supercritical CO_2 bio-oil from reaction samples at 250 (●

<InlineShape10>
) and 280 °C (○

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).

Bio-oils extracted with supercritical CO_2 from reaction products obtained in the initial heating period of the supercritical alcoholysis at 250 °C and 280 °C shows fatty acid ester contents between 50 wt.% and 74 wt.%, respectively. These results are consistent with fatty acid esters concentrations found in hexane bio-oils. Supercritical CO_2 Bio-oils with up to 74 wt.% fatty acid ester were obtained from the reaction products of the supercritical alcoholysis at 280 °C and 1 min. of reaction time. A lower fatty acid ester content (66 wt.%) was analyzed in the supercritical CO_2 bio-oil extracted from reaction products obtained at 250 ºC and 20 min of reaction time. Supercritical alcoholysis reactions carried out at both reaction temperatures and 30 min. shows a reduction in the fatty acid ester content in comparison with lower reaction times.

Fatty acid methyl esters exhibit a high solubility in CO_2 at the operating conditions tested in this work (140 bar and 40 °C) [27]. Polar and heavy compounds in the reaction products samples like non-converted acylglycerides, pigments, glycolipids, have low solubility in CO_2 [16, 27] which explains the higher selectivity of CO_2 to extract fatty acid esters. On the other hand,

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a high fatty acid esters in the solvent phase could promote a co-solvency effect increasing the solubility of acylglycerides, pigments and oligomers [15, 26].

Reaction products remaining in the high pressure column after the CO_2 extraction were extracted also with liquid hexane. Figure 5 and 6 shows a typical GC analysis of the supercritical CO_2 bio-oil and soluble hexane products present in the high pressure column after the CO_2 extraction. GC analysis shows fatty esters were completely removed by CO_2, while free fatty acids remained in the extractor together with other compounds of higher molar mass.

Patil et al. [10] observed a fatty acid ester content of about 80 wt.% in the direct supercritical methanolysis of *Nannochloropsis* sp. (69.8 wt.% water) at 280 °C and 30 min of reaction time working with a higher methanol to algae mass ratio (in the order of 10 g/g). The fatty acid esters content reported by the authors corresponded to a refined product obtained by solid phase extraction [10]. It is well known that the molar ratio of methanol to lipids is a relevant factor in the supercritical transesterification technology [7, 26, 27]. A high alcohol to oil molar ratio is necessary to attain homogenous operation and to shift the chemical equilibrium toward the production of fatty esters [26]. In this work, it was used a mass ratio of 3 methanol/dry algae which means a methanol/oil molar ratio ca. 423:1, assuming that the 20 wt.% of the microalgae neutral lipids have a mean molar mass of 885 g/mol. High alcohol to oil molar ratios are also normally employed in the low pressure direct transesterification of microalgae [7]. For instance, Ehimen et al. [32] used methanol to lipids molar ratios between 105:1 and 524:1 in the direct transesterification of chlorella biomass with an acid catalyst.

Regarding the supercritical alcohol transesterification of vegetable oils, several studies have shown an important effect of the reaction temperature in the fatty acid esters yield. As an example, Silva et al. [34] studied the biodiesel production from soybean oil using supercritical ethanol in a continuous microtube reactor. It was observed working with a molar ratio of 20 to 1 ethanol to oil and 25 min. of reaction time a fatty acid esters yield of 19 wt.% at 250 °C that increased to 53 wt.% at 325 °C and similar operating conditions [33]. Later, Silva et al. [34] shows the supercritical ethanol processing of Jatropha curcas with a mass ratio of 1:1 ethanol to oil produced fatty acid ester yields of 25 wt.% and 38 wt.% for a residence time of 21 min. and reaction temperatures of 250 °C and 275 °C, respectively. Abdala et al. [36] studied the supercritical alcohol transesterification of waste cooking oils in a continuous reactor at different reaction conditions using a mass ratio 1:1 of ethanol to oil. The authors founded at 200 bar and 40 min. of residence time a fatty acid ester yield of 34 wt.% at 275 °C and it increased up to 46 wt.% at 325 °C [35].

The mass of hexane soluble products at 280 ºC was nearly constant over the reaction time pointing out fatty acid esters would further reacted towards other hexane soluble products. According to Quesada-Medina and Carrillo [36] both fatty esters and the triglycerides can be degraded to oligomers at high temperature due to the thermal linear dimerization of monounsaturated fatty acids to produce acyclic structure of dimers. Polyunsaturated fatty esters are more susceptible of degradation producing a mixture of the monocyclic and six-membered cyclic dimers [36]. Silva et al. [34] observed in the biodiesel production from refined soybean oil with supercritical ethanol at 200 bar, 325 °C and 45 min. of residence time a low decomposition of fatty acid ester (< 5 wt.%). Vieitez et al. [37] studied the decomposition of fatty acid esters in the continuous supercritical alcohol transesterification of soybean oil at 200 bar and different temperatures from 250 °C to 375 °C. The authors showed the decomposition rate is highly dependent on temperature, and mainly on the nature and unsaturation degree of the alkyl chains. At 250 °C the fatty acid ester content remain almost

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constant (2.5 % decomposition) while at 350 °C the decomposition increase to 14.5 % [37]. In this work it was observed the fatty acid esters in the bio-oils tend to decrease with the reaction time at 280 °C indicating fatty esters could be reacting with other components present in the microalgae biomass loaded to the reactor and converted to non-polar products soluble in hexane and partially soluble in CO_2.

The water present in the biomass also plays an important role because the hydrolysis reaction competes with the methanolysis. Nevertheless, the GC analysis of the bio-oil samples showed a minor concentration of free fatty acids (≈1.5%). The processed biomass exhibited a water content of 25 wt.% which means a molar ratio of about 82 water/oil. The relative higher alcohol concentration in the system with respect to water promotes the production of esters over free fatty acids [26]. Furthermore, in the supercritical ethanol transesterification of used frying oils it was observed the addition of 5 wt.% water led to improve the fatty acid ester yields [35]. However, greater concentrations of water had non-significant effects in the system [35]. Regarding results obtained in this work a complete drying of the original wet biomass (80 wt.% water) is not necessary because humidity values of 25 % yield a reasonable selectivity to achieve high fatty esters over fatty acids ratios in the reaction products.

3.3 Biodiesel yields

Figure 7 shows the fatty esters yields obtained in the direct methanolysis of *N. oleoabundans* respect to the initial dry mass of processed microalgae. The maximum fatty esters yield was obtained at 280 ºC during the heating time period (≈11 wt.% based on dry microalgae processed) and thereafter it decreased with the reaction time at this temperature. On the other hand, the fatty ester production increased with the reaction time when the methanolysis was carried out at 250 ºC. A maximum of ≈9 wt.% of fatty esters on a dry biomass basis is attained after 30 min. All runs showed a complete conversion of the neutral lipids present in *N. oleoabundans* (no-triglycerides were detected in the GC-analysis). However, a maximum yield of ≈56 wt.% through the fatty esters production respect to the initial neutral lipids content was obtained in the range of operating conditions studied in this work. Reddy et al. [38] studied the direct conversion of *Nannochloropsis salina* using supercritical ethanol conditions. The authors obtained a maximum yield of 67 wt.% respect to the initial lipids present in the microalgae working at 265 °C, 20 min of reaction time and using a mass to volume ratio of 1 to 9 g mL^{-1} algae/ethanol [38].

In order to test and validate the operating conditions studied in this work, a direct supercritical alcoholysis of *N. oleoabundans* was carried out in a batch reaction cell of 41 mL using 5.5 g of microalgae, at 280 °C, and 10 min. of reaction time using an alcohol/mass ratio of 2.14 methanol/ dry microalgae. A fatty acid ester yield of 10.5 wt.% respects to the initial biomass processed was obtained in the duplicated experiments. Also, results indicated an hexane bio-oil production higher than the initial neutral lipid in the biomass (≈30 wt.% with respect to the dry microalgae).

Similar fatty acid esters yields for other microalgae species were reported using the direct supercritical alcohol technology. Levine et al. [9] studied a two steps process consisting in hydrolysis of wet biomass and subsequent supercritical ethanol treatment to obtain the fatty esters. The authors obtained in the hydrolysis of *Chlorella vulgaris* a wet solid (46 wt.% water) that retained 87 wt.% of the initial lipids from which a 67 wt.% were converted to fatty acids working at 280 °C during 120 min of reaction time. Further processing of the wet solid by

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supercritical ethanol at 325 °C during 120 min and with a mass ratio of 8.3 of ethanol/dry hydrolysis solids produced a FAEE content of 58.7 wt.% in the crude biodiesel meaning a fatty ester yield of 51 wt.% respects to the initial lipids. Further studies reported by the authors [33] showed a considerable improvement on the esterification reaction yields of the fatty acids present in wet hydrochars (79 wt.% of fatty acids were converted to esters) at 275 ºC and 150 min, using a 5/1 ethanol to fatty acids molar ratio. The authors observed a clear degradation of the unsaturated fatty acids at reaction temperatures higher than 280 ºC and 90 min of reaction time.

According to different authors [6,7], the non-soluble in hexane or CO_2 reaction products could be further treated with water in order to obtain a potential nutrient source that can be recycle to grow more lipid-reach microorganisms. Valdez et al. [31] studied the hydrothermal liquefaction of *Nannochloropsis sp* and reported the recovery of nearly 80 % of the initial phosphorus and nitrogen into the aqueous phase. Toor et al. [39] proposed to use the solid residues of microalgae liquefaction as an animal feed additive because of the high nutrient value of these products. Lehmann [40] suggested the used of the solid charcoal residue obtained in thermochemical conversion technologies for fertilizers and carbon sequestration to reduce carbon dioxide emissions and produce carbon negative-biofuels.

The supercritical CO_2 fractionation of the direct alcohol supercritical reaction products could be a feasible technique to obtain a refined biodiesel from microalgae biomass (high monounsaturated fatty acid esters concentration). It is a green technology [14,16] that can be an interesting alternative in biorefineries of second generation in order to avoid the used of hydrocarbons in the separation processes.

4. Conclusions

The direct supercritical methanolysis of partially dried *N. oleoabundans* (25 wt.% water) was carried out at 250 ºC / 280 ºC and increasing reaction times up to 30 min. A conversion of the initial dry biomass higher than 30 wt.% toward hexane soluble products was attained at 280 °C and different reaction times. However, a maximum fatty acid ester content of 35 wt.% was analyzed in hexane bio-oils (reaction products processed at 280 ºC after the heating time). On the other hand, reaction products that were extracted with supercritical CO_2 yielded between 12 wt.% and 17 wt.% of bio-oil respect to the initial biomass processed with up to 74 wt.% of fatty acid esters (280 °C and 10 min.) pointing out it is an interesting technology to fractionate biodiesel from the reaction products obtained in the direct supercritical alcoholysis of microalgae. Based on the neutral lipid content of the initial biomass, biodiesel yields of ≈50 wt.% were obtained under the operating conditions studied in this work.

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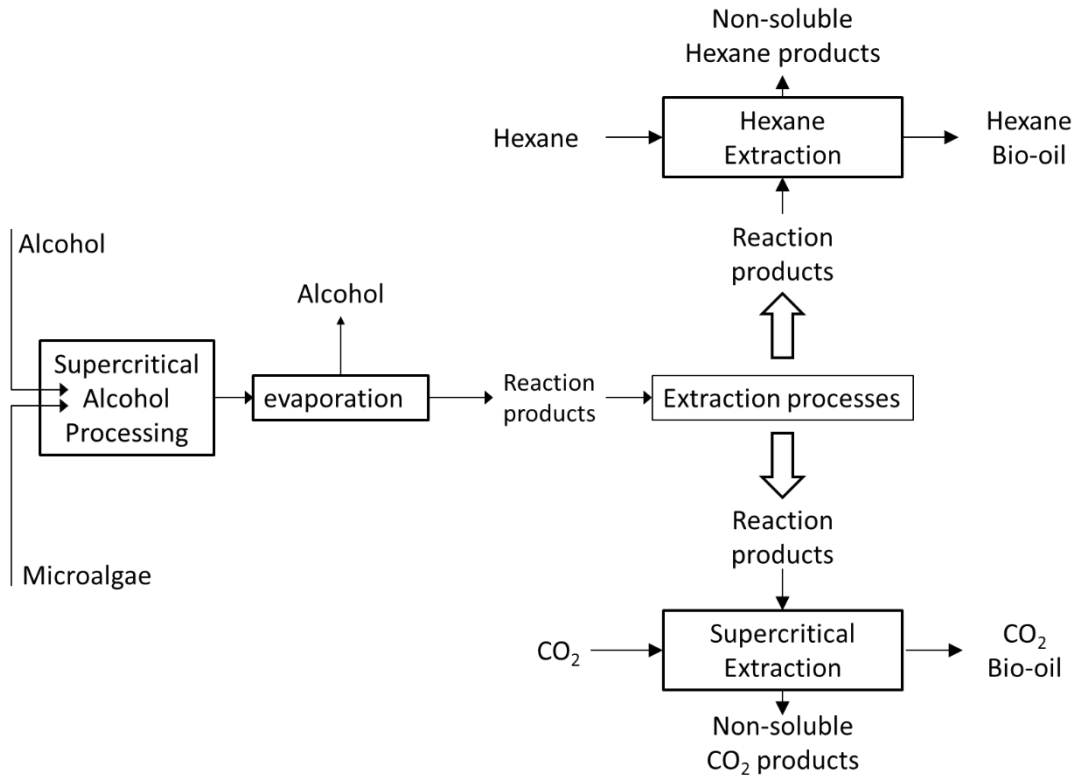

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Figure 1. Schematic diagram of the experimental procedure.

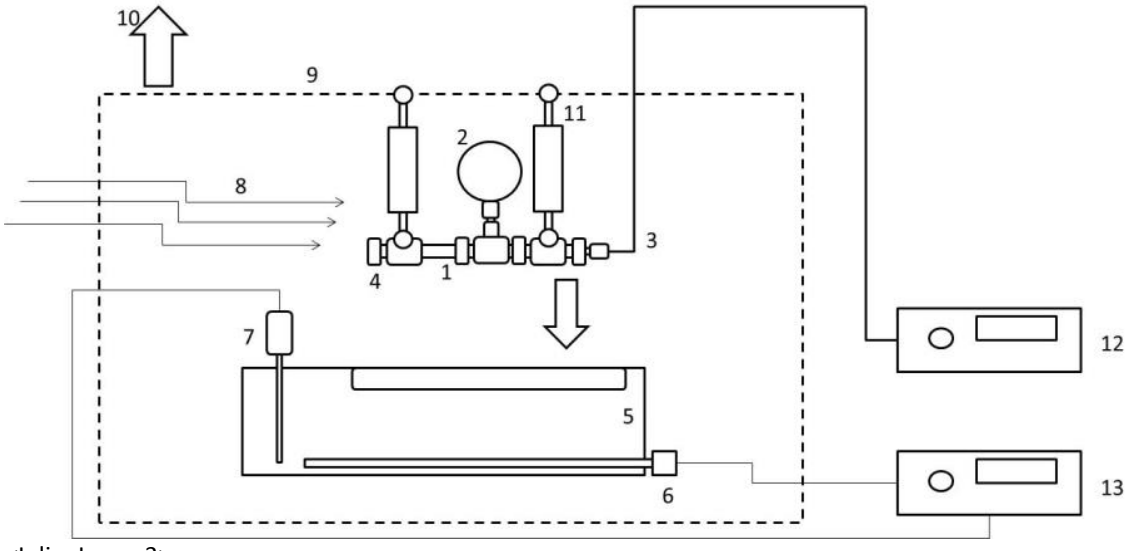

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Figure 2. Reactor set up used in the supercritical methanolysis of algal biomass. 1. Reactor, 2. Pressure gauge, 3. Reactor temperature sensor, 4. Screw cap, 5. Tin heating bath, 6. Electrical heating element, 7. Bath temperature sensor, 8. Air cooling system, 9. Air thermostatic bath, 10. Relief cooling air, 11. Clamping arm and immersion device, 12. Reaction temperature register, 13. Tin heating controller.

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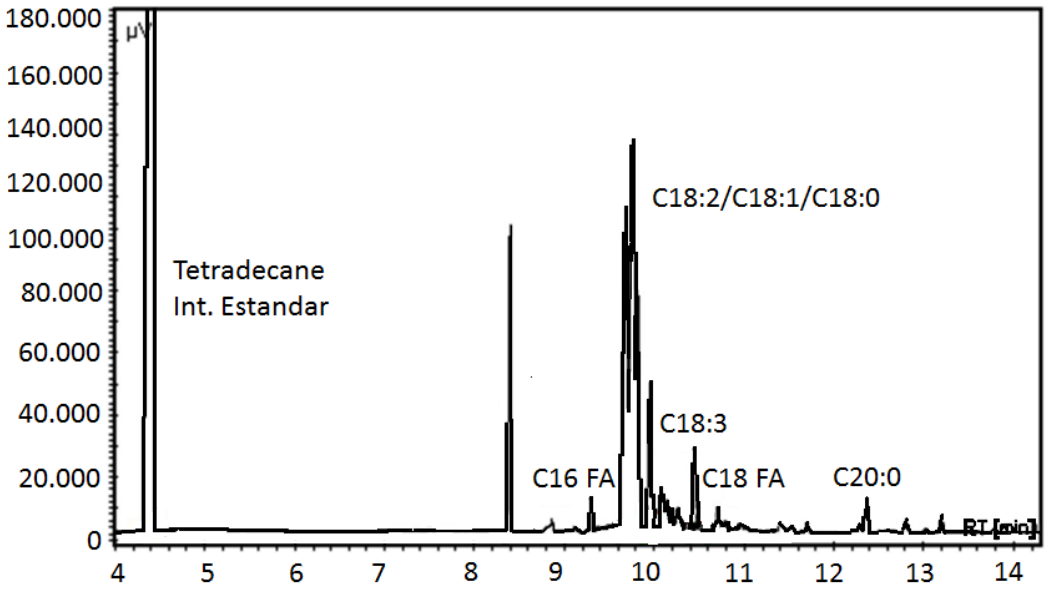

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Figure 5. GC analysis of the bio-oil CO_2-extract fractionated at 40 ºC and 140 bar. Bio-oil sample obtained by supercritical methanolysis at 280 ºC and 10 min reaction time. C16:1 methyl palmitate; C16 FA palmitic acid; C18:0 methyl stearate; C18:1 methyl oleate; C18:2 methyl linoelate; C18:3 methyl linolenate; DFE Degraded fatty esters; C18 FA oleic acid; C20:0 methyl eicosanoate.

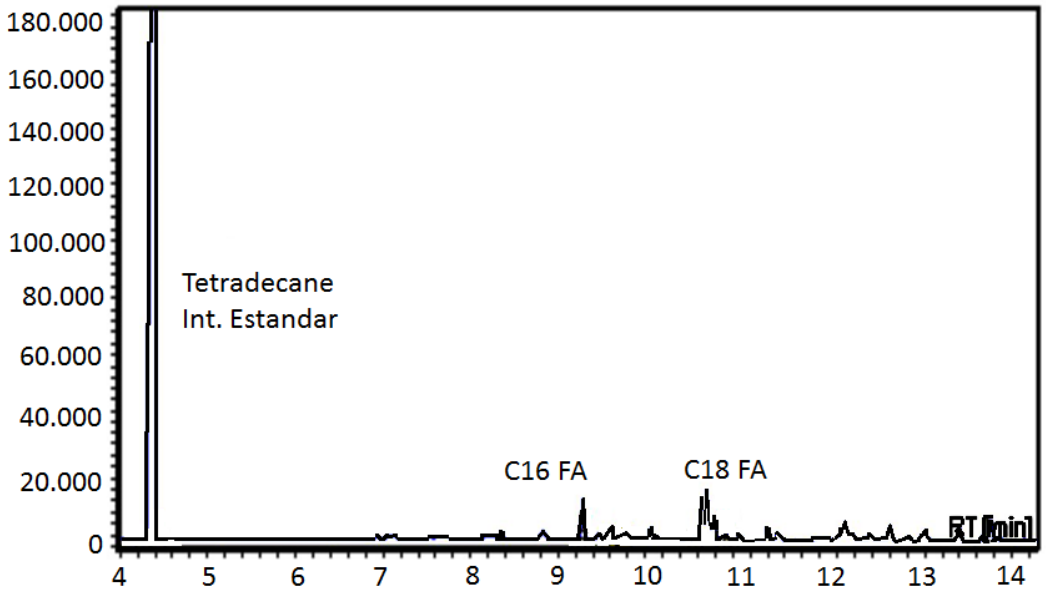

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Figure 6. GC analysis of the bio-oil CO_2-raffinate fractionated at 40 ºC and 140 bar. Bio-oil sample obtained by supercritical methanolysis at 280 ºC and 10 min reaction time. C16:1 methyl palmitate; C16 FA palmitic acid; C18:0 methyl stearate; C18:1 methyl oleate; C18:2 methyl linoelate; C18:3 methyl linolenate; DFE Degraded fatty esters; C18 FA oleic acid; C20:0 methyl eicosanoate.

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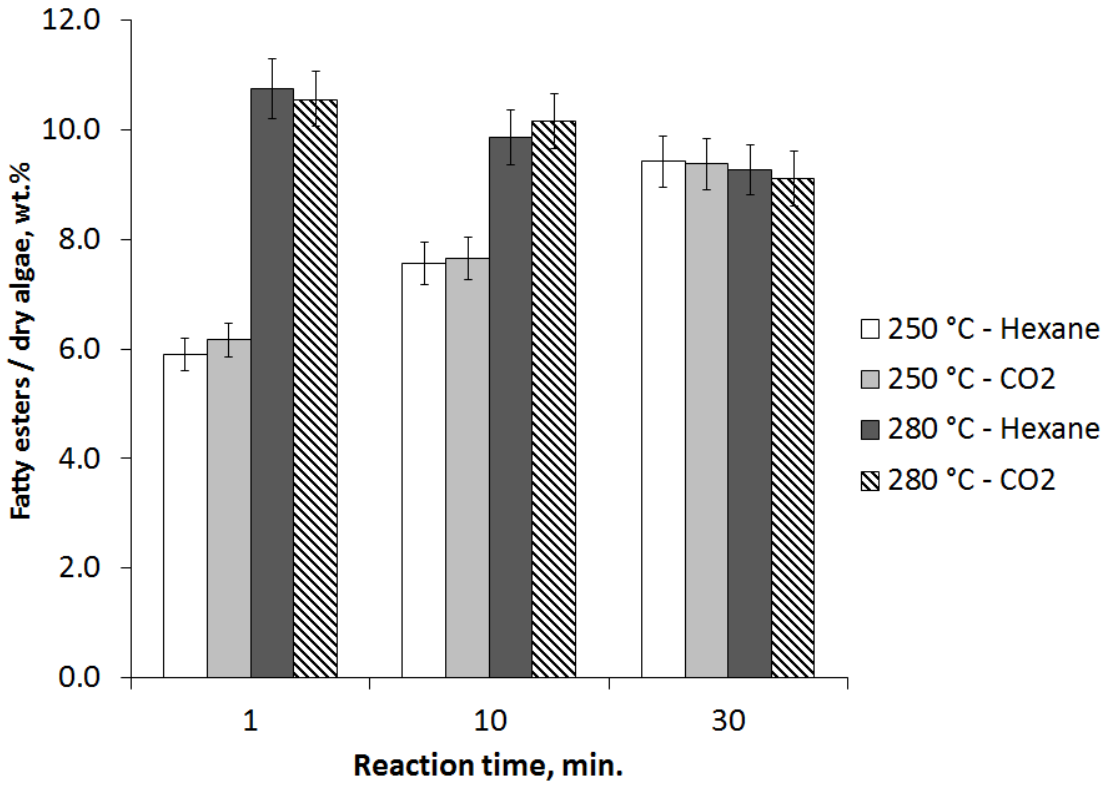

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Figure 7. Biodiesel yields obtained in the direct supercritical methanol transesterification of the lipids present in *N. oleoabundans*. Results reported for the different operating conditions carried out in the supercritical alcohol process

Table 1. Main fatty acids composition of *N. oleoabundans* lipids determined in GC/MS analysis

Main fatty acids	(Area, %)
C16:0	19.3
C18:0	5.2
C18:1n9c	47.3
C18:2n6c	16.2
C18:3n3	5.9
C20:0	0.3
C22:0	0.2

Table 2. GC-MS analyses of the bio-oil samples obtained in the supercritical methanolysis of *N. oleoabundans* at 250 ºC and 20 min of reaction time.

Components	time (min)	Area (%) Hexane bio-oil	Area (%) CO2 bio-oil
Methyl palmitate	8.4	12.33	12.84
Hexadecanoic acid	9.3	1.26	0.24
Methyl linolenate	9.7	5.94	6.48
Methyl oleate	9.8	41.22	41.8
Methyl linoelate	9.9	22.90	23.41
Methyl stearate	10	5.83	6.32
Degraded unsaturated fatty esters	10.1	6.90	7.1
Octadecanoic acid	10.98	1.13	0.21

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Methyl eicosanoate	12.4	0.81	1.33
Adipic acid, bis (2-ethylhexyl) ester	13.6	0.21	0.24
Monopalmitin	15	0.62	0.01
Monolein	17	0.85	0.02