FATTY ACIDS RECOVERY FROM MICROALGAE FOR BIODIESEL PRODUCTION

R. Montero[a,b], P. Hegel[a], C. Popovich[b], C. Damiani[b], S. Pereda[a] *, P. Leonardi[b]

[a] PLAPIQUI, Universidad Nacional del Sur,
CONICET, Bahía Blanca, Argentina

[b] CERZOS, Universidad Nacional del Sur,
CONICET, Bahía Blanca, Argentina.

Email: spereda@plapiqui.edu.ar

Abstract. The freshwater microalga *Neochloris oleoabundans* has been recognized as a promising oil feedstock, due to its capacity to accumulate lipids. Particularly, recent studies pointed out the potential of *N. oleoabundans*, cultured in sea water or in anaerobically digested dairy manure, to produce triglycerides with high content of monounsaturated fatty acids. Taking into account these results, the aim of the present study was to investigate the supercritical methanolysis of *N. oleoabundans* to recover fatty acids as biodiesel. In this work the fatty acids recovery is studied in a batch stainless steel reactor (14 cc). A methanol to algae mass ratio of 2.7 g/g, and different temperatures (533 K and 553 K) were analyzed against the reaction times in order to evaluate the crude bio-oil production and its biodiesel content. Fatty esters yields up to 10 wt% respect to the initial biomass processed were obtained.

Keywords: Supercritical methanol, microalgae, biodiesel

1. Introduction

Microalgae can significantly contribute to replace petroleum derived liquid fuels because it grows extremely rapidly and high oil contents have been reported in several varieties. Moreover, the fuels produced from microalgae oils do not compromise the production of food, as it is the case with oil crops[1]. Popovich et al. [2] carried out a lipid analysis in *Neochloris oleoabundans* (*N. oleoabundans*) cultured in seawater to evaluate its potential as raw material for biofuel production. The authors concluded that this microalgae is a good oil source to produce biodiesel due to its capacity to accumulate neutral lipids (20 wt%) with a major concentration of monounsaturated fatty acids[2]. Similarly, Levine et al. [3] studied the culture of *N. oleoabundans* in anaerobically digested dairy manure concluding also that the lipids produced by this species are suited for biodiesel production.

Nowadays, biodiesel is produced from refined vegetable oils derived from crops by an alkali-catalyzed transesterification process. Briefly, in this process the mixture of alcohol + oil + catalyst is stirred vigorously during two hours at 340 K and atmospheric pressure to produce fatty esters (biodiesel) and glycerol as by product[4]. However, it requires practically anhydrous vegetable oils with a free-fatty acid content lower than 0.5 wt% and also moisture free alcohols in order to obtain high reaction yields. The addition of more alkaline catalyst to the system compensates for higher acidity levels, but the resulting soaps cause the formation of gels, which interferes in the glycerol separation [5].

The biodiesel production by supercritical alcohols has been proposed as an efficient alternative technology to process raw lipids feedstock with high free fatty acids and water contents [6]. This method is carried out at temperatures around 598 K and pressures higher than 150 bar with a high excess of alcohol (alcohol to oil molar ratio in the order 40 to 1). At supercritical conditions, the reactive mixture is homogenous avoiding the mass transfer limitations present in the alkali process due to the liquid-liquid partial miscibility of alcohols and triglycerides [7]. Thus, the reaction rate increases because of the high temperature and triglycerides can

be converted completely toward fatty ester in less than 30 min. Moreover, the purification step is simpler because of the absence of catalyst. These advantages compensate for the higher capital cost associated to the process [8].

The direct supercritical methanolysis of algal biomass has been proposed recently as an alternative energy efficient technology and economical route for algal biodiesel production. Patil et al. [9] worked in the simultaneous extraction and transesterification of the lipids contained in wet algal biomass (*Nannochloropsis* sp.) by supercritical methanol. Levine et al. [10] proposed a two steps catalyst-free biodiesel production process involving lipid hydrolysis of *Chlorella vulgaris* and subsequent supercritical ethanol *in-situ* esterification. More recently, Levine et al. [11] also studied a two step process involving the hydrolysis of *Chlorella protothecoides* at 220-250 ºC and the esterification of the fatty acids using subcritical ethanol (215 ºC) in the presence of rare-earth metal triflate catalyst.

Recent studies pointed out the potential of *N. oleoabundans* cultured in sea water to produce high quality biodiesel[2]. Based on these results, the aim of the present study was to investigate the methanolysis of the lipids present in *N. oleoabundans* by a single-step supercritical process. This method is of interest because of its tolerance to process a high water content biomass as is the case of the microalgae. A direct methanolysis of the partially dehydrated biomass (25 wt% of water) was carried out in a batch reactor. The operating conditions were set according to previous works [9-12], a methanol/algae mass ratio of 2.3 g/g, temperatures between 523 K and 553 K and reaction times between 10 min. and 40 min. Depending on the operating conditions up to 11 wt% of the original biomass was converted to biodiesel (on a dry basis).

2. Materials and methods

2.1 Materials

Neochloris oleoabundans algal biomass was obtained according to the method described below. The *N. oleoabundans* algal sample had an initial water content of 80 wt.% with a neutral lipid content of ca. 20 wt% on a dry weight basis [2]. Methanol (99.6 wt.%) was purchased from Ciccarelli SA. For the 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 Algal strain and culture conditions

Neochloris oleoabundans (strain UTEX 1185 from the culture collection of the University of Texas, Austin) was used for this study. Cells were acclimated to marine conditions, through successive transfers in modified SWES (seawater + soil extract + salts) medium for 3weeks. The medium was composed of aged and filtered (0.45 μm Millipore) seawater from Bahía Blanca Estuary (38º 45'S, 62º 22'W), enriched with $NaNO_3$ (3.5mM), K_2HPO_4 (0.117mM), soil extract and micronutrient solution. The latter was prepared according to f/2 medium [13].The seawater salinity was 30 PSU and contained natural concentrations of NO_3 (24.6 μM), PO_4 (2.85 μM) and NH_4 (7.81 μM). The medium was autoclaved and its pH was adjusted to 8.0. Flasks with 3 L of culture were used for the experiments. Cells grew under a continuous bubbling of air (500-700 cm^3/min.) at 25-26 ºC. Moreover, an enriched air stream containing 1% CO_2 was fed every day during 4 h. Light was supplied by cool-white fluorescent tubes in a 16:8 light:dark photoperiod in order to get an average light irradiance of 168 μmol photons m^{-2} s^{-1}. In order to produce the required biomass, first an inoculum of 40 x 10^6 cells mL^{-1} was resuspended in 18 L of SWES medium for 19 days; after that the log-phase culture finished, it was harvested by centrifugation (10 min at 3600g) and transferred to 18 L of nitrogen-free medium SWES for 6 days.

2.3 Direct methanolysis of algal biomass

First, the biomass was rinsed with distilled water and dried in a convection oven at 60 ºC during 6 h. The final water content in the biomass of 25 wt% was determined by a gravimetric analysis (Sartorious moisture analyser MA 35).

The supercritical methanolysis were carried out in a stainless steel batch reactor of 14 cm^3, assembled with a thermocouple (± 1.5 K) and a pressure gauge (± 2.5 bar) as described in Figure 1. The reactor was loaded with methanol (≈2.94 g) and algal biomass (≈1.28 g) in a 2.3 methanol/algae mass ratio. After shaken the

reactants, it was submerged in a pre-heated tin bath to achieve the desired reaction temperature (523 K and 553 K). The operating conditions studied in this work were selected based on previous works [9-12]. Once initiated the heating procedure, reaction temperatures were reached in ~20 min. and ~30 min. at 523 K and 553 K, respectively. After the reaction time was completed the cell was cooled at room temperature in a water bath.

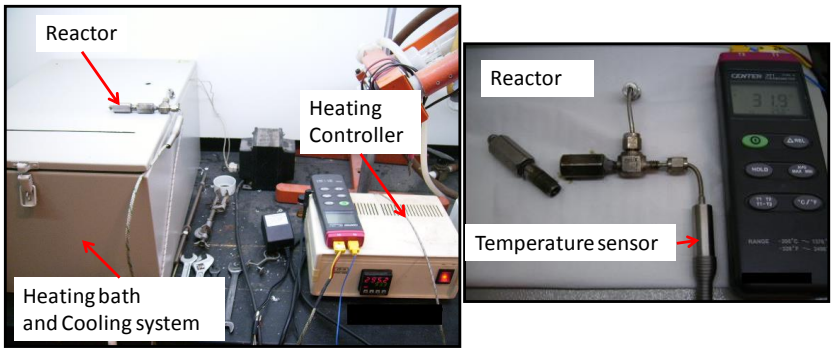

Figure 1. Experimental setup for the supercritical methanolysis of algal biomass.

Figure 2 shows the general procedure carried out to separate the reaction products. Briefly, the gas produced in the reaction was removed from the cell after the system was cooled at room temperature. Afterwards, the cell contents were transferred into a 100 mL round-bottom flask using acetone to clean the cell. Volatiles and excess methanol were separated under vacuum in a rotary evaporator operated at 343 K. Then, the oily-hexane soluble products were recovered by a twofold extraction with 25 mL of hexane and centrifugation at 3200 g. The non-volatile reaction products soluble in hexane were considered in this work as bio-oil, being further analyzed by GC to determine its fatty ester concentration. The bio-oil, water soluble and biodiesel yields were evaluated on a dry biomass basis. The present experiments were not replicated since the amount of biomass available was limited. However, the biomass sample size required to achieve good reproducibility was evaluated on the supercritical methanolysis of soy powder. This evaluation showed that for the size of samples used in this work the relative error in the gravimetric yields to bio-oil production were between 3 % and 9 %.

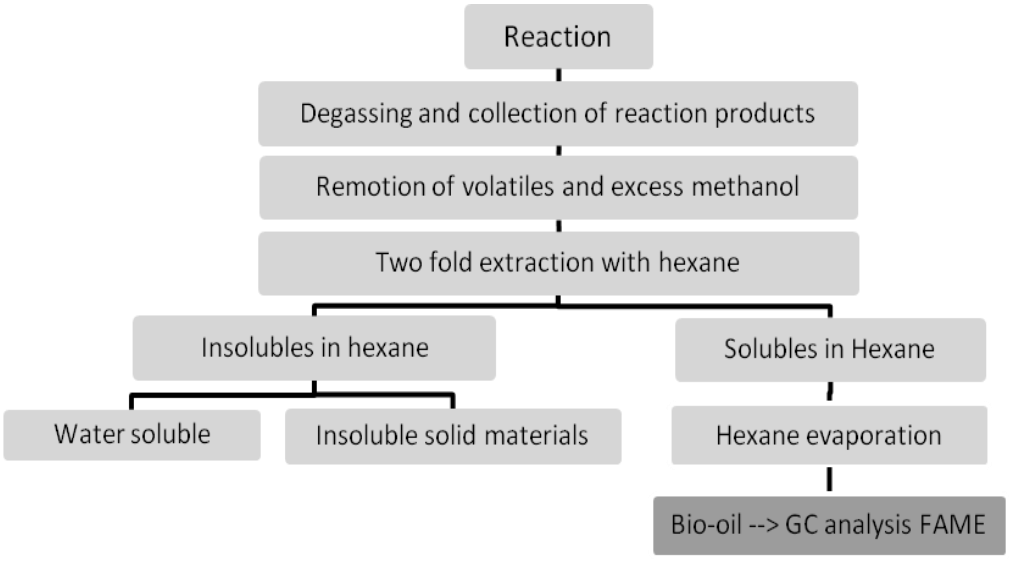

Figure 2. Procedure for the separation of direct supercritical methanolysis products.

3. Results and discussion

The bio-oil obtained in the direct methanolysis experiments showed a dark-brown color. Hexane dissolutions of these components were amber and they were more intense as the operating temperature increased. The solution of reaction products soluble in water presented a clear amber color. The solid products (i.e. non-soluble in hexane and water) after drying in a vacuum oven was a black powder.

Figure 3 shows the bio-oil yields obtained in the supercritical methanol transesterifications of *N. oleoabundans*. As it can be observed the bio-oil obtained at a given temperature was nearly independent of the reaction time. The absolute differences in the values of bio-oil yield are not significant because the errors associated to the experiments are in the same order. A barely higher yield was achieved at 553 K with respect to the reaction carried out at 523 K. From the results it can be noted the heating time to get the reaction temperature was enough to produce a given amount of bio-oil that remains constant during the rest of the reaction time. The fraction of products soluble in hexane that was obtained in the experiments is certainly higher than the neutral lipid content of the processed biomass. These results point out that, besides triglycerides, also other compounds like phospholipids or glycolipids could also be reacting with methanol and/or water.

According to Dote et al. [14], hexane soluble products can be regarded also as "hydrocarbons". These authors studied the liquefaction of *Botryococcus braunii* at high temperatures (473-613 K) and the reaction products were separated by dichloromethane and successively by hexane. They found [14] that the hydrocarbon fraction after the liquefaction was also greater than the lipids initially present in the material. More recently, Valdez et al. [15] defined the hexane soluble products obtained in the liquefaction of *Nannochloropsis* sp. as light "bio-crude". The authors reported a light bio-crude yield in the order of 20 wt% working at 573 K during 10 to 30 min., while the biomass had a 14 wt% of initial lipid content [15]. Patil et al. [9] followed a similar experimental procedure to the one used in this work to isolate the fatty esters from the reaction products. However, the authors only reported the fatty ester concentration in the bio-oil obtained in the reactions.

The mass of water soluble products was inferred from the mass of bio-oil and solid residue. As in the case of bio-oil production, it was not observed significant variations of the water soluble reaction products yield with temperature and/or reaction time. A mean value of 32 wt% of water soluble products yield respect to the dry biomass processed was obtained. High content of phosphorus and nitrogen could be present in aqueous soluble products whose recovery is important because both are desired to facilitate nutrient recycling in the microalgae biomass production [10,15].

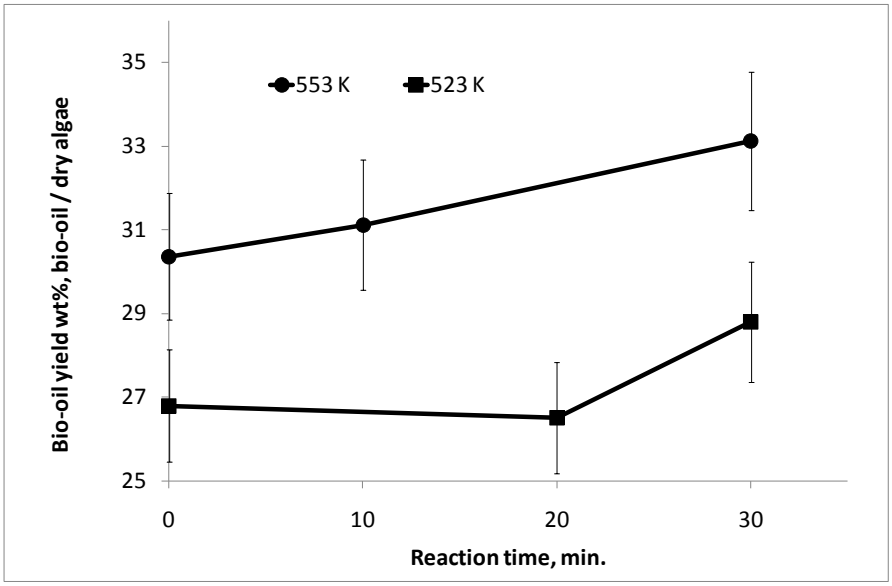

Figure 3. Bio-oil yield obtained in the direct supercritical methanol treatment of *N. oleoabundans* against the reaction time.

Figure 4 shows the fatty ester content in the bio-oil. In contrast to the bio-oil yield behavior, the fatty ester content is influenced by both, the temperature and reaction time. At 553 K, a 43 wt% of fatty ester was obtained in the bio-oil at the initial reaction time (heating period). Then, the fatty ester content decreased till reaching a 22 wt% of esters at 30 min. Clearly fatty esters were converted to other reaction products. The mass of bio-oil as well as the water soluble products were nearly constant over the reaction time. Thus, it is possible that the fatty esters were polymerized to heavy hydrocarbon products that remain in the bio-oil, but they were not detected in the GC analysis because of their high molar mass.

On the other hand, an increment in the fatty esters content of the bio-oil with the reaction time was observed when working at 523 K. After the heating period, a fatty ester content in the order of 10 wt% was found and it increased up to 26 wt% and 29 wt% after 20 min and 30 min., respectively.

Patil et al. [9] observed a fatty ester content of around 80 wt% in the direct supercritical methanol transesterification of *Nannochloropsis* sp. (69.8 wt% water) after 30 min. of reaction time working also at 523 K, but with a higher methanol to algae mass ratio (in the order of 10 g/g). However, the authors used a silica column to purify the hexane soluble products obtained in the reaction. Therefore, the reported fatty esters yields correspond to a refined bio-oil obtained from a fractionation by solid phase extraction. The experimental protocol, besides the different microalgae processed, could explain the lower yields obtained in this work respect to those results reported previously [9].

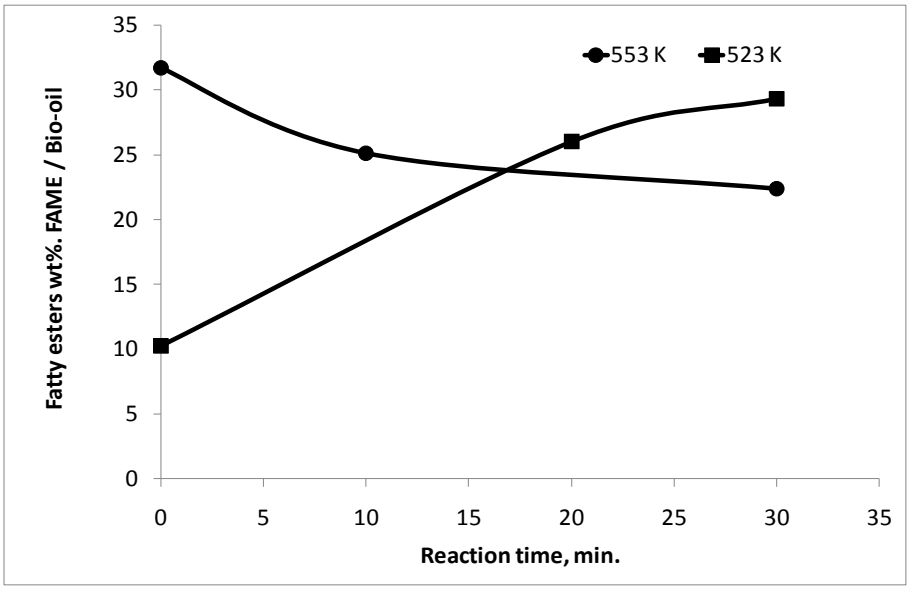

Figure 4. Fatty ester content in the bio-oil determined from GC analysis against the reaction time for both reaction temperatures.

The molar ratio of methanol to lipids is another relevant factor in the biodiesel production by the supercritical alcohol technology [6,7]. In the transesterification of vegetable oils a high molar ratio of alcohol to oil is necessary to obtain a single reaction phase and to shift the chemical equilibrium toward the production of fatty esters. However, there is a general agreement in the literature [6,7,16] that fatty ester contents in the biodiesel increased with increasing molar ratios up to 40 to 1 methanol to oil, after which the conversion remain constant. In this work, it was used a mass ratio of 2.3 methanol / algae which implicates a molar ratio in the order of 424 methanol / oil (estimated assuming a molar mass of 885 g/mol for 20 wt% biomass neutral lipids). Therefore, the alcohol used in this work should be adequate to shift the reaction towards the biodiesel production from the point of view of the transesterification. Anyway, it should be noted that high molar ratios beyond 100 methanol / oil could also implicate a reduction of the reaction rate because of a lower triglyceride concentration [16].

The water present in the original biomass also plays an important role because the hydrolysis reaction competes with the methanolysis. The biomass processed exhibit a final water content of 25 wt% which means a molar ratio in the order of 82 water / oil. The relative higher alcohol concentration in the system with

respect to water may promote the production of esters [10, 16]. Moreover, the processing of the original wet biomass (80 wt% water) entails a reduction of biomass loaded to the reactor. Also, higher water contents in the reaction system implicates an increased of the methanol to wet algae mass ratio in order avoid the fatty acids production. Thus, the costs associated with drying may be compensated by a greater production capacity per unit of reactor volume.

Figure 5 shows the biodiesel yields obtained in the direct transesterification of *N. oleoabundans* respect to the initial algae material processed, which obviously follows the same trend as the fatty esters content in the bio-oil. The maximum fatty esters yield was obtained at 553 K during the heating time period and thereafter it decreased with the reaction time at this temperature. On the other hand, the fatty ester production increased with the reaction time working at 523 K to attain an 8 wt.% of fatty esters on a dry biomass basis 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 50 wt.% with respect to the initial biomass oil content was obtained in the range of operating conditions studied in this work.

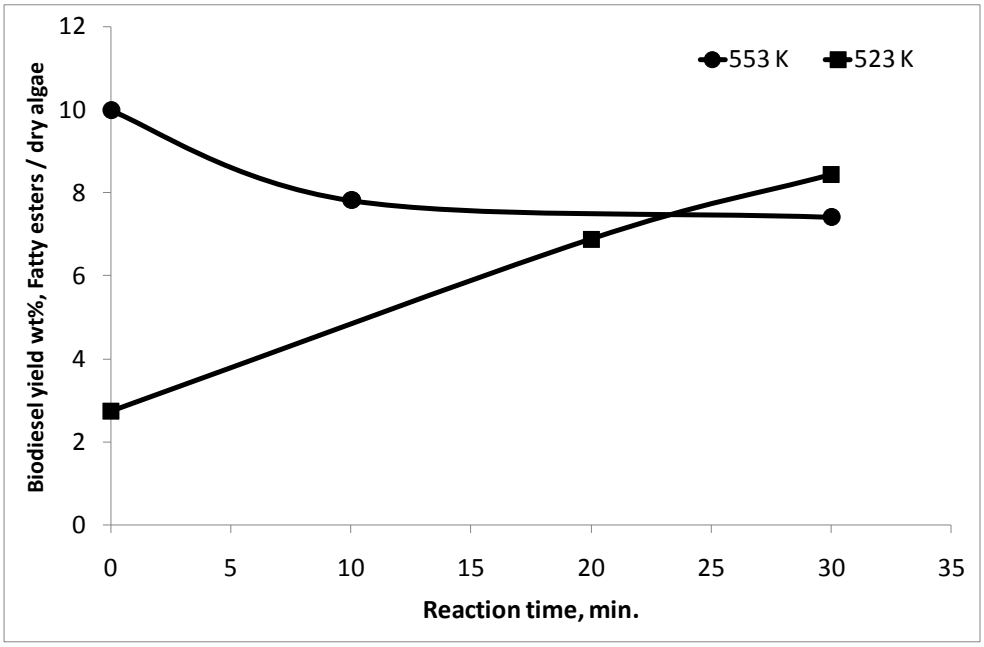

Figure 5. Biodiesel yields obtained in the direct supercritical methanol transesterification of the lipids present in *N. oleoabundans*.

In a previous work on the direct supercritical methanol processing *N. oleoabundans* [12] was obtained a fatty ester yield of 11 wt% respects to the processed biomass. In this early study ca. 5.5 g of biomass (15 wt% water) was processed with a mass ratio of 2.14 methanol / algae in a batch reactor (41 mL capacity) operating the reactor at 533 K during 20 min. The results reported in this work agree well with the previous results[12] in which the initial neutral lipids were converted in around 55 wt% toward fatty esters.

Also, similar biodiesel yields for other microalgae varieties were reported using the direct supercritical alcohol technology. Levine et al. [10] studied a two step 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 553K during 120 min. of reaction time. Further processing of the wet solid by supercritical ethanol at 598 K 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.

Results obtained in this work point out a bio-oil production higher than the initial neutral lipid in the biomass (~30 wt% with respect to the dry microalgae). However, the operating conditions have to be carefully controlled to maximize the fatty ester content in the bio-oil because a reaction temperature higher than 523 K during prolonged reaction times caused fatty esters degradation. A similar phenomenon was also

reported in the literature [9]. Residual water soluble products can be recycled as nutrient for the microalgae cultures because of its high nitrogen and phosphorus content, adding value to the byproducts obtained in the reaction [15]. The recovery of fatty esters from the bio-oil by different technologies as vacuum distillation, solid adsorption or supercritical CO_2, could be feasible options to obtain a refined high quality biodiesel and a residual bio-oil of important heating value [14] that can be used as fuel oil in furnaces or boilers for heat generation or upgraded to lighter hydrocarbon fractions.

4. Conclusions

The direct supercritical methanolysis of partially dehydrated *N. oleoabundans* (25 wt% water) was carried out at 523 K and 553 K and increasing reaction times up to 40 min. 60 wt.% of the original biomass was converted towards water and hexane soluble products. The supercritical alcohol treatment produced a 30 wt% bio-oil yield respect to the initial biomass with ca 32 wt.% of fatty esters after the heating time necessary to attain 553 K(15 min.). Based on the initial biomass lipid content, a biodiesel yield of 50 wt% was obtained under the better conditions studied in this work. Besides higher biodiesel yields are desirable, further refining processing of the bio-oil could lead to the production of high quality biodiesel and a green fuel-oil.

Acknowledgements

This study was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET), PIP 112-200801-00234; Secretaría de Ciencia y Tecnología de la Universidad Nacional del Sur, PGI TIR and Agencia Nacional de Promoción Científica y Tecnológica, PICT-2010-0959.

References

[1] Y. Christi, Biodiesel from microalgae, Biotechnology Advances 25 (2007) 294-306.
[2] C.A. Popovich, C. Damiani, D. Constenla, A.M. Martínez, H. Freije, M. Giovanardi, S. Pancaldi, P.I. Leonardi, *Neochloris oleoabundans* grown in enriched natural seawater for biodiesel feedstock: Evaluation of its growth and biochemical composition, Bioresource Technology 114 (2012) 287-293.
[3] R.B. Levine, M.S. Costanza-Robinson, G.A. Spatafora, *Neochloris oleoabundans* grown on anaerobically digested dairy manure for concomitant nutrient removal and biodiesel feedstock production, Biomass and Bioenergy 35 (2011) 40-49.
[4] A. Demirbas, Biodiesel: a realistic fuel alternative for diesel engines. London: Springer; 2008.
[5] B. Freedman, E.H. Pryde, T.L. Mounts, Variables Affecting the yields of fatty esters from transesterified vegetable oils, JAOCS, 61 (1984) 1638-1643.
[6] D. Kusdiana, S. Saka, Effects of water on biodiesel fuel production by supercritical methanol treatment, Bioresource Technology 91 (2004) 289–295.
[7] P. Hegel, A. Andreatta, S. Pereda, S. Bottini, E.A. Brignole, High pressure phase equilibria of supercritical alcohols with triglycerides, fatty esters and cosolvents, Fluid Phase Equilibria 266 (2008) 31–37.
[8] M.S. Diaz, S. Espinosa, E.A. Brignole, Model-Based Cost Minimization in Noncatalytic Biodiesel Production Plants, Energy & Fuels 23 (2009) 5587–5595.
[9] P.D. Patil, V.G. Gude, A. Mannarswamy, S. Deng, P. Cooke, S. Munson-McGee, I. Rhodes, P. Lammers, N. Nirmalakhandan, Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions, Bioresource Technology 102 (2011) 118–122.
[10] R.B. Levine, T. Pinnarat, P.E. Savage, Biodiesel Production from Wet Algal Biomass through in Situ Lipid Hydrolysis and Supercritical Transesterification Energy & Fuels 24 (2010) 5235–5243.
[11] R.B. Levine, A.A. Bollas, M.D. Durham, P.E. Savage, Triflate-catalyzed (trans)esterification of lipids within carbonized algal biomass, Bioresource Technology 111 (2012) 222–229.
[12] G. Soto, P. Hegel, C. Popovich, C. Damiani, S. Pereda, P. Leonardi, Biodiesel production from microalgae by supercritical methanolysis, 10th International Symposium on Supercritical Fluids, San Francisco CA, USA, 2012.

[13] Guillard, R.R.L., 1973. Division rates. In: Stein, J.R. (Ed.), Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, pp. 289–311.
[14] Y. Dote, S. Sawayama, S. Inoue, T. Minowa, S. Yokoyama, Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction, Fuel 73 (1994) 1855-1857.
[15] P.J. Valdez, M.C. Nelson, H.Y. Wang, X.N. Lin, P.E. Savage, Hydrothermal liquefaction of *Nannochloropsis* sp.: Systematic study of process variables and analysis of the product fractions, Biomass and Bioenergy 46 (2012) 317-331.
[16] T. Pinnarat, P. E. Savage, Assessment of noncatalytic biodiesel synthesis using supercritical reaction conditions, Ind. Eng. Chem. Res. 47 (2008) 6801–6808.
[17] P. Valle, A. Velez, P. Hegel, G. Mabe, E.A. Brignole, Biodiesel production using supercritical alcohols with a non-edible vegetable oil in a batch reactor, J. of Supercritical Fluids 54 (2010) 61–70.
[18] K. Bunyakiat, S. Makmee, R. Sawangkeaw, S. Ngamprasertsith, Continuous Production of Biodiesel via Transesterification from Vegetable Oils in Supercritical Methanol, Energy & Fuels 20 (2006) 812-817.
[19] M.N. Varma, G. Madras, Synthesis of Biodiesel from Castor Oil and Linseed Oil in Supercritical Fluids, Ind. Eng. Chem. Res. 46 (2007) 1-6.