17th International Conference on Environmental Science and Technology

Athens, Greece, 1 to 4 September 2021

Biodiesel Properties from *Chlorella sorokiniana* Bio-Oil Cultivated Heterotrophically with Industrial By-Products

KOKKALIS A.[1], KASITEROPOULOU D.[2], PAPADOPOULOU A.[2], METSOVITI M.N.[2], MPESIOS A.[2], GOUGOULIAS N.[3], SPILIOTIS X.[2], PAPAPOLYMEROU G.[2]

[1] GRINCO S.A., Industrial Area of Larisa, Makrychori Larisas, TK 41303, Greece

[2] Dept. of Environmental Studies, University of Thessaly, Gaiopolis, Larissa, T.K. 41500, Greece

[3] Dept. of Agrotechnology, University of Thessaly, Gaiopolis, Larissa, T.K. 41500, Greece

*Corresponding author: Papapolymerou George

e-mail: papapoly@uth.gr

Abstract

The fatty acid (FA) distribution of bio-oil derived from *Chlorella sorokiniana* and the biodiesel basic properties were examined. *C. sorokiniana* was cultivated heterotrophically in two growth media: a) glycerol and inorganic salts (GLIN) and b) glycerol and anaerobic digestrate (GLAD). The cultivation took place in 42 L bioreactors. The bio-oil was extracted from the biomass collected. Extraction was performed using a mixture of n-hexane and isopropanol in a 3:2 ratio. The fatty acid (FA) distribution was determined in a gas chromatograph by converting the bio-oil in biodiesel. The fatty acid distribution covered chain lengths from C10 to C26. The great proportion of the FA were of medium chain FA C16-C18 constituting about 85% and 53% of the total fatty acids of the GLIN and GLAD treatments respectively. Also, the distribution of saturated, monounsaturated and polyunsaturated FA differed. The basic properties of the biodiesel such as the density, the kinematic viscosity, the acid value, the cetane number, the iodine value and the heating value were within the range of the respective values from biodiesel obtained from seed oils and differences in the properties of the two treatments were explained in terms of differences in FA distribution.

Keywords: biodiesel properties, *Chlorella sorokiniana*, heterotrophic, FA distribution

1. Introduction

Microalgae are unicellular photosynthetic organisms that use light and carbon dioxide, with higher photosynthetic efficiency than plants, for the production of biomass. Some microalgae species can also grow and multiply heterotrophically in the absence of light if an organic carbon source becomes available (Mata *et al*., 2010). Microalgae are rich in proteins and lipids. The microalgae growth rate as well as the protein and lipid content are influenced by many parameters, such as the cultivated species, the temperature, the concentration and type of carbon source used, the ratio of carbon to nitrogen, the medium pH, the concentration of potassium, phosphorus and micronutrients, dissolved oxygen availability and the mixing rate (Gouveia & Oliveira, 2009).

For biodiesel production, except for a high lipid productivity a favorable fatty acid profile of the lipids is needed as well. Currently, biodiesel is mainly produced from used oils and various seed oils such as sunflower and cotton seed oils. Vegetable and seed oils contain fatty acid mostly from about C12:0 to about C24:0. Used oils contain a small amount of animal fats which have a lower fatty acid chain length. The fatty acid (FA) distribution such as chain length and the degree of saturation influence the properties of the biodiesel obtained through the transesterification process.

In this study fatty acid methyl esters (FAME) obtained from the transesterification of bio-oil extracted from the biomass of *C. sorokiniana*, were cultivated in two different growth media and growth modes were examined for their basic properties in order to compare them with the values required for the commercial biodiesel and thus determine its suitability in a potential future application. Therefore, *C. sorokiniana* was cultivated heterotrophically, a) in a semi-batch mode with glycerol, as the source of carbon, and inorganic salts as the source of N, P and micronutrients and b) in a batch mode with glycerol and 40% anaerobic digestrate (AD) as a source of additional carbon and N, P and micronutrients.

2. Materials and Methods

2.1 Bioreactors

The cultivation of *C. sorokiniana* was carried out in glass cylindrical bioreactors of 42L capacity each that were filled to 80% of their volume. Air was continuously provided to each bioreactor though a perforated network of piping placed at the bottom of the bioreactor tank. The pH was adjusted manually as needed with the use of HCl or NaOH solutions. The bioreactors, the glass tubing and the culture medium were sterilized before use.

2.2 Inoculum preparation

The microalgae species *C. sorokiniana* (SAG strain 211-31) was obtained from Culture Collection of Algae from the University of Göttingen in Germany (EPSAG). The cultivation medium used for the preparation of the inoculum was the Basal Medium (= ES "Erddekokt + Salze"). Each bioreactor was inoculated with a standard quantity of 250 mL of *C. sorokiniana* inoculum of 0.5 absorbance.

2.3 Materials

Crude glycerol was used as the carbon source. It was obtained from a local biodiesel production plant. Its methanol was removed and its composition was 86% glycerin, 0.5% methanol, 7.5% water, 3.5% MONG (Non-Glycerin Organic Matter) and 2.5% ash. Anaerobic digestrate was provided by a local biogas production plant. It was first filtered through three successive sieves and then centrifuged at 4000 rpm for 10 minutes. It was then sterilized by boiling for about 10 minutes. Following its cooling the macronutrient content of the AD was determined. Nitrogen in AD is present only in the form of ammonium ions while carbon comes from the partly digested organic material. The carbon content was calculated according to the Walkley-Black method so that the Co/No ratios could be estimated. The organic carbon and ammonium ion concentrations of the AD (100 %) were determined and were equal to 1130 mg/l and 1.95 g/l respectively. So, the initial concentration of elemental nitrogen is equal to 878 mg/l.

2.4 Method of Lipid Extraction and Fatty Acid Analysis

The *C. sorokiniana* biomass was collected partly by sedimentation and partly by centrifugation at 4000 rpm. It was dried at 45 °C in an air-circulating oven until it attained a constant weight. Subsequently it was pre-treated in a planetary mill at 200 rpm for 10 min. Following this pre-treatment extraction of the bio-oil followed. This was carried out in a horizontal extraction apparatus (tehtnica ZELEZNIKI EV-402) at 300 rpm for 48 hours. A solvent mixture of n-hexane and isopropanol in a 3:2 ratio was used. The ratio of solvent to biomass was 10:1 (v/w). Following extraction, the biomass was filtered out and the solvent was evaporated.

The bio-oil was subjected to transesterification and the fatty acid distribution was determined by gas chromatography.1 μl of the FAME (Fatty Acid Methyl Ester) phase was injected into an Agilent Gas Chromatographer Model 6890 N. Analysis of the FAME distribution was performed according to the EN 14103 method. The density and the viscosity of the FAME were determined using the EN ISO 3675 and the EN ISO 3679 methods respectively while, the flash point and the acid value were determined using the EN ISO 3104 and the EN 14104 methods respectively.

2.5 Estimation of FAME properties

The saponification number (SN) and the iodine value (IV) were calculated theoretically from the FA distribution using the equations suggested by Kalayasiri *et al*. (1996) and validated by Azam *et al*. (2005). Similarly, the cetane number (CN) was evaluated from the theoretical equation suggested by Krisnangkura (1986) and the higher heating value (HHV) from the equation suggested by Demirbas (1998).

$$SN = 560\sum_{i=1}^{i=n}\frac{\%Wi}{MWi} \quad (1)$$

$$IV = 254\sum_{i=1}^{i=n}\frac{N(\%Wi)}{MWi} \quad (2)$$

$$CN = 46.3 + \left(\frac{5458}{SN}\right) - (0.225IV) \quad (3)$$

$$HHV = 49.43 - (0.041SN) - (0.015IV) \quad (4)$$

Where, %Wi is the % weight of each FA, N is the number of double bonds and MWi is the molecular weight of the respective FAME.

2.6 Treatments

Two treatments in the cultivation of *C. sorokiniana* were used. In the 1st treatment (GLIN) *C. sorokiniana* was cultivated in a semi-batch mode with the glycerol added in equal amounts divided in ten equal intervals during the cultivation period. Total amount of carbon added was 13.2 g/l. An additional amount of nitrogen in the form of nitrate equal to 27.7 mg/l was added in the middle of the GLIN cultivation. Total nitrogen in the GLIN treatment was 108.2 mg/l of which 52.8 mg/l was in the form of ammonium and 55.4 mg/l in the form of nitrate. The initial Co/No treatment was 122:1 and, except for the carbon, all other macro and micro nutrients were from inorganic salts. In the second treatment (GLAD), *C. sorokiniana* was cultivated in a batch mode with glycerol and 40% anaerobic digestrate (AD) as a source of additional carbon and all of the N, P and micronutrients. Co was equal to 7.4 g/l and No, in the form of ammonium, was equal to 351 mg/l. Therefore, the initial Co/No in the GLAD treatment was equal to 21:1. The temperature and pH in both treatments were kept the same and equal to 30 ± 1 °C and 7±0.3 respectively.

3. Results and Discussion

Table 1 shows the FA distribution of *C. sorokiniana* cultivated with glycerol and inorganic macro and micro nutrients (GLIN). Table 2 shows the FA distribution of *C. sorokiniana* cultivated with glycerol and 40% anaerobic digestrate (GLAD).

Table 1 Fatty acid distribution in the GLIN treatment (Glycerol + Inorganic salts)

FA	%	FA	%
C10:0	0.4	C18:0	1.7
C10:1	0.9	C18:1	43.9
C12:0	0.7	C18:2	2.5
C12:1	2.4	C18:3	2.7
C14:0	5.6	C20:0	0.3
C14:1	2.6	C20:1	0.2
C16:0	20.1	C22:0	0.5
C16:1	12.1	C22:1	0.3
C16:2	1.9	C24:0	1.2

Table 2 Fatty acid distribution in GLAD treatment (Glycerol + AD)

FA	%	FA	%
C10:0	3.1	C18:1	17.9
C10:1	3.6	C18:2	4.9
C12:0	2.9	C18:3	3.8
C12:1	4.2	C20:0	4.6
C14:0	7.1	C20:1	1.9
C14:1	6.9	C22:0	1.8
C16:0	7.4	C22:1	2.5
C16:1	15.5	C24:0	1.4
C16:2	2.1	C26:0	6.6
C18:0	1.8		

With Respect to the % SFA, % MUFA and the % PUFA it can be seen from Tables 1 and 2 that the % SFA appears to affect FAME properties. Increasing the degree of saturation leads to a decrease in ignition delay (Schönborn *et al.*, 2009). The ignition delay is related to the cetane number (CN). As the ignition delay increases the CN number decreases and the CN number is an indication of the quality of the biodiesel produced (Azam *et al.*, 2005). Therefore, increasing the degree of saturation should increase the CN number of the fuel. Also, increasing the chain length of FA decreases both the iodine value and the saponification number (Folayan *et al.*, 2019) and therefore increases the cetane number. The degree of saturation affects the iodine value. As the degree of saturation increases the iodine value decreases. However, low iodine values lead to biodiesel which is more combustible but it is not suitable for colder climates as it has rather poor flow properties at low temperatures (Schönborn *et al.*, 2009).

The respective biodiesel properties are shown on Table 3.

Table 3 Properties of the obtained biodiesel

Property*	Limits	GLIN	GLAD
Density	860-900	878	881
Viscosity	3.5-5.0	3.77	3.86
Flash point	>101	147	141
Acid value	<0.5	0.25	0.29
Saponification Number (SN)		201.8	206.3
Iodine Value (IV)	<120	71.3	72.9
Cetane number (CN)	>51	57.3	56.4
Higher Heating value (HHV)	>35	40.1	39.9

* Units: Density: (15 °C) kg/m^3, Viscosity: (40 °C) mm^2/s, Flash Point: °C, Acid Value: mg KOH/g Iodine Value: g I/100 g FAME and Higher Heating Value: MJ/kg.

Figure 1 shows the histogram for the distribution of SFA, MUFA and PUFA, while figure 2 shows the distribution of chain lengths between C10-

C14, C16-C18 and >C18, for the two treatments namely, GLIN and GLAD.

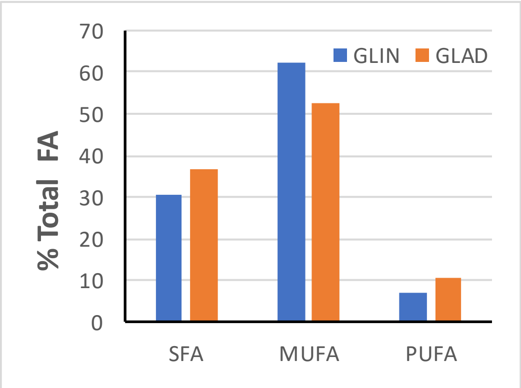

Figure 1. Histogram of the distribution according to saturation-unsaturation of the FA.

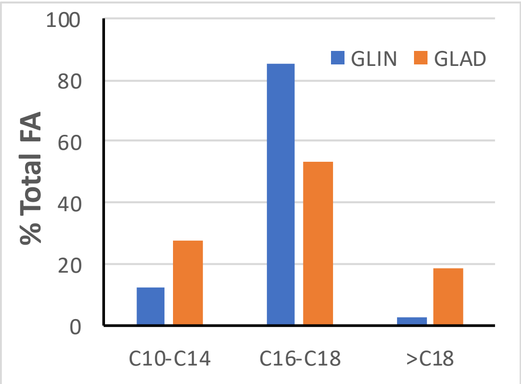

Figure 2. Histogram of the distribution according to the chain length of the FA.

It can be seen by examining table 3 and the two figures that although the GLAD treatment produces 6.2% more SFA than the GLIN treatment its CN and HHV are lower than the corresponding ones of the GLIN treatment because the GLAD treatment produces 15.2% more short chain FA (C10-C14) than the GLIN treatment and the net effect is an increase, as can be seen from table 3, of both the SN and IV values in the GLAD treatment. Despite these differences in FA distribution, both treatments, although very different, produce similar FAME properties well within the acceptable limits.

4. Conclusions

The FA distribution during the cultivation of *C. sorokiniana* depends on the treatment. The two cultivation treatments of this study are just an example of the many treatments possible. The treatment does affect the FAME properties but for both treatments they are the range of accepted values for biodiesel produced from vegetable and seed oil. Therefore, cultivation should instead focus on enhancing lipid productivities as well as on lipid extraction cost in order to make competitive a future application for biodiesel production. Because glycerol prices fluctuate a great deal in the market, other cheap carbon sources can be investigated. Among other options is the cultivation using 100% AD, which in this moment has zero market value. Other options are mixotropic cultivation and the use of genetically modified microalgae strains.

Acknowledgements

This research was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE - INNOVATE (project code: T1EDK-01580).

References

Azam M.M., Waris A. and Nahar N.M. (2005), Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India, *Biomass Bioenergy*, **29** (4), 293-302.

Demirbaş A. (1998), Fuel properties and calculation of higher heating values of vegetable oils, *Fuel*, **77**(9-10), 1117-1120.

Folayan A.J., Anawe P.A.L., Aladejare A.E. and Ayeni A.O. (2019). Experimental investigation of the effect of fatty acids configuration, chain length, branching and degree of unsaturation on biodiesel fuel properties obtained from lauric oils, high-oleic and high-linoleic vegetable oil biomass, *Energy Reports*, **5,** 793-806.

Kalayasiri, P., Jayashoke, N. and Krisnangkura, K. (1996). Survey of seed oils for use as diesel fuels. *J. Am. Oil Chem. Soc.* **73** (4), 471–474.

Krisnangkura K. (1986), A simple method for estimation of cetane index of vegetable oil methyl esters, *J. Amer. Oil Chem. Soc.*, **63**(4), 552-553.

Mata, T.M., Martins, M. M. and Caetano, N.S. (2010), Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, **14**, 217-232.

Gouveia L. and Oliveira A.C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology & Biotechnology,* **36** (2): 269-274 (2009).

Schönborn A., Ladommatos N., Williams J., Allan R. and Rogerson J. (2009). The influence of molecular structure of fatty acid monoalkyl esters on diesel combustion. *Combust Flame*, **156**(7), 1396–1412.