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ASSESSMENT OF MICROALGAE BIODIESEL FUELS USING A FUEL PROPERTY ESTIMATION METHODOLOGY

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Abstract. *Recently, depleting supplies of petroleum and the concerns about global warming are drawing attention to alternative sources of energy. In this context, advanced biofuels, derived from non edible superior plants and microorganisms, are presented as promising options for the transportation sector. Biodiesel, which is the most prominent alternative fuel for compression ignition engines, have a large number as potential feedstock, such as plants (e.g., soybean, canola, palm) and microorganism (i.e., microalgae, yeast, fungi and bacterium). In order to determine their potential, most studies focus on the economic viability, but few discuss the technical viability of producing high quality fuels from such feedstock. Since the fuel properties depend on the composition of the parent oil, and considering the variability of the fatty acid profile found in these organisms, it is clear that the fuels derived may present undesarible properties, e.g., high viscosity, low cetane number, low oxidative estability and poor cold flow properties. Therefore, it is very important to develop ways of analysing the fuel quality prior to production, specially considering the high cost of producing and testing several varieties of plants and microorganisms. In this aim, this work presents the use of fuel properties estimation methods on the assessment of the density, viscosity, cetane number and cold filter plugging point of several microalgae derived biofuels, comparing then to more conventional biodiesel fuels. The information gathered with these methods helps on the selection of species and cultivation parameters, which have a high impact on the derived fuel quality, and have been succesfuly employed on the Center for Research and Development of Sustainable Energy. The results demonstrate that some species of microalgae have the potential to produce high quality biodiesel if cultivated with optimised conditions, associeted with the possibilty of obtaining valuable long chain polyunsaturated fatty acids. It reafirms the concept of "designed fuels", which aims on the reduction of compression ignition engines pollutant emissions.*

Keywords*: Biodiesel, microalgae, estimation methods, fuel properties, designed fuel.*

1. INTRODUCTION

Biofuels are one of many potential alternatives for substituting conventional petroleum derived fuels, which are not renewable and which contribute to global warming due to the large amount of carbon dioxide emitted in their life cycle. Biodiesel is the most prominent option among the potential substitutes of Diesel fuels. It is obtained by the reaction of transesterification of oils and fats, which consists on the reaction of glycerides with an alcohol, in the presence of a catalyst, producing monoalkyl esters (fatty acid esters).

The biodiesel feedstock include animal fats, residual oils, superior plants oils (*e.g.*, soybean, canola) and more recently microorganism, *i.e.*, microalgae, yeast, fungi and bacterium (Meng *et al.*, 2008). The composition of the parent oil varies depending on the location and source, and for plants and microorganisms also on the cultivation conditions. Among the oleaginous microorganisms considered for biodiesel production, microalgae have received greatest attention, since microalgae cultures present many advantages when compared to conventional biofuels or fossil fuels, which include (Hu *et al.*, 2008):

- high growth rate;
- high lipid yields;
- tolerance to saline and waste water as culture medium and nutrient source;
- valuable by-products and co-products generation (e.g., pigments, biopolimers, proteins, polyunsaturated fatty acids)

- Higher annual biomass productivity than terrestrial plants;
- Tolerance to marginal lands not suitable for agriculture;

In what concerns the economic feasibility, however, the development of microalgal cultivation is still on check. In despite of some authors' optimistic results (Chisty, 2007), recent studies have demonstrated the need of extracting other valuable by-products in order to guarantee economical feasibility (Brennan and Owende, 2010, Williams and Laurens, 2010). Microalgae produce several different high-value bioactives, including long chain fatty acids, mostly polyunsaturated such as ω-3 and ω-3, pigments, and high yields of proteins and carbohydrates (Satyanarayana *et al.*, 2010).

Many microalgae species contain omega-3 poly unsaturated fatty acids which can be purified to provide a high valuable food supplement. Eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA) are the most important of these fatty acids, with applications in treatment of heart and inflammatory diseases; asthma, arthritis, migraine headache and psoriasis (Harun *et al.*, 2010). EPA and DHA recovery may solve the economical feasibility issue of microalgae biodiesel, even doe they are not suitable for fuel production, due to very low oxidative stability and poor ignition properties. Considering the fact that the production of these compounds tend to displace the production of short chain fatty acid esters suitable for biodiesel, it is necessary to consider this trade-off while selecting species and cultivation parameters.

Most of the effort on Biodiesel fuels research has been focused on lowering the production costs, which is justified by the competition with relatively low cost conventional fuels. However, in order to create a real alternative to Diesel fuels, appropriated fuel properties must be guaranteed. Different biodiesel standards are being established in various countries, such as United States, Europe, Brazil, South Africa and Australia (Knothe, 2006), and the compliance with such standards is required for commercialization.

As stated by Knothe (2005), the properties of the various individual esters that compose biodiesel determine its overall properties. Thus by estimating fatty acid esters' properties and applying mixing rules, it is possible to estimate physical and fuel properties (Yuan *et al.*, 2003). Estimating the physical and fuel properties is a cheap and fast way to assess fuel quality, prior to expensive production and characterization processes. It helps therefore setting cultivation conditions and on the selection of species for improved fuel quality and high EPA or DHA recovery.

In this context, the present works aim to assess the quality of microalgae biodiesel and its employability when coupled with the recovery of EPA and DHA, based on the application of fuel properties estimation methods, as an initial step on the assessment of microalgal oil employability in biodiesel production. The fatty acid profile of two commonly studied microalgae, *Nannochloropsis* sp. and *Phaeodactilun tricornutum*, was has been gathered in literature, as well as more conventional oils (i.e., soybean and canola), and the data used for fuel property estimation.

In order to estimate the properties of the different microalgal-derived biodiesel, a conjunction of different methods is necessary. The use of such methods is supported by many researchers, as an important step on the development of advanced fuels (Yuan *et al.*, 2003, Ramos *et al.*, 2009, Francisco *et al.*, 2010), the majority of which focus on the estimation of density, viscosity, cetane number and cold filter plugging points. These properties are reconised as critical for biodiesel quality, and depend mostly on the composition of the feedstock (Knothe, 2005). Thus, the present work aims to estimate these properties for the feedstocks selected.

Information gathered by such methods has been successfully used in the Center for Research and Development of Sustainable Energy at Federal University of Paraná, on both the search for more adapted species and on the comparison of cultivation conditions.

2. MATERIALS AND METHODS

2.1. Estimation methods

The estimation of fuel properties may be performed in very different ways. Thermodynamic methods tend to be too complex and demand very extensive databases of physic-chemical properties. Thus, most studies employ statistical methods based on multi-variable regressions, such as group-contribution based methods (e.g., Ceriani *et al.*, 2007, Yuan *et al.*, 2003), and sometimes empirical relations (e.g., Clemens, 1996, Ramos et al., 2009). Such methods tend to be simpler, and even though their accuracy also depends on the data employed generally only the fatty acid profile is required for estimation.

The density of a fuel (ρ, in g/l) may be estimated through a simple arithmetic combining rule (Eq. 1), such as described in Poling *et al.* (2000). The density of individual esters (ρ_i) must be know, as well as the molar fraction of each ester (y_i)

$$\rho_m = \sum_i y_i \rho_i \tag{1}$$

In order to estimate the viscosity of the esters, the methodology proposed by Ceriani *et al.* (2007) was used method for dynamic viscosity (η, in mPa.s) estimation, which is very useful at low temperatures. This method consists of a group contribution method with a perturbation term and a correction term, and it was specially developed and optimized for fatty compounds. Thus, the Eq.2 following equation may thus be used for the estimation of the viscosity of the neat fatty acid esters.

$$\ln \eta_i = \sum_k N_k \left(A_{1k} + \frac{B_{1k}}{T} - C_{1k} \ln T - D_{1k} T \right) + \left[M_i \sum_k N_k \left(A_{2k} + \frac{B_{2k}}{T} - C_{2k} \ln T - D_{2k} T \right) \right] + Q \qquad (2)$$

where the constants A_{1k}, B_{1k}, C_{1k}, D_{1k}, A_{2k}, B_{2k}, C_{2k} and D_{2k} are parameters obtained by Ceriani *et al.* (2007) from the regression of experimental data, T is the absolute temperature, k represents each group in the molecule (CH_3, CH_2, CH= and COO), M_i is the molecular weight of the molecule and Q is the correction term, dependent on temperature and the molecular structure, given by the following relations.

$$Q = \varepsilon_1 q + \varepsilon_2 \qquad (3)$$

$$q = -0.3157 + \frac{9.324}{T} + 0.054 \ln T - 0.00007812T \qquad (4)$$

$$\varepsilon_1 = -5291.2 + 354 N_c \qquad (5)$$

$$\varepsilon_2 = 0.1984 - 0.0512 N_{cs} \qquad (6)$$

where q is a function of the temperature T optimized based on the experimental data reported on the original work, N_c is the total number of carbons in the molecule of the FAE and N_{cs} is the number of carbon atoms in the alcoholic part of the molecule (1 for methyl esters, 2 for ethyl esters, etc.).

With viscosity of each FAE calculated, it's then possible to estimate the viscosity of the mixture. Allen *et al.* (1999b), proposed a mixing rule that uses the mass fraction of the FAEs (w_i) and the viscosity of each component. More precise relations were reported, as the Grunberg-Nissan equation, described by Yuan *et al.* (2009), which is considerably more complex. Therefore, for a first estimation the following equation is appropriated.

$$\ln \eta = \sum_i w_i \ln \eta_i \qquad (7)$$

The international standards set limits to the cinematic viscosity (ν) of the fuels, at 40ºC. Thus, it is necessary estimate the density at this temperature, since $\nu = \eta/\rho$. The density may be estimated, using the modified Raquett equation, recommended by Reid et al. (1987).

The quality of ignition is normally expressed in terms of the cetane number (CN) of the fuels. Recent works (i.e., Ramos *et al.*, 2009, Francisco et al., 2010 and Garcia et al., 2010), have employed the equation proposed by Clemens (1996), as follows.

$$CN = \sum x_i \cdot CN_i \qquad (8)$$

where CN is the cetane number of the fuel, x_i is the mass fraction of each individual ester and CN_i is the cetane number of the individual ester. Thus, detailed data of individual esters is required.

The cold filter plugging point (*CDPP*, in ºC) is a property correlated to cold performance. It is related to the crystallization of saturated esters in the fuel, and have been successfully correlated to the adimensional long chain saturated (*LSCF*, Eq. 9) proposed by Ramos *et al.* (2009). Both Francisco *et al.* (2010) and Garcia *et al.* (2010) have employed this method, since it much simpler than those based on the thermodynamic of phase change. The correlation, shown in Eq. 10, presented a coefficient of correlation R^2 = 0,966, according to Ramos *et al.* (2009).

$$LCSF = 0{,}1 \cdot C_{16} + 0{,}5 \cdot C_{18} + 1 \cdot C_{20} + 1{,}5 \cdot C_{22} + 2 \cdot C_{24} \qquad (9)$$

$$CFPP = 3{,}1417 \cdot LCSF - 16{,}477 \qquad (10)$$

where C_{16}, C_{18}, C_{20}, C_{22} and C_{24} represent the mass percentage of saturated fatty esters with 16 to 24 carbons. The Eq. 10 is an empirical relation, which is valid for methyl esters.

2.2. Fatty acid profiles

The composition of the microalgae oils was gathered in the literature. Hu and Gao, (2006) reported the fatty acid profile of the *Nannochloropsis* sp. under different cultivation conditions. This microalga was chosen since it is a potential feedstock for EPA recovery (Zitelli *et al.*, 1999). The other microalgae selected for this work was the diatom *Phaeodactylum tricornutum*, which is also a potential feedstock for EPA recovery (Yongmanitchai, 1991). The fatty acid profiles of this microalga were obtained from Alonso et al., (2000). For this work, the data concerning three different Nitrate concentrations ($NaNO_3$), for each microalgae, was gathered (details on the culture medium available in Hu and Gao, 2006 and Alonso *et al.*, 2000), and are presented in Tab.1, and the fatty acids separed in classes: saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (SFA, MUFA, PUFA, respectively).

Table 1. Fatty acid profiles (% w/w) of microalgae under different conditions. The fatty acids are classified by the number of carbons and unsaturations (*i.e.*, C16:1 has 16 carbons and one unsaturation).

		Nannochloropsis sp.[a]			*Phaeodactylum tricornutum*[b]		
		NaNO3 (μM)			NaNO3 (μM)		
		150	600	3000	2000	5000	10000
SFA	C12:0				1[c]	5[c]	7[c]
	C14:0	4	4	4	9	8	8
	C16:0	38	34	23	27	12	11
	C18:0						
MUFA	C14:1						
	C16:1	28	24	23	30	23	23
	C18:1	16	13	4	4	2	2
	C20:1			3			
	C22:1						
PUFA	C16:2				5	4	5
	C16:3				10	9	10
	C18:2	3	4	7	2	5	3
	C18:3						
	C20:4	1	3	4	0	1	0
	C20:5	8	16	30	15	28	25
	C22:6				1	2	2
	others		1	2	8	6	6

[a] Hu and Gao, 2006. [b] Alonso *et al.* 2000. [c] Reported as unknown short chain fatty acids, considered as C12:0 for estimation purposes.

The long chain unsaturated fatty acids, C20:4, C20:5 and C22:6 may not be used to produce biodiesel since the correspondent esters would present low oxidative stability and poor ignition quality. Since this compounds are very valuable dietary supplements with many positive health effects the tendency is separate and sell this components apart from the biodiesel produced. Therefore, this work considers this separation. The composition of the oil useful for biodiesel production is presented in Tab. 2, along with the composition of more conventional oils employ as feedstock for biodiesel.

2.3. Other data

The data concerning pure fatty acid esters were gathered from the literature. The Scifinder® Database contained the data density values necessary for the estimation of the fuel density with Eq.1. The viscosity was estimated with the data from Ceriani *et al.* 2007, while the CN_i were obtained from the data report by Knothe (2005 and 2008). The CN of the methyl ester of C16:2 and C16:3 were not available in the literature, and were thus considered to be the same of C18:2 and C18:3, which does not compromise severally the accuracy of the estimations, due to the low yield of these compounds in the analyzed oils.

3. RESULTS AND DISCUSSION

The fatty acid profile of the parent oil determines the biodiesel properties. Thus, changes in the oil composition may influence negatively the biodiesel performance. In one hand, the presence of SFA affects positively the ignition quality and oxidative stability, while has a negative impact on cold flow properties (and thus, on the *CFPP*). On the other hand, unsaturated fatty acids have an opposite effect, decreasing the ignition quality, oxidative stability and improving cold flow properties (Knothe, 2005 and Ramos *et al*. 2009). Thus, finding species of plants or microalgae with fatty acid profiles that solve the trade-off between these tendencies constitutes the challenge of finding the ideal biodiesel composition in terms of fuel properties (Knothe 2008, Pinzi *et al.*, 2009).

Table 2. Composition of the oils suitable for biodiesel production. The microalgae oils were considered to have their long chain PUFA removed.

		Nannochloropsis sp.			Phaeodactylum tricornutum			Soybean[a]	Palm[a]	Colza[a]
		NaNO3 (μM)			NaNO3 (μM)					
		150	600	3000	2000	5000	10000			
SFA	C12:0				1	7	10			
	C14:0	4	5	6	10	12	12			
	C16:0	43	43	36	31	18	16	11	37	5
	C18:0							4	7	2
MUFA	C16:1	32	30	36	34	34	34			
	C18:1	18	17	6	5	2	3	25	47	33
	C20:1			5						9
	C22:1									23
PUFA	C16:2				5	5	7			
	C16:3				11	13	15			
	C18:2	3	5	11	2	8	4	54	9	20
	C18:3									8
	Legend	N1	N2	N3	PH1	PH2	PH3	S	P	C

[a] Ramos et al., 2009.

In order to compare the

3.1. Estimated density

The result of density estimation is show in Fig. 1. All the fuels presented acceptable densities according to the norms and resolutions that the limits for this property (UNE-EN 14214, ASTM 6751, ANP 255). The palm oil derived biodiesel presents the lower density due to its high content of short chain saturated fatty acid.

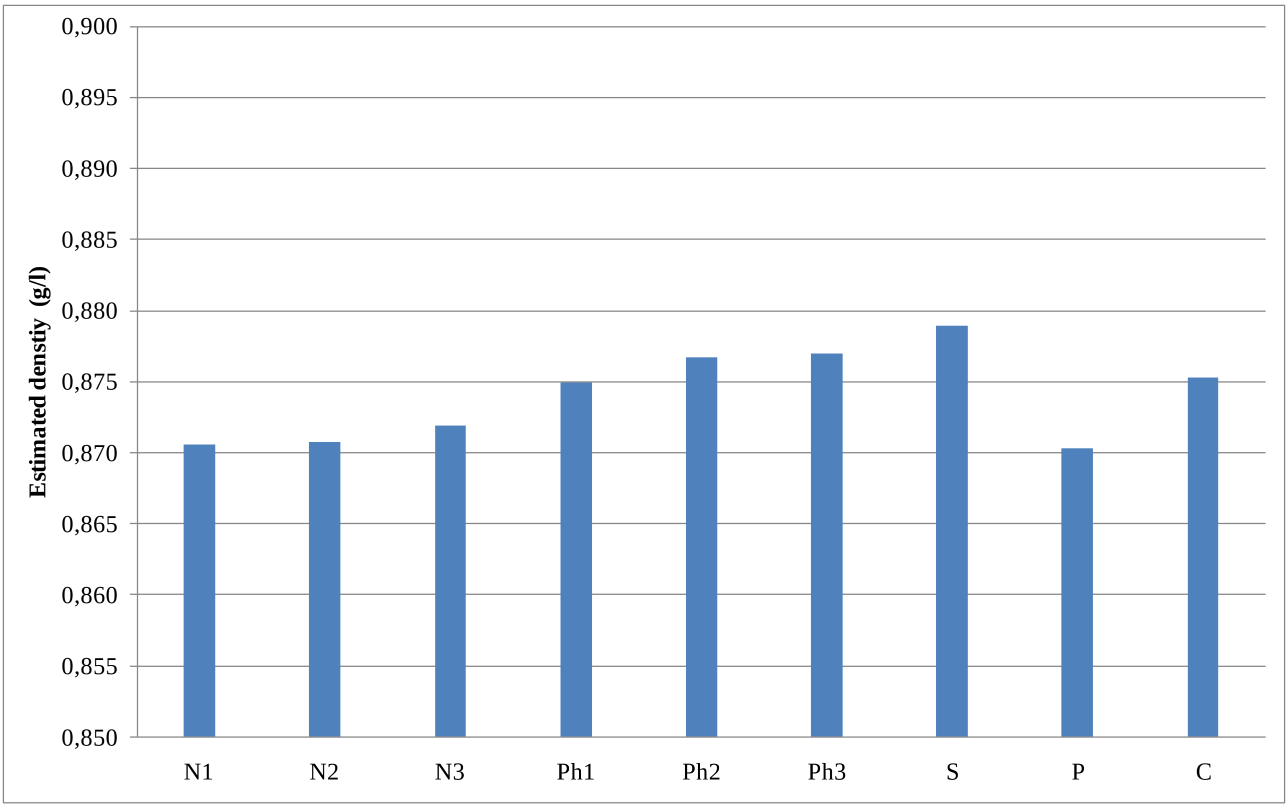

Figure 1. Estimated densities for the different feedstocks.

3.2. Estimated viscosity

The estimated viscosity is presented in Fig. 2. This property is important for both injection and pulverization and it is correlated to pollutant emissions.

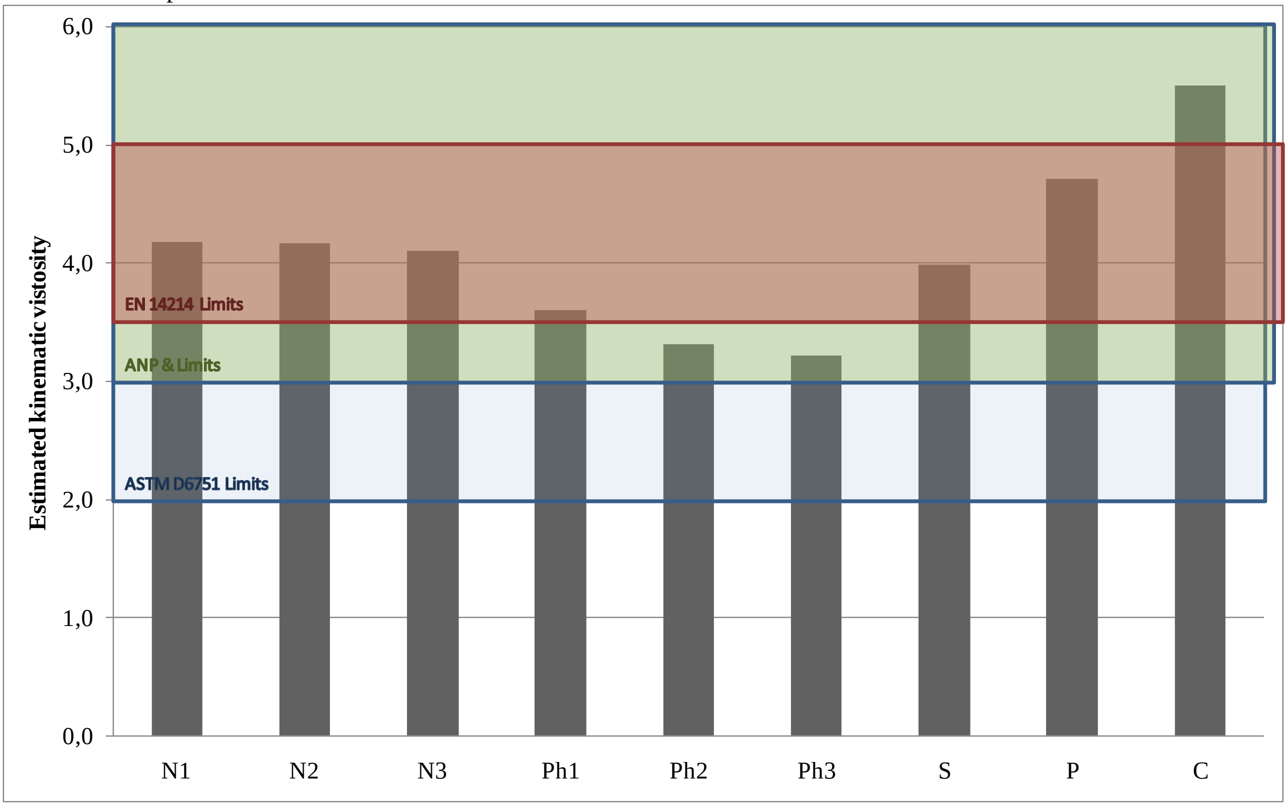

Figure 2. Estimated viscosity and normative limits.

As shown in Fig. 2, the viscosity of the Ph2 and Ph3 samples are not compatible with the limits determined by the EN 14214, what demonstrates the influence of the cultivation conditions on the fuel properties. This biodiesel fuel would need additives in order to respect such norm.

Colza biodiesel with the studied composition presented very high viscosity which is not in compliance with the norm. This may be caused by the overestimation since the method employed was not formulated taking into account on long chain unsaturated fatty acids that are present in this biodiesel (22% of C22:1). The other compositions meet the viscosity requisites without further problems.

2.3. Cetane number

The cetane number is a critical property since it determines the ignition quality. It is directly connected to pollutant emissions (Graboski and Mccormick, 1998, Lapuerta *et al.*, 2008). Thus, it is of great importance that the biodiesel present high CN, according to the norms. The results of the estimation are presented in Fig. 3. The soybean diesel is the only one under the European norm limit (minimum CN = 51). This is due to high content of PUFA found in soybean oil. All microalgae derived biodiesel fuels present high CN, tanks to high yields of saturated esters (between 37% and 48%). The influence of cetane number on pollutant emission is very strong even doe limits are absents on the Brazilian norm (ANP 7).

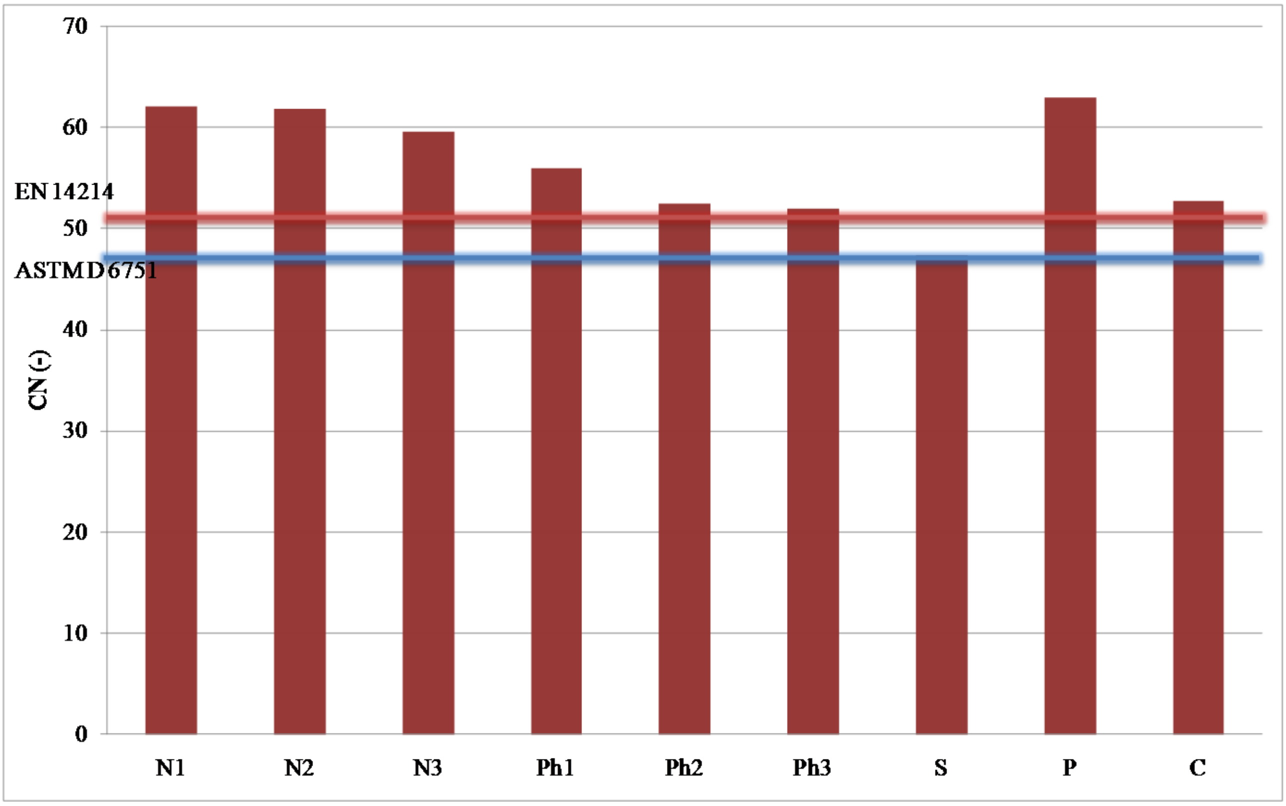

Fig 3. Estimated cetane numbers. Soybean is the only under outside the normative limits.

3.4. Estimated Cold Filter Plugging Point

The estimated of the CFPP are present in Tab.3. All fuels presented values under 0ºC, except palm fuels. Thus, depending on the region, these fuels may be used without concern. The norms normally require regional limits to this property. For the Brazilian climate, all microalgae would present good performance.

Table 3. Estimated cold filter plugging points (ºC)

Names	N1	N2	N3	Ph1	Ph2	Ph3	S	P	C
CFPP (ºC)	-3,01	-2,98	-5,23	-6,77	-10,83	-11,55	-7,17	5,87	-12,43

6. CONCLUSIONS

The microalgae oil composition studied are in compliance with the requirements of the norms (except Ph2 and Ph3, in terms of viscosity). Therefore, these microalgae would present acceptable fuel properties, which is one of the main factors that define the technical feasibility of biodiesel. The variation of properties depending on the cultivation conditions may produce undesirable results, as illustrated here by the different Nitrate concentrations.

Estimation methods are very useful tools for assessing the quality of biodiesel fuel prior to its production. Estimation help the search of ideal properties, in what Knothe (2008) denominated “designer” biodiesel. This concept may be applied to microalgae biodiesel, searching for economical viability, with the recovery of valuable PUFA and the production of high quality renewable fuels.

5. ACKNOWLEDGEMENTS

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