



Effect of pretreatments and catalytic route in the quality and productivity of biodiesel obtained from secondary sludge

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ABSTRACT

The production of biodiesel from waste activated sludge by the in-situ transesterification of its lipid content is studied in this work, comparing both basic and acidic catalytic routes. The acid mechanism is chosen as the optimum one, considering that the high percentage of free fatty acids strongly limits the yields obtained by the basic route, due to the saponification competence. Different pre-treatments are proposed (sonication, microwaves, sieving) to enhance the extraction rate, identified as the limiting step of the process. Sludge drying requirements are also analysed, concluding that water contents below 20% (weight base) does not lead to significant decreases in the transesterification activity. The acidic reaction is also chosen once the quality of the final mixture as alternative biofuel is analysed. Almost all the biodiesel properties (higher heat value, iodine number, kinematic viscosity, density, and long-chain saturated factor and cetane number) are in the optimum ranges proposed by the international normative, concluding that pretreatments do not damage the final product. Optimum results were obtained applying microwaves, with a final yield of 46.7% of FAMES, which is a clear improvement on the previous literature of biodiesel obtaining from sewage sludge.

1. Introduction

Sewage sludge is the waste produced in the highest proportion in wastewater treatment plants (WWTPs), with an expected increasing trend in the future because of the increasing world population and the growing requirements in terms of wastewater treatment [1,2]. Different alternatives have been proposed not only for sludge management (landfill, incineration) but also for its upgrading (composting, anaerobic digestion). According to the Sustainable Development philosophy, recycling technologies must prevail over revalorization ones. Thus, research must be focused on processes to produce value-add products from waste streams.

The starting point of new recycling approaches must be the high carbon content in sewage sludge. The secondary sludge (waste activated sludge, WAS) has a more homogeneous composition than the first one. Consequently, it is the most attractive fraction to be used as raw material. Although the biological treatments of each WWTP condition the composition [3], in general terms WAS contains around 20% of lipids [4], in the same order of magnitude of other lipid raw materials, such as soybean and sunflower oils, and cottonseed, which contain 15–35% of these biomolecules [5]. Most of these lipids are glycolipids, with a very

low content of sterol and oils, and around 40% of them corresponds to C16 and C18 [6], an optimum range for their transformation into liquid fuels (biodiesel).

Biodiesel is commonly produced through catalytic esterification of free fatty acids or triglycerides transesterification using alcohol (methanol or ethanol) [7]. In general, the high cost of the raw material conditions its economic viability, promoting the development of processes based on waste oils or different raw material enriched in lipids. Good results with both acid and alkali catalysis are reported, demonstrating the high variability of this procedure [8,9]. NaOH and KOH are suggested as base catalysts, obtaining fast reactions (2–8 h) and a final high-quality product working at 40–60 °C and using methanol as solvent. However, they also catalyse saponification reactions in the presence of free fatty acids [10]. By comparison, H₂SO₄, H₃PO₄ and HCl are the typical acids proposed for this reaction. They produce slower reactions than basic catalysts, requiring higher temperatures. On the contrary, they prevent saponification since they catalyse transesterification and esterification reactions [9,11]. Heterogeneous catalysis has been also studied, obtaining promising results with different zeolites [12], metal oxides [13], acid resins [14], clays [15], and magnetic solids [16], among others.

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The use of WAS as feedstock implies several challenges, highlighting its high-water content (WAS typically contains less than 3% of solids). This percentage can increase up to 30% if it is dried, using different types of physical processes [17]. Water promotes soap formation, reducing the transesterification yields [7], but the complete drying of these large volumes needs high thermal energy consumptions, which jeopardises the economic viability of the whole process.

In addition, a previous fatty acids extraction from cells to the reaction medium is required since most of them take part in the internal cell structure of microorganisms. It has been demonstrated that lipids can be extracted by using organic solvents [9]. The use of common reactants from extraction and transesterification procedures allows suggesting the one-step process (*in-situ*). The low efficiency in the extraction step limits the goodness of this approach [4,18,19]. Moreover, it requires the use of homogeneous catalysts because of the mass transfer limitations that would imply the required solid-solid contact if a heterogeneous catalyst is used.

Thus, some authors propose a two-steps approach, with a previous liquid-liquid [20] or solid-liquid extraction [21], using severe conditions and more complex solvents. Nevertheless, a multi-step process is not the optimum alternative, either from an environmental or from a techno-economical point of view (long reaction times, harsh operation conditions, high solvent volumes required, etcetera).

Therefore, the advance in this methodology implies optimizing the *in-situ* procedure, which requires improving the efficiency of the extraction step [4]. At this point, introducing sludge pre-treatments can play a relevant role. These physical processes produce cell disruption and the subsequent lipid release to the reaction medium. The literature proposes some quite expensive alternatives, such as hydrothermal liquefaction [22,23]. However, some interesting cheaper physical approaches were successfully applied to similar feedstock (mainly algae). They could be good options for the sewage sludge, but the literature in this field is very scarce. The application of microwaves (MW) aligns the macromolecules with the electric field, causing the rupture of hydrogen bonds, which result in the disintegration and hydrolysis of the raw materials and the subsequent lipid release. The use of MW has been corroborated as an interesting way to improve the efficiency obtained with a conventional reactor in more than 70% [24]. Martínez-Guerra et al. [25] also propose this methodology, highlighting the relevant results obtained at low power levels and low solvent/reactant ratios. Sonication uses cavitation to produce small bubbles in the liquid, generating microturbulence and shock waves. Because of this, the bubbles implode; produce the cell wall rupture and the lipids release. Sonication (US) has been proposed by Gonze et al. [26], obtaining an increase in the organic matter solubilized between 23 and 62% as a function of the conditions. Despite this work specifically evaluates the US in sludge flocs, the aim of this pre-treatment is related to increasing the efficiency in an aerobic reactor, but not in a transesterification process. To sum up, there is a gap of knowledge about the effects of any of these pre-treatments in the lipid extraction from sewage sludge, as well as their effect on the yields and biodiesel quality obtained.

Based on this background, this work aims to optimize the biodiesel production from waste activated sludge by *in-situ* transesterification, highlighting three main novelties. Firstly, the study of the effect of different pretreatments in lipid extraction. Secondly, the identification of the balance between sludge moisture and biodiesel production. Finally, the analysis of the main physicochemical properties of the resulting mixture to identify the optimum conditions as a function of the fuel quality.

2. Materials and methods

2.1. Chemicals

Sulfuric acid (96%, Sigma Aldrich), potassium hydroxide ($\geq 85\%$, Sigma Aldrich), methanol ($\geq 99.8\%$, VWR) and *n*-hexane (97%, VWR)

were used without any further purification. Standard used for the identification and quantification of fatty acid methyl esters (FAMES) was supplied by Supelco (mix of 37 FAMES from C₄ to C₂₄).

2.2. Sample preparation and characterization

The sludge was collected from a municipal WWTP in Oviedo, Spain. This plant combines anaerobic, anoxic, and aerobic zones within the bioreactor to remove simultaneously nitrogen, phosphorous and organic matter, a typical flow diagram of most WWTP ("NIPHO" activated sludge). Samples correspond to the waste stream obtained in the biological reactors after being pre-concentrated by a flotation treatment (from around 0.5 to 3%). The waste activated sludge (WAS) was stored at 4 °C and dried at 105 °C for 24 h before use.

Total lipid content (TL; g lipid·g⁻¹ dry sludge) was determined by liquid-liquid extraction, according to the method proposed by Siddiquee and Rohani [27], using chloroform ($\geq 99.8\%$, J.T. Baker), with a dry sludge/solvent ratio equal to 0.04 g mL⁻¹ at 75 °C for 4 h. After extraction, the sample was filtered, and residual particles of the filtrate fraction were removed by centrifugation, using a Kubota 6500 centrifuge at 3000 rpm (6500×g) for 5 min. As to the supernatant, the solvent was removed under vacuum using a Heidolph VV 2000 rotavapor at 70 °C, obtaining the lipids as the remaining oil. Weighting the oil fraction allows calculating the lipid yield by dry sludge on a mass basis (g·g⁻¹).

2.3. Experimental procedure

Reactions were carried out in glass reactors submerged in a thermostat water bath at 60 °C for 24 h, under stirring and using methanol as solvent. The raw material, WAS sludge, does not suffer any further purification. NaOH was selected as the basic catalyst, being used at four different mass fractions of methanol: 4%, 30%, 50% and 70%. The methanol/sludge ratio was 10:1 according to the optimized parameters obtained by Wu et al. [28]. In the case of acid catalysis, sulfuric acid in methanol (4% v/v) was mixed with the sludge in a ratio of 20:1, according to the process optimized in our previous work [4]. Each experiment has been repeated at least twice to guarantee the reproducibility of the results.

Samples were centrifuged at 3000 rpm (6500×g) for 5 min. In the case of the acid procedure, NaCl is previously added to guarantee that the reaction stops by neutralizing the catalyst as Na₂SO₄. The supernatant recovered by centrifugation (free of sludge and Na₂SO₄, recovered as a solid due to its low solubility in methanol) was warmed up to 70 °C in a rotatory evaporator to reduce the total volume to 10 mL. This re-concentration of the samples helps the FAMES extraction (by four cycles of 5 mL of hexane) and their analyses. The effect of water content on the biodiesel yield was studied modifying the drying time to have samples from 0 to 80% of moisture content. Reactions were carried out with 4% (volume ratio) of H₂SO₄ under the same conditions, already explained.

Microwaves (MW), sonication and particle sieving were checked as pre-treatments to maximize the lipid extraction. The effect of MW was studied at 2450 MHz, checking the influence of the pre-treatment time from 0 to 10 min. Concerning sonication, this pre-treatment was optimized in our previous work, obtaining a maximum yield after applying 50 min of ultrasounds at 10 kHz [4]. Regarding particle sieving, the sludge has been divided into three fractions: 50–100 μm; 100–255 μm; and 255–350 μm. The three different ranges were tested without any pre-treatment. In addition, they were also checked after being exposed to ultrasound and microwaves. In all cases, the experimental conditions were the same, 60 °C of reaction temperature, for 24 h under stirring, with 4% (volume ratio) of H₂SO₄ as the catalyst and methanol to sludge ratio of 20:1.

2.4. FAMES analysis

FAMES produced were identified and quantified to determine the biodiesel quality and yield. The qualitative analysis of FAMES was analysed using a SHIMADZU GC-MS QP2010 Plus with a TRB-5MS (30 m × 0.25 mm × 0.25 μm). 1 μL of the sample was injected with a split ratio of 30. Injection port temperature was kept at 260 °C, MS source at 200 °C and interface temperature 250 °C. The column temperature was operated to start at 100 °C, kept for 5 min, then raised from 100 °C to 240 °C with a rate of 4 °C·min⁻¹, and held at this temperature for 30 min.

The quantitative analysis was carried out using a SHIMADZU GC2010 with an FID detector and a CP-Sil 8 C B (30 m × 0.25 mm × 0.25 μm) column using a FAMES standard commercial sample for the calibration. The detector temperature was 260 °C, and the temperature program was the same as for the qualitative analysis.

Yield expressed as g FAME/g lipid, was calculated as the ratio between the sum of the concentrations of all the detected FAMES (in g·L⁻¹) and the total lipid concentration (TL), according to the following expression:

$$\phi_{FAME} = \frac{\sum C_{i, FAME}}{TL} \cdot 100 \quad (1)$$

2.5. Fuel properties

The relative weight of saturated and unsaturated esters was analysed considering the concepts of “Long chain saturated factor” (LCSF), “saponification value” (SV) and “degree of unsaturation” (DU), according to the following equations suggested in the literature [29,30]:

$$LCSF = 0.1 \times C16 + 0.5 \times C18 + 1 \times C20 + 1.5 \times C22 \quad (2)$$

$$SV = 540 \cdot \sum \frac{x_i}{MW_i} \quad (3)$$

$$DU = MUSFA\% + 2 \times PUSFA\% \quad (4)$$

In these equations, “C_i” corresponds to the weight percentage of each saturated fatty acids transformed into FAMES (C16:0, C18:0, C20:0 and C22:0); whereas “x_i” and “MW_i” indicate the percentage and the molecular weight of each methyl ester, respectively. “MUSFA” and “PUSFA” are the acronyms of monounsaturated fatty acids and polyunsaturated fatty acids, respectively.

The most interesting properties of oils obtained are defined based on the results of these previous values, obtaining the cold filter plugging point (CFPP), the iodine number (IN), the density (ρ, kg·m⁻³), kinematic viscosity (η_k, mm²·s⁻¹), the “higher heating value” (HHV, MJ·kg⁻¹), and the cetane number (CN), according to the following expressions previously used in the literature [31–34]:

$$CFPP = 3.1417 \times LCSF - 16.477 \quad (5)$$

$$IN = 254 \cdot \sum \frac{x_i \cdot DB_i}{MW_i} \quad (6)$$

$$\ln(\eta_k) = \sum (x_i \cdot (-12.503 + 2.496 \cdot \ln(MW_i) - 0.178 \cdot DB_i)) \quad (7)$$

$$\rho_i = \sum \left(x_i \cdot \left(0.8463 + \frac{4.9}{MW_i} + 0.0118 \cdot DB_i \right) \right) \quad (8)$$

$$HHV = \sum \left(x_i \cdot \left(46.19 - \frac{1794}{MW_i} - 0.21 \cdot DB_i \right) \right) \quad (9)$$

$$CN_1 = 61.1 + 0.0882 \cdot M + 0.133 \cdot P + 0.152 \cdot S - 0.101 \cdot PL - 0.039 \cdot O - 0.243 \cdot L - 0.395 \cdot LL \quad (10)$$

$$CN_2 = 46.3 + \frac{5458}{SN} - 0.225 \cdot IV \quad (11)$$

$$CN_3 = \sum (x_i \cdot (-7.8 + 0.302 \cdot MW_i - 20 \cdot DB_i)) \quad (12)$$

where “x_i”, “MW_i”, and “DB_i” are the percentage, the molecular weight, and the number of double bonds of each methyl ester, respectively, and “DB_i” is the number of double bonds.

3. Results and discussion

3.1. Comparison of basic and acid catalysis

3.1.1. Biodiesel production

According to the literature, base-catalysed transesterification is faster than acid one [17,28]. Checking if this statement is also applied to the WAS transesterification, the first experiments were carried out comparing the in-situ transesterification at 60 °C using 4% of NaOH with the previously reported results with the same percentage of H₂SO₄ [4]. Results after 5 h of reaction are shown in Fig. 1, observing that yields at acid conditions are more than 100 times higher than with the basic ones (from 0.21 to 22.2%, respectively). As explained in the experimental section, each experiment was carried out twice, showing the negligible differences plotted by the error bars in Fig. 1.

Results discard a slower but more productive reaction when using the NaOH since samples at longer times (24 h) do not demonstrate any further evolution (final yield 1.1%). Two concomitant reasons explain this result: the base-catalysed reaction requires a higher catalyst concentration than the acid one, and the initial lipid mixture extracted has a very low percentage of triglycerides and a high proportion of free fatty acids (FFA). It highlights that the reaction of these FFA (esterification) only occurs at acid conditions. On the contrary, basic ones produce soap, so acid catalysis is proposed in the bibliography if the percentage of FFA is higher than 1% [35]. Reactions with different NaOH percentages were carried out to check the first hypothesis (30, 50 and 70%). Fig. 1 compares the final yields. As the NaOH increases, the yield also does, these increments being more marked below 50% NaOH. Thus, the basic transesterification requires higher catalytic loading than the acid one. However, even working with a high NaOH concentration, the maximum FAMES yield obtained is significantly lower than the value obtained by the acid mechanism. This fact reveals a limiting role of lipid composition, suggesting a high percentage of FFA, at the expense of triglycerides. As mentioned before, these FFA undergo saponification at basic conditions, instead of esterification.

The presence of FFA in a relevant amount in the lipid composition of

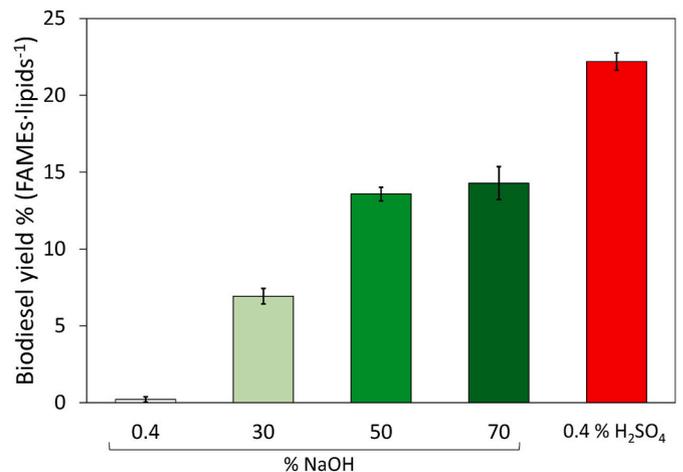


Fig. 1. Biodiesel yield obtained after 5 h of alkali and acid catalysed *in situ* transesterification of dried WAS at 60 °C.

sludge has been previously corroborated by *Jardé and co-workers* [36]. Considering the high concentration of acylglycerides in living microorganisms, the hydrolysis of microbial cells explains the absence of tri-, di-, and monoglycerides in high percentage. This hydrolysis is the first step of the anaerobic digestion of lipids, producing the decomposition of acylglycerides into free fatty acids. According to the literature, this reaction occurs after a low period of anaerobic conditions, whereas the following steps (acidogenesis, acetogenesis and methanogenesis) require severe conditions [37]. This short period is congruent with the transportation and storage time, a possible justification of these results. This partial degradation can strongly modify the percentage of free lipid content. Thus, the maximum FFA of the total lipids in this type of raw material can reach up to 65%, whereas the amount of glyceride fatty acids can be even lower than 10% [38]. Considering the experimental data, the amount of esters obtained from these FFA in the sludge is close to 10% (9.8%), so the acid catalysis is justified. This calculation was done comparing the maximum FAMES reached with acid and base conditions related to the total amount of lipids obtained after extraction with chloroform ($20.4 \pm 1.5\%$), according to the methodology proposed by *Siddiquee and Rohani* [27]. Comparing the experimental results with this statement (basic catalysis maximum yield of 14.3%, expressed as mass of FAME divided by lipid content), it can be assumed that basic transesterification has reached its maximum. On the contrary, there is a high improvement potential for the acid one. At this point, it must be highlighted that lipids have been not detected in the reaction media after the acid catalysis, suggesting that the main limitation is related to the lipid extraction and not to the esterification step.

3.1.2. Biodiesel quality

In addition, to evaluate the productivity, the results of basic and acid catalysis must be compared considering the biodiesel quality. The complete FAMES distribution analysed in terms of saturation level and the number of carbons distributions are included in [Table 1](#).

There are stark differences, both in terms of saturation percentage and carbon chain length. Thus, acid catalysis produces a high amount of saturated FAMES, whereas 50% of the FAMES obtained with the base catalysis corresponds to polyunsaturated ones. Moreover, biodiesel obtained by acid catalysis has a more similar chemical composition (with hydrocarbons from 12 to 18 carbon atoms) concerning the mineral diesel than the basic ones [39]. However, the NaOH catalysed biodiesel presents a high concentration of long-chain esters (more than 20 carbon atoms); increasing this ratio as the NaOH concentration increases.

These differences determine the final quality of the biodiesel, being the basis to calculate most of its physic-chemical properties, as is summarized in [Table 2](#). In this table, two examples of first-generation biodiesel were included, to compare the results obtained with the sludge to others using more typical raw materials (*Jatropha* and Barley straw), as well as the values required by the standard specification for biodiesel

Table 1

Analysis of FAMES distribution and composition as function of the type and percentage of catalysts used for the WAS transesterification. All values are given in percentage (%) and correspond to the average value of, at least, two samples ($SD < \pm 4.3\%$ for all the data).

		30% NaOH	50% NaOH	70% NaOH	4% H ₂ SO ₄
Type of FAMES	Saturated	10.8	11	12.9	68.1
	Monounsaturated	36.9	40.6	37.3	13.3
	Polyunsaturated	52.3	48.4	49.7	18.5
Fatty Acid Length	< C14	5.9	0	0	0.6
	C14	2.2	7.5	1.1	2.3
	C15	2.5	10.4	17.1	2.3
	C16	27.1	15.8	16.2	37.2
	C17	3.3	–	–	1
	C18	41.6	11	10	35
	C20	3.3	20.3	14.9	20.9
	> C20	14.1	35	40.6	0.6

fuel by ASTM D975 and EN 590.

The SV is a parameter related to the average molecular weight of biodiesel obtained. It must have a value lower than 500 mg g^{-1} , according to the ASTM standards. Thus, all the samples have correct values for this parameter, observing a decreasing trend as the basic amount increases (from 197 to 184) and similar values if comparing acid and base catalysis. These values are in all the cases lower than the reported for first-generation biodiesel, but with differences minor than 10%.

There is no specific requirement related to the DU value since this is not a final property of biofuels. However, its value correlates with different relevant parameters (as would be detailed in [section 3.4](#)). Typically, highly saturated esters have worse fuel properties than those enriched in unsaturated ones [32]. Concerning experimental data, there is not a clear effect of NaOH percentage on this magnitude, with values almost constant and close to 131. This value is nearly three times higher than the value obtained with the acid catalysis (50.36), which indicates a high increase in the percentage of saturated fatty esters. According to the data obtained in the literature, there is no good agreement about the optimum range, reporting biodiesel with good properties and DU values close to 30 and 103. These discrepancies support the idea of not being a key parameter in the final quality if the other values are balanced.

CFPP is a critical property to determine the low-temperature operability of biodiesel fuels, with different minimums as a function of the climate of each area. Considering the values obtained, all these biodiesels could be used in Spain, for example, since the most restrictive CFPP required, according to the RD1088/2010, is $-10 \text{ }^\circ\text{C}$ for winter. A positive effect of increasing the percentage of NaOH is observed for this parameter, whereas in acid catalysis, this temperature is very high, requiring other additives to decrease it and allow its marketing. These high values are also observed with the first generation of biodiesel, as in the case of fuel produced from *Jatropha* ($CFPP = 39.5 \text{ }^\circ\text{C}$, see [Table 2](#)), being one of the main causes of the limitation about its commercialization as a pure fuel, being limited to defined percentages.

The fuel trend to oxide and polymer is also relevant, being studied by the “iodine number” (IN). As shown in [Table 2](#), all these biodiesels would be far from the maximum allowed, the value obtained after the acid catalysis being the lowest one and observing a relevant decrease as the percentage of NaOH increases. In any case, literature reports high levels of this parameter with other raw materials, a direct consequence of the high oxygen content of FAMES (27.3 and $1 \text{ g I}_2/\text{g}^{-1}$ biodiesel, for barley straw and *Jatropha*, respectively, see [Table 2](#)).

In terms of the kinematic viscosity, the international standards (ASTM D6751) are laxer than the European ones (EN14214), allowing biodiesel in the range from 1.9 to $6.0 \text{ mm}^2 \text{ s}^{-1}$ and from 3.5 to $5 \text{ mm}^2 \text{ s}^{-1}$, respectively. Samples indicate that only the most severe base conditions and the acid ones produce an optimum biodiesel, considering the ASTM standard. However, none of them has enough quality to be commercialized in Europe, being the acid one the closest to the minimum value. It highlights that biodiesel is marketed mixed with mineral fuel. Thus, the better qualities of this biodiesel, the higher percentage of renewable fuel that can be introduced in these mixtures without affecting the final properties.

As to the density, nothing is said in the ASTM, whereas an optimum range from 860 to 900 kg m^{-3} is detailed in the EN14214. All the samples are out of this optimum range, being the biodiesel obtained by acid transesterification the one with the lowest value (916 kg m^{-3}). These two properties (kinematic viscosity and density) have better values with first-generation biodiesel than with the sludge-derived one, suggesting that a refinement of the esterification reaction is required. Concerning the HHV, very similar values are obtained in all the cases, being much closer to the HHV of mineral diesel (49.65 MJ kg^{-1}) than typical biodiesel obtained by transesterification of pure raw materials [41].

Maybe the most interesting parameter is the cetane number (CN), a property that indicates the ability of the fuel to auto-ignite, in such a way that the lower CN, the higher ignition delay. A higher pollution level and

Table 2

Summary of main physic-chemical properties of fuel obtained after activated sludge transesterification as function of the catalyst used. The standard deviation between the two replicated trials in each case is lower than 5.8% for all the data.

	30% NaOH	50% NaOH	70% NaOH	4% H ₂ SO ₄	Jartropha ^a	Barley straw ^b	ASTM D975	EN 590
Degree of unsaturation (DU)	133.1	128.9	131.2	50.4	102.4	30.04	–	–
Long chain saturated factor (LCSF)	0.8	0.3	0.4	19.5	17.8	9.8	–	–
Cold Filter Plugging Point (CFPP, °C)	–13.8	–15.6	–16.5	43.9	39.5	14.4	–	–
Saponification value (SV, mgKOH·g ⁻¹)	197	186	184	195	204	215	<500	–
Iodine number (IN, gI ₂ ·100 g ⁻¹ _{biodiesel})	144.3	121.4	117.9	70.9	100	27.3	25	25
Density at 15 °C (ρ, kg·m ⁻³) _{rowhead}	927	920	919	916	878	860	890	860–900
Kinematic viscosity at 40 °C (η _v , mm ² ·s ⁻¹)	1.3	1.8	1.9	2.6	4.9	3.8	1.9–6.0	3.5–5.0
Higher heating value (HHV, MJ·kg ⁻¹)	39.5	39.9	40.0	39.8	41.1	39.2	–	–
Cetane number (CN ₁)	45.8	55.5	55.8	69.1	55	65.6	47	51
Cetane number (CN ₂)	43.8	50.7	51.8	60.6	–	–	–	–
Cetane number (CN ₃)	45.6	54.8	56.1	62.2	–	–	–	–

- Not reported.

^a Data from [29].

^b Values adapted from [40].

non-homogeneous combustion are the main consequences of this delay. There are different experimental correlations relating the CN number to the chemical composition. Since none of them is specifically determined to sewage sludge, the most cited ones [32–34] have been compared (Table 2).

Similar trends are obtained with all the experimental samples, despite the equation used, with only relevant discrepancies between CN₁ and the other two correlations in the specific case of the acid sample. These differences can be due to the presence of a high amount of some FAMES not considered by this equation. Based on this fact, this correlation has been discarded. Although the minimum CN required depends on the engine design, mineral diesel has standard CN values from 48 to 50 [42]. A minimum CN of 51 is required to market biodiesel, considering the EN14214. According to these values, the biodiesel obtained with 30% of NaOH could not be commercialized, whereas NaOH percentages higher than 50% have not significant effect in this property obtaining, in any case, higher values with the acid catalysis. These values are in good agreement with values proposed in the literature for other raw materials, despite the method to estimate them [29,40].

To sum up, acid transesterification produces not only a high amount of FAMES but also biodiesel with better properties as fuel than with the basic catalysis. However, several parameters are out of the standard limits, such as IN, LCSF, and η. All these parameters are directly related to the initial composition of fatty acids, suggesting that the extraction step conditions these values. On the other hand, the typical percentages of free fatty acids indicate that improving the acid catalysis is possible. The study has been completed based on these conclusions, proposing different pretreatments to improve the extraction; and analysing the results from two complementary points of view: productivity and fuel quality.

3.2. Effect of pre-treatments in the biodiesel yield

3.2.1. Sludge humidity

The efficiency of the most typical pretreatments improving the subsequent lipids extraction is strongly affected by the presence of water, with positive or negative effect as a function of the technology used. Thus, their impact must be compared when it is applied to dry sludge and when the wet one (initial humidity 80%). This study is not so evident since water can have positive effect on pretreatments, but also a negative role in the transesterification, masking these improvements. The role of humidity in the *in-situ* transesterification was studied to isolate these effects, analysing samples with different water percentage and comparing the results in Fig. 2.

Different humidity percentages were obtained by controlling the drying time from 0 to 24 h (time required to the total dried). Thus, the amount of water is experimentally measured in each case. The negative effect of water in the transesterification process is observed, according to

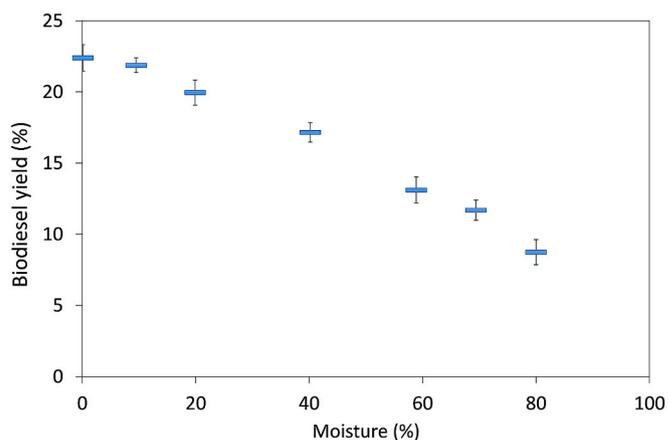


Fig. 2. Influence of water content on the biodiesel yield obtained by *in situ* transesterification with acid catalyst (H₂SO₄, 4%, volume ratio).

the decreasing trend of FAMES yield at increasing percentages of moisture, with a total 58% reduction comparing results with dry and 80% wet sludge. The most interesting analysis, however, is to identify if there is any influence of this parameter in the biodiesel composition. Table 3 shows the distribution of FAME profiles grouped by families. An almost perfect concordance among all the experiments is observed, with average values (all the moistures considered) of 86.4 ± 2.8% and 10.9 ± 3.2% for saturated and monounsaturated fatty esters, respectively. Concerning the carbon chain length, all biodiesel samples are enriched in the C17 and C19 fraction, with typical values of 47.2 ± 1.8% and 32.1 ± 1.6%, respectively. All the physical-chemical properties indicate these insignificant differences, with deviations lower than 5% for all the parameters. Taking these results into account, the role of water in transesterification is limited to productivity, but it does not affect biodiesel quality. Thus, the effects of different pretreatments on dry and wet sludge are comparable.

Previous literature suggests the use of microwaves (MW) and sonication (US) as two of the most significant pretreatments that maximize the lipid extraction of biological systems [43]. The wet sludge after the pretreatment was also dried to carry out the *in-situ* transesterification at the optimum conditions to remove the negative role of water in transesterification. Considering the previous results, both pretreatments are applied to the acid catalysis. Final yields obtained after 24 h of *in situ* extraction-transesterification applied to the WAS after the MW pretreatment are compared in Fig. 3.

Pretreatments produce a relevant improvement, with a common trend despite the presence or absence of water. A high increase in the final yields is observed after low pretreatment times, reaching a

Table 3

Analysis of FAMES distribution and composition as function of the initial moisture. Results corresponding to values after 5 h of acid catalysed transesterification at 60 °C. All the data shown correspond to the average values of two trials, having a maximum standard deviation of $\pm 3.7\%$.

		0%	10%	20%	40%	60%	70%	80%
Type of FAMES (%)	Saturated	86.3	87.4	84.1	86.6	83.6	87.4	92.5
	Monounsaturated	12.0	10.9	10.0	12.8	10.9	10.7	6.1
	Polyunsaturated	1.7	1.7	5.9	0.6	5.5	1.9	1.4
Fatty Acid Length (%)	< C14	1.4	1.4	1.2	1.3	1.3	1.4	2.3
	C15	3.5	3.6	3.1	3.9	3.5	3.5	5.0
	C16	2.9	2.8	2.5	3.0	2.9	2.9	3.3
	C17	47.2	47.8	45.0	47.0	46.5	49.2	55.0
	C18	3.9	4.2	2.9	1.9	5.0	4.4	3.3
	C19	33.5	32.7	32.6	32.7	30.3	31.5	24.3
	> C20	7.6	7.6	12.7	10.1	10.5	7.2	6.9

Microwaves (MW) and Sonication (US).

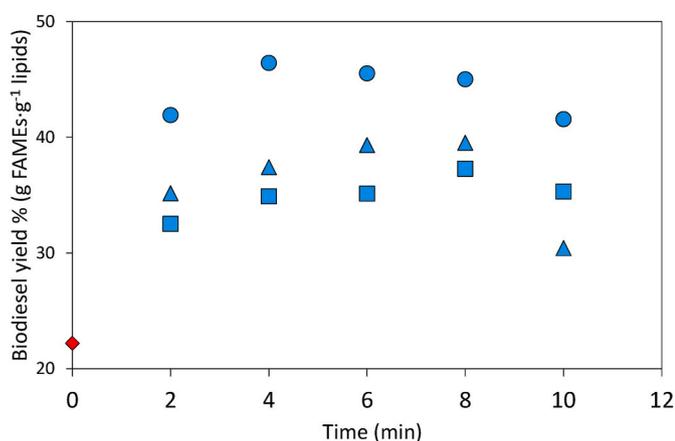


Fig. 3. Effect of pretreatment time (MW) on biodiesel yield by *in situ* transesterification with 4% (volume ratio) as catalyst at 60 °C after 24 h of reaction. Comparison between (●) dry sludge; (■) wet sludge; and (▲) wet sludge and dried before transesterification. Results compared with previous value obtained without pretreatment (◆). (Each experiment was carried out twice with a deviation lower than 3%; error bars are not shown since they are smaller than the symbol size).

maximum after which the yields decrease. Times longer than 10 min were not evaluated, considering these results. As there were no fatty acids in the samples after 24 h, differences must be analysed considering how the MW time affects the extraction capacity.

A negative effect of MW on the structure of lipids has been reported in the literature, highlighting the partial oxidation that can produce long times of microwaves in different types of foods [44]. This point has been checked by analysing the lipids obtained after liquid extraction without suffering transesterification once the samples were pre-treated with different MW times. GC-MS spectra were compared with the spectra obtained without any pretreatment, without observing any displacement in the retention times, which would be the expected consequence of any oxidation or degradation process. On the contrary, a continuous increase in the peak's intensity is observed, from non-pre-treated to 4 min of MW samples, whereas longer MW times produce a decrease in all the signals. These results are congruent with a negative effect of MW on the proteins taking part in the cell membranes, resulting in different spatial configurations of these polymers that hinder the lipid extraction. This effect has been previously observed in the literature [45].

A significant role of water in these reactions has been observed. Thus, the dried sludge pre-treated produces the maximum yield after 4 min (46.7%, more than 50% of the yield obtained without pretreatment), whereas this maximum requires double-time with the wet sample (37.3%). This fact is justified since water molecules can absorb microwaves at this frequency (2450 MHz), preventing the total absorption by the sludge. In addition, water can cause the partial degradation of

triglycerides into diglycerides, monoglycerides and even free fatty acids, affecting the transesterification capacity [46]. The difference observed with the wet sludge if the sample is dried after the pretreatment or not (maximum yields of 39.5 and 37.3%, respectively) is congruent with the negative effect of water in transesterification. Thus, not all the extracted lipids are transformed into FAMES when using the wet sludge (square points in Fig. 3).

The improvement obtained by MW can be due to two synergetic causes. On one hand, a thermal effect, which leads to the heating of the sludge; on the other hand, a non-thermal effect due to the alignment of the polarized parts of the molecules with the electric field, causing the break of hydrogen bonds [43]. The result obtained after 4 min of pretreatment (46.7%) is compared to the one with the same total pretreatment time, but applied at short intervals of 30 s (to prevent heating the sludge), to evaluate the relative influence of both effects. The maximum yield obtained at these conditions is very similar (46%), discarding a relative role of thermal effect at this short time. This yield, according to the total lipid content of this sludge (20.4%), corresponds to the valorisation of the 10% of total solids, i.e., the dry sludge.

In the case of sonication, water is also negative, decreasing the final FAMES products because water molecules absorb the US and reduce the amount of energy efficiently applied to the sludge. Thus, final values after 5 h of *in situ* transesterification are 42, 31.4 and 26.7%, for dry sludge, wet sludge, and wet sludge + drying before reaction, respectively. No significant evolution is observed by increasing the reaction time up to 24 h, concluding that 5 h is enough to complete the transesterification.

All these experiments have been carried out without any previous sieving of the sludge particles. A broad range of particles sizes is expected, considering the origin of this raw material and the flocculation trend of microorganisms. The particle size is a relevant parameter since it restricts the surface/volume ratio and the contact with the solvent, which can condition the pretreatment and extraction efficiency. It can be assumed that if the size of these flocs does not influence their lipid content, the smallest floc size, the highest contact, and extraction efficiency. According to this hypothesis, once the sludge has been dried, big particles have been discarded. Thus, sludge sewage has been divided into three fractions: 50–100 μm ; 100–255 μm ; and 255–350 μm and the FAME yield is analysed, applying the previously optimized pretreatment conditions. These fractions correspond to three typical ranges considered by the international standards (ISO 3310/1), with a relative weight of 17.4, 19.1 and 21.2%, respectively. It must be pointed out that this sieve was carried out with natural sewage, without modifying the original size by any milling procedure, since milling could alter the lipids yield. Results obtained are plotted in Fig. 4, comparing with the corresponding ones without doing any size classification. The analysis must be done in terms of the mass of FAMES divided by the total mass of dry sludge since the amount of lipid in each fraction can be different, and the assumption of 20.4% of TL content can be inaccurate.

Results obtained in the absence of pretreatment indicate that the

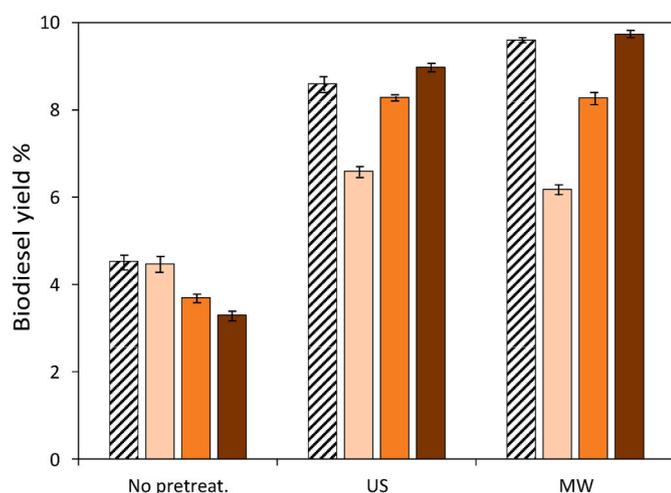


Fig. 4. Effect of sludge particle size on the biodiesel yield obtained after 4 h of *in-situ* transesterification catalysed by H_2SO_4 . Results correspond to no sieving (black bars); 50–100 μm (light orange); 100–255 μm (orange); 255–350 μm (brown)

reaction conditions are not severe enough to produce the extraction of those lipids contained in the large particle size fractions, with an almost total correspondence between values without sieving and the 50–100 μm fraction (4.53 and 4.5%, respectively). Once the pretreatments are introduced, values without sieving are closer to those obtained with the large fraction (255–350 μm), indicating that these techniques are very efficient for the extraction of lipids contained in these big flocs. The relevant differences among different sizes suggest different lipid content. This point was corroborated by the extraction with chloroform, according to the standard procedure proposed by Siddiquee [27], observing that the large fraction is enriched in lipids, in comparison to the smallest one: 17, 24 and 29%, for 50–100, 10–255 and 255–350 μm , respectively.

To sum up, from the point of view of productivity, the best results are obtained with microwaves applied to dried sludge, not requiring the previous sieve. This result suggests that the lipid composition depends on the particle size in such a way that the extraction procedure is efficient enough to produce the lipid extraction, even for the largest particles. In this situation, 46.6% of the TL are transformed in FAMES,

whereas this value only increases up to 47.8% applying the particle sieving.

3.3. Effect of pre-treatments in the biodiesel quality

The direct application of pretreatments to dry sludge can affect the biodiesel quality since some lipids can be damaged by the direct impact of US or MW, producing a different distribution of final FAMES. Table 4 summarizes the most meaningful parameters at the optimum conditions of each pretreatment, as well as the FAMES distribution. In general, all the pretreatments produce an increase in the saturated FAMES percentage, and this value reaches its maximum when applying MW and US to the wet sludge (87.9 and 89.6%, respectively). This increase produces a decrease in the polyunsaturated FAMES, whereas the monounsaturated ones remain almost constant.

Concerning the fatty acid length, all the pretreatments produce an increase in the percentage of C16 and C18, with a significant decrease in the C20 fraction. When MW is applied, results obtained with the wet sludge dried before the transesterification are like the dry ones for the short acid lengths (from C12 to C16), whereas results are more comparable to values obtained with the wet sludge for long acid chains (higher than C16). In the case of the US, this analysis is not so clear, but results obtained with the wet + drying sample are, in general, as the wet sample, suggesting that the presence of water is more relevant in this case.

As to the physicochemical properties of FAMES as fuels, the oxidative stability strongly improves with all the pretreatments, obtaining better results (lower iodine numbers) with the US than with MW. A relevant decrease in the density is also obtained, with values close to 909 kg m^{-3} in all the cases, with the lowest values after applying the US. This decrease is due to the high percentage of short-chain acids and the corresponding decrease in C20 and higher compounds (in comparison to samples without pretreatment). However, the values obtained are still out of the optimum range (850–900 kg m^{-3} according to EN standards). The quality in terms of kinematic viscosity also improves it, the obtained values being in the average of the allowed range. It highlights that, even inside this range, low values can produce a lack of lubrication, whereas too high ones can damage the fuel pumps, so a good equilibrium is essential. No significant differences in terms of saponification value and higher heat value were observed, with continuous values of $200 \text{ mg NaOH} \cdot \text{g}^{-1}$ and 39.8 MJ kg^{-1} . In global terms, both pretreatments imply an enhancement in the biodiesel quality without observing significant

Table 4

Detailed analysis of biodiesel composition and fuel properties as function of pretreatments applied. Results correspond to optimum conditions of each technique. All the characterization data shown correspond to the average of two trials, having a maximum standard deviation of $\pm 3.8\%$.

		No pretreatment			US			
			MW dry	MW wet	MW Wet + drying	US dry	US wet	US Wet + drying
Type of FAMES (%)	Saturated	68.1	86.5	87.9	86.9	86.1	89.6	88.4
	Monounsaturated	13.3	11.6	10.6	10.8	12.3	9.6	10.2
	Polyunsaturated	18.5	1.9	1.5	2.3	1.5	0.7	1.4
Fatty Acid Length (%)	< C14	0.6	1.0	2.1	0.9	0.9	0.9	1
	C14	2.3	3.1	3.4	2.7	2.9	3	3.1
	C15	2.3	2.5	0.7	2.5	2.2	2.6	2.5
	C16	37.2	48.5	48.6	47.2	49.6	50.9	48.7
	C17	1	0.8	0.9	1.1	1.0	0.8	1.1
	C18	35	39.3	38.9	38.7	36.7	37.7	38.6
	C20	20.9	2.8	4.4	5.8	5.7	3.4	4.0
	> C20	0.6	1.9	0.9	1	0.9	0.6	1.0
Long chain saturated factor (LCSF)		19.5	23.4	25.4	25.1	23.8	23.8	24.5
Cold Filter Plugging Point (CFPP, °C)		43.9	57.1	60.5	62.4	58.3	58.2	60.5
Saponification value (SV, $\text{mgKOH} \cdot \text{g}^{-1}$)		195	200	199	199	199	200	200
Iodine number (IN, $\text{gI}_2 \cdot 100 \text{ g}^{-1} \text{ biodiesel}$)		70.9	17.1	13.3	15.6	15.2	10.4	13.1
Density at 15 °C (ρ , $\text{kg} \cdot \text{m}^{-3}$)		916	909.8	909.2	909.3	909.4	909.0	909.2
Kinematic viscosity at 40 °C (η_k , $\text{mm}^2 \cdot \text{s}^{-1}$)		2.6	4.1	4.3	4.2	4.2	4.4	4.3
Higher heating value (HHV, $\text{MJ} \cdot \text{kg}^{-1}$)		39.8	39.8	39.8	39.8	39.8	39.8	39.8
Cetane number (CN ₂)		60.6	72.0	72.9	72.4	72.4	73.4	72.9
Cetane number (CN ₃)		62.2	73.3	74.4	74.1	73.9	74.7	74.3

differences between results at optimum conditions. Values obtained are like those proposed in the literature for biodiesel obtained from different raw materials, such as *Jatropha* or *Jajoba* [29,47] and also quite similar to those proposed with primary sludge [48].

A similar analysis for samples obtained after sieving the sludge produces the main results of FAMES composition summarized in Table 5.

In general, the isolation of different particles sizes produces an increase in the amount of saturated compounds (up to 95% of the total FAMES), with a slight decrease in the percentage of FAMES derived from C19 and C17 fatty acids and an enrichment of C15 ones. This fact is more relevant for the largest particles (255–350 μm). Because of these results, the fuel properties are more unbalance than without any particulate sieving. Thus, density is higher than the allowed one, reaching values up to 910 kg m^{-3} , and the high heater value decreases to 37 MJ kg^{-1} . On the other hand, there is a positive decrease in the iodine number, with values from 6 to 10 g g^{-1} . To sum up, no improvement in the biodiesel productivity is reached, discarding the classification of wet activated sludge, neither from the yield point of view nor from the quality one.

3.4. Correspondence between evolution of fuel properties

Fig. 5 illustrates the evolution of some fuel properties as a function of the density, the kinematic viscosity, and the degree of unsaturation. A direct correspondence between LCSF and the two physical properties analysed (density and kinematic viscosity) is observed, with opposite trends. The LCSF decreases as the density increases, whereas its evolution is proportional to the kinematic viscosity. In both cases, the slopes are very soft. These trends are stronger when the analysis includes the results of the particulate size. In the case of the dependence on DU percentage, almost complete independence can be assumed, at least in the considered range.

More marked results are obtained for IN, with a relevant increase as the density and the degree of unsaturation increase, and an opposite trend concerning the kinematic viscosity. The best correspondence is obtained when results are analysed as a function of the degree of unsaturation. The correlation between density and the iodine number was previously reported in the literature for different first-generation raw materials [29].

Despite the dispersion among experimental data, it can be concluded that iodine number is a very sensitive factor, and different FAMES distributions can produce relevant changes in this value, whereas they can be masked for some variables related to the total distribution. In good agreement, a very good fit of experimental results and degree of unsaturation is observed.

The cetane number decreases with the density and, with a lower rate, with the degree of unsaturation. The analysis of the degree of unsaturation is not so conclusive. According to the high slopes observed cetane numbers higher than 86 are required to guarantee a density lower than

the maximum allowed by the ASTM standards. This value would be too high to consider the fuel as conventional biodiesel (the standard recommend biodiesel from $\text{CN} = 47$). In parallel to this behaviour, the evolution of CPFF (not shown) does not follow any clear trend with any of these parameters but, in general, it increases as the density does. Thus, it is not possible to reach simultaneously good density and cloud point values with a pure sample of this fuel, and biodiesel obtained by this transesterification must take part of mixed biodiesel before its commercialization.

Finally, the almost flat evolution of the only parameter that can be correlated to this property, the degree of unsaturation, is congruent with the low variability of the HHV. As previously mentioned, all the samples show a very similar HHV, being in the optimum range in all the cases.

To sum up, experimental correlations chosen for these studies give congruent values and correspondences among them. Thus, despite not being defined for this type of raw material, they are useful to predict the quality of biodiesel obtained by analysing its FAME composition. The best correlations related to cetane number are obtained using the correlation proposed by Ardabili et al. [33], labelled as CN_3 . This model is proposed as the most accurate one for this type of raw material. On the other hand, the correct correspondence despite the treatments corroborates that the only effect of microwaves and sonication is related to the amount of lipids available to suffer transesterification, without producing any other change that could affect the quality properties.

4. Conclusions

Initial comparison between base and acid catalysts for the *in-situ* sewage sludge transesterification allows concluding that the high percentage of free fatty acids (9.8%) prevents the optimum activity of base catalyst (NaOH). In both cases, lipid extraction is identified as the limiting stage of the process, obtaining promising improvements when using pretreatments.

The best results are obtained with 4 min of MW applied to dried sludge, with an increase in the FAMES production of 110% (from 22.1 to 46.7%), whereas sonication produces a slightly lower improvement (up to 42%). These upgrades can condition the viability of this approach since it improves the results published for this raw material. On the contrary, sludge seizing produces only slight differences, discarding preferential fractions for the lipid deposits.

The role of water was studied, concluding that a maximum of 20% of the moisture is allowed to prevent significant decreases in the transesterification activity, being this conclusion supported by analysis with and without pretreatments. This fact reinforces the potential of this raw material since no total dried is required (the principle negative aspect of previous studies).

Acid catalysis produces better biodiesel than basic one, both in terms of saturation percentage and carbon chain length. Therefore, this fuel

Table 5

Detailed analysis of biodiesel composition and fuel properties when reactions are carried out after a previous sieving of the sludge. Results corresponding to reactions without any pretreatment and applying microwaves (MW) and sonication (US). All the values shown correspond to the average results after two analyses, having a maximum standard deviation considering all the characterization data of $\pm 4\%$.

		No pre-treatment			MW			US		
		Particle size (μm)			50–80	100–255	255–350	50–80	100–255	255–350
Type of FAMES (%)	Saturated	50–80	100–255	255–350	50–80	100–255	255–350	50–80	100–255	255–350
	Monounsaturated	95.2	94.4	94.3	94.9	95.6	93.1	90.8	93.2	95.7
	Polysaturated	3.9	4.7	4.9	4.5	3.7	6.3	8.4	6.3	3.7
Fatty Acid Length (%)	< C14	1.0	0.8	0.8	0.6	0.7	0.6	0.7	0.5	0.6
	C15	4.1	6.3	6.6	7.3	6.8	7.2	4.8	5.1	6.8
	C16	3.2	3.2	3.4	2.4	20.0	19.2	2.9	19.5	19.5
	C17	0.9	0.8	0.8	0.7	0.9	3.3	0.9	3.5	0.8
	C18	49.1	40.6	36.8	35.9	28.3	27.9	38.8	28.6	29.3
	C19	5.9	16.5	22.3	25.5	20.4	20.4	23.6	20.4	20.2
	C20	33.6	29.6	27.1	25.6	21.4	20.3	26.1	20.9	21.3
	> C20	3.3	2.9	2.8	2.5	2.1	1.68	2.9	1.9	2.0

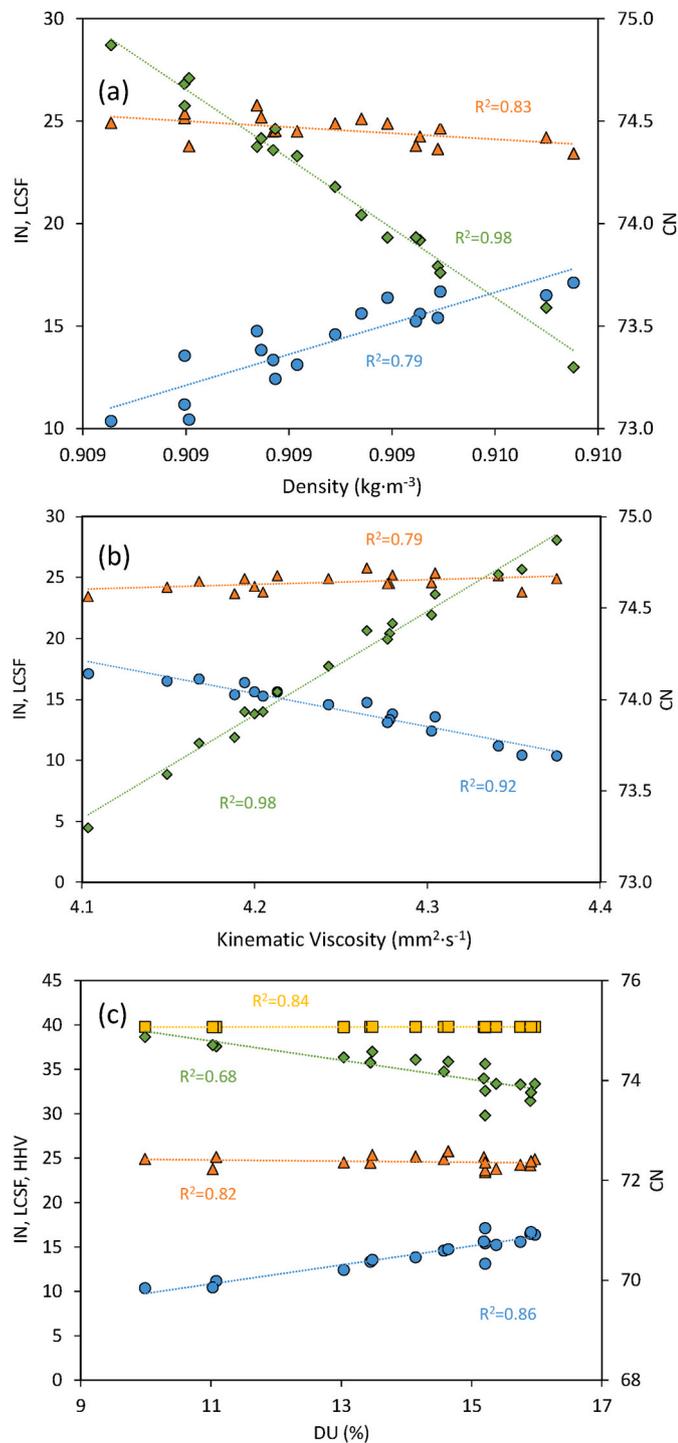


Fig. 5. Experimental correlation between different fuel properties and the corresponding biodiesel physical parameters: (a) density; (b) kinematic viscosity; (c) degree of unsaturation. Symbols corresponds to high heater value, HHV (■); iodine number, IN (●); longer chain saturated factor, LCSF (▲), cetane number, CN (◆), and high heater value, HHV (■).

has better values of cetane number and saponification value. Applying pretreatments, the amount of C12–C16 FAMES increases, obtaining better oxidation properties (measured by the Iodine number) and higher heat capacity values, reaching typical values of most of the fuels obtained from different lipid-rich raw materials.

The good correlation obtained between FAMES composition and biodiesel properties allow identifying a fast and easy way to predict the fuel properties of biodiesel obtained from other sludges as function of

composition values.

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