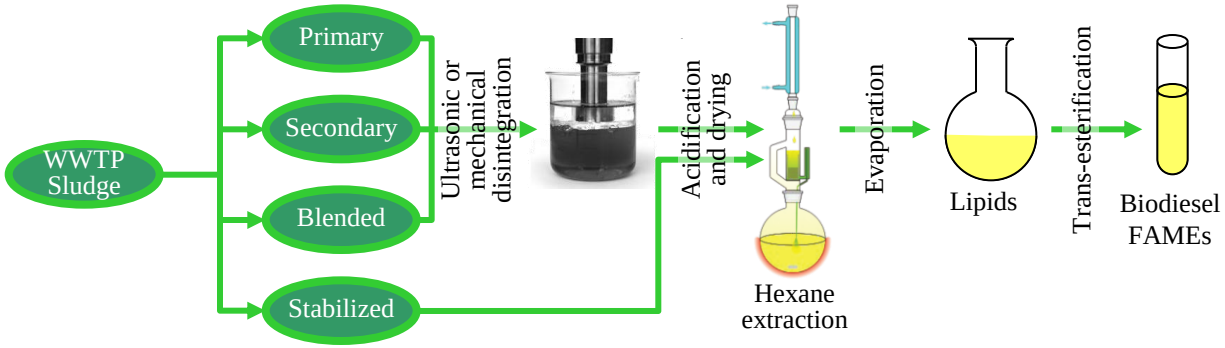
***Highlights (for review)**

- One of the first attempts made in Europe to convert municipal sludge to biodiesel.
- First attempt to optimise the yield of lipids extracted with pre-treatments.
- First attempt to compare four sludge of different characteristics from the WWTP.
- Primary sludge has a conversion to FAME of 19% in dried sludge basis.
- Pre-treatments do not affect essentially conversion and composition of FAME.

Graphical Abstract (for review)



Effects of pre-treatments on the lipid extraction and biodiesel production from municipal WWTP sludge

Magdalena Olkiewicz [a], Agustí Fortuny [b], Frank Stüber [a], Azael Fabregat [a], Josep Font [a], Christophe Bengoa [a,*]

[a] Departament d'Enginyeria Química, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Spain.

[b] Departament d'Enginyeria Química, Universitat Politécnica de Catalunya, Av. Víctor Balaguer S/N, 08800 Vilanova i la Geltrú, Spain.

* Corresponding author. Tel.: +34-977-558619; fax: +34-977-559667.
E-mail address: christophe.bengoa@urv.cat

Abstract

Biodiesel production is currently limited due to high raw material costs. The potential of using sludge from municipal wastewater treatment plants as an alternative lipid feedstock was investigated. Four different types of sludge (primary, secondary, blended and stabilised) were tested in lipid extraction by Soxhlet using hexane, and biodiesel production by acid catalysis. To improve the extraction efficiency, the influence of pre-treatment methods (ultrasonic and mechanical disintegration) and duration of these treatments were investigated. Finally, the effect of sludge acidification with concentrated HCl was also evaluated. The pre-treatment methods did not increase significantly the amount of extracted lipid as well as biodiesel yield. Previous sludge acidification showed lower yield of lipids from primary, secondary and blended sludge. However, the amount of saponifiable lipids was higher, giving the overall

biodiesel yield almost unchanged. Among the four sludges tested, primary sludge achieved the greatest lipid and biodiesel yields, 27% and 19% respectively, on the basis of dry sludge. The highest biodiesel yields obtained from blended, secondary and stabilised sludge amounted to 15%, 4% and 2% respectively, on the basis of dry sludge. No significant influence of the pre-treatments and acidification on the fatty acid composition was found. At least 8 fatty acids were determined, with a predominance of palmitic (C16:0), stearic (C18:0) and oleic acid (C18:1). The comparison of sludge fatty acids profile with common biodiesel feedstocks showed suitability of WWTP sludge for production of biodiesel.

Key-words

WWTP municipal sludge; biodiesel; lipid extraction; sludge acidification; ultrasonication; mechanical disintegration.

1. INTRODUCTION

Biodiesel is one of the most promising renewable fuels as it is biodegradable, less toxic than fossil diesel, compatible with current commercial diesel engine and refuelling technology, and it has low emission profile. Additionally, it has excellent lubricating properties and it could provide energy density similar to diesel [1-4]. Biodiesel is generally produced by transesterification of vegetable oils or animal fats, yielding fatty acids methyl esters (FAMEs) from the lipid fraction. The production of biodiesel in the EU increased from 3.6 (2005) to 10.7 billion litres in 2010 [5]. However, nowadays the competitive potential of biodiesel is limited due to high cost of common lipid feedstocks (soybean, canola, rapeseed, sunflower, palm, and coconut oils), which constitutes 70-85% of the overall biodiesel production cost, strongly influencing the final price of this biofuel [2, 3, 6, 7]. In fact, the production of biodiesel decreased by 10% in 2011 as compared to 2010 [5]. In addition, lack of agricultural lands for growing biodiesel feedstocks limits biodiesel expansion and has contributed to the

increase of food prices over the past few years, raising the concerns of food shortage versus fuel crisis [3]. Thus, there is an urgent need to find an alternative, cheaper feedstock, non-edible, readily available and in large quantities.

In contrast, municipal sewage sludge that is gaining more attention nowadays in biodiesel production can meet the requirements of lipid feedstock [3, 4, 8]. Sewage sludge is a waste, formed during treatment of wastewater in wastewater treatment plants (WWTPs) that needs specific treatment before disposal and represents a major cost in WWTP operation. In addition, WWTPs annually produce higher amounts of sludge due to the expansion of urbanised and industrialised areas. Each year, higher quantities of sludge are produced and the number is estimated to increase from 10 million tons (2005) to 13 million tons in 2020 in the whole of EU [9]. Additionally, dry sludge could comprise up to 30 wt% of lipids [10, 11, 12], which could be converted into FAMEs. Recent studies have indicated that lipid contained in sewage sludge could be potential feedstock for biodiesel [1, 6, 7, 11, 13]. Nevertheless, production of biodiesel from sludge poses great challenges for fast commercialisation. The optimisation of lipid extraction is a major challenge that may affect the economy of the process [7].

It has been demonstrated that ultrasonic pre-treatment [14, 15] and acid hydrolysis [16] are able to increase the lipid extraction yield from biologic samples but their utilisation to improve lipid extraction from sludge has not been reported. These pre-treatments are able to release the lipids from other macromolecules which are not available to solvent in bonded form. Therefore, the utilisation of the sludge pre-treatments could also improve the efficiency of extraction. The most common methods of sludge pre-treatment are ultrasonication and mechanical disintegration, commonly used to enhance biogas production [17, 18, 19, 20]. Ultrasonic energy is able to disintegrate sludge flocs and disrupt large organic particles, breaking down bacterial cell wall and releasing intracellular substances and extracellular polymeric substances into aqueous phase [14, 15, 17, 20]. Mechanical disintegration is used to

reduce size of the sludge particles, disintegrate cells and release organic components into sludge [18, 19]. On the other hand, acid hydrolysis of sludge is another pre-treatment method used to increase the solubility of the organic matter contained within sludge and thus to reduce the amount of sludge and improve its dewaterability [21]. As sewage sludge is a processed sample, in which lipid can be bonded to proteins, carbohydrates and/or minerals, the proposed pre-treatment methods could facilitate the extraction of lipids by sludge disintegration.

The purpose of this study was to investigate the influence of sludge type (primary, secondary, blended and stabilised) and sludge pre-treatments (ultrasonic and mechanical), combined with or without sludge acidification, on the yield of lipid extracted as well as biodiesel (FAMEs) produced. Finally, the composition of FAMEs was determined and compared with common biodiesel feedstocks.

2. MATERIALS AND METHODS

2.1. Chemicals

Lipid extraction experiments were conducted using hexane of laboratory reagent grade (ref: 208752) and magnesium sulphate monohydrate (ref: 434183) purchased from Sigma-Aldrich. Fuming hydrochloric acid (ref: 84418) used for sludge acidification was purchased from Fluka. Transesterification experiments were carried out using hexane (ref: 34859), anhydrous methanol (ref: 322415) and sulphuric acid (ref: 33974) from Sigma-Aldrich at the highest purity available. Sodium chloride (ref: 71379), sodium bicarbonate (ref: S6297) and anhydrous sodium sulphate (ref: 239313) were provided by Sigma-Aldrich. Standard used for identification and quantification of fatty acid methyl ester (FAMEs) was supplied by Supelco (37 component FAMEs mix, ref: 47885-U). Analytical standards of free fatty acids (FFA) were provided by Sigma-Aldrich (C12 ref: L556, C14 ref: 70082, C15 ref: 96125, C16 ref: P0500, C16:1 ref: P9417, C18 ref: S4751, C18:1 ref: O1008, and C18:2 ref: L1376. High

Performance Liquid Chromatography (HPLC) grade toluene (ref: 650579) used for preparation of FFAs solution was also provided by Sigma-Aldrich.

2.2. Sludge collection, handling and characterisation

Primary, secondary, blended and stabilised sludge were collected from the municipal WWTP in Reus (Tarragona, Spain) with a capacity to process near 25,000 m^3 of wastewater per day. Primary sludge was collected after partial gravity thickening. Secondary sludge, produced by an activated sludge process, was collected after partial thickening by flotation. Blended sludge was collected after the combination of primary and secondary at a ratio of 65:35, v/v in the feed of the anaerobic reactor. Stabilised sludge, produced by an anaerobic digestion was sampled after belt filter press dewatering. Sludge samples were taken every 2-3 weeks and the sampling was done four times. The samples were immediately delivered to the laboratory and stored at 4ºC prior to use (maximum storage time 7 days).

Each sample of received sludge was characterised in order to determine total solids (TS) and volatile solids (VS) content, both according to standard method 2540G [22]. Chemical oxygen demand (COD) was measured in a UV-spectrophotometer (DINKO UV-VIS 800 spectrophotometer) according to standard method 5220D [22]. The Sludge characteristics are given in Table 1. As the sludge composition varies during the wastewater treatment and depends on the specific treatment applied, therefore the stabilized sludge gave the largest content of TS and VS due to the water elimination by filter press system, and the primary sludge gave higher quantity of TS, VS and COD than blended and secondary.

2.3. Pre-treatment of sludge samples

Before the extraction, primary, secondary and blended sludge were pre-treated using ultrasonic and mechanical disintegration methods. Due to its solid appearance, anaerobically stabilized sludge was used as received without previous disintegration.

The ultrasonic disintegration experiments were carried out using the procedure previously described elsewhere [23]. The mechanical disintegration experiments were carried out using a mechanical homogenizer (Taurus, Turbo-rotation system) at 600 W of the input of energy at room temperature. 200 ml of sludge was used for each test of each disintegration method. In order to optimise the disintegration time, the blended sludge was disintegrated by both methods for 5, 10, 15 and 20 min.

After sludge disintegration, one part of disintegrated samples and one part of untreated samples were acidified till pH 2 at ambient temperature. That pH was attained by the addition of approximately 0.3 mL of concentrated HCl to the sample of 20 mL of sludge, which afterwards was used directly in the lipid extraction experiment.

To evaluate the effect of sludge disintegration, the blended sludge after each pre-treatment was characterized by Scanning Electron Microscopy (SEM (Jeol JSM-6400)) to observe the appearance of the floc size. For this purpose, a drop of each sludge sample was deposited on the support, dried at room temperature and then coated under vacuum with a gold layer before examination.

2.4. Extraction of lipids

In order to compare the influence of pre-treatment methods, the extraction experiments were carried out using untreated, ultrasonically disintegrated and mechanically disintegrated primary, blended and secondary sludge with and without acidification. The stabilized sludge was subjected to lipid extraction with and without sludge acidification. According to standard procedure the lipid was extracted from acidified untreated sludge 5520E [22], and the utilisation of acidified untreated sludge in the extraction was used as a reference method. Before the extraction, the samples were dried by adding magnesium sulphate monohydrate according to standard method 5520E [22]. The mixture was stored in a desiccator at room temperature overnight. The lipid extraction procedure was carried out in a Soxhlet apparatus

using hexane as a solvent, according to standard method 5520E [22]. After the lipid extraction, the hexane was removed from the flask using a rotary evaporator. The flask, containing the lipids, was stored in a desiccator overnight and weighed the next day. The yield of extracted material was determined gravimetrically and expressed as weight of lipid extracted per weight of dry sludge. After the quantification, the lipids were dissolved in hexane, and kept frozen at -20ºC until further analysis.

2.5. Lipid and biodiesel analysis

The amount of FFA in extracted lipid was determined using an Agilent gas chromatograph 6890GC with a flame-ionization detector (GC-FID). Separation was achieved in an Agilent HP-INNOWax column (19091N-133) using helium as a carrier gas. The injection volume of a sample was 1.5 mL with a split ratio 100:1. The oven temperature programme began at 60 ºC, holding for 2 min and increased by 10 ºC/min to 200 ºC, and then increased by 10 ºC/min to 240 ºC, holding for 12 min. The detector and injector temperature were set at 250ºC for the duration of the analysis.

The lipids were converted into FAMEs (biodiesel) through acid catalysis transesterification/esterification using a modified version of Christi's method [10] and the FAMEs were analysed by GC-FID and GC-MS as described elsewhere [10]. The results of GC-FID were used to estimate the amount of saponifiable (trans/esterifiable) material in the lipid fraction and hence the maximum mass of biodiesel (FAMEs) that could yield. The compounds which could not be identified by GC-FID are presented as others. The other compounds identified by GC-MS are described in Section 3.1.3.

3. RESULTS AND DISCUSSION

3.1. Influence of sludge type

3.1.1. Lipid extraction yield

The lipid yield extracted from the four type of sludge tested is illustrated in Table 2. The values represent the average of at least three different samples collected in WWTP during several months. Irrespective of the sludge pre-treatment and acidification, the primary sludge achieved the greatest lipid yield (27%) followed by blended (21%), secondary (9%) and stabilised (9%). This fact was predictable because the composition of primary sludge consists essentially of organic matter originated from raw wastewater, which is a combination of floating grease and solids (the highest lipid fraction). On the other hand, secondary sludge is composed mainly of microbial cells and suspended solids produced during the aerobic biological treatment of the primary treated wastewater; the lipid fraction comes mainly from extracellular polymeric substances and cell membrane of microorganisms. As blended sludge is a mixture of primary and secondary, with a higher fraction of the first one, the result is slightly lower than of the primary. Finally, in the case of stabilised sludge, it comes from anaerobic digestion process of blended sludge, during which the organic matter is degraded into intermediary products then converted into methane. However, stabilised sludge gave the same yield of lipid as secondary sludge. This is due to possible co-extraction of non-lipid fraction which contributes to the increase in gravimetric yield (see Section 3.1.2.).

3.1.2. Biodiesel (FAME) yield

Total lipid content extracted from sludge is not the real one that could be converted to FAMEs-biodiesel. Lipid fraction extracted from sludge using non-polar solvent can consist not only of acyglycerols, free fatty acids and some waxes (saponifiable lipids), but also of hydrocarbons, pigments, trepans, linear alkyl benzenes, polycyclic aromatic hydrocarbons, sterols and other waxes [8, 24]. Only acyglycerols and free fatty acids that represent the saponifiable part of lipids are suitable for biodiesel (transesterifiable/esterifiable to FAMEs). Hydrocarbons, other non-polar substances (non-lipids) that could be co-extracted with hexane, and also part of lipids like some waxes and sterols are considered as non-saponifiable lipids.

Non-saponifiable lipids are not convertible into biodiesel and represent lipid contaminants. Therefore, the yields of saponifiable lipids and overall biodiesel yields produced from the four sludge types were analysed and the results are presented in Table 3. Irrespectively of sludge pre-treatments and acidification the primary sludge achieved the greatest saponifiable yield (69%) followed by blended (56%), secondary (41%) and stabilised sludge (14%). Thus, the overall biodiesel yields were 18%, 11%, 4% and 1% for primary, secondary, blended and stabilized untreated sludge, respectively. Comparison between stabilised and secondary sludge indicates that although the amount of lipids extracted from both was the same (Table 2), the secondary gave a higher overall biodiesel yield owing to a much larger amount of saponifiable matter in the lipid extracted. This shows that an extraction from stabilised sludge produce lipids heavily contaminated with non-saponifiable material, causing a lower productivity of biodiesel. Among the sludge tested, primary sludge has the higher quantity of extractable lipids and additionally it has also the best quality of lipids that are able to form FAMEs. For these reasons, primary sludge can be considered a better feedstock for biodiesel production than other type of sludge generated in WWTPs. On the other hand, the elimination of lipids from primary sludge can significantly reduce the amount of lipids in blended sludge which is the feed of the anaerobic digester. It is known that significant amount of lipids can negatively affect anaerobic digestion process [25]. Therefore, the extraction of lipids from primary sludge to produce biodiesel will additionally improve the performance of the anaerobic digester and the production of biogas.

3.1.3. Fatty acids composition

Fatty acid compositions of biodiesel produced from sludge are presented in Tables 4. All types of sludge have a significant amount of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). These results are confirmed by the comparison with the

composition of human faecal fatty acids, dominated by C16:0, C18:0 and C18:1 and by kitchen wastes, dominated by C16:0, C18:0, C18:1 and C18:2 [24].

The significant difference was found in secondary sludge as compared to primary and blended. Secondary sludge which comes from biological process in the presence of microorganisms contains high amounts of C16:1 (11.6%) but not of C15:0 (0.3%) which both are considered to be bacterial [24]. In contrast, the amount of C16:0 is lower (21.2%) than the amount in primary (39.0%) and blended (40.7%) sludge. Additionally, the amount of "others" is the highest (16.9%). On the other hand, primary sludge had lower amount of "others", identified by GC-MS, which mainly consist of methyl 10-hydroxyhexadecanoate, methyl 13-methyltetradecanoate, methyl 12-methyl-tetradecanoate, methyl 15-methylhexadecanoate, methyl-14methylhexadecanoate benzenoacetic acid methyl ester and benzenopropanoic acid methyl ester; or other substances like 1-decene, 1-tetradecene and cyclotetradecane. The composition of blended sludge is very similar to primary sludge, the highest difference was found in the amount of oleic acid (C18:1) and "others". Finally, in the case of stabilised sludge, the first acid detected was palmitic acid (C16:0).

3.1.4. Comparison of sludge oil with other biodiesel feedstock

As primary sludge achieved the best yield of lipid and the highest overall yield of biodiesel, the fatty acid composition of primary sludge was compared to common biodiesel feedstocks as shown in Figure 1 [26, 27]. It can be observed that the most of fatty acids found in the sludge are the same as compared to other feedstocks; palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). The important difference is observed in the lower amount of C18:2 and the absence of C18:3 in sludge fatty acids profile as compared to the profiles of soybean and rapeseed. This fact is an advantage because these polyunsaturated fatty acids can undergo reactions such as auto-oxidation due to the bis-allylic position of the carbon double bonds, provoking a destabilisation of biodiesel [28, 29]. The

polyunsaturated fatty acids in the biodiesel from primary sludge constitute only 7%, as compared to 61%, 31% and 30% in the biodiesel from soybean, rapeseed and waste cooking oil, respectively. This fact is an interesting benefit because feedstock rich in saturated or monounsaturated fatty acids gives biodiesel higher oxidation stability [26, 29].

On the contrary, the level of saturated fatty acids found in the sludge (65%) is much higher than the saturation level of other biodiesel feedstocks, and it may present a problem for the cold flow properties of biodiesel. The significant amounts of saturated fatty compounds, increasing the temperature at which a liquid biodiesel, when cooled, becomes cloudy due to formation of crystals and solidification of saturates. However, the cold flow problem can be overcome by using branched chain alcohols instead of methanol in the reaction of transesterification [26, 28] and/or by the presence of branched-chain and hydroxy fatty acid monoalkyl esters [10]. Actually, branched-chain and hydroxy fatty acid, identified by GC–MS, exist in sludge lipids and were included as "others" in Tables 4 (see Section 3.1.3.). Thereby, the cold flow properties of biodiesel produced from primary sludge could be even better because of the presence of other fatty acid methyl ester.

3.2. Influence of the acidification

Table 2 also shows the results of lipid yields from the four sludge types with and without acidification. Irrespective of sludge pre-treatments, the acidification gave slightly lower yield of lipid extracted from primary, secondary and blended sludge as compared to the sludge without acidification. Although in this study, the sludge was acidified at ambient temperature, just by adjusting pH until 2, the sludge hydrolysis accrued giving more dissolved DQO after sludge acidification (increase from 3 g/L to 3.8 g/l, blended sludge). After sludge acidification the hydrolysis could release lipids from proteins and/or carbohydrates giving more esterifiable (saponifiable) lipids (Table 3), and leaving the polar compounds unextracted. Furthermore, the phospholipids, triglycerides, wax esters and sterol esters found in the sludge may have

been also hydrolyzed into FFAs [30] leaving the polar fraction (glycerol, alcohol and phosphate group) unextracted, resulting in a decrease in gravimetric yield. On the other hand, the lipid yield of stabilised sludge increased after acidification. It could be due to release the lipid bonded to the mineralised matter, which contains traces amounts of macromolecular compounds, causing a slight higher yield.

The results of FFA analysis confirmed that sludge acidification increases the amount of FFA in the lipid extracted from all sludge types. The lipid extracted from untreated primary, blended, secondary and stabilised sludge contained 48.2%, 42.8%, 23.4% and 8.9% of FFA (on the basis of lipid), respectively. After sludge acidification the amount of FFA in extracted lipids increased to 60.3%, 50.1%, 27.2% and 9.9% for primary, blended, secondary and stabilised sludge respectively. The larger increase of FFA in primary and blended sludge as compared to secondary and stabilised is related to the conversion of insoluble soaps, present in primary sludge, into FFAs upon exposure to an acidic environment [10]. As a result of higher amount of FFAs in the lipid extracted after sludge acidification, the amount of saponifiable lipids also increased, and the higher increase is observed for primary and blended sludge (Table 3). These results show that an extraction from acidified sludge produces lipids less contaminated with non-saponifiable material. However, the overall biodiesel yield increased only slightly in the case of primary, blended and stabilised sludge. Secondary sludge gave lower overall biodiesel yield after sludge acidification owing to a lower amount of lipid extracted.

As shown in Tables 4a, 4b and 4d, irrespectively of sludge pre-treatments, the effect of sludge acidification of primary, secondary and stabilized sludge in general shows increase in the amount of palmitic acid (C16:0) and decrease in the amount of oleic acid (C18:1). In the case of blended sludge the opposite situation occurs. However, the difference is not essential (Table 4c).

3.3. Influence of the pre-treatments

3.3.1. Optimisation of pre-treatment time on the lipid extraction yield

In order to optimise the duration of pre-treatments, untreated blended sludge was disintegrated by ultrasonic and mechanical method for 5, 10, 15 and 20 minutes. For both pre-treatment methods, the maximum lipid yield was achieved after 10 minutes of sludge pre-treatments, giving 23% and 22% of lipid (based on dry sludge) for ultrasonic and mechanical pre-treatment, respectively. A further increase in the pre-treatment time until 15 and 20 minutes indicates a decrease in the extraction efficiency to a value close to untreated sample, 21% (based on dry sludge). After the first 10 minutes of the pre-treatment, the recovery of lipid was enhanced by the disruption or disintegration of flocs and/or the lysis of the bacterial cells, thus improving the availability of the organic matter to the solvent. However, maintaining the sludge disintegration more than 10 min could increase the size of particle gradually, due to re-flocculation of the particles by the appearance of new linkages of the organic matter from intracellular and extracellular substances which initially were released [17]. According to the results, 10 min of pre-treatment for both, ultrasonic and mechanical disintegration was chosen as the pre-treatment duration for this study.

3.3.2. Evaluation of the pre-treatments

The lipid, saponifiable and biodiesel yields obtained from primary, secondary and blended sludges by difrent pre-treatment methods are presented in Table 2 and 4. Surprisingly, the sludge pre-treatments were not able to enhance substantially neither the yield of lipids nor saponifiable and biodiesel yields, irrespective of sludge acidification. The ultrasonic and mechanical pre-treatment methods were chosen because of their capacity to disintegrate sludge, what may lead to better homogenization of the sample and better penetration of the solvent into the sample [15]. Furthermore, the methods are able to disintegrate the sludge flocs, break down bacterial cell wall and release the lipid present in extracellular polymeric

substances of bacterial sludge flocs [17]. As secondary sludge comes from aerobic biological process, it is composed mainly of microbial cells and suspended solids, the slight improvement in the extraction end transesterification yields after sludge pre-treatment is observed in some cases of secondary and blended sludge. However, the differences are not significant (Table 2 and 4). Comparing both pre-treatment methods, in general ultrasonic treatment gave better results of lipid, saponifiable and biodiesel yields than mechanical. Nevertheless, again the differences are not significant.

In order to observe the effect of sludge disintegration the photos of the microscopic appearance of the blended sludge floc were taken and are illustrated in Figure 2. There is only a slight difference on the microscopic appearance of the sludge floc between acidified (Figure 2b) and untreated sludge (Figure 2a). The acidification had almost no effects on the floc size; although the floc texture seems to be a little bit looser, the structure and the size of floc is basically the same as the original sludge. Bigger differences can be observed between untreated and treated sludge. As it can be seen in Figure 2a, the untreated sludge has big and incoherent floc structure, while after pre-treatments, Figure 2c and 2d, the appearance of floc structure is compacted and smaller in size due to the disintegration process. The same differences were reported elsewhere [31]. Comparing both pre-treatments, in the case of ultrasonication the floc texture is more separated than the structure of mechanically pre-treated sludge, due to a better disintegration of sludge floc and decrease in particle size by ultrasonic treatment.

As shown in Tables 4, there is no significant influence of the pre-treatments on the fatty acid profiles of primary (Table 4a), secondary (Table 4b) and blended (Table 4c) sludge.

3.4. Statistical evaluation

In order to identify statistically significant differences in the results of lipid, saponifiable and biodiesel yields between different pre-treatment methods and sludge acidification a two-way

363 analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$) was performed on
364 primary, blended, and secondary sludge. The results of the test revealed no significant
365 interaction effect ($p >>> 0.05$) of the two factors investigated on the lipid, saponifiable and
366 biodiesel yields for primary, blended and secondary sludge. Furthermore, the test for the three
367 types of sludge (primary, blended and secondary) indicated no significant differences ($p >>>$
368 0.05) on the lipid, saponifiable and biodiesel yields between three pre-treatments tested
369 (untreated, ultrasonic, mechanical). However, the results of the test indicated significant
370 difference on the lipid yield between acidified and non-acidified secondary sludge ($p =$
371 0.0039). Primary ($p = 0.1164$) and blended ($p = 0.1595$) sludge did not show the significant
372 influence of acidification on the lipid yield. On the contrary, the saponifiable yield was found
373 to be significantly affected by the acidification of primary ($p = 0.0397$) and blended ($p =$
374 0.0049) sludge while secondary sludge did not show significant differences ($p = 0.4451$).
375 Finally, the results of the test indicated significant difference on the biodiesel yield between
376 acidified and not acidified secondary ($p = 0.0066$) and blended ($p = 0.0315$) sludge while
377 primary sludge did not show significant differences ($p = 0.1443$).
378 On the other hand, to identify statistically significant differences between the data with and
379 without acidification obtained from stabilised sludge, a *t-test* at the 0.05 significance level was
380 performed. The results of the test showed that there is no significant differences between
381 acidified and non-acidified sludge on the lipid ($p = 0.0992$), saponifiable ($p = 0.3333$) and
382 biodiesel ($p = 0.0917$) yields.
383

384 **3.5. Comparison with other processes of production of biofuels**

385 In the best scenario of this study, the production of biodiesel from sewage sludge can reach a
386 maximum of 19% based on dry matter. The best alternatives to compete with biodiesel
387 production to make fuels from the volatile matter of dry sludge are direct combustion,
388 gasification, pyrolysis and liquefaction. These processes are able to produce the thermal

decomposition of the organic matter and to transform this organic matter into bio-fuels [32]. Among this processes, liquefaction is gaining more attention due to better condition of low temperature and pressure [33].

The treatment of dried sewage sludge by liquefaction in water at 340ºC, catalyzed by sodium carbonate was able to produce heavy oils with a conversion of 42% based on dry matter [34]. Moreover, the same results were obtained in a continuous pilot plant treating 5 tons/day of dewatered sludge [35]. Recently, sewage sludge was processed by deoxy-liquefaction in supercritical ethanol producing up to 55% of bio-oil at 400ºC in methanol [32] and until 46% of conversion to bio-oil at 380ºC and 7.5 MPa in acetone [33]. Since bio-oil and biodiesel are biofuels with different characteristics, only the heating value of both of them can be compared to have an idea of the energy that can be recovered. The heating value of bio-oils was in the range of 35 to 39 MJ/kg [32, 33], comparable to 39.5 MJ/kg from biodiesel [8]. The composition of the bio-oils depends on the solvent used during process, but more than a hundred of substances are usually identified. For instance, when acetone is used as solvent in hydrothermal liquefaction, the bio-oils are essentially composed by ketones. On the other side, when methanol or ethanol is used, some esters are obtained [33], until 25% of the bio-oil, that are also found in biodiesel. Finally, regarding to biodiesel production cost from sludge, different studies estimated cost of 0.83-0.85 $/L [6, 11], although the yields of conversion to biodiesel were 7 and 10% based on dry sludge, lower than the 19% obtained in this study.

4. CONCLUSIONS

The pre-treatment methods tested in this study are not able to increase significantly the amount of extracted lipid as well as biodiesel yield. Thus, the pre-treatments are not suitable for biodiesel production from municipal sewage sludge. The sludge acidification showed lower extraction efficiency as compared to non-acidified sludge, while the amount of saponifiable lipid was higher, and giving the overall biodiesel yield almost unchanged.

Gas chromatography analysis of the FAMEs indicated a similarity between the fatty acid compositions of the four sludge evaluated. All types of sludge have a significant amount of palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1), which are essential for the production of biodiesel.

The results have shown that all types of municipal sludge produced during wastewater treatment are a potential source of suitable lipid for the production of biodiesel. Among the four sludge tested, the primary sludge achieved the greatest lipid (27% based on dry sludge) and biodiesel (19% based on dry sludge) yield. Thus, primary sludge is the most beneficial lipid feedstock for biodiesel production. Furthermore, it is possible to take advantage of the excess sludge, reusing it as a source of lipid for the production of biodiesel and, consequently, lowering the WWTP operation cost.

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Figure captions

Figure 1. Comparison of sludge's fatty acids with other biodiesel feedstocks (Sludge: this work; Soybean and Rapeseed: Canacki et al. (2008) [26]; Mixed fat, Waste cooking oil and Crude palm oil: Melero et al. (2012) [27])

Figure 2. Microscopic appearance of the blended sludge floc: a) untreated sludge, b) acidified sludge, c) after 10 min of ultrasonic treatment (50W), d) after 10 min of mechanical treatment (600W).

Table 1. Characteristics of four types of sludge used in the present study.

	Sludge [a]			
	Primary	Secondary	Blended [b]	Stabilised
Total solids (TS), %	4.2 ± 1.2	3.2 ± 0.7	3.1 ± 0.7	25.3 ± 4.4
Volatile solids (VS), %	3.3 ± 0.9	2.8 ± 0.5	2.5 ± 0.6	15.6 ± 2.8
Chemical oxygen demand (COD), g/L	64.1 ± 11.1	44.1 ± 7.5	46.0 ± 4.9	n.m.

[a] Each value is the average of at least 3 samples collected on different days.

[b] Primary and secondary at a ratio of 65:35, v/v.

n.m.: not measured

Table 2. Amount of lipid fraction extracted from different types of sludge by different pre-treatment methods (Lipid yield (%) on the basis of dry sludge).

		Sludge [a]			
Acidification	Pre-treatment	Primary	Secondary	Blended [b]	Stabilised
Not acidified	Untreated	27 ± 1	9 ± 1	21 ± 1	9 ± 1
	Ultrasonic	27 ± 1	10 ± 1	23 ± 3	-
	Mechanical	26 ± 1	9 ± 1	22 ± 3	-
Acidified	Untreated	25 ± 1	7 ± 1	20 ± 1	10 ± 2
	Ultrasonic	25 ± 1	8 ± 1	22 ± 2	-
	Mechanical	25 ± 1	8 ± 1	20 ± 2	-

[a] Each value is the average of at least 3 samples collected on different days.

[b] Primary and secondary at a ratio of 65:35, v/v.

Table 3. Transesterification yield obtained from different types of sludge by different pre-treatment methods (Saponifiable yield (%) on the basis of lipid, Biodiesel yield (%) on the basis of dry sludge).

Acidification	Pre-treatment	Sludge [a]							
		Primary		Secondary		Blended [b]		Stabilised	
		Saponifiable	Biodiesel	Saponifiable	Biodiesel	Saponifiable	Biodiesel	Saponifiable	Biodiesel
Not acidified	Untreated	69 ± 7	18 ± 1	41 ± 6	4 ± 1	56 ± 8	11 ± 1	14 ± 1	1 ± 1
	Ultrasonic	70 ± 5	19 ± 2	42 ± 5	4 ± 1	53 ± 6	12 ± 1	-	-
	Mechanical	65 ± 10	17 ± 2	42 ± 5	4 ± 1	60 ± 5	13 ± 1	-	-
Acidified	Untreated	76 ± 3	19 ± 1	42 ± 4	3 ± 1	64 ± 8	13 ± 2	15 ± 1	2 ± 1
	Ultrasonic	75 ± 4	19 ± 1	45 ± 4	4 ± 1	68 ± 6	15 ± 3	-	-
	Mechanical	74 ± 6	19 ± 2	43 ± 2	3 ± 1	67 ± 6	14 ± 2	-	-

[a] Each value is the average of at least 3 samples collected on different days.

[b] Primary and secondary at a ratio of 65:35, v/v.

Table 4a. Fatty acids composition of primary sludge for each pre-treatment (%, w/w).

	Pre-treatment					
Fatty acid	Untreated	Ultrasonic	Mechanical	Untreated acidified	Ultrasonic acidified	Mechanical acidified
C12:0	0.3	0.3	0.3	0.3	0.3	0.2
C14:0	3.0	3.1	3.0	3.0	2.8	2.8
C15:0	0.2	0.2	0.2	0.1	0.1	0.1
C16:0	39.0	39.7	38.8	43.3	47.0	44.6
C16:1	1.1	0.4	0.4	0.3	0.2	0.2
C:18:0	14.1	14.1	13.8	15.7	17.4	16.4
C18:1	29.9	30.3	30.7	25.9	21.3	24.7
C18:2	7.2	7.4	7.8	6.0	4.8	5.7
Others	5.2	4.5	5.0	5.4	6.1	5.3

Table 4b. Fatty acids composition of secondary sludge for each pre-treatment (%, w/w).

	Pre-treatment					
Fatty acid	Untreated	Ultrasonic	Mechanical	Untreated acidified	Ultrasonic acidified	Mechanical acidified
C12:0	0.1	0.0	0.0	0.2	0.1	0.0
C14:0	2.9	1.9	2.8	3.6	2.7	1.9
C15:0	0.3	0.1	0.3	0.4	0.3	0.2
C16:0	21.2	20.7	20.5	24.7	25.7	22.3
C16:1	11.6	11.3	11.8	10.2	10.7	10.8
C:18:0	9.5	9.9	9.1	10.4	10.1	8.7
C18:1	29.5	30.6	29.0	26.0	26.0	28.8
C18:2	8	8.6	7.3	7.7	6.9	6.5
Others	16.9	16.9	19.2	16.8	17.5	20.8

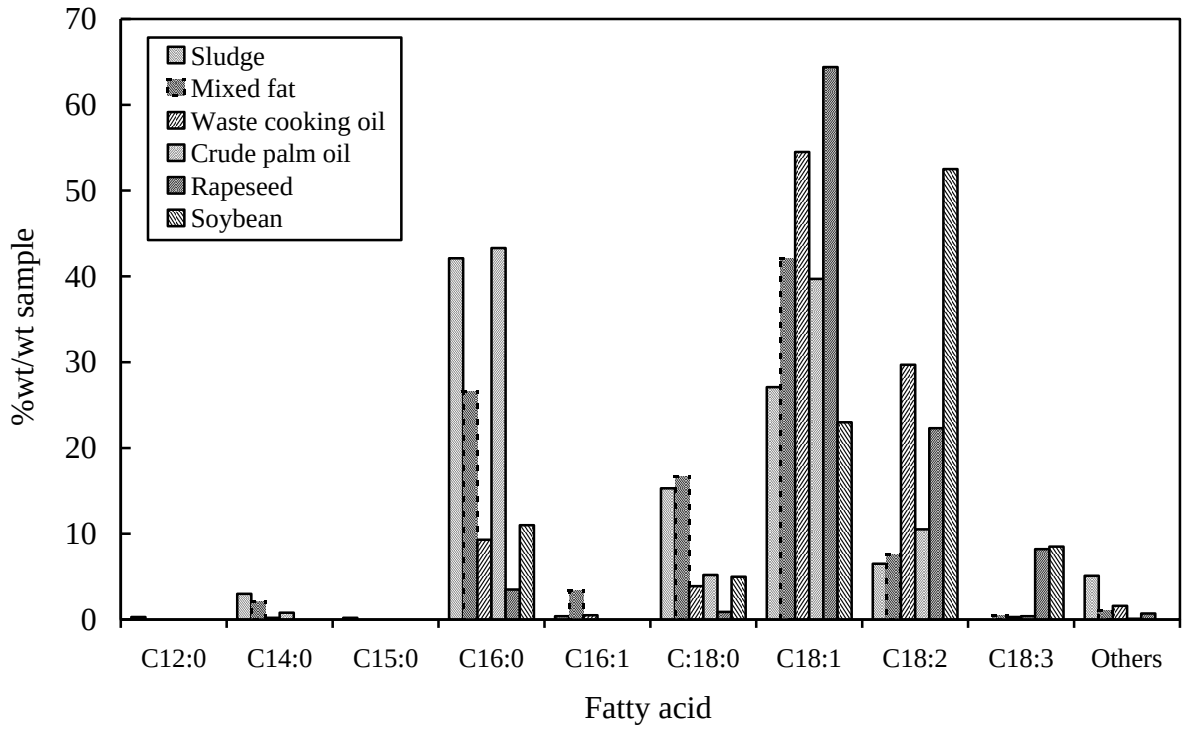
Table 4c. Fatty acids composition of blended sludge for each pre-treatment (%, w/w).

	Pre-treatment					
Fatty acid	Untreated	Ultrasonic	Mechanical	Untreated acidified	Ultrasonic acidified	Mechanical acidified
C12:0	0.3	0.2	0.4	0.3	0.2	0.2
C14:0	3.2	3.3	3.4	2.8	3.0	3.0
C15:0	0.3	0.2	0.3	0.2	0.1	0.1
C16:0	40.7	40.6	41.1	38.7	38.8	37.9
C16:1	2.0	2.1	1.6	1.8	1.5	1.4
C:18:0	13.3	12.8	13.7	14.3	14.5	14.1
C18:1	24.6	26.1	24.4	27.1	26.4	28
C18:2	5.2	4.8	4.6	5.1	5.1	5.3
Others	10.4	9.9	10.5	9.7	10.4	10.0

Table 4d. Fatty acids composition of stabilised sludge (%, w/w).

	Pre-treatment	
Fatty acid	Untreated	Untreated acidified
C12:0	0.0	0.0
C14:0	0.0	0.0
C15:0	0.0	0.0
C16:0	30.8	33.8
C16:1	2.5	2.1
C:18:0	10.3	10.7
C18:1	36.2	35.1
C18:2	9.5	8.8
Others	10.7	9.5

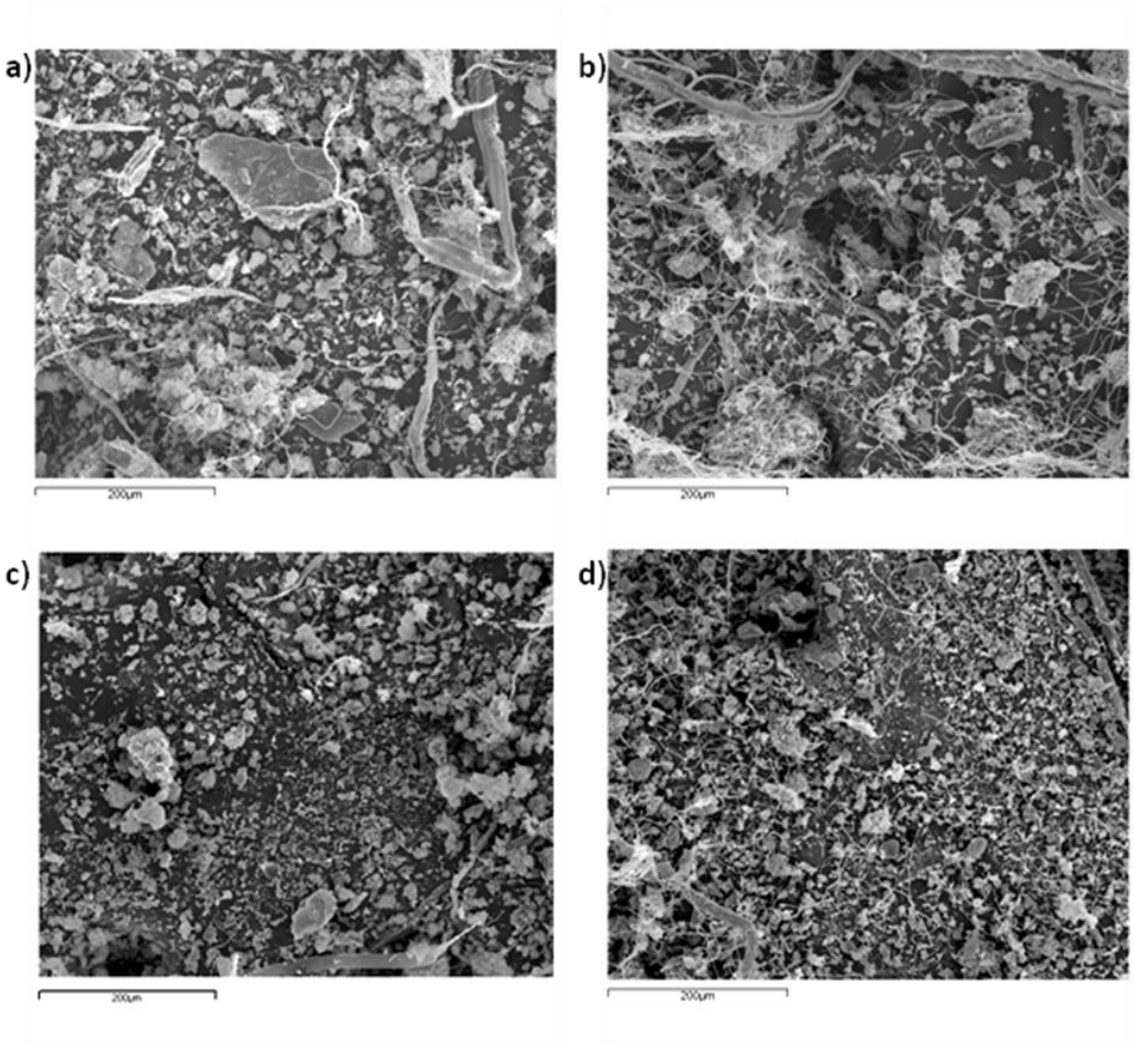
Figure 1


70
60
50
40
30
20
10
0
%wt/wt sample
Sludge
Mixed fat
Waste cooking oil
Crude palm oil
Rapeseed
Soybean
C12:0
C14:0
C15:0
C16:0
C16:1
C:18:0
C18:1
C18:2
C18:3
Others
Fatty acid

1

Figure 2

1



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