Abstract

Secondary sludge from municipal wastewater treatment plant is proposed as a promising alternative lipid feedstock for biodiesel production. A deep study combining different type of raw materials (sludge coming from the oxic, anoxic and anaerobic steps of the biological treatment) with different technologies (liquid-liquid and solid-liquid extractions followed by acid catalysed transesterification and in situ extraction-transesterification procedure) allows a complete comparison of available technologies. Different parameters – contact time, catalyst concentration, pretreatments – were considered, obtaining more than 17 % FAMEs yield after 50 min of sonication with the in situ procedure and 5 % of H₂SO₄. This result corresponds to an increment of more than 65 % respect to the best results reported at typical conditions. Experimental data were used to propose a mathematical model for this process, demonstrating that the mass transfer of lipids from the sludge to the liquid is the limiting step.

Keywords: liquid-liquid extraction; solid-liquid extraction; in situ transesterification; 2nd generation biofuels; sludge sonication
1. Introduction

The rise in oil price, the fossil fuels depletion, and, even more markedly, the environmental and climate problems associated with their combustion, are promoting the development of renewable fuels. Among the different alternatives currently available, biodiesel highlights as one of the most promising ones since it is biodegradable, less toxic than fossil fuels and provides similar energy density than the mineral one, but improving its lubricating properties (Revellame et al., 2010; Xue et al., 2006). In addition, its ignition point is considerable higher than the diesel one, making it easy and safe to manipulate it (Anuar and Abdullah, 2016; Shahid and Jamal, 2011).

Chemically, biodiesel is a mixture of monoalkyl esters of long chain fatty acids, commonly called fatty acid methyl esters (FAME). Industrially, it is currently obtained by transesterification of vegetable oils or animal fats with methanol, obtaining a product known as “first generation” biofuel (Atabani et al., 2012; Shahid and Jamal, 2011). However, the competitive potential of biodiesel is limited due to the high cost of these lipid feedstocks. This fact, as well as ethical issues related to the competition between energy and food industry, have triggered the search for inedible, inexpensive and, if possible, residual raw materials, making up the “second generation” biodiesel (Hajjari et al., 2017). The use of oleaginous microorganisms, those that accumulate lipid droplets in their cells, reaching dry lipid percentages up to 25 % (Koutb and Morsy, 2011), is an attractive alternative. However, the high consumption nutrients and the specific needs of their growth (light, temperature) can discourage its cultivation for this specific aim. On the other hand, the microorganisms used in biological treatments of a wastewater treatment plants (WWTPs) have relevant concentration of triglycerides, and constitute the solid phase of sludge streams usually considered as a waste (Kumar et al., 2016; Mondala et al., 2009).

Due to the urbanisation and industrialisation, quantities of sewage sludge produced increase year on year, being considered as the main waste of these plants. It is forecasted that
approximately 13 million tonnes of sludge will be produced in the European Union in 2020 (Comission, 2010). Its treatment and disposal implies an important cost, in both, economic and environmental terms (Dufreche et al., 2007). Therefore, sewage sludge is an available and cheap feedstock that has attracted attention during the last decade (Dufreche et al., 2007; Kumar et al., 2016; Olkiewicz et al., 2014). Particular characteristics of these sewage sludge (high humidity, heterogeneous and few reproducible composition, etc.), makes difficult its fast commercialisation, being no possible the direct application of conditions previously optimised for the first generation biofuels. Thus, many efforts are nowadays focused on the study and standardisation of this process.

In this context, the optimisation of lipid extraction is a major challenge that determines the economy of the process (Kargbo, 2010). Thus, several researchers have proposed different alternatives, such as the liquid-liquid extraction, the solid-liquid extraction and the in situ transesterification (Dufreche et al., 2007; Kwon et al., 2012; Mondala et al., 2009; Olkiewicz et al., 2014; Pokoo-Aikins et al., 2010; Revellame et al., 2010; Siddiquee and Rohani, 2011; Willson et al., 2010). The two first ones, liquid-liquid and solid-liquid extractions, require the use of organic solvents, without agreement about the optimum ones, although interesting results using toluene, chloroform, hexane, methanol and ethanol are published (Dufreche et al., 2007; Kwon et al., 2012; Pokoo-Aikins et al., 2010; Siddiquee and Rohani, 2011). However, reported results are difficult to compare because many different conditions were tested and, to the best of our knowledge, there is not a systematic study comparing the different available techniques. Consequently, general conclusions are difficult to withdraw, being difficult to predict the behaviour of other sludges.

As to the transesterification, acid catalysis is the most frequently used procedure, mainly using sulphuric acid, obtaining higher biodiesel yields in comparison with results with basic catalyst (Olkiewicz et al., 2016). Despite that classical transesterification of pure oils is industrially carried out using basic materials, when the raw material is a waste, the presence of free fatty
acids in a basic medium promotes the saponification, obtaining a non-desired product that hinders the separation and purification of the biodiesel fraction. Recent studies also propose the enzymatic catalysis or the non-catalytic transesterification, when reaction is done under subcritical conditions (Kwon et al., 2012; Pourzolfaghar et al., 2016).

Analysing all these previous results, one of the main conclusion is that acid transesterification is very efficient, and results are mainly conditioned by the lipid extraction step. As consequence, some authors propose different alternatives to enhance this step, being the sonication one of the most promising pretreatment. Sonication technology is based on the introduction of high intensity sound waves in the sludge, creating bubbles that implode, breaking the cell walls and releasing the intracellular content, including the lipids, into the medium. This technology has been previously used for obtaining biodiesel from algae or biogas from sludge (Ruffino et al., 2015; Tran et al., 2012; Wolski, 2012). However, the few studies applied to this aim are not conclusive enough (Olkiewicz et al., 2015; Olkiewicz et al., 2012)

Taking into account this entire context, biodiesel yields reported in the literature using secondary sludge as raw material vary greatly from one study to another. Therefore, we consider that a systematic comparison of the results obtained applying the three lipid extraction techniques to a specific secondary sewage sludge is of key interest for both understanding the process and being able to propose efficient technologies for this purpose. Once the raw material is the same for all the treatments, and after the transesterification of the obtained lipids, tracking down conclusions would be easy and useful.

The main aim of this work is to present a deep comparison among biodiesel yields obtained by applying the three different techniques – liquid-liquid extraction, solid-liquid extraction, and in situ transesterification – to the same type of secondary sludge. Three different raw materials were used, from oxic, anoxic and anaerobic zone (sampled directly from the corresponding reactor) and results were compared with those obtained from the floating sludge (common pretreatment for these sludges). Industrially, only floating sludge adds up (taking samples
directly from the reactors before being concentrated by decantation or floating is economically and technically unviable). However, the individual study of each sludge fraction allows analysing if the sludge nature has any effect in the final efficiency, suggesting an independent pre-concentration of the most interesting fraction to maximize the biodiesel yield. The effect of catalyst concentration as well as the role of sludge pretreatment by sonication was also analysed.

2. Materials and Methods

2.1. Chemicals

n-Hexane (97%) and sulphuric acid (96%) were purchased from Sigma-Aldrich. Sodium chloride (99.5%) and methanol (≥ 99.8) were purchased from Panreac. A mixture of 37 reference fatty acid methyl esters (FAMEs) was supplied by Supelco (ref. 47885-U), and it was used for identification and quantification purposes (in the GC-MS and GC-FID analyses).

2.2. Sample collection and preparation

Secondary sludge samples were collected from the municipal wastewater treatment plant (WWTP) in Villapérez-Oviedo (Asturias, NW Spain). The block diagram of this plant, which has a capacity to process 8500 L/s, is summarized in Figure 1, indicating the steps where the four different types of secondary sludge (oxic, anoxic, anaerobic and floating ones) are sampled. Considering the global process of this WWTP, primary sludge was discarded because of their low potential capacity (this sludge mainly correspond to solid particles, inorganic chemicals and free fatty acids that can suffer saponification). Samples were taken weekly during one month (4 batches) and stored at 4°C prior to use. The sludge from the oxic, anoxic and anaerobic zones were individually pre-treated following with the aim to reduce the water content and to prepare the samples for the extraction and transesterification. Sludges were settled for 24 h, after which the supernatant was removed.
The resulting sludge was centrifuged at 3000 rpm for 10 min using a Kubota 6500 centrifuge.

Dewatered sludge was dried at 100 °C for 24 h and the desiccated sludge was crushed into a fine powder (with particle size ranging from 150 to 255 μm) in order to prepare a homogeneous suspension for the following steps. These dried sludge samples were used to the solid-liquid extraction and \textit{in situ} transesterification studies. In the case of liquid-liquid extraction, the sludges were only subjected to the settling process. This procedure has been previously reported in the literature, observing a relevant decrease in water content (less than 5 % in the final sample) (Melero et al., 2015; Mondala et al., 2009). In the case of floating sludge, and due to its low water content, the first step (settling) was not performed; they were directly used for the liquid-liquid extraction and centrifuged, dried and crushed before the solid-liquid extraction or \textit{in situ} transesterification.

All the samples were characterised before and after the pretreatment, in order to analyse the morphological changes introduced by the initial pre-processing. Total solid content (TS) was analysed according to the standard method 2540G (Rice et al., 2012). Lipid contents were measured by gas chromatography using a mass spectrometer detector (GC-MS) - in a Shimadzu Q2010 Plus – after total extraction with chloroform, following the typical procedure reported in the literature (Siddiquee and Rohani, 2011). Data reported in the \textit{Results section} corresponds to the average value obtained after two analyses with each sample, without observing any variability among sludge from different batches. All the standard deviations are included in the results, being in all the cases lower than 1 %.

2.3. \textit{Liquid-liquid extraction}

Liquid-liquid technique consists of using an organic phase to extract the lipid phase from the suspended flocules. Among the different solvents proposed in the literature, chloroform and toluene were discarded because of environmental concerns (despite their high extraction capacity). From the non-polar organic solvents, hexane was chosen based on economic and
technical reasons, having a low cost, high immiscibility with water and a high capacity to extract non-polar saponifiable lipids, the base to obtain the biodiesel. This solvent is extensively studied for these treatments (Melero et al., 2015; Olkiewicz et al., 2014; Siddiquee and Rohani, 2011). In addition to hexane, other more polar organic mixture of solvents were also proposed for the lipid extraction of secondary sewage, adding methanol or acetone to the hexane, suggesting that the mixture of them helps to disrupt the lipid membrane of microorganisms (Dufreche et al., 2007; Zhang et al., 2016; Zhu et al., 2012). However, the use of these solvents was discarded because of the considerable decrease in the selectivity towards saponifiable material reported in previous works (Dufreche et al., 2007).

According to the Scheme 2, in the case of liquid-liquid extraction, the sludges were used after the sedimentation step, with the exception of floating sludge, which was used without any previous pre-treatment. Sequential liquid-liquid extraction of lipids was performed in a separatory funnel at ambient temperature using a hexane/sludge ratio of 2:1, according to the best conditions determined by Dufreche and co-workers (Dufreche et al., 2007). Extraction was repeated four times, mixing the organic phases obtained in each step. Hexane was removed using a rotatory evaporator at 70 °C and the samples were dried at 105 °C for one hour and stored in a desiccator. The lipids obtained were weighed in order to determine the extraction yield based on the dried sludge used.

2.4. Solid-liquid extraction

Lipid extraction from dried sludge was performed using a Soxhlet apparatus using hexane as solvent. Ethanol and methanol were also proposed for this aim, but they were discarded because of their high polarity, which could enhance the extraction of non-saponifiable lipids, increasing the purification costs of all the process (Pokoo-Aikins et al., 2010). The lipid extraction was carried out with a hexane/dried sludge ratio 10:1 for four hours and 8-9 extraction cycles per hour, according to the procedure previously reported by Willson and
co-workers (Willson et al., 2010). After extraction, the hexane was removed following the same procedure as in the liquid-liquid extraction, the final oil phase was weight, and the extraction yield estimated based on the dried sludge used:

\[
Lipids \, (\%) = \frac{Oil \, phase \, weight \, (g)}{Dry \, sludge \, weight \, (g)} \cdot 100
\]

2.5. Lipids transesterification and FAMEs analysis

Biodiesel production from extracted lipids was carried out following the modified Christie’s method through acid catalysis (Christie, 2003). 20 mg of lipids were dissolved in 1 mL of hexane and 2 mL of sulphuric acid in methanol (1% v/v). Resulting mixture was heated at 55 °C for 24 h, after which 5 ml of 5% sodium chloride were added and the FAMEs were extracted twice with 5 mL of hexane.

FAME analysis was carried out using a Shimadzu gas chromatography 2010 equipped with a flame ionization detector (GC-FID) and a capillary column CP-Sil 8CB (30 m x 0.25 mm x 0.25µm), using helium as carrier gas. 1 µL of sample was injected at 260 °C, with a split ratio equal to 30. The FID detector was set at 260 °C. The GC oven temperature program was: 100 °C as initial temperature for five minutes, increased to 240 °C at slope of 4 °C/min, and held at this temperature for 30 min. A mixture of 37 reference fatty acid methyl esters (FAMEs) was used for instrument calibration and the qualitative correspondence between standards and experimental samples was corroborated by GC-MS using a Shimadzu QP2010 Plus, using the same column and analysis method than in the GC-FID.

2.6. In situ transesterification

Reaction were carried out based on the procedure proposed by Revellame and co-workers (Revellame et al., 2010). 1.5 g of dried sludge were mixed with 37.5 mL of sulphuric acid in methanol (4% v/v) and the mixture was introduced into sealed glass reactions vials. The mixture was kept at 55 °C in a stirring bath (100 oscillations per minute –opm-) to ensure the
total suspension of the solids during the reaction. After 24 hours at these conditions, and once the sample reach ambient temperature, it was centrifuged at 3000 rpm for 5 min. The supernatant was recovered and the solid residue was re-suspended in 5 mL of methanol and centrifuged at 3000 rpm for 5 min. The volume of both supernatants was reduced to a final volume of around 9 mL using a rotary evaporator. Finally, the FAMEs were extracted four times with 5 mL of hexane, which was removed using a rotatory evaporator at 70 °C. Samples were analysed by GC-FID, using the same procedure explained for the other transesterifications.

3. Results and Discussion

3.1. Sludge characterization

Characterization results are summarized in Table 1. It must be noted that samples from oxic, anoxic and anaerobic areas are taken directly from the reactor, and no after any settling process. In good agreement with the suspended solids concentrations at which these stages are configured, total solids (TS) values are very similar in all the cases, with concentrations around 0.3 %. On the contrary, sludge obtained after the floating treatment is almost nine times more concentrated, reaching the typical value of sludge streams after this kind of treatments (around 3 %). These differences in the TS are less remarkable after pretreatment, reaching values higher than 4 % in all the cases and obtaining the maximum with the floating sludge, despite of the absence of decantation step (5.4 %).

Concerning to the lipids content, results obtained after total extraction using chloroform as solvent, and following the procedure previously explained, indicate that sludge from the floating sludge zone achieved the greatest lipid content (19.4 %), whereas the minimum amount was detected in the analysis of the anaerobic region (16.2 %). In any case, the differences were not very significant, with fluctuations close to 3 %. It must be remarked than total lipid content cannot completely transformed into FAMEs-biodiesel, because it consists...
not only of acyl-glycerols, free fatty acids and some waxes (saponifiable lipids), but also of many other types of sterols, alkyl benzenes, etc. that are not suitable for biodiesel (Jardé et al., 2005; Pastore et al., 2013). This fact introduces the concept of saponifiable lipids. This parameter is indirectly calculated based on the final amounts of FAME produced when the 100 % of conversion is ensured. As different conversions were obtained by each technique, a previous value cannot be estimated.

3.2. Comparison of biodiesel production by liquid-liquid, solid-liquid and in situ techniques

Preliminary analyses were carried out in order to identify the most effective technique to maximize the biodiesel yield from each fraction of sludge. As it was mentioned in the introduction section, there is not good agreement about the optimum procedure, suggesting that the kind of sludge plays a key role in the final yields.

In a first approach, the liquid-liquid lipid extraction was considered, using hexane as organic solvent. Considering that liquid-liquid technique is a two-step process, results from the extraction and after the transesterification can be reported separately. Thus, preliminary results obtained after four consecutive cycles of one hour of extraction at room temperature are summarized in Table 2. As it is observed, very low lipid percentages were obtained, conditioning the results of the subsequent transesterification step. In good agreement, only a small amount of FAMEs was detected when floating sludge was used as raw material (0.86 % of FAMEs yield). Despite that previous results in the literature are not conclusive, these values are far from the optimum ones reported in previous papers. One of the highest value using same procedure is reported by Olkiewicz and co-workers, reaching almost 27 % of lipids (Olkiewicz et al., 2014). However, it must be taken into account that these authors consider primary sludge as raw material. The higher amount of free lipids in this kind of sewage makes easier the lipid extraction, whereas the extraction of lipids from a cell membrane is more difficult. In good agreement, results reported by Dufreche and co-workers suggest, for similar
type of sludge, a maximum extraction using hexane of 1.94 % (Dufreche et al., 2007). This result is obtained working with a solvent/solid ratio of 40:1 at 100 °C, considerable more severe conditions in comparison with the 2:1 ratio and room temperature used for this work. Solid-liquid extraction was also studied, considering extraction for four hours in hexane once the sludge was previously dried. These preliminary results are also reported in Table 2, indicating the amount of lipids obtained with each kind of sewage and the percentage of FAMEs after the transesterification step. A clear improvement is observed in all the cases, highlighting the values obtained with the floating sludge (more than one order of magnitude in all the cases). Besides, results obtained with the floating sludge and the solid-liquid extraction method are comparable with those previously reported in the literature by Dufreche and co-workers (Dufreche et al., 2007), with a lipid yield after extraction with hexane around 1.9 %. These results are justified because the mass transfer is improved by this technique by two complementary effects: on the one hand, the huge amount of cycles maximize the contact between solvent and sludge; on the other hand, the high temperature enhances the lipid extraction (Dufreche et al., 2007). Very similar results were obtained for the lipid extraction step when using sewage from oxic and anaerobic areas, (0.9 and 1.0 %, respectively). The higher extraction obtained with the anoxic sewage is explained by the expected higher amount of free fatty acids (FFAs) present in this area, reaching an extraction level comparable with the floating sludge (1.6 and 1.8 %, respectively). However, results obtained after the transesterification step (a relevant high FAME concentration with the anaerobic sample despite of the lower lipid extraction) suggest a relevant difference between lipids obtained from each fraction. Considering that all the values correspond to the same reaction conditions (24 h, 1 mL of methanol, 2 mL of sulphuric acid), the difference lower than 0.7 % in the lipid yield between anaerobic and floating sludge does not justify the relative increase of more than 48 % in the FAMEs yield (15.9 and 23.6 %, with anaerobic and floating sludge, respectively).
For understanding these differences, FAME profile of the biodiesel produced was analysed, obtaining the distribution shown in Figure 2a. In general, the esters of acids with 16 and 18 carbon atoms predominate regardless of the fraction studied, in good agreement with the distribution reported for other authors (Mondala et al., 2009; Olkiewicz et al., 2014; Revellame et al., 2010; Siddiquee and Rohani, 2011). According to the identification, main fractions correspond to palmitic (C16:0), palmitoleic (C16:1), estearic (C18:0) and oleic acids (C18:1), with less than 3 % of other polyunsaturated fatty acids. This fact represents an advantage over the use of vegetable-based raw materials, since these compounds are very vulnerable to autoxidation, decreasing the oxidative stability of biodiesel (Saluja et al., 2016). If the distribution among results obtained with different sewage fractions is compared, the C16/C18 proportion (considering all the acids together) is almost constant with all the fractions (from 1.05 to 1.15) observing a slightly majority of the shortest ones. The exception is the case of the anaerobic zone, with a prevalence of longer FAMEs (C16/C18 ratio of 0.9). On the other hand, if all the esters are analysed as function of their unsaturated/saturated character, similar results were obtained for all the sludge fractions (45 % of unsaturated and 55 % of saturated), except with the sewage from the anaerobic region, with more than 60 % of saturated compounds. These results suggest that saturated lipids are easier to transform into FAMEs, having a more saponifiable character. In fact, considering that no time evolution was observed after 24 hours, complete conversion of all the saponifiable lipids is supposed and the percentage of these lipids can be calculated based on the following expression:

\[
\text{Saponifiable (\%)} = \frac{\text{FAMEs (g)}}{\text{Lipid (g)}} \times 100
\]

Experimental results are congruent with a percentage of saponifiable lipids in the dry sludge close to 0.2 % in the case of anaerobic sludge, 0.1 % for the anoxic ones and 0.05 % for the aerobic sewage; in the same order as the amount of FAMEs obtained. This sequence is in good agreement with previous results reported by Olkiewicz and co-workers (Olkiewicz et al., 2012).
A study of the influence of extraction time and the amount of lipid obtained was carried out trying to optimise the conditions to maximise this yield. Different extraction times were considered, from 1 to 8 hours, being the final results obtained plotted in Figure 3. This study was only carried out with the floating sludge because of their more promising results obtained in the preliminary tests. As it was previously observed in similar studies (Olkiewicz et al., 2014), the yield of lipids fast decreases when working with extraction times shorter than 4 hours, whereas longer times do not mean any relevant improvement in the total yield of lipid extracted. Considering that the maximum amount of lipids of this raw material is 19.6 % (previously determined by chloroform extraction), it can be concluded that using hexane as extraction solvent only around the 9 % of the total lipids can be extracted (1.75 g lipids / 100 g dry sludge). Results of transesterification step are congruent with the evolution of lipids extracted with the time, obtaining an increasing trend with a maximum production of 0.4 g FAMES / 100 g dry sludge, which corresponds to the transesterification of 26.8 % of the total lipid extracted. These results were obtained after 24 h of reaction, but the temporal profile (almost flat in the last hours) discards any relevant effect of longer reaction times.

The last technique studied is the in situ extraction and transesterification, using sulphuric acid in methanol solvent (4 % v/v). Taking into account that in situ procedure does not distinguish between extraction and transesterification steps, results directly show the final FAME concentration obtained as function of the sludge fraction used. There is a relevant improvement in the final yield (wt_{FAMES}/wt_{dry sludge}) obtained with all the fractions, reaching a minimum of 1.7 % with sludge from oxic area and a maximum of 2.1 % for floating sludge (in comparison with the 0.4 % obtained at optimum conditions with the solid-liquid procedure).

These results are obtained with 4 % of acid catalyst, being congruent with the typical values reported in the literature for this type of raw material. With the aim to identify if these conditions are the optimal, the effect of catalyst loading was studied, in the range of 1 to 8 %, being the results plotted in Figure 4. As in the previous case, only floating sludge were
considered for this study because of their preliminary results at a fixed concentration. As it can be observed, there is an exponential relation between the acid catalyst and the final yield obtained when this concentration is lower than 4%, whereas values higher than 5% do not have any relevant effect. This behaviour, previously observed in the literature by several authors, (Dufreche et al., 2007; Mondala et al., 2009; Olkiewicz et al., 2014) (Revellame et al., 2010), was justified by the secondary reactions (formation of estolides, polymers of fatty acid esters) that are promoted by high temperatures or strong acid conditions. So, 4% of sulphuric acid is defined as the optimum one and, after 24 h of reaction, 2.1% of FAME yield was obtained. This result corresponds to a global extraction and transesterification of more than 10.7% of the total lipid content of these floating sludges.

In order to compare the potential quality of biodiesel obtained by solid-liquid and in situ procedures, the composition of the fatty acid obtained is plotted in Figure 2b. As it could be expected, similar carbon range were obtained, but with significant differences between these FAMEs and those obtained using solid-liquid method. After the in situ treatment, a higher concentration of unsaturated FAME is detected, in good agreement with the polar character of methanol (Dufreche et al., 2007; Willson et al., 2010). Globally, more than 66% of unsaturated compounds were obtained in all the cases after the in situ treatment (mainly monounsaturated), being the highest percentage obtained with the floating sludge. On the contrary, this value was lower than 55% with the solid-liquid procedure. It is reported that an excess of saturated FAMEs has a negative effect on the global quality of fuel obtained. Thus, when this amount exceeds 60%, the biodiesel has a bad behaviour at low temperatures, being able to form crystals from the solidification of the saturated components (Olkiewicz et al., 2014). Other relevant conclusion of these analyses is the similar distribution of acids obtained despite the type of sludge; suggesting that this methodology is more robust and profile obtained is less conditioned by the nature of the initial raw material.
According to all these results, the *in situ* process is chosen as the optimum one, obtaining not only the highest conversion (more than 5 times higher than the maximum one obtained with the solid-liquid extraction) but also the highest FAME quality and more reproducible results. However, results obtained with the solid-liquid technique cannot be discarded, so this procedure is also considered in the following experiments, with the aim of identify if final yields can be improved by the pretreatment of the initial samples.

3.3. **Effect of sludge pretreatment on the final FAME yield**

Considering that previous results suggest that the limiting step is the lipid extraction, pretreatment of initial samples was considered, with the aim to break the complex structure of microorganisms (mainly the cell wall) and make easier the contact between solvent and lipids. Despite the scarce references to these procedures, with only some studies carried out by Olkiewicz (Olkiewicz et al., 2015), sonication was chosen as pretreatment technique because of the promising results reported for the lipid extraction from microalgae (Dong et al., 2014; Gerde et al., 2012; Lee et al., 2010; Ranjan et al., 2010). Thus, the effect of sonication was studied for the floating sludge fraction (best results in the preliminary analyses), using a resonance of 10 kHz and analysing the influence of time from 10 to 120 minutes. Results are reported in Figure 5, plotting the final FAMEs yields obtained after the solid-liquid and *in situ* processes.

According to Figure 5, maximum FAMEs yield was reached after 50 minutes of sonication, not observing any relevant improvement at longer pretreatment times. These results suggest that the maximum cell fragmentation at these conditions is reached after this time, so the maximum amount of accessible lipids is exposed to the solvent and catalyst. In good agreement, results after transesterification improve more than 65 % respect to the corresponding data without pretreatment: from 0.4 to 0.7 % with the solid-liquid phase, and from 2.1 to 3.46 % with the *in situ* method. This improvement confirms that the extraction is
the limiting step of the process and suggests that this pretreatment makes accessible some lipids that cannot be extracted even using a polar solvent (without pretreatment).

Previous data are analysed after 24 h reaction time, in both cases. However, if the temporal evolution is compared, the key role of this pretreatment is more evident. Thus, as example, the temporal evolution with and without pretreatment and applying the in situ method is plotted in Figure 6. The effect of sonication is clearly observed since the first moments, observing an exponential trend that reaches the maximum after only 16 h (instead of the 24 h needed to reach the maximum in absence of sonication).

3.4. Kinetic study

As it was previously mentioned, results of the previous sections were analysed at the end of each method, to make easier the comparison. However, in the case of “in situ” transesterification, samples were analysed during all the process, obtaining the FAMEs’ temporal evolution. The pseudo-first order dependence on reaction time, as well as the same asymptotic behaviour for the experiments performed in absence and in presence of sonication, and even the improvement detected for the sonicated samples, suggest that the in situ transesterification is controlled by mass transfer instead of the chemical reaction. In the same way, no lipids were detected in the liquid phase, also suggesting that once the lipid is transferred to the liquid phase, it is transformed into FAMEs. Therefore, it is possible to assume that the asymptotic FAME yield reached in both cases (Fig. 6) correspond to the total concentration of saponifiable lipids (1.5 g saponifiable TG/100 g dry sludge; 4.23 g FAME/100 g dry sludge). Under the mass transfer control assumption and considering the vessel as a stirred batch reactor, the unsteady state mass balance to the triglycerides follows the resulting equation:

\[ -\frac{dX_{TG,s}}{dt} = k_{SL} \cdot a_p \cdot (X_{TG,s} - K_{eq,TG} \cdot C_{TG,L}) \]  \[ [1] \]
Where \( X_{TG,s} \) is the concentration of triglycerides in the solid phase (mg of TG per mg of dry solid), and \( C_{TG,L} \), their concentration in the liquid phase, whereas \( k_{SL} \) is the solid-liquid mass transfer coefficient, “\( a_p \)” is the interfacial area per volume, and \( K_{eq,TG} \) is the equilibrium constant for the distribution of the TG between the sludge and the liquid. Considering that the reaction is very fast, as evidenced the above-mentioned analysis, the concentration of triglycerides in the liquid phase is negligible.

An integration of the equation [1] under these assumptions, will lead to the following expression:

\[
\ln \left( \frac{X_{TG,s}}{X_{TG,s,0}} \right) = -k_{SL} \cdot a_p \cdot t \tag{2}
\]

In this expression, the denominator corresponds to the initial concentration of saponifiable lipids in the sludge, considering that both curves trend to an asymptotic value, this parameter can be calculated from the final concentration of FAME in the liquid phase (1.5 g TG saponifiable/100 g dry sludge).

The solid-liquid interfacial area per unit volume of solid is determined using the following expression, where “\( C_v \)” is the volume fraction of solids in the slurry and “\( d_{32} \)” is the Sauter-mean particle diameter determined by a Zetasizer nano Instrument (Malvern Instruments Ltd., UK).

\[
a_p = \frac{6 \cdot C_v}{d_{32}}
\]

\( C_v \) parameter was determined to be 0.09, whereas the \( d_{32} \) particle diameter was 170 \( \mu \)m for the floating sludge. It must be remarkable that no significant changes in these parameters were observed comparing the samples before and after the reaction, so the \( a_p \) coefficient was considered as constant with a value of 3176.5 m\(^{-1}\).

The Scientist\textsuperscript{®} software was used to fit the experimental results to the proposed mechanism, obtaining apparent constant values of 0.037 and 0.12 h\(^{-1}\), for reaction without and with
pretreatment, respectively. These results correspond to $k_{SL}$ values of $1.2 \times 10^{-5}$ and $3.8 \times 10^{-5}$ m/s, respectively. These values are congruent with the range of values defined in the literatures as typical of processes limited by the mass transfer between solid particles and the liquid phase. (Levenspiel, 1972). In both cases, the correlation index was higher than 0.995, indicating a great correspondence between experimental and fitted data, as it can be observed in Fig 6 as dotted lines. As it was expected, the improvement of sonication is clearly observed in the mass transfer constant values, with a value almost 50% higher under sonication.

4. Conclusions

Promising results were obtained with floating sludge as raw material to obtain biodiesel by in situ procedure. Liquid-liquid procedure was discarded because of its low efficiency whereas the solid-liquid approach is conditioned by a lower quality of FAMEs obtained. Once reaction was optimized, 2.1% of FAMEs yield was obtained after 24 h by in situ reaction with 4% of $\text{H}_2\text{SO}_4$. This result increase considerably by using sonication as pretreatment, obtaining a final yield of 3.5% after 16 h. Results are congruent with a model limited by the mass transfer of lipids from the sludge particle to the liquid, being congruent with the highlighted improvement observed when using sonication as pretreatment.

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References


biodiesel by simultaneous extraction and conversion of lipids. Energ. Convers. Manage., 103, 111-118.


Figure captions

Figure 1. Process of urban wastewater treatment of the WWTP of Villapérez (Oviedo, Spain)

Figure 2. FAME composition of the biodiesel produced by (a) solid-liquid extraction method and (b) in situ transesterification. Oxic sludge, anoxic sludge, anaerobic sludge.

Figure 3. Process yields at different extraction times using floating sludge as raw material by solid-liquid extraction. Results corresponding to the (a) extraction step and (b) transesterification process.

Figure 4. Effect of catalyst concentration on FAMES yield by in situ transesterification with floating sludge as raw material.

Figure 5. Effect of sonication time on FAMES yield, solid-liquid extraction and in situ transesterification, with floating sludge as raw material.

Figure 6. FAME yield versus reaction time by in situ transesterification of floating sludge: without pretreatment and 50 minutes of ultrasound as pretreatment. Dotted lines correspond to model fitting.
Figure 1. Process of urban wastewater treatment of the WWTP of Villapérez (Oviedo, Spain)
Figure 2. FAME composition of the biodiesel produced by (a) solid-liquid extraction method and (b) in situ trasesterification.

C12:1 C14:0 C15:0 C16:1 C16:0 C17:1 C17:0 C18:3 C18:1 C18:0 C20:1 C20:2 C20:0

% wt/wt sample

(a) (b)
Figure 3. Process yields at different extraction times using floating sludge as raw material by solid-liquid extraction. Results corresponding to the (a) extraction step and (b) transesterification process.
Figure 4. Effect of catalyst concentration on FAMES yield by *in situ* transesterification with floating sludge as raw material.
Figure 5. Effect of sonication time on FAMEs yield, • Solid-liquid extraction and ■ in situ transesterification, with floating sludge as raw material.
Figure 6. FAME yield versus reaction time by in situ transesterification of floating sludge: • without pretreatment and ◆ 50 minutes of ultrasound as pretreatment. Dotted lines correspond to model fitting.
Table 1: Moisture content, total solid concentration and lipid content (dry sludge basis) of the considered sludge samples.

<table>
<thead>
<tr>
<th>Sludge sample</th>
<th>Moisture (%)</th>
<th>Total Solids (%)</th>
<th>Lipids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>After decantation</td>
</tr>
<tr>
<td>Oxic zone</td>
<td>99.7 ± 0.1</td>
<td>0.30 ± 0.02</td>
<td>0.79 ± 0.06</td>
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<tr>
<td>Anoxic zone</td>
<td>99.7 ± 0.1</td>
<td>0.29 ± 0.01</td>
<td>0.55 ± 0.07</td>
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<tr>
<td>Anaerobic zone</td>
<td>99.7 ± 0.1</td>
<td>0.28 ± 0.01</td>
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<tr>
<td>Floating sludge</td>
<td>97.3 ± 0.1</td>
<td>2.63 ± 0.06</td>
<td>2.64 ± 0.06</td>
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<tr>
<td></td>
<td>Lipid yield</td>
<td>FAMEs yield</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% lipids/dry sludge (w/w)</td>
<td>% FAMEs/lipids(w/w)</td>
<td></td>
</tr>
<tr>
<td><strong>Liquid-Liquid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxic zone</td>
<td>0.03 ± 0.04</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anoxic zone</td>
<td>0.05 ± 0.04</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anaerobic zone</td>
<td>0.02 ± 0.03</td>
<td>0</td>
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<tr>
<td>Floating sludge</td>
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<td>0.86 ± 0.03</td>
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<tr>
<td><strong>Solid-Liquid</strong></td>
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<tr>
<td>Oxic zone</td>
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<td>Anoxic zone</td>
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<td>Anaerobic zone</td>
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<td>15.90 ± 0.05</td>
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<tr>
<td>Floating sludge</td>
<td>1.75 ± 0.03</td>
<td>23.55 ± 0.02</td>
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