Direct liquid-liquid extraction of lipid from municipal sewage sludge for biodiesel production

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Abstract

Municipal sludge from wastewater treatment plants is a promising lipid feedstock for biodiesel production as it contains a significant amount of lipids. However, the energy necessary to remove its high water content is a major inconvenience for scaling up because of the high associated cost. In addition, the expensive conventional sludge drying methods are not effective enough for for lipid recovery, thus reducing the potential biodiesel production. This study explores an alternative method, the direct sequential liquid-liquid extraction, which was performed in a batch mixer-settler reactor at room temperature, using hexane as a solvent, after previous sludge acidification showed significant increase in the lipid efficiency. The optimisation study demonstrated that, after three stages, 91% of lipid from primary sludge was recovered. The optimised extraction gave slightly higher lipid (27%, dry sludge) than the standard method (25%, dry sludge), supporting the suitability of the proposed process. Finally, this work demonstrates that the residual lipid-extracted sludge is still a good feedstock for energy production via anaerobic digestion. Anyway, the economic and environmental aspects of biodiesel production from sewage sludge could be improved.

Key-words

Sewage sludge; liquid-liquid extraction; acidification; lipid; biodiesel.

1. INTRODUCTION

The global continuous growth of energy demand poses urgent problem due to the fossil fuels depletion, as they currently represent about 75% of all energy consumed worldwide [1]. One of the most promising renewable fuels proposed as an alternative is biodiesel that can be directly used with current engine and refuelling technology [1-3]. However, the competitive potential of biodiesel is currently limited by the price of the common lipid feedstocks, which constitutes 70-85% of the overall biodiesel production cost, thus strongly influencing the final price of this biofuel and raising the concerns of food shortage versus fuel crisis [1]**.**

In turn, municipal sewage sludge from wastewater treatment plants (WWTPs) is gaining more attention nowadays as a lipid feedstock for the production of biodiesel as the dry sludge can contain up to 30 wt% of lipids [1-6]. In fact, sewage sludge is a waste that needs specific treatment before disposal and represents a major cost in the WWTP operation. In addition, the WWTPs annually produce higher amounts of sludge due to the expansion of urbanised and industrialised areas. Therefore, the sewage sludge can be envisaged as a relatively cheap, readily available, and in abundance feedstock, which can make the biodiesel production profitable. Furthermore, it is one possible alternative to take advantage of the excess sludge, reusing it as a source of lipid for the production of biodiesel, consequently lowering the WWTP operation cost. Nevertheless, the production of biodiesel from sludge poses great challenges for a fast commercialisation. The main challenge to be faced by biodiesel production from waste sludge is an efficient lipid extraction from water, as water can account for up to 95-98 wt %, so dewatering and drying constitute more than 50% of total biodiesel production cost [4]. This makes the production very expensive and difficult the scale-up due to the cost of the energy necessary for water removal step.

Most of the literature reports only the utilisation of dry sludge in the extraction of lipid by an organic solvent [3-4, 6]. Recently, some works have used dewatered primary [5] and secondary sludge [7] by centrifugation, but the energy of dewatering still constitutes 14% of total biodiesel production cost [4]. On the other hand, the direct transesterification of sewage sludge into biodiesel has been also reported “in situ” on dry [2, 4] and dewatered sludge [7]. Interestingly, the biodiesel yield obtained from dewatered sludge was about 20% lower than from dried sludge [7]. The “in situ” process can reduce the time and amount of solvent, however, after transesterification, a solvent recovery step is then needed, adversely affecting the overall cost of biodiesel.

Moreover, water elimination from biomass by conventional thermal drying or freeze-drying results in the loss of valuable organic compounds [8-9]. This fact can also provoke the loss of lipids in sewage sludge hence decreasing biodiesel production yield. Nevertheless, the influence of sludge drying on the lipid extraction efficiency has not been yet evaluated. Therefore, the effect of common sludge drying methods on the lipid extraction efficiency as well as the fatty acids composition still needs to be examined.

Surprisingly, the direct liquid-liquid extraction has neither been reported, so the sludge drying and dewatering would thus become unnecessary. Thus, the main objective of this study was to explore this alternative and to demonstrate its feasibility. Three types of sludge generated in WWTPs were tested. Optimisation of liquid-liquid extraction was studied varying the ratio sludge/hexane, time of contact, and number of consecutive batch extraction steps in order to get the most favourable process. In addition, as the residual sludge after lipid extraction is still a potential biomass for energy recovery, the residual sludge can be used as feed for anaerobic digestion, which is widely implemented in municipal WWTPs. Therefore, the lipid-extracted sludge was subjected to anaerobic digestion to check out its potential for biogas generation. Finally, a simplified energy consumption estimation of the biodiesel production via liquid-liquid extraction was conducted.

2. MATERIALS AND METHODS

2.1. Reagents

The transesterification/esterification experiments were carried out using anhydrous methanol and sulfuric acid from Sigma-Aldrich at the highest purity available. Standard used for identification and quantification of fatty acid methyl esters (FAMEs) was supplied by Supelco (37 component FAMEs mix, ref: 47885-U). For the free fatty acids FFAs analysis, 0.5 M potassium hydroxide volumetric solution was purchased from Fluka. All other solvents and reagents were high performance liquid chromatography grade and analytical reagent grade provided by Sigma-Aldrich.

2.2. Sludge collection and handling

Primary, secondary and blended sludge were collected from the municipal WWTP in Reus (Tarragona, Spain) with a capacity to daily process 25000 m^3 of wastewater. Fig. 1a shows a schematic diagram of the WWTP, illustrating the step where these different types of sludge are generated. 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. The collected sludges were immediately stored at 4ºC prior to use. Because the sludge properties could be changed during long storage time, fresh sludge was always used for each experiment.

The inoculum used in anaerobic digestion tests was sludge collected from a mesophilic anaerobic digester in the same facility.

2.3. Sludge drying

2.3.1 Primary sludge – evaluation of drying methods

According to standard method 5520E [10], sludge was dried using magnesium sulfate monohydrate but without previous acidification. Using the referenced method, the sludge sample was considered as completely dried.

Oven drying method was conducted using an universal oven ULE400 (Memmert GmbH, Germany) at two different temperatures, 105ºC for 2 days based on standard method 2540G [10] or 70ºC for 3 days [3].

Freeze-drying method was conducted by using the method presented elsewhere [2]. At first, sludge was centrifuged and then allowed to freeze for 2 days at -20ºC. Afterwards, the frozen sludge was freeze-dried in an automatic vacuum freeze dryer, model FT33-A (Armfield Limited) for 2 days.

In the sun drying method, the sludge sample was left outside for 10 days, where the temperature was in the range of 25-35ºC.

Drying under fume hood was performed based on the method presented elsewhere [6]. The sludge was first centrifuged and then put in a fume hood for 4 days at ambient temperature.

Approximately 500 mL of sludge was used for the drying procedures, except for the standard method. After all drying methods, sludge was crushed to fine particles. To determine the final moisture content, 1 g of crushed sludge was placed in the oven at 105ºC and dried until reaching constant weight.

2.3.2 Drying of primary, secondary and blended sludge by standard method

The sludges were dried using magnesium sulfate monohydrate according to standard method 5520E [10] with previous acidification. The reference method was used for a comparison study to a novel liquid-liquid extraction from acidified liquid sludge.

2.4. Lipid extraction

2.4.1 Extraction of lipids from dried sludge

The extraction after drying was carried out in a Soxhlet apparatus using hexane as a solvent according to standard method 5520E [10]. After extraction, the hexane was removed using a rotary evaporator at 40ºC under vacuum at 50 mbar. Then, the remnant lipid fraction was stored in a desiccator overnight and weighed the next day to determine the extraction yield.

2.4.2 Liquid-liquid extraction of lipids from primary, secondary and blended sludges

Sequential liquid-liquid extraction of lipids was performed in a batch mixer-settler reactor with mechanical agitation (330 rpm), at ambient temperature, using hexane as solvent and 200 mL of sludge. The effect of previous sludge acidification to pH=2 was evaluated. This pH was attained by addition of approximately 3 mL of concentrated HCl to the sample of 200 mL of sludge. The experimental setup for the liquid-liquid extraction is presented in Fig. 1b. Until nine consecutive extraction stages were conducted, in which the sludge, after settling, was extracted again with additional fresh solvent. The mechanical settling was performed at 60 rpm for 15 min for primary and blended sludge and for 30 min for secondary sludge. After each extraction stage, the hexane phase was filtered using a 2-4 μm filter paper in order to eliminate the remaining solid particles and then dried over anhydrous sodium sulfate. Later, hexane was removed using a rotary evaporator at 40ºC under vacuum at 50 mbar and reused for the consecutive stage. Lipids were stored in a desiccator overnight and weighed the next day to determine the extraction yield.

2.5. Anaerobic digestion of lipid-extracted sludge

As depicted in Fig. 1, primary sludge after lipid liquid-liquid extraction was subjected to anaerobic digestion directly and after residual solvent evaporation. The residual hexane was removed using a rotary evaporator at 40ºC under vacuum at 50 mbar. The sludge was anaerobically digested at 33ºC under mesophilic conditions [11]. Lipid-extracted sludge (LES) and evaporated lipid-extracted sludge (ELES) were digested in order to evaluate the impact of the remaining solvent on biogas production.

Anaerobic digestion test was conducted in 120 mL serum bottles in triplicate. Digested sludge was used as inoculum and, although acclimation is not strictly required, an anaerobic semi-continuous plant was set to adapt inoculum to a more stable temperature, 33ºC. The optimal digestion conditions were assured with anaerobic basic medium addition [11].

Then, ELES and LES were used as substrates. Substrate to inoculum ratio was fixed to 0.5:1 in a VS base. Deionised water was added to reach a final volume of 80 mL and the reactors were closed with a septum and an aluminium crimp. Finally, the reactors were purged with nitrogen to assure anaerobic conditions and placed into an oven at 33 ºC. Blank assays were prepared without substrate addition, and its biogas production was subtracted from the reactors fed with the substrates. Biogas production was volumetrically measured by liquid displacement. The experiment was considered completed after 25 days, when biogas production was negligible.

Biogas composition was analysed using an Agilent gas chromatograph (6890GC) equipped with a thermal conductivity detector and a Porapak Q 50/80 packed column (CP99960C). Both methane and carbon dioxide were quantified, and the results expressed as methane percentage in a two component mixture.

Volatile fatty acids (VFA) were analysed in the soluble phase by gas chromatography using a flame-ionization detector (GC-FID). The method was performed according to Agilent Application Note 228-398 using a HP-INNOWax column (19091N-133).

2.6. Lipid and biodiesel analysis

The content of free fatty acids (FFAs) was analysed according to section 9.1 of European standard method EN ISO 660 (2009). Due to the predominance of palmitic acid in the sludge lipids, the results of FFA content were expressed as equivalent to palmitic acid.

The lipids were converted into FAMEs (biodiesel) through acid catalysed transesterification/esterification using a modified version of Christi's method [4], i.e., with hexane instead of toluene. This method was chosen because of the high amount of FFAs in the sludge lipid fraction. The FAMEs were analysed by GC-FID according to Agilent Application Note 228-398 using a HP-INNOWax column (19091N-133). For the calibration of the method, a 37 component FAMEs standard mixture was used (Supelco: 47885-U). The samples were also subjected to GS-MS analysis (G1099A/MSD5973) using a HP-FFA column (19091F-433). The results of the GC-FID were used to estimate the amount of saponifiable (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 3.4.

3. RESULTS AND DISCUSSION

3.1. Sludge characterisation

Each sample of received sludge was analysed in triplicate in order to determine the total solids (TS) and volatile solids (VS) contents according to standard method 2540G, and lipid content according to standard method 5520E [10]. The results in Table 1 show that TS and VS contents were very similar for all types of sludge tested. On the other hand, the lipid contents showed clear differences between the sludges. Thus, primary sludges achieved the greatest lipid fraction, followed by blended and secondary. Primary sludge mainly consists of organic matter from non-treated raw wastewater, so it is a combination of floating grease and solids; instead, the secondary sludge is mainly composed of microbial cells and suspended solids produced during the aerobic biological treatment of the primary treated wastewater. Thus, it is expected that primary sludge gives the highest lipid fraction as most of this fraction is originally formed by fats whereas lipids from secondary sludge come from the cells after breaking their structure. As blended sludge is a mixture of primary and secondary, with a higher fraction of the first one, it results in the intermediate lipid content.

Comparing both primary sludges, some differences in the TS, VS and lipids can be observed (Table 1). As the primary sludges were collected in different days, this indeed implies variations in their composition. The fluctuations may be the result of climate changes or by deviations in the amount and quality of the wastewater received in the WWTP.

3.2. Effect of sludge drying methods

The influence of the conventional sludge drying methods on the moisture, lipid and biodiesel yields is illustrated in Table 2. Comparing with $MgSO_4 \cdot H_2O$ drying, thereafter the standard method, the other drying methods showed a negative effect on both lipids extracted and saponifiable matter recovery, thereby decreasing the potential biodiesel yield.

The content of final moisture in the sludge is an important factor that explains the adverse effect on the lipid extracted as well as biodiesel produced. Water contained within the biomass has a tendency to shield lipids from the extracting solvent. As seen in Table 2, the final moisture content in the sludge depends on the temperature of the drying method. At high temperature (70ºC, 105ºC), the content of moisture is low, but always higher than that of the $MgSO_4 \cdot H_2O$ method. Furthermore, it was observed for experiments with oven at 70ºC, freeze-dried, fume hood, and sun drying that the solid particles were more compacted, even after grinding. The water content could surround the sludge particles and thus inhibite the good penetration of hexane inside the solid particle. As Table 2 shows, in general, the greater the amount of moisture contained in the sludge, the lower the amount of extracted and esterified lipids.

Additionally, it can also be noted in Table 2 that the drying methods at high temperature (70ºC, 105ºC) had a negative impact on lipid composition giving lower saponifiable matter, thereby decreasing the potential biodiesel yield when compared to standard method. Despite the lower temperature used for fume hood and sun drying, the lipid content extracted from these dried sludge decreased to 12.3% and 11.4%, respectively, again compared to standard $MgSO_4{\cdot}H_2O$ method, 26.3%. Finally, freeze-drying also showed a significant loss of extracted lipids (11.2%) but, in contrast, the rate of saponifiable matter was higher (57.3%) than those from oven at 70ºC (53.9%), sun (45.5%) and fume hood (44.8%) drying methods. The low lipid content extracted from dried sludge at low temperature and freeze-drying reported here are in agreement with Cordero Esquivel et al. (1993). They reported that biomass drying by both freeze-drying and oven drying at low temperature (30 ºC) caused an approximately 70% loss of total lipid content [8].

The biodiesel yield regarding $MgSO_4{\cdot}H_2O$ method showed a decrease in all methods, -17% for oven at 105ºC, -53% for oven at 70ºC, -65% for freeze-dryer, -71% for fume hood and -73% for sun drying. On the other hand, the values of biodiesel obtained from primary sludge dried by oven at 70ºC (8.8%) and fume hood (5.5%) compares well to those reported elsewhere [3, 6].

The influence of sludge drying methods on the fatty acid composition was also studied and the results are collected in Table 3. The same fatty acids were found for all methods showing a significant amount of palmitic (31.1 to 49.4%), oleic (18.3 to

32.6%) and stearic (8.3 to 15.8%) acids in the sludge biodiesel. The most important differences in the composition were observed for palmitic, palmitoleic, stearic, oleic and linoleic acids. In detail, oven at 105ºC for two days gave the fatty acid composition almost identical with respect to $MgSO_4 \cdot H_2O$ method. The other methods showed an increase in the fraction of oleic, linoleic and palmitoleic acids, counterbalanced by a decrease of palmitic and stearic acids. This trend, where the fraction of saturated fatty acids decreased while the fraction of unsaturated fatty acids increased, is particular for the sludge. Usually, unsaturated fatty acids are more unstable and readily oxidized than saturated ones.

Definitely, usual sludge drying methods adversely affect the yield of extracted lipids as well as the lipid saponifiable matter, consequently reducing the potential for biodiesel production. FAMEs composition of biodiesel is also modified, too. Among the methods tested, oven drying at 105ºC could be the best option for subsequent biodiesel production, giving 15.7% of biodiesel produced from a dried sludge basis. Unfortunately, thermal drying is not cost effective for a large scale application.

3.3. Sequential liquid-liquid extraction

As above commented, it is surprising that liquid-liquid extraction has not yet been applied to lipid fraction recovery from sludge as this is a fair alternative allowing the scale-up into a continuous process. In first place, sequential batch liquid-liquid extraction was performed to examine its feasibility and evaluate the effect of operation variables. The determination of the partition coefficient for some chosen experiments will allow the scale-up of the extraction step in a continuous process.

3.3.1. Effect of sludge acidification

Despite the fact that primary sludge was found to contain the highest lipid content as compared to secondary and blended (Table 1), the liquid-liquid extraction was also studied for these sludges, in order to evaluate the suitability of the novel extraction method for all type of sludge generated in WWTPs.

The first step to be evaluated was the effect of previous sludge acidification with concentrated hydrochloric acid. It is expected that the acidification will facilitate the extraction of lipids from processed samples, so as well the amount of saponifiable lipid, thereby the biodiesel yield.

Fig. 2 shows the results of the sequential liquid-liquid extraction for acidified and non-acidified primary, secondary and blended sludges. The accumulated lipid yield was continually increasing in each extraction stage in all cases. As it was expected, primary sludge achieved the highest lipid yield followed by blended and secondary, irrespective of sludge acidification. Sludge acidification highly increased the lipid yield in each extraction stage. Nonetheless, this trend is more evident in the case of primary and blended sludge. In the last extraction stage, the lipid yield obtained from primary sludge was 26.6% and 13.0% for acidified and non-acidified samples, respectively, whereas blended sludge gave 19.1% and 10.7 for acidified and non-acidified samples, respectively. Secondary sludge achieved the lowest lipid yield, a meagre 6.3% and 5.1% for acidified and non-acidified sludge, respectively. The high difference between the values obtained for primary and blended sludge, with and without acidification owns to the fact that municipal wastewater contains fatty acids from commercial soaps, potassium and sodium from household cleaning products, cosmetics, lubricant and coatings. During primary treatment, the physico-chemical process leads to a rapid formation of relatively insoluble calcium and magnesium salts precipitating during the primary wastewater treatment, which remain in the primary sludge [5]. For this reason, the acidification was responsible of the conversion of insoluble soaps into FFAs that are soluble in the extract solvent, increasing the lipid yield and the saponifiable matter [5]. Since the secondary sludge does not contain insoluble soaps that could be converted into FFAs, which significantly raises the lipid content, the lipid fraction in secondary sludge mainly comes from microorganism cells. Thus, the acidification can only release by acid hydrolysis some additional lipids bonded to the cells, slightly increasing the lipid yield.

The results of FFAs analyses in primary sludge showed that, after sludge acidification, the FFAs content increased from 39.2% to 68.7% (on the basis of lipids) showing a good agreement with previous literature data [5]. Moreover, the increase of FFAs content resulted on significant increase of saponifiable (esterifiable) lipids (from 45.3% to 70.0%), which accounts for the rise of biodiesel production from 5.9% to 18.6% (on the basis of dry sludge).

It should be noted that the final lipid yield obtained by liquid-liquid extraction from acidified primary sludge (26.6%, Fig. 2) was higher than the yield obtained by standard $MgSO_4{\cdot}H_2O$ method (25.2%, Table 1). Therefore, for the first time a process that can be easily scaled-up, i.e. liquid-liquid extraction, is able to extract all lipid contained in the primary sludge as the standard method does.

On the other hand, the acidified blended and secondary sludge gave lower lipid yield than that attained by standard $MgSO_4{\cdot}H_2O$ method. The absolute lipid yields obtained by the liquid-liquid extraction from acidified blended and secondary sludges were 19.1% and 6.3%, respectively (Fig. 2). These values are 10% and 20% less, respectively, than those achieved by the standard $MgSO_4{\cdot}H_2O$ method (Table 1). Hence, the liquid-liquid extraction from acidified blended and secondary sludge is not so effective to extract lipids present in these sludges. Because liquid-liquid extraction from acidified primary sludge is more favourable than from acidified blended and secondary, the optimization of liquid-liquid extraction was conducted over the primary sludge.

3.3.2. Optimisation of liquid-liquid extraction from primary sludge

The extraction optimisation from acidified primary sludge was carried out using a combination of different time of contact in each stage (20, 40 and 60 min) and different sludge to hexane volume ratio (4:1, 2:1, 1:1 and 1:2). The other operative conditions were maintained constant, i.e. 200 mL of sludge, 9 consecutive extractions, 330 rpm agitation speed and ambient temperature.

Fig. 3 shows the results of the optimisation of the lipid extraction. In all cases, the accumulated yields of lipids increased with consecutive extraction stages, reaching a constant value at the last stages of the extraction. The best value of the accumulated yield of lipids at the last stage of extraction was 29.6% (based on dry sludge), attained for the experiment with a sludge to hexane volume ratio 1:2. The 1:1 volume ratio was able to achieve 29.5%, the 2:1 gave 28.8% and the 4:1 only 28.1%. As expected, the lower the amount of solvent, the lower the extraction efficiency achieved.

The contact time is also of great importance. For the sludge to hexane volume ratios 4:1, 2:1 and 1:1, the lipid yield grew as the contact time increased in each extraction stage, always reaching the best results for 60 min, 28.1%, 28.8%, and 29.5%, respectively. In turn, 40 min of extraction time allowed attaining 26.3%, 27.8% and 28.4% of lipids for sludge to hexane volume ratios 4:1, 2:1 and 1:1, respectively. The lowest lipid yield was obtained for 20 min of extraction time giving 22.9%, 26.7% and 26.6% of lipids for sludge to hexane volume ratio 4:1, 2:1, 1:1, respectively. On the contrary, the results using sludge to hexane volume ratio 1:2 did not show any influence of the extraction time. Beyond the third stage, the accumulated lipid yields remained practically unaltered, reaching 27% of lipids based on dry sludge. This value represents 91% of the attainable lipid recovery.

Independently of the extraction time, as it was expected, the yield of lipids increased after each extraction stage when increasing the amount of solvent. However, for volume ratios of 2:1, 1:1 and 1:2, 60 min of extraction time did not show significant difference between the lipid yields, after the third stage of extraction. This suggests that the quantity of hexane used for a ratio 2:1 for 60 min was enough to achieve 27% of lipids based on dry sludge (91% of the attainable lipid value).

Overall, in order to reach at least 91% of lipids present in the primary sludge, only three consecutive extraction stages were needed. This extraction efficiency was achieved for 60 min (2:1, 1:1, 1:2, sludge:hexane) as well as for 20 and 40 min (1:2, sludge:hexane). Taking into account the minimization of solvent used, the best operation conditions are 60 min using a 2:1 sludge:hexane ratio. On the other hand, minimizing the extraction time, the best operation conditions are a 1:2 sludge:hexane ratio for 20 min of extraction time in each stage.

3.3.3. Scale-up of liquid-liquid extraction process

Cost-effective production of biodiesel requires continuous operation plants. Therefore, design and scale-up of continuous processes must be done from batch data and operation. Lipid recovery data, starting from acidified primary sludge, obtained through batch liquid-liquid extraction experiments allow determining partition coefficients in a wide range of process conditions. Fig. 4 presents an example of the equilibrium curve obtained in the experiment with these operative conditions: 200 mL of sludge, 400 mL of hexane, 20 minutes for each extraction, 9 consecutive extractions with fresh hexane, 330 rpm of agitation speed and ambient temperature. As the liquid-

liquid equilibrium thermodynamic diagrams are then available, application of design methods for typical extraction equipment gives optimised solvent to feed flowrate ratio and number of stages in continuous operation.

3.4. Biodiesel produced from primary sludge by liquid-liquid extraction

The results of lipid transesterification from liquid-liquid extraction were compared with the results from drying by standard $MgSO_4 \cdot H_2O$ method in order to verify that the process did not affect the yield of biodiesel and the composition of FAMEs. The optimised liquid-liquid extraction gave 26.7 ± 0.1% of lipid (on the basis of dry sludge), the saponifiable obtained after transesterification was 72.0 ± 3.0% (on the basis of lipid) and the value of biodiesel was 19.2 ± 0.1% (on the basis of dry sludge). These values are higher than those obtained by standard method (25.2 ± 0.2% of lipid, 69.7 ± 0.7% of saponifiable and 17.6 ± 0.2% of biodiesel).

Based on the present research, i.e. experimental biodiesel yield of 19.2% from dry primary sludge basis, the annual biodiesel potential, theoretically produced from primary sludge generated at WWTP of Reus (1922 ton/year of dry primary sludge generation), was estimated to be 369 ton. Speculating the biodiesel production from wastewater produced from all Spanish population, 6 hm^3/day, the annual biodiesel potential was estimated at 88664 ton. This value may replace approximately 15% of current biodiesel production in Spain [12].

The FAMEs composition of biodiesel produced from standard and liquid-liquid extraction methods is presented in Table 3. At least 12 fatty acids were identified for both methods, ranging from C12 to C22 with a predominance of palmitic, oleic and stearic acids. As it can be observed in Table 3, the composition of the two biodiesel is the same, the differences observed being not essential. This fact is critical as the acidification and liquid-liquid extraction did not affect the characteristics of the biodiesel produced, making the liquid-liquid extraction technology viable.

In addition, the properties of biodiesel strongly depend on the fatty acid composition. The fact that the amount of polyunsaturated fatty acids found in the sludge lipids is really low, around 2% of linoleic acid (C18:2), is an advantage in comparison to the common vegetable oil feedstocks, which contain a large amount of polyunsaturated fatty acids. The polyunsaturated fatty acids are very susceptible to auto-oxidation, resulting in a poor oxidation stability of the biodiesel. On the other hand, the high level of saturated fatty acids found in the sludge, more than 60%, could represent a problem for the cold flow properties of biodiesel, when it becomes cloudy due to formation of crystals and solidification of saturated compounds. This could be solved by the presence of branched-chain and hydroxy fatty acid monoalkyl esters [13-14]. Actually, these compounds exist and were included as “Others” in Table 3. This fraction was identified by GC-MS (data not shown) and mainly consists of hydroxy and oxy fatty acids and branched-chain fatty acid methyl esters. This suggests that, despite the high amount of saturated fatty acids, the cold flow properties of biodiesel produced from primary sludge could be even better because of the presence of other fatty acids methyl ester.

3.5. Economic estimation of biodiesel production from primary sludge by liquid-liquid extraction (laboratory case).

The economic evaluation of biodiesel production cost from municipal sewage sludge has been already carried out elsewhere [4]. This study estimated the price of biodiesel about 0.83$/L, taking into account the cost of sludge dewatering and subsequent drying, which represent about 42-53% of the overall biodiesel production cost. However, in order to avoid the influence of currency, the energy required for the production of 1kg of FAMEs is a better parameter to estimate the final cost [5]. In this study, the minimum specific energy demand was estimated to be 17 MJ/kg_{FAMEs} but the result was given without considering the energy needed for sludge dewatering, which should have been added to this value.

In the present study, in order to perform the economic evaluation of biodiesel production from primary sludge by liquid-liquid extraction, all different process operations involving energy demand were included: agitation during extraction and settling, evaporation of the extract solvent, heating of the esterification mixture, evaporation of the product mixture, and separation of FAMEs by solvent extraction. Table 4 shows the values used to calculate the specific energy demand and the results of the economic estimation of biodiesel production based on the experimental results for the following extraction conditions: 60 min, 2:1 sludge to hexane volume ratio. As shown in Table 4, the energy demand and the price per litre of FAMEs depend on the number of extraction stages, varying between 60.95 MJ/kg_{FAMEs}, 1.88 €/LFAMEs (1 stage) to 290.15 MJ/kg_{FAMEs}, 8.94 €/L_{FAMEs} (9 stages). In a continuous process, no more than three extraction stages should be used to gain 99% of lipids. In addition, scaling-up of the process from lab-scale to industrial plant should reduce the price.

It should be also stated that the values calculated in the present study are final, including all different operation steps in the production of biodiesel from primary liquid sludge, and any additional cost of drying or dewatering are not necessary to include it in the final cost. On the other hand, costs of methanol, hexane and HCl used in the overall process were not accounted as it was an energetic balance calculation.

Finally, it must be noted that the production of biodiesel from primary sewage sludge reduces the amount of sludge generated at the WWTP facility, which should subsequently be managed and disposed as a waste. As the above is a major cost in the

WWTP operation, this saving should be taken into consideration when calculating the final price of biodiesel.

3.6. Anaerobic digestion of lipid-extracted primary sludge

As residual sludge after lipid extraction still contains a large amount of organic matter, this lipid exhausted sludge was subjected to anaerobic digestion in order to evaluate the remnant potential for biogas generation. Fig. 5 shows the biogas production during anaerobic digestion of evaporated lipid-extracted sludge (ELES), i.e. hexane free, and lipid-extracted sludge (LES). The biogas measure was converted at standard conditions (0ºC and 1 atm) and is given as the volume of biogas per gram of VS fed (mL_{Biogas}/g_{VS}). Biogas production from ELES reached 365 ± 10 mL_{Biogas}/g_{VS}, whereas LES only reached 31 ± 4 mL_{Biogas}/g_{VS}. This huge difference, over tenfold, can be attributed to the presence of hexane in LES. In a mass balance, it was calculated that solvent still represented approximately 9% of the volume in LES. Furthermore, a VFA analysis revealed a concentration of 12.0 ± 0.1 mol/m^3 in the reactor with LES, while no VFA were detected in reactors with ELES. A value over the range 6.7-9.0 mol/m^3 has been reported to be toxic for methanogenic microorganisms, stopping the biogas production [15].

Methane content in biogas from ELES was 62%, whereas in LES was barely a 31%. The theoretical methane production based on sludge composition was estimated, following Buswell’s equation [11], in 486 mL_{CH4}/g_{VS} for sludge after lipid extraction. Based on the experimental methane production, biodegradability (expressed as the ratio measured to theoretical methane production) resulted 47% and 4% for ELES and LES, respectively. The 47% of biodegradability is in line with the conversion that can be expected from highly particulated and structured organic matter [11].

Hence, it can be concluded that the lipid-extracted sludge can be easily anaerobically digested with good biogas production, although the elimination of residual hexane is required. In the proper conditions, this solvent could be recovered and reused for the extraction step. As the anaerobic digestion is widely installed in WWTPs, the hexane free residual sludge after lipid extraction could be returned to the WWTP to be anaerobically stabilised giving additional energy in form of biogas.

4. CONCLUSIONS

Common sludge drying methods decrease the yield of lipids as well as the saponifiable fraction, thus reducing the biodiesel production. In addition, they require high energy input. The proposed alternative, liquid-liquid extraction using hexane, is feasible and compares well with those classical methods. Previous sludge acidification improves lipid and subsequently biodiesel yields. The FAMEs obtained from liquid extracted lipids are similar to those obtained by standard method.

The cost of the proposed liquid-liquid extraction process and the lipid yield depend on the number of extraction stages. The scale-up of the process should allow reducing the final biodiesel price, as the cost of drying is eliminated. Finally, the lipid extracted sludge can be used to produce biogas by anaerobic digestion, avoiding the generation of a new sludge. The biogas obtained maintains a similar composition, i.e. quality, than that coming from raw excess sludge.

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Figure captions

Figure 1. Diagram of the wastewater treatment plant (a) and schematic diagram of the experimental liquid-liquid extraction setup carried out in the present study (b).

Figure2. Effect of sludge acidification on the lipid yield. Conditions: 1:1 sludge to hexane volume ratio, each stage extraction time - 20 min.

Figure 3. Effect of extraction time on the lipid yields with different sludge to hexane volume ratio.

Figure 4. Equilibrium curve lipid in hexane – lipid in sludge. 200 mL of acidified primary sludge, 400 mL of hexane, 20 minutes for each extraction, 9 consecutive extractions, 330 rpm of agitation speed and ambient temperature.

Figure 5. Biogas production from lipid-extracted sludge with and without evaporation process. Batch reactors, 33ºC and 25 days.

Table 1. Characteristics of sludge used for different experiments in this work.

Sludge type	Experiment type	TS (%)	VS (%)	Lipid (a) (%)
Primary (b)	Sludge drying	3.9 ± 0.1	2.9 ± 0.1	26.3 ± 0.5
Primary (c)	Liquid-liquid extraction	3.4 ± 0.1	2.7 ± 0.1	25.2 ± 0.2
Secondary (c)	Liquid-liquid extraction	3.8 ± 0.1	3.2 ± 0.1	7.7 ± 0.1
Blended (c)	Liquid-liquid extraction	3.5 ± 0.1	2.7 ± 0.1	21.1 ± 0.2

(a) Extraction according to standard $MgSO_4 \cdot H_2O$ method, lipid yield on the basis of dry sludge

(b) Lipid extracted from not acidified sludge

(c) Lipid extracted from acidified sludge

Values are means ± SD, n = 3

Table 2. Effect of drying method on the moisture content, lipid and transesterification yields.

Sludge drying method	Moisture (%)	Lipid [(a)] (%)	Saponifiable [(b)] (%)	Biodiesel [(a)] (%)
$MgSO_4 \cdot H_2O$	0.0 ± 0.0	26.3 ± 0.5	71.8 ± 2.4	18.9 ± 0.6
Oven at 105ºC	3.4 ± 0.0	26.5 ± 0.2	59.1 ± 0.6	15.7 ± 0.2
Oven at 70ºC	6.0 ± 0.4	16.4 ± 0.1	53.9 ± 0.8	8.8 ± 0.1
Freeze-dryer	6.6 ± 0.4	11.2 ± 0.3	57.3 ± 0.8	6.4 ± 0.1
Fume hood	7.6 ± 0.2	12.3 ± 0.1	44.8 ± 1.4	5.5 ± 0.2
Sun	10.8 ± 1.2	11.4 ± 0.2	45.5 ± 0.1	5.2 ± 0.1

(a) Lipid and biodiesel yield on the basis of dry sludge

(b) Transesterification yield on the basis of lipid

Values are means ± SD, n = 3

Table 3. Fatty acids composition of biodiesel produced from primary sludge (average of 3 experiments).

	(%) weight/weight$_{sample}$ (SD < 0.1)							
	Primary sludge 1[a]						Primary sludge 2[b]	
FAME from fatty acid	$MgSO_4$	105 ºC	70 ºC	Fre.-dryer	F. hood	Sun	$MgSO_4$	Liq-liq
Lauric (C12:0)	0.8	0.8	1.0	1.0	1.0	1.0	1.0	1.1
Myristic (C14:0)	4.6	4.8	4.5	3.4	4.1	4.1	4.1	4.3
Pentadecanoic (C15:0)	0.6	0.7	0.6	0.4	0.5	0.5	0.5	0.5
Palmitic (C16:0)	48.5	49.4	38.1	27.4	31.6	31.1	42.8	41.0
Palmitoleic (C16:1)	1.2	1.3	1.9	3.2	2.2	2.6	2.5	2.5
Heptadecanoic (C17:0)	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.4
Stearic (C18:0)	15.6	15.8	12.1	8.3	9.6	9.6	13.4	12.6
Oleic (C18:1)	18.3	18.3	28.8	39.6	32.8	32.6	23.3	25.7
Linoleic (C18:2)	2.1	0.6	3.4	7.2	5.3	5.2	1.9	2.0
Arachidic (C20:0)	0.4	0.2	0.4	0.3	0.3	0.3	0.4	0.4
Eicosenoic (C20:1)	-	-	0.5	0.6	0.7	0.7	0.3	0.3
Behenic (C22:0)	0.5	0.6	0.5	0.4	0.4	0.4	0.5	0.4
Others	7.0	7.1	7.9	7.9	11.2	11.7	9.0	8.8

[a] Primary sludge used for drying experiment

[b] Primary sludge used for liquid-liquid extraction experiment

Table 4. Energy and economic evaluation of biodiesel production from primary municipal sludges through liquid-liquid extraction of lipids.

Process	Basis for energy calculation	Energy values
Extraction: Mixing	200 mL sludge, 100 mL hexane, 330/2000 rpm, 50 W, 60 min/stage	nº stages × 29.700 kJ
Extraction: Settling	200 mL sludge, 100 mL hexane, 60/2000 rpm, 50 W, 15 min/stage	nº stages × 1.350 kJ
Extraction: Evaporation of hexane	ΔH_{vap}: 0.335 kJ/g, ρ: 0.655 g/mL, 100 mL/stage	nº stages × 21.94 kJ
Reaction: Heating of methanol	C_p: $2.53 \cdot 10^{-3}$ kJ/g·K, ρ: 0.792 g/mL, 2 mL, T: 323.15K	0.120 kJ
Reaction: Evaporation of methanol	ΔH_{vap}: 1.099 kJ/g, ρ: 0.792 g/mL, 2 mL	1.741 kJ
Separation FAMEs by hexane	ΔH_{vap}: 0.335 kJ/g, ρ: 0.655 g/mL, 10 mL	2.194 kJ

Extraction stage	1	2	3	4	5	6	7	8	9
FAMEs recovered (g)	0.94	1.35	1.53	1.60	1.62	1.63	1.64	1.65	1.66
Total Energy (kJ)	57	110	163	216	269	322	375	428	481
Specific Energy (MJ/kg_{FAME})	60.95	81.50	106.41	135.15	166.56	197.33	228.68	259.44	290.15
Price [a,b] (€/L_{FAME})	1.88	2.51	3.28	4.16	5.13	6.08	7.04	7.99	8.94

[a] Energy price: 0,126 €/kW·h

[b] Density of FAMEs (biodiesel): 0.88 kg/L

Figure 1

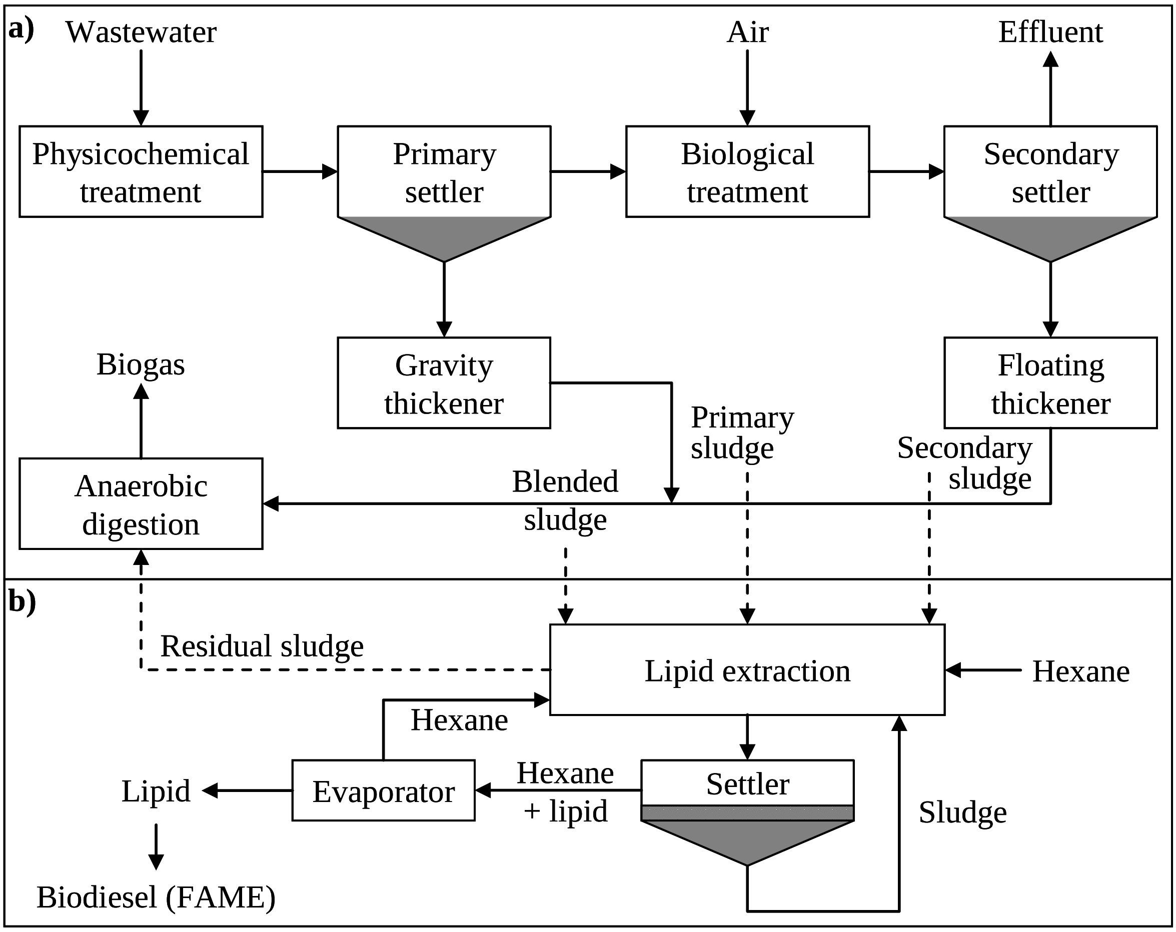

a)
Wastewater
Air
Effluent
Physicochemical treatment
Primary settler
Biological treatment
Secondary settler
Gravity thickener
Floating thickener
Biogas
Primary sludge
Secondary sludge
Anaerobic digestion
Blended sludge
b)
Residual sludge
Lipid extraction
Hexane
Hexane
Lipid
Evaporator
Hexane + lipid
Settler
Sludge
Biodiesel (FAME)

Figure 2

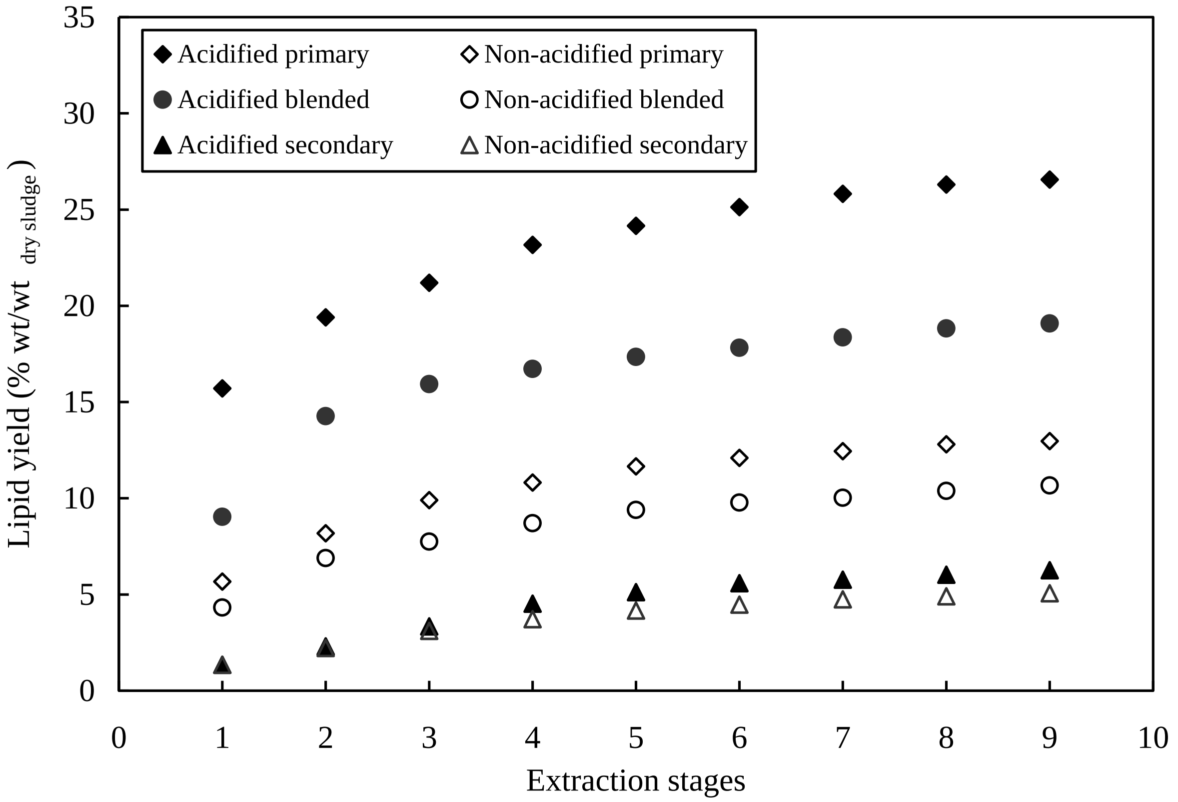

Acidified primary
Non-acidified primary
Acidified blended
Non-acidified blended
Acidified secondary
Non-acidified secondary
Lipid yield (% wt/wt dry sludge)
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6
7
8
9
10
Extraction stages

Figure 3

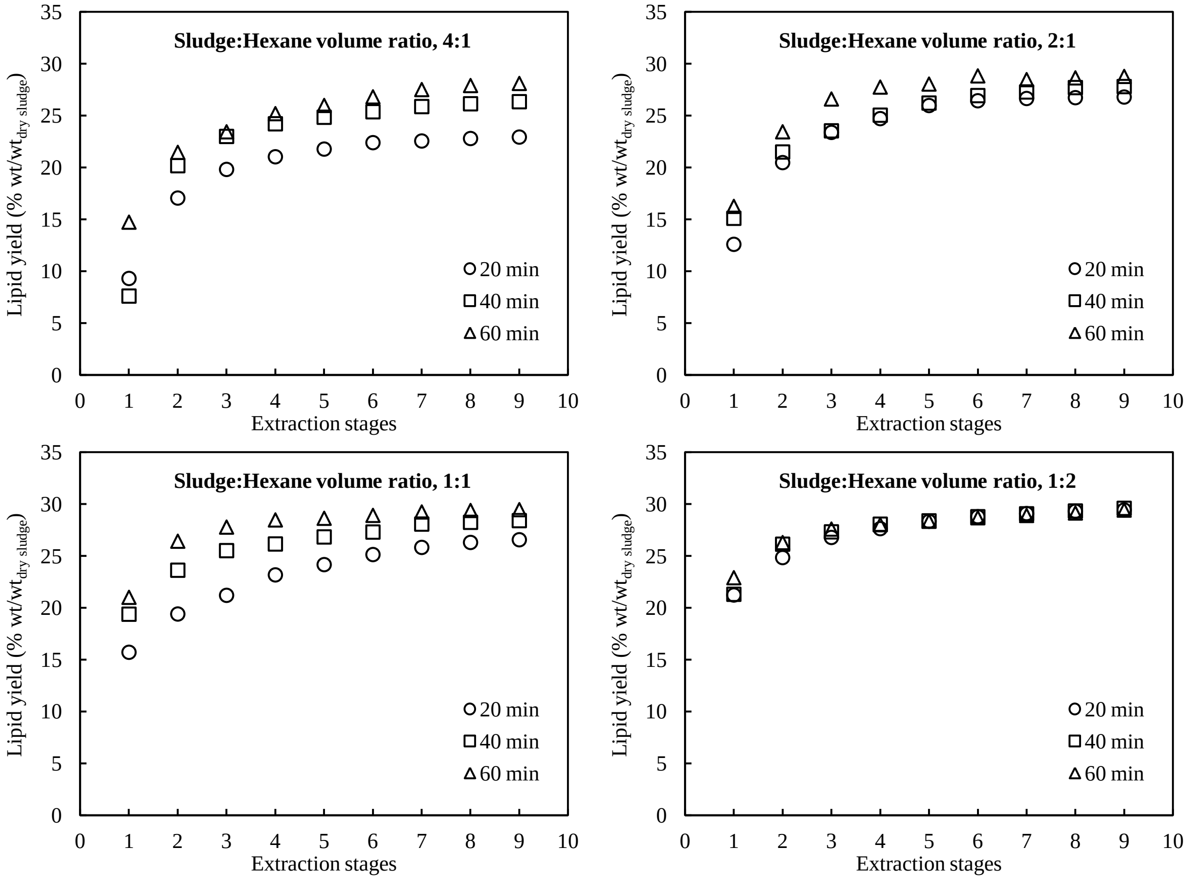

Sludge:Hexane volume ratio, 4:1
Sludge:Hexane volume ratio, 2:1
Sludge:Hexane volume ratio, 1:1
Sludge:Hexane volume ratio, 1:2
Lipid yield (% wt/wt$_{dry\ sludge}$)
Extraction stages
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6
7
8
9
10
○ 20 min
□ 40 min
△ 60 min

Figure 4

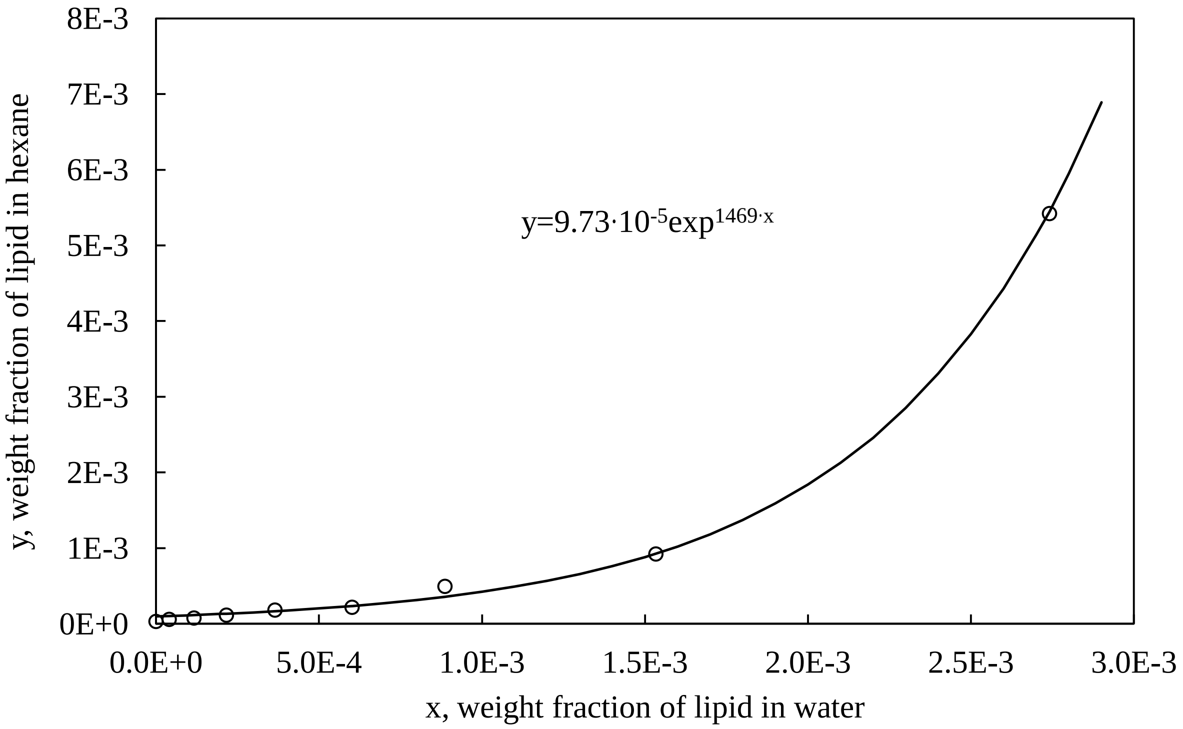
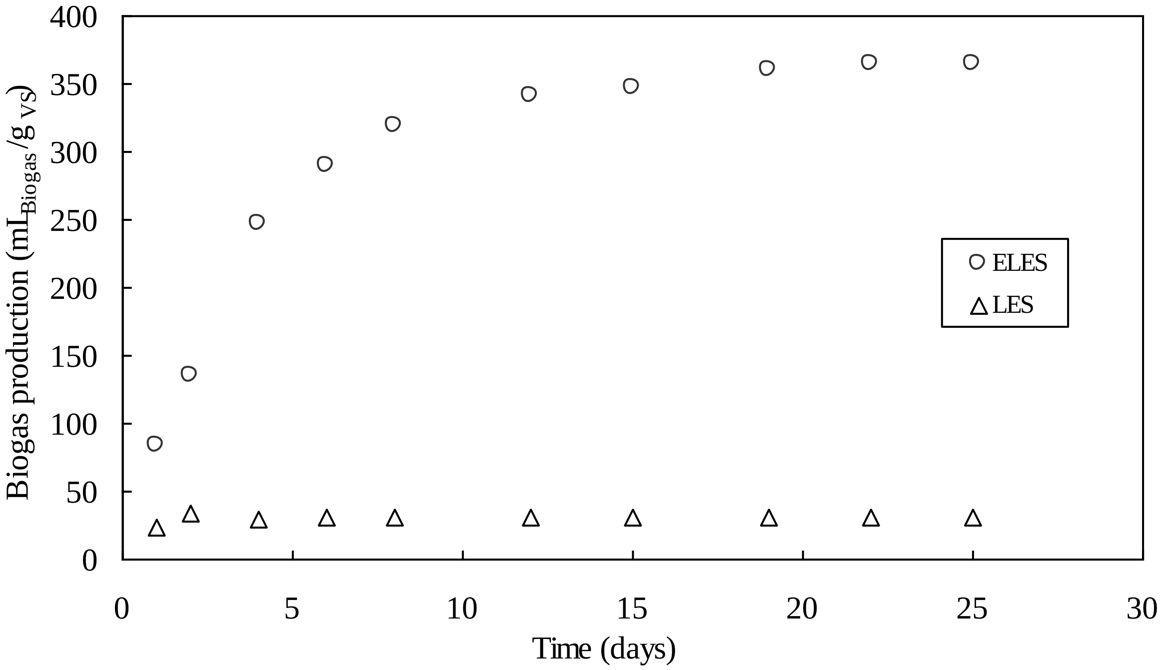

$y=9.73\cdot10^{-5}\exp^{1469\cdot x}$
y, weight fraction of lipid in hexane
8E-3
7E-3
6E-3
5E-3
4E-3
3E-3
2E-3
1E-3
0E+0
0.0E+0
5.0E-4
1.0E-3
1.5E-3
2.0E-3
2.5E-3
3.0E-3
x, weight fraction of lipid in water

Figure 5


Biogas production (mL_{Biogas}/g_{VS})
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Time (days)
○ ELES
△ LES

***Highlights (for review)**

- Sludge drying methods affect the lipid yield, reducing biodiesel production.
- Direct lipid extraction from liquid sludge was successfully developed.
- The method gave high lipid and biodiesel yields after previous sludge acidification.
- Residual lipid-extracted sludge is still a good biomass for biogas production.

Graphical Abstract (for review)