Efficient extraction of lipids from primary sewage sludge using ionic liquids for biodiesel production

Magdalena Olkiewicz [a], Natalia V. Plechkova [b], Azael Fabregat [a], Frank Stüber [a], Agustí Fortuny [c], Josep Font [a], Christophe Bengoa [a,*]

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

[b] The QUILL Centre, School of Chemistry and Chemical Engineering, The Queen's University of Belfast, Stranmillis Road, Belfast, BT9 5AG, Northern Ireland, U.K.

[c] Departament d'Enginyeria Química, Universitat Politècnica 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

This study proposes a novel method to extract lipids from wet primary sludge for biodiesel production using ionic liquids. Tetrakis(hydroxymethyl)phosphonium chloride and widely used 1-butyl-3-methylimidazolium methylsulfate were evaluated to extract lipids from raw and dried sludge (96% and 2%, wt. water content, respectively) and compared to the conventional Soxhlet method using organic solvents. Both these ionic liquids showed suitability for lipid extraction

from raw sludge, giving even better results than expected from dried sludge. The [C_4mim][$MeSO_4$] ionic liquid reached 18.5% and 26.9% of lipids, 14.1% and 18.4% of biodiesel from dried and raw sludge, respectively. The [$P(CH_2OH)_4$]Cl ionic liquid gained 23.4% and 27.6% of lipids, 17.0% and 19.8% of biodiesel from dried and raw sludge respectively, reaching comparable results to the conventional Soxhlet method (27.2% of lipids, 19.4% of biodiesel). Therefore, the proposed ionic liquid process is efficient in lipid extraction directly from wet primary sludge, eliminating the expensive step of sludge drying and the use of volatile organic solvents. Under the optimised extraction conditions using [$P(CH_2OH)_4$]Cl ionic liquid and raw sludge (1:5 sludge (g/TS):IL (cm^3) ratio, 100 ºC and 3 h), the obtained yield of lipids and biodiesel amounted to 25.7% and 21.1%, respectively. Additionally, lipid extraction using [$P(CH_2OH)_4$]Cl ionic liquid also precipitates cellulosic material, which allows for direct and easy cellulose-based co-product recovery, giving high additional value to the process. Consequently, the economic and environmental aspects of biodiesel production from sewage sludge could be improved.

Keywords

Ionic liquid, Primary sludge, Lipid extraction, Biodiesel, Cellulosic material

1. INTRODUCTION

The energy demand from fossil fuels for transportation has been increasing during the last few years, and it will be the strongest growing energy demand sector in the future. However, the expected depletion of fossil fuels and the environmental problems associated with their combustion limit their utilisation in the future [1]. Therefore, the necessity of using alternative renewable fuels, with no environmental impact, is currently increasing. Biodiesel is one of the most promising renewable fuels in road transport, proposed as an alternative to fossil diesel.

However, the competitive potential of biodiesel is currently limited by the high price of common lipid feedstocks, which constitutes between 70-85% of the overall biodiesel production cost, furthermore the cultivation of edible vegetable oils for biofuels raises the concerns of food shortage, which competes with fuel production [1,2]

Nowadays, due to a considerable amount of lipids, municipal wastewater sludge has been receiving progressive attention as a promising non-edible lipid feedstock for biodiesel production [2-9]. In fact, sewage sludge is a waste that needs specific treatment before disposal and represents a major cost in a wastewater treatment plant (WWTP) operation. Therefore, the sewage sludge can be envisaged as a relatively cheap, readily available and non-edible feedstock, which can make biodiesel production profitable. Furthermore, the use of sludge as a source of lipid for biodiesel production is also an alternative to exploit the excess of waste sludge.

Nevertheless, the main challenge to be faced by biodiesel production from waste sludge is the efficiency of lipid extraction from water, which can reach up to 95-98 wt %, as dewatering and drying constitutes more than 50% of the total biodiesel production cost [2,3]. Thus, the cost of energy necessary to eliminate the water, before lipid extraction, is a main limitation for scaling up. Despite this fact, the published data so far have reported only on the utilisation of dried or dewatered sludge in lipid extraction or *in situ* transesterification using organic solvents [2-7,9-11]. Solely, one previous study demonstrated the feasibility of lipid extraction from raw sludge (~96% of water) by direct liquid–liquid extraction using hexane as a solvent [8]. On the other hand, health, security, and regulatory problems related to the use of volatile organic solvents are also very important issues.

In recent years, ionic liquids (ILs) have attracted significant attention for their use as green replacements for harmful volatile organic solvents due to their non-volatile character, excellent chemical and thermal stability, potential recoverability, and design possibilities [12,13]. The

use of ionic liquids for lipid extraction from dry biomass has been successfully studied [14-18]. In addition, the application of ionic liquids to extract lipids from wet biomass has been also noted [14,19]. It was suggested that direct dissolution of wet biomass by ionic liquids could lead to the recuperation of all organic components due to the dissolution of hard cell walls composed mainly by cellulose [19,20]. Hence, the rôle of ionic liquids in the lipid extraction is not only to replace organic solvents, but also the ability to dissolve wet biomass and thereby the possibility to recover other valuable components such as proteins and polysaccharides as cellulose. As municipal primary sludge, apart from lipids, contains proteins and high amount of cellulose, mainly from waste toilet paper [21-23], the recovery of all valuable materials will give added value to the process.

All of the studies about lipid extraction by ionic liquids which are listed above focused on the microalgae biomass and the utilisation of imidazolium-based ionic liquids. The high cost of imidazolium-based ionic liquids [15,24] could limit their availability and suitability for this purpose. On the other hand, phosphonium-based ionic liquid was recently used to extract lipids from microalgae, *Chlorella vulgaris* and *Nannochloropsis oculata* [25]. Furthermore, phosphonium-based ionic liquids offer the advantage of commercial availability (manufactured on a multi-ton scale) and low prices [24]. Nevertheless, the application of ionic liquids for the lipid extraction from sewage sludge as well as the use of phosphonium-based ionic liquids for this purpose have not yet been reported in literature.

Therefore, the aim of this study was to investigate for the first time the feasibility of ionic liquids to extract lipids from wet primary sewage sludge as a green and potentially energy saving system for the production of biodiesel. The comparison of performance between phosphonium and widely used imidazolium ionic liquids was evaluated and compared to the conventional Soxhlet method. The lipid extraction from wet (raw) sludge was evaluated and compared with that from dried sludge, in order to decide on the most economically favourable

process. Additionally, the recovery of other valuable components from primary sludge (cellulose and proteins) was also investigated.

2. MATERIALS AND METHODS

2.1. Reagents

Ionic liquids, 1-butyl-3-methylimidazolium methyl sulfate (>95% purity, 1.21 g cm^{-3} density), [C_4mim][$MeSO_4$] and tetrakis(hydroxymethyl)phosphonium chloride (hydrated ionic liquid, 80% in water, 1.34 g cm^{-3} density), [$P(CH_2OH)_4$]Cl were supplied by Sigma-Aldrich. Transesterification experiments were carried out using anhydrous methanol and sulfuric acid from Sigma-Aldrich, at the highest purity available. Greater than 99% purity sodium chloride, sodium bicarbonate and sodium sulfate anhydrous were also provided by Sigma-Aldrich. Standards used for identification and quantification of fatty acid methyl esters (FAMEs) were supplied by Supelco (37 component FAMEs mix). All other solvents and reagents were high performance chromatography grade and analytical reagent grade provided by Sigma-Aldrich.

2.2. Sludge collection, handling and preparation

Primary sludge was collected from the municipal wastewater treatment plant (WWTP) in Reus (Tarragona, Spain) with a capacity to process near 25,000 m^3 of wastewater per day. The collected sludge was immediately delivered to the laboratory and stored at 4 ºC prior to use. Depending on the experimental design, primary sludge was either used as received (raw sludge, 96% water content) or in dried form (dried sludge, 2% residual water content). The sludge was dried for 2 days at 105 ºC [8].

2.3. Analysis of sludge composition by conventional methods

Total solids (TS), volatile solids (VS) and ash content were analysed according to standard methods 2540B and 2540E respectively [26].

Protein determination was carried out by the Lowry method [27], when the sludge sample was first pretreated by heating with 2 M sodium hydroxide at 100 ºC for 10 min. The absorbance was measured at 750 nm.

The total carbohydrate amount was quantified by the phenol-sulfuric acid method of Dubois [28]. The absorbance was measured at 480 nm.

Lipid extraction was carried out in a Soxhlet apparatus using hexane as a solvent according to standard method 5520E [26]. After extraction, the hexane was removed using a rotary evaporator, the lipids were stored in a desiccator overnight and weighed the next day. The lipid yield was determined gravimetrically and expressed as gram of extractable lipids per gram of dry sludge.

2.4. Lipid extraction using ionic liquids

The primary sludge (dried or raw) and ionic liquid in ratio 1 g TS equivalent to 10 cm^3 ionic liquid were added to a round bottomed flask (50 cm^3) fitted with a condenser. The mixture was heated at 100 ºC in an oil bath for 24 h under magnetic stirring, 500 rpm. After the reaction completed, further procedure was carried out according to our previous study [25]. The optimisation study using $[P(CH_2OH)_4]Cl$ ionic liquid and raw sludge was carried out with varying extraction times (½, 1, 3, 6, 12 and 24 h), extraction temperatures (25, 40, 60, 80 and 100 ºC) and sludge:IL ratios (1:5, 1:10, 1:20; g/TS: cm^3/IL).

2.5. Lipid transesterification and FAMEs analysis

The lipids were converted into FAMEs (biodiesel) through acid catalysis transesterification/esterification using 1v% of sulphuric acid in methanol according to the

150 procedure previously described [8]. Then, the FAMEs were analysed by GC–FID as described
151 previously [8]. The results of GC–FID were used to estimate the amount of saponifiable
152 (transesterifiable/esterifiable to FAMEs) material in the lipid fraction and hence the maximum
153 mass of biodiesel (FAMEs) that could be yielded.
154
155 **2.6. Recovery of precipitated solid**
156 After lipid extraction from raw sludge by ionic liquid, the precipitated solid was recovered for
157 further analysis from the IL/methanol/sludge's water solution by centrifugation (6000 rpm, 10
158 min) and subsequent removal of the aqueous supernatant. The recovered solid was washed with
159 methanol (3×10 cm^3), followed by centrifugation and removal of supernatant. Then the
160 remaining methanol was evaporated in a rotary evaporator and the resulting solid was heated
161 overnight at 105 ºC and afterwards the solid was weighted in order to calculate the yield of the
162 precipitated components. The precipitated solid was further analysed in order to determine the
163 VS and ash content.
164
165 **2.7. FTIR analysis**
166 The precipitated solid, dried sludge, extracted lipids and also cellulose standard were analysed
167 by Fourier Transform Infrared (FTIR) spectroscopy for a comparison study, as described
168 previously [25].
169
170 **2.8. Proteins in ionic liquid**
171 Proteins, which are polar in nature, could be extracted into the polar ionic liquid,
172 $[P(CH_2OH)_4]Cl$, and subsequently recovered. After lipid extraction from raw sludge and
173 precipitation of sludge components, the presence of protein was tested by application of the

174 Lowry method [27] to the recovered ionic liquid solution. The absorbance was measured at 500
175 nm.
176
177 **2.9. Metals in ionic liquid**
178 0.5 cm^3 of recovered ionic liquid was tested for metal content after microwave digestion with 2
179 cm^3 of HNO_3. An inductively coupled plasma-optical emission spectrometry (ICP-OES)
180 SPECTRO ARCOS was employed to measure the metal ion (Fe, Cu, Mg, Na, K, Ca, Al, Zn,
181 Sr, Ba, Co, Ni, Mn, Cr, Pb and Cd) concentrations in the digested ionic liquid solution.
182
183 **2.10. Ionic liquid analysis after extraction**
184 After lipid extraction from raw sludge by $[P(CH_2OH)_4]Cl$ ionic liquid and precipitation of
185 sludge components, the ionic liquid was recovered and its structure was checked by ^1H NMR
186 spectroscopy as described in our previous study [25].
187
188 **3. RESULTS AND DISCUSSION**
189 **3.1. Lipid extraction from sewage sludge by using ionic liquids**
190 The hydrophilic and water miscible ionic liquids were used in this study due to their high
191 capacity to extract lipids by the dissolution of biomass hydrophilic compounds, leaving lipids
192 insoluble [15]. $[C_4mim][MeSO_4]$ was selected as an imidazolium-based ionic liquid due to its
193 high suitability for lipid extraction from microalgae biomass [15,16]. $[P(CH_2OH)_4]Cl$ was
194 chosen as a phosphonium-based ionic liquid which is inexpensive, and commercially available,
195 which showed a great capacity to extract lipids from wet biomass [25].
196 Fig. 1(a)-(c) shows the extraction steps with dried sludge (2% residual water content), using the
197 example of $[P(CH_2OH)_4]Cl$ ionic liquid. Dried sludge, Fig. 1(a), was dissolved by ionic liquid
198 resulting in gel formation, Fig. 1(b), due to the higher viscosity of the solution increasing after

199 sludge dissolution. Then, after methanol addition to the system, phase separation took place,
200 Fig. 1(c), precipitating some of the sludge components (cellulose and/or proteins), giving the
201 white colour of the precipitate, and leaving the less dense lipids insoluble. The same happened
202 when [C_4mim][$MeSO_4$] ionic liquid was used, but with the difference of a dark colour of final
203 precipitated fibrous material, Fig. 2.
204 Fig. 1(d)-(f) shows the extraction steps using raw sludge (96% water content), also as an
205 example of [$P(CH_2OH)_4$]Cl ionic liquid. In the case of raw sludge, Fig. 1(d), the sludge was
206 dissolved by ionic liquid because the colour of the solution changed, and at the same time
207 precipitation of sludge components occurred giving a white fibrous material, Fig. 1(e), leaving
208 the less dense lipids insoluble. Further addition of methanol to the system did not show any
209 changes, Fig. 1(f). This suggests that the water contained within raw sludge plays the rôle of a
210 polar solvent, providing separation of all components at the end of the process. On the contrary,
211 the [C_4mim][$MeSO_4$] ionic liquid shows a distinct behaviour without a colour change of the
212 solution and with the precipitation of dark material, Fig. 2.
213 In both cases (dried or raw sludge), the lipids are not dissolved by ionic liquids and float because
214 of a lower density than the ionic liquids solution. However, the upper lipid phase in Fig. 1(c)
215 and Fig. 1(f) cannot be easily observed as the lipid content in the whole mixture is far too low.
216 The lipid phase can be observed after hexane addition, which dissolves floating lipids as
217 depicted in Fig. 2(a).
218

219 **3.2. Comparison of the different strategies of extraction**

220 **3.2.1. Evaluation of the ionic liquids in lipid extraction from raw and dried sludge**

221 As already discussed, the elimination of water from sewage sludge is a limiting step in the
222 production of biodiesel from these wastes. Therefore, the possibility of using wet (raw) sludge

in lipid extraction by ionic liquids was investigated. The standard extraction method for a sludge sample using Soxhlet apparatus and hexane as a solvent was used as a comparison study.

Table 1 shows the lipid extraction yields obtained by both ionic liquids from raw and dried sludge and by the standard Soxhlet method. In the case of dried sludge, the extraction using both ionic liquids did not achieve the lipid yield obtained by the standard method. The lowest lipid yield was obtained using $[C_4mim][MeSO_4]$ ionic liquid (18.5%) followed by $[P(CH_2OH)_4]Cl$ ionic liquid (23.4%), while the standard Soxhlet method gave the lipid yield 27.2%, on the basis of dry sludge. In the case of extraction from raw sludge, the lipid yield for both ionic liquids increased as compared to the equivalent extractions from dried sludge, reaching the same yield of lipids as the standard method. The $[C_4mim][MeSO_4]$ ionic liquid was able to extract 26.9% of lipids and the $[P(CH_2OH)_4]Cl$ ionic liquid 27.6% of lipids, on the basis of dry sludge, suggesting that the ionic liquid extraction from raw sludge is able to extract all lipids present in the primary sludge.

The lower lipid content extracted from dried sludge by both ionic liquids as compared to the standard Soxhlet method can be explained by the high viscosity of ionic liquids, especially of the $[C_4mim][MeSO_4]$ ionic liquid. Pure ionic liquids are not able to extract lipids from biomass efficiently because of their high viscosity, hampering a good contact with biomass [16]. Therefore, the $[C_4mim][MeSO_4]$ ionic liquid, whose viscosity (213 mPa·s, 25 ºC) is much higher than the hydrated $[P(CH_2OH)_4]Cl$ ionic liquid (35 mPa·s, 25 ºC), gave the lowest lipid yield. On the other hand, in the case of extraction from raw sludge, the high water content (96%) served as a polar solvent which dissolved the hydrophilic ionic liquids, decreasing the ionic liquid's viscosity, thereby enhancing its contact with sludge particles and thus enhancing lipid efficiency. Additionally, the presence of water in the raw sludge allows the precipitation of cellulose and/or proteins, other valuable sludge components, thereby omitting the use of methanol (as commented on in the Section 3.1). This suggests that the water contained within

raw sludge plays a key rôle in the ionic liquid extraction process, in two different ways: as a solvent for decreasing ionic liquid viscosity and thereby enhancing lipid efficiency; and as a polar solvent for the regeneration of other valuable sludge components (cellulosic material and/or proteins). Therefore, the use of methanol is not necessary for separation in the case of raw sludge. The experiment was also conducted without methanol addition, showing no changes in lipid and in biodiesel yields.

Comparing both ionic liquids, the $[C_4mim][MeSO_4]$ ionic liquid produced a dark colour precipitate, Fig. 2(b), suggesting that complete sludge dissolution and regeneration of clean precipitate did not take place. This can be explained by the ability of some ionic liquids to extract inorganic species (ashes). The analysis of metals in both ionic liquids after extraction from raw sludge confirmed that the $[P(CH_2OH)_4]Cl$ ionic liquid is able to dissolve ashes, resulting in the recuperation of clean precipitate, whereas the $[C_4mim][MeSO_4]$ ionic liquid did not show this ability. The $[P(CH_2OH)_4]Cl$ ionic liquid after extraction contained 8% metals, based on the dry sludge used, with a predominance of Ca, Fe, Na, Mg, and K, among others. On the contrary, the $[C_4mim][MeSO_4]$ ionic liquid contained only 1% of metals, based on dry sludge used, of which only Na, Ca, Mg, K were detected. In addition, the mass of precipitated solid after lipid recovery from raw sludge using $[P(CH_2OH)_4]Cl$ and $[C_4mim][MeSO_4]$ ionic liquid amounted to 43% and 72% respectively, on the basis of dry sludge. These results confirm that some part of the sludge components were still dissolved in the $[P(CH_2OH)_4]Cl$ ionic liquid. Whereas, the $[C_4mim][MeSO_4]$ ionic liquid, besides the lipids extraction, left all other components in the sludge, provoking the dark precipitate.

3.2.2. Influence of ionic liquids extraction on the saponifiable lipids and biodiesel composition from raw and dried sludge.

Only the saponifiable part of the total lipids extracted from the sludge can be converted into FAMEs-biodiesel. Thus, the yields of saponifiable lipids and overall biodiesel obtained from primary sludge by the ionic liquids and the standard Soxhlet method were analysed and the results are presented in Table 1.

In the case of dried sludge, the ionic liquid methods were able to extract lipids with slightly higher saponifiable matter than the standard method. The $[C_4mim][MeSO_4]$ ionic liquid extraction showed the highest fraction of saponifiable lipids (76.2%) followed by $[P(CH_2OH)_4]Cl$ ionic liquid which gave 72.3%, a value not significantly different than that of Soxhlet extraction (71.2%). Although ionic liquids extractions from dried sludge gave similar ($[P(CH_2OH)_4]Cl$) or higher ($[C_4mim][MeSO_4]$) yields of saponifiable lipids, the biodiesel yields for both ionic liquids were lower than obtained by Soxhlet method due to much lower lipid yields. Comparing both ionic liquids in the extraction from dried sludge, $[P(CH_2OH)_4]Cl$ ionic liquid gave higher yield of biodiesel than $[C_4mim][MeSO_4]$ ionic liquid (Table 1).

For raw sludge, the $[C_4mim][MeSO_4]$ ionic liquid method resulted in significant reduction of saponifiable matter as compared to dried sludge, from 76.2% to 68.3% for dried and raw sludge respectively, giving a lower result than the Soxhlet method. Whereas, the $[P(CH_2OH)_4]Cl$ ionic liquid extraction gave 71.9% of saponifiable lipids, the result approximates to the extraction from dried sludge (72.3%) and to the standard Soxhlet method (72.2%). Although ionic liquid extractions from raw sludge gave lower ($[C_4mim][MeSO_4]$) or similar ($[P(CH_2OH)_4]Cl$) yields of saponifiable lipids as compared to the equivalent extraction from dried sludge, the overall biodiesel yields were higher due to a much higher amount of total lipid extracted from the raw sludge (Table 1). However, only the $[P(CH_2OH)_4]Cl$ ionic liquid extraction from raw sludge achieved the biodiesel yield as high as standard Soxhlet method, 19.8% and 19.4% respectively, on the basis of dry sludge.

296 The FAME profiles of biodiesel produced from lipid extracted by both ionic liquids were very
297 similar to those of the lipid obtained by the standard Soxhlet method. The same fatty acids were
298 found for all methods with a predominance of palmitic (34%-45%), oleic (23%-34%) and
299 stearic (11%-14%) acids in sludge biodiesel. These fatty acids are comparable with those
300 obtained by direct liquid-liquid extraction using hexane [8].
301
302 **3.3. Optimisation of extraction parameters using raw sludge**
303 As the extraction by $[P(CH_2OH)_4]Cl$ ionic liquid from raw sludge gave higher lipids and
304 biodiesel yields with additional recuperation of other sludge components, the optimisation of
305 extraction conditions was performed using this ionic liquid.
306
307 **3.3.1. Effect of time**
308 The experiments of the influence of extraction time were conducted at 100 ºC using 1:10
309 sludge:IL ratio (g/TS: cm^3/IL). Table 2 shows the study of different extraction time on lipids,
310 saponifiable lipids and biodiesel yields. As can be seen in Table 2, the higher the extraction
311 time, the higher the amount of extracted lipids. In contrast to the lipid yield, the amount of
312 saponifiable lipids decreased with increased extraction time. This suggests that higher
313 extraction time was able to extract more lipids, but the lipid fraction was more contaminated
314 with non-saponifiable matter which is not convertible into biodiesel. Thus, the biodiesel yields
315 remained almost unchanged for all extraction times tested, 19.8% in average. On the other hand,
316 as described in the observation part of Table 2, the extraction time of 3 h showed better
317 recuperation of precipitated material due to better sludge dissolution, giving purer cellulosic
318 material than for 1 h and ½ h of extraction time (the results are discussed in further detail in
319 Subsection 3.4.1). Finally, the extraction time did not affect the FAME profile of biodiesel (data
320 not shown).

3.3.2. Effect of temperature

The experiments of the influence of extraction temperature were conducted for 3 h using 1:10 sludge:ionic liquid ratio (g/TS: cm^3/IL). The results of lipids, saponifiable lipids and biodiesel yields are presented in Table 2. As shown, the lower the temperature of process, the lower the amount of extracted lipids. On the other hand, the reduction of temperature showed an increase in the amount of saponifiable lipids, demonstrating that the higher temperature of extraction gave more lipids contaminated with non-saponifiable matter, which is not convertible into biodiesel. Despite the changes in the amount of extracted lipids and saponifiable fraction, the biodiesel yields remained almost unchanged between 40-100 ºC. The extraction at ambient temperature (25 ºC) resulted in the lowest lipid and biodiesel yields, decreasing by about 20% as compared to higher temperatures.

In order to optimise only lipid extraction for biodiesel production, the best operation temperature would be 40 ºC. However, taking into account the recuperation of other value-added sludge components, as described in the observation part of Table 2, the best temperature condition should be 100 ºC (the results are discussed in further detail in Subsection 3.4.1). Finally, the FAME profile of biodiesel was not affected by the extraction temperature (data not shown).

3.3.3. Effect of sludge:IL ratio

The experiments of the influence of sludge to IL ratio (g/TS: cm^3/IL) were conducted at 100 ºC for 3 h and the results are presented in Table 2. As shown in the table, there is no significant influence of the amount of ionic liquid on the lipids, saponifiable and biodiesel yield. Comparable results were obtained for all IL volumes tested. Therefore, the amount of ionic liquid in the lipid extraction from sewage sludge to produce biodiesel can be reduced to 1:5

346 sludge:IL ratio (g/TS: cm^3/IL). Additionally, the 1:5 sludge:IL ratio did not affect the
347 precipitate, Table 2. Finally, the FAME profile of biodiesel was not affected by the sludge:IL
348 ratio (data not shown).
349
350 **3.4. Characterisation of post reaction components after the $[P(CH_2OH)_4]Cl$ ionic liquid**
351 **extraction from raw sludge**
352 **3.4.1. Precipitated solid**
353 Table 3 shows the amount of precipitate and its ash and VS content after different conditions of
354 ionic liquid extraction. Comparing the extractions at 100 ºC for 1 h, 3 h and 24 h, the extraction
355 duration of 3 h reached the highest amount of precipitated material which contained a similar
356 content of VS as 24 h. Despite the fact that the reaction for 1 h at 100 ºC gave a similar amount
357 of precipitate to 24 h, the VS content was lower, giving precipitate with a higher ash content
358 (dark solids were visible in the precipitate, Table 2) compared to the reactions for 3 h and 24 h.
359 The influence of temperature on the precipitated solids obtained after extraction for 3 h
360 demonstrated that the higher the temperature of process the higher the amount of precipitated
361 solid. Furthermore, below 100 ºC, the VS content in the precipitates decreased, giving darker
362 material, as described in the observation part of Table 2.
363 The precipitate (3 h, 100 ºC) was analysed by FTIR and compared to the analysis of intact
364 sludge before extraction and cellulose standard sample (see Fig. S1 of Supplementary Material).
365 Based on the FTIR data presented in Supplementary Material, it could be stated that the solid
366 precipitated after ionic liquid extraction contains not only cellulose but also proteins. The
367 purified proteins can be further used in bulk chemical market and the cellulose for producing
368 bioethanol or other chemicals.
369
370 **3.4.2. Sludge composition**

371 Table 4 shows the mass balance of primary sludge composition obtained by the conventional
372 method and the ionic liquid methods. The amount of lipid extracted by the conventional method
373 and ionic liquid methods are similar. The differences are found in the quantity of proteins and
374 ashes in the precipitate. The protein content analysed in the ionic liquid after extraction gave
375 lower amount of proteins than total proteins analysed by the conventional method (Table 4).
376 The lower protein content was counterbalanced by the higher amount of precipitated cellulosic
377 material (carbohydrates) after both ionic liquid extractions, confirmed by the FTIR analysis
378 (presence of proteins in precipitate, Fig. S1(a) of Supplementary Material). Thus, the amount
379 of proteins in the precipitate was calculated from the difference between the total proteins
380 obtained by the conventional method and proteins analysed in ionic liquid phase. Furthermore,
381 the quantity of ashes analysed in the precipitate after both ionic liquid extractions is much lower
382 than the amount of ashes in the sludge determined by standard method. This suggests that the
383 ionic liquid is able to dissolve some sludge ashes, reducing the ash content in the precipitated
384 cellulosic material. This was confirmed by the analysis of metals, 7% and 8% in the ionic liquid
385 after extraction for 3 h and 24 h respectively, based on dry sludge used (also commented on in
386 the Subsection 3.2.1). Based on the results presented in Table 4, the cellulose content in the
387 precipitate after ionic liquid extraction for 3 h and 24 h was 61% and 67% respectively.
388
389 **3.5. Energetic evaluation of lipid extraction from raw sludge using $[P(CH_2OH)_4]Cl$ ionic**
390 **liquid**
391 The advantage of using ionic liquid to extract lipids from sewage sludge is not only the
392 elimination of volatile organic solvent but also the possibility of lipid extraction directly from
393 liquid sludge, avoiding the expensive step of sludge drying, and the ionic liquid stability to be
394 reused (data presented in Fig. S2 of Supplementary Material). In addition, the proposed ionic
395 liquid process also eliminates the primary sludge pre-treatment by using strong corrosive acid

which is needed in the classical direct extraction from liquid sludge using hexane, previously reported [8]. On the other hand, the disadvantage of the ionic liquid extraction is the miscibility of the ionic liquid with sludge water. At first sight, it would appear that using wet sludge will result in an energy saving, as the need for drying the sludge is eliminated. However, this is deceptive, as the water now has to be eliminated at the end of the process (by drying the ionic liquid) rather than at the beginning in order to reuse the ionic liquid.

To compare the proposed lipid extraction from liquid primary sludge using ionic liquid with the extraction from liquid primary sludge using organic solvent (*i.e.* hexane) [8], the simplified energetic estimation of the two processes was performed using heating processes, including the heat for recycling solvents through their distillation (detailed data is presented in Table S1 of Supplementary Material). The direct lipid extraction using hexane is performed at ambient temperature therefore, the heating process is eliminated. In the case of ionic liquid extraction, the extraction must be performed at least at 40 ºC to extract all suitable lipids. Although higher amount of solvent is required in the classical hexane extraction to recover at least 90% of lipids, the ionic liquid extract all lipids but the process needs more energy in order to recuperate the ionic liquid from water. The energy required to extract 1 kg of lipids was estimated to be 43 MJ/kg_{lipid} and 238 MJ/kg_{lipid} for hexane and ionic liquid extraction respectively.

The separation of hydrophilic ionic liquids from water for a large scale application (omitting evaporation or distillation which requires a large amount of energy) is still a challenge under investigation [25]. Future investigation is required to well understand and improve the whole process: recovery of cellulose, proteins and metals from the ionic liquid, and the ionic liquid reusability. Afterwards, an appropriate energetic evaluation of the whole process would be performed.

4. CONCLUSIONS

Both ionic liquids show a high potential for direct lipid extraction from raw primary sludge. The ionic liquids were able to extract as high amount of lipids as organic solvent used in standard Soxhlet method. However, only the $[P(CH_2OH)_4]Cl$ ionic liquid reached the same biodiesel yield as the standard method. The FAME profile of biodiesel was not affected by the ionic liquid. There is significant potential using the $[P(CH_2OH)_4]Cl$ ionic liquid for an improved biodiesel production process. The commonly used volatile organic solvents can be successfully replaced by the non-volatile ionic liquid, the expensive sludge drying step can be eliminated, and the ionic liquid can be recycled in this treatment. Additionally, the $[P(CH_2OH)_4]Cl$ ionic liquid was able to recover the cellulose and proteins, together with lipids in one step; which is not the case in organic solvent extraction. The recovery of all valuable components from waste excess sludge can therefore provide a valuable alternative solid waste management strategy in WWTPs.

Acknowledgements

The authors wish to acknowledge Jaume Cabré from the public company Gestió Ambiental i Abastament S.A. (WWTP of Reus, Spain) for his kind collaboration during this project. Magdalena Olkiewicz also thanks the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) of Catalan Government (Spain) for the pre-doctoral scholarship. Financial support for this research was provided by the Spanish Ministerio de Educación y Ciencia and the FEDER, grant (CTM2011-23069). Natalia Plechkova thanks the industrial advisory board of QUILL.

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Figure captions

Fig. 1. Photographs of the extraction steps using $[P(CH_2OH)_4]Cl$, where the experiments shown in (a), (b), and (c) use dried sludge; (d), (e), and (f) use raw sludge. (a) and (d) represent sludge before the experiment. (b) and (e) show sludge dissolved in the ionic liquid after 24 h at 100^0C. (c) and (f) show the systems after methanol addition, when the separation of dissolved components occurred.

Fig. 2. Photographs illustrating the differences after extraction using $[P(CH_2OH)_4]Cl$ and $[C_4mim][MeSO_4]$ from raw and dried sludge: (a) separation of the phases after methanol addition, (b) recovered solids after each extraction.

533 **Table 1.** Extraction and transesterification/esterification yields from raw and dried sludge for
534 each extraction method. IL extraction conditions: 24 h, 100 ºC, 1:10 sludge:IL ratio (g/TS:
535 cm^3/IL).

Sludge type	Extraction methods	Lipid[(a)] / %	Saponifiable[(b)] / %	Biodiesel[(a)] / %
Raw sludge	$[C_4mim][MeSO_4]$	26.9 ± 1.0	68.3 ± 1.2	18.4 ± 0.3
	$[P(CH_2OH)_4]Cl$	27.6 ± 0.6	71.9 ± 1.3	19.8 ± 0.1
Dried sludge[*]	Standard Soxhlet	27.2 ± 0.4	71.2 ± 0.6	19.4 ± 0.2
Dried sludge[**]	$[C_4mim][MeSO_4]$	18.5 ± 1.2	76.2 ± 1.7	14.1 ± 0.3
	$[P(CH_2OH)_4]Cl$	23.4 ± 0.5	72.3 ± 0.6	17.0 ± 0.1

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

[(b)] Transesterifiable/esterifiable lipid yield on the basis of lipid

[*] Sludge dried by $MgSO_4 \cdot H_2O$ according to the standard method 5520E [26]

[**] Sludge dried at 105 ºC for 2 days

Values are means ± SD, n = 3

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538 **Table 2.** Influence of different extraction variables on the results from raw sludge using $[P(CH_2OH)_4]Cl$.

Extraction conditions		Lipid[(a)] / %	Saponifiable[(b)] / %	Biodiesel[(a)] / %	Observations
Effect of time / h 100 ºC, 1:10 sludge:IL ratio (g/TS: cm^3/IL)	½	23.9 ± 0.2	81.2 ± 0.5	19.4 ± 0.1	Dark solids in precipitate
	1	24.6 ± 0.9	80.4 ± 0.9	19.8 ± 0.2	Dark solids in precipitate
	3	25.1 ± 0.9	80.6 ± 0.7	20.2 ± 0.2	White fibrous precipitate
	6	25.2 ± 0.7	78.5 ± 1.3	19.8 ± 0.3	White fibrous precipitate
	12	25.8 ± 0.6	76.7 ± 0.1	19.8 ± 0.1	White fibrous precipitate
	24	27.6 ± 0.6	71.9 ± 0.3	19.8 ± 0.1	White fibrous precipitate
Effect of temperature / ºC 3 h, 1:10 sludge:IL ratio (g/TS: cm^3/IL)	100	25.1 ± 0.9	80.6 ± 0.7	20.2 ± 0.2	White fibrous precipitate
	80	24.0 ± 0.1	82.6 ± 0.2	19.8 ± 0.1	Dark solids in precipitate
	60	23.8 ± 0.2	84.2 ± 0.2	20.1 ± 0.1	Dark solids in precipitate
	40	23.1 ± 0.4	86.4 ± 0.1	20.0 ± 0.1	Dark solids in precipitate
	25	19.2 ± 0.6	83.9 ± 1.0	16.1 ± 0.2	More dark solids in precipitate
Fffect of sludg:IL ratio (g/TS: cm^3/IL) 3 h, 100 ºC	1:20	24.3 ± 1.0	81.0 ± 0.8	19.7 ± 0.2	White fibrous precipitate
	1:10	25.1 ± 0.1	80.6 ± 0.7	20.2 ± 0.2	White fibrous precipitate
	1:5	25.7 ± 1.2	82.1 ± 0.5	21.1 ± 0.1	White fibrous precipitate

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

[(b)] Transesterifiable/esterifiable lipid yield on the basis of lipid

Values are means ± SD, n = 3

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Table 3. Composition of precipitate after $[P(CH_2OH)_4]Cl$ extraction, 1:10 sludge:IL ratio (g/TS: cm^3/IL).

Extraction conditions		Precipitated solid[(a)] / %	Ashes[(b)] / %	VS[(b)] / %
Time / h	Temperature / ºC			
1	100	43.1 ± 0.7	17.4 ± 0.3	82.6 ± 0.3
3	25	37.5 ± 0.9	17.4 ± 0.5	82.6 ± 0.5
	40	39.0 ± 1.4	17.9 ± 0.9	82.1 ± 0.8
	60	39.1 ± 1.1	17.6 ± 0.7	82.4 ± 0.7
	80	41.0 ± 1.0	18.6 ± 0.9	81.4 ± 0.9
	100	48.0 ± 0.8	14.4 ± 0.6	85.7 ± 0.6
24	100	43.1 ± 1.2	15.3 ± 1.4	84.7 ± 1.4

[(a)] on the basis of dry sludge

[(b)] on the basis of precipitated solid

VS – volatile solids

Values are means ± SD, n = 3

Table 4. Comparison of the sludge composition obtained by the conventional and $[P(CH_2OH)_4]Cl$ methods (g/100g, dry sludge).

	Raw sludge[a]	IL extraction			
		3 h, 100 ºC		24 h, 100 ºC	
		IL phase	Precipitate	IL phase	Precipitate
Lipids	27.2 ± 0.4	25.1 ± 0.9[b]		27.6 ± 0.6[b]	
Proteins	24.2 ± 1.4	12.1 ± 0.9	12.1[c]	16.6 ± 1.2	7.6[c]
Carbohydrates	26.2 ± 2.6	-	29.0[d]	-	28.9[d]
Ashes	20.1 ± 0.4	13.2[e]	6.9 ± 0.4	13.5[e]	6.6 ± 0.9
Total	97.7	98.4		100.8	

[a] All analyses according to conventional methods

[b] Lipids in separate upper phase after IL extraction

[c] Calculated from the difference between total proteins obtained by the conventional method and proteins analysed in the IL phase

[d] Calculated from the difference between the precipitate and the proteins in precipitate

[e] Calculated from the difference between total ashes obtained by the conventional method and ashes analysed in the precipitate

Values are means ± SD, n = 3

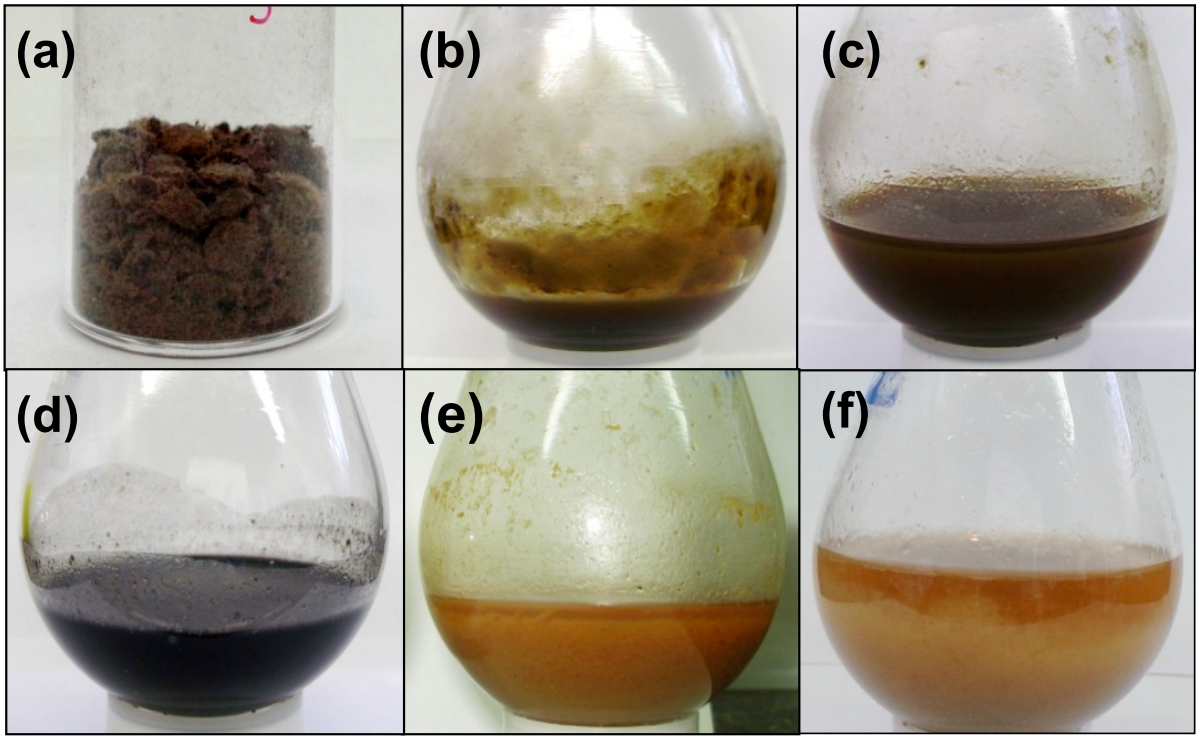

Fig. 1. Photographs of the extraction steps using $[P(CH_2OH)_4]Cl$, where the experiments shown in (a), (b), and (c) use dried sludge; (d), (e), and (f) use raw sludge. (a) and (d) represent sludge before the experiment. (b) and (e) show sludge dissolved in the ionic liquid after 24 h at 100^0C. (c) and (f) show the systems after methanol addition, when the separation of dissolved components occurred.

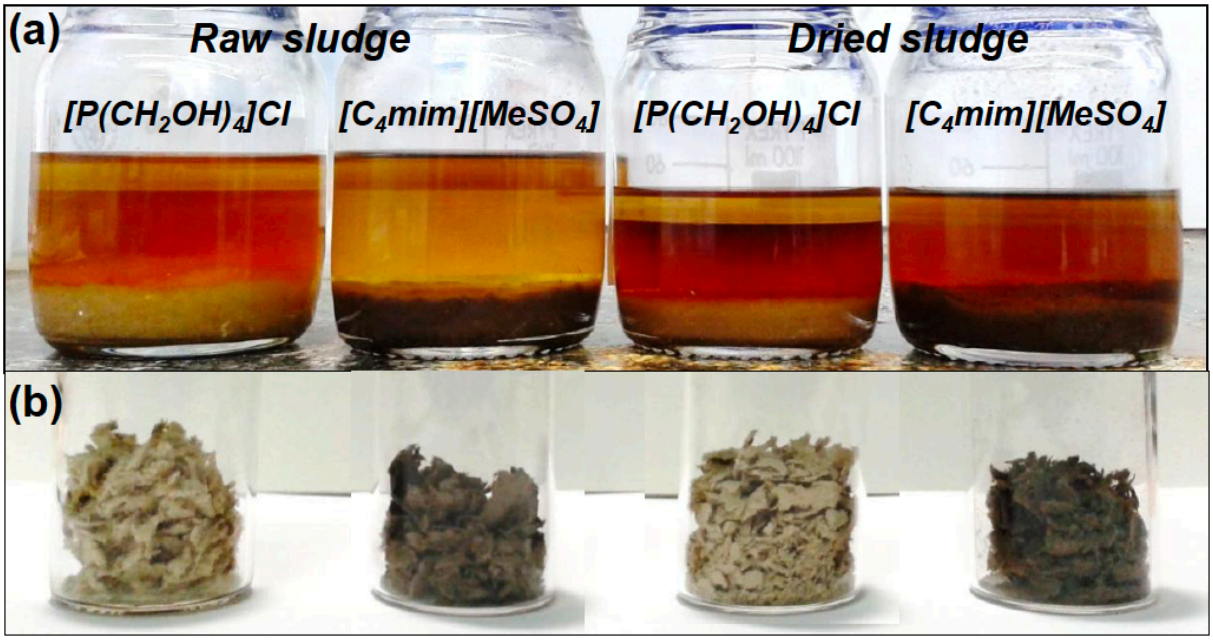

Fig. 2. Photographs illustrating the differences after extraction using $[P(CH_2OH)_4]Cl$ and $[C_4mim][MeSO_4]$ from raw and dried sludge: (a) separation of the phases after methanol addition, (b) recovered solids after each extraction.

Supplementary material

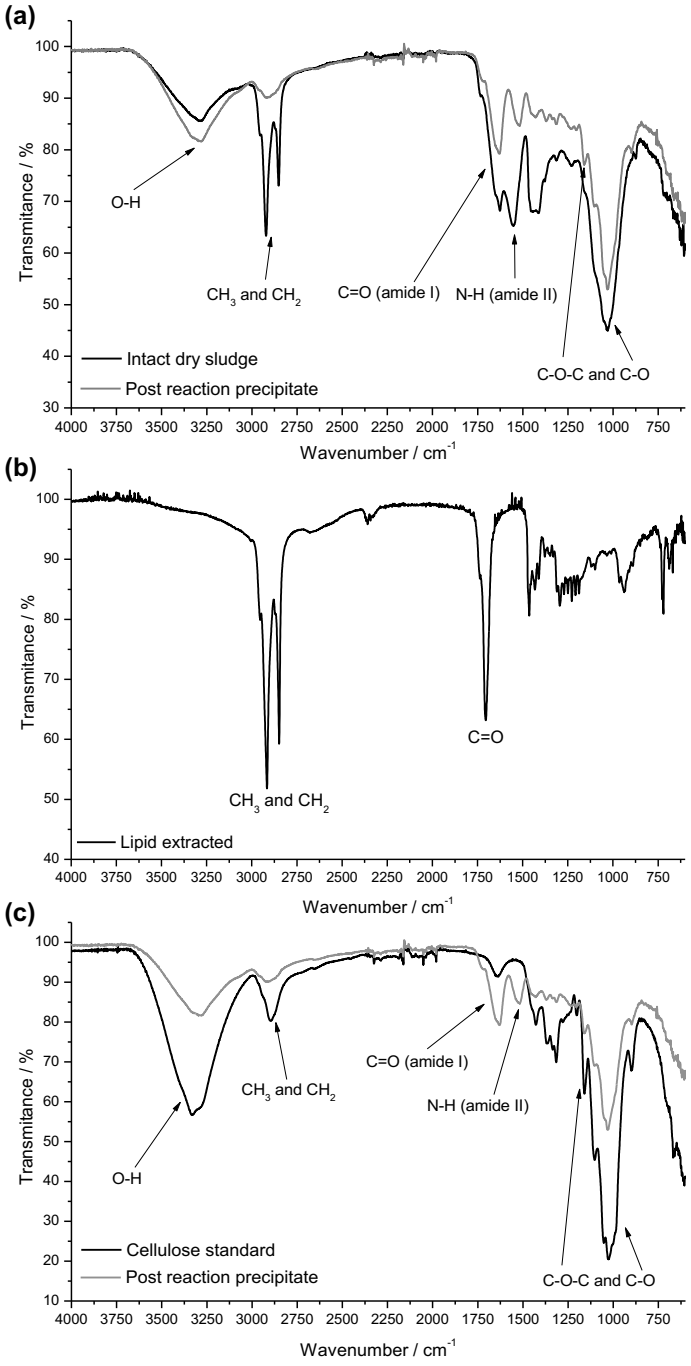

Fig. S1. FTIR spectra of (a) intact dry sludge (black line), precipitated solid after ionic liquid extraction for 3 h at 100 ^{0}C (grey line), (b) lipid extracted using ionic liquid method for 3 h at 100 ^{0}C, (c) cellulose standard (black line), precipitated solid after ionic liquid extraction for 3 h at 100 ºC (grey line).

In order to estimate changes in the main organic components of sludge (lipid, protein and carbohydrates), the FTIR analysis of intact sludge before extraction, post reaction precipitate, extracted lipids and cellulose standard sample were performed, Fig. S1. The lipids, protein and carbohydrates can be easily identified by their characteristic absorbance in different frequency regions: 1700–1750 cm^{-1} for C=O groups in lipid esters (glycerides) as well as in the free fatty acids, and 2800–3000 cm^{-1} for lipid acyl chains; 1500–1700 cm^{-1} for peptide amide groups of proteins; 1000–1200 cm^{-1} for C–O and C–O–C groups of carbohydrates (Olkiewicz et al., Green Chem. 17 (2015) 2813–2824).

As shown in Fig. S1(b), the infrared spectrum of lipids gave the characteristic absorption bands at 1710 cm^{-1} (assigned as the C=O stretching vibration of the ester functional group) and between 2800–3000 cm^{-1} (assigned as the CH_3 and CH_2 stretching vibration in lipid acyl chain). The peak at 1710 cm^{-1} is particularly associated with free fatty acids (FFAs) (Dong et al., Food Anal. Methods 8 (2015) 857-863), suggesting a high content of FFAs in the lipids.

As a result of lipid extraction, the absence of two absorption peaks at 2800–3000 cm^{-1} in post reaction precipitate can be observed in Fig. S1(a). The precipitate also gave less intensified absorption bands in the protein region of 1480–1736 cm^{-1} as compared to intact sludge. These changes suggest that part of proteins also precipitated after ionic liquid extraction. The broad peak around 1035 cm^{-1} (assigned as the C–O stretching vibration of carbohydrates) is present in both; intact sludge and post reaction precipitate. The difference is the appearance of a peak at 1160 cm^{-1} in the spectra of post reaction precipitate, assigned as the C–O–C stretching vibration particularly associated with cellulose (Champagne and Li (2009) Bioresour. Technol. 100 5700–5706; Olkiewicz et al., Green Chem. 17 (2015) 2813–2824). Fig. S1(c) confirms the presence of a peak at 1160 cm^{-1} in the spectra of cellulose standard, showing that the only difference between the spectra of cellulose standard and post reaction precipitate is the absorption bands in the protein region.

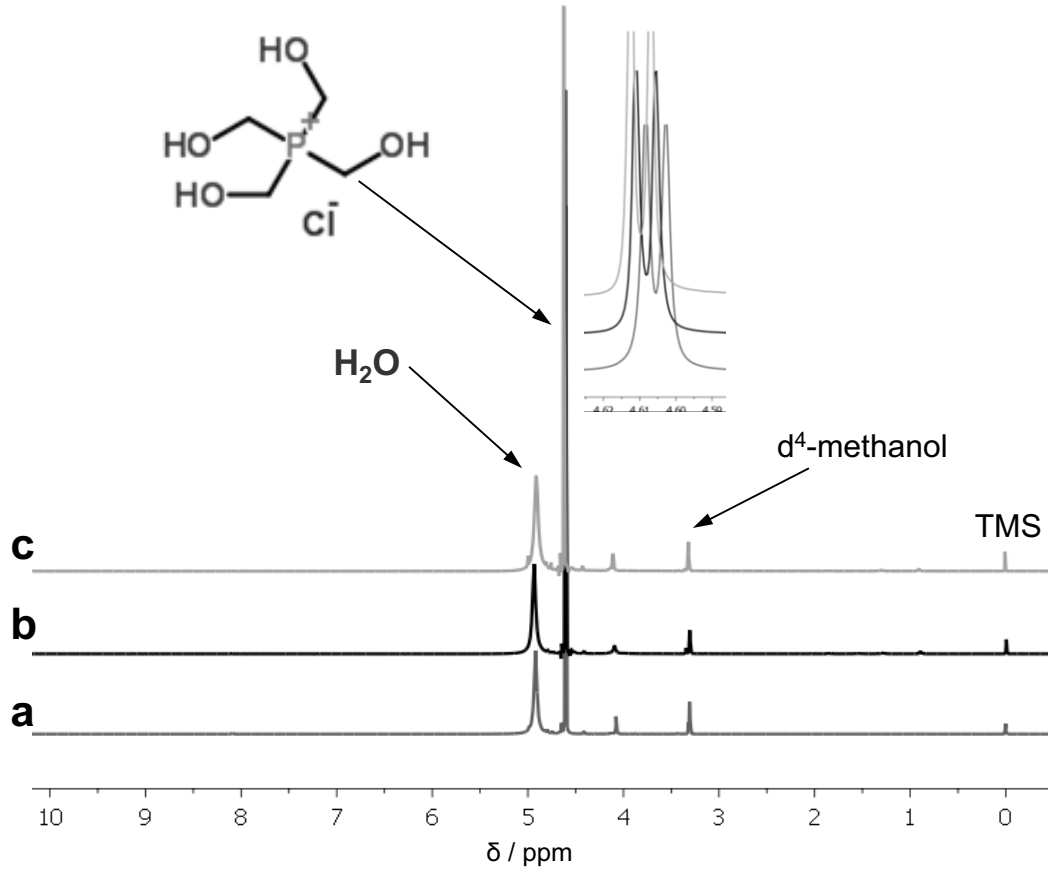

Fig. S2. ^{1}H NMR spectra (d^{4}-methanol) of [P(CH$_2$OH)$_4$]Cl, 80% aqueous solution: (a) fresh ionic liquid, (b) recovered ionic liquid after sludge treatment for 24 h at 100 ºC, and (c) recovered ionic liquid after sludge treatment for 3 h at 100 ºC.

The stability of the [P(CH$_2$OH)$_4$]Cl ionic liquid after extraction from raw sludge at 100 ºC for 3 h and 24 h was checked by ^{1}H NMR spectroscopy, comparing fresh ionic liquid with the ionic liquid after sludge treatment. As shown in Fig. S2, the same peaks were found for fresh ionic liquid and the ionic liquid after both treatments. The doublet at 4.6 ppm (J = 2.2 Hz) is due to PCH$_2$ of the ionic liquid cation, and the singlet at 4.9 ppm is due to water. This suggests that the ionic liquid does not decompose, and can be fully recycled. We obtained the same result when the ionic liquid was used for recovery of lipids from microalgae biomass (Olkiewicz et al., Green Chem. 17 (2015) 2813–2824).

Table S1. Energy requirements for lipid extraction from 100 ml of raw primary sludge (water content 96%) using hexane and $[P(CH_2OH)_4]Cl$ ionic liquid as a solvent.

Process	**Lipid extracted** (g)	**Ionic liquid** ml (KJ)	**Hexane** ml (KJ)	**Water** ml (KJ)	**Total Energy** KJ	**Specific energy** MJ/kg_{lipid}
Hexane extraction[(a)]	1.02	-	150 (44**)	-	44	43
Ionic liquid extraction[(b)]	1.12	25 (11)	-	96 (7*) (249**)	267	238

[(a)] Sequential liquid-liquid extraction, 3 extraction stages, 2:1 sludge:hexane volume ratio, extraction 1 h in each stage (total time 3h), ambient temperature, lipid recovery 91% of total lipids (Olkiewicz et al., Fuel Process. Technol. 128 (2014) 331–338).

[(b)] Ionic liquid extraction, 2:0.5 sludge:ionic liquid volume ratio, extraction time 3 h in each at 40 ºC, total lipid recovery.

* Energy required for heating the water contained into raw sludge to 40 ºC.

**Energy required for heating and evaporating the hexane from lipids and the water from ionic liquids.

The energy needed for heating and evaporating the relevant solvents was calculated using equations of Sensible and Latent Heat. Starting temperature for all solvents was ambient temperature, *i.e.* 20 ºC. Specific heat capacity for the ionic liquid was assumed to be the same as for water. Although in the ionic liquid experimental procedure hexane was used to recuperate the isolated lipids for exact calculation of the yield, this step was not included in the energy calculation. For commercial application the upper lipid phase, formed after ionic liquid extraction, can be easily separated by decantation and directly used.