PRODUCTION OF BIODIESEL FROM SLUDGE GENERATED IN MUNICIPAL WASTEWATER TREATMENT PLANTS.

Magdalena Anna Olkiewicz

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DOCTORAL THESIS

Department of Chemical Engineering

UNIVERSITAT ROVIRA I VIRGILI

Tarragona
2015

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DOCTORAL THESIS

Supervised by Dr. Christophe Bengoa

Department of Chemical Engineering

CREPI research group

UNIVERSITAT ROVIRA I VIRGILI

Tarragona

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I, Dr. Christophe Bengoa, associate professor in the Department of Chemical Engineering of the Rovira i Virgili University,

CERTIFY:

That the present study, entitled "PRODUCTION OF BIODIESEL FROM SLUDGE GENERATED IN MUNICIPAL WASTEWATER TREATMENT PLANTS", presented by Magdalena Anna Olkiewicz for the award of the degree of Doctor, has been carried out under my supervision at the Department of Chemical Engineering of this university, and that it fulfils all the requirements to be eligible for the International Doctorate Label.

Tarragona, 9th June 2015

Dr. Christophe Bengoa

"One never notices what has been done; one can only see what remains to be done."

"Człowiek nigdy nie ogląda się na to, co zrobione, ale na to patrzy, co ma przed sobą do zrobienia."

Maria Skłodowaska-Curie

UNIVERSITAT ROVIRA I VIRGILI
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Summary

Biodiesel is one of the most promising renewable fuels proposed as an alternative to fossil diesel. Biodiesel is predominantly produced from vegetable edible oils; more than 95% of the world's biodiesel is produced from edible vegetable oils. However, the competitive potential of biodiesel is currently limited by the high price of the common lipid feedstocks, which constitutes between 70-85% of the overall biodiesel production cost, strongly influencing the final price of this biofuel. In addition, the excessive cultivation of edible oil seeds for biodiesel raises the concerns of food shortage, which competes with fuel production. Therefore, a low-cost and non-edible feedstock is required in order to reduce the production cost and to facilitate competitiveness with petroleum diesel.

This thesis investigates the utilization of municipal wastewater sludge as a lipid feedstock for the production of biodiesel. As municipal sewage sludge is an inevitable waste, generated in large quantities during treatment of wastewater, the cost of biomass production is eliminated. Therefore, the sewage sludge can be envisaged as a non-cost, readily available in large quantities and non-edible feedstock, which can make the 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 generated in WWTPs.

Lipid extraction from sewage sludge is a first step in the biodiesel production from these wastes. Therefore, the optimisation of lipid extraction from sewage sludge is a major challenge that may affect the economy of the whole process. Thus, the valorisation of sewage sludge for biodiesel production is focused predominantly on the lipid extraction from these wastes and further on the biodiesel production from extracted lipids.

The first study realised in this thesis evaluates the suitability of four sludge types generated in WWTPs (primary, secondary, blended and stabilised) for biodiesel production by lipid extraction using hexane and further lipids analysis. Moreover, the effect of sludge pre-treatments (ultrasonic, mechanical and acidification) on the yield of lipid extracted as well as biodiesel produced is also investigated, as the pre-treatments are able to increase the lipid yield from other biologic samples. The results demonstrate that the ultrasonic and mechanical pre-treatments of sludge are not useful for lipid extraction and thus for biodiesel production. However, an extraction from acidified sludge produces lipids less contaminated with non-saponifiable material, non-convertible to biodiesel. Among the four sludges tested, primary sludge achieved the greatest lipid and biodiesel yields, 27% and 19% respectively, on the basis of dry sludge. The highest biodiesel yields obtained from blended, secondary and stabilised sludge amounted to 15%, 4% and 2% respectively, on the basis of dry sludge. Furthermore, the comparison of sludge fatty acids profile with common biodiesel feedstocks showed suitability of the municipal sewage sludge for biodiesel production.

Although the sewage sludge is a promising lipid feedstock for biodiesel production, 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 lipid recovery, thus reducing the potential biodiesel production. In this context, the present work investigates the classical direct liquid–liquid extraction from raw sludge using hexane in order to eliminate the expensive step of sludge drying and dewatering and thus to decrease the overall biodiesel production cost. This study explores and improves the direct liquid–liquid extraction in order to demonstrate its feasibility, as this alternative can be easily scaled up

using technology currently available. The results indicate that the proposed direct liquid-liquid extraction of lipids from liquid sludge is feasible after previous sludge acidification. The optimisation study demonstrates that, after three extraction stages, 91% of lipid from primary sludge was recovered. The optimised extraction gave slightly higher yield of lipids (27%, dry sludge) and biodiesel (19%, dry sludge) than the standard method (25% and 18% respectively), supporting the suitability of the proposed process. Finally, this work also demonstrates that the residual lipid-extracted sludge is still a good feedstock for energy production via anaerobic digestion.

Since the lipid extraction from liquid sewage sludge is feasible, the expensive sludge drying step can be eliminated, and therefore the overall biodiesel production cost can be reduced. In order to get final price of the biodiesel produced, the economic feasibility of biodiesel production via direct liquid–liquid extraction of lipids from sludge is studied by the simulation of process scale up. This part of the thesis was performed in collaboration with SUSCAPE group (Sustainable Computer Aided Process Engineering), they designed modelled and simulated the continuous process based on the previous laboratory scale data. The results indicate that the biodiesel production via direct liquid-liquid extraction of lipids from primary sludge is economically feasible and more cost-effective than from dry sludge. Under the optimised extraction parameters, the break-even price of biodiesel was estimated to be 1232 $/t, being economically competitive with the current cost of fossil diesel.

Although the technology currently available (liquid-liquid extraction using hexane) allows easy extraction of lipids from sewage sludge, the use of ionic liquids as a green alternative solvent is also investigated in order to improve the environmental impact of the process. The first attempt of using ionic liquids in this thesis work was performed on microalgae biomass during the research stay at QUILL group (Queen's University Ionic Liquid Laboratories) in Belfast. The protocol of using phosphonium-based ionic liquid for lipid extraction from microalgae has been well developed, investigating the influence of the ionic liquid on the lipid and biodiesel yields. After the acquired knowledge at QUILL, the application of ionic liquids to extract lipids from liquid sewage sludge, as a green and potentially energy saving system for biodiesel production, was studied for the first time in this thesis work. The non-volatile ionic liquids also show a high potential for direct lipid extraction from liquid primary sludge, giving results comparable to the classical method. The $[P(CH_2OH)_4]Cl$ ionic liquid demonstrates a high potential reaching 27.6% of lipids and 19.8% of biodiesel based on dry sludge, the results comparable to the standard method (27.2% of lipids, 19.4% of biodiesel). Additionally, the tetrakis(hydroxymethyl)phosphonium chloride, $[P(CH_2OH)_4]Cl$, is very promising due to its ability to recover the cellulose and proteins, together with lipids in one step, giving another advantage over the organic solvents.

Finally, this thesis also investigates the synthesis of biodiesel from extracted lipids using Brønsted acidic ionic liquids as an alternative catalyst, capable to overcome the problems related to conventional catalysts. The Brønsted acidic ionic liquids with an alkane sulfonic acid group show a good catalytic performance for the conversion of sludge lipids into biodiesel. Among the ionic liquid tested, $[mimC_4SO_3H][SO_3CF_3]$ is selected as the best catalyst due to its high catalytic performance and purer biodiesel obtained than the equivalent ammonium ionic, $[N_{6\,6\,6}(C_4SO_3H)][SO_3CF_3]$. The yield of biodiesel reached 90% (based on saponifiable lipids) under the optimised reaction conditions In addition, the ionic liquid has a good reusability and can be easily separated from the biodiesel.

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Chapter 1

Introduction

1. The present world's energy and petroleum scenario

Energy has become a crucial factor for humanity. Nowadays, modern society consumes large amounts of energy to maintain a high standard of living and to ensure economic growth and development. As the world population and industrialization continues to grow, humanity will consistently consume more energy year after year. As we consider the future, the expansion of the global economy, the development of new technologies, new industry and an increase in the world's population, it becomes obvious there will be an increase in the demand for world energy. According to the International Energy Agency, the world will need 37% more energy in 2040 than today [1]. Therefore, we are entering a phase of very high energy consumption growth. The majority of the demand will come from developing countries such as China and India, where the increased energy requirement is driven by strong economic growth [2,3].

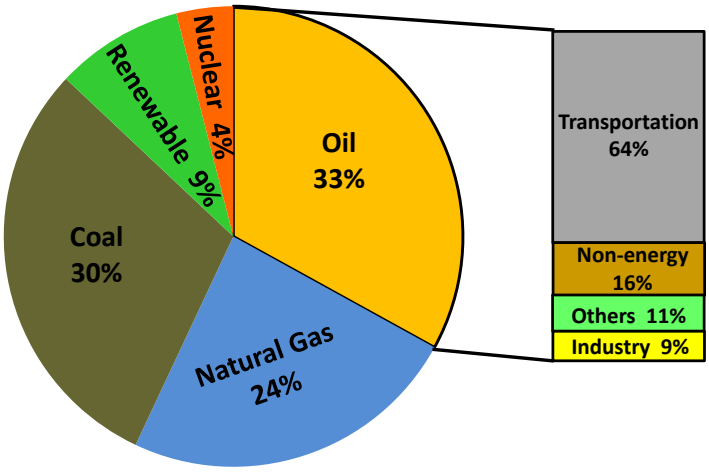

Figure 1. World primary energy consumption by fuel in 2013 [4] and oil consumption by energy sector in 2012 [5].

As shown in Figure 1, currently 87% of all energy consumed worldwide is from fossil fuel sources. The fossil fuel sources are non-renewable, and will be depleted in near future. It is predicted that coal reserves will be available up to 2112 while oil and gas will be depleted by 2040 and 2042, respectively [6]. In addition, the environmental problems associated with excessive usage of fossil fuels such as air pollution, greenhouse gas emissions (GHG), and global warming, consequently limits the utilization of these sources in the future.

At present, within the fossil fuel sources, crude oil represents 33% of world primary energy consumption and it is the leading source of world's energy. As shown in Figure 1, 64% of the world oil demand is consumed by the transportation sector. Globally, this sector is the second largest energy consuming sector, after industrial sector, of which 80% is road transport [2,7]. The demand for petroleum (oil) fuels in the transportation sector has been growing over recent years, and in the future will become the strongest growing energy demand sector, mainly because of steadily increasing numbers of cars around the world, need for personal travel and freight transport. Thus, the demand for fossil oil resources will be rising, which consequently implies the rise in crude oil prices [3,7]. This is also attributed to the fossil fuels vanishing and diminishing, thus becoming harder to extract, for example oil has to be drilled deeper and in more complex environments. As can be seen in Figure 2, the prices of petroleum fuels, i.e., diesel fuel, are driven by the cost of crude oil. The prices have been increasing over the past years and are expected to increase steadily in the future [3,7]. Therefore, the availability of diesel fuel and

gasoline, two major forms of liquid fuels, at prices that are attractive to consumers will be continually decreasing.

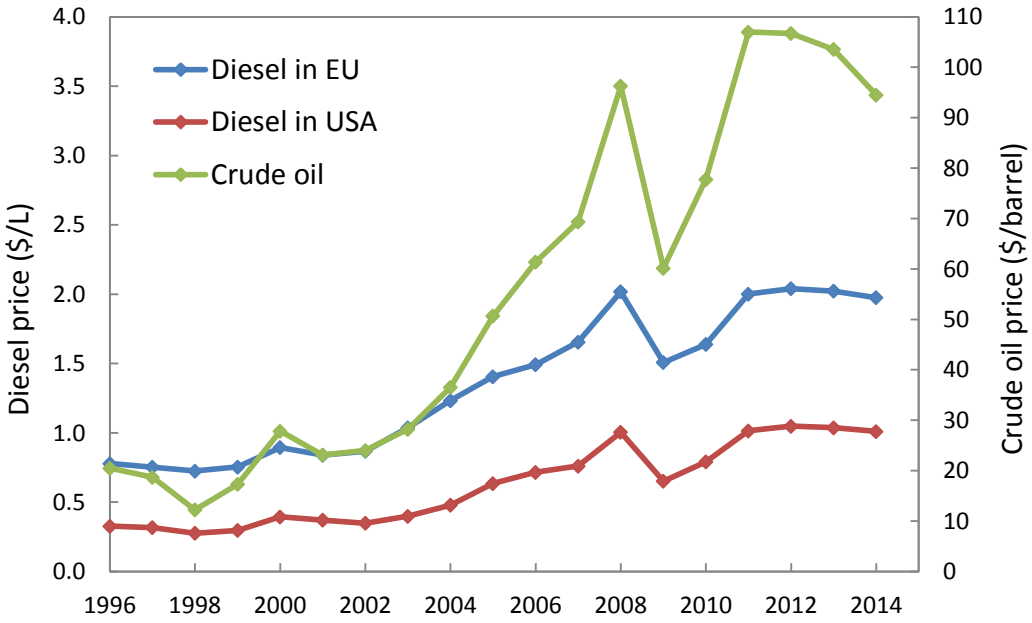

Figure 2. Automotive diesel and crude oil prices between 1996 and 2014 [8,9]. European Union (EU) includes Germany, Italy, France and the United Kingdom. Crude oil includes EU and USA.

In the current context of rising oil prices, growing demand for fuels in the transportation sector, expected depletion of fossil fuels reserve and environmental problems associated with their combustion, the need for alternative renewable fuels with minimal or no environmental impact is currently rising.

2. Renewable fuels for the transportation sector

In order to reduce the excessive use of petroleum in the transportation sector (more than 95% energy consumption in this sector comes from petroleum products [2,10,11] and the carbon dioxide emission (transportation sector accounts for 27% of total world CO_2 emission in 2011, which primarily comes from road transport [7], the use of liquid biofuels from renewable sources is a promising solution. Biofuels are fuels derived from biomass, which can play an important role in reducing CO_2 and all greenhouse gas (GHG) emissions in the transport sector, and represent an alternative to replace at least a portion of the petroleum fuels market. Current and future biofuels production could have important environmental and ecological impacts [11-13]. Furthermore, at present, liquid biofuels are recognized as the most promising alternatives to petroleum fuels in the transportation (gasoline and diesel) as they can be used directly in conventional engine without the need of major modifications [10,13].

Nevertheless, biofuels still represent a small proportion when compared to petroleum products. At present, biofuels provide around 3% of road transport globally [12]. In the European Union (EU) ,the share of biofuels in road transport fuels was around 5% in 2011, an increase of two percent point when compared to 2008 [10,12]. From the EU perspective, as part of the European energy climate policy, a political decision reached has played a fundamental role in the expansion of biofuels. The EU's 2009 directive on renewable energy [14] established a

mandatory target of a 20% share of energy from renewable sources by 2020, and particularly in relation to the transportation sector, the target states that 10% of the final energy consumption in transport should be met by renewable energy by 2020. Under the recent law, first generation biofuels (from crops grown on agricultural land) should account for no more than 7% of final energy consumption in transport by 2020, in order to offset that switched to biofuel production. This is to stimulate the development of alternative, so called second generation biofuels from non-food feedstock, like waste or straw. In relation to this target, liquid biofuels will make a substantial contribution [10-12].

Although different types of liquid biofuels for transportation are recognized, the most important and produced on an industrial scale are bioethanol (substitute for gasoline) and biodiesel (substitute for diesel) [10,12,15].

The present thesis is focused on biodiesel, environmentally friendly and renewable fuel, produced predominantly from vegetable oils or to a lower extent from animal fats.

3. Biodiesel

3.1 Biodiesel origin and characteristics

The possibility of using vegetable oils as an engine fuel was demonstrated in 1900 when Rudolf Diesel's first engine ran on peanut oil. However, further development of vegetable oils as a fuel was suspended due to a wide availability and the low price of petroleum at this time. Additionally, the high viscosity of pure vegetable oils was recognized as a main drawback when used to fuel diesel engine. In 1937, George Chavanne patented the "Procedure for the transformation of vegetable oils for their uses as fuels"; the high viscosity of vegetable oils was decreased by their triglycerides transesterification reaction with methanol or ethanol. However, the full exploration of biodiesel only started in the 1980s, when the renewable energy sources started to gather more attention due to the world's preoccupation in relation to the huge emission of greenhouse gases and recognized depletion of fossil fuel reserves [2,15].

Presently, biodiesel is produced through the transesterification reaction of vegetable oils or animal fats with alcohol (usually methanol) in the presence of a catalyst (usually base), which yields fatty acid methyl esters (FAMEs). This renewable fuel represents an excellent alternative to conventional diesel, as it has chemical properties similar to conventional diesel, and it is compatible with current commercial diesel engine and refuelling technology. Biodiesel may be effectively used as both, blend with conventional diesel fuel and in a pure form. Additionally, biodiesel possesses significant environmental benefits; it is highly biodegradable (four times faster than conventional diesel), nontoxic, safe for storage and handling, it burns much cleaner then petroleum diesel and therefore reduces most exhaust emission (CO_2, CO, hydrocarbons, particulate, except NO_x) and essentially eliminates emissions of SO_x and sulfates as it does not contain sulphur [16-19].

3.2. Biodiesel production

As shown in Figure 3, biodiesel production has grown significantly over the past years. The world biodiesel production reached approximately 22 million tonnes in 2012. In the same year, the EU produced 8.8 million tonnes of biodiesel. The EU is the world's major player in biodiesel production, accounting for 40% of the global production in 2012. Furthermore, biodiesel represents more than 70% of total biofuels production in the EU, 73% in 2009 and 71% in 2011 [11,20]. Moreover, the biodiesel production is rapidly growing in other non

EU countries, such as Argentina, Brazil, USA, China, Thailand and Indonesia, hence the world production is rising fast [21]. Nonetheless, in the EU, for the first time, a decline in biodiesel production was reported in 2011 (Figure 3). The decrease in the EU production is a result of increased imports from other countries such as Argentina and Indonesia. This change in European biodiesel production can be attributed to the high prices of biodiesel feedstock (vegetable oils), the economic crisis and therefore the reduction of tax exemptions and subsidies for biodiesel production by Governments. Without tax exemptions and subsidies, biodiesel production is not cost-effective, forcing biodiesel suppliers to import lower-cost biofuels [11].

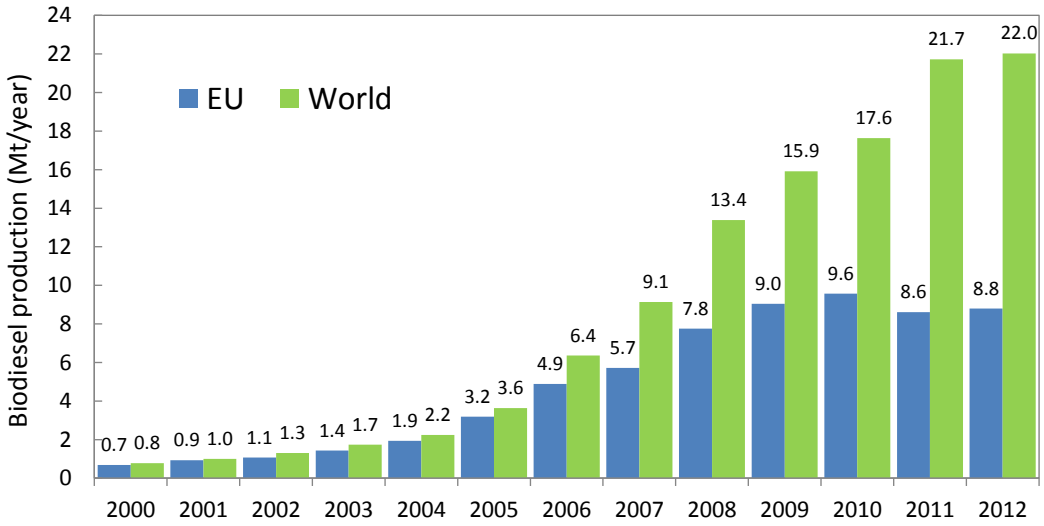

Figure 3. Biodiesel production in the World and in the European Union (EU) between 2000 and 2012 [17,21].

3.3. Biodiesel feedstock

Biodiesel is predominantly produced from vegetable edible oils; more than 95% of the world's biodiesel is produced from edible oils such as rapeseed (84%), sunflower oil (13%), palm oil (1%), soybean oil and others (2%) [2,18]. The increasing demand for the biodiesel feedstock has strongly contributed to increasing the global vegetable oil market over the past years. However, correspondingly, the vegetable oil prices have increased substantially [2,11]. Therefore, the major economic factor which currently limits the biodiesel production is the high cost of biodiesel feedstock, which constitutes 70-85% of the overall biodiesel production cost, strongly influencing the final price of this biofuel [2,11,19].

In addition, the excessive cultivation of edible oil seeds for biodiesel raises the concerns of food shortage, which competes with fuel production. As more vegetable oils are converted to fuels less are available for food, which could have a direct impact on the food price increases over the past few years. Therefore, the continued use of edible oils for biodiesel would lead to food starvation especially in the poor countries, and could also contribute to environmental problems, such as destruction of vital soil resources, deforestation, and usage of much of the available arable land [2,18,22].

Regarding the high cost of edible vegetable oils and the concern of food vs. fuel crisis, the search for low-cost and non-edible feedstock is receiving progressive interest. The most attention has been paid to non-edible plant oils (e.g. jatropha, castor, neem, karanja), microalgae, waste cooking oil and now municipal sewage sludge. Non-edible plant oils are grown in marginal and waste lands with no possibility of land use competing with food

production. However, their excessive cultivation would cause deforestation and destruction of the ecosystem [18]. Microalgae, has long been considered as a promising alternative to vegetable oils, however the production of biodiesel using microalgae is still economically challenging due to high cost associated to biomass production (cultivation/harvesting) [23]. Waste cooking oil is also a promising option because the production step is eliminated, however the major issue is associated with the collection infrastructure and logistics in order to generate sufficient quantities, as the sources are generally scattered [2].

This thesis is focused on the utilization of municipal sewage sludge as a lipid feedstock for the production of biodiesel. As municipal sewage sludge is an inevitable waste, generated in large quantities during treatment of wastewater, the cost of biomass production is eliminated. Therefore, the sewage sludge can be envisaged as a non-cost, readily available and non-edible feedstock, which can make biodiesel production profitable.

4. Municipal sewage sludge

Sewage sludge is a residual material left behind from the treatment of municipal wastewater that includes: household waste liquid from toilets, baths, showers, kitchens, sinks and so forth that is disposed of via sewers. Sewage sludge is a complex heterogeneous mixture of microorganisms, undigested organics such as paper, plant residues, oils and faecal material, inorganic materials and moisture [24].

4.1. Sewage sludge generation and management

As shown in Figure 4, a typical municipal wastewater treatment plant (WWTP) produces two main types of sludge, primary and secondary, with significant differences between their compositions.

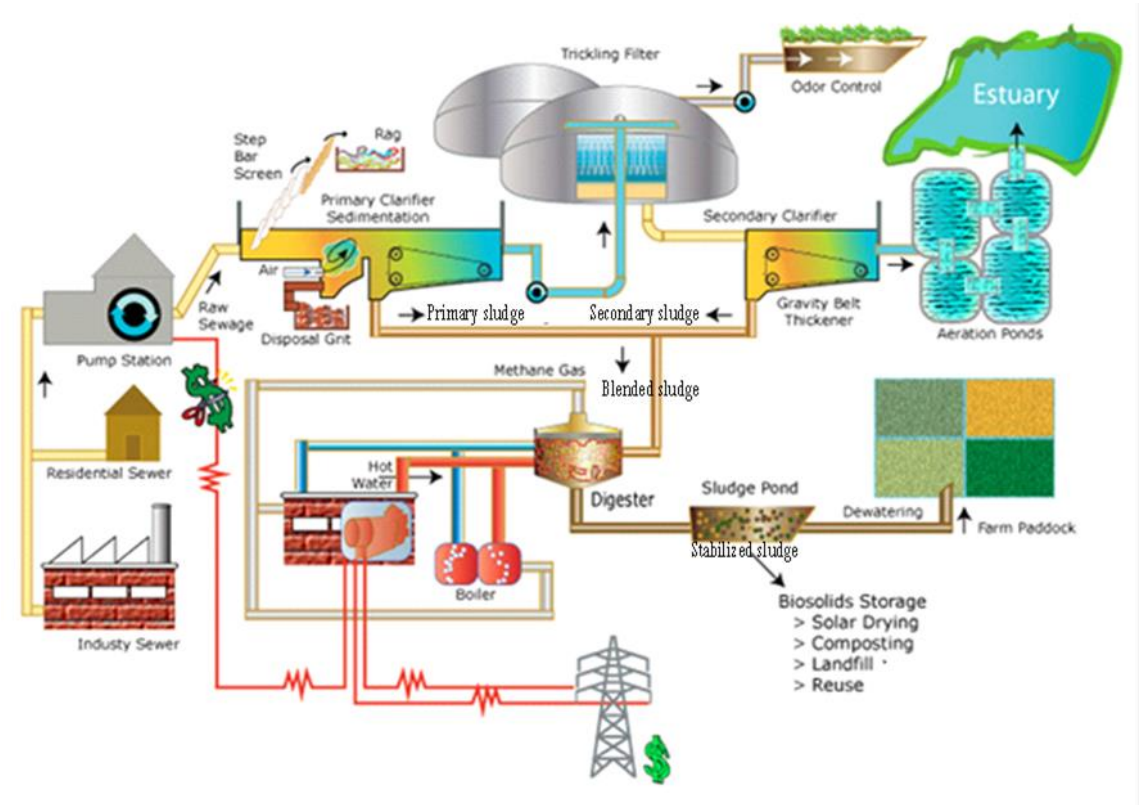

Figure 4. Classical wastewater treatment plant (WWTP) scheme.

The primary sludge is a combination of floating grease and solids, mainly simple organic components (cellulose, lipids, and proteins), collected at the bottom of the primary settler after screening and grit removal. The secondary or activated sludge is a complex biomass, composed mainly of microbial cells and suspended solids produced during the aerobic biological treatment of primary treated wastewater and collected in the secondary settler [19,24-27]. The remainder of secondary sludge, after thickening is mixed with thickened primary sludge and a blend of them is the by-product after wastewater treatment, which usually consists of 60% primary and 40% secondary sludge [27]. In most of the municipal WWTPs, the blended sludge feeds anaerobic digester to reduce the level of pathogens, odours and solids, after which process stabilized sludge is obtained.

At present, approximately 120 million tonnes of dry sewage sludges are produced annually among the USA (7.1 Mt), China (30 Mt), Japan (70 Mt) and the EU (10.2) [28,29]. Furthermore, this already huge sludge production by WWTPs is expected to increase in the future due to the expansion of urbanised and industrialised areas together with the growing number of WWTPs. As an example of the EU (Figure 5), higher quantities of sludge are produced each year, and the number is estimated to increase from 10 million tons (2005) to 13 million tons in 2020.

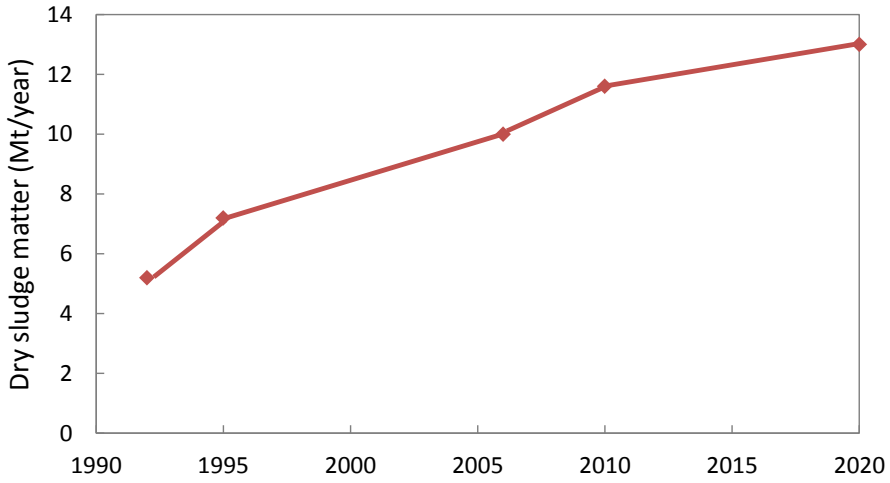

Figure 5. Sewage sludge production in the European Union [30].

Sewage sludge is an inevitable waste, formed during treatment of wastewater which further needs specific treatment before disposal and represents a major cost in WWTP operation, between 50% and 60% of the total cost [27,31]. Additionally, the disposal of sewage sludge poses formidable environmental challenges. The conventional way of sludge disposal via incineration, land application as fertilizer, landfill or ocean disposal has the potential of releasing toxic substances and heavy metals into the environment. [19,25]. Consequently, more stringent sludge disposal criteria has been legislated and for example ocean dumping is banned worldwide [24]. Therefore, the valorisation of raw untreated sludge for renewable energy recovery, to achieve more sustainable sludge management strategy, is being increasingly studied.

In the context of fossil oil depletion, the production of bio-oil from sewage sludge via thermal technologies such as pyrolysis and liquefaction has been investigated. However, the complexity of bi-oil produced by these methods complicates the chemical processes needed to refine it [24,32,33]. On the other hand, there is a

promising potential in the utilization of sewage sludge as a source of lipid feedstock for biodiesel production [25,32,34,35].

4.2. Sewage sludge as a promising lipid feedstock

Nowadays, municipal sewage sludge has been receiving progressive attention as an alternative biodiesel feedstock due to the considerable amount of lipids, up to 30 wt.% based on dry sludge, suitable for biodiesel production [24,32].

Besides the suitable lipid fraction, sewage sludge is considered as potential lipid feedstock for biodiesel production due to its advantages over the conventional vegetable oils such as:

- Sewage sludge is non-edible lipid feedstock, therefore the competition with the food market is eliminated.
- Sewage sludge is cheap or practically costless feedstock, as it is a waste generated in WWTPs, therefore the cost of biomass production and land requirement is eliminated.
- Sewage sludge is readily available and in large quantities as the waste is steadily generated in abundance in WWTPs.

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, consequently lowering WWTP operation cost.

5. Lipid extraction from sewage sludge

Production of biodiesel from sludge poses great challenges for effective commercialisation. Lipid extraction from sewage sludge is a first step in the biodiesel production from these wastes. Therefore, the optimisation of lipid extraction is a major challenge that may affect the economy of the process [19,26].

5.1. Conventional extraction using organic solvents

At present, the main method to extract lipids from sewage sludge is conventional organic solvent extraction. Some studies also investigated the application of subcritical methods for this purpose [32,35-37], however extreme subcritical conditions are not cost-effective for large scale production [38]. In organic solvent extraction, extensive studies were performed to select the best solvents system. Several solvents were investigated such as chloroform, toluene, hexane, methanol and ethanol, alone or in the mixture [31,32,38,39]. The selection of the appropriate solvent should be based on various properties such as polarity, immiscibility with water, boiling point, cost and environmental issue [38]. According to this, chloroform and toluene are not environmentally friendly and the use of solvent mixture is not beneficial because the recovery of solvent from mixture is difficult and more energy intensive [38]. Regarding the polarity, it is well known that non-polar solvents are the best for this purpose because they are able to extract non-polar/saponifiable lipids (glycerides and free fatty acids) convertible to biodiesel. On the other hand, polar solvents result in a high amount of extractable fraction due to the extraction of polar lipid and non-lipid fraction, giving a low percentage of saponifiable lipids, thereby increasing biodiesel contamination. Moreover, the final cost of biodiesel production

using polar solvents (methanol, ethanol) is more expensive than using non-polar solvents (hexane and toluene) [31]. Regarding the environmental aspect of the process, hexane is much more environmentally friendly than toluene [31,38]. The preliminary study done for this doctoral thesis [40], demonstrated that toluene and hexane gave comparable results in term of lipids and biodiesel yields. Consequently, taking into account the safety, environmental issues and higher immiscibility with water, hexane was selected for further study in this thesis.

Sewage sludge lipids come from direct adsorption of lipids (oil, greases, fats and long chain fatty acids) from domestic wastewater in the sludge and/or from the phospholipids in the cell membranes of microorganisms, their metabolites and by-product of cell lysis [38]. Due to the differences in lipid fraction as well as in the sludge type (primary, secondary, blended, stabilized), it was important to evaluate the influence of sludge type on the lipid and biodiesel yield in order to find the most suitable sludge fraction for biodiesel production. Moreover, it has been demonstrated that pre-treatments (ultrasonic and acid hydrolysis) are able to increase the lipid yield from biologic samples but their utilisation to improve lipid extraction from sludge has not been reported. Thus, it is believed that these pre-treatments could also help in lipid extraction from sewage sludge. In these contexts, the first study in the valorisation of sewage sludge for biodiesel production realised in this thesis focused on the influence of sludge type (primary, secondary, blended and stabilised) and sludge pre-treatments (ultrasonic, mechanical and acidification) on the yield of lipid extracted as well as biodiesel produced, the results are presented and discussed in Chapter 2.

If commercial opportunities are to be realised, the sludge valorisation should be focused on the minimization of biodiesel production cost in order to be competitive with current petroleum diesel. The main challenge to be faced by industrial 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 [25,32]. 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 [25,27, 29,32,34-37,38,41]. Moreover, water elimination from biomass by conventional thermal drying or freeze-drying results in the loss of valuable organic compounds [42,43]. This fact can also provoke the loss of lipids in sewage sludge hence decreasing biodiesel yield. Therefore, the first part of the work presented in Chapter 3 evaluates the influence of different sludge drying methods on the lipid and biodiesel yields. The second and the most important part of Chapter 3 investigates the classical direct liquid–liquid extraction from raw sludge, so the sludge drying and dewatering would thus become unnecessary, reducing the production cost. This study explores and improves the direct liquid–liquid extraction in order to demonstrate its feasibility, as this alternative can be easily scaled up using technology currently available.

Since the feasibility of lipid extraction from liquid sewage sludge was demonstrated in Chapter 3, the expensive sludge drying step can be eliminated, and therefore the overall biodiesel production cost can be reduced. However, in order to confirm the stated hypothesis, the full study of economic feasibility of biodiesel production using wet process (direct extraction from liquid sludge) and its comparison with dry process (extraction from dry sludge) is needed. Furthermore, cost-effective production of biodiesel requires continuous operation plant. Therefore, thanks to the SUSCAPE group (Sustainable Computer Aided Process Engineering), the continuous process was design, modelled and simulated based on the batch data and operation presented in Chapter 3,

considering the technology currently available. The economic feasibility study is based on the simulation of the plant in AspenHysys V8® and the economic results are presented in Chapter 4. The simulation of process scale up leads to full techno-economic evaluation of the plant and finally the cost of biodiesel produced. Finally, the optimised biodiesel production process from liquid sludge is compared to the processes using dry sludge (also simulated in this study) in order to decide on the most economically favourable process.

5.2. Ionic liquids as an extracting solvent

The conventional lipid extraction processes from biomass are based on organic solvents; however health, security, and regulatory problems related to the use of volatile organic solvents are also very important issues. In this context, it is reasonable to explore the possibility of using new, more environmentally friendly solvents for lipid extraction.

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 (except at low pressures and high temperature), excellent chemical and thermal stability, potential recoverability, and design possibilities [44]. ILs are defined as salts that are in liquid state at below 100 ºC. The use of ILs for lipid extraction from dry biomass has been successfully studied [45-49]. In addition, the application of ILs to extract lipids from wet biomass (cost of biomass drying is omitted) has been also noted [45,50]. It was suggested that direct dissolution of wet biomass by ILs could lead to the recuperation of all organic components [50,51]. Hence, the role of ILs 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. However, all of the studies about lipid extraction by ILs which are listed above focused on the microalgae biomass and the utilisation of imidazolium-based ILs. The high cost of imidazolium-based ILs [46,52] could limit their availability and suitability for this purpose. On the other hand, phosphonium-based ILs offer the advantage of commercial availability (manufactured on a multi-ton scale) and low prices [52].

As the extraction of lipids from microalgae biomass using ILs are well known, the first attempt of using phosphonium-based ionic liquid was performed on microalgae biomass during the research stay at QUILL group (Queen's University Ionic Liquid Laboratories) in Belfast. The protocol of using phosphonium-based ionic liquid for lipid extraction from microalgae has been well developed, investigating the influence of the ionic liquid on the lipid and biodiesel yields. This work is presented in Chapter 5 of the thesis.

After the acquired knowledge at QUILL, the application of ILs to extract lipids from liquid sewage sludge, as a green and potentially energy saving system for biodiesel production, was studied for the first time and the work is presented in Chapter 6.

6. Biodiesel synthesis

Conversion of sewage sludge into biodiesel includes two steps: lipid extraction (described in the above Section 1.5) and further conversion of extracted lipids into biodiesel, discussed in this Section.

Usually, when conventional feedstock, i.e., vegetable oils, is used, the biodiesel-fatty acid methyl ester (FAME) is synthesised through transesterification of triglicerides with methanol in the presence of basic catalyst, as presented in Figure 6 (a). When high amount of free fatty acids (FFAs) is present in the feedstock, usually in

alternative feedstock (e.g., Jatrhopha oil, microalgae, waste cooking oil, sludge lipids), biodiesel can be also produced from FFAs by esterification reaction with methanol, but acid catalyst must be used, Figure 6 (b).

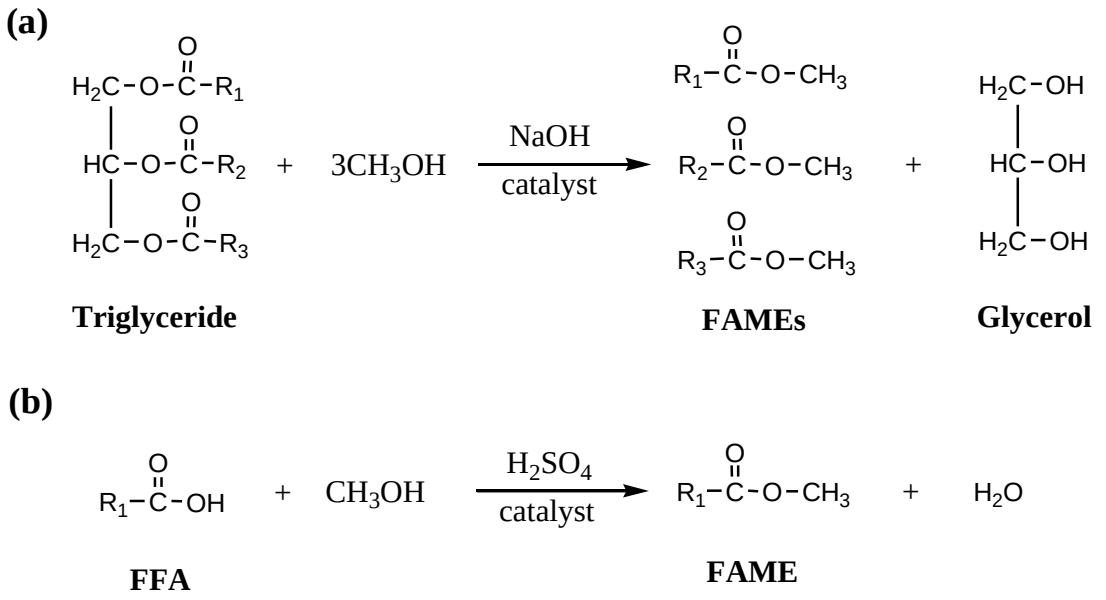

Figure 6. Transesterification of triglyceride (a) and esterification of FFA (b), here R_1, R_2 and R_3 are different or the sane long aliphatic hydrocarbon groups.

6.1. Conventional catalysts

The production of biodiesel form sludge lipids as well as from other low-cost feedstock poses significant processing problems in standard biodiesel manufacturing due to the high free fatty acids (FFAs) content, which in the case of the sludge, can account for up to 65% of the lipid content [39]. The high FFA content of feedstock is known to reduce biodiesel yield significantly when using a conventional basic catalyst (e.g., NaOH) because of soap formation and thus difficulties involved in separating and purifying the product. Acid catalysts (e.g., H_2SO_4) can be used for simultaneous esterification of FFAs and transesterification of glycerides without any soap formation, however the transesterification of glycerides is much slower and the reaction time is much longer to achieve a high conversion. Homogenous acid and base catalysts are corrosive and difficult to remove after the reaction; a large amount of wastewater is produced due to the separation and purification of products and catalyst [53,54]. In order to overcome these problems, clean and promising catalysts are required.

6.2. Ionic liquids as a catalyst

Recent developments have demonstrated that the use of ionic liquids (ILs) in biodiesel production is a promising alternative for efficient green preparation of biofuels. ILs have a great potential for biodiesel production due to the ease of product isolation from the reaction, the possibility of reusing the catalyst and the low environmental impact [53-55]. Several publications have shown that both acidic and basic ILs can function as a good catalyst for transesterification and/or esterification reactions to obtain biodiesel [56-58]. However, in the case of sludge lipids, because of high FFAs content, acidic ILs are preferred. The use of Brønsted acidic ILs as a catalyst for the production of biodiesel from lipid feedstocks with high FFAs content was successfully studied [56,59-62].

Therefore, ILs were shown to have enormous potential for the production of biodiesel from low-cost feedstock as a green replacement for corrosive acid catalysts.

Although extensive studies have been performed in this area, the use of ILs as a catalyst for the esterification of wastewater sludge lipids is absent from literature. Thus, the work presented in Chapter 7 investigates a possible application of Brønsted acidic ILs for the production of biodiesel from sludge lipids.

7. Objectives of the thesis

The overall objective of this thesis is the valorisation of municipal sewage sludge as an alternative lipid source for the production of biodiesel. The sewage sludge valorisation is focused firstly on the lipid extraction from these wastes and further on the biodiesel production from extracted lipids. As the optimisation of lipid extraction from sewage sludge is a major challenge that may affect the economy of the whole process, the main purpose of this work is to investigate different strategies of lipid extraction from sewage sludge, in order to develop a more efficient extraction process. The secondary purpose of this study was to optimise the synthesis of biodiesel from extracted lipids using an alternative to the conventional process.

In order to achieve the main objective, the following specific objectives have been formulated:

- To investigate the influence of sludge types and sludge pre-treatments on the yield of lipid extracted as well as biodiesel (FAMEs) produced. Finally, to compare the composition of FAMEs determined with common biodiesel feedstocks (Chapter 2).
- To study the direct liquid–liquid extraction of lipids from liquid sludge in order to eliminate the expensive step of sludge drying and dewatering and thus to decrease the overall biodiesel production cost. To explore and to optimise this alternative in order to demonstrate its feasibility (Chapter 3).
- To study the economic feasibility of biodiesel production via direct liquid–liquid extraction of lipids from sewage sludge by the simulation of process scale up in order to get the final cost of biodiesel produced (Chapter 4).
- To investigate the feasibility of using alternatives to the conventional organic solvents, i.e., ionic liquids, to extract lipids from liquid sewage sludge as a green and promising new solvent system in lipid extraction for the production of biodiesel (Chapter 6). Additionally, to investigate the feasibility of novel ionic liquid process to extract lipids from other than sludge, non-edible lipid feedstock, i.e. microalgae (Chapter 5).
- To evaluate a possible application of Brønsted acidic ionic liquids with an alkane sulfonic acid functional group as a catalyst for the synthesis of biodiesel from sludge lipids, the low-cost waste feedstock contained a huge amount of free fatty acids. (Chapter 7).

8. Thesis outline

The document is organized into eight chapters outlined below:

- Chapter 1 presents the general introduction and the objectives of this work.

- Chapter 2 investigates the influence of sludge types and sludge pre-treatments on the yield and characteristics of lipid extracted as well as biodiesel produced. This work has been published in the *Fuel* journal.
- Chapter 3 explores the direct liquid–liquid extraction of lipids from liquid sludge in order to eliminate the expensive step of sludge drying and dewatering, optimises this alternative and demonstrates its feasibility. This work has been published in the *Fuel Processing Technology* journal.
- Chapter 4 presents the full study of economic feasibility of biodiesel production via proposed wet lipid extraction process (direct liquid–liquid extraction) by the simulation of biodiesel plant in order to get the final cost of the biodiesel produced. This work is presently under review in the *Computers &Chemical Engineering*.
- Chapter 5 presents the work done during a research stay at the QUILL group in Belfast, when the feasibility of novel phosphonium-based ionic liquid (alternatives to organic solvents) process to extract lipids from non-edible lipid feedstock, i.e. microalgae was studied. This work has been published in the *Green Chemistry* journal.
- Chapter 6 reports the application of ionic liquids, an alternative to the conventional organic solvents, to extract lipids from liquid municipal sewage sludge as a green and promising new solvent system in lipid extraction for the production of biodiesel from these wastes. This work is presently under review in the *Separation & Purification Technology* journal.
- Chapter 7 investigates the application of Brønsted acidic ionic liquids as a catalyst for the synthesis of biodiesel from sludge lipids, the low-cost waste feedstock contained a huge amount of free fatty acids. This work is presently under review in the *Applied Catalysis B: Environmental* journal.
- Chapter 8 presents the main conclusions of this thesis and outlines suggestions for future work in this field.

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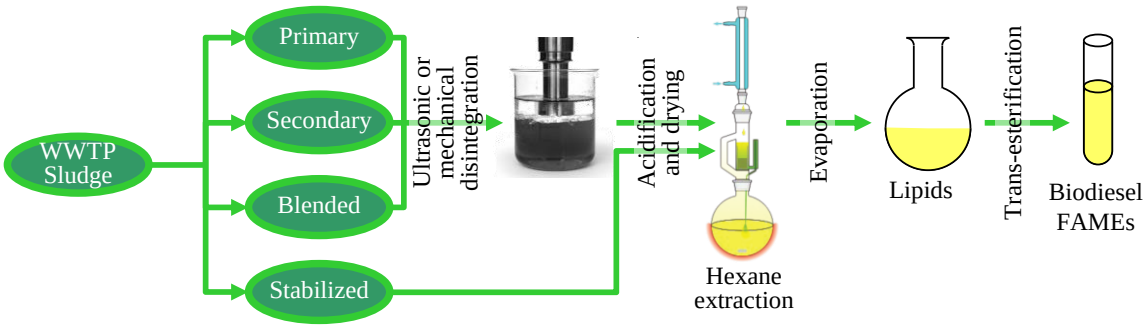
Chapter 2

Effects of pre-treatments on the lipid extraction and biodiesel production from municipal WWTP sludge [1]



ABSTRACT

Biodiesel production is currently limited due to high raw material costs. The potential of using sludge from municipal wastewater treatment plants as an alternative lipid feedstock was investigated. Four different types of sludge (primary, secondary, blended and stabilised) were tested in lipid extraction by Soxhlet using hexane, and biodiesel production by acid catalysis. To improve the extraction efficiency, the influence of pre-treatment methods (ultrasonic and mechanical disintegration) and duration of these treatments were investigated. Finally, the effect of sludge acidification with concentrated HCl was also evaluated. The pre-treatment methods did not increase significantly the amount of extracted lipid as well as biodiesel yield. Previous sludge acidification showed lower yield of lipids from primary, secondary and blended sludge. However, the amount of saponifiable lipids was higher, giving the overall biodiesel yield almost unchanged. Among the four sludges tested, primary sludge achieved the greatest lipid and biodiesel yields, 27% and 19% respectively, on the basis of dry sludge. The highest biodiesel yields obtained from blended, secondary and stabilised sludge amounted to 15%, 4% and 2% respectively, on the basis of dry sludge. No significant influence of the pre-treatments and acidification on the fatty acid composition was found. At least 8 fatty acids were determined, with a predominance of palmitic (C16:0), stearic (C18:0) and oleic acid (C18:1). The comparison of sludge fatty acids profile with common biodiesel feedstocks showed suitability of WWTP sludge for production of biodiesel.

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1. Introduction

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

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

It has been demonstrated that ultrasonic pre-treatment [14,15] and acid hydrolysis [16] are able to increase the lipid extraction yield from biologic samples but their utilisation to improve lipid extraction from sludge has not been reported. These pre-treatments are able to release the lipids from other macromolecules which are not available to solvent in bonded form. Therefore, the utilisation of the sludge pre-treatments could also improve the efficiency of extraction. The most common methods of sludge pre-treatment are ultrasonication and mechanical disintegration, commonly used to enhance biogas production [17,18,19,20]. Ultrasonic energy is able to disintegrate sludge flocs and disrupt large organic particles, breaking down bacterial cell wall and releasing intracellular substances and extracellular polymeric substances into aqueous phase [14,15,17,20]. Mechanical disintegration is used to reduce size of the sludge particles, disintegrate cells and release organic components into sludge [18,19]. On the other hand, acid hydrolysis of sludge is another pre-treatment method used to increase the solubility of the organic matter contained within sludge and thus to reduce the amount of sludge and improve its dewaterability [21]. As sewage sludge is a processed sample, in which lipid can be bonded to proteins, carbohydrates and/or minerals, the proposed pre-treatment methods could facilitate the extraction of lipids by sludge disintegration.

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

2. Materials and methods

2.1. Chemicals

Lipid extraction experiments were conducted using hexane of laboratory reagent grade (ref: 208752) and magnesium sulphate monohydrate (ref: 434183) purchased from Sigma-Aldrich. Fuming hydrochloric acid (ref: 84418) used for sludge acidification was purchased from Fluka. Transesterification experiments were carried out using hexane (ref: 34859), anhydrous methanol (ref: 322415) and sulphuric acid (ref: 33974) from Sigma-Aldrich at the highest purity available. Sodium chloride (ref: 71379), sodium bicarbonate (ref: S6297) and anhydrous sodium sulphate (ref: 239313) were provided by Sigma-Aldrich. Standard used for identification and quantification of fatty acid methyl ester (FAMEs) was supplied by Supelco (37 component FAMEs mix, ref: 47885-U). Analytical standards of free fatty acids (FFA) were provided by Sigma-Aldrich (C12 ref: L556, C14 ref: 70082, C15 ref: 96125, C16 ref: P0500, C16:1 ref: P9417, C18 ref: S4751, C18:1 ref: O1008, and C18:2 ref: L1376. High Performance Liquid Chromatography (HPLC) grade toluene (ref: 650579) used for preparation of FFAs solution was also provided by Sigma-Aldrich.

2.2. Sludge collection, handling and characterisation

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

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

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

[(a)] Each value is the average of at least 3 samples collected on different days.
[(b)] Primary and secondary at a ratio of 65:35, v/v.
n.m.: not measured

Each sample of received sludge was characterised in order to determine total solids (TS) and volatile solids (VS) content, both according to standard method 2540G [22]. Chemical oxygen demand (COD) was measured in a

UV-spectrophotometer (DINKO UV-VIS 800 spectrophotometer) according to standard method 5220D [22]. The Sludge characteristics are given in Table 1. As the sludge composition varies during the wastewater treatment and depends on the specific treatment applied, therefore the stabilized sludge gave the largest content of TS and VS due to the water elimination by filter press system, and the primary sludge gave higher quantity of TS, VS and COD than blended and secondary.

2.3. Pre-treatment of sludge samples

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

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

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

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

2.4. Extraction of lipids

In order to compare the influence of pre-treatment methods, the extraction experiments were carried out using untreated, ultrasonically disintegrated and mechanically disintegrated primary, blended and secondary sludge with and without acidification. The stabilized sludge was subjected to lipid extraction with and without sludge acidification. According to standard procedure the lipid was extracted from acidified untreated sludge 5520E [22], and the utilisation of acidified untreated sludge in the extraction was used as a reference method.

Before the extraction, the samples were dried by adding magnesium sulphate monohydrate according to standard method 5520E [22]. The mixture was stored in a desiccator at room temperature overnight. The lipid extraction procedure was carried out in a Soxhlet apparatus using hexane as a solvent, according to standard method 5520E [22]. After the lipid extraction, the hexane was removed from the flask using a rotary evaporator. The flask, containing the lipids, was stored in a desiccator overnight and weighed the next day. The yield of extracted material was determined gravimetrically and expressed as weight of lipid extracted per weight of dry sludge. After the quantification, the lipids were dissolved in hexane, and kept frozen at –20ºC until further analysis.

2.5. Lipid and biodiesel analysis

The amount of FFA in extracted lipid was determined using an Agilent gas chromatograph 6890GC with a flame-ionization detector (GC–FID). Separation was achieved in an Agilent HP-INNOWax column (19091N-133) using helium as a carrier gas. The injection volume of a sample was 1.5 mL with a split ratio 50:1. The oven temperature programme began at 60 ºC, holding for 2 min and increased by 10 ºC/min to 200 ºC, and then increased by 10 ºC/min to 240 ºC, holding for 12 min. The detector and injector temperature were set at 250ºC for the duration of the analysis.

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

3. Results and discussion

3.1. Influence of sludge type

3.1.1. Lipid extraction yield

The lipid yield extracted from the four type of sludge tested is illustrated in Table 2. The values represent the average of at least three different samples collected in WWTP during several months.

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

		Sludge [(a)]			
Acidification	Pre-treatment	Primary	Secondary	Blended [(b)]	Stabilised
Non-acidified	Untreated	27 ± 1	9 ± 1	21 ± 1	9 ± 1
	Ultrasonic	27 ± 1	10 ± 1	23 ± 3	-
	Mechanical	26 ± 1	9 ± 1	22 ± 3	-
Acidified	Untreated	25 ± 1	7 ± 1	20 ± 1	10 ± 2
	Ultrasonic	25 ± 1	8 ± 1	22 ± 2	-
	Mechanical	25 ± 1	8 ± 1	20 ± 2	-

[(a)] Each value is the average of at least 3 samples collected on different days.
[(b)] Primary and secondary at a ratio of 65:35, v/v.

Irrespective of the sludge pre-treatments and acidification, the primary sludge achieved the greatest lipid yield (27%) followed by blended (21%), secondary (9%) and stabilised (9%). This fact was predictable because the composition of primary sludge consists essentially of organic matter originated from raw wastewater, which is a combination of floating grease and solids (the highest lipid fraction). On the other hand, secondary sludge is composed mainly of microbial cells and suspended solids produced during the aerobic biological treatment of the primary treated wastewater; the lipid fraction comes mainly from extracellular polymeric substances and cell membrane of microorganisms. As blended sludge is a mixture of primary and secondary, with a higher fraction

of the first one, the result is slightly lower than of the primary. Finally, in the case of stabilised sludge, it comes from anaerobic digestion process of blended sludge, during which the organic matter is degraded into intermediary products then converted into methane. However, stabilised sludge gave the same yield of lipid as secondary sludge. This is due to possible co-extraction of non-lipid fraction which contributes to the increase in gravimetric yield (see Section 3.1.2.).

3.1.2. Biodiesel (FAME) yield

Total lipid content extracted from sludge is not the real one that could be converted to FAMEs-biodiesel. Lipid fraction extracted from sludge using non-polar solvent can consist not only of acyglycerols, free fatty acids and some waxes (saponifiable lipids), but also of hydrocarbons, pigments, trepans, linear alkyl benzenes, polycyclic aromatic hydrocarbons, sterols and other waxes [8,24]. Only acyglycerols and free fatty acids that represent the saponifiable part of lipids are suitable for biodiesel (transesterifiable/esterifiable to FAMEs). Hydrocarbons, other non-polar substances (non-lipids) that could be co-extracted with hexane, and also part of lipids like some waxes and sterols are considered as non-saponifiable lipids. Non-saponifiable lipids are not convertible into biodiesel and represent lipid contaminants. Therefore, the yields of saponifiable lipids and overall biodiesel yields produced from the four sludge types were analysed and the results are presented in Table 3.

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

Acidification	Pre-treatment	Sludge [(a)]							
		Primary		Secondary		Blended [(b)]		Stabilised	
		Saponifiable	**Biodiesel**	**Saponifiable**	**Biodiesel**	**Saponifiable**	**Biodiesel**	**Saponifiable**	**Biodiesel**
Non-acidified	Untreated	69 ± 7	18 ± 1	41 ± 6	4 ± 1	56 ± 8	11 ± 1	14 ± 1	1 ± 1
	Ultrasonic	70 ± 5	19 ± 2	42 ± 5	4 ± 1	53 ± 6	12 ± 1	-	-
	Mechanical	65 ± 10	17 ± 2	42 ± 5	4 ± 1	60 ± 5	13 ± 1	-	-
Acidified	Untreated	76 ± 3	19 ± 1	42 ± 4	3 ± 1	64 ± 8	13 ± 2	15 ± 1	2 ± 1
	Ultrasonic	75 ± 4	19 ± 1	45 ± 4	4 ± 1	68 ± 6	15 ± 3	-	-
	Mechanical	74 ± 6	19 ± 2	43 ± 2	3 ± 1	67 ± 6	14 ± 2	-	-

[(a)] Each value is the average of at least 3 samples collected on different days.
[(b)] Primary and secondary at a ratio of 65:35, v/v.

Irrespectively of sludge pre-treatments and acidification the primary sludge achieved the greatest saponifiable yield (69%) followed by blended (56%), secondary (41%) and stabilised sludge (14%). Thus, the overall biodiesel yields were 18%, 11%, 4% and 1% for primary, secondary, blended and stabilized untreated sludge, respectively. Comparison between stabilised and secondary sludge indicates that although the amount of lipid extracted from both was the same (Table 2), the secondary gave a higher overall biodiesel yield owing to a much larger amount of saponifiable matter in the lipid extracted. This shows that an extraction from stabilised sludge produce lipids heavily contaminated with non-saponifiable material, causing a lower productivity of biodiesel. Among the sludge tested, primary sludge has the higher quantity of extractable lipids and additionally it has also the best quality of lipids that are able to form FAMEs. For these reasons, primary sludge can be considered a better feedstock for biodiesel production than other type of sludge generated in WWTPs. On the other hand, the elimination of lipids from primary sludge can significantly reduce the amount of lipids in blended sludge which is the feed of the anaerobic digester. It is known that significant amount of lipids can negatively affect anaerobic

digestion process [25]. Therefore, the extraction of lipids from primary sludge to produce biodiesel will additionally improve the performance of the anaerobic digester and the production of biogas.

3.1.3. Fatty acids composition

Fatty acid compositions of biodiesel produced from sludge are presented in Tables 4. All types of sludge have a significant amount of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). These results are confirmed by the comparison with the composition of human faecal fatty acids, dominated by C16:0, C18:0 and C18:1 and by kitchen wastes, dominated by C16:0, C18:0, C18:1 and C18:2 [24].

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

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

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

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

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

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

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

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

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

3.1.4. Comparison of sludge oil with other biodiesel feedstock

As primary sludge achieved the best yield of lipid and the highest overall yield of biodiesel, the fatty acid composition of primary sludge was compared to common biodiesel feedstocks as shown in Figure 1 [26,27]. It can be observed that the most of fatty acids found in the sludge are the same as compared to other feedstocks; palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). The important difference is observed in the lower amount of C18:2 and the absence of C18:3 in sludge fatty acids profile as compared to the profiles of soybean and rapeseed. This fact is an advantage because these polyunsaturated fatty acids can undergo reactions such as auto-oxidation due to the bis-allylic position of the carbon double bonds, provoking a destabilisation of biodiesel [28,29]. The polyunsaturated fatty acids in the biodiesel from primary sludge constitute only 7%, as compared to 61%, 31% and 30% in the biodiesel from soybean, rapeseed and waste cooking oil, respectively. This fact is an interesting benefit because feedstock rich in saturated or monounsaturated fatty acids gives biodiesel higher oxidation stability [26,29].

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

included as "others" in Tables 4 (see Section 3.1.3.). Thereby, the cold flow properties of biodiesel produced from primary sludge could be even better because of the presence of other fatty acid methyl ester.

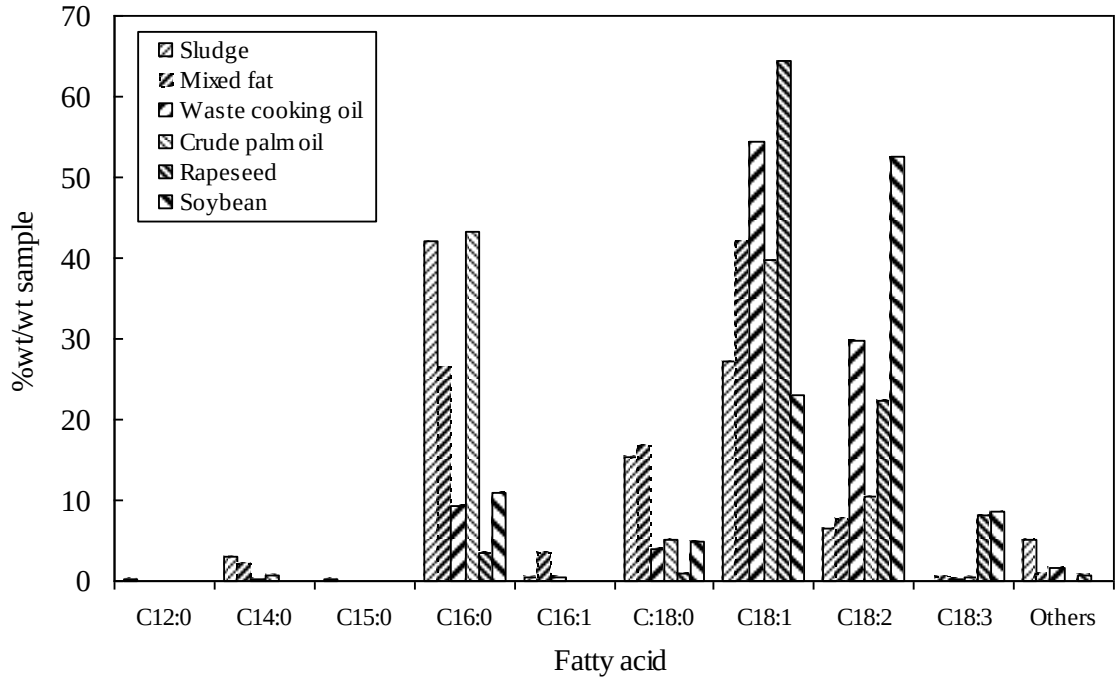

Figure 1. Comparison of sludge's fatty acids with other biodiesel feedstocks (sludge: this work; soybean and rapeseed: Canacki and Sanli [26]; mixed fat, waste cooking oil and crude palm oil: Melero et al. [27]).

3.2. Influence of the acidification

Table 2 also shows the results of lipid yields from the four sludge types with and without acidification. Irrespective of sludge pre-treatments, the acidification gave slightly lower yield of lipid extracted from primary, secondary and blended sludge as compared to the sludge without acidification. Although in this study, the sludge was acidified at ambient temperature, just by adjusting pH until 2, the sludge hydrolysis accrued giving more dissolved DQO after sludge acidification (increase from 3 g/L to 3.8 g/l, blended sludge). After sludge acidification the hydrolysis could release lipids from proteins and/or carbohydrates giving more esterifiable (saponifiable) lipids (Table 3), and leaving the polar compounds unextracted. Furthermore, the phospholipids, triglycerides, wax esters and sterol esters found in the sludge may have been also hydrolyzed into FFAs [30] leaving the polar fraction (glycerol, alcohol and phosphate group) unextracted, resulting in a decrease in gravimetric yield. On the other hand, the lipid yield of stabilised sludge increased after acidification. It could be due to release the lipid bonded to the mineralised matter, which contains traces amounts of macromolecular compounds, causing a slight higher yield.

The results of FFA analysis confirmed that sludge acidification increases the amount of FFA in the lipid extracted from all sludge types. The lipid extracted from untreated primary, blended, secondary and stabilised sludge contained 48.2%, 42.8%, 23.4% and 8.9% of FFA (on the basis of lipid), respectively. After sludge acidification the amount of FFA in extracted lipids increased to 60.3%, 50.1%, 27.2% and 9.9% for primary, blended, secondary and stabilised sludge respectively. The larger increase of FFA in primary and blended sludge as compared to secondary and stabilised is related to the conversion of insoluble soaps, present in primary

sludge, into FFAs upon exposure to an acidic environment [10]. As a result of higher amount of FFAs in the lipid extracted after sludge acidification, the amount of saponifiable lipids also increased, and the higher increase is observed for primary and blended sludge (Table 3). These results show that an extraction from acidified sludge produces lipids less contaminated with non-saponifiable material. However, the overall biodiesel yield increased only slightly in the case of primary, blended and stabilised sludge. Secondary sludge gave lower overall biodiesel yield after sludge acidification owing to a lower amount of lipid extracted.

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

3.3. Influence of the pre-treatments

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

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

3.3.2. Evaluation of the pre-treatments

The lipid, saponifiable and biodiesel yields obtained from primary, secondary and blended sludges by different pre-treatment methods are presented in Table 2 and 3. Surprisingly, the sludge pre-treatments were not able to enhance substantially neither the yield of lipids nor saponifiable and biodiesel yields, irrespective of sludge acidification. The ultrasonic and mechanical pre-treatment methods were chosen because of their capacity to disintegrate sludge, what may lead to better homogenization of the sample and better penetration of the solvent into the sample [15]. Furthermore, the methods are able to disintegrate the sludge flocs, break down bacterial cell wall and release the lipid present in extracellular polymeric substances of bacterial sludge flocs [17]. As secondary sludge comes from aerobic biological process, it is composed mainly of microbial cells and suspended solids, the slight improvement in the extraction and trans/esterification yields after sludge pre-treatment is observed in some cases of secondary and blended sludge. However, the differences are not significant (Table 2 and 3). Comparing both pre-treatment methods, in general ultrasonic treatment gave better results of lipid, saponifiable and biodiesel yields than mechanical. Nevertheless, again the differences are not significant.

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

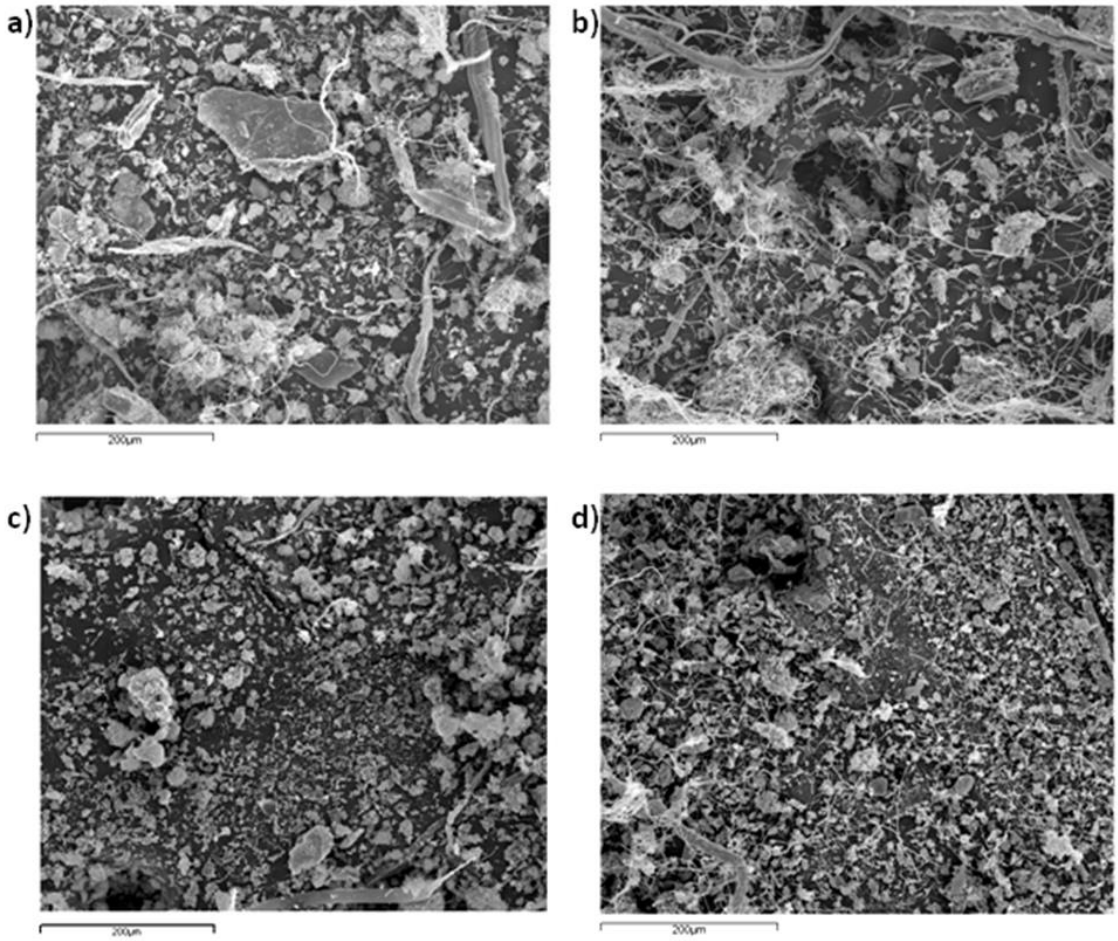

Figure 2. Microscopic appearance of the blended sludge floc: (a) untreated sludge, (b) acidified sludge, (c) after 10 min of ultrasonic treatment (50 W), (d) after 10 min of mechanical treatment (600 W).

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

3.4. Statistical evaluation

In order to identify statistically significant differences in the results of lipid, saponifiable and biodiesel yields between different pre-treatment methods and sludge acidification a two-way analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$) was performed on primary, blended, and secondary sludge. The results of the test revealed no significant interaction effect ($p >>> 0.05$) of the two factors investigated on the lipid, saponifiable and biodiesel yields for primary, blended and secondary sludge. Furthermore, the test for the three types of sludge (primary, blended and secondary) indicated no significant differences ($p >>> 0.05$) on the lipid, saponifiable and biodiesel yields between three pre-treatments tested (untreated, ultrasonic, mechanical). However, the results of the test indicated significant difference on the lipid yield between acidified and non-acidified secondary sludge ($p = 0.0039$). Primary ($p = 0.1164$) and blended ($p = 0.1595$) sludge did not show the significant influence of acidification on the lipid yield. On the contrary, the saponifiable yield was found to be significantly affected by the acidification of primary ($p = 0.0397$) and blended ($p = 0.0049$) sludge while secondary sludge did not show significant differences ($p = 0.4451$). Finally, the results of the test indicated significant difference on the biodiesel yield between acidified and non-acidified secondary ($p = 0.0066$) and blended ($p = 0.0315$) sludge while primary sludge did not show significant differences ($p = 0.1443$).

On the other hand, to identify statistically significant differences between the data with and without acidification obtained from stabilised sludge, a *t-test* at the 0.05 significance level was performed. The results of the test showed that there is no significant differences between acidified and non-acidified sludge on the lipid ($p = 0.0992$), saponifiable ($p = 0.3333$) and biodiesel ($p = 0.0917$) yields.

3.5. Comparison with other processes of production of biofuels

In the best scenario of this study, the production of biodiesel from sewage sludge can reach a maximum of 19% based on dry matter. The best alternatives to compete with biodiesel production to make fuels from the volatile matter of dry sludge are direct combustion, gasification, pyrolysis and liquefaction. These processes are able to produce thermal decomposition of the organic matter and transform this organic matter into bio-fuels [32]. Among these processes, liquefaction is gaining more attention due to better condition of low temperature and pressure [33].

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

although the yields of conversion to biodiesel were 7% and 10% based on dry sludge, lower than the 19% obtained in this study.

4. Conclusions

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

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

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

Acknowledgements

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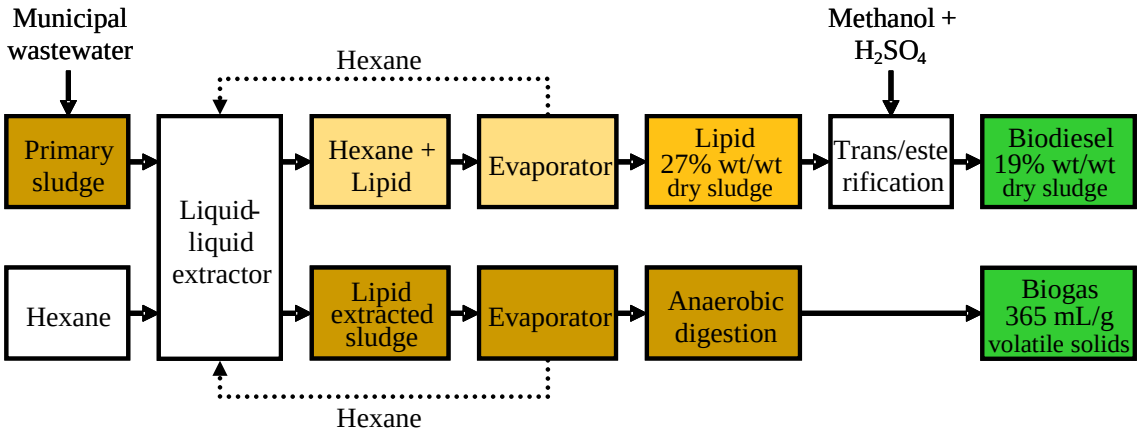
Chapter 3

Direct liquid–liquid extraction of lipid from municipal sewage sludge for biodiesel production [1]



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 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.

[1] M. Olkiewicz, M.P. Caporgno, A. Fortuny, F. Stüber, A. Fabregat, J. Font, C. Bengoa, Direct liquid–liquid extraction of lipid from municipal sewage sludge for biodiesel production, *Fuel Processing Technology* 128 (2014) 331–338.

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 to 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 sludges [7] by centrifugation, but the energy of dewatering still constitutes 14% of the 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 sludges [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 acid 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 acid (FFA) 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.

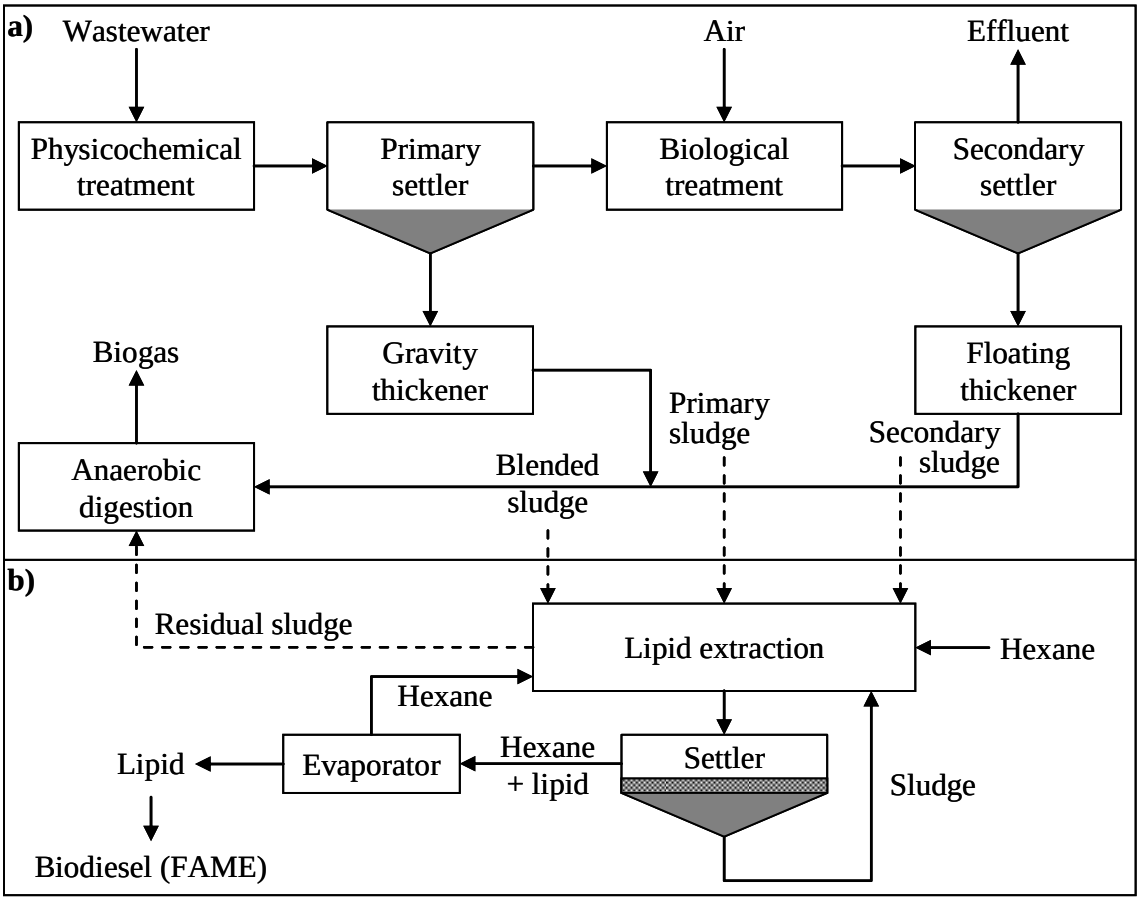


Figure 1. Diagram of the wastewater treatment plant (a) and schematic diagram of the experimental liquid–liquid extraction setup carried out in the present study (b).

2.2. Sludge collection and handling

Primary, secondary and blended sludges were collected from the municipal WWTP in Reus (Tarragona, Spain) with a capacity to daily process 25,000 m^3 of wastewater. Figure 1a shows a schematic diagram of the WWTP, illustrating the step where these different types of sludges 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 4°C 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, 105°C for 2 days based on standard method 2540G [10] or 70°C 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 −20°C. 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 25–35°C.

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 500mL 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 an oven at 105°C and dried until reaching constant weight.

2.3.2. Drying of primary, secondary and blended sludges 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 40°C 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 the 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 Figure 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 15min for primary and blended sludges 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 40°C 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 Figure 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 40°C under vacuum at 50 mbar. The sludge was anaerobically digested at 33°C 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, 33°C. 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°C. 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 were expressed as methane percentage in a two component mixture. Volatile fatty acids (VFAs) 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 esterification/transesterification 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 FAME 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 solid (TS) and volatile solid (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 sludges 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.

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

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.

Table 2. Effect of drying method on the moisture content, lipid and esterification/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

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 (70°C, 105°C), 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 70°C, freeze-dried, fume hood, and sun drying that the solid particles were more compact, even after grinding. The water content could surround the sludge particles and thus inhibit 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 (70°C, 105°C) 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 70°C (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 is in agreement with Cordero Esquivel et al. [8]. They reported that biomass drying by both freeze-drying and oven drying at low temperature (30°C) 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 105°C, −53% for oven at 70°C, −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 70°C (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 105°C 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. FAME composition of biodiesel is also modified, too. Among the methods tested, oven drying at 105°C 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.

Table 3. FAME 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

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 the 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 types 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, as well as the amount of saponifiable lipid, thereby the biodiesel yield.

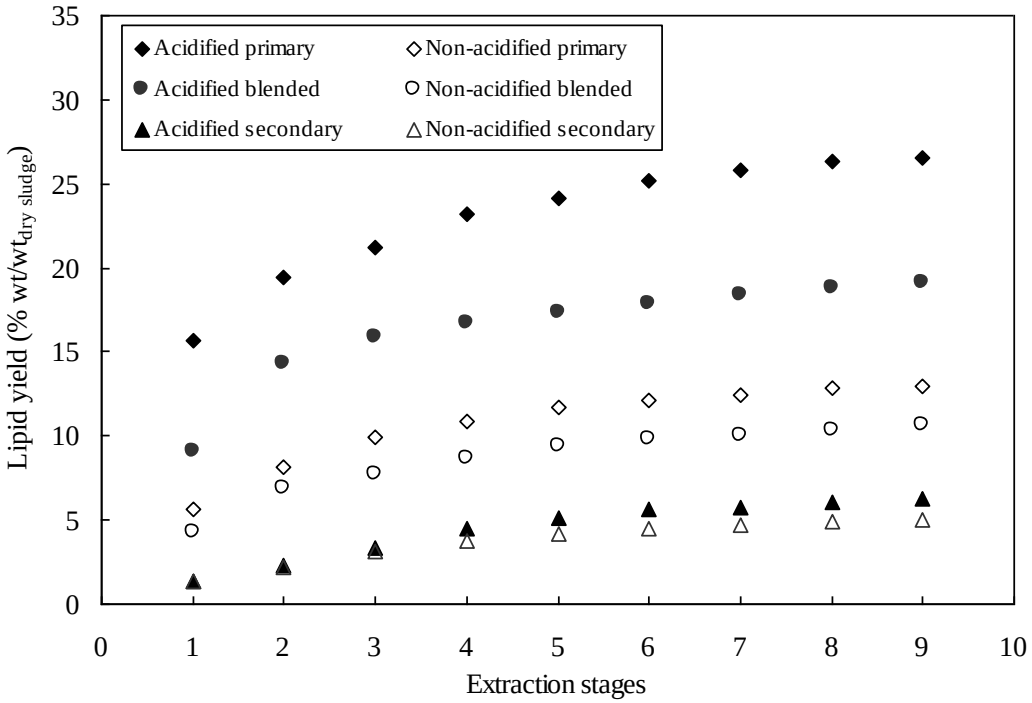


Figure 2. Effect of sludge acidification on the lipid yield. Conditions: 1:1 sludge to hexane volume ratio, each stage extraction time — 20 min.

Figure 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 sludges. 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 sludges, respectively. The high difference between the values obtained for primary and blended sludges, 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 FFA analyses in primary sludge showed that, after sludge acidification, the FFA 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 FFA 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%, Figure 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 sludges 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 (Figure 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 sludges 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 times of contact in each stage (20, 40 and 60 min) and different sludges 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, and 330 rpm agitation speed and ambient temperature.

Figure 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.

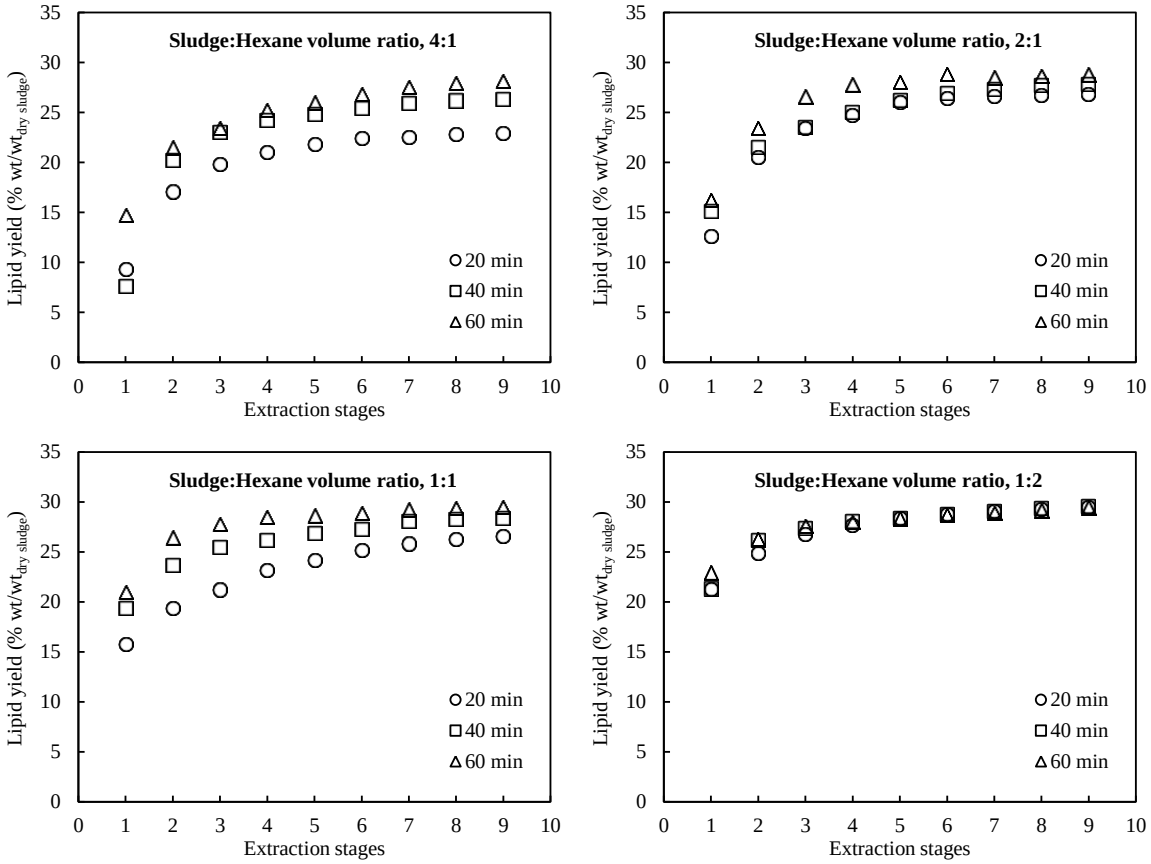


Figure 3. Effect of extraction time on the lipid yields with different sludges to hexane volume ratio.

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 ratios 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, 60min 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) aswell as for 20 and 40min (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 the determination of partition coefficients in a wide range of process conditions. Figure 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 min for each extraction, 9 consecutive extractions with fresh hexane, and 330 rpm of agitation speed and ambient temperature. As the liquid–liquid equilibrium thermodynamic diagrams are then available, the application of design methods for typical extraction equipment gives optimised solvent to the feed flow rate ratio and number of stages in continuous operation.

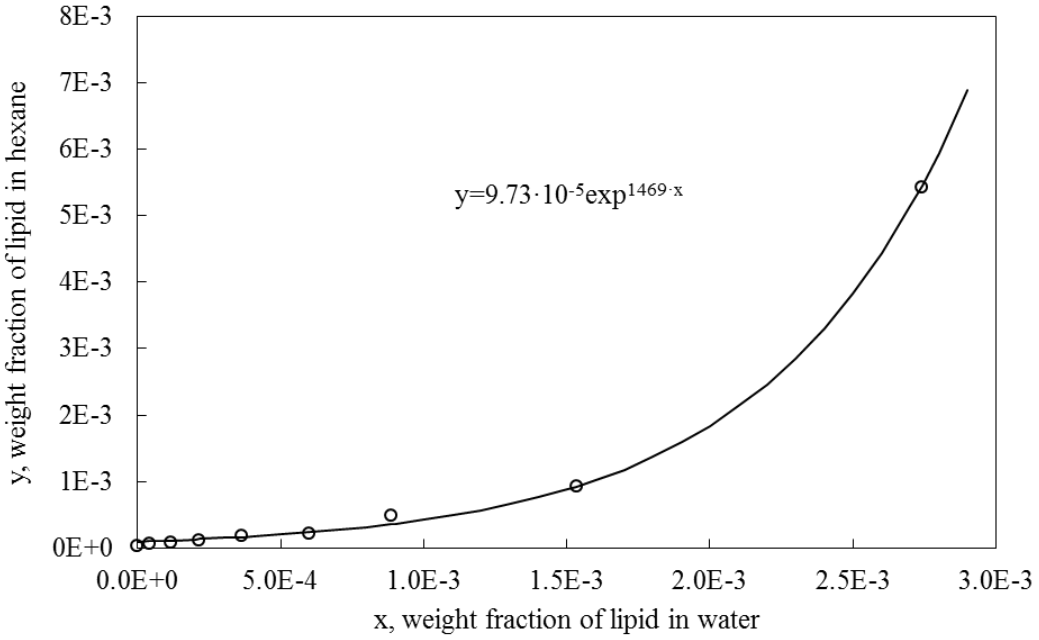


Figure 4. Equilibrium curve lipid in hexane–lipid in sludge. 200 mL of acidified primary sludge, 400 mL of hexane, 20 min for each extraction, 9 consecutive extractions, and 330 rpm of agitation speed and ambient temperature.

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 t/year of dry primary sludge generation), was estimated to be 369 t. Speculating the biodiesel production from wastewater produced from all Spanish population, 6 h m^3/day, the annual biodiesel potential was estimated at 88,664 t. This value may replace approximately 15% of current biodiesel production in Spain [12]. The FAME

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 the 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 acid 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 1 kg 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 $€/L_{FAMEs}$ (1 stage) and 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, the scaling-up of the process from lab-scale to industrial plant should reduce the price.

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*

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 is not necessary to be included 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. Figure 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 (0°C 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 VFAs were detected in reactors with ELES. A value over the range of 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 486mL_{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].

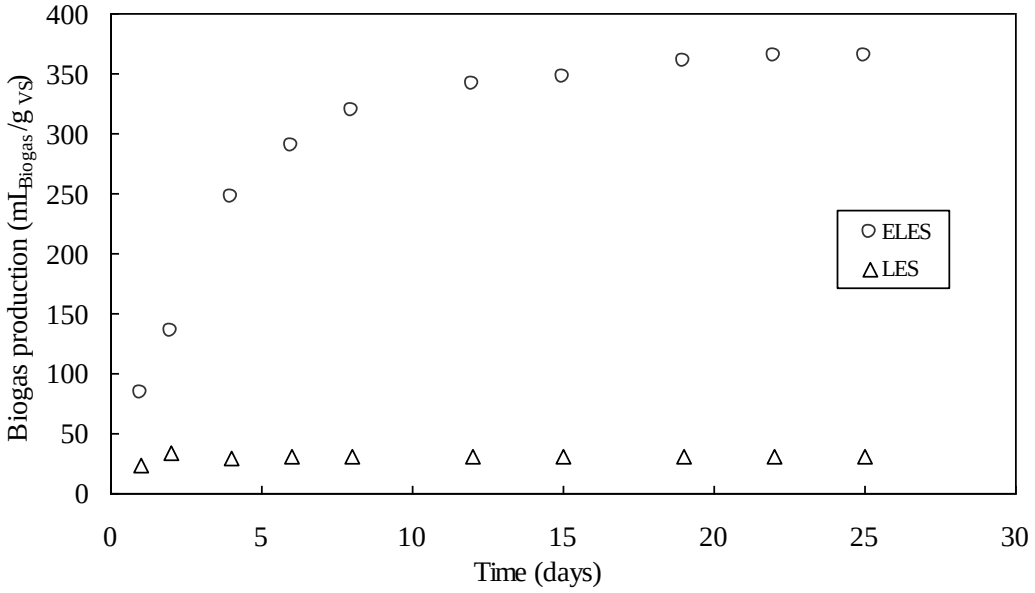


Figure 5. Biogas production from lipid-extracted sludge with and without evaporation process. Batch reactors, 33°C and 25 days.

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 depends 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.

Acknowledgements

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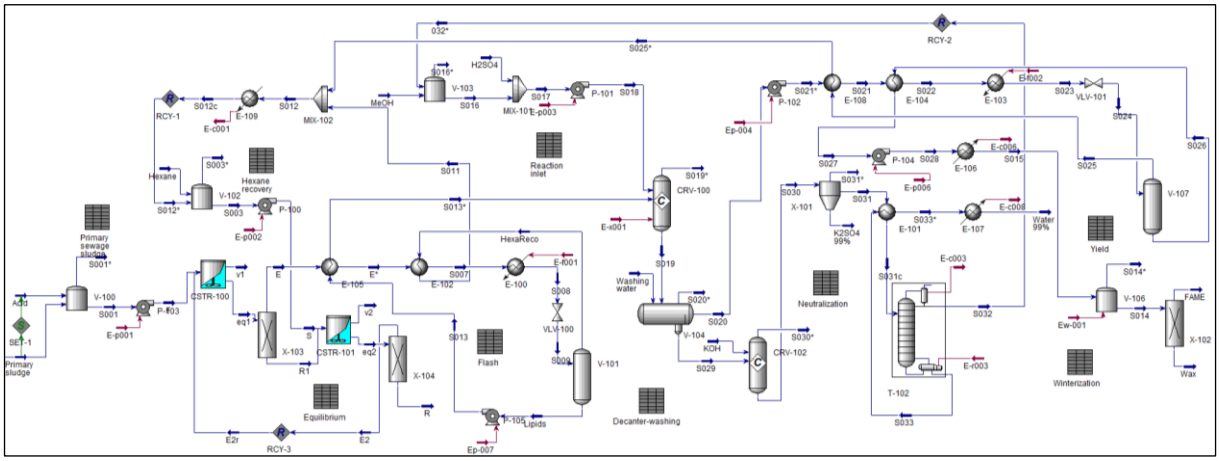
Chapter 4

Scale-up and economic analysis of biodiesel production from municipal primary sewage sludge [1]



ABSTRACT

Municipal wastewater sludge is a promising lipid feedstock for biodiesel production, but the need to eliminate the high water content before lipid extraction is the main limitation for scaling up. This study evaluates the economic feasibility of biodiesel production directly from liquid primary sludge based on experimental data at laboratory scale. Computational tools were used for the modelling of the process scale up and the different configurations of lipid extraction to optimise this step, as it is the most expensive. The operational variables with higher influence in the cost were the extraction time and the amount of solvent. The optimised extraction process had a break-even price of biodiesel of 1232 $/t, being economically competitive with the current cost of fossil diesel. The proposed biodiesel production process from waste sludge eliminates the expensive step of sludge drying, lowering the biodiesel price.

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1. Introduction

Nowadays, around 80% of all the energy consumed worldwide is obtained from fossil fuel sources [1]. However, the availability of fossil fuels at an acceptable price for society is continuously decreasing, mainly due to the fossil fuels vanishing [2]. The expected depletion of fossil fuels and the environmental problems associated with their combustion, will limit their use. Therefore, the search for new materials and processes for production of renewable energy has been intensified and a huger research effort is done in this direction.

Biodiesel is one of the most promising renewable fuels, apart from being renewable, it is biodegradable, less toxic, may generate similar amount of energy to fossil diesel and can be directly used with current engine and refuelling technology/infrastructure without major modification [3-5]. Biodiesel is a mixture of fatty acids methyl esters (FAMEs), the result of the transesterification reaction of lipids with methanol. Although it was demonstrated that biodiesel can be produced from a variety of raw materials or wastes: vegetable oils, animal fats, waste oils, oleaginous microorganisms such as bacilli, fungi, yeast or microalgae [5,6], current technology are based on using mainly vegetable oils [6]. However, the high cost of vegetable oils which constitutes between 70-85% of the overall biodiesel production cost, strongly influences the final price of this biofuel, limiting its expansion [3-5,7]. Furthermore, the cultivation of edible oilseeds for biofuels raises the concerns of food shortage, which competes with fuel production [5,6].

The possibility of using municipal sewage sludge as non-edible lipid feedstock is gaining more attention due to the large amounts of wastewater sludge generated in the developed countries, and high amount of lipids contained within these wastes, up to 30 wt% [8-10]. Each year, higher quantities of sludge are produced and the number is estimated to increase from 10 million tons (2005) to 13 million tons in 2020 in the EU [11]. Furthermore, the sludge formed during treatment of wastewater needs specific management before disposal and represents a major cost in wastewater treatment plant (WWTP) operation [8,12]. Therefore, the sewage sludge can be envisaged as a low-cost, readily available in abundance and non-edible feedstock, which can make biodiesel production profitable. Recent studies have indicated that the lipid contained in sewage sludge could be a potential feedstock for biodiesel production [4,7-10,12-15]. Nevertheless, the cost of energy necessary to eliminate the high water content (95-98 wt %), before lipid extraction, is the main limitation to scale-up, as dewatering and drying constitutes more than 50% of the total biodiesel production cost [7,8]. On the other hand, previous research demonstrated the feasibility of lipid extraction from liquid sludge (~96% of water) by direct liquid–liquid extraction using hexane as a solvent [9]. Since the production of biodiesel from liquid sewage sludge is feasible, the expensive sludge drying step can be eliminated, and therefore the overall biodiesel production cost can be reduced. However, in order to confirm the stated hypothesis, the economic feasibility of the wet process (direct use of liquid sludge) and its comparison with dry process (use of dry sludge) has to be done.

Economic analysis of the production of biodiesel from dry sewage sludge has already been reported. Dufreche et al. (2007) [8] estimated the cost of biodiesel production from dry sludge by direct *in situ* transesterification, without the extraction step, to be 933 $/t. However, in this research short-cut economic methods were used without giving details about the cost methods used. A more detailed breakdown of estimated costs also for *in situ* transesterification of dry sludge was calculated by Mondala et al. (2009) [7], based on data published by others, *e.g.*, the cost of sludge drying was taken from Dufreche et al. (2007) [8]. They obtained a break-even price of

biodiesel of 970 $/t. Pokoo-Aikins et al. (2010) [16] presented a full economic feasibility study, based on process design and simulation, to choose the best option to produce biodiesel from dry sewage sludge, using two-step process: preliminary extraction from dry sludge, evaluating four solvents (hexane, toluene, methanol and ethanol), and subsequent conversion of the lipids into biodiesel. The results indicated that hexane and toluene were cheaper solvents with a cost of 868 and 838 $/t of biodiesel, respectively. These excellent results were obtained considering that dry sludge was free of cost, charging the sludge drying to WWTPs. Certainly, if sludge drying were also taken into consideration, the final price of biodiesel would increase significantly.

In short, on the one hand, in the aforementioned economic studies, some assumptions were underestimated and in some cases not all process steps were considered for the estimation of the final biodiesel cost. Therefore, to fairly estimate the biodiesel production cost from sewage sludge, all assumption must be taken with a constructive criticism and include realistic values. On the other hand, the biodiesel production from wastewater sludge has a promising future but it is still in research stage. Therefore, further large scale studies are required to realize the benefits of this new biotechnology.

The purpose of this research is to critically review the biodiesel production from sewage sludge using the know-how acquired at bench scale experimentally work. Laboratory scale data obtained in our previous study [9], where the feasibility of lipids extraction directly from liquid sludge was demonstrated, is analysed by computational tools in order to carry out the scale up of this novel biodiesel production process. In particular, the lipid extraction from liquid primary sludge is optimised by using computational tools to model the process performance and the economic evaluation of the process alternatives. Process options are envisaged to estimate a realistic scenario considering the technology currently available. Finally, the optimised biodiesel production process from liquid sludge is compared to the *in situ* and two-step processes using dry sludge (also simulated in this study) in order to decide on the most economically favourable process.

2. Methods

A production plant with a capacity of around 4000 t/year of biodiesel produced from primary sewage sludge is studied. The capacity of the facility will depend on the sewage sludge availability. In this sense, a nearby urban waste water treatment plant (WWTP) to feed 60 m^3/h of primary sewage sludge was considered. This set-up (Figure 1) can eliminate the cost of transporting the sludge feedstock into the biodiesel production facility, which therefore was not taken into account in the economic study as well as the cost of raw sludge, which is a waste generated during treatment of wastewater. The proposed process aims to improve the biodiesel production from sewage sludge, *i.e.* lipid extraction process, by the elimination of the energy intensive step of sludge dewatering and drying and also the elimination of the heating process during extraction. Particularly, the process developed is compared with those described in other works, whose main differences are: on the one hand, the use of sludge previously dehydrated, with the consequent increased costs of the raw material, that in some assessments seem to be understated or dismissed [7,8,16,17]; and on the other hand, the use of heating during extraction, which also increases the cost of the process [8,16,17]; and finally, the conversion of all lipids into biodiesel [17], since based on experimental studies, approximately 70-85% of lipids can be converted into biodiesel (saponifiable lipids) [9,12]. The economic evaluation of the process and its potential alternatives is performed based on the previous results experimentally tested [9].

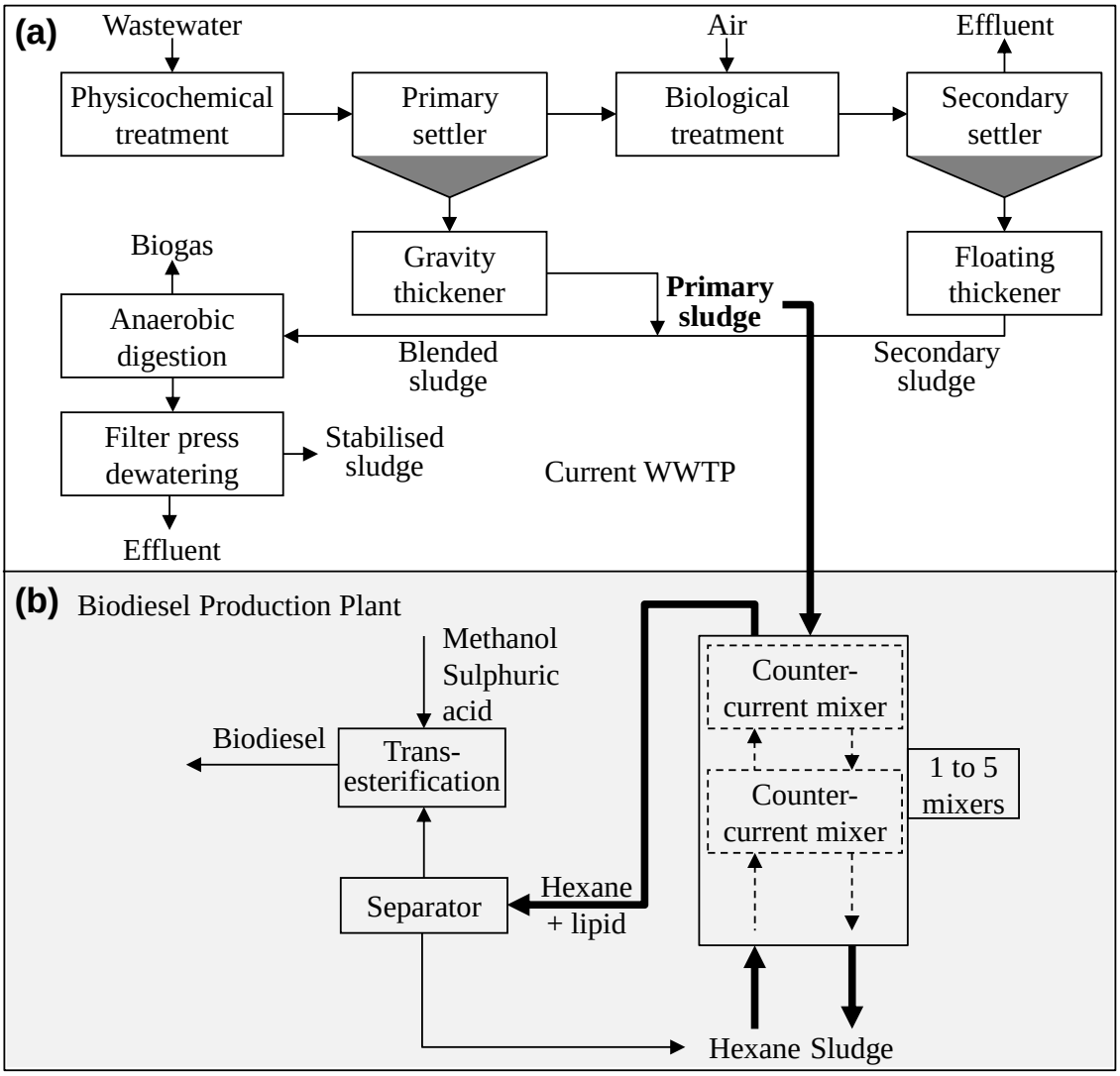


Figure 1. Scheme of the current WWTP of Reus (Tarragona, Spain) and the biodiesel production process.

2.1. Approaches and assumptions

2.1.1. Primary sludge

The calculations of the economic feasibility study were performed with the data of the primary sludge collected from the municipal WWTP in Reus (Tarragona, Spain) with a capacity to process near 25,000 m^3 of wastewater per day which serves 200,000 inhabitants. The WWTP of Reus produces an average of 135 m^3/day of primary sludge. For the calculations, the flow rate was approximated to 60 m^3/h to assimilate the production of a big town, as for example the WWTP near Barcelona, which serves approximately to 2 million inhabitants.

Figure 1(a) shows a schematic diagram of the WWTP in Reus (Tarragona, Spain), illustrating the sludge generation in the WWTP facility. The primary sludge was sampled after partial gravity thickening. To determine the average characteristics of the primary sludge, the total solids (TS), ash and lipid contents were analysed according to standard methods 2540B, 2540E and5520E, respectively [18]. Proteins were quantified following the Lowry method [19]. Carbohydrates were quantified following the phenol–sulfuric acid method [20]. The primary sludge composition used for the analysis in AspenHysys V8® simulator is listed in Table 1. The lipids

content was 27.2% on the basis of dry sludge. This value could allow recovering 11 g of lipids from each kilogram of liquid (96% water content) coming from a primary sewage sludge.

Table 1. Characteristics of primary sewage sludge collected from the municipal WWTP in Reus (Tarragona, Spain).

	Weight % [a]
Water	96.0
Total solids	4.0
Ash [b]	21.8
Protein [b]	26.0
Carbohydrates [b]	25.0
Lipids [b]	27.2
Free Fatty Acids [c]	76.8
Triglycerides [c]	3.2

[a] *average of 3 determinations of 3 sludge samples collected on different days.*
[b] *on the basis of dry sludge.*
[c] *on the basis of lipids.*

2.1.2. Lipid extraction from liquid primary sludge

The calculations of the economic feasibility study were performed using the experimental values obtained in laboratory with a bench scale experimental device [9]. The sequential liquid-liquid extraction of lipid was conducted in a batch mixer-settler reactor with mechanical agitation (330 rpm) at ambient temperature, using hexane as a solvent. After extraction, settling was performed at 60 rpm for 15 min during which hexane containing lipids were separated from aqueous sludge. In the experimental procedure, nine consecutive extractions stages were carried out. In the simulation of the process scale up, the lipid extraction takes place in a liquid-liquid extraction series of mixers, where hexane is used as solvent, but in a counter-current system, as depicted in Figure 1(b). A maximum of five mixers were modelled in the AspenHysys V8® simulator, as more than 90% of lipids were extracted after 5 consecutive extraction stages in the experimental study [9]. According to the experimental study, in order to extract all lipids from liquid sludge, sludge acidification until pH 2, prior to extraction is required [9]. In order to minimize the addition of acid, sludge acidification to pH 4 was also studied. The sludge was acidified to pH 2 and pH 4 by addition of concentrated HCl. Due to the primary sludge pH varies between 5.8 and 6.5, according to experimental results, it was assumed that the required concentration of HCl in liquid sludge is approximately 1.5% or 0.8 % v/v to attain the pH 2 and pH 4, respectively.

After sludge acidification, the composition of lipids consists normally of 20% non-saponificable and 80% saponifiable lipids (convertible to biodiesel). As shown in Table 1, the saponifiable lipid fraction consists mainly of free fatty acids (FFAs) and traces of triglycerides (TG), suggesting that the main reaction during the conversion of sludge lipids into biodiesel is esterification of FFAs.

Based on the laboratory experiments, six different process configurations (Table 2) were selected to perform the comparative economic study with the aim to optimise the number of extraction stages and extraction conditions, to find the most economically favourable.

Table 2. Configurations of the lipid extraction process.

Configuration	Sludge/hexane volume ratio	pH	Mixing time (min)	Fitting equation		R^2	
				Concentration		Concentration	
				Low	High	Low	High
CS1	1/2	2	20	y=0.2554x+7e-5	y=0.0001exp(1483.6x)	0.916	0.989
CS2	1/1	2	60	y=0.8968x-0.0001	y=0.0002exp(1302.3x)	0.967	0.990
CS3	2/1	2	60	y=3.9716x-0.0016		0.997	
CS4	1/1	4	20	y=7e-5exp(956.94x)		0.954	
CS5	2/1	4	20	y=-238.81x2+4.3902x-0.0046		0.980	
CS6	2/1	4	60	y=3.9716x-0.0016		0.997	

2.1.3. Biodiesel production

Due to the very high content of FFAs in the sludge lipids (Table 1), the acid catalysis esterification/transesterification was selected to convert saponifiable lipids into biodiesel. Acid catalyst, *i.e.*, H_2SO_4, is used in the simultaneous esterification of FFAs and transesterification of glycerides avoiding soap formation, which takes place in the case when conventional alkali catalyst is used (*e.g.*, NaOH). The assumptions applied in the esterification/transesterification reaction were taken from Zhang et al. (2013) [17], modifying the temperature of the reaction.

According to Zhang et al. (2013) [17], the sludge lipids conversion is considered as 99% under the following conditions: 6:1 methanol to lipids molar ratio, 1% (v/v) of H_2SO_4 as catalyst in methanol, temperature reaction of 50ºC and 4 hours of residence time. However, the laboratory experimental test applied to the primary sludge lipids, using these conditions but taking into account the correction for saponifiable lipids (*i.e.*, 6:1 methanol to saponifiable lipids molar ratio), gave only 88% of reaction efficiency. An increase in the temperature to 60 ºC, showed an increase in the reaction efficiency to approximately 99%. According to the experimental results, the reaction was simulated at 60 ºC.

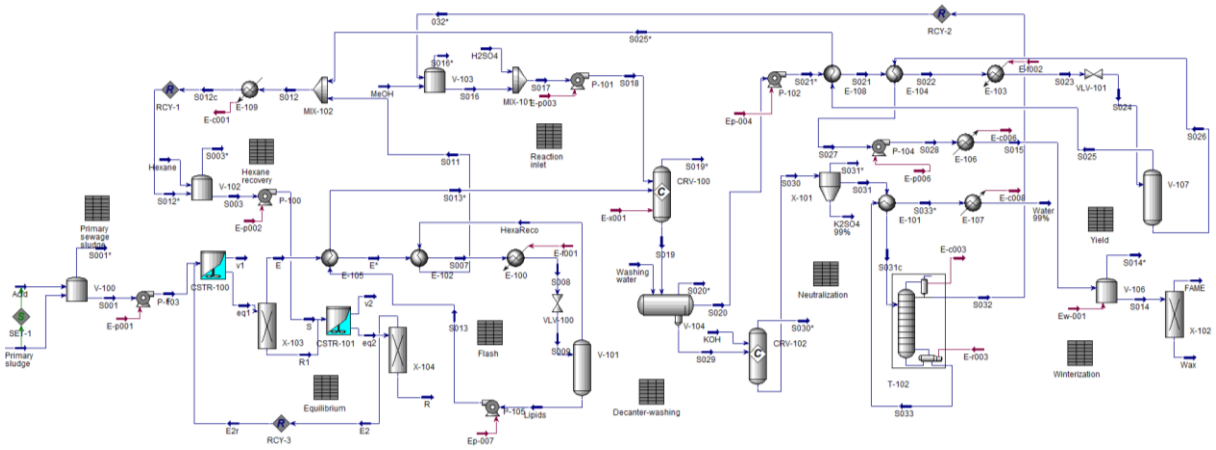
As only the saponifiable part of sludge's lipids is convertible into biodiesel, the separation of non-saponifiable lipids was performed after biodiesel production, in the purification step, as commented on in subsection 2.2.2 of this paper.

2.2. Process simulation

The economic characterization approach is based on the simulation of the plant in AspenHysys V8®, in a continuous process. Although most of the chemical components involved in the simulation are defined in the AspenHysys® component library, some compounds have been defined as hypothetical solids, as potassium sulphate, the inorganic matter or ash content in the sludge and the organic matter different from lipids, for which average molecular weights and mass densities are defined. Moreover, certain compounds are used in representation of similar substances. More precisely, a mixture of M-palmitate and M-oleate was selected to represent the biodiesel product, the palmitic acid represents the FFA content, the triolein plays the role of triglycerides and a saturated fatty acid ester was selected to represent the non-saponifiable lipids (*i.e.*, cetyl

palmitate). The palmitic acid was selected due to the predominance of this acid in the sludge lipids and thus in the biodiesel produced [9,10]. The primary sewage sludge (and its lipids composition) was simulated according to the characterization made by Olkiewizc et al. (2014) [9] (Table 1). The Peng-Robinson Soave (PRSV) equation of state was the fluid package selected to predict all physicochemical properties.

The detailed flow diagram obtained directly with AspenHysys® simulation package, and the characterization of the main material streams involved can be seen in Figure 2. The simulation is based on the current available technologies. The process simulation is structured in two main sections: the lipid extraction from the liquid (96% water content) primary sludge and the acid-catalyzed esterification/transesterification process. In the following sections a detailed description of both process sections will be explained.



Stream name	Primary sludge	F	E	R	S	S012	S013	S015	S017	S019	S020	S025	S031	S032	FAME 98%	Water 98%	K_2SO_4 99%
Temperature (°C)	25.0	24.9	25.9	31.7	60.6	61.8	89.3	35.0	59.6	60.0	61.3	168.9	41.4	69.7	5.0	35.0	41.4
Pressure (kPa)	101.3	115.0	105.0	105.0	120.0	80.0	101.3	101.0	101.0	101.0	101.0	80.0	101.0	101.0	101.3	110.0	101.0
Mass Flow (kg/h)	60000.0	60319.6	19925.3	59858.6	19464.9	19454.1	588.4	501.0	292.1	880.6	618.2	117.2	610.6	224.2	406.4	386.5	11.2
Components mass fractions																	
H_2O	0.9600	0.9549	0.0000	0.9623	0.0001	0.0001	0.0000	0.0001	0.0360	0.0409	0.0019	0.0098	0.6376	0.0466	0.0001	0.9804	0.0001
Palmitic acid	0.0084	0.0084	0.0183	0.0024	0.0000	0.0000	0.6167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Triolein	0.0004	0.0003	0.0008	0.0001	0.0000	0.0000	0.0257	0.0003	0.0000	0.0002	0.0002	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000
Rest Biomass	0.0205	0.0204	0.0000	0.0205	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ash	0.0086	0.0085	0.0000	0.0086	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Non-saponifiable lipids	0.0022	0.0022	0.0048	0.0006	0.0000	0.0000	0.1609	0.1887	0.0000	0.1075	0.1531	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000
HCl	0.0000	0.0053	0.0000	0.0053	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
n-Hexane	0.0000	0.0000	0.9762	0.0000	0.9993	0.9993	0.1967	0.0218	0.0000	0.1314	0.1872	0.8943	0.0000	0.0000	0.0169	0.0000	0.0000
Methanol	0.0000	0.0000	0.0000	0.0001	0.0004	0.0004	0.0000	0.0008	0.9425	0.2593	0.0140	0.0702	0.3598	0.9534	0.0010	0.0156	0.0000
H_2SO_4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0215	0.0071	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
M-Palmitate	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.7582	0.0000	0.4346	0.6191	0.0244	0.0000	0.0000	0.9446	0.0000	0.0000
M-oleate	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0301	0.0000	0.0171	0.0244	0.0003	0.0000	0.0000	0.0371	0.0000	0.0000
Glycerol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000	0.0000	0.0026	0.0000	0.0000	0.0040	0.0000
KOH	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
K_2SO_4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999

Figure 2. Flow diagram of the AspenHysys® simulation model for the biodiesel production process from liquid primary sludge (wet route).

2.2.1. Lipid extraction from liquid primary sludge

The primary sludge is stabilized with acid before the extraction (V-100), as commented on in subsection 2.1.2. After that, the lipid extraction takes place in a liquid-liquid extraction series of mixers (CSTR-100, 101), where hexane is used as solvent in a counter-current system. The equilibrium and operating data were taken from the experiments carried out by Olkiewicz et al. (2014) [9]. In order to find the number of stages that optimize the economic results, different alternatives are modelled regarding the working pH for the sludge, residence time during the extraction stage and the ratio between sludge and hexane, depending on the ratio of hexane used

(Table 2). The design of the extraction is based on a short-cut method comparable to the McCabe-Thiele stepwise calculation for distillation columns [21]. A constant flow rate of feed solvent and extraction solvent is assumed, and the solute concentrations are given as the weight ratios of solute to feed solvent and extraction solvent in the raffinate and the extract phases, respectively. The compositions (raffinate and extract) of each extraction stage (operation line) are calculated with the equilibrium curve equation obtained by the fit of experimental data. The curve fitting equations and their coefficients of determination for each extraction configuration are presented in Table 2.

The raffinate composed by the rest of biomass and more than 96% water can be recycled to the WWTP for further processing and exploitation. As demonstrated by Olkiewicz et al., 2014 [9], the residual lipid extracted sludge can be easily anaerobically digested (the process widely installed in WWTPs, Figure 1(a)) with good biogas production, avoiding the generation of a new waste sludge. On the other hand, the extract is led to an equilibrium-flash separator V-101 where over 99% of hexane is recovered and recycled to the extraction.

2.2.2. Biodiesel production

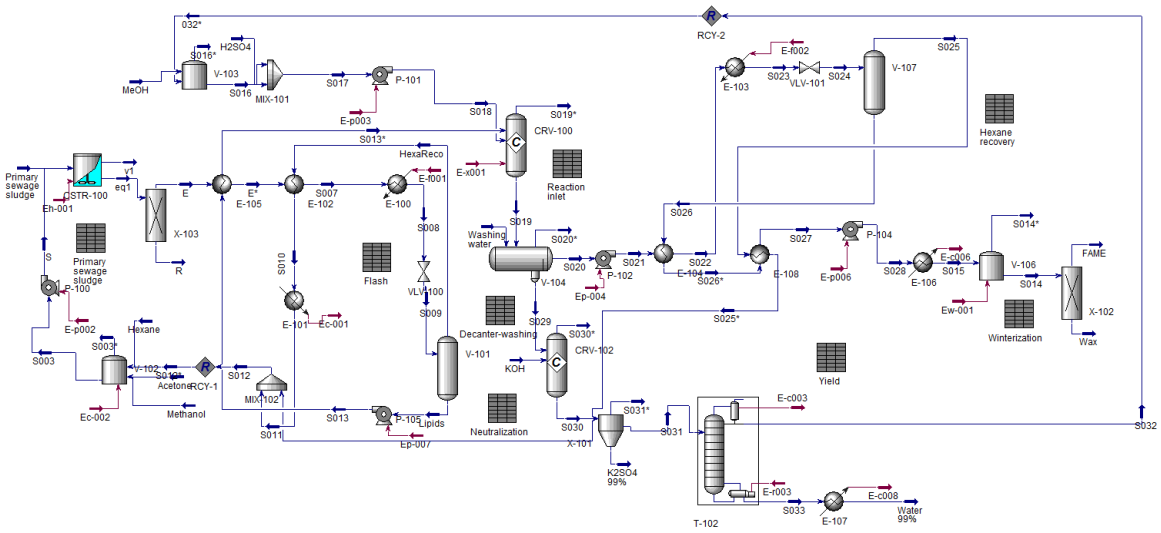
Acid catalyzed reaction system is proposed for the production of FAME using methanol as reactant. Under these conditions, two reactions take place (CRV-100): the acid esterification of the FFA to give FAME and water, and the acid transesterification of the triglycerides to obtain FAME and glycerol. Based on experimental results, near 99% is achieved for the sludge's lipids esterification/transesterification under the conditions described in subsection 2.1.3.

The products stream is forwarded to a decanter (V-104) where the contact with washing water forces the separation of two-phases. The light phase is conducted to a flash separation (V-107) to reduce further the amount of hexane and traces of methanol that accompanied the obtained biodiesel. The heavy phase includes water, methanol, a low quantity of glycerol as by-product of the transesterification reaction, and the acid used as catalyst. To recover the methanol for its recycling as excess reagent in the esterification/transesterification reaction, the heavy phase is first neutralized (CRV-102) by the addition of potassium hydroxide obtaining a salt, *i.e.*, potassium sulphate, that is removed (X-101) and that may be considered as a valuable by-product. Then, the neutralized stream is forwarded to a distillation column (T-102) to recover the methanol (79%) and to obtain a stream of water with traces of glycerol (0.4%) that might be reused in the wastewater treatment plant. As the obtained biodiesel contains also non-saponifiable lipids, a crystallization fractionation is applied to split biodiesel into a liquid (low-melting point) and a solid fraction with high melting point, *i.e.*, sterols and/or waxes, achieving a product of more than 98% of FAME. Particularly, the traditional fractionation consists of two stages, the crystallization under strictly controlled cooling rate combined with gentle agitation, and the separation by filtration [22]. This process was simulated by units V-106 and X-102 (see Figure 2), to coarsely estimate the costs derived from the energy and equipment requirements.

On the other hand, during the design of the process, energy integration strategies were applied in order to reduce the energy consumption in certain stages of the process that were specially energy consuming. For example, during the hexane recovery and the product purification the streams that leave the separation units at high temperature are used to exchange heat with the input streams so the heating and cooling requirements are reduced.

2.3. Dry routes alternatives

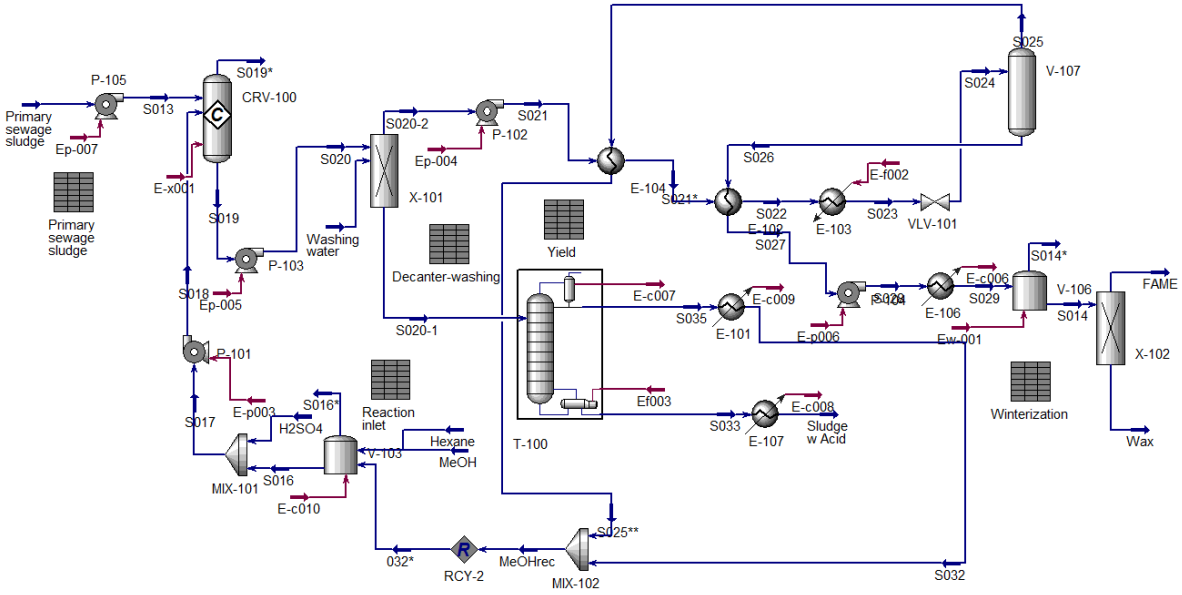
The wet extraction route, optimised in this study, is compared with the dry route extraction where the sewage sludge is previously dried. The sewage sludge drying process was modelling in a similar way to that used for microalgae biomass harvesting, which consists in a two-staged dewatering process: centrifugation from a concentration of 5 to 20% of solids, and drying in a spray dryer till 95% of solids [23]. Two dry routes are considered in the economic comparison. On the one hand, the conventional dry route based on operating data given by Zhang et al. (2013) [17] uses hexane in a ratio of 10 L/kg dry sludge in a mixture with methanol and acetone 3:1:1, achieving 96% of extraction efficiency at 50ºC and a residence time of 1 hour. After the solvent recovery, the esterification/transesterification reaction is carried out using the same approaches as in the wet route process. On the other hand, a dry route alternative is also assessed where the extraction and the acid catalysed reaction take place simultaneously, called *in situ* transesterification. This alternative was simulated using the operation data detailed by Mondala et al. (2009) [7] including a mixture of 12:1:3.3 methanol:sludge:hexane mass ratio and 5% (v/v) of H_2SO_4 as catalyst in methanol. This alternative reduces the number of equipment involved and eventually the reduction of the costs associated is foreseen. The solvent recovery and product purification phases for both dry extractions alternatives are similar to the described for the wet route, with the only difference of impossibility of by-product formation during the *in situ* process. In this way, simulating the three alternatives under common operational basis, *i.e.*, dry sludge composition, drying expenses, economic parameters, diesel specifications, etc., the results obtained are valuable especially in terms of comparison.



Stream name	Primary sludge	E	R	S	S012	S013	S015	S017	S019	S020	S025	S031	S032	FAME 99%	Water 98%	K_2SO_4 99%
Temperature (ºC)	24.9	50.0	30.2	46.6	46.6	119.9	35.0	60.3	60.0	60.5	169.3	41.1	69.6	5.0	35.0	41.1
Pressure (kPa)	101.3	105.0	105.0	120.0	70.0	101.3	101.0	101.0	101.0	101.0	80.0	101.0	101.0	101.3	110.0	101.0
Mass Flow (kg/h)	2526.0	18612.1	1988.3	18074.4	17983.3	652.3	662.5	401.9	1054.2	685.9	23.5	857.9	315.2	536.6	542.7	15.4
Components mass fractions																
H2O	0.0500	0.0000	0.0643	0.0001	0.0001	0.0000	0.0004	0.0353	0.0456	0.0027	0.0693	0.6347	0.0448	0.0005	0.9773	0.0000
Palmitic acid	0.1996	0.0268	0.0105	0.0009	0.0009	0.7398	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Triolein	0.0083	0.0011	0.0004	0.0000	0.0000	0.0309	0.0003	0.0000	0.0002	0.0003	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000
Rest Biomass	0.4868	0.0000	0.6185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ash	0.2033	0.0000	0.2583	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Non-saponifiable lipids	0.0520	0.0068	0.0027	0.0000	0.0000	0.1931	0.1900	0.0000	0.1195	0.1836	0.0015	0.0000	0.0000	0.0000	0.0000	0.0000
Acetone	0.0000	0.2134	0.0100	0.2208	0.2208	0.0064	0.0015	0.0002	0.0040	0.0061	0.1357	0.0001	0.0003	0.0018	0.0000	0.0000
n-Hexane	0.0000	0.5369	0.0253	0.5557	0.5554	0.0238	0.0070	0.0000	0.0147	0.0226	0.4627	0.0000	0.0000	0.0087	0.0000	0.0000
Methanol	0.0000	0.2149	0.0101	0.2224	0.2226	0.0046	0.0022	0.9429	0.3031	0.0120	0.2907	0.3628	0.9549	0.0027	0.0189	0.0000
H2SO4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0215	0.0082	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
M-Palmitate	0.0000	0.0001	0.0000	0.0001	0.0001	0.0015	0.7684	0.0000	0.4837	0.7434	0.0395	0.0000	0.0000	0.9486	0.0000	0.0000
M-oleate	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0303	0.0000	0.0190	0.0293	0.0005	0.0000	0.0000	0.0374	0.0000	0.0000
Glycerol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0000	0.0000	0.0024	0.0000	0.0000	0.0038	0.0000
KOH	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
K2SO4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.9999

Figure 3. Flow diagram of the AspenHysys® simulation model for the biodiesel production process from dry primary sludge (dry route, conventional).

The detailed process flow diagrams obtained with AspenHysys® simulation package, and the characterization of the main material streams involved are presented in Figure 3 and Figure 4 for the conventional and *in situ* dry routes respectively.



Stream name	Primary sludge	S017	S019	S020-1	S020-2	S025	S026	S029	S032	FAME 99%	Sludge Acid
Temperature (ºC)	24.9	37.2	50.0	60.2	25.0	149.2	37.2	35.0	35.0	10.0	35.0
Pressure (kPa)	101.3	69.0	101.3	102.0	101.0	70.0	69.0	101.0	101.0	101.0	103.0
Mass Flow (kg/h)	2526.0	43337.9	45863.9	36470.8	9436.5	8732.8	43337.9	703.8	29957.2	505.1	6513.6
Components mass fractions											
H2O	0.0500	0.0012	0.0046	0.0069	0.0000	0.0000	0.0012	0.0000	0.0002	0.0000	0.0377
Palmitic acid	0.1996	0.0001	0.0011	0.0000	0.0054	0.0003	0.0001	0.0678	0.0000	0.0000	0.0000
Triolein	0.0083	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0030	0.0000	0.0037	0.0000
Rest Biomass	0.4868	0.0000	0.0268	0.0337	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1888
Ash	0.2033	0.0000	0.0112	0.0141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0788
Non-saponifiable lipids	0.0520	0.0001	0.0029	0.0000	0.0142	0.0003	0.0001	0.1865	0.0000	0.0000	0.0000
n-Hexane	0.0000	0.1931	0.1825	0.0000	0.8869	0.9559	0.1931	0.0312	0.0000	0.0087	0.0000
Methanol	0.0000	0.6994	0.6596	0.8212	0.0321	0.0346	0.6994	0.0005	0.9998	0.0008	0.0000
H2SO4	0.0000	0.1044	0.0986	0.1240	0.0000	0.0000	0.1044	0.0000	0.0000	0.0000	0.6943
M-Palmitate	0.0000	0.0018	0.0122	0.0000	0.0592	0.0088	0.0018	0.6839	0.0000	0.9495	0.0000
M-oleate	0.0000	0.0000	0.0004	0.0000	0.0021	0.0001	0.0000	0.0270	0.0000	0.0375	0.0000
Glycerol	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003

Figure 4. Flow diagram of the AspenHysys® simulation model for the *in situ* biodiesel production process from dry primary sludge (dry route, *in situ*)

2.4. Automated economic evaluation

The characterization of the process presented in this work is performed by the automated environmental evaluation tool (AEET) programmed in Matlab® R2010b [24]. In this tool the calculations are grouped into different modules, more precisely, it includes an inventory module that retrieves data from the process simulation related with the inputs and outputs of materials and energy, while the economic module computes the capital investment, operating costs and profitability indicators. The automation of the procedure makes the AEET a powerful tool for the evaluation of any process by the acquisition of realistic data from the simulation case. Besides, it allows performing additional analysis, emphasizing among others, generation and discrimination of alternatives [25], retrofit and sensitivity analysis and coupling with external optimization algorithms [24].

The profitability analysis module includes the calculation of the net present value (NPV) and the discounted payback period. However, taking into account that the purpose of this study is to determine whether the primary sludge can be a feasible feedstock for biodiesel production, the break-even price (BEP) is computed because it allows an easy comparison with the biodiesel main competitor, *i.e.*, fossil diesel. The calculations of the capital and production costs are based on Spain/European Union conditions (6% rate of interest for the capital investment and a plant life span of 20 years were assumed).

The total capital investment includes the fixed capital cost and the working capital cost, where the second is usually a fraction of the first (15% is used in this work). The fixed capital cost consists of the total bare module capital cost, the contingencies and fees and the auxiliary facilities cost. In this work, the equipment module costing technique is used to estimate the total bare module capital cost of the plant. This technique relates all costs back to the purchase cost of equipment evaluated for some base conditions. The deviation from these base conditions are handled using multiplying factors that depend on the equipment type, the system pressure and the materials of construction.

The total manufacturing cost includes three different items: direct manufacturing cost (*i.e.*, raw materials, labour fees, utilities, maintenance and repairs, operating supplies, laboratory charges and patents and royalties), fixed manufacturing cost (*i.e.*, overheads, packaging, storage, local taxes, insurances and depreciation), and general expenses (*i.e.*, administration, distribution and selling, and research and development). Detailed information about the equipment module costing technique used to estimate the total bare module of the plant can be found in the literature [26,27]. For more detailed information, the prices of all raw materials, utilities used and by-product are listed in Table 3.

Table 3. Raw materials, subproducts and utilities prices.

Raw materials [a]	
Hexane (99.5%)	463 $/t
Methanol (99.9%)	597 $/t
Sulfuric acid (99.0%)	94 $/t
Hydrochloric acid (35.0%)	90 $/t
Potassium hydroxide	900 $/t
Acetone (99.5%)	1300 $/t
By-product [a]	
Potasium sulfate	150 $/t
Utilities [b]	
Electricity	25.8 $/GJ
LP steam	4.86 $/GJ
MP steam	5.61 $/GJ
HP steam	7.81 $/GJ
Fuel	2.5 $/GJ
Cooling water (5-15ºC)	0.25 $/GJ
Makeup water	0.06 $/t

[a] *ICIS. Trusted market intelligent for the global chemicals, energy and fertilizer. Accessed the 18.04.2015. <https://www.icis.com>*
[b] *Torres et al., Fuel 111 (2013) 535–542.*

3. Results and discussion

The results of cost-effectiveness of the production of biodiesel, directly linked to the extraction of lipids are discussed in this section. Initially, the results of the production of biodiesel obtained from the different extraction configurations are evaluated (Section 3.1). Then, the economic results of the six extraction alternatives studied are discussed (Section 3.2). After that, the details of the best configuration are presented (Section 3.3). Finally, a comparison with production of biodiesel from dried sewage sludge, *i.e.*, conventional dry route and *in situ* dry route, and BEPs comparison are debated (Section 3.4 and 3.5).

3.1. Effect of the configurations of lipid extraction on biodiesel production

The origin of the biomass used to produce biodiesel, *i.e.*, municipal sewage sludge, makes that only few parameters can be modified to improve the final production. The most important point is the extraction of lipids from sewage sludge, because the reaction of esterification/transesterification is not optimised and its procedure is fixed. For this reason, the election of the best configuration of extraction is crucial to make the process economically feasible. The optimisation of the extraction was performed for six different configurations, presented in Table 2. These configurations were selected as the best alternatives regarding the experimental results obtained in laboratory work [9].

Figure 5 presents the annual values of biodiesel production obtained for the six configurations depending on the number of mixers used for the extraction of lipids. As it can be seen all configurations present the same behaviour, the production of biodiesel grows with the number of mixers used during the extraction stages. The optimisation was limited to 5 mixers as the costs of the process of extraction is directly related to the number of mixers and, the increase in the quantity of produced biodiesel by the last units is much smaller in comparison with the increase of the costs (law of diminishing returns). As stated in subsection 2.1.2., more than 90% of lipids were extracted after 5 consecutive extraction steps in the laboratory work.

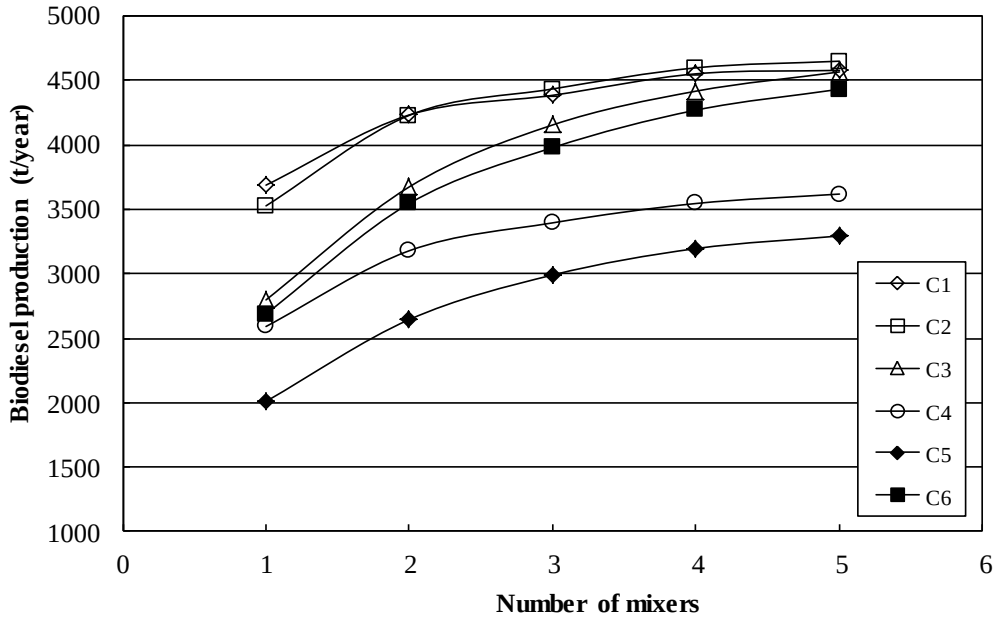


Figure 5. Biodiesel production *vs* number of stages in the extraction of lipid from primary sewage sludge.

As shown in Figure 5, the highest productions of biodiesel were attained with 5 mixers for the configurations CS1, CS2 and CS3, approximately 4600 tonnes of biodiesel per year. Slightly lower amount, approximately 4400 of biodiesel per year, was achieved by configuration CS6. Comparing the extraction configuration CS3 with CS6, where the only difference is the sludge pH, it can be concluded that the increase of pH from 2 to 4 does not have significant impact on the biodiesel production. Additionally, the use of less acid in the process will reduce the biodiesel production cost (discussed in further detail in section 3.2). The lowest results were obtained in the case of CS4 and CS5, approximately 3600 and 3300 tonnes of biodiesel per year, respectively. These two configurations have a mixing time of only 20 min, suggesting that the extraction time seems to be the most important parameter. The lower the extraction time, the lower the yield of lipid extracted and, therefore the lower the amount of biodiesel produced. However, in the case of CS1, also 20 min of mixing, the time is compensated by the higher amount of solvent, ratio sludge/hexane of 1/2. This configuration is able to extract high quantities of lipids, but by using a high quantity of solvent, which is not economically favourable for the process (discussed in further detail in section 3.2). The detailed values of biodiesel production for each configuration in each extraction stage are also presented in Table 4 and discussed in the following section.

3.2. Optimisation of lipid extraction configuration

In order to find the most profitable extraction conditions in biodiesel production from liquid primary sludge, the value of break-even price (BEP), for all configurations tested at each number of mixers was evaluated.

The BEP is defined as:

$$BEP\ (\$/t) = \frac{Manufacturing\ cost\ (\$/y) - By\text{-}product\ sales\ (\$/y)}{Production\ of\ biodiesel(t/y)} \quad (1)$$

Where the manufacturing costs is the total manufacturing cost as defined in section 2.4; by-products sales are the revenue from the sale of all by-products generated during the process, *i.e.*, potassium sulfate; production of biodiesel is tonnes of biodiesel produced per year.

Figure 6 presents the evolution of the break-even price for the six configurations studied as a function of the number of mixers used. The six configurations show exactly the same behaviour, the BEP decreases from one to two mixers, giving a minimum value for two mixers and then increases constantly until the five mixers. As shown in Table 4, independently of the configuration used, the total manufacturing costs increase faster in each stage than the production of biodiesel. The low increment of biodiesel production from 2 to 5 mixers is not compensated by the high increase in the manufacturing costs, resulting in continuous increase of BEP from 2 to 5 mixers. Furthermore, as can be seen in Table 4, using more extraction stages (mixers) entails also the increase of total investment cost, which consequently prolongs the payback period, *i.e.*, time to achieve benefits by the plant. As shown in Figure 6, the results of BEP indicates that for all tested configurations, two extraction stages (mixers) are sufficient to extract enough lipids to make biodiesel production profitable. Although additional mixers increase the amount of lipids and, thus the amount of biodiesel in the process, the installation of more than two mixing equipment is not cost-effective.

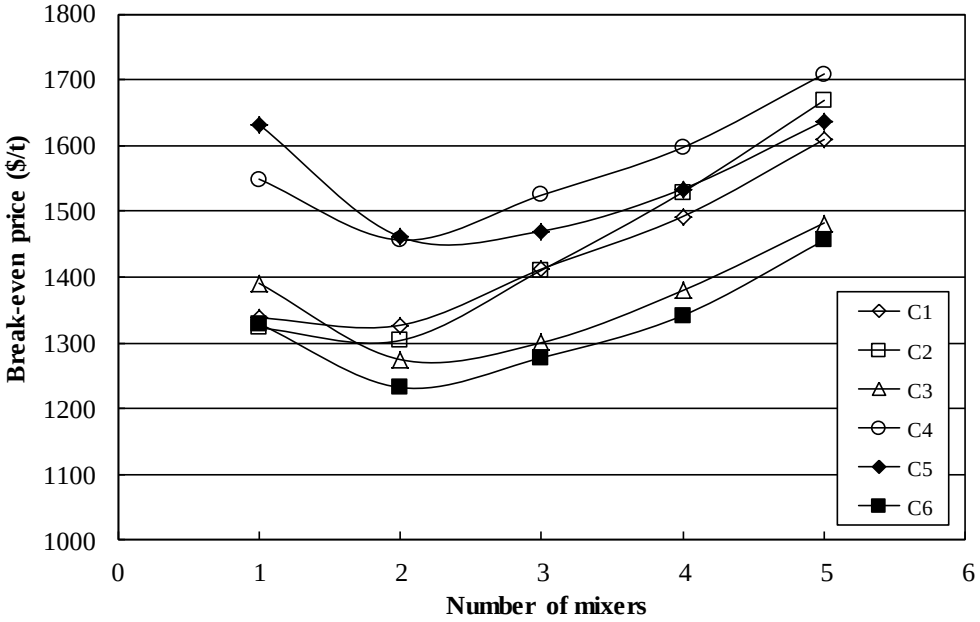


Figure 6. Break-even price of biodiesel production *vs* number of stages in the extraction of lipid from primary sewage sludge.

Table 4. Biodiesel production, total manufacturing and investment costs, by-product sales and break-even price for different extraction configurations.

Conf.	Item	Number of mixers 1	2	3	4	5
CS1	Biodiesel production (t/y)	3688	4233	4385	4551	4578
	Total manufacturing costs ($/y)	4949762	5633345	6210885	6811035	7387242
	Total investment costs ($)	11102888	12017556	12820760	13794481	14672841
	By-products sales ($/y)	15239	17425	18197	18733	18844
	Break-even price ($/t)	1338	1327	1412	1492	1610
CS2	Biodiesel production (t/y)	3527	4230	4434	4598	4649
	Total manufacturing costs ($/y)	4680052	5530978	6272897	7050857	7778452
	Total investment costs ($)	9606309	10744879	11743775	12938432	14026381
	By-products sales ($/y)	14516	17408	18403	18828	19136
	Break-even price ($/t)	1323	1304	1411	1529	1669
CS3	Biodiesel production (t/y)	2798	3675	4155	4417	4566
	Total manufacturing costs ($/y)	3901921	4698688	5419636	6112023	6787240
	Total investment costs ($)	6457913	7456932	8422813	9369721	10308893
	By-products sales ($/y)	11515	15253	17098	18107	18788
	Break-even price ($/t)	1391	1274	1300	1380	1482
CS4	Biodiesel production (t/y)	2591	3179	3395	3545	3619
	Total manufacturing costs ($/y)	4024948	4643102	5191317	5677963	6199331
	Total investment costs ($)	9144633	9896187	10614320	11317334	12021778
	By-products sales ($/y)	10664	13083	14091	14588	14892
	Break-even price ($/t)	1549	1456	1525	1597	1709
CS5	Biodiesel production (t/y)	2010	2645	2991	3196	3294
	Total manufacturing costs ($/y)	3289494	3876531	4409168	4916490	5407762
	Total investment costs ($)	6049257	6708422	7334908	7948455	8554410
	By-products sales ($/y)	8273	10980	12307	13068	13558
	Break-even price ($/t)	1632	1461	1470	1534	1638
CS6	Biodiesel production (t/y)	2689	3546	3979	4270	4432
	Total manufacturing costs ($/y)	3585196	4385208	5099454	5746372	6470870
	Total investment costs ($)	6446752	7455447	8407842	9356264	10295163
	By-products sales ($/y)	11071	14719	16517	17572	18241
	Break-even price ($/t)	1329	1232	1277	1342	1456

As presented in more detail in Table 4, the lowest BEP (1232 $/t) and therefore the best profitability was obtained by the configuration CS6 using two mixers. This configuration works using 50% solvent than CS2 and CS4 and four times less solvent than CS1. Consequently, the cost of total manufacturing for the CS6 was lower, mainly due to the lower amount of solvent that has to be handled (and recovered, and recycled). Despite the fact that the biodiesel production was increased by 19% in the case of CS1 and CS2 compared to CS6, the manufacturing cost increased by about 28% and 26% respectively, leaded to higher BEP. In addition, the total investment cost related mainly to storage and separation was also lower in the case of CS6 than the CS1, CS2 and CS4. Although the cost of mixing equipment, in the case of CS1 and CS4 was lower than for the best configuration (CS6), due to the lower residence time which permits to use smaller equipment, the cost of other investments was much higher resulting in higher overall investment cost. The detailed economic results for each configuration using two mixers are shown in Table 5. Continuing with the comparison, the configuration CS5, where the only difference is lower residence time with respect to CS6, allows a reduction in total manufacturing cost of 12% and also in investment cost of 10%, Table 4. However, the reduction of extraction time resulted in a significant decrease of biodiesel production by around 25%, giving therefore higher BEP than the best configuration (CS6).

Table 5. Disaggregated results for the different configurations with two mixers.

		CS1: sludge/hexane: 1/2, pH 2, 20min.	CS2: sludge/hexane: 1/1, pH 2, 60min.	CS3: sludge/hexane: 2/1, pH 2, 60min.	CS4: sludge/hexane: 1/1, pH 4, 20min.	CS5: sludge/hexane: 2/1, pH 4, 20min.	CS6: sludge/hexane: 2/1, pH 4, 60min.
Investment costs ($)	Total bare module	6812288	6090856	4227046	5609765	3802745	4226204
	Centrifugation and drying	0	0	0	0	0	0
	Reactors	179065	179090	166297	153018	138719	163057
	Distillation columns	687713	687707	687394	687061	686716	687314
	Flush & other separation equipment	612684	476573	388766	463642	375108	386897
	Mixing units	977908	1237273	1064023	796084	683097	1062606
	Heat exchangers	484640	474594	467108	475722	467202	466835
	Pumps	63038	58780	54858	57398	53303	54652
	Storage	3807240	2976840	1398600	2976840	1398600	1398600
	Fix. cap. costs (bare, cont. aux.)	10450049	9343373	6484289	8605380	5833410	6482997
	Working capital	1567507	1401506	972643	1290807	875012	972450
	Total investment costs	12017556	10744879	7456932	9896187	6708422	7455447
Manufacturing costs ($/y)	Raw materials	949707	944993	890353	592408	531224	638338
	Utilities	403374	511108	370253	266042	170500	367623
	Steam	245705	149246	97817	155733	86911	95290
	Cooling	2905	2890	2531	2169	1826	2437
	Electricity	154557	358767	269724	107984	81633	269722
	Makeup water	206	206	181	155	130	174
	Fuel (spray dryer)	0	0	0	0	0	0
	Operation labour	661500	661500	661500	661500	661500	661500
	Direct manufacturing cost	2014581	2117601	1922106	1519950	1363224	1667461
	Overhead (fix. & gen. exp. costs)	3618764	3413377	2776582	3123152	2513307	2717746
	Total manufacturing costs	5633345	5530978	4698688	4643102	3876531	4385208
	Biodiesel production (t/y)	4233	4230	3675	3179	2645	3546
	Break-even price (BEP) ($/t)	1327	1304	1274	1456	1461	1232

Looking at the configuration CS3, which has only lower pH than the configuration CS6, the investment cost was almost equal but the manufacturing cost was slightly increased by about 7% due to the higher amount of acid

required. The increased of manufacturing cost for this configuration was not compensated by the insignificant rise of biodiesel production (≈ 4%), thus having higher BEP than CS6. It can be concluded that the optimized configuration, (*i.e.*, CS6), does not only allow to minimize the biodiesel production cost, but also to reduce the solvent and acid use, making the process less detrimental for equipment and environment.

3.3. Details of the best configuration

The optimized extraction process (*i.e.,* configuration CS6 with two mixers) allows the plant to produce 3546 tonnes of biodiesel per year, with the minimum biodiesel cost of 1232 $/t. The biodiesel production plant is divided into four processes: extraction (lipid extraction from liquid sludge), recovery (recovery of lipids from solvent), reaction (biodiesel production), and purification (biodiesel separation, purification and catalyst neutralization). In order to establish the cost requirement of each process in the whole biodiesel production plant, the detailed economic analysis was distributed and the results are presented in Table 6.

Table 6. Disaggregated results for the optimal configuration CS6[1] as a function of the process steps.

		Unit ($)				
	Item	Extraction	Recovery	Reaction	Purification	Total
Investment costs ($)	Total bare module	2270511	410244	316032	1229418	4226204
	Centrifugation and drying	0	0	0	0	0
	Reactors	0	0	129214	33843	163057
	Distillation columns	0	0	0	687314	687314
	Flush & other separation equipment	249299	63451	0	74147	386897
	Mixing units	942909	96465	9563	13670	1062606
	Heat exchangers	0	239677	0	227158	466835
	Pumps	26143	10652	4035	13823	54652
	Storage	1052160	0	173100	173340	1398600
	Fix. cap. costs (bare, cont. aux.)	3482963	629315	484793	1885927	6482997
	Working capital	522444	94397	72719	282889	972450
	Total investment costs	4005408	723712	557512	2168816	7455447
Manufacturing costs ($/y)	Raw materials	271648	0	313255	53435	638338
	Utilities	269715	44350	60	53497	367623
	Steam	0	44346	0	50944	95290
	Cooling	0	0	59	2378	2437
	Electricity	269715	5	1	1	269722
	Makeup water	0	0	0	174	174
	Fuel (spray dryer)	0	0	0	0	0
	Operation labour	270000	40500	155250	195750	661500
	Direct manufacturing cost	811363	84850	468565	302683	1667461
	Overhead (fix. & gen. exp. costs)	1312587	210534	440997	753629	2717746
	Total manufacturing costs	2123950	295384	909562	1056311	4385208

[1] *ratio sludge/hexane 2/1, pH 4, 2 mixers and 60 min of mixing*

Regarding the total investment cost, the extraction step is responsible for 54% of the total investment followed by purification (29%), recovery (10%) and reaction (7%). With respect to manufacturing cost, the extraction step is also the most expensive, representing 48% of the total, while purification, recovery and reaction account for 24%, 7% and 21% respectively. This economic analysis of biodiesel production demonstrates that the proposed direct extraction from liquid sludge is the most cost intensive step for the investment as well as for the manufacturing of the process. Therefore, any improvement of the lipid extraction step would have a high impact on the final profitability of the process and finally on the biodiesel price. On the other hand, the purification step is the second expensive one due to the high amount of water used for the separation and purification of the final product. It is well known that in the conventional synthesis of biodiesel, using acid or basic catalyst, the

separation and purification of final product from catalyst is difficult and required high amount of energy [28,29]. Thus, the improvement of the separation step by using better catalyst for the reaction, easy to separate and reuse, could also reduce the final cost of biodiesel production from liquid sludge. However, this implies the employment of new biodiesel manufacturing technologies based on *e.g.*, heterogeneous catalysis [29] or ionic liquid catalyst [28], capable to overcome the problems related to conventional catalyst but that, to our knowledge, are not ready to be commercially used (or are not economically attractive).

3.4. Comparison with dry routes processes

In order to decide on the most economically favourable process, the economic data of the wet route *i.e.*, the optimized biodiesel production process with extraction configuration CS6, was compared to conventional dry route (extraction from dry sludge and consequent conversion of lipids into biodiesel) and to *in situ* dry route (direct production of biodiesel from dry sludge). The detailed process flow diagrams were modelled with AspenHysys® simulation package, and the characterization of the main material streams involved are presented in Figure 2, Figure 3 and Figure 4 for wet, dry conventional and dry *in situ* routes, respectively.

Table 7. Comparison of optimal configuration CS6[1] (wet route) with dry routes.

	Item	Process ($)		
		CS6 (wet route)	Dry route (conventional)	Dry route (*in situ*)
Investment costs ($)	Total bare module	4226204	9780923	11290902
	Centrifugation and drying	0	6400000	6400000
	Reactors	163057	189218	1196930
	Distillation columns	687314	688299	316706
	Flush & other separation equipment	386897	384994	437067
	Mixing units	1062606	255744	339856
	Heat exchangers	466835	420554	265841
	Pumps	54652	43513	56962
	Storage	1398600	1398600	2277540
	Fix. cap. costs (bare, cont. aux.)	6482997	15003935	17320243
	Working capital	972450	2250590	2598036
	Total investment costs	7455447	17254525	19918280
Manufacturing costs ($/y)	Raw materials	638338	978234	3910752
	Utilities	367623	2617825	5263030
	Steam	95290	558431	3075539
	Cooling	2437	28423	156467
	Electricity	269722	92086	92363
	Makeup water	174	245	21
	Drying: Fuel (spray dryer) and Electricity (centrifuge)	0	1938641	1938641
	Operation labour	661500	717750	508500
	Direct manufacturing cost	1667461	4313809	9682283
	Overhead (fix. & gen. exp. costs)	2717746	5174616	6574965
	Total manufacturing costs	4385208	9488425	16257248
	Biodiesel production (t/y)	3546	4700	4425
	Break-even price (BEP) ($/t)	1232	2014	3674

[1] *ratio sludge/hexane 2/1, pH 4, 2 mixers and 60 min of mixing*

As shown in Table 7 the wet route process is much more cost-effective than both dry routes. With respect to investment cost, both dry routes have a total investment cost around twice higher than the wet route. The wet route process requires large equipment size for lipid extraction step due to large volume of liquid sludge associated, and consequently with a higher mixing cost. However, the cost of dewatering and drying equipment

in the dry routes is six times higher as compared to mixing equipment used in wet route, making the total investment cost of wet route more profitable. Regarding the manufacturing cost, dry processes are also more costly, with the total manufacturing cost twice higher in the case of dry conventional and approximately 4 times higher in the case of dry *in situ* (in both cases compared with the wet route). The much higher manufacturing cost is mainly related to the cost required for sludge drying which represents about 20% (334 $/t of biodiesel) and 10% (314 $/t of biodiesel) of the total biodiesel cost for dry conventional and dry *in situ*, respectively. As can be seen in Table 7, although the biodiesel production is higher in both dry routes, owing to more efficient extraction of lipid from dry sludge than from wet one, the much larger increase of manufacturing cost results in higher BEP as compared to wet process. Therefore, the proposed process of biodiesel production using directly liquid sludge is more cost-effective than the conventional dry or *in situ* dry routes, giving the break-even price of 1232 $/t , 1656 $/t, 3145 $/t, respectively.

Comparing the dry routes, the conventional two-step dry route is more profitable than the *in situ* dry route. This result is in agreement with other authors who demonstrated that two-step process is more energetically favourable than the one-step *in situ* [12,17].

It is interesting to compare the BEP of biodiesel produced following the *in situ* approach in this work (3145 $/t) with the BEP obtained by Mondala et al. (2009) [7] (970 $/t), as they differ widely. On the one hand, the cost of sludge drying used in the present study according to Lassing et al. (2008) [23] was higher than the cost presented by Mondala et al. (2009) [7], who based their calculation on the cost of sludge drying given by Dufreche et al. (2007) [8]. Both authors did not described details about the method and equipment used for drying, presenting such a favourable drying cost (2.1 $/t of wet sludge) as compared to other authors, 4.3 and 52.2 $/t of wet sludge reported by Lassing et al. (2008) [23] and Kwon et al. (2012) [4], respectively. Therefore, the sewage sludge drying process was modelled in a similar way to the totally defined procedure by Lassing et al. (2008) [23], increasing the BEP as compared to the BEP obtained by Mondala et al. (2009) [7] for the same process. On the other hand, the purification of the final product from non-saponifiable lipids, which was performed in the present study, but was not mentioned in other works [7,8,17] also contributes to the cost raising. Furthermore, the costs of raw materials presented by Mondala et al. (2009) [7] are also lower than the presented here and should be included in order to compare the approaches. Finally, regarding the *in situ* studies [7,8], only economic evaluation was performed, however the whole process was not modelled and simulated. The simulation of process scale up leads to get a full techno-economic evaluation of the plant and finally more realistic cost of biodiesel are obtained.

3.5. Break-even prices comparison

The optimised BEP of biodiesel produced from liquid primary sludge was estimated to be 1232$/t. This value is comparatively lower than the current price of fossil diesel in Europe, estimated to be 1445 $/t on March 2015 [30]. Therefore, it is evident that the biodiesel obtained from municipal primary sludge has a high potential to economically compete with fossil diesel.

On the other hand, it is interesting to compare the BEP of biodiesel from sewage sludge to that from microalgae, the alternative biodiesel feedstock that has been investigated extensively for a very long time but whose industrialisation is not yet economically viable [31]. The BEP of biodiesel produced from sewage sludge is lower

than the obtained from microalgae in different studies; 1344 $/t calculated with the best scenario available [23], 2953 $/t for a biodiesel with microalgae produced in open ponds [32], and 5700 $/t obtained in an exhaustive study [31]. As municipal sewage sludge is a waste, provided for free from WWTP, the cost of biomass production is eliminated; which is not the case in microalgae biodiesel manufacturing, where biomass production accounts for 65% of the overall cost [31].

4. Conclusions

The detailed techno-economic study indicates that the proposed biodiesel production process from liquid primary sludge is economically feasible and more cost-effective than alternatives from dry sludge. The required biodiesel selling price for the optimised lipid extraction step was estimated to be 1232 $/t, which is lower than the current cost of fossil diesel and the cost of biodiesel produced from microalgae. Thus, the municipal sludge has a large potential as a cost-competitive, plentiful and non-edible feedstock for biodiesel production. Additionally, further improvement of the proposed process (especially lipid extraction from liquid sludge and biodiesel purification) could lower the biodiesel price even more.

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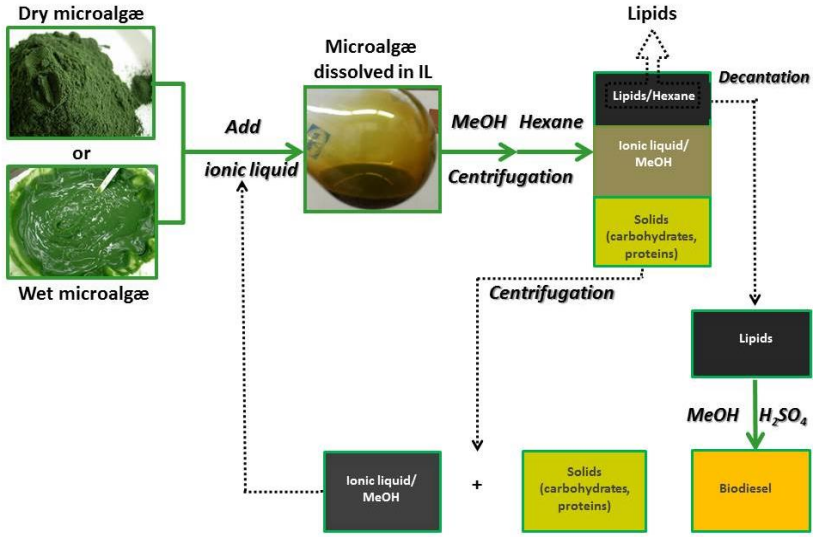
Chapter 5

A novel recovery process for lipids from microalgæ for biodiesel production using a hydrated phosphonium ionic liquid [1]



ABSTRACT

The use of a hydrated phosphonium ionic liquid, $[P(CH_2OH)_4]Cl$, for the extraction of microalgæ lipids for biodiesel production, was evaluated using two microalgæ species, *Chlorella vulgaris* and *Nannochloropsis oculata*. The ionic liquid extraction was compared to the conventional Soxhlet, and Bligh & Dyer, methods, giving the highest extraction efficiency in the case of *C. vulgaris*, at 8.1%. The extraction from *N. oculata* achieved the highest lipid yield for Bligh & Dyer (17.3%), while the ionic liquid extracted 12.8%. Nevertheless, the ionic liquid extraction showed high affinity to neutral/saponifiable lipids, resulting in the highest fatty acid methyl esters (FAMEs)-biodiesel yield (4.5%) for *C. vulgaris*. For *N. oculata*, the FAMEs yield of the ionic liquid and Bligh & Dyer extraction methods were similar (>8%), and much higher than for Soxhlet (<5%). The ionic liquid extraction proved especially suitable for lipid extraction from wet biomass, giving even higher extraction yields than from dry biomass, 14.9% and 12.8%, respectively (*N. oculata*). Remarkably, the overall yield of FAMEs was almost unchanged, 8.1% and 8.0%, for dry and wet biomass. The ionic liquid extraction process was also studied at ambient temperature, varying the extraction time, giving 75% of lipid and 93% of FAMEs recovery after thirty minutes, as compared to the extraction at 100 °C for one day. The recyclability study demonstrated that the ionic liquid was unchanged after treatment, and was successfully reused. The ionic liquid used is best described as $[P(CH_2OH)_4]Cl \cdot 2H_2O$, where the water is not free, but strongly bound to the ions.

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1. Introduction

The expected depletion of fossil fuels, which are currently the major sources of all energy consumed worldwide, and the environmental problems associated with their combustion, will limit their utilisation in the future.[1] In order to meet the continuous growth of the world's energy demand, alternative renewable fuels with minimal environmental impact are needed. One of the most promising renewable fuels proposed as an alternative to fossil oil is biodiesel, which is biodegradable, has low toxicity, and low emission profiles.[1–4] Additionally, biodiesel can be easily used with current engine and refuelling technology without major modification.[1,4] Biodiesel production has increased dramatically in the past decade, increasing the demand for lipid feedstocks, such as vegetable oils and animal fats. Therefore, the competitive potential of biodiesel is currently limited by the price of common lipid feedstocks, which represents more than 75% of the overall biodiesel production cost, strongly influencing the final price of this biofuel, and raising the concerns of a food versus fuel crisis.[1,3,4]

In order to overcome the competition between energy and food, non-edible microalgæ as a lipid feedstock are receiving increasing attention. Microalgæ have long been considered as a promising alternative to vegetable oils for biodiesel production, due to their extremely rapid biomass productivity, their capability to accumulate higher amounts of lipid than conventional oil crops, and their minimal land requirement.[1–3] Under suitable culture conditions, some microalgæ species are able to accumulate up to 50%–70% of lipids per dry biomass, with much of this as neutral lipid adequate for biodiesel production.[5,6]

Despite the advantages of microalgæ over the oil crops, the production of biodiesel using microalgæ is still economically challenging, due to the fact that among the steps required for the production of microalgæ biodiesel, lipid extraction (including microalgæ drying) is the most costly, and has a significant energy demand. Any improvement on the extraction step would have a direct impact on the economic and environmental impact of the process.[7,8] Furthermore, the lipid extraction step is particularly important because it is connected to cell disruption. Although higher lipid yield was reported after microalgæ pre-treatment by various cell disruption methods,[9] the additional energy then required leads to an economically infeasible process.[2,10]

The most applied method to extract lipids from microalgæ is organic solvent extraction. The Soxhlet method using hexane as a solvent,[11] and Bligh & Dyer's method using a mixture of trichloromethane and methanol,[12] are the two most commonly used methods.[4,13] However, these methods are commonly applied to dry biomass, which is also their drawback, owing to the high cost associated with microalgæ drying.[7,8] Additionally, it was reported that the extraction of lipids from wet biomass using organic solvents has an adverse effect on the extraction efficiency because of the water barrier.[14] Furthermore, health, security, and regulatory problems related to the use of volatile organic solvents are also very important issues.[2,4]

Ionic liquids have been utilised in a large number of chemical reaction types, as may be seen in books by Wasserscheid and Welton,[15] and Freemantle,[16] and a review on the industrial applications of ionic liquids.[17] Ionic liquids have attracted significant attention for their use as green replacements for harmful volatile organic solvents due to their design properties,[18] non-volatile character (except at low pressures and high temperatures),[19] excellent chemical and thermal stability, and potential recoverability.[17]

Some ionic liquids have superior properties for dissolution of cellulosic biomass.[13,20,21] As dissolution takes place at a molecular level,[22] ionic liquids should have merit at preventing cellulose from crystallisation, and removing hemicelluloses and lignin. However, high temperature is still needed in most cases.

As the cell walls of microalgæ are composed mainly of cellulose, ionic liquids are also considered to be effective solvents for microalgæ dissolution. Thus, the role of ionic liquids in the microalgæ lipid extraction is not only to replace organic solvents, but also to utilise their ability to simultaneously dissolve the biomass, and thus disrupt the cellular structure of the microalgæ.[23–25] As first, the feasibility of lipid extraction using mixture of ionic liquid and polar organic solvent was demonstrated.[8,13,26,27] However lower extraction efficiency was found when wet biomass was used.[26] More recently, various ionic liquids were directly applied to microalgæ biomass evaluating the effect of ionic liquids mixtures,[23] molten salt/ionic liquid mixtures,[24] and ultrasound applications.[25] Although, in these studies, the lipid yield was improved using ionic liquids alone or in admixture, as compared to conventional organic solvents, dry biomass was still used.

The direct dissolution of wet microalgæ using chloride ionic liquids has been studied by Teixeira,[8] where higher lipid yields than the conventional Bligh & Dyer method were also reported. Unfortunately, the chloride ionic liquids used so far are solid at room temperature. To melt them and make them suitable for treatment of algæ, it is necessary to heat them above 100 °C. It should also be possible to treat them with supercooled ionic liquids, but these are normally highly viscous.

Marine microalgæ have recently been shown to dissolve directly without heating in polar ionic liquids (such as 1-ethyl-3-methylimidazolium methyl hydrogenphosphonate, $[C_2mim][MeO(H)PO_2]$ (incorrectly named as methylphosphate in the original paper).[28] According to Fujita et al., the main advantage, when polar ionic liquids are used, is that there is no need to dry the microalgæ, so energy can be saved.[28]

All of the studies stated above focussed on the utilisation of 1,3-dialkylimidazolium ionic liquids,[29] however the high cost of these ionic liquids[13] could limit their availability. They all also focussed on using the hydrogen-bonding ability of the anion to penetrate the structure; here we introduce a cation deliberately selected to possess significant hydrogen-bonding potential. Therefore, this study investigates the utilisation of a specific phosphonium ionic liquid, $[P(CH_2OH)_4]Cl$ (which is commercially available as an aqueous solution and is inexpensive compared to 1,3-dialkylimidazolium ionic liquids, as it is used commercially as a flame retardant),[30] to extract lipids from microalgæ biomass. Importantly, this cation contains four –OH functionalities, meaning it will be a very effective hydrogen bonding moiety. The influences of the ionic liquid solution on the lipid extraction efficiency, saponifiable lipid content and fatty acids composition were investigated in comparison to conventional methods. The lipid extraction from wet biomass was evaluated and compared with that from dry biomass, in order to decide on the most economically favourable process.

In this work, heating was applied to ensure that full dissolution occurred, but actually the focus of our paper is on recuperation of lipids and the other dissolved products and their analysis, applying a novel ionic liquid aqueous system. Additionally, milder conditions (stirring at ambient temperature) and ionic liquid recycling have been successfully tested.

2. Experimental

2.1. Chemicals

Lipid extraction experiments were conducted using tetrakis(hydroxymethyl)phosphonium chloride ionic liquid– $[P(CH_2OH)_4]Cl$ solution (80% w/w in water), hexane (purity >97%), trichloromethane (purity >99.9%) and methanol (purity >99.9%), all supplied by Sigma-Aldrich. Transesterification experiments were carried out using anhydrous methanol (purity >99.8%), sulfuric acid (purity >99.999%) and methyl heptadecanoate (purity >99.0%) from Sigma-Aldrich at the highest purity available. Sodium chloride, sodium hydrogencarbonate and anhydrous sodium sulfate (all >99% purity) were also provided by Sigma-Aldrich. The standard used for identification and quantification of fatty acid methyl esters (FAMEs) was supplied by Supelco (a 37-component FAMEs mix).

2.2. Microalgæ cultivation, harvest and drying

Nannochloropsis oculata and *Chlorella vulgaris* were cultivated at AlgoSource's production unit (Alpha Biotech) near Saint-Nazaire, France. The production system is made of raceways in greenhouses with thermal control. Harvesting is achieved via centrifugation at around 5000 rpm. The *C. vulgaris* was dried in an oven using an air flux at 40 °C for 24 h. For this study, *C. vulgaris* was received in dried form and *N. oculata* in harvested (dewatered) form. Then, dewatered *N. oculata* was allowed to freeze for two days at −20 °C, followed by freeze-drying in a vacuum freeze dryer (Armfield Limited, model FT33-A) for two days. Depending on the experimental design, *N. oculata* was either used in freeze-dried form, or as received in dewatered form. In order to determine moisture content in received *C. vulgaris* and *N. oculata*, and in freeze-dried *N. oculata*, 1 g of biomass was placed in a conventional oven at 105 °C and dried to obtain constant weight. The experiment was performed in triplicate and the water content was 71.7 ± 0.3%, 6.8 ± 0.1% and 6.3 ± 0.1% for dewatered *N. oculata*, freeze-dried *N. oculata* and dried *C. vulgaris* respectively. In addition, experiments using wet biomass (ca. 98% of water) were performed (not reported here because of the difficulty of sample reproducibility), and produced results similar to those found for dewatered samples.

2.3. Lipid extraction

For all extraction procedures, the lipid yield was determined gravimetrically and expressed by eqn (1),

$$\frac{\text{Lipid}}{\%} = \frac{\left(\frac{\text{Lipid}}{\text{g}}\right)}{\left(\frac{\text{Microalgæ}}{\text{g}}\right) \times \frac{\text{TS}}{\%}} \times 100 \qquad (1)$$

where Lipid is the amount of extracted lipids, Microalgæ is the amount of microalgæ used for extraction, and TS is the total solid content of microalgæ used for extraction.

2.3.1. Soxhlet lipid extraction

Lipids were extracted from microalgæ biomass in a Soxhlet extractor using hexane as a solvent. Before the extraction, dried *C. vulgaris* or freeze-dried *N. oculata* was firstly mashed and ground using a mortar and pestle until the sample was as homogenous as possible. The biomass (ca. 1 g) was transferred into a cellulose thimble

(Filter Lab, ANOIA S.A.) which was plugged with glass wool to avoid the scattering of the solid. Hexane (200 cm^3) was added into a round-bottomed flask of known weight. The heating process was monitored to allow 80 cycles of the extraction in ca. 7 h. After the lipid extraction, the hexane was removed from the flask using a rotary evaporator. The flask, containing the lipids, was stored in a desiccator overnight and weighed the next day.

2.3.2. Lipid extraction via Bligh & Dyer's method

Lipids were extracted from microalgæ biomass using a modified method of Bligh & Dyer (B&D).[12] Dried *C. vulgaris*, or freeze-dried *N. oculata*, (1 g) was blended with distilled water (10 cm^3). Wet *N. oculata* (1 g, total solids equivalent) was blended with distilled water (ca. 7 cm^3), supplementing the water already contained in the wet biomass (72%, wt). The lipids were extracted by shaking the prepared sample for 5 min with a solvent mixture of trichloromethane–methanol (1 : 2, v/v; 45 cm^3) in a separating funnel. Then trichloromethane (15 cm^3) and distilled water (15 cm^3) were added to form a two-phase system. The trichloromethane layer, containing the lipids, was separated. The extraction was repeated once more by adding further trichloromethane (15 cm^3) to the remaining solution of cells suspended in water and methanol system. The trichloromethane layers were combined, washed with distilled water (2 × 10 cm^3), dried over anhydrous sodium sulfate, and the solvent removed in a rotary evaporator. The lipids were stored in a desiccator overnight, and weighed the next day.

2.3.3. Lipid extraction using ionic liquid

The mixture of biomass (dried *C. vulgaris* or freeze-dried *N. oculata* or wet *N. oculata*) and $[P(CH_2OH)_4]Cl$ in a ratio of 1 g total dry solids equivalent to 10 cm^3 ionic liquid was heated at 100 °C for 24 h with magnetic stirring. The conditions were selected to ensure the reaction went to completion, and the obtained lipid yield was compared to conventional Soxhlet and B&D methods. The mixture was then cooled to room temperature and methanol (10 cm^3) was added, precipitating the dissolved components and leaving the less dense lipids insoluble. The experimental steps, using the example of *N. oculata* are presented in Figure 1.

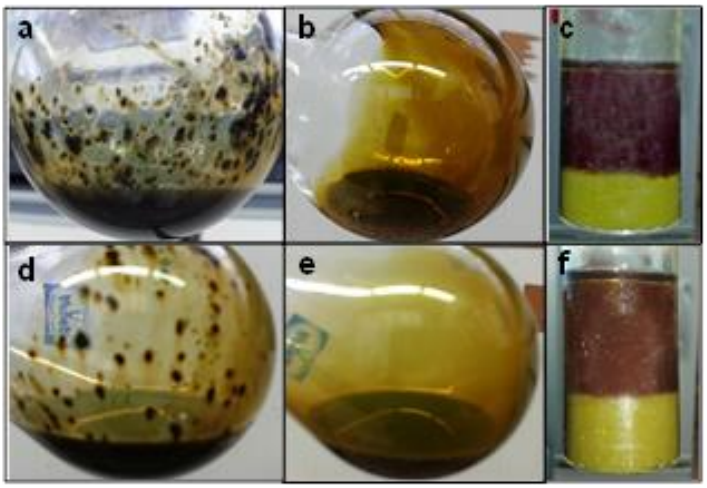

Figure 1. Photographs of the extraction steps using $[P(CH_2OH)_4]Cl$, from *N. oculata*. The experiments shown in (a), (b), and (c) use dry biomass; (d), (e), and (f) use wet biomass. (a) and (d) represent biomass and ionic liquid just before the experiment. (b) and (e) show biomass dissolved in the ionic liquid after 24 h at 100 °C. (c) and (f) show the produced triphasic systems after methanol addition, where the bottom layer contains the precipitated components, the middle layer contains the ionic liquid and methanol. On careful examination of (c) and (f), the upper lipid phase can also be observed.

The upper lipid phase in Figure 1(c) and 1(f) cannot be seen as the lipid content in the whole mixture system is far too low, with the lipids (ca. 0.14 cm^3; droplets) being dispersed in the solvent mixture (20 cm^3; IL–methanol 1 : 1 v/v).

In order to calculate the exact lipid yield, hexane (5 cm^3) was added three times to the biphasic mixture of ionic liquid–biomass–methanol. The (upper) hexane phase, containing lipids, was separated by centrifugation, combined, and washed with distilled water (2 × 10 cm^3) to remove any traces of polar compounds. Then, the hexane phase was dried over anhydrous sodium sulfate and evaporated in a rotary evaporator. The lipids were stored in a desiccator overnight and weighed the next day. The same procedure was applied for the study of ionic liquid extraction at ambient temperature from wet *N. oculata*, varying only the time of contact between microalgæ and ionic liquid (24 h, 12 h and 0.5 h).

2.4. Lipid transesterification and FAMEs analysis

The lipids extracted from the microalgæ were converted into FAMEs-biodiesel through acid catalysed transesterification/esterification using a modified version of Christie's method[31] (i.e. with hexane instead of toluene, and using methyl heptadecanoate as an internal standard). The FAMEs produced by transesterification were analysed using an Agilent gas chromatograph 6890GC with a flame-ionisation detector (GC-FID), according to Agilent Application Note 228-398 using a HP-INNOWax column (19091N-133).[32] For the calibration of the method, a 37-component FAMEs standard mixture was used. Results of the GC-FID were used to calculate the amount of saponifiable (transesterifiable) material in the lipid, eqn (2), and hence the yield of biodiesel (FAMEs) produced from dry microalgæ biomass, eqn (3). The compounds which could not be identified by GC-FID are represented as "others".

$$\frac{\text{Saponifiable}}{\%} = \frac{\left(\frac{\text{FAMEs}}{\text{g}}\right)}{\left(\frac{\text{Lipid}}{\text{g}}\right)} \times 100 \qquad (2)$$

where FAMEs is the total FAMEs produced after transesterification, determined by the GC-FID run, and Lipid is amount of lipid used for transesterification.

$$\frac{\text{FAMEs}}{\%} = \frac{\text{Lipid}}{\%} \times \frac{\text{Saponifiable}}{\%} \qquad (3)$$

where Lipid is the lipid content in dry microalgæ, eqn (1), and Saponifiable is the FAMEs content in the lipid, eqn (2).

2.5. Microscopic visualisation

In order to evaluate the degree of microalgæ dissolution in ionic liquid, after each ionic liquid treatment, the morphologies of the dissolved *N. oculata* were observed by optical microscopy (NIKON, model TE2000-E). The reaction aliquots were diluted five times in water to precipitate the microalgæ components, giving better visualisation, according to the procedure described by Teixeira.[8] The cells amount, length and area, as an average of three different micrographs of the same sample, were quantified using an ImageJ programme.

2.6. Ionic liquid recycle

After lipid extraction from wet *N. oculata*, the ionic liquid solution was separated from the precipitated microalgæ components by centrifugation. The supernatant liquid, containing ionic liquid, methanol and residual microalgæ water, was evaporated to remove the methanol and some of the residual water, and the structure of the recovered ionic liquid was checked by NMR (Varian NMR System 400). The extraction of lipids using recycled ionic liquid was tested using the same procedure (vide supra), but now at ambient temperature for 30 min. In order to compare the lipid yield obtained from recycled ionic liquid, the lipids from a blank control sample were extracted following the same procedure, but using water instead of ionic liquid.

2.7. FTIR spectroscopic analysis

As shown in Figure 1(c) and 1(f) after methanol addition, the dissolved microalgæ cell components precipitated. This solid, after extraction of the lipids, was washed with methanol, and then the remaining methanol was evaporated in a rotary evaporator to yield dry microalgæ biomass. Both the solid and the extracted lipids were analysed by Fourier Transform Infrared (FTIR) spectroscopy. These samples, without any further preparation, were directly scanned using a Fourier Jasco FT/IR-600 Plus spectrometer with a diamond golden gate ATR (GS10542, Specac Ltd) reflectance cell.

3. Results and discussion

3.1. Effect of extraction methods on the lipid yield

Total lipid contents, extracted from the two types of microalgæ using Soxhlet, B&D and ionic liquid methods are presented in Table 1. All extraction experiments were performed at least twice.

Table 1. Extraction and transesterification yields from each species for each extraction method. Values are means ± SD; n ≥ 2.

		Yield / %		
Microalgæ species	Extraction method	Lipid [a]	Saponifiable [b]	FAMEs [a]
C. vulgaris	Soxhlet	3.6 ± 0.7	65.1 ± 0.4	2.4 ± 0.1
	Bligh & Dyer	6.9 ± 0.6	39.7 ± 0.8	2.7 ± 0.1
	$[P(CH_2OH)_4]Cl$	8.1 ± 0.3	55.8 ± 1.2	4.5 ± 0.1
N. oculata [c]	Soxhlet	9.1 ± 0.3	54.3 ± 0.6	4.9 ± 0.1
	Bligh & Dyer	17.3 ± 0.2	48.7 ± 0.4	8.4 ± 0.1
	$[P(CH_2OH)_4]Cl$	12.8 ± 0.3	63.5 ± 1.0	8.1 ± 0.1

[a] *Lipid and FAME yield on the basis of dry biomass*
[b] *Transesterification yield on the basis of lipid*
[c] *Freeze-dried form used for the experiments*

In case of *C. vulgaris*, the lowest lipid yield was obtained by Soxhlet (3.1%) followed by the B&D (6.9%) method. The low lipid content extracted by Soxhlet as compared to B&D can be explained by the difference in polarity between the solvents used. In Soxhlet extraction, hexane was used as the extracting solvent, since it is well know that it is able to extract the mainly non-polar (neutral) lipids – but not polar lipids, which are the main

component of the microalgæ cell membrane. In contrast, the polar nature of the trichloromethane–methanol mixture used in the B&D method additionally induces the extraction of the polar lipids such as phospholipids and glycolipids, thereby increasing total lipid content.[33,34] It has also been demonstrated that this trichloromethane–methanol mixture can extract a greater amount of neutral lipids than hexane alone, attributed to presence of polar methanol.[14] Because some neutral lipids are bound to polar lipids and/or to proteins in the cell membrane, they are not easily available to non-polar solvents in this form. Polar solvents, like methanol, can disrupt this bonding and liberate neutral lipids which are otherwise dissolved in the organic phase.[35] Thus, the B&D extraction was able to extract polar lipids and more neutral lipids giving higher lipid yield than the Soxhlet method.

For *C. vulgaris*, the method using ionic liquid gave the highest extraction efficiency (8.1%), which was 1.2 and 2.3 times higher than B&D and Soxhlet methods, respectively (see Table 1). These results indicate that using organic solvents is not sufficient to extract all the microalgæ lipids: this may be ascribed to limited diffusion of organic solvents through the cell walls of the microalgæ into the inner cells.[2] In contrast, the water-miscible ionic liquid, $[P(CH_2OH)_4]Cl$, could mediate the efficient extraction of lipids from microalgæ by dissolving the hydrophilic content of the biomass, releasing lipids from the cells. The lipids do not dissolve in the ionic liquid, but float during the dissolution process (because they have a lower density than the ionic liquid solution). It has already been reported that hydrophilic and water miscible ionic liquids have a higher capacity to extract lipids because the lipids do not dissolve in the ionic liquids, and remain insoluble,[13] and it has been proposed that the high extraction efficiency may be due to the efficient disruption of the cellular structure of microalgæ caused by the biomass dissolution.[25] In contrast, Choi et al. also reported that, during ionic liquid extraction, the cell walls are modified, affected by the ions, liberating inner material (including lipids) without destroying the cell walls.[23] But, because lipids are not soluble in many ionic liquids, then they cannot be directly extracted: hence, they must be liberated after destruction or modification of the cell walls.

Like *C. vulgaris*, the extraction from *N. oculata* also showed the lowest lipid yield for Soxhlet extraction (9.1%). However, in contrast to *C. vulgaris*, the B&D method extracted more lipids from *N. oculata* than the ionic liquid method, giving lipid yields of 17.3% and 12.8% respectively. This difference could be attributed to the different cell wall resistance of each microalgæ species. As the mechanism of solvent extraction by Soxhlet and B&D is likely to be diffusion of lipids across the cell wall into a solvent of high lipid affinity,[34,35] the permeability of the cell walls will affect extraction efficiency. Elsewhere, *Chlorella sp.* was found to have a cell wall with higher resistance to cell disruption methods than *Nannochloropsis sp.*[36] Because of the apparent greater permeability of the *N. oculata* cell walls, the solvent extraction methods were more efficient for lipid extraction than for *C. vulgaris*. Ionic liquid extraction gave only 1.4 times more lipids than Soxhlet, and gave 1.3 times less lipids than B&D. Furthermore, *N. oculata* is known to contain a really high content of polar lipids.[35] Thus, the higher extraction efficiency for B&D could be due to their higher affinity for the polar trichloromethane–methanol mixture, hence being capable of extracting a higher content of polar lipids and/or co-extraction of the non-lipid fraction.

3.2. Effect of extraction methods on the saponifiable (transesterifiable) lipids

The total lipid content extracted from microalgæ biomass does not correspond to that which can be converted to FAMEs-biodiesel, and the microalgæ biodiesel productivity is often confused with total lipid productivity. Total

lipid content extracted from microalgæ consists of non-polar (neutral) lipids, polar lipids (complex phospholipids and glycolipids) and sometimes non-lipids (proteins and carbohydrates) which could be co-extracted depending on the solvent used.[35] Neutral lipids are comprised of acylglycerols, free fatty acids (saponifiable lipids) and also hydrocarbons, ketones, sterols, and pigments (non-saponifiable lipids). Only the saponifiable part of neutral lipids is suitable for biodiesel (transesterifiable to FAMEs). Non-saponifiable neutral lipids together with polar lipids and non-lipid fraction, if present, are not convertible to biodiesel and represent lipid contaminants (non-saponifiable matter).[33,35] Thus, the extraction process selected should not only be lipid-specific, but also selective towards neutral/saponifiable lipid fraction. Therefore, the yields of saponifiable lipids and overall FAMEs yields produced from *C. vulgaris* and *N. oculata* by the extraction methods used, were analysed and the results are presented in Table 1. All transesterification experiments were performed at least twice.

In the case of *C. vulgaris*, Soxhlet extraction showed the highest fraction of saponifiable lipids, 65.1%. As was stated before, hexane used in Soxhlet extraction has a high affinity towards neutral lipids, generating a high content of saponifiable molecules. In contrast, the B&D method gave the lowest saponifiable fraction in the extracted lipids, 39.7%. Although the B&D extraction obtained the greatest total lipid yield, it produces lipids highly contaminated with non-saponifiable matter. Hence, for *C. vulgaris*, B&D and Soxhlet were able to give similar FAMEs yield from dry biomass. On the other hand, ionic liquid extraction also produced a high yield of saponifiable material (55.8%), higher than the B&D method, but lower than Soxhlet. Nevertheless, the overall FAMEs yield produced from dry biomass was the highest one, suggesting that the ionic liquid extraction method gave not only a larger amount of lipid, but also had a high affinity for neutral/saponifiable lipids.

The results for *N. oculata* showed the same tendency for Soxhlet and B&D extractions, giving greater amounts of saponifiable material in lipids extracted by Soxhlet than by B&D, 54.3% and 48.7% respectively. However, in this case, the much higher yield of total lipids extracted by B&D resulted in higher overall FAMEs yield produced from dry biomass as compared to Soxhlet extraction. Furthermore, although the B&D method appeared to produce more FAMEs than the ionic liquid extraction, the difference in yield is insignificant ($<3\sigma$); statistically, they produced the same value. Thus, for both microalgæ studied, the ionic liquid extraction produced equivalent or better yields than the B&D procedure with dry biomass, and was always significantly better than the Soxhlet process.

3.3 Effect of wet biomass in Bligh & Dyer and ionic liquid extraction

As already discussed, the energy consumption for drying microalgæ is extremely high, and should be avoided. The elimination of biomass drying, and also organic solvent use, from lipid extraction processes could lead to significant energy and cost savings. Therefore, the possibility of using wet microalgæ biomass for lipid extraction by the ionic liquid $[P(CH_2OH)_4]Cl$ was investigated. The experiment was conducted using *N. oculata* because this microalgæ was received as freshly harvested (71.7% water content). The B&D method was used as a standard method for comparison with ionic liquid extraction because it is known to be applicable to any tissue containing water up to 80%,[4] whereas Soxhlet requires the use of dry biomass. Figure 2 shows the lipid and FAMEs yields on the basis of dry biomass (DB) and saponifiable fraction in extracted lipids obtained by the B&D and ionic liquid methods. The results demonstrated the same tendency in difference between dry and wet biomass for both extraction methods. When wet biomass was used, lipid yield increased significantly from 17.3% to 19.5% and from 12.8% to 14.6% for B&D and ionic liquid, respectively. In contrast, the saponifiable

matter in extracted lipids decreased by about 10%. The B&D method gave 48.7% and 38.3% of saponifiable lipids for dry and wet biomass, respectively, whereas the ionic liquid method gave 63.5% and 55.1% of saponifiable lipids for dry and wet biomass, respectively. However, despite the changes in the amount of extracted lipids and saponifiable fraction, the FAMEs yield remains effectively constant for both methods, independently of wet or dry biomass use. A slight difference can be observed for the B&D procedure, which gave 8.4% and 7.5% of FAMEs using dry and wet biomass, respectively: the ionic liquid method yielded 8.1% and 8.0% of FAMEs using dry and wet biomass, respectively.

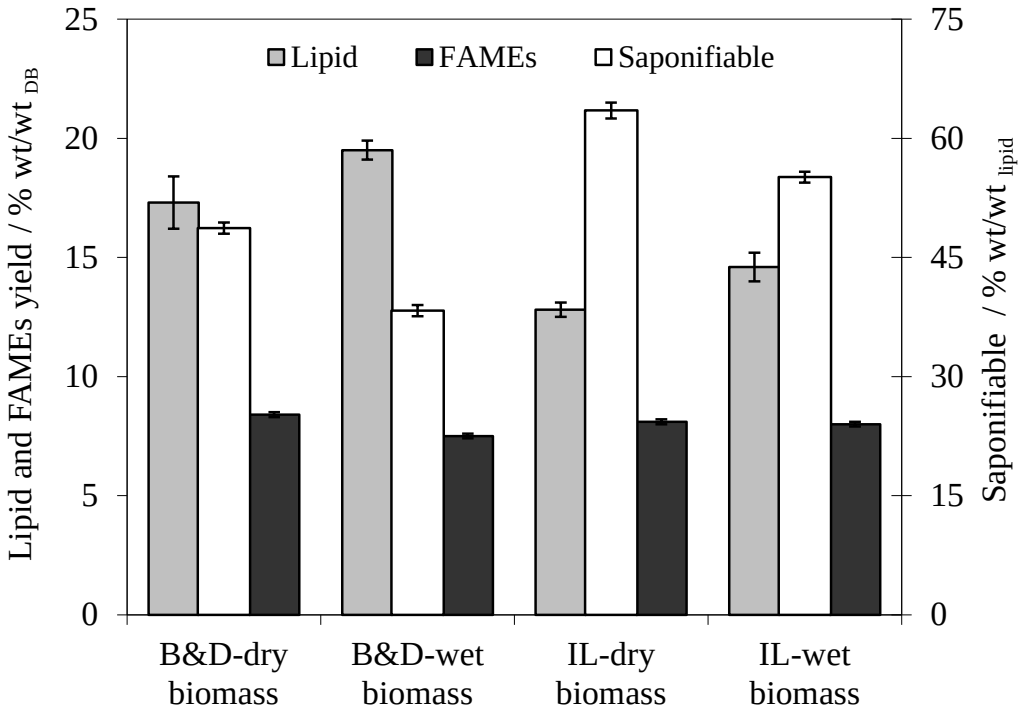

Figure 2. Extraction and transesterification yields using B&D and $[P(CH_2OH)_4]Cl$ extraction methods for *N. oculata*; dry biomass (DB): freeze-dried *N. oculata* (6.8% water content), wet biomass: dewatered *N. oculata* (71.7% water content).

This tendency for the B&D method in lipid, saponifiable and FAMEs yields has been noted elsewhere.[37] In marked contrast, the influence of wet microalgæ on the lipid and FAMEs yield using ionic liquids has not been observed previously. The only possibly related data are from the work of Young et al., who reported (in contrast to the results reported here) that wet biomass gave 25% lower extraction efficiency than dry biomass, using a $[C_2mim][MeSO_4]$-methanol system (where $[C_2mim]^+$ = 1-ethyl-3-methylimidazolium).[26] Moreover, their work did not evaluate the FAMEs yield, which is important for final biodiesel production. Their results are in direct opposition to ours, where wet biomass gained 16% more lipids than dry, but they were using a different ionic liquid, so there should be no expectation of similar conclusions. Other authors reported the utilisation of related imidazolium ionic liquids for wet microalgæ biomass dissolution, demonstrating that water content had a huge importance in microalgæ cell lyses. They reported that ionic liquids are able to dissolve dry and wet biomass but complete cell lysis was noted only for wet biomass, indicating a hydrolysis reaction.[8]

The results demonstrated that for both B&D and ionic liquid extraction methods, harvested biomass without drying can be successfully used with the same level of FAMEs recovery. However, comparing both methods,

ionic liquid extraction gave higher saponifiable content, decreasing lipid contamination which is important for biodiesel application.

3.4. Effect of extraction methods on the fatty acids composition

The properties of biodiesel strongly depend on its fatty acid composition. Thus, it was important to evaluate the influence of the ionic liquid used on that fatty acid composition. The fatty acid profiles of biodiesel (FAMEs) produced from the lipids of *C. vulgaris* and *N. oculata* are presented in Tables 2 and 3, and representative chromatograms are depicted in Figure 3(a) and 3(b), respectively.

Table 2. FAMEs profile obtained from *C. vulgaris* by each extraction method[a].

	(%) w/w		
Fatty acids	Soxhlet	B&D	$[P(CH_2OH)_4]Cl$
Undecylic (C11:0)	1.40	1.03	0.90
Lauric (C12:0)	0.00	0.43	0.17
Myristic (C14:0)	0.21	0.00	0.69
Pentadecanoic (C15:0)	0.28	0.18	0.18
Palmitic (C16:0)	21.87	24.76	21.33
Palmitoleic (C16:1)	9.18	14.03	13.64
Heptadecenoic (C17:1)	12.08	9.09	8.76
Stearic (C18:0)	0.85	0.63	1.16
Oleic (C18:1)	8.32	9.05	8.12
Linoleic (C18:2)	8.28	7.24	7.67
α-Linolenic (C18:3)	26.25	21.66	25.28
Others	11.29	11.89	12.10

[a] *Values are means ± SD, n ≥2, SD < 0.7*

Table 3. FAMEs profile obtained from *N. oculata* by each extraction method[a].

	(%) w/w				
		B&D		$[P(CH_2OH)_4]Cl$	
Fatty acids	Soxhlet	Dry	Wet	Dry	Wet
Lauric (C12:0)	0.34	0.27	0.11	0.18	0.58
Myristic (C14:0)	3.66	4.75	5.13	3.68	5.00
Pentadecanoic (C15:0)	0.24	0.35	0.37	0.28	0.36
Palmitic (C16:0)	6.59	14.08	15.86	9.35	14.49
Palmitoleic (C16:1)	19.82	25.15	31.46	20.99	26.02
Stearic (C18:0)	0.39	0.65	0.32	0.54	0.42
Oleic (C18:1)	1.89	2.47	2.12	2.26	2.15
Linoleic (C18:2)	1.30	1.56	1.20	1.43	1.07
γ-Linolenic (C18:3)	0.90	0.81	0.76	0.89	0.62
Eicosatrienoic (C20:3)	3.19	3.03	1.49	3.22	1.66
Arachidonic (C20:4)	5.22	3.92	3.25	5.02	3.94
Eicosapentaenoic (C20:5)	49.85	37.77	32.93	45.59	39.01
Docosahexaenoic (C22:6)	0.99	0.90	0.79	1.02	0.96
Others	5.63	4.29	4.21	5.55	3.73

[a] *Values are means ± SD, n ≥2, SD < 0.7*

The fatty acid profile of *C. vulgaris* was dominated by palmitic (16 : 0), palmitoleic (C16 : 1), heptadecenoic (C17 : 1), oleic (C18 : 1), linoleic (C18 : 2) and linolenic (C18 : 3n3) acids, and no significant differences were

found between the extraction methods tested. In case of *N. oculata*, also the same fatty acids were identified for all extraction methods, with a predominance of palmitic (16 : 0), palmitoleic (C16 : 1) and eicosapentaenoic acid (C20 : 5n3). Beside the slight differences, the *N. oculata* profiles of FAMEs produced from lipid extracted using the ionic liquid method were very similar to those of the lipid obtained by conventional Soxhlet and B&D methods, showing no significant influence of the ionic liquid on the fatty acids composition.

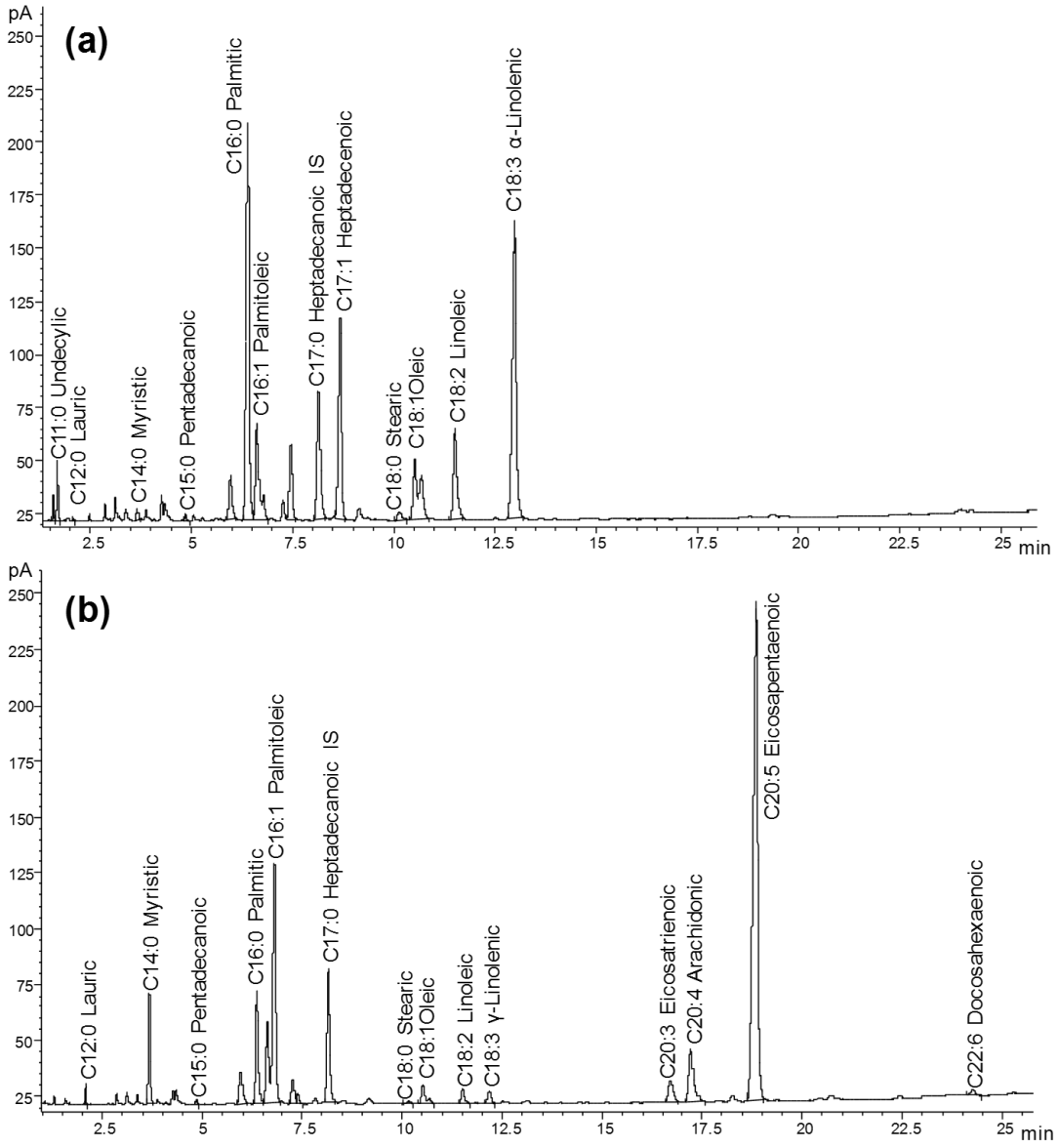

Figure 3. Representative gas chromatograms of FAMEs produced from the lipid extracted by the ionic liquid method from (a) *C. vulgaris*, and (b) *N. oculata* – wet biomass.

On the other hand, some notable differences can be observed between dry and wet biomass of *N. oculata* (Table 3), suggesting the influence of microalgæ drying and/or water content. The extraction from wet biomass for both methods, in general, increased the amount of palmitic and palmitoleic and decreased the amount of eicosapentaenoic acid. These changes influence the total polyunsaturated fatty acid content, which is essential to biodiesel properties. The polyunsaturated fatty acids are very susceptible to auto-oxidation, resulting in poor oxidative stability of the biodiesel.[38] The total polyunsaturated fatty acid content in FAMEs obtained from dry

biomass was 48.0% and 57.2% as compared to 40.4% and 47.3% from wet biomass for B&D and ionic liquid methods, respectively. Based on these results, it can be stated that the use of wet biomass can imply some improvement in the oxidative stability of biodiesel. This could be optimised by working on the extraction conditions for a more suitable selectivity, or by modifying culture conditions and/or cultivated strains so as to promote accumulation of intracellular lipids better suited for biodiesel application.

3.5. Ionic liquid extraction under mild conditions

In order to significantly reduce the energy consumption during ionic liquid extraction, the procedure was carried out at ambient temperature, varying the extraction time. The lipid yield extracted from wet *N. oculata* at ambient temperature and the degree of microalgæ dissolution in ionic liquid were compared to the results obtained at 100 °C for 24 h. All extraction experiments were performed at least twice.

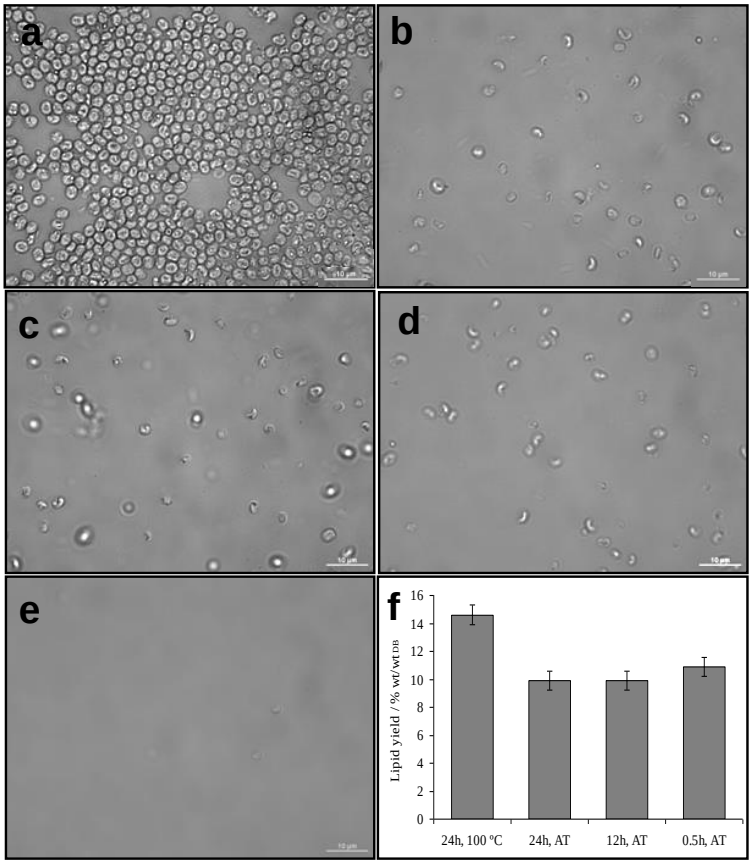

Figure 4. Microscopic images of wet *N. oculata*: (a) untreated intact microalgæ, and then after ionic liquid treatment for (b) 0.5 h, ambient temperature, (c) 12 h, ambient temperature, (d) 24 h, ambient temperature, and (e) 24 h, 100 °C. (f) corresponds to lipid yields for these treatments. Optical micrographs are all at 1000× magnification.

As shown in Figure 4(f), the highest lipid yield was obtained for the extraction condition of 24 h, 100 °C. This condition also showed complete microalgæ dissolution due to the absence of microalgæ cells, Figure 4(e). More than 99% of cells disappeared after the ionic liquid treatment, as compared to the intact microalgæ shown in Figure 4(a). On the other hand, the extractions at ambient temperature, regardless of treatment time, gave lower lipid yields than at 100 °C. This can be also observed on the micrographs of the corresponding treatment time – Figure 4(b)–4(d) – when the number of cells decreased compared to intact microalgæ, Figure 4(a), but were

higher than for 100 °C, Figure 4(e). For these three treatment times at ambient temperature, about 85% of cells disappeared, indicating partial dissolution. Furthermore, the cell size and area decreased and the cell walls changed their shape, giving also some empty-looking cells. This suggests that, at ambient temperature, the microalgæ cells did not fully dissolve but were mostly disrupted liberating inert material. Thus, the lipid yields at ambient temperature were lower than at 100 °C, but still high enough, giving as high as 75% of lipid recovery after 0.5 h. Furthermore, the saponifiable lipids were also analysed for the lipid extracted at ambient temperature for 0.5 h (68.0%), giving a higher saponifiable content compared to extraction at 100 °C for 24 h (55.1%). Although the extraction at ambient temperature for 0.5 h gave lower lipid yield, it gave the lipids with lower contamination of non-saponifiable matter, which is important for biodiesel application. Hence, the final FAMEs yields were very similar for both conditions: 7.4% (ambient temperature, 0.5 h) and 8.0% (100 °C, 24 h) based on dry biomass.

These results demonstrated that the lipid was successfully extracted from wet microalgæ using $[P(CH_2OH)_4]Cl$, without heating, for only 0.5 h. These conditions lead to significant energy, and thereby cost, saving for biodiesel production from microalgæ biomass as compared to conventional extraction processes.

3.6. Ionic liquid recycling

The possibility of ionic liquid recycling is also a very important issue for an energy saving system. For that reason, the recycling of the ionic liquid, and its reuse for subsequent lipid extraction, was investigated.

Firstly, the stability of the ionic liquid solution was checked by ^1H NMR spectroscopy, comparing fresh ionic liquid with the ionic liquid after microalgæ treatment. As can be seen in Figure 5, the ionic liquid is stable even after microalgæ treatment at 100 °C.

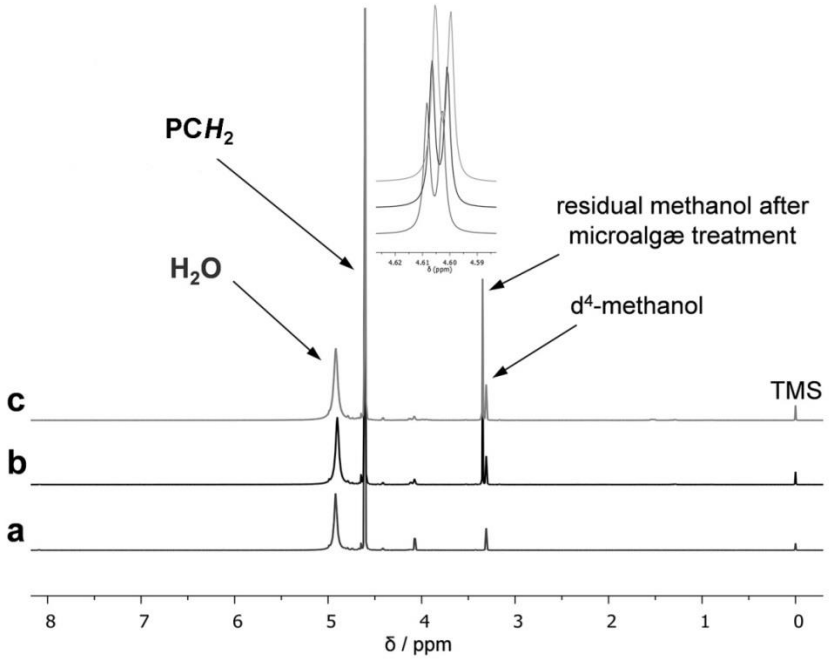

Figure 5. ^1H NMR spectra (d^4-methanol) of $[P(CH_2OH)_4]Cl$, 80% aqueous solution: (a) fresh ionic liquid, (b) recovered ionic liquid after microalgæ treatment, 0.5 h, room temperature, and (c) recovered ionic liquid after microalgæ treatment, 24 h, 100 °C.

The same peaks were found for fresh ionic liquid and the ionic liquid after treatment. The doublet at 4.6 ppm is due to PCH_2 of the ionic liquid cation, and the singlet at 4.9 ppm is due to water. No additional peaks were observed in the recycled ionic liquid, suggesting that the ionic liquid does not decompose significantly, and can be fully recycled.

The recycling of the ionic liquid and its reuse was investigated by performing four runs at ambient temperature for 0.5 h. The ionic liquid was recovered after each run (see Experimental). The lipid was subsequently extracted using the recovered ionic liquid from the previous run. The saponifiable lipid content and final FAMEs yields were also examined for each run. The recyclability data are shown in Figure 6.

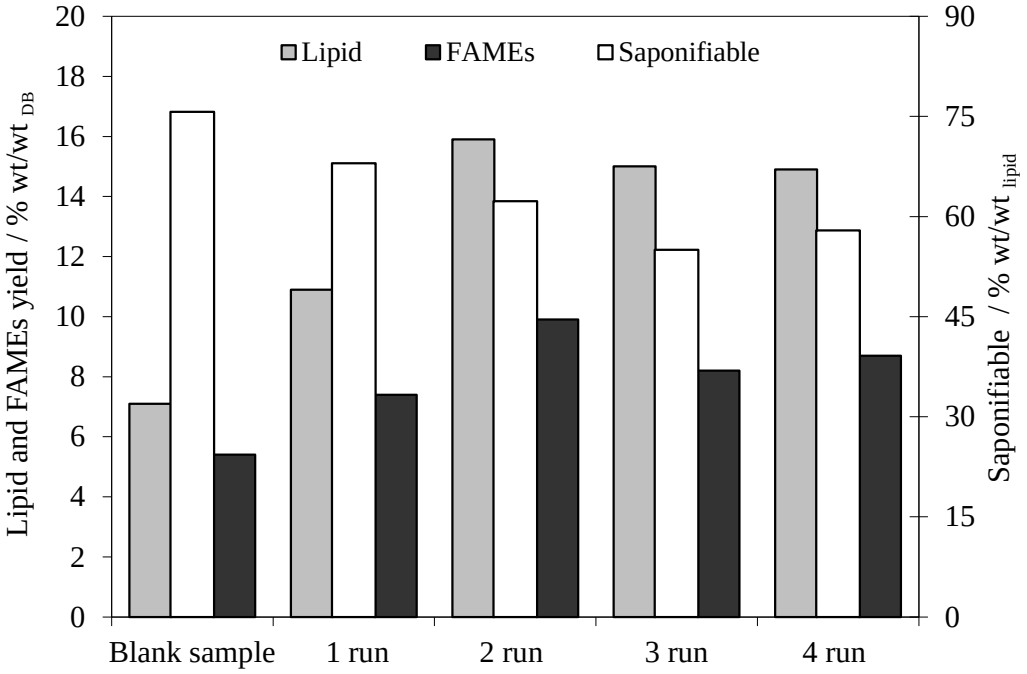

Figure 6. Ionic liquid recycling for lipid extraction at ambient temperature for 0.5 h. Influence of the ionic liquid recycling on the saponifiable lipids and biodiesel yields.

All ionic liquid runs showed higher lipid extraction and FAMEs efficiency than the blank control sample, demonstrating the potential of this process for microalgæ cell disruption, releasing the inert material and thus the lipids. As can be observed in Figure 6, the lipid yield even increased after ionic liquid reuse. However, the saponifiable lipid content decreased, giving only a slightly higher FAMEs yield as compared to run 1. As the recovered ionic liquid solution was not totally clean due to the dissolution of some other microalgæ component which did not precipitate, the possible co-extraction of non-saponifiable matter could occur, giving higher extraction efficiency but lower saponifiable content in the extracted lipids.

Based on the result analysis, $[P(CH_2OH)_4]Cl$ is stable after microalgæ treatment, and can be reused without decreasing the lipid and biodiesel efficiency.

3.7. Energetic evaluation of the ionic liquid recovery

A significant energy tariff on the conventional system is the energy intensive pre-treatment step of microalgæ disruption, needed in conventional lipid extraction methods using organic solvents. This is eliminated when

using an ionic liquid, as the microalgæ cell walls are dissolved or dispersed, and do not need the mechanical disruption to release the lipids. At first sight, it would appear that using wet biomass will also result in an energy saving, as the need for drying the biomass 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. Nevertheless, Teixeira[8] demonstrated that the energy demand for an ionic liquid extraction (using the ubiquitous, but expensive, $[C_4mim]Cl$)[39] from wet microalgæ (including ionic liquid recycle by water elimination) is around 0.4 MJ kg^{-1}, as compared to 4–9 MJ kg^{-1} for the conventional solvent processes. Moreover, it is energetically more favourable to reduce the water content of a wet ionic liquid than to remove water from a structurally complex biological system (due to the large wetted surface area, and related capillary forces between cells).[8] When a hydrophilic ionic liquid is used for disruption of microalgal biomass, both water maintained in wet biomass and water released by the breaking of microalgæ cells walls end up in the ionic liquid, so this water will have to be removed eventually before the ionic liquid could be recycled. Overall, various factors influence the energy needed for water removal: the process used for removal, the media from which the water is eliminated, and the type of bond between the water and biomass. For example, Xu et al. stated that microalgæ after mechanical dehydration (30% total solid) can only be dried using a thermal way because the water which left is mainly intracellular water, difficult to eliminate, except for the case where the cell walls are broken.[40] This is the case when ionic liquids are used, where the cell walls are broken, then the cell disruption step can be eliminated, in opposite to conventional solvent processes.

In order to get an exact energy estimation, simulation of the whole ionic liquid process should be performed using industrial equipment. This is a subject for a separate engineering study. However, there is an example in the literature where the process of lipid extraction from microalgæ is simulated using 1-butyl-3-methylimidazolium chloride, but the separation of a hydrophilic ionic liquid from water (omitting evaporation or distillation which requires a large amount of energy) is a challenge. Gutowski et al. demonstrated that the addition of the kosmotropic (water-structuring) salt, $K_3[PO_4]$, to an aqueous solution of a hydrophilic ionic liquid, produced an aqueous biphasic system, forming an upper ionic liquid-rich phase and a lower $K_3[PO_4]$-rich phase.[41] Moreover, Haerens et al. demonstrated that an ionic liquid solution can be concentrated by membrane separation,[42] but the highest concentration obtained in this study was only 30% of ionic liquid in water. After water addition from microalgæ to the ionic liquid in this study, a solution with a concentration of ca. 67% is formed.

3.8. Characterisation of the precipitate after ionic liquid extraction

The precipitated solid after ionic liquid extraction was analysed by FTIR spectroscopy, and the data compared to the spectra of the microalgæ biomass before extraction. In order to estimate changes in the main microalgæ components (lipids, proteins and carbohydrates), the FTIR analysis of extracted lipids was also performed. The FTIR spectra of *N. oculata* are presented in Figure 7. The lipids, protein and carbohydrates can be easily identified by their characteristic absorbances: 1500–1700 cm^{-1} for peptide amide groups of proteins, 1700–1750 cm^{-1} for C=O groups in lipid esters, and 2800–3000 cm^{-1} for lipid acyl chains, and 1000–1200 cm^{-1} for C–O and C–O–C groups of carbohydrates.[43]

As shown in Figure 7(b), the infrared spectra 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 vibrations in the lipid acyl chains).[43,44] In the spectra of intact biomass, Figure 7(a), the band of the lipid ester group is not clearly seen because of some overlapping with amide I band at 1636 cm^{-1}, which appears in the spectral range 1762–1590 cm^{-1} (assigned mainly as the C=O amide stretching vibration of proteins). Overall, the infrared spectra suggest that some proteins were liberated from microalgæ cells after ionic liquid extraction and are present in precipitated solids.

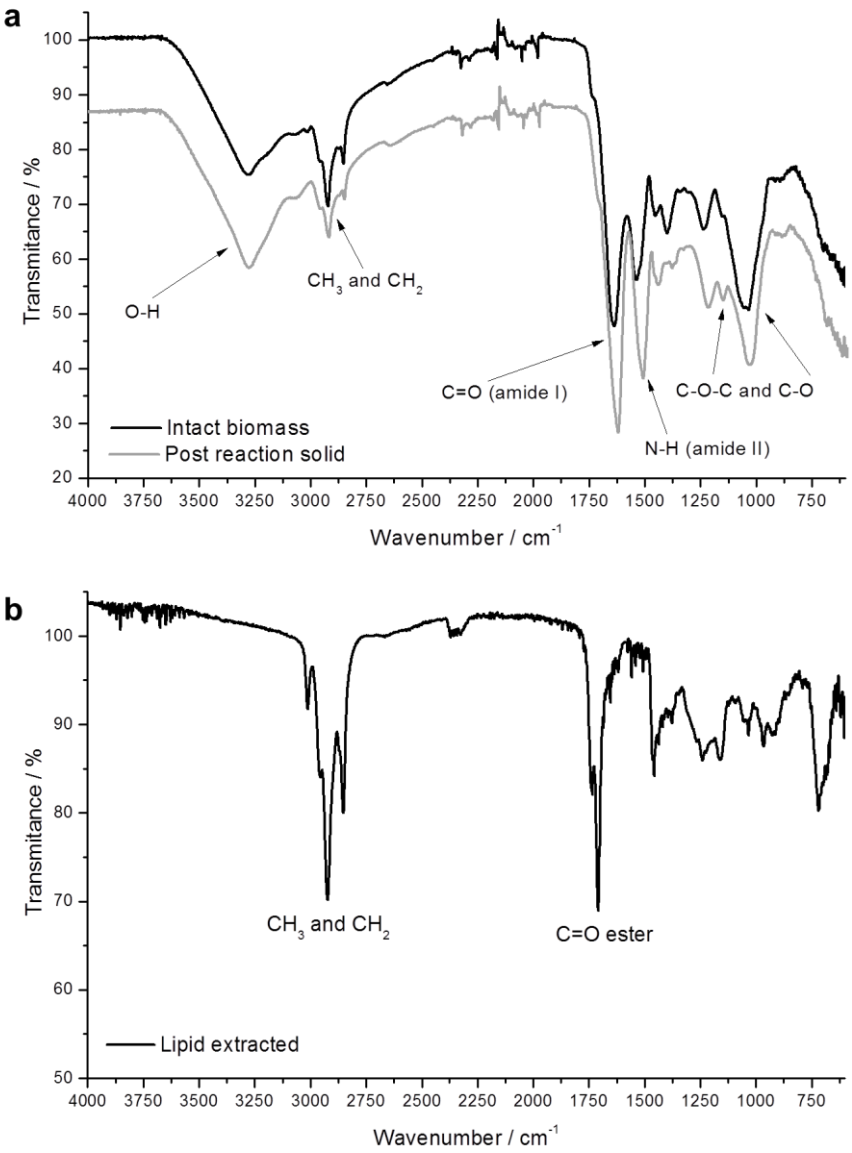

Figure 7. FTIR spectra of *N. oculata*, and its post-extraction precipitates, and lipids obtained after ionic liquid extraction from wet biomass. (a) Intact biomass – freeze-dried form (black line), precipitated solid after ionic liquid extraction, 100 °C, 24 h (grey line). (b) Lipid extracted using ionic liquid method.

As shown in Figure 7(a), the characteristic bands of polysaccharides are found in the spectral range of around 1190–940 cm^{-1}. The broad peak around 1035 cm^{-1} (assigned to the C–O stretching vibration of carbohydrates) is present in both the intact microalgæ and precipitate. The difference in the appearance of the peak at 1160 cm^{-1},

in the spectra of the precipitate, is tentatively assigned as the C–O–C stretching vibration particularly associated with cellulose.[44] The microalgæ cell walls of species like *C. vulgaris* and *N. oculata* are mainly composed of polysaccharides such as cellulose, hemicellulose and pectin.[36]

Based on the data obtained from FTIR analysis, it could be stated that the solid precipitated after ionic liquid extraction contains proteins from cells and cellulosic materials from cell walls of microalgæ. This suggests that the main microalgæ components (lipids, proteins and polysaccharides) could be recovered in one process.

3.9. Is a hydrated ionic liquid actually an ionic liquid?

A remarkable explosion in the ionic liquid literature (ca. 5000 papers are now published per year) has resulted in many inconsistencies: even the definition of an ionic liquid has many variations. Two decades ago, when our factual database was severely limited and our theoretical understanding was underwhelming, the common definition of an ionic liquid was a liquid, comprised entirely of ions, which melted at or around 100 °C. This dates back to a paper published in 1914, by Paul Walden,[45] who stated about the first recognised ionic liquid $[EtNH_3][NO_3]$, "The general picture of these organic salts at low temperatures (below, or around 100 °C) thus corresponds to the experiences made with inorganic (single, non-complexing) molten salts at much higher temperatures approximately between 300 and 600 °C)". After over 25 000 publications, this naïve, but for the time insightful, vision of the nature of ionic liquids is being revisited, extending the definition to include all tradition molten salts.[46] In parallel, a class of solvents related to ionic liquids, known as deep eutectic solvents, was developed by Abbott.[47,48] These contain molecular components, typically one anion, one cation, and two moles of a Lewis base; in some cases their structure may contain the molecular components tightly bound to either of the ions (this closely resembling ionic liquids in many of their properties, and if the binding is tight, they may actually be ionic liquids), in others the molecular components may be essentially free. Ohno, in a series of papers,[49,50] has shown that "hydrated ionic liquids" (i.e. ionic liquids containing a small amount of water) have unique properties, distinct from simple ionic liquids, and very different from aqueous salt solutions, and our own work has demonstrated that phosphonium carboxylates, in the presence of small amounts of water, capture carbon dioxide more efficiently (both physically and chemically) than any reported simple ionic liquid.[51,52] So, with this background in mind, it is important to consider whether the solvent used in this work, an 80% w/w solution of tetrakis(hydroxymethyl)phosphonium chloride, $[P(CH_2OH)_4]Cl$, in water can be described as an ionic liquid, or an aqueous solution of a salt. The formulation of the solvent used in this work is that of the commercial product, $[P(CH_2OH)_4]Cl{\cdot}2.64H_2O$, and the question can be posed as "is the water bound to the ions, or is it free water?". Figure 8 shows the infrared spectrum of the neat solvent, and Figure 9 shows the infrared spectrum of this solvent to which an aliquot of water has been added in order to double the water content, $[P(CH_2OH)_4]Cl{\cdot}5.28H_2O$. Deconvolution of the bands between 3600 and 3000 cm^{-1} yields evidence for two distinct types of water. The 80 : 20 IL–water composition shows a strong band at 3197 cm^{-1}, with a weak shoulder at 3464 cm^{-1}, in an approximate 5 : 1 ratio, corresponding respectively to bound water, with a lesser amount of less tightly bound water – neither is identical to free water.[53] The diluted solution, $[P(CH_2OH)_4]Cl{\cdot}5.28H_2O$, shows slightly shifted bands at 3195 cm^{-1} and 3412 cm^{-1}, in the respective ratios of ca. 1 : 1. This corresponds to bound and not-so-tightly bound water, occurring at positions similar, but not identical, to those observed in $[P(CH_2OH)_4]Cl{\cdot}2.64H_2O$. These data show that the water present in the ionic liquid used in this study is not "free", and is strongly hydrogen-bonded to the ions present (either the anion

and/or the cation), allowing us with our current state of understanding to describe the system as being an ionic liquid, and clearly not an aqueous solution. It most closely resembles a solution of half a mole of water in a mole of a hydrated ionic liquid, $[P(CH_2OH)_4]Cl \cdot 2H_2O$.

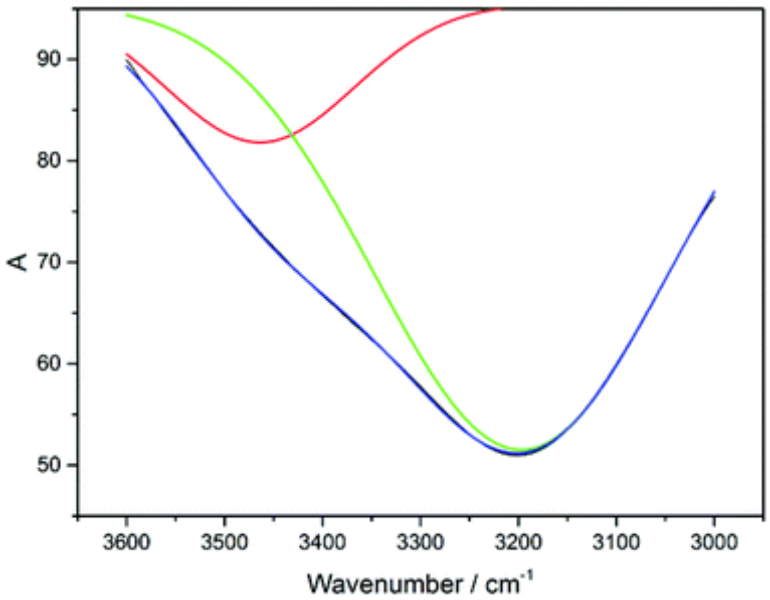

Figure 8. The infrared spectrum of $[P(CH_2OH)_4]Cl \cdot 2.64H_2O$ in the O–H stretching region: the red and blue bands represent the deconvolution of the experimental data (the black curve); the blue curve is the sum of the red and green bands. R^2 for the fit is 0.9998. The area under the green curve is five times larger than that under the red curve.

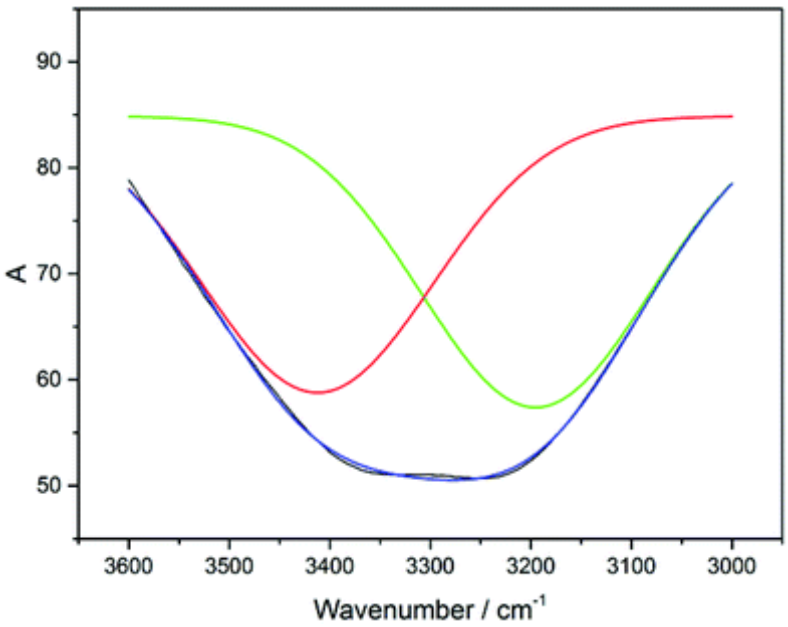

Figure 9. The infrared spectrum of $[P(CH_2OH)_4]Cl \cdot 5.28H_2O$ in the O–H stretching region: the red and blue bands represent the deconvolution of the experimental data (the black curve); the blue curve is the sum of the red and green bands. R^2 for the fit is 0.9991. The area under the green curve is slightly larger (1.06) than that under the red curve.

4. Conclusions

This work has demonstrated for the first time the potential of an unusual hydrated phosphonium ionic liquid, $[P(CH_2OH)_4]Cl \cdot 2H_2O$, for lipid extraction from two different microalgæ species, *N. oculata* and *C. vulgaris*:

there is significant potential in these observations for an improved biodiesel production process. The commonly used volatile organic solvents can be successfully replaced by the involatile ionic liquid. The energy intensive pre-treatment step of microalgæ disruption, needed in conventional lipid extraction methods, can also be eliminated due to the high dissolution ability of the ionic liquid, resulting in the efficient disruption of microalgæ cell walls.

$[P(CH_2OH)_4]Cl$ has been studied for possible toxicity and carcinogenetic activity, since it is used extensively as a flame retardant for cotton fabrics.[54] This study, for the US Department of Health and Human Services, concluded that no neurotoxicity or any other signs of clinical toxicity were observed. There was no evidence of carcinogenicity of $[P(CH_2OH)_4]Cl$ in either sex of F344/N rats given 3.75 or 7.5 mg kg^{-1}, in male B6C3F1 mice given 7.5 or 15 mg kg^{-1}, or in female B6C3F1 mice given 15 or 30 mg kg^{-1}. $[P(CH_2OH)_4]Cl$ demonstrated no mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537, with or without metabolic activation. There are no other extant reports of the toxic effects of $[P(CH_2OH)_4]Cl$.

In addition, this study has clearly demonstrated that the ionic liquid procedure can be used to extract lipid directly from wet microalgæ under mild conditions. Furthermore, the ionic liquid can be recycled in this treatment, and was successfully reused. Hence, the economics of the process can be further improved by eliminating microalgæ disruption and drying, and the heating step during extraction. In addition, the possible recovery of other valuable microalgæ components during the ionic liquid extraction of lipids was also noted, which will be an additional factor in the economics of this process.

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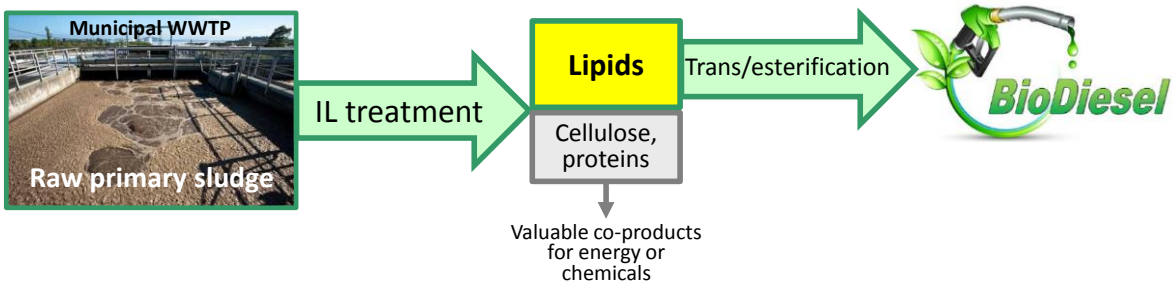
Chapter 6

Efficient extraction of lipids from primary sewage sludge using ionic liquids for biodiesel production [1]



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.

[1] M. Olkiewicz, N.V. Plechkova , A. Fabregat , F. Stüber, A. Fortuny, J. Font, C. Bengoa, Efficient extraction of lipids from primary sewage sludge using ionic liquids for biodiesel production, *Separation & Purification Technology*, submitted (May 2015).

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 mixture of sludge (dried or raw) and ionic liquid in ratio 1 g TS equivalent to 10 cm^3 ionic liquid was heated at 100 ºC 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 and the FAMEs were analysed by GC–FID as described previously [8]. The results of GC–FID were used to estimate the amount of saponifiable (transesterifiable/esterifiable to FAMEs) material in the lipid fraction and hence the maximum mass of biodiesel (FAMEs) that could be yielded.

2.6. Recovery of precipitated solid

After lipid extraction from raw sludge by ionic liquid, the precipitated solid was recovered for further analysis from the IL/methanol/sludge's water solution by centrifugation (6000 rpm, 10 min) and subsequent removal of the aqueous supernatant. The recovered solid was washed with methanol (3×10 cm^3), followed by centrifugation and removal of supernatant. Then the remaining methanol was evaporated in a rotary evaporator and the resulting solid was heated overnight at 105 ºC and afterwards the solid was weighted in order to calculate the yield of the precipitated components. The precipitated solid was further analysed in order to determine the VS and ash content.

2.7. FTIR analysis

The precipitated solid, dried sludge, extracted lipids and also cellulose standard were analysed by Fourier Transform Infrared (FTIR) spectroscopy for a comparison study, as described previously [25].

2.8. Proteins in ionic liquid

Proteins, which are polar in nature, could be extracted into the polar ionic liquid, $[P(CH_2OH)_4]Cl$, and subsequently recovered. After lipid extraction from raw sludge and precipitation of sludge components, the presence of protein was tested by application of the Lowry method [27] to the recovered ionic liquid solution. The absorbance was measured at 500 nm.

2.9. Metals in ionic liquid

0.5 cm^3 of recovered ionic liquid was tested for metal content after microwave digestion with 2 cm^3 of HNO_3. An inductively coupled plasma-optical emission spectrometry (ICP-OES) SPECTRO ARCOS was employed to

measure the metal ion (Fe, Cu, Mg, Na, K, Ca, Al, Zn, Sr, Ba, Co, Ni, Mn, Cr, Pb and Cd) concentrations in the digested ionic liquid solution.

2.10. Ionic liquid analysis after extraction

After lipid extraction from raw sludge by $[P(CH_2OH)_4]Cl$ ionic liquid and precipitation of sludge components, the ionic liquid was recovered and its structure was checked by ^1H NMR spectroscopy as described in our previous study [25].

3. Results and discussion

3.1. Lipid extraction from sewage sludge by using ionic liquids

The hydrophilic and water miscible ionic liquids were used in this study due to their high capacity to extract lipids by the dissolution of biomass hydrophilic compounds, leaving lipids insoluble [15]. $[C_4mim][MeSO_4]$ was selected as an imidazolium-based ionic liquid due to its high suitability for lipid extraction from microalgae biomass [15,16]. $[P(CH_2OH)_4]Cl$ was chosen as a phosphonium-based ionic liquid which is inexpensive, and commercially available, which showed a great capacity to extract lipids from wet biomass [25].

Figure 1(a)-(c) shows the extraction steps with dried sludge (2% residual water content), using the example of $[P(CH_2OH)_4]Cl$ ionic liquid. Dried sludge, Figure 1(a), was dissolved by ionic liquid resulting in gel formation, Figure 1(b), due to the higher viscosity of the solution increasing after sludge dissolution. Then, after methanol addition to the system, phase separation took place, Figure 1(c), precipitating some of the sludge components (cellulose and/or proteins), giving the white colour of the precipitate, and leaving the less dense lipids insoluble. The same happened when $[C_4mim][MeSO_4]$ ionic liquid was used, but with the difference of a dark colour of final precipitated fibrous material, Figure 2.

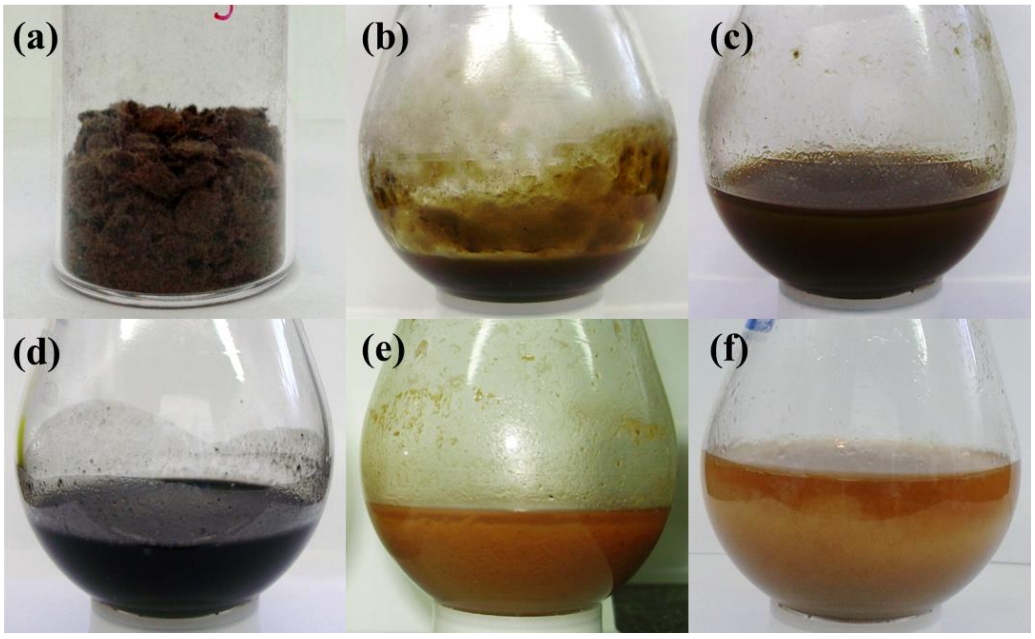


Figure 1. Photographs of the extraction steps using $[P(CH_2OH)_4]Cl$, where the experiments shown in (a), (b), and (c) use dried sludge; (d), (e), and (f) use raw sludge. (a) and (d) represent sludge before the experiment. (b) and (e) show sludge dissolved in the ionic liquid after 24 h at 100 ^{0}C.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

Figure 1(d)-(f) shows the extraction steps using raw sludge (96% water content), also as an example of $[P(CH_2OH)_4]Cl$ ionic liquid. In the case of raw sludge, Figure 1(d), the sludge was dissolved by ionic liquid because the colour of the solution changed, and at the same time precipitation of sludge components occurred giving a white fibrous material, Figure 1(e), leaving the less dense lipids insoluble. Further addition of methanol to the system did not show any changes, Figure 1(f). This suggests that the water contained within raw sludge plays the rôle of a polar solvent, providing separation of all components at the end of the process. On the contrary, the $[C_4mim][MeSO_4]$ ionic liquid shows a distinct behaviour without a colour change of the solution and with the precipitation of dark material, Figure 2.

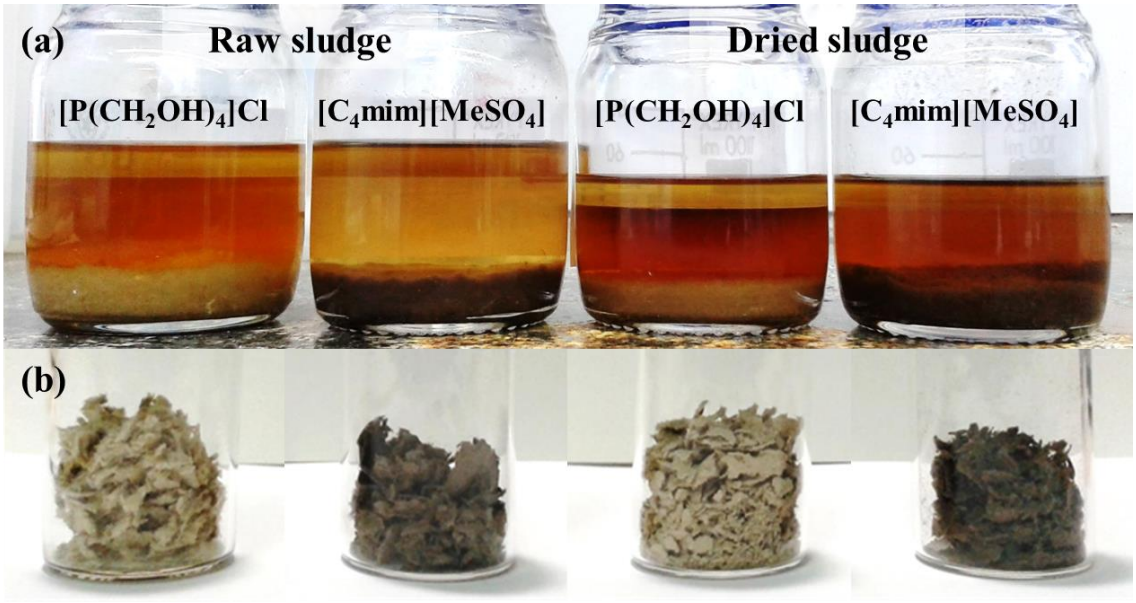


Figure 2. Photographs illustrating the differences after extraction using $[P(CH_2OH)_4]Cl$ and $[C_4mim][MeSO_4]$ from raw and dried sludge: (a) separation of the phases after methanol addition, (b) recovered solids after each extraction.

In both cases (dried or raw sludge), the lipids are not dissolved by ionic liquids and float because of a lower density than the ionic liquids solution. However, the upper lipid phase in Figure 1(c) and Figure 1(f) cannot be easily observed as the lipid content in the whole mixture is far too low. The lipid phase can be observed after hexane addition, which dissolves floating lipids as depicted in Figure 2(a).

3.2. Comparison of the different strategies of extraction

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

As already discussed, the elimination of water from sewage sludge is a limiting step in the 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.

Table 1. Extraction and transesterification/esterification yields from raw and dried sludge for each extraction method. IL extraction conditions: 24 h, 100 ºC, 1:10 sludge:IL ratio (g/TS: 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

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, Figure 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

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.

The FAME profiles of biodiesel produced from lipid extracted by both ionic liquids were very similar to those of the lipid obtained by the standard Soxhlet method. The same fatty acids were found for all methods with a predominance of palmitic (34%-45%), oleic (23%-34%) and stearic (11%-14%) acids in sludge biodiesel. These fatty acids are comparable with those obtained by direct liquid-liquid extraction using hexane [8].

3.3. Optimisation of extraction parameters

As the extraction by $[P(CH_2OH)_4]Cl$ ionic liquid from raw sludge gave higher lipids and biodiesel yields with additional recuperation of other sludge components, the optimisation of extraction conditions was performed using this ionic liquid.

3.3.1. Effect of time

The experiments of the influence of extraction time were conducted at 100 ºC using 1:10 sludge:IL ratio (g/TS: cm^3/IL). Table 2 shows the study of different extraction time on lipids, saponifiable lipids and biodiesel yields. As can be seen in Table 2, the higher the extraction time, the higher the amount of extracted lipids.

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

In contrast to the lipid yield, the amount of saponifiable lipids decreased with increased extraction time. This suggests that higher extraction time was able to extract more lipids, but the lipid fraction was more contaminated with non-saponifiable matter which is not convertible into biodiesel. Thus, the biodiesel yields remained almost unchanged for all extraction times tested, 19.8% in average. On the other hand, as described in the observation part of Table 2, the extraction time of 3 h showed better recuperation of precipitated material due to better sludge dissolution, giving purer cellulosic material than for 1 h and ½ h of extraction time (the results are discussed in further detail in Subsection 3.4.1). Finally, the extraction time did not affect the FAME profile of biodiesel (data 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, the lower the amount of ionic liquid, the larger the yield of lipids and biodiesel. The yield of saponifiable lipids for 1:20 and 1:10 sludge:IL ratio were almost unchanged, while the ratio 1:5 reached the highest yield of saponifiable and thus the highest yield of biodiesel. As the ionic liquid contains 20% water, the higher the amount of ionic liquid, the higher the amount of water in the system, decreasing the extraction efficiency and making the lipid fraction more contaminated with non-saponifiable matter, further reducing biodiesel yield. Based on the results, the amount of ionic liquid in the lipid extraction from sewage sludge to produce biodiesel can be reduced to 1:5 sludge:IL ratio (g/TS: cm^3/IL). Additionally, the 1:5 sludge:IL ratio did not affect the precipitate, Table 2. Finally, the FAME profile of biodiesel was not affected by the sludge:IL ratio (data not shown).

3.4. Characterisation of post reaction components after the [P(CH2OH)4]Cl ionic liquid extraction

3.4.1. Precipitated solid

Table 3 shows the amount of precipitate and its ash and VS content after different conditions of ionic liquid extraction.

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
	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

Comparing the extractions at 100 ºC for 1 h, 3h and 24 h, the extraction duration of 3 h reached the highest amount of precipitated material which contained a similar content of VS as 24 h. Despite the fact that the reaction for 1 h at 100 ºC gave a similar amount of precipitate to 24 h, the VS content was lower, giving precipitate with a higher ash content (dark solids were visible in the precipitate, Table 2) compared to the reactions for 3 h and 24 h. The influence of temperature on the precipitated solids obtained after extraction for 3 h demonstrated that the higher the temperature of process the higher the amount of precipitated solid. Furthermore, below 100 ºC, the VS content in the precipitates decreased, giving darker material, as described in the observation part of Table 2.

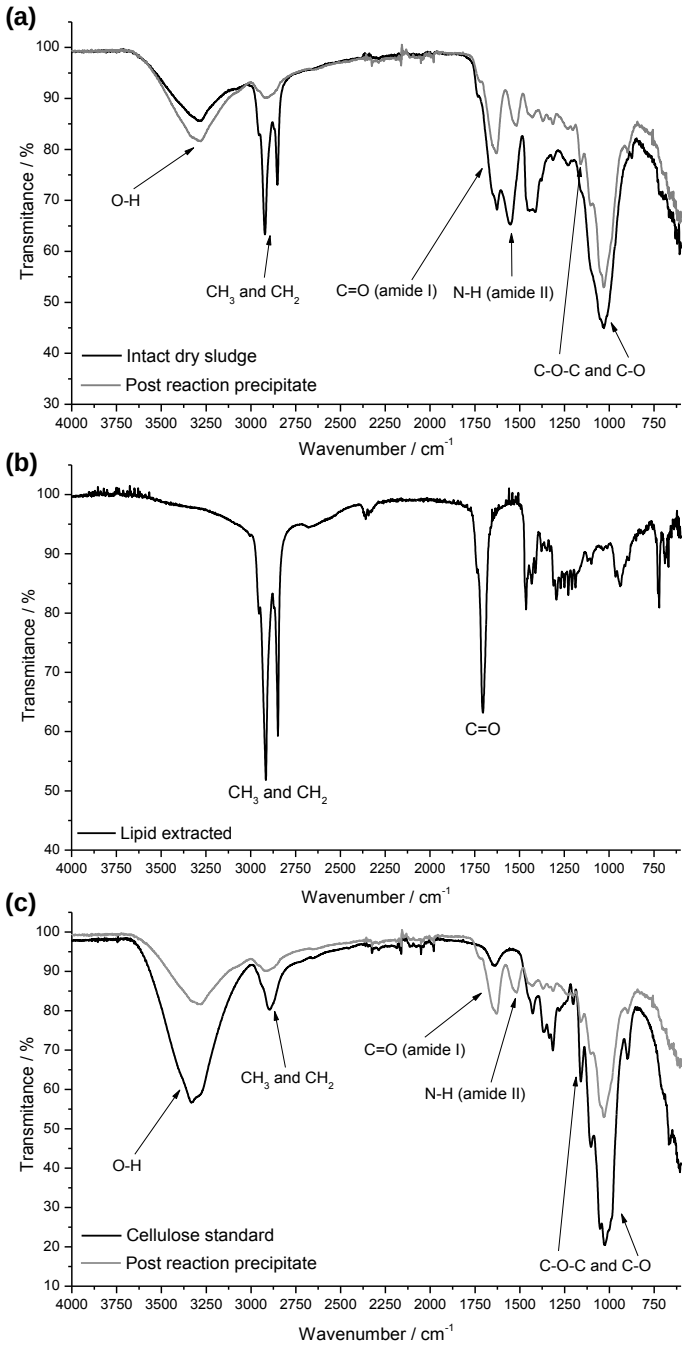


Figure 3. FTIR spectra of (a) intact dry sludge (black line), precipitated solid after ionic liquid extraction for 3 h at 100 ^{0}C (grey line), (b) lipid extracted using ionic liquid method for 3 h at 100 ^{0}C, (c) cellulose standard (black line), precipitated solid after ionic liquid extraction for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

The precipitate (3 h, 100ºC) was analysed by FTIR and compared to the analysis of intact sludge before extraction, Figure 3(a). In order to estimate changes in the main organic components of sludge (lipid, protein and carbohydrates), the FTIR analysis of extracted lipids and cellulose standard sample were also performed (Figure 3(b) and Figure 3(c), respectively). 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 [25].

As shown in Figure 3(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 [29], suggesting a high content of free fatty acids in the lipids. The high amount of free fatty acids was also found in the lipid extracted directly from raw acidified primary sludge by using hexane [8].

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 Figure 3(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 [22,25]. Figure 3(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.

Based on the FTIR data, it could be stated that the solid precipitated after ionic liquid extraction contains not only cellulose but also proteins. The purified proteins can be further used in bulk chemical market and the cellulose for producing bioethanol or other chemicals.

3.4.2. Sludge composition

Table 4 shows the mass balance of primary sludge composition obtained by the conventional method and the ionic liquid methods. The amount of lipid extracted by the conventional method and ionic liquid methods are similar. The differences are found in the quantity of proteins and ashes in the precipitate. The protein content analysed in the ionic liquid after extraction gave lower amount of proteins than total proteins analysed by the conventional method (Table 4). The lower protein content was counterbalanced by the higher amount of precipitated cellulosic material (carbohydrates) after both ionic liquid extractions, confirmed by the FTIR analysis (presence of proteins in precipitate, Figure 3(a)). Thus, the amount of proteins in the precipitate was calculated from the difference between the total proteins obtained by the conventional method and proteins analysed in ionic liquid phase. Furthermore, the quantity of ashes analysed in the precipitate after both ionic liquid extractions is much lower than the amount of ashes in the sludge determined by standard method. This suggests that the ionic liquid is able to dissolve some sludge ashes, reducing the ash content in the precipitated cellulosic material. This was confirmed by the analysis of metals, 7% and 8% in the ionic liquid after extraction for 3 h and 24 h respectively, based on dry sludge used (also commented on in the Subsection 3.2.1). Based on

the results presented in Table 4, the cellulose content in the precipitate after ionic liquid extraction for 3 h and 24 h was 61% and 67% respectively.

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

		IL extraction			
		3 h, 100 ºC		24 h, 100 ºC	
	Raw sludge[(a)]	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[(c)]	6.9 ± 0.4	13.5[(c)]	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
Values are means ± SD, n = 3

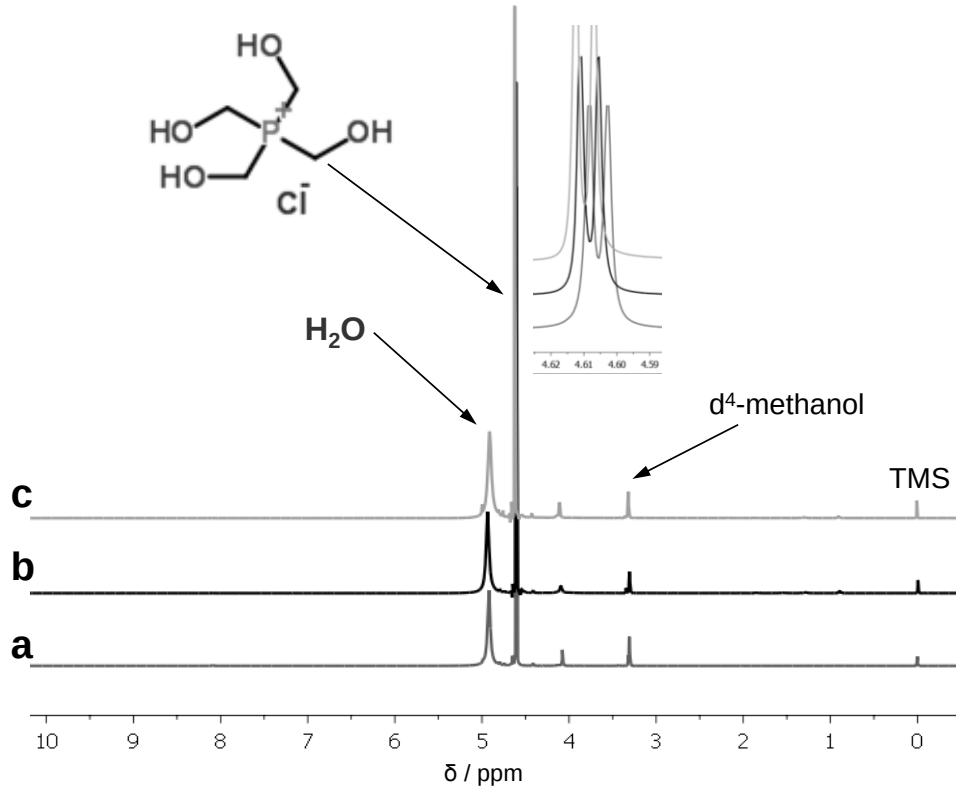


Figure 4. ^{1}H NMR spectra (d^4-methanol) of $[P(CH_2OH)_4]Cl$, 80% aqueous solution: (a) fresh ionic liquid, (b) recovered ionic liquid after sludge treatment for 24 h at 100 ºC, and (c) recovered ionic liquid after sludge treatment for 3 h at 100 ºC.

3.5. Ionic liquid characterisation after extraction

The stability of the $[P(CH_2OH)_4]Cl$ ionic liquid after extraction at 100 ºC for 3 h and 24 h was checked by ^1H NMR spectroscopy, comparing fresh ionic liquid with the ionic liquid after sludge treatment. As can be seen in Figure 4, the same peaks were found for fresh ionic liquid and the ionic liquid after both treatments. The doublet at 4.6 ppm 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 [25].

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.

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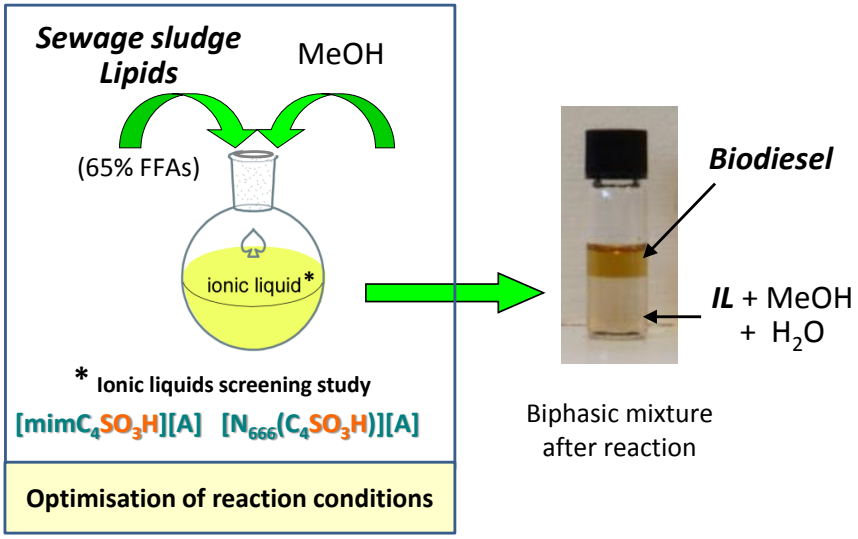
Chapter 7

Biodiesel production from sewage sludge lipids catalysed by Brønsted acidic ionic liquids [1]



ABSTRACT

Production of biodiesel from sewage sludge lipids catalysed by six different Brønsted acidic imidazolium and long chain ammonium ionic liquids; both with an alkane sulfonic acid group and with different acidities of anion, was investigated. Among the ionic liquid tested, 4-(3-methylimidazolium)butanesulfonic acid trifluoromethanesulfonate, $[mimC_4SO_3H][SO_3CF_3]$, was selected as the best catalyst due to its high catalytic performance and purer biodiesel obtained than the equivalent ammonium ionic liquid, 4-(trihexylammonium)butanesulfonic acid trifluoromethanesulfonate, $[N_{6\,6\,6}(C_4SO_3H)][SO_3CF_3]$. The influence of different reaction variables on the biodiesel yield was studied using $[mimC_4SO_3H][SO_3CF_3]$ as a catalyst. The yield of fatty acid methyl esters (biodiesel) reached 90% (based on saponifiable lipids) under the following optimised conditions: 10:1 molar ratio of methanol to saponifiable lipids, 7% ionic liquid catalyst (based on lipids), 100 ºC and 5 h. In addition, the ionic liquid has a good reusability and can be easily separated from the biodiesel. These acidic ionic liquids were found to be efficient catalysts for the synthesis of biodiesel from low-cost and non-edible feedstock like sewage sludge lipids.

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1. Introduction

The need to use alternative renewable fuels, while minimising the environmental impact, is currently increasing due to the rise in oil price, the depletion of sources of fossil fuels and the environmental problems associated with their combustion. Biodiesel is one of the most promising renewable fuels proposed as an alternative to fossil diesel that can be directly used with current engine and refuelling technologies without major modification [1,2]. Biodiesel is generally produced by transesterification of vegetable oils or animal fats with methanol, yielding fatty acids methyl esters (FAMEs) from the lipid fraction. However, the competitive potential of biodiesel is currently limited by the high price of the common lipid feedstocks, which constitutes between 70-85% of the overall biodiesel production cost, strongly influencing the final price of this biofuel [1,3,4]. Furthermore, the cultivation of edible vegetable oils for biofuels raises the concern of food shortage, which competes with fuel production [1]. Therefore, a low-cost and non-edible feedstock is required in order to reduce the production costs and to facilitate competitiveness with petroleum diesel.

Among non-edible feedstocks, municipal sludge lipids are gaining more attention nowadays as a promising lipid source which can make biodiesel production profitable [3-7]. As municipal sewage sludge is a waste, formed during the treatment of wastewater, it is a possible alternative source of lipids for the production of biodiesel, consequently lowering the wastewater treatment plant (WWTP) operation costs.

Nevertheless, the production of biodiesel form sludge lipids as well as from other low-cost feedstock poses significant processing problems in standard biodiesel manufacturing due to the high free fatty acids (FFAs) content, which in the case of the sewage sludge, can account for up to 70% of the lipid content [4,7]. The high FFA content of feedstock is known to reduce biodiesel yield significantly when using a conventional basic catalyst (*e.g.*, NaOH) because of soap formation and thus difficulties involved in separating and purifying this by-product. Acid catalysts (*e.g.*, H_2SO_4) can be used for simultaneous esterification of FFAs and transesterification of glycerides without any soap formation, however the transesterification of glycerides is much slower and the reaction times are much longer to achieve a high conversion. Homogenous acid and base catalysts are corrosive and difficult to remove after the reaction; a large amount of wastewater is produced due to the separation and purification of products and catalyst [2,8]. In order to overcome these problems, clean and promising new catalysts are required.

Recently, room-temperature ionic liquids (ILs) have been widely recognised as green replacements for volatile organic solvents and a relatively clean and promising catalyst in a variety of applications [8-10]. ILs are defined as salts that are in liquid state at below 100 ºC. They possess important attributes such as a wide liquid range, non-volatility (except at low pressures and high temperature), high catalytic activity, excellent chemical and thermal stability, potential recoverability, design possibilities, and ease of separation of the products from reactants [9-12]. Recent developments have demonstrated that the use of ILs in biodiesel production is a promising alternative for efficient green preparation of biofuels. ILs have a great potential for biodiesel production due to the ease of product isolation from the reaction, the possibility of reusing the catalyst and the low environmental impact [2,8,10]. Several publications have shown that both acidic and basic ionic liquids can function as a good catalyst for transesterification and/or esterification reactions to obtain biodiesel [13-15]. However, in the case of sewage sludge lipids, because of high FFAs content, acidic ILs are preferred. The use of Brønsted acidic ILs as a catalyst for the production of biodiesel from lipid feedstocks with high FFAs content

was successfully studied [13,16-19]. Therefore, ILs were shown to have enormous potential for the production of biodiesel from low-cost feedstock as a green replacement for corrosive and volatile acid catalysts. Although extensive studies have been performed in this area, the use of ILs as a catalyst for the esterification of sewage sludge lipids is absent from the biodiesel related literature.

Thus, the purpose of this research was to investigate a possible application of Brønsted acidic ILs with an alkane sulfonic acid functional group for the production of biodiesel from sludge lipids. Six different ILs were prepared and their effects as catalysts on the synthesis of biodiesel from sludge lipids was examined, and compared to conventional acid catalysts. The reaction conditions were optimised and the influence of the ILs on the FAMEs composition of biodiesel was also evaluated.

2. Materials and methods

2.1. Reagents

All reagents used in this study were supplied by Sigma–Aldrich. 1,4-butanesultone (purity ≥99%), 1-methylimidazole (purity ≥99%), trihexylamine (purity 96%), trifluoromethanesulfonic acid (purity 98%), sulphuric acid (purity 99.999%) and lithium(I) bistriflamide (purity ≥99%) were used to synthesise the ILs. The synthesis of biodiesel was carried out using anhydrous methanol (purity 99.8%) and sulfuric acid (purity 99.999%) at the highest purities available. Standards, 37 component FAMEs mix and methyl nonadecanoate (purity ≥99.5%) were used for the identification and quantification of fatty acid methyl esters (FAMEs). Analytical standards of C12:0, C14:0, C15:0, C16:0, C16:1, C18:0, C18:1, and C18:2 were used for free fatty acids (FFAs) determination. Oleic acid, triolein, monoolein, diolein and triolein were the standards used for identification of the lipid classes by TLC. All other solvents and reagents were high performance liquid chromatography grade and analytical reagent grade.

2.2. Synthesis of NH(SO2CF3)2

Bis{(trifluoromethyl)sulfonyl}amine, $NH(SO_2CF3)_2$ was prepared by the addition of sulfuric acid (15.0 g, 145.3 mmol) to lithium(I) bistriflamide (15.0 g, 52.3 mmol) in 60 cm^3 round-bottom tube and connected to a Kugelrohr apparatus. The mixture was heated under vacuum (60 °C, 0.1 mbar) and the product was sublimed out of the reaction vessel. White crystals where obtained (14.05 g, 50.2 mmol, 96 %; T_m = 52-54 °C).

2.3. Synthesis of the catalysts

2.3.1. Brønsted acidic imidazolium-based IL

The imidazolium-based ILs were prepared by the method previously reported in literature [20] with minor modifications. 1-methylimidazole (0.2 mol) and 1,4-butanesultone (0.2 mol) were added to a round bottomed flask fitted with a condenser and stirred vigorously at 80 ºC for 10 h under nitrogen purge. The obtained solid zwitterion was washed with toluene followed by diethyl ether and then dried under vacuum overnight (yield: 92%). Then, a stoichiometric amount of acid (H_2SO_4, CF_3SO_3H, $NH(SO_2CF_3)_2$) was added dropwise to the zwitterion and stirred overnight at 60 ºC under reflux condenser and nitrogen purge. The resultant viscous ILs were dried under vacuum before their characterisation by ^1H NMR spectroscopy (Varian NMR System 400) and before their use as a catalyst.

2.3.2. Brønsted acidic ammonium-based IL

Trihexylamine (0.2 mol) and 1,4-butanesultone (0.2 mol) were joined together in a round bottomed flask. They formed a biphasic mixture. The magnetic stirrer was added to the mixture and it was stirred forcing two layers to mix. The mixture was stirred at 130 °C under reflux condenser and nitrogen purge for 21 h. The reaction was stopped when white solid was formed within the whole volume of the reaction mixture preventing the reaction mixture from stirring. The reaction was stopped. The reaction mixture was washed with diethyl ether yielding a zwitterion white solid which was further dried under vacuum overnight (yield: 67%). The ILs was prepared by adding different acids, using the same procedure described above (Subsection 2.3.1), increasing only the temperature of the reaction to 80 °C.

2.4. Characterisation of the sewage sludge lipids

Lipids were extracted from municipal primary sludge by direct liquid-liquid extraction with sludge acidification as described previously [4].

Water content in the extracted lipids was determined according to the European standard method EN ISO 8534 [21].

The free fatty acids (FFAs) content in the extracted lipids were analysed according to the method described by [7].

The amount of saponifiable (trans/esterifiable) matter, *i.e.* the total amount of lipids that can be converted into biodiesel (FAMEs), in the extracted lipids was analysed using a modified version of Christi´s method [4], where the lipids were converted into FAMEs using acid catalyst. Then, the FAMEs (biodiesel) produced were determined according to the European standard method EN 14103 [22], using an Agilent gas chromatograph 6890GC with a flame-ionisation detector (GC-FID), equipped with a HP-INNOWax column (19091N-133). The amount of saponifiable lipids was expressed by eqn. (1),

$$\frac{\text{Saponifiable}}{\%} = \frac{\left(\frac{\text{FAMEs}}{\text{g}}\right)}{\left(\frac{\text{Lipid}}{\text{g}}\right)} \times 100 \qquad (1)$$

where FAMEs is the total FAMEs produced after trans/esterification by the reference method, determined by the GC-FID run, and Lipid is the amount of lipids used for trans/esterification.

The sludge lipids were also analysed by Thin Layer Chromatography (TLC) and by Fourier Transform Infrared (FTIR) spectroscopy to qualitatively understand its composition. The lipids were dissolved in hexane and spotted on a TLC plate which was then developed using a mobile phase of hexane/diethyl ether/acetic acid (60:40:1, v/v/v). The separated compounds were visualised using iodine vapour and identified by using authentic standards. For the FTIR analysis, the lipid sample was directly scanned using a Fourier Jasco FT/IR-600 Plus spectrometer with a diamond golden gate ATR (GS10542, Specac Ltd) reflectance cell.

2.5. Biodiesel (FAMEs) synthesis and analysis

As only the saponifiable part of the total lipids can be converted into biodiesel, the yield of biodiesel produced was expressed based on saponifiable lipids by eqn. (2),

$$\frac{\text{FAMEs}}{\%} = \frac{\left(\frac{\text{FAMEs}}{\text{g}}\right)}{\left(\frac{\text{Lipid}}{\text{g}} \times \frac{\text{Saponifiable}}{\%}\right)} \times 100 \quad (2)$$

where FAMEs is the FAMEs produced after each reaction, determined by the GC-FID run. Lipid is the amount of lipid used for each reaction and Saponifiable is the content of saponifiable in the lipids (the total FAMEs that could yield from the lipid), eqn. (1).

2.5.1. Screening of the ILs

For each experiment 1 g of sludge lipids was first preheated to the designed temperature, followed by the addition of methanol (1 cm^3) and catalyst (0.2 mmol). The reaction was carried out at 40 ºC and 60 ºC for ½ h in a sealed tube, under magnetic stirring at 500 rpm. After completion of the reaction, the mixture was cooled to room temperature and was observed to be biphasic with the desired product (biodiesel) remaining mostly in the upper phase, depending on the IL used. Then, the product was washed by adding 4 cm^3 of cool water, followed by further centrifugation of the mixture (5 min, 3600 rpm). The bottom layer contained water, IL and excess methanol and the upper layer contained biodiesel which was collected and analysed according to the European standard method EN 14103 [22] as described in Section 2.4. The yield of biodiesel (FAMEs) produced was calculated by eqn (2).

2.5.2. Optimisation study of different reaction parameters

The optimisation study was performed always using 1 g of sludge lipids preheated to the designed temperature before the addition of a known amount of methanol and [mimC_4SO_3H][SO_3CF_3] catalyst. The reaction was performed under various conditions to study the effect of the amount of methanol (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 cm^3), the amount of catalyst (3, 5, 7, 9 and 12%, based on the mass of lipids), the reaction temperature (25, 40, 60, 80, 100 and 120 ºC) and the reaction time (½, 1, 2, 5 and 10 h). Further procedures were performed as described above, in Subsection 2.5.1.

3. Results and discussion

3.1. Characterisation of the sludge lipids

Only the saponifiable part of the total lipids extracted from the sludge can be converted into biodiesel, *i.e.* the fatty acid methyl esters (FAMEs). Thus, it was important to determine the yields of saponifiable lipids and its composition. As shown in Table 1 the sludge lipids contains negligible water content, a very high amount of free fatty acids (65.1%) and 68.6% of saponifiable lipids, which can be converted into biodiesel. The saponifiable lipid fraction consists of 94.9% of FFAs and trace amounts of glycerides, suggesting that the main reaction during the conversion of sludge lipids into biodiesel is the esterification of the FFAs. This composition was confirmed by the FTIR and TLC analyses, presented in Figure 1. As shown in Figure 1(a), the infrared spectrum of the lipids gave the characteristic absorption bands between 2800–3000 cm^{-1} (assigned as the CH_3 and CH_2 stretching vibration in lipid acyl chain) and at 1710 cm^{-1} (assigned as the C=O stretching vibration of the free fatty acids group) while, the peak at 1739 cm^{-1} (assigned as the C=O stretching vibration of the ester glycerides group) [6,23] is scarcely seen, confirming a high content of free fatty acids in the lipids.

Table 1. Basic properties of primary sludge lipids and its saponifiable matter.

	Composition	wt%
Sludge lipids	Moisture	0.2 ± 0.0
	FFAs	65.1 ± 0.9
	Saponifiable	68.6 ± 2.0
Saponifiable lipids	FFAs	94.9 ± 0.9
	Glycerides*	5.1 ± 0.9

** Estimated by difference*
Values are means ± SD, n = 3

TLC analysis also demonstrated a low content of glycerides giving a less intensive and smaller spot than for the FFAs, Figure 1(b). Among the glycerides, only the presence of di- and tri-glycerides was confirmed by the TLC analysis. Additionally, besides the other scarcely visible spots on the TLC plate, the lipid sample shows an intensive spot at the top of the developed TLC plate. Apart from the saponifiable lipids (FFAs and glycerides), non-saponifiable lipids (or non-lipids) are also present in the sludge lipids [6,7]. Thus, the intensive spot at the top of the TLC plate could be attributed to non-saponifiable mater (hydrocarbons, wax esters and/or cholesterol esters) as demonstrated by Revellame et al. [24].

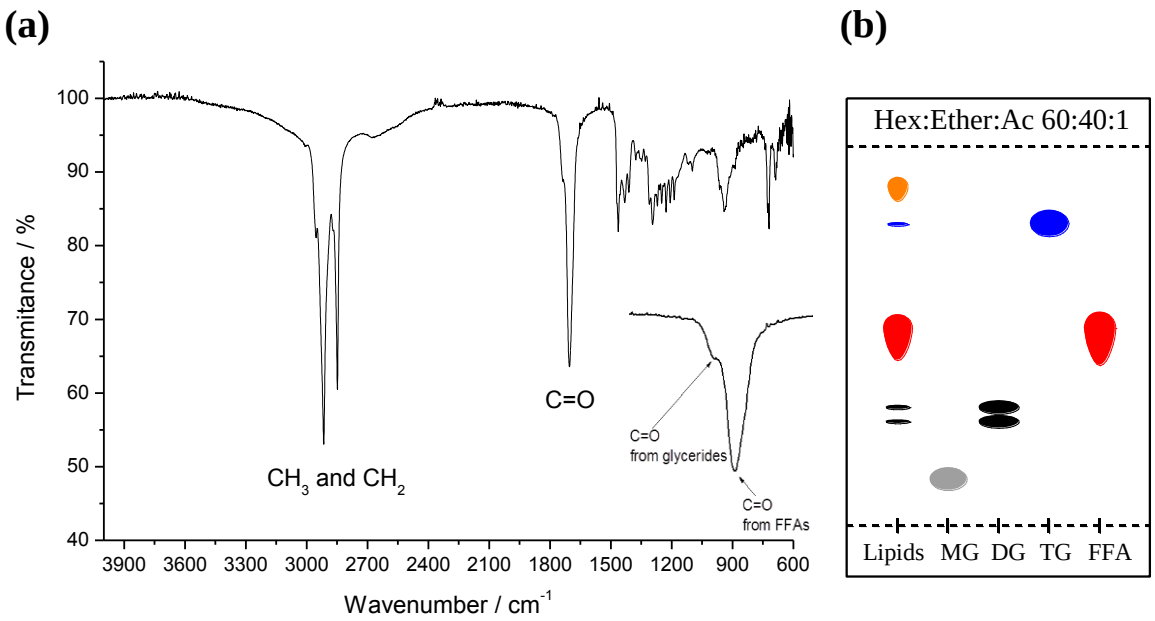

Figure 1. FTIR and TLC analysis of sludge lipids: (a) FTIR spectra of sludge lipids; (b) TLC sludge lipids and standards.

3.2. Characterisation of the IL catalysts

Brønsted acidic ILs with an alkane sulfonic acid group (−SO_3H) were especially selected for this study due to their much better catalytic activity in esterification and/or transesterification than ILs without the −SO_3H functional group [17,18]. Six different imidazolium and long chain ammonium Brønsted acidic ILs, both with butanesulfonic functional group and with different acidity of anion, were synthesised and their chemical structures are depicted as shown in Figure 2. The ILs were characterised by ^{1}H NMR spectroscopy (as given below) confirming the structure of the respective ILs.

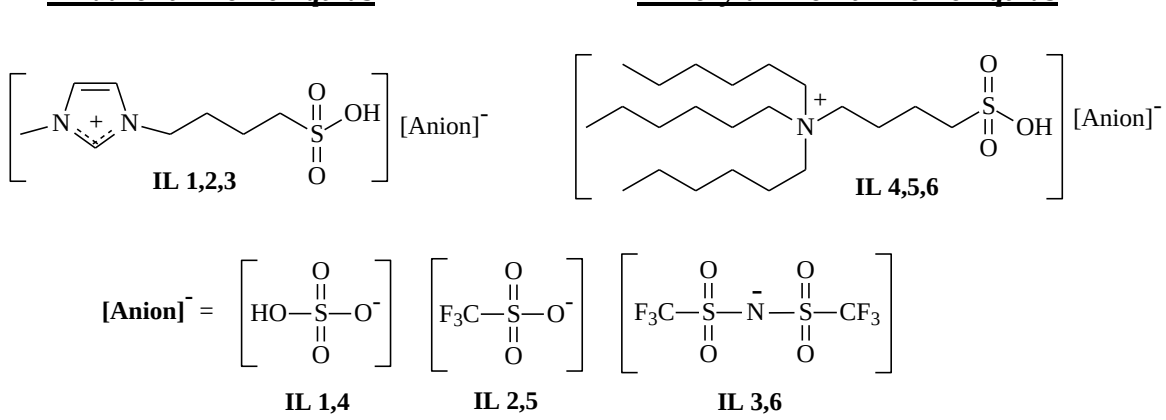

Figure 2. Structures of ionic liquids prepared and used in this study.

IL 1: 4-(3-methylimidazolium)butanesulfonic acid hydrogensulfate, [mimC_4SO_3H][HSO_4]; ^{1}H NMR spectrum (400 MHz, d^1-methanol): δ/ppm = 1.79-1.86 (m, 2H, CH_2), 2.03-2.13 (m, 2H, CH_2), 2.90 (t, 2H, CH_2-SO_3H, *J* = 7.5), 3.96 (s, 3H, N-CH_3), 4.30 (t, 2H, N-CH_2, *J* = 7), 7.61 (s, 1H, CH), 7.69 (s, 1H, CH), 8.98 (s, 1H, N-CH-N).

IL 2: 4-(3-methylimidazolium)butanesulfonic acid trifluoromethanesulfonate, [mimC_4SO_3H][SO_3CF_3]; ^{1}H NMR spectrum (400 MHz, d^1-methanol): δ/ppm = 1.99-2.09 (m, 2H, CH_2), 2.26-2.36 (m, 2H, CH_2), 3.13 (t, 2H, CH_2-SO_3H, *J* = 7.5), 4.18 (s, 3H, N-CH_3), 4.52 (t, 2H, N-CH_2, *J* = 7.1), 7.83 (s, 1H, CH), 7.91 (s, 1H, CH), 9.2 (s, 1H, N-CH-N).

IL 3: 4-(3-methylimidazolium)butanesulfonic acid bis{(trifluoromethyl) sulfonyl}amide, [mimC_4SO_3H][$N(SO_2CF_3)_2$]; ^{1}H NMR spectrum (400 MHz, d^1-methanol): δ/ppm = 1.76-1.86 (m, 2H, CH_2), 2.03-2.13 (m, 2H, CH_2), 2.87-2.90 (t, 2H, CH_2-SO_3H, *J* = 7.4), 3.96 (s, 3H, N-CH_3), 4.29 (t, 2H, N-CH_2, *J* = 7.2), 7.60 (s, 1H, CH), 7.68 (s, 1H, CH), 8.96 (s, 1H, N-CH-N).

IL 4: 4-(trihexylammonium)butanesulfonic acid hydrogensulfate, [N_{666}(C_4SO_3H)][HSO_4]; ^{1}H NMR spectrum (400 MHz, d^1-trichloromethane): δ/ppm = 0.88 (t, 9H, 3CH_3), 1.28-1.37 (m, 18H, 3(CH_2-CH_2-CH_2)), 1.58-1.70 (m, 6H, 3CH_2), 1.84-1.96 (m, 4H, 2CH_2), 3.00-3.35 (m, 2H, CH_2-SO_3H and 8H, 4CH_2-N).

IL 5: 4-(trihexylammonium)butanesulfonic acid trifluoromethanesulfonate, [N_{666}(C_4SO_3H)][SO_3CF_3]; ^{1}H NMR spectrum (400 MHz, d^1-trichloromethane): δ/ppm = 0.88 (t, 9H, 3CH_3), 1.28-1.36 (m, 18H, 3(CH_2-CH_2-CH_2)), 1.56-1.72 (m, 6H, 3CH_2), 1.82-1.95 (m, 4H, 2CH_2), 2.99-3.30 (m, 2H, CH_2-SO_3H and 8H, 4CH_2-N).

IL 6: 4-(trihexylammonium)butanesulfonic acid bis{(trifluoromethyl) sulfonyl}amide, [N_{666}(C_4SO_3H)][$N(SO_2CF_3)_2$]; ^{1}H NMR spectrum (400 MHz, d^1-trichloromethane): δ/ppm= 0.88 (t, 9H, 3CH_3), 1.29-1.36 (m, 18H, 3(CH_2-CH_2-CH_2)), 1.53-1.70 (m, 6H, 3CH_2), 1.78-1.88 (m, 4H, 2CH_2), 3.00-3.25 (m, 2H, CH_2-SO_3H and 8H, 4CH_2-N).

3.3. Biodiesel production using different catalysts

3.3.1. Screening of the ILs

The catalytic activity of the six ILs in the esterification of sludge lipids with methanol was tested in comparison to H_2SO_4 as an industrial catalyst. The reaction was conducted at 40 ºC and 60 ºC for ½ h using 1 g of sludge lipids, 1 cm^3 of methanol and 0.2 mmol of catalyst.

As shown in Table 2, for all catalysts tested the FAMEs yield increased with a temperature increase from 40 ºC to 60 ºC, showing that the reaction is temperature-dependent. Irrespective of the reaction temperature, the conventional catalyst, *i.e.* H_2SO_4, gave a higher reaction yield than with the IL catalysts. However, the IL catalysts possess some advantages over the conventional catalyst, such as the facility for product separation from the reaction, the possibility of reusing the catalyst and the lower environmental impact [2,8,10].

Table 2. Effect of ILs and temperature on the FAMEs yield.

Catalyst	Temperature / ºC	FAMEs yield / %
H_2SO_4	40	66.2
	60	86.1
$[minC_4SO_3H][HSO_4]$	40	59.1
	60	73.1
$[minC_4SO_3H][SO_3CF_3]$	40	64.7
	60	77.0
$[mimC_4SO_3H][N(SO_2CF_3)_2]$	40	63.3
	60	76.5
$[N_{666}(C_4SO_3H)][HSO_4]$	40	61.0
	60	79.0
$[N_{666}(C_4SO_3H)][SO_3CF_3]$	40	63.4
	60	79.3
$[N_{666}(C_4SO_3H)][N(SO_2CF_3)_2]$	40	56.8
	60	76.3

Reaction conditions: sludge lipids 1 g, methanol 1 cm^3, catalyst 0.2 mmol, reaction time ½ h.

The catalytic performances of the ILs containing anions with different acidities of the conjugate acid ($[HSO_4]^-$, $[SO_3CF_3]^-$, $[N(SO_2CF_3)_2]^-$) were compared to select the better performing anion. The IL anion plays a key role in the Brønsted acidic nature of the ILs; the more acidic the conjugate acid of the anion, the stronger the IL's acidity [18,25] however, this does not necessarily result in a higher catalytic activity [25]. In the case of imidazolium-based ILs, the ILs with the most acidic conjugate acid of the anions gave a higher yield of FAMEs than the weaker inorganic conjugate acid of the anion for both temperatures tested. In general, the catalytic activity of the imidazolium-based ILs for the esterification of sludge lipids increased in the following order: $[mimC_4SO_3H][HSO_4] < [mimC_4SO_3H][N(SO_2CF_3)_2] < [mimC_4SO_3H][SO_3CF_3]$. Although the conjugate acid of the $[N(SO_2CF_3)_2]^-$ anion is slightly more acidic than $[SO_3CF_3]^-$, the IL with $[SO_3CF_3]^-$ anion reached higher FAMEs yield at 40 ºC, but similar at 60 ºC (Table 2). In the case of ammonium-based ILs, the IL containing the, *i.e.* $[N(SO_2CF_3)_2]^-$ anion, gave the lowest FAMEs yield for both temperatures tested. The IL with $[SO_3CF_3]^-$anion reached the highest FAMEs yield at 40 ºC and it gave equal results to the IL containing $[HSO_4]^-$ anions at 60 ºC reaction temperature. In general, the catalytic activity of the ammonium-based ILs for the esterification of sludge lipids increased in the following order: $[N_{6\,6\,6}(C_4SO_3H)][N(SO_2CF_3)_2] < [N_{6\,6\,6}(C_4SO_3H)][HSO_4] < [N_{6\,6\,6}(C_4SO_3H)][SO_3CF_3]$. These results indicated that for both, imidazolium and ammonium ILs used in this study, the IL containing $[SO_3CF_3]^-$ anion showed the highest catalytic activity,

gaining the highest FAMEs yield (Table 2). Additionally, the ILs containing $[SO_3CF_3]^-$ anion are more stable than the others tested [14], which is important for IL recycling. Furthermore, the price of ionic liquids with the $[SO_3CF_3]^-$ anion is much cheaper than $[N(SO_2CF_3)_2]^-$ones.

Comparing the cation composition of the ILs, with the $[SO_3CF_3]^-$ anion, it can be observed in Table 2, that the catalytic efficiency of the ILs depends on the temperature. At 40 ºC the IL with imidazolium cation achieved slightly higher FAMEs yield than the IL with ammoniumcation, 64.7% and 63.4% respectively. The effect of increasing the reaction temperature to 60 ºC, showed an increase in the FAMEs yield for both ILs and an increase in the activity of $[N_{6\,6\,6}(C_4SO_3H)][SO_3CF_3]$ IL which gave slightly better results than $[mimC_4SO_3H][SO_3CF_3]$, 79.3% and 77.0% respectively. However, $[N_{6\,6\,6}(C_4SO_3H)][SO_3CF_3]$ was not selected for further study due to the presence of other unidentified peaks in the GC chromatograms of the FAMEs produced, as described in Subsection 3.3.2. For that reason, in this study, $[mimC_4SO_3H][SO_3CF_3]$ was selected as the best catalyst for the reaction and was used in the following experiments to optimise different reactions parameters.

3.3.2. Effect of various ILs catalyst on the FAME composition

The influence of IL catalysts on the FAME composition of biodiesel in comparison to a conventional H_2SO_4 catalyst was evaluated and the results are shown in Table 3.

Table 3. FAME composition of biodiesel produced from primary sludge lipids by different catalysts (%, w/w).

FAME from fatty acid	H_2SO_4	$[mimC_4SO_3H]$			$[N_{666}(C_4SO_3H)]$		
		$[HSO_4]$	$[SO_3CF_3]$	$[N(SO_2CF_3)_2]$	$[HSO_4]$	$[SO_3CF_3]$	$[N(SO_2CF_3)_2]$
Lauric (C12:0)	1.25 ± 0.01	1.32 ± 0.08	1.32 ± 0.07	1.25 ± 0.4	1.11 ± 0.08	1.21 ± 0.12	1.11 ± 1.25
Myristic (C14:0)	3.59 ± 0.01	3.63 ± 0.06	3.63 ± 0.02	3.65 ± 0.10	3.43 ± 0.13	3.49 ± 0.03	3.42 ± 0.04
Pentadecanoic (C15:0)	0.53 ± 0.01	0.53 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.51 ± 0.02	0.52 ± 0.01	0.52 ± 0.01
Palmitic (C16:0)	39.75 ± 0.01	39.57 ± 0.20	39.72 ± 0.29	39.89 ± 0.27	38.07 ± 1.50	37.80 ± 1.60	38.38 ± 0.98
Palmitoleic (C16:1)	1.65 ± 0.01	1.63 ± 0.01	1.64 ± 0006	1.63 ± 0.05	1.56 ± 0.10	1.58 ± 0.05	1.57 ± 0.02
Heptadecanoic (C17:0)	0.40 ± 0.01	0.42 ± 0.04	0.41 ± 0.02	0.41 ± 0.01	0.34 ± 0.02	0.34 ± 0.01	0.35 ± 0.01
Stearic (C18:0)	15.32 ± 0.08	15.06 ± 0.09	15.12 ± 0.08	14.93 ± 0.16	14.52 ± 0.18	14.88 ± 0.35	15.02 ± 0.55
Oleic (C18:1)	27.38 ± 0.52	27.31 ± 0.58	27.27 ± 0.54	27.28 ± 0.19	26.59 ± 0.92	27.12 ± 0.49	26.92 ± 1.14
Linoleic (C18:2)	3.85 ± 0.08	3.90 ± 0.01	3.82 ± 0.05	3.78 ± 0.14	3.65 ± 0.07	3.73 ± 0.01	3.66 ± 0.05
Arachidic (C20:0)	0.53 ± 0.11	0.58 ± 0.11	0.55 ± 0.01	0.53 ± 0.11	0.78 ± 0.14	0.55 ± 0.04	0.57 ± 0.04
Eicosenoic (C20:1)	0.56 ± 0.13	0.50 ± 0.07	0.59 ± 0.09	0.55 ± 0.13	0.68 ± 0.14	0.50 ± 0.12	0.58 ± 0.10
Others	5.19 ± 0.45	5.55 ± 0.06	5.39 ± 0.29	5.57 ± 0.35	8.74 ± 1.31	8.27 ± 1.25	7.92 ± 1.96

Average of the results obtained at 40 ºC and 60 ºC, sludge lipids 1g, methanol 1 cm^3, catalyst 0.2 mmol, reaction time ½ h.

The FAME profiles of biodiesel produced by each IL catalyst are very similar to each other and to those of the conventional H_2SO_4 catalyst. The same fatty acid methyl esters were found for all catalysts tested with a predominance of methyl esters from palmitic (38%-40%), oleic (27%) and stearic (15%) acids, showing no influence of the catalyst on the FAME composition. However, an important difference is observed in the content

of "others". It was found to be present in a much larger amounts in the three ammonium-based ILs tested as compared to the other catalysts (Table 3). For all imidazolium-based IL catalysts the "others" are exactly the same as for the H_2SO_4 catalyst; the same peaks were obtained in the GC chromatograms. This fraction of "others" mainly consists of hydroxy and oxy fatty acids and branched-chain fatty acid methyl esters [4]. On the other hand, for all the ammonium-based IL catalysts, the percentage of "others" was increased by an unidentified compound; broad peak present only in the chromatograms of the FAMEs produced by the ammonium-based ILs. For that reason, further investigation on trihexylsulfobutaneammonium-based ILs is necessary, not only to improve its synthesis but also to check its stability, physical properties and impurity content.

3.4. Optimisation study of different reaction parameters

The influence of different reaction variables on the biodiesel yield was studied using $[mimC_4SO_3H][SO_3CF_3]$ as a catalyst and the results are shown in Figure 3.

3.4.1. Effect of the amount of methanol

The amount of methanol is a very important factor that affects the yield of biodiesel. Although the stoichiometric molar ratio for esterification and transesterification reaction is 1:1 and 3:1 (methanol to lipids) respectively, it is well known that excess alcohol is required to shift the reaction equilibrium towards the products and produce more FAMEs (this is because the reactions are reversible). Thus, the influence of methanol on the reaction was studied in the range of 0.5 to 3.5 cm^3 which corresponds to approximately 5:1 and 35:1 methanol to saponifiable lipids molar ratio, respectively. The experiments with different amounts of methanol were conducted at 40 ºC for ½ h using 1 g of sludge lipids and 7% (0.2 mmol) of the catalyst, based on the mass of lipids. As shown in Figure 3(a), the FAMEs yield increased considerably from 44% to 65% by increasing the amount of methanol from 0.5 to 1 cm^3. Further increases in the amount of methanol to 2.5 cm^3 showed only a slight increase in the FAMEs yield, giving 68% for 2 and 2.5 cm^3 of methanol. When the amount of methanol was increased beyond 2.5 cm^3, the yield of FAMEs decreased slightly (Figure 3(a)). This concurred with previous findings in literature, because too much methanol was found to cause a dilution in the concentration of IL [13,18,19,25]. Additionally, the use of a larger amount of methanol would not facilitate IL recycling due to the separation of a larger amount of alcohol from the dissolved ILs. Therefore, the slight increase in the FAMEs yield beyond 1 cm^3 of methanol was negligible and 1 cm^3 of methanol (approximately 10:1 methanol to saponifiable lipids molar ratio) was selected as the optimal amount of methanol for the reaction.

3.4.2. Effect of the amount of catalyst

The catalyst amount is also a very important factor that affects the reaction. The number of sulfonic acid group ($-SO_3H$) should be in proportion to the reaction in order to obtain a high conversion. Figure 3(b) shows the effects of various concentrations of $[mimC_4SO_3H][SO_3CF_3]$ catalyst (expressed as a percentage based on the mass of lipids) on the esterification of sludge lipids with methanol. The experiments were carried out varying the catalyst concentrations between 3% and 12% while other parameters were fixed at 40 ºC, ½ h using 1 g of sludge lipids and 1 cm^3 of methanol. Figure 3(b) clearly shows that an increase of the amount of catalyst from 3% to 7% resulted in an increase in the FAMEs yield, from 49% to 65%. However, when the amount of catalyst exceeded 7%, no increase in conversion was observed, *e.g.* for 9% and only a slight increase was observed for 12% of

catalyst (66% of FAMEs yield). Thus, considering the reaction rate and the cost of the catalyst, the optimum amount of catalyst was found to be 7%.

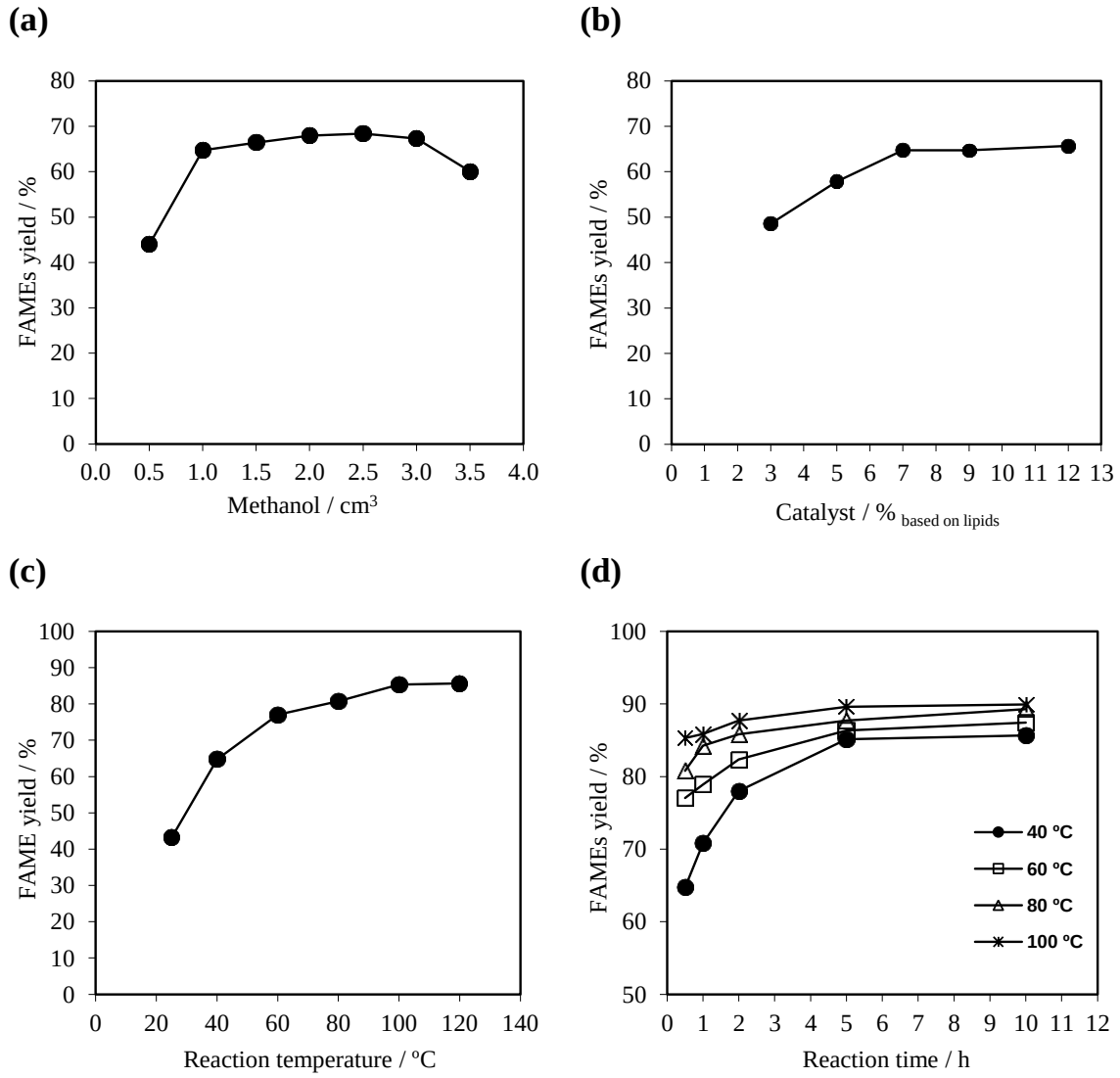

Figure 3. FAMEs (biodiesel) yield *vs.* reaction variables when [mimC$_4$SO$_3$H][SO$_3$CF$_3$] IL was used as a catalyst: (a) effect of methanol amount (reaction conditions: sludge lipids 1 g, catalyst 7%, reaction temperature 40 ºC, reaction time ½ h); (b) effect of catalyst amount (reaction conditions: sludge lipids 1 g, methanol 1 cm^3, reaction temperature 40 ºC, reaction time ½ h); (c) effect of temperature (reaction conditions: sludge lipids 1 g, methanol 1 cm^3, catalyst 7%, reaction time ½ h); (c) effect of time at different temperatures (reaction conditions: sludge lipids 1 g, methanol 1 cm^3, catalyst 7%).

3.4.3. Effect of reaction temperature

The reaction temperature is another important parameter which influences the reaction and the yield of FAMEs product. As demonstrated in Subsection 3.3.1, a higher reaction temperature gave higher FAMEs yield. Thus, the effect of temperature on the reaction was investigated in the temperature range of 25-120 ºC for a period of ½ h using 1 g of sludge lipids, 1 cm^3 of methanol and 7% of [mimC$_4$SO$_3$H][SO$_3$CF$_3$] catalyst. As shown in Figure 3(c), when the reaction temperature was 25 ºC only 43% of the saponifiable lipid was converted into FAMEs. The FAMEs yield increased sharply with a rise in temperature, reaching 85% at 100 ºC. However, further increases in temperature had no significant effect on the product yield, (86% at 120 ºC). Furthermore, at 120 ºC

the reaction system exhibited a very dark brown colour. This suggests that increasing the reaction temperature improves the catalytic activity, but only to a certain extent.

3.4.4. Effect of reaction time at different temperatures

Figure 3(d) shows the effect of the reaction time on the FAMEs yield at different temperatures. The experiments were conducted using the established optimal parameters of 1 g of sludge lipids, 1 cm^3 of methanol, 7% of $[mimC_4SO_3H][SO_3CF_3]$ catalyst. As shown in Figure 3(d), the reaction rate and the yield of FAMEs were enhanced with increased reaction temperature. For all temperatures tested, the FAMEs yield increased steadily with reaction time up to 5 h and remains steady afterwards. The reaction moved closer to equilibrium after 5 h, and the product yield did not increase significantly when the reaction time was prolonged to 10 h. The maximum yield of 90% was achieved at 100 ºC for 5 h and 10 h. Therefore, 100 ºC and 5h was selected as the optimum reaction conditions for the esterification of 1g of sludge lipids with 1 cm^3 of methanol catalysed by using 7% of $[mimC_4SO_3H][SO_3CF_3]$ ionic liquid.

3.5. Evaluation of the IL as a catalyst for biodiesel production

The above results demonstrated that the Brønsted acidic IL $[mimC_4SO_3H][SO_3CF_3]$ was effective in catalysing the esterification of wastewater sludge lipids with methanol, reaching 90% of biodiesel under the optimum reaction conditions. Therefore, the cheap and non-edible lipid feedstock containing almost only FFAs, *i.e.* sludge lipids, was successfully converted into biodiesel by the green catalyst. Furthermore, previous studies demonstrated that $[mimC_4SO_3H][SO_3CF_3]$ IL also have a high potential for catalysing the transesterification of waste cooking oil, lard and glycerides feedstocks, giving 99% of biodiesel yield in both cases, however a much larger amount of methanol and catalyst were used than in this study [14,26]. This clearly demonstrates that the IL is able to efficiently catalyse the conversion of different lipid feedstocks, especially waste feedstock, into biodiesel.

Additionally, one of the reasons that render ILs as green catalysts is their recyclable nature. After completion of the esterification of sludge lipids, the IL is easily separated from the biodiesel product by decantation (Subsection 2.5.1). Then the excess methanol and the water formed during esterification must be removed from the IL by distillation and hence the IL could be reused in further reactions. The catalytic stability of Brønsted acidic ILs with an alkane sulfonic acid group has been widely studied in literature, demonstrating excellent reusability in biodiesel synthesis even up to 10 times [13,17,18,25]. In this study, the IL was reused only once under the optimised reaction conditions determined above, and the yield of biodiesel only slightly decreased from 90% to 89%, suggesting its stability. Additionally, the stability of the recovered IL, after first and second reaction, was also checked by ^{1}H NMR spectroscopy. No additional peaks were found in the ^{1}H NMR spectra of the recovered IL as compared to the initial IL, confirming its stability. The results of the ^{1}H NMR of recovered IL were the same as for IL2, see Section 3.2.

4. Conclusions

The six Brønsted acidic ILs with an alkane sulfonic acid group tested in this study showed similar catalytic performance for the conversion of sludge lipids into biodiesel. However, the IL containing $[SO_3CF_3]^-$ anion revealed a higher biodiesel yield than the ILs with other anions tested for both imidazolium and ammonium ILs.

The imidazolium IL, [mimC_4SO_3H][SO_3CF_3] was selected as a better catalyst due to an easier, less time and energy intensive, synthesis and a purer biodiesel than for the equivalent ammonium IL. [mimC_4SO_3H][SO_3CF_3] catalyst was efficient for the synthesis of biodiesel from wastewater sludge lipids, reaching a yield of 90% under the optimum reaction conditions. Compared with conventional sulfuric acid catalyst, the Brønsted acidic IL possesses many advantages such as the facility for product separation from the reaction, catalyst reusability, the possibility of converting different lipid feedstock into biodiesel without the saponification problem and the low environmental impact. Therefore, an efficient catalyst was provided for the green synthesis of biodiesel from low-cost waste feedstocks, such as wastewater sludge lipids or waste cooking oils. Furthermore, the utilisation of sludge lipids, non-edible and low-cost feedstock, for biodiesel production can solve both the economic and environmental problems associated with the biodiesel process and waste management in WWTPs.

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Chapter 8

Conclusions and future work

1. Conclusions

The main conclusions arising from the results provided by this thesis are listed below:

- Municipal sewage sludge produced during wastewater treatment is a potential source of suitable lipids for the production of biodiesel. Among the four types of sludge generated in WWTPs, the primary sludge is the most beneficial lipid feedstock for biodiesel production due to the highest amount of lipids extracted and the highest amount of biodiesel produced.
- Sludge pre-treatments (ultrasonic and mechanical disintegration) are not useful for lipid extraction and thus for biodiesel production. However, an extraction from acidified sludge produces lipids less contaminated with non-saponifiable material, non-convertible to biodiesel.
- Common sludge drying methods, which require a high energy input, decrease the yield of lipids as well as the saponifiable fraction, thus reduce the biodiesel production.
- Direct liquid-liquid extraction of lipids from liquid sludge is feasible after previous sludge acidification and compares well with the conventional method. The liquid-liquid extraction process eliminates the cost associated with sludge drying, reducing the cost of production of biodiesel.
- The scale-up and economic analysis of the process indicates that the biodiesel production via direct liquid-liquid extraction of lipids from primary sludge is economically feasible and more cost-effective than from dry sludge. Under the optimised extraction parameters, the break-even price of biodiesel was estimated to be 1232 $/t, being economically competitive with the current cost of fossil diesel.
- The technology currently available (classical organic solvent, i.e. hexane, and liquid-liquid extraction) allows easy extraction of lipids from municipal primary sludge. Nevertheless, the non-volatile ionic liquids, alternatives to the conventional volatile organic solvents, also have a high potential for direct lipid extraction from liquid primary sludge, giving results comparable to the classical method.
- Especially, the tetrakis(hydroxymethyl)phosphonium chloride, $[P(CH_2OH)_4]Cl$, is very promising due to its ability to recover the cellulose and proteins, together with lipids in one step, giving another advantage over the organic solvents. Additionally this ionic liquid is also an efficient solvent to extract lipids from microalgae, another alternative to edible oil crops, to produce biodiesel.
- The Brønsted acidic ionic liquids with an alkane sulfonic acid group show a good catalytic performance for the conversion of sludge lipids into biodiesel. Compared with conventional sulfuric acid catalyst, the ionic liquids possesses many advantages such as the facility for product separation from the reaction, catalyst reusability, the possibility of converting different lipid feedstock into biodiesel without the saponification problem and the low environmental impact.

2. Future work

As a result of the knowledge gained in this thesis, proposals for possible future work are outlined below:

- As a first recommendation for the proposed biodiesel production via direct extraction of lipids from liquid primary sludge, further investigation to improve lipid extraction and biodiesel purification technologies is required in order to decrease the biodiesel production cost.
- As demonstrated in this thesis, the lipid concentration in secondary sludge is too low to be economically viable as a feedstock for biodiesel production. However, the lipid enhancement in secondary sludge is possible when the bacteria present in the sludge are subjected to stress conditions (deficiency in nitrogen, oxygen or micronutrients), producing lipid storage compounds. Therefore, future work should be focused on the selection and optimisation of a strategy to increase the concentration of lipids in secondary sludge. In this way, the possibility of biodiesel production from secondary sludge will increase the overall yield of biodiesel produced from municipal sewage sludge.
- This work preliminary demonstrated (Chapter 3) the feasibility of anaerobic digestion of lipid-extracted sludge to produce biogas as this process is widely used in municipal WWTPs. Therefore, future work should also concentrate on the implementation of biogas production from lipid-extracted sludge in the biodiesel process, to evaluate the remaining potential for biogas generation. In this context, the assessment of feasibility of biogas production from exhausted sludge, together with biodiesel from primary, as well as, from lipid enhanced secondary sludge, aims for a higher positive energy balance in respect to current WWTP. However, the WWTP should be re-engineered in order to modify processes.
- Regarding the utilisation of ionic liquids in lipid extraction from sewage sludge, it would be interesting to evaluate the feasibility of ionic liquids to extract lipids from lipid enhanced secondary sludge. The use of ionic liquids as a solvent could disrupt cell walls of secondary sludge bacteria and improve the lipid efficiency as the lipids are mainly intracellular.
- Another topic worthy of development in the future, is to design an ionic liquid in such a way to be suitable to work as both, solvent and catalyst. In this context, the *in situ* production of biodiesel from wet sewage sludge would be possible, minimising the time, amount of solvent and number of equipment involved.

Appendix

About the author

Magdalena Olkiewicz was born in Konin, Poland, in 1983. She obtained her M.Sc. degree in Chemistry, specialising in Environmental Chemistry and Pedagogy in Chemistry, in 2008 at the Chemistry Faculty of Adam Mickiewicz University in Poznań, Poland. During her university education, she was selected under the Erasmus Programme and under the Erasmus Lifelong Learning Programme. In 2005 as an Erasmus student, she spent six months in the Chemistry Department of Loughborough University, United Kingdom. In 2007, under Erasmus Lifelong Learning Programme, she did a six month internship in the Research and Development Department (Medicinal Chemistry Group in the Drug Discovery area) of PharmaMar S.A. in Colmenar Viejo, Madrid, Spain. After receiving her M.Sc. degree, she moved to Madrid, where she was employed as a laboratory technician by Vivotecnia Research S.L. in Tres Cantos, Madrid, Spain. In January 2011 she was awarded a predoctoral contract for the formation of research personnel, FI Programme of Autonomous Government of Catalonia, to undertake the Ph.D. research at the Department of Chemical Engineering of Rovira i Virgili University in Tarragona, Spain. During her Ph.D., she experienced a three months research stay at the QUILL Research Centre of Queen's University Belfast, Northern Ireland, United Kingdom. Research topic: Biodiesel production using Ionic Liquids.

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PRODUCTION OF BIODIESEL FROM SLUDGE GENERATED IN MUNICIPAL WASTEWATER TREATMENT PLANTS.
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